

**GENETIC STUDIES OF RICE (*Oryza sativa*) FOR IMPROVEMENT
OF YIELD AND AROMA IN TOGO**

By

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DECLARATION

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

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ABSTRACT

In Togo, the quantity of rice produced is very low compared to the needs of the country. Farmers and consumers prefer aromatic rice. The main objective of this research was to increase the local production and supply farmers with aromatic and higher yielding varieties. It was conducted to lay the foundation of a rice breeding programme in Togo. Therefore, the research investigated farmers' preferences and production constraints, established a germplasm collection and studied its genetic diversity, characterized the germplasm for yield and aroma, developed aromatic and high yielding F₁ hybrids and estimated genetic parameters. The methods used to achieve the set goals consisted of focus group discussions and questionnaire survey to record information from farmers, collection of accessions around the country through prospecting survey, genetic diversity studies (notably principal components and cluster analyses), phenotypic and genotypic characterization of the germplasm for aroma and yield, as well as crosses of the parents identified (using the North Carolina Design II) and genetic analysis [notably Genotype by Environment (GxE) interactions, combining ability and genetic variance components] of yield and its component traits at four locations. From the participatory rural appraisal, absence of aroma in the new varieties introduced was the main reason for which farmers failed to adopt them. Their major preferences were presence of aroma, yield and earliness. Birds, insects and iron toxicity were their major production constraints. Collected germplasm comprising of 50 accessions were grouped, after diversity study using 30 agro-morphological traits and 5,736 informative Single Nucleotide Polymorphism (SNP) markers, in four and five clusters respectively, with 13 distinct samples of IR 841, the most widely cultivated aromatic rice variety in the country. Three aromatic (IR 841_Sot, Orylux 5 and Chapeau vert) and six high yielding (Chinoivi, Sipi, Berice 21, Gambiaca_Dan, Londo londo and Wita 4) parents were selected from the characterization, and used respectively as male and female parents to develop 18 F₁ progeny

families. The evaluation of the 18 F₁s and their nine parents in the four agro-ecological zones of the country showed large phenotypic variability in the performance of the 27 genotypes for yield and its component traits. The hybrid N3A1 had the highest yield across the sites: 13 t/ha. Significant GxE interactions were detected for the four traits: grain yield, number of panicles per plant, number of grains per panicles, and 100-grain weight. The site Adéta produced more stable yield and number of panicles per plant but had the lowest performance for all the traits. All the traits were highly heritable (> 0.85) except for yield. The parent N5 had a significant general combining ability (GCA) for number of grains per panicle, and the hybrids N1A1 and N6A3 had significant specific combining ability (SCA) for 100-grain weight. No parent nor hybrid had significant GCA nor SCA for yield, showing the challenges to develop F₁ hybrid rice varieties for farmers with these nine parents used. However, the progeny families from the parent N5 *viz* N5A1, N5A2 and N5A3 will be advanced using single seed descent selection method.

DEDICATION

To all who believed in me, especially my mother **TEKPO Adzovi Ahouéfa**, my wife **SAMARI Dédé Edwige Nicole**, my children **Luc Aniel** and **Emeraude Florelle**, and my brothers **Kossi Kataora**, **Kodjo Akonta** and **Enyo Atéyaba**. Kindly find through the achievement of this work, my infinite gratitude.

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TABLE OF CONTENTS

DECLARATION	i
ABSTRACT	ii
DEDICATION	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	xi
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvi
CHAPTER ONE: GENERAL INTRODUCTION	1
CHAPTER TWO: LITERATURE REVIEW	5
2.1. Biology of rice	5
2.1.1. Taxonomy	5
2.1.2. Botanical description	6
2.2. Origin and geographical distribution of cultivated rice	7
2.3. Rice production	10
2.3.1. Ecology and cropping systems	10
2.3.2. Distribution of rice	13
2.3.3. Global production and consumption	14
2.3.4. Production in Togo	15
2.3.5. Production constraints	17
2.3.5.1. Biotic constraints	17
2.3.5.2. Abiotic constraints	18
2.4. Importance of rice	18
2.4.1. Nutritional value of rice	18
2.4.2. Medicinal importance of rice	20
2.4.3. Economic importance of rice	21
2.4.4. Ethno-botany	21
2.5. Rice yield	22
2.5.1. Rice yield over time	23
2.5.2. Rice yield components	24
2.5.3. Genes controlling rice yield and its component traits	24
2.6. Rice aroma	26

2.6.1. Volatile compounds associated with aromatic rices	26
2.6.2. Genetics of rice aroma	27
CHAPTER THREE: STATUS OF RICE VARIETIES ADOPTION, FARMERS’	
PREFERENCES AND PRODUCTION CONSTRAINTS IN TOGO	29
3.1. Introduction	29
3.2. Materials and Methods	30
3.2.1. Documentation of varieties introduced from 1980 to 2013	30
3.2.1.1. Establishment of a list of varieties introduced by the NARS	30
3.2.1.2. Collection of information on varieties introduced by the	
NAES/farmers	31
3.2.2. Assessment of farmers’ reasons of failure of adoption of varieties	
introduced, their preferred traits and production constraints	33
3.2.2.1. Focus group discussions	34
3.2.2.2. Questionnaire survey	34
3.3. Results	36
3.3.1. Varieties introduced by the NARS	36
3.3.2. Varieties introduced by the NAES and farmers	38
3.3.3. Status of adoption of the varieties introduced in Togo	39
3.3.4. Farmers’ reasons of failure of adoption of introduced varieties	45
3.3.5. Farmers’ varietal preferences	47
3.3.6. Rice production constraints	51
3.4. Discussion	55
3.5. Conclusion	61
CHAPTER FOUR: COLLECTION AND GENETIC DIVERSITY ANALYSIS OF	
RICE GERMPLASM	62
4.1. Introduction	62
4.2. Materials and Methods	62
4.2.1. Germplasm collection	62
4.2.1.1. Material used for collection	62
4.2.1.2. Methodology	63
4.2.1.3. Data collected	63
4.2.1.4. Data analysis	63
4.2.2. Multiplication of the accessions	64

4.2.3. Agro-morphological characterization of the accessions	64
4.2.3.1. Location	64
4.2.3.2. Plant materials used	64
4.2.3.3. Experimental design	65
4.2.3.4. Conduction of the trial	65
4.2.3.5. Data collected	65
4.2.3.6. Data analysis	67
4.2.4. Molecular analysis	67
4.3. Results	69
4.3.1. Accessions collected and their descriptive statistics	69
4.3.2. Agro-morphological characterization	71
4.3.2.1. Discriminative traits	71
4.3.2.2. Correlations between discriminative traits	73
4.3.2.3. Principal components	74
4.3.2.4. Accessions' clusters	76
4.3.3. Molecular characterization	79
4.3.3.1. Informative SNPs	79
4.3.3.2. Principal components	80
4.3.3.3. Genotypes' clusters	81
4.3.4. Comparison between phenotypic and SNP data	83
4.4. Discussion	84
4.5. Conclusion	89
CHAPTER FIVE: PHENOTYPIC AND MOLECULAR SCREENING OF THE	
ACCESSIONS FOR YIELD AND AROMA	91
5.1. Introduction	91
5.2. Materials and Methods	92
5.2.1. Plant materials	92
5.2.2. Sensory evaluation for aroma	93
5.2.2.1. Methodology	93
5.2.2.2. Data collected	93
5.2.3. Evaluation of yield and its component traits	93
5.2.3.1. Location	93
5.2.3.2. Experimental design	94

5.2.3.3. Methodology	94
5.2.3.4. Data collected	94
5.2.4. Molecular characterization	94
5.3. Results	97
5.3.1. Aromatic accessions identified by sensory evaluation	97
5.3.2. Analysis of variance of yield and its component traits	98
5.3.3. Distribution of markers for grain quality in the germplasm	99
5.3.4. Distribution of yield and its components markers in the germplasm	100
5.3.5. Correlations between aroma and yield components traits	101
5.3.5.1. At phenotypic level	101
5.3.5.2. At molecular level	102
5.3.7. Parents selected	104
5.4. Discussion	105
5.5. Conclusion	107
CHAPTER SIX: GENETIC ANALYSIS OF YIELD AND ITS COMPONENT	
TRAITS	108
6.1. Introduction	108
6.2. Materials and Methods	109
6.2.1. Crosses	109
6.2.1.1. Location	109
6.2.1.2. Parents	109
6.2.1.3. Mating design	110
6.2.1.4. Methodology	110
6.2.2. Evaluation of the genotypes	111
6.2.2.1. Plant material	111
6.2.2.2. Locations	111
6.2.2.3. Pot experiments	112
6.2.2.4. Data collected	112
6.2.3. Data analysis	113
6.3. Results	113
6.3.1. Combined analysis of variance for yield and its component traits	113
6.3.2. Performance of parents and their F1 Hybrids	115
6.3.3. Genotype by environment interaction analysis for yield and its	

components	118
6.3.4. Phenotypic correlations	121
6.3.5. Estimate of genetic parameters of yield and its component traits	122
6.4. Discussion	124
6.5. Conclusion	126
CHAPTER SEVEN: GENERAL CONCLUSION AND RECOMMENDATIONS	127
BIBLIOGRAPHY	131
APPENDICES	138

LIST OF TABLES

Table 2.1: Taxonomy of rice (<i>Oryza</i> spp)	5
Table 2.2: Area, average production and yield of rice recorded in Togo from 2012 to 2016	16
Table 2.3: Composition per 100 g of edible portion of milled Asian rice ..	19
Table 2.4: Available genes controlling some traits related to rice yield and its components	25
Table 3.1: Sites of focus group discussions on rice varieties introduced by NAES and farmers	32
Table 3.2: List of rice varieties introduced in Togo by the NARS from 1980 to 2013 and presented to farmers for adoption through PVS approach	37
Table 3.3: List of rice varieties introduced in the study area by NAES and farmers	38
Table 3.4: List of introduced rice varieties unknown, abandoned or lost by farmers in the study area	40
Table 3.5: List of introduced rice varieties currently cultivated by a few farmers in the study area	41
Table 3.6: Main reasons for non-adoption and some farmers' preferred traits of the introduced rice varieties.....	46
Table 3.7: List of farmers' varietal preferences per site	48
Table 3.8: Farmers' perception on their major rice varietal preferences	50
Table 3.9: Relationship between farmers' perception on their major rice varietal preferences in rainfed and irrigated lowlands	51
Table 3.10: List of biotic and abiotic constraints per site	52
Table 3.11: Farmers' perception on their major production constraints	53
Table 3.12: Relationship between farmers' perception on their major constraints in rainfed and irrigated lowlands	54
Table 4.1: Agro-morphological traits evaluated	66
Table 4.2: Information on the accessions collected	70
Table 4.3: Summary statistics of the 11 quantitative traits evaluated	72
Table 4.4: Wilk's lambda criterion, F value and p-value for 25 variables evaluated	73

Table 4.5: Correlation matrix for the 11 discriminative traits	74
Table 4.6: Eigenvalue and variance explained by the principal components (PCs)	75
Table 4.7: Contribution of the 11 discriminative traits to the first eight principal components	76
Table 4.8: Summary statistics on the 21,253 SNP markers used for the characterization of the 50 rice genotypes	79
Table 5.1: Rice accessions used for phenotypic and molecular screening for yield and aroma.....	92
Table 5.2: SNP markers used for the molecular screening of the 50 rice accessions	96
Table 5.3: Aromatic accessions identified after the sensory evaluation	97
Table 5.4: Analyse of variance using REML for yield and its component traits	98
Table 5.5: Performance of the eight higher yielding accessions in yield and its component traits	99
Table 5.6: Rice grain quality SNP alleles and their frequencies in the germplasm	100
Table 5.7: Correlation matrix of the caryopsis scent, yield and its component traits	102
Table 5.8: Correlation coefficients between eight SNP markers linked to yield and aroma	103
Table 5.9: Yield, fragrance and their markers alleles for eight rice accessions.....	104
Table 5.10: Parents selected for the development of aromatic and yielding lines	105
Table 6.1: Characteristics of the nine accessions used as parents in the crosses	109
Table 6.2: Eighteen rice F ₁ hybrids and their nine parents tested on four locations in Togo	111
Table 6.3: Summary of the results of combined ANOVA of the yield and its component traits on four sites in Togo	114

Table 6.4: ANOVA for AMMI model for the yield and its component traits in four locations.....	114
Table 6.5: Correlation matrix presenting Pearson coefficient between yield and its component traits collected on F ₁ hybrids and their parents	121
Table 6.6: F probability for yield and its component traits from NCII design ANOVA	122
Table 6.7: GCA estimates of the nine parents for yield and its component traits	122
Table 6.8: SCA estimates of the 18 F ₁ hybrids for yield and its component traits	123
Table 6.9: Genetic variance components of yield and its component traits	123

LIST OF FIGURES

Figure 2.1: The rice plant	6
Figure 2.2: Schematic representation of the evolutionary pathways of Asian and African cultivated rices	9
Figure 2.3: African rice producing area	13
Figure 2.4: Average production (in tons) and average yield (in tons per hectare) of major cereals in Togo from 2012 to 2016	16
Figure 2.5: QTL for rice yield and its components in 4 populations derived from an <i>indica x indica</i> cross	25
Figure 3.1: Number of introduced rice varieties currently cultivated per site and per actor of introduction	42
Figure 3.2: Distribution of the introduced rice varieties in terms of the actors of their introduction as well as their current status of cultivation ...	44
Figure 3.3: Number of sites at which each rice varietal preference was mentioned by a group of farmers	49
Figure 3.4: Number of sites at which each production constraints was mentioned by a group of farmers	53
Figure 4.1: Number of accessions collected per site	69
Figure 4.2: Scree plot with Eigenvalues (a) and proportion of variance explained (b) by the principal components	74
Figure 4.3: Evolution of the variance explained by the number of clusters	76
Figure 4.4: Dendrogram of 50 rice accessions from Togo based on 11 discriminative agro-morphological traits using average linkage method	77
Figure 4.5: Minimum spanning tree presenting the 50 rice accessions from Togo in four clusters based on 11 discriminative traits	78
Figure 4.6: Variance explained by the principal components	80
Figure 4.7: Dendrogram showing the clusters of the 50 rice genotypes collected in Togo based on 5,736 SNP markers	82
Figure 5.1: Distribution of five yield SNP alleles in 50 rice genotypes collected in Togo	101
Figure 6.1: Geographical and agro-ecological zone of the four sites of experiment.....	112

Figure 6.2: Performance of F ₁ hybrids and their parents for number of panicles per plant	115
Figure 6.3: Performance of F ₁ hybrids and their parents for grains per panicle	116
Figure 6.4: Performance of F ₁ hybrids and their parents for 100-grain weight	117
Figure 6.5: Performance of F ₁ hybrids and their parents for grain yield	117
Figure 6.6: AMMI plot showing the GxE interaction for number of panicles per plant	119
Figure 6.7: AMMI plot showing the GxE interaction for number of grains per panicle	119
Figure 6.8: AMMI plot showing the GxE interaction for 100-grain weight	120
Figure 6.9: AMMI plot showing the GxE interaction for grain yield	121

LIST OF ABBREVIATIONS

AGD	Analysis of Genetic Designs
AMMI	Additive Main effect and Multiplicative Interactions
AMOVA	Analysis of molecular variance
ARICA	Advanced rice for Africa
CCA	Canonical correlation analysis
CGIAR	Consultative group on International agricultural research
CYMMIT	International Maize and wheat improvement center
DNA	Deoxyribo Nucleic Acid
DSID	Direction of statistics, information and documentation (in Togo)
F1	First hybrid generation after fertilization
FAO	Food and Agriculture Organization of United Nations
GBS	Genotyping by sequencing
GPS	Global positioning system
GRISP	Global rice science partnership
GXE	Genotype by environment interaction
IFPRI	International food policy research institute
INERA	Institut de l'Environnement et de Recherches Agricoles
IRRI	International rice research institute
ITRA	Togolese agriculture research institute
LSD	Least significant difference
NAES	National agriculture extension system
NARS	National agriculture research system
NERICA	New rice for Africa
NPK	Nitrogen – Phosphorous – Potassium
PCA	Principal components analysis
PIC	Polymorphism information content
PVS	Participatory varietal selection
ROPPA	West Africa network of farmers' organisations
SES	Standard evaluation system
SNP	Single nucleotide polymorphism
SPSS	Statistical package for social science
SSA	Sub Saharan Africa

CHAPTER ONE: GENERAL INTRODUCTION

Rice is the main source of food for the largest number of people on Earth (GRISP, 2013), which provides 27% of energy intake and 24% of dietary protein to people in the third world countries (FAO, 2004). This cereal is produced and consumed in 38 countries in Sub-Saharan Africa (SSA) (GRISP, 2010) where it is a staple food for many countries (AfricaRice, 2011a). In West and Central Africa, the demand for rice is growing at about 6% per year, faster than anywhere else in the world (ROPPA, 2005). However, as reported by AfricaRice (2011a), no SSA country was self-sufficient in rice in 2010. However, the continent has the human, physical and economic resources to produce enough rice to feed its populations. In 2009, Africa imported 10 million tons of milled rice costing US\$5 billion (AfricaRice, 2011a). This represents a third of milled rice stocks available on the world market (GRISP, 2013). Depending on imports is a very risky, expensive and unsustainable situation that may lead to food insecurity and civil instability in some African countries, as it happened for the food crisis in 2008 (AfricaRice, 2011b). So, relying on imports is not at all a sustainable strategy. Therefore, to address this situation efforts are being made by researchers to develop varieties with high yield potential, and adapted to local conditions. Rice research on the continent led by AfricaRice is making important progress in the development of high yielding varieties adapted to local conditions. A great achievement is the development of 60 rainfed/irrigated lowland and 18 upland interspecific varieties known under the label of New Rice for Africa (NERICA) (WARDA/FAO/SAA, 2008). These varieties developed from *Oryza glaberrima* x *Oryza sativa* crosses are well adapted and high yielding varieties. Most of them are early maturing (103 days at maturity for NERICA L4) and high yielding (5 to 7 t/ha) with a good 1000-grain weight of 33 grams for NERICA L5. Many NERICA varieties have been adopted by farmers and released in many SSA countries (FAO, 2008).

Beyond the NERICAs, efforts are being made by AfricaRice and National Agriculture Research Systems (NARS) to increase rice productivity in Africa. In the last 5 years, AfricaRice has developed together with the NARS across the continent, a series of new lines with very good performance. These lines have been tested for their adaptability and agronomic performance as well as in participatory varietal selection (PVS) procedures in many countries through a strong partnership under the “Africa Rice breeding task force”. In 2013, five of these promising lines have been named under the Advanced Rice for Africa (ARICA) label as ARICA 1 to ARICA 5.

It is then very important to notice that despite these satisfactory results, most African countries are still not self-sufficient in rice. However, rice production predictions are encouraging for Africa. As estimated by AfricaRice (2011a), on the one hand, SSA countries’ rice paddy production will have increased from 18.4 million tons (11.9 million tons of milled rice) in 2010 to 46.8 million tons (30.4 million tons of milled rice) by 2020; and on the other hand, the needs for rice consumption from 19.8 million tons in 2010 to 35.0 million tons by 2020. In view of that, many SSA countries will reach near rice self-sufficiency (cover 90% of their needs) by 2020. Among these countries ten have been reported to become self-sufficient by 2020 (AfricaRice, 2011a).

In Togo, rice is the third most important cereal in terms of production after maize and sorghum. In 2016, local rice production was estimated at 137,106 tons of paddy with an average yield of 1.76 t/ha (DSID, 2017). Rice is grown in three main production ecologies: upland, rainfed lowlands, and irrigated lowlands. In terms of surface areas covered, the major production ecology is the rainfed lowlands. Irrigated lowlands are the least but contribute enormously in the local production. Up to 90% of annual rice production in Togo is obtained from rainfed lowlands (60%) and irrigated lowlands (30%). This is because the highest yields are obtained in irrigated conditions, and rice is produced twice in the year. The local

production covers only half the population needs. Thus, about the same quantity of rice produced in Togo is imported annually to cover the needs (Aboa *et al.*, 2007).

Furthermore, the demand for rice consumption is growing because of its nearly daily use in the population's nutrition. Rice consumption per capita per year has been estimated in 2009 at 21 kg of milled rice (GRISP, 2013). Both of *Oryza sativa* and *O. glaberrima*, as well as interspecific varieties such as NERICAs are being grown, with a larger proportion of *O. sativa* varieties. The improved varieties cultivated by farmers are introduced from IRRI (International Rice Research Institute) and AfricaRice because of lack of a proper breeding programme in the country. Rice research mainly led in the country by the National agriculture research institute ITRA (*Institut Togolais de recherche agronomique*) is limited to introductions and adaptability tests of varieties. No activity on the development of rice varieties is conducted so far by ITRA.

IR 841, an aromatic variety introduced in the late 1980s is the most widely cultivated. This is because it is well adapted to local conditions and also because of its aroma. Several socio-economic surveys reported that farmers and consumers prefer aromatic varieties more than non-aromatic ones. Thus, IR 841 despite its introduction in 1980s is still preferred by farmers and consumers than the new introduced varieties among which some are even higher yielding but non-aromatic (like the NERICAs). In the local conditions, IR 841 is 6 t/ha yield potential and 120 days variety while NERICA-L14 has been reported to yield 7t/ha in 108 days. Unfortunately, due to technical issues for seeds maintenance, the popular variety IR 841 has become contaminated and its yield has become low.

In view of the above situation, there is a need to provide Togolese farmers with new high yielding and aromatic irrigated and rainfed lowland varieties. For that purpose, prominence must be given to locally well adapted accessions and high performing introduced varieties. This is because many varieties with good adaptability and yield potential have been

introduced by ITRA. Also, the National agriculture extension system (NAES) and farmers themselves have introduced varieties from neighbouring countries which were found to be high performing. So, it is important to gather all these materials as well as the currently cultivated aromatic varieties in a germplasm collection and exploit their genetic potential to develop high yielding and aromatic varieties for farmers and consumers, as they all like aromatic varieties. It is also important to assess the purity of the samples of IR 841 grown in the main sites of rice production across the country.

Therefore, the main objective of this work is to contribute to increase rice production in Togo through the development of aromatic and high yielding varieties.

Specifically, it aims to:

- create an inventory of introduced rice varieties for irrigated and rainfed lowland conditions, and record of failure of adoption by farmers, farmers preferred traits and production constraints;
- establish germplasm collection and determine the genetic diversity existing among the germplasm;
- characterize rice accessions for aroma and yield (by phenotypic evaluation and with molecular markers) and identify parents for crosses; and
- develop and evaluate F_1 hybrids for the estimation of genetic parameters.

The activities underlying the above stated objectives have been carried out following appropriate methodology for each of them as described in subsequent chapters in this thesis.

CHAPTER TWO: LITERATURE REVIEW

2.1. Biology of rice

2.1.1. Taxonomy

Rice (*Oryza* spp.) in general is a plant belonging to the Branch of Spermaphytes. It is a Monocotyledon of the Order of Poales. The Genus *Oryza* belongs to the Family of Poaceae, the sub family of Panicoideae and the Tribe of *Oryzaceae* (Anonymous, 1991). The summary information on rice (*Oryza* spp.) taxonomy is shown in Table 2.1.

Table 2.1: Taxonomy of rice (*Oryza* spp)

Taxon	Name
Kingdom	<i>Plantae</i>
Division	<i>Magnoliophyta</i>
Class	<i>Liliopsida</i>
Order	<i>Poales</i>
Family	<i>Gramineae or Poaceae</i>
Tribe	<i>Oryzaceae</i>
Genus	<i>Oryza</i>
Species	Spp.

About 25 species (Morishima, 1984; Vaughan, 1994; Brar and Khush, 2003) or 23 species (Sié *et al.*, 2009) in which only two are cultivated (*O. sativa* from Asia and *O. glaberrima* from Africa) have been reported. *O. sativa* is the most cultivated around the world and contributes to nearly the whole global production (Juliano, 1994; National Bureau of Plant Genetic Resources, 2011).

2.1.2. Botanical description

Rice plant is an annual herbaceous and self-pollinated more or less pubescent with a round erect stalk (except floating varieties), arranged in clump covered by leaves and presenting at the tip flowers in forms of panicle (Anonymous, 2002).

The roots system is made up of abundant tiny roots in aquatic varieties, and with much bigger and deeper diameter in rainfed varieties (Anonymous, 2002). The secondary roots possess absorbent hairs (Sié *et al.*, 2009). After germination, each grain will give a whole plant of rice with many tillers forming a clump. The number of tillers resulting from one germinated grain can attain thirty at vegetative stage. A limited number of tillers (about 15 tillers) will produce panicles (Anonymous, 2002). The Figure 2.1 shows a plant of rice.

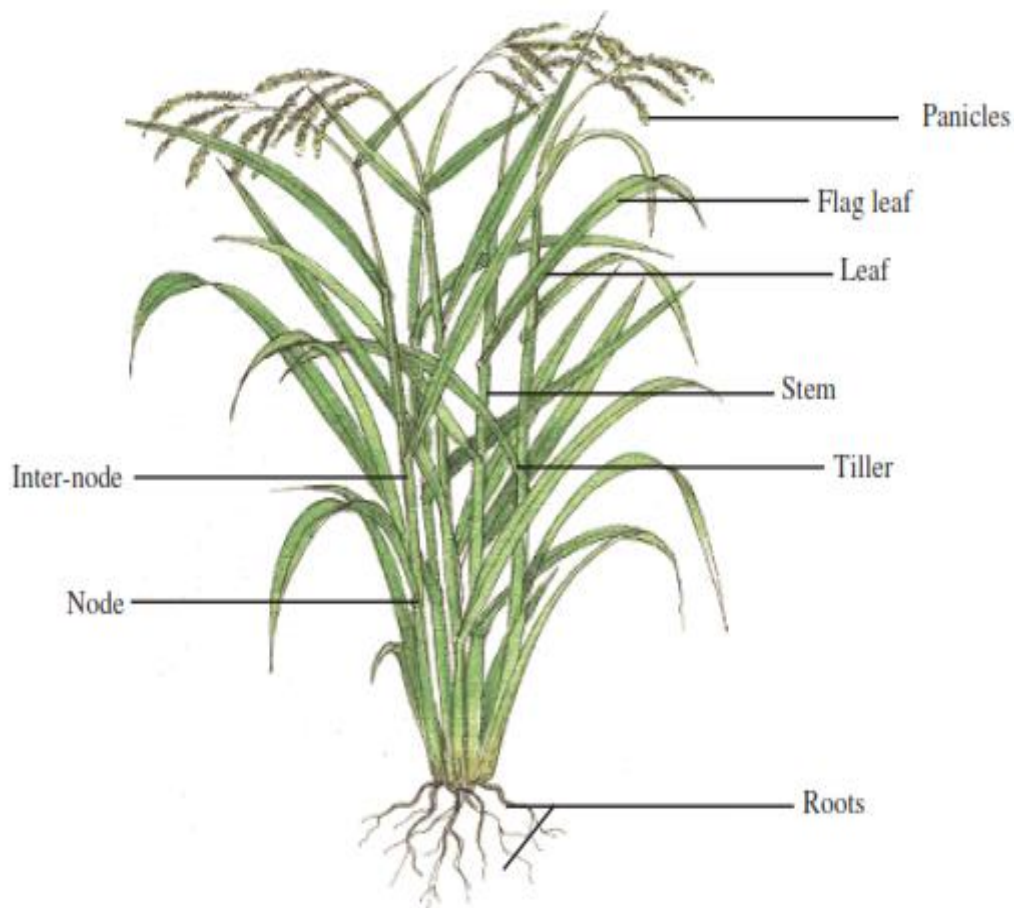


Figure 2.1: The rice plant

Source: Sié *et al.* (2009)

The rice plant produces from the soil many tillers. Each of them is made up of a certain number of nodes and internodes in a successive order (Sié *et al.*, 2009). The length of each tiller goes from 0.6 to 6 m (floating rice) (Anonymous, 2002).

To the nodes are inserted leaves and buds that can generate other tillers. The leaves are made up of a cover and a limb. The cover envelops the whole internodes and in some cases, the following node. It is a link between the node and the limb. The last leaf under the panicle is called the flag leaf (Sié *et al.*, 2009).

The stalk presents at the top a panicle which can be 20 to 30 cm long. *O. glaberrima* panicle is erect whereas *O. sativa* one is curved. Each panicle is made up of 50 to 300 spikelets which will become the grains. The grain obtained is a caryopsis enveloped in two glumella which is called paddy (Anonymous, 1991; Anonymous 2002). The 1000-grain weight of the paddy varies from 20 to 45 g (Anonymous, 2002). As regard to the life cycle of rice varieties, one can distinguish early mature varieties (90 to 120 days), normally mature varieties (120 to 150 days), and late mature varieties (more than 150 days) (FAO, 2011).

The African rice plant presents specific botanical traits which enable one to distinguish it. According to Carney (2001), the African rice plant presents an erect panicle with no or very low secondary branching. Its glumella presents an extension (beard) (Vido, 2011). Porteres (1955) added that *O. glaberrima* is characterized by round and truncated ligula whereas *O. sativa*'s one is long, pointed and bifid.

2.2. Origin and geographical distribution of cultivated rice

Among the 25 species of rice, the origin and geographical distribution of only the two cultivated species of rice *O. sativa* and *O. glaberrima* have been reported. According to many authors, information gathered from genetic diversity studies, historical and archaeological evidences and geographical distribution have been used in the identification of the centres of

origin as well as the centres of diversity of both species (National Bureau of Plant Genetic Resources, 2011). Even though the Asian origin of *O. sativa* and the African origin of *O. glaberrima* are generally known, the geographical site of the domestication of *O. sativa* in Asia is not clearly defined (Juliano, 1994).

It has been reported so far that Asian rice domestication has been done independently in China, in India and in Indonesia, hence the three races of *O. sativa*: *sinica* (also called *japonica*), *indica*, and *javanica* (also called *bulu* in Indonesia) (Juliano, 1994). About the centre of diversity of *O. sativa*, the foothills of the Himalayas, Chhattisgarh, Jeypore Tract of Orissa, North-eastern India, northern parts of Myanmar and Thailand, Yunnan Province of China, etc have been mentioned by authors (National Bureau of Plant Genetic Resources, 2011).

According to Carpenter (1977), the African rice's origin is the central delta of the River Niger. Mayer and Bonafant (1973) reported that *O. glaberrima* has been domesticated from the Delta of the river Niger with two secondary origin centres in Senegambia and in the mountains of Guinea. This precision evokes the hypotheses of *O. glaberrima* domestication from the wild species *O. barthii* A. Chev. (Mayer and Bonafant, 1973), or from the annual species *O. breviligulata* (syn. *O. barthii*) which has been domesticated from the perennial species *O. longistaminata* (Anonymous, 1991 and 2002). Based on the historical, botanical and genetic studies, a few authors have indicated in 1973 that *O. glaberrima* has been developed from Asian rice, and that the wild annual species *O. barthii*, derived as hybrid from the two cultivated species (Mayer and Bonafant, 1973). Recent works have shown the link which could exist between *O. sativa* and *O. glaberrima* (Figure 2.2).

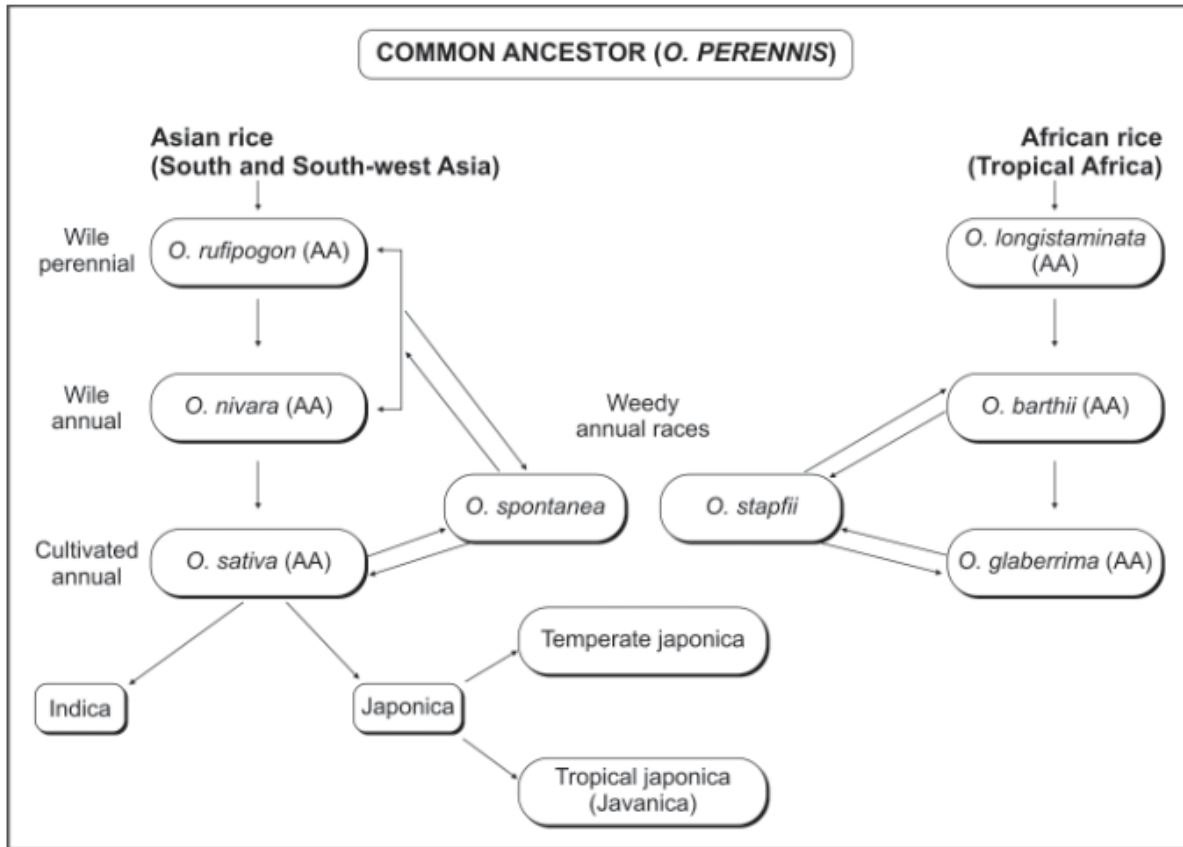


Figure 2.2: Schematic representation of the evolutionary pathways of Asian and African cultivated rices (Source: DB-MST and MEF/GI, 2011)

Today, rice is cultivated on all the continents (Anonymous, 2002). According to Chang (1983), *O. sativa* species has been rapidly spread from its tropical (South and south-east Asia) and sub-tropical (South and South-west China) origins to higher altitudes and latitudes in Asia, this so recently (2300 years ago) in the case of Japan. It has been introduced into very far areas like West Africa, Northern America, and Australia during the last six centuries. Its cultivation in South Carolina in United States of America (USA) dates back to 1690 (Adair, 1972). As indicated by Anonymous (2002), Malaysian navigators have introduced the Asian rice from Indonesia to Madagascar around the 4th century. It is important to know that Asian rice has been introduced in Europe where it is cultivated from the 8th century in Portugal and in Spain, and later in the 9th or 10th century in Southern Italy (Lu and Chang, 1980). If the

Asian rice species has been spread and cultivated all over the world, the African rice species is confined to Africa.

O. glaberrima's propagation from its origin was made following many ways. Vido (2011) has indicated that many evidences have shown a plural introduction in the time as well as in the space. From these evidences, the author has stated that rice was introduced from its origin to other areas following three main ways: the way North-South, the way East-West in its southern part from places located in the area called rice civilisation area, and the way West-East on its southern part from Slaves coast to the island Sao Tome and Principe. Many authors of travel stories have mentioned rice cultivation along rivers in West Africa since the early 17th century. Arrandea (1998) stated that the extension of *O. glaberrima* in West Africa was probably done 2000 years ago. Nowadays, *O. glaberrima* cultivation is confined in West Africa in a marginal production system (Anonymous, 2002), reflecting its net decline on the benefit of Asian rice (Sié, 1991).

2.3. Rice production

2.3.1. Ecology and cropping systems

Rice production ecologies are mainly based on the level of water supply. Thus, two main types of production ecologies are distinguished *viz*: the rainfed and the aquatic systems (irrigated systems with complete water control conditions, as well as submersion flood systems) (Anonymous, 1991; Jacquot *et al.*, 1997; Anonymous, 2002).

In rainfed rice production systems, rains are the source of water. Ground water near to the soil surface can be useful in this system. Rainfed upland and rainfed lowlands are distinguished. GRISP (2013) defined rainfed lowland rice as the one grown in banded fields that are flooded with rainwater for at least part of the cropping season to water depths that exceed 100 cm for no more than 10 days. According to the same author, upland rice is grown under dryland

conditions (no ponded water) without irrigation and without puddling (harrowing or rototilling under shallow submerged conditions), usually in non banded fields. For IFPRI (2002), about 52 million ha of rainfed lowlands worldwide supply about 19% of the world's rice production.

According to Wopereis *et al.* (2009), in rainfed lowlands, small to moderate topographic differences can have important consequences for water availability, soil fertility, and flooding risk. The unpredictability of rainfall often results in field conditions that are too dry or too wet. Besides imposing water-related stresses on crop growth, these conditions prevent timely and effective management operations such as land preparation, transplanting, weed control, and fertilizer application (GRISP, 2013).

Upland rice is encountered in Asia, Africa and Latin America (FAO, 1997). In fact, on the 151 million hectares of rice producing areas worldwide in 2004, upland rice represents about 13% for 4% of global production (GRISP, 2013). It is the main rice production ecology in West Africa and Latin America where it respectively represents 50% and 75% of rice producing areas (IFPRI, 2002). For the same author, in Central and West Africa, the rice belt of Africa, upland areas represent about 35% of the area under rice cultivation and employ about 70% of the region's rice farmers. Based only on rainfalls, upland rice production is highly limited by rainfall's irregularity, soil infertility and toxic minerals (Iron, Aluminium, etc) (FAO, 1997). The lowest yields are often recorded in upland rice systems.

In irrigated rice production system, water level is controlled for irrigation as well as for drainage. Water availability and management can enable two cycles of rice production yearly on the same plot. About 79% of rice producing areas worldwide are irrigated (IFPRI, 2002). The best yields going from 3 to 9 tons per hectare are mentioned and more than 75% of global production is recorded in irrigated rice systems (GRISP, 2013). In West Africa, 10.5% of the rice cultivation areas constitute the irrigated rice production ecology (Maclean *et al.*, 2002).

According to GRISP (2013), worldwide, about 93 million ha of irrigated lowland rice provide 75% of the world's rice production. Some 56% of the world's irrigated areas of all crops are in Asia, where rice accounts for 40 to 46% of the irrigated area of all crops (GRISP, 2013). According to IFPRI (2002) rice occupies 64 to 83% of the irrigated area in Southeast Asia, 46–52% in East Asia, and 30–35% in South Asia. From the same source, at the field level, rice receives up to two to three times more water per hectare than other irrigated crops, but an unknown portion of the water losses is reused by other fields downstream. In many irrigated areas, rice is grown as a monoculture with two crops a year. However, significant areas of rice are also grown in rotation with a range of other crops, including 15–20 million ha of rice-wheat systems (GRISP, 2013).

Submersion rice production system without water level control is mostly encountered in flooded lowlands. It represents 33% of global rice producing areas (IFPRI, 2002). Authors have reported high water level going from 1 to 5 m occurring sometimes during the production cycle. Adapted rice varieties elongate their stalk depending on the height of the water level (CNUCED, 2004).

African rice is mainly cultivated in two ecologies: upland and rainfed lowland. The accessions are classified into two ecological groups: upland type and floating type. Ndjondjop *et al.* (1996) has distinguished two main agro-ecotypes:

- One erect, early maturing and photoperiod insensitive which is adapted in strictly rainfed or moderately flooded conditions,
- One floating, late maturing and photosensitive which is cultivated in flooded lowlands.

However, some accessions of *O. glaberrima* known as upland types are also adapted to flooded lowland conditions, and vice versa. Nowadays, African rice is rarely cultivated in pure. It is often associated with Asian rice which is more and more encountered throughout the continent (Nayar, 2012).

2.3.2. Distribution of rice

Rice is a very plastic plant which can be cultivated in dry or irrigated conditions (Mayer and Bonefond, 1973). Nowadays, rice is cultivated around the continents in 117 countries (GRISP, 2013). Its producing countries are located between the 40° South (centre of Argentina) to the 53° North (North east of China) latitudes (Juliano, 1994). Inter tropical Asia is the main producing region worldwide (Mayer and Bonefond, 1973; Anonymous, 2002). According to Juliano (1994), Arrandea (1998), and Anonymous (2002), rice is grown on lands going from sea level (mostly rivers' plains) to 3000 m of altitude like on the Himalaya Mountain in Nepal. China, Indonesia, India, Thailand, Pakistan, Vietnam and Japan are the main producers all over the world (FAO, 2007).

In Africa, essentially Asian rice varieties are produced in 38 countries in the south of the Sahara (GRISP, 2010). However, as originated from Africa, *Oryza glaberrima* is still cultivated in some areas even if its production is in consistent decline. It is essentially cultivated in West Africa. Its production is localised from the South western region of Mauritania and central region of Mali to the whole Gulf of Guinea. Moreover, the producing area is extended from the Senegalese coast to the South-western part of Chad (Figure 2.3).

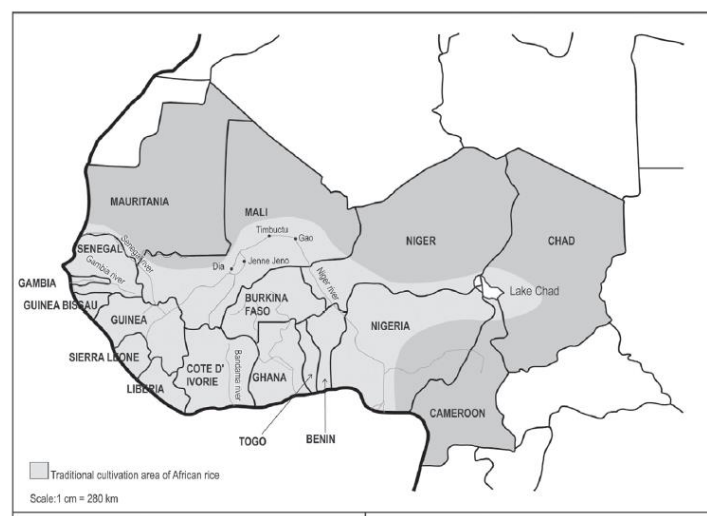


Figure 2.3: African rice producing area
Source: Nayar (2012)

2.3.3. Global production and consumption

Rice is nearly (90%) all produced in Asia. Its global production totalled 696 million tons in 2010. This production has been harvested on 162 million hectares (GRISP, 2013). From the same source, rice, wheat, and maize are the three leading food crops in the world; together they directly supply more than 42% of all calories consumed by the entire human population. Wheat is the leader in area harvested each year with 225 million hectares (ha) in 2009, followed by maize and rice, both with 159 million ha. Human consumption in 2009 accounted for 78% of total production for rice, compared with 64% for wheat and 14% for maize.

Rice accounts for 19% of total crop area harvested worldwide and about 144 million farms have been reported with the vast majority in developing countries. Of the three major crops, rice is by far the most important in terms of human consumption in low and lower-middle income countries. It provided 19% of global human per capita energy and 13% of per capita protein in 2009 (GRISP, 2013).

On the consumption side, the only countries outside Asia where rice contributes more than 30% of caloric intake are Madagascar, Sierra Leone, Guinea, Guinea-Bissau, and Senegal (countries with population less than 1 million are excluded) (IFPRI, 2002). The same source added that on the production side, the only countries outside Asia where rice accounts for more than 30% of total crop area harvested are Madagascar; Sierra Leone and Liberia in West Africa, plus Suriname, Guyana, French Guiana and Panama in Latin America. The world's largest rice producers by far are China and India (GRISP, 2013). Although its area harvested is lower than India's, China's rice production is greater due to higher yields because nearly all of China's rice area is irrigated, whereas less than half of India's rice area is irrigated (IFPRI, 2002). After China and India, the next largest rice producers are Indonesia, Bangladesh, Vietnam, Thailand, and Myanmar. These seven countries all had average production in 2006-2010 of more than 30 million tons of paddy. The next highest country on the list, the

Philippines, produced only a little more than half of that. Collectively, the top seven countries account for more than 80% of world production (GRISP, 2013).

Despite Asia's dominance in rice production and consumption, rice is also very important in other parts of the world. In Africa, for example, rice has been the main staple food (defined as the food, among the three main crops, that supplies the largest amount of calories) for at least 50 years in parts of western Africa (Guinea, Guinea-Bissau, Liberia, Sierra Leone) and for some countries in the Indian Ocean (Comoros and Madagascar) (FAO, 2015). On balance, in Africa, production has grown rapidly, but rice consumption has grown even faster, with the balance being met by increasing quantities of imports. Western Africa is the main producing sub region, accounting for more than 40% of African production in 2006-2010 (FAO, 2015). In terms of individual countries, the leading producers of paddy (2006-2010) are Egypt (6.1 million tons), Madagascar (4.1 million tons), and Nigeria (3.9 million tons) (GRISP, 2013).

2.3.4. Production in Togo

In Togo, rice is cultivated in all the five regions throughout rainfed upland, rainfed lowlands and irrigated lowlands production systems (Aboa, 2005 ; Aboa *et al.*, 2007). Rainfed lowlands contribute about 60% to the national production, whereas this is 30% for irrigated lowlands and only 10% for upland systems (Aboa, 2005). The irrigated lowlands sites are: Zio valley in Mission Tové and Mono Valley in Agomé Glozou (Maritime region), Kpélé Tutu and Sodo (Plateaux region), Oti valley in Koumbeloti, and Tantigou (Savannas region). Another important on-going irrigated lowlands development in the Zio Valley (Maritime region) is in Djagblé. Irrigated plains development is also on-going around the country. Important sites are Avétonou and Kpélé Djokpé (Plateaux region), Lama Tessi (Central region), Guerin Kouka, Mô plain and Kara plain (Kara region), and Sadori and Mango (Savannas region).

Rice is the third cereal in terms of annual average production after Corn and Sorghum in Togo (DSID, 2017). In 2016, national rice production was estimated at 137,106 tons of paddy whereas maize and sorghum productions were respectively 826,896 and 272,776 tons respectively. From 2012 to 2016, all the cereals productions declined (Figure 2.4a). However, the highest average yield is recorded with rice (Figure 2.4b).

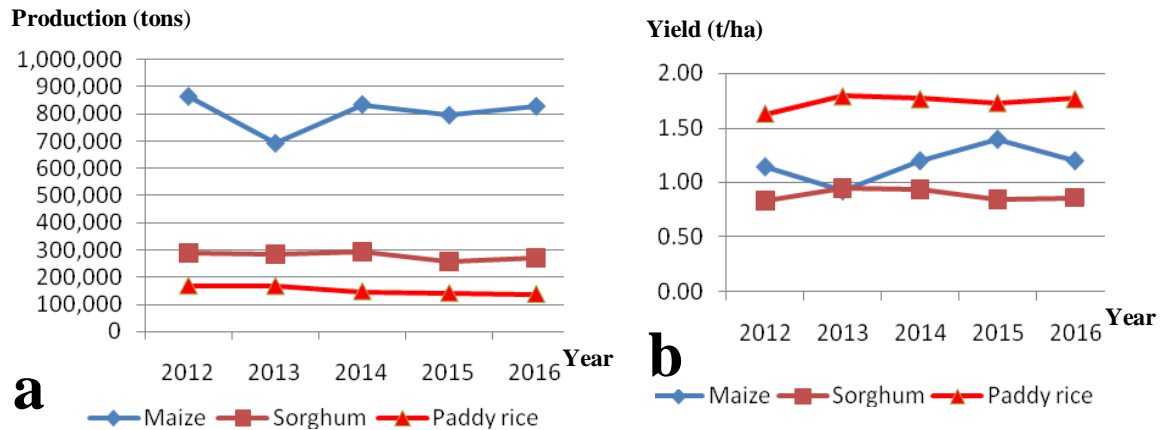


Figure 2.4: (a) Average production (in tons) and (b) average yield (in tons per hectare) of major cereals in Togo from 2012 to 2016

The average yields of cereals produced in Togo (Maize, Sorghum, Rice, Millet and Fonio) are very low (less than 2 t/ha) (DSID, 2017). Data on fields' areas sown with rice, average production and yield of rice recorded from 2012 to 2016 are presented in Table 2.2.

Table 2.2: Area, average production and yield of rice recorded in Togo from 2012 to 2016

	2012	2013	2014	2015	2016
Surface area (ha)	104,043	92,239	86,515	81,601	82,914
Average production (t)	169,273	168,326	147,930	140,952	137,106
Average yield (t/ha)	1.63	1.79	1.76	1.73	1.76

Source: DSID (2017)

The average annual production displayed above covers only half the needs of the Togolese population. Thus, about the same quantity is imported to satisfy the needs. In 2010, rice imports in Togo have been estimated at 74,800 tons when the local production was 110,100 tons of paddy rice which are equivalent to 73,000 tons of milled rice. In the same year, 100 tons of rice have been exported from the country (GRISP, 2013; FAO, 2015). In the last ten years, demand of rice has been increasing, and although domestic production is increasing, much rice is imported. It appears that working to increase the yield in local conditions will be very important in reducing rice importation in Togo.

2.3.5. Production constraints

Two main types of constraints are distinguished in rice cultivation. These are known as biotic and abiotic constraints.

2.3.5.1. Biotic constraints

Biotic stresses are caused by alive organisms. Weeds, rodents, birds, insects, and pathogens (causing diseases) are the main biotic factors encountered in rice production.

In Togo, the main rice diseases encountered are rice blast (caused by the fungus *Magnaporthe grisea*) and rice bacterial leaf blight (caused by the bacterium *Xanthomonas oryzae* pv. *oryzae*) (Aboa *et al.*, 2007). Rice weeds inventory made by Pocanam (2005) enables to check off 35 to 68 species as rice weeds depending upon the ecosystems in Togo. The most harmful are: *Echinochloa colona* (L.) Link in irrigated sites, *Lersia hexandra* Sw in rainfed lowlands, and *Digitaria horizontalis* Willdenowen in upland sites. Manual or chemical weeding is used to eradicate weeds on the fields. Rice is the host plant for hundreds of insect species among which about thirty are economically important (Anonymous, 2002). *Orseolia oryvira* is the

major insect found in rice fields across the country. This insect causes rice gall midge. Moreover, birds are the major biotic constraint that contribute a lot in rice yield reduction in Togo (Aboa *et al.*, 2007).

2.3.5.2. Abiotic constraints

Abiotic constraints are constraints that are not triggered by alive organisms. Many abiotic constraints are reported: Drought, flooding, iron toxicity, soil poverty, plant lodging, etc. Mainly, they are linked to climate and soil.

According to Chen and Murata (2002) and Herdt (1991), the main abiotic constraints reported in rice production are iron toxicity and drought.

In Togo, Aboa (2005) indicates that absence of water control (irrigation), absence of appropriate inputs, lack of adequate equipment and financial assistance are also abiotic constraints limiting rice production in our sub-region. For this author, all these constraints can be addressed by the farmer.

2.4. Importance of rice

Both cultivated rice species are useful for many purposes. They are not used only as food but also at medicinal, economical and socio-cultural levels.

2.4.1. Nutritional value of rice

Rice is the single largest food source for the poor (GRISP, 2013). According to GRISP (2010), rice is the staple food for 3.5 billion persons worldwide. In Asia, it is synonymous with food and it is the source of one quarter of global per capita energy (GRISP, 2013).

According to FAO (2004), rice contributes to 20% of the global alimentary energy. Throughout the last forty years, annual rice consumption per capita has been increased at 40% going from 61.5 kg in 1961 to 85.9 kg in 2002 (FAO, 2004).

According to DB-MST and MEF/GI (2011), rice is a nutritious cereal crop, used mainly for human consumption. It is the main source of energy and is an important source of protein providing substantial amounts of the recommended nutrient intake of zinc and niacin (Table 2.3). However, rice is very low in calcium, iron, thiamine and riboflavin and nearly devoid of beta-carotene. Anonymous (1991) indicated that milled rice is richer in carbohydrates and proteins than paddy rice.

Table 2.3: Composition per 100 g of edible portion of milled Asian rice

Calories (Kcal)	345.0	Phosphorus (mg)	160.0	Riboflavin (mg)	0.06
Moisture (g)	13.7	Minerals (g)	0.6	Thiamine (mg)	0.06
Carbohydrates (g)	78.2	Amino acids (mg)	1.09	Niacin (mg)	1.9
Protein (g)	6.8	Calcium (mg)	10.0	Folic acid (mg)	8.0
Fat (g)	0.5	Iron (mg)	0.7	Copper (mg)	0.14
Fibre (g)	0.2	Magnesium	90.0		

Source: National Bureau of Plant Genetic Resources (2011)

It is reported that there is no significant difference in the energetic value of milled rice and white rice, this varying from 350 to 360 calories per 100 g. However exclusive consumption of white rice may lead to Beriberi because it lacks vitamins, notably thiamine (Anonymous, 1991).

African rice is well appreciated and preferred as food in its production areas. According to Sié (2007), Asian rice is digested faster than African rice. This may be due to the eventual difference noted in the nutrients composition of the two species.

Both African and Asian rices are consumed as different meals. Since colonial time, African rice is consumed in form of paste. There are many changes in the mode of cooking in that

time compared to the way it is cooked now. In old days rice ground before its uses. Nowadays, rice grains can be boiled and consumed with vegetable sauce, soup or with stew. Rice can also be used as porridge with milk. Finally, African rice was used in Axim (current Ghana) to make bread after being reduced into flour. This flour was also used like maize flour to make paste after its fermentation (Vido, 2011).

Apart from rice uses as human food; it is also used to make alcohol, starch and its by-products, oil, medicines, fuel, etc. Moreover rice transformation by-products are used to feed animals, and its straw to make papers. According to GRISP (2013), rice contains many compounds in the grains that promote shiny hair and good skin. Several countries are now making face washes, liquid shower soaps, and hair products from rice, including Japan, Republic of Korea, the Philippines, and Thailand. Also, in Thailand and the US, milk is made from rice for lactose-intolerant people.

2.4.2. Medicinal importance of rice

Chinese state that the black rice is successfully used to reinforce the organism. So this rice is called ‘blood reinforcement rice’ or ‘medicinal rice’ (Li and Lai, 1989). In Kerela in India, the Navara variety is used to reactivate nerves in case of paralysis (Chopra, 1933). The use of the rest of rice after decortications is helpful to human good health (Ventura, 1977). Good results were obtained by using rice cooking water in the treatment of diarrhoea (Molla *et al.*, 1985; Wong, 1981; Rivera *et al.*, 1983; Tribelhorn *et al.*, 1986). GRISP (2013) states that certain parts of some varieties are used as medicines in the traditional treatment of illnesses in West Africa.

2.4.3. Economic importance of rice

Rice production is one of the most important economic activities on Earth (GRISP, 2013). However, its market is one of the smallest in the international market compared to the other cereals. This is due to the fact that the rice production around the world is locally consumed. Only 7% of all rice production is exported from its country of origin (GRISP, 2013). Every year, about 113 million tons of wheat and 80 million tons of maize are subjected to international transactions all over the world against 25 million tons for rice. This represents only 5 to 6% of the total annual production (FAO, 2004). These data concern the cumulated production of the two cultivated species. Specific information on each of the species is not available. If available, this information should indicate the contribution of each species in the international transactions.

African rice, with its cultivation is in net decline (Sié, 1991) is mostly auto-consumed. A small part is commercialized on local markets. Today, thanks to research works undertaken by AfricaRice on this species, a particular attention is given to it beyond Africa. For instance, recently AfricaRice has exported some kilograms of *O. glaberrima* to the USA. This may be an important and promising revolution for the continent.

In Togo, although higher costs are mentioned in rice production, rice cultivation is the most profitable compared to the other cereals (Aboa *et al.*, 2007).

2.4.4. Ethno-botany

GRISP (2013) reported that rice cultivation was once the basis of the social order and occupied a major place in Asia's religions and customs. Rice is still sometimes used to pay debts, wages and rent in some Asian rural areas.

Social and cultural importance of rice has been reported mainly on *O. glaberrima*. Since the beginning of African rice cultivation, this cereal is really important for the populations that

produce it. In fact, the ethnographical studies of Brydon (1981) have showed the cardinal place of African rice in the agriculture in West Africa. This author has reported that the sowing, the harvest as well as the first consumption of rice can only happen after the accomplishment of appropriate ceremonies to divinities. Vido (2011) added that this is the life cycle of the cereal which is used to regulate the agricultural year and the dates of other ceremonies to divinities. The cereal is also used in the ceremonies as main food, and even more to reinforce a lady that has given birth.

In many localities where *O. glaberrima* is cultivated; the cereal is subjected to a traditional feast just after harvest. From this feast, *O. glaberrima* can be consumed because before the feast, rice consumption is forbidden. Brydon (1981) reported that two weeks after the ceremonies preceding African rice harvest, the new rice is offered to « Ayapo », the main divinity of « Avatime » to implore his/her benediction and to allow the consumption of the newly harvested rice.

Nowadays, in the Kpele and Danyi regions in Togo, a traditional feast is annually celebrated for African rice. This feast is called « Ewemozan », meaning « the Ewe's rice feast » (Ewe is the name of the ethnical group that people South west of BENIN, South of Togo and South east of Ghana). Many authors have mentioned similar celebrations around West Africa.

2.5. Rice yield

Global rice production more than tripled between 1961 and 2010, with a compound growth rate of 2.24% per year. This increase was slightly greater than that for wheat (2.02% per year), but substantially less than that for maize, which grew at 2.71% per year. Most of the increase in rice production was due to higher yields, which increased at an annual average rate of 1.74%, compared with an annual average growth rate of 0.49% for area harvested. In absolute terms, paddy yields increased at an annual average rate of 51.1 kg/ha per year,

although this rate of increase has declined in both percentage and absolute terms (FAO, 2015).

From the same source, yields are much higher in East Asia and the United States than in most of the rest of the world. Africa has the lowest average yield and the historical growth rate in yield there has also been lower, with some indication of an increase in growth rate during more recent years.

Anyway, rice yields are highly limited by several biotic and abiotic stress as well as climate changes effects.

2.5.1. Rice yield over time

The world average rice yield in 1960, the product of thousands of years of experience, was about 2.0 t/ha. For rice, the revolution began with the release by IRRI of the high-yielding semi-dwarf variety IR8 in 1966 (IFPRI, 2002). Rice farming yields began to rise dramatically and even better high yielding varieties (HYVs) became available over subsequent years. Astonishingly, in only 40 more years, as the Green Revolution spread, rice yield doubled, reaching 4.0 t/ha in 2000 (IFPRI, 2002). The rice varieties and technologies developed during the Green Revolution have increased yields in some areas to between 6.0 and 10.0 t/ha (GRISP, 2013). Nowadays hybrid rice varieties have been reported to yield more than 10 t/ha. Some Egyptian hybrid varieties named *Bio 434*, *Bio 404* and *Egyptian hybrid one* have been reported to yield 17.3, 16.3, and 15.6 t/ha respectively (Personal communication). Moreover, FAO (2015) has reported that rice yield growth of 1.0–1.2% annually beyond 2020 will be needed to feed the still-growing world and keep prices affordable.

2.5.2. Rice yield components

Grain yield in rice is a complex trait multiplicatively determined by its three component traits (Xing and Zhang, 2010). These are panicle number per plant (or per unit area), the number of filled grains per panicle, and grain weight. Some breeders use spikelet number per unit area and grain weight (AfricaRice, 2010b; Acquah, 2012).

All of the rice yield component traits are typically quantitative traits (Xing and Zhang, 2010). Number of panicles is dependent on the ability of the plant to produce tillers (tillering ability), including primary, secondary, and tertiary tillers. Number of grains per panicle can also be attributed to two subcomponents: number of spikelets, which is mainly determined by the numbers of primary and secondary branches, and seed setting rate of the spikelets. Grain weight is largely determined by grain size, which is specified by its three dimensions (length, width, and thickness), and the degree of filling (Xing and Zhang, 2010).

2.5.3. Genes controlling rice yield and its component traits

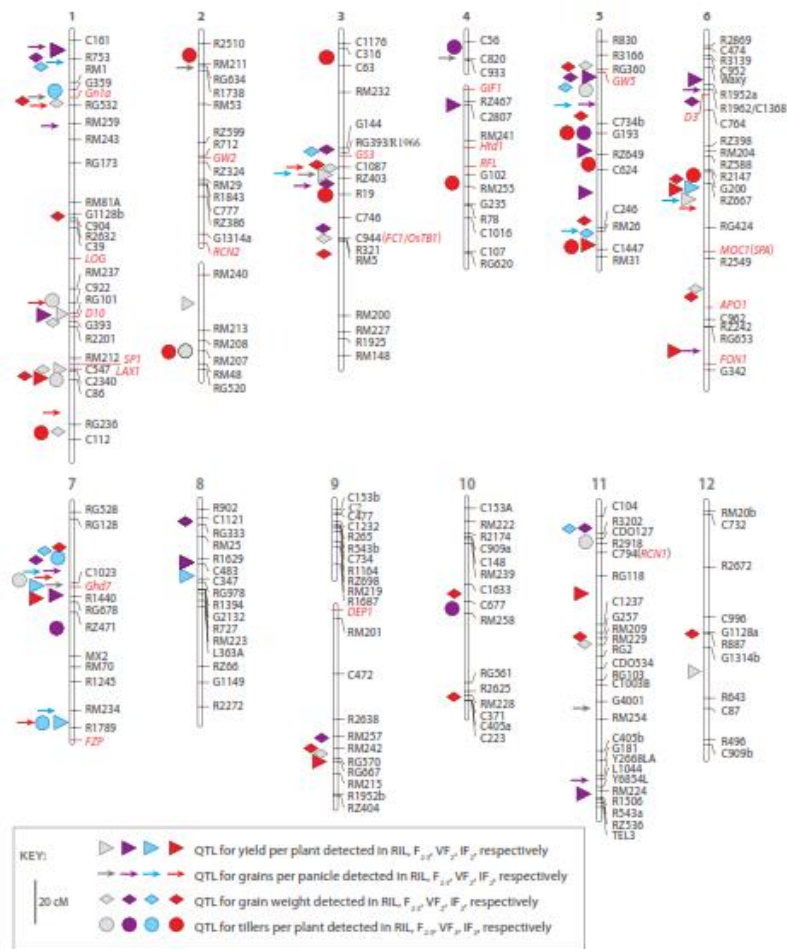
The inheritance of quantitative traits classically involves multiple genes, each having a small effect that is sensitive to environmental changes. These traits are known in general as having low heritability and thus have earned the reputation of being difficult to investigate. However, the development of molecular markers, genome mapping, and QTL analysis technologies has greatly facilitated the investigation of genetic bases of quantitative traits (Acquah, 2012).

According to Xing and Zhang (2010), in rice, researchers have constructed high-density genetic linkage maps based on restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers. Mapping populations specifically designed for dissecting the genetic bases of yield traits via QTL mapping have been constructed, producing large amounts of data leading to the identification of hundreds of QTLs for yield traits. Figure 2.5 displays an example where four populations derived from *indica x indica* cross have been

used for QTL mapping (Xing and Zhang, 2010). Moreover, some genes controlling yield components or their subcomponents have been identified (Table 2.4).

Table 2.4: Available genes controlling some traits related to rice yield and its components

Traits	Gene	Traits	Gene
Tillering ability	<i>MOC1</i>	Spikelet number	<i>APO1</i>
Flowering time	<i>RCN2</i>	Grain length	<i>GS3</i>
Panicle initiation	<i>LAX1</i>	Grain number per panicle	<i>Ghd7</i>
Establishment of floral meristem	<i>FZP</i>	Grain width and weight	<i>GW2 and GW5</i>
Panicle development	<i>RNC1</i>	Grain filling	<i>GIF1</i>



Source: Xing and Zhang (2010)

Figure 2.5: QTL for rice yield and its components in 4 populations derived from an *indica x indica* cross

2.6. Rice aroma

Scented or aromatic rice is preferred in some areas and draws a premium price in certain specialty markets. Consumers in Asia and some West African countries prefer rices with strong aroma. They feel that rice without a distinctive aroma is like food without salt. In the point of view of a few consumers in Europe, a trace of aroma is an objectionable trait, because for them any scent signals spoilage and contamination (Efferson, 1985). However, most of the high-quality preferred varieties in major rice growing countries are aromatic.

2.6.1. Volatile compounds associated with aromatic rices

Bullard and Holguin (1977) detected more than 100 volatile compounds in rice among which 70 were identified. However, from an odour point of view, they found that no individual compound had the characteristic aroma of uncooked rice. Thus, it can be concluded that aroma in rice is formed by a blend of various volatiles (Singh *et al.*, 2000).

The volatile compounds from cooked rice were analyzed by Yajima *et al.* (1978). They found the isolated volatile fraction represented 4.8 ppm by weight of the cooked rice. Of the volatile weight, 66% was in the acid fraction, 33% in the neutral fraction and 1% in the basic fraction. They identified over 100 compounds, which included 13 hydrocarbons, 13 alcohols, 16 aldehydes, 14 ketones, 14 acids and 8 esters.

Buttery *et al.* (1982) isolated and identified 2-acetyl-1-pyrroline as an important compound contributing to the aromatic odour. They suggested that 2-acetyl-1-pyrroline was a major contributor to the aroma in several of the Asian aromatic rice varieties. Lin *et al.* (1990), Tanchotikul and Hsieh (1991), and Ahmed *et al.* (1995) confirmed the reports of Buttery *et al.* (1983), and Paule and Powers (1989) that 2-acetyl-1-pyrroline was the characteristic odour of aromatic rice varieties.

According to Singh *et al.* (2000), most volatiles recorded so far from cooked non-aromatic and aromatic rice are the same except for their relative proportion. Even 2-acetyl-1-pyrroline is present in both types of rice. Therefore, there is need to find out optimum proportions of volatile compounds forming the pleasant smell of aromatic rice. Moreover, in aromatic varieties pleasant aroma is not only associated with cooked rice. Quite often these varieties emit aroma in the field at the time of flowering. Moreover, it has been also reported that pre-harvest (environment, cultural methods) and postharvest (drying, milling, storage, and cooking methods) activities may affect the aroma, as much as genetic factors.

2.6.2. Genetics of rice aroma

The genetics of the aroma characteristic is somewhat complex. Bemer and Hoff (1986) concluded that a single recessive gene controlled the aromatic nature of Della. This gene was located on chromosome 8 as determined by RFLP technology (Ahn *et al.*, 1992). Several others (Ghose and Butany, 1952; Sood and Siddiq, 1978; Reddy and Reddy, 1987) suggested the aroma characteristic was a single recessive gene. In contrast, Kadam and Patankar (1983) results suggested a single dominant gene. Dhulappanavar and Mensinkai (1969) interpreted their result to indicate two dominant aroma genes that interacted in a duplicate or complimentary manner. Reddy and Sathyanarayanaiah (1980) concluded that there were three complementary recessive aroma genes. Dhulappanavar (1976) suggested there were four complementary recessive aroma genes. Pinson (1994) suggested that one of the reasons for the confusion over the aroma gene was that different rice cultivars were analysed. He looked at six cultivars to evaluate the type of aroma genes involved. Pinson (1994) found that Jasmine 85, A-301, Della-X and PI45917 each contained a single gene for aroma and that they were allelic. Amber and Dragon Eyeball 100 contained two aroma genes, a novel gene plus one allelic to the gene in A-301, Della-Z, Jasmine 85 and PI 457917. Buttery *et al.*

(1986) suggested that the difference between aromatic and non-aromatic rice was not the presence or absence of 2-acetyl-1-pyrroline but to a difference in the quantity of the chemical in the grain. Multiple alleles of a single aroma gene could produce slightly different alteration in the same enzyme that resulted in rice with different aromatic intensities.

In sum, it is admitted that aroma in rice is controlled by a single recessive nuclear gene called *fgr* gene, located on the chromosome 8 of rice genome (Dong *et al.*, 2000).

Any breeding programme needs an easy assay to follow the inheritance. Sood and Siddiq (1978) developed an assay for determining the scent from plant material by adding Potassium hydroxide (KOH) to the plant sample, which released the aroma. Jin *et al.* (1996) used the RAPD technique to find a marker associated with the aroma gene. Ahn *et al.* (1992) used RFLP methods to tag the aroma gene. Mittal *et al.* (1995) evaluated 322 races and Basmati cultivar for aroma. They found 177 stocks had strong aroma and 28 had mild aroma. The characteristic for aroma was heterogeneous for 117 lines.

Unfortunately, aromatic or scented cultivars have often undesirable agronomic characters, such as low yield, susceptibility to pests and diseases, and strong shattering (Berner and Hoff, 1986). Therefore, breeding aromatic varieties with high yield potential is one of the major breeding objectives in many rice improvement programmes.

CHAPTER THREE: STATUS OF RICE VARIETIES ADOPTION, FARMERS' PREFERENCES AND PRODUCTION CONSTRAINTS IN TOGO

3.1. Introduction

In developing varieties for farmers, an important step is farmers' involvement in order to identify together with them their varietal preferences and the production constraints that they are facing. These are key steps for easy adoption of new lines. In Togo, the rice breeding programme is limited so far to varieties introduction and adaptability tests. Most of the varieties introduced are not adopted by farmers. Only the aromatic variety IR 841 introduced from IRRI in the 1980s is widely cultivated around the country despite introduction and participatory varietal selection (PVS) of more than 30 varieties among which some are even higher yielding and non aromatic, in the past ten years. The involvement of farmers in the varietal selection from their vegetative stage on the field to their taste (after cooking) through the PVS approach (AfricaRice, 2010b) was supposed to make easy and even increase farmers' adoption rate of the tested varieties. Unfortunately, more than ten PVS have been implemented in ten areas involving more than 200 farmers in the past ten years without any variety adopted by farmers.

Based on the precedent information, it clearly appears that rice growers have good reasons to fail to adopt the varieties introduced. Documenting these reasons is crucial in building a proper breeding programme in Togo. Also, of great importance is information on farmers' preferred traits and rice production constraints. All these will guide breeders in developing varieties for farmers.

Therefore, the objectives of this research were to:

- make an inventory of all the varieties introduced in the study area from 1980 to 2013,

- document the current status of rice varieties adoption in Togo and investigate the main reasons for non-adoption of new rice varieties by farmers,
- identify and assess the perception of farmers on their major preferred traits and rice production constraints; and
- compare farmers' perceptions on their major preferred traits and rice production constraints in rainfed lowlands and irrigated lowlands.

3.2. Materials and Methods

Before assessing the reasons of the introduced varieties adoption failure, it is imperative to establish a list of the introduced varieties from 1980 to 2013. This was done in two steps. Firstly, secondary data on the introduced varieties were collected from the National Agriculture Research System (NARS) through literature review, and secondly, information through focus group discussions and questionnaire survey by farmers and officers from National Agriculture Extension System (NAES).

3.2.1. Documentation of varieties introduced from 1980 to 2013

3.2.1.1. Establishment of a list of varieties introduced by the NARS

Secondary data on the varieties introduced from 1980 to 2013 were collected through literature review. Research activities reports and publications by ITRA (*Institut Togolais de Recherche Agronomique*) were reviewed to collect data such as name of variety, year of introduction, source of introduction, Institution that introduced the variety, site of PVS, and ecology of production. With the information collected, a list of varieties introduced in Togo by the NARS from 1980 to 2013 were prepared using Microsoft Excel.

3.2.1.2. Collection of information on varieties introduced by the NAES and farmers

In Togo, because of the borders' porosity many rice varieties have been introduced by the NAES workers or by farmers themselves. These varieties are cultivated in a few farmers' fields especially in the bordering areas. So, the list of varieties introduced by the NARS was completed with the varieties introduced by the NAES and farmers. The collection of information on such varieties was done around the country in the main rice producing areas (with a sample of rice growers and NAES workers) through focus group discussions as presented below.

➤ Survey area

The focus group discussion was conducted in the five Regions of Togo in two main rice producing areas per Region, thus a total of ten sites were covered in this study. These are the main irrigated sites (four) and the major rainfed lowlands (six) as presented in the Table 3.1.

➤ Materials

A semi-structured guide elaborated with topics related to introduced varieties were used to collect information from the sample of farmers at each site, as shown in Appendix 3.1.

➤ Farmers involved

Each focus group was composed of 15 farmers per site. For all the six rainfed lowland sites, the 15 farmers were chosen from two to ten villages around the sites. The same was applied for the Zio irrigated site which covers three villages. For the other irrigated sites, all the 15 farmers were chosen in the same village where the site is located, because of the fact that the

sites are small and located in only one village. In total, for the ten sites, 150 farmers (15 farmers x 10 sites) were involved in the focus group discussions.

The target farmers were chosen by the NAES workers (*Institut de Conseil et d'Appui Technique, ICAT*) using the list of rice growers. The sampling was based on the number of years of cultivation by farmers (at least ten years), gender (female and male) as well as farmer's age (> 40 years old). Data on number of villages and farmers involved are presented in Appendix 3.2.

Table 3.1: Sites of focus group discussions on rice varieties introduced by NAES and farmers

Regions	N°	Sites	Ecology	Map
Savannas	1	<i>Tone</i>	Irrigated	
	2	<i>Oti</i>	Irrigated	
Kara	3	<i>Binah</i>	Rainfed lowlands	
	4	<i>Dankpen</i>	Rainfed lowlands	
Central	5	<i>Tchamba</i>	Rainfed lowlands	
	6	<i>Sotouboua</i>	Rainfed lowlands	
Plateaux	7	<i>Est Mono</i>	Rainfed lowlands	
	8	<i>Kpélé</i>	Rainfed lowlands	
Maritime	9	<i>Zio</i>	Irrigated	
	10	<i>Bas Mono</i>	Irrigated	

➤ **Methodology**

The discussion was led by a facilitator. At the four sites of the Maritime and Plateaux Regions, the discussions were had in Ewe (local language spoken in the South of Togo), and in French on Sotouboua (Central Region), Binah and Dankpen (Kara Region) sites. At the other sites, recourse to a translator was needed to make the discussions easy. Therefore, the discussions were done in Kotokoli, Tchokossi and Moba respectively at Tchamba (Central Region), Oti and Tone (Savannas Region) sites. A semi-structured guide was used for the focus group discussions.

➤ **Data collected and analysis**

From the focus group discussions, information on varieties introduced by the NAES and farmers were collected. These were the names of the varieties, the ecology of production, the year of introduction, the person/institution responsible for the introduction, the source of introduction, and the area of introduction. The above information were compiled by using Microsoft Excel.

3.2.2. Assessment of farmers' reasons of failure of adoption of varieties introduced, their preferred traits and production constraints

With the established list of introduced varieties, farmers' reasons of their non-adoption, their varietal preferences, and rice production constraints were assessed through focus group discussions and questionnaire survey at all the ten sites.

3.2.2.1. Focus group discussions

The survey was undertaken at the same ten sites as mentioned above in *paragraph 3.2.1.2 (section Survey areas)*. A semi-structured guide covering topics related to the introduced varieties' traits and the main reasons of their non-adoption, farmers' varietal preferences, and the main biotic and abiotic constraints occurring, has been used for the focus group discussions. The same set of the 15 farmers per site, as indicated in *paragraph 3.2.1.2 (section Farmers involved)* were involved at each of the ten sites for this study. As well, the same methodology as presented in *paragraph 3.2.1.2 (section Methodology)* was used.

Data collected included the name of introduced varieties that are said to be lost, abandoned, unknown or still grown; their major agro-morphological traits that are preferred by farmers. Also, farmers were asked to mention the main reasons for their non-adoption of the varieties. The phenotypes of the main traits as preferred by farmers were also collected, as well as the main abiotic and biotic constraints occurring on their fields. The preferred traits and the production constraints which were mentioned by farmers on more than half of the ten sites, were considered respectively as the major preferred traits (and their phenotypes) and the major constraints. In mentioning their preferences and constraints, recourse to a vote was done to get consensus among farmers.

Microsoft Excel was used to compile all the information collected from the focus group discussions.

The focus group discussion guide for this survey is presented in Appendix 3.3.

3.2.2.2. Questionnaire survey

A questionnaire survey was conducted at the same sites where the focus group discussions were conducted, with the same farmers. This was to deepen the farmers' opinion on the

introduced varieties and their perceptions on the major varietal preferences, as well as on the major rice production constraints they pointed out.

➤ **Materials**

From the focus group discussions, a questionnaire was developed on the major farmers' varietal preferences and rice production constraints. The content of the questionnaire is presented in Appendix 3.4.

➤ **Methodology**

Interview with individual farmers by surveyors using the questionnaire was carried out in each of the ten areas. *Likert* scale of the level of importance was used in assessing separately farmers' perceptions on the major varietal preferences and production constraints that came out from the focus group discussions. The scores of this scale were as follows:

1- Very important, 2- Important, 3- Not so important and 4- Not important.

➤ **Data collected and analysis**

The level of importance of each major varietal preference and production constraint as perceived by farmers individually was collected. The collected data was analysed using the statistical software SPSS 17.0. The mean score of each preference or constraint was used to identify the average level of importance of each trait around the country. In addition, Chi-square test was run to determine the relationship between the perception of farmers in rainfed lowlands and that of their counterparts on irrigated lowlands sites for each of their major varietal preferences and production constraints.

3.3. Results

3.3.1. Varieties introduced by the NARS

Table 3.2 is a summary of the varieties that were tested for adaptation by the NARS after being introduced in Togo, and presented to farmers for adoption mostly through PVS (participatory varietal selection) approach.

In total 38 varieties were reported to have been introduced from IRRI (two varieties), WARDA (11 varieties), AfricaRice (11 varieties), CNS-Mali (five varieties), China (three varieties), and other unknown sources (six varieties). Apart from the varieties IR 841 and Sorad introduced respectively in 1980 and 1985, the rest were introduced between 2005 to 2013. For 18 varieties, information on the sites where the PVS was implemented could not be identified. Only one variety (NERICA 4) was identified as an upland variety whereas 20 and 17 varieties were introduced and tested respectively in irrigated and rainfed lowland sites.

Table 3.2: List of rice varieties introduced in Togo by the NARS from 1980 to 2013 and presented to farmers for adoption through PVS approach

N°	Name of varieties	Source of introduction	Year of introduction	Sites of PVS	Type of ecology	References
1	IR 841	IRRI	1980	All the Regions	Rainfed lowlands	ITRA, 2007
2	Accravi	Not found	1985	Est Mono	Rainfed lowlands	ITRA, 2008
3	TGR 1	ADRAO	2005	Not found	Rainfed lowlands	ITRA, 2008
4	TGR 203	ADRAO	2005	Not found	Rainfed lowlands	ITRA, 2008
5	TGR 405	ADRAO	2005	Not found	Rainfed lowlands	ITRA, 2008
6	TGR 75	ADRAO	2005	Not found	Rainfed lowlands	ITRA, 2006
7	WAB 638-1	ADRAO	2005	Not found	Rainfed lowlands	ITRA, 2006
8	WAS 161	ADRAO	2005	Not found	Rainfed lowlands	ITRA, 2006
9	TOVE 1	Not found	2005	Not found	Rainfed lowlands	ITRA, 2006
10	NERICA L14	ADRAO	2006	Binah, Kpélé, Tone	Irrigated	ITRA, 2007
11	NERICA L19	ADRAO	2006	Kpélé	Irrigated	ITRA, 2007
12	NERICA L20	ADRAO	2006	Not found	Irrigated	ITRA, 2007
13	NERICA L41	ADRAO	2006	Not found	Irrigated	ITRA, 2007
14	NERICA L42	ADRAO	2006	Not found	Irrigated	ITRA, 2007
15	Er You 725	China	2007	Kozah	Rainfed lowlands	Akata, 2008
16	Jin You 38	China	2007	Not found	Rainfed lowlands	Akata, 2008
17	Pel Liang You 3076	China	2007	Not found	Rainfed lowlands	Akata, 2008
18	IR 46	IRRI	2007	Not found	Rainfed lowlands	ITRA, 2007
19	SAHEL 108	AfricaRice	2007	Not found	Irrigated	ITRA, 2009
20	ITA 212	Not found	2007	Not found	Rainfed lowlands	ITRA, 2007
21	BOUAKE 189	Not found	2007	Not found	Rainfed lowlands	ITRA, 2007
22	Dapaong	Not found	2007	Not found	Rainfed lowlands	ITRA, 2007
23	ITA 324	Not found	2007	Not found	Rainfed lowlands	ITRA, 2007
24	Nerica 4	AfricaRice	2011	Tone	Upland	ITRA, 2007
25	IDSA 64	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
26	BASMATI 370	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
27	IR 72	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
28	IR 50	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
29	ORYLUX 1	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
30	ORYLUX 3	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
31	ORYLUX 4	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
32	ORYLUX 5	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
33	ORYLUX 3	AfricaRice	2013	Maritime, Plateaux	Irrigated	ITRA, 2013
34	KOGONI 91.1	CNS-Mali	2013	All the Regions	Irrigated	ITRA, 2013
35	DKA 1	CNS-Mali	2013	All the Regions	Irrigated	ITRA, 2013
36	DKA-M-11	CNS-Mali	2013	All the Regions	Irrigated	ITRA, 2013
37	DKA-M-13	CNS-Mali	2013	All the Regions	Irrigated	ITRA, 2013
38	SIK 353 A-10	CNS-Mali	2013	All the Regions	Irrigated	ITRA, 2013

3.3.2. Varieties introduced by the NAES and farmers

From the focus group discussions held on the ten sites with a total of 150 farmers and NAES workers, a list of 18 varieties was established (Table 3.3). These varieties were mainly introduced by farmers from neighbouring countries between 1995 and 2013.

Table 3.3: List of rice varieties introduced in the study area by the NAES and farmers

N ^o	Name of the varieties	Introduction sites	Actors	Year	Mean	Source of introduction	Type of ecology
1	Anachen	Binah	Nord TOGO project	2006	Buying	Unknown	Lowlands
2	Londo Londo	Binah	A parliamentarian	2013	Buying	Unknown	Lowlands
3	Gambiaca	Binah, Dankpen, Oti	A farmer	2010	Buying	Benin	Lowlands
4	Kpive-sa	Est Mono	A farmer	2007	Buying	Unknown	Lowlands
5	Sorad	Est Mono	Sorad	1995	Gift	Unknown	Lowlands
6	Chinoivi	Kpélé	A Chinese	1995	Gift	China	Irrigated
7	Mossi	Oti	A farmer	2009	Buying	Burkina Faso	Lowlands
8	Timbou	Oti	A Burkinabe	2011	Buying	Burkina Faso	Irrigated
9	Awini	Sotouboua	A farmer	2006	Buying	Unknown	Lowlands
10	Datcha	Sotouboua	A farmer	1995	Buying	Unknown	Lowlands
11	Djama	Tchamba	A farmer	2013	Buying	Unknown	Lowlands
12	Ibo	Tchamba	A farmer	2013	Buying	Unknown	Lowlands
13	Lobo Lobo	Tchamba	A farmer	2013	Buying	Unknown	Lowlands
14	Alandine	Tone	A farmer	2011	Exchange	Burkina Faso	Lowlands
15	Ablayibo	Zio	A farmer	1997	Gift	Ghana	Irrigated
16	Chapeau vert	Zio	A farmer	1995	Gift	Ghana	Irrigated
17	Sipi	Zio	A farmer	2011	Gift	AfricaRice	Irrigated
18	Berice 21	Zio	A farmer	2011	Gift	AfricaRice	Irrigated

On the 18 varieties introduced by the NAES and farmers in the study area, 14 varieties (77.78%) were introduced by farmers. The NAES called Sorad and Nord Togo Project as well as a parliamentarian, a Chinese and a Burkinabe (who are not rice growers) have also introduced in their respective areas, one variety each. On the ten sites covered by the study, no variety introduced by NAES or farmers was reported on the site of Bas Mono. On the contrary, the variety Gambiaca was introduced at three sites (Binah, Dankpen, Oti) by farmers.

It clearly appears that four varieties were introduced in Zio area; three in Binah, Oti and Tchamba areas; two varieties in Est Mono and Sotouboua; and only one variety in Dankpen, Kpélé, and Tone. For 50% of the varieties the source of origin is unknown, and for the rest, vaguely known. The variety Chinoivi was introduced by a Chinese national from China. Three varieties (Alandine, Mossi and Timbou) were taken from Burkina Faso; Chapeau vert and Ablayibo from Ghana; and Gambiaca from Benin. Finally, the varieties Berice 21 and Sipi were said to be introduced by farmers from the Africa rice center (Africarice) in the Zio valley. The varieties were introduced mainly through the means of buying seeds (for 61.11% of the varieties), through gifts (for 33.33% of the varieties); and through seed exchanges (for 5.56% of the varieties). Briefly, 33.33% of the varieties were presented to be adapted to irrigated lowland conditions whereas 66.67% to rainfed lowland conditions.

3.3.3. Status of adoption of the varieties introduced in Togo

In sum, a total of 56 introduced varieties were listed from the NARS (38 varieties in Table 3.2), and the NAES and farmers (18 varieties in Table 3.3) reports. The list of these 56 introduced varieties was presented to the same panel of 150 farmers around the ten sites through focus group discussions to assess their current status of adoption by farmers. From the 56 varieties listed, 25 varieties (44.64%) were said to be unknown by farmers, only the variety Sipi (1.79%) was reported to be abandoned by farmers, seven varieties (12.50%) were said to be lost (Table 3.4), and 23 varieties (41.07%) were still cultivated by a few farmers in the study areas (Table 3.5).

Table 3.4: List of introduced rice varieties unknown, abandoned or lost by farmers in the study area

N°	Name of varieties	Status	Sites of	Actor
1	BASMATI 370	Unknown	Zio and Kpélé	NARS
2	BOUAKE 189	Unknown	All the Regions	NARS
3	Dapaong	Unknown	All the Regions	NARS
4	DKA 1	Unknown	All the Regions	NARS
5	DKA-M-11	Unknown	All the Regions	NARS
6	DKA-M-13	Unknown	All the Regions	NARS
7	Er You 725	Unknown	Sarakawa	NARS
8	IDSA 64	Unknown	Zio and Kpélé	NARS
9	IR 46	Unknown	All the Regions	NARS
10	IR 50	Unknown	Zio and Kpélé	NARS
11	IR 72	Unknown	Zio and Kpélé	NARS
12	ITA 212	Unknown	All the Regions	NARS
13	ITA 324	Unknown	All the Regions	NARS
14	Jin You 38	Unknown	All the Regions	NARS
15	KOGONI 91.1	Unknown	All the Regions	NARS
16	ORYLUX 2	Unknown	Zio and Kpélé	NARS
17	ORYLUX 3	Unknown	Zio and Kpélé	NARS
18	ORYLUX 4	Unknown	Zio and Kpélé	NARS
19	ORYLUX 5	Unknown	Zio and Kpélé	NARS
20	Pel Liang You 3076	Unknown	All the Regions	NARS
21	SAHEL 108	Unknown	All the Regions	NARS
22	SIK 353 A-10	Unknown	All the Regions	NARS
23	TOVE 1	Unknown	All the Regions	NARS
24	WAB 638-1	Unknown	All the Regions	NARS
25	WAS 161	Unknown	All the Regions	NARS
1	Sipi	Abandoned	Zio	A farmer
1	Anachen	Lost	Binah	Nord Togo
2	Djama	Lost	Tchamba	A farmer
3	Mossi	Lost	Oti	A farmer
4	TGR 1	Lost	All the Regions	NARS
5	TGR 405	Lost	All the Regions	NARS
6	TGR 75	Lost	All the Regions	NARS
7	Timbou	Lost	Oti	A Burkinabe

NARS = National agriculture research system

All the 25 varieties unknown by farmers were introduced by the NARS. Moreover, 4 varieties (57.14%) introduced by the NAES and Farmers on the one hand, as well as 3 varieties (42.86%) introduced by the NARS on the other hand, were lost by farmers respectively in Binah (one variety), Oti (two varieties) and Tchamba (one variety) for the first group; and in all the regions for the second group.

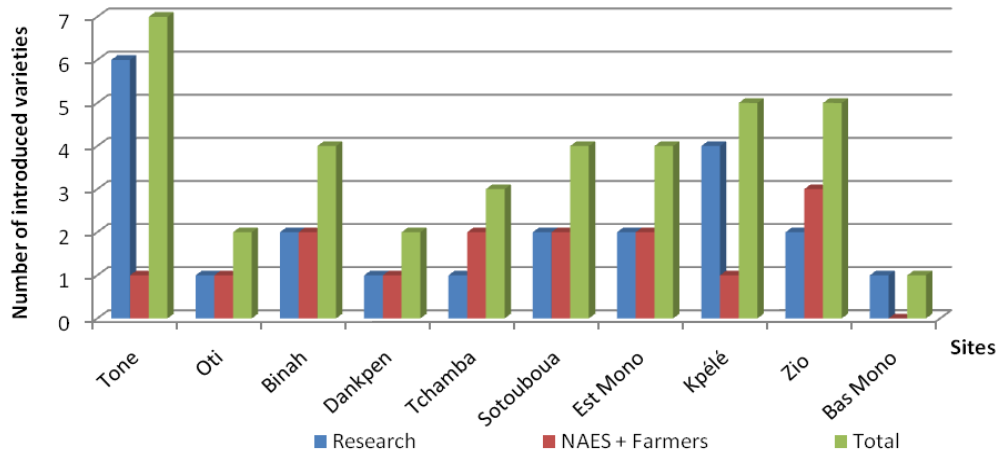
Table 3.5: List of introduced rice varieties currently cultivated by a few farmers in the study area

N°	Name of varieties	Sites of cultivation	Actor of introduction
1	Ablayibo	Zio	A farmer
2	Accravi	Est Mono	NARS
3	Alandine	Tone	A farmer
4	Awini	Sotouboua	A farmer
5	Berice 21	Zio	A farmer
6	Chapeau vert	Zio	A farmer
7	Chinoivi	Kpélé	A Chinese
8	Datcha	Sotouboua	A farmer
9	Gambiaca	Binah, Dankpen, Oti	A farmer
10	Ibo	Tchamba	A farmer
11	IR 841	All the Regions	NARS
12	Kpive-sa	Est Mono	A farmer
13	Lobo Lobo	Tchamba	A farmer
14	Londo Londo	Binah	A parliamentarian
15	Nerica 4	Tone	NARS
16	NERICA L14	Binah, Kpélé, Tone	NARS
17	NERICA L19	Kpélé	NARS
18	NERICA L20	Tone	NARS
19	NERICA L41	Tone	NARS
20	NERICA L42	Tone	NARS
21	ORYLUX 1	Zio and Kpélé	NARS
22	Sorad	Est Mono	Sorad
23	TGR 203	Sotouboua	NARS

NARS = National agriculture research system

Among the 23 introduced varieties currently cultivated in the study area, only the variety IR 841 introduced by the NARS is cultivated in all the ten locations of the study. Nine other varieties were introduced by the NARS. These are Nerica 4, Nerica L20, Nerica L41 and Nerica L42 in Tone; Accravi in Est Mono; Nerica L19 in Kpélé; TGR 203 in Sotouboua; Orylux 1 in Zio and kpélé; and Nerica L 14 in Binah, Kpélé and Tone. Thus ten varieties among the 38 introduced by the NARS are still being cultivated like 13 varieties introduced by the NAES and farmers over the 18 varieties listed.

The following graph (Figure 3.1) presents the number of introduced varieties currently cultivated per site and per actor of introduction.



NAES = National agriculture extension system, Research = NARS = National agriculture research system

Figure 3.1: Number of introduced rice varieties currently cultivated per site and per actor of introduction

At least one introduced variety was still cultivated in each of the ten locations of the study. Only the variety IR 841 was introduced in Bas Mono by the NARS and was being cultivated. This variety was the only one which was widely cultivated in all the ten locations. In terms of number of varieties cultivated, Tone comes first with seven introduced varieties, followed by Kp  l  and Zio with five varieties; Binah, Sotouboua, Est Mono with four varieties; Tchamba with three varieties; Oti and Dankpen with two varieties; and Bas Mono with only one variety (the variety IR 841).

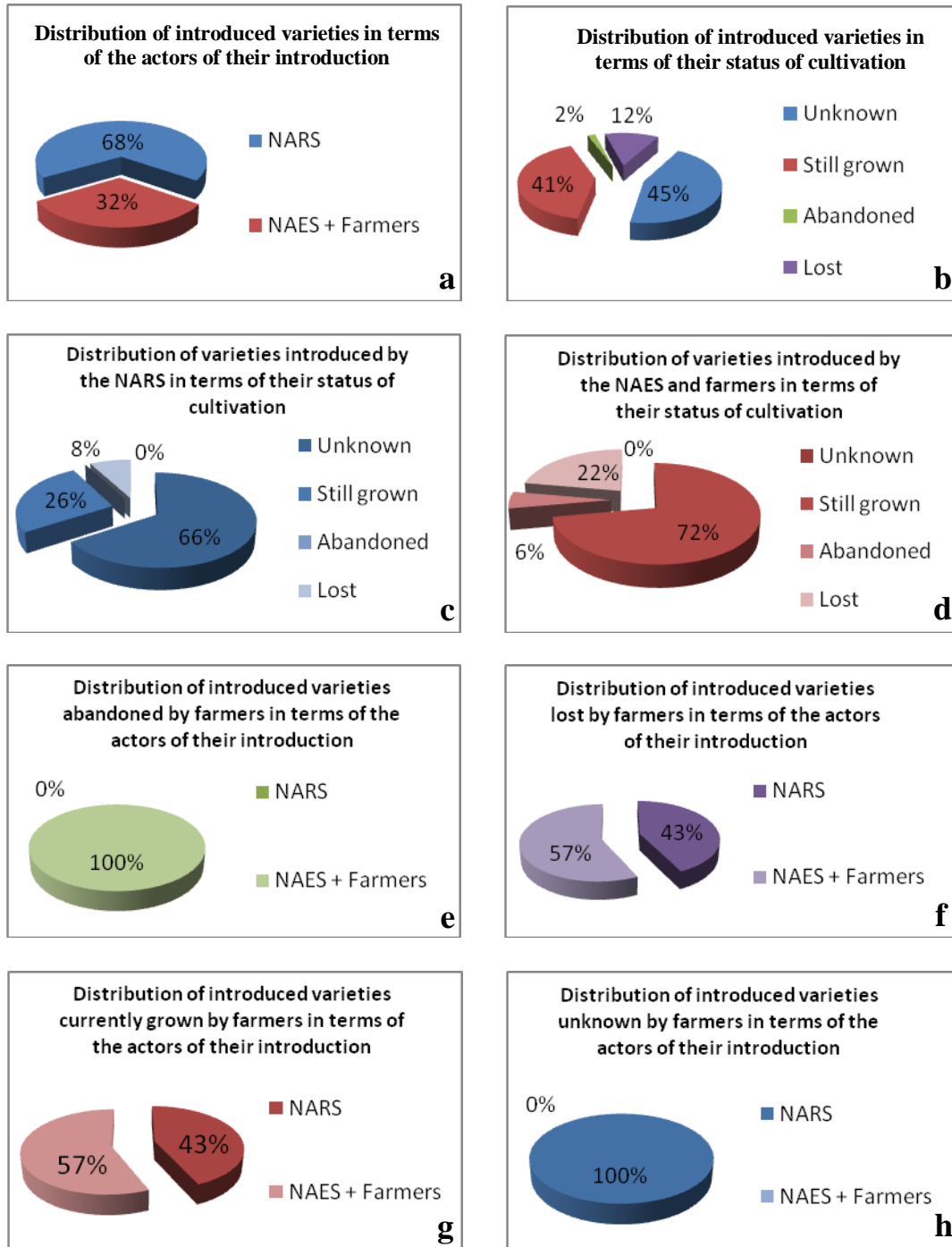
Of the seven varieties cultivated in Tone, six were introduced by the NARS. Likewise in Kp  l  where four varieties introduced by the NARS were still being cultivated over five varieties were recorded. On the rest of the sites, only one or two varieties introduced by the NARS were still being cultivated. Moreover, about the varieties introduced by NAES and farmers, only Zio farmers were cultivating three (Ablayibo, Berice 21, and Chapeau vert). At the nine other sites, one or two varieties introduced by NAES and farmers were still being cultivated except Bas Mono where no variety was introduced by NAES and farmers.

Among the varieties introduced by the NARS which were still being cultivated, IR 841 was cultivated on the ten sites suggesting an adoption rate of 100% by the 150 farmers involved in the study, Nerica L14 on 3 sites (Tone, Binah and Kpél ) was adopted by 25% of farmers, Orylux 1 on two sites (Kp l  and Zio) was adopted by 10% of farmers, and the seven other varieties on only one site each were adopted by less than 5% of farmers. On the other hand, the variety Gambiaca was the only one introduced by the NAES and farmers, and which was being cultivated on more than one site. This variety was encountered in the northern part of Togo in three locations of the study areas: Oti, Binah and Dankpen.

The following graphs (Figure 3.2) summarize in percentage, the distribution of the varieties introduced in terms of the actors of their introduction and their current status of cultivation. From Figure 3.2a, the NARS has introduced more than two times varieties (68%) in the study area than the NAES and farmers taken together (32%). About half of the varieties introduced (41%) were still being grown by farmers. A little more (45%) was unknown by farmers who reported only 2% of the varieties as abandoned and 12% as lost (Figure 3.2b). From the varieties introduced by the NARS, 66% was unknown by farmers, and none was abandoned (Figure 3.2c). On the contrary Figure 3.2d shows that 72% of the varieties introduced by the NAES and farmers were still being grown and only 22% were lost.

All the varieties abandoned by farmers were introduced by the NARS and farmers (Figure 3.2e). Likewise, all the varieties unknown by farmers were introduced by the NARS (Figure 3.2h). Taking all the varieties that were still being grown by farmers, on the one hand, and those reported to be lost on the other hand, the largest proportion was for the NAES and farmers (57%) in both cases (Figure 3.2f and 3.2g).

In view of the above results, IR 841 was the only one variety cultivated in both ecologies of rainfed and irrigated lowlands, and across all the ten sites of the study area. Thus, this variety is the only one adopted by all the farmers involved in the study in Togo.



NARS = National agriculture research system, NAES = National agriculture extension system

Figure 3.2: Distribution of the introduced rice varieties in terms of the actors of their introduction as well as their current status of cultivation

Taking the 38 varieties introduced by the NARS from 1980 to 2013, only IR 841 can be said to be adopted in the ten locations, giving only 2.63% of such varieties adopted in the study area.

3.3.4. Farmers' reasons of failure of adoption of introduced varieties

The main reasons of non-adoption of varieties were according to the farmers their non-preferred phenotypes expressed by the varieties. They also reported that the 31 varieties they knew (abandoned, lost or cultivated) had some good traits they preferred. Both non-preferred and preferred traits were collected as main reasons of adoption failure and good traits for breeding programme respectively as shown in Table 3.6.

Eight main reasons were stated by farmers across the ten sites for not having adopted the varieties introduced. These are absence of aroma, too short or too tall height, lateness, susceptibility to drought, small grain size, spontaneous grain shattering, and stony grain after cooking.

Absence of aroma comes out as the main reason stated by farmers in all the ten locations for not having adopted 28 varieties over the 31 (90.32%). Only three varieties Chapeau vert, IR 841 and Orylux 1 were not discredited for aroma because these varieties are aromatic. As far as the other reasons are concerned, seven, five, four, four, three, two and two varieties have been rejected by farmers due to their susceptibility to drought (on three sites), lateness (six sites), short height (four sites), tall height (six sites), small grain (two sites), spontaneous grain shattering (two sites) and stony grain after cooking (two sites) respectively.

Table 3.6: Main reasons for non-adoption and some farmers' preferred traits of the introduced rice varieties

N°	Name of genotype	Sites	Reasons of non-adoption						Good traits									
			Absence of aroma	Short height	high height	Lateness	Susceptibility to drought	Short grain	Spontaneous grain shattering	Stony grain after cooking	Yield	Earliness	Presence of aroma	Resistance to drought	Resistance to diseases	Resistance to weeds	Good taste after cooking	Good tillering
1	Ablayibo	Zio	x							x								
2	Accravi	Est Mono	x							x								
3	Alandine	Tone	x	x							x							
4	Anachen	Binah	x	x							x							
5	Awini	Sotouboua	x	x		x					x							
6	Berice 21	Zio	x							x								
7	Chapeau vert	Zio								x	x	x				x	x	
8	Chinoivi	Kpélé	x	x				x			x							
9	Datcha	Sotouboua	x			x										x		
10	Djama	Tchamba	x							x								
11	Gambiaca	Binah, Dankpen, Oti	x		x	x							x					
12	Ibo	Tchamba	x													x		
13	IR 841	All the sites								x	x	x				x	x	
14	Kpive-sa	Est Mono	x			x												x
15	Lobo Lobo	Tchamba	x		x	x	x										x	
16	Londo Londo	Binah	x							x								
17	Mossi	Oti	x											x				
18	Nerica 4	Tone	x					x		x	x							
19	NERICA L14	Binah, Kpélé, Tone	x					x		x	x							
20	NERICA L19	Kpélé	x					x		x	x							
21	NERICA L20	Kpélé	x					x		x	x							
22	NERICA L41	Kpélé	x					x		x	x							
23	NERICA L42	Kpélé	x					x		x	x							
24	ORYLUX 1	Zio								x	X	x				x	x	
25	Sipi	Zio	x															x
26	Sorad	Est Mono	x		x									x				
27	TGR 1	Sotouboua	x							x								
28	TGR 203	Sotouboua	x					x						x				
29	TGR 405	Sotouboua	x					x							x			
30	TGR 75	Sotouboua	x							x								
31	Timbou	Oti	x			x								x				

Accession Awini was rejected for four reasons (absence of aroma, short height, lateness and spontaneous grain shattering) whereas accession Lobo lobo was rejected for absence of aroma, tall height, lateness and susceptibility to drought. Farmers gave no reasons to reject varieties Chapeau vert, IR 841 and Orylux 1. IR 841 is the widely cultivated in all regions. Chapeau vert and Orylux 1 are still cultivated in the Zio valley.

Even though 28 introduced varieties were not widely cultivated by farmers, some good traits were identified in these varieties. Nine good traits have been listed by farmers: high yield, earliness, presence of aroma, resistance to drought, resistance to diseases, resistance to weeds, good taste, good tillering, and presence of awn (strongly limits birds damage at maturity). Out of the 31 varieties, 17 varieties (54.84%) were high yielding, ten varieties (32.26%) were early maturing, seven varieties (22.58%) were resistant to drought, four varieties (12.90%) had good taste after cooking, three varieties (9.68%) were aromatic, three varieties (9.68%) expressed good tillering ability, two varieties (6.45%) were resistant to diseases, two varieties (6.45%) were resistant to weeds, and only one variety (3.23%) had awns. About 71% of the varieties (22 varieties) were said had at least one of these good traits.

3.3.5. Farmers' varietal preferences

A variable number of preferences came out from the focus group discussions. 11 preferences were listed in Kpélé, ten in Zio, nine in Bas Mono, Dankpen and Tchamba, eight in Tone, six in Binah, Oti and Sotouboua, and five in Est Mono (Table 3.7).

Table 3.7: List of farmers' varietal preferences per site

Tone	Oti	Binah	Dankpen	Tchamba
1 Aromatic	1 Aromatic	1 Aromatic	Adapted to all ecology	1 Aromatic
2 Good germination	2 Early maturing	2 Early maturing	2 Aromatic	2 Early maturing
3 Good tillering	3 High yield	3 High yield	3 Early maturing	3 High yield
4 Heavy panicle	4 Medium height	4 Long grain	4 High yield	4 Long grain
5 Long grain	5 Resistant to drought	5 Medium height	5 Long grain	5 Medium height
6 Medium height	6 Resistant to weeds	6 White grain	6 Sticky grain	6 Resistant to birds
7 Not sticky grain			7 Swelling grain	7 Resistant to drought
8 Swelling grain			8 Tall height	8 Swelling grain
			9 White grain	9 White grain
Sotouboua	Est Mono	Kpélé	Zio	Agomé Glozou
1 Aromatic	1 Easy to shell	1 Aromatic	1 Aromatic	1 Aromatic
2 Early maturing	2 Good taste	2 Early maturing	2 Early maturing	2 Early maturing
3 High yield	3 High yield	3 Good taste	3 Good tillering	3 Good taste
4 long grain	4 Long grain	4 Good tillering	4 High yield	4 Good tillering
5 Medium height	5 Medium height	5 High yield	5 Heavy panicle	5 High yield
6 Resistant to diseases		6 Greenish grain at maturity	6 Long grain	6 Long grain
		7 Heavy panicle	7 Medium height	7 Medium height
		8 Long grain	8 Soft after cooking	8 Resistant to birds
		9 Medium height	9 Solid stalk	9 Resistant to drought
		10 Resistant to lodging	10 White grain	
		11 Sticky grain		

Generally, 24 agro-morphological and organoleptic traits were recorded from farmers in all of the ten sites as their preferences.

By cross checking farmers' preferences listed per site, it appears that four preferences are common to nine sites. These are presence of aroma (all the sites except Est Mono), high yield (all the sites except Tone), long grain (all the sites except Oti), and medium height (all the sites except Dankpen where the preference is for tall plants). Earliness was noted at eight sites (all the sites except Tone and Est Mono). For the other preferences listed by farmers, two preferences were common to farmers at four sites, four preferences at three sites, and two

preferences at two sites. Eleven preferences were specific to only one site as presented in Figure 3.3.

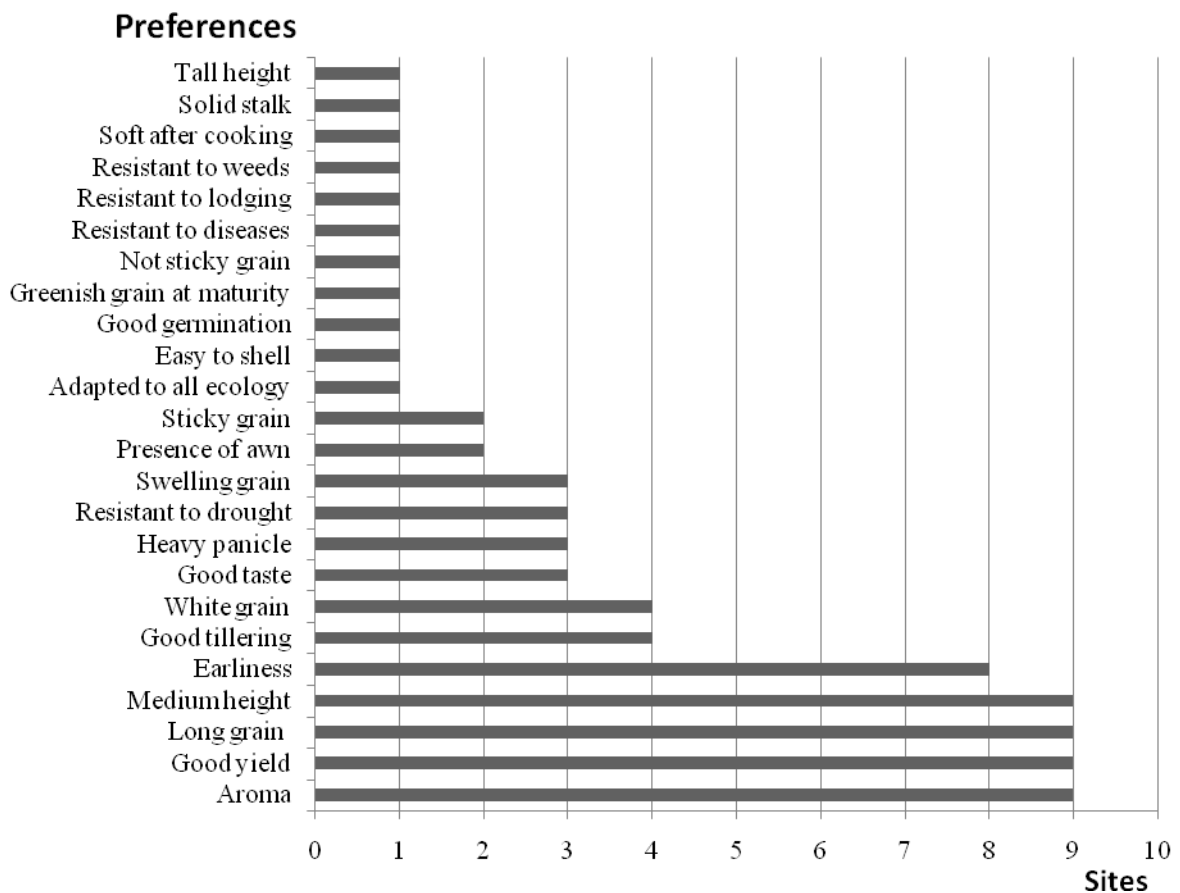


Figure 3.3: Number of sites at which each rice varietal preference was mentioned by a group of farmers

From the above results obtained from focus group discussions, the main preferences on which a national breeding programme should be based are the most common preferences to farmers on more than the half of the sites. Thus, the four preferences mentioned on nine sites (presence of aroma, high yield, long grain, and medium height) as well as the one preference mentioned on eight sites (earliness) are considered as the major preferences for which farmers' perception in terms of importance has been assessed following *Likert* scale through a questionnaire survey. Table 3.8 shows the obtained results.

Table 3.8: Farmers' perception on their major rice varietal preferences

Preferences	Levels of importance*				Total farmers	Mean score	Implication
	1	2	3	4			
Presence of aroma	76	34	10	30	150	2	Important
Good grain yield	78	28	16	28	150	2	Important
Long grain	26	5	6	113	150	3	Not so important
Medium height	8	9	54	79	150	3	Not so important
Earliness	53	15	32	50	150	3	Not so important

* Likert scale: 1 – Very important, 2 – Important, 3 – Not so important, 4 – Not important

The assessment of farmers' perception on their major preferences based on mean scores of levels of importance showed that *aroma* and *high yield* were important to farmers in the country. The other three preferences: *long grain*, *medium height*, and *earliness* were not so important.

Relationship between the perception of farmers growing rice in rainfed lowlands and those of irrigated lowlands for these preferences were assessed. Table 3.9 shows comparison between the two ecologies for each of farmers' preferences. The two ecologies differed significantly for only earliness.

Table 3.9: Relationship between farmers' perception on their major rice varietal preferences in rainfed and irrigated lowlands

Preferences	Sites	Data	Levels of importance				Total valid cases	Calculated X ²	Df
			1	2	3	4			
Presence of aroma	Rainfed	<i>Observed</i>	50	20	8	12	150	0.14	3
		<i>Expected</i>	45.6	20.4	6.0	18.0			
	Irrigated	<i>Observed</i>	26	14	2	18			
		<i>Expected</i>	30.4	13.6	4.0	12.0			
High grain yield	Rainfed	<i>Observed</i>	60	15	10	5	150	5.16	3
		<i>Expected</i>	46.8	16.8	9.6	16.8			
	Irrigated	<i>Observed</i>	18	13	6	23			
		<i>Expected</i>	31.2	11.2	6.4	11.2			
Long grain	Rainfed	<i>Observed</i>	17	3	2	68	150	2.09	3
		<i>Expected</i>	15.6	3.0	3.6	67.8			
	Irrigated	<i>Observed</i>	9	2	4	45			
		<i>Expected</i>	10.4	2.0	2.4	45.2			
Medium height	Rainfed	<i>Observed</i>	4	3	43	40	150	8.69	3
		<i>Expected</i>	4.8	5.4	32.4	47.4			
	Irrigated	<i>Observed</i>	4	6	11	39			
		<i>Expected</i>	3.2	3.6	21.6	31.6			
Earliness	Rainfed	<i>Observed</i>	38	10	18	24	150	15.38*	3
		<i>Expected</i>	31.8	9.0	19.2	30.0			
	Irrigated	<i>Observed</i>	15	5	14	26			
		<i>Expected</i>	21.2	6.0	12.8	20.0			

Df = degree of freedom, * significant difference, $X^2 = 13.277$ for $df = 3$ at $p < 0.01$

3.3.6. Rice production constraints

Farmers in nine locations observed the presence of oily stains on the soil surface as the symptom of iron toxicity. In total, 12 constraints in all the ten sites were raised by farmers among which six were abiotic (Iron toxicity, flooding, drought, plant lodging, soil poverty, and spontaneous grain shattering) and six were biotic (birds, insects, diseases, rodents, weeds, and termites) as shown in Table 3.10.

Table 3.10: List of biotic and abiotic constraints per site

Tone	Oti	Binah	Dankpen	Tchamba
1 Birds	1 Birds	1 Birds	1 Birds	1 Birds
2 Diseases	2 Diseases	2 Diseases	2 Drought	2 Diseases
3 Drought	3 Flooding	3 Drought	3 Flooding	3 Insects
4 Flooding	4 Insects	4 Flooding	4 Insects	4 Iron toxicity
5 Insects	5 Rodents	5 Iron toxicity	5 Iron toxicity	5 Rodents
6 Iron toxicity	6 Weeds	6 Plant lodging	6 Termites	6 Termites
7 Rodents		7 Spontaneous grain shattering	7 Weeds	7 Weeds
8 Weeds		8 Termites		
		9 Weeds		

Sotouboua	Est Mono	Kpélé	Zio	Bas Mono
1 Birds	1 Birds	1 Birds	1 Birds	1 Birds
2 Diseases	2 Drought	2 Diseases	2 Diseases	2 Diseases
3 Drought	3 Insects	3 Drought	3 Drought	3 Flooding
4 Flooding	4 Iron toxicity	4 Flooding	4 Flooding	4 Insects
5 Insects	5 Termites	5 Insects	5 Insects	5 Iron toxicity
6 Iron toxicity	6 Weeds	6 Iron toxicity	6 Iron toxicity	6 Weeds
7 Rodents		7 Rodents	7 Plant lodging	
8 Termites		8 Termites	8 Rodents	
9 Weeds			9 Soil poverty	
			10 Weeds	

The constraint, birds was encountered by farmers at all the ten sites as one of their major constraints. Iron toxicity, insects, and weeds were encountered at all the sites except at Oti, Binah, and Kpélé respectively. Diseases and flooding were encountered at all the sites except Dankpen and Est Mono for the diseases, and except Tchamba and Est Mono for flooding. The constraint, drought, was reported on seven sites, rodents and termites on six sites, plant lodging on two sites, soil poverty (Zio) and spontaneous grain shattering (Binah) on only one site as shown on Figure 3.4.

Nine major constraints were identified: birds, insects, iron toxicity, weeds, diseases, flooding, drought, rodents, and termites. From Table 3.11, drought is the only one constraint which is important for farmers.

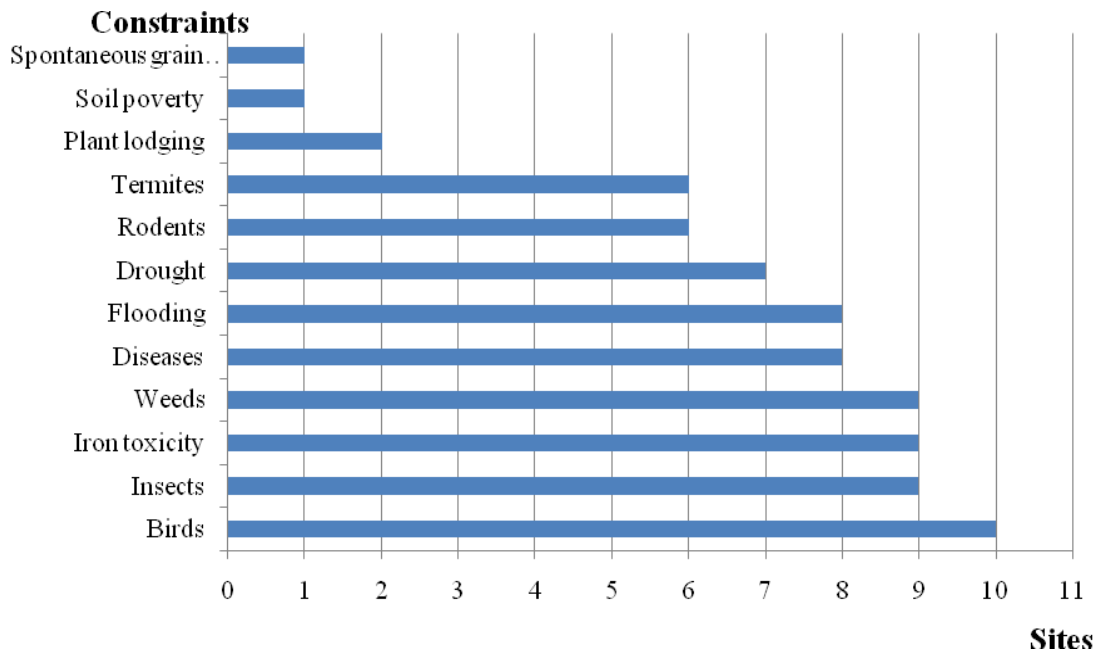


Figure 3.4: Number of sites at which each production constraint was mentioned by a group of farmers

Table 3.11: Farmers’ perception on their major production constraints

Constraints	Levels of importance*				Total farmers	Mean score	Implication
	1	2	3	4			
Drought	63	28	19	40	150	2	Important
Birds	9	31	48	62	150	3	Not so important
Insects	30	7	49	64	150	3	Not so important
Iron toxicity	19	11	29	91	150	3	Not so important
Weeds	21	49	38	42	150	3	Not so important
Diseases	42	19	45	44	150	3	Not so important
Flooding	19	16	7	108	150	3	Not so important
Rodents	7	11	18	114	150	4	Not important
Termites	6	6	36	102	150	4	Not important

* Likert scale: 1 – Very important, 2 – Important, 3 – Not so important, 4 – Not important

As shown in table 3.12, a significant difference was noted for the constraints iron toxicity, diseases, and rodents. This means that farmers in rainfed lowlands have a different appreciation of these constraints in terms of level of importance than their counterparts working in irrigated sites.

Table 3.12: Relationship between farmers' perception on their major constraints in rainfed and irrigated lowlands

Constraints	Sites	Data	Levels of importance				Total valid cases	Calculated X ²	Df
			1	2	3	4			
Drought	Rainfed	Observed	41	17	8	24	150	6.41	3
		Expected	37.8	16.8	11.4	24.0			
	Irrigated	Observed	22	11	11	16			
		Expected	25.2	11.2	7.6	16.0			
Birds	Rainfed	Observed	1	18	33	38	150	0.37	3
		Expected	5.4	18.6	28.8	37.2			
	Irrigated	Observed	8	13	15	24			
		Expected	3.6	12.4	19.2	24.8			
Insects	Rainfed	Observed	12	3	35	40	150	5.95	3
		Expected	18.0	4.2	29.4	38.4			
	Irrigated	Observed	18	4	14	24			
		Expected	12.0	2.8	19.6	25.6			
Iron toxicity	Rainfed	Observed	16	10	14	50	150	40.5*	3
		Expected	11.4	6.6	17.4	54.6			
	Irrigated	Observed	3	1	15	41			
		Expected	7.6	4.4	11.6	36.4			
Weeds	Rainfed	Observed	19	29	23	19	150	0.75	3
		Expected	12.6	29.4	22.8	25.2			
	Irrigated	Observed	2	20	15	23			
		Expected	8.4	19.6	15.2	16.8			
Diseases	Rainfed	Observed	32	1	27	30	150	20.05*	3
		Expected	25.2	11.4	27.0	26.4			
	Irrigated	Observed	10	18	18	14			
		Expected	16.8	7.6	18.0	17.6			
Flooding	Rainfed	Observed	7	10	3	70	150	9.77	3
		Expected	11.4	9.6	4.2	64.8			
	Irrigated	Observed	12	6	4	38			
		Expected	7.6	6.4	2.8	43.2			
Rodents	Rainfed	Observed	5	9	12	64	150	21.93*	3
		Expected	4.2	6.6	10.8	68.4			
	Irrigated	Observed	2	2	6	50			
		Expected	2.8	4.4	7.2	45.6			
Termites	Rainfed	Observed	1	5	24	60	150	11.84	3
		Expected	3.6	3.6	21.6	61.2			
	Irrigated	Observed	5	1	12	42			
		Expected	2.4	2.4	14.4	40.8			

Df = degree of freedom, * significant difference, $X^2 = 13.277$ for $df = 3$ at $p < 0.01$

3.4. Discussion

The National Agriculture Research System (NARS) in Togo provides rice growers with high yielding rice varieties since 1970s. With the lack of varieties development activities, the NARS resorted to introductions from International research institutions such as IRRI, AfricaRice, CNS-Riz, etc. The study identified 38 varieties that have been introduced and promoted to rice growers by the NARS from 1980 to 2013. The major approach used by the NARS in promoting the varieties to farmers was the PVS. In this approach, farmers are involved in the selection of the varieties in the rice field. The selection is done by farmers in two stages of the rice development: tillering and maturity. So, PVS is known to be a good approach to speed up farmers' adoption of new varieties since they have been involved in the selection process (Sié *et al.*, 2009).

However, from the first introductions, IR 841 developed by IRRI is still the most widely cultivated around the country (adopted by 100% of farmers). It was introduced in 1980, and therefore it is an old variety. The variety named Sorad is also an old variety (introduced in 1985). About 95% of the varieties (introduced by the NARS) have been introduced from 2005 to 2013. This means that the NARS tried to replace the old varieties by new performing ones. But unfortunately, farmers have not adopted the new varieties. That is why IR 841 is still cultivated everywhere around the country, though its yield is lower than some new introduced ones (especially NERICAs). Therefore, farmers as well as the NAES workers tried to change the approach by introducing by themselves varieties they found or heard to be higher yielding from neighbouring countries or from research institutes abroad. The study identified 18 varieties introduced by farmers and the NAES workers in the study area. Among these, only two have been introduced from AfricaRice (Berice 21 and Sipi). Up to 14 varieties (77.78%) have been introduced by farmers between 1995 and 2013 in all the sites except Bas Mono. This makes sense in that Bas Mono is a new irrigated site where rice was not the major crop.

So, most of the farmers have adopted rice production after the development of the irrigated site by the Government in 2011. The new rice growers in that area were accompanied by the NARS, that is why the only one variety grown so far in Bas Mono is IR 841, compared to the nine other sites where at least two varieties are grown. Despite the varieties introduced by farmers, IR 841 is still the most widely cultivated, because of its aroma, good tillering ability and resistance to drought.

Moreover, only one variety introduced by farmers has been found in three sites (the variety Gambiaca in Binah, Dankpen and Oti), the rest being strictly localised in their introduction sites. The three sites Binah, Dankpen and Oti are all in the Northern regions of Togo. They are close to each other, and close to Burkina Faso (especially Oti). Having in mind that Gambiaca has been reported to be an improved variety created by ADRAO in 1970 and released in Burkina Faso (FAO, 2008), this variety would have been introduced from Burkina Faso in the nearest site (Oti) where it might have spread to Dankpen and Binah. Apart from Gambiaca, all the 13 other varieties introduced by farmers were confined in the introduction sites where they were grown even in small scale. As they are not largely cultivated, it means that all the farmers in the sites do not prefer them to IR 841. This can be understood since the introduction is straightforward (without any evaluation for adaptability) and the choice of the variety made by only one person (the farmer). In addition, from the obtained results, Zio comes as the site where four varieties have been introduced by farmers. This seems to be normal because Zio is the largest irrigated site across the country where rice has been grown since 1980s. Its closeness to Ghana is also a favourable factor in easier introduction of varieties by farmers.

Even though the NARS has introduced varieties (68%) more than twice as much as the NAES and farmers (32%), on the 23 varieties that are still cultivated, 43% has been introduced by the NARS and 57% by the NAES and farmers. These parts represent only 26% of the NARS

introductions and 72% of the NAES and farmers' introductions. Moreover, 45% of the introduced varieties are unknown by farmers. This represents 66% of the NARS introductions. Such situation implies that the NARS was not able to provide farmers with 66% of the varieties they introduced. The probable cause may be financial support in doing the adaptability tests and conducting PVS with farmers. This is also because the NARS was not able to cover all the agro-ecological zones with all the varieties they proposed to farmers. In addition, the NAES which is supposed to link the NARS with the farmers is limited in actions. So, much work has to be done to enhance both the NARS and the NAES. A little more than 40% of the introduced varieties are said to be still cultivated. This includes ten varieties introduced by the NARS and 13 varieties by the NAES and farmers. It appears that in the study areas, the varieties that are cultivated have been fifty-fifty introduced by the two actors of introduction. This situation reveals a serious weakness of the NARS which should be the major actor to provide farmers with good varieties. Furthermore, this shows a poor functioning of the national quarantine service.

Only one variety (Sipi) introduced by farmers in the Zio valley has been reported to be abandoned because of its stony grain after cooking. No variety introduced by the NARS has been abandoned by farmers, meaning that even though the NARS has limited actions, the few that are introduced were well done. A few varieties have been lost (three and four varieties respectively from NARS and the NAES and farmers). It happened that farmers lose varieties when seed is not renewed over time. However, for the NARS to lose a variety is a serious lack of professionalism. It is a result of a lack of proper seed storage conditions and a bad management of germplasm maintenance in fields.

Among these 23 varieties currently grown; only the variety IR 841 is cultivated in the ten locations. Farmers have adopted IR 841 because it is aromatic. They all go for aromatic varieties because such varieties, even though they are low yielding than some non-aromatic

ones, are sold at a higher price. Therefore, with aromatic varieties, farmers incomes increase more than the non-aromatic ones. Apart from IR 841, among the varieties introduced by the NARS and which are still cultivated, Nerica L-14 in three sites (Tone, Binah and Kpél ), and Orylux 1 in two sites (Kp l  and Zio) are the most cultivated (adopted by 25% and 10% of farmers respectively), though they are in very small proportion compared to IR 841 (Nerica L-14 because of its yield, and Orylux 1 because of its aroma). The highest number of varieties cultivated per site, has been recorded in the locations where the NARS has their stations like Tone (seven varieties), Kp l  and Zio (five varieties). Because of the presence of the research stations (PVS sites), farmers on these sites are more likely to be easily involved in PVS and have access to new varieties.

Across the ten sites in the study area, farmers stated eight reasons for which they have rejected the varieties. These are absence of aroma, short height, tall height, lateness, susceptibility to drought, small grain, spontaneous grain shattering, and stony grain after cooking. The main reason identified was the absence of aroma, because this has been identified for 28 varieties (out of the 31 varieties) in all the ten sites. In addition, the three aromatic varieties IR 841, Chapeau vert, and Orylux 1 have not been rejected by farmers. This confirms that the presence of aroma is essential for farmers to adopt a variety. Therefore, it is more understandable that the only one variety adopted by all the farmers is aromatic (IR 841). Furthermore, considering the number of reasons for which the varieties have been rejected, ten varieties have been rejected for only absence of aroma. These varieties are Ablayibo, Accravi, Berice 21, Djama, Ibo, Londo londo, Mossi, TGR 1, TGR 75 and Timbou. These varieties can be improved for aroma. The rest were rejected for more than one reason. Also, nine desirable traits presented by the varieties have been pointed out by farmers. These were high yield, earliness, presence of aroma, resistance to drought, resistance to diseases, resistance to weeds, good taste, good tillering, and presence of awn. In sum, this study

enabled the identification of eight undesirable traits (reasons of non-adoption) and nine desirable traits. Both undesirable and desirable traits must be considered in a breeding programme. As farmers prefer aromatic varieties, prominence should be given to the three aromatic varieties IR 841, Chapeau vert, and Orylux 1 which also combine high yield, good tillering ability, resistance to drought, and good taste after cooking.

In total, 24 agro-morphological traits have been stated by farmers as their preferences in the ten sites of the study. These should be taken into account in the rice national breeding programme with emphasis on the five major traits identified which are common to more than eight sites. The five major traits were: presence of aroma, high yield, long grain, medium height, and earliness. After their assessment by farmers following the *Likert* scale of importance, the traits presence of aroma and high yield are important. The three other preferences are not so important. This confirms the importance of aromatic varieties for rice growers, knowing that the most important trait targeted by both breeders and farmers is high yield. However, it is important to consider all the five preferences in developing varieties for farmers in Togo. About the same preferences, the Chi-square test revealed a significant difference in the farmers' perception on earliness in rainfed and irrigated lowlands. Having in mind the irregularity of rainfall often followed by severe drought recorded in the last years in the rainfed lowlands, it is clear that earliness should be perceived as more important for farmers in rainfed lowland sites than their counterparts in irrigated lowlands sites where water is not a challenge.

The similar perception on presence of aroma, high yield, long grain, and medium height by farmers indicates the relevance of these traits for farmers. So rice breeders in Togo should work on them.

As considered for farmers' preferences, the 12 production constraints listed by farmers around the ten sites will be taken into account in the rice breeding programme in Togo. These are:

Iron toxicity, flooding, drought, plant lodging, soil poverty, spontaneous grain shattering, birds, insects, diseases, rodents, weeds, and termites. Much emphasis will be put on the nine major constraints identified: birds, insects, iron toxicity, weeds, diseases, flooding, drought, rodents, and termites. The assessment of their perception by farmers using *Likert* scale of importance shows that the only one constraint which is important for farmers is drought. Knowing that with climate changes, drought is now more severe in rainfed lowlands, this result is understandable. On the contrary, the constraints, rodents and termites are not important for farmers. Such constraints occur rarely and can be mitigated easily. The same applies to the six other constraints (birds, insects, iron toxicity, weeds, diseases, and flooding) which are perceived by farmers as not so important. However, after the Chi-square test, the constraints diseases, iron toxicity, and rodents show a significant difference in terms of farmers' perception in rainfed and irrigated lowlands. This result makes sense knowing that impact of diseases differs from rainfed lowlands to irrigated lowland sites. For instance, rice blast disease which is the most important rice disease in Togo (Sy, 1991; Kpemoua and Dantsey Barry, 2003) is more severe in rainfed lowlands than irrigated lowlands due to the lack of water sometimes in the rainfed lowlands. Therefore, diseases can be said to be one of the main constraints for farmers in rainfed lowlands contrary to the farmers in irrigated lowlands. As for diseases, iron toxicity is mainly found in rainfed lowlands where water level is very difficult to control. Knowing that iron toxicity occurs when water is not well drained, this constraint is rarely found in irrigated sites where irrigation water is well controlled. Therefore, it makes sense that farmers in rainfed lowland conditions perceive iron toxicity as a main constraint while those in irrigated lowlands take it as a minor one. Rodents too are perceived the same way, because they cause more damage in rainfed lowlands than in irrigated lowlands where the water flow constitutes an obstacle to the rodents' movements in the fields.

3.5. Conclusion

This study had the merit to present clearly the inventory on rice varieties introduced in Togo from 1980 to 2013. In the ten locations covered, 56 varieties were reported to be introduced by the NARS (38 varieties), the NAES and farmers (18 varieties). Only ten of the varieties introduced by the NARS are still cultivated in the study area. Even though the NARS implemented the PVS approach to facilitate the adoption of the varieties, only one variety (IR 841) has been adopted by 100% of farmers (only 2.63% of varieties introduced by the NARS), the nine others being adopted by less than 25% of farmers in less than four locations. Most of the introduced varieties have not been adopted by farmers because they lack aroma. In fact, presence of aroma, grain yield, long grain, medium height and earliness were the major preferred traits pointed out by farmers. Their major production constraints were birds, insects, iron toxicity, weeds, diseases, flooding, drought, rodents and termites. In these preferences and constraints, presences of aroma and grain yield on the one hand, and drought on the other hand, have been perceived by farmers as their important preferred traits and production constraints respectively. However, significant differences have been noticed for farmers' perception on earliness, iron toxicity, diseases and rodents' damages in rainfed lowlands compared to irrigated lowlands.

CHAPTER FOUR: COLLECTION AND GENETIC DIVERSITY ANALYSIS OF RICE GERMPLASM

4.1. Introduction

Developing new varieties for farmers requires, after assessing their preferences and constraints, gathering locally adapted genetic materials for the establishment of a germplasm collection. The study of the genetic diversity within the established germplasm is critically important.

In Togo, the rice germplasm has been lost due to the inappropriate seed storage conditions and the pressure of several stresses in the field (drought, diseases, insects and rodents). Moreover, the only one variety adopted by farmers has been reported to be contaminated and therefore is declining in yield. From the foregoing, this research aimed to:

- collect accessions from farmers around the country in the major irrigated and rainfed lowlands; and
- study the genetic diversity in the germplasm at phenotypic and genotypic levels.

4.2. Materials and Methods

4.2.1. Germplasm collection

Any variety introduced or local accession currently cultivated by farmers were collected through a prospecting survey on ten sites across the country.

4.2.1.1. Material used for collection

A data collection form was used to record information on the materials and their donors. A Global positioning system (GPS) device was used for reading the geographical coordinates (longitude, latitude, altitude) of the collection sites.

4.2.1.2. Methodology

As the prospecting survey was made in December (after harvest), paddy grains and panicles were collected from farmers. The accessions were collected with the consent of farmers. The National extension service (NAES) workers were involved in the collection. About 50 g of grain or ten panicles were collected for each accession and packed in paper envelopes. The envelopes were numbered. The most cultivated aromatic variety IR 841 was collected in each location.

4.2.1.3. Data collected

Passport data related to the site, the accessions and the provider farmer. These are: Site's name, site's geographical coordinates (longitude, latitude and altitude), accession local name, sample number, type of the collected material (panicle or grain), date of collection, Region/province/village, agro-ecological zone, rice ecology (irrigated or rainfed lowlands, upland), sample quantity (number of collected panicles or weight of grains collected), farmers' names, and farmers' gender (Male or female).

Seeds of 11 introduced varieties were requested from AfricaRice to be used as checks during phenotyping. These were: ARICA 1, ARICA 2, ARICA 3, WITA 4, NERICA L14 and NERICA L19 (for yield), Orylux 1, Orylux 3, Orylux 4, Orylux 5, Orylux 6 (for aroma).

4.2.1.4. Data analysis

The list of the collected accessions and the requested varieties from the NARS was made with Microsoft Excel. This list summarizes the main information collected. The descriptive statistics of the information collected was done.

4.2.2. Multiplication of the accessions

The collected materials were purified and multiplied to obtain sufficient quantities of seeds for subsequent research. Seeds multiplication and purification was done on ITRA station in Ativémé (Togo) under water control conditions.

About 5 g of grains of each accession were sown in a nursery. Twenty-one days after nursery establishment, the plants were transplanted in rows with one seedling per hill on plots of 5 m² (5m x 1m). The distance between rows was 20 cm, and also 20 cm within rows and 50 cm between plots. The accessions were inspected throughout the crop cycle and any off-types removed. The experiment was conducted following the technical recommendations of AfricaRice for irrigated rice production (Wopereis *et al.*, 2009).

From the above activity, sufficient purified seeds were harvested. The results are presented in Appendix 4.1.

4.2.3. Agro-morphological characterization of the accessions

4.2.3.1. Location

The experiment was conducted on station under irrigated conditions in Mission Tové located at 30 km from Lomé (Togo).

4.2.3.2. Plant materials used

The plant material used composed of 50 purified accessions and varieties (requested from AfricaRice).

4.2.3.3. Experimental design

An alpha lattice design with 3 replications was used. In this design, each replication of the 50 treatments was divided into ten incomplete blocks of five plots per block in which the treatments (accessions and checks/control) were randomly distributed. A 5 m² plot (5 m x 1 m) containing five rice rows (of 5 m each) represented the experimental unit. The distance within rows was 20 cm and the one within rice plants on the same row was also 20 cm, thus each plot had a total of 125 plants. Moreover, the distance within plots was 50 cm, and between replications was 1 m. Only the plants in the three middle rows of each plot were evaluated.

4.2.3.4. Conduction of the trial

The establishment of the trial began with the land preparation which consisted of clearing, ploughing and harrowing. The seedlings were transplanted 21 days after planting with one plant per hill. A fertilizer containing N₁₅P₁₅K₁₅ was applied before transplanting at 200 kg/ha. After that, 100 kg/ha of urea containing 46% N was applied in two fractions: 35 kg/ha at early tillering stage (25 days after transplanting) and 65 kg/ha at panicle initiation (60 days after transplanting). Furthermore, three manual weedings were done. No pest management was done. However, the field was surrounded by a wire mesh to avoid rodents' damages. At maturity stage, the field was guarded to prevent birds' invasion.

4.2.3.5. Data collected

A total of 30 characters (19 qualitative and 11 quantitative) were collected by using the *Descriptors for wild and cultivated rice* (Bioversity International, 2007) and the *Standard evaluation system* (IRRI, 2002). Table 4.1 shows the list of variables collected.

Table 4.1: Agro-morphological traits evaluated

Qualitative traits				Quantitative traits			
N°	Traits	Document	Code	N°	Traits	Document	Code
1	Population uniformity	Descriptor	PU	1	Culm number	Descriptor	CN
2	Seedling vigor at 21 DAT	SES	Vg 21	2	Main heading	Descriptor	MH
3	Vegetative vigor at 42 DAT	SES	Vg 42	3	Leaf length	SES	LL
4	Leaf blade colour	SES	LBC	4	Flag leaf length	Descriptor	FLL
5	Leaf blade pubescence	SES	LBP	5	Number of panicles/plant	Descriptor	NPP
6	Collar colour	SES	CC	6	Culm length	SES	CL
7	Ligule shape	SES	LS	7	Maturity	SES	Mat
8	Culm habit	Descriptor	CH	8	Panicle length	SES	PnL
9	Flag leaf attitude	SES	FLA	9	Grain yield	SES	Yld
10	Panicle exsertion	SES	Exs	10	Grain length	Descriptor	GL
11	Culm lodging resistance	Descriptor	CLR	11	100-grain weight	SES	GW
12	Panicle axis	SES	PnA				
13	Panicle shattering	Descriptor	PS				
14	Leaf senescence	SES	Sen				
15	Awns presence	SES	An				
16	Awn colour	SES	AnC				
17	Caryopsis pericarp color	Descriptor	CPC				
18	Caryopsis scent	Descriptor	CS				
19	Phenotypic acceptability	SES	Pacp				

The evaluation scales as presented for each qualitative trait in the *Descriptor* or in the *Standard evaluation system* is in Appendix 4.2.

Moreover, the presence or absence of aroma in the accessions was evaluated in the laboratory following the technique developed by IRRI (1971) described as follows (Singh *et al.*, 2000). One brown rice gram was placed into centrifuge tube (50 ml round bottom). About 20 ml distilled water was added. The tubes were then covered with aluminium foil. The samples were placed in a boiling water bath for 30 minutes. The cooked samples were allowed to cool and the presence of aroma was determined for every sample by smelling. The samples were scored as strongly aromatic, moderately aromatic, slightly aromatic and non-aromatic. The aromatic variety IR 841 was used as check for comparison.

4.2.3.6. Data analysis

The 30 traits were considered together in Discriminant analysis using the Statistical package for social science SPSS 17.0. The stepwise selection method was used. The measurement of the variables' contribution to the discriminatory power of the model is based on the test statistics of Wilks' Lambda criterion (Daulfrey, 1976). Then, variables with least contribution were removed. The significance level of the Wilks' lambda for retaining or adding a discriminative variable was set at 0.15. In addition, canonical correlation analysis (CCA) was accomplished with the same software to assess the relationship strength between the variables retained after the Discriminant analysis. Spearman correlation coefficients (r_s) were used for that purpose. Significance level set at $p=0.001$, 0.01 and 0.05..

Furthermore, Principal components analysis (PCA) using the software GenStat 9.2 was run with the variables retained after the Discriminant analysis. The Elbow method after Scree test helped to identify the efficient number of principal components (PCs). Cluster analysis, especially hierarchical classification based on Euclidean distance and the average link method was run by the same software to identify phenotypic groups among the collection. The efficient number of clusters was determined with the Elbow method when plotting the percentage of variance explained by the clusters against the number of clusters. This method looks at the percentage of variance explained as a function of the number of clusters: one should choose a number of clusters so that adding another cluster does not give much better modelling of the data.

4.2.4. Molecular analysis

The germplasm was characterized using high density genotyping platform to identify SNP (single nucleotide polymorphism) markers. This component of the research was conducted in the laboratory equipped with genotyping by sequencing (GBS) platform at BeCA-ILRI in

Kenya. DNA was extracted from leaves of 21 days old seedlings of each of the 39 accessions and the 11 checks. Genotyping was based on 21,250 SNP markers randomly located across the rice genome.

The internal protocol required for DNA extraction as well as the sequencing methodology as undertaken in BeCA-ILRI was applied. GBS technology consisting in the sequencing and the alignment of the whole genome of each accession studied enabled the use of the SNP markers to determine the polymorphism between accessions throughout the entire genome. Four variables were collected: the score of each marker per accession, the frequency of homozygote and heterozygote, polymorphism information content (PIC), and the call rate. The collected data was analysed using the KDCCompute platform accessible on <https://kdcompute.igss-africa.org/kdcompute>.

Based on data of the markers' call rate and average polymorphism information content (PIC), the non polymorphic markers were removed for the analyses. Principal components analysis (PCA) was performed in order to identify the main components explaining the genotypic variation in the collection. The Elbow method based on the scree plot was used in the identification of the efficient number of principal components (PCs). Moreover, cluster analysis was run for grouping similar genotypes.

4.3. Results

4.3.1. Accessions collected and their descriptive statistics

From the prospecting survey conducted in the ten locations of the study, 61 accessions were collected from 30 villages. Throughout the seeds multiplication on the field, 22 accessions were discarded for not germinating. In the end, 39 accessions were retained (Table 4.2). Among these 39 accessions, 48.72% were collected in form of panicle and 51.28% as paddy grain. Moreover, 23.08% are reported to be grown under irrigated lowlands conditions, and 76.92% in rainfed lowland conditions.

The variety IR 841 introduced by the NARS and which is the most widely cultivated around the country was collected in all the ten locations. A total of 13 samples of this variety were collected. Two different samples of each of the four accessions Gambiaca, Kpive-sa, Lobo lobo and Sorad have also been collected. Only the two varieties IR 841 and NERICA L-19 were found and collected from all the varieties introduced by the NARS.

Among the collections, seven accessions were brought by farmers without names. There were four accessions from Binah, two from Dankpen and one from Tchamba. These were named with the code given to each accession during the collection.

In sum, in terms of the number of accessions collected per site, only one accession was collected in Bas Mono, two in Tone and Kpélé, three in Oti and Zio, four in Tchamba and Sotouboua, six in Dankpen, and seven in Binah and Est Mono as shown on Figure 4.1.

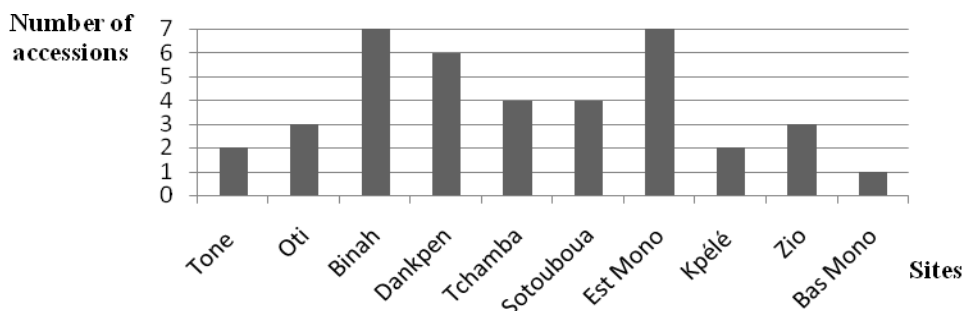


Figure 4.1: Number of accessions collected per site

To the list of the 39 accessions collected, the 11 varieties brought from AfricaRice were added to make a list of 50 accessions which were characterized.

Table 4.2: Information on the accessions collected

N°	Accession name	Site	Agro-ecological area	Ecology	Collected form
1	Alandine	Tone	Dry savannas	Irrigated	Paddy grain
2	IR 841_Ton	Tone	Dry savannas	Irrigated	Paddy grain
3	IR 841 TC_Oti	Oti	Dry savannas	Irrigated	Paddy grain
4	IR 841 TH_Oti	Oti	Dry savannas	Irrigated	Paddy grain
5	Mossi	Oti	Dry savannas	Irrigated	Paddy grain
6	Londo londo	Binah	Dry savannas	Rainfed	Paddy grain
7	Pagou 2	Binah	Dry savannas	Rainfed	Paddy grain
8	Pagou 3	Binah	Dry savannas	Rainfed	Panicle
9	IR 841 TC_Bin	Binah	Dry savannas	Rainfed	Panicle
10	IR 841_Bin	Binah	Dry savannas	Rainfed	Panicle
11	Pagou 8	Binah	Dry savannas	Rainfed	Panicle
12	Pagou 9	Binah	Dry savannas	Rainfed	Panicle
13	IR 841 TC_Dan	Dankpen	Dry savannas	Rainfed	Panicle
14	Mandi	Dankpen	Dry savannas	Rainfed	Panicle
15	Kouka 3	Dankpen	Dry savannas	Rainfed	Panicle
16	Kouka 5	Dankpen	Dry savannas	Rainfed	Panicle
17	Gambiaca_Dan	Dankpen	Dry savannas	Rainfed	Panicle
18	IR 841 TH_Dan	Dankpen	Dry savannas	Rainfed	Panicle
19	IR 841 TC_Tch	Tchamba	Wet savannas	Rainfed	Paddy grain
20	Lobo Lobo	Tchamba	Wet savannas	Rainfed	Panicle
21	Lobo Lobo_2	Tchamba	Wet savannas	Rainfed	Panicle
22	Tcham 4	Tchamba	Wet savannas	Rainfed	Panicle
23	IR 841_Sot	Sotouboua	Wet savannas	Rainfed	Paddy grain
24	Ibo	Sotouboua	Wet savannas	Rainfed	Panicle
25	Awini	Sotouboua	Wet savannas	Rainfed	Panicle
26	IR 841 TC_Sot	Sotouboua	Wet savannas	Rainfed	Panicle
27	IR 841_EM	Est Mono	Wet savannas	Rainfed	Paddy grain
28	Sorad	Est Mono	Wet savannas	Rainfed	Paddy grain
29	Kpive-sa	Est Mono	Wet savannas	Rainfed	Paddy grain
30	Sorad_2	Est Mono	Wet savannas	Rainfed	Paddy grain
31	IR 841 TC_EM	Est Mono	Wet savannas	Rainfed	Paddy grain
32	Kpive-sa_2	Est Mono	Wet savannas	Rainfed	Paddy grain
33	Gambiaca_EM	Est Mono	Wet savannas	Rainfed	Panicle
34	Chinoivi	Kpélé	Forest	Rainfed	Paddy grain
35	NERICA L-19_Kpé	Kpélé	Forest	Rainfed	Paddy grain
36	SIPI	Zio	Littoral	Irrigated	Paddy grain
37	BERICE 21	Zio	Littoral	Irrigated	Paddy grain
38	Chapeau vert	Zio	Littoral	Irrigated	Panicle
39	IR 841_BM	Bas Mono	Littoral	Irrigated	Paddy grain

4.3.2. Agro-morphological characterization

4.3.2.1. Discriminative traits

The 30 variables have shown different degree of variation across the 50 accessions in their pattern and amount. For the 19 qualitative traits, no variation has been reported for the following five traits: population uniformity, seedling vigour, vegetative vigour, panicle axis, and panicle shattering for which all the accessions presented respectively homogeneity, normal vigour, extra vigour, droopy panicle, and very low (<1%) grain shattering. So these five traits have not been considered for the Discriminant analysis. Therefore the 25 remaining traits were used.

About leaf blade colour, 84% of the accessions were light green and 16% dark green. The ninety-eight percent of the accessions had green collar whereas only 2% showed purple collar. Two types of caryopsis pericarp colour were found in the germplasm: 14% were white and 86% were light brown. Only 8% of accessions were awned, 94% had an acute ligule, 68% pubescent leaf blade, 26% erect culm, 76% a strong resistant culm, and 80% a fair phenotypic acceptability (against 10% of poor acceptability). Sixty-two percent of the accessions were non aromatic, 24% strongly scented and 14% were moderately scented. Fifty-two percent of the accessions had erect flag leaves, 42% intermediate, and 6% horizontal. In terms of panicle exertion, 14% of accessions were well exerted, 32% moderately exerted, 30% just exerted, 22% partly exerted, and 2% enclosed. About leaf senescence, 48% of accessions were late and 52% intermediate.

Table 4.3 shows summary statistics of the quantitative traits. The number of tillers (culm number CN) ranged from 6.70 to 14.96 for 5.12 to 14.12 panicles per plant (NPP). Number of days to flowering ranged from 57.85 to 97.70 days, whereas number of days to maturity ranged from 87.39 to 127.13 days. Leaf length (LL) and flag leaf length (FLL) ranged respectively from 35.97 and 25.24 cm to 65.40 and 46.92 cm. The culm length (CL) varied

from 91.54 to 154.19 cm while the panicle length (PnL) ranged from 16.65 to 23.65 cm, and the grain length (GL) from 5.12 to 14.12 mm. The minima of 100-grain weight (GW) and the grain yield (Yld) were respectively 1.97 g and 2.19 t/ha, for maxima of 2.95 g and 7.63 t/ha.

Table 4.3: Summary statistics of the 11 quantitative traits evaluated

<i>Traits</i>	Code	Minimum value	Maximum value	Mean value	Standard deviation
Culm number	CN	6.70	14.96	10.44	1.78
Main heading (days)	MH	57.85	97.70	77.57	6.53
Leaf length (cm)	LL	35.97	65.40	49.86	7.04
Flag leaf length (cm)	FLL	25.24	46.92	34.27	4.51
Number of panicle/plant	NPP	5.12	14.12	8.94	1.70
Culm length (cm)	CL	91.54	154.49	125.55	14.19
Maturity (days)	Mat	87.39	127.13	107.57	6.64
Panicle length (cm)	PnL	16.65	23.65	20.08	1.38
Grain yield (t/ha)	Yld	2.19	7.63	4.45	1.31
Grain length (mm)	GL	5.12	14.12	8.94	1.70
100-grain weight (g)	GW	1.97	2.95	2.66	0.17

From the results of the discriminant analysis, 11 traits showed significant discriminative power in differentiating the accessions with a p-value for Wilks' lambda ranging from 0.00000 to 0.12756 (Table 4.4).

The 11 discriminative variables were: culm number (CN), number of panicle per plant (NPP), ligule shape (LS), leaf blade pubescence (LBP), phenotypic acceptability (Pacp), leaf length (LL), culm length (CL), 100-grain weight (GW), culm habit (CH), flag leaf attitude (FLA), and flag leaf length (FLL). The highest discriminative power was recorded for the culm number (CN), the number of panicle per plant (NPP), the ligule shape (LS), and the leaf blade pubescence (LBP) respectively with F values 31.6017; 28.607; 12.956; and 10.310 (and associated p-values 0.00000; 0.00000; 0.00001; and 0.00006).

Table 4.4: Wilk's lambda criterion, F value and p-value for 25 variables evaluated

Variables	Codes	Wilks' lambda	F	df1	df2	p-value
Culm number	CN	0.699	31.617	2	147	0.00000
Number of panicle/plant	NPP	0.720	28.607	2	147	0.00000
Ligule shape	LS	0.850	12.956	2	147	0.00001
Leaf blade pubescence	LBP	0.877	10.310	2	147	0.00006
Phenotypic acceptability	Pacp	0.938	4.891	2	147	0.00878
Leaf length	LL	0.938	4.877	2	147	0.00889
Culm length	CL	0.946	4.194	2	147	0.01692
100-grain weight	GW	0.946	4.161	2	147	0.01746
Culm habit	CH	0.960	3.096	2	147	0.04818
Flag leaf attitude	FLA	0.970	2.304	2	147	0.10344
Flag leaf length	FLL	0.972	2.088	2	147	0.12756
Culm lodging resistance	CLR	0.979	1.572	2	147	0.21115
Leaf blade colour	LBC	0.980	1.465	2	147	0.23438
Panicle exertion	Exs	0.989	0.786	2	147	0.45775
Leaf senescence	Sen	0.991	0.660	2	147	0.51855
Grain yield	Yld	0.992	0.561	2	147	0.57207
Collar colour	CC	0.997	0.203	2	147	0.81625
Panicle length	PnL	0.998	0.132	2	147	0.87646
Main heading	MH	0.998	0.124	2	147	0.88348
Maturity	Mat	0.998	0.114	2	147	0.89267
Awns presence	An	1.000	-	2	147	1.00000
Awn colour	AnC	1.000	-	2	147	1.00000
Caryopsis pericarp color	CPC	1.000	- 0.000	2	147	1.00000
Caryopsis scent	CS	1.000	-	2	147	1.00000
Grain length	GL	1.000	-	2	147	1.00000

Significant p value for Wilk's Lambda < 0.15

4.3.2.2. Correlations between discriminative traits

The correlations existing among the 11 agro-morphological discriminative traits were estimated based on Spearman's coefficient. The traits leaf blade pubescence (LBP) and Ligule shape (LS), culm habit (CH) and LS, phenotypic acceptability (Pacp) and LS, as well as flag leaf attitude (FLA) and CH were significantly negatively correlated at $p=0.05$. Positive significant correlations were detected between seven pairs of attributes at $p=0.05$. At $p=0.01$, 8 couples of traits presented the highest positive correlation coefficients of 0.945 for culm number (CN) and number of panicles per plant (NPP), 0.544 for flag leaf length (FLL) and leaf length (LL), 0.486 for culm length (CL) and LL, 0.347 for CL and FLL, 0.266 for 100-

grain weight (GW) and CL, 0.263 for LBP and CL, 0.238 for NPP and FLL, and 0.217 for LBP and LL (Table 4.5).

Table 4.5: Correlation matrix for the 11 discriminative traits

		LS	CH	FLA	Pacp	CN	LL	FLL	NPP	CL	GW	
Leaf blade pubescence	LBP	-.196*	.096	-.089	.164*	.160	.217**	.031	.151	.263**	.109	
Ligule shape	LS		-.173*	.173*	-.176*	-.111	-.053	-.139	-.103	-.042	.092	
Culm habit	CH			-.197*	.042	-.016	.100	.042	-.033	-.103	-.012	
Flag leaf attitude	FLA				-.137	-.098	.182*	.197*	-.073	.186*	.120	
Phenotypic acceptability	Pacp					.149	.114	.054	.123	.138	-.048	
Culm number	CN						.131	.179*	.945**	.054	-.124	
Leaf length	LL							.544**	.174*	.486**	.047	
Flag leaf length	FLL								.238**	.347**	.063	
Number of panicles/plant	NPP									.105	-.128	
Culm length	CL										.266**	
100-Grain weight	GW											1

* Significant at $p < 0.05$, ** significant at $p < 0.01$

4.3.2.3. Principal components

Table 4.6 shows the results for principal components analysis. The optimal number of principal components identified was five based on the Eigenvalues of the PCs and using Elbow method with the Scree plot (Figure 4.2).

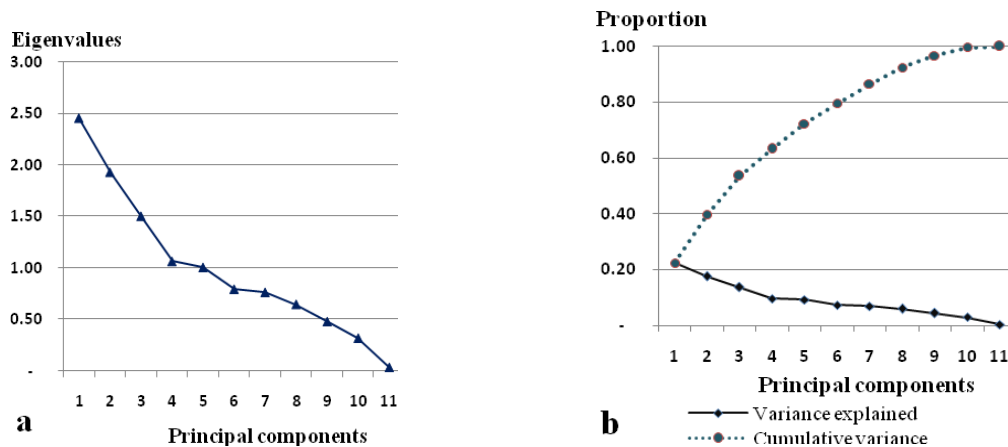


Figure 4.2: Scree plot with Eigenvalues (a) and proportion of variance explained (b) by the principal components

Table 4.6: Eigenvalue and variance explained by the principal components (PCs)

PCs	Eigenvalue	Difference	Variance explained	Cumulative variance
1	2.453	0.525	0.2230	0.2230
2	1.928	0.426	0.1753	0.3983
3	1.502	0.438	0.1365	0.5348
4	1.064	0.058	0.0967	0.6315
5	1.006	0.211	0.0914	0.7229
6	0.795	0.030	0.0722	0.7951
7	0.765	0.117	0.0696	0.8647
8	0.648	0.167	0.0589	0.9236
9	0.481	0.160	0.0437	0.9673
10	0.321	0.284	0.0292	0.9965
11	0.037		0.0034	0.9999

The first five PCs accounted for 72.29% of total variation among the accessions. PC1 explained 22.30%. PC2 explained 17.53%, PC3 with 13.65%, PC4 with 9.67%, and PC5 with 9.14% of variation.

The first component (PC1) was negatively and highly associated with Leaf length (LL), culm length (CL) and flag leaf length (FLL) with respective loadings of – 0.446, - 0.394 and – 0.392. The traits culm number (CN) and number of panicle/plant (NPP) even though presenting high loading values on the PC1 (- 0.406 and - 0.423 respectively) presented higher loading values on PC2 with which they were positively associated. PC2 was positively associated with CN, NPP and negatively with flag leaf attitude (FLA) respectively with 0.450, 0.423, and – 0.422. The only trait highly associated with PC3 was ligule shape (LS). The traits 100-grain weight (GW) and leaf blade pubescence (LBP) contributed highly to PC4 with negative loadings of –0.717 and 0.468 respectively. Culm habit (CH) with – 0.667 and phenotypic acceptability (Pacp) with 0.515 explained more the PC5 (Table 4.7).

Table 4.7: Contribution of the 11 discriminative traits to the first eight principal components

Traits	Principal components							
	1	2	3	4	5	6	7	8
Culm habit (CH)	- 0.030	0.112	- 0.431	- 0.129	- 0.667	- 0.196	0.375	- 0.319
Culm length (CL)	- 0.394	- 0.335	- 0.079	- 0.219	0.238	- 0.013	- 0.012	0.187
Culm number (CN)	- 0.406	0.450	0.336	0.075	- 0.062	- 0.003	0.067	- 0.048
Flag leaf attitude (FLA)	- 0.085	- 0.422	0.272	0.283	0.085	0.260	0.156	- 0.747
Flag leaf length (FLL)	- 0.392	- 0.312	- 0.024	0.268	- 0.298	- 0.115	- 0.248	0.148
100-grain weight (GW)	- 0.052	- 0.131	0.305	- 0.717	- 0.140	- 0.286	- 0.403	- 0.288
Leaf blade pubescence (LBP)	- 0.290	0.087	- 0.267	- 0.468	0.291	0.438	0.370	- 0.060
Leaf length (LL)	- 0.446	- 0.347	- 0.140	0.063	- 0.138	- 0.069	0.114	0.206
Ligule shape (LS)	0.154	- 0.223	0.446	- 0.088	0.061	- 0.423	0.673	0.226
Number of panicle/plant (NPP)	- 0.423	0.423	0.346	0.080	- 0.081	0.006	0.051	- 0.023
Phenotypic acceptability (Pacp)	- 0.173	0.159	- 0.343	0.161	0.515	- 0.651	- 0.029	- 0.315
Eigenvalue	2.45	1.93	1.50	1.06	1.01	0.80	0.77	0.65
Variance explained (%)	22.30	17.53	13.65	9.67	9.14	7.22	6.96	5.89
Cumulative variance (%)	22.30	39.83	53.48	63.15	72.29	79.51	86.47	92.36

4.3.2.4. Accessions' clusters

From the Figure 4.3, the 50 accessions were grouped into 4 clusters.

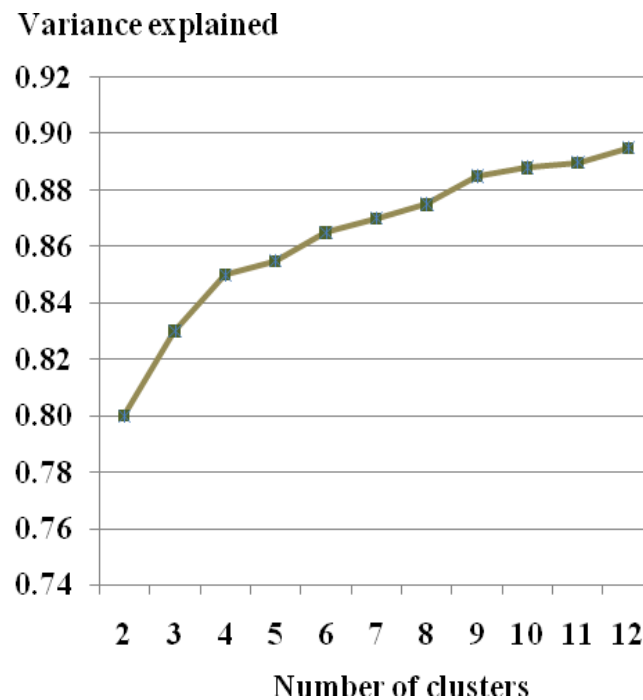


Figure 4.3: Evolution of the variance explained by the number of clusters

Cluster analysis result for the fifty accessions is represented by Figure 4.4. The four clusters: cluster I, II, III and IV were composed by seventeen, nine, sixteen and eight accessions respectively.

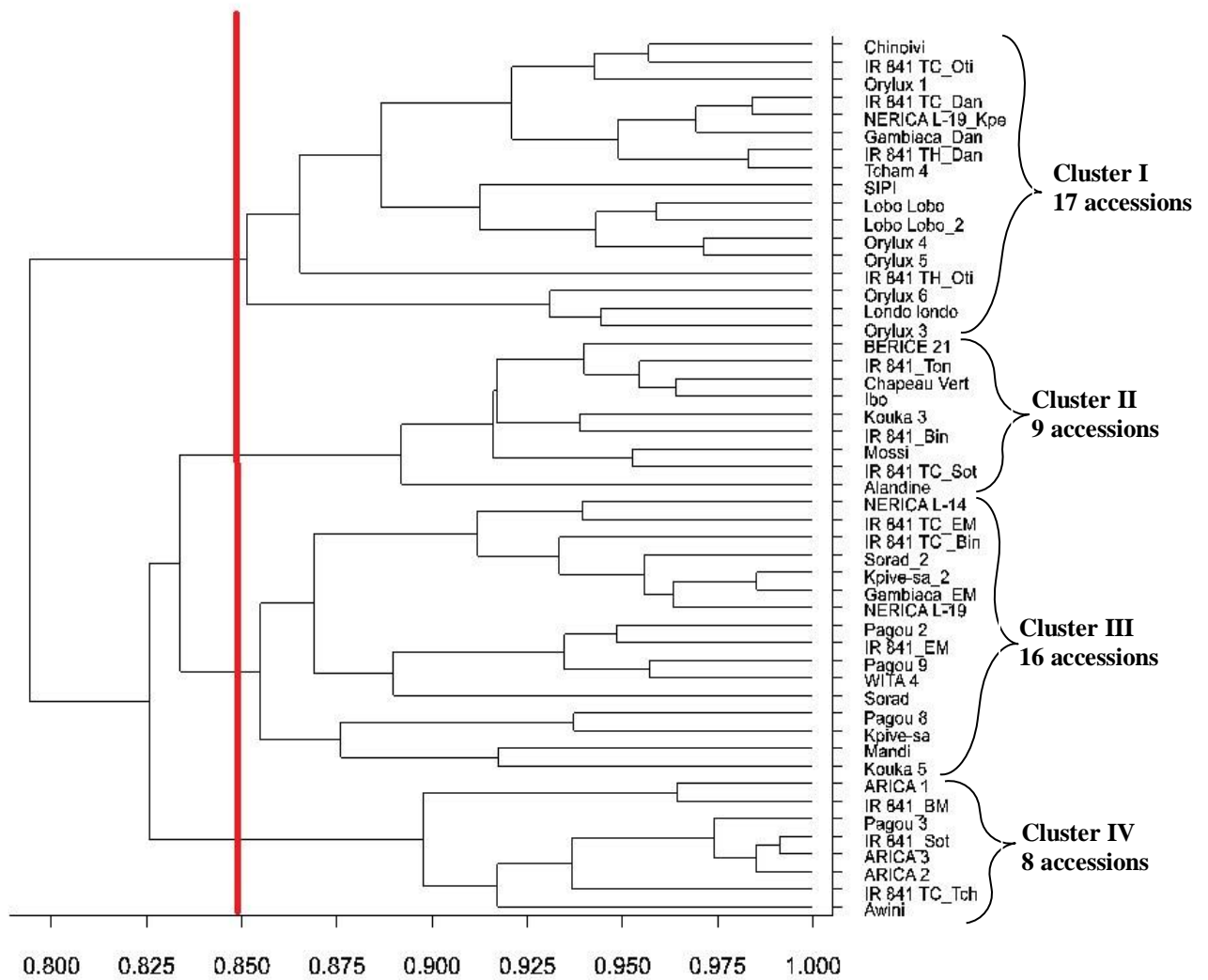


Figure 4.4: Dendrogram of 50 rice accessions from Togo based on 11 discriminative agro-morphological traits using average linkage method

No cluster was specific to the geographical origin or to the aromatic status of the accessions. All the aromatic accessions were distributed throughout the four clusters. The five Orylux varieties (Orylux 1, 3, 4, 5 and 6) belonged to Cluster I, the accession Chapeau vert to cluster II whereas the 13 samples of IR 841 were found across the four clusters: four in cluster I and three in each of the clusters II, III and IV. About the six yielding checks, ARICA 1, 2 and 3 were in cluster IV while NERICA L-14, L-19 and WITA 4 shared cluster III. For the four accessions collected in two different samples, three couples shared the same cluster: Sorad and Sorad_2, Kpive-sa and kpive-sa_2 in cluster III, Lobo lobo and Lobo lobo_2 in cluster I, reversely to Gambiaca_Dan and Gambiaca_EM which were found in cluster I and cluster III respectively. The accession NERICA L-19_Kpe (in cluster I) collected in Kpélé did not share the same cluster as NERICA L-19 (cluster III) taken from AfricaRice as check. Moreover, the seven non-named accessions were scattered across the four clusters (Figure 4.5).

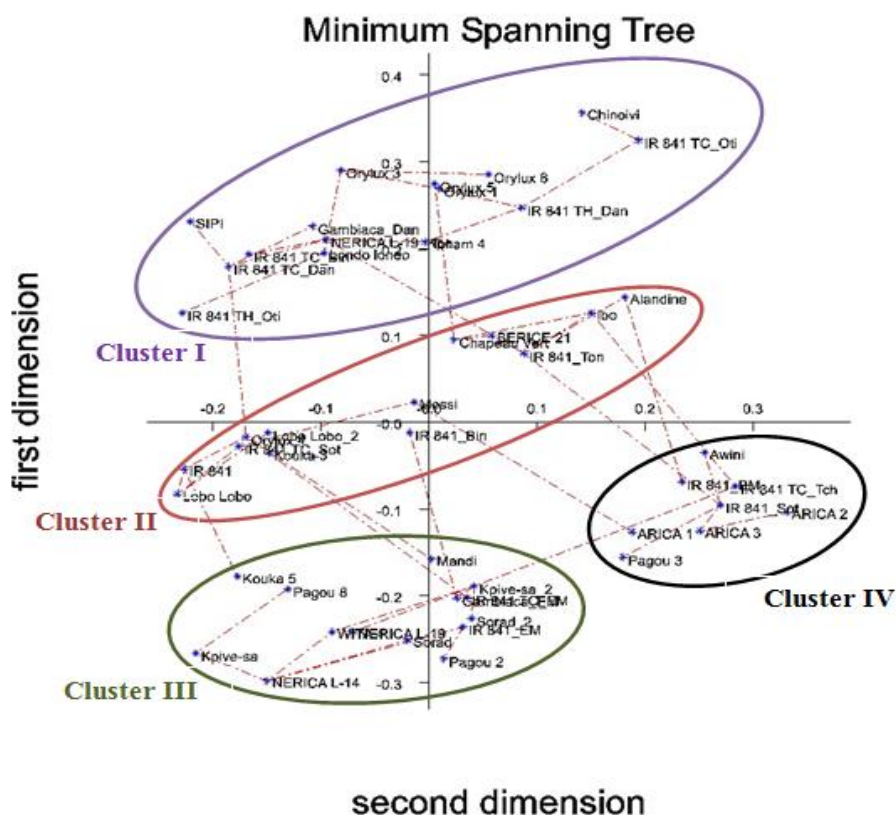


Figure 4.5: Minimum spanning tree presenting the 50 rice accessions from Togo in four clusters based on 11 discriminative traits

Cluster I was characterized by awnless accessions, cluster II by accessions with high number of tillers (10 to 15), cluster III by accessions with light green leaves, and cluster IV by accessions with semi erect culm, short panicle and low yield (2.40 to 4.69t/ha).

4.3.3. Molecular characterization

The 50 accessions have been characterized with 21,250 SNP markers randomly distributed across the rice genome. From this molecular characterization, informative markers were identified and used for principal components (PC) and cluster analyses.

4.3.3.1. Informative SNPs

Table 4.8 displays summary statistics on the 21,250 SNPs used in the characterization.

Table 4.8: Summary statistics on the 21,250 SNP markers used for the characterization of the 50 rice genotypes

	Call rate	Average PIC	Frequency of homozygote reference	Frequency of homozygote SNP	Frequency of heterozygote
Minimum	0.33	0.02	0	0	0
Maximum	1	0.5	0.98	0.98	0.6
Mean	0.84	0.23	0.55	0.41	0.03

It is shown from the above table that the polymorphic information content ranged from 0.02 to 0.5 with an average mean of 0.23. As regard to the alleles' frequencies, the higher average mean was for homozygote reference with 0.55 followed by the homozygote SNP with 0.41 and a very low mean frequency was recorded for heterozygote: 0.03.

By sorting out the average polymorphism information content and the call rate, and setting them at 0.3 to 0.5 and 0.9 to 1.0 respectively, 5,736 informative markers were identified and

considered for the principal components and cluster analyses. By considering these 5,736 informative SNPs, the frequency of homozygote reference ranged from 0.02 to 0.85 with an average mean of 0.50, the frequency of homozygous SNP went from 0.02 to 0.85 with an average mean of 0.46, while the frequency of heterozygote was from 0 to 0.60 with an average mean of 0.04.

4.3.3.2. Principal components

With the help of the plot of the PCs against the variance explained and using the Elbow method, the optimal number of principal components to be considered in this study is six (Figure 4.6). From this plot, the top six principal components accounted for 87.5% of the total variance existing within the genotypes. They respectively contributed 29%, 19%, 17.5%, 9%, 7% and 6% of the total variance.

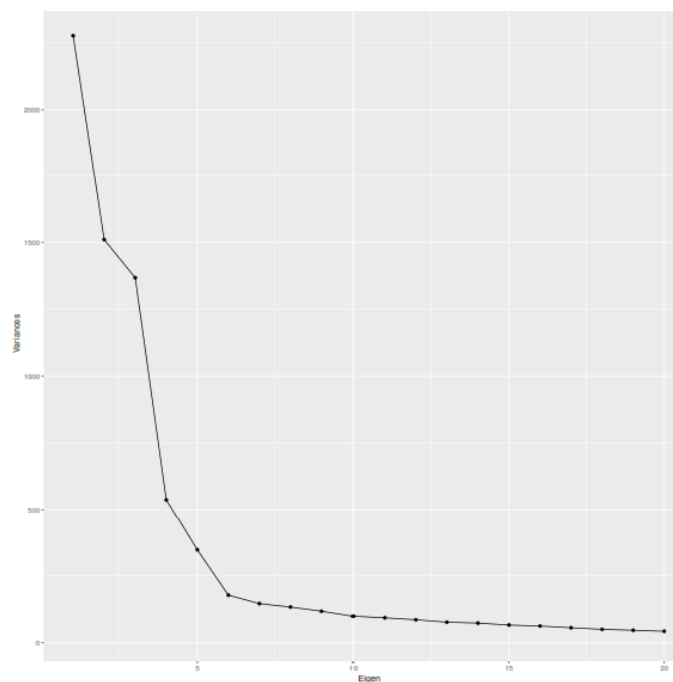


Figure 4.6: Variance explained by the principal components

The projection of the genotypes' coordinates was shown based on the 5,736 informative SNP markers, that the genotype NERICA L-14 was highly negatively associated with the top three PCs and highly positively associated with the fourth one with respective scores as follows: -161.96, -75.15, -101.21 and 32.50. High and positive association to PC1 was noticed for WITA 4 with a score of 66.48. Likewise, Gambiaca_EM, Pagou 2, IR 841 TC_Oti and Ibo were highly positively associated with PC1, PC2, PC3, PC5 and PC6.

4.3.3.3. Genotypes' clusters

Figure 4.7 shows the dendrogram of the 50 accessions based on SNP markers. The 50 genotypes grouped into five clusters (A, B, C, D and E) and one outlier (Kouka 5) at the height of 73 (the genotype Kouka 5 is not included in any cluster). Moreover, no duplicate was observed.

In terms of number of genotypes in each of the clusters, 12 genotypes belonged to Cluster A, four genotypes to cluster B, eight genotypes to cluster C, 16 genotypes to cluster D and nine genotypes to Cluster E.

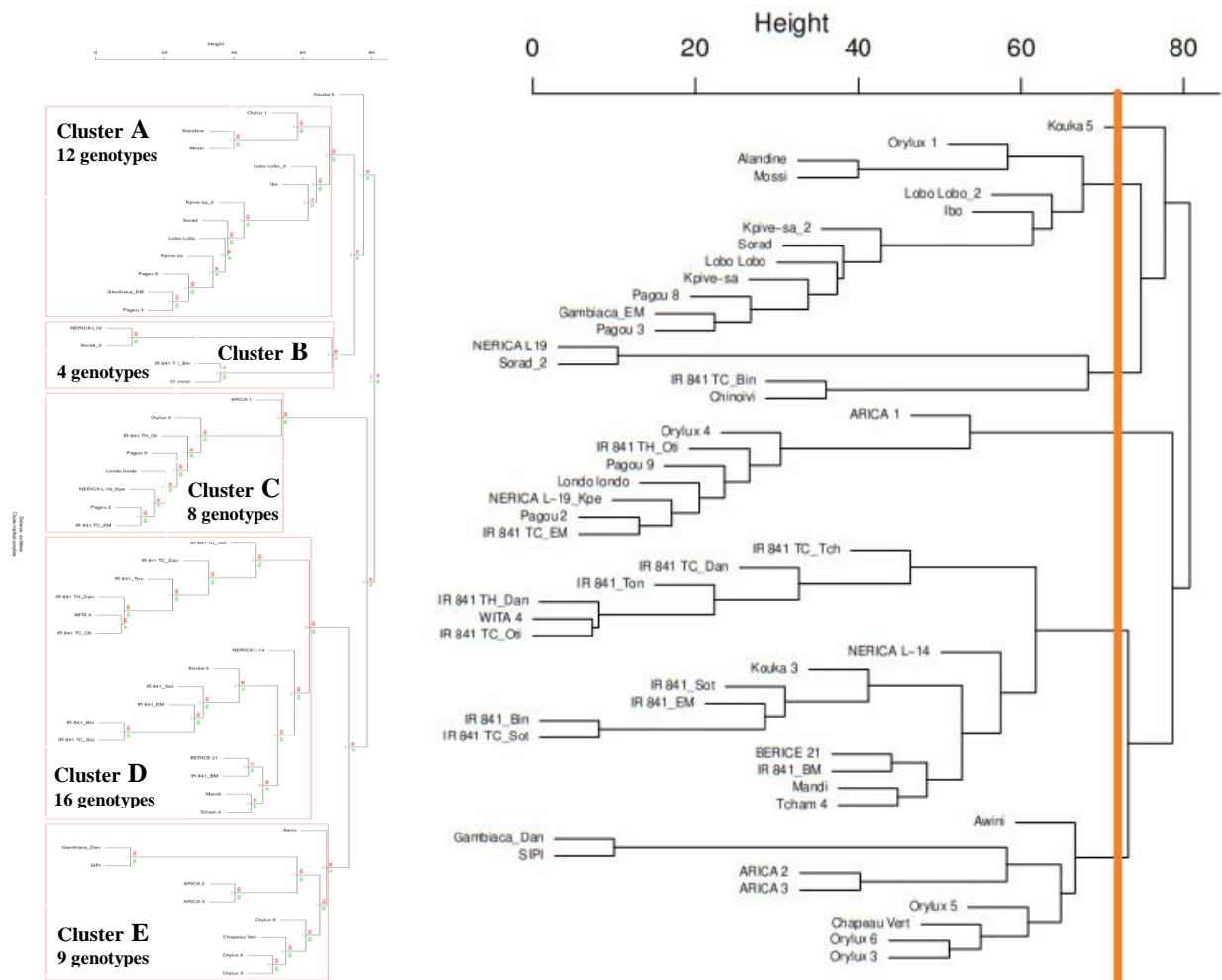


Figure 4.7: Dendrogram showing the clusters of the 50 rice genotypes collected in Togo based on 5,736 SNP markers

The clustering did not show any geographical distribution pattern of the genotypes. The aromatic genotypes were scattered across the five clusters: Orylux 1 in cluster A; IR 841 TC_Bin in cluster B; Orylux 4, IR 841 TH_Oti and IR 841 TC_EM in cluster C; ten samples of IR 841 in Cluster D; and Chapeau vert, Orylux 3, Orylux 5 and Orylux 6 in Cluster E.

The six high yielding checks were distributed in four clusters as follows: Cluster B (NERICA I-19), Cluster C (ARICA 1), cluster D (NERICA L-14 and WITA 4), and cluster E (ARICA 2 and ARICA 3). This means that NERICA L14 and NERICA L-19 did not share the same cluster. Also, the accession NERICA L-19_Kpé collected from farmers in Kpélé belonging to cluster C did not share the same cluster as NERICA L-19 brought from AfricaRice as check.

For each of the 4 accessions collected in two different samples, Lobo lobo and Lobo lobo_2, Kpive-sa and Kpive-sa_2 on the one hand were included in cluster A; and on the other hand Sorad (cluster A) and Sorad_2 (cluster B), Gambiaca_EM (cluster A) and Gambiaca_Dan (cluster E) were in separate clusters.

In addition, apart from kouka 5 which was not included in any cluster, the six non-identified accessions collected were members of cluster A (Pagou 3 and Pagou 8), cluster C (Pagou 2 and Pagou 9), and cluster D (Kouka 3 and Tcham 4).

4.3.4. Comparison between phenotypic and SNP data

Important differences were observed between the results of phenotypic and genotypic data analyses. As regard to the principal components analysis, five PCs explaining 72.29% of total variation among the 50 accessions were identified as optimal number of PCs based on the 11 discriminative phenotypic traits. With markers data, six PCs were identified as optimum and they explained 87.5% of total variation in the 50 accessions. Based on phenotypic data, the 50 accessions were grouped into four clusters without any outlier, whereas for the molecular data five clusters and one outlier came out from the cluster analysis. Accessions such as Sorad

(Sorad and Sorad_2), the five Orylux varieties (1, 3, 4; 5 and 6), the 2 NERICA varieties (L-14 and L-19), the 3 ARICA varieties (1, 2 and 3) which were for each set in the same clusters as regard to the phenotypic data, were scattered across the five clusters observed with molecular data.

Although divergence in the accessions belonging to clusters is high, the comparison between the results of phenotypic and molecular data analysis revealed concordance on the fact that the two samples of the accessions Kpive-sa (Kpive-sa and Kpive-sa_2) and Lobo lobo (Lobo lobo and Lobo lobo_2) shared the same clusters for both types of data. The same applied in that both types of results confirm the non-membership of the two samples of Gambiaca (Gambiaca_Dan and Gambiaca_EM) and NERICA L-19 (NERICA L-19 and NERICA L-19_Kpe) to the same cluster. Also, both types of data confirmed the non-existing relationship between, on the one hand the geographical origin of the accessions and their clusters, and on the other hand the aromatic or yielding status of the accessions and their clusters.

By taking into account the phenotypic performance of the members of the five clusters resulting from markers data analysis, cluster B is characterized by light green leaf blade, cluster C with light brown caryopsis pericarp, cluster E with a very strong culm lodging resistance, whereas no particular phenotype was associated with the clusters A and D members. The outlier Kouka 5 presented a very long awn differentiating it from the other genotypes.

4.4. Discussion

The prospecting survey made as part of this study enabled the collection of 38 accessions that were characterized. Among this collection, all the varieties reported to be introduced were not found. Only variety IR 841 introduced by the NARS was found in all the ten locations as it is adopted by farmers and thus collected in 13 samples. All the other varieties introduced by the

NARS were not brought by farmers. This would be because such varieties were grown in very small scale and by very few farmers. However, 14 accessions introduced by the farmers were collected. Among these 14 accessions, Gambiaca, Kpive-sa, Lobo lobo and Sorad were collected in two samples in different villages, the rest being collected in only one sample. Furthermore, seven accessions were collected without name in Binah, Dankpen and Tchamba. According to farmers these accessions appeared in their rice fields as off-types that presented high yield potential. Therefore, they kept some grain for production. For most of these accessions farmers have reported to grow them for two to five years. Such accessions would have evolved from probable mutation of cultivated rice varieties in these areas, or would be another accession mixed up in the seeds that were introduced. The last situation could easily occur in the varieties' introduced by farmers for which no seeds purity test is implemented. Nevertheless, these accessions represent genetic materials to be exploited in many ways as well as the 11 high yielding varieties brought from AfricaRice.

The 50 accessions gathered for this study did not vary at all for plant uniformity, seedling vigour, vegetative vigour, panicle axis and panicle shattering. The first three characters are tightly linked to the best quality of the seeds. A good specific purity and good germination power ensure the best uniformity and vegetative vigour of the plants. However, the droopy panicle (panicle attitude) and very low grain shattering (< 1%) observed at all the accessions indicated that the 50 accessions were *O. sativa* or interspecific (with *O. sativa* as one parent). This is because *O. glaberrima* is characterized with erect panicle, truncated ligules and a high degree of spontaneous grain shattering (Porteres, 1956; Carney, 2001; and Vido, 2011) and, having in mind that seven accessions were collected without any information from their growers (non-identified accessions) they were likely to be from the African species of rice (*O. glaberrima*) which were largely cultivated in the same areas where the non-identified accessions were collected. The above observation has been confirmed with molecular data.

From the markers alleles as well as the clustering results, the seven non-identified accessions displayed the same alleles and shared the same clusters with the well-known *O. sativa* accessions such as Orylux varieties (Sié *et al.*, 2010).

Even though 25 phenotypic traits showed important variability across the 50 accessions, the discriminant analysis based on the significance of the Wilk's lambda identified only 11 traits which explained better the variability in the germplasm. These were five qualitative and six quantitative traits [culm number (CN), number of panicle per plant (NPP), ligule shape (LS), leaf blade pubescence (LBP), phenotypic acceptability (Pacp), leaf length (LL), culm length (CL), 100-grain weight (GW), culm habit (CH), flag leaf attitude (FLA), and flag leaf length (FLL)], suggesting that both types of traits have contributed enough to the variability observed in the 50 accessions. The trait yield which varied from 2 to 7 t/ha showed no significance to the discriminant analysis, however its two components (number of panicle per plant and 100-grain weight) were discriminative.

The estimates of correlations among the 11 discriminative traits revealed significant positive correlation between culm number and number of panicle per plant. This result makes sense since each culm bears one panicle. Therefore, the higher the culm number, the higher the number of panicles per plant. The positive significant correlation between these two traits is quite normal. However, positive correlations were also detected among culm length (CL), leaf length (LL) and flag leaf length (FLL). Likewise, similar correlations existed between number of panicles per plant (NPP) and flag leaf length. Such results mean that the higher the NPP, the longer the FLL, and then, the longer the LL and the CL. These results confirmed those of Ranawake *et al.* (2013) who reported significant positive correlations between culm number and number of panicles per plant on the one hand, and culm length and leaf length on the other hand. Also, Ashrafuzzaman *et al.* (2009) mentioned positive association between flag leaf length and yield components such as number of panicles per plant.

According to Acquaaah (2012), rice as self-pollinated plant is highly homozygous. The summary statistics of the 21,250 SNP markers used to characterize the 50 accessions confirmed that assertion with very low to null frequencies of heterozygotes (0.03 as average frequency).

The 11 discriminative traits contributed to the five PCs identified as optimum and that explained 72.29% of total variation in the germplasm. Traits such as leaf length and culm length, flag leaf length contributed highly to PC1. Culm number, number of panicles per plant and flag leaf attitude composed the higher part of the second PC. For PC3, ligule shape was the highest contributing trait. PC4 was mainly explained by leaf blade pubescence and 100-grain weight, while PC5 was mainly explained by culm habit and phenotypic acceptability. Even though all the discriminative traits contributed to the five PCs, not too much variability were explained by the five PCs (72.29%). Compared to the PCs from molecular data, the six optimal PCs explained 87.50% of total variation. Therefore, much more variation was explained by molecular data than by the agro-morphological data. The difference observed seems to be logical because phenotypic data are subject to environmental effects.

As reported by Pisani *et al.* (2007), incongruence in the dendrogram from molecular data and the one from agro-morphological data were observed. With phenotypic data, four clusters (I, II, III, and IV) were identified whereas with molecular data, five clusters (A, B, C, D and E) and one outlier were identified. The molecular data is more precise because it is not influenced by environmental effects. Therefore, it makes sense that the non-identified accession Kouka 5 came as an outlier, since it showed a very long awn (6 cm) which made it very peculiar from the other 49 accessions.

The results also showed based on the 11 discriminative traits that all the five Orylux varieties, the two NERICAs (L-14 and L-19) as well as the three ARICAs and three accessions (collected in two different samples) shared the same clusters whereas with the 5736 SNP

markers, it was not the case. This could be explained by the fact that these varieties would share the same phenotypes regarding the 11 discriminative traits considered for the analysis, and in view of the higher number of SNP markers (5 736) located across all the 12 chromosomes of the rice genome, different alleles at each marker would be noticed. This raised the hypothesis that the 11 discriminative traits would not be highly associated with the informative markers used. This confirmed also the disparity observed in the phenotypic characteristics of the clusters I, II, III and IV (phenotypic based) on the one hand and A, B, C, D and E (molecular based) on the other hand. Clusters I, II, III and IV were respectively characterized by awnless; high culm number; light green leaves; and semi erected culm, short panicle and low yield. Conversely, clusters A, B, C, D and E were characterized by no particular trait; light green leaves; light brown caryopsis pericarp; no particular trait; and very strong culm lodging resistant accessions. Clusters III and B shared the same phenotypic characteristics but presented only three accessions in common. Apart from the differences observed between the results from phenotypic and genotypic data analyses, some similarities were noticed. Both types of data did not present any duplicate, any link between the clusters and the geographical origin of the accessions, nor any specificity of the clusters as regard the aromatic status of the accessions. The results of the two samples Lobo lobo, and Kpive-sa being in the same clusters, as well as the two samples of Gambiaca not sharing the same cluster were confirmed by the two types of data. Then, the accessions Lobo lobo and Kpive-sa respectively collected in Tchamba and Est Mono in two samples have the same origin, and would have been polluted or mutated over time. This would apply to the divergence between NERICA L-19 (from AfricaRice) and NERICA L-19_Kpé (collected from farmers in Kpélé). On the contrary, the two samples of Sorad and Gambiaca were distinct from the molecular information. These two samples are therefore very different. It is understandable because several accessions have been named Sorad from the name of the national Extension service

Sorad which have provided farmers across the country with new varieties introduced by the NARS. About the disparity between the two samples of Gambiaca, these two samples were collected in different areas: Dankpen (Northern region) and Est Mono (Southern region). So, it might have been introduced from diverse sources (probably from Burkina Faso and Benin). In addition, the molecular data gave more clarification about the 13 samples of IR 841 (the most cultivated aromatic variety across the country). Ten of the 13 samples of IR 841 clustered together, showing a high level of similarity among these ten accessions. It is important to mention that none of the 13 accessions were 100% similar. This indicated that pollution and possibly mutation occurred from one site to another over time. This could also be explained by the high differences shown by the phenotypic clustering of these 13 accessions (they were all scattered across the four clusters in four, three, three and three accessions). Moreover, the sensory evaluation of aroma revealed different levels of strength of aroma in the 13 accessions. The three accessions of IR 841 not included in the same cluster as the ten others were: IR 841 TH_Oti, IR 841 TC_EM and IR 841 TC_Bin. They were highly different from the original race of IR 841 introduced in Togo in 1980. In consequence, the ten IR 841 accessions clustered together would have undergone slight changes from their original seeds.

4.5. Conclusion

This study facilitated the establishment of a local germplasm collection of 50 accessions. The agro-morphological characterization of this germplasm differentiated the 50 accessions into four clusters on the basis of 25 traits in which 11 discriminative traits were identified. Likewise, 5,736 informative SNP markers contributed to the distinction in the 50 accessions in five clusters. In sum, with both types of data, no duplicates were observed. The 13 samples of the aromatic variety IR 841 are distinct suggesting the hypothesis of a contamination of this

variety across the country. With their specific characters, each one of the clusters and their accessions can be exploited in the development of a desired variety. In this, both of phenotypic and genotypic based clusters must be considered. As well, the types and strength of correlations estimated between traits will be for a great help in the use of indirect selection. Among the 25 traits which presented variation in the accessions, the main target traits of this research (aroma and yield) were discarded for their very low discriminative power.

CHAPTER FIVE: PHENOTYPIC AND MOLECULAR SCREENING OF THE ACCESSIONS FOR YIELD AND AROMA

5.1. Introduction

For almost all the breeding programmes, grain yield is always the major target trait since the grain is the source of income. Then, it is important to breed also for traits that improve the grain quality. High quality grain is more expensive than low quality grain. So, combine yield to grain quality ensures an increase in the plant growers' income. In rice production, aroma is an important grain quality trait which improves the value of rice. Aromatic rice is said to be for rich households, because it is more expensive than the non-aromatic one. In Togo, the participatory rural appraisal conducted in 2016 in ten rainfed and irrigated lowlands (identified as major rice producing areas across the country) revealed that apart from grain yield, fragrance (aroma) was a very important trait for farmers and consumers. This so important that majority of rice growers in Togo prefer the cultivation of the aromatic variety IR 841. Accordingly, across the country, cultivation of non-aromatic varieties is decreasing. Unfortunately, due to contamination, IR 841 also is declining in yield.

Therefore, it became imperative to develop new aromatic lines for farmers. This study focused on that purpose and aimed to:

- identify the aromatic accessions in the germplasm,
- identify high yielding accessions in the germplasm,
- estimate the correlations between aroma, yield and its components, and
- select parents for hybridization.

5.2. Materials and Methods

5.2.1. Plant materials

The local collection of 39 rice accessions as well as 11 elite varieties from AfricaRice making a total of 50 entries was screened for both presence of aroma and grain yield at phenotypic and molecular levels (Table 5.1).

Table 5.1: Rice accessions used for phenotypic and molecular screening for yield and aroma

N°	Accession name	Source	N°	Accession name	Source
1	Alandine	Tone	26	Awini	Sotouboua
2	IR 841_Ton	Tone	27	IR 841 TC_Sot	Sotouboua
3	IR 841 TC_Oti	Oti	28	IR 841_EM	Est Mono
4	IR 841 TH_Oti	Oti	29	Sorad	Est Mono
5	Mossi	Oti	30	Kpive-sa	Est Mono
6	Londo londo	Binah	31	Sorad_2	Est Mono
7	Pagou 2	Binah	32	IR 841 TC_EM	Est Mono
8	Pagou 3	Binah	33	Kpive-sa_2	Est Mono
9	IR 841 TC_Bin	Binah	34	Gambiaca_EM	Est Mono
10	IR 841_Bin	Binah	35	Chinoivi	Kpélé
11	Pagou 8	Binah	36	SIPI	Zio
12	Pagou 9	Binah	37	BERICE 21	Zio
13	IR 841 TC_Dan	Dankpen	38	Chapeau vert	Zio
14	Mandi	Dankpen	39	IR 841_BM	Bas Mono
15	Kouka 3	Dankpen	40	WITA 4	AfricaRice
16	Kouka 5	Dankpen	41	ARICA 1	AfricaRice
17	Gambiaca_Dan	Dankpen	42	ARICA 2	AfricaRice
18	IR 841 TH_Dan	Dankpen	43	ARICA 3	AfricaRice
19	IR 841 TC_Tch	Tchamba	44	NERICA L-14	AfricaRice
20	Lobo Lobo	Tchamba	45	NERICA L-19	AfricaRice
21	Lobo Lobo_2	Tchamba	46	Orylux 1	AfricaRice
22	Tcham 4	Tchamba	47	Orylux 3	AfricaRice
23	Datcha	Sotouboua	48	Orylux 4	AfricaRice
24	IR 841_Sot	Sotouboua	49	Orylux 5	AfricaRice
25	Ibo	Sotouboua	50	Orylux 6	AfricaRice

5.2.2. Sensory evaluation for aroma

Phenotypic evaluation for presence of aroma in the accessions was performed using sensory evaluation methods as described by Singh *et al.* (2000).

5.2.2.1. Methodology

One brown rice grain was placed into centrifuge tube (50 ml round bottom). About 20 ml distilled water was added. The tubes were then covered with aluminium foil. The samples were placed in a boiling water bath for 30 minutes. The cooked samples were allowed to cool and the presence of aroma was determined for every sample by smelling. The scented variety IR 841 was used as check for comparison. A panel of five persons was involved in this sensory evaluation.

5.2.2.2. Data collected

The samples were scored following the evaluation scale of presence or absence of aroma presented in the *Descriptors for wild and cultivated rice* (Bioversity International, 2007) as follows: 0 – non-scented, 1 – slightly scented, 2 – moderately scented, 3 – strongly scented. For each accession, the mean score from the five evaluations were recorded.

5.2.3. Evaluation of yield and its component traits

The 50 entries were evaluated in the field for yield and its component traits.

5.2.3.1. Location

The trial was conducted on station in Mission Tové under irrigation. The station is located at 35 km from Lomé.

5.2.3.2. Experimental design

The experiment was conducted following an alpha lattice design with three replications. Each replication of the 50 plots was divided into ten incomplete blocks of five plots per block in which the accessions were randomly distributed. The experimental unit was represented by a 5 m² plot (5 m x 1 m) containing five rice rows (of 5 m each). Each plot was made of a total of 125 plants (20 cm within rows and 20 cm within rice plants on the same row). The distance within plots was 50 cm, and between replications was 1 m.

5.2.3.3. Methodology

The trial was conducted following the technical recommendations of AfricaRice for irrigated rice production (Wopereis *et al.*, 2009). Yield was evaluated by weighting the harvest obtained on the 5 m² at 14% of moisture content and estimating the production on 1 ha.

5.2.3.4. Data collected

Data on Yield (Yld) and its three component traits: number of panicle per plant (NPP), number of grain per panicle (NGP) and 100-grain weight (GW) were collected as mentioned in the standard evaluation system (SES) (IRRI, 2002).

5.2.4. Molecular characterization

Like for the phenotypic evaluation of the 50 accessions for aroma and yield, the molecular screening of the same set of accessions and for the same purpose was done. This activity was conducted in Intertek laboratory in Sweden.

A set of 35 SNP markers for grain quality traits among which three are specific to presence or absence of aroma were used, in addition to five SNP markers for yield and its component traits (Table 5.2).

The SNP marker BADH 2.1-7 identifies two different sequences corresponding to two different alleles. One is a sequence of 13 base pairs AAAAGATTATGGC and the other a sequence of five base pairs TATAT. The homozygote carrying the allele AAAAGATTATGGC is not scented as well as the heterozygous carrying both alleles, as rice aroma gene *fgr* is recessive. On the contrary, the homozygote with the allele TATAT is scented. For the SNP markers for yield and other grain quality traits, information on the favourable allele was not yet available (see information on the following web pages: <http://gsl.irri.org/genotyping/trait-based-genotyping/10-snp-panel> and https://docs.google.com/spreadsheets/d/10g_BGU1w0ArL0kV49_Wgv8YWiK1ZILnu4wBJVzsta5A/view#gid=110970023).

The protocol for DNA isolation and sequencing methodology in the Intertek laboratory in Sweden was applied. The number of alleles and their frequencies are presented in summary statistics. The presence of the desired alleles for aroma was used to characterize the accessions and identify the aromatic accessions.

Based on the phenotypic data, descriptive analysis was run as regard to the sensory evaluation of aroma using Microsoft Excel on the one hand, and analysis of variance (ANOVA) using the linear mixed model (REML) with block set as random using GenStat 9.2 for the evaluation of yield and its component traits on the other hand. For the variables with a high significant p-value (0.01), Duncan's multiple means comparison test was performed based on the least significant difference (LSD) between the accessions means. The LSD value was given by $LSD \text{ value} = t\text{-value} \times s.e.d$ with s.e.d the average standard error of difference, and the t-value given by the PQRS (Probabilities, Quantiles and Random Samples) package from

the degree of freedom (df). From the results of the multiple means comparison, strongly scented on the one hand, and high yielding accessions on the other hand were selected as parents.

Canonical correlations between the traits were estimated with the statistical package for social sciences SPSS 17.0 considering the Spearman correlation coefficient to assess the strength of relationship between each pair of traits.

The SNP alleles were summarized with their frequencies across the genotypes. Alleles' information were transformed into scores to compute Spearman's correlation estimates with the SPSS 17.0. Homozygotes therefore were scored 0 and 1 while the score of 2 was recorded for heterozygotes.

Table 5.2: SNP markers used for the molecular screening of the 50 rice accessions

N ^o	SNP name	Traits	N ^o	SNP name	Traits
1	BADH2.1-7	Aroma	21	id5005867	Chalkiness
2	BadH2_3bp_5UTR	Aroma	22	id8006321	Chalkiness
3	BadH2_3bp_E12	Aroma	23	id10002943	Chalkiness
4	chalk5_721	Grain quality	24	id11010447	Chalkiness
5	chalk5_576	Grain quality	25	Waxy-54	Amylose
6	chalk5_485	Grain quality	26	Waxy-55	Amylose
7	chalk5_215	Grain quality	27	Waxy-1	Amylose
8	GS3	Grain quality	28	Waxy-59	Amylose
9	SSIIa – SNP	Grain quality	29	Waxy-37	Amylose
10	SSIIa_SNP3F2	Grain quality	30	Waxy-2	Amylose
11	Waxy exon 6	Grain quality	31	Waxy-36	Amylose
12	Wx-GBSS-ex10	Grain quality	32	Waxy-3	Amylose
13	Waxy	Grain quality	33	Alk-11	Gelatinization
14	id1000223	Chalkiness	34	Alk-56	Gelatinization
15	id1009557	Chalkiness	35	Alk-9	Gelatinization
16	id2009229	Chalkiness	36	IRM-45	Yield potential
17	id3008613	Chalkiness	37	IRM-47	Yield potential
18	id4007289	Chalkiness	38	IRM-50	Yield potential
19	id4008855	Chalkiness	39	Gn1a-44	Number of grains per panicle
20	id5002468	Chalkiness	40	GS5-07_100bp	Grain width and weight

5.3. Results

5.3.1. Aromatic accessions identified by sensory evaluation

Nineteen accessions (38%) were identified as aromatic over the 50 accessions screened for presence or absence of aroma through sensory evaluation as described by Singh *et al.* (2000). Following the evaluation scale, 31 accessions (62%) were scored 0 (meaning that no aroma was perceived after the sensory evaluation). None of the accessions were scored 1 (slightly scented), 12 accessions (24%) were scored 2 (moderately scented), and seven accessions (14%) scored 3 (strongly scented) as presented in Table 5.3.

Table 5.3: Aromatic accessions identified after the sensory evaluation

N°	Accession name	Source	Score	Implication
1	IR 841_Sot	Sotouboua	3	Strongly scented
2	Chapeau vert	Zio	3	Strongly scented
3	Orylux 1	AfricaRice	3	Strongly scented
4	Orylux 3	AfricaRice	3	Strongly scented
5	Orylux 4	AfricaRice	3	Strongly scented
6	Orylux 5	AfricaRice	3	Strongly scented
7	Orylux 6	AfricaRice	3	Strongly scented
8	IR 841_Ton	Tone	2	Moderately scented
9	IR 841 TC_Oti	Oti	2	Moderately scented
10	IR 841 TH_Oti	Oti	2	Moderately scented
11	IR 841 TC_Bin	Binah	2	Moderately scented
12	IR 841_Bin	Binah	2	Moderately scented
13	IR 841 TC_Dan	Dankpen	2	Moderately scented
14	IR 841 TH_Dan	Dankpen	2	Moderately scented
15	IR 841 TC_Tch	Tchamba	2	Moderately scented
16	IR 841 TC_Sot	Sotouboua	2	Moderately scented
17	IR 841_EM	Est Mono	2	Moderately scented
18	IR 841 TC_EM	Est Mono	2	Moderately scented
19	IR 841_BM	Bas Mono	2	Moderately scented

From Table 5.3, all the five accessions obtained from AfricaRice for aroma presented a strong fragrance. Only the two accessions IR 841_Sot and Chapeau vert were also strongly scented. The rest of aromatic accessions were moderately scented.

5.3.2. Analysis of variance of yield and its component traits

The analysis of variance using linear mixed model with block as random revealed no significant differences between the accessions and replications' means as far as number of grains per panicle is concerned. Highly significant differences were shown between replications but not between accessions for the number of panicles per plant. It was for 100-grain weight and grain yield that highly significant differences between accessions were detected. Therefore, least significant difference (LSD) value was calculated to compare the means of the accessions for grain yield and 100-grain weight. In addition, significant differences between replications were detected for 100-grain weight (Table 5.4).

Table 5.4: Analysis of variance using REML for yield and its component traits

Traits	Source of variation	Wald statistic	df	Wald/df	Chi probability	Average s.e.d	LSD value
<i>Number of grains per panicle</i>	Replications	10.33	2	5.16	0.006	23.690	
	Accessions	60.77	49	1.24	0.121		
<i>Number of panicles per plant</i>	Replications	37.15	2	18.58	<0.001	2.330	
	Accessions	49.53	49	1.01	0.452		
<i>100-grain weight</i>	Replications	15.72	2	7.86	<0.001	0.183	0.481
	Accessions	85.5	49	1.74	<0.001		
<i>Grain yield</i>	Replications	0.5	2	0.25	0.777	1.300	3.410
	Accessions	101.85	49	2.08	<0.001		

df = degree of freedom, s.e.d = standard error of difference, LSD = least significant difference

Using the LSD value, the significantly similar accessions were grouped based on 100-grain weight and grain yield means. For both of the traits, two distinctive groups were identified with some accessions sharing the two groups. The two groups were marked as “a” and “b”. Accessions sharing both groups were marked with “ab”. The highest yield as well as the highest 100-grain weight was represented in group “a”. So the accession Gambiaca_Dan in the group “a” had the highest 100-grain weight (2.95 g). The lowest 100-grain weight was recorded for accession Tcham 3 (2.00 g) which is the single accession in group “b”. The eight

highest yielding accessions were as follows: Sipi (7.63 t/ha), Gambiaca_Dan (7.39 t/ha), IR 841_Sot (7.03 t/ha), Londo londo (6.95 t/ha), Berice 21 (6.57 t/ha), WITA 4 (6.49 t/ha), Chinoivi (6.29 t/ha) and Sorad (5.78 t/ha). Table 5.5 displays the means of these eight accessions for yield and its component traits.

Table 5.5: Performance of the eight higher yielding accessions in yield and its component traits

Accessions' name	NPP	NGP	GW	Yld
<i>Sipi</i>	8.28	141	2.62	7.63
<i>Gambiaca_Dan</i>	6.41	140	2.82	7.39
<i>IR 841_Sot</i>	11.74	86	2.79	7.03
<i>Londo londo</i>	10.55	96	2.76	6.95
<i>Berice 21</i>	9.06	111	2.62	6.57
<i>WITA 4</i>	11.01	90	2.62	6.49
<i>Chinoivi</i>	11.42	78	2.70	6.29
<i>Sorad</i>	7.72	122	2.45	5.78

NPP = number of panicles per plant, *NGP* = Number of grains per panicle, *GW* = 100-grain weight (g), *Yld* = grain yield (t/ha)

5.3.3. Distribution of markers for grain quality in the germplasm

The alleles and their frequencies as distributed across the 50 genotypes are presented in Table 5.6. Among these 35 SNP markers, were included three specific to rice aroma: BADH 2.1-7, BadH2_3bp_E12 and BadH2_3bp_5UTR. Among the three SNP specific to presence or absence of aroma in the rice grain, only the marker BADH 2.1-7 was polymorphic across the 50 accessions, identifying both homozygotes and the heterozygote. The markers BadH2_3bp_E12 and BadH2_3bp_5UTR were monomorphic showing respectively the allele GAA and GTC for all the individuals. Based on the favourable allele of the marker BADH 2.1-7, 20 accessions were identified as being aromatic. These 20 accessions included the 19 found to be aromatic after the sensory evaluation and Berice 21 which is an accession

introduced by farmers in the Zio Valley. The two heterozygotes were ARICA 1 and WITA 4 collected from AfricaRice and used as checks.

Table 5.6: Rice grain quality SNP alleles and their frequencies in the germplasm

SNP ID	Code*		Alleles			Missin g call	SNP ID	Code*		Alleles			Missing call
	1	2	1:1	1:2	2:2			1	2	1:1	1:2	2:2	
id1000223	C	A	17	3	30		Waxy-3	T	C	23	4	23	
id1009557	C	A	50	0	0		Alk-11	A	T	14	1	35	
id2009229	C	A	50	0	0		Alk-56	A	G	14	1	35	
id3008613	G	A	1	0	49		Alk-9	T	G	2	0	46	2
id4007289	C	G	4	2	44		GS3	T	G	35	2	13	
id4008855	G	A	28	2	20		SSIIa – SNP	GC	TT	24	1	25	
id5002468	G	C	1	0	49		Waxy exon 6	C	A	5	1	44	
id5005867	G	A	50	0	0		Wx-GBSS-ex10	T	C	23	4	23	
id8006321	C	A	0	0	50		Waxy	T	G	15	1	34	
id10002943	C	A	30	1	19		chalk5_576	G	A	28	4	18	
id11010447	A	T	29	1	2	18	Chalk5_721	A	G	29	3	18	
Waxy-54	T	C	32	1	17		chalk5_485	A	T	28	3	17	2
Waxy-55	A	G	32	1	17		chalk5_215	C	G	30	3	17	
Waxy-1	T	G	15	1	34		SSIIa_SNP3F2	G	A	30	3	17	
Waxy-59	A	G	27	2	21		BADH 2.1-7**	3	4	20	2	28	
Waxy-37	C	T	28	2	20		BadH2_3bp_E12	-	GAA	0	0	50	
Waxy-36	C	T	43	1	6		BadH2_3bp_5UTR	-	GTC	0	0	50	

*1 and 2 represent the base or sequence of the SNP **BADH 2.1-7:: 3 = TATAT and 4 =AAAAGATTATGGC

5.3.4. Distribution of yield and its components markers in the germplasm

The screening of the 50 genotypes for yield was made using five markers: IRM-45, IRM-47, IRM-50 identified by IRRI for yield potential, Gn1a-44 for number of grains per panicle, and GS5-07_100 bp for grain width and weight. The results showed no variation across the 50 genotypes in the base pairs representing the alleles of the markers IRM 50 and Gn1a-44 (linked to number of grain per panicle) for which all the genotypes presented homozygosity G:G and T:T respectively.

All the genotypes were homozygotes for the marker IRM-45. Forty eight individuals (96%) has the genotype C:C and only 2% the alternative T:T. The latter was Tcham 3 characterized by small panicles. This result translated the positive contribution of the allele C to the yield.

As far as the marker IRM-47 is concerned, both homozygotes (G:G and T:T) as well as the heterozygote G:T were observed in the germplasm in respective proportion of 22%, 74% and 4% of the genotypes. The results on marker GS5-07_100 bp followed the same pattern (26% for G:G, 70% for T:T and only 4% for G:T) as shown on the Figure 5.1.

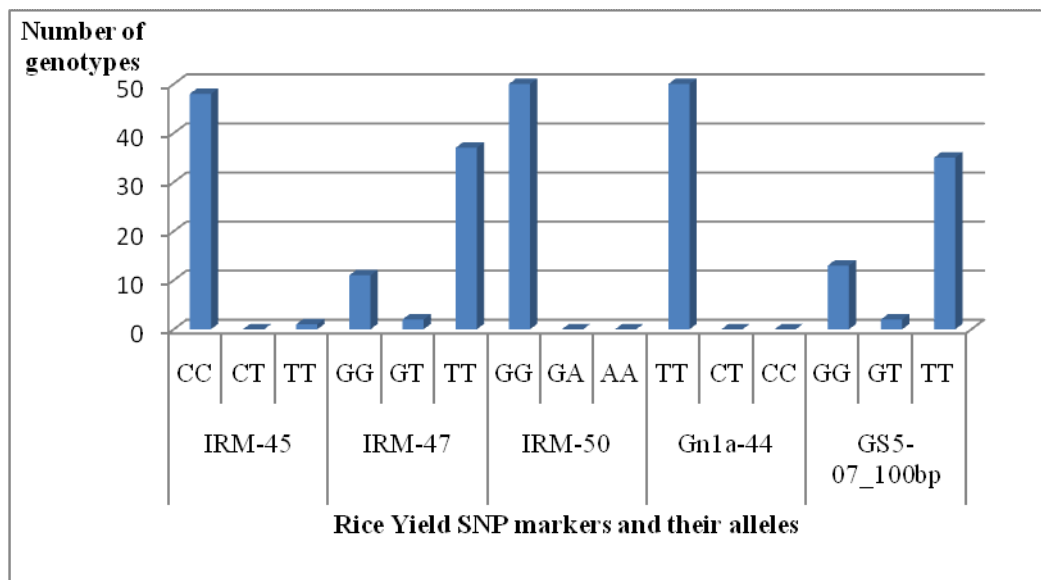


Figure 5.1: Distribution of five yield potential SNP alleles in 50 rice genotypes collected in Togo

5.3.5. Correlations between aroma and yield components traits

5.3.5.1. At phenotypic level

The strength of relationship between caryopsis scent (CS), yield (Yld) and its component traits assessed through significance test of Spearman correlation revealed that the trait 100-grain weight showed no significant correlation with all the other traits, though this correlation is positive.

In Table 5.7, number of grains per panicle (NGP) was correlated with a high significance level to all the other three traits. It was highly and positively correlated with yield (Yld), negatively correlated with number of panicles per plant (NPP), and positively correlated with caryopsis scent (CS) respectively with Spearman correlation coefficients of 0.731; -0.475 and 0.495. Grain yield was positively correlated with number of panicles per plant (0.100), and significantly to caryopsis scent (0.373). Conversely, a negative non-significant correlation was detected between number of panicles per plant and caryopsis scent (-0.194).

Table 5.7: Correlation matrix of the caryopsis scent, yield and its component traits

	<i>NPP</i>	<i>NGP</i>	<i>GW</i>	<i>CS</i>
<i>Grain yield (Yld)</i>	.100	.731**	.164	.373**
<i>Number of panicle per plant (NPP)</i>		-.475**	.068	-.194
<i>Number of grain per panicle (NGP)</i>			.053	.495**
<i>100-grain weight (GW)</i>				.102
<i>Caryopsis scent (CS)</i>				1.000

** significant at $p=0.01$

5.3.5.2. At molecular level

The Spearman correlation coefficients and their significance levels were estimated between eight SNP markers specific to aroma (three markers: BADH 2.1-7, BadH2_3bp_E12 and BadH2_3bp_5UTR) and yield and its component traits (five markers: IRM-45, IRM-47, IRM-50, Gn1a-44 and GS5-07_100bp). From the results, no significant correlation was shown between each pair of markers as presented in Table 5.8. However negative correlations were detected between the marker for aroma BADH2.1-7 and two markers for yield IRM-45 and GS5-07_100bp. Similarly, GS5-07_100bp was negatively correlated with IRM-45, both being markers for yield.

Table 5.8: Correlation coefficients between eight SNPs linked to yield and aroma

	BADH2.1-7	BadH2_3bp_E12	BadH2_3bp_5UTR	IRM-45	IRM-47	IRM-50	Gn1a-44	GS5-07_100bp
BADH2.1-7	1.000	..003	0.250	-0.176	0.019	0.002	0.170	-0.125
BadH2_3bp_E12		1.000	0.030	0.040	0.040	0.070	0.020	0.080
BadH2_3bp_5UTR			1.000	0.130	0.050	0.060	0.140	0.120
IRM-45				1.000	0.065	0.150	0.030	-0.228
IRM-47					1.000	0.020	0.070	0.014
IRM-50						1.000	0.110	0.210
Gn1a-44							1.000	0.200
GS5-07_100bp								1.000

5.3.6. Comparison between phenotypic and molecular data

The results coming from phenotypic and molecular data analyses were similar for some aspects and not for others. The highest similarity (95%) was the concordance in the identification of the 19 aromatic accessions with both sensory evaluation results and markers information. As far as yield and its component traits are concerned, congruence in the low phenotypic variability observed in number of grains per panicle and the monomorphism of the marker Gn1a-44 (linked to this trait) was observed. Also, the accession Tcham 3 showing the lowest yield was reported to be the only accession carrying the genotype T:T for the yield potential marker IRM-45. Negative correlations were detected between yield component traits and caryopsis scent by both phenotypic and marker data, though no significance was detected from the marker data analysis.

High dissimilarity was also observed in comparing phenotypic and molecular results. The variety Berice 21 reported to be non-scented displayed the allele TATAT indicating that it would be aromatic. Moreover, significant correlations were observed within the phenotypes but not between the markers. The negative correlation between BADH 2.1-7 (aroma) and GS5-07_100bp (grain width and weight) would have suggested negative correlation between caryopsis scent and 100-grain weight which on the contrary was positive. The same applied to the negative correlation between GS5-07_100bp (grain wigth and weight) and IRM-45 (yield

potential), and between BADH 2.1-7 (aroma) and IRM-45 (yield potential) on the one hand, and the positive correlation observed between 100-grain weight and yield, and between caryopsis scent and yield.

In sum, the eight yielding accessions identified from the phenotypic evaluation were detected as regard to the polymorphic SNPs used for the molecular screening. In Table 5.9 are presented the yields recorded as well as the alleles carried by these eight accessions.

Table 5.9: Yield, fragrance and their markers alleles for eight rice accessions

Accessions	Yield (t/ha)	Fragrance	BADH2.1-7	IRM-45	IRM-47	GS5-07_100 bp
SIPI	7.63	Non scented	AAAAGATTATGGC:AAAAGATTATGGC	C:C	G:G	T:T
Gambiaca_Dan	7.39	Non scented	AAAAGATTATGGC:AAAAGATTATGGC	C:C	T:T	G:G
IR 841_Sot	7.03	Scented	TATAT:TATAT	C:C	T:T	G:G
Londo londo	6.95	Non scented	AAAAGATTATGGC:AAAAGATTATGGC	C:C	T:T	T:T
BERICE 21	6.57	Non scented	TATAT:TATAT	C:C	T:T	T:T
WITA 4	6.49	Non scented	TATAT:AAAAGATTATGGC	C:C	T:T	T:T
Chinoivi	6.29	Non scented	AAAAGATTATGGC:AAAAGATTATGGC	C:C	T:T	G:G
Sorad	5.78	Non scented	AAAAGATTATGGC:AAAAGATTATGGC	C:C	G:G	T:T

From the table, all the eight high yielding accessions are similar for IRM-45, but not for GS5-07_100bp.

5.3.7. Parents selected

Considering the eight high yielding accessions and the seven strongly scented accessions from the above results, nine accessions (three for aroma and six for yield) were selected as parents for the development of aromatic and high yielding lines. Table 5.10 presents the list of these nine accessions. Apart from the target trait aroma and yield, accession Chinoivi and WITA 4 were also early maturing. IR 841_Sot combined both the traits yield and aroma.

Table 5.10: Parents selected for the development of aromatic and yielding lines

Name	Target traits	Yield (t/ha)	Source
<i>Sipi</i>	Yield	7.63	Farmers
<i>Gambiaca_Dan</i>	Yield	7.39	Farmers
<i>Londo londo</i>	Yield	6.95	Farmers
<i>Berice 21</i>	Yield	6.57	Farmers
<i>WITA 4</i>	Yield	6.49	AfricaRice
<i>Chinoivi</i>	Yield	6.29	Farmers
<i>IR 841_Sot</i>	Aroma	7.03	Farmers
<i>Chapeau vert</i>	Aroma	5.72	Farmers
<i>Orylux 5</i>	Aroma	5.63	AfricaRice

5.4. Discussion

The results of this study clearly shown a close link between phenotypic and molecular information on aroma. The 19 accessions identified as scented by sensory evaluation were confirmed as such by the BADH2.1-7 marker allele's information. However, the variety Berice 21 expressed the allele for presence of aroma but identified as non-scented, showing that in some cases the marker can separate from the gene. There was therefore 95% agreement between the phenotypic and molecular data. This means that the sensory evaluation to detect presence of aroma as designed by Singh *et al.* (2000) is effective. However, it is still subjective and cannot be fully reliable and substitutable to biochemical evaluation or molecular screening. Improved and highly reliable methods in detecting presence of aroma in rice consist in the use of electronic nose as done by Xian-zhe (2009). Moreover, the different degrees of aroma observed by sensory evaluation in the aromatic accessions could not be explained by molecular data for the reason that two markers over three used for aroma were monomorphic.

The non-significant difference for number of grains per panicle could be explained and supported by the monomorphism observed on the marker Gn1a-44 which is linked to that trait. Also, 100-grain weight and yield for which the accessions were significantly different are linked to the markers RM-47, RM-45 and GS5-07_100bp which were polymorphic. This

confirmed the tight link between markers and these traits, even though they are highly influenced by environment. Yield is controlled by many genes with high interactions within them. For the very small set of five markers used to screen for yield, all the eight more yielding accessions identified in this study carried the same allele C for the marker IRM-45, suggesting that this allele would be important in increasing yield. This assumption has been confirmed by the results of Tcham 3 having the lowest yield and carrying the T allele. Therefore, the two alleles C and T observed on the marker IRM-45 would be good for increasing and decreasing yield respectively. However, the variability observed in the alleles for IRM-47 and GS5-07_100bp regarding the eight high yielding accessions does not enable the conclusion on the probable contribution of their alleles.

From the correlation estimates, yield and number of grains per panicle in this study were positively and significantly correlated. This result is similar to those reported by Ashrafuzzaman *et al.* (2009) who reported significant and positive correlations between yield and its component traits. According to Berner and Hoff (1986), aromatic cultivars have often undesirable agronomic characters, such as low yield, susceptibility to pests and diseases, and strong shedding. Conversely, in this study, positive and significant ($p=0.05$) correlation has been observed between caryopsis scent and grain yield on the one hand, and between caryopsis scent and number of grains per panicle. These results however were not confirmed by the marker data. As suggested by Berner and Hoff (1986), in this study the marker BADH2.1-7 (for aroma) was negatively correlated with IRM-45 (for yield potential) and GS5-07_100bp (for grain width and weight). The non-significance observed in the correlations for all the markers would suggest that they are not linked. Similarly, the negative correlation observed between GS5-07_100bp (for grain width and weight) and IRM-45 (for yield potential) would be explained by the non-linkage between these two markers, and not by

the negative interaction between the two markers. This is more obvious while taking the phenotypic results for which 100-grain weight and the yield were positively correlated.

The combination of phenotypic and molecular data led to the efficient selection of the parents in view of the fact that much of the information on the aroma and yield potential of the accessions are complementary.

At the end of this study, nine accessions were selected as parents. Among them, IR 841_Sot collected from farmers in Sotouboua expressed a very strong aroma. The same was recorded for accession Chapeau vert collected from farmers in Mission Tové. Among all the 13 samples of IR 841 collected, it was only the IR 841_Sot that expressed strong aroma. The same accession gave a higher yield compared to the 12 others. Moreover, accessions such as Sipi, Berice 21, Gambiaca_Dan, Londo londo and Chinoivi gave high yield performance. In addition, Chinoivi was early maturing as WITA 4 and Orylux 5.

5.5. Conclusion

The present study focusing on the identification of accessions that are aromatic and high yielding revealed from the sensory evaluation that 19 accessions out of the 50 were scented. The molecular screening of the germplasm led to the identification of 20 accessions carrying the favourable allele for presence of aroma. After cross checking the two lists, all the 19 accessions identified from sensory evaluation were common to both of the lists, making 95% similarity in the two types of results. The three polymorphic markers used to screen the germplasm for yield potential have also supported the data collected from the field. Even though as much similarity has not been noted for the correlations between the traits on the one hand, and the markers on the other hand, the complementarity of both types of results led to the efficient selection of nine parents among which three were aromatic and six were high yielding.

CHAPTER SIX: GENETIC ANALYSIS OF YIELD AND ITS COMPONENT TRAITS

6.1. Introduction

The needs in rice consumption in Togo have been estimated to be double of the local production. Knowing that resorting to imports to meet this demand is a very risky and unsustainable strategy (GRISP, 2013), actions must be made to reach self-sufficiency in rice production. Among these, the development of lines that meet farmers and consumers expectations is fundamental. From previous research, farmers and consumers prefer aromatic varieties. With the climate changes and the evidence that the best yields are recorded in irrigated and rainfed lowland conditions, developing scented and high yielding rice lines for rainfed and irrigated lowlands conditions would contribute significantly in the increase of the local production and reduce the gap between the supply and the demand.

It is also important in developing such lines, to estimate the genetic parameters (genotype by environment interactions and genetic variance component) which are useful tools for the breeder for making good decisions and progress in the breeding activities. The present study focused on the development of F₁ hybrids from aromatic, and high yielding parents; and the multi-location evaluation of the hybrids and their parents for the estimation of genetic parameters. It aims to: (i) assess in four locations the performance of the hybrids and their parents regarding yield and its component traits, (ii) estimate correlations between these traits, (iii) assess the genotype x environment (GXE) interactions for these traits, and (iv) determine genetic variance components for the same traits.

6.2. Materials and Methods

Two activities were conducted as part of this study. These are: crosses of parents as well as F₁ hybrids and their parents evaluation in pots (at multi-location trials).

6.2.1. Crosses

6.2.1.1. Location

The crosses were made on station in Mission Tové. The station is located at 35 km in the north from Lomé. Water control conditions were available.

6.2.1.2. Parents

Nine accessions were used as parents for the crosses. These were selected for yield (six accessions) and for aroma (three accessions). The six high yielding accessions were used as female parents while the three aromatic accessions were pollen donors (used as male parents) as mentioned in the Table 6.1 hereunder presented.

Table 6.1: Characteristics of the nine accessions used as parents in the crosses

Code	Name	Type of parent	Target traits	Source
N1	<i>Chinoivi</i>	Female	Yield	Farmers
N2	<i>Sipi</i>	Female	Yield	Farmers
N3	<i>Berice 21</i>	Female	Yield	Farmers
N4	<i>Gambiaca</i>	Female	Yield	Farmers
N5	<i>Londo londo</i>	Female	Yield	Farmers
N6	<i>Wita 4</i>	Female	Yield	AfricaRice
A1	<i>IR 841</i>	Male	Aroma	Farmers
A2	<i>Orylux 5</i>	Male	Aroma	AfricaRice
A3	<i>Chapeau vert</i>	Male	Aroma	Farmers

6.2.1.3. Mating design

The set of six female parents was mated to the set of three male parents in a North Carolina Design II (NCII). Therefore, 18 crosses were made (six females x three males) from the nine parents. As described by Nduwumuremyi *et al.* (2013), NCII is a factorial mating scheme, essentially a two-way ANOVA in which the variation is partitioned between females, males and their interactions.

6.2.1.4. Methodology

Crosses involved emasculating the spikelets of the female parents, collecting pollen from the male parents, and putting it in the emasculated spikelet. An emasculator adapted from a vacuum cleaner as described by Lamo *et al.* (2010) was used. Before emasculating, the spikelets were cut in their upper part. Then the emasculator was applied to remove the pollen from the cut spikelets.

The parents were sown in pots in such a way that the flowering dates of the female and the male coincided. On each female panicle, only 15 spikelets were used for the crosses, the rest were removed. Just after the emasculation, the emasculated spikelets were covered with paper envelopes. Emasculation was made early in the morning or late in the afternoon. The pollen was collected in one day time following the opening of the flowers, from 10 am to noon. The pollination method involved shaking male panicles shedding pollen on the emasculated spikelets of the female parent. Once pollination was done, the pollinated spikelets were immediately covered with paper envelop. In case of success, the F₁ hybrid seeds were harvested 30 days after the pollination.

6.2.2. Evaluation of the genotypes

The 18 F₁ hybrid families and their nine parents were evaluated at four locations across the country, corresponding to the four agro-ecological zones.

6.2.2.1. Plant material

A total of 27 entries composed of the 18 progeny families and their nine parents were used as plant material for the evaluation. The list of the names of the 18 F₁ hybrids and their nine parents is presented in the Table 6.2.

Table 6.2: Eighteen F₁ hybrids and their nine parents tested on four locations in Togo

Code	Name	Type of material	Code	Name	Type of material
N1	Chinoivi	Female parent	N1A2	Chinoivi/Orylux 5	F ₁ hybrid
N2	Sipi	Female parent	N2A2	Sipi/Orylux 5	F ₁ hybrid
N3	Berice 21	Female parent	N3A2	Berice 21/Orylux 5	F ₁ hybrid
N4	Gambiaca_Dan	Female parent	N4A2	Gambiaca_Dan/Orylux 5	F ₁ hybrid
N5	Londo londo	Female parent	N5A2	Londo londo/Orylux 5	F ₁ hybrid
N6	Wita 4	Female parent	N6A2	Wita 4/Orylux 5	F ₁ hybrid
A1	IR 841_Sot	Male parent	N1A3	Chinoivi/Chapeau vert	F ₁ hybrid
A2	Orylux 5	Male parent	N2A3	Sipi/Chapeau vert	F ₁ hybrid
A3	Chapeau vert	Male parent	N3A3	Berice 21/Chapeau vert	F ₁ hybrid
N1A1	Chinoivi/IR 841_Sot	F ₁ hybrid	N4A3	Gambiaca_Dan/Chapeau vert	F ₁ hybrid
N2A1	Sipi/IR 841_Sot	F ₁ hybrid	N5A3	Londo londo/Chapeau vert	F ₁ hybrid
N3A1	Berice 21/IR 841_Sot	F ₁ hybrid	N6A3	Wita 4/Chapeau vert	F ₁ hybrid
N4A1	Gambiaca_Dan/IR 841_Sot	F ₁ hybrid			
N5A1	Londo londo/IR 841_Sot	F ₁ hybrid			
N6A1	Wita 4/IR 841_Sot	F ₁ hybrid			

6.2.2.2. Locations

The trials were conducted at four locations (Kara, Sotouboua, Adéta and Mission Tové) across the country. Figure 6.1 presents the names of the sites, their agro-ecological zones as well as geographical region and coordinates.

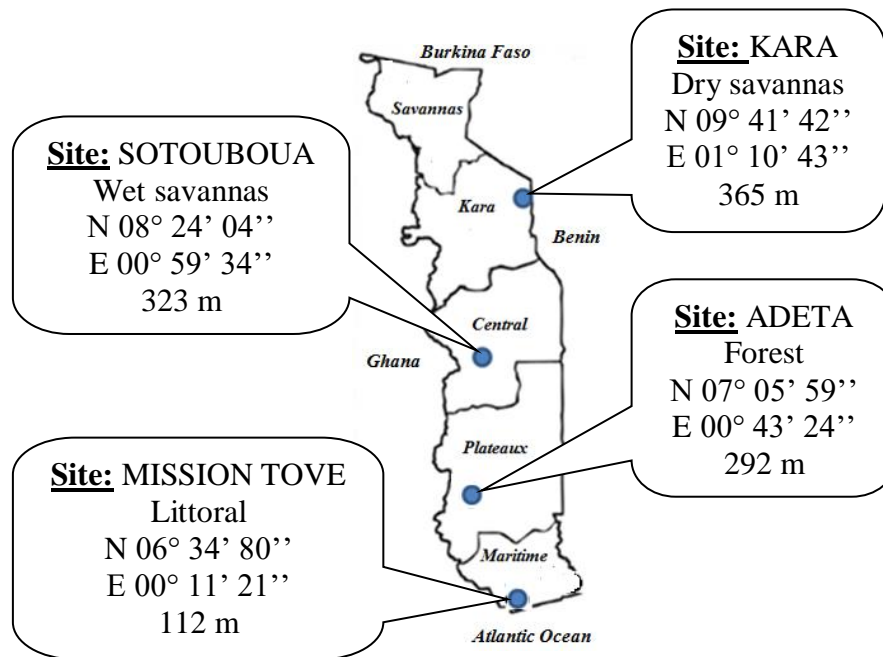


Figure 6.1: Geographical and agro-ecological zones of the four sites of experiment

6.2.2.3. Pot experiments

The seeds of the nine parents and their 18 F₁ hybrids were sown in pots. The pots had a volume of 10 l. They were 20 cm in diameter and height of 30 cm. For each of the 27 entries, five pots (representing replications) were used per site, and arranged following the crosses pattern in a completely randomised design (CRD). Only one grain was sown per pot. Fertilizers N₁₅P₁₅K₁₅ and Urea (46%N) as well as manual weeding were applied as recommended by Wopereis *et al.* (2009). Water supply was essentially rainfall. However, the plants were watered if needed. For each entry, panicles were harvested one by one in paper envelops.

6.2.2.4. Data collected

Yield (Yld) and its component traits: number of panicles per plant (NPP), number of grains per panicle (NGP) and 100-grain weight (GW) were collected following the *Standard evaluation system* (IRRI, 2002).

6.2.3. Data analysis

The significance of the differences in the performance of the F₁ hybrids and their parents was assessed through ANOVA using the software GenStat 9.2. The genotype by environment interaction (GXE) was also estimated using AMMI (Additive Main Effect and Multiplicative Interaction) analysis with the same software.

Correlations among the traits were estimated with the SPSS 17.0. The Spearman correlation coefficient was used with significance levels for $p = 0.05$, $p = 0.01$ and $p = 0.001$.

The Plant breeding tools (PBtools) developed by IRRI was used in the determination of the genetic variance components of the traits and their heritability. The software AGD (Analysis of genetic designs) developed by CIMMYT was used to estimate general and specific combining ability of the parents and the offsprings respectively.

6.3. Results

From the 18 crosses made between the sets of six female and three males, different F₁ hybrids seeds quantities were collected depending on the success rate of the crosses. The data of F₁ hybrids seeds quantity as collected in this study are presented in Appendix 5.1.

6.3.1. Combined analysis of variance for yield and its component traits

The yield and components data collected on the four sites were pooled together for analysis of variance. Table 6.3 presents the main results.

For all the four variables, highly significant differences were found among the genotypes, the sites as well as their interactions.

Table 6.3: Summary of the results of combined ANOVA of the yield and its component traits on four sites in Togo

Trait	Source of variation	Df	F	F pr	Average s.e.d	LSD	CV (%)
Number of panicles/plant (NPP)	Sites	3	129.73	<0.001	0.52	1.022	30.4
	Genotypes	26	10.01	<0.001	1.351	2.655	
	Sites.genotypes	78	5.95	<0.001	2.701	5.31	
Number of grains/panicle (NGP)	Sites	3	66.89	<0.001	1.585	3.116	13.4
	Genotypes	26	23.46	<0.001	4.118	8.094	
	Sites.genotypes	78	9.17	<0.001	8.236	16.189	
100-grain weight (GW)	Sites	3	41.30	<0.001	0.0761	0.1496	23
	Genotypes	26	24.52	<0.001	0.1978	0.3888	
	Sites.genotypes	78	5.56	<0.001	0.3956	0.7776	
Grain yield (Yld)	Sites	3	218.23	<0.001	0.31	0.609	29.2
	Genotypes	26	11.76	<0.001	0.805	1.583	
	Sites.genotypes	78	9.76	<0.001	1.611	3.166	

df = degree of freedom, F pr = probability value of significance, s.e.d = standard error of difference, LSD = least significant difference, CV = coefficient of variation

The lowest coefficient of variation was recorded for NGP (13.4%) whereas NPP gave the highest (30.4%). Table 6.4 presents the ANOVA table for AMMI model for all the four traits.

Table 6.4: ANOVA for AMMI model for the yield and its component traits in four locations

Source	df	NPP		NGP		GW		Yld	
		F	F_prob	F	F_prob	F	F_prob	F	F_prob
Treatments	107	10.80	0.00	14.16	0.00	11.12	0.00	16.30	0.00
Genotypes	26	10.39	0.00	23.29	0.00	24.42	0.00	11.91	0.00
Environments	3	63.88	0.00	75.99	0.00	26.54	0.00	150.46	0.00
Replications	16	2.11	0.01	0.87	0.60	1.55	0.08	1.47	0.11
Interactions	78	6.18	0.00	9.10	0.00	5.53	0.00	9.88	0.00
IPCA 1	28	10.50	0.00	11.46	0.00	9.87	0.00	16.72	0.00
IPCA 2	26	4.67	0.00	8.79	0.00	3.74	0.00	6.51	0.00
Residuals	24	2.78	0.00	6.69	0.00	2.42	0.00	5.56	0.00

F_prob = probability value for significance, IPCA = Interaction principal components, NPP = number of panicles per plant, NGP = number of grains per panicle, GW = 100-grain weight, Yld = grain yield

All the sources of variation were significantly different at $p=0.01$ for all the four traits except for replications in the NPP, NGP and Yield.

6.3.2. Performance of parents and their F₁ Hybrids

6.3.2.1. Number of panicles per plant (NPP)

The performance of the genotypes is presented in Figure 6.2.

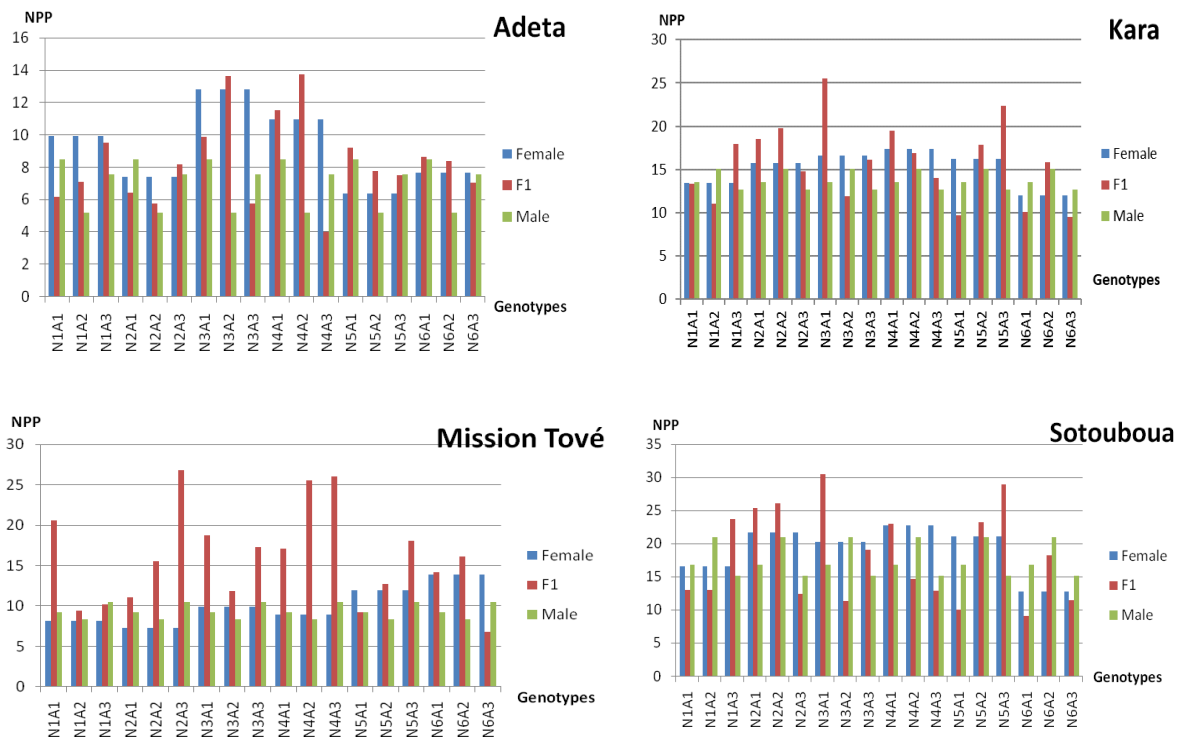


Figure 6.2: Performance of F₁ hybrids and their parents for number of panicles per plant

The most performing genotype across the four sites was N3A2 in Sotouboua with about 30 panicles per plant while the least was N4A3 in Adéta. The best parent across the sites was N3 in Adéta. Some hybrids did not perform as well as their parents.

6.3.2.2. Number of grains per plant (NGP)

Figure 6.3 shows the number of grains per panicle for each genotype per site. The best parent for this trait was A1 in Kara and Sotouboua. Hybrids N2A3 and N3A3 in Mission Tové recorded the highest NGP among the hybrids across the sites.



Figure 6.3: Performance of F₁ hybrids and their parents for number of grains per panicle

6.3.2.3. 100-grain weight (GW)

For 100-grain weight, the parents performed better than most of the hybrids (Figure 6.4). N1 in Adéta and N6 in Kara and Sotouboua were the best genotypes. However, the hybrid N6A3 was the best in Mission Tové.

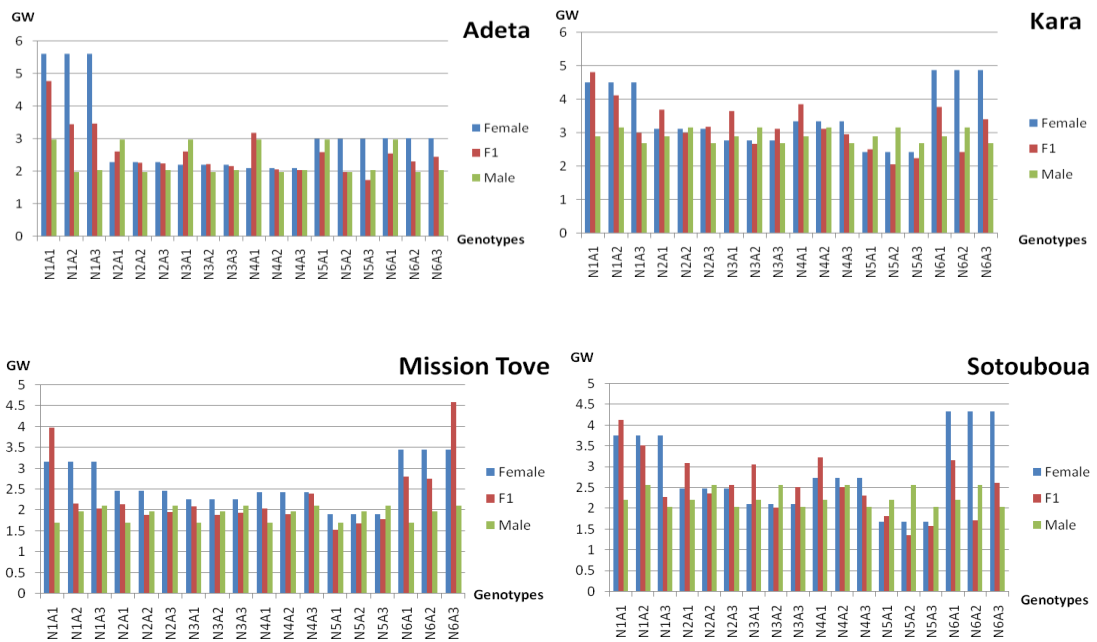


Figure 6.4: Performance of F₁ hybrids and their parents for 100-grain weight

6.3.2.4. Grain yield (Yld)

The hybrids N3A1 in Kara with about 15 t/ha and N4A1 in Sotouboua with about 13t/ha expressed the highest yield across the sites.



Figure 6.5: Performance of F₁ hybrids and their parents for grain yield

6.3.3. Genotype by environment interaction analysis for yield and its components

The interaction between the average performance of the genotypes and the sites was assessed using the AMMI analysis for each variable. All the sources of variation (treatments, replication, genotypes, environment, as well as their interactions) showed significance. Then, the scores of genotypes and the sites were used for the AMMI plot.

6.3.3.1. Number of panicles per plant (NPP)

On the AMMI plot the horizontal axis represents the average performance of the genotypes across the sites while the vertical one explains the GxE interaction. Therefore, the genotypes and sites close to both blue lines are more stable than those away from the two lines. A genotype on the left of the vertical line presents lower performance, whereas the one on the right is higher performing. Similarly, the genotype far away from the horizontal line presents a higher GxE interaction.

Perceived as such, the site Mission Tové showed the greater GXE while the site Adéta was more stable but lower in performance. The higher performing genotype for number of panicles per plant was N5A3 followed by N4A1 which was more stable across the sites. N6A3 had the lowest performance though it was very stable (Figure 6.6).

6.3.3.2. Number of grains per panicle (NGP)

For the number of grains per panicle, the best genotype was N5A2. It showed the highest performance and was very stable across the sites. The greatest GxE was recorded in Mission Tové as shown in Figure 6.7.

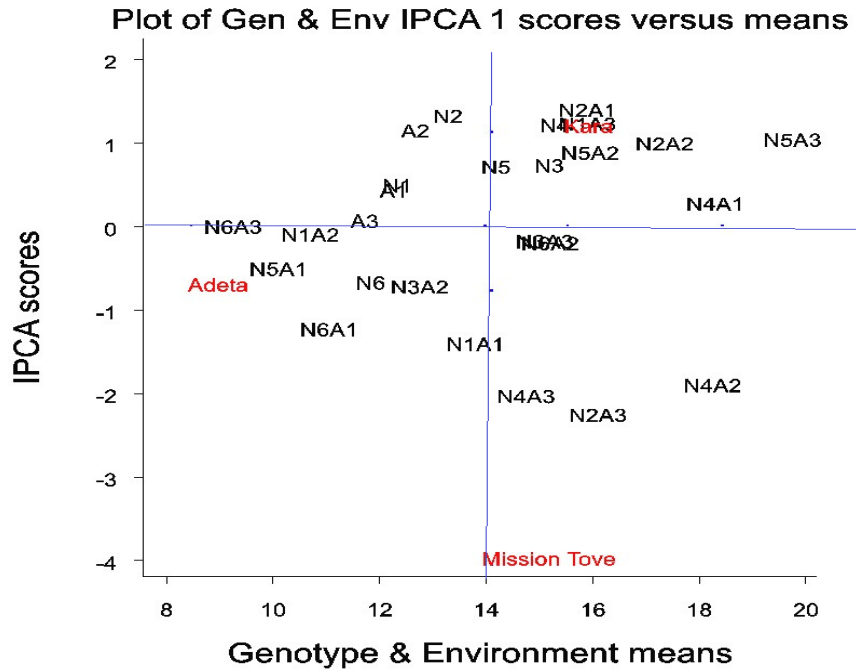


Figure 6.6: AMMI plot showing the GxE interaction for number of panicles per plant

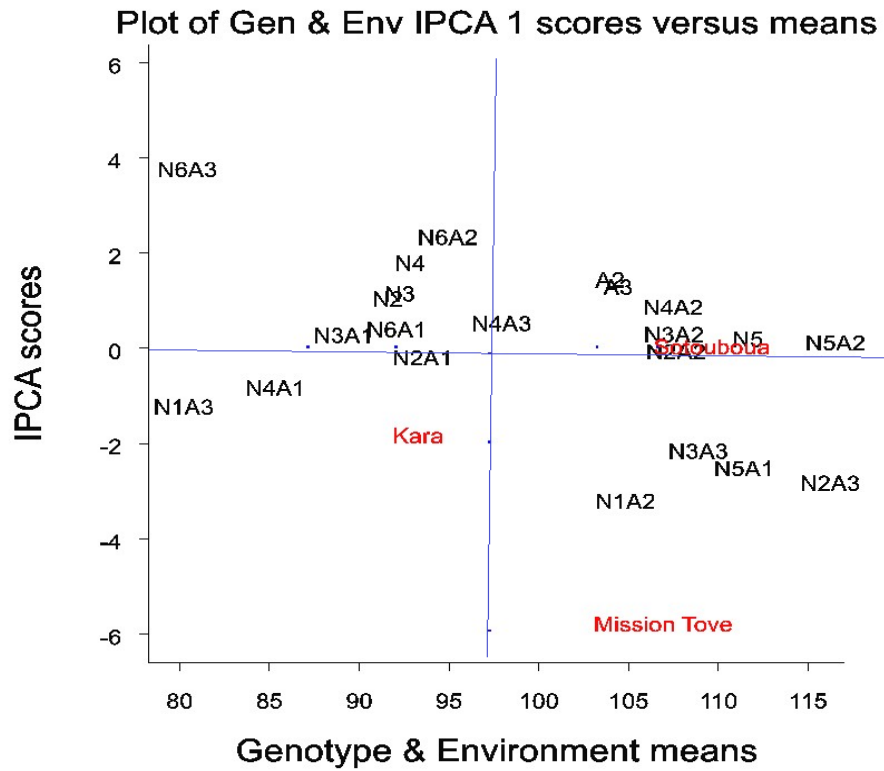


Figure 6.7: AMMI plot showing the GxE interaction for number of grains per panicle

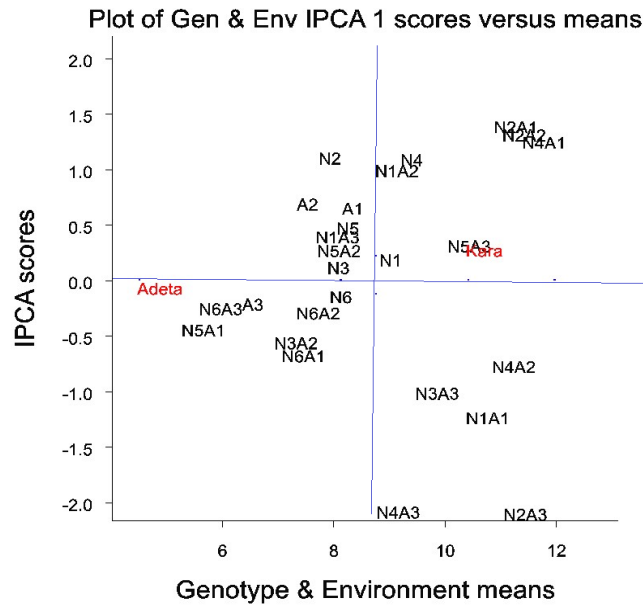


Figure 6.9: AMMI plot showing the GxE interaction for grain yield

6.3.4. Phenotypic correlations

The correlation estimates between yield and its component traits indicated a negative and significant correlation between 100-grain weight and both number of panicles per plant ($p=0.05$) and number of grains per panicle ($p=0.01$). As presented in the Table 6.5, grain yield was significantly and positively correlated to number of panicles per plant ($p=0.01$). Non-significant and negative correlation was detected between yield and 100-grain weight.

Table 6.5: Correlation matrix presenting Pearson coefficient between yield and its component traits collected on F₁ hybrids and their parents

	NGP	GW	Yld
Number of panicles per plant (NPP)	.138	-,424*	,809**
Number of grains per plant (NGP)		-,724**	.039
100-grain weight (GW)			-.015
Grain yield (Yld)			1

6.3.5. Estimate of genetic parameters of yield and its component traits

The ANOVA for NCII design considering all the sites revealed significance for the genotypes and the female parents on all the sites. The male parents were not significantly different for number of panicles per plant and grain yield across the four sites as reported in Table 6.6.

Table 6.6: F probability for yield and its component traits from NCII design ANOVA

Source	Df	NPP	NGP	GW	Yld
Site	3	2.1E ⁻⁴⁵	7.6E ⁻³³	1.2E ⁻¹⁶	6.4E ⁻⁵⁶
Replications	4	0.01	0.31	0.27	0.31
Genotypes	17	9.1E ⁻³¹	6.7E ⁻⁴⁷	1.0E ⁻⁴²	2.1E ⁻²⁹
Male	2	0.91	1.1E ⁻¹⁸	2.2E ⁻¹³	0.00
Female	5	2.3E ⁻¹⁴	2.4E ⁻³⁶	3.4E ⁻³¹	1.8E ⁻²⁰
Male x female	10	9.4E ⁻²⁵	3.1E ⁻¹³	7.3E ⁻¹⁷	5.2E ⁻¹⁶
Site x genotypes	51	4.3E ⁻³¹	1.2E ⁻⁴²	6.1E ⁻²⁶	1.1E ⁻⁴³
Site x male	6	0.00	2.9E ⁻²¹	1.4E ⁻⁰⁹	3.1E ⁻¹³
Site x female	15	1.4E ⁻¹¹	2.6E ⁻²⁵	6.2E ⁻¹⁹	1.6E ⁻¹⁴
Site x male x female	30	7.5E ⁻²⁸	2.1E ⁻²²	2.4E ⁻¹¹	5.8E ⁻³⁷

Df = Degree of freedom, NPP = number of panicles per plant, NGP = Number of grains per panicle, GW = 100-grain weight (g), Yld = grain yield (t/ha)

The General and specific combining ability effects of the nine parents were estimated for the four variables (Tables 6.7 and 6.8 respectively). Over the nine parents, only N5 had a significant general combining ability with a GCA value of 12.24 for number of grains per panicle. All the parents had very low GCA values for the three other traits. Negative values of GCA were reported.

Table 6.7: GCA estimates of the nine parents for yield and its component traits

Genotypes	NPP	NGP	GW	Yld
N1	-0.19	-9.18	0.45	-0.11
N2	0.15	4.35	-0.06	0.99
N3	0.18	1.60	-0.15	0.53
N4	0.22	-1.81	-0.05	0.76
N5	0.01	12.24	-0.39	-0.81
N6	-0.37	-7.20	0.20	-1.35
A1	0.002	-4.36	0.16	0.001
A2	0.0005	3.51	-0.13	0.002
A3	0.006	0.85	-0.03	0.03

Table 6.8: SCA estimates of the 18 F₁ hybrids for yield and its component traits

F1 Hybrids	NPP	NGP	GW	Yld
N1A1	-0.537	-1.37942	0.60347	1.189522
N2A1	0.282573	-0.71201	0.028281	0.574818
N3A1	3.292661	-0.91566	0.008322	3.827512
N4A1	1.365423	-0.88216	0.115547	1.319288
N5A1	-2.35484	0.788805	-0.15211	-3.22354
N6A1	-1.73069	1.375687	-0.11795	-0.88296
N1A2	-1.98016	1.80495	0.198358	-0.44588
N2A2	0.957599	-0.44961	-0.02943	0.723818
N3A2	-1.19976	0.081557	-0.11256	-2.91099
N4A2	1.342146	0.772076	-0.06849	0.774538
N5A2	0.36854	-0.51351	-0.0835	-0.78704
N6A2	0.201278	-0.307	-0.30199	-0.62146
N1A3	0.440622	-1.71309	-0.36587	-1.53638
N2A3	0.37568	1.772543	-0.05677	0.717718
N3A3	-0.10575	1.058844	-0.04515	-0.66524
N4A3	-0.28723	-0.14386	-0.09583	-1.32846
N5A3	2.137575	1.440648	-0.14242	1.522459
N6A3	-2.56866	-2.07878	0.61811	-0.04946

NPP = number of panicles per plant, NGP = Number of grains per panicle, GW = 100-grain weight (g), Yld = grain yield (t/ha)

Globally, the genotypes had non-significant SCA values except, for 100-grain weight where the hybrids N1A1 and N6A3 showed significant values. Ten hybrids had negative SCA for yield. The highest SCA value was expressed by the hybrid N3A1 with 3.827 for yield. Furthermore, genetic variance components were estimated for the traits (Table 6.9).

Table 6.9: Genetic variance components of yield and its component traits

Variable	NPP	NGP	GW	Yld
Male Variance	0.02	31.80	0.05	0.02
Female Variance	0.50	89.57	0.15	1.59
Male x Female Variance	5.42	12.55	0.15	0.01
Genotype Variance	5.86	114.03	0.32	1.26
Additive Variance	23.44	456.13	1.26	5.03
Dominance Variance	21.68	50.21	0.58	0.02
Environmental Variance	6.22	86.80	0.13	3.98
Broad Heritability	0.88	0.85	0.93	0.58
Narrow Heritability	0.46	0.77	0.64	0.56

NPP = number of panicles per plant, NGP = Number of grains per panicle, GW = 100-grain weight (g), Yld = grain yield (t/ha)

Apart from yield, all the other traits were highly heritable with broad sense heritability estimates above 0.85. The trait NGP had a high heritability with the narrow sense heritability estimated at 0.77 whereas NPP had a narrow sense heritability of 0.46 and GW with 0.64. Yield had a moderate heritability with a narrow sense equal to 0.56. Additive variance was moderate in number of panicles per plant, for which dominance variance was also moderate. For yield, relatively high additive variance was recorded, as for NGP and GW.

6.4. Discussion

The multilocal evaluation of the 18 F₁ hybrids and their nine parents showed significant phenotypic variability among the genotypes in their performance for number of panicles per plant, number of grains per panicle, 100-grain weight and grain yield. The highest yield across the sites was recorded for the hybrid N3A1 (13 t/ha). The same hybrid expressed the highest performance in NPP with 30 panicles per plant. However, no particular genotype was the best in each location. Different genotypes performed depending on the site and for each of the four traits studied. For these four traits, the genotypes were significantly different at each location and across the four locations. Roughly, the genotypes performed well in Sotouboua and less in Adéta where the lowest performances were recorded. This result can be explained by the significant GXE interaction revealed from the AMMI analysis.

The GxE interaction contributed in the identification of the site Adéta as the most stable site for yield and number of panicles per plant though it presented the lowest performance among all the sites. This is linked to environmental factors such as rainfall and soil quality, knowing that the rainfall was not good in Adéta. Even though the evaluation was conducted in pots, water supply was essentially rainfall and the soil sample of each site was used. Moreover, Mission Tové was the site where the highest GXE interaction was recorded for NGP and NPP.

From this study, the NC II design ANOVA revealed no significant difference between the male parents for NPP and yield; only the female parents were significantly distinct for all the traits. Therefore, for the GCA estimates, all the male parents had very low GCA (close to zero) for NPP and Yield. Then the parents did not combine well for the two traits. In consequence all the hybrids did not show significant SCA values for NPP and yield. Likewise, no significant SCA values were recorded for the trait NGP. Only the parent N5 had a significant GCA estimate for NGP. Also, the hybrids N1A1 and N6A3 were the only ones that had significant SCA for GW. Generally, very low GCA and SCA values were recorded for the traits. Even more negative values were found, suggesting a negative effect of the combination of two parents on their offspring (for negative GCA), and very low performance than the performance expected for the hybrid (for negative SCA).

The estimation of genetic variance components showed by all the traits were highly heritable, except yield which presented moderate heritability of 0.56. The dominance variance was almost null for yield; therefore, the narrow sense heritability was equal to the broad sense heritability. This makes sense knowing that yield is controlled by many genes with minor effects. For yield, the low GCA observed can be explained by the low additive variance recorded. With the moderate heritability and the low GCA effects expressed by all the parents for yield, reasonable progress can be made through selection of yield in the genotypes used. The same applied to the number of panicles per plant which also showed moderate narrow sense heritability. For number of grains per panicle and 100-grain weight, which showed 0.77 and 0.64 narrow sense heritability respectively, more important progress (compared to yield and number of panicles per plant) can be made using the same genotypes.

Number of grains per panicle was highly heritable (with narrow sense heritability of 0.77). Therefore, the parent N5 which had a significant GCA for that trait can be used in improving

this trait. With the high narrow sense heritability, increase in the genotypes performance for this trait is possible (Acquaah, 2012).

Yield and its component traits in this study were positively correlated except 100-grain weight which was negatively correlated with all the three other traits. Research conducted by Ashrafuzzaman *et al.* (2009), also concluded on the positive correlation between yield and its component traits. However, the negative correlation shown by GW may be due to the presence in the evaluated material of aromatic varieties as reported by Berner and Hoff (1986) to have undesirable agronomic characters such as low yield.

Results from the current study suggest that the grain yield improvement in Togo with the genotypes used should focus on all the yield component traits, with which reasonable progress can be made.

6.5. Conclusion

At the end of this study, the F_1 hybrids presented diverse performance translated by significant differences in their means across the four locations. Most of them had best performance than their parents. The GXE interaction was significant for the genotypes as well as for the sites as regard to the four traits evaluated. Adéta came out as the most stable site for yield and NPP even though the lowest performances of the genotypes were recorded over there. Mission Tové presented the highest GXE interaction. The correlation between the traits was positive except with grain weight. Only the parent N5 had a significant GCA for NGP. Moreover, the entire traits had relatively high additive variance. Then, the progeny families coming from the parent N5 should be advanced. The hybrids N1A1 and N6A3 had significant SCA for GW however no parent or hybrid had significant GCA and SCA for yield. This shows the impossibility to develop high yielding F_1 hybrid cultivars with the 27 genotypes.

CHAPTER SEVEN: GENERAL CONCLUSION AND RECOMMENDATIONS

Rice production in Togo must be doubled to reach self-sufficiency. The NARS is making efforts with the available resources to provide farmers with new technologies. This research contributed in the development of new aromatic and higher yielding lines that is going to meet both farmers and consumers' preferences. It went from the inventory of locally adapted varieties, farmers' preferred traits and production constraints; the collection of accessions, their study for genetic diversity and screening for the selection of parental materials; to the development and evaluation of F₁ hybrids in multi-locations for estimation of genetic parameters.

From the results of the participatory appraisal, contribution to knowledge was made in knowing farmers' preferences for the rice varieties they really want to grow in order to notably improve their income. Farmers in Togo prefer aromatic and high yielding varieties with a medium height, early maturity and long grain. Such information are indispensable in developing varieties for farmers, especially when the breeder expects a higher rate of adoption.

Aside the above findings, this study enabled to report some of the limits of the NARS in Togo. In fact, one of the roles of the NARS is to provide farmers with performing varieties that correspond to their needs. The NARS in Togo plays this role through introduction of varieties from International research institutions, adaptability tests of the varieties, and PVS. This is a quite good procedure knowing that there is no varietal development activity in the country. In addition, none of the varieties introduced by the NARS has been abandoned by farmers, even if they have not been fully adopted. Also, the highest number of cultivated varieties introduced by the NARS has been recorded in the sites close to the research stations.

Having in mind that the only one variety widely cultivated across the country was introduced by the NARS, it is obvious that the NARS impacts positively farmers' varietal choices; even if much has to be done to fully meet farmers demand.

As revealed by the results, the NARS failed to meet farmers demand since the varieties recently introduced have been rejected by farmers compared to IR 841 (adopted by 100% of farmers) introduced in 1980s. It seems that the NARS is not able to replace this variety. With no doubt, this situation is a reasonable consequence of the limits of resorting to introductions to meet farmers varietal needs, instead of developing varieties for them based on their preferences. This failure of the NARS explains the involvement of farmers in introducing varieties by themselves in such a way that the currently cultivated rice varieties in the study are provided fifty-fifty from the NARS and the farmers. This is also the failure of the national quarantine service which even seems to not exist. Another failure of the NARS is noted in its inability to present to farmers 66% of the varieties introduced. Financial reasons could be stated to explain such situation. However, the NARS should rely on the NAES to relay their results to farmers. So, the situation translates a bad functioning of the link between the NARS and the NAES.

Moreover, farmers should no longer replace the NARS in introducing varieties. So, they must be sensitized on the dangers of uncontrolled introductions among which can be noticed the introduction of dangerous pathogens with the seeds. This takes back to the issue of the national quarantine service. This service should be strengthened to enable it control well seeds introductions in the country. Remembering that at the Zio site where more than four varieties have been introduced by farmers, a well-organised quarantine service is required to sensitize farmers and avoid anarchic introductions. As well, such institution will help on Bas Mono site where only IR 841 is grown to keep the site free of anarchic introductions. Finally, the NARS

needs to strengthen its collaboration with the NAES in order to let this institution lend a helping hand in relaying its technologies to farmers.

In view of the above, it is a big challenge in Togo to meet farmers' varietal needs. However, this is a key step in achieving self-sufficiency. For this purpose, the NARS should build a proper rice breeding programme for the development of varieties to farmers. As this study has started, prominence has been given to locally adapted varieties to develop the first generation of aromatic and high yielding rice lines in Togo. The PVS approach should still be implemented to speed up the adoption of the new varieties.

Progress made in this study has been the establishment and the assessment of the genetic diversity in the germplasm of 50 accessions. No duplicate has been identified with both of phenotypic and molecular information, meaning that all the 50 accessions, including the 13 samples of IR 841, were distinct, suggesting contamination or mutation of this variety. This engages the responsibility of the NARS and the seeds technical services in a good control of the registered seeds production. As well, farmers and NAES are also responsible to promote the use of quality registered seeds, knowing that the larger number of rice growers use their own harvest or rice grain bought in the market as seeds. All these actors should be encouraged in the production, control, promotion and use of high quality and pure seeds. Farmers must be trained by the NAES on the importance of the use of registered seeds. More distinct samples of the variety IR 841 would have been collected if the study area was extended. So, this should hail the NARS to investigate the reasons of such contamination of IR 841 and work to purify the variety since it is the most widely grown so far.

All the 50 accessions in the germplasm have specific characteristics that may be considered for the development of new lines, even though they have been grouped in clusters. No specificity regarding the origin and the aromatic or yielding status of the accessions was noticed to be related to the clusters. For their best exploitation, results of phenotypic and

molecular clusters must be used in the choice of the accessions for hybridisation. Similarly, the type and strength of the correlations among these traits could help in the use of indirect selection.

As the focus of this study was to develop high yielding and aromatic lines for farmers, a phenotypic and molecular screening of the 50 accessions was performed to select parents. From this screening the phenotypic and molecular results for detecting aromatic accessions were similar at 95%. This means that the sensory evaluation method used, even though it is subjective is effective. Nineteen accessions were then identified as aromatic material. Eight yielding accessions also were identified using the phenotypic and molecular methods. At the end of this study, nine parents were identified in which three were aromatic and six high yielding. The crosses within these nine parents in a 6x3 NC II mating design have enabled the estimation of the genetic variance components of the 18 offspring and their nine parents. Briefly, only the parent N5 had a significant GCA effects for NGP, and two hybrids N1A1 and N6A3 a significant SCA effects for GW. As far as yield is concerned, no significant GCA nor SCA effects were recorded. This confirmed that no heterotic F₁ hybrids can be developed from crosses of the parents used. The traits evaluated were highly heritable, so the material can be useful in improving these traits. Furthermore, the significance of the GXE interaction suggests that the genotypes should be tested in many locations for the best estimation of the genetic variance components.

At the end of this research, the farmers' preferences and constraints are known, a local germplasm of 50 accessions has been established and its diversity assessed, nine parents have been identified in this germplasm and used in the development of 18 F₁ hybrids. The only one parent N5 that presented significant GCA for NGP will be used for further breeding activities. The three progeny families obtained with this parent, namely N5A1, N5A2 and N5A3 will be advanced using single seed descent approach.

BIBLIOGRAPHY

- Aboa, K., Dantsey-Barry, H., Kpemoua, K. E. (2007) Le riz (*Oryza* spp.). In: Agbobli, C. A., Adomefa, K. and Labare, K. eds. *Situation de référence pour les principales céréales au Togo: Riz, maïs, mil, sorgho*. Lomé, ITRA, pp.39–68 (107p.)
- Aboa, K. (2005) Création et amélioration variétale du riz. In: Agbobli, C. A., and Adomefa, K. eds. *Actes de restitution des acquis de recherches*. Lomé, ITRA, pp.52–57 (147p.)
- Acquaah, G. (2012) *Principles of plant genetics and breeding*. 2nd ed. London, Wiley-Blackwell, 740p.
- Adair, C. R. (1972) Production and utilization of rice. In: Houston, D. E. eds. *Rice chemistry and technology*. United States, Saint Paul MN, pp.1–5.
- AfricaRice (2010) *Participatory Varietal Selection of Rice – The Technician’s Manual*. Cotonou, AfricaRice, 120p.
- AfricaRice (2010) *New breeding directions at AfricaRice: Beyond NERICA*. Cotonou, AfricaRice, 28p.
- AfricaRice (2011) *Boosting Africa’s Rice Sector: A research for development strategy 2011–2020*. Cotonou, AfricaRice, 84p.
- AfricaRice (2011) *Lessons from the rice crisis: policies for food security in Africa*. Cotonou, AfricaRice, 32p.
- Ahmed, S. A., Borua, I., Sarkar, C. R., and Thakur, A. C. (1995) *Volatile componen (2-acetyl-1-pyrroline) in scented rice*. Proceedings of the Seminar on Problems and Prospects of Agricultural Research and Development in North-east India. Assam Agricultural University, Jorhat, India, 27-28 November 1995, pp.55-57.
- Ahn, S. N., Bollich, C. N., and Tanksley, S. D. (1992) RFLP tagging of a gene for aroma in rice. *Genetics*. 84: pp.825 -828.
- Akobundu, I. O., and Agyakwa, C. W. (1989) *Guide des adventices d’Afrique de l’Ouest*. Ibandan, IITA.
- Anonymous (1991). *Mémento de l’agronome*. Paris, Ministère de la coopération et du développement, pp.687-716 (1635 p.)
- Anonymous (2002) *Mémento de l’agronome*. Paris, CIRAD–GRET, pp.799–811 (1687 p.)
- Arrandea, P. (1998) *Le riz irrigué*. Tome 1, Paris, Maisonneuve et Larose, 321p.
- Ashrafuzzaman, M., Islam, M. R., Ismail, M. R., Shahidullah, S. M., and Hanafi, M. M. (2009) *Evaluation of six aromatic rice varieties for yield and yield contributing characters*. *Int. J. Agric. Biol.* 11: pp.616–620.

- Bemer, D. K. and Hoff, B. J. (1986) Inheritance of scent in American long grain rice. *Crop Science* 26: pp.876-878.
- Bioversity International (2007) *Descriptors for wild and cultivated rice (Oryzaspp.)*. Bioversity International, Rome, 72p.
- Bullard, R. W. and Holguin, G. (1977) Volatile components of unprocessed rice (*Oryza sativa*). *Journal of Agriculture and Food Chemistry*. 25: 99p.
- Buttery, R. G., Ling, L. C., Juliano, B. O. and Tumbaugh, J. (1982) Cooked rice aroma and 2-acetyl-1-pyrroline. *Journal of Agriculture and Food Chemistry*. 31: pp.823-826.
- Buttery, R. G., Juliano, B. O. and Ling, L. C. (1983) Identification of rice aroma compound 2-acetyl-1-pyrroline in pandan leaves. *Chemistry and Industry London*, 23: pp.478-479.
- Buttery, R. G., Ling, L. C., and Mon, T.R. (1986) Quantitative analysis of 2-acetyl-1-pyrroline in rice. *Journal of Agriculture and Food Chemistry*. 34: pp.112-114.
- Brar, D. S., and Khush, G. S. (2003) Utilization of wild species of genus *Oryza* in rice improvement. In: Nanda, J. S., and Sharma, S. D. eds. *Monograph on Genus Oryza*. pp.283-309.
- Brydon, L. (1981) Rice, yams and chiefs in Avatime: speculations on the development of social order. *Africa* 51: pp.659-677.
- Carney, J. (2001) African rice in the columbian exchange. *The Journal of African History*, vol. 42, n°3, 2001, pp.377-396.
- Carpenter, A. J. (1977) The history of rice. In: Buddenbagen J. W., and Presley, G. J. eds. *Rice in Africa*. London, Academic press.
- Chang, T. T. (1983) The origins and early cultures of the cereal grains and food legumes. In: Keightley, D. N. ed. *The origins of Chinese civilization*. Berkeley, University of California Press, pp.65 – 94.
- Chen T. H., and Murata N. (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Current Opinion in Plant Biology*. 5: pp.250-257.
- Chopra, R. N. (1933) Indigenous drugs of India. In: Quisumbing, E. Ed. *Medicinal plants in the Philippines*. Quezon, Katha Publishers.
- CNUCED (2004) *Rapport 2004 sur les pays les moins avancés : Commerce international et réduction de la pauvreté*. New York, Nations Unies, 422p.
- Daulfrey S., (1976) Principal component analysis. *Concepts and Techniques in Modern Geography*. 8: p.50.

- Dhulappanavar, C.V. (1976) Inheritance of scent in rice. *Euphytica* 25: pp.659-662.
- Dhulappanavar, C. V., and Mensinkai, S.W. (1969) Inheritance of scent in rice. *Karnataka University Journal*. 14: pp.125-129.
- Dong J. Y., Tsuzuki, E., and Terao, H. (2000) Inheritance of aroma in four rice cultivars (*Oryza sativa* L.). *International Rice Research Notes* 25: p.2.
- DSID (2017) *Evolution des superficies, de la production et des rendements des principales cultures vivrières au Togo*. Direction des statistiques agricoles et de la documentation, Lomé. p. 2.
- Efferson, J. N. (1985) Rice quality in world markets. Conference Title: Rice Grain Quality and Marketing, presented at the International Rice Research Conference. pp.1-13
- FAO (1997) *Elaboration d'un plan national de relance de la filière riz*. Final report, vol. 1 and 2, FAO/Projet TCP/BEN/5613 (A), Cotonou.
- FAO (2004) Production year book. Vol.58. Rome, 4 p.
- FAO (2007) *Perspectives de recolte et situation alimentaire*. Bulletin d'information N° 6 de décembre 2007, SMIAR, 41p.
- FAO (2008) *West African catalogue of plant species and varieties*. Rome, 109p.
- FAO (2011) *Guide pratique pour la gestion intégrée de la production du riz irrigué*. Projet GCP/RAF/453/SPA, Juillet 2017, 10p.
- FAO (2015) *World food and agriculture*. FAO statistical pocket book. Rome. 236p.
- Ghose, R. L. M., and Butany, W. T. (1952) Studies on the inheritance of some characters in rice (*Oryza sativa* L.). *Indian Journal of Genetics and Plant Breeding*. 12: pp.26-30.
- GRISP (2010) *CGIAR Thematic Area 3: Sustainable crop productivity increase for global food security A CGIAR Research Program on Rice-Based Production Systems*. IRRI, AfricaRice, CIAT, 267p.
- GRISP (2013). *Rice almanac Fourth Edition : Source book for one of the most important economic activities on earth*. IRRI, AfricaRice, CIAT. 283 p.
- Herdt, R. W. (1991) Research priorities for rice biotechnology. In: Khush G. S., and Toenniessen G. H. eds. *Rice Biotechnology, Biotechnology in Agriculture*. Wallingford, International Rice Research Institute - CAB International, pp.19-54.
- International Food Policy Research Institute (2002). *Green Revolution: Curse or Blessing?* Washington, IFPRI
- Institut Togolais de recherche agronomique (2011). *Rapport d'activités de recherche, campagne 2009/2010*. ITRA. 124 p.
- IRRI (2002) *Standard evaluation system (SES)*. Laos Banos, IRRI.

- Jacquot, M., Clément, G., Guesquière, A., Glazmann, J. C., Guiderdoni, G., and Tharreau, D. (1997) Les riz. In: Charrier, A., Jacquot, M., Hamon, S., and Nicolas, D. eds. *L'amélioration des plantes tropicales*. Paris. ORSTOM/CIRAD, pp.533-564
- Jin, Q., Vanavichit, S., and Trakoolrung, S. (1996) Identification and potential use of a RAPD marker for aroma in rice. *Journal of Genetics and Breeding*. 50: pp.367-370.
- Johnson, D. E. (1997) Les adventices en riziculture en Afrique de l'Ouest.
- Juliano, B. O. (1994) *Le riz dans la nutrition humaine*. Rome, FAO - IRRI, 180p.
- Kadam, B. S., and Patankar, V. K. (1983) Inheritance of aroma in rice. *Chron. Bot.* 4: 32p.
- Kpémoua, K. E., and Dantey Barry, H. (2003) Gestion intégrée et participative du RYMV et des maladies fongiques du riz en écosystèmes irrigué et de bas fond au Togo. Report. Lomé, ITRA, pp.20–25.
- Lamo J., Tongoona P., Derera J., and Laing M. D. (2010) A household vacuum cleaner for anther removal in rice flowers: University of KwaZulu-Natal design (Short communication). *Crop management and physiology*. 4p.
- Li, B.-J., Lai, L. Z. (1989). *The study on the breeding of « Black superior rice » by using biotechniques*. SABRAO, pp. 289 – 291
- Lin, C. F., Hsieh, R. C. Y and Hoff, B. J. (1990) Identification and quantification of the pop-corn-like aroma in Louisiana aromatic Della rice (*Oryza sativa*). *J. Food Sci.* 35: pp.1466-1467.
- Lu, J. J., and Chang, T. T. (1980) Rice in its temporal and spatial perspective. In: Luh B. S. ed. *Rice: Productio and utilization*. Westport, AVI Publishing Co, pp.1–74.
- Maclean, J. L., Dawe, D. C., Hardy, B., and Hettel, G. P. (2002) *Rice almanac*. Los Baños, IRRI, 253p.
- Mayer, J., and Bonefond, R. (1973) *La riziculture paysanne. Amélioration possible*. Paris, Secrétaire d'Etat aux Affaires Etrangères, 215p.
- Mittal, U. K., Preet, K., Singh, D., Shukla, K. K., and Saini, R. G. (1995) Variability for aroma in some land races and cultivars of scented rice. *Crop Improvement*. 22: pp.109-112.
- Molla, A. M., Ahmed, S. M., and Greenough, W. B. (1985). *Rice-based oral rehydration solution decreases the stool volume in acute diarrhea*. Bull, WHO, p 63; pp. 751–756.
- Morishima, H. (1984) Species relationships and the search for ancestors. In: Tsunoda, S., and Takahashi, N. eds., *Biology of Rice*. Amsterdam, Elsevier.

- National Bureau of Plant Genetic Resources [Project Coordinating and Monitoring Unit, Ministry of Environment and Forests] (2006) *Biology of Rice (Oryza sativa L.) in India*. New Dehli. 63p.
- Nayar, N. M. (2012) Evolution of the African Rice: A Historical and Biological Perspective. *Crop Science*, 52: pp.505–516.
- Ndjiondjop, M. N., Lorieux, M., Sequier J., Fargette D., Rever8at G., Second G., and Ghesquiere A. (1996) Application des marqueurs moléculaires et de la cartographie génétique à l'utilisation du potentiel de l'espèce africaine de riz cultivé (*O. glaberrima* Steud.) en amélioration des plantes. Ln: ADRAO. ed. *Hybridations interspécifiques*. Bouaké, ADRAO, pp.123–139
- Nduwumuremyi, A., Tongoona, P., and Habimana, S. (2013) Mating designs; helpful tool for quantitative plant breeding analysis. *Journal of Plant Breeding and Genetics*. 01 (03), pp.117-129
- Paule, C. M., and Powers, J. J. (1989) Sensory and chemical examination aromatic and non aromatic rices. *Journal of Food Science*. 54: pp.343-346.
- Pinson, S. R. M. (1994) Inheritance of aroma in six cultivars, *Crop Sci*. 34: pp.1151-1157.
- Pisani, D., Benton, M. J., and Wilkinson M. (2007) Congruence of morphological and molecular phylogenies. *Springer*, DOI 10.1007/s10441-007-9015-8. 13p.
- Pocanam, Y. (2005) Gestion des mauvaises herbes dans les rizières au Togo. In: Agbobli, C. A., and Adomefa, K. eds. *Actes de restitution des acquis de recherches*. Lomé, ITRA, pp.58–63 (147 p.)
- Porteres, R. (1955). Historique sur les premiers échantillons d'*Oryza glaberrima* Seudt. recueillis en Afrique, *Journal d'Agriculture Tropicale et de Botanique Appliquée*, Tome II, n°10-11, 1955, pp.535-537.
- Porteres, R. (1956) Taxonomic agrobotanique des riz cultivés *O. sativa* Linne. et *O. glaberrima* Steudel, *Journal d'Agriculture Tropical et de Botanique Appliquée*. 3: pp.341-384.
- Reddy, P. R., and Sathyanarayanaiah, K. (1980) Inheritance of aroma in rice. *Indianational Journal of Genetics and Plant Breeding*. 40: pp.327-329.
- Reddy, V. D., and Reddy, G. M. (1987) Genetic and biochemical basis of scent in rice (*Oryza sativa*). *Theories and Applications in Genetics*. 73: pp.699-700.
- Ranawake, A. L., Amarasingha, U. G. S., and Dahanayake, N. (2013) Agronomic characters of some traditional rice (*Oryza sativa* L.) cultivars in Sri Lanka. *Journal of University Ruhuna*, Vol. 1, N° 1, ISSN 2345-9387, 7p.

- Rivera, E. J., Aligui, G. L., Taaca, A., Juliano, B. O., and Perez, C. M. (1983) The use of «am» (rice water) among cases of acute gastroenteritis in children. *Philippines Journals.*, p.32
- ROPPA (2005) *Pour un développement durable des filières riz en Afrique de l'Ouest.* Campagne riz du ROPPA
- Sié, M., (1991) *Prospection et évaluation génétique des variétés traditionnelles de riz (Oryza sanvaL et O. glaberrima Steud) du Burkina Faso.* Thesis, Abidjan, Faculté des Sciences et Techniques de l'Université Nationale de Côte d'Ivoire, 118p.
- Sié, M. (2007) Un pays où la recherche n'évolue pas, n'avance pas. Sidwaya, n° 5831, 21 february 2007, p.17.
- Sié, M., Dogbé, S., Diatta, M. (2009) *Participatory varietal selection of rice: the Technician's Manual.* Cotonou, AfricaRice, 126 p.
- Sié, M., Moukoumbi, Y., Dogbé, S., Toulou, B., Manful, J., and Afokpé, P. (2010) *Soixante nouvelles variétés de riz de type NERICA pour la riziculture de bas-fond/irriguée: NERICA-L1 à -L60.* Cotonou, AfricaRice. 134 p.
- Sié, M., Dogbé, S., Toulou, B., Manful, J., and Afokpé, P. (2010) *Nouvelles variétés de riz de type Orylux : Orylux 1, Orylux 2, Orylux 3, Orylux 4, Orylux 5, Orylux 6.* Cotonou, AfricaRice, 24p.
- Singh, R.K., Singh, U.S., and Khush, G. S. (2000) *Aromatic rices.* New Delhi, Oxford and IBH publishing Co. Pvt. Ltd., 300p.
- Sood, B. C., and Siddiq, E.A. (1978) A rapid technique for scent determinations in rice. *Indianational Journal of Genetics and Plant Breeding.* 38: pp.2268-2271.
- Sy, A. A. (1991) *Recherche sur la pyriculariose.* Bouaké, ADRAO, pp.31-34.
- Tanchotikul, U., and Hsieh, T. C. Y. (1991) An improved method for quantification of 2-acetyl-1-pyrroline a popcorn-like aroma, in aromatic rice by high-resolution gas chromatography/mass spectrometry/selected ion monitoring'. *Journal of Agriculture and Food Chemistry.* 39: pp.944-947
- Tribelhorn, R.E., O'Deen, L.A., Harper, J.M. and Fellers, D.A. (1986) *Investigation on extrusion for ORT samples.* Boulder, CO, USA, Colorado State University Research Foundation. 70 pp.
- Vaughan, D. A. (1994) *The wild relatives of rice.* Manila, IRRI.

- Ventura, B. (1977) Rice bran utilization in the prevention and treatment of dental decay (caries). In Proceedings. Rice Byproducts Utilization International Conference, Valencia, Instituto de Agroquímica y Tecnología de Alimentos, pp.215-218.
- Vido A. A. (2011) Le riz africain (*Oryza glaberrima* Steudel), un aspect de l'histoire rurale de la Côte de l'Or ou l'extension de la zone de « civilisation du riz » (XVII^e-XVIII^e Siècles). *Revue Ivoirienne d'histoire*, 19; pp.41–60
- WARDA/FAO/SAA (2008) *NERICA: the New Rice for Africa – a Compendium*. Cotonou, WARDA, 240p.
- Wong, H. B. (1981) *Rice water in treatment of infantile gastroenteritis*. *Lancet*, pp.102-103.
- Wopereis, M., Defoer, T., Idinoba, T., Diack, S., and Dugué, M.J. (2009) *Curriculum for participatory learning and action research (PLAR) for irrigated rice management (IRM) in inland valleys of Sub-Saharan Africa. Technician manual*. Cotonou, Africarice, 41p.
- Xian-Zhe Z. And Yu-Bin L. (2009) Rapid identification of rice samples using an electronic nose. *Springer*. Vol. 6-3, pp.290-297.
- Xing, Y., and Zhang, Q. (2010) Genetic and molecular bases of rice yield. *Annual review of plant biology*. DOI: 10.1146/annurev-arplant-042809-112209, 26p.
- Yajima, I., Yanai, T., and Nakamura, M. (1978) Volatile flavour components of cooked rice. *Agriculture and Biology*. Chem. 42: pp.1229-1233.

APPENDICES

Appendix 3.1: Semi-structured guide for focus group discussions on the inventory of varieties introduced by NAES and farmers

Participatory rural appraisal (PRA)

Inventory of rice varieties introduced by the NAES and farmers in Togo

Focus group discussion Guide

A- General information

Date:/...../.....

Location:

Region:

Agro-ecological zone:

Focus group number:

Rice production ecology:

Number of farmers involved:

Research team:

1- DEWA Kassa Messan Koussakana, PhD student

2-

3-

Appendix 3.2: Number of villages and farmers involved in the PRA

Site	Region	Agro-ecological area	Production ecology	Number of farmers			Villages involved	Number of farmers		
				Male	Female	Total		Male	Female	Total
Kpélé	Plateaux	Forest	Rainfed	7	8	15	Bémé	5	5	10
							Tutu	2	3	5
Zio	Maritime	Littoral	Irrigated	9	4	13	Mission Tové	8	1	9
							Kovié	1	1	2
							Assomé	0	2	2
Tone	Savanes	Dry savanna	Irrigated	2	12	14	Tantigou barrage	2	12	14
Oti	Savanes	Dry savanna	Irrigated	10	6	16	Koumbeloti	10	6	16
Dankpen	Kara	Dry savanna	Rainfed	14	0	14	Kouka	5	0	5
							Namab	2	0	2
							Koudjokponkpon	2	0	2
							Takpapebou	1	0	1
							Kroupime	2	0	2
							Nambi	2	0	2
Binah	Kara	Dry savanna	Rainfed	16	0	16	Pagouda	2	0	2
							Tchikawa	5	0	5
							Asséré	3	0	3
							Lama Tessi 1	2	0	2
							Pramhroè	1	0	1
							Pessaré	1	0	1
							Kagnissi	2	0	2
Tchamba	Centrale	Wet savanna	Rainfed	13	4	17	Koutchoni	4	0	4
							N'Tchourou	2	0	2
							Amoutou	3	1	4
							Alibi 1	2	3	5
							Sidaba	2	0	2
Sotouboua	Centrale	Wet savanna	Rainfed	15	0	15	Sotouboua	4	0	4
							Adjengré	2	0	2
							Dalanda	2	0	2
							Yemaboua	1	0	1
							Lama Wéré	1	0	1
							Tinlao	1	0	1
							Fondah	1	0	1
							Tchébébé	1	0	1
							Gnimda	1	0	1
							Baga	1	0	1
Est Mono	Plateaux	Wet savanna	Rainfed	11	3	14	Ananikopé	3	2	5
							Seiboukopé	3	0	3
							Djokpé	3	0	3
							Amédéka	2	1	3
Bas Mono	Maritime	Littoral	Irrigated	8	8	16	Agomé Glozou	8	8	16
10	5	4	2	105	45	150	40	105	45	150

Appendix 3.3: Semi-structured guide for focus group discussions on the reasons of the non adoption of the varieties introduced, farmers' preferences and production constraints

Participatory rural appraisal (PRA)

Assessment of rice production constraints, farmers' preferences and reasons of the failure of adoption of rice varieties introduced in Togo from 1980 to 2013

Focus group discussion Guide

A- General information

Date:/...../.....

Location:

Region:

Agro-ecological zone:

Focus group number:

Rice production ecology:

Number of farmers participants:

Research team:

1- DEWA Kassa Messan Koussakana, PhD student

2-

3-

2- Which performing introduced varieties have been lost or abandoned and why?

N°	Name of the varieties	Status	Why lost or abandoned?	Traits to be improved	Yield performance	Aromatic?

3- Which ones do you want Research to improve and which trait(s)?

N°	Name of the varieties	Trait 1	Trait 2	Trait 3

4- Other reasons of introduced varieties adoption failure

1- Why do you not adopt some introduced varieties?

2- What do you suggest to mitigate these problems?

3- Do you have something else to add?

Appendix 3.4: Questionnaire on farmers' perception on their major preferences and constraints

Participatory rural appraisal (PRA)

Assessment of rice production constraints and farmers' varietal preferences

Individual Questionnaire

C- General information/Informations générales

Date:/...../..... Surveyer name/Nom de l'enquêteur:

Village/Village: Canton/Canton :

Prefecture/Préfecture : Region/Région :

Geographical coordinates/Coordonnées géographiques:

Longitude/Longitude :.....E Latitude/Latitude : N

Altitude/Altitude :m

D- Household Characteristics/Caractéristiques du ménage

Farmer Code number/Code du producteur :

Name of farmer/Nom et prénoms du producteur:

Sex/Sexe : Age/Age:

Major occupation/Fonction principale :

Secondary occupation/Fonction secondaire :

Marital status/Statut matrimonial:

Education level/Niveau d'études :

Number of years of rice production/Nombre d'années d'expérience en matière de production de riz : ans

Number of children/Nombre d'enfants

1. Income sources/Sources de revenus

What are your income sources/Quelles sont vos sources de revenus:

1-)

2-)

3-)

4-)

5-)

How important is rice production income for you/Quel est le pourcentage de contribution des recettes de vente de riz dans tout ton revenu ? :.....%

What is the global amount of your yearly rice production income in/Quel est le montant total de tes revenus annuels de vente de riz en :

2015 ? : FCFA 2014? :FCFA 2013?:FCFA

How much cost 1 ha of rice production?/Combien coûte la production d'un hectare de riz?:

.....FCFA

2- Income expenditure/Utilisation du revenu

For which needs do you spend your income?/Pour quels besoins dépensez-vous votre revenue?

Needs/Besoins	Percentage of income used/Pourcentage du revenu dépensé
Food/Nourritures	
Clothes/Vêtements	
Drink/Boisson	
Farm inputs/Intrants agricoles	
Education/Education	
Health/Santé	
Others/Autres	

E- Cropping systems/Système de culture

1- Crop types and their uses/Types de culture et leurs utilisations

How many farms do you hold ?/Combien de champs de riz avez vous?

Kindly fill the information gap below for each rice farm ?/Veuillez recueillir les informations suivantes pour chaque champ de riz

Farm 1/Champ 1

Type of production ecology/Type d'écologie de production :

1-Irrigated/Irrigué 2-Rainfed lowlands/Bas-fonds 3-Upland/Pluvial strict

Cropping system/Système de culture :

1-Intercropping/Culture intercalaire 2-Associated culture/Culture associée 3-Monoculture

If 1 precise the order of crops/Si 1 précisez l'ordre des cultures

1..... 2..... 3..... 4.....

If 2 associated with which crops/Si 2 associé à quelles cultures?

1..... 2..... 3..... 4.....

Surface area of the rice farm ?/Superficie du champ de riz? ha

Production obtained in/Production obtenues en:

2015 kg 2014kg 2013kg

Farm 2/Champ 2

Type of production ecology/Type d'écologie de production :

1-Irrigated/Irrigué 2-Rainfed lowlands/Bas-fonds 3-Upland/Pluvial strict

Cropping system/Système de culture :

1-Intercropping/Culture intercalaire 2-Associated culture/Culture associée 3-Monoculture

If 1 precise the order of crops/Si 1 précisez l'ordre des cultures

1..... 2..... 3..... 4.....

If 2 associated with which crops/Si 2 associé à quelles cultures?

1..... 2..... 3..... 4.....

Surface area of the rice farm ?/Superficie du champ de riz? ha

Production obtained in/Production obtenues en:

2015 kg 2014kg 2013kg

Farm 3/Champ 3

Type of production ecology/Type d'écologie de production :

1-Irrigated/Irrigué 2-Rainfed lowlands/Bas-fonds 3-Upland/Pluvial strict

Cropping system/Système de culture :

1-Intercropping/Culture intercalaire 2-Associated culture/Culture associée 3-Monoculture

If 1 precise the order of crops/Si 1 précisez l'ordre des cultures

1..... 2..... 3..... 4.....

If 2 associated with which crops/Si 2 associé à quelles cultures?

1..... 2..... 3..... 4.....

Surface area of the rice farm ?/Superficie du champ de riz? ha

Production obtained in/Production obtenues en:

2015 kg 2014kg 2013kg

How do you use your production?/Que faites-vous de vos récoltes de riz?

1-Autoconsumption/Autoconsommation 2-Sellings/Ventes 3- both/les deux

If 3 which proportion of the production is sold ?/Si 3 quelle proportion de la récolte est vendue?(in percentage/en pourcentage)

Other uses of the rice produced (socio-cultural aspect)/Autres utilisation de la récolte du riz (aspect socio-culturel)

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2- Rice varieties grown/Variétés de riz cultivées

N°	Name of the variety Nom de la variété	Source of introduction Source d'introduction	Good traits Bons caractères	Traits to be improved Caractères à améliorer
		Who/Qui? Year/Année Mode/Mode		
		Who/Qui? Year/Année Mode/Mode		
		Who/Qui? Year/Année Mode/Mode		

3- Source of seed and conservation/Source et conservation des semences

N°	Name of the variety Nom de la variété	Source of seeds Source des semences	Seeds uses Utilisation des semences	Seeds conservation Conservation des semences
		Who/Qui? Price/Prix au kg	Per year/Par an ? Kg/ha used/utilise?	Where/Où? How/Comment?
		Who/Qui? Price/Prix au kg	Per year/Par an ? Kg/ha used/utilise?	Where/Où? How/Comment?
		Who/Qui? Price/Prix au kg	Per year/Par an ? Kg/ha used/utilise?	Where/Où? How/Comment?
		Who/Qui? Price/Prix au kg	Per year/Par an ? Kg/ha used/utilise?	Where/Où? How/Comment?

F- Planting and Crop management/Gestion de la culture du riz

In which month do you usually start your rice production activities?/Dans quel mois commencez-vous d'habitude à préparer le sol?

How many labour do you make ?/Combien de labour faites-vous?

With which equipment?/Avec quell outil?

Which type of planting?/Quel type de semis? 1-Semis direct 2-Repiquage

How many weedings do you make?/Combien de désherbages faites-vous du semis à la récolte?

Type of weeding used ?/Types de désherbages utilisés ?.....

1-Manuel 2-Chimique 3-les deux

If 3 how ?/Si 3 à quelle période pour chaque type de désherbage ?

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Do you use fertilizer ?/Utilisez-vous l'engrais ? 1-Yes/Oui 2-No/Non

If 1 which one do you use ?/Si 1 quels types d'engrais utilisez-vous ?.....

1-NPK15-15-15 2-Urea/Urée 3-Both/les deux

Which quantity of fertilizer do you apply per ha/Quelle quantité utilisez-vous par hectare de votre rizière ? NPK15-15-15kg/ha UréeKg/ha

Who is your workforce ?/Qui constituent vos mains d'œuvres ?

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G- Farmers' production constraints/Contraintes de production

N°	Constraints	Level of importance*	Impact/ Consequences	Proposition de solution/ Proposed solution	Qui doit le faire?/ Who must do it?

* evaluated following *Likert* scale of importance: 1- Very important, 2- Important, 3- Not so important and 4- Not important

H- Farmers' varietal preferences

N°	Traits/ caractères	Desired phenotype/ Phénotype désiré	Level of importance*	Reasons/ Raisons

* evaluated following *Likert* scale of importance: 1- Very important, 2- Important, 3- Not so important and 4- Not important

I- Perceptions on climatic change/Perception sur les changements climatiques

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J- Perceptions of farmers on rice aroma/Perception des producteurs sur l'arome

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Appendix 4.1: Quantity of seeds obtained after multiplication of the collected accessions

N°	Name of accession	Quantity (Kg)	N°	Name of accession	Quantity (Kg)
1	<i>Chinoivi</i>	0.96	26	<i>Tcham 4</i>	0.89
2	<i>SIPI</i>	2.00	27	<i>Orylux 3</i>	1.50
3	<i>BERICE 21</i>	2.00	28	<i>IR 841_Sot</i>	1.79
4	<i>Orylux 6</i>	2.50	29	<i>Lobo Lobo_2</i>	2.32
5	<i>ARICA 1</i>	1.00	30	<i>Awini</i>	1.43
6	<i>IR 841_Ton</i>	1.25	31	<i>IR 841 TC_Sot</i>	2.38
7	<i>NERICA L-14</i>	2.27	32	<i>IR 841_EM</i>	2.75
8	<i>IR 841 TH_Oti</i>	2.50	33	<i>Sorad</i>	1.25
9	<i>Chapeau Vert</i>	2.08	34	<i>Kpive-sa</i>	2.86
10	<i>IR 841 TC_Oti</i>	2.08	35	<i>Sorad_2</i>	1.92
11	<i>Mossi</i>	2.73	36	<i>IR 841 TC_EM</i>	1.61
12	<i>Kouka 3</i>	2.32	37	<i>Kpive-sa_2</i>	1.54
13	<i>IR 841 TC_Dan</i>	2.00	38	<i>Gambiaca_EM</i>	2.88
14	<i>Gambiaca_Dan</i>	2.50	39	<i>IR 841_BM</i>	2.68
15	<i>IR 841 TH_Dan</i>	2.38	40	<i>WITA 4</i>	2.14
16	<i>Londo londo</i>	2.50	41	<i>Alandine</i>	2.50
17	<i>Pagou 2</i>	2.68	42	<i>ARICA 2</i>	1.63
18	<i>Pagou 3</i>	1.50	43	<i>ARICA 3</i>	1.43
19	<i>IR 841 TC_Bin</i>	2.14	44	<i>NERICA L-19_Kpe</i>	1.36
20	<i>IR 841_Bin</i>	2.08	45	<i>NERICA L-19</i>	2.68
21	<i>Pagou 8</i>	1.56	46	<i>Orylux 1</i>	2.74
22	<i>Pagou 9</i>	2.50	47	<i>Ibo</i>	2.86
23	<i>IR 841 TC_Tch</i>	2.08	48	<i>Orylux 4</i>	2.08
24	<i>Lobo Lobo</i>	2.31	49	<i>Orylux 5</i>	2.68
25	<i>Mandi</i>	1.34	50	<i>Kouka 5</i>	1.25

Appendix 4.2: Evaluation scale used for the recording data on the 19 qualitative agro-morphological traits

N°	Qualitative trait	Code	Document	Scale							
1	Population uniformity	PU	<i>Descriptor</i>	1 – Homogenous	2 – Heterogenous						
2	Seedling vigor at 21 DAT	Vg 21	<i>SES</i>	1 - Extra vigorous	3 – Vigorous	5 – Normal	7 – Weak	9 - Very weak			
3	Vegetative vigor at 42 DAT	Vg 42	<i>SES</i>	1 - Extra vigorous	3 – Vigorous	5 – Normal	7 – Weak	9 - Very weak			
4	Leaf blade colour	LBC	<i>SES</i>	1 - Light green	2 – Green	3 - Dark Green	4 - Purple tips	5 - Purple	6 - Purple blotch	7 – Purplecale	
5	Leaf blade pubescence	LBP	<i>SES</i>	1 – Glabrous	2 – Intermediate	3 – Pubescent					
6	Collar colour	CC	<i>SES</i>	1 - Light Green	2 – Green	3 – Purple					
7	Ligule shape	LS	<i>SES</i>	1 - Acute to acuminate	2 – Cleft	3 – Truncate					
8	Culm habit	CH	<i>Descriptor</i>	1 - Erect (<15°)	3 - Semi-erect (~20°)	5 - Open (~40°)	7 - Spreading (>60–80°)	9 – Procumbent			
9	Flag leaf attitude	FLA	<i>SES</i>	1 – Erect	3 – Intermediate	5 – Horizontal	7 – Descending				
10	Panicle exertion	Exs	<i>SES</i>	1 - Well exerted	3 - Moderately well exerted		5 - Just exerted	7 - Partly exerted	9 – Enclosed		
11	Culm lodging resistance	CLR	<i>Descriptor</i>	1 - Very weak	3 – Weak	5 - Intermediate	7 – Strong	9 - Very strong			
12	Panicle axis	PnA	<i>SES</i>	1 – Straight	2 – Droppy						
13	Panicle shattering	PS	<i>Descriptor</i>	1 - Very low (<1%)	3 - Low (~3%)	5 - Moderate (~15%)		7 - High (~35%)	9 - Very high (>50%)		
14	Leaf senescence	Sen	<i>SES</i>	1 - Late and slow	5 – Intermediate		9 - Early and fast				
15	Awns presence	An	<i>SES</i>	0 – Absent	1 - Short and partly awned		5 - Short and fully awned		7 - Long and partly	9 - Long and fully	
16	Awn colour	AnC	<i>SES</i>	0 – Awnless	1 - Straw	2 – Gold	3 – Brown	4 – Red	5 – Purple	6 – Black	
17	Caryopsis pericarp color	CPC	<i>Descriptor</i>	1 – White	2 - Light brown	3 - Speckled	4 – Brown	5 – Red	6 - Variable	7 - Purple	
18	Caryopsis scent	CS	<i>Descriptor</i>	0 - Non-scented	1 - slightly scented	2 – Moderately scented		3 – Strongly scented			
19	Phenotypic acceptability	Pacp	<i>SES</i>	1 – Excellent	3 – Good		5 – Fair	7 – Poor	9 – Unacceptable		

Appendix 5.1: Quantity of seeds harvested from the crosses between the six high yielding and three aromatic parents

F1 hybrids	Number of seeds (grains)	F1 hybrids	Number of seeds (grains)
N1A1	53	N4A2	43
N2A1	52	N5A2	21
N3A1	84	N6A2	25
N4a1	100	N1A3	23
N5A1	37	N2A3	78
N6A1	28	N3A3	62
N1A2	39	N4A3	73
N2A2	41	N5A3	34
N3A2	27	N6A3	54