GENETIC DIVERSITY OF *ORYZA* SPECIES IN NIGER; SCREENING AND BREEDING FOR RESISTANCE TO *RICE YELLOW MOTTLE VIRUS* (RYMV)

By

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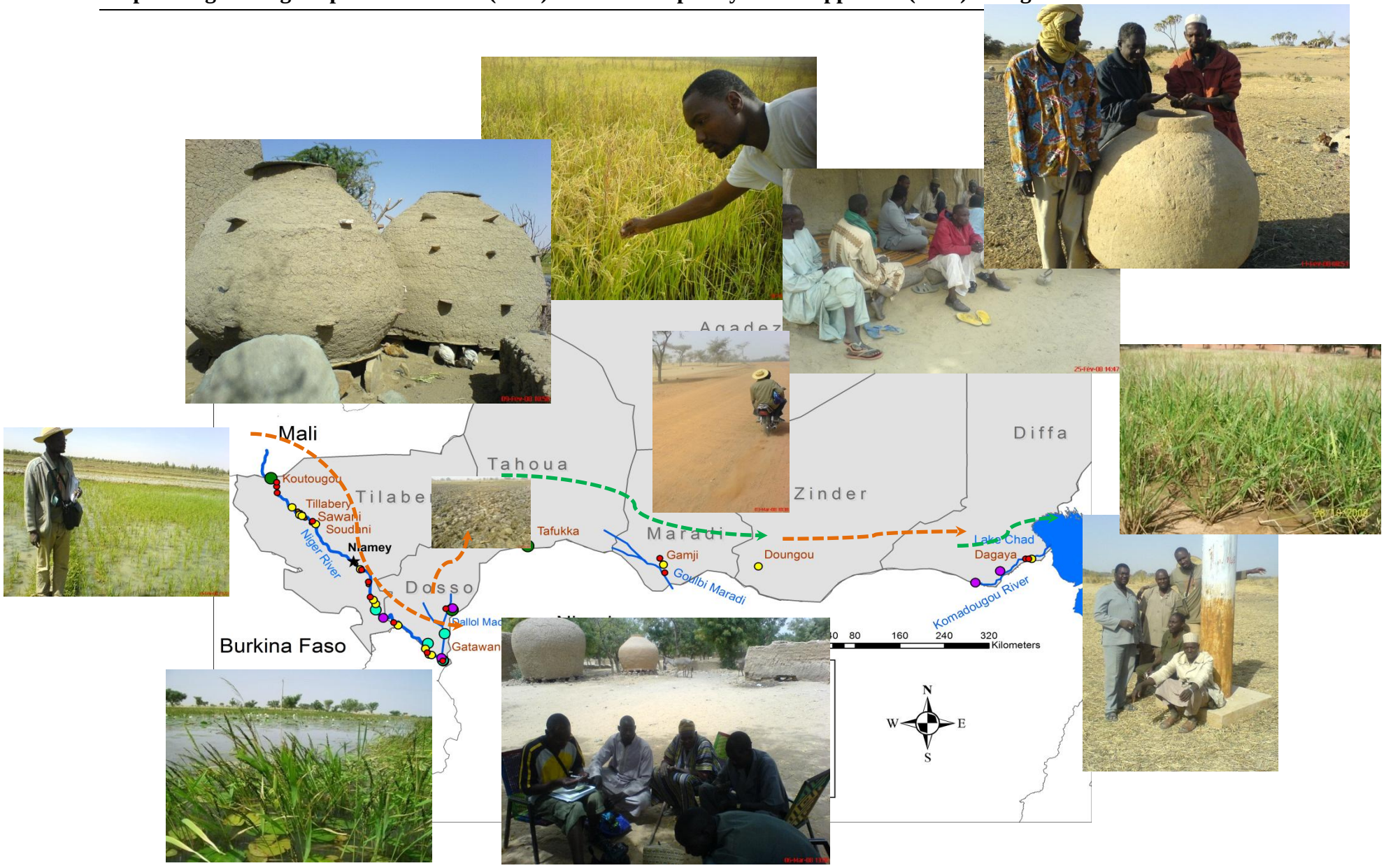
Trip during a rice germplasm collection (2008) and a Participatory Rural Appraisal (2009) in Niger



Thesis Abstract

Rice is a staple food in many West African countries, including Niger. However, both regional and national rice production have failed to meet demand due to several constraints, among which is the *Rice yellow mottle virus* (RYMV). Moreover, attempted intensification of rice cultivation and the introduction of modern cultivars are encouraging farmers towards abandoning local landraces for high yielding, but often susceptible varieties. The study was primarily oriented towards rice pre-breeding, and identifying priorities for rice breeding in Niger in relation to farmers' preferences and their environment. A secondary aim was the development and evaluation (for release at the regional level) of new breeding lines with resistance to RYMV. This study aimed to:

1) Establish farmers' perception of rice varieties as well as the main constraints on rice production in Niger and particularly those posed by RYMV;

2) Create a collection of rice species from Niger for ex- situ conservation, and to determine the phenotypic variability within this collection;

3) Determine the genetic diversity and population structure of the collection;

4) Screen the collection for resistance to RYMV, so that new sources of resistance could be detected;

5) Improve five elite varieties from West Africa for resistance to RYMV using marker-assisted selection (MAS).

The germplasm collection and PRA of this study were conducted in 2008 and 2009 in Niger, while the field and the laboratory researches were conducted in 2008 and 2009 at the Africa Rice Center (AfricaRice) in Benin.

For the PRA, data was obtained from a semi-structured group discussion carried out in 14 villages, individual questioning of 153 farmers and visits to farmers' field and storage facilities. The local farmers' union was the only formal seed dissemination system. Seed exchanges between farmers and the use of seeds from previous harvests were important. The RYMV and the bacterial leaf blight (BLB) were cited as the prevalent biotic stresses in the irrigated agrosystem,

where the varieties IR1529-680-3 and Waihidjo were found to be the most popular. Flood, birds and hippopotamus were the most damaging agents in the lowland cropping system, and the landrace Degaulle/ D5237 was the preferred variety. Apart from the yield, farmers preferred varieties with good grain quality (milling quality and good taste), high market value, stress tolerance (drought, flood, disease, birds, rodents), and those recommended by the local farmers' association. These findings should be included in breeding goals, seed production and dissemination systems.

During collection, a total of 270 rice accessions were assembled, comprising the two cultivated rice species *Oryza sativa* L. and *O. glaberrima* Steud. and its two wild relatives *Oryza barthii* A. Chev. and *O. longistaminata* Chev. et Roehr. The region of the Niger River and its tributary (the Dallol Maouri) provided the majority (80.7%) of the accessions. Apart from a few wild *O. barthii* accessions, the accessions found around Lake Chad and the Komadougou river (South-East) were also collected in the Niger River area. Farmers' naming and ecological classification of rice varieties was generally consistent. Three major phenotypic groups were found during the field trials, and the overall phenotypic variability of the collection (as measured by the Shannon-Weaver Diversity Index) was relatively high. There was no significant difference in diversity between the main eco-geographical zones of collection, as well as between the identified phenotypic groups, suggesting a high level of germplasm exchange between the regions in Niger.

From the collection, 264 accessions were genotyped from the collection using 18 well distributed SSR markers and two main genetic compartments were detected, comprising *O. sativa* subsp. *indica* varieties and *O. glaberrima* and its wild relative *O. barthii* and *O. longistaminata*. The *O. sativa* group in Niger was divided into irrigated and floating rice, bound by lowland rice. The wild progenitor *O. barthii* was widespread but without any clear genetic differentiation from *O. glaberrima*, probably due to the presence of admixtures within the collected samples of *O. barthii*. Allelic diversity was relatively high, despite the geographical distance from the centre of domestication of African rice, and the points of entry of Asian rice to Africa. The findings reflect the underuse of Niger's rice landraces genetic potential for rice breeding, given that all the "improved" varieties released during the last 25 years in Niger were clustered together on the dendrogram.

The response of a set of the rice collected from Niger and some accessions from Mali to inoculation by RYMV was evaluated using five different virus isolates from Niger (3), Benin (1) and Burkina Faso (1). All rice varieties were susceptible to the disease. However, depending on the virus strain, a few *O. glaberrima* accessions displayed partial resistance, similar to the highly resistant TOG5681. Allelic research based on primers derived from the *RYMV1* gene revealed one accession with allele *rymv1-3*, and two accessions with allele *rymv1-4*, and one accession with a different resistance gene. The implications of the finding were discussed and a strategy proposed for breeding varieties with a comprehensive resistance to RYMV.

After three generations of backcrossing, the major resistance gene of the variety Gigante was successfully introgressed into five elite rice varieties of West Africa by Marker-Assisted Backcross (MABC). The newly developed BC_3F_3 progenies were screened for resistance to RYMV in farmers' fields in Guinea and Mali and also under controlled conditions in a screenhouse in Benin. As shown by low virus content and level of disease incidence, low tiller number and plant height reduction, the transferred gene was fully functional in the new genetic background. Moreover, some lines also displayed a high level of resistance to rice blast (*Pyricularia oryzae*) and stem borer infestation in Guinea. Four of those lines are in the second year of multi-location trial in seven West African countries. Therefore, effective deployment of the newly developed varieties, coupled with good cultural practices, should reduce the damaging effects of RYMV in lowland and irrigated rice cropping systems and thereby increase the income of small scale farmers from rice cultivation.

Declaration

I, Mounirou El-Hassimi Sow declare that:

i. The research reported in this thesis, except where otherwise *indica*ted, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain any other persons' data, pictures, graphs or information unless specifically acknowledged as being sourced from other persons.

iv. This thesis does not contain any other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been rewritten but the general information attributed to them has been referenced;

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…… Date……

As the candidate's supervisors, we agree to the submission of this thesis.

…… Date……

Professor Mark Laing (Supervisor)

…… Date……

Dr Marie Noëlle Ndjiondjop (Co-supervisor)

Acknowledgments

Praise to the Almighty Lord that give me the chance to achieve this exercise.

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Dedication

To my Mums Hajia Oumou Diallo, Aminatou Dahi, and Salamata Elhaji Arbonkana, whom we call affectionately "Inna", "Minnay" and "Salay", you have been infinite sources of Love in the shade of this exceptional Dad of mine! May God bestow you a special place in Al Djannatul Firdaous!

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I am so Lucky to have all of you in my life!

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Thesis Introduction

The importance of rice

Rice is a staple food for more than half of the world's population. It is also the fastest growing food crop in developing countries in Sub-Saharan Africa (SSA) due to demographic, professional and social changes. The annual rice demand in Africa is growing at a rate of 6% and regional production fail to meet the demand. Therefore, Africa spent around four billion USD on rice importation in 2009 (Diagne et al., 2010). An increase of 2.5 to 3% per year is projected for global rice prices until 2017 (USDA, 2008). The rice sector in Africa employs nearly 70 million people including farmers, processors and traders. West Africa is the main rice producer and rice consumer of the continent (Fig. 1).

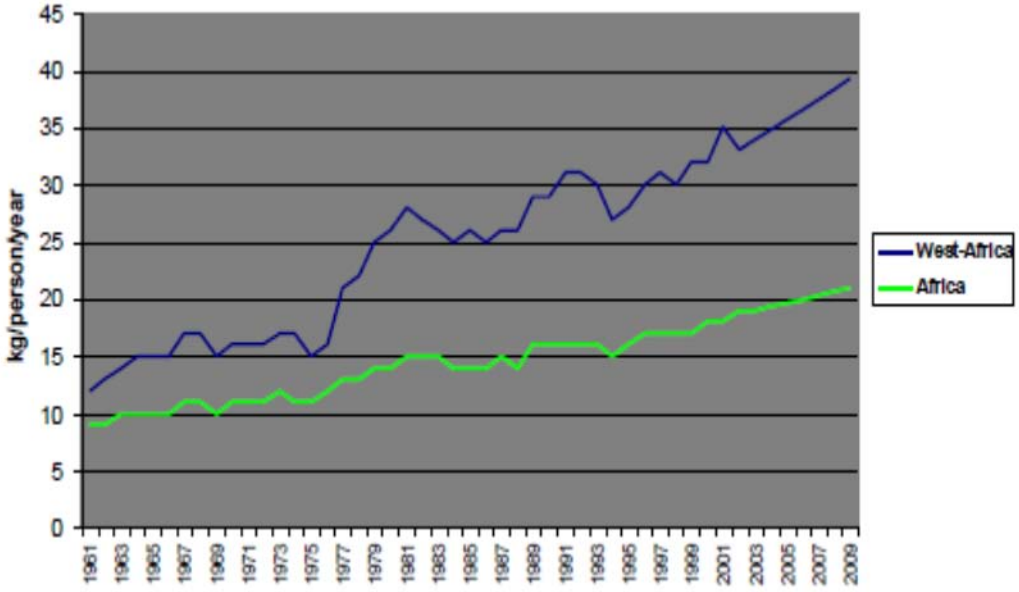

Figure I.1: Evolution of rice consumption in West Africa as compared to the rest of the continent. Source: (Diagne et al., 2010)

In Niger, rice is the third important crop after pearl millet and sorghum, but constitutes the most rapidly increasing food crop, following the continental and global trend. Rice is grown on more than 30,000ha, using two main agrosystems (irrigated and lowland) and production systems (traditional versus modern) (Alfari et al., 2006; Faivre-Dupaigre et al., 2006). The national production was estimated at 132,000 tons in 2009. However, with a per capita consumption of over 20 kg/year; production failed to meet the growing demand, requiring imports of rice, at a

cost of over 35 million USD per year (Sido, 2010). The Niger's production has been stagnant over the last decade. This is due to several constraints including rice policies, and biotic and abiotic stresses, of which the *Rice yellow mottle virus* (RYMV) is the most damaging.

Rice genetic resource conservation

Plant genetic resources are the key component in the improvement of agricultural production. The natural genetic variation of these genetic resources, acquired over centuries of adaptation to local conditions, confers on plants the ability to survive in difficult ecological conditions (Malik and Singh, 2006). Of the 23 rice species in the world, only two species, *Oryza sativa* L. and *O. glaberrima* STEUD., are cultivated (Vaughan, 1994). Several global efforts have been conducted to collect African rice genetic resources for ex- situ conservation in the genebanks of the Africa Rice Center (AfricaRice), the International Rice Research Institute (IRRI) and the Institut de la Recherche pour le Developpement (IRD) (Bezançon et al., 1977; Second et al., 1977; Chang, 1984). Nevertheless, several cases of genetic erosion of rice genetic resources have been reported mainly due to natural disasters (drought, flood, typhoons, etc) or agricultural intensification leading to the replacement of landraces by modern varieties (Akimoto et al., 1999; Morin et al., 2002; Teklu and Hammer, 2006). Crucially, there is no record of a formal collection of rice genetic resources collection from Niger, nor is there any germplasm from Niger in the AfricaRice genebank, despite the fact that the species *O. glaberrima* was domesticated along the Niger River and is endemic to West Africa (Vaughan, 1994)

Rice yellow mottle virus

The virus was first reported in Kenya, but within the last 20 years, it has been reported in all rice growing regions of Africa and Madagascar (Bakker, 1970; Kouassi et al., 2005). The appearance of the disease in the Sahel coincides with the intensification of rice cultivation in the region and the introduction of high yielding Asian cultivars of which varieties IR1529 and BG90-2 are grown in Niger. Both are now commonly used as checks for susceptibility to RYMV in routine screening for resistance to the virus (WARDA, 2001; Basso et al., 2010). In Niger, yield losses up to 68% have been reported (Reckhaus and Adamou, 1989) and the Niger's rice production is increasing slowly despite major investments due to the convergence of several factors, of which RYMV is a major one (Faivre-Dupaigre et al., 2006).

Molecular breeding

Unlike conventional breeding (which relies on phenotypic variation between plants for selection), molecular breeding relies on molecular markers (Semagn et al., 2006). This process, known as Marker-Assisted Selection (MAS), takes advantage of the absence of environmental effect on the natural variation observed in the DNA. Thus, selection can be conducted at early stages of the plants' development and conducted accurately on the gene of interest, using Polymerase Chain Reaction (PCR) and genome sequencing (Farooq and Azam, 2002). Several major genes' resistance to RYMV were discovered and mapped on the rice genome (Ndjiondjop, 1999; Albar et al., 2003; Thiémélé et al., 2010). Thus, closely linked molecular markers are available for MAS for RYMV resistance to improve local popular, but susceptible rice varieties.

Farmers' preferences

Although yield and resistance to the factors decreasing yield are the main concerns of most plant breeders, several high yielding crop varieties developed by researchers have failed to be adopted by farmers (Witcombe et al., 2003). Participatory Rural Appraisals (PRA) were used in several countries or regions to assess farmers' preferences prior to starting a breeding program. Thereafter, a Participatory Varietal Selection (PVS) can be used to ensure that improved varieties actually meet the farmers' preferences (Sthapit et al., 1996).

Synopsis and Research objectives

Resistance to RYMV has become a priority for rice breeders at the AfricaRice due to its devastating effects on rice in Africa. USAID under its project "End Hunger in Africa" funded AfricaRice to introgress a high resistance gene against RYMV into elite rice varieties of four West African countries (Burkina Faso, Gambia, Guinea and Mali), using MAS. The overall strategy was to tackle the disease, firstly on a short and medium-term through the use of genetic and genomic tools developed on rice; and secondly in the long term through the use of genetic diversity and development of lines with comprehensive quantitative resistance. This study formed part of this project.

The overall goal of the research was to limit the disastrous effects of RYMV in Niger and West African lowland rice agrosystems, through the use of genetic diversity and breeding for RYMV resistance using MAS. A further purpose was to document the state of rice diversity in Niger, so that an appropriate conservation plan could be implemented.

The specific objectives of the research were to:

i. Generate formal data on farmers' perception of rice varieties, main constraints on rice production and the status of RYMV in the main rice growing areas of Niger;
ii. Make the first extensive collection of rice landraces in Niger, representing the genetic variability of rice species and landraces in the country;
iii. Study the extent, partitioning and genetic structure of the collection;
iv. Establish the phenotypic variability, and genetic value of the collection for future breeding programs;
v. Screen the collection for resistance to RYMV, looking for new sources of resistance;
vi. Convert some elite West African rice cultivars for resistance to RYMV using MAS.

Thesis plan

The following organization has been chosen for the thesis:

Thesis Introduction

Chapter One: Literature review

Chapter Two: Farmers' perception of rice varieties, major constraints to rice production and RYMV in the region of Tillabéry, West Niger

Chapter Three: Collection and agro-morphological variability of rice species from Niger

Chapter Four: Genetic diversity and population structure of *Oryza* species from Niger

Chapter Five: Screening a rice collection from Niger for resistance to RYMV

Chapter Six: Efficiency of MABC to improve the resistance of five West African elite rice cultivars to RYMV

Thesis overview

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Chapter 1: Literature review

1.1 Rice in the world and in Africa

Rice is the staple food of more than half of the world's population (Zeigler and Barclay, 2008). However, the world's rice production is largely unequally partitioned, with Asia producing more than 90% of the global production, of which China and India are leaders. Africa is far behind (Figure 1.1A). Indeed, rice supplies nearly 75% of the calorific uptake of Asians, 21% of global per capita energy and 15% of per capita protein (MacLean et al., 2002; Khush, 2005). Despite being far behind Asia, yearly rice production in Africa has increased to 3.81% from 1961 to 2006, while yearly rice consumption has increased by 4.25% during the same period (WARDA, 2008a). This situation, coupled with the rapid increase of Africa's population and food preferences changing from other coarse cereals to rice led to a 5.84% increase of rice consumption in the area. Thus, annually one third of global rice on the international market (40% of Africa rice demand), is being imported into Africa (Figure 1.1B) at a cost of US$4 billion for a region where more than 40% of the population lives on less than $1 a day (AfricaRice, 2009; Mohapatra, 2010). The increase in rice production in Africa is mainly due to augmentation of rice cropping areas, rather than yield improvement, the progression of which is very low (Figure. 1.3A). The rate of increase fastest around 1985; corresponding to a period when most West African countries invested in the development of huge irrigated rice production schemes. In Africa, rice production and demand are also disproportional between countries and regions. Egypt, Madagascar, Nigeria, and Cote d'Ivoire are African leaders in rice production, while West Africa is responsible for 65% of the continent's production and East Africa 30%. Southern and Western Africa are the biggest rice consumers, with annual demands growing, to 11.58% and 6.55%, respectively, between 2001-2005 (WARDA, 2008a).

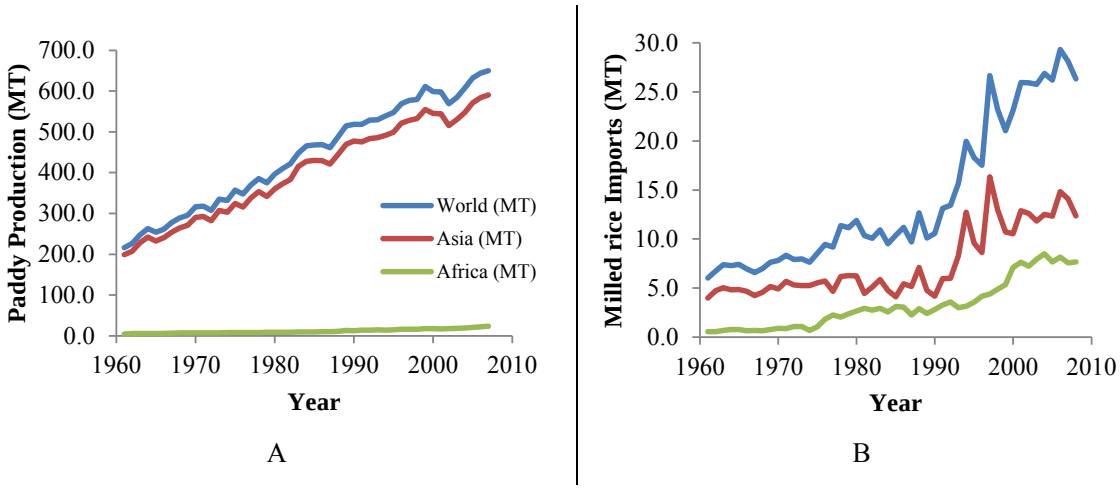


Figure 1.1: Evolution of paddy rice production (A) and milled rice imports (B) in the world, in Asia and in Africa. Source: (IRRI, 2009)

1.2 Rice in West Africa

West African rice production areas have grown beyond the general expansion of rice production on the continent, but still fail to fill the gap between production and demand in the region. Thus, West Africa relies on importations for 42% of its rice demand. The major rice importers of West Africa are Nigeria, Senegal and Cote d'Ivoire. The rising demand for rice consumption in West Africa can be attributed to changes in people's food preferences due to increased urbanization, coupled with changes in the incomes of urban dwellers (MacLean et al., 2002). However, increased rice consumption in the area is not evenly shared. Rice consumption rate increased by 46.87% in Benin, 14.03% in Mali, 7.46% in Cote d'Ivoire between 2001 and 2005. However, rice consumption reduced in other countries such as the Gambia, Burkina Faso and Niger (WARDA, 2008a). But this decrease in rice consumption can be attributed more to lack of capacity to provide enough rice, rather than real desire to reduce the rice consumption.

1.3 Geography of Niger

Niger is a landlocked country of 1,267,000 km^2. It is bordered to the north by Algeria and Libya, the east by Chad, the west by Mali and Burkina Faso, and south by Benin and Nigeria (Figure 1.2) Depending on the annual amount of rainfall, four main climatic zones, oriented from south to north, are found in Niger:

- The Sahelian- sudanean zone, with rainfall ranging from 600 to more than 800 mm representing 1% of the territory. This zone covers part of the Niger River in the region of Dosso and the Dallol Maouri watercourse;

- The Sahelian zone with 350 to 600 mm rain per year, which covers 10% of the country's surface;

- The Sahelian-Saharan zone with 150 to 350 mm rain per year, which represents 12% of the country;

- The Saharan zone, with less than 150 mm of rain per year, which covers 77% of the country surface.

Thus, agriculture is practicable only on a maximum of 23% of the country's lands, depending on the yearly rainfall, of which only 11% is really suited to cropping.

The average day temperature ranges from 31°C to 41°C but, temperatures fall below 20°C at night.

The Sahelian-saharan zone is primarily dedicated to raising livestock. In 2008, the population of camels, cattle, goats, sheep and horses were estimated at 33,443,169 million heads in Niger (FAO, 2010). Agriculture accounts for 41% of the gross domestic product (GDP), 44% of the exports and employs 90% of the labour force, of which 83.8% live in rural area. The agricultural production is dominated by pearl millet, sorghum and rice. The country is the foremost producer of millet and sorghum in West Africa (Sido, 2010).

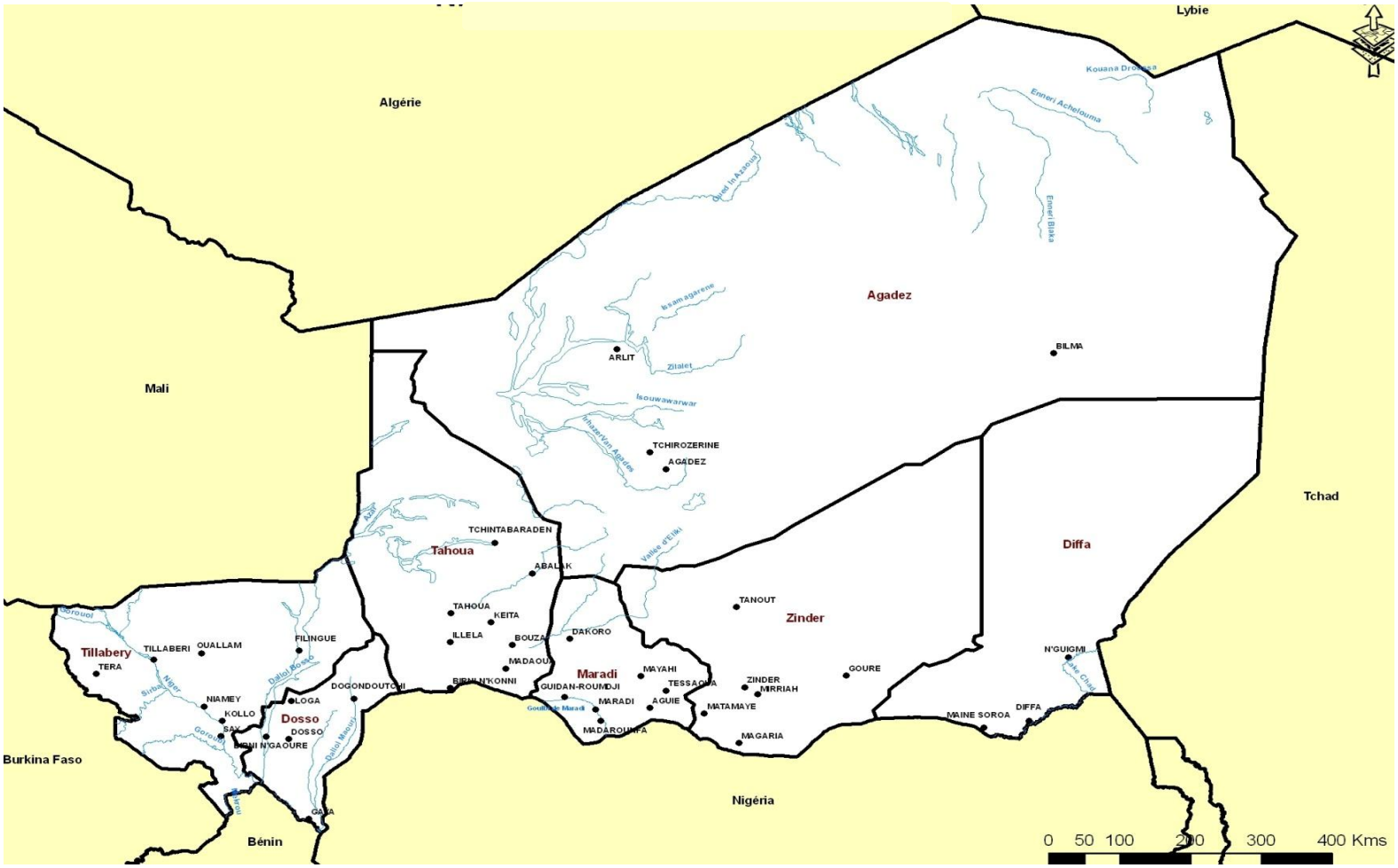


Figure 1.2: Administrative region, Main and seasonal watercourses of Niger (source: National Institute of Statistics of Niger, 2009). The watercourses represented in the North of the country are fossils or underground due to desertification

1.4 Production and evolution of rice in Niger

In Niger, rice is mainly produced along the Niger River in the south-west part of the country in the region of Tillabéry, Dosso and Niamey, as well as along the Komadougou River (south-east) in the Diffa region. It is only rarely produced in the Maradi, Tahoua and Zinder regions (central-south). Three main rice agrosystems are found in Niger (Bonkoula and Miezan, 1982; Sido, 2010):

- Rainfed lowland rice with free or semi- controlled submersion, on the flooding basin of the Niger and Komadougou rivers, as well as on the seasonal watercourses and marshlands (Dallols, Goulbis, etc.) in central-south regions;

- Deepwater to floating agrosystems, in the rivers (Niger and Komadougou), with water levels higher than 1.5 m;

- Irrigated rice in huge irrigated schemes, with full water control and irrigation channels, under the control of local farmers' unions and the "Office National des Aménagements Hydro-Agricoles" (ONAHA), there are few small private irrigated areas using water-pumps.

Attempts are being made to introduce rainfed upland rice production into the region of Dosso and Maradi (Sido, 2010). The production, as well as the yield, depends on the system utilized by the farmer. The first two systems are cropped in the traditional way, while the last is modernized with mechanization of land preparation, improved seed utilisation, and the use of fertilizers, pesticides and herbicide applications. The traditional system, covering 29,013.6 ha, is cropped once a year during the rainy season with yields around 0.7 to 1.3 ton per ha, whereas the 41 irrigated schemes under the control of ONAHA cover 8,706.7 ha. Two croppings are conducted per year on 6,500 ha, with yields between 4 and 5 tons per ha. Private irrigated areas, currently representing 1,500 ha, yield up to 3 tons per ha (Alfari et al., 2006; Sido, 2010).

Since 1991, the national rice production figure has been estimated to range between 55,000 and 70,000 tons per year, but this has taken into account only production on irrigated lands. Thus, very little change in the cultivated area and the yield were reported the last decade (Figure 1.3B). A dropping-off of rice production during 1999-2000 can be attributed to a major outbreak of RYMV, together with the lack of fertilizers and chemicals produced by an international embargo placed on Niger (due to a military coup) during this period. An increase of the national

production in 2009 can be attributed to a slight increase of the irrigated area and the distribution of fertilizers and pesticides under a project sponsored by the World Bank.

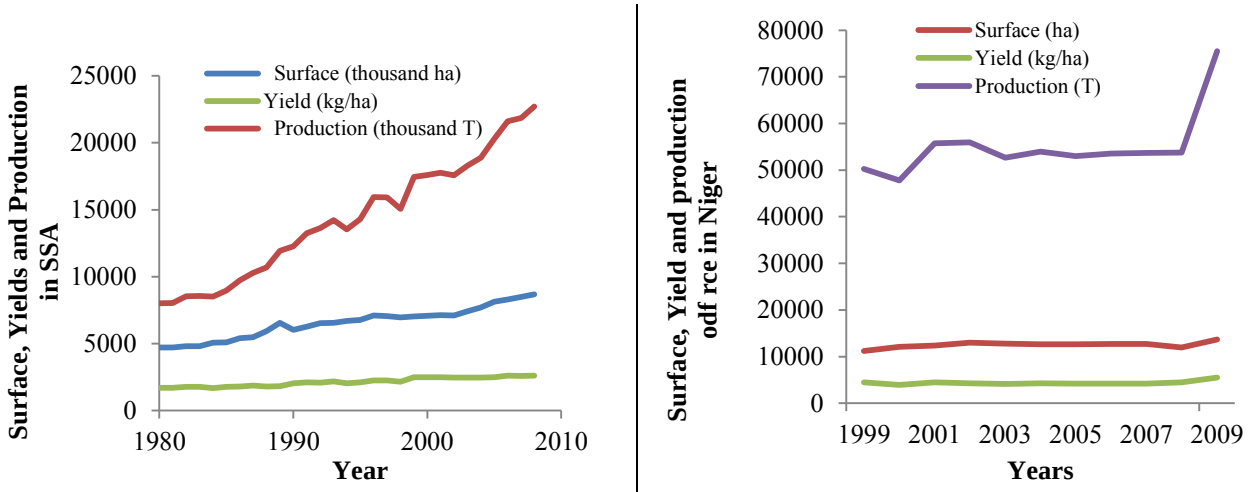


Figure 1.3: Evolution of paddy rice production, rice growing surfaces and yield in Africa (A) (IRRI, 2009) and in Niger (B) (Sido, 2010)

Recently, a study reported that the traditional rice growing system is producing around 62,030 tons per year along the Niger River (Alfari et al., 2006), thus the new figure of national rice production could be considered as 132,030 t in 2009. However, this production is far from filling the gap between the national production and demand, which has quadrupled between 1999 and 2005 reaching 71399862.1 USD, as shown in Table 1.1, (Faivre-Dupaigre et al., 2006). In addition, during the same period, rice constituted only 1.7% of the primary agricultural sector and 2.5% of the cereal production, and accounted for an amount between one third and half of the national balance of trade. Thus, demand for rice is growing rapidly in the Niger, both in towns and rural areas, where at times, millet and sorghum are not available (drought years) (Sido, 2010).The per capita consumption of rice in Niger ranged from 2.4 kg to 52.7 kg depending of the income of the households, with an average of more than 20 kg (Faivre-Dupaigre et al., 2006).

Table 1.1: Changes in levels of rice importations in value (USD) and weight as national imports into Niger between 1995 and 2005

Period	Cost (USD)	Share in total national imports
1995	14,845,074	-
1996	20,466,506	6.9
1997	35,717,858	10.4
1998	40,820,238	9.6
1999	29,877,347.4	7.7
2000	34,395,850.2	8.6
2001	57,987,839.8	12.2
2002	66,325,569.6	12.1
2003	40,405,410.7	7
2004	45,894,653.2	6.8
2005	71,399,862.1	-

Source: Data adopted from Customs Service adapted (Sido, 2010)

1.5 Constraints on rice production

1.5.1 The unexploited potential

Several inconsistencies exist about information relating to the potentially irrigable land in Niger. Three estimates have been provided: 219,000 ha, 270,000 ha and 400,000 (Faivre-Dupaigre et al., 2006), but 270,000 ha is the figure generally accepted, of which 150,000 ha lies along the Niger River, 38,000 ha along marshes and lakes in the south-central regions of the country, 20,000 ha around the lake Chad and the Komadougou, and the remaining in marginal zones of the northern region (Bonkoula and Miezan, 1982; Sido, 2010). However, only 13,500 ha are fully exploited, while the rest is either cultivated traditionally, or not exploited at all. Moreover, Niger is the only country among the countries crossed by the Niger River, which does not have a dam built across it. Thus, controlling water is also a challenge in improving the national rice production. In addition to the control of water, agricultural mechanization, poor management and maintenance of existing infrastructure, the absence of a strong formal seed system and fertilizer supply system,

and several inappropriate agricultural policies all provide restraints limiting rice production in Niger (Sido, 2010).

1.5.2 Abiotic stresses

Apart from some pockets of salinity, and iron toxicity in the area of the Dallol Maouri in the region of Dosso, along the Komadougou river in the region of Diffa, and (rarely) in some irrigated schemes of the Niger River, drought and flood constitute the main abiotic stress in the traditional lowland agrosystem in Niger. The soil conditions have been studied in Niger and à large variability has been found. Along the Niger River soils are pseudo-gley developed on river sediments (Bonkoula and Miezan, 1982). Due to the lack of an important permanent water courses in the area, the central- south zone is characterized by medium to low-fertility soils in the regions of Maradi and Tahoua, with water pH ranging from moderately acid to neutral, while soils are acid in the region of Zinder. On the other hand, soils in the region of Diffa (Lake Chad and Komadougou River areas) are basic on the irrigated scheme, but acidic or neutral in traditional farmers' fields (Masunaga, 1994).

1.5.3. Biotic stresses

Pests

During the rainy season several insect pests of rice can be found. The African rice stem borer (*Chilo zacconius* B., Lepidoptera, Pyralidae) is the most frequent rice pest in Niger, while the whitefly *Aleurocybotus indicus* D. and S. (Homoptera, Aleyrodidae) and the beetle *Trichispa sericea* G. (Chrysomelidea, Hispiniae) are the most damaging. Finally, incidences of *Nymphula sp* (Leptoceridae Neoprla) have also been reported. There is no major pest occurrence in rice crops grown during the dry season cropping (Sido, 2010).

Fungal diseases

There is no major incidence of fungal disease on rice in Niger. Minor incidences of brown spots due to *Gerlachia orysae* (G. and M.) have been reported at levels ranging from 10 to 35% (Sido, 2010). Traces of leaf blast due to *Magnaporthe grisea* have been noticed in some irrigated schemes, but the incidence remains insignificant, possibly due to high temperatures and low relative humidity.

Bacterial diseases

Bacterial leaf blight (BLB) due to *Xanthomonas oryzae pv. oryzae* Ishiyama (Anonymous 1990) is the second most important disease inflicting irrigated rice in Niger. The disease has been reported on 21 irrigated rice schemes along the Niger (south-west) and the Komadougou (south-east) rivers, with its prevalence reaching 70% in farmers' fields (Séré et al., 2005b). The genetic diversity of a collection of 48 isolates of BLB from Niger was determined revealing that only 45.8% were pure *Xanthomonas oryzae* pv. *oryzae*. There was co-infection with *Xanthomonas oryzae* pv. *oryzicola* Fang et al., *Pseudomonas fuscovaginae* Miyajima et al. or *Pseudomonas syringae* pv. *syringae* Van Hal. At times, combinations of three pathogens have been detected (Onasanya et al., 2010). Yield losses range from 15 to 25% (Sido, 2010).

1.6 Viral disease: *Rice yellow mottle virus* (RYMV)

The RYMV is the only viral disease of rice reported in Niger, but it is the most important and damaging rice disease in the country (Basso et al., 2010). This *Sobemovirus* was first reported in Kenya (Bakker, 1970) and has progressively conquered the entire African continent and Madagascar. The virus is a T = 3 icosahedral particle of 25-28 nm in diameter, formed by 180 assembled subunits of 26-kDa coat protein. The particle, composed of approximately 20% RNA, 80% protein and containing no lipids or carbohydrates, is stabilized by divalent cations (Ca2+), pH, protein-protein interaction, salt bridges between proteins and RNA (Opalka et al., 2000). The genome of RYMV is composed of a single-stranded, positive-sense RNA, whose length varies around 4,452 nucleotides, depending of the strain, of contains only four open reading frames (ORF1, ORF2a, ORF2b, and ORF4) (Kouassi et al., 2005) coding for proteins involved in different part of the viral reproductive and infectious cycle (Figure 1.4).

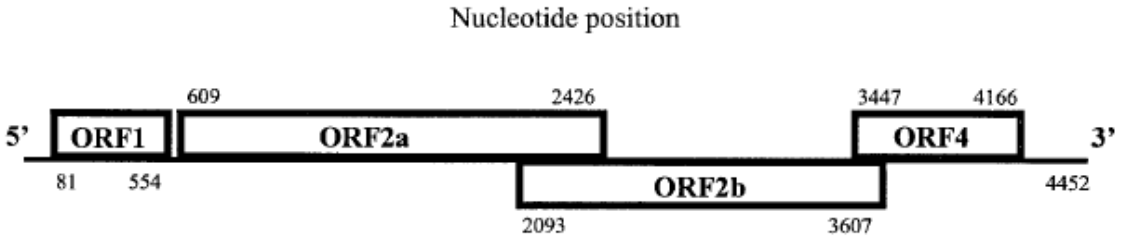


Figure 1.4: Genome organization of RYMV. ORF1 code for a 17.8 kDa protein involved in the virus movement, ORF3: code a 13.7 kDa protein, ORF4: code for a protein of 26 kDa (Fargette et al., 2004)

Bonneau et al. (1998) found that the P1 protein coded by ORF1 is involved mainly in the infection process and also in the virus spread in the plant. The ORF2a and ORF2b polyproteins (Serine protease-like and viral protein, genome-linked (VPg), RNA dependent RNA polymerase) are involved in virus replication. The sequence of these components is well conserved within the genus, which has therefore been used for phylogenic studies (Abubakar et al., 2003; Fargette et al., 2004; Traoré et al., 2005). The CP gene implicated in the virus encapsidation is also essential to full infection. Additionally, it is involved in cell-to-cell, long distance movement and systemic infection (Brugidou et al., 1995).

1.6.1. Occurrence and incidence of RYMV

The RYMV was first reported in Niger by Reckhaus and Adamou (1986), and then appeared in all major irrigated rice schemes in Niger, with yield losses ranging from 56-68%. The high yielding Asian rice cultivar IR1529 released following the creation of large irrigated schemes in Niger, to promote a green revolution, has been reported to be highly susceptible (Reckhaus and Adamou, 1989). Along with the widespread cultivation of the susceptible variety BG90-2, these two varieties may be the major cause of RYMV epidemic outbreaks in the country, and also in several Sahelian countries (WARDA, 2001).

The disease has been reported in all African rice lowland agrosystems and is associated with major losses all over the continent. Between 1975 and the late 1990s, RYMV has been observed in all rice lowland agrosystems of West African countries (WARDA, 2001). As in Niger, irrigated rice schemes in Mali were cropped with BG90-2, losses ranging from 64-100% have been reported (Sy et al., 1993). In Sierra Leone, losses of 82% have been reported (Taylor et al., 1990). Losses of 0.4 to 1.6 tons per ha were reported in Burkina Faso (WARDA, 2001). The RYMV was first found to infect only lowland rice varieties, but later, the virus was also reported in upland agrosystems (Awoderu et al., 1987). In central Africa, the virus has been reported in Chad and Cameroon (Traoré et al., 2001); in Eastern Africa, its centre of origin, the disease has been detected in Kenya, Tanzania, Rwanda and Malawi (Thottappilly, 1992; Banwo, 2003; Ndikumana et al., 2011). Madagascar has also been seriously affected, to a point where farmers have abandoned their fields (Reckhaus and Randrianangaly, 1990).

Lack of resistance in the Asian rice varieties and crop uniformity could explain the deep impact of RYMV during the 1990's. Indeed, 70 to 90% of the area in each infected West African country

was planted with the same high-yielding varieties (BG90-2, IR1529, Bouaké189, IR8, IR54 and IR65) that are highly susceptible to RYMV.

1.6.2. Epidemiology, transmission and symptoms of RYMV

Both cultivated rice species in Africa, *Oryza glaberrima* Steud. and *O. sativa* L. can be infected by RYMV. Additionally, the wild African rice species, *O. longistaminata*, and *O. barthii*, and several wild grasses such as *Echinochloa colona* L., *Panicum repens* L., *Erasgrotis tenuifilia* (Rich.) Hochst. ex Steud., and *Dinebra retroflexa* (Vahl) Panzer, are probable hosts of the virus (Bakker, 1971; Konate et al., 1997). From these sources, the virus can be introduced into a field by several vectors, including both insects and mammals. Beetles belonging to the Chrysomelidea family such as *Sesselia pusilla* G. (Chrysomelidae, Galerucinae), *Chaetocnema pulla* C. (Chrysomelidea, Halticinia), *Trichispa sericea* G. (Chrysomelidea, Hispiniae), *Dicladispa viridicyanea* K. (Chrysomelidea, Hispinae), and the grasshopper, *Conocephalus merumontanus* S. (Orthoptera, Tettigonidae), can transmit the virus in a semi-persistent but not circular manner, 1 to 8 days after feeding on an infected plant (Bakker, 1971; Hibino, 1996; Abo and Sy, 1998). Mammals such as grass rats *Arvicanthis niloticus* D., domestic cows *(Bos spp*.), and donkeys (*Asinus spp*.) are vectors of RYMV (Sarra and Peters, 2003). Moreover, in infected fields, the virus's dispersal can be facilitated by the wind through contact between plant leaves (Sarra et al., 2004). These authors found that contamination of healthy plants was almost ten times higher in plots with a density of 33 plants m^{-2} than in plots with 16 plants m^{-2}. In addition, the virus can also be transmitted mechanically by farmers' contaminated hands during weeding, as well as through farming equipment such as sickles, and through injuries of young plants during transplanting (Abo and Sy, 1998; Abo et al., 2000). Although, RYMV has been detected in all seed parts of several rice genotypes at high rates (65 to 100%), there is no evidence of seed transmission (Konate et al., 2001). However, rice seedbeds were pointed out as primary sources of RYMV infections (Traoré et al., 2006).

The type of symptoms, as well as the delay of symptom development, depends on the mode of transmission, the occurrence of vectors, the period of infection and the rice variety involved. Generally, one to two weeks after infection small, yellowish-green, oblong to linear spots appear on leaves. Later, the symptoms strengthen on infected leaves, with darker spots developing in the centre of the yellow mottles. Depending on the severity and the rice variety, old leaves turn into

yellow, or orange (Bakker, 1974). However, the symptoms are more severe on seedlings under 20 days old, which may stop growing and die. Even if plants survive from 20 to 50 days after transplanting, yield components such as tillering, plant height (stunting), and flowering are reduced. In addition, panicles are poorly exserted and may be sterile (Bakker, 1974; Awoderu, 1991). Later infection (more than 50 days after planting) displays only few symptoms on the leaves but flowers and seeds will be normal. On susceptible genotypes earlier infected plants usually die, whereas in more resistant varieties, the symptoms may not be distinctive (Ndjiondjop et al., 1999).

In susceptible varieties, the virus can multiply easily and spread all over the plant. Opalka et al. (1998) studied RYMV multiplication and movement in susceptible *O. sativa* rice varieties using Western immuno-blotting, Northern blotting, and electron microscopy. After mechanical inoculation of a few leaves, RYMV RNA and coat protein monitoring established that six days are sufficient for the virus to colonise all the leaves. Virus particles are abundant in all tissue including epidermal, mesophyll, bundle sheath, and vascular parenchyma cells. The findings suggest that the virus migrates towards vessels after having weakened pit membranes by absorbing the Ca2+ necessary for its stability. Vacuoles have been found to be the preferable compartment for viral maturation, because of the acidic pH, that favoured Ca^{2+} binding to virus particles (Brugidou et al., 2002).

Since RYMV is transmitted by various insect vectors, their occurrence may influence epidemics onsets and development. Heinrichs et al. (1997) studied the seasonal occurrence of RYMV in lowland rice in Côte d'Ivoire and found that infected plants were severely affected in the July and August plantings, when insect pest occurrence was at its most populous. However, there was no correlation between the disease incidence and insect population. Following the symptoms described above and the dominant mode of virus dispersal in place, different patterns of disease distribution can be observed in the infected fields: entirely or almost entirely infected fields may be surrounded by partially or non- infected fields; variable numbers of distinct patches of infected zones spread throughout the infected field (Sarra and Peters, 2003).

1.6.3. Variability and evolution of the virus

Several serological, pathogenicity, and molecular studies have been conducted to assess RYMV diversity and distribution in Africa. Konate et al. (1997) have classified West African Sudano-

Sahelian area RYMV isolates, into three distinct serogroups (S1, S2 and S3), using two polyclonal antisera and seven monoclonal antibodies' reactivity in ELISA. The first serogroup is widely distributed in West Africa, while the second and the third serogroups are located in the Sudano-Savannah and the Savannah zones, respectively. Furthermore, the correlation of these serogroups using a set of differential rice varieties postulated that RYMV strains are closely related and can be defined by their agro-ecological zone. They display variability in coat protein and pathogenicity. Similar grouping has been found by using double immuno-diffusion gel assay based on twenty six polyclonal antisera to assess the serological diversity of 42 West African RYMV isolates (Séré et al., 2005a). The authors found that the first two groups- S1 and S2- were widely distributed across West Africa. However, isolates from forest zones were different from the savannah ones. In Côte d'Ivoire, for example S1 and S2 occurred alone in the Sahelo-Soudanian (North) and in the Forested (West) zones, respectively, while both coexisted in the northern Guinean savanna (N'Guessan et al., 2001). In cases of mixed infection, S2 always dominated over S1, both in viral capsid and replication.

Additionally, broad molecular and phylogeographical studies of RYMV on the continent were conducted. The molecular typing was based either on the sequences of the ORF4 coding for the coat protein (CP) and the ORF1 coding for the movement protein P1, or on the full genome sequences. Six major serotypes were revealed and were assigned into six clades which have differentiated, from East to West Africa (Table 1.2). The West African strains were found to be less divergent than Central African ones, which were themselves less divergent than East African strains (Pinel et al., 2000; Fargette et al., 2002; Abubakar et al., 2003; Fargette et al., 2004; Traoré et al., 2005).

It has been suggested that RYMV appeared for the first time in East Africa around 200 years ago (Fargette et al., 2008a), and later in West Africa, following a progressive dissemination and differentiation toward the western side of the continent. Furthermore, with a mutation rate of 4 x 10^4 and 8 x 10^4 nucleotides (nt)/site/year, RYMV was found to evolve as rapidly as 50 animal viruses (Fargette et al., 2008b). This ability of the virus could explain the inconsistencies observed between the genetic differentiation and the spatial separation of isolates. For example, virus isolates S1 from Niger and Benin (West Africa) are closer to isolates S1 from Cameroon (Central Africa) than isolates found in the bordering country, Burkina Faso (Traoré et al., 2005).

Man-mediated dispersal of the virus in the continent could also explain the process (Traoré et al., 2009). However, evidence of recombination in RYMV was found in East Africa, indicating that genetic evolutionary factors under variable selection pressures, as well as lineages, contributed to RYMV evolution (Pinel-Galzi et al., 2009).

Table 1.2: Repartition of RYMV isolates on the African continent

Areas	**Serotypes**	**Clades**
West Africa	S1, S2, S3	3, 4, 5 and 6
Central Africa	S1	3
East Africa	S4, S5, S6	1 and 2

Sources: (Abubakar et al., 2003; Traoré et al., 2005)

1.6.4. Screening for resistance to RYMV

Although breeding for comprehensive resistance does not necessarily require a good source of resistance, the time needed for a good response to selection depends on the initial frequencies of desirable traits in the target population. Therefore, screening for resistance aims to provide parents with a high level of genetic frequencies for desirable traits. The screening process requires (1) a good method for inoculum isolation and multiplication, (2) a good experimental design, (3) an efficient inoculation technique and (4) a valuable notation method to assess the different responses of the material to be screened.

Generally, RYMV can be easily isolated from any infected plant in an infected field on the basis of the symptoms of its leaves. Samples are stored in ice boxes during collection and later in freezers for long term conservation. The virus can be then extracted following the technique described by Fauquet and Thouvenel (1977). Leaves are ground in a phosphate buffer (0.1 M KH_2PO_4, 0.1 M Na_2HPO_4, adjusted to pH 7.2) using 1:10 (Ndjiondjop et al., 1999) or 1:15 ratio (w:v) (Séré et al., 2005a). The homogenate is then filtrated and added with 5 mg ml^{-1} carborundum powder (Konate et al., 1997), which functions as an abrasive to facilitate mechanical infection. The sap can be used for inoculation for 14 days (Ndjiondjop et al., 1999; N'Guessan et al., 2001) to 21 days (Séré et al., 2005a) for old and susceptible rice seedlings such

as BG90-2, Bouake-189, IR64 or IR8 for inoculum propagation. From these susceptible plants, the final inoculum is prepared by extracting viruses two or four weeks after infection, depending on the intensity of the symptoms developed, following the same procedure described above. For experiments involving the virus, the inoculum concentration is determined and dilutions made when necessary. Plants to be tested are inoculated on the last expanded leaf two to three weeks after sowing. In the screenhouse, as well as in a field, the intensity of the infection could be recorded, using the Standard Evaluation Scale (SES) of IRRI (2002), where score 1 for no symptoms and plants considered to be highly resistant (HR), score 3 for sparse dots or streaks considered as partially resistant (PR), score 5 for visible mottling on green to light-green leaves considered as Intermediate (I), score 7 for yellowing considered as susceptible (S) and stunting, and score 9 for necrosis and sometimes plant death, for varieties considered to be highly susceptible (HS) (Konate et al., 1997; Rakotomalala et al., 2008).

In addition to the visual evaluation of symptom intensity, other phenotypic symptoms are usually being recorded. Ghesquière et al. (1997) recorded plant height and number of tillers used at two growing stages (40 days post-inoculation and plant maturity), heading date, number of grain per panicle, fertility, and grain weight. Similar data was also collected by Albar et al. (1998). In addition to the disease severity score and the yield, the chlorophyll content of leaves can also be evaluated using a SPAD chlorophyll meter (Onasanya et al., 2004).

Moreover, the virus content of infected plants can be evaluated to assess the resistance or tolerance of a rice variety. Double Antibody Sandwich ELISA (DAS- ELISA) tests using polyclonal antibodies, can be used (Albar et al., 1998) or monoclonal antibodies (MAbs) in triple antibody sandwich ELISA (TAS- ELISA) (N'Guessan et al., 2000). The utilization of ELISA can separate resistant accessions from tolerant ones, where the plant, can grow normally, despite a high virus titre.

1.6.5. Management of RYMV

Outbreaks of RYMV epidemics are due mainly to the increased use of intensive irrigation, fertilizer, and pesticide utilisation, changing cultural practices such as crop monoculture, and transplanting, that induces injuries, therefore increases mechanical contamination. Therefore, as is the case with most plant diseases, an integrated control programme is required, including good cultural practises, vector controls, genetic engineering and breeding for resistant varieties.

Cultural practices

Presently, there is no rice variety that combines both high resistance to RYMV and desirable agronomic traits. Thus, farmers have to manage RYMV severity in existing cultivars in order to maintain their production. The first aim of good cultural practices is to reduce inoculum around the field by destroying any potential source of inoculum of the virus. Dykes, irrigation canals and swamps around the field should be cleansed of wild rice species, and host grass species that could be reservoirs of the virus. Additionally rice plants exhibiting disease symptoms should be eliminated. Mechanical infection of seedlings is more likely to occur during transplantation, thus direct sowing should limit initial infections. Even if farmers prefer indirect sowing, changing seedbed sites can limit early contamination, as rice seedbeds are recognized as primary sources of RYMV infection (N'Guessan et al., 2000). Moreover, seedling exchanges between farmers from different irrigation schemes, as well as pasturage of domestic animals on farms have been cited for increasing the transmission of the virus (Sarra, 2005). Since vector occurrence is seasonal, shifting of sowing dates in a double cropping system will also decrease initial infection. Effective use of insecticides can control vector populations. In addition, biological control of vectors using plant natural substances has been investigated and promising results have been obtained using Neem (*Azadirachta indica* L.) extracts in controlling some Coleoptera and grasshoppers (WARDA, 2001).

Breeding for resistance to RYMV

Good cultural practices can greatly be enhanced by the use of resistant rice cultivars. Resistant rice cultivars can be obtained through genetic engineering or conventional breeding.

Genetic engineering

Two forms of genetic engineering have been tested to control the incidence of RYMV. Firstly, post-transcriptional gene silencing was used, based on the concept of pathogen-derived resistance, which assumed that the products of pathogen genes may disrupt pathogenesis. A trans-gene encoding for the RNA-dependent RNA polymerase of a S1 Central Africa isolate of RYMV was introduced in susceptible cultivars Bouaké 189, ITA 212, and BG90-2 to generate independent transgenic lines (Pinto et al., 1999). Unfortunately, the resistance was stable only over three generations in some plants providing complete suppression of virus multiplication.

Secondly, transgenic rice plants derived from susceptible cultivars such as TP309 and BG90-2 and expressing the coat protein and the P1 protein involved in viral movement were created (Pinto et al., 1999). However, the resistance manifested by a delay of a week for virus replication, was not stable; this may be because of the coat protein expression in trans stimulated the infection (Kouassi et al., 2006). To date, RYMV transgenic resistance has been less durable than natural resistance (Sorho et al., 2005).

Breeding for resistance to RYMV: successes and failure

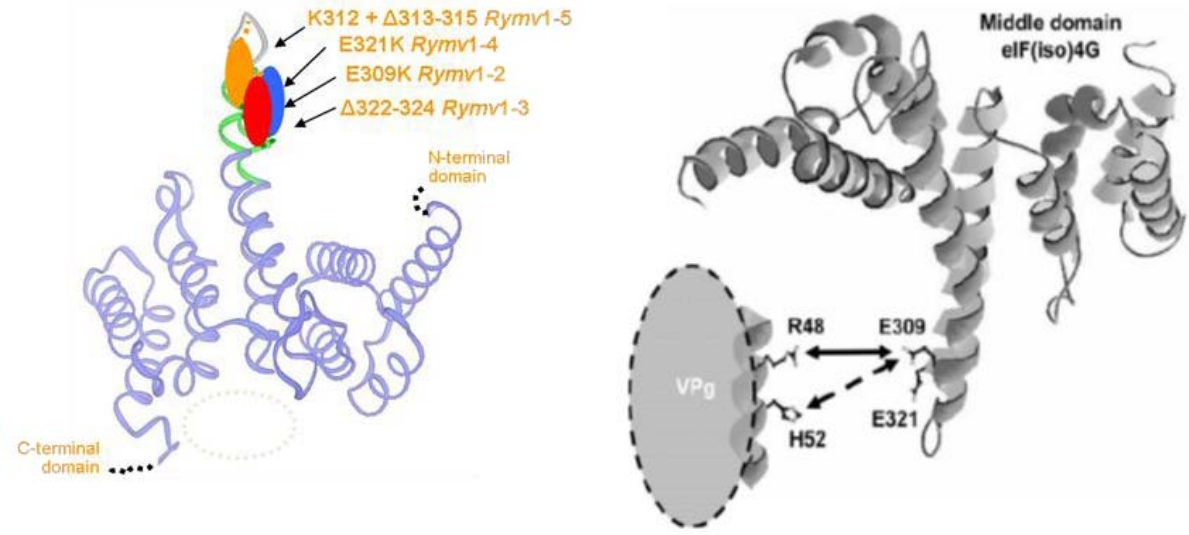
Since the late 1980's the International Institute of Tropical Agriculture (IITA, Nigeria), and the Africa Rice Center (ex- WARDA) have been using resistant rice varieties found during different screenings, in their breeding programs. The resistance of *O. glaberrima* accessions such as Tog5674, Tog5681 and the partial resistance of Moroberekan (*O. sativa* subspp. *japonica*) have been crossed with high yielding susceptible lowland *Oryza sativa* varieties (ITA212, ITA22, ITA304, ITA230 and ITA306). Improvements have been made for some agronomic traits as well as resistance to RYMV (IITA, 1986). In the late 1990 the partial resistance of *O. sativa* subspp. *japonica* cv. Azucena and the high level of resistance of *O. sativa* subspp. *indica* cv. Gigante, and *O. glaberrima* cv. TOG 5681 were identified. Albar et al. (1998) used a doubled haploid (DH) population of Azucena/IR64 (IR64 as susceptible parent) to show that the partial resistance of Azucena is controlled polygenically by 15 QTLs, detected on seven chromosomal segments. The major QTL on found on Chromosome 12 is in epistasic interaction with Chromosome 7 (Pressoir et al., 1998; Ahmadi et al., 2001). This explained 36% of the quantitative variation of virus content in the infected plants. The effects were assessed by introgression into the susceptible background of IR64. The derived near-isogenic-lines (NILs) expressed tolerance characterised by mild symptoms, despite accumulation of the virus; and a partial, seedling-resistance consisting of a delay of one week in virus accumulation and symptom expression (Ioannidou et al., 2003). In addition, it was established that the high resistance of TOG5681 and Gigante, characterized by no viral coat protein or RNA accumulation, the absence of symptoms and the failure of cell to cell movement by RYMV, is conferred by a single recessive gene localized on the long arm of chromosome 4 (Ndjiondjop, 1999; Ndjiondjop et al., 2001). Furthermore, the physical map and positional cloning of that resistance gene named *RYMV1*, linked to the microsatellite marker RM252, and flanked by RM273 and RM241, proved that an isoform of the eukaryote translation

initiation complex protein eIF(iso)4G was involved (Albar et al., 2003; Albar et al., 2006). The same authors found a wide variability in the sequence of that gene, but only two *O. sativa* cultivars were found to be resistant. The cultivars Gigante and Bekarosaka bear both the *rymv1-2* allele (Albar et al., 2006; Rakotomalala et al., 2008), while susceptible rice varieties bear *rymv1-1* allele. This resistance found in *O. sativa* varieties is conferred by a point mutation leading to the substitution of a glutamic acid by a lysine at Codon 309 of the central domain of eIF(iso)4G. Contrary to this, a wide range of resistant accessions were found in the African rice *O. glaberrima*. Accessions TOG5681 bear *rymv1-3*, while TOG5674 contains *rymv1-5* and finally TOG5672 carries both the allele *rymv1-4* and a new resistant gene *RYMV2* has been found in *O. glaberrima* accession TOG7291 (Albar et al., 2006; Thiémélé et al., 2010).

Generally, this type of monogenic resistance implying interaction between the pathogen and its host proteins involves a vertical gene. It provides protection only against non-matching auto-infection, and is useless against matching allo-infection (Robinson, 1995). Effectively, some rare, naturally-occurring RYMV isolates capable of overcoming host resistance have been observed (Konate et al., 1997; Sorho et al., 2005). These virulent or "Resistance-breaking isolates" have been reported in proportions ranging from 9% to 15% (Traoré et al., 2006). Furthermore, in experiments, the high resistance of Gigante and the partial resistance of Azucena have been overcome after serial mechanical inoculations (Fargette et al., 2002). The full-length sequences of isolates before and after acquiring virulence revealed that only one mutation at position G1729T in the putative VPg domain is responsible for the virulence (Hebrard et al., 2006). In addition to this, studies revealed other patterns of nucleotide substitution leading to the emergence of virulent isolates, as shown in Figure 1.4, and direct interaction with the plant elF(iso)4G1 factors was demonstrated (Pinel-Galzi et al., 2007; Hébrard et al., 2008; Traoré et al., 2010). Recently, it was formally accepted that only virus isolates with a threonine at Codon 49 of the VPg could overcome the *rymv1-3* allele. These virus groups are called "T" types. Virus isolates with glutamic acid at the same position (so called "E" types) are more likely to overcome the *rymv1-2* allele (Traoré et al., 2010). In addition, to this it was found that the virulence capacity was highly dependent on the amount of virus inoculated and on the mode of transmission (Sorho et al., 2005). Furthermore, these authors found that isolates' virulence is not affected or counter-selected after serial passages in susceptible cultivars, even when mixed with avirulent quasi-isogenic wild type isolates. However, though recent studies confirmed the low prevalence of virulent isolates;

they also reflected the ability of some RYMV isolates to overcome the *rymv1-2* allele (Poulicard et al., 2009).

Presently, three varieties WITA8, WITA9 and WITA10, developed by AfricaRice and partially resistant to RYMV, have been released in some West African countries (Amancho et al., 2009).



A B

Figure 1.5: Modelling of the eukaryotic translation initiation factor eIF(iso)4G protein of Chromosome 4 of rice. Sites conferring allelic variation of the resistance to RYMV are highlighted (A) and interaction with the virus VPg shown (B). Source: (Hébrard et al., 2008)

The promises of Marker-Assisted Selection (MAS)

Conventional breeding has had a great impact on the improvement of various crop species (Collard and Mackill, 2008). Conventional breeding relies on phenotypical differences observed among individuals in a population. However, phenotypic variation can be highly environment-dependant, and the stage of development of the plant can interfere with the evaluation of the target trait. Moreover, with regard to traits like disease resistance or abiotic stresses, reliable evaluations can be difficult due to the lack of common and accessible methods, and especially if the trait is under recessive gene control (Babu et al., 2004; Semagn et al., 2006a). Developed during the eighties, MAS is commonly used in crop improvement. Marker-assisted selection is defined by Wikipedia as "the indirect selection process where a trait of interest is selected, not based on the trait itself but on a marker linked to it"

(http://en.wikipedia.org/wiki/Marker_assisted_selection). The technique has become widespread with the development of molecular markers and genome sequencing of various plants, leading to the molecular characterization and mapping of several genes involved in plants' resistance to stresses (Ribaut and Hoisington, 1998). Indeed, when using molecular markers, MAS relies on variation observed on the genetic sequence of individuals, thus it is less prone to environmental changes, and is detectable at any development stage. Thus, MAS purports to offer gains of time, precision and low cost as compared to conventional breeding. However, MAS has been effective mainly on the improvement of traits under monogenic and oligogenic control and where a reliable phenotyping method is available. Thus, MAS aims only at improving conventional breeding, not challenging it. The purpose of plant breeding is to develop new cultivars that accumulate more advantageous genetic combinations, and MAS is a powerful tool that can be utilised to enhance in this endeavour (Collard and Mackill, 2008). Semagn et al. (2006a) described the following steps as basics required prior to implementing a MAS program:

- Identification of molecular markers linked to the traits of interest (for common traits, a literature search can provide the answer)

- Testing the applicability and reliability of the markers in predicting the traits in related families (also referred to as marker validation).

- Producing clear and simple protocols for assaying the markers.

- Modifying the breeding strategy to optimize the procedure. For example: Marker-assisted backcross (MAB) could allow recovering of the genome of the interesting parent more rapidly.

In the case of RYMV, the recessive resistance gene found in Gigante has been mapped, sequenced and positional cloned (Ndjiondjop, 1999; Albar et al., 2003; Albar et al., 2006). The gene was mapped on the long arm of the Chromosome 4 near microsatellite marker RM252 (1.8 cM), flanked by at 2.3 cM by RM273 and at 2.4 cM by RM241 (Figure 1.6).

Various authors have worked on RYMV and its interaction with rice. Thus, reliable genotyping protocols are available, but need to be validated. The donor variety Gigante belongs to the *indica* subgroup of *O. sativa* adapted to lowland ecology, which is the area of worst occurrence of RYMV. Most of rice in West Africa is produced in lowland and irrigated agrosystems, thus

Gigante could easily be used as donor in a MAB program to improve lowland and irrigated rice cultivars in West Africa.

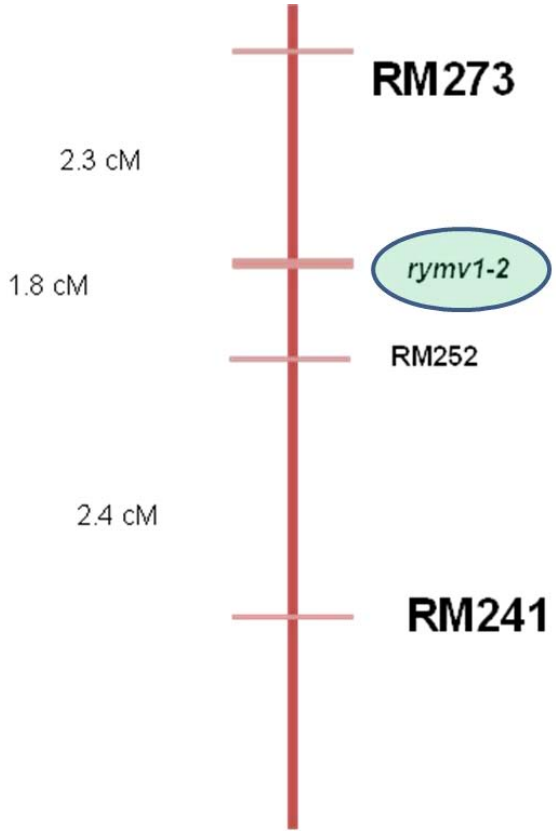


Figure 1.6: Schematic representation of the area containing the *rymv1-2* resistance gene on rice Chromosome 4

1.7 Rice taxonomy

Rice is a grammineae belonging to the genus *Oryza*, the family of Gramineae and the tribe Oryzeae. The taxonomy within the genus *Oryza* has been the subject of debate between scientists for a long time. Tateoka (1963) divided the genus into six complexes, comprising twenty one species, based on cytogenetic, genome symbols, morphological studies and geographic distribution. Later, four species complexes were proposed: *Oryza sativa*, *O. officinalis*, *O. meyeriana* and *O. ridleyi*, containing 23 species, based on the genomic constitution and chromosome number (Vaughan, 1994; Vaughan et al., 2003). Twenty six species were reported for the same complexes by Sharma et al. (2003). *Oryza*'s main morphological traits are sterile lemmas, narrow, linear herbaceous leaves and bisexual spikelets (Vaughan et al., 2003).

1.8 Rice domestication

Of the species of these complexes, only *O. sativa* L., and *O. glaberrima* Steud.. (two annual species part of the *O. sativa* complex, itself composed by six others wild species), are cultivated. The two species sprang from a common ancestor around 5000 years B.C and were domesticated in Asia and Africa, respectively (Chang, 1976). *O. sativa*, widely cultivated in the tropical and temperate zones of all the continents except Antarctica, was domesticated in the Indo-China area from the annual species O. *rufipogon*, itself derived from the perennial wild species O. nivara. Similarly, *O. glaberrima*, which is endemic to Africa, was domesticated 3500 year ago, in the upper delta of the Niger River and later in Guinea and Sene-Gambia (all in West Africa), from the wild annual species *O. barthii* Chev. The latter is itself derived from the wild perennial *O. longistaminata* Chev(Chang, 1977). et Roehr. (Portères, 1956; Chang, 1976). Unlike the Asian rice, African rice did not move far from its centre of domestication and moreover, it has been supplanted by the high yielding *O. sativa* cultivars even in Africa. *Oryza sativa* arrived in Africa in the 15^{th} and 17^{th} centuries, carried by either Arab or Portuguese traders (Porteres, 1956). Thus Africa is the only continent growing the two cultivated rice species. Cultivated rice species of the genus *Oryza* and their ancestral species are diploid (2n= 24) and possess the A genome, while other wild species are diploid or tetraploid (2n= 48) but distributed into genomes B, C, D and E (Morinaga, 1963; Khush, 1997). In addition to cytogenetic and morphologic methods, molecular biology techniques were also used during the 1990's to assess the accurate delineation of the rice complexes. Abe et al. (1999) used restriction fragment length polymorphisms (RFLP) to assess the phylogenetic relationships of eleven *Oryza* species. They classified the genus into two large clusters: A-genome species on the one hand and B-, BC-, C- and CD-genome species on the other. The same conclusion has been reached using DNA sequences, called Miniature Inverted-repeated Transposable Elements (MITEs) as molecular markers, combined with Amplified Fragment Length Polymorphisms (AFLP) to analyse genetic variation in fifty three *Oryza* species (Park et al., 2003). Many others authors have used RAPDs (Random Amplified Polymorphic DNA) to confirm the accuracy of the classification (Ishii et al., 1996 Yu and Nguyen, 1994).

1.9 Diversity of cultivated rice

Several studies have also been conducted to establish the genetic relationships within cultivated rice varieties. The Asian cultivated rice *O. sativa* can be divided into groups of subspecies, based on geographical distribution (continental or insular), agro-morphological traits (grain size, awn

presence or absence, drought resistance, etc), biochemical reactions (potassium chlorate sensibility, phenol coloration, etc) and DNA sequencing (one chloroplast and two nuclear regions). The first group, named *O. sativa* subspecies *indica*, was domesticated on the Southern and Southern-West Indian side (Indochina) of the Himalaya Mountains. The second group is the *O. sativa* subspp. *japonica*, which was domesticated in southern China (Oka, 1958; Morishima, 1984; Takahashi, 1984; Londo et al., 2006). *Oryza sativa* subspp. *japonica* varieties are widely spread and cultivated mainly in southern China, Indonesia, Southeast Asia, Africa and America, while *O. sativa* subspp. *indica* is commonly cultivated in the lowland agrosystems of tropical Asia and Africa (Khush, 1997). Additionally Matsuo (1952) suggested three groups called A, B and C with *O. sativa* subspp. *japonica* varieties (A) mostly grown in tempered lowland area of Japan, *O. sativa* subspp. *indica* varieties (C) widely cultivated in tropical lowland ecosystems of India, and *O. sativa* subspp. *javanica* varieties (B) adapted to the tropical upland of Java. The most accepted classification of the Asian rice was done by Glaszmann (1987), using isozymes. The author recognized six varietal groups, of which two major groups, I and VI, corresponded to subspp. *O. sativa* subspp. *indica* and *O. sativa* subspp. *japonica* respectively, while deepwater rices were clustered into groups III and IV and finally, minor groups II and V corresponded respectively to Aus. and Basmati aromatic rice. This classification was confirmed by the use of molecular markers (Garris et al., 2005). Five distinct groups were found, where deepwater varieties rejoined the *O. sativa* subspp. *indica* groups. In addition to this, the tropical and temperate forms of *O. sativa* subspp. *japonica* were clearly detected.

Despite genetic and phenotypic variation found in *O. glaberrima* and its wild relatives, there is still no subdivision like *O. sativa*. Two main forms were detected in *O. glaberrima*: an upright form adapted to upland cultivation and a deepwater type (Bezançon et al., 1977). Moreover, this author has studied the adventitious form of *O. barthii* in areas where *O. glaberrima* was grown. This "weedy" type was called O. *stapfii* Rosch., and could have been the result of natural hybridization between the cultivated rice species and *O. barthii*. However, due to the absence of clear morphological differentiation, compared to the wild species, several authors have proposed including it in the *O. barthii* species (Portères, 1956; Morinaga, 1963; Bezançon, 1993). As in the case of the Asian rice, the classification of the cultivated rice in Africa has been studied using microsatellites (Semon et al., 2005). Five groups were found: two groups corresponding to Asian rice subgroups *indica* and *japonica*, two groups corresponding to the floating and upright forms

of *O. glaberrima*, and finally a group corresponding to different phenotypic combinations created due to ecological adaptation. The relatively narrow diversity of *O. glaberrima* may be explained by its geographical isolation and its restriction to West Africa. Moreover, the region is flat and characterized by limited environmental variations.

1.10 Diversity estimation methods

Several techniques have been used to determine variability in crop species. However all the techniques are based on genetic markers that have been classified into three groups (Semagn et al., 2006a).

- Phenotypical markers based on agro-morphological trait differences between plants,

- Biochemical markers, based on proteins or their activity as detected through gene expression,

- Molecular markers based on the detection of DNA polymorphisms between individuals.

1.10.1. Phenotypic markers

Phenotypical traits were the first generation of markers to be used for diversity studies, both on animals and plants. Tateoka, (1963) used spikelet and awns length to differentiate wild forms within the *Oryza* complex, based on their geographical origin. Later, several agro-morphological traits were used to estimate the genetic diversity in African rice species (Chang et al., 1977; Second et al., 1977). Similarly, phenotypic markers were used to study the genetic diversity of tomato landraces from Greece (Terzopoulos and Bebeli, 2010). Phenotypic traits allow for visual assessments, based on a direct observation of the trait of interest. Several documents describing traits to be measured and measurement methods have been developed (IRRI, 2002; Bioversity International et al., 2007). However, phenotypic markers are cost effective and reliable only when skilled personnel are available.

1.10.2. Biochemical markers

Biochemical markers have greatly enhanced the improvement of various traits of some crops especially where phenotypical traits or molecular markers are not efficient. Biochemical markers can be grouped into two types, including metabolic-based markers and protein-based markers. The first type is based on products from the secondary metabolism of plants, such as polyphenols (De Vicente et al., 2004). Thus, biochemical analysis methods are required to accomplish this

kind of study. Plants like tea, coffee and tobacco have benefited from this technique. Catechins were successfully used to classify Kenyan and Western Himalayan tea cultivars (Magoma et al., 2000; Karthigeyan et al., 2008). Apart from classifying the varieties into groups, the content of catechins could be used as an *indica*tor of the quality of tea. The second type is based the detection of protein activity. Isozymes are the most used and the most beneficial to both Asian (Glaszmann, 1987) and African rice studies (Second, 1985; Bezançon et al., 1989). The technique is based on the detection of enzymes, diverging in nucleotide sequence but catalyzing the same chemical reaction. Although biochemical markers were very useful in assessing genetic diversity of many important crops, they have been supplanted by molecular markers, which are considered to be more reliable and repeatable to as confirmation of visual or phenotypical assessment of variation in plant populations.

1.10.3. Genomic Markers

Genomic markers are identifiable DNA sequences that can be used as landmarks to orientate the genome of individuals. They are associated exclusively with a segment of the genome, and follow a Mendelian mode of inheritance across generations (Semagn et al., 2006a). Genomic markers detect variation between individuals based on changes due to mutations, substitutions or deletions within the DNA sequence. The relationship between the genomic marker and the genomic segment (which can be considered to be the target trait) depend on the distance between them: The more tightly they are linked, the greater their probability to segregate together. The distance between a genomic marker and a target trait is calculated based on frequencies of recombination between them in centimorgan (cM). Three categories of genomic markers were proposed by Semagn et al. (2006a), based on the mode of transmission (biparental nuclear inheritance, maternal nuclear inheritance, maternal organelle inheritance, or paternal organelle inheritance), the mode of gene action (dominant or codominant markers) and finally, the method of analysis (hybridization-based or PCR-based markers).

PCR-based markers

With the automation of the Polymerase Chain Reaction (PCR), and the advances in genome sequencing, PCR-based markers have become the commonly used genomic markers. Moreover, the development of site-targeted PCR techniques from known DNA sequences and several thousand Simple Sequence Repeats (SSRs) or microsatellites were developed for rice (Temnykh

et al., 2000; Temnykh et al., 2001) and have become the most commonly used genotyping tool for rice. These sequences are usually 1 to 6 base pairs of non-coding DNA, inherited co-dominantly and created by mutation. Microsatellites are often used in population genetic studies, gene mapping and marker-assisted selection. Forward and Reverse sequences of SSRs that will recognize and hybridize the target region of the DNA template could be added to the PCR mix reaction and the final product separated on agarose or acrylamide gel. The high mutations rate of the neutral region of the genome permits the detection of several alleles for a given SSR (Langridge and Chalmers, 2005). Microsatellites were used for the last global study of the two cultivated rice species (Garris et al., 2005; Semon et al., 2005).

Several variants of microsatellites exist. Inter-simple sequence repeat amplification (ISSR), for instance, is a PCR-based amplification of DNA using a single primer developed from the interval sequence between two microsatellite sequences. In this case the target to be amplified is the variable region between the sequences, and is conducted without prior knowledge of the sequence (Wu et al., 2004). Another but dominant and arbitrary PCR-based marker is RAPD (random amplified polymorphic DNA). This method is difficult to reproduce.

The latest generation of PCR-based genomic markers involves Single Nucleotide Polymorphisms (SNPs). This type of marker is based on detecting single base pair changes between species or paired chromosomes. They are widely and densely distributed in *Oryza sativa* L. genome, with a rate of 0.65% in the *O. sativa* subspp. *indica* and *O. sativa* subspp. *japonica* subspecies (Liu and Zhang, 2006). The SNP rate is 0.65%, indicating a bright future for this technique in rice.

Hybridization-based Markers

Restriction fragment length polymorphism (RFLP) is the most widely used hybridization-based molecular marker. It uses restriction enzymes to digest the DNA fragment into sequence pieces, which are then separated using a gel electrophoresis system. From the gel, the sequences are transferred to a membrane via the Southern blot procedure, involving probes with RNA and finally revelation using autoradiography (Semagn et al., 2006a). Presently, this technique has been overtaken by the PCR-based techniques. A derived technique is the amplified fragment length polymorphism (AFLP), which combines the enzymatic digestion of RFLP with the

amplification of PCR-based. This technique generates lots of variation and has been used in diversity studies and gene mapping.

1.11 Farmers' preferences for rice traits

The development of new high yielding rice varieties, well adapted to African agrosystems cannot have an appreciable impact unless the selection takes into account farmers' expectations. While, in some areas farmers have readily adopted new varieties, in others areas newly developed varieties have failed to meet farmers' needs. In the Sikasso region of Mali, farmers' reluctance to adopt varieties newly developed by breeders has been reported (Efisue et al., 2008). This could go towards explaining their low yield despite the release of high yielding, "improved" cultivars. Contrary to this, the variety SAHEL108, released in the Senegal River Valley was so successful that the rice cropping area was multiplied by tenfold within 10 years and farmers from the bordering country Mauritania adopted it voluntarily (WARDA, 2008b). In India, a few varieties developed through conventional breeding have failed to satisfy farmers, where within a few years; participatory plant breeding has succeeded (Witcombe et al., 2003). Thus, any breeding program should study the target environment through the eyes and perceptions of the target people, and design appropriate varieties, based on their preferences.

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Chapter 2: Farmers' perception of rice varieties, major constraints on rice production and *Rice yellow mottle virus* (RYMV) in the region of Tillabéry, West Niger

2.1 Abstract

The region of Tillabéry is the main rice cropping area of Niger and includes both irrigated and lowland rice. In spite of large investments, rice production has stagnated due to several factors including seed sector and biotic stresses. This study was conducted in 2009 to investigate those factors through the eyes of farmers. Semi-structured group discussions were carried out in 14 villages, 153 farmers were individually subjected to a questionnaire, and farmers' fields and storage facilities were visited. Fields were larger in private irrigation and lowland agrosystems compared to those in irrigation schemes. The local farmers' union was the only formal seed dissemination system. Apart from this, farmers conducted seed exchanges between themselves as well as utilizing seed left over from the previous harvest. The *Rice yellow mottle virus* (RYMV) and Bacterial Leaf Blight (BLB) were cited as the most prevalent biotic stresses in the irrigated agrosystems, with the varieties IR1529-680-3 and Waihidjo considered to be the most popular. Floods, birds and hippopotamuses were the most damaging agents in lowland agrosystems and the landrace Degaulle/ D5237 was the preferred variety.

Keywords: farmer perceptions; seed; Participatory Rural Appraisal (PRA); constraints, BLB, RYMV; West Africa, rice

2.2 Introduction

Rice constitutes the third most important food crop in Niger after pearl millet and sorghum, but represents the most rapidly increasing crop in term of demand and consumption. However, the national rice production does not match the demand, causing rice imports to grow from 40,000 tons in 1995 to 210,000 tons in 2005, at a cost of 71.4 million USD, for a country where nearly 60% of the population lives below the poverty line (Sido, 2010; WFP, 2010). Rice is produced mainly in the region of Tillabéry covering 91,199 km^2 over a total of 1,267,000 km^2. It is the home to 16.44% of the 15.2 million people of Niger (Faivre-Dupaigre et al., 2006; INS, 2010). The region of Tillabéry is bordered to the north by Mali, the north-west by the region of Tahoua, the east by the region of Dosso, the west by Burkina Faso and the south by Benin. With 100,000 ha of arable, irrigable lands, the region of Tillabéry contains nearly half of the irrigable land of Niger; in addition to 420km of the 550km that the Niger River runs through the country. In

addition to this, the river has seven major tributaries in the region, and there are a number of marshes in the same area (Seybou and Kodako, 2004).

In the rice production area of Tillabéry can be found all three of the main agrosystems of rice in the country. Firstly, the region contains 29 irrigated rice schemes that cover 7,432 ha (85.3% of the national irrigated schemes) with double cropping each year. The average yield in this agrosystem is 3.5 to 4.5 tons per hectare (Seybou and Kodako, 2004; Alfari et al., 2006). This intensive system, under the control of local farmer unions, and supervised by the "Office National des Aménagements Hydro-Agricoles" (ONAHA), currently produces 30,000 to 35,000 tons per year. Additionally, private irrigated system with individual water pumps are found in the area. The irrigated ecosystems are planted with improved *Oryza sativa* varieties only. Secondly, traditional rice growing in the Tillabéry region accounts for about 62.13% of lowland rice production, including lowland deepwater and floating agrosystems along the Niger River. Yields are low, between 0.9 and 1.5 tons per hectare with a total yearly production around 55,631 tons (Alfari et al., 2006). The rainfed lowland agrosystems are sown with both improved and traditional *O. sativa* varieties, while the deepwater and floating agrosystems are sown with *O. glaberrima*, and a few *O. sativa* varieties.

People in the south-eastern region of Tillabéry started growing rice relatively recently; whereas rice has been the staple food for people in its western region for centuries. Indeed, The Wogo and Songhai (Gaoboro) people are traditionally rice growers. The Niger River splits the area into many islands, where floating basins were used for rice cropping (Bonkoula and Miezan, 1982). During the 1980's, the Government of Niger invested in the development of large irrigated areas for rice cropping intensification. These irrigated areas were cropped with high yielding Asian rice cultivars IR1529 and BG90-2. This was followed by the emergence of several diseases (Rechans, 1983; Reckhans and Adamou, 1986) of which the RYMV was, first reported in the country in 1984 (Reckhaus and Adamou, 1986). The Asian rice cultivars have been identified as the causes of the first RYMV outbreaks. The susceptibility of those cultivars resulted in yield losses reaching 58-68% (Reckhaus and Adamou, 1989; WARDA, 2001). Presently the virus has been reported on more than 12,000 ha of rice along the Niger (West) and the Komadougou (East) Rivers, more than 1000 km distant from the first sites where RYMV was reported (Basso et al., 2010).

During a rice germplasm collection conducted as part of this thesis in Niger in 2008, major changes in rice cultivation practices were observed in the homeland of the Wogo people in the region of Tillabéry. Firstly, more than 80% of the *O. glaberrima* landraces identified by Bonkoula and Miezan (1982) had disappeared in the canton of Sinder. Secondly, the *O. sativa* variety IR1529-680-3 (which is highly susceptible to RYMV) was still being cultivated on most of the irrigated schemes, in spite of the release of RYMV tolerant rice varieties, WITA8 and WITA9, developed by AfricaRice and released in 1997 by the National Institute of Agricultural Research in Niger (INRAN). A farmer's variety of *O. sativa* named "Kassimo" or "Waihidjo" was grown on 60% of the irrigated rice fields of the region (personal communication). This variety was said to have been derived from IR1529-680-3 by mass selection made by a farmer of the village of Darbani. Surprisingly, the variety had not been tested, nor disseminated by the INRAN or the ONAHA; prior to its being grown on a large scale. Several questions have risen from this situation: (a) Why were farmers still growing the RYMV susceptible IR1529, despite the availability of RYMV tolerant varieties ?; (b) Why did RYMV tolerant rice varieties, such as WITA 8 and WITA 9, were not adopted by farmers for replacing the RYMV susceptible IR1529-680-3 ?; (c) Which were the farmers' preferred rice varieties in the region of Tillabéry and what were the main preferred traits interest for adoption by farmers ?; (d) What were the seed dissemination channels ?

Farmers' reluctance to adopt new and improved varieties has also been reported in other countries. In Ethiopia, for instance, a study found that in some areas farmers continued to use susceptible varieties even when disease- resistant varieties were made available by scientists (Kiros-Meles and Abang, 2008). The key explanation for this attitude was their interest in other criteria, unfortunately not recognized by researchers. Similarly in Ghana, cassava growers were found to retain disease-susceptible landraces, despite the availability of the disease-resistant, improved varieties. Farmers were not aware of the disease-resistance ability of the improved varieties (Manu-Aduening et al., 2007). Next to disease and pest resistance, crop agronomic traits can also drive farmers' choices. In this way, maize farmers in Nepal refuse to adopt "improved" varieties without the desired traits of earliness, responsiveness to low fertility soils, grain colour, etc (Witcombe et al., 2003). In some Nepali villages, rice farmers preferred cultivars with white caryopsis for various reasons ranging from culinary practicality to social prestige (Sthapit et al., 1996). Fortunately, rice varieties accepted by farmers in Nepal were successfully developed

through participatory research at the same time as the above information on farmers' perceptions and preferred traits were identified (Joshi and Witcombe, 2003). Among participatory research methods, Participatory Rural Appraisal (PRA) is a powerful tool to rapidly accumulate information on rural issues, with farmers' involvement. The PRA involves a tight multidisciplinary collaboration between scientists first, and then with farmers. The PRA team aspires to be directly informed by target rural communities using several exploratory techniques including iteration, survey, cross-checking and on-site visits (Bhandari, 2003; Efisue et al., 2008).

To understand the reasons behind the inconsistencies observed in the region of Tillabéry, a PRA on farmers' perception and their management of the main constraints in three rice agrosystems (particularly RYMV), and their preferred traits in rice, as well as their management of local varietal diversity, was conducted. The primary goal of this study was to gather information on farmers' perception of main constraints to rice production, and the seed sector in the region of Tillabéry. The information could be used to maximize the impact of new varieties, in order to reduce the damaging effects of RYMV on irrigated and lowland rice of the region and thereby improve the livelihoods of the farmers.

2.3 Material and Methods

2.3.1. Sampling and interview techniques

The PRA team was comprised of the principal investigator, a field technician from INRAN with a background in genetic resource management and sociological surveys, and the agricultural extension officer of the place to be visited. Prior to the survey, a meeting was conducted with a social scientist of INRAN and a senior rice breeder to finalize the questionnaire (Appendix 2.1) and choose the villages to be included in the survey. A trip was then organized to visit farmers' field of 20 villages in Tillabéry region during the harvest season. The team then selected 14 villages representative of the three rice agrosystems used in the region were chosen and six to eighteen farmers were interviewed per village.

Several techniques, including iteration, probing, direct observation and pairwise ranking, were used for the study (Efisue et al., 2008). Semi-structured interviews were conducted first, to obtain community level information, and then 153 farmers were interviewed individually. Additionally, fields were revisited during the preparation of seedbeds in a second cropping period and rice seeds were collected.

2.3.2. Data analysis

An Access® database was used to capture the data generated by the PRA and SPSS 17.0 software was used to analyse the dataset. Means, and frequencies were computed and significance tests performed, whenever necessary.

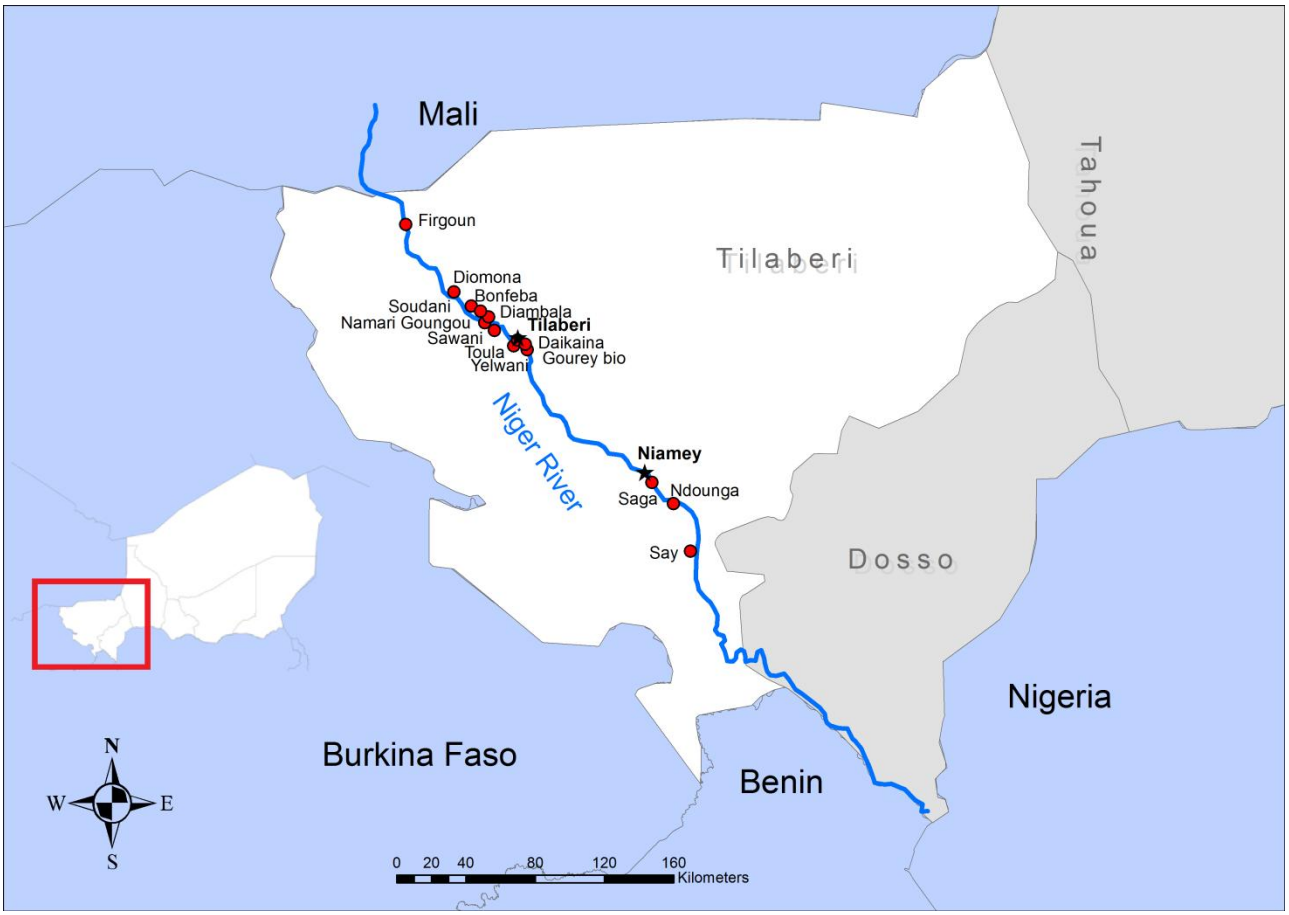

Figure 2.1: Map of the region of Tillabéry (also writing Tilaberi,Niger Republic) showing the villages included in the survey

2.4 Results

2.4.1. Socio-economic characteristics

One hundred and fifty three farmers from 14 villages participated in the survey. The number of farmers ranged from six (village of Soudani) to 18 (village of Yelwani). Ninety eight percent of the farmers were male, and only 2% were female (Table 2.2). The age of farmers ranged from 22 to 78, with a mean of 47.8±9.6 years and a median of 48 years. Prior to our survey, the time spent in a village by a farmer, ranged from 15 to 78 years; with a mean of 47.7±9.9 years and a median

of 47 years. 129 farmers (84.3%), of which three females, were members of their local farmers' union found in all villages with irrigated areas under the supervision of the ONAHA. The duration of their membership varied from one to 35 years, with a mean of 14±8.6 years. About 94.1% of the farmers considered agriculture to be their main activity, 6% considered it as secondary activity, and 3.2% as tertiary activity. About 4% were domestic workers or labourers and 1.3% pursued other activities (Table 2.1). 15% of the farmers considered farming as a secondary activity and 1.3% considered it to be a tertiary activity, while 29.4% cited trade and 25.5% cattle-breeding as their secondary activity. More than half of the target population (53.6%) have participated to a training in agriculture, and 51% have participated to a rice growing workshop.

Table 2.1: Economic occupations of the interviewed farmers in Tillabery Region, Niger Republic

	Main activities		Secondary activities		Tertiary activities	
Activities	Count	Percent	Count	Percent	Count	Percent
Farming	144	94.10%	8	6.02%	1	3.22%
Domestic Work	3	1.96%	1	0.75%	0	0.00%
Trade	1	0.70%	45	33.83%	12	7.80%
Artisan	0	0.00%	8	6.02%	2	6.45%
Labour	3	2.00%	15	11.28%	5	16.12%
Other	2	1.30%	17	12.78%	4	12.90%
Cattle-breeding	0	0.00%	39	29.32%	6	319.35%
Total	153	100.00%	133	86.93%	31	20.30%

Others: builders, carpenter, fisherman, porter, warehouseman, security guard, school teacher.

Most of the farmers had a source of information on agriculture, while 3.9% had no information source. The source of agricultural information included the local farmers' union (88.3%), the agricultural extension service of the nearest town (5.3%), and other fellow farmers (2.6%). About 3.9% of interviewed farmers did not have any source of agricultural information, and thus relied solely on their traditional practices. In addition, 88.2% of the farmers accessed credit, in form of fertilizers, pesticides and seeds, through their local farmers' union.

2.4.2. Cropping systems and post-harvest losses

Two main rice agrosystems (namely the lowland and the irrigated agrosystems) were found in the region of Tillabéry. The lowland agrosystem included floating rice in deepwater, and lowland

(paddy) rice production, with water levels less than 0.5 m. The irrigated agrosystem included large scale irrigation schemes under the control of local farmers' unions, assisted by ONAHA's agricultural extension officer, and private irrigation fields. The sample used in this survey was largely dominated by large scale irrigated rice with ONAHA (86.3%), followed by floating rice (5.2%), private irrigated rice (4.6%) and finally, lowland rice (3.9%) as shown in Table 2.2. Because of the predominance of irrigated agrosystems in this region, the transplantation of 20 day old seedlings during the rainy season and 30 to 40 day old young plants during the cool period, were the main sowing methods. In addition to this, a few farmers had started transplanting in the lowland fields, because they found it easier for weeding and harvesting. Contrary to this, farmers in the deepwater agrosystem still practiced broadcast seeding. The size of the fields varied from 0.17 ha to more than 3 ha, with a variation across agrosystems. In irrigated rice, fields ranged between 0.17 ha and 1 ha. The majority of farmers on large scale irrigated schemes had 0.25 ha, while private farmers had at least 0.5 ha. Lowland farmers possessed even bigger fields of 1 ha to more than 3 ha.

Prior to planting, all farmers used animal traction for field preparation. In irrigated agrosystems used for double cropping, the first season starts in June-July, while the second season starts in December-January. On the other hand, in the lowland agrosystem, a single cropping is practiced every year from June-July to November-December. Most farmers planted only rice (90.2%), while a few farmers (9.8%) combined rice with okra (*Abelmoschus esculentus* Moench) and rosella (*Hibiscus sabdariffa* L.). Half of the farmers (79.8%) practiced one to two weedings a season, while 17.6% weeded three times and 2.6% four times. Only farmers in irrigated agrosystems used the herbicide Londax™ for weed control, at rates ranging from 50 g ha^{-1} to 200 g ha^{-1} (71.9% used 100g ha^{-1}). Fertilizer was applied as NPK and urea, mostly at 200 to 300 kg ha^{-1} (66.5%). Some farmers (13.2%) used less than 200 kg ha^{-1}, while 13.8% used more than 350 kg ha^{-1} and 9% used 400 to 500 kg ha^{-1}.

Table 2.2: Number of participant farmers per village, agrosystem and gender in the Tillabéry Region, Niger Republic

		Ecology				Gender		
District	Villages	Irrigated (ONAHA)	Lowland	Floating (Deepwater)	Irrigated (Private)	Male	Female	Total
Dargol	Yelwani	18	0	0	0	17	1	18
Dessa	Diomona	14	1	1	0	16		16
Sakoira	Bonfeba	10	0	0	0	10		10
	Namari Goungou	15	0	0	0	15		15
	Diambala	15	0	0	0	15		15
Ayorou	Firgoune	8	1	0	0	9		9
Saga	Saga	7	0	0	0	7		7
Tillabéry	Daikaïna	10	0	0	0	10		10
	Toula	11	0	0	0	10	1	11
Say	Say	10	0	0	0	9	1	10
Sinder	Gourey Bio	1	2	2	4	9		9
	Sawani	0	2	2	3	7		7
	Soudani	3	0	3	0	6		6
N'Dounga	N'Dounga	10	0	0	0	10		10
	Total	132	6	8	7	150	3	153
	Percentage	(86.3%)	(3.9%)	(5.2%)	(4.6%)	(98%)	(2%)	(100%)

A Few farmers (15.7%) use organic manure. No fertilizer was applied to lowland rice. Farmers used rice varieties with cycles ranging from three to seven months, depending to the variety and the agrosystem. Long cycle varieties were found in lowland deepwater agrosystems. Rice was harvested with traditional serrated knives by 79.7% of the farmers, whereas 20.3% used sickles. Traditional granaries were still being used for storage by 31.4% of farmers, whereas 68.6% stored their harvest in rooms in their houses. The majority of farmers (52.2%) reported postharvest losses, due mainly to rats (61%), termites (13.4%), mould (8.5%), combinations of those three factors (13.4%) and thieves (3.7%). Besides rice, the majority of farmers grew pearl millet (88.2%), sorghum (44.4%), beans (23.5%), onion (10.5%), sesame (9.8%), maize (7.2%), and tobacco (3.9%). Onion, sesame and tobacco were planted mainly as cash crops.

2.4.3. Farmers' seed source and variety preferences

The three main sources of seed supply are summarized in Table 2.3. At the community level, the main source of seeds was found to be the local farmers' union, which represented 78.6%. The local farmers' union itself was provided certified seed by the farm of Saguia (57.2%) under the supervision of the ONAHA. However, nearly all the farmers' unions that where visited complained of the high level of contamination in seed bags from this source. The seed farm was supplied foundation seeds by INRAN, which is also in charge of varietal creation, testing and releasing of new breeding lines developed by the AfricaRice and partners, and controlling and certifying seed quality. The same percentage of farmers (64.2%) used seeds from their previous harvest or borrowed seeds from fellow farmers in the same village. Thus, using their own seed and exchanging of seeds by farmers within villages constituted the predominant informal seed systems, followed by seed exchange between villages (28.6%). On rare occasions, some farmers received seed directly from the INRAN, probably during Participatory Varietal Selection (PVS) processes, prior to varietal release. There was no evidence of any private seed company in the rice sector in Niger.

Table 2.3: Main seed sources of 153 farmers in the region of Tillabéry

	First Seed Source (%)	Second Seed Source (%)	Third Seed Source (%)	Total (%)
Certified seed (Saguia farm-ONAHA)	57.2	-	-	57.2
Local farmers' union	14.3	64.3		78.6
Previous harvest	21.4	14.3	28.6	64.3
Other farmer (same village)	7.1	21.4	35.7	64.2
Other farmers (different village)	-	-	28.6	28.6
INRAN	-	-	7.1	7.1

Local farmers' unions rely mainly on selected farmers for seed multiplication. Thus, 81% of the farmers recognized the presence of seed producers in their community. The number of seed producers ranged from zero, in Gourey Bio and Sawani, to 49 in Saga, with an average of 12 producers per union. However, none of the producers was found to produce seeds for lowland rice and floating rice. Surprisingly, there were no seed producers at the farmers' union of N'Dounga and very few at Namari Goungou, despite the presence and effectiveness of large scale

irrigated schemes there since 1976 and 1980, respectively. Among the interviewed farmers, nearly one in five (17 %) had participated in a seed production training. A Chi-square test performed on the data set showed significant difference (P = 0.04) between the proportion of farmers that attended, at least one seed production training and those without any formal seed production experience. None of the interviewed farmers at Daikaina and Toula had ever attended seed production training, despite the existence of local seed producers on irrigated farms (Figure 2.2). The farmers' unions of the irrigated farms of Diomona, Say and Saga had the highest number of trained seed producers, while the farms of N'Dounga had no trained seed producers. The seed production training was conducted either by the ONAHA (9.8%), the local farmers union (3.3%), the project "PAFRIZ" (2.0%), or the INRAN (1.9%).

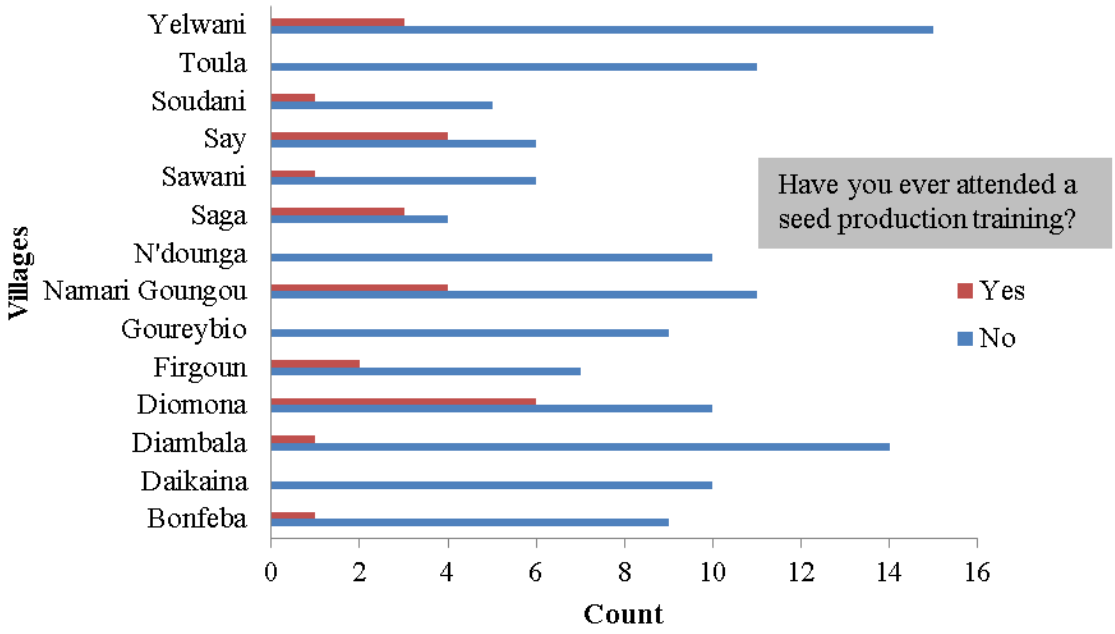

Figure 2.2: Number of farmers that attended seed production training per village in the Tillabéry Region, Niger Republic

The majority of farmers (59.5%) cultivated only one rice variety per season, 31.4% of the farmers cultivated two varieties, 8.5% three varieties and few farmers (0.7%) up to four rice varieties per season.

Depending on the agrosystem, some rice varieties were more frequently grown by farmers than others (Table 2.4). Most farmers in irrigated ecology preferred the released variety IR1529-680-3

(30.7%) and its derivative variety, Waihidjo, also called Kassimo, (35.3%). This variety is said to have been "breed" by a farmer from Darbani, a village near Diambala, in the district of Sakoira. Apparently, farmers adopted Waihidjo readily because of its phenotypic similarity with IR1529, distinguished only by the presence of awns on the grains. These two varieties were cropped on 15.8 and 17% of the surface of the interviewed farmers' fields.

Table 2.4: Variety status, area planted to the variety and species of the main rice varieties grown in the region of Tillabéry, Niger Republic

Rice varieties cropped	Status[1]	Frequency (%)[2]	Area planted (%)	Species	Agrosystem
Waihidjo (Kassimo)[3]	NR	35.3	17.0	*sativa*	irrigated
IR1529	R	30.7	15.8	*sativa*	irrigated
Degaulle/ D52	L/R	17.6	23.7	*sativa*	lowland
NERICAL 39	R	13.7	7.5	*sativa*	irrigated
Gambiaka	R	9.2	4.5	*sativa*	irrigated
Kardjikoyo[3]	NR	7.2	3.4	*sativa*	irrigated
WITA 8	R	6.5	3.7	*sativa*	irrigated
Mala	L	5.9	7.6	*glaberrima*	lowland
Dogonbaraw	NR	5.2	4.4	*sativa*	both
NERICAL 49	R	4.6	2.1	*sativa*	irrigated
Barkanaye	L	3.3	2.6	*glaberrima*	lowland
Hara Massay	L	2.6	2.6	*glaberrima*	lowland
WITA 9	R	1.3	1.1	*sativa*	irrigated
Maï alewa	NR	1.3	0.6	*sativa*	irrigated
Pontompormi	R	1.3	0.9	*sativa*	irrigated
Bassirou Mo[3]	NR	1.3	0.4	*sativa*	irrigated
Motafiya	NR	0.7	0.2	*sativa*	irrigated
Soumana Gourara	R	0.7	0.2	*sativa*	irrigated
Mo Aro	NR	0.7	0.2	*sativa*	irrigated
Locki	NR	0.7	1.0	*sativa*	irrigated
TGR 48	R	0.7	0.5	*sativa*	irrigated

[1]Status: NR: not released, R: officially released, L: landrace
[2]Total more than 100 because of multiple responses.
[3] Varieties “bred” by a local farmer

Similarly two other varieties, Kardjikoyo and Bassirou Mo, "bred" by other farmers, were grown by 7.2 and 1.3% of the farmers, respectively. It is important to highlight that no agronomic or resistance studies have been conducted on any of those three farmers' varieties, despite their

importance on irrigated perimeters. The newly released lowland New Rice for Africa (NERICA) varieties NERICAL39 and NERICAL49 were grown by 13.7 and 4.6% of the farmers on 7.5 and 2.1% of the surface.

Two varieties WITA 8 and WITA 9, released in 1997 because of their tolerance to the *Rice yellow mottle virus*, were grown by 6.5 and 1.3% of the interviewed farmers, respectively on 3.7 and 1.1% of the surface. The lowland agrosystem was largely dominated by the variety Degaulle, with 17.6% of the farmers and 23.7% of the lands cultivated by the farmers interviewed. In addition, the lowland agrosystem was also cropped to three *O. glaberrima* landraces, namely Mala, Barkanaye and Hara Massay.

Farmers were asked for their reasons for the choice of two of their most recently adopted rice varieties. Results are summarized in Table 2.5. Grain yield was found to be the main target trait for adopting a new rice variety by farmers (33.5%), followed a combination both high yield and good taste (10.2%), novelty (9.2%) and good taste (8.7%). High market value rice was also appreciated (5.4%), along with good milling quality (4.9%). Only 2.9 and 3.9% of the farmers accepted new rice varieties due to resistance to diseases and flood, respectively.

Table 2.5: The different reasons considered by farmers in adopting new rice varieties in the region of Tillabéry, Niger Republic

Reasons for adoption	Percentage of farmers
High Yield	33.5
High yield + good taste	10.2
Novelty (newness)	9.2
Good taste	8.7
High market value	5.4
Good milled quality	4.9
Flood tolerant[1]	3.9
Heritage[1]	3.4
Disease tolerant[2]	2.9
Bird tolerant	2.4
Adapted to agrosystem[1]	1.5
Short Cycle[2]	0.5

[1]: Reasons specific to lowland agrosystem; [2]:Reasons specific to irrigated agrosystem

Some lowland farmers (3.4%) continued growing varieties due to heritage (they inherited them from their parents) while few others (1.5%) grown deepwater floating rice varieties due of their adaptation to this agrosystem.

Similar to the questions about rice varieties grown in the region of Tillabéry, each farmer was asked about the two rice varieties abandoned from cultivation. As shown in in Table 2.6, IR1529-680-3 was the most abandoned variety, with a frequency of 34.6%, followed by WITA 8 (18.3%) and Waihija at17.6%.

Table 2.6: The frequency of rice varieties abandoned by farmers in the region of Tillabéry, Niger Republic

Variety	Species	Status	Cited as 1st abandoned variety (%)	Cited as 2nd abandoned variety (%)
IR1529-680-3	*sativa*	R	34.6	21.0
Waihija / Kassimo	*sativa*	NR	17.6	12.8
NERICAL 39	*sativa*	R	5.2	4.6
Gambiaka	*sativa*	R	0.7	2.3
WITA 8	*sativa*	R	18.3	20.9
NERICAL 49	*sativa*	R	-	2.3
Dogonbaraw	*sativa*	NR	-	2.3
Degaulle / D5237	*sativa*	R/ L	2.7	1.2
Mala	*glaberrima*	L	2.6	-
WITA 9	*sativa*	R	3.9	9.3
Kardjikoyo	*sativa*	NR	-	1.2
Barkanaye	*glaberrima*	L	3.3	1.2
BG 90-2	*sativa*	R	2.6	5.8
Hainia	*glaberrima*	L	1.3	5.8
Daddari	*glaberrima*	L	1.3	2.3
Pontompormi	*sativa*	NR	0.7	2.3
Hara Massay	*glaberrima*	L	0.7	1.2
Bazaye (IR 54)	*sativa*	R	2.6	1.2
TC 10	*sativa*	R	-	1.2
Locki	*sativa*	NR	2.0	1.2

R: released, NR: not released, L: landrace

It was followed by the RYMV tolerant variety WITA 8 (18.3 and 20.9%). In addition to this, Waihidjo, the derivative of IR1529-680-3, was also rejected by 17.6 and 12.8% of the participating farmers.

Surprisingly the newly released interspecific lowland varieties NERICAL39 (5.2 and 4.6%) and NERICAL49 (2.3%) were also cited as recently abandoned varieties. The old high yielding but RYMV susceptible BG 90-2 was also abandoned by 2.6 and 5.8% of the farmers. The variety Gambiaka held the smallest proportion of abandonment among varieties released in the area during the last 10 years.

Similarly, a few lowland varieties were abandoned recently, probably because most of them were already being abandoned from thirty years ago, with the creation of large irrigation schemes.

Farmers were also asked to cite two reasons leading them to shift from the varieties they had been growing to the new varieties. Results are summarized in Table 2.7. The susceptibility of a variety to disease was cited by 27.6% as the first reason and by 22.1% as second reason for changing it. Two varieties, IR1529-680-3 and BG 90-2, were cited as high yielding but disease-susceptible varieties. Similarly, some farmers (mainly in Bonfeba, Namari Goungou, and Diambala) cited disease-susceptibility as the main reason leading them to abandon NERICAL39 and NERICAL49, after just one season of testing. The second reason for changing a rice cultivar was the availability of a new one with a higher yield (18.6% and 11.6%). However, 11.2% and 9.3% of the farmers declared themselves capable of abandoning their varieties just to test novelty. That finding confirmed the influence of local farmers' unions in varietal testing and acceptance by farmers. Some varieties were abandoned by farmers because of seed degeneration and failure to renew the seed stock. Some farmers cited IR1529-680-3 and Degaulle as good examples of seed-degenerated varieties.

The variety Waihidjo, that was disseminated by simple seed exchange between farmers and found on 60% of the irrigated rice areas in the region of Tillabéry, was reported to have poor grain filling ability (5.9 and 10.5%), and had thus poor value after milling. Similarly, WITA 8 was rejected by several farmers' unions because of its poor milled quality (4.6 and 11.6%). This caused grain depreciation, with a resultant low market value, and hence showed itself opposite to the criteria of adoption of 1.5% of the interviewed farmers (Table 2.5).

Table 2.7: Reasons leading farmers to abandon their rice varieties in the Region of Tillabéry, Niger Republic

	Reason Abandoned 1st (%)	Reason abandoned 2nd (%)
Low yield	18.4	11.6
Disease susceptibility[2]	27.6	22.1
Bird susceptibility	2.6	4.7
Drought[1]	3.3	4.7
Red pericarp[1]	1.3	2.3
Too much straw[2]	1.3	2.3
High fertilizer requirement[2]	1.3	5.8
High shattering incidence[1]	1.3	2.3
Seed degeneration	9.2	2.3
Flood[1]	3.3	3.5
Long cycle[1]	0.7	1.2
Tasteless[2]	1.3	1.2
Bad grain filling	5.9	10.5
To test novelty	11.2	9.3
Rice intensification[1]	1.3	-
Bad milled quality[2]	4.6	11.6
Seed shortage[1]	3.9	2.3
Disease susceptibility + low high	1.3	2.3

[1]: specific to lowland agrosystem, [2]: specific to irrigated agrosystem

Other farmers abandoned WITA 8 and WITA 9 because of the relative importance of the straw, compared to the yield at harvest. Some other grain qualities like the colour of the pericarp and the taste were also identified by farmers of irrigated rice agrosystem. Red pericarp colour was cited as a rejection criteria (1.3 and 2.3%), while aromatic and low to medium starch were desirable. Finally, long cycle varieties (0.7 and 1.2%), and varieties requiring large quantities of fertilizer (mainly IR1529-680-3) were not appreciated by farmers in irrigated rice agrosystem.

In the lowland agrosystem, the main reasons for changing varieties were drought (3.3 and 4.7%), mainly at an early vegetative stage, before the raising of water levels. In addition to this, some farmers (3.3 and 3.5%) abandoned rice varieties that could not grow fast enough to match the rapid increase of water levels during the rainy seasons. Seed shortage due to food insecurity led

3.9 and 2.3% of the farmers to consume their seeds and thus be forced to "abandon" a lowland rice variety that they still preferred (Table 2.7).

2.4.4. Farmers' perception of RYMV and other major constraints to rice production

The main constraints to rice production as perceived by farmers' communities in the region of Tillabéry are summarised in Table 2.8. The perception of constraints varied significantly between agrosystems, as shown by a Chi-square test ($\chi 2$ = 30.12, df = 18 and P = 0.03). RYMV was perceived by 31% of the farmers as the main disease in irrigated rice and also 33.3% across all agrosystems. Bacterial Leaf Blight (BLB) caused by *Xanthomonas Oryzae* species was cited as the second most important disease in irrigated ecosystems. Incidence of the whitefly due to *Aleurocybotus indicus* D. and S. (Homoptera, Aleyrodidae) and stem borers (*Chilo zacconius* B., Lepidoptera, Pyralidae) were perceived by farmers (10.3%) to be equally serious problems in irrigated agrosystems. Birds, spiders, salinity and other damage-inducing factors, were cited as minor constraints.

In the traditional lowland agrosystem, birds, hippopotamuses and natural disaster (drought and flood) were perceived as contributing equally to reduce rice production. Across all agrosystems evaluated, stem borer and RYMV were perceived to be more damaging than to birds, BLB and hippopotamuses.

Table 2.8: Main constraints on rice production as perceived by farmers in Tillabéry,Niger Republic

Main constraints	Irrigated agrosystem	Lowland agrosystem	Across agrosystems	Total% within agrosystem
Birds	6.9	33.3	11.1	9.8
BLB	27.6	-	11.1	22.0
Borers	10.3	-	33.3	14.6
Drought/ Flood	-	33.3	-	2.4
Hippopotamus	-	33.3	11.1	4.9
RYMV	31.0	-	33.3	29.3
Salinity	3.4	-	-	2.4
Spiders	3.4	-	-	2.4
Unknown	6.9	-	-	4.9
Whitefly	10.3	-	-	7.3

χ^2 = 30.12, df = 18 and P = 0.03. BLB: bacterial leaf blight, RYMV.

Some characteristics such as local names, main symptoms, period of infection or infestation, causes, and management strategies of biotic and abiotic stresses as cited by farmers are summarised in Table 2.9. Overall, farmers were aware of stresses affecting rice (as revealed by the description), as well as the period of infection/infestation. Concerning diseases (RYMV, BLB), the local naming refers usually to a visual description of the resulting symptoms. However, the causes of the disease were not accurate and no farmers mentioned a description of bacteria or viruses during the survey. More surprisingly, only two out of eleven heads of local agricultural offices (ONAHA) interviewed, mentioned the word "virus" and "bacteria" when describing the causal agent of the two most important diseases. Like the farmers, they cited "worms", either in the soil or in the plant, to be the cause of RYMV and BLB. Consequently, when contacted by farmers about RYMV or BLB in their fields, they systematically recommended using pesticides (mainly Dimethoate and Furadan). The latter was banned from the European Union, partially in the United Sates and possibly in Kenya for health and environmental reasons (http://en.wikipedia.org/wiki/Furadan). This inappropriate practice also increased production costs for farmers, and thus reduced their incomes.

Table 2.9: Summary of main biotic and abiotic constraints on rice production as described by farmers in the region of Tillabéry, Niger Republic

Constraint name	Effect on rice	Causes	Time of infection	Control measures	Local names (LN)	Translation of LN
Birds	Suction of spikelets	Birds, drought	Milk stage, maturity	Guardians (two rice bags/guardian/season	Sassa, Tsounsayé	Birds
Bacterial Leaf Blight (BLB) (*Xanthomonas Oryzae s*pp.)	Drying and rotting of leaves, milky drops	Soil worms, unknown	Tillering, booting	Apply ash, pesticides, elimination of diseased-plants	Bouharora, Wiharora, Goura	Kill in the water
Borers *(Chilo zacconius B.)*	Srying of panicles, rotting of the "heart"	Plant worms, butterflies	Booting, milk stage	Generally none, sometimes pesticides	Djindegoro, Bon kwaraye, kofo, Goro-goro, Kolo-wiyo	Neck sting, white head, "heart killer"
Drought	Drying and death of seedling	God	Seeding, tillering	Late sowing	Kogay	Drought, lack of water
Flood	Rotting of plants and death	God	Seeding, tillering	Early sowing	Hari ngayan	"Eaten" by water
Hippopotamus *(Hippopotamus amphibious* L.*)*	Destruction of fields, feeding of plants	Overprotected by the government	From tillering to harvest	Guardians (60 USD /guardian/sector	Ba nga	Hippopotamus
Pests	Injuries to leaves and spikelets	God, good rainy season	Booting, milk stage	Generally none, sometimes pesticides	A nga riya, doyzo, fara	Insects, grasshopper
***Rice yellow mottle virus* (RYMV)**	Yellowing of leaves, height reduction, blackish and empty panicles	Worms, high humidity, "white fertilizer" (urea), unknown	Tillering after application of urea	Apply ash from millet glume and glumelles, drying fields, pesticides, elimination of diseased-plants, sometimes none, inefficient	Ola, Olalo, Tiguiro, Larabo, Dori Sayo, Tchoukki, Bellayze tchira	The yellow one, the dwarf, the Arabic man, the Touareg man, the one with light complexion
Salinity	Discoloration and death of leaves, yield reduction, low tillering	Salt in the soil	Anytime	Apply millet glume and glumelle, organic manure	Sosso	Bicarbonate
Spiders *(species unknown)*	Trap seedling, reduce vigour	God	Seedling	Destruction of cobwebs, none	Dadara	Spider
Unknown	Whitening of seedlings' leaves	Unknown	Seedling	Apply ash, usually none	Kofa	Unknown
Weedy rice *(species unknown)*	Invades fields, seed degeneration	Transformation of rice over time to wild rice	Anytime, but visible at heading	Elimination of invasive plants (usually young farmers could not differentiate from rice)	Sombay	Wild rice, *O. barthii*
Whitefly *(Aleurocybotus indicusD. And S.)*	Honeydew on leaves and sheath	Flies, high density transplanting,	Booting	Apply ash, drying fields, sometimes none	Katou, You	Honey

Besides consulting local agricultural offices, farmers also used local knowledge such as applying ash, applying millet glumes and lemma, and organic manure for disease management. Individually, a large majority (90.2%) of the interviewed farmers had experienced RYMV in their rice crops, thus a significant difference in farmers' experience of RYMV was found (Table 2.10). The survey showed that RYMV was more important on the irrigated perimeter under the control of the ONAHA (96.2%), followed by the lowland (83.3%) and finally the private irrigation sector (71.4%). Contrary to this, the incidence was lower in floating rice ecologies (12.5%).

Table 2.10: Percentage of farmers (%) who experienced RYMV in each ecology in Tillabéry, Niger Republic

		Have you experienced RYMV?		Total (%)
		NO (%)	*YES (%)*	
Agrosystems	Irrigated (ONAHA)	5 (3.8)	127 (96.2)	132 (100)
	Lowland	1 (16.7)	5 (83.3)	6 (100)
	Floating	7 (87.5)	1 (12.5)	8 (100)
	Irrigated (Private)	2 (28.6)	5 (71.4)	7 (100)
Total (%)		15 (9.8)	138 (90.2)	153 (100)

$\chi^2 = 63.12$, df = 3 and $P \leq 0.001$; Lowland was split into floating and lowland, while Irrigated was split into the large perimeter under the ONAHA, and private irrigation.

For the majority of farmers (91.6%), the disease symptoms started just after the application of urea, about 15 to 30 days after transplanting, corresponding to the tillering stage. Table 2.11 summarizes the common recognized symptoms of RYMV. Most of the farmers cited the yellowing of the leaves (65.9%), explaining the naming of the disease, Larabo or Olalo, meaning "the Arabic" or the "yellow one". Some farmers, named it after the second more cited symptoms, the stunting (15%), Tiguiro or Tchoukki meaning "the one that is static", thus "the dwarf".

Several farmers cited the aquatic grass *Echinochloa pyramidalis* Hitchc. & Chase (called "Bourgou" in Sonraï language), the two wild rice species *O. barthii* ("Sombay") and *O. longistaminata* ("Baou"), and rice crop debris as hosts of the disease. For some, the disease was caused by "worms", humidity, the white fertilizer (urea), and for others, God. For 34.8% of the farmers, the disease usually started on plants near the field's borders, while 26.8% did not recognize any pattern to the epidemic. For a few farmers (8%), the disease started around the

irrigation channels near the irrigation riser pipes, while 15.2% cited the middle of the field as the starting point. The same proportion found that the epidemic started at the place in the field where the previous harvest was threshed or the lowest point of the field where water usually stagnates.

Table 2.11: Farmers' descriptions (%) of RYMV symptoms in the Tillabéry Region, Niger Republic

Symptoms	Percentage of farmers (%)
Yellowing	65.9
Stunting	15.0
Panicle sterility	8.1
Black spikelets	3.7
Shortened leaves	2.9
Low tillering	1.5
Poor panicle exsertion	1.5
Infectiousness	0.7
Drying of leaves	0.7

In order to assess farmers' awareness of the probable implications of pests in the transmission of RYMV, they were asked about the period of high pest prevalence in the field. 85.6% of the farmers noticed high pest population during the rainy season, compared to the dry season. Moreover, 86.3% of the farmers noticed that pest populations were more important in irrigated fields than to lowland fields. Additionally, only 19% of the farmers took action against pests by applying pesticides on the advice of the local ONAHA extension officer. The remaining declared that they had not seen the necessity of action. Although 95.1% of the farmers noticed insects feeding in seedbeds, only 11.5% of the farmers linked the rise of RYMV epidemics to pest proliferation. The majority declared no correlation between RYMV appearance and pest populations. They cited previous crop debris, bad field and channel cleaning, early sowing, heat, high humidity, high levels urea, organic manure, weeds, etc. Some even cited God, reasoning that epidemics can arise randomly.

Farmers were then questioned about, their management of seedbeds. A large majority (98.6%) have never changed the place of their seedbeds. 80.5% abandoned the remaining rice in the seedbeds until the next season. Only 9% claimed they would destroy these plants and clean the seedbed.

Once RYMV symptoms were spotted in a field, 56.9% of the farmers took some action, including drying the field, then applying ash, destroying diseased plants, or simply notifying the ONAHA extension officer, who generally recommended applied Dimethoate or Furadan. The remaining farmers admitted to taking no action either because they deemed it unnecessary or hopeless. Indeed, yield losses due to RYMV were estimated to range from 50 to 60% and 55.1% of the farmers, while 13.2% estimated it to range between 70 and 80%. Thus, contrary to curative action, nearly all farmers (97.8%) agreed to prevent RYMV, after a season of attacks. Fields were cleaned, early-ploughed and dried (57.8%) to eliminate the disease. For 20% of the farmers, stubble-burning of previously infected sites as well as threshing sites is necessary to prevent a new attack (Table 2.12). However, a few farmers (2.2%) have no prevention strategies. The failure to use varietal resistance as an RYMV prevention strategy was confirmed by the fact that only 2.8% of the farmers declared having tested a cultivar primarily because of its tolerance to RYMV.

Table 2.12: Percentage of farmers employing RYMV management strategies

RYMV management strategy	Percentage of farmers adopting prevention strategy (%)
Field drying and cleaning between two cropping	32.3
Early ploughing and drying	25.5
Apply organic manure	13.3
Stubble-burning infected places and threshing places	20
Apply pesticide before transplanting	6.7
No prevention strategy	2.2
Using resistant varieties	0

2.5 Discussion

2.5.1. Position of information and gender in the rice sector

Participatory Rural Appraisal was successfully used to study farmers' perception of several agricultural issues in many countries (Manu-Aduening et al., 2007; Efisue et al., 2008; Kiros-Meles and Abang, 2008). In this study, very few women were represented in the 153 rice farmers interviewed in semi-structured discussions and individual questionnaires. This is due to the fact that women in Niger, unlike in Mali (Efisue et al., 2008), are not involved in rice cultivation, but are principal actors in the post-harvest activities, processing, parboiling and selling as was also

observed in Benin (Zossou et al., 2010). The few women in our study had been exploiting their rice fields for less than 10 years and use daily workers. Farmers relied mostly on well-organized local unions for agricultural information, input supply and access to credit. Unfortunately, those local farmers' unions lack motivating and innovative methods to continuously remind to farmers of crucial information on rice production improvement, with respect to the sowing calendar, use of certified seeds, transplanting densities, fertilizer doses and periods of application, good cropping practices, etc. In Mali, on the other hand, no Non-Governmental Organisations (NGOs) were found to assist local farmers' unions in disseminating relevant information (Efisue et al., 2008). However, the farmers unions are supported by innovative information and communication media such as learning videos and radio broadcasts that have been developed and made available by AfricaRice and its partners, to facilitate technology transfer to African farmers (Van Mele et al., 2010). The efficiency of these tools was established in improving rice cultivation in Mali and Bangladesh (Van Mele, 2006; Bentley and Van Mele, 2011) and also in enhancing rice parboiling capacities of women in Benin (Zossou et al., 2009). Besides improving farmers' livelihoods, such tools could benefit rice farmers in Niger in terms of environmental and health awareness, especially regarding the excessive use of fertilizers, herbicides and pesticides, in a context of global water scarceness. For example, resistance to Londax, the main herbicide used by Tillabéry rice farmers, has been reported on different weeds in California, including the smallflower umbrella sedge, the ricefield bulrush, and the Californian arrowhead (UCDavis, 2010). This is an indicator of problems that may occur with this herbicide in Niger in the future.

2.5.2. Seed sector and varietal preferences

The seed farm of Saguia, under the responsibility of the ONAHA, is the main and only source of certified rice seed for local farmers' unions, themselves united into a federation of rice farmers' unions of Niger. In contrast to the Central valleys of Oaxaca in Mexico, where no formal maize seed supply exists (Badstue et al., 2006), in Tillabéry, each local union relied on its own seed producers for seed multiplication and delivery. It is clear that the lack of other seed companies competing with the Saguia Farm has had a negative impact on the rice seed sector of Niger. However, this monopoly cannot be the sole explanation for the shortage of certified seeds to reach small scale farmers in Niger. Despite the existence of a formal institutional frame composed of extension workers, farmers, farmers' associations, agro-input dealers, researchers and NGO's, improved seed has also failed to efficiently reach small-scale farmers in Guinea

(Okry et al., 2011). Similarly to Guinea, the rice seed sector in Niger has undergone changes during the last twenty years, from state-led intervention, to farmers' associations-led intervention, but with less impact than expected. In addition to the lack of competition, this situation is affected by the shortage of qualified seed inspectors, and a clear and strict national seed policy to ensure access to quality seed for smallholders. Furthermore, the promotion and creation of small private seed enterprises would gradually enhance farmers' access to seed. Such a course of action was adopted in northern Cameroon, where farmers were trained both for seed production and enterprises management, and were linked to the national extension services for regular counselling (Guei et al., 2011). Consequently the national seed production nearly doubled and; the yield of rice rose fourfold. If implemented in Niger, this revitalized seed system would complement the local informal seed system, as pointed out by Almekinders et al. (1994).

Understanding farmers' perceptions of rice varieties and varietal preferences are crucial in order for breeding to have the expected impact on communities' development. Farmers in the irrigated agrosystem of Tillabéry preferred rice variety was IR1529 mainly because of its high yield. Additionally, this variety was said to have good grain quality (good taste and milling quality), and thus a high market value. Such characteristics met the goals of both the producers (in this case male farmers, who were willing to pay production charges and get benefits) and the post-harvest processors (in this case female traders). In some regions, rice sector actors did not find as many desired traits in one variety. Rice farmers and processors in the Long region of Vietnam preferred varieties IR1529-680-3 and IR50404, the first because of its grain quality and culinary properties and the second for its yield (Ngoc Chi et al., 2007). The variety IR1529-680-3 was also cited as the most abandoned recently, mainly because of its high susceptibility to RYMV and its high input requirements. Indeed this variety has been identified as one of the main causes of RYMV epidemics in Niger (WARDA, 2001). Participatory Varietal Selection (PVS) is a powerful tool for rapid delivery of promising varieties to farmers (Joshi and Witcombe, 2002). This tool was used to deliver both WITA8; an RYMV tolerant variety, and interspecific NERICAL39 and 49 to farmers. However, the impact of WITA8 was mitigated due to a high frequency of grain breakage during milling, while the interspecific lowland NERICAs faced disease susceptibility problems. The PVS process should be improved and if doubts persist, a pilot scale distribution of the variety could be introduced in the system. The farmers' informal

seed system will then disseminate or reject it, as was successfully done for upland rice in Ghana (Dorward et al., 2007).

2.5.3. Perception of main constraints on Rice production and RYMV in Niger

Farmers' perception in Niger of the main constraints on rice production were consistent with the findings of other studies (Reckhaus, 1983; Abo et al., 1998; WARDA, 2001; Traoré et al., 2009) and identified RYMV and BLB (Rechans,1983; Reckhaus and Adamou,1986,1989) as the main diseases of irrigated rice agrosystems in Niger, followed by pests (whitefly and stem borers), while birds, hippopotamus and drought/flood were the main constraints in the lowland agrosystem. Some of theses threats to rice production were previsously reported for other African rice agrosystems by Balasubramanian et al. (2007). In the Gambia, hippopotamuses were reported to have been destructive to important rice fields (Jawo, 2010). In Tillabéry too, hippos were cited among the most destructive ravager of rice fields. In this study farmers also listed pests such as stem borers, spiders, and whitefly as factors limiting rice production. Indeed, stem borers and whiteflies have been reported as major insect pests in Mali, Niger and other West African countries (Heinrichs and Barrion, 2004). Pests have also been reported to limit food production of other crops such as vegetables in Botswana (Obopile et al., 2008) and cassava in Ghana (Manu-Aduening et al., 2007). As with farmers in Botswana, rice farmers in Niger relied on pesticides to control crop pests. Finally, the lack of water or its excess caused serious food insecurity in Niger, for example in 2010 5,300 ha of crops were destroyed by flooding (UN, 2010).

Although, the cause of the RYMV remained a mystery to farmers, their naming of the disease was consistent with scientific naming despite several synonyms (all derived from the two main characteristics of the disease "yellowing" and "stunting"). On the contrary, farmers in ten villages in Ghana have no clear and constant naming for Cassava Mosaic Disease (CMD) (Manu-Aduening et al., 2007). The periods of appearance, as well as the symptoms were consistent with the summary of RYMV's symptoms as compiled by several authors (Bakker, 1970; Abo et al., 1998; Banwo, 2003). However, farmers had not worked out the relationship between the pests and RYMV. However, they recognized the role of weed and wild rice species in the disease cycle. Similarly, cassava farmers of Ghana did not recognize the vectoring role of the whitefly *Bemisia tabaci* G. (Homoptera, Aleyrodidae) to the CMD, but cited other insects (Manu-

Aduening J.A., Lamboll R.I., Mensah G.A., Gibson R.W. (2007). In Tillabéry, some of the methods of transmission of RYMV were well understood and priority was given to prevention through stubble burning and cleaning. However, the role of rice seedbeds was neglected, and the same ploughs were used in a given area. The farmers were not aware of the involvement of cows, donkeys and rice seedbeds in the movement and persistence of the disease, despite other sanitation practices (Sarra and Peters, 2003; Sarra et al., 2004; Traoré et al., 2006).

Although the informal seed sector is active, the formal seed sector should be restructured, opened to NGOs and small private enterprises and a national seed policy implemented. Additionally, the staff of the seed farm of Saguia, seed producers of local farmers' association, as well as seed inspectors, should be trained in seed production and policies.

Prior to any PVS, rice researchers should screen promising lines in controlled conditions against the main diseases (RYMV and BLB), so that susceptible lines can be discarded. Those lines, as well as popular varieties, IR1529/Waihidjo and Degaulle/ D5237, should be bred for resistance to RYMV and BLB. In addition, Degaulle/ D5237 could also be improved to withstand flood, by introgressing the gene SUB1 (Xu et al., 2006). If varieties "bred" by one farmer, are widely adopted by fellow farmers and become popular, they deserve the attention of researchers for basic study, including genetic studies on their resistance to disease and pest, agronomic requirements, etc.

Rice farmers in the region of Tillabéry are aware that RYMV is the main disease that poses a threat to their production. Moreover, they recognize the importance of good cropping practices and apply some prevention methods. However, more training on diseases, integrated pest management and environmental issues is needed for both farmers and ONAHA extension officers.

If implemented, these recommendations may contribute towards increasing rice production in the region and improving farmers' livelihoods.

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Appendix 2.1: Summary of the Questionnaires used during the PRA

A- Group discussion questionnaire for the community in each village (2 pages):

I. Varietal diversity in the village and main seed sources

In this section farmers were asked to provide information on the varieties they knew: name, status (still cultivated or not), type of variety, duration of the variety in the village, cycle and characteristics, seed sources, presence/absence of seed producers in the village, seed training or not

II. Farmers perception of major constraints to production

In this section farmers were asked to name, described and give information (causes, actual methods of management, stage of development) of the main constraints to their production

B- Individual questionnaire for farmers (4 pages):

I. General information on cultural practices (date and type of sowing, ecology, size of the field, type and quantity of fertilizer applied, etc.)

II. Socio-economic information (gender, age, ethnical group, educational level, membership, source of agricultural information, activities, etc.)

III. Varietal diversity (source of seed, training in seed production, number of varieties cropped per season, 3 last varieties adopted and 3 last varieties abandoned and reasons

IV. Perception main diseases and RYMV (local names, description, causes and stage of development of main diseases known by the farmer, etc. RYMV experience, causes, main symptoms, period and place of apparition in the field, period of insects' presence, which ecology, management options, correlation with symptoms, sowing type, varietal attitude in relation to RYMV attacks, etc.

V. Farmers' preferred traits in rice (preferred traits naming, justification and pairwise ranking).

C- Questionnaire for heads of irrigated schemes (3 pages)

I. Varietal diversity in the village and main seed sources

In this section, heads of irrigated scheme were individually questioned about the varieties that have been grown or still being grown on their areas: name, status (still cultivated or not), type of variety, duration of the variety in the village, cycle and characteristics, seed sources, presence/absence of seed producers in the area, seed training or not supplied to the farmers.

II. Perception of major constraints to production

In this section heads of irrigated schemes were asked to name, described and give information (causes, actual methods of management, stage of development, local names) of the main disease on their irrigated areas. They were also asked about the availability or not of training to farmers about main disease about which farmers are usually seeking advice from them. Finally, heads of irrigated schemes were asked to cite the bad cultural practices they often notice from farmers in their area and give any useful information to improve this survey.

Chapter 3: Agro-morphological variability of rice species collected from Niger

3.1 Abstract

A collection of rice species from Niger was undertaken in all the rice growing regions and agrosystems of the country. A total of 270 rice accessions were assembled, which included 177 accessions of *O. sativa,* 65 *O. glaberrima,* 27 *O. barthii* and 1 *O .longistaminata*. The periphery of the Niger River and its tributary, the Dallol Maouri, provided the majority (80.7%) of the accessions. Many of the accessions found around the Lake Chad and the Komadougou River (South-East) were already included in those from the Niger River, except for some wild *O. barthii* accessions. Drought, insects, weeds, birds and the diseases such as *Rice yellow mottle virus* and bacterial leaf blight (BLB) were the most cited constraints to rice production. The naming of rice varieties by farmers was generally consistent within regions, but rarely across regions, as well as their classification of rice varieties by agrosystem suitability.The agro-morphological characterization of the collection pointed out three phenotypic groups where *O. longistaminata*, floating *O. glaberrima* and late maturing floating *O. sativa* clustered in one group. The second group included lowland *O. barthii* and *O. glaberrima* accessions, while the last was a mixture of irrigated *O. sativa* and lowland accessions of the two cultivated rice species. The overall phenotypic variability of the collection, as measured by the Shannon-Weaver diversity index was relatively high (H' = 0.69). There was no significant difference in diversity between main eco-geographical zones of collection or between the identified phenotypic groups, suggesting a substantial germplasm exchange between regions in Niger.

Keywords: genetic resources, rice, Niger, phenotypic characterization, collection

3.2 Introduction

Globally, plant breeding has played an important role in the yield increase and quality improvement of many crops essential to human and animal nutrition. However, the widespread adoption rate of modern cultivars, combined with changing patterns in cropping, such as changes in cultural practices, crop intensification and sometimes natural disasters, tend to induce erosion of genetic diversity of cultivated crop species. This threatens long term global agricultural production (Hammer and Teklu, 2008). Indeed, preserving the genetic diversity of plants is important for the survival of species as well as their evolution in a rapidly changing environment, but also for small-scale farmers to be able to produce food under various climatic, biotic and

edaphic factors. Additionally, that observed diversity is essential for future breeding programs (Gao, 2003). Generally, genetic erosion refers to the quantitative reduction of discrete representatives of a species in a region or ecosystem (Solbrig, 1991). However, for plant breeders, genetic erosion refers to the decrease of genetic variability, due to the loss of entities carrying specific genes or alleles (Esbern, 1999). By extension, it could also be defined as the disappearance of named varieties in regions or ecosystems where they have been reported previously (Hammer et al., 1996).

Two methods are being used to prevent the loss of genetic variability in crop species:

- *In-situ* conservation, based on farmers' involvement, has the advantages of keeping the species in their natural ecosystems or appropriately managed ecology. In that way, the crop species will evolve in their local environment and will thus undergo natural selection and adaptation (Tin et al., 2001);

- *Ex-situ* conservation, which involves collecting and storing seeds, cultured tissues or cloned DNA fragments in a genebank. However, seed collection is the most commonly used. Based on the duration of the conservation and the use of the material, a base collection, an active collection or a working collection could be established (Balick, 1991).

To support the global effort of plant genetic resource conservation, several world collections were developed for various crops, including pigeon pea (Reddy et al., 2005), and pearl millet (Bhattacharjee et al., 2007). Additionally, several national and regional collecting expeditions have been undertaken in several countries. These have included both cultivated crops (Lasa et al., 2001; Reddy et al., 2005; Mahalakshmi et al., 2007) and related wild species (Nooryazdan et al., 2010; Shakhatreh et al., 2010). These collections were evaluated for agro-morphological traits, documented, stored and are now being used to broaden breeding programs.

Likewise, collections have been conducted in many countries and regions to collect, evaluate and store rice genetic resources. Only two of the 23 species comprising the genus *Oryza* are cultivated (Vaughan et al., 2003), while the remaining are wild species. However, because of their adaptation to harsh biotic and abiotic environmental conditions, these wild species bear traits that may be useful for the improvement of cultivated rice (Khush, 1997) and they have therefore also been included in the conservation of the genus *Oryza*. The cultivated Asian rice

Oryza sativa L. was domesticated in Asia, around 10,000 years ago from the wild annual O. *rufipogon*, followed by a later, differentiation into two main subspecies: *indica* adapted to tropical and subtropical floating, lowland and irrigated agrosystems, and *japonica* adapted to temperate and tropical upland ecosystems (Chang, 1984; Khush, 1997). These two main subgroups have been differentiated based on morphological traits such as grain shape, apiculus hair length, leaf width and colour, or through biochemical assays for reaction to phenol (Kovach et al., 2007). In addition, isozymes have been used to classify the species into six varietal groups with *indica* and *japonica* as major groups (Glaszmann, 1987). With the advent of rice genome sequencing, simple sequence repeats (SSR) and microsatellites have been used to divide *O. sativa* into five groups consistent with the structure obtained using the isozymes (Garris et al., 2005).

African rice, *O. glaberrima*, was domesticated 3,500 year ago in the inner delta of the Niger River and later in the West African coastal regions (Portères, 1956; Chang, 1976; Second, 1985). It was domesticated from the annual wild species *O. barthii*, itself derived from perennial wild species *O. longistaminata* (Sarla and Mallikarjuna Swamy, 2005). Several studies have concluded that African rice is less diverse than Asian rice (Chang et al., 1977; Second, 1985; Barry et al., 2007a). Nevertheless, three subgroups, including a floating photosensitive ecotype (adapted to deepwater agrosystem), a non-floating ecotype (adapted to rainfed lowland agrosystem) and an upright ecotype (adapted to rainfed upland agrosystem) have been identified in African rice accessions using SSR markers (Semon et al., 2005). While Asian rice is being cultivated in all other parts of the world, Africa is the only continent where the two cultivated rice species have been cropped together since the 15th or the 17th century (Linares, 2002). Major rice collections were assembled during the sixties in most African countries and seeds were stored with three major research institutions: IRAT-ORSTOM, IITA, IRRI and the Japanese Institute of Genetics (Oka, 1977). Recently, collection of cultivated rice species was conducted in West Africa (Barry et al., 2007b; Nuijten et al., 2009), but Niger was not included, although the country is the nearest bordering of the primary centre of diversification of *O. glaberrima*, on the downstream part of the Niger River. On the other hand, rice species in Guinea, Mali and Nigeria were collected, studied and stored in genebanks (Bezançon et al., 1977; Semon et al., 2005; Barry et al., 2007b). In addition, in 2007 research conducted online on the AfricaRice Genetic Resources Unit database, which revealed that only 32 accessions had ever been collected from Niger, (1982), but were no longer available from 2007 to 2010. Thus, Niger did not benefit from

the global effort to conserve local rice diversity *ex-situ*, although rice is the primary crop of people along the Niger River and other marshes from the central-south to the south-eastern part of the country around the Lake Chad and the Komadougou River. Moreover, major irrigated areas were constructed in the country, promoting high yielding *O. sativa* rice cultivars at the expense of *O. glaberrima*. Rice agrosystems in Niger include a diverse range, including rainfed lowland, deepwater flooding and irrigated (Bonkoula and Miezan, 1982). The present study was undertaken firstly, to create the first exhaustive collection of rice species from Niger, and secondly to evaluate the collection for agro-morphological traits, as well as identifying their geographical and ecological distribution.

3.3 Material and Methods

3.3.1. Germplasm collection

Seven of the eight regions of Niger were visited. Villages were chosen randomly along different eco-geographical zones where rice is cropped (Appendix 3.1). A distance of more than 2,000 km was travelled in the course of seed collection, largely by canoe and boat in the region of the Niger River, and by motorcycle and car in the others collection areas.

Four main zones were visited; (1) the zone of the Niger River; (2) the zone of the Dallol Maouri, a seasonal tributary of the river, in the south-west; (3) the central south zone along the marshes of Goulbi of Maradi, and Lake Madarounfa; and (4) the zone of Lake Chad and the Komadougou River in the south-eastern region of the country. In each zone three rice agrosystems are practiced, namely floating-deepwater (more than 1.5 m depth), rainfed lowland (less than 1 m depth), with semi-controlled immersion, and irrigated paddy rice with full water control.

The village as a finite entity was the basis of the sampling strategy. In each village a meeting was held with farmers and the community leaders (chief, mayor, religious leader, town councillor, etc), and the local representative of agricultural services and the farmers' cooperative. First, a comprehensive inventory of varieties known to farmers was made. From the list, each accession was called and two to four farmers were asked to bring seed samples hereafter referred to as "accessions". The community was then asked to identify the accession by consensus. If the samples brought were mixed, the farmers were asked to re-sample within the provided samples some seeds presenting the identified accessions. Farmers who brought the accessions were interviewed in public to complete the passport data of the accessions. If a listed accession was

recognized as disappeared or abandoned, questions were asked of the community about the reasons that led to the disappearance or abandonment. In addition to this, farmers were also asked to advise the collection team about another village where the absent accessions might be found. Generally, an identified accession was re-sampled again in one to two villages following its first appearance in order to confirm the name. However, if a name, already recorded, appeared later (in very distant villages, eco-geographical zones or ethnic groups), it was sampled again. Additionally, if an accession already identified and sampled appeared again under a different name in another village, it was re-sampled and all related information was taken again from the farmer. Overall 202 rice accessions were collected, of which nine samples of wild *O. barthii* were collected at sites far from any cultivated rice farm.

The collection was then brought to AfricaRice, where the seeds of each identified sample were visually separated from off-types to constitute "pure" samples for each identified accession. If the off-types derived from a sample did not correspond to any identified accessions, they were kept. The accessions were then coded using the initials of the region where they were collected, and followed by a number corresponding to the order of collection (e.g. TH3 corresponded to the third accession collected in the region of Tahoua, while DF13 was the thirteenth sample collected in the region of Diffa). The kept off-types were named after their samples code followed by an alphabetic letter (e.g. DS14-E was the fifth off-type in the fourteenth sample collected in the region of Dosso). Finally, 370 rice accessions, including 168 off-types derived from each sample, plus 5 checks were grown in the first trial in 2008 (a purification-characterisation trial). From the purification-characterization field, 270 accessions, comprising 202 accessions and 68 ‘unique’ off-types were selected for the second field trial in 2009 (characterisation trial). This study then focused on the 270 accessions, which represent the observed phenotypic diversity of rice in Niger, plus eight Control varieties, including four *O. sativa* varieties, comprising two irrigated *indica* (IR64, B6144), one traditional deep-water floating *indica* variety (RAM63), and one upland *japonica* variety (Moroberekan), together with two *O. glaberrima* varieties including the upland CG14 and the lowland TOG7106, and two interspecific varieties (NERICA14 (upland) and NERICAL41 (lowland)).

3.3.2. Field trials

The purified accessions, as well as all related unique off-types and seven control varieties (Total N= 370) were sown at the AfricaRice research station (Togoudo, Benin) during the rainy season of 2008. The field was regularly watered if there was no rain. An augmented experimental design with five replicated checks (ITA212, NERICA4, NERICA14, B6144 and TOG7106) in ten blocks was used. When enough seed was available, the elementary plot for each accession was 0.6 x 1.5 m with 3 rows, with a spacing of 200 mm between and within rows and a distance of 400 mm maintained between plots, at the rate of 3 plants per hill by direct seeding. Thinning to one plant/hill was done 20 days after sowing (DAS). Fertilizer rate used was NPK (15-15-15) at 200 kg ha^{-1} applied just after thinning. Urea was also applied at a rate of 100 kg ha^{-1} three weeks after thinning. Regular weeding was done when necessary. Data was recorded on five to 10 plants of the inner row from seedling stage to harvest. For each accession, panicles of five well-identified plants from the inner row were individually harvested and the remaining ten were bulk-harvested.

From the 2008 purification-characterization field, 270 accessions were selected after eliminating accessions that were similar in the field. Accessions and the eight control varieties were directly sown during the rainy season of 2009 in plastic buckets of 5 litre capacity at a rate of two plants per bucket. Buckets were laid out in a randomized complete block design (RCBD) with two replications. For each accession, three buckets was used per replication. Thus, a total of 12 plants were sown per accession. Fertilizers were applied as NPK (15-15-15) at 20 DAS, and Urea was applied at panicle initiation. The trial was watered during days without rain. Only 44 traits that were found discriminating our accessions, after a factorial analysis on the previous year's data set, were recorded on five plants per accession.

3.3.3 Data collection and analysis

All the data was recorded using a descriptor for wild and cultivated rice species (Bioversity International et al., 2007). During the trials 44 agro-morphological traits were measured (Appendix 3.2). A factorial analysis was performed on the data set, and the 34 traits that contributed the most to the different factors (when the correlation was > 0.45) were selected for analyses.

In addition to data collection, the accessions were classified into *O. sativa* L. or *O. glaberrima* Steud., based mainly on the ligules length and shape. *O. sativa* species have long ligules while *O. glaberrima* has short ligules (Sarla and Mallikarjuna Swamy, 2005). Similarly, awns length, texture and consistency, as well as grain length (Besancon, 1993) (Appendix 3.3) were used to classify the intermediate forms into *O. glaberrima* or *O. barthii*, in addition to the samples of *O. barthii* collected in wild populations far from cultivated fields.

A Pearson Principal Component Analysis (PCA) was performed on the standardized quantitative data (18 traits), followed by the assignment of the different accessions into groups by Agglomerative Hierarchical Clustering (ACH). Dissimilarities were computed based on the Euclidian distance and aggregation of accessions was based on the Ward method (Ward, 1963). Discriminant Analyses (DA) were conducted using groups identified by cluster analysis plus eco-geographical zones, agrosystems, and species as categorical variables. Thirty four traits (18 quantitative and 16 qualitative traits) were used. These analyses were performed using XL-STAT 2010 application package.

The diversity of phenotypic traits was estimated using the Shannon-Weaver Index (Shannon, 1948), computed under Microsoft Excel 2010 application package. The Shannon Index was computed of all the 44 traits. However, only the results from the 33 most expressive traits (15 quantitative and 18 qualitative traits) were presented. Quantitative continuous data were transformed into categorical data by creating for each phenotypic trait classes based on either the rice descriptors of Bioversity, IRRI, and AfricaRice, or as described by Sanni et al. (2008). When information was not available, three classes were created, based on the mean, median and quartiles. The Shannon Diversity Index H' was computed as:

$$H' = -\sum_{i=1}^{k} P_i \log_2 P_i$$

Where, k: the number of phenotypic classes for a character and Pi, the relative abundance of individuals in the ith class, calculated as the proportion of individuals in the phenotypic class i to the total number of individuals (N). However, to keep the value of the Shannon-Weaver Index between 0 and 1, H' was divided by its maximum value, $\log_2 k$ (Abdi et al., 2002; Sanni et al., 2008).

3.4 Results

3.4.1 Collection

A total of 270 rice seed samples were collected in seven regions of Niger. Fifty villages were visited, with 1 km distance between the two closest villages (Gatawani Béri and Gatawani Kaina in the region of Dosso, south-west) and around 1500 km between the two farthest villages (Koutougou, in the region of Tillabéry, south-west, and Dagaya in the region of Diffa, near Lake Chad, south-east). The collection area is situated mainly in the Sahel zone with rainfall ranging from 200 to 600 mm per year. However, a third of the Niger River zone and the whole Dallol Maouri area fall into the Sudanean Savannah zone, with annual rainfall ranging from 600 to 900 mm.

As seeds were sampled from granaries, 59% of the collected samples contained off-types seeds, ranging from 1 to 9 mixtures of seeds types, with a mean of four different types per accession. After, the purification field trial in 2008, 68 off-types were kept in the collection. These off-types often corresponded to varieties that farmers said had disappeared. Sometimes they were intermediate forms between wild and cultivated rice. In addition to cultivated rice samples and intermediate mixed forms, 10 wild rice samples corresponding to nine *O. barthii* and one *O. longistaminata* were also collected. Surprisingly, in the village of Mill, region of Diffa, Lake Chad zone, old women were found to harvest natural populations of *O. barthii* growing along marshes. The habitat of collected rice samples ranged from floating rice in deepwater (more than 1.5 m), to rainfed lowland rice with variable water level up to 1 m and fully irrigated paddy rice. After the initial purification and field evaluation of 370 accessions (202 samples, plus 168 off-types) in 2008, a subset of 270 accessions was selected for further characterization. However, these accessions were not evenly distributed from the collecting area (Table 3.1). About 27% of the samples were collected from 18 villages in the region of Tillabéry, which is also located along the Niger River. Thus, 63% of the collection was made along the Niger River. The proportion of accessions collected around the region of Diffa (5 villages) was 9.6%, while the region of Tahoua (2 villages), Maradi (3 villages) and Zinder (1 village) accounted for 5.9%, 2.6% and 1.5%, respectively.

The habitats for the collected rice samples were rainfed lowland rice with water level up to 1 m, floating rice with water depth of more than 1.5 m, and fully irrigated paddy rice. Lowland,

floating and irrigated rice accessions represented 58.5%, 21.1% and 20.4%, respectively (Table 3.1). Overall, the same agrosystems were repeated in all the main eco-geographical zones, but not in all the regions. Zinder and Maradi did not have floating rice accessions, while there was no irrigated rice in the village visited in the region of Zinder. The most important genetic erosion, defined as the disappearance of named varieties in regions or agrosystems where they have been reported before, was in Tillabéry (Table 3.1), mainly in the Canton of Sinder (homeland of Wogo people), and in the Koutougou, (homeland of the Songhai people). Both communities are traditional rice growers. In many villages of Tillabéry, the collection team was clearly told that any named accessions that could not be found in Sinder or Koutougou must be considered as definitely lost. Moreover, only 20% of the accessions found (all were *O. glaberrima* species) and listed in Sinder by Bonkoula and Miezan (1984) were still cultivated at the time of this collection. Drought or flood were cited as reasons for their disappearance in Sinder, while the people in Koutougou claimed that the floating variety D5237 introduced during the fifties by the colonial administration, was the main cause of the disappearance of the landraces. The variety D5237 was later named Degaulle, but displaced the real Degaulle, because of its white grains (the original Degaulle has red grains), the ease of husking and a much higher market-value. Disease was not cited as a reason for abandoning landraces in any village during the collection.

According to farmers, the cropping cycle of the varieties ranged from three to five months around the areas of Lake Chad and the central south zone, ranged from three to six months in the Dallol Maouri zone and up to seven months the Niger River zones. Additionally, they classified 60.6%% of the accessions as tall varieties with plant height over 1 m. 26.7% were classified as medium-height varieties, with heights ranging between 0.5-1 m, and finally 12.8% were classified as short varieties with plant heights less than 0.5 m.

New varieties originated mainly from neighbouring villages, farmers and the local farmers' union, when available. The farmers' union also works with the national seed dissemination institutions, started by the national agricultural research institute (INRAN), and the national irrigated areas office (ONAHA). However, some new accessions were introduced from neighbouring countries. For example, in the region of Dosso, some new varieties have come from Benin and Nigeria, while some accessions of Tillabéry originated from Mali and rarely from Burkina Faso. In the villages visited in the central south area, Nigeria constituted the most frequent source of new

varieties, while in the region of Diffa, Niger come first, followed by Nigeria and occasionally Chad.

Table 3.1: Distribution of the accessions from the different regions and eco-geographical zones of Niger

Eco-geographical zones	Regions	Villages Number	Accessions per agrosystem				Abandoned accessions
			Lowland	Irrigated	Floating	Total collected	
Niger River	INRAN*	-	0	10	2	12	-
	Tillabéry	18	30	15	28	73	21
	Dosso	18	68	20	9	98	10
Dallol Maouri	Dosso	4	29	1	5	35	2
Lake Chad	Diffa	5	13	5	8	26	3
Central- South	Maradi	3	4	3	0	7	1
	Tahoua	1	10	1	5	16	1
	Zinder	1	4	0	0	4	3
	Total collected	50	158	55	57	270	41

* Accessions collected at INRAN were counted among those of the Niger River zone, because more than half of the irrigated areas lie along the river and use INRAN varieties.

During the community meeting, farmers were also asked to rank the most important constraints on rice production in their areas (Table 3.2). In all the regions in rainfed lowland and floating deep-water agrosystems, drought at the seedling stage before the setting of the full season, and the filling up of valleys, was cited as the most important constraint in 23.8% of the villages. The main cause for this was the irregularities in the rainfall patterns before the season would fully settle, and the necessity for the plant to reach a certain stage before the flood. In lowland and irrigated agrosystems, insects and telluric worms were cited as the most damaging constraints in 27.9% of the villages at seedling and reproductive stages. Disease described as RYMV and Bacterial Leaf Blight (BLB) were reported in lowland and irrigated rice, in 16.4% of the villages of all the areas, except the central south zone. Finally, birds, hippopotamuses, fishes and flood were reported as major constraints by 13.4%, 4.2% and 4.2%, respectively (Table 3.2).

Table 3.2: Main constraints on rice production, as described by farmers during the germplasm collection in 2008 in Niger

Constraints	Prevalence (%)	Stage	Agrosystem	Region
Drought	23.8	seedling	L and F	all
Insects and worms	27.9	seedling and reproductive	L and I	all
Weeds (Striga)	6.2	vegetative	L	all
Birds	13.4	milky stage and maturity	all	all
Hippopotamus	4.2	all stages	L and I	Tillabéry
Herbivorous fishes	3.8	seedling	L	
Diseases (RYMV, BLB)	16.4	vegetative	L and I	Tillabéry, Dosso, Diffa
Flood	4.2	seedling + vegetative	L	Tillabéry, Dosso

L: lowland, I: irrigated, F: floating, RYMV: *Rice yellow mottle virus*, BLB: bacterial leaf blight

The local naming of rice accessions in most of the cases referred to morphological characteristics of plants or seeds. For example "Maï Adda" in Hausa language means "the one with a machete", referring to the shape of the extremity of the grain, recalling the extremity of a machete. Another example is "Waïhidjo", meaning "the bride" in Zarma language because of the highly droopy panicles and the upright status of the flag leaf, which recalled to farmers "a veiled decent bride". However, the meaning can also be related to agronomic traits. For example, the accession "Aysi a filla", means in Zarma-Songhai, "I will never do it again", referring to the long cycle of the variety, so long that the farmer will promise himself not to replant it again. Another example is the accession "Hawrou ga koungou", meaning in Zarma "eat to satiate" because if not, the cooked rice will melt during the night due to its high starch content. Sometimes, accessions were simply named after the person who brought them into the village, or the farmer who was supposed to have developed them. For example, the most popular lowland variety was named "Degaulle" because of its tall height comparable for farmers to the former French president General Charles Degaulle. The most popular irrigated rice variety, IR1529-680-3, is also called "Saga", because it was released in the irrigated area of Saga first, before spreading in the country. With few exceptions the farmers' naming was consistent in each region, but not across regions.

3.4.2. Agro-morphological characterization

The phenotypic variability was assessed, using 44 traits, (of which 18 are quantitative traits and 16 are qualitative traits) and recorded for each of the 270 rice accessions. The only sample of *O. longistaminata* was collected as a rhizome in a small bucket with soil and transplanted later. Thus, from the purification field trial, 25 accessions were identified as *O. barthii*, 177 accessions as *O. sativa*, 67 accessions as *O. glaberrima* and finally 1 sample as *O. longistaminata* (Table 3.3).

Table 3.3: Summary of the repartition of the accessions per species and zone of collection

	O. sativa	*O. glaberrima*	*O. barthii*	*O. longistaminata*	Total per zone
Niger River	137	32	12	1	182
Dallol Maouri	15	13	7	0	35
Lake Chad	15	6	5	0	26
Central-South	10	14	3	0	27
Total per species	177	65	27	1	270

The descriptive statistics including the range, the mean and the coefficient of variation of the 18 quantitative traits is summarized in Table 3.3. The coefficient of variation was particularly high for the length of awns (145.8%), ranging from awnless accessions (34% of the collection), to 100 mm length for some accessions. In contrast to this, the lowest coefficient of variation was recorded for the length of grains (11.2%). The days to maturity followed by the first heading date had the highest standard deviation, displaying the broad range of cropping cycles in the collection, while the width of plant leaves and the weight of 100 grains display the narrowest ranges.

Table 3.4: Summary statistics of the 18 quantitative continuous traits measured on 270 accessions from Niger

Trait	Min.	Max.	Mean	SE	CV (%)	SD
Seedling height (m)	0.2	0.8	0.4	0.0	0.3	0.1
Leaf Length (mm)	176.5	755.6	426.8	6.6	255.9	109.4
Leaf Width (mm)	7.0	22.8	11.7	0.2	296.8	3.5
Flag Leaf Length (mm)	147.5	660.0	322.4	5.8	297.7	96.2
Flag Leaf Width (mm)	7.0	26.6	13.8	0.2	294.2	4.1
Panicle Length (mm)	162.0	303.4	231.7	1.9	135.3	31.4
CuTN (number)	6.0	66.0	16.1	0.6	58.3	9.4
CuHM (m)	0.3	2.0	1.1	0.01	0.2	0.2
Ligule Length (mm)	2.8	34.8	14.1	0.5	56.2	7.9
CuDN (mm)	2.7	9.9	4.9	0.1	17.9	0.9
PaNup (number)	5.0	45.0	13.3	0.4	54.2	7.2
1st Heading (days)	38.0	240.0	86.4	2.2	43.1	37.3
Maturity (days)	62.0	261.0	116.8	2.4	33.8	39.5
Awn Length (mm)	0.0	100.0	12.1	1.1	145.9	17.7
100GWgt (g)	1.3	3.7	2.6	0.0	14.7	0.4
Grain Length (mm)	5.1	10.9	8.6	0.1	11.2	1.0
Grain Thickness (mm)	1.1	3.0	1.8	0.0	12.0	0.2
Grain Width (mm)	1.8	3.3	2.6	0.0	11.6	0.3

CuTN: culm tiller number, CuHM: culm height at maturity, CuDN: culm diameter at nodes, PaNup: panicle number per plant, 1stHead: days from sowing to 1st heading, Maturity: days to maturity, 100GWgt: 100 grain weight.

Similarly, the distribution and frequencies of the 16 qualitative traits were also computed and an example of the abundance and distribution of spikelets borne on secondary branches of the panicles as well as the distribution of the different coloration of the caryopsis of accession are given in Figure 3.1.

Most of the collection (70.7%) produced dense panicles, with two to three secondary branches, per primary branch, while 16.7% developed three to four secondary branches per primary branch, and finally 12.6% of the collection grew sparse secondary branches with most spikelets borne directly on primary branches. With regard to the pericarp colour of the caryopsis, 37.9% of the accessions were brown, 34.2% were white, 15.6% were red or purple and 12.3% were light brown or whitish.

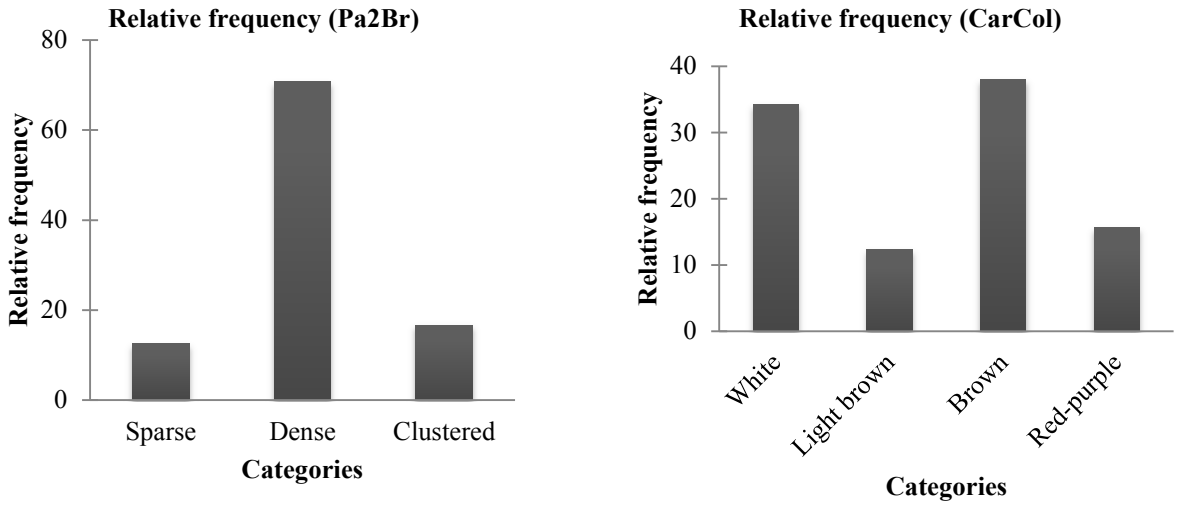

Figure 3.1: Distribution of secondary branching of panicles (Pa2Br) Sparse: 1 secondary branch, most of the spikelets borne directly on primary branches; Dense: 2-3 secondary branches, 50% of spikelets borne directly on primary branches; Clustered: 3-4 secondary branches per primary branch, All spikelets on secondary branches, giving a clustered appearance) and frequencies of the caryopsis (CarCol) coloration in the collection of 270 rice accessions from Niger.

Table 3.5 shows summary of the 4 principal components from PCA performed on the 18 quantitative traits measured on 270 accessions. PC1 and PC2 explained 28.86% and 17.39% of the total variation, respectively. The main traits that had the highest loading scores (and hence contributed most for the differences) were flag leaf width and length, leaf blade width and length, culm number and panicle length for PC1, and flag leaf length, culm length and diameter, maturity, and heading dates for PC2.

Table 3.5: Factor loadings of the 18 quantitative characters for the first four principal components and the percentage variance accounted for each component

Traits	PC1	PC2	PC3	PC4
Seedling Height (cm)	0.17	0.22	0.01	0.39
Leaf Length (cm)	**0.60**	0.55	0.34	-0.10
Leaf Width (cm)	**0.88**	-0.10	0.04	-0.01
Flag Leaf Length (cm)	**0.62**	**0.60**	0.17	-0.14
Flag Leaf Width (cm)	**0.89**	-0.09	0.02	-0.02
Panicle Length (cm)	**0.64**	0.29	0.21	-0.19
CuTN (number)	**0.71**	0.06	-0.22	0.26
CuHM (cm)	0.48	**0.59**	0.20	0.00
Ligule Length (cm)	**-0.66**	0.40	0.28	-0.09
CuDN (mm)	0.02	**0.67**	-0.22	0.09
PaNup (number)	**0.73**	0.05	-0.23	0.26
1stHead (days)	-0.45	**0.75**	-0.30	0.23
Maturity (days)	-0.48	**0.74**	-0.29	0.23
Awn Length (mm)	0.37	-0.25	-0.06	-0.08
100GWgt (g)	-0.14	-0.16	**0.70**	0.15
Grain Length (mm)	-0.15	0.34	0.53	-0.31
Grain Thickness (mm)	-0.32	0.01	**0.61**	0.40
Grain Width (mm)	0.18	-0.23	0.26	**0.77**
Variability (%)	28.86	17.39	10.31	7.55
Cumulative (%)	28.86	45.25	56.56	64.11

Culm number (CuTN), Culm length (CuHM), Culm diameter at basal internode (CuDN), Panicle number per plant (PaNup), First heading (1stHead), Maturity (Mat), 100 grain weight (100GWgt),

The first two PCs were plot (Figure 3.2) and a two to three grouping patterns were noticed. However, the groups were not clearly defined.

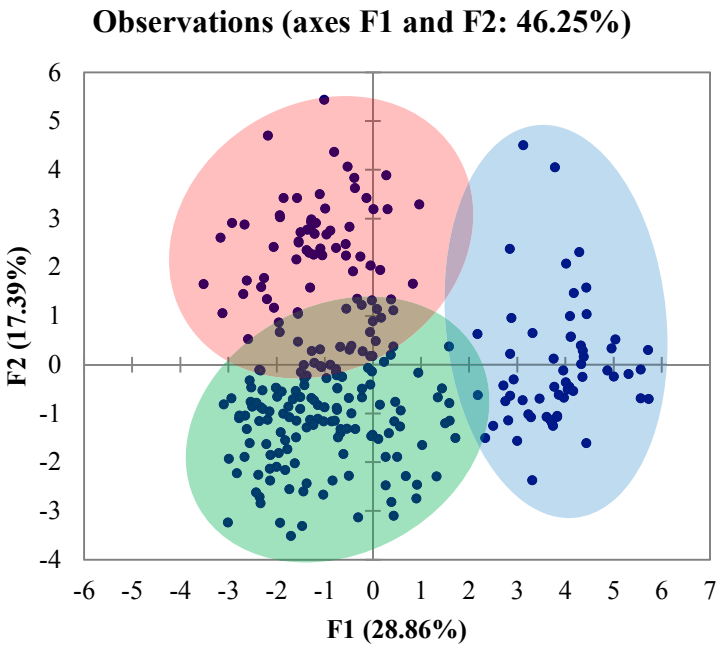

Figure 3.2: Plot of PC1 (23.86%) and PC2 (17.39%) from principal component analysis of 270 accessions from Niger plus 8 checks, evaluated with 18 quantitative traits.

The four PCs were used to classify the accessions using a hierarchical agglomerative clustering (ACH) method, based on Euclidian distance dissimilarities. Three phenotypic groups were identified (Figure 3.3 and Appendix 3.4). The first group of 66 accessions, coded as Gp1, consisted of 52 accessions of *O. sativa*, equally shared between floating and lowland agrosystems, 13 accessions of *O. glaberrima*, of which 10 are floating and 3 are lowland ecotypes, and 1 floating sample of *O. longi staminata*. The floating check RAM63 and the lowland variety TOG7106 were also in Gp1. Individuals within Gp1 are erect with a weak anthocyanin presence on the outer surface of the basal leaf sheath of seedlings. They have also intermediate leaf length with narrow width, very long ligules, thick culms, medium tillering ability. Additionally, plants of the Gp1 present semi-compact, awnless panicles or very short awns. Most of them are photosensitive accessions with late first headings and extended days to maturity. The second group, Gp2, consisted of 56 accessions, of which 35 accessions of *O. glaberrima* (six floating and 29 lowland types), 16 accessions of *O. barthii* (three floating ecotypes and 13 lowland ecotypes).

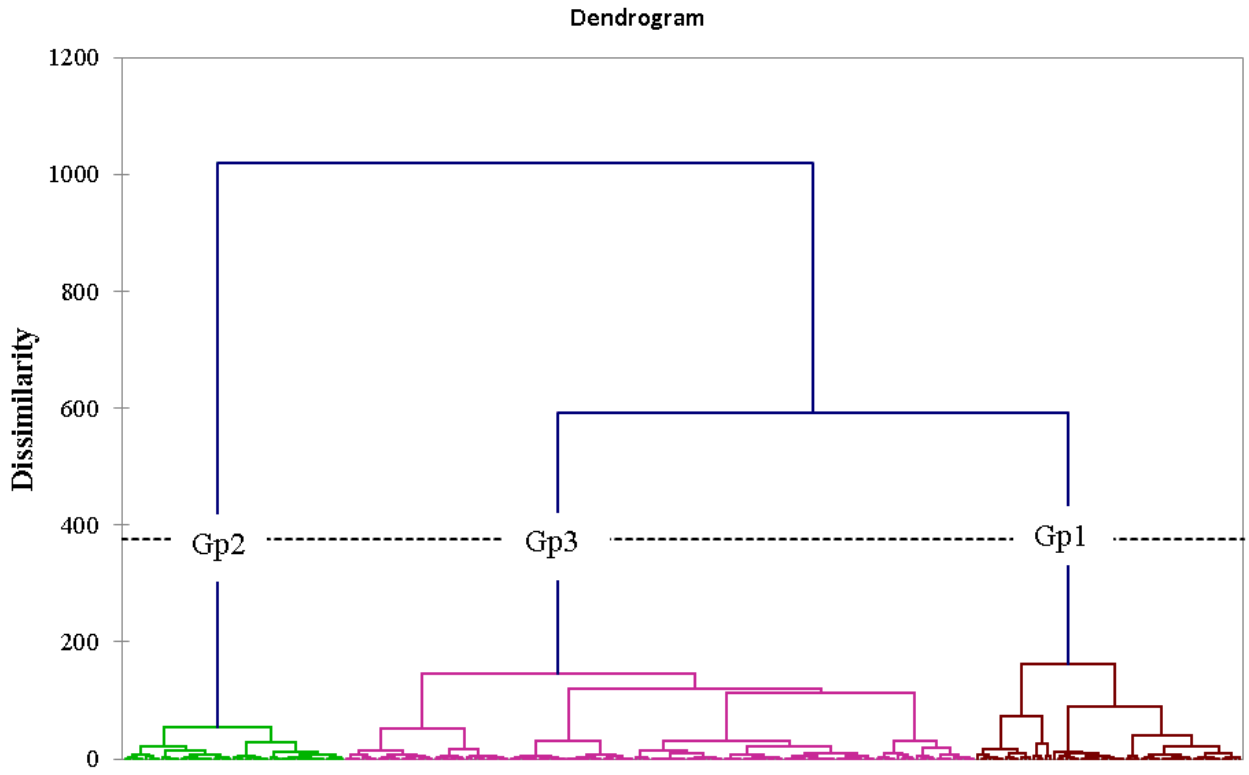

Figure 3.3: Agglomerative hierarchical (ACH) dendrogram of 270 accessions from Niger and 8 checks, based on Euclidian distance dissimilarities of 18 quantitative traits

The main characteristics of the Gp2 are short crop cycles of 51 days from sowing to flowering and 78 days to maturity, short, yellowish green ligules, and long leaves. In addition, they have high tillering ability, broad flag leaves, and long spreading panicles with most of the spikelets borne directly on primary branches. Near maturity, the grains are wide and have a purple apex as well as long awns. The last phenotypic group, Gp3, is constituted of 156 accessions. The majority (126 accessions) are *O. sativa* species, including checks varieties IR64, B6144, NERICAL41, NERICA14 and Moroberekan. All the irrigated ecotypes in the overall collection, except for TY51-F, clustered into this group. Twenty one accessions of *O. glaberrima*, most of them lowland ecotypes, and the check variety CG14, were also in Gp3. Nine wild *O. barthii*, including the two pure wild types harvested far from any farm, fell into this group. Plants in Gp3 are dwarf or semi-dwarf plants, with short erect leaves, and flag leaves pubescent only on the upper surface, medium ligule length with yellowish green auricles and light green collars. They have a medium

tillering ability and medium crop cycle length. Panicles are either semi-compact or droopy and clustered with low grain shattering.

The discriminant analysis performed using the 3 groups obtained with cluster analysis as categorical variable and the 34 quantitative and qualitative traits as explanatory variables assigned 99.6% of the accessions into their predefined a *priori* group (Figure 3.4a). The first factor was associated with five quantitative traits (leaf width, flag leaf width, ligule length, days to first heading and days to maturity) and four qualitative traits: leaf blade attitude (erect or droopy), ligule shape (obtuse or rounded), apiculus coloration of the lemma (purple apex) and awn colour (purple). The second factor is strongly correlated to leaf and flag leaf length, and plant height.

Discriminant analysis conducted using eco-geographical zones (Figure 3.4b) assigned 94.1% of the accessions into their respective groups. All the accessions collected around the Lake Chad region were mixed with the accessions collected from other eco-geographical zones.

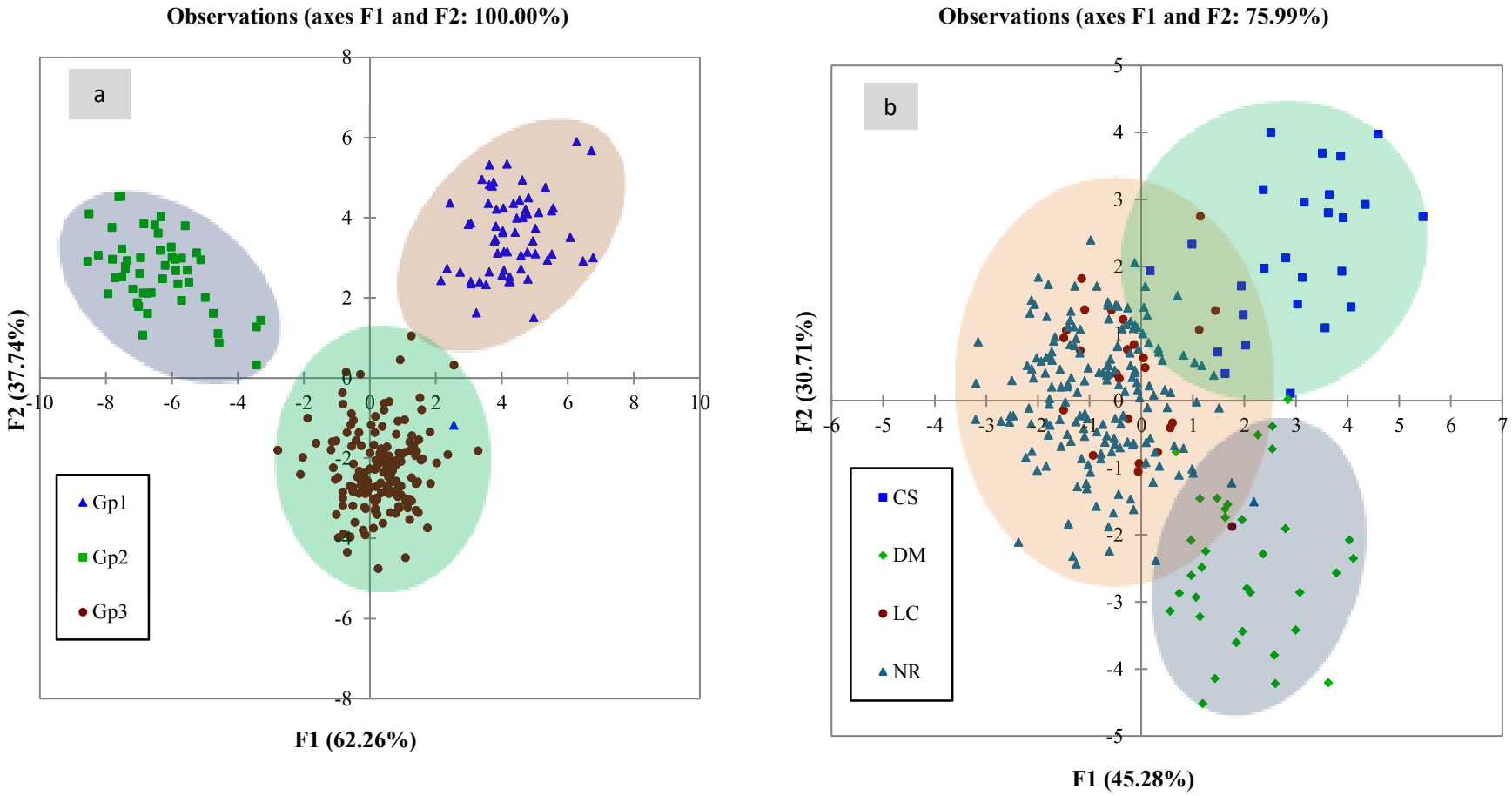

Figure 3.4: Discriminant Analyses (DA) (1) conducted on the 270 accessions collected from Niger: (a) DA based on the 3 groups (Ggroup 1 = Gp1; Group 2 = Gp2; group 3 = Gp3) identified through cluster analysis; (b) DA based on eco-geographical zones of the collection (CS: Central-South, DM: Dallol Maouri, LC: Lake Chad, NR: Niger River)

This suggested that the phenotypic variation of rice in that region is included in that of the Niger River, with some exceptions and remaining accessions were mixed with the accessions from other eco-gegraphical zones.

The discriminant analysis performed using the different rice growing agrosystems, as provided by farmers, assigned 97.8% of the accessions into their predefined groups (floating, lowland or irrigated; Figure 3.5a). Compared to the other categorical variables, however, the level of separation among these 3 different agrosystems was low.

Finally, the exactness of the allocation of accessions into different species was confirmed using the assigned species as a categorical variable. The discriminant analysis assigned 99.6% of the accessions into their correct species (Figure 3.5b)

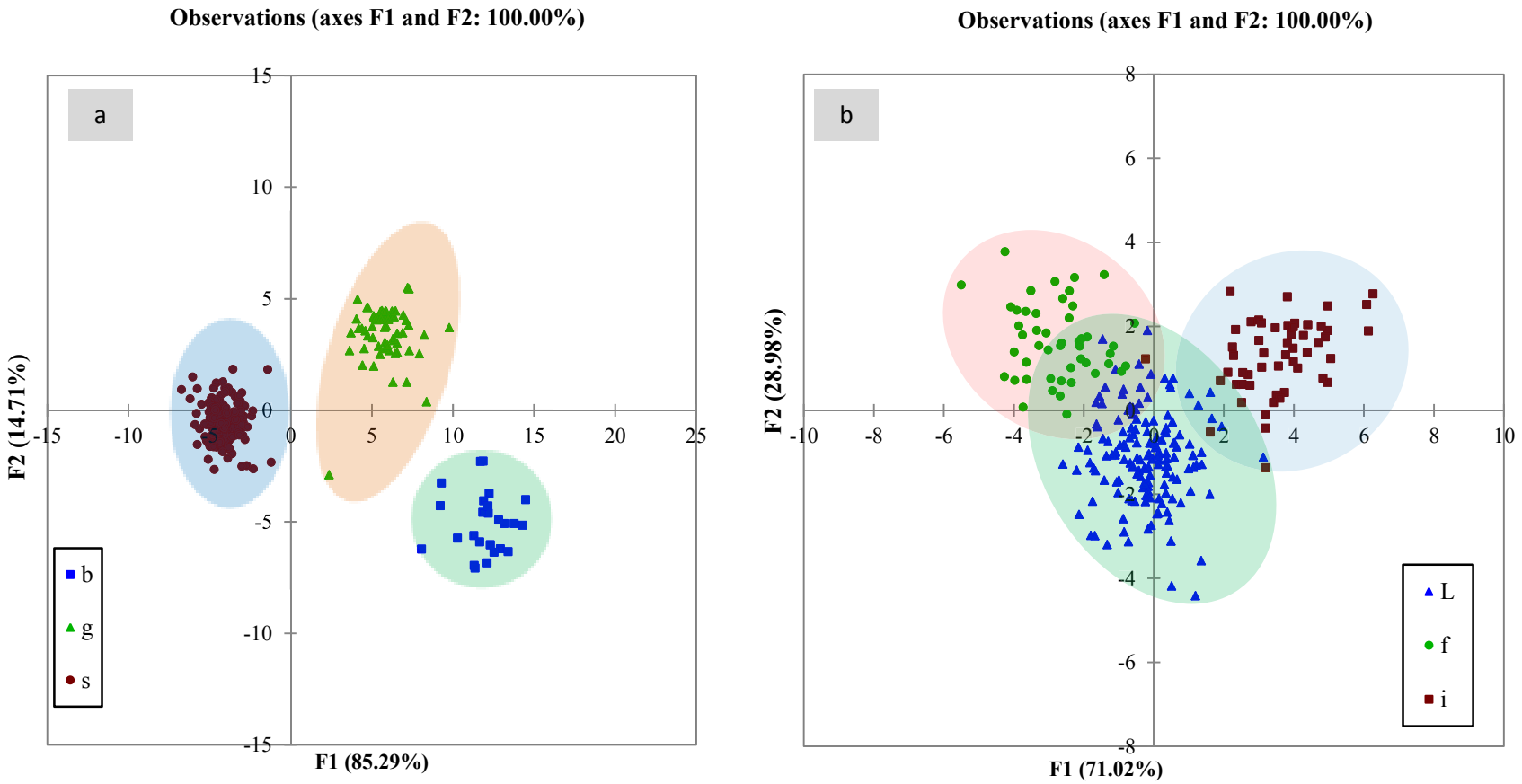

Figure 3.5: Discriminant analyses (DA) conducted on the 270 accessions collected from Niger: (a) DA based on the species (b = *O. barthii;* g = *O. glaberrima;* s = *O. sativa*) determined using morphological traits; (b) DA based on the agrosystem (f = floating; L = lowland; i = irrigated) as provid-ed by farmers during the collection.

3.4.3 Phenotypical diversity of the collection

The Shannon-Weaver Diversity Index was computed on 33 characters, of which 15 quantitative traits were coded as described in Appendix 3.2, along with 18 qualitative traits. The diversity index was calculated for the entire collection first (overall H'), and was then partitioned by the 4 eco-geographical zones of collection, and three groups were identified through cluster analysis (Table 3.6). The overall Shannon Index for the 33 characters ranged from 0.16 for the weight of 100 grains, to 0.98 for the outer diameter of the basal portion of the plants' main culm (CuDN), with an average of 0.65. Pairwise comparisons of the mean Shannon Index showed no statistical differences among the collection zones or the phenotypic groups. However, the diversity index of specific characters was different from one zone to another or from one group to another. While there was no significant variation in the panicle length and the weight of 100 grains in the 27 accessions from the central-south zone or the 52 accessions of the phenotypic group Gp2 (H' = 0), the diversity of panicles was H'= 0.40 for the Niger River zone and the phenotypic group H'= 0.41 in Gp3. Additionally, Gp1 had the highest diversity value for 100 grain weight (H'= 0.37) and the Niger River had the highest among the zones of collection (H'= 0.18), in addition to plant height (H'=0.67).

While the variability was high for caryopsis pericarp colour, flag leaf attitude, panicle main axis attitude, culm habit, and panicle shattering, regardless of the zone of collection, or the phenotypic groups, the diversity of days to first heading and days to maturity were very high, and stable only between eco-geographical zones. H' was very high in Gp3 for both characters but was weak in Gp2 and Gp1. The variability of the colours of flowers' stigma and the lemma of grains was lower in Gp2, and higher in the other groups. Grain length presented the highest diversity (H' = 0.96) in the phenotypic Gp1 and in the region of the Lake Chad.

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Table 3.6: Estimates of the Shannon-Weaver Diversity Index of the 33 traits of rice collection from Niger by eco-geographical zones and phenotypic groups detected by the hierarchical agglomerative clustering (ACH)

Traits	Eco- geographical zones				Phenotypic groups			Overall H'
	CS	DM	LC	NR	Gp1	Gp2	Gp3	
ColAC	0.51	0.84	0.70	0.69	0.80	0.67	0.62	0.73
LfL	0.29	0.48	0.51	0.52	0.47	0.36	0.42	0.54
LfW	0.46	0.53	0.62	0.70	0.61	0.25	0.65	0.68
FLL	0.47	0.49	0.32	0.55	0.50	0.43	0.44	0.56
FLW	0.60	0.78	0.47	0.61	0.53	0.61	0.42	0.67
PanL	0.00	0.28	0.27	0.40	0.34	0.00	0.41	0.36
CuTN	0.70	0.71	0.66	0.72	0.64	0.66	0.65	0.74
CuHM	0.31	0.53	0.61	0.67	0.61	0.43	0.58	0.65
LiL	0.68	0.94	0.89	0.89	0.58	0.47	0.89	0.93
CuDN	0.61	0.99	0.89	0.98	0.50	0.91	0.84	0.98
PaNup	0.81	0.85	0.56	0.89	0.84	0.70	0.80	0.91
1stHead	0.67	0.78	0.86	0.96	0.17	0.24	0.88	0.96
Maturity	0.65	0.68	0.68	0.81	0.05	0.12	0.83	0.83
100GWgt	0.00	0.12	0.15	0.18	0.37	0.00	0.06	0.16
GrLn	0.55	0.65	0.89	0.82	0.96	0.49	0.73	0.80
CarCol	0.68	0.76	0.85	0.93	0.82	0.52	0.87	0.92
LsbaC	0.59	0.57	0.37	0.59	0.33	0.61	0.49	0.61
LsaC	0.41	0.49	0.42	0.48	0.35	0.53	0.49	0.48
Lba	0.74	0.83	0.49	0.37	0.21	0.94	0.15	0.51
LBp	0.57	0.58	0.84	0.59	0.77	0.85	0.44	0.64
LBpbS	0.35	0.35	0.46	0.17	0.27	0.35	0.17	0.26
AuC	0.09	0.16	0.20	0.17	0.12	0.16	0.18	0.18
CoC	0.33	0.40	0.20	0.28	0.10	0.48	0.24	0.31
LiS	0.72	0.74	0.69	0.67	0.54	0.52	0.67	0.73
LiC	0.27	0.23	0.20	0.51	0.40	0.09	0.52	0.44
CuHb	0.66	0.59	0.69	0.67	0.64	0.47	0.64	0.69
FlaL	0.78	0.88	0.77	0.82	0.85	0.75	0.75	0.84
StC	0.71	0.51	0.68	0.80	0.68	0.25	0.81	0.77
LemAC	0.70	0.65	0.78	0.67	0.62	0.35	0.65	0.72

Eco-geographical zones: CS: Central-south, DM: Dallol Maouri, LC: Lake Chad, NR: Niger River.
Coleoptile anthocyanin coloration (ColAC), Leaf blade length (LfL), Leaf blade width (LfW), Flag leaf length (FLL), Flag leaf width (FLW), Panicle length (PanL), Culm number (CuTN), Culm length (CuHM), Ligule length (LiL), Culm diameter at basal internode (CuDN), Panicle number per plant (PaNup), First heading (1stHead), Maturity (Mat), 100 grain weight (100GWgt), Grain Length (GrLn), Caryopsis pericarp colour (CarCol), Basal leaf sheath colour (LSbaC), Leaf sheath anthocyanin colouration (LSaC), Leaf blade attitude (LBa), Leaf blade pubescence (LBp), Leaf blade pubescence on blade surface (LBpbS), Auricle colour (AuC),Collar colour (CoC), Ligule shape (LiS), Ligule colour (LiC), Culm habit (CuHb), Flag leaf attitude

(late observation) (FLaL), Stigma colour (StC), Lemma colour of apiculus (LemAC).

Table 3.5: (continued)

Traits	Eco- geographical zones				Phenotypic groups			Overall H'
	CS	DM	LC	NR	Gp1	Gp2	Gp3	
AwD	0.78	0.67	0.79	0.68	0.55	0.86	0.68	0.73
AwC	0.50	0.59	0.63	0.53	0.43	0.55	0.50	0.57
PmaA	0.93	0.92	0.92	0.93	0.95	0.83	0.88	0.96
PaSh	0.87	0.83	0.88	0.76	0.73	0.96	0.76	0.69
Mean	0.54	0.62	0.60	0.64	0.54	0.52	0.59	0.65
Standard Error	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04

Eco-geographical zones: CS: Central-south, DM: Dallol Maouri, LC: Lake Chad, NR: Niger River.
Awns distribution (AwD), Awns coloration (AwC), Panicle attitude of main axis (PMaA), Panicle shattering (PaSh)

3.5 Discussion

3.5.1 Collection

The village, the administrative region or the eco- geographical zone have been used as the basis for collecting plant genetic resources of a crop in an entire country or region for ex- situ conservation, by several authors, on various crops including sorghum, lentil and rice (Sultana et al., 2006; Barry et al., 2007b; Barro-Kondombo et al., 2008). Moreover, in the case of a crop like rice, which is adapted to humid environments, a collection following hydrographical maps of the target area could be most relevant. The collection of rice species in Niger, which was conducted by following the distribution of villages along the main watercourses and marshes. Only the region of Agadez, (which is mainly desertic) was not visited. There was a significant difference in the number of accessions collected from one region to another. The majority of the collection (35.6%) originated from the region of Dosso along the Niger River and in the Dallol Maouri watercourse, against 27% collected in Tillabéry, the main rice growing area of the country. This situation could be explained by the fact that the collection process, for logistic reasons, started with the region of Dosso. Therefore, as an accession was collected only in the first villages in which it was found, most of the accessions were collected in Dosso. The second explanation is that villages around the two dominant hydrographical formations of the region of Dosso were visited (the Niger River and the Dallol Maouri); while none of the tributaries of the Niger River were visited in the region of Tillabéry. And finally, during the collection in Tillabéry, several villages with large irrigated areas under the supervision of the ONAHA were avoided because of

the predominance of modern *O. sativa* rice varieties released by INRAN. With a few exceptions, all the varieties found in Dosso also existed in Tillabéry. The most remarkable exception was "El Sambera" found in the region of Dosso and in Maradi under another name, but not in the other regions. The accession was supposed to have originated in Nigeria, as with most of the accessions found in the central-south zone.

Farmers have no notion of the existence of two formal species (*O. glaberrima* and *O. sativa*) in the cultivated rice. They classify the accessions into "irrigated rice" for most of the improved rice varieties and "lowland rice" for some floating and lowland accessions, or "river or marsh rice" for old *O. glaberrima* accessions. However, they were aware of the existence of the two wild African relatives, the annual *O. barthii* ("Sombay" in Zarma-Songhai language (West), "Fouremmi" in Kanuri language (East) and the perennial *O. longistaminata* ("Baou" in Zarma-Songhai language). Several studies reported that local people in the Niger Delta in Mali (Second et al., 1977) and in Tillabéry in Niger (Bonkoula and Miezan, 1982) harvested and consumed *O. longistaminata* and *O. barthii*, respectively. A similar trend was also observed during the collection in the region of Diffa, around the Komadougou River, but not in the Niger River valley.

Farmers classify drought and insects as the main constraints on production, mainly in the lowland and irrigated agrosystems at seedling and reproductive stages. Several authors have reported that drought is one of the major limitations to rice production worldwide (Courtois et al., 2000; Manickavelu et al., 2006), while stem borers and rice bugs have been cited as major pests on rice in Africa. In Africa, drought is mainly due to unequal distribution of rainfalls in time and space, and poor soil conditions (Balasubramanian et al., 2007). The effects of drought are more damaging at the reproductive stages, mainly during the irreversible reproductive processes like flowering. During the vegetative stage, the plant may recover from drought (Yue et al., 2006). In addition to insects and drought, farmers ranked weeds and diseases as harmful in the collection areas, mainly in irrigated and lowland agrosystems. Apart from field invasions, weeds are responsible for nitrogen loss up to 25% in West Africa (Becker and Johnson, 2001), while RYMV and BLB are recognized as the most damaging diseases in Niger (Basso et al., 2010).

The naming of rice varieties by farmers in Niger is usually with reference to plant morphology, particular agronomic trait, the name of the person who has brought it to the village, the name of

the original village, etc. This is similar to that of rice growers in the Gambia (Nuijten and Almekinders, 2008). Generally, farmers' naming of rice varieties was consistent in a region, even if sometimes there was a direct translation of the name from one language or a misspelling of the name: for example, the accession "Waihidjo" in Zarma-Songhai, meaning "the bride", was called "Amaria" ("the bride") by Haussa people. Another example is the accession "Degaulle" in the region of Tillabéry that was called "Dikkol" in the region of Diffa, more than 1200 km away. This could be explained by the relatively small number of ethnic groups and dialects in Niger (nine dialects), of which Hausa and Zarma are spoken by more than 80% of the population. Naming of varieties after animals, synonyms or multiple names for a single accession, as was the case in Lao PDR (Appa Rao et al., 2002) were found, but very rarely.

3.5.2 Phenotypic variation and diversity

Agro-morphological traits have been widely used to evaluate plant genetic resource collections. The phenotypic diversity of the collections of durum wheat (*Triticum durum* Desf) landraces from Tunisia, and wild barley (*Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell.) from Jordan, were studied using 11 and 13 agro-morphological traits, respectively (Shakhatreh et al., 2010; Sourour et al., 2010). Similarly, the phenotypic variation of 880 rice landraces from Cote d'Ivoire was studied using 13 traits (Sanni et al., 2008). Thus, phenotypic characterisation of plants, in addition to providing useful information on the real value of accessions for breeding programs, also provides information on the spatial distribution and structure of the variability. Such information is useful for optimal exploitation of crop genetic resources.

The collection of rice from Niger was very variable in terms of the measured traits. The collection was characterized by the preponderance of very late maturing accessions, with 36% requiring more than 145 days from sowing to maturity. Likewise, the height of 36.4% of the accessions was between 1060 and 1200 mm and 60% had a light brown caryopsis colour. Those observations denoted the relative importance of traditional varieties and landraces in the collection. Indeed, Sanni et al. (2008) reported that rice landraces from Cote d'Ivoire were tall and late maturing, and Oka (1977) reported that some rice varieties from Africa were sensitive to photoperiod, and had brown pericarp colour. Similarly, a collection of rice landraces from Burkina Faso contained a majority of late maturing accessions (68%) with light brown to brown pericarps (Sié et al., 1998).

Although the number of accessions varied among the four eco-geographical zones, there was a clear pattern of morphological adaptation in the collection. The DA also revealed that the variability of the accessions collected around the Lake Chad area was similar to that of the accessions collected along the Niger River, except for a very few *O. barthii* accessions specific to the Lake Chad region. These accessions were collected in wild populations, far from cultivated fields. They had spread-out culms (inclination of the base of the main culm from vertical > 60-80°), were very early maturing (first heading 38 to 41 days after sowing in four experiments), with few long grains (> 100 mm) on the panicles and very long rigid awns (> 80 mm). Similar observations of grain features have been made on *O. barthii* accessions from Mali (Bezançon et al., 1977). No wild populations of *O. barthii* were collected far from cultivated rice fields along the Niger, because the region contained huge irrigated areas cropped with *O. sativa* species only. With the high growth rate of the human population (2.9% per year), farmers were obliged to occupy the remaining refuges of the wild species. Such a phenomenon of the disappearance of wild species was also observed for the Asian wild rice, O. *rufipogon* Griff., in Thailand (Akimoto et al., 1999). Additionally, most of the *O. barthii* accessions of the Niger River zone had semi-erect to open culms (20-40°), and were early to medium maturing and had medium length grains with, long, but less rigid awns. The rice cropping intensification along the Niger River, by allowing proximity between cultivated rice fields and wild species, had probably promoted gene flow and natural hybridization between *O. sativa* and *O. barthii*. Gene flow was observed in Asia between *O. sativa*, wild O. *rufipogon*, and the weedy rice *O. sativa* f. *spontanea* (Chen et al., 2004). The gene flow could explain the reduction in grain size and the openness of culms, as well as the lengthening of the crop cycle of the collected *O. barthii* accessions in the Niger River zone. According to Second (1985), the population of adventive *O. barthii* found in the inland delta of the Niger River (East) in Mali was different from *O. barthii* found in the western part of the Mali. The same author made a similar observation concerning the differences in the isozymes profiles of *O. barthii* populations on the western side of Lake Chad (in Niger) and those on the eastern and the southern side (Chad and Nigeria). The inland delta of the Niger River is believed to be the first centre of domestication of African rice from its wild progenitor, *O. barthii*, while the Lake Chad area is believed to have played an important role in the domestication process through the concept of a "non-centre", where wild accessions could have been taken from the region of the Lake Chad, and domesticated and spread far from it (Harlan, 1975).

The passport data of the collected accessions with regard to the agrosystems provided by the farmers was coherent, and the majority of the accessions were grown in rainfed lowland agrosystems (less than 1 m depth). For farmers, the difference from the irrigated agrosystem is clear, because most of the irrigated varieties were dwarf to semi-dwarf, and therefore could not stand the unpredictable water depth which depends on the amount of rainfall and the duration of the rainy season. The irrigated rice was dominated by large-scale irrigation schemes as part of the national food security program. The national rice breeding program has focused on breeding only for this agrosystem. Therefore, to improve their production, farmers have started using some semi-dwarf to intermediate height varieties to the lowland agrosystem which explain the mixture of the accessions from the two agrosystems. Deepwater (up to 1.5 m) and floating (more than 1.5 m) agrosystems constitute an extension of lowland agrosystem. Depending of the amount of rainfall and the level of flooding of the river, the cultivated surface varies between these systems. Thus, there is a continuum between the two agrosystems, while no such continuum exists between the floating and irrigated agrosystems. Certainly, morphological characters such as culm height and cycle from sowing to maturity readily distinguish the two groups of ecotypes. Such separation between agrosystems has been also observed in Mali and Nigeria (Second, 1985; Akpokodje et al., 2001).

The grouping of the accessions provided by the hierarchical classification gave three phenotypic groups. p1, included floating *O. glaberrima* and long cycle *O. sativa*, clustered with *O. longistaminata*. Barry et al. (2007b) found a similar grouping of lowland *O. glaberrima* accessions and photoperiodic *O. sativa* in a rice collection from maritime Guinea. Likewise, photosensitive rice accessions have been found in Nigeria in both *O. sativa* and *O. glaberrima* (Aladejana and Faluyi, 2007). However, these authors considered this photosensitivity to be a problem blocking the production of two crops per year, whereas in Niger farmers found it convenient because it allows them to partition their work during the one rainy season of the year for 4-5 months. Their production system consists of sowing the fields early during the rainy season, and then forgetting them until, after the harvest of other crops, mainly because there is no need for weeding due to the high water level. In contrary most lowland *O. glaberrima* varieties are early maturing. The clustering of a majority of *O. sativa* in the same group as some lowland *O. glaberrima* in Gp3 was consistent with the findings of Semon et al. (2005). These authors analysed 198 accessions of *O. glaberrima* from different African countries and found that, due to

gene flow between the two cultivated species, some accessions of *O. glaberrima* clustered within a group of *O. sativa* species.

The phenotypic variability found in the collection was high, regardless of the phenotypic group or the zone of collection. This could be explained by the existence of the same agrosystems in the different regions, and also at the village level, as has been found for sorghum in Burkina Faso (Barro-Kondombo et al., 2008). A further explanation could be the existence of a good system of seed exchange and variety circulation within the rice growing community of Niger. The overall mean of the Shannon Diversity Index was lower than the diversity index found in six populations of wild barley from Jordan using 13 phenotypic traits (Shakhatreh et al., 2010). Conversely, the mean Shannon index of the collection from Niger was higher than that calculated for a collection of 880 rice accessions from Cote d'Ivoire, studied using 13 phenotypical traits (Sanni et al., 2008). However, the Shannon indexes for panicle and leaf length were higher in Cote d'Ivoire as compared to Niger, while the indexes for leaf width and 100 grain weight were similar in the two countries. The indexes from Niger were higher than Cote d'Ivoire for grain length, leaf pubescence, tiller number, and days to maturity. These differences of Shannon index values for rice accessions from Niger and Cote d'Ivoire can be explained by the role of the Niger River valley region as the primary centre of African rice domestication, and Lake Chad being a well-recognized region of wild *O. barthii* variability. Thus, the collection of Niger was more variable in term of different rice species and agrosystems, while the collection of Cote d'Ivoire was constituted by *O. sativa* varieties only. For example, the low value of H' for leaf blade pubescence in Cote d'Ivoire was probably due to the predominance of hair on the leaves of *O. sativa*, while *O. glaberrima* can have both hairless and glabrous leaves (Chang et al., 1977), thus increasing the variability of the Shannon index's value.

Overall, a high level of agro-morphological variability was found in Niger, regardless of the zone of collection. Tillabéry constitutes the primary centre of rice cultivation in Niger. Nevertheless, rice cultivation is conquering secondary zones progressively, probably because of recurrent food insecurity in Niger due to rainfall irregularities affecting other crops, and because the irrigated rice schemes encourage external investments (DREF, 2010; World Bank, 2010). However, the first series of rice cropping intensifications(1975-1985) resulted in the disappearance of several *O. glaberrima* varieties in Tillabéry, as revealed by a comparison of current accessions with

accessions collected in the area in 1982 (Bonkoula and Miezan, 1982). The same trend possibly exists for Diffa (Lake Chad) where temporal collection data for comparison is not available. Thus, the present collection of rice accessions from Niger for *ex-situ* conservation is timeous and essential, in order to safeguard the remaining few landraces, as well as the wild ancestors, *O. barthii* and *O. longistaminata*. Both could be useful in improving cultivated rice using the same model as has been used on *O. sativa* by backcrossing it with the wild Asian rice ancestor O. *rufipogon*. A backcross population derived from a cross between an O. *rufipogon* accession and *O. sativa* subsp. *indica* variety was used to capture several characters including the gene responsible for flowering time (Thomson et al., 2003), the red colour of the pericarp and several QTLs related to yield (McCouch et al., 2007). Additionally, Chen et al. (2009) used the African wild perennial *O. longistaminata* to map QTLs for sterility and plant height. Cultivated African rice has been used to map several genes, including, a gene responsible for the size of grains (Li et al., 2004), a *Rice stripe necrosis virus* (RSNV) resistance locus, and yield component QTLs (Gutierrez et al., 2010) and a second RYMV resistance gene (Thiémélé et al., 2010). More collection should be conducted on wild populations from the Lake Chad eco-geographical zone before the expansion of irrigated rice agrosystems is undertaken. In addition, a deep study of the ecosystem of wild rice and its interaction with local people, regarding their practices, knowledge and utilisation should be conducted, as a prerequisite for the planning of an *in-situ* conservation scheme. Such a study was performed to plan a conservation strategy for Vietnamese landraces (Fukuoka et al., 2006). The researchers found that farmers' management strategy tends to decrease the phenotypic diversity, thus a large collection is needed to capture the regional variability of their landraces. In contrast to this, Barry et al. (2007a) found that the agrosystems of Guinea were suitable for *in-situ* conservation, involving villages with a small number of farmers because most of the genotypic variability (up to 50%) of a village was found within farms.

For a better understanding of the distribution of *Oryza* species in Niger, the collection should be genotyped using appropriate molecular markers to establish the genetic diversity and genetic structure of the varieties in relation to the different regions, species or eco-geographical zones. A survey of the genetic variability, similar to that of on-farm varieties in some villages in Guinea, could be performed in Diffa, assimilating wild populations around marshes as farms, around villages. In addition to this, the genetic diversity at village level could be studied in other regions

and a long-term germplasm conservation plan should be developed. The phenotypic variability showed in this study could be of great value for rice breeding in Niger.

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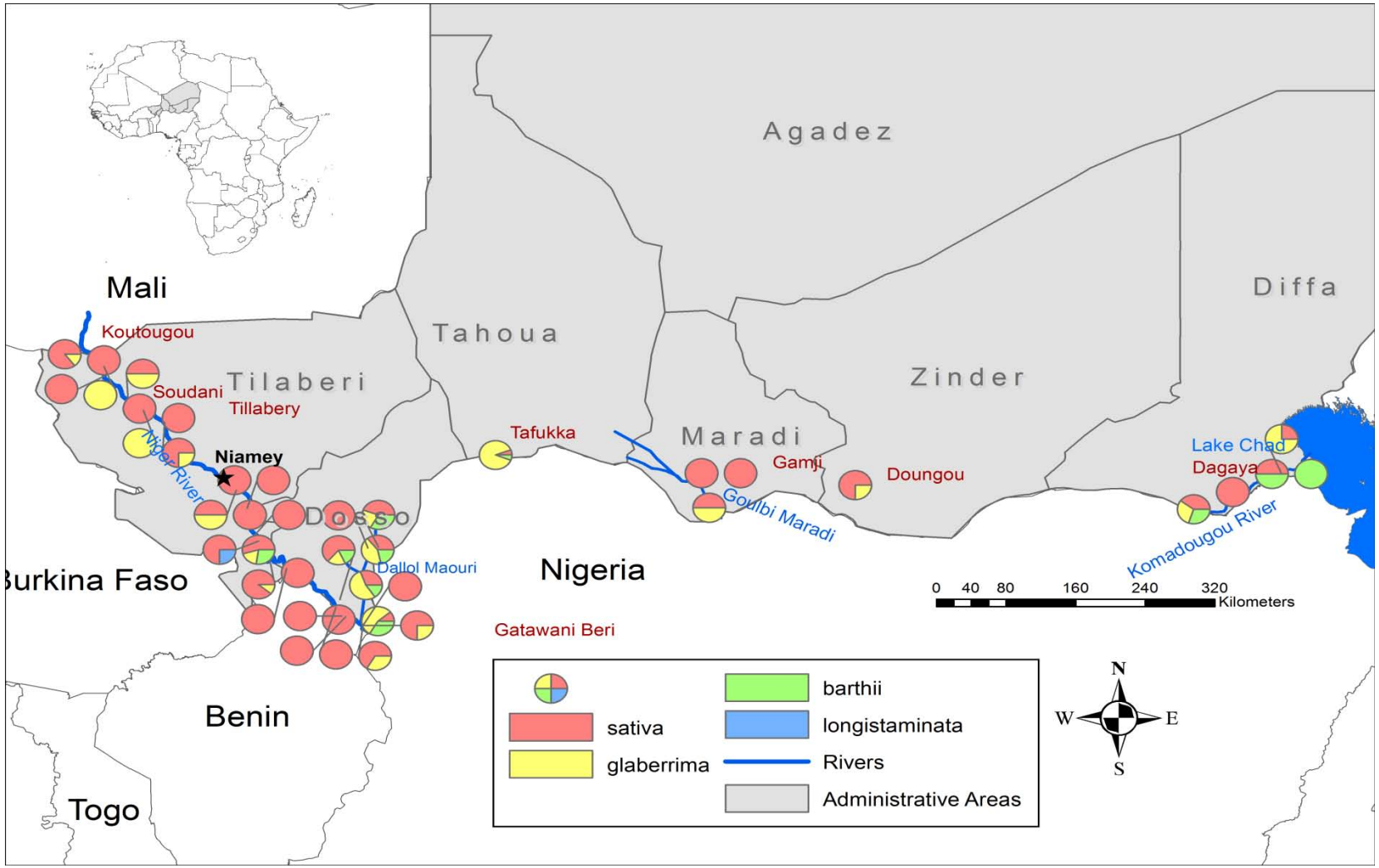


Appendix 3.1: Maps of villages in Niger where seeds samples were collected. The colour of the points indicated the proportion of each rice species sampled in a village

Appendix 3.2: Summary of the 44 phenotypic traits used for characterzing the rice collection in Niger

Trait	Abbreviation	Number of phenotypic classes	Source
Coleoptile anthocyanin coloration	ColAC	5	Bioversity et al., (2007)
Seedling height (m)	SeedH	3	Bioversity et al., (2007)
Leaf blade length (mm)	LfL	5	Bioversity et al., (2007)
Leaf blade width (mm)	LfW	3	Bioversity et al., (2007)
Flag leaf length (mm)	FLL	5	Bioversity et al., (2007)
Flag leaf width (mm)	FLW	3	Bioversity et al., (2007)
Panicle length (mm)	PanL	5	Bioversity et al., (2007)
Culm number	CuTN	3	Bioversity et al., (2007)
Culm length (m)	CuHM	9	Bioversity et al., (2007)
Culm diameter at basal internode (mm)	CuDN	2	Bioversity et al., (2007)
Panicle number per plant	PaNup	3	Bioversity et al., (2007)
Awn length (cm)	AwnL	6	Bioversity et al., (2007)
Lemma and palea colour	LPCoL	11	Bioversity et al., (2007)
Caryopsis pericarp colour	CarCol	7	Bioversity et al., (2007)
Basal leaf sheath colour	LSbaC	4	Bioversity et al., (2007)
Leaf sheath anthocyanin colouration	LSaC	4	Bioversity et al., (2007)
Leaf blade intensity of green colour	LBigC	4	Bioversity et al., (2007)
Leaf blade attitude	LBa	3	Bioversity et al., (2007)
Leaf blade pubescence	LBp	3	Bioversity et al., (2007)
Leaf blade pubescence on blade surface	LBpbS	4	Bioversity et al., (2007)
Auricle colour	AuC	6	Bioversity et al., (2007)
Collar colour	CoC	5	Bioversity et al., (2007)
Ligule shape	LiS	8	Bioversity et al., (2007)
Ligule colour	LiC	6	Bioversity et al., (2007)
Flag leaf attitude (early observation)	FLaE	4	Bioversity et al., (2007)
Culm habit	CuHb	5	Bioversity et al., (2007)
Culm lodging resistance	CuLoR	5	Bioversity et al., (2007)
Flag leaf attitude (late observation)	FLaL	4	Bioversity et al., (2007)
Stigma colour	StC	5	Bioversity et al., (2007)
Lemma colour of apiculus	LemAC	9	Bioversity et al., (2007)
Awns distribution	AwD	6	Bioversity et al., (2007)
Awns coloration	AwC	9	Bioversity et al., (2007)
Panicle attitude of main axis	PMaA	4	Bioversity et al., (2007)
Panicle attitude of branches	PBrA	5	Bioversity et al., (2007)

Appendix 3.2: (continued)

Trait	Abbreviation	Number of phenotypic classes	Source
Panicle secondary branching	Pa2Br	4	Bioversity et al.,(2007)
Panicle exsertion	PaEx	5	Bioversity et al.,(2007)
Panicle shattering	PaSh	5	Bioversity et al.,(2007)
Ligule length (mm)	LiL	3	Computed median, mean and quartiles
Grain width (mm)	GrWdh	3	Computed median, mean and quartiles
Grain thickness (mm)	GrThk	3	Computed median, mean and quartiles
First heading (days)	1stHead	5	Sanni et al. (2008)
Maturity (days)	Mat	5	Sanni et al. (2008)
100 grain weight (g)	100GWgt	3	Sanni et al. (2008)
Grain Length (mm)	GrLn	3	Sanni et al. (2008)

Appendix 3.3: Grain features of some accessions from the rice collection from Niger

Accession TY41: *O. sativa* with white, medium-length grain

Accession TY13*: O. sativa* with white, round grain

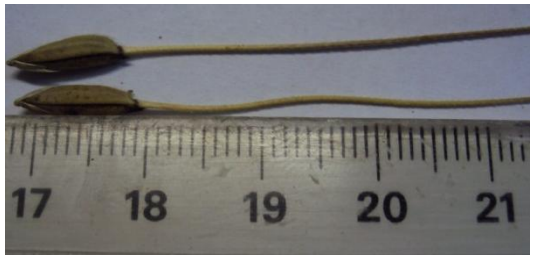
Accession DS17-A: *O. glaberrima* with short awn

Accession TY13-B: *O. sativa* with brown, medium-length grain

Accession TY35-E*: O. sativa* grain with short awns, white, medium-length

Accession TY55: *O. glaberrima* with long sterile lemma

O. glaberrima with long awns



Accession DF13 : « true » *O. barthii*

« false » *O. barthii* : natural interspecific

Appendix 3.4: Phenotypic groups of 270 accessions from Niger, and 8 checks obtained from an Agglomerative Hierarchical Clustering (ACH) analsysi with 18 agro-morphological quantitative traits

Accession Code	Phenotypic groups	Species	Ecology
Longist.	1	*longistaminata*	Floating
DS62-A1	2	*barthii*	Lowland
DS72-C	2	*barthii*	Lowland
DF20-H	2	*barthii*	Lowland
DS34-K	2	*barthii*	Lowland
DS63-C	2	*barthii*	Lowland
DF13	3	*barthii*	Lowland
DS14-C	2	*barthii*	Lowland
DS66-C	3	*barthii*	Floating
DS1-B	2	*barthii*	Floating
DS6-A1	2	*barthii*	Floating
DS1-C	2	*barthii*	Lowland
DS11-A1	2	*glaberrima*	Lowland
DS14-B	2	*barthii*	Lowland
TH5-1	2	*barthii*	Lowland
DS47-B	3	*glaberrima*	Lowland
DS52-E	3	*glaberrima*	Floating
TY7-J	2	*barthii*	Lowland
DF17-B	3	*barthii*	Lowland
DS34-E	2	*barthii*	Lowland
DF23	2	*barthii*	Floating
TY51-F	2	*sativa*	Irrigated
DS9-B	2	*barthii*	Lowland
DS2-E	2	*barthii*	Lowland
TY53	2	*glaberrima*	Floating
DS14-E1	2	*glaberrima*	Lowland
TY7	2	*glaberrima*	Lowland
TH5-B	2	*glaberrima*	Lowland
TH5-E	2	*glaberrima*	Lowland
DF12	1	*glaberrima*	Lowland
DS3-A	2	*glaberrima*	Lowland
DS83-C	2	*glaberrima*	Lowland
TH1-B	2	*glaberrima*	Floating
DS34-B	2	*glaberrima*	Lowland
MD1-A	2	*glaberrima*	Lowland
DS11-A	2	*glaberrima*	Lowland
DS47-C3	2	*glaberrima*	Lowland
DS70-C	2	*glaberrima*	Lowland
DS45-B	2	*glaberrima*	Floating
DS47-C2	2	*glaberrima*	Lowland
DS62-B	2	*glaberrima*	Lowland
TY8-D1	3	*glaberrima*	Lowland
DS14-F2	2	*glaberrima*	Lowland

DS34-C1	2	*glaberrima*	Lowland
DS14-F1	2	*glaberrima*	Lowland
DS11-B	2	*glaberrima*	Lowland
TY50	3	*glaberrima*	Floating
TH7	2	*glaberrima*	Lowland
DS34-A	2	*glaberrima*	Lowland
TH6	2	*glaberrima*	Lowland
DS4-B1	2	*glaberrima*	Lowland
TH1-A1	2	*glaberrima*	Lowland
TH5	2	*glaberrima*	Lowland
TY7-D	2	*glaberrima*	Lowland
TH1-A	2	*glaberrima*	Floating
TY1	1	*sativa*	Floating
TY3	3	*sativa*	Lowland
TY4	3	*sativa*	Lowland
TY5	3	*sativa*	Lowland
TY6	3	*sativa*	Lowland
TY7-B	3	*barthii*	Lowland
TY7-E	3	*barthii*	Lowland
TY9	3	*sativa*	Lowland
TY10	1	*sativa*	Lowland
TY10-A	1	*sativa*	Lowland
TY10-E	1	*sativa*	Lowland
TY10-E1	3	*sativa*	Lowland
TY10-J	1	*sativa*	Lowland
TY10-J1	1	*sativa*	Lowland
TY11	3	*sativa*	Lowland
TY14	3	*sativa*	Lowland
TY15	3	*sativa*	Lowland
TY16	3	*sativa*	Lowland
TY17	3	*sativa*	Lowland
TY18	3	*sativa*	Lowland
TY19	3	*sativa*	Lowland
TY20	3	*sativa*	Lowland
TY21	3	*sativa*	Lowland
TY23	3	*sativa*	Irrigated
TY24	3	*sativa*	Lowland
TY25	3	*sativa*	Irrigated
TY26-C	1	*glaberrima*	Floating
TY27	1	*sativa*	Lowland
TY28	3	*sativa*	Lowland
TY30	1	*sativa*	Floating
TY30-1	1	*sativa*	Floating
TY31	3	*sativa*	Floating
TY33	3	*glaberrima*	Floating
TY35	1	*sativa*	Floating
TY35-A	1	*sativa*	Floating

TY35-B	1	*sativa*	Floating
TY36	3	*sativa*	Lowland
TY37	3	*glaberrima*	Floating
TY38	1	*sativa*	Floating
TY39	1	*sativa*	Floating
TY39-F	1	*sativa*	Floating
TY40	1	*sativa*	Floating
TY41	3	*sativa*	Lowland
TY41-F	1	*sativa*	Lowland
TY41-G	1	*sativa*	Lowland
TY41-G1	1	*sativa*	Lowland
TY42	1	*sativa*	Floating
TY43	1	*sativa*	Lowland
TY45	1	*glaberrima*	Floating
TY45-A	1	*glaberrima*	Floating
TY46	1	*sativa*	Lowland
TY48	1	*sativa*	Floating
TY49	3	*sativa*	Irrigated
TY50-C	1	*glaberrima*	Floating
TY51	3	*sativa*	Irrigated
TY51-C	3	*sativa*	Irrigated
TY52	3	*sativa*	Floating
TY56	1	*glaberrima*	Floating
TY57-A	3	*glaberrima*	Floating
TY62	1	*sativa*	Lowland
TY63	1	*glaberrima*	Floating
TY63-B	1	*sativa*	Floating
TY60	3	*sativa*	Irrigated
TH1	2	*glaberrima*	Floating
TH1-C	2	*glaberrima*	Floating
TH2	1	*glaberrima*	Floating
TH3	3	*sativa*	Lowland
TH4	3	*glaberrima*	Lowland
TH5-B1	2	*glaberrima*	Lowland
TH5-C	2	*glaberrima*	Lowland
TH7-A	2	*glaberrima*	Lowland
MD1	3	*sativa*	Lowland
MD2	2	*sativa*	Lowland
MD3	2	*sativa*	Lowland
MD4	1	*sativa*	Lowland
MD5	2	*sativa*	Lowland
MD7	2	*sativa*	Lowland
ZR1	3	*glaberrima*	Lowland
ZR1-C	3	*sativa*	Lowland
ZR2	3	*sativa*	Lowland
ZR4	3	*sativa*	Lowland
NY1	3	*sativa*	Irrigated

NY2	1	*sativa*	Irrigated
NY3	3	*sativa*	Irrigated
NY4	3	*sativa*	Irrigated
NY5	3	*sativa*	Irrigated
NY6	3	*sativa*	Irrigated
NY8	3	*sativa*	Irrigated
NY9	3	*sativa*	Irrigated
NY10	3	*sativa*	Irrigated
NY11	3	*sativa*	Irrigated
NY12	3	*sativa*	Irrigated
NY14	3	*sativa*	Irrigated
DF1	1	*sativa*	Floating
DF1-1	1	*sativa*	Floating
DF1-A	3	*sativa*	Floating
DF1-B	1	*sativa*	Floating
DF2	3	*sativa*	Irrigated
DF3	3	*sativa*	Irrigated
DF4	3	*sativa*	Irrigated
DF5	3	*sativa*	Lowland
DF6	3	*sativa*	Irrigated
DF11	1	*sativa*	Lowland
DF11-A	1	*glaberrima*	Lowland
DF11-B	1	*glaberrima*	Lowland
DF14	1	*sativa*	Lowland
DF15	3	*barthii*	Lowland
DF16-F	3	*sativa*	Floating
DF16-C	3	*glaberrima*	Floating
DF17	1	*glaberrima*	Floating
DF18	1	*sativa*	Irrigated
DF18-F	3	*sativa*	Irrigated
DF19	3	*sativa*	Lowland
DF20	3	*sativa*	Lowland
IN	1	*sativa*	Floating
DS1	1	*sativa*	Floating
DS2	1	*sativa*	Lowland
DS3	3	*sativa*	Lowland
DS4	3	*sativa*	Lowland
DS5	3	*sativa*	Lowland
DS6	1	*sativa*	Floating
DS8	1	*sativa*	Lowland
DS9	3	*sativa*	Lowland
DS10	3	*sativa*	Lowland
DS11-E	2	*glaberrima*	Lowland
DS12	3	*sativa*	Lowland
DS13	3	*sativa*	Lowland
DS14	3	*sativa*	Lowland
DS14-E	3	*glaberrima*	Lowland

DS15	3	*sativa*	Lowland
DS15-B	3	*glaberrima*	Lowland
DS15-D	3	*glaberrima*	Lowland
DS15-E	3	*glaberrima*	Lowland
DS16	3	*sativa*	Lowland
DS17	3	*sativa*	Lowland
DS18	3	*sativa*	Irrigated
DS19	3	*sativa*	Irrigated
DS20	1	*sativa*	Lowland
DS22	3	*sativa*	Irrigated
DS23	3	*sativa*	Irrigated
DS24	3	*sativa*	Irrigated
DS25	3	*sativa*	Irrigated
DS28	3	*sativa*	Irrigated
DS29	3	*sativa*	Floating
DS30	3	*sativa*	Irrigated
DS31	3	*sativa*	Irrigated
DS33	3	*sativa*	Lowland
DS34	3	*sativa*	Lowland
DS34-F1	3	*glaberrima*	Lowland
DS34-H	3	*barthii*	Lowland
DS35	1	*sativa*	Floating
DS36	3	*sativa*	Lowland
DS37	3	*sativa*	Lowland
DS38	3	*sativa*	Lowland
DS39	1	*sativa*	Floating
DS40	3	*sativa*	Floating
DS41	3	*sativa*	Lowland
DS42	3	*sativa*	Lowland
DS43	3	*sativa*	Lowland
DS44	3	*sativa*	Lowland
DS45	3	*sativa*	Irrigated
DS46	3	*sativa*	Lowland
DS46-F	3	*sativa*	Lowland
DS47	3	*sativa*	Lowland
DS47-C	3	*glaberrima*	Lowland
DS48	3	*sativa*	Lowland
DS49	3	*sativa*	Irrigated
DS50	1	*sativa*	Lowland
DS51	3	*sativa*	Irrigated
DS51-A	3	*sativa*	Irrigated
DS52	1	*glaberrima*	Floating
DS53	3	*sativa*	Lowland
DS54	1	*sativa*	Floating
DS55	3	*sativa*	Irrigated
DS56	1	*sativa*	Lowland
DS57	3	*sativa*	Lowland

DS57-F	3	*sativa*	Lowland
DS57-E	3	*sativa*	Lowland
DS58	3	*sativa*	Lowland
DS60	3	*sativa*	Lowland
DS61-B	3	*sativa*	Lowland
DS62	3	*sativa*	Lowland
DS62-A	3	*glaberrima*	Lowland
DS63	3	*sativa*	Lowland
DS63-A	3	*sativa*	Lowland
DS64	3	*sativa*	Lowland
DS65	1	*sativa*	Lowland
DS68	3	*sativa*	Lowland
DS68-A	3	*sativa*	Lowland
DS69	3	*sativa*	Lowland
DS70	3	*sativa*	Lowland
DS72	3	*sativa*	Lowland
DS72-A	3	*barthii*	Lowland
DS72-B1	3	*glaberrima*	Lowland
DS72-D	3	*barthii*	Lowland
DS74	3	*sativa*	Lowland
DS75	1	*sativa*	Lowland
DS76	1	*sativa*	Lowland
DS78	1	*sativa*	Lowland
DS79-A	1	*sativa*	Floating
DS80	3	*sativa*	Floating
DS81	3	*sativa*	Lowland
DS82	3	*sativa*	Lowland
DS83	3	*sativa*	Lowland
DS83-B	3	*glaberrima*	Lowland
DS84	1	*sativa*	Lowland
DS85	1	*sativa*	Floating
DS86	3	*sativa*	Lowland
DS87	3	*sativa*	Lowland
DS87-E	3	*glaberrima*	Lowland
DS87-G	3	*sativa*	Lowland
DS87-I	3	*sativa*	Lowland
DS88	3	*sativa*	Lowland
DS88-B	3	*sativa*	Lowland
DS89	3	*sativa*	Lowland
CG14	3	*glaberrima*	Upland
Moro	3	*sativa*	Upland
IR64	3	*sativa*	Irrigated
RAM63	1	*sativa*	Floating
B6144	3	*sativa*	Irrigated
TOG7106	1	*glaberrima*	Floating/Lowland
NERICAL41	3	Interspecific	Lowland
NERICA 14	3	Interspecific	Upland

Chapter 4: Simple sequence repeat (SSR) marker-based genetic diversity and population structure of *Oryza* accessions from the Niger Republic

4.1 Abstract

Rice genetic resources conservation and evaluation is vital to secure material for breeding programs. A Niger-wide collection of *Oryza* species was conducted in 2008 and agro-morphological studies performed. For a better understanding of the level of the genetic diversity, its structuration and partitioning within eco-geographical zones, the use of molecular markers is essential. Two hundred and sixty four accessions, identified in the field as 173 *O. sativa,* 65 *O. glaberrima,* 25 *O. barthii* and 1 *O. longistaminata* were genotyped with 18 SSR markers. A total of 178 alleles were detected, with a mean of 9.89 alleles per locus, polymorphism information content (PIC) value was 0.65 and heterozygosity was 0.14. Two main well differentiate genotypic groups were identified, corresponding to *O. sativa* and African rice species. The *O. sativa* group in the country was divided into irrigated and floating rice, bound by lowland rice. African rice species group was composed of *O. glaberrima* accessions, the only *O. longistaminata* and wild progenitor *O. barthii*, but without any clear genetic differentiation probably due to the presence of admixtures within the collected samples of *O. barthii*. Five accessions, that could be natural interspecific accessions were too admixed to be classified to any of the two well differentiate groups. The eco-geographical distribution of the diversity in the countries pointed out a good germplasm exchange in Niger. The findings were discussed, and propositions made for the next step towards conservation of *Oryza* species in Niger.

Keywords: *O. glaberrima*, *O. sativa*, *O. barthii*, Conservation, Diversity, Niger

4.2 Introduction

Africa is the only continent where the two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud., have grown side-by-side for centuries (Porteres, 1956). *Oryza sativa* L., composed by two subspecies (subspp.); *indica* and *japonica*, was domesticated in Asia about 10,000 years ago, and reached African coasts between the 15th and 17th centuries (Purseglove and Harlan, 1976; Chang, 1984). *O. glaberrima*, endemic to Africa, was domesticated an estimated 3,500 years ago in West Africa, around the upper and inland delta of the Niger River in Guinea, and later in the coastal region of Senegambia (Portères, 1956; Chang, 1976; Bezançon, 1993).

However, Second (1985) suggested that the area around the Lake Chad may have played a more important role in rice domestication than the inland delta.

In the Republic of Niger, rice is the favourite crop of people along the Niger River, especially in the western part of the country. Rice cultivation was discussed as occurring in the region of Maradi (central-south) and Lake Chad (south-east) as early as the 16th century by Jean-Léon de Médicis (1488-1548), a diplomat and explorer from the Mediterranean basin, who was also known as 'Leon L'Africain' (Bonkoula and Miezan, 1982). Progressively, *O. sativa* varieties introduced from Asia has replaced *O. glaberrima* in many rice growing areas of Africa, leading to the disappearance of local rice varieties (Chang, 1984). From the 1970's, several international research centres such as the International Rice Research Institute (IRRI), the Africa Rice Center (formally WARDA) and the IRD (formally ORSTOM) as well as the International Institute of Tropical Agriculture (IITA) and the International Center for Tropical Agriculture (CIAT) have conducted bio-prospecting expeditions, and have collected and stored thousands of *Oryza* samples from all around the world (Chang, 1984; Bezançon et al., 1989; Semon et al., 2005). However, only 32 accessions from Niger, collected in 1982, were reported on the website of the AfricaRice Center's genebank (http://www.africarice.org/wagis/default.asp). These accessions were not available from 2007 to 2009 when WARDA moved from Cote d'Ivoire to Mali, then to Benin. Thus, Niger did not benefit from the global effort to conserve local rice diversity *ex-situ*.

To understand and make better use of the potential of the conserved rice accessions, several studies used different techniques to assess the genetic diversity, both overall in Africa, and in specifically in some countries of Africa or regions within African countries. Initially, isozyme markers were used to evaluate the genetic variability, as well as the relationship between cultivated rice and related wild species (Second, 1985; Bezançon, 1993). With the advent of DNA molecular markers,Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphisms (AFLP) and Simple Sequence Repeats (SSR) have become widely used (Second, 1985; Garris et al., 2005; Nuijten et al., 2009). Semon et al. (2005) studied the population structure of *O. glaberrima* accessions from 12 African countries using SSR markers. Forty two percent of their collections were accessions from the countries crossed by the Niger River, of which 80% were from Nigeria, but none were from the Niger Republic. Additionally, the genetic diversity of *O. glaberrima* has been studied in Maritime Guinea and in

Mali (Barry et al., 2007; Ndjiondjop et al., 2010). However there remains a serious lack of information on the genetic profile of the *Oryza* species in Niger.

During the last 30 years, the Republic of Niger, like other countries from the Sahel region, has developed huge irrigated areas where only high-yielding *O. sativa* varieties are cultivated. Progressively, those irrigated perimeters are remodelling the rice ecosystem of the country by continuously leading to the substitution of the indigenous rice varieties by high-yielding Asian rice varieties. Recently, rice varieties from Egypt were brought to Niger by a private rice company and are already being grown in farmers' fields in some parts of Niger. In Niger, rice is grown in three ecosystems: the irrigated ecosystem along the Niger and the Komadougou Rivers; the rainfed lowland ecosystem with controlled or semi-controlled immersion along the rivers and seasonal watercourses (Dallols and Goulbis) and lakes (Lakes Chad and Madarounfa); and the rainfed lowland with deep immersion and floating systems (Bonkoula and Miezan, 1982). The overlapping of these ecosystems, coupled with the association of different rice species and varieties may have led to the particular partitioning of rice genetic variability in Niger.

This study was initiated to conduct exhaustive rice collection in Niger and fill in the information gap about rice variability in the main eco-geographical rice cropping zones of Niger. It might also provide insight regarding the evolution of *O. glaberrima* in its centre of diversification. Therefore, the first exhaustive collection of rice species was conducted in Niger in early 2008. The goals of this study were: (1) to understand and study the genetic variation and genetic structure of rice in Niger; and (2) to reduce the genetic erosion of local landraces by establishing a baseline for both *in-situ* and/or *ex-situ* conservation.

4.3 Material and Methods

4.3.1. Plant material

In early 2008, a total of 202 samples, hereafter referred to as "accessions", were collected from 51 villages, which represented 7 of the 8 rice growing regions in Niger. The collecting trip focused on the Niger River Valley (west and south-west), the Dallol Maouri watercourse valley (south-west), the marshlands and Goulbi of Maradi (central-south), and the Komadougou River Valley (south-east) (Appendix 4.1). In each village, rice varieties were inventoried and a seed sample collected from garrets. Within accessions, seeds were purified visually and then a "purification-characterization" phenotyping trial was conducted in the 2008 rainy season.

Morphological features such as ligule length, abundance and distribution of spikelets borne on secondary branches of the panicle, and the attitude of the main axis of the panicle were used to differentiate *O. sativa* from *O. glaberrima* (Sano and Sano, 1984). Additionally, plant culm attitude (openness), grain length, awn length and texture were also used to differentiate *O. glaberrima* from adventive *O. barthii* Chev. off-types found in the rice samples. Five plants were individually harvested for each accession. The final collection was 370 accessions, but only 321 accessions were used in this study, after the elimination of duplicates. The collection was composed of cultivated rice and 26 wild rice samples, of which 9 were pure *O. barthii* accessions, collected far from any cultivated rice field; 16 were weedy *O. barthii* types isolated from rice samples collected with farmers, and one sample was *O. longistaminata* Chev. et Roehr. collected in deepwater. Pure and weedy *O. barthii* types were regrouped together as the samples of *O. barthii* in this study. Eight varieties such as RAM63 (a floating, photosensitive traditional *O. sativa* subsp. *indica* from Mali), IR64 and B6144 (irrigated *O. sativa* subsp. *indica* varieties, from the Philippines and Indonesia respectively), Moroberekan and Nipponbare (upland *O. sativa* subsp. *japonica* varieties, respectively, from Guinea and Japan), Basmati 307 (an irrigated aromatic *O. sativa* variety from India), NERICA 7 (an upland interspecific variety from AfricaRice) and CG14 (an upland *O. glaberrima* from Cote d'Ivoire) were added to the collection as reference varieties.

4.3.2. DNA extraction and genotyping

DNA was extracted by bulking about equal amounts of leaf tissues from 4 plants per accession using the CTAB protocol as described by Rustericci et al. (2000). DNA concentration was by running aliquots of DNA samples on a 1% agarose gel that contained 0.3 µg/mL of ethidium bromide. Twenty six SSR markers, previously used for studying the Generation Challenge Programme rice core collection (CIRAD, Montpellier), were chosen for this study. PCR mix consisted of 1x colorless GoTaq Reaction Buffer (Promega, M7921, Madison, WI, USA), 200 µM of each dNTP (dATP, dGTP, dCGT, dTTP), 0.2 µM of each of the M13-tailed forward and reverse primer, 0.2 µM of a universal M13 fluorescently labelled dye at 700 nm or 800 nm, 1 unit of taq DNA polymerase, and 20 ng template DNA. The 5' end of each forward primer was labelled with a M13 fluorescent dye. The amplification was performed in a T-Gradient thermocycler (Biometra, Goettingen, Germany)

The PCR products were separated on a 5.5% denaturant polyacrylamide gel on a LiCor IR2 DNA Sequencer. Alleles were called using the Geneprofiler (Version 4.05) software package. Five positive control (reference set) DNA samples (bulked-rice DNA samples of known allele sizes received from CIRAD, Montpellier) and three size markers were loaded on the same gel to serve as size references.

4.3.3. Data analysis

A total of 321 accessions and 8 control varieties were initially genotyped with 26 SSR markers. Eighteen markers were maintained after dropping the markers with more than 10% missing data. Fifty seven accessions with identical genotypes were also excluded from the dataset. Thus, the different statistical analyses were performed on the dataset consisting of 264 accessions and 18 SSR markers. Firstly, the software GenAlex (version 6.2) (Peakall and Smouse, 2006), was used to eliminate redundant accessions, with the same genotypic profile for all the markers. Secondly, a genetic dissimilarity matrix was computed based on the simple matching index using the DARwin (Version 5.0.156) software (Perrier et al., 2003).

The distance matrix was then used as input data for constructing a dendrogram using the Neighbour Joining method. Genetic diversity parameters of the collection, such as the number of alleles (Na), heterogeneity (due to heterozygosity and/or bulking of two homozygote genotypes), polymorphic information content (PIC), and gene diversity were calculated using PowerMarker Version 3.25 (Liu and Muse, 2005).

Analysis of Molecular Variance (AMOVA) was used to partition the total genetic variation into among and within population components using Arlequin Version 3.5 (Excoffier and Lischer, 2010). An admixture model-based clustering method was used to infer population structure using the software package STRUCTURE software version 2.3.3 (Pritchard et al., 2000). Allele frequencies were assumed to be correlated and loci were assumed to be unlinked (Falush et al., 2003). The number of populations tested (K) varied from 1 to 12, with ten repetitions each. The procedure was based on a burn-in period of 50,000 iterations and 200,000 iterations of the MCMC. Five independent simulations were performed. The real K value was determined using the method proposed by Evanno et al. (2005), based on the second-order rate of change of likelihood of data between consecutive K values. Accessions with probability of 70% of membership were assigned to the same group while those with lower probability memberships in

any single group were assigned to an ‘‘admixed’’ group. Finally, *O. glaberrima* accessions were separated from *O. barthii* and comparison of related diversity indexes was made.

4.4 Results

4.4.1. Overall Genetic Diversity

The total number of alleles per SSR ranged from 5 for RM124 and RM510 to 18 for RM25. The 18 SSRs detected a total of 178 alleles, and the average was 9.9 alleles per locus. PIC ranged from 0.31 to 0.91, and the average was per loci was 0.65. Sixteen out of the 18 markers had a PIC higher than 0.5; thus only 2 markers, RM124 and RM316, were not highly informative. The heterogeneity (Ho) varied from 0.08 to 0.30, with a mean of 0.14. The gene diversity (D) fluctuated between 34 and 92%, with a mean (D) of 69%. The mean frequency of the major allele (maF) at each locus ranged from 0.17 to 0.80, with a mean of 0.41 (Table 4.1).

Table 4.1: Genetic diversity of the 264 rice accessions from Niger genotyped with 18 SSR markers

Marker	**Total (N = 264)**				
	Na	**maF**	**D**	**Ho**	**PIC**
RM1	16.00	0.31	0.81	0.17	0.79
RM5	8.00	0.33	0.72	0.13	0.67
RM11	11.00	0.25	0.84	0.11	0.82
RM19	11.00	0.34	0.79	0.15	0.77
RM25	18.00	0.16	0.92	0.25	0.91
RM44	7.00	0.46	0.60	0.09	0.52
RM124	5.00	0.80	0.34	0.11	0.31
RM154	13.00	0.28	0.83	0.17	0.81
RM215	9.00	0.33	0.77	0.11	0.74
RM237	9.00	0.48	0.64	0.10	0.58
RM271	13.00	0.26	0.78	0.16	0.75
RM287	12.00	0.17	0.86	0.30	0.84
RM316	7.00	0.78	0.38	0.09	0.36
RM431	7.00	0.44	0.66	0.13	0.60
RM447	7.00	0.38	0.70	0.22	0.65
RM510	5.00	0.60	0.53	0.08	0.44
RM1227	10.00	0.49	0.65	0.14	0.60
RM14643	10.00	0.47	0.68	0.08	0.63
Total	178.00				
Mean	9.89	0.41	0.69	0.14	0.65

N : Number of accessions, maF : major allele frequency, Na : mean number of alleles per locus, D : mean gene diversity rate per locus, Ho : heterogeneity rate per locus, PIC : polymorphism information content

4.4.2 Structure of the diversity

The ad hoc statistics ΔK showed a higher likelihood value at K=2, supporting the presence of two genotypic groups or populations (Figure 4.1). The first genotypic group (Gg1) comprised of 173 accessions of *O. sativa* and 2 accessions *O. glaberrima.* Individuals of this group shared more than 98% of their genomes in common. The second genotypic group (Gg2) consisted of 86 accessions of *O. glaberrima, O. barthii* and *O. longistaminata*, sharing also 98% of common genomes. Five accessions had a membership probability < 0.7% and were assigned into a mixed genotypic group (Gg-NA). The genetic diversity indices for the two groups obtained from the model-based population structure analysis are summarised in Table 4.2 and inferred ancestry values presented in Appendix 4.2. Group 1 had higher average number of alleles, heterogeneity and gene diversity than group 2. The separation of the African rice genotypic group (Gg2) into *O. glabberima* subgroup (Gg-glab) and *O. barthii* subgroup (Gg-barthii) showed that the two groups had a similar PIC, and gene diversity, but differed in other parameters. Additionally, the Ho of RM5, RM11 and RM19 markers were nil in Gg2-*barthii*. The frequency of the major allele was higher in *O. glaberrima* and *O. barthii*, as compared to the *O. sativa* group Gg1. However, only a few alleles were found to be specific to Gg2 with markers RM19, RM124, RM215, and RM510 and very few to Gg1 with RM510, and RM1227. Thus, the genetic differentiation was mostly associated with relative differences in allelic frequencies.

Genetic distances were computed as dissimilarities based on the simple matching dissimilarity index. Very distant accessions had an index value of 1, whereas closely related accessions had a dissimilarity index = 0.03. The generated matrix was used for factorial analysis and the first two axes explained 31.3% of the total SSR variations among accessions. A plot of Axis-1 (25.0%) and Axis-2 (6.3%) revealed 2 major groups (Figure 4.2) and the pattern of groupings was basically the same as that of the model-based population partition at K=2. Nearly all accessions assigned to a population at K=2 were in the same group in the factorial analysis, with the mixed group being intermediate between the 2 groups. However, there was much more within group variation for accessions that belong to Group 1, as confirmed in a subsequently drawn tree using the Unweighted Neighbour Joining Method (UNJM) (Fig 4.3). This suggests that there was a

hidden sub-structure in that cluster that was not detected by STRUCTURE. Therefore, population structure analysis was again performed only for accessions from Group 1. The ad hoc statistics ΔK showed a higher likelihood value at K=2, suggesting the presence of two subgroups within Group 1, as shown by the tree derived from UNJM (Figure 4.4). The first sub-group (Gg1-1) consisted of 54.4% of accessions from Gg1 and included floating and photosensitive traditional *O. sativa* accessions of which reference variety, RAM63, from Mali and two cultivars released in Niger in 1952; a cultivar labelled D5237 (also called "white Degaulle" as opposite to "red Degaulle", both named after the former French president the General Charles De Gaulle, because of his tall height) and in 1959, a cultivar called "Santane Diofor"; also clustered into that group. Cultivar D5237 is a deepwater variety, whereas Satane Diofor is a very plastic variety, which can adapt to various hydrological conditions. The second sub-group (Gg1-2) included accessions collected from the irrigated and rainfed lowland ecosystems. The irrigated control varieties IR64, B6144, as well as the upland Moroberekan, the interspecific NERICA7 and Nipponbare are also in this subgroup.

Twelve accessions, were too admixed to be assigned to any of the two genetically distinct sub-populations of Gg1, and were assigned into a mixed group (Gg1-NA). These 12 accessions shared 49% and 51% of genome of Gg1-1 and Gg1-2, respectively. They were essentially irrigated rice accessions, of which the aromatic reference variety Basmati 370. Accessions like "Gambiaka" and "Taiwan" already designated by farmers as "perfumed varieties" clustered also into this subgroup. These 12 accessions shared, 49% and 51% of genome of Gg1-1 and Gg1-2, respectively. However, they were scattered within the ecological groups observed in the genetic tree, without any clear relationship (Figure 4.4).

Table 4.2: Genetic diversity among the two groups composed by individuals sharing more than 70% ancestry in Niger, and genetic diversity among the two species within Gg2 assessed at 18 SSR loci

Marker	Gg1 (N = 173)					Gg2 (N = 86)					Gg2-*glab*. (N = 60)					Gg2-*barthii* (N = 25)				
	Na	**maF**	**D**	**Ho**	**PIC**	**Na**	**maF**	**D**	**Ho**	**PIC**	**Na**	**maF**	**D**	**Ho**	**PIC**	**Na**	**maF**	**D**	**Ho**	**PIC**
RM1	14.0	0.36	0.79	0.20	0.77	8.0	0.89	0.21	0.07	0.21	8.0	0.91	0.18	0.07	0.17	5.0	0.83	0.30	0.08	0.28
RM5	8.0	0.50	0.64	0.15	0.58	3.0	0.92	0.14	0.05	0.13	3.0	0.94	0.11	0.07	0.11	2.0	0.88	0.21	0.00	0.19
RM11	9.0	0.38	0.75	0.12	0.72	3.0	0.57	0.52	0.05	0.42	3.0	0.51	0.54	0.07	0.43	3.0	0.71	0.43	0.00	0.37
RM19	11.0	0.26	0.80	0.18	0.77	5.0	0.96	0.07	0.05	0.07	5.0	0.95	0.10	0.07	0.10	1.0	1.00	0.00	0.00	0.00
RM25	17.0	0.20	0.90	0.25	0.90	11.0	0.42	0.76	0.20	0.74	10.0	0.38	0.77	0.24	0.75	8.0	0.54	0.67	0.13	0.65
RM44	7.0	0.68	0.49	0.12	0.46	4.0	0.92	0.16	0.02	0.15	4.0	0.92	0.14	0.02	0.14	3.0	0.89	0.20	0.04	0.19
RM124	4.0	0.73	0.43	0.15	0.38	4.0	0.95	0.10	0.03	0.10	3.0	0.95	0.10	0.03	0.09	3.0	0.94	0.11	0.04	0.11
RM154	11.0	0.25	0.83	0.18	0.81	7.0	0.82	0.32	0.11	0.31	5.0	0.81	0.32	0.07	0.31	6.0	0.82	0.32	0.20	0.31
RM215	7.0	0.37	0.74	0.12	0.71	5.0	0.95	0.10	0.03	0.10	4.0	0.97	0.07	0.03	0.06	3.0	0.90	0.18	0.04	0.17
RM237	5.0	0.52	0.61	0.08	0.54	6.0	0.78	0.37	0.11	0.35	5.0	0.82	0.31	0.12	0.29	5.0	0.67	0.52	0.08	0.49
RM271	9.0	0.36	0.69	0.16	0.63	8.0	0.39	0.73	0.16	0.68	8.0	0.40	0.73	0.19	0.69	6.0	0.40	0.70	0.12	0.65
RM287	10.0	0.26	0.82	0.31	0.79	9.0	0.48	0.58	0.23	0.49	6.0	0.52	0.56	0.22	0.47	6.0	0.52	0.60	0.28	0.52
RM316	5.0	0.78	0.37	0.10	0.34	4.0	0.76	0.39	0.08	0.36	4.0	0.72	0.45	0.10	0.40	4.0	0.86	0.25	0.04	0.24
RM431	7.0	0.52	0.62	0.13	0.56	4.0	0.66	0.49	0.12	0.42	4.0	0.68	0.48	0.13	0.42	3.0	0.60	0.51	0.08	0.41
RM447	7.0	0.55	0.62	0.20	0.57	4.0	0.50	0.55	0.21	0.45	3.0	0.57	0.52	0.20	0.42	4.0	0.54	0.57	0.24	0.49
RM510	5.0	0.86	0.26	0.07	0.25	2.0	0.94	0.11	0.07	0.10	2.0	0.92	0.14	0.08	0.13	2.0	0.98	0.04	0.04	0.04
RM1227	7.0	0.73	0.41	0.10	0.36	6.0	0.80	0.35	0.15	0.34	6.0	0.81	0.34	0.18	0.32	5.0	0.78	0.38	0.04	0.36
RM14643	9.0	0.70	0.49	0.06	0.47	6.0	0.89	0.20	0.10	0.20	6.0	0.88	0.23	0.12	0.22	4.0	0.92	0.15	0.08	0.15
	152					**99.0**					**89**					**73**				
	8.44	**0.50**	**0.63**	**0.15**	**0.59**	**5.50**	**0.76**	**0.34**	**0.10**	**0.31**	**4.94**	**0.76**	**0.34**	**0.11**	**0.31**	**4.06**	**0.77**	**0.34**	**0.09**	**0.31**

N : Number of accessions ; maF : major allele frequency; Na : mean number of alleles per locus; D : mean gene diversity rate per locus; Ho : mean heterogeneity rate per locus; PIC : polymorphism Information content; Gg2 (*O. glaberrima* and *O. barthii*, the one sample of *O. longistaminata* was excluded for this estimation)

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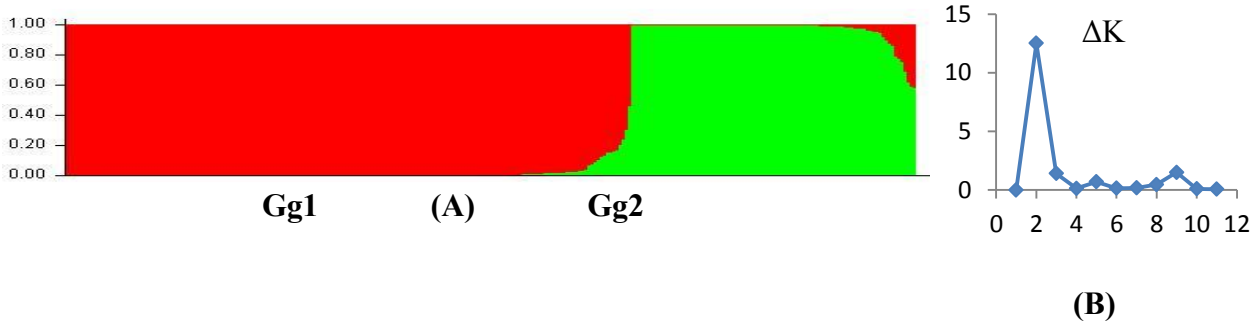

Figure 4.1: (A): Ancestries of 272 rice accessions estimated from 18 SSR loci using Structure. (B): number of populations detected from computing ΔK

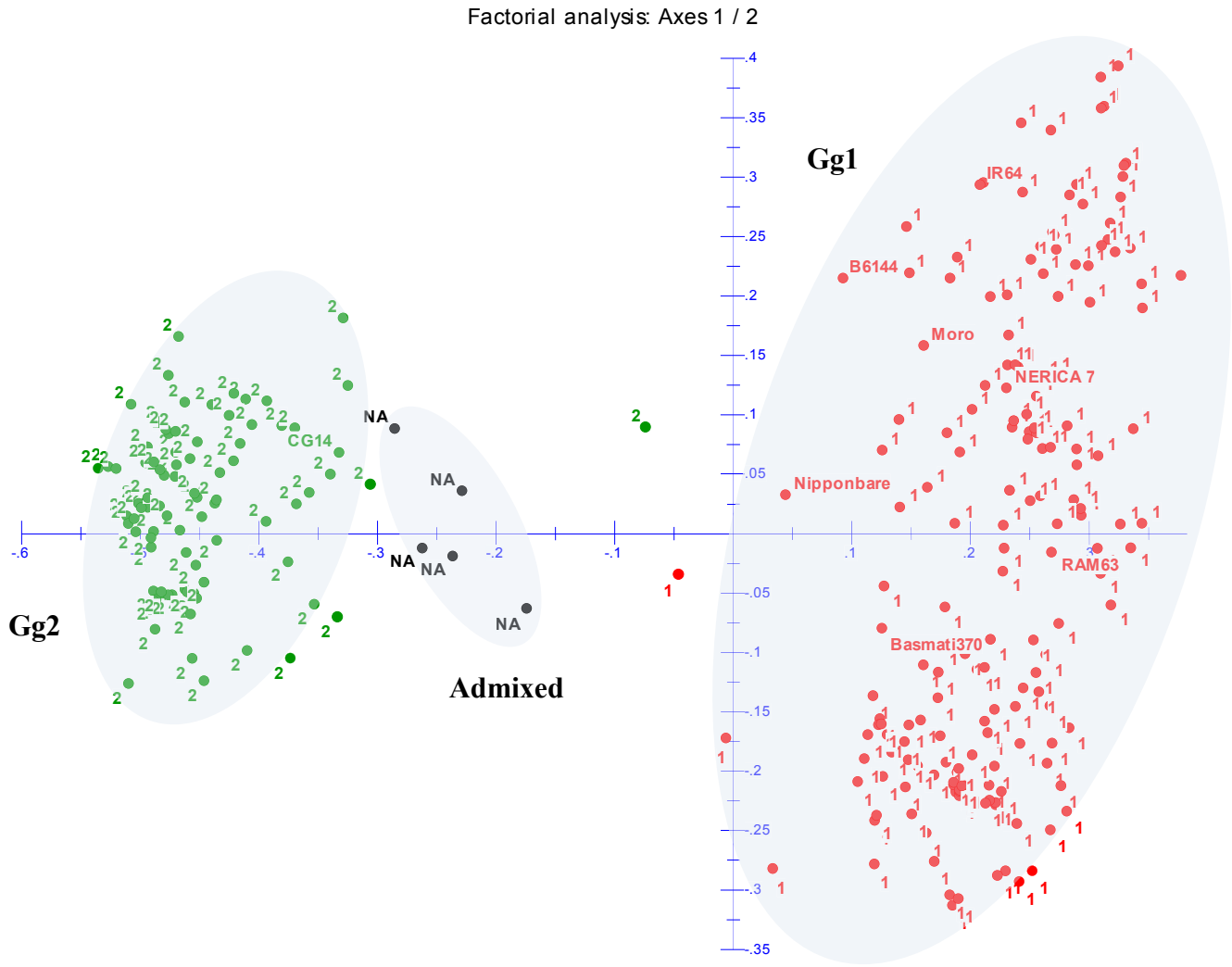

Figure 4.2 : Factorial analysis of correspondence of 272 rice accessions displaying two main groups, and admixed plotted on Axis 1 and Axis 2. Gg1 (labelled 1 in red), Gg2 (labelled 2 in green) and admixed (labelled NA). Axis 1 (25.01) and 2 (6.29)

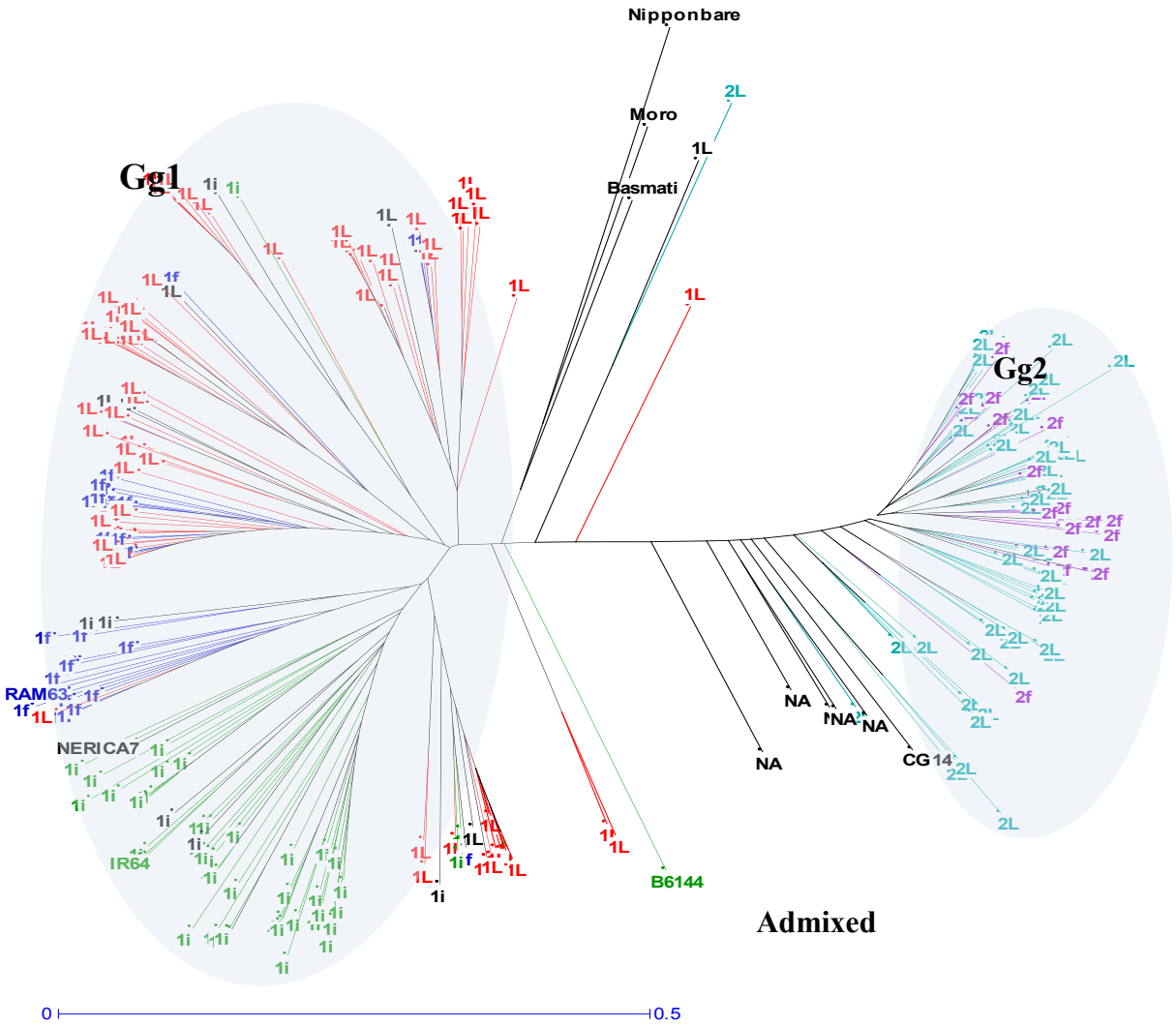

Figure 4.3: Unrooted tree of simple matching distances, based on neighbour joining, of 272 rice accessions at 18 SSR loci. The genetic groups detected by STRUCTURE displayed following the ecology (f: floating, i: irrigated and L: lowland). For Gg1: Floating is labelled in deep blue, Irrigated in green and Lowland in red; For Gg2, *O. glaberrima* is colored in light blue and *O. barthii* in purple

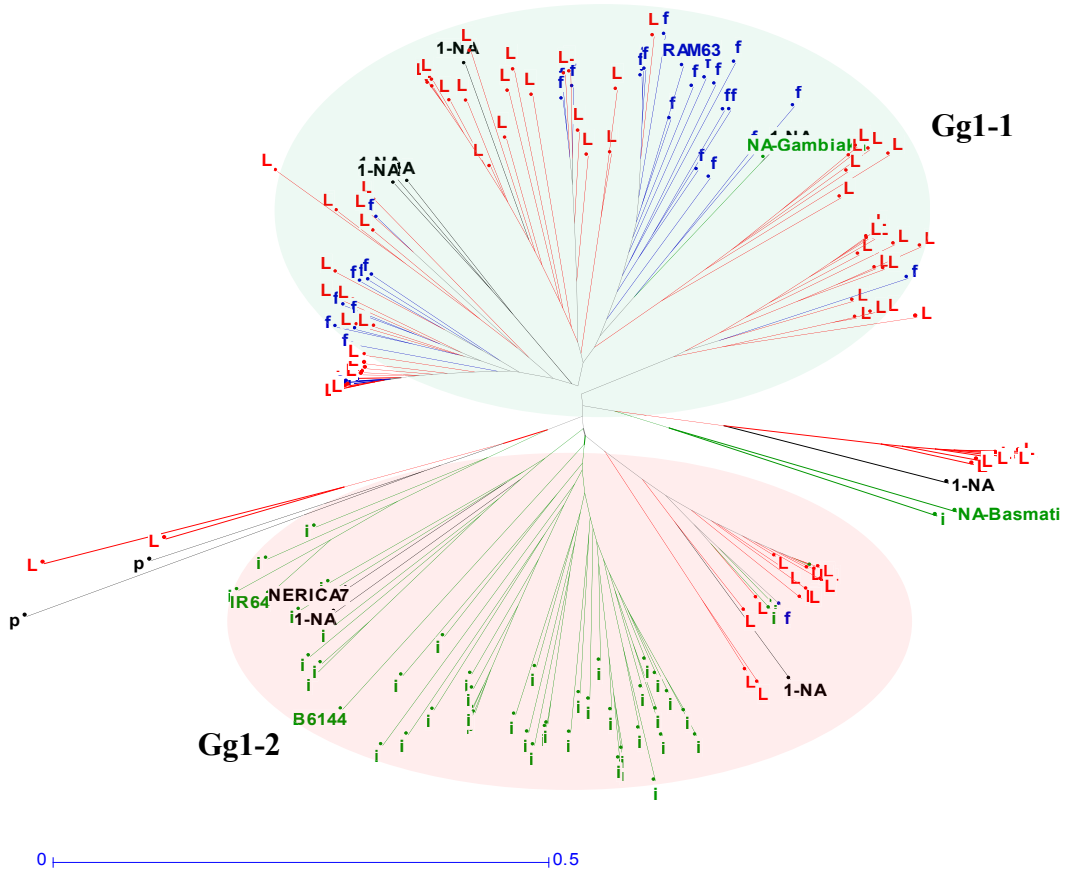

Figure 4.4: Unrooted tree of simple matching distances, based on neighbour joining, of 180 rice accessions of Gg1 at 18 SSR loci. Ecologies are differentiated by colour and letter: f= Floating is in deep blue, i = Irrigated in green and L= Lowland in red. Admixed genotypes are labelled 1-NA

4.3. Distribution of the Diversity

The populations were not equally distributed in the main rice-growing eco-geographical zones of the country (Table 4.3). The majority of the accessions of each group were found in the zone of the Niger River (72% of the collection). Indeed, the river zone represented 13.64% of the accessions from Gg1-glab., thus 60% of the total collection of *O. glaberrima*, followed by the Central-South region containing 5.30 %. Likewise, the two sub groups of *O. sativa* accessions were also well-represented in the Niger River zone, followed by the Dallol Maouri zone for Gg1-1. Gg 1-2 was under-represented in this area. Accessions of Gg2-barthii were also important across the Niger River and the Lake Chad, but very few come from the Dallol Maouri's region. The admixed group created between the two main groups (NA) originated mainly from the Niger River and Lake Chad, while the admixed between the within the *O. sativa* group (Gg1-NA) was evenly split across the different zones, except for the region of Lake Chad.

Table 4.3: Eco-geographical distribution (%) of the populations of rice accessions from the the Niger Republic

	Eco-geographical zones				
Subgroups	N	Niger River	Lake Chad	Central-South	Dallol Maouri
Gg1-1	98	27.27	3.41	1.89	4.55
Gg1-2	63	20.08	1.52	1.52	0.76
Gg1-NA	12	3.41	1.14	0.00	0.00
Gg2-glab.	60	13.64	1.89	5.30	1.89
Gg2-barthii	25	7.20	1.89	0.38	0.00
NA	5	0.38	0.00	1.14	0.38
Total	263*	71.97	9.85	10.23	7.58

* : The *O. longistaminata* accession was not included in the total

Genetic diversity parameters were also computed per eco-geographical zones. The observed heterogeneity, Ho, was similar for all zones. Pairwise differentiation between the different eco-geographical zones, as per Wright (1978) pointed out a moderate genetic differentiation between the Central-South and the others zones (Table 4.4). However, differentiation was low between the other three zones and very low between the Niger River and the Lake Chad zones.

Table 4.4: Genetic diversity and pairwise differentiation (Fst) among the rice cropping zones of the Niger Republic

	Diversity			Differentiation		
Eco-geographical zones	N	Na	Ho	Niger River	Lake Chad	Central-South
Niger River	191	9.06	0.15	-	-	-
Lake Chad	26	5.78	0.14	0.011	-	-
Central-south	27	5.06	0.14	0.064**	0.056*	-
Dallol Maouri	20	5.83	0.13	0.020	0.020	0.073*
Overall	264	9.89	0.14			

N: number of accessions; Na: mean number of alleles per locus; Ho: mean heterogeneity rate per locus; * and **: F_{ST} values significant at $p \leq 0.05$and $P \leq 0.001$, respectively

As expected, pairwise genetic differentiation between the two main groups, Gg1 and Gg2, was highly significant (Table 4.5). Furthermore, the admixed group NA was found to be moderately differentiated from the two main groups and its mean heterogeneity value was four and six times higher than Gg1 and Gg2. The same trend in the differentiation was observed between the subgroups related to the two main groups. Except for the two subgroups of Gg2, all the pairwise differentiations were significant. Gg2-glab was more differentiated from all the Gg1 subgroups

than Gg2-barthii. However, both were more differentiated from the irrigated subgroup Gg1-2 than the floating subgroup Gg1-1, composed mainly of traditional floating and lowland rice. The least differentiation was observed between the admixed Gg1-NA and the floating rice sub-cluster Gg1-1. However, the admixed Gg1-NA, was found to be the most diverse between sub-clusters with Ho = 0.38 and Gg2-barthii was the least diverse.

Table 4.5 : Genetic diversity and pairwise differentiation (Fst) among the two main populations of rice accessions sharing more than 70% ancestry, and their related subpopulations

Diversity				Differentiation			
Cluster	N	Na	Ho	Gg1	Gg2		
Gg1	173	8.44	0.15	-	-		
Gg2	86	5.50	0.10	0.405**	-		
NA	5	3.67	0.64	0.185**	0.146**		
Global	264	9.89	0.14				
Sub-cluster				Gg1-1	Gg1-2	Gg1-NA	Gg2-glab
Gg1-1	98	6.56	0.12	-	-	-	-
Gg1-2	63	6.83	0.15	0.181**	-	-	-
Gg1-NA	12	4.89	0.38	0.079**	0.11**	-	-
Gg2-glab.	60	4.94	0.11	0.438**	0.492**	0.337**	-
Gg2-barthii	25	4.06	0.09	0.407**	0.465**	0.299**	0.022

N: number of accessions; Na: mean number of alleles per locus; Ho: mean heterogeneity rate per locus; **: F_{ST} values respectively significant at $P \leq 0.001$

The partitioning of the observed genetic variance of the collection into different hierarchical levels (eco-geographical zones, ecologies, genetic groups and sub-groups) using AMOVA, revealed that significant genetic variation was found: between groups (50%) and within groups (35.5%), and also within subgroups (Table 4.6). Thus, accessions in each subgroup were highly differentiated (54.6% of the total molecular variance).

Table 4.6: Eco-geographical partitioning of the Molecular Variance of the 264 Accessions

Source of variation	df	Estimated variance	Percentage of variation
(a)			
Between zones of collection	3	-0.207	-2.451
Ecologies within each zone	8	0.953	11.283**
Groups within ecologies	6	3.477	41.167**
Within groups	500	4.223	50**
Total	517	8.446	100
(b)			
Between groups	1	2.792	35.5**
Between sub-populations within groups	2	0.774	9.8**
Accessions within subgroups	488	4.294	54.6**
Total	491	7.861	100

**: Values significant at $P \leq 0.001$

4.5 Discussion

4.5.1 Overall genetic diversity

The maintenance of crop genetic resources for evaluation and use in breeding programmes is crucial to improving agricultural production. Rice genetic resource conservation and evaluation has been conducted in several countries and regions of the world (Chang, 1984; Yawen et al., 2003; Barry et al., 2007b; Sanni et al., 2008; Girma et al., 2010), but no accessions from Niger were included in these studies. Therefore direct comparisons of the level of rice genetic diversity found in this study with others may be hazardous, because of differences in plant material composition (population size and different species proportion), as well as the number and types of SSR markers involved. As expected, *O. sativa* samples were more diverse than *O. glaberrima* and *O. barthii*, whether mixed together or evaluated separately. The same trend was observed by Barry et al. (2007b) when studying the genetic diversity of the two cultivated rice species in maritime Guinea using 170 accessions. The overall mean number of alleles, Na = 12 and polymorphism information content, a PIC = 0.81, were found with 11 SSRs loci by this author. Likewise an Na = 11.8 and a PIC = 0.67, were found with 169 SSR SSRs loci by Garris et al. (2005). They studied a world collection of 234 samples of *O. sativa* species, figures which were higher than the Na = 9.89 and the PIC = 0.59 found in the present study. This higher PIC, compared to Niger, could be explained by the existence of the two main subspecies (*indica* and

japonica), with high overall diversity in the world and Guinea samples, while there was only the *indica* subspecies in Niger. However, the gene diversity in Niger (D = 0.69) was similar to D = 0.7 found in the world collection of *sativa* species and the mean heterozygosity, Ho = 0.14, was twice as high as that of the collection of rice accessions of Maritime Guinea.

Regarding *O. glaberrima* accessions, the number of alleles found in the collection from Niger (Na=4.94) was higher than that found in the Maritime Guinea (Na=0.45). In contrast, a large number of alleles (Na= 9.4) was found in a collection of 198 rice accessions from 12 Africa countries (without Niger), when analysed at 93 SSR loci (Semon et al., 2005). Likewise, the African collection of *O. glaberrima* had slightly high PIC value (0.34), compared to that of the Niger collection presented here (PIC= 0.31), while the gene diversity was higher in Niger.

Girma et al. (2010) studied Ethiopian wild rice using inter-simple sequence repeat amplification (ISSR) and found for their sample of *O. barthii* a gene diversity value of 0.18, four times lower than the value of gene diversity (D=0.77) within the *O. barthii* sample from Niger. The number of allele per locus (Na = 4.06) in this study was double that found in a collection of 240 accessions of wild O. *rufipogon*, collected in China and south-eastern Asia, when they were evaluated by 24 pairs of SSR primers (Li et al., 2006). However, this collection of *O. rufipogon* displayed greater gene diversity. In addition, the value of gene diversity observed in the sample of *O. barthii* from Niger was higher that those from populations of *O. rufipogon* and *O. officinalis* from China, that were evaluated with seven SSR markers (Gao and Zhang, 2005).

4.5.2 Population structure and distribution

The results from the model-based population structure analysis showed the presence of two main genotypic groups. Nearly all *O. sativa* accessions belong to Group 1, while Group 2 consisted of O. *glaberrima* and its two wild relatives, *O. barthii* and *O. longistaminata*. The *O. sativa* accessions in the present study were only *O.s.* subsp. *indica*. The lack of *japonica* subspecies in the current study was not surprising because of the hot and dry climate of Niger. Indeed, in 1965 an attempt was made to introduce *japonica* varieties originating from Taiwan, but without success (Bonkoula and Miezan, 1982).

Rice cultivation in Niger is dominated by lowland production, with low to deep-water ecology (water level < 500 mm), floating ecology (water level up to 5 m) and irrigated ecology (full

controlled water level). Thus, *O. sativa* subspp. *indica* (Gg1-2), corresponding to Group I identified by Glaszmann, (1987), was well represented in irrigated and lowland ecologies with both traditional accessions, landraces. Floating photosensitive *O. sativa* accessions (corresponding to Group III and IV identified by Glaszmann) were also cultivated in the country along the Niger River and other seasonal watercourses. In addition to local floating landraces like the "red Degaulle", intensive releases of deep-water and floating rice were achieved before the independence of the country. Some varieties of the subgroup Gg1-1, like Sintane Diofo and D5237, are representative of these varieties. The latter cultivar was found to be the most important floating rice variety of the country in the production area in the western part of the Niger River, while varieties El-Sambera and Sountan, two other floating accessions, were the most important in the south-eastern part of the Niger River region.

The Gg2 group was composed of *O. glaberrima* (Gg2-glab.) cultivated only in lowland and floating ecologies, plus the closely related wild annual *O. barthii* (Gg2-barthii) and one sample of *O. longistaminata*, which is the perennial ancestor from which *O. barthii* was supposed to have been derived. Apart from ecological information, genetic data was unable to separate the "floating" ecotype from the "non-floating" described by Porteres (1970) and confirmed later (Semon et al., 2005). The floating ecotype of *O. glaberrima* was cultivated mainly in Tillabéry, bordering Mali, while the "non-floating" varieties appeared progressively in the proximity of Nigeria. However, few accessions of the "floating" glaberrima were found in the last village of Niger before entering Nigeria. In contrast, only a few accessions of glaberrima remained in the valley of the Komadougou Yobé, in the region of Lake Chad. Most of the *glaberrima* accessions from the central-south region, bordering Nigeria, were also 'non-floating" types. However, a few floating *glaberrim*a varieties were still cultivated in Tafukka, a village near the city of Konni, very far from the Niger River and close to the Nigerian border. Apart from a few villages on the Niger River, this village had the largest number of accessions of *O. glaberrima*. It was probably due to germplasm exchange between autochthones and relatives living in the region of the Niger River in Niger or Nigeria.

Our data also revealed the presence of admixed accessions between *O. sativa* and *O. glaberrima* in different regions (the Niger River, the Dallol Maouri and in the village of Tafukka). Despite

the sterility barrier, such natural recombination between the two cultivated species was widely reported. After the first case of identified natural interspecific variety in Guinea (Barry et al., 2007b), a study conducted recently on 315 rice samples from seven West-African countries revealed the occurrence of natural interspecific hybrids between the African and Asian rice in farmers' fields, leading to the emergence of a new genetic group (Nuijten et al., 2009). It was suggested that spontaneous backcrossing events were driving the process. Such possibilities were suggested long before the creation of New RIce for Africa (NERICA) by AfricaRice (Pham, 1992; Ghesquiere et al., 1997; Jones et al., 1997). Similarly natural hybrids have occurred between *O. sativa* and *O. barthii*, the wild progenitor of African rice. Their progenies were called O. *stapfii*. Chev. but in the absence of clear discriminating morphological traits and a continuous variation of traits between the two parents, progenies were subsequently classified with the barthii species (Chevalier, 1932; Bezançon, 1993). Thus, the prevalence of weedy *O. barthii* types (collected near cultivated rice fields) in our *O. barthii* samples could explain the low differentiation observed between Gg2-glab. and Gg2-*barthii* in the present study. Another hypothesis is that the marker set used in this study could not separate *O. glaberrima* from *O. barthii*, with most of their genome being common. To confirm this information, the genetic data of 2757 accessions genotyped with 50 SSR by the GCP to constitute its rice core collection was downloaded from Internet and analysed to test the concept (http://gcpcr.grinfo.net/index.php?app=datasets&inc=files_list). Similar to our study, *O. glaberrima*, *O. barthii* and *O. glumaepatula* (a floating South American wild rice species) were not separated by this set of markers, in the same way *O. sativa* was not separated from its wild ancestors, the annual O. *rufipogon* and the perennial *O. nivara*. A more recent study was conducted to investigate the domestication history of *O. glaberrima* using multiple sequences from 14 genes (Li et al., 2011). Analyses of the population structure of 20 accessions of *O. glaberrima* and 20 accessions of *O. barthii* species, from 16 African countries showed that some *O. barthii* samples, originating from the supposed centres of domestication of *O. glaberrima* (Niger River upper delta and Sahelian rivers), shared a high level of ancestry and clustered with the *O. glaberrima* group, which was homogenous, regardless the inferring methods applied.

A farmer variety collected in Tafukka is named "Chinkafa Djado", meaning "rice of the warthog" in Hausa. It has intermediate spikelets and awns characteristics similar to *O. barthii* and has very

high shattering incidence. This variety could be considered to be the result of either a natural interspecific between the two species or a variety in the process of domestication. Chen et al. (2004) reported gene flow frequencies to vary from 0.011% to 0.046%, from cultivated rice to weedy forms of *O. sativa*; and 1.21% to 2.19% from cultivated rice to wild rice O. *rufipogon*, under field conditions.

When partitioned, most of the observed genetic variance remained between the two main groups and within related subgroups, suggesting that accessions were highly differentiated within subgroups. Additionally, 12 accessions were admixed between the two sativa subgroups. However, they are randomly scattered and have no grouping pattern, and may correspond to independent gene flow event in farmers' field.

The overlapping of the three different rice ecosystems in Niger and the flat geographical pattern of the country provide the most plausible hypothesis explaining the low to moderate genetic differentiation between the main eco-geographical zones. High levels of seed exchange take place within, and between farmers in the regions, regardless of whether the accession being traditional or improved. Rice cropping intensification has reached all rice growing areas, except for some villages in the central-south zone. Agricultural extension officers have been disseminating new, improved, high-yielding varieties to replace landraces because the country is facing the third major food insecurity situation during the last decade. This could explain the very low genetic differentiation observed between the Niger River region and the Lake Chad region despite the large geographical distance (more than 1000 km). However, regarding the small number of samples collected across the Lake Chad zone, the diversity could be considered as relatively high and deserving of particular attention due to the presence of various endangered natural populations of wild *O. barthii* with long and well-filled grains. Moreover, they were very early maturing, thus could be used in breeding programmes for earliness and various traits. Such an approach has been demonstrated to be efficient in the improvement of *O. sativa*, from using genes from its wild progenitor O. *rufipogon* for numerous traits (McCouch et al., 2007).

The present study is the first reporting an exhaustive collection and evaluation of rice species from the major rice-growing regions of Niger. The analysis of the extent of the diversity revealed that a relatively high genetic diversity of *O. glaberrima* could be found, even far from the centre of diversification. The interpenetration of different ecologies and the easiness of varietal

movement promoted the dual cultivation of the two cultivated rice species, together with the wild relative, *O. barthii* species. This provides the opportunity for the occurrence of natural interspecies hybridizations. The study also revealed that Niger has much more rice diversity than the few *O. sativa* varieties currently used in modern breeding programmes in Niger. Indeed, all the varieties released the last 25 years are clustered in the Gg1-2 subgroup. Thus modern rice breeding is not exploiting the full genetic potential of Niger rice germplasm. Therefore, great progress could be made by widening the gene pool of the nationalofficial rice breeding programmes with the glaberrima and barthii compartments. The hierarchical partitioning of the genetic diversity according to the main eco-geographical zones, and genetic structures revealed that most of the variance was distributed between groups, within subgroups and within ecologies, suggesting that analyses of a collection should consider different species of rice accessions and ecologies rather than eco-geographical zones to capture the maximum diversity.

This collection is stored for *ex-situ* conservation but this provides only a short-term solution. Therefore, prior to initiating a long-term rice breeding plan, a thorough study of rice diversity in some villages should be undertaken, in order to prioritise the social network involved in the maintenance and the evolution of this diversity. This may provide a framework for an appropriate *in-situ* conservation programme.

4.7 References

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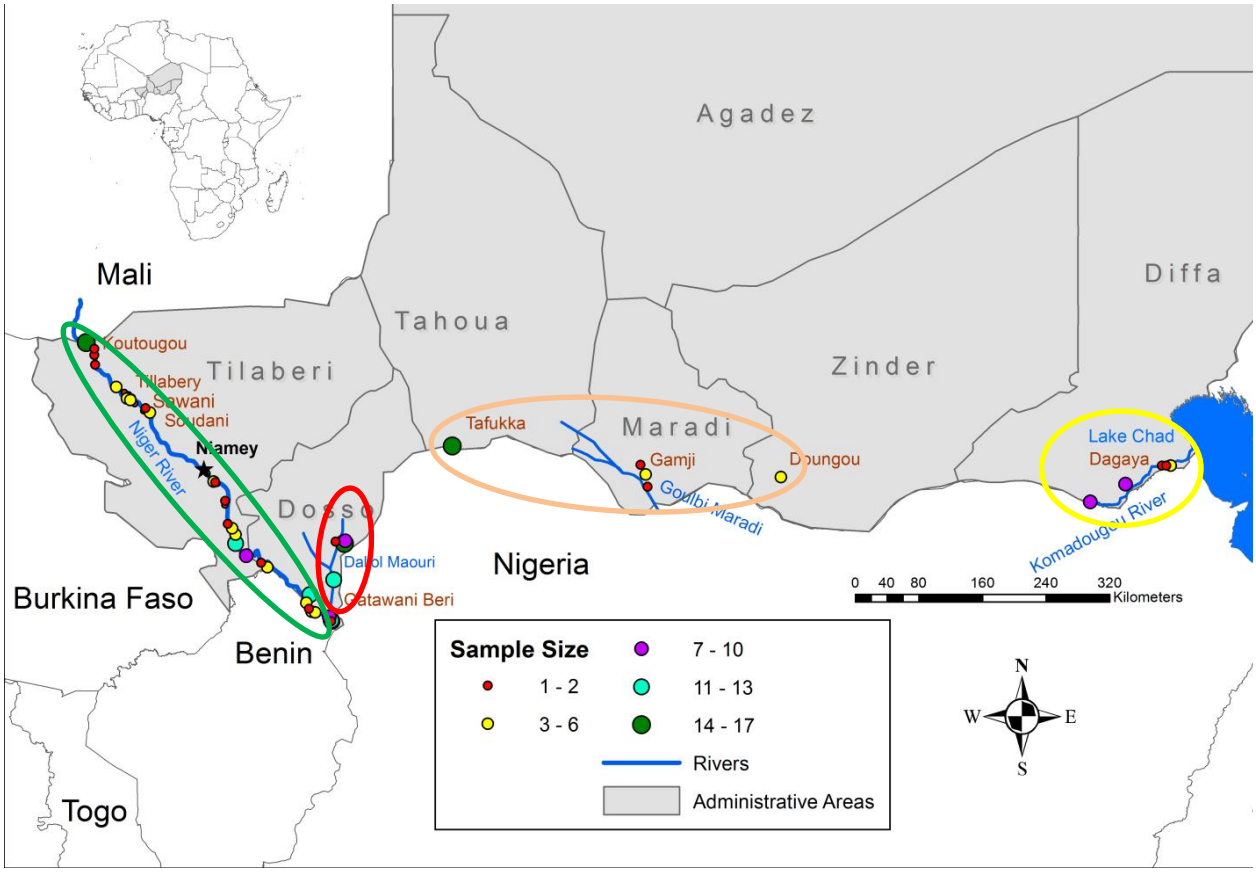
Appendix 4.1: map of Niger, showing areas where rice accessions were collected in Niger. Niger River zone is circled in green, Dallol Maouri zone is circled in red, Central South zone is circled in orange, and Lake Chad zone is circled in yellow



Appendix 4.2: Population structure of the collection from Niger derived from STRUCTURE, computed from 18 SSR markers data. The maximum likelihood was found assuming K=2

ID	Accession Code	Species	Inferred ancestry of individuals	
			Gg1 (if value > 0.7) Average distances between individuals : 0.633	Gg2 (if value > 0.7) Average distance between individuals: 0.345
1	Longist.	*longistaminata*	0.002	0.998
2	DS62-A1	*barthii*	0.003	0.997
3	DS72-C	*barthii*	0.002	0.998
4	DF20-H	*barthii*	0.005	0.995
6	DS34-K	*barthii*	0.007	0.993
7	DS63-C	*barthii*	0.002	0.998
8	DF13	*barthii*	0.027	0.973
9	DS14-C	*barthii*	0.016	0.984
10	DS66-C	*barthii*	0.022	0.978
11	DS1-B	*barthii*	0.002	0.998
12	DS6-A1	*barthii*	0.003	0.997
13	DS1-C	*barthii*	0.007	0.993
14	DS11-A1	*glaberrima*	0.122	0.878
15	DS14-B	*barthii*	0.002	0.998
16	TH5-1	*barthii*	0.003	0.997
17	DS47-B	*glaberrima*	0.002	0.998
18	DS52-E	*glaberrima*	0.002	0.998
19	TY7-J	*barthii*	0.006	0.994
20	DF17-B	*barthii*	0.005	0.995
21	DS34-E	*barthii*	0.004	0.996
22	DF23	*barthii*	0.006	0.994
23	TY51-F	*sativa*	0.998	0.002
24	DS9-B	*barthii*	0.002	0.998
25	DS2-E	*barthii*	0.055	0.945
26	TY53	*glaberrima*	0.002	0.998
28	DS14-E1	*glaberrima*	0.012	0.988
30	TY7	*glaberrima*	0.841	0.159
31	TH5-B	*glaberrima*	0.002	0.998
32	TH5-E	*glaberrima*	0.408	0.592
34	DF12	*glaberrima*	0.002	0.998
36	DS3-A	*glaberrima*	0.224	0.776
37	DS83-C	*glaberrima*	0.015	0.985
38	TH1-B	*glaberrima*	0.002	0.998
39	DS34-B	*glaberrima*	0.001	0.999
40	MD1-A	*glaberrima*	0.044	0.956
41	DS11-A	*glaberrima*	0.103	0.897
43	DS47-C3	*glaberrima*	0.037	0.963
44	DS70-C	*glaberrima*	0.014	0.986
45	DS45-B	*glaberrima*	0.011	0.989
46	DS47-C2	*glaberrima*	0.002	0.998

49	DS62-B	*glaberrima*	0.028	0.972
50	TY8-D1	*glaberrima*	0.998	0.002
51	DS14-F2	*glaberrima*	0.002	0.998
53	DS34-C1	*glaberrima*	0.006	0.994
54	DS14-F1	*glaberrima*	0.002	0.998
56	DS11-B	*glaberrima*	0.003	0.997
57	TY50	*glaberrima*	0.008	0.992
58	TH7	*glaberrima*	0.007	0.993
61	DS34-A	*glaberrima*	0.002	0.998
62	TH6	*glaberrima*	0.002	0.998
63	DS4-B1	*glaberrima*	0.001	0.999
70	TH5	*glaberrima*	0.005	0.995
71	TY7-D	*glaberrima*	0.024	0.976
72	TH1-A	*glaberrima*	0.018	0.982
76	TY4	*sativa*	0.995	0.005
78	TY6	*sativa*	0.997	0.003
81	TY7-B	*barthii*	0.002	0.998
82	TY7-E	*barthii*	0.002	0.998
86	TY10	*sativa*	0.997	0.003
87	TY10-A	*sativa*	0.998	0.002
89	TY10-E	*sativa*	0.998	0.002
90	TY10-E1	*sativa*	0.997	0.003
92	TY10-J	*sativa*	0.997	0.003
93	TY10-J1	*sativa*	0.998	0.002
94	TY11	*sativa*	0.974	0.026
96	TY14	*sativa*	0.998	0.002
97	TY15	*sativa*	0.998	0.002
98	TY16	*sativa*	0.999	0.001
99	TY17	*sativa*	0.999	0.001
100	TY18	*sativa*	0.998	0.002
101	TY19	*sativa*	0.998	0.002
102	TY20	*sativa*	0.998	0.002
103	TY21	*sativa*	0.998	0.002
105	TY23	*sativa*	0.998	0.002
106	TY24	*sativa*	0.999	0.001
107	TY25	*sativa*	0.998	0.002
108	TY26-C	*glaberrima*	0.381	0.619
109	TY27	*sativa*	0.998	0.002
110	TY28	*sativa*	0.998	0.002
112	TY30	*sativa*	0.998	0.002
113	TY30-1	*sativa*	0.997	0.003
114	TY31	*sativa*	0.998	0.002
116	TY33	*glaberrima*	0.002	0.998
118	TY35	*sativa*	0.998	0.002
119	TY35-A	*sativa*	0.998	0.002
120	TY35-B	*sativa*	0.998	0.002
121	TY36	*sativa*	0.997	0.003

122	TY37	*glaberrima*	0.002	0.998
123	TY38	*sativa*	0.998	0.002
124	TY39	*sativa*	0.998	0.002
125	TY39-F	*sativa*	0.996	0.004
126	TY40	*sativa*	0.998	0.002
127	TY41	*sativa*	0.998	0.002
128	TY41-F	*sativa*	0.91	0.09
129	TY41-G	*sativa*	0.998	0.002
130	TY41-G1	*sativa*	0.998	0.002
131	TY42	*sativa*	0.998	0.002
133	TY43	*sativa*	0.998	0.002
135	TY45-A	*glaberrima*	0.008	0.992
136	TY46	*sativa*	0.998	0.002
137	TY48	*sativa*	0.998	0.002
138	TY49	*sativa*	0.997	0.003
139	TY50-C	*glaberrima*	0.002	0.998
140	TY51	*sativa*	0.998	0.002
141	TY51-C	*sativa*	0.998	0.002
142	TY52	*sativa*	0.998	0.002
143	TY56	*glaberrima*	0.049	0.951
144	TY57-A	*glaberrima*	0.002	0.998
145	TY62	*sativa*	0.997	0.003
146	TY63	*glaberrima*	0.002	0.998
147	TY63-B	*sativa*	0.998	0.002
149	TY60	*sativa*	0.998	0.002
150	TH1	*glaberrima*	0.006	0.994
151	TH1-C	*glaberrima*	0.002	0.998
152	TH2	*glaberrima*	0.003	0.997
153	TH3	*sativa*	0.849	0.151
154	TH4	*glaberrima*	0.31	0.69
155	TH5-B	*glaberrima*	0.005	0.995
156	TH5-C	*glaberrima*	0.003	0.997
157	TH7-A	*glaberrima*	0.009	0.991
158	MD1	*sativa*	0.933	0.067
159	MD2	*sativa*	0.998	0.002
160	MD3	*sativa*	0.998	0.002
161	MD4	*sativa*	0.997	0.003
162	MD5	*sativa*	0.998	0.002
164	MD7	*sativa*	0.417	0.583
165	ZR1	*glaberrima*	0.002	0.998
166	ZR1-C	*sativa*	0.998	0.002
167	ZR2	*sativa*	0.987	0.013
168	ZR4	*sativa*	0.998	0.002
169	NY1	*sativa*	0.998	0.002
170	NY2	*sativa*	0.997	0.003
171	NY3	*sativa*	0.997	0.003
172	NY4	*sativa*	0.997	0.003

173	NY5	*sativa*	0.997	0.003
174	NY6	*sativa*	0.995	0.005
176	NY8	*sativa*	0.997	0.003
177	NY9	*sativa*	0.984	0.016
178	NY10	*sativa*	0.998	0.002
179	NY11	*sativa*	0.992	0.008
180	NY12	*sativa*	0.996	0.004
182	NY14	*sativa*	0.998	0.002
183	DF1	*sativa*	0.998	0.002
184	DF1-1	*sativa*	0.998	0.002
185	DF1-A	*sativa*	0.994	0.006
186	DF1-B	*sativa*	0.998	0.002
187	DF2	*sativa*	0.994	0.006
188	DF3	*sativa*	0.999	0.001
189	DF4	*sativa*	0.998	0.002
190	DF5	*sativa*	0.997	0.003
191	DF6	*sativa*	0.998	0.002
194	DF11	*sativa*	0.998	0.002
195	DF11-A	*glaberrima*	0.008	0.992
196	DF11-B	*glaberrima*	0.002	0.998
198	DF14	*sativa*	0.997	0.003
199	DF15	*barthii*	0.142	0.858
200	DF16-F	*sativa*	0.998	0.002
201	DF16-C	*glaberrima*	0.002	0.998
202	DF17	*glaberrima*	0.004	0.996
204	DF18	*sativa*	0.997	0.003
205	DF18-F	*sativa*	0.998	0.002
206	DF19	*sativa*	0.997	0.003
207	DF20	*sativa*	0.998	0.002
210	IN	*sativa*	0.998	0.002
211	DS1	*sativa*	0.998	0.002
212	DS2	*sativa*	0.998	0.002
213	DS3	*sativa*	0.998	0.002
214	DS4	*sativa*	0.998	0.002
215	DS5	*sativa*	0.998	0.002
216	DS6	*sativa*	0.998	0.002
217	DS8	*sativa*	0.998	0.002
218	DS9	*sativa*	0.847	0.153
219	DS10	*sativa*	0.998	0.002
221	DS11-E	*glaberrima*	0.002	0.998
222	DS12	*sativa*	0.975	0.025
223	DS13	*sativa*	0.997	0.003
224	DS14	*sativa*	0.54	0.46
225	DS14-E	*glaberrima*	0.005	0.995
226	DS15	*sativa*	0.998	0.002
228	DS15-B	*glaberrima*	0.002	0.998
229	DS15-D	*glaberrima*	0.001	0.999

230	DS15-E	*glaberrima*	0.002	0.998
231	DS16	*sativa*	0.867	0.133
232	DS17	*sativa*	0.764	0.236
233	DS18	*sativa*	0.997	0.003
234	DS19	*sativa*	0.999	0.001
235	DS20	*sativa*	0.998	0.002
237	DS22	*sativa*	0.997	0.003
238	DS23	*sativa*	0.998	0.002
239	DS24	*sativa*	0.998	0.002
240	DS25	*sativa*	0.987	0.013
242	DS28	*sativa*	0.998	0.002
243	DS29	*sativa*	0.994	0.006
244	DS30	*sativa*	0.924	0.076
245	DS31	*sativa*	0.998	0.002
247	DS33	*sativa*	0.998	0.002
248	DS34	*sativa*	0.98	0.02
249	DS34-F1	*glaberrima*	0.002	0.998
250	DS34-H	*barthii*	0.004	0.996
251	DS35	*sativa*	0.969	0.031
252	DS36	*sativa*	0.998	0.002
253	DS37	*sativa*	0.998	0.002
254	DS38	*sativa*	0.977	0.023
255	DS39	*sativa*	0.998	0.002
256	DS40	*sativa*	0.998	0.002
257	DS41	*sativa*	0.998	0.002
258	DS42	*sativa*	0.998	0.002
259	DS43	*sativa*	0.995	0.005
260	DS44	*sativa*	0.992	0.008
261	DS45	*sativa*	0.997	0.003
262	DS46	*sativa*	0.997	0.003
263	DS46-F	*sativa*	0.997	0.003
264	DS47	*sativa*	0.997	0.003
265	DS47-C	*glaberrima*	0.002	0.998
266	DS48	*sativa*	0.998	0.002
267	DS49	*sativa*	0.998	0.002
268	DS50	*sativa*	0.998	0.002
269	DS51	*sativa*	0.998	0.002
270	DS51-A	*sativa*	0.996	0.004
271	DS52	*glaberrima*	0.003	0.997
272	DS53	*sativa*	0.997	0.003
273	DS54	*sativa*	0.998	0.002
274	DS55	*sativa*	0.995	0.005
275	DS56	*sativa*	0.998	0.002
276	DS57	*sativa*	0.998	0.002
277	DS57-F	*sativa*	0.699	0.301
278	DS57-E	*sativa*	0.99	0.01
279	DS58	*sativa*	0.998	0.002

281	DS60	*sativa*	0.997	0.003
282	DS61-B	*sativa*	0.997	0.003
283	DS62	*sativa*	0.982	0.018
284	DS62-A	*glaberrima*	0.039	0.961
286	DS63	*sativa*	0.998	0.002
287	DS63-A	*sativa*	0.998	0.002
288	DS64	*sativa*	0.995	0.005
289	DS65	*sativa*	0.997	0.003
293	DS68	*sativa*	0.967	0.033
294	DS68-A	*sativa*	0.998	0.002
295	DS69	*sativa*	0.998	0.002
296	DS70	*sativa*	0.995	0.005
297	DS72	*sativa*	0.987	0.013
298	DS72-A	*barthii*	0.008	0.992
299	DS72-B1	*glaberrima*	0.212	0.788
300	DS72-D	*barthii*	0.007	0.993
301	DS74	*sativa*	0.998	0.002
302	DS75	*sativa*	0.872	0.128
303	DS76	*sativa*	0.997	0.003
305	DS78	*sativa*	0.998	0.002
306	DS79-A	*sativa*	0.998	0.002
307	DS80	*sativa*	0.998	0.002
308	DS81	*sativa*	0.963	0.037
309	DS82	*sativa*	0.899	0.101
310	DS83	*sativa*	0.998	0.002
311	DS83-B	*glaberrima*	0.085	0.915
312	DS84	*sativa*	0.996	0.004
313	DS85	*sativa*	0.979	0.021
314	DS86	*sativa*	0.998	0.002
315	DS87	*sativa*	0.998	0.002
316	DS87-E	*glaberrima*	0.004	0.996
317	DS87-G	*sativa*	0.997	0.003
318	DS87-I	*sativa*	0.982	0.018
319	DS88	*sativa*	0.997	0.003
320	DS88-B	*sativa*	0.997	0.003
321	DS89	*sativa*	0.834	0.166
322	CG14	*glaberrima*	0.248	0.752
323	Moro	*sativa*	0.989	0.011
324	IR64	*sativa*	0.979	0.021
325	RAM63	*sativa*	0.998	0.002
501	B6144	*sativa*	0.984	0.016
502	Basmati 370	*sativa*	0.975	0.025
507	Nipponbare	*sativa*	0.799	0.201
508	NERICA 7	Interspecific	0.997	0.003

Red colour: accessions too admixed to be classified into one group or another. Green colour: reference accessions

Chapter 5: Resistance to *Rice yellow mottle virus* (RYMV) in a rice germplasm collection from Niger

5.1 Abstract

The *Rice yellow mottle virus* (RYMV) is the most damaging disease in the rice agrosystems in Niger. To search for new sources of resistance to RYMV, a rice collection from Niger and some accessions from Mali were screened for resistance. Five different virus isolates from Niger (3), Benin (1) and Burkina Faso (1) were used for inoculation. The assessment was based on visual disease symptom scoring, virus content by ELISA and secondary disease-related traits such as chlorophyll content of leaves, and plant height. It was found that most rice accessions were susceptible to the disease. However, a few *O. glaberrima* accessions displayed a level of resistance similar to the highly resistant TOG5681, but this depended upon the virus strain. Their resistance was characterised by the absence of symptoms, low chlorosis and limited plant height reduction. Allelic research based on primers derived from the *RYMV1* gene revealed one accession with allele *rymv1-3*, and two accessions with allele *rymv1-4*, while there was no matching for one highly resistant accession, suggesting the presence of another resistance gene. The RYMV isolate BF1 from Burkina Faso was more aggressive than the three isolates from Niger, which were in turn found to be more aggressive than the isolate from Benin.

Keywords: RYMV, Niger, rice collection, screening, differential reaction

5.2 Introduction

Rice yellow mottle virus (RYMV) is caused by a *Sobemovirus* and is the most harmful disease of irrigated and lowland rice ecology in Africa (Abo et al., 1998; Banwo, 2003). Less than twenty years after the first report of the virus in Kenya (Bakker 1920), RYMV has been reported in all rice growing ecosystems of Africa (WARDA, 2001; Kouassi et al., 2005). The virus emerged in East Africa approximately 200 years ago, and has undergone evolution into at least six groups, and a rapid dispersion from Eastern to Western Africa (Abubakar et al., 2003; Traoré et al., 2005; Fargette et al., 2008a). The virus is transmitted by numerous biotic vectors among which are: insects belonging to the Chrysomelidae family (Nwilene et al., 2009), farmers' hands, rats, cows and donkeys (Sarra and Peters, 2003), as well as abiotic vectors like infected soils, farming tools, water and even wind through contact between plants (Sarra et al., 2004; Traore et al., 2008). The disease is seedling-borne, but it is not seedborne (Konate et al., 2001). Yield losses of 56-68%

(Reckhaus and Adamou, 1986) have been reported in Niger and up to 100% in Mali (Sy et al., 1993). On susceptible plants, the virus symptoms are yellowish or orange stripes on leaves, severe stunting and tiller reduction, poor panicles exsertion, spikelet sterility and plant death, depending on the rice variety and the period of infection (Nwilene et al., 2009). Due to the importance of man in the dissemination of the virus, chemicals were found ineffective in controlling its spread (Traoré et al., 2009).Good cultural practices provided an important way to manage the disease, especially when combined with varietal resistance. Several studies have attempted to identify natural sources of resistance to RYMV in *O. sativa* and *O. glaberrima*, and *O. barthii* (Thottappilly and Rossel, 1993; Coulibaly et al., 1999). The gene that provides for strong and stable natural resistance to RYMV (*RYMV1*) has been found in two *O. sativa* subspp. *indica* varieties, Gigante (Ndjiondjop et al., 1999) and Bekarosaka (Rakotomalala et al., 2008) and also in several accessions of *O. glaberrima* (Ndjiondjop et al., 1999; Thiémélé et al., 2010). A recessive gene has been identified as coding for the eukaryotic translation initiation factor eIF(iso)4G (Albar et al., 2006). Five allelic forms of this gene have been identified. The allele *rymv1-1* has been found in *O. sativa* variety IR64 and *O. glaberrima* variety TOG5673. This allele confers susceptibility, while the other four alleles confer resistance. The allele *rymv1-2* was identified in *O. sativa* varieties Gigante and Bekarosaka, the alleles *rymv1-3* and *rymv1-4* were found in *O. glaberrima* varieties TOG5681 and TOG5672, respectively (Albar et al., 2006); and allele *rymv1-5* was found in *O. glaberrima* variety TOG5674 (Thiémélé et al., 2010). The allele *rymv1-1* confers susceptibility, while the other four alleles confer resistance. Another resistance gene (*RYMV2*) has also been identified in O. glaberrima accession TOG7291. Partial resistance has been found in the *O. sativa* subspp. *japonica* variety Azucena (Albar et al., 1998). The natural resistance gene, *rymv1-2,* was found to be more stable than this partial resistance, and a transgenic resistance, containing a transgene encoding for the RNA-dependent RNA polymerase of the virus (Pinto et al., 1999; Sorho et al., 2005). However, resistance-breaking (RB) isolates of the virus have been reported under both experimental screenhouse conditions and in farmers' fields (Fargette et al, 2002; Traoré et al., 2006). A single substitution in the genome-linked viral protein VPg was reported to confer to a wild virus strain the ability to overcome the resistance of *rymv1-2* (Hébrard et al., 2006).

Very few studies have been published on RYMV in Niger. Reckhaus and Adamou (1989) noted that RYMV exists in the major irrigated rice areas in Niger, with yield losses reaching up to 65%.

A recent study revealed that three major pathogenic groups of RYMV (high pathogenicity, intermediate and low pathogenicity) existed in Niger (Basso et al., 2010).The variety *O. sativa* subspp. *indica*, IR1529-680-3, the most widely spread cultivar in irrigated areas of Niger, is highly susceptible to the disease, while WITA8 and WITA9 are tolerant only in some areas and susceptible in others. However, the study by Basso et al., (2010) evaluated only a few rice varieties released in Niger. Therefore, this study investigated the response of a set of 175 accessions from a rice collection from Niger to inoculation with multiple strains of RYMV. The aim was to search for new sources of resistance to RYMV to be introgressed into a rice breeding program in the country.

5.3 Material and Methods

5.3.1. Plant materials and inoculations

The plant material consisted of 175 accessions from Niger, 52 accessions from Mali, 2 resistant checks (Gigante and TOG5681) and 2 susceptible checks (IR64 and Bouaké189). The 175 accessions from Niger were randomly selected among the collections made in the country early in 2008. This collection included 139 traditional landraces, as well as modern *O. sativa* varieties, 32 traditional *O. glaberrima* landraces, and 4 wild *O. barthii* accessions.

RYMV isolates were multiplied by mechanical inoculation onto the susceptible variety IR64 for wild types of the virus, or varieties Gigante and TOG5681 for RB-isolates, as described by Ndjiondjop et al. (1999). Infected frozen leaves sourced from the Pathology Unit of AfricaRice were ground in a phosphate buffer (0.1 M KH_2PO_4, 0.1 M Na_2HPO_4, pH 7.2) using a ratio of 1:10 (w:v). The mixture, with carborundum dust (600 mesh) added as an abrasive, was used directly to inoculate two week old seedlings of the susceptible varieties by finger-rubbing young leaves with the inoculum. Two weeks later, infected leaves were harvested and used to prepare the inoculum needed for the different trials.

Four disease evaluation trials were conducted under screenhouse conditions of which 3 trials were conducted at AfricaRice in Cotonou, Benin and one trial at IRD, Montpellier, France. The first trial involved 227 accessions (175 accessions from Niger and 52 accessions from Mali), plus a susceptible (IR64) and resistant (Gigante) check. Three plants per accession were inoculated, with the two checks grown between every 20 accessions. The RYMV isolate B27 that belongs to the S1 group (Thiémélé et al., 2010) was used to inoculate the plants in Trial 1. Trial 2 had three

experiments and was conducted on 155 accessions from Niger (the worst 20 accessions from Trial 1 were discarded), plus the 2 susceptible and 2 resistant checks described above. However, one accessions did not germinate, thus data were recorded and analysed for 154 accessions. Accessions were randomly assigned to five blocks, arranged into an Augmented Design, with three pots per accession per experiment and three plants per pots. In Experiment One, all plants were inoculated with the RYMV isolate Ng122; in Experiment Two plants were inoculated with Isolate Ng144; Experiment Three was run as a negative control and all plants were inoculated with distilled water and carborundum dust. The two virus strains have been reported to induce different symptoms on the susceptible IR64 and resistant TOG5681 and Gigante checks.

In Trial 3, a subset of 24 resistant accessions (eight were *O. sativa*) that were the most resistant in the previous trials were screened against three RYMV isolates (Ng117b, Ng122 and Ng144) from Niger using a split plot design, with virus isolates as the main plots and the genotypes as subplots. Three replications, of three pot each, each containing three plants, were used for each virus strain. A fourth replication was only inoculated with distilled water and carborundum.

In Trial 4, a subset of 25 accessions (of which only one was *O. sativa*) that were also the most resistant in the previous trials were also evaluated at IRD, Montpellier,France using the RYMV isolate BF1, sourced from Burkina Faso. Only 8 accessions were common between Trial 3 and Trial 4. They were selected because they were resistant to Isolate B27 in Trial 1, and Isolates Ng122 and Ng144 in Trial 2. For each accession ten plants were inoculated. Varieties IR64 and Gigante were grown between every seven accessions, as a susceptible and resistant checks, respectively.

5.3.2. Data collection and statistical analysis

For all the experiments, a visual evaluation of RYMV symptoms was made using the Standard Evaluation System of IRRI (2002) with a 1-9 visual scale: 1 for no symptoms and plants considered as highly resistant (HR); 3 for sparse dots or streaks, considered as partially resistant (PR); 5 for visible mottling on green to light-green leaves, considered as Intermediate (I); 7 for generalised yellowing and stunting, considered as susceptible (S); 9 for necrosis, and plant death, considered as highly susceptible (HS) (Konate et al., 1997). Depending on the trial, the visual rating was recorded every week from 14 to 42 or 49 days after inoculation (DAI).

In Trials 1,2 and 3 the chlorophyll content of leaves (ChcL) was measured, with 10 points per accession, either at 42 DAI (Trial 1 and 2), or every week from 14 to 42 DAI (Trial 3), with a SPAD 502 chlorophyll Meter (Minolta C. Co. Osaka, Japan) (Esfahani et al., 2008). Likewise, Plant height (PHgt) was also recorded on 3 plants per accession at 42 DAI in Trials 1, 2 and 3 (Onasanya et al., 2006). Accessions that displayed a high level of resistance (disease score equal to 1) during the first trial were further evaluated for the virus content using an enzyme-linked immunosorbent assay (ELISA), as described by Séré et al. (2007). The last fully expanded leaf was harvested, ground in a coating buffer (Na_2CO_3, $NaHCO_3$, pH 9.6) at 1:10, w:v) and the homogenized sap was directly adsorbed to microtitre plates overnight, followed by blocking with 1% bovine serum albumin. Double serial dilutions of antisera were made and bound antibody was detected with goat anti-rabbit serum, conjugated to alkaline phosphatase (Sigma-Aldrich Co, MO, USA). The bound conjugate was detected using a p-nitrophenyl phosphate solution (1 mg ml^{-1}) and the optical density was read with a spectrophotometer at 405 nm. A sample was considered to be negative when the absorbance value was lower than the average absorbance of the negative control.

The software package SAS version 9.1 (SAS Institute Inc., Cary, NC) was used to analyse the data sets. First, an analysis of variance (ANOVA) was performed to compare differences in virulence among virus isolates. In addition, a generalized linear model (GLM) for multinomial data procedure was used to analyse discrete data, such as the disease symptom scores. The odds ratio estimates the strength of association between the response of interest (in this case disease scores), which stipulated that resistant accessions should have low disease symptoms and thus low scores). Microsoft Office Excel 2007 was also used to draw charts and compute the area under a symptoms progression curve (AUSPC) estimated using the formula:

$AUSPC = \sum [(S_i+S_{(i+1)}-2)(t_{(i+1)}- t_i)]/2$, ($S_i$ is the symptom score at date t_i)

Finally, the AUSPC, rPHgt, and rChcL were used to perform a principal component analysis using the software GenStat version 14.

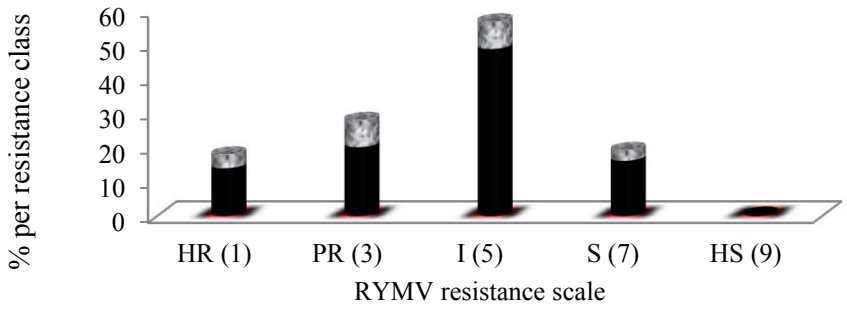
5.3.5. Genotyping

From Trials 4 and 2, 11 resistant accessions were investigated using primers allel-specific primers that were designed to amplify the three resistant alleles of *RYMV1* gene. DNA was extracted from infected plant leaves as described by Edwards et al. (1991). All the 11 accessions selected for genotyping were *O. glaberrima*; therefore only alleles for *rymv1-3*, *rymv1-4* and *rymv1-5* were targeted in this study. PCR was performed as described by Thiémelé et al. (2010), using eight primer pairs, of which two primers were specific for each allele (F18 and R16 for *rymv1-3;* F17 and R15b for *rymv1-4;* R18 and F6 for *rymv1-5),* while the remaining two primers (F5 and R3) were common for all three alleles. Amplified products were separated on 2.5% stained with 5% ethidium bromide (BET).

5.4 Results

The disease score made at 42 DAI in Trial 1 ranged from total absence of symptoms (HR) to severe symptoms, including plant death (HS) (Figure 5.1a). The majority of the accessions (65.6%), including the control IR64, displayed at least visible mottling or light green leaves (S > 5) and 0.4% were highly susceptible. The remaining 34.0% were either symptomless (14.1%, including the control Gigante) or exhibited green leaves with sparse dots or light streaks (20.3%). Of the 175 rice accessions from Niger 13.1% were symptomless, 16% were partially resistant (PR), 537% intermediate (I), 16.6% susceptible and 0.6% highly susceptible (HS) (Figure 5.1b). Whereas for the accessions from Mali, none were HS, 3.1% were susceptible and 4% were symptomless.

A



b

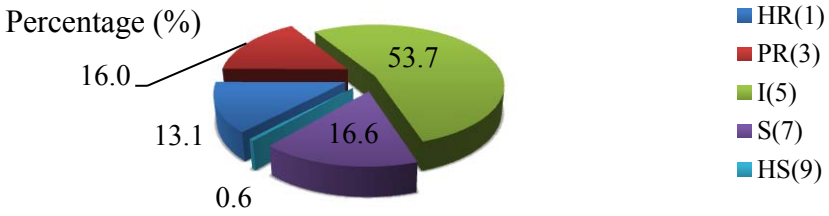


Figure 5.1: (a) Frequency distribution for disease scores 42 days after inoculation on 175 rice accessions from Niger and 52 accessions from Mali after inoculation with RYMV isolate B27 in Trial 1. Accessions from Niger are in black, while accessions from Mali are in grey. (b) Frequency distribution for the disease for accessions from Niger only in Trial 1. HR (1) Highly resistant, PR (3) partially resistant, I (5) intermediate, S (7) susceptible, HS (9) highly susceptible. Numbers in bracket indicates the disease score

Half the number of the *O. barthii* accessions (2 of 4) and *O. glaberrima* accessions (14 of 32) from Niger displayed no symptoms, while only a few accessions of *O. sativa* (6 of 139) were symptomless, reflecting the high resistance of African rice species as compared to Asian rice. The percentage of reduction on plant height (rPHgt) and chlorophyll content of leaves (rChcL) ranged from 4.5 to 100% and 0.4 to 100%, respectively (Table 5.1). The highest values were observed for the accession TY14, while the lowest reduction of ChcL was observed for *O. barthii* accession DF23 and *O. glaberrima* accession TY33. The lowest reduction of PHgt was observed for *O. sativa* accession DS22 and *O. glaberrima* TH7-A. Twenty two resistant accessions from Niger, three resistant accessions from Mali plus the two checks (IR64 and Gigante) and the highly susceptible *O. sativa* accession TY14 were tested for virus content using ELISA (Table

5.1). Four samples (one *O. barthii*, one *O. glaberrima* and two *O. sativa*) that did not develop any symptoms contained a significant amount of the virus, similar to that of susceptible check IR64 and accession TY14.

Table 5.1: Summary of response of the most resistant accessions from Niger and Mali inoculated with the RYMV strain B27 at 42 DAI during Trial 1.

Accession	**Score 21DAI**	**Score 42DAI**	**rPHgt(%)**	**rChcL(%)**	**DO ELISA**	**ELISA**	***O.* Species**
DF13	1	1	24.3	26.7	0.092	+	*barthii*
DF16	3	1	8.4	9	0.07	-	*glaberrima*
DF23	1	1	22.9	0.4	0.078	-	*barthii*
DS12	3	1	18	16.7	0.057	-	*sativa*
DS17-E	3	1	26.3	12.8	0.061	-	*glaberrima*
DS1-C	1	1	15.2	2.8	0.095	+	*sativa*
DS22	1	1	0.3	19.8	0.091	+	*sativa*
DS37	3	1	14.2	6.2	0.058	-	*sativa*
DS52-E	3	1	7.9	11.4	0.05	-	*glaberrima*
DS86	1	1	22.5	11.4	0.075	-	*sativa*
DS89	3	1	4.9	34.5	0.06	-	*sativa*
RAM 68	1	1	34.1	28.8	0.059	-	*glaberrima*
RAM19	1	1	35.3	20.2	0.062	-	*glaberrima*
RAM73	1	1	5.3	40.6	0.076	-	*glaberrima*
TH1	1	1	8.1	21.8	0.068	-	*glaberrima*
TH4	1	1	16.2	3.2	0.072	-	*glaberrima*
TH5-C	1	1	7.3	35.6	0.073	-	*glaberrima*
TH7-A	3	1	4.5	28.8	0.067	-	*glaberrima*
TY11	3	1	13.9	28	0.057	-	*sativa*
TY14	7	9	100	100	0.105	+	*sativa*
TY33	1	1	5.8	0.8	0.072	-	*glaberrima*
TY33-A	1	1	24.6	8.8	0.091	+	*glaberrima*
TY45	1	1	8.8	12.9	0.069	-	*glaberrima*
TY50	1	1	14.1	20.7	0.081	-	*glaberrima*
TY56	1	1	9.9	2.7	0.083	-	*glaberrima*
ZR1	3	1	20.4	31.8	0.05	-	*glaberrima*
Gigante	1	1	9.2	2.3	**0.076**	-	*sativa*
IR64	7	7	27.2	30.3	**0.105**	+	*sativa*
Control -					0.089		
Control +					0.102		

OD = optical density, ELISA- Enzyme Linked Immunosorbent Assay, DAI = Days After Inoculation

During Trial 2, the first symptoms appeared within seven DAI on susceptible varieties (TY62, IR64, and Bouake189). Symptoms were overall more severe in accessions inoculated with the normal isolate Ng122 than the virulent isolate Ng144. This was evident from the high number of accessions that showed higher disease scores (Figure 5.2). . Additionally, 19 accessions were highly resistant to Ng144, while 15 accessions were fully resistant to Ng122, and showed no symptom. Moreover, eight *O. glaberrima* accessions (DS72-A, TH1, DF 12, TH1 -B, DF17, DS11-B1, DS52 and TY7-D) gave similar responses to the two RYMV isolates. Surprisingly, the isolate Ng122 induced symptoms on the variety Gigante at 21 DAI, while the variety TOG5681 remained highly resistant during the entire screening period with both RYMV isolates.

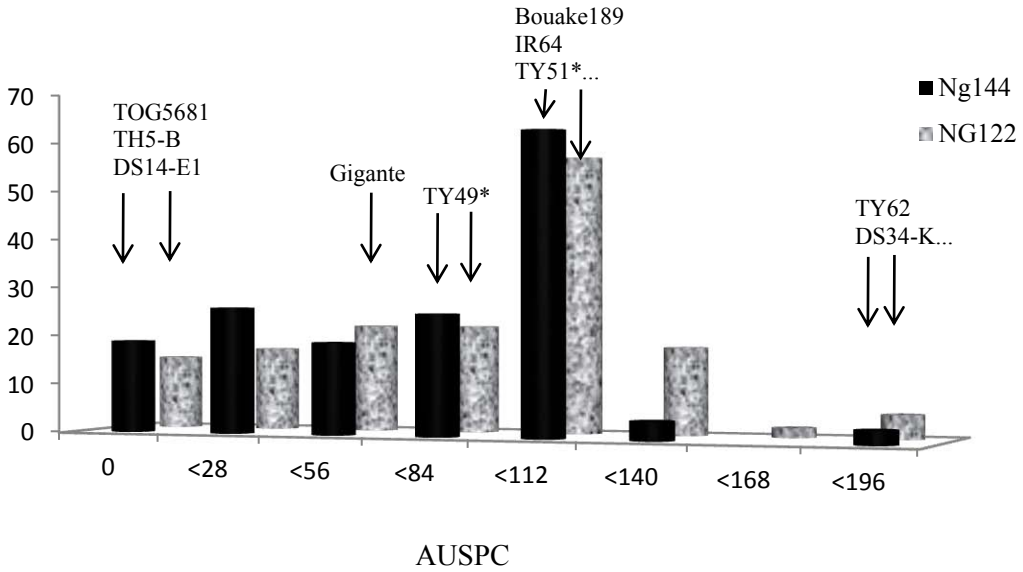


Figure 5.2: Area under a symptoms progression curve (AUSPC) for 154 accessions from Niger inoculated with virus isolates Ng122 (grey) and Ng144 (black) during Trial 2. Control varieties are displayed side by side with some accessions from Niger. *TY51 corresponds to a farmer-bred variety known as Kassimo that is believed to have been derived from IR1529-680-3 (here coded as TY49).

A combined analysis of variance (ANOVA) was performed on the data related to plant height and chlorophyll content of leaves at 42 DAI (Table 5.2). Regardless of the RYMV isolates, the accessions differed in the chlorophyll content of their leaves ($F = 2.1$, $df = 153$, $P \leq 0.01$).

Similarly, the RYMV isolates caused different results ($F = 26.3$, $df = 2$, $P \leq 0.01$). However, the interaction between isolates and accessions was not significant.

Table 5.2: ANOVA tables for chlorophyll content and plant height for 154 rice accessions from Niger inoculated with RYMV isolates Ng122 and Ng144 during Trial 2.

Chlorophyll content at 42DAI					
Source of Variation	**DF**	**Type III SS**	**Mean Square**	**F Value**	**p-value**
Bloc (Isolate)	12	223.38	18.61	1.17	0.3405
Check	4	331.27	82.82	5.21	0.0021
Accession	153	5099.43	33.33	2.1	0.006
Isolate*Check	8	149.38	18.67	1.17	0.3418
Isolate*Accession	294	3540.90	12.04	0.76	0.8855
Isolate	2	1143.88	571.94	26.3	0.0094
Plant height at 42 DAI					
Source of Variation	**DF**	**Type III SS**	**Mean Square**	**F Value**	**p-value**
Bloc (Isolate)	12	1347.77	112.31	0.98	0.4819
Check	4	10294	2573.48	22.56	<.0001
Accession	153	100615	657.61	5.76	<.0001
Isolate*Check	8	3794.65	474.33	4.16	0.0014
Isolate*Accession	302	62443	206.77	1.81	0.0176
Isolate	2	12202	6101.20	55.32	0.012

DAI – Days After Inoculation

In addition in Trial 2, the accessions responded differently to each virus isolate with respect to the plant height, as shown by the significant interaction between isolates and accessions ($F = 1.81$, $df = 302$, $P \leq 0.01$). Therefore, for plant height, the least square means (LS mean) of 154 accessions inoculated with virus isolates Ng122 and Ng144 were compared to the non-inoculated control using the "sliced effects" procedure during a combined analysis. Results of most resistant accessions are summarized in Table 5.3.

Table 5.3: Summary of combined analysis showing resistant accessions from Niger and susceptible and resistant varieties inoculated with RYMV isolates Ng122 and Ng144 (42 DAI) during Trial 2.

Accessions	*O.* Species	rChcL (%)	p-value	rPHgt (%)	p-value	Score 42 DAI	AUSPC
DS45-C	*glaberrima*	33.1	0.492	31.6	0.210	1	0
DS72-A	*barthii*	17.0	0.712	54.3	**0.300**	1	0
TY53	*glaberrima*	10.4	0.735	8.2	0.704	1	0
TH1[1]	*glaberrima*	8.6	0.689	56.7	**0.262**	1	0
DS14-E1	*glaberrima*	12.8	0.029	8.6	0.044	1	0
DF12	*glaberrima*	12.0	0.810	10.8	**0.348**	1	0
DS83-C	*glaberrima*	-9.0	0.880	25.4	0.033	1	0
TH1-B	*glaberrima*	1.5	0.983	5.4	**0.185**	1	0
IN2	*glaberrima*	5.6	0.963	32.7	0.363	1	0
DS14-E	*glaberrima*	17.8	0.742	56.1	0.000	1	0
DS14-F1	*glaberrima*	17.0	0.588	39.4	0.098	1	0
DF17	*glaberrima*	-11.3	0.644	22.3	**0.001**	1	0
TH1-A1	*glaberrima*	8.3	0.386	91.7	0.185	1	0
DS11-B1	*glaberrima*	26.2	0.139	38.2	**0.574**	1	0
DS52-E[1]	*glaberrima*	-7.9	0.644	33.6	**0.009**	1	0
TY7-D	*glaberrima*	3.2	0.985	19.3	**0.584**	1	0
TH1-A	*glaberrima*	15.0	0.818	50.0	0.030	1	0
DF23[1]	*barthii*	28.7	0.883	8.5	0.011	1	0
MD1-A	*glaberrima*	-2.4	0.261	35.5	0.803	1	0
DS14-D1	*glaberrima*	7.3	0.975	-7.3	0.063	1	0
DS14-C1	*glaberrima*	5.7	0.943	9.2	0.368	1	0
TH5	*glaberrima*	5.8	0.006	24.6	0.539	1	0
DS14-C	*barthii*	16.4	0.666	11.1	0.140	1	0
Gigante	*sativa*	33.2	0.005	19.4	0.011	1	0
TOG5681	*glaberrima*	20.2	0.190	13.3	0.119	1	0
IR64	*sativa*	29.7	0.001	27.8	0.001	7	140
Bouake189	*sativa*	15.5	0.193	32.0	0.014	5	112

rChcL (%) : Reduction of chlorophyll content of leaves; rPHgt (%) : Reduction of plants height;

AUSPC: Area under symptoms progression curve estimated using the formula : AUSPC = $\sum [(S_i+S_{(i+1)}-2)(t_{(i+1)} - t_i)]/2$, (S_i is the symptom score at date t_i).

Meaning of p-value < 0.05: If there is difference in plant height or chlorophyll content between inoculated and non-inoculated plants of an accession, then the accession should be considered as susceptible. Whereas if p-value > 0.05, there is no significant difference between inoculated and non-inoculated plants of the accessions. The accession should then be considered as resistant.

Accessions in bold were resistant to both isolates of the virus, while the others were only resistant to one of the strain.

[1]Accessions resistant during Trials 1 and 2.

Regarding the combined analysis and sliced effect, for resistant accessions, there should not be significant differences between the inoculated and non-inoculated accessions. Therefore, the F probability should not be significant at 0.05%. However, some accessions with no discoloration symptoms had significantly different means regarding the related traits. This suggested that the disease scores alone were not sufficient to assess the effects of RYMV on rice plants. The disease scores, as well as computed variables like AUSPC, rPHgt (%),%rChcL (%) were used to perform a Principal Component Analysis (PCA) (Figure 5.3).

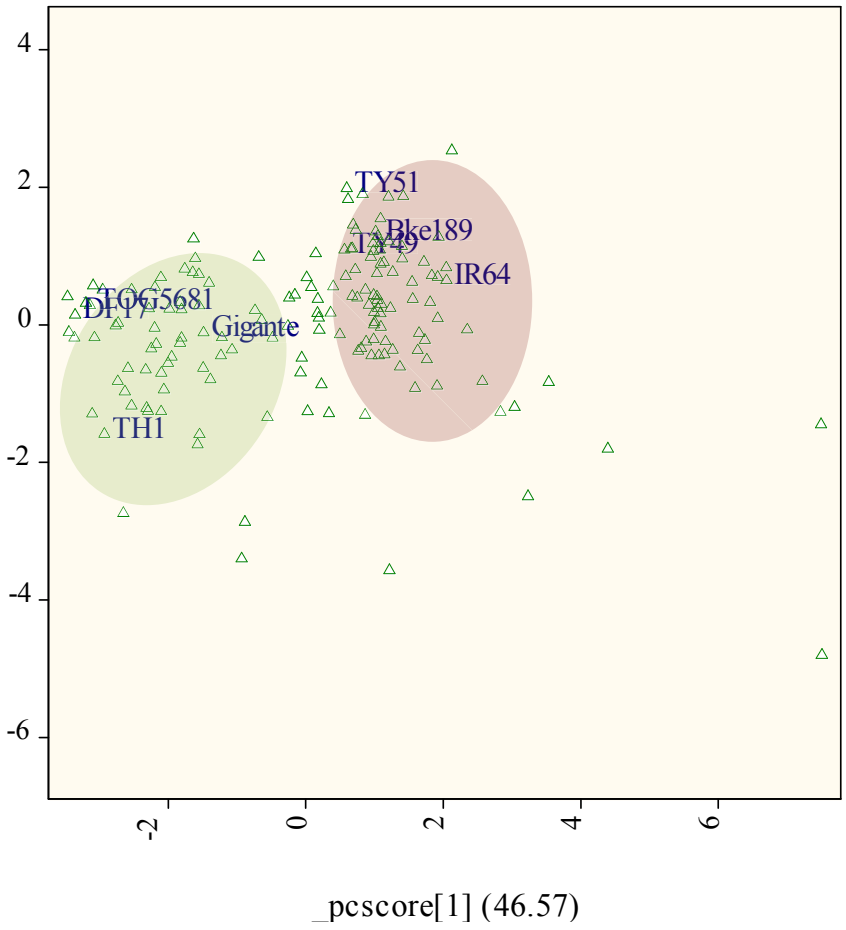


Figure 5.3: Plot of PC1 (46.6%) and PC2 (14.5%) from principal component analysis of 154 accessions from Niger plus 4 checks (IR64 and Bouake189 as susceptible checks; TOG5681 and Gigante as resistant checks) after inoculation with RYMV isolates Ng122 and Ng144 during Trial 2. IR64 and Bouake189 are in red font, Gigante and accessions resistant to Ng144 are in green, accessions resistant to Ng122 only are in orange, TOG5681 and accessions resistant to both isolates are in purple

The PCA separated resistant accessions from susceptible ones and confirmed previous results by showing that both TY49 (IR1529-680-3) and TY51, the two most widely grown varieties in the irrigated areas were susceptible to RYMV.

The last two trials involved 41 accessions divided into 2 groups of 24 and 25 accessions, respectively. Eight accessions found resistant to RYMV isolates B27, Ng122 and Ng144 were common to both Trials 3 and Trial 4. In both trials, the first symptoms appeared 6 DAI on IR64. With Isolate BF1, 14 of the 25 accessions also displayed symptoms as quickly as IR64, four accessions exhibited symptoms two days later, three accessions presented symptoms two weeks after inoculation, one accession (TH4) at 18 DAI, two accessions (TH1-B and TH1-C) at 21DAI and finally one *O. glaberrima* accession, coded DS14-E1, was symptomless even after 28 DAI.

For Trial 3, regarding the three RYMV isolates from Niger, the response of the 24 accessions ranged from highly susceptible (IR64, DS1-C, etc) to highly resistant (Tog5681, DS52-E, etc). All the *O. sativa* varieties displayed symptoms within 14 DAI, while *O. glaberrima* and *O. barthii* varieties displayed symptoms progressively. The analysis of variance showed different levels of significance between the rice accessions, the RYMV isolate and their interaction (Table 5.4A). The level of disease reaction was significantly different among some of the accessions ($P \leq 0.05$), for all the six chosen dates, while only the disease score at 14 DAI resulted in a significant difference between RYMV isolates ($P \leq 0.05$). However, the interaction between accessions and isolates was not significant at that date, whereas it was significant at 21 and 28 DAI ($P \leq 0.001$).

Concerning the chlorophyll content of leaves, the interaction between accessions and isolates was significant ($P \leq 0.001$) only from 14 to 28 DAI (Table 5.4B). Beyond 28 DAI, the interaction lost its significance. The same trend was observed for Isolate effect. Additionally, the Accession effect become non-significant three weeks after inoculation. Finally, at 42 DAI, plant height was significantly different, but RYMV isolates was well as their interaction, isolate x accessions, were not significant.

Table 5.4: Analysis of Variance (ANOVA) of multiple parameters and interactions between RYMV isolates and rice accessions. (A) Disease score and, (B) the chlorophyll content of leaves at 14, 21, 28, 35, 42 and 49 DAI and plant height at 42 DAI of 24 rice accessions from Niger and three control varieties, inoculated with three differential virus isolates Ng117b, Ng122 and Ng144 also from Niger during Trial 3.

(A) Source of variation	DF	*p-value*					
		14DAI	21DAI	28DAI	35DAI	42DAI	49DAI
Accession	26	0.010*	0.000*	0.001*	0.000*	0.000*	0.000*
Isolate	2	0.044*	0.339ns	0.031*	0.725ns	0.999ns	0.376ns
Accession*Isolate	52	0.798	0.000*	0.063ns	0.000*	0.181ns	0.356ns

Disease Score at 14, 21, 28, 35 and 42 days after inoculation (DAI).

(B) Source of variation	DF[1]	*p-value*					
		ChcL14	ChcL21	ChcL28	ChcL35	ChcL42	PHgt42
Accession	26	0.000*	0.000*	0.088ns	0.784ns	0.000*	0.000*
Isolate	3	0.000*	0.000*	0.001*	0.244ns	0.007*	0.070ns
Accession*Isolate	78	0.000*	0.000*	0.008*	0.443ns	0.528ns	0.162ns

ChcL: Chlorophyll content of leaves at 14, 21, 28, 35 and 42 days after inoculation (DAI); PHgt: Plant height (PHght) at 42 DAI. * p-value significant at $P \leq 0.05$.

[1] The degree of freedom for chlorophyll content is 3, because in the value of chlorophyll content from replications of the 3 virus isolates were compared to those from the control, non-inoculated replication, thus N=4.

The odds ratio was estimated for pairwise comparison of disease scores among accessions in Trial 3 with the control variety Gigante (Table 5.5 and Figure 5.4). For example, the check Gigante had 23.4 chances to exhibit reduced symptoms, and therefore to be more resistant than IR64, whereas its chance to display reduced symptoms in comparison with TOG5681 was < 0.01. The *O. sativa* accession DS86 was 15.5 times likely to be more resistant than IR64, and had 0.66 chances to be less diseased than Gigante. The resistance of Gigante at 49 days after inoculation was similar to that of TOG5681, while it was 1.5 times higher than TH4, twice as high as the resistance of TY11, 10 times that of TH1-C, 12 and times that of TY46. However, the *O. glaberrima* accessions ZR1, TY56, TY45, TY33, TH7-A, TH1, and TH5-C were more resistant than Gigante.

Table 5.5: Pairwise comparisons of disease scores for Gigante against 15 other accessions 49 days after inoculation with RYMV isolates Ng117b, Ng122 and Ng144 during Trial 3. Only a few accessions and varieties were selected as examples.

1st accession	2nd accession	Odds ratio
Gigante vs	IR64[1]	23.4
	TH1[1]	0.1
	TH1-C[1]	10.9
	DS86[3]	0.6
	TH4[1]	1.5
	TH5-C[3]	0.2
	TH7-A[3]	0.1
	TY11[1]	2.1
	TY33[3]	0.1
	TY45[3]	0.5
	TY46[1]	12.1
	TY50[2]	0.0
	TY56[3]	0.1
	TY8-D1[3]	0.1
	TOG5681[2]	0.0
	ZR1[3]	0.2

[1] accessions less resistant than Gigante, [2] accessions with resistance similar to that of Gigante, [3] accessions more resistant than Gigante

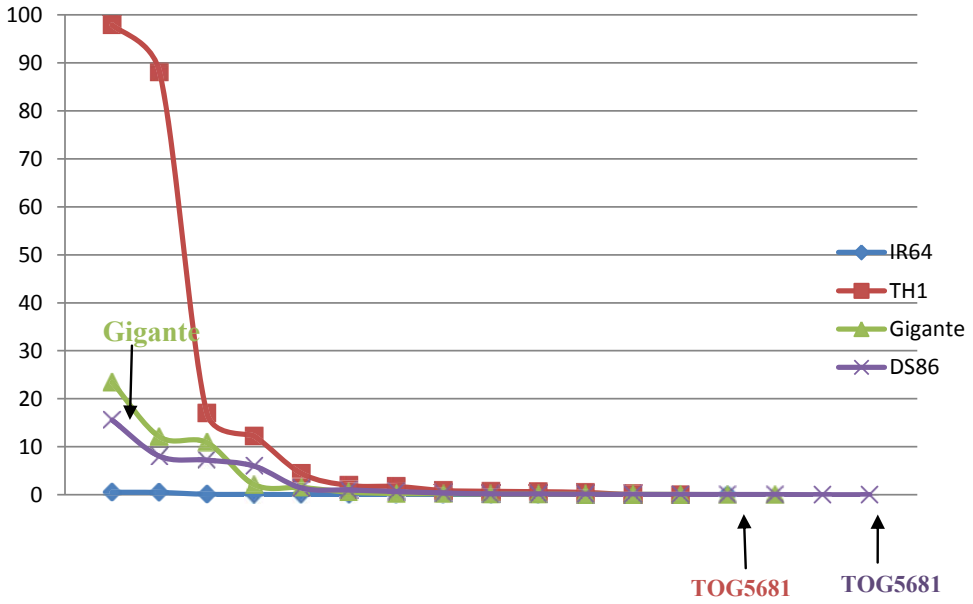


Figure 5.4: The odds ratio of the disease score of the two control varieties, IR64 and Gigante, compared to the odds ratio of two accessions, TH1 and DS86. The Y axis represents the likelihood. TH1 = *O. glaberrima* and DS86 = *O. sativa.*

In addition to the comparison between pairs of rice accessions, the odds ratio was also used for pairwise comparison of the three RYMV isolates (Table 5.6). The difference between virus strains was only significant for the two first weeks following the inoculation. The likelihood of the isolate Ng117b inducing lower disease scores in comparison to Ng144 was 6.03, therefore Ng117b was less aggressive in this experiment. In addition to this, the chances for Ng117b to induce mild symptoms were lower compared to the probability of Ng122, itself less destructive than Ng144. Thus the isolate Ng144 was the most dangerous on used in this series of experiments.

Table 5.6: Pairwise comparison of the likelihood of the three virus isolates to induce less symptoms to 24 rice accessions from Niger during Trial 3.

Isolates	Days of scoring				
	14DAI	21DAI	35DAI	42DAI	49DAI
Ng117b vs Ng144	6.03	0.72	1.18	1.34	0.60
Ng117b vs Ng122	0.60	0.20	0.00	1.01	0.11
Ng144 vs Ng122	0.10	0.27	0.00	0.76	0.18
p-value (isolates)	<.0.05*	0.339ns	0.725ns	0.999ns	0.376ns

* p-value significant at 0.05

Unlike isolate Ng122, both isolates Ng144 and Ng117b were able to break the resistance of Gigante and TOG5681 (Table 5.7). Thus, the ranking for RYMV resistance depends on the RYMV isolate, and the ranking of RYMV isolates depends on the rice accessions. RYMV isolate Ng117b was very virulent (vv) on Gigante and partially virulent (pv) on TOG5681. The converse was true for Ng144. Isolate Ng122 was avirulent to both Gigante and TOG5681. All the 3 RYMV isolates in this study were virulent (v) or pv to IR64. The majority of the 24 accessions displayed vertical resistance (VR), whereas 11 accessions displayed horizontal resistance or unmatched partially VR. Vertical Resistance is resistance that is differentially expressed against different virulent isolates while horizontal resistance provides a constant ranking of quantitative resistance against all isolates. Partial Vertical Resistance is quantitative Vertical Resistance, where a differential resistance response is observed to different isolates. Whilst Partial Vertial Resistance remains unmatched by a virulent isolate of the pathogen, it cannot be distinguished from Horizontal Resistance. RYMV Isolate Ng117b was virulent or very virulent to all the

O. sativa accessions; while isolate Ng122 was avirulent to 6 of the 10 *O. sativa* accessions. Furthermore, there was a constant ranking (C) of the *O. sativa* accessions, except for Gigante, which failed into two different classes, probably due to virulence x the Vertical Resistance interaction. Despite, this observation, the intermediate to susceptible phenotypes of the some *O. sativa* accessions, were less subjected to rank change than some *O. glaberrima* accessions (TH4 and TY45 also subject to virulence x vertical resistance interaction) and the *O. barthii* accession DF23.

Table 5 7: Disease reactions for 24 accessions and 3 checks (IR64, Gigante and TOG5681) 28 days after inoculation (DAI) with 3 RYMV isolates (Ng177b, Ng144 and Ng122) from Niger during Trial 3.

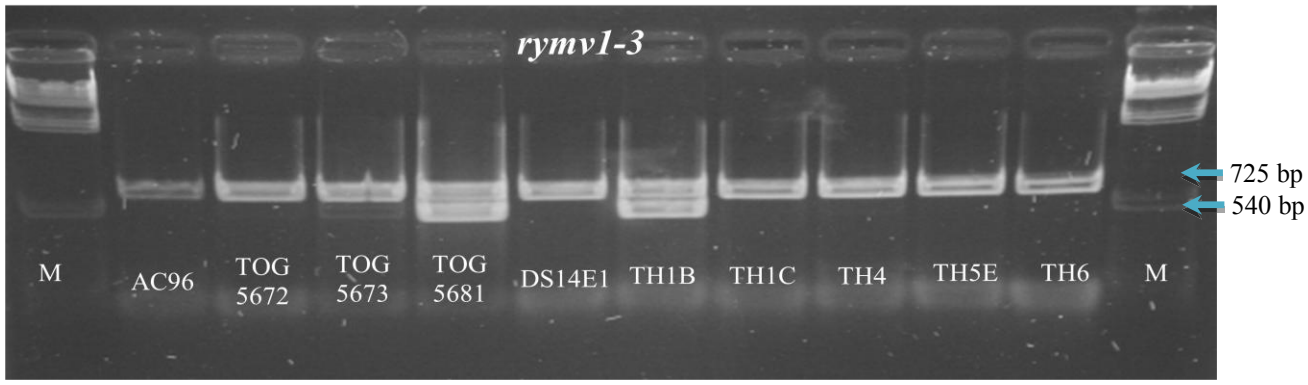
Accession		virus Isolates				virus Isolates							Form of Resistance
		Ng117b	Ng144	Ng122		Ng117b		Ng144		Ng122			
Code	Species	Score	Score	Score		%rChcL		%rChcL		%rChcL		Pr > F	
IR64	*O. sativa*	7	5	7	C	29.7	v	21	pv	32.4	v	0.003	VR
Gigante	*O. sativa*	5	5	1	DD	40.6	vv	26.4	pv	0.5	av	<.0001	VR
TOG5681	*O. glab.*	3	5	1	D	29.7	pv	41.9	vv	1.3	av	<.0001	VR
DF13	*O. barthii*	3	1	1	C	18.4	pv	14.3	av	7.7	av	0.244ns	HR or unmatched pVR
DF23	*O. barthii*	3	5	1	D	29.1	pv	51.1	vv	18.2	pav	<.0001	VR
DS1-C	*O. barthii*	5	5	5	C	23.4	pv	18	pav	59.9	vv	<.0001	VR
DS12	*O. sativa*	5	3	3	C	29.7	pv	27	pv	17.3	pav	0.008	VR
DS22	*O. sativa*	5	5	3	C	36.1	vv	30.5	vv	24.8	pv	0.002	VR
DS37	*O. sativa*	3	5	3	C	23.8	pv	28.1	pv	8.9	av	0.019	VR
DS47	*O. sativa*	5	3	5	C	23.5	pv	10.7	av	18.4	pav	0.084ns	HR or unmatched pVR
DS47-B	*O. glab.*	5	5	3	C	26.4	pv	28.9	pv	14.3	pav	0.016	VR
DS52-E	*O. glab.*	3	3	1	C	24.2	pv	25.2	pv	14.7	pav	0.073ns	pVR
DS86	*O. sativa*	5	5	5	C	31.5	v	30.7	v	13.3	pav	0.001	VR
DS89	*O. sativa*	5	5	5	C	48.8	vv	26.1	pv	31.8	v	<.0001	VR
TH1	*O. glab.*	3	3	1	C	6	av	25.2	pv	11.4	av	0.059ns	HR or unmatched pVR
TH1-C	*O. glab.*	5	7	7	C	25.8	pv	37.4	vv	47	vv	<.0001	VR
TH4	*O. glab.*	3	3	7	DD	15.1	pav	22.1	pav	46.8	vv	<.0001	VR
TH5-C	*O. glab.*	5	3	1	D	31.9	v	23.1	pv	23.6	pv	0.004	pVR
TH7-A	*O. glab.*	1	3	3	C	14.4	pav	22.6	pav	23.3	pav	0.026	HR or unmatched pVR
TY11	*O. sativa*	5	3	5	C	24.6	pv	18.9	pav	23.7	pav	0.039	HR or unmatched pVR
TY33	*O. glab.*	1	3	3	C	8.4	av	19.4	pav	16.9	pav	0.596ns	HR or unmatched pVR
TY45	*O. glab.*	5	3	1	D	38.7	vv	10.8	av	12.5	pav	0.002	pVR
TY46	*O. sativa*	5	5	5	C	36.1	vv	37.9	vv	40.9	v	<.0001	HR or unmatched pVR
TY50	*O. glab.*	3	1	1	C	25.4	pv	13.4	av	12.7	av	0.062ns	HR or unmatched pVR
TY56	*O. glab.*	3	1	1	C	39.2	vv	14.9	av	13.5	pav	0.001	HR or unmatched pVR
TY8-D1	*O. glab.*	3	1	3	C	22.6	pv	9.8	av	17.5	pav	0.104ns	HR or unmatched pVR
ZR1	*O. glab.*	5	5	3	C	28.4	pv	24.2	pv	13.2	v	0.005	HR or unmatched pVR

ns: no significant at 0.05. %rChcL: percentage of reduction of chlorophyll content of leaves

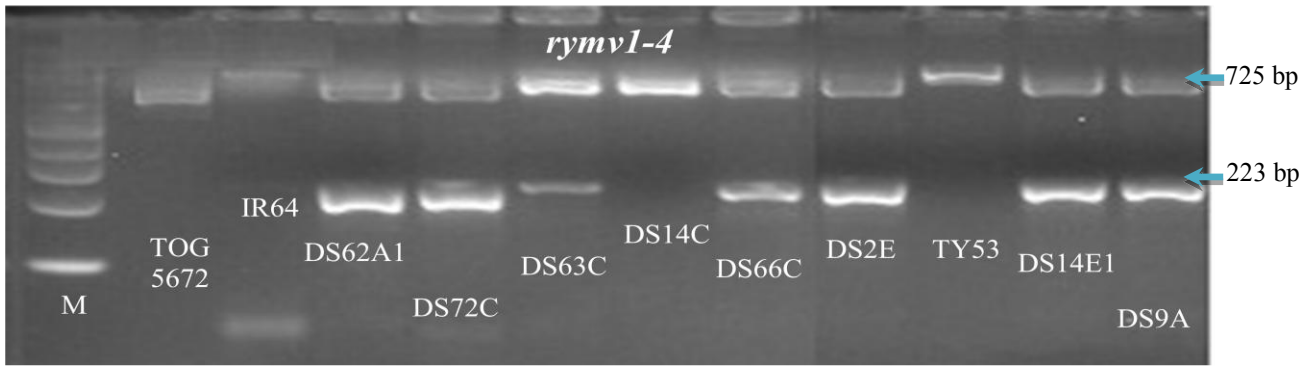
Very constant	DD - 2 classes different
Constant to 1 level	D - 2 classes different, 1 either way
Differential over 2 levels	C - constant ranking
Differential 2 levels to 1	

same class across all three races	<15 = av avirulent
similar but at least one class different	16-22=pav partially avirulent
at least 2 classes different	23-29=pv partially virulent
at least three classes different between 2 races	30-36=v virulent
	>36=vv very virulent

Among the 11 resistant *O. glaberrima* accessions genotyped with *RYMV1* allele specific markers (*rymv1-3*, *rymv1-4* and *rymv1-5*), one accession (TH1-B) had an allele similar to the *rymv1-3* from TOG5681 (Figure 5.5A). This accession was highly resistant with a disease score of 1 at 42 days after inoculation with both Ng122 and Ng144, 1.5% reduction in plant height and and a 5.4% reduction in chlorophyll content. However, this accession was only partially resistant (disease score of 3) to BF1 at 21 days after inoculation. Two accessions (TY53 and DS14-C) appeared to possess the TOG5672-like allele *rymv1-4* (Figure 5.5B) and they were also highly resistant at 42 days after inoculation both with Ng144 and Ng122. The remaining accessions had none of the three allelic patterns suggesting another allele or gene involvement. No accessions with *rymv1-5* were found (gel image not presented)



A



B

Figure 5.5: DNA banding patterns of resistant accessions genotyped with *RYMV1* allelic specific markers *rymv1-3* (A) and *rymv1-4* (B) and separated on 2.5% of Agarose gels. Accession TH1-B has the allele *rymv1-3* and accessions DS14-C and TY53, the allele *rymv1-4*. M: size marker

5.5 Discussion

The majority of the rice accessions in the present study were found to be susceptible to the different RYMV isolates. Although direct comparisons of RYMV resistance of the accessions used in the current study with previous studies conducted elsewhere are not reliable for different reasons (due to differences in sample sizes, species composition, genetic differences among accessions, difference in RYMV isolates used for disease evaluation, the experimental designand the method of inoculation), the use of the same reference varieties such as Gigante, IR64 and TOG5681 make comparison possible. Overall, the present study confirmed the high susceptibility of most of *O. sativa* accessions compared with most *O. glaberrima* accessions. Only seedling stage resistance was found in *O. sativa* accessions, becausenone of the *O. sativa* accessions were found to be highly resistant until 49 days after inoculation while a few accessions of *O. glaberrima* remained resistance throughout the disease scoring period. The susceptibility of most *O. sativa* varieties to RYMV has been reported by Rakotomalala et al. (2008) after screening a rice collection from Madagascar. In addition to this, none of the accessions from Niger and Mali were found to have comparable level of resistance as that of *O. sativa* varieties Gigante and Bekarosaka. Nevertheless, the *indica* accession DS86 expressed a high level of resistance to RYMV isolate B27, an intermediate phenotype to Ng177b, Ng122 and Ng144. However, it was susceptible to RYMV isolate BF1 at 21DAI. In contrast to this, a popular modern irrigated variety, IR1529-680-3 (coded as TY49) from IRRI, and a farmer variety TY51 named "Kassimo" or "Waihidjo", both covering more than 80% of the rice irrigated areas of the country were found to be susceptible to RYMV during all the trials. Indeed, the susceptibility of the IR1529-680-3 had been reported earlier (Reckhaus and Adamou, 1989), more than 10 years after it was released. This variety is suspected to be the main cause of the first outbreak of the RYMV epidemic in Niger in 1984 (WARDA, 2001). The Kassimo variety occupied 60% of rice cropping area in Tillabéry in 2008 (data from ONAHA- Tillabéry, 2008) and was recently said as highly susceptible (Issaka, et al., 2012). Therefore the combination of these two varieties could explain the recent importance of RYMV in the region (Chap. 2). The relatively larger proportion of *O. glaberrima* accessions with high levels of resistance compared to *O. sativa* was in agreement with several other studies (Coulibaly et al., 1999; Ndjiondjop et al., 1999; Thiémélé et al., 2010). Indeed, the gene *RYMV1* has shown a high allelic variability in *O. glaberrima* compare to *O. sativa*, whiles the resistance gene *RYMV2*, was found only in *O. glaberrima* (Albar et al.,

2006; Thiémélé et al., 2010). Thiemélé et al. (2010) predicted the occurrence of new resistance genes or alleles in the African rice. Although some of the resistant accessions found in the present study were similar to that of TOG5681 and TOG5672, they could be useful in breeding, in terms of other phenotypic traits. Indeed, TOG5681 has already been used as a donor parent to the lowland NERICAs developed by AfricaRice (Sié et al., 2008). Thus its genetic and phenotypic potential are being used, while any of the new resistant accessions have not yet been used in any breeding program. However, caution should be taken when using this kind of monogenic resistance because of the high selection pressure it applies on RYMV populations and "fortuitous" molecular incident, such as a single mutation, can lead to the emergence of new virulent strains as it happened with the resistance gene Cf4 of tomato to the fungus Cladosporium fulvum (Joonsten et al., 1994) and *rymv1-2* in rice (Hébrard et al., 2006). Moreover, molecular evidence revealed that RYMV's evolution is as faster as animal viruses (Fargette et al., 2008b). Therefore priority should be given to combining monogenic resistance with partial or intermediate resistance. Plants with partial polygenic resistance cause a delay in the appearance of symptoms. The development of the disease will therefore be reduced and subsequently its re-transmission to neighbouring plants and the increase of the epidemic in the field (Van Der Plank, 1966). The interaction between the vertical resistance identified in the RYMV-rice pathosystem suggest caution when using this type of gene in breeding programmes, because of the risk of rising of virulent pathotypes (Robinson, 1995).

The use of several pathogenically different virus strains was helpful in identifying such accessions. Some of our *O. sativa* varieties and most of the *O. glaberrima* varieties could be used as parents to develop comprehensive resistance to RYMV. Another interesting point suggested by the present study was the non-significance of the virus isolate on both the disease score and the reduction of chlorophyll content of leaves over time. In addition, the likelihood of one virus strain inducing more or less symptoms than another, were also low over time. This *indica*tes that short periods should be used for disease score assessment post-inoculation. The effect of the time of infection of three rice varieties, with different levels of resistance to RYMV were assessed in a screenhouse by inoculating rice plants at their seedling, tillering, booting and flowering stages (Onwughalu et al., 2011). The observation period varied between inoculation and harvest. The level of sterility of spikelets was not related to the period of infection, while the difference in the mean yield and the plant height were significant for plants inoculated at the seedling stage, even

in the susceptible cultivar. Ndjiondjop et al. (1999) observed symptoms from 14 to 62 days post infection while Thiémelé et al. (2010) recorded symptoms up to 12 weeks post inoculation. However, in this series of experiments at 14 DAI the disease score, as well as the virus content assessed by ELISA, were able to separate the highly resistant Gigante and TOG5681 from the susceptible IR64 and the partially resistant Azucena.

The response to inoculation depends on the RYMV isolates used. Earlier studies reported that RYMV isolates from Niger belong mainly to the S1 serological group, but are related to a Central African lineage, although the country is located in West Africa (Traoré et al., 2005).

Ng117b and Ng122 appear to be more aggressive than Ng144, because of their ability to induce more severe symptoms in the susceptible IR64. However, the RYMV isolate BF1, belonging to the S2 group, was the most aggressive, not only based on its ability to damage IR64, but also the rapidity with which symptoms appeared. Presently a country-wide study of the genetic and pathological structure of RYMV is ongoing and the first results *indica*ted that both S1-West Africa and S1-Central Africa strains of RYMV are present in the Niger, within four major pathotypes (Issaka, 2011; Issaka 2012). Additionally, "T" type strains, many of which are virulent to the resistance allele *rymv1-3*, were found predominantly in Niger, while a few "E" types that are virulent to *rymv1-2* were also found (Traoré et al., 2010). Most of the few "T" and "E" isolates virulent to *rymv1-2*, were also virulent to *rymv1-3* in Niger (Issaka, 2011)

The secondary traits measured in this study, namely chlorophyll content of leaves, plant height, and AUSPC, have also been successfully used by several authors in the screening for resistance to RYMV (Albar et al., 1998; Coulibaly et al., 1999; Séré et al., 2008); as well as, in the management of nitrogen levels in rice (Esfahani et al., 2008). However, the measurement of the chlorophyll content of leaves was subject to variations due to the position of the SPAD-meter and the number of points taken on the plant leaf for the same plant, the age of the plant used for the notation, and the intensity of the sunshine during the period of the recording. This technology needs a standardization protocol to solve these problems.

The screening of a large collection of rice accessions from Niger and Mali revealed the overall vulnerability of most rice varieties to RYMV in these countries. In particular, most rice production is being undertaken in irrigated and lowland areas, cropped mainly with *O. sativa*

varieties, most of which are highly susceptible to RYMV. However, the survey has unlocked the hidden potential of the few remaining traditional landraces and wild relatives of the country for breeding for comprehensive resistance to RYMV. Those findings, coupled with the pending information on the structure of the viral population will make a great impact on rice breeding for resistance to RYMV in Niger. Furthermore, the sequencing of the rice genome and resulting applications, such as genome-wide selection (GWS) and marker-assisted recurrent selection (MARS), offer great opportunities to successfully combine both monogenic and polygenic modes of resistance in rice breeding programs.

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Chapter 6: Efficiency of Marker-Assisted Backcross (MABC) in Improving the Resistance of Five West African Elite Rice Cultivars to *Rice yellow mottle virus* (RYMV)

6.1 Abstract

The *Rice yellow mottle virus* is causing severe damage in African rice agrosystems. To improve the resistance of rice varieties to RYMV, the major resistance gene *rymv1-2* of Gigante was successfully introgressed into five elite rice varieties of West Africa using a Marker–Assisted Backcross (MABC) technique, using three generations of backcrossing. Newly developed BC_3F_3 progenies were evaluated for resistance to RYMV in farmers' fields, both in Guinea and Mali; and the BC_3F_4 generation was screened under controlled conditions in a screenhouse in Benin. The improved progenies with the *rymv1-2* introgression showed low virus titre and level of disease incidence, low tiller numbers, and little reduction in plant height. Some of the improved lines also displayed high levels of resistance to fungal blast and stem borer infestation in Guinea. Regarding agronomic traits, some progenies were similar to the recurrent parent. However, the genetic contribution of the donor was still high (at least 21.3%) and further backcrosses were suggested to increase the recurrent parent's genome contribution. Adequate deployment of the newly developed varieties, coupled with good cultural practices, will reduce the damaging effects of RYMV and therefore increase small scale farmers' income.

Keywords: Marker-assisted selection, Rice, RYMV, backcross, West Africa, Gigante, *rymv1-2*

6.2 Introduction

West Africa is the largest producer, consumer and importer of rice in Africa. Production is dominated by rainfed lowland (33%) and irrigated cropping systems (20%). Despite, concerted efforts to improve rice productivity in West Africa, a decrease in average rice yield has been recorded over the last decade. The phenomenon is due to several reasons: (1) structural and policy problems such as the widespread lack of quality seed and mineral fertilizer, poor land preparation, and labour inefficiency; (2) abiotic stresses, including drought, flood, disparities in rainfall, salinity, low phosphorus availability, and (3) biotic constraints such as weeds, blast, Bacterial Leaf Blight (BLB), African Rice Gall Midge (AfRGM) and *Rice yellow mottle virus* (RYMV) (Sere et al., 2005; Nwilene et al., 2006; Balasubramanian et al., 2007; AfricaRice, 2009). The RYMV is endemic to Africa and Madagascar (Abo et al., 1998). It was first described in Kenya (Bakker, 1970). Since then, the virus has been reported in all West, Central and East

African lowland, upland and irrigated rice cropping systems (Awoderu, 1991; Traoré et al., 2001; WARDA, 2001; Banwo, 2003; Kouassi et al., 2005). The virus induces yellowish or orange streaking of leaves due to chlorophyll degradation, severe stunting of plants and a reduction in tiller, incomplete panicle exsertion, spikelet sterility and even plant death depending on the rice variety and the period of infection. In farmer's fields, losses due to RYMV are variable, ranging from 64 to 100% in Mali (Sy et al., 1993), 84 to 97% in Sierra Leone (Taylor et al., 1990), 56 to 68% in Niger (Reckhaus and Adamou, 1986), 50% in Kenya (Bakker, 1970) and 19 to 44% in Burkina Faso (Séré, 1991). The virus is mostly transmitted by insects that belong to the Chrysomelidae family (Nwilene et al., 2009). Other vectors include farmers' hands, rats, cows and donkeys (Sara and Peters, 2003), as well as abiotic vectors such as infected soils, farming tools, water, and even wind through contact between plants (Sarra et al., 2004; Traore et al., 2008). Due to the complex mode of transmission of the disease, chemicals are only efficient to reduce the insect vectors pressure, but do not provide for adequate control of RYMV. Good cultural practices are ineffective in controlling the disease (Kouassi et al., 2005). Control of the disease through the development of transgenic plants has been investigated and some transgenic lines with high levels of RYMV resistance have been obtained, but those lines displayed only seedling stage resistance (Pinto et al., 1999; Sorho et al., 2005). A high level of natural resistance gene has been found in a variety variety Gigante, which belongs to an Oryza sativa subspecies (subsp.) indicaand in some other accessions of African cultivated rice, O. glaberrima (Ndjiondjop et al., 1999; Thiémélé et al., 2010). Additionally, partial resistance has been found in a japonica variety, Azucena (Albar et al., 1998). The RYMV resistant gene RYMV1 in Gigante was mapped on the long arm of the Chromosome 4, between microsatellite markers RM252 and RM273 (Ndjiondjop et al., 1999; Albar et al., 2003). The positional cloning of the resistance gene proved that the isoform of the eukaryotic translation initiation factor 4G (eIF(iso)4G) was involved in the resistance, which involved either amino acid substitutions or short amino-acid deletions in the middle domain of the encoding protein (Albar et al., 2006). Moreover, five different alleles of the resistance gene RYMV1 have been characterized, of which the resistance allele for Gigante is called rymv1-2. This recessive rymv1-2 allele is characterized by a substitution of a lysine (K) for a glutamic acid (E) in susceptible cultivars at position 309 of eIF(iso)4G (Albar et al., 2006; Thiémélé et al., 2010). However, resistant breaking virulent (Vir) strains of the virus have been reported both in experimental conditions after serial inoculations of the resistant variety Gigante

by a non-virulent strain (wild type: WT) of the virus (Sorho et al., 2005), and naturally in the field (Traoré et al., 2006). This virulence of the virus is due to a single substitution in the genome-linked viral protein VPg (Hébrard et al., 2006). However, recently the accumulation rates of both WT and Vir strains of the virus have been monitored and results suggest that both genetic and competitiveness constraints contribute to the limited ability of RYMV to develop virulence to rymv1-2 (Poulicard et al., 2009). Thus, only a small proportion of isolates (9% to 15%) are virulent against the resistance in the field (Pinel et al., 2006; Traoré et al., 2006). Consequently, this natural resistance is still of great interest in breeding for RYMV resistance.

With the sequencing of the rice genome and the development of thousands of Simple Sequence Repeats (SSR) markers, also called microsatellites, rice breeding has gained in speed and accuracy when transferring one or more genes of interest from a donor into an elite variety (recurrent parent) through marker-assisted backcrossing (MABC) (Temnykh et al., 2001; McCouch et al., 2002; Semagn et al., 2006). The transfer can be more precise and more effective when using markers located within the resistance gene (Lüttge et al., 2008). MABC has been widely used in the improvement of elite cultivars (recurrent parent), because it offers a quick possibility in identifying individuals that contains the gene of interest with the highest proportion of the recurrent parent genome, as compare to conventional backcross selection. The objective of this study was, therefore, to transfer the rymv1-2 gene from Gigante into 5 rice elite cultivars widely grown in West Africa but susceptible to RYMV using MBAC

6.3 Material and methods

6.3.1. Plant material and populations development

Five elite cultivars of *O. sativa*, including three indica subsp. varieties, namely IR64, Sahelika, WAT310 (adapted to irrigated and lowland ecologies); and two japonica subsp., namely FKR28 and IR47 (both adapted to tropical upland conditions) were used in this study. The five varieties are widely grown in West Africa but display susceptibility to RYMV and they were recommended by National Agricultural Research Scientist (NARS) from Mali, Gambia, Burkina Faso and Guinea. Gigante, bearing the resistance gene *rymv1-2* that confers resistance to RYMV (Ndjiondjop et al., 1999; Albar et al., 2006), was used as the donor parent. The five elite cultivars were used as females and Gigante was used as the male to develop the F_1s. The F_1 plants were then backcrossed to their female parent (recurrent parent) to generate the BC_1F_1 generation.

Successive backcrossing was conducted until the BC_3F_1 generation was produced. Markers were used to confirm whether F_1s were true-to-type with the expected alleles from both parents, for checing the introgression of the *RYMV*1 gene from the donor into the recurrent parents in the backcross generations, and also for background selection as described in Figure 6.1. The selected BC_3F_1 were self-pollinated for two generations to produce the BC_3F_3 population. One thousand three hundred and fifty four lines that were homozygous for the recessive *rymv1-2* allele were finally developed. (Table 6.1).

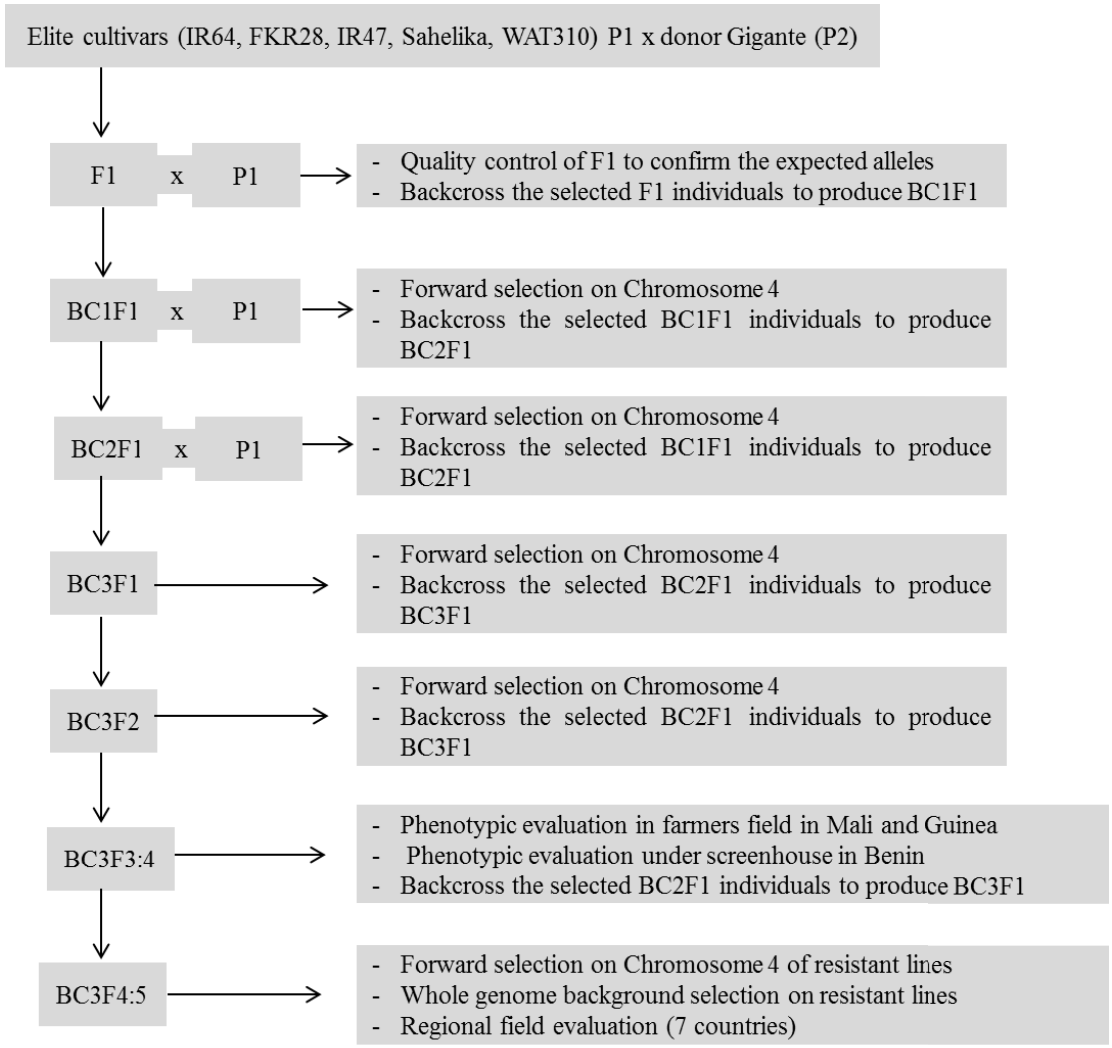


Figure 6.1: Schematic representation of *RYMV1* resistance gene introgression into elite rice cultivars through marker-assisted backcrossing. For practical reasons, background selection was done only at BC3F4, instead of BC_2F_1 and BC_3F_1.

6.3.2. Genotyping and marker-assisted selection

The microsatellite marker RM252 that mapped about 1.8 cM from the resistance *RYMV1* gene (Albar et al., 2003) and two closest flanking markers RM273 (2.3 cM on one side of the chromosome) and RM241 (2.4 cM on the other side of the chromosome), were used for foreground selection. First, all individuals in each cross were genotyped with RM252 and those which were heterozygote for this marker were selected. Second, all heterozygote individuals for RYMV1 gene were then genotyped with RM241 and RM273. On the basis of genotypes at the target marker and two flanking markers, Frisch et al., (1999) outlined the methodology to be used in selecting the best desirable genotype for MABC. For each population, we adopted their methodology and classified the genotypes in to one of the following 4 types: (a) Type 1 (individuals heterozygous for RM252 and homozygous for the recurrent parent alleles at both RM241 and RM273); (b) Type 2 (individuals heterozygous for RM252 and homozygous for the recurrent parent allele either for RM241 or RM273); (c) Type 3 (individuals heterozygous for RM252 and heterozygote for the recurrent parent allele at both flanking markers), and (e) Type 4 (individuals homozygous for the recurrent parent allele at the target locus; i.e., they were not a carrier of the target allele). For each foreground selection cycle, the most desirable individuals were type 1, followed by type 2 and finally by type 3. The selected individuals were backcrossed with the recurrent parent for further generations. Later, 69 to 80 additional SSR markers that were polymorphic between the recurrent and donor parents in each crossing were used to estimate the proportion of parental genome in the progenies.

DNA was extracted from two week old seedlings using a Cetyl Trimethylammonium Bromide (CTAB) protocol described by Murray and Thompson (1980). PCR were performed in a total reaction volume of 25 µl that consisted of about 15 ng DNA, 1x PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$ and 10 mM Tris-HCl, pH 8.3), 0.4 µM of each of the forward and reverse, 200 µM each dNTP (Boerhinger Mannheim) and 0.5 U Taq DNA polymerase (New England BioLabs, UK). Amplifications were carried out in a MyCycler thermal cycler (Bio-Rad, PA, USA) using the following programs: 5 min at 94 °C followed by 34 cycles of 1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C, with a final extension of 5 min at 72 °C. The amplified products were either separated on 3% agarose with ethidium bromide (BET) staining and visualized on an AlphaImager gel documentation system (now part of Cell Biosciences, Inc. CA, USA) or on 5%

denaturing poly-acrylamide gel electrophoresis (PAGE) with silver staining (Temnykh et al., 2000).

6.3.3. Virus isolates propagation and inoculation

Two simultaneous disease evaluation experiments were first conducted under artificial inoculation at the "Institut d'Economie Rural" (IER) research station of Niono, part of the "Office du Niger" in Mali and under natural infestation at the "Institut de la Recherche Agronomique de Guinée" (IRAG) station of Sérédou in Guinea. For the experiment in Mali, the virus was multiplied by mechanically inoculating a susceptible cultivar IR64 and a sap from ground leaves was used to artificially infect two week old young plant prior to being transplanted in the field. In Guinea, young seedlings were directly transplanted in a naturally infested lowland field. Fields in most West African countries were infected by a virus strain belonging to the serotype S1 (N'Guessan et al., 2001; Traoré et al., 2005; Onasanya et al., 2006).

Eighty three resistant progenies from and their parents were screened under controlled screenhouse conditions at AfricaRice, in Benin. The RYMV isolate B27 from Benin that belongs to the S1 strain (Thiémélé et al., 2010) and is widespread in West Africa (Traoré et al., 2005) was used. The virus was multiplied by mechanically inoculating a susceptible cultivar IR64 as described by Ndjiondjop et al. (1999). , Infected frozen leaves were ground in a phosphate buffer (0.1 M KH_2PO_4, 0.1 M Na_2HPO_4, pH 7.2) using 1g:10 ml ratio. The mixture, with added carborundum (600 mesh) as an abrasive, was used to inoculate two week old seedlings of the susceptible cultivar by gently rubbing young leaves. Two weeks later, infected leaves were harvested and used to prepare the inoculum needed for the experiment. Both the inoculum preparation and the inoculation were done as described above.

6.3.4. Experimental designs of field trials

In Guinea, 1353 BC_3F_3 lines (Table 6.1) were planted in 16 randomized blocks. The parents (WAT310, Sahelika, IR64, IR47, FKR28 and Gigante), plus a local cultivar CK21 were replicated in each block to serve as checks. Disease evaluation was conducted by transplanting seedlings in to a naturally infested lowland field. In Mali, 421 BC_3F_3 lines (Table 6.1) were randomized into 14 blocks. Their parents (IR64 and Gigante), Azucena BG90-2 and WAT310, were used as checks and were replicated in each block. Both experiments in Mali and Guinea were conducted in 2009 using Augmented Experimental Design (AED), with plot size of 0.6 m x

2.5 m with three rows per entry, a spacing of 0.3 m between rows, 0.25 m between plants within the same row and 0.5 m plots, at the rate of one plant per hill. Rice seedlings were transplanted into the field 21 days after sowing, followed by basal application of 200 kg per ha^{-1} NPK. In addition, 50 kg ha^{-1} of urea was applied 21 days after transplanting and then at panicle initiation. All other agronomic practices were done as per standard recommendation for rice production (Vergara, 1984).

The third experiment was conducted under screenhouse at the AfricaRice in Cotonou, Benin. A total of 83 BC_3F_4 lines from 4 populations (Table 6.1) that best performed from the disease evaluation in the field experiments were artificially inoculated with the virus isolate B27. As with the field experiment, an augmented design was split in 3 blocks with 5 checks (the recurrent parents and Gigante). The design was replicated three times: two were inoculated with the virus, while the third was inoculated with only water and used as a negative control. Seeds for each family were sown directly into 1 litre pots and thinned to three plants per pot prior to inoculation. NPK was applied 20 days after sowing (DAS) and Urea 40 DAS.

Table 6.1: Populations developed and tested for resistance to *Rice yellow mottle virus* (RYMV) in various conditions in three West African countries

Crosses	Number of BC_3F_3 families	Field test in Mali	Field test in Guinea	Screenhouse test in Benin*
FKR28/Gigante	67	-	67	13
IR64/Gigante	871	421	871	38
Sahelika/Gigante	46	-	46	4
WAT310/Gigante	23	-	23	-
IR47/Gigante	346	-	346	28
Total	1353	421	1353	83

* : seeds used in Benin were derived from the BC_3F_3, thus they corresponded to BC_3F_4

6.3.5. Data collection and analysis

For all the experiments, priority was given to scoring the disease symptoms, based on a 1-9 symptom severity scale: 1 for absence of symptoms and plants considered to be highly resistant; 3 for sparse dots or streaks; 5 for visible mottling on green to light-green leaves; 7 for yellowing

and stunting; 9 for necrosis and sometimes plant death (Konate et al., 1997). Additionally, in the field experiments, important agronomic traits were recorded such as the number of tillers, plant height, date of flowering, date of maturity, grain weight/plant. Finally, resistance to other diseases occurring naturally in the experimental fields were also recorded, such as blast, *Diopsis spp.* and borers' attacks (IRRI, 2002). From the Experiment in Mali, some highly resistant families (81 out of the 95 HR accessions) and Gigante were tested for virus titre. Fresh leaves were ground in distilled water, carborundum (600 mesh) added and used to inoculate two week old seedlings of the susceptible variety BG90-2. Five plants of BG90-2 were inoculated with the sap prepared with the leaves of each BC_3F_3 family. The ability of the different families to induce or deflect RYMV symptoms on BG90-2 were recorded 21 DAI.

Regarding the screenhouse experimentation, in addition to the visual evaluation of RYMV symptoms at 28 and 42 DAI, the disease incidence (DI) was evaluated as the percentage of infected tillers per pot. Related traits, such as the chlorophyll content of leaves (ChcL) were measured with a SPAD 502 chlorophyll Meter (Minolta C. Co. Osaka, Japan) (Esfahani et al., 2008). Ten points were measured on leaves per pot. Plant height (PHgt) and tiller number (TilN) were recorded at 28 and 42 DAI (Onasanya et al., 2006).

Plant lines that displayed good resistance (disease score equal to 1) during the different trials were further evaluated for their virus content using an enzyme-linked immunosorbent assay (ELISA) as described by Sere et al. (2007). The last fully expanded leaf was harvested, ground in a coating buffer (Na_2CO_3, $NaHCO_3$, pH 9.6) at 1:10, w:v) and the homogenized sap was directly adsorbed to microtitre plates overnight, followed by blocking with 1% bovine serum albumin. Double serial dilutions of antisera were made and bound antibody was detected with goat anti-rabbit serum conjugated to alkaline phosphatase (Sigma-Aldrich Co, MO, USA). The bound conjugate was detected using a p-nitrophenyl phosphate solution (1mg/lml) and the optical density was read with a spectrophotometer at 405 nm.

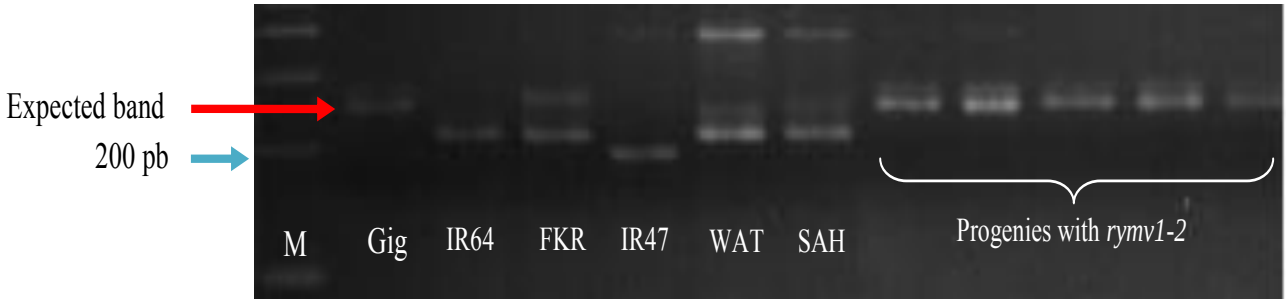
The software package SAS version 9.1 was used to compute correlations between the traits. Analysis of variance (ANOVA) for mean comparisons among genotypes was also done with Genstat 11th Edition. Additionally, a principal component analysis (PCA) was performed using the software Statistica version 7.1. Finally, the Graphical GenoType, GGT version 2.0 (Van Berloo, 2008) was used to estimate the proportion of each parent in the resistant progenies.

6.4 Results

1353 BC_3F_3 families derived from the five backcross populations were developed through MAS and screened for resistance to RYMV in three countries under different conditions. Screening of all the progenies and parents was carried out in Guinea

6.4.1 Marker-Assisted Selection

Different proportions of each type were obtained, but emphasis was given to select double homozygote lines, which contained the expected band for RM252 and homozygous for recurrent parent alleles at both RM273 and RM241. Such parents were globally low (5 to 23% depending to the cross and the generation). Generally, five to ten plants per crossing combination were selected and backcrossed with the recurrent parent to produce further generation. A typical gel profile obtained during the selection process is represented in Figure 6.2.



M: size marker, Gig: Gigante, FKR: FKR28, WAT: WAT310, SAH: Sahelika

Figure 6.2: Expected patterns of parental lines and RYMV resistant progenies amplified with RM252 (on 3% Agarose gel)

6.4.2. Field evaluation in Guinea

A field evaluation of the developed progenies, related parents and a local check variety was conducted under natural RYMV infection in 2009. The proportion of highly resistant (HR) BC_3F_3 families (Score 1), was 45.1%, and those with slight sparse dots of streaks on the green leaves, that could be considered as partially resistant (PR) (Score 3) to the disease represented 53.4%, 1.6% of the progenies presented visible mottling on light green leaves (score 5) and were considered Intermediate (I). The cultivar FKR28 was the most resistant among the recurrent parents (Figure 6.3D), followed by IR47 (Figure 6.3C) and IR64 (Figure 6.3E), while Sahelika (Figure 6.3B) and WAT310 (Figure 6.3F) were the most susceptible. The donor parent Gigante

did not display symptoms during this experiment. Additionally, there were no families that displayed severe susceptibility symptoms (Figure 6.3A). The local check variety CK21 displayed symptoms ranging from moderately resistant to susceptible during this trial.

However, the overall performance of the parents and progenies under natural RYMV infection varied depending on the crossing combination (Figure 6.3 A-F). Firstly, the combination of IR47\Gigante had the highest proportion (47%) of HR progenies, but also the highest proportion (9.7%) of Intermediate progenies (Figure 6.3C). This was followed by combination with IR64, which provided 45.9% of the HR lines (Figure 6.3E). Despite the low proportion of HR progenies (17.4), the cross involving WAT310 had the highest percentage of R progenies (82.6%), followed by the cross involving Sahelika, with 78.3% of R, and then by FKR28 with 67.2% of R progenies.

A

Disease Severity score	Progenies size (lines)	Proportion (%)
1 (HR)	610.0	45.1
3 (PR)	722.0	53.4
5 (I)	21.0	1.6
7 (S)	-	-
Total	1353.0	100.0

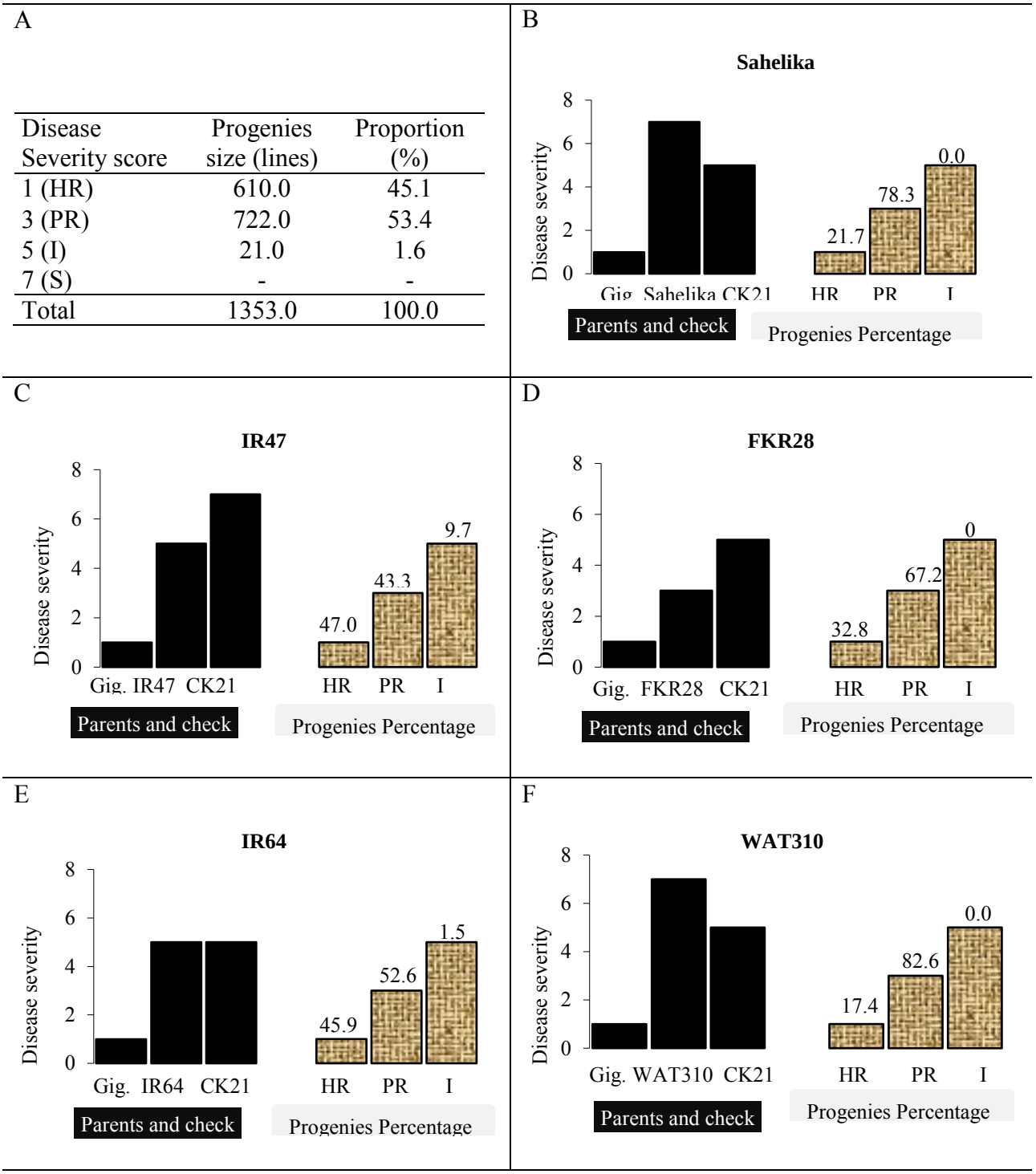


Figure 6.3: Summary of overall RYMV severity in Guinea. (A); Disease severity on parents and proportion of HR = highly resistant progenies, PR = partially resistant progenies and I = Intermediate progenies: the cross involving Sahelika (B), IR47 (C), FKR28 (D), IR64 (E) and WAT310 (E). The charts represent the severity of the disease and the proportion of progenies for the related disease score. CK21 is a local check variety

Occurrence of other major rice diseases and major pests was also noticed and their incidence recorded during the trial in Guinea. Only 0.48% of the lines did not develop blast disease. The majority of genotypes showed small brown specs of pin-point size or larger brown specks without sporulating centers (34.7%) or small roundish to slightly elongated necrotic gray spots, about 1-2 mm in diameter with a distinct margin, mostly observed on upper leaves. Some lines (12.8%) developed typical blast lesions on less than 4% of leaf area while more severe disease symptoms were observed on 6.5% of the rice lines.

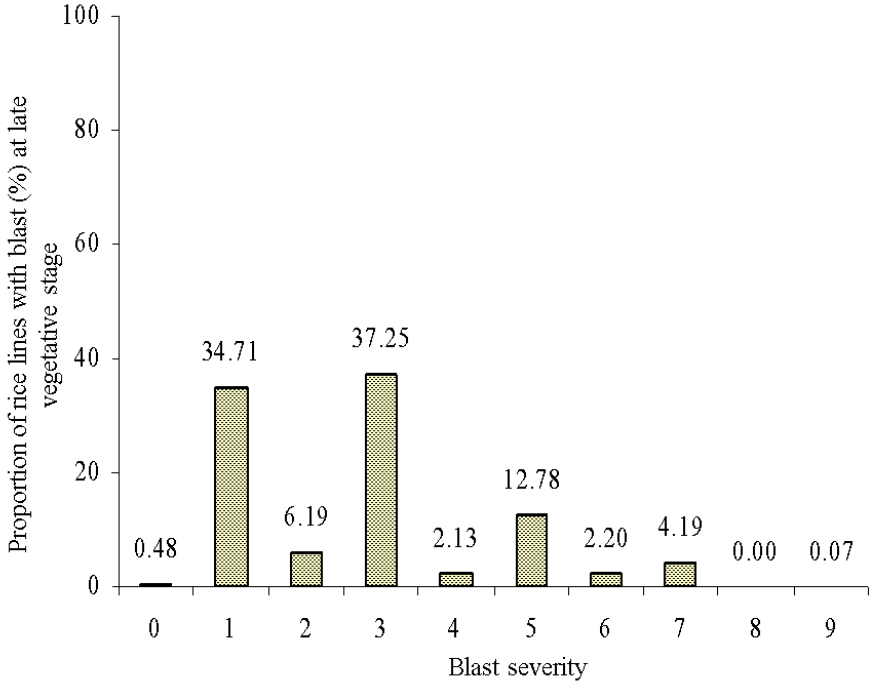


Figure 6.4: Occurrence and severity of blast disease on 1353 B C_3F_3 rice lines during their screening for resistance to RYMV in Guinea

Blast Severity Scale: 0 = No lesions observed; 1 = Small brown specks or pin-point size or larger brown specks without sporulating centre; 2 = Small roundish to slightly elongated necrotic gray spots, about 1-2 mm in diameter with a distinct brown margin. Lesions are mostly found on the lower leaves; 3 = Lesion type is the same as in scale 2, but a significant number or lesions are on the upper leaves; 4 = Typical susceptible blast lesions 3 mm or larger, infecting less than 4% of the leaf area; 5 = Typical blast lesions infecting 4-10% of the leaf area; 6 = Typical blast lesions infecting 11-25% of the lea area; 7 = Typical blast lesions infecting 26-50% of the leaf area; 8 = Typical blast lesions infecting 51-75% of the leaf area and many leaves dead; 9 = more than 75% leaf area affected (IRRI, 2002)

Diopsis spp. and stem borers were less abundant than blast. Both pests were of minor incidence on the BC_3F_3 families and their severity was random on the different parental combinations. Only a few families (0.14%) suffered more than 30% damage due to *Diopsis* species, while 97.9% of the progenies were healthy (Figure 6.4). Similarly, only 0.07% of the progenies were injured by stem borers, against 94.4% of plants without any symptoms (Figure 6.5).

Important agronomic traits were recorded and correlation analysis was performed (Table 6.2). The correlation analysis of traits determined that RYMV severity was significantly (1 %) correlated with all the traits except with the number of days to flowering, where the correlation, despite being non-significant was negative. There was strongest positive correlation between RYMV and blast ($r = 79\%$, $p > 0.01\%$), but the correlation between pests and diseases was not important. There was a negative correlation between the numbers of days from sowing to the emergence of flowers and traits like blast severity, and grain weight. The number of tillers was also negatively correlated with the crop duration (Table 6.2)

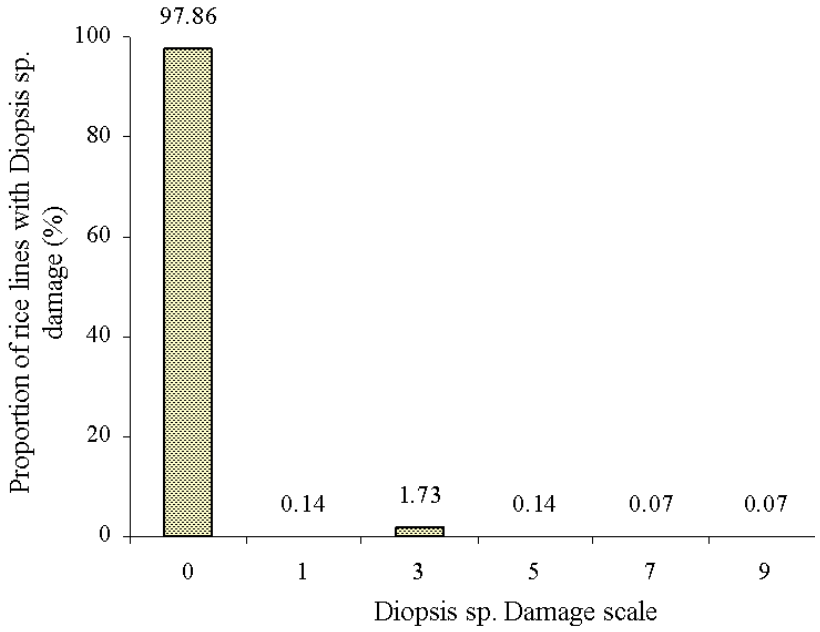


Figure 6.5: *Diopsis spp.* damage on 1353 BC_3F_3 rice families in Guinea.

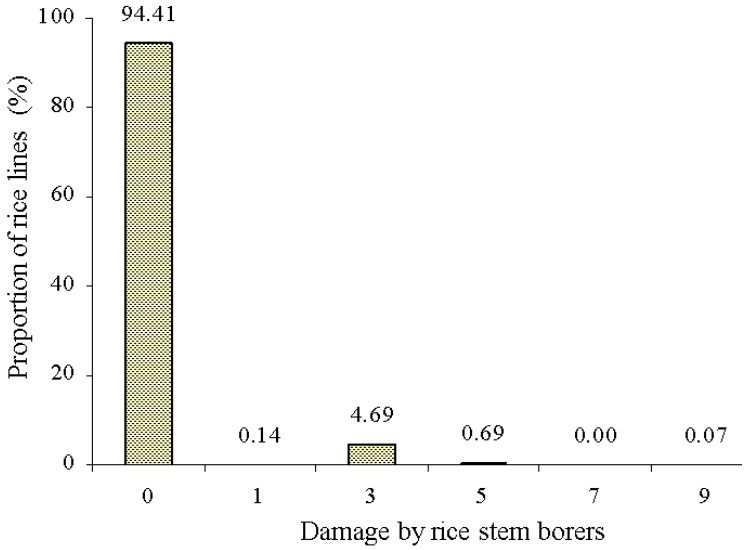


Figure 6.6: Rice stem borer damage on 1353 BC_3F_3 rice families in Guinea.

Table 6.2: Correlation analysis between disease and pests severity and some agronomic traits of the BC_3F_3 generation in Guinea

Parameter	Plant height (mm)	Tillers number	Days to flowering	Days to maturity	RYMV	Blast	Other borers	*Diopsis* spp.
Tillers number	0.18 **							
Days to flowering	0.01 ns	-0.00ns						
Days to maturity	0.14 **	-0.06 *	0.15 **					
RYMV	0.08 **	0.09 **	-0.02 ns	0.08 **				
Blast	0.17 **	0.05*	-0.07 *	0.10 **	0.79 **			
Other borers	0.02 ns	0.07 **	0.05 *	0.03 ns	0.26 **	0.24**		
Diopsis spp.	-0.04 ns	-0.02 ns	0.15 **	0.05 ns	0.11 **	0.12**	0.55**	
Grain weight/plant (g)	0.22 **	0.07 **	-0.01 ns	-0.02 ns	0.11 **	0.08 **	0.02 ns	-0.05 ns

RYMV = *Rice yellow mottle virus*, ** = significantly at 0.01, * = significantly at 0.05, ns = non-significant

6.4.3. Field evaluation in Mali

Except the resistant parent Gigante, all the other checks (IR64, Azucena and BG90-2) displayed RYMV symptoms (Figure 6.7). The recurrent parent IR64 was susceptible (S) and showed general mottling with pale yellow leaves, with a severe stunting (score 7), while the local check

BG90-2 and the variety WAT310 were highly susceptible (HS) and some of these plants even died during the trial. The partial resistance of Azucena was confirmed. As shown in Figure 6.7, about 22% of the BC_3F_3 families were symptomless and considered to be highly resistant, 67.5% were partially resistant, 4.3% were susceptible and 5.9% were highly susceptible.

The first symptoms appeared less than eight days after inoculation in 0.7% of the lines, including IR64, BG90-2 and WAT310. Symptoms appeared gradually for other lines (Figure 6.8), while 22.5% of the families, including Gigante did not show any symptoms at all during the experiment. The majority of the lines (76.4%), presented first symptoms between 12 and 21 DAI

The incidence of the disease was assessed from the number of infested tillers per hill for each plant family. The mean incidence of infected tillers per hill was 83% for early diseased plants (< 8 DAI), 86.1% when the symptoms appeared 12-15 DAI, and 16.7% of infected tillers for late diseased plants (> 30 DAI).

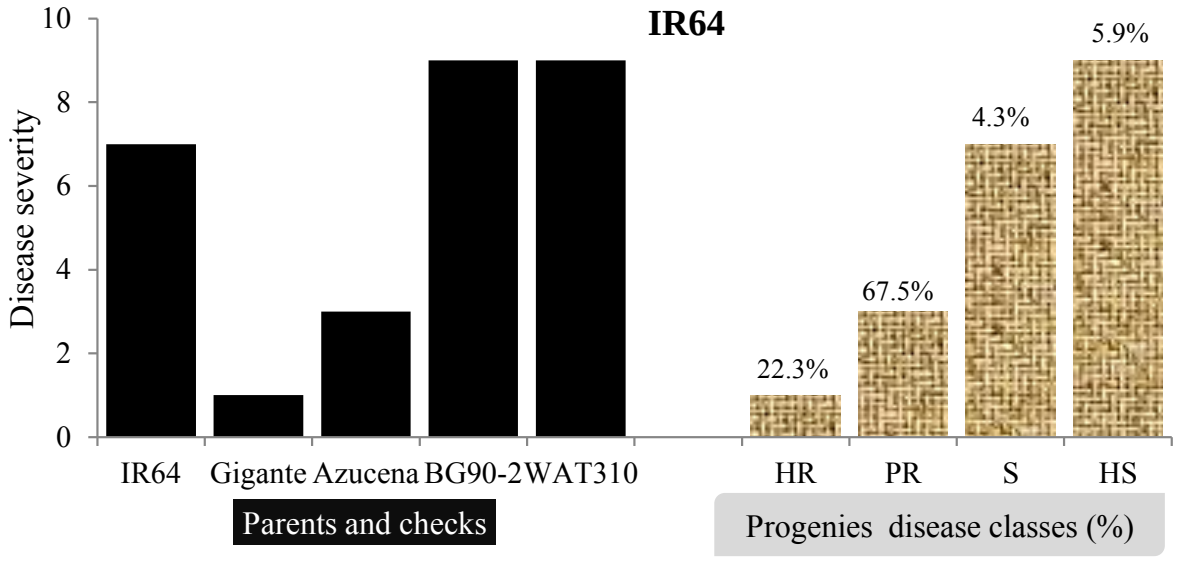


Figure 6.7: Summary of overall RYMV severity and proportion of BC_3F_3 families in Mali

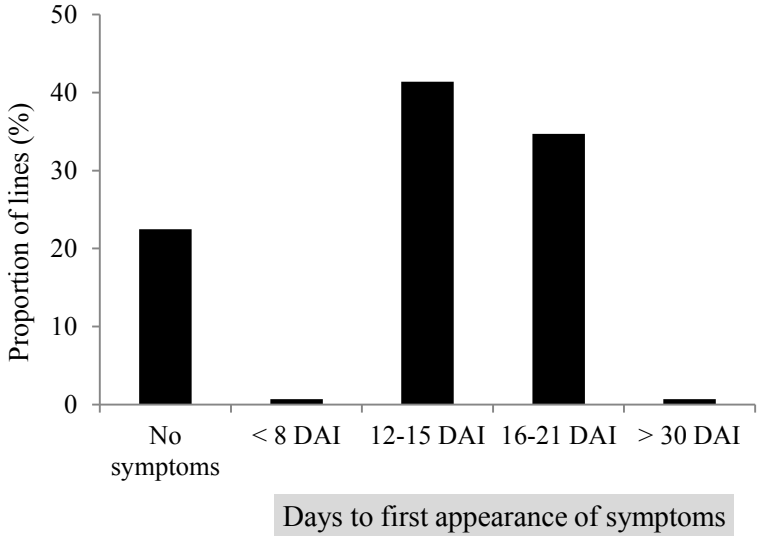


Figure 6.8: Days to appearance of first symptoms of RYMV during the experiment in Mali. The ordinate axis represents the percentage of individuals for each period.

Sap from 81 of the 95 HR lines in Mali, were used to inoculate BG90-2, and symptoms recorded at 21 DAI. Symptoms were induced by sap from 54.3% of the highly resistant families including Gigante, while sap from 45.7% of the progenies did not induce symptoms on the susceptible BG90-2.

Some agronomic data were recorded for the highly resistant families and their parents, and Azucena. The results are summarized in Table 6.3. Most of the progeny's crop cycles were longer than the mean of both parents, but their height was intermediate. IR64's crop cycle was delayed by RYMV inoculation, compared to the non-inoculated control. Most progeny's panicles were slightly longer than both parents, but the weight of 1000 grains was the same as Gigante, and slightly lower than IR64.

Table 6.3: Summary statistics of four agronomic traits measured on 81 BC_3F_3 families and parents in Mali

		IR64 Inoculated	IR64	Gigante	BC_3F_3
Days to maturity	Min	119	106	115	106
	Max	136	115	126	163
	Mean	126	109	120	138
	SD	8.6	4.7	5.5	1.44
	CV	6.8	4.3	4.6	0.09
Plant height (mm)	Min	610.0	800	1170	592
	Max	700.0	980	1260	1680
	Mean	647	887	1210	1040
	SD	47	90	46	26
	CV	7.3	10.1	3.8	0.2
Panicle length (mm)	Min	152	229	225	158
	Max	190	250	241	1290
	Mean	172	239	232	247
	SD	19	11	8	13
	CV	11.13	4.4	3.5	0.5
1000 grain weight (g)	Min	7.8	21.6	20	5.3
	Max	10	22.7	21	25
	Mean	9.2	21.6	20.7	20.2
	SD	1.2	1.0	6	0.3
	CV	13.2	4.6	3	0.1

Finally, a PCA was performed on the agronomic data. The PCA is shown in Fig.6.9. Axis 1 (42.72%) and Axis 3 (18.65%), reflect a relative variability of the BC_3F_3 families compared to their parents (IR64 and Gigante) and the partially resistant check Azucena. Overall, the resistance gene *rymv1-2* was successfully introgressed and functional in the progenies compared to the inoculated IR64 sample. However, some phenotypic characteristics like plant height, 1000 grain weight and cycle from sowing to maturity seemed closer to Gigante compared to the recurrent parent IR64. Nevertheless, some families like L155, L138 and L154 were more similar to IR64, with heavy panicles, short plant height and a short crop cycle.

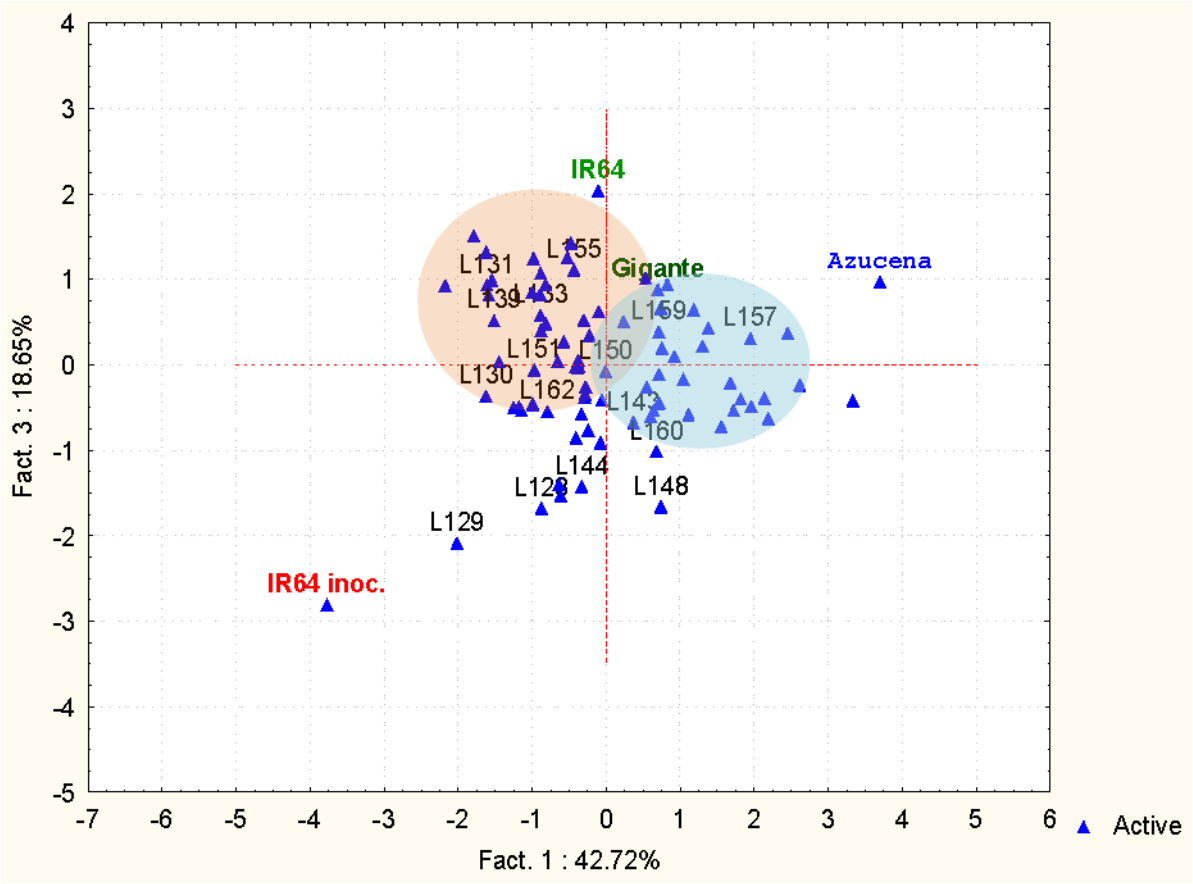


Figure 6.9: PCA of 81 highly resistant BC_3F_3 progenies derived from a cross IR64\Gigante, evaluated in Mali for resistance to RYMV and four agronomic traits

6.4.4. Screenhouse evaluation in Benin

From the experiments in Guinea and Mali seeds from the best 83 BC_3F_3 families from the different crossing combinations were selected. Forty five were selected from the trial in Guinea and 36 were common to both the Mali and Guinea trials. Thirty eight families were derived from the cross involving IR64, 28 families from IR47, 4 families from Sahelika and 13 families from the FKR28 combination. These BC_3F_4 lines were then screened with their parents, and 2 control variaties WAT310 and Azucena, against the RYMV isolate B27. The first RYMV symptoms appeared 6 DAI on IR64 and Sahelika, 8 DAI on FKR28 and 14 DAI on IR47. Similarly, the appearance of first symptoms was very variable among related BC_3F_4 lines. The ANOVA of the different traits recorded during the trial (disease incidence evaluated as the percentage of infected tiller per hill, leaves chlorophyll content measured with a SPADmeter, tiller number per plant and plant height) are summarized in Table 6.4. The reaction of the progenies and parents to

inoculation with RYMV isolate B27 was highly variable, as illustrated by the disease incidence significance (F = 6.99, df = 89, P = 0.000). A similar observation was conducted for the reduction of the number of tiller per plant (F = 3.58, df = 89, P = 0.000) and the reduction of plant height (F = 2.67, df = 89, P = 0.000), but the level of chlorophyll content reduction was not significant. The incidence of the disease overtime was significant for the two dates of notation (F = 16.64, df = 1, P = 0.000). The other traits did not reflect significant differences. The highest level of disease was recorded for IR64 and the line L17, derived from the variety Sahelika.

Table 6.4: Analysis of variance (ANOVA) of four data measured on 83 BC_3F_4 lines and their 6 parents

Source of variation	DF	F- ratio			
		DI (%)	rChcL (%)	rPHg t(%)	rTilN (%)
Variety (V)	89	6.99 **	1.02 ns	2.67 **	3.58 **
Replication	1	0.39 ns	1.20 ns	0.03 ns	0.29 ns
Date (D)	1	16.64 **	0.45 ns	1.48 ns	0.09 ns
V x D	89	6.28 **	1.00 ns	19.03 **	1.54 **

DI: Disease incidence, rChcL: reduction of chlorophyll content, rPhgt: reduction of plant height, rTilN: reduction of tiller number. **: Significant at 1% level ($P \leq 0.001$); ns = non-significant.

However, the interaction Variety x Dates was significant for all the traits except the reduction of chlorophyll content. During the experiment, 20 lines and Gigante displayed high resistance, with disease incidence ranging from 11.1% to 27.8%, while 38 lines and IR47 showed 33.33% to 50% disease incidence and were considered moderately resistant. Finally, 25 lines and the remaining recurrent parents were susceptible, with disease incidence ranging from 55.6 to 88.9%.

The virus content of the highly resistant and moderately resistant lines was assessed by ELISA, and significant differences were found. Moreover, the virus content in some moderately resistant lines was found to be higher than in certain highly resistant lines and inversely. Thus, the number of highly resistant lines (Appendix 6.1) increased to 36 (cf. Appendix 6.2) based on the ELISA test.

The genetic proportion of each parent in the constitution of the progenies' genome was investigated using 69 to 80 SSR markers well distributed on the rice genome. Analysis of the data revealed difference of genome content among progenies overall and also among progenies from the same cross. The average introgression of Gigante in all progenies was much higher than the

6.25% expected for a BC_3 generation. For example, it was as high as 21.3% in the cross with IR47 (Figure 6.10). This estimate covered 1492.8 cM of the genome.

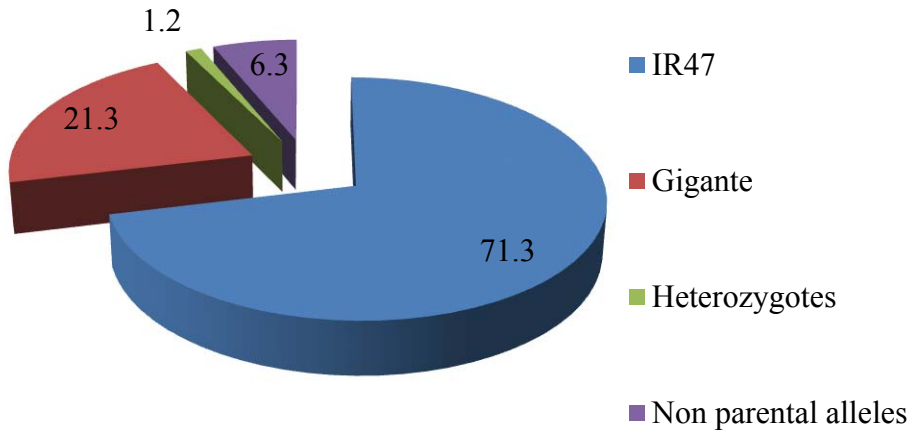


Figure 6.10: Genome content for BC_3F_4 progeny L54, derived from a cross IR47 x Gigante (estimated)

6.5 Discussion

Breeding for resistance to RYMV is one of the main goals of most of the rice breeding programs in the West Africa. However, the nature of the resistance genes (recessive), combined with the variable virulence of the virus makes selection for RYMV resistance complex and time consuming. However, numerous reports have suggested that MAS using SSR markers, tightly linked to a major resistance gene could produce a more efficient genetic gain than using phenotypical selection, especially when the trait involved is assessed in a qualitative approach (Francis et al., 2003). MAS has been used to transfer agronomic traits, especially resistance to pathogens, on several crops including, tobacco, maize, tomato and rice (Yi et al., 2009), (Johnson et al., 2002; Yang and Francis, 2005; El Mohtar et al., 2007; Ribaut and Ragot, 2007). For rice, the development of the breeding lines for such selection depends greatly on the crossability between the parents involved, because there are several sterility barriers in rice, even within the two main subspecies (*indica* and *japonica*) of the *O. sativa* species (Harushima et al., 2002). Thus, in the present study, intraspecific barriers, coupled with the ability to synchronize (or not) the flowering times of the different parents, and finally, the amount of spikelet emasculated from

each crossing combination could explain the disparity in the numbers of BC_3F_3 families produced for each cross. Moreover, random genetic recombination, during meiosis, even at low frequencies, could have influenced the expected pattern of the two flanking markers, thus explaining the low level of double homozygotes observed (Semagn et al., 2006). The populations developed in this study were diverse enough to detect all the different types of profile obtained when the MAS procedure was conducted on the basis of genotypes at the target locus and two flanking markers loci (Frisch et al., 1999).

More progeny families were screened in Guinea than in Mali because of the availability of favourable climatic conditions to screen for the disease under natural conditions the whole year, whereas Mali has only one rainy season per year. Because of the wet and green climatic conditions of Guinea, grasses serving as reservoirs for the virus and insect vectors grow throughout the year. Besides, rice seedbeds were reported to be a source of primary infection by RYMV (Traoré et al., 2006), thus, the seedlings were naturally exposed to the disease in the nursery before transplanting. However, to ensure infection during the period of the experimentation in Mali, mechanical inoculation with sap prepared from diseased plants collected around the site of the experiment has preceded the transplanting of the seedling in the field. Therefore, a much higher inoculum level of RYMV could be assumed in the experiment in Mali compared to Guinea. Consequently, symptoms were earlier and more severe for Mali in comparison to Guinea. A similar observation was made by Fargette et al. (2002b) that associated the symptom intensity to high virus content in infected plants.

The different recurrent parents did not react similarly to the disease in Guinea. Varieties WAT310 and Sahelika were susceptible, while IR64 and IR47 were intermediate and FKR28 was partially resistant. Varieties IR47 and FKR28 are *O. sativa* cv. *japonica* and some previous studies had reported that some *japonica* varieties are partially resistant to RYMV (Rakotomalala et al., 2008), including IR47 (WARDA, 2001; Soko et al., 2010) and Moroberekan (Heinrichs et al., 1997). In addition, a major QTL for partial resistance was mapped onto Chromosome 12 of the *japonica* cv. Azucena (Albar et al., 1998). The resistance of Azucena was confirmed during the trial in Mali with a delay in symptom expressions (12DAI) and mild disease expression. High levels of resistance, characterised by a lack of symptoms and an undetectable level of virus, were only reported in two *O. sativa* cv. *indica* varieties: Gigante and Bekarosaka (Ndjiondjop et al.,

1999; Rakotomalala et al., 2008). The *indica* variety IR64, however, is well known to be highly susceptible to RYMV, from many field and screenhouse screening trials, and displayed symptoms early as six DAI (Albar et al., 1998; Sorho et al., 2005; Thiémélé et al., 2010). The same trend was observed during the trial in Mali, probably because of the high inoculum pressure. Direct comparison of incidence of RYMV on developed progenies in Guinea and Mali is risky due to the knowledge gap of the RYMV's population structure, distribution and pathogenicity in Guinea and the eco-climatic differences between the two countries. Yield losses of 100% have been reported in Mali (Sy et al., 1993). Onasanya et al. (2006) reported the presence of two main pathotypes of RYMV in Mali, including highly pathogenic isolates as well as a probable interaction between the two existing pathotypes. The high disease pressure in Mali compared to Guinea was confirmed by the increase of the disease score of IR64 from Intermediate symptoms in Guinea to Highly susceptible in Mali. The susceptibility of WAT310 also increased, along with that of the local control BG90-2. Some varieties sharing the same pedigree as WAT310 were rated as Susceptible in previous studies (Zouzou et al., 2008) and BG90-2 and several *indica* varieties were cited as highly susceptible to RYMV (Kouassi et al., 2005).

Early research suggested that screening for resistance to RYMV should not focus on visual symptoms only (Coulibaly et al., 1999). For example, a major QTL for RYMV resistance detected by Albar et al. (1998) also affects plant morphology. Additionally, yield reduction due to panicle sterility and poor panicle exsertion, tiller number, reduction of chlorophyll and disease incidence were also used to assess the resistance of rice varieties to RYMV (Awoderu, 1991; Onasanya et al., 2004; Onasanya et al., 2006; Sere et al., 2008; Soko et al., 2010). Except for the chlorophyll content of leaves (found to be not significant in the present study), all the other traits were found to be in agreement with previous studies. Moreover, delays in plant maturity and changes in 1000 grain weight were consistent with relating to reproductive losses due to RYMV in *O. sativa* species (Onwughalu et al., 2011a).

Although, isolates of RYMV virulent against the gene *rymv1-2* have been reported to occur in farmer's fields (Traoré et al., 2006), the resistance of Gigante was not overcome during our field trials in Mali and Guinea. This was probably due to the low prevalence of this RYMV isolate

(Pinel et al., 2006; Traoré et al., 2006). This result supports the finding of Poulicard et al. (2009), that RYMV has a limited ability to develop virulence to *rymv1-2*.

In addition to RYMV, leaf blast severity and stem borer prevalence were also recorded in the trial run in Guinea, because the country's climatic conditions (high humidity, medium temperature, good vegetal coverage and long period of leaf wetness) are ideal for the pathogens and insect pests. These conditions were found to be optimal for blast development in the USA (Greer and Webster, 2001). Leaf blast has been found to be the most damaging in association with RYMV, because RYMV suppresses blast resistance, probably. Teng, (1994) reported that the causal agent of blast, *Magnaporte grisea*, induces disease at the development stage of rice, as RYMV does. Therefore, the synergy between the two pathogens, particularly during early infection would be more harmful than their individual effects.

The advantage of using MABC approach to gene introgression is to allow fast recovery of the recurrent parent's genome. However, the proportion of the donor parent, Gigante, was still high in the BC_3F_4 progenies, despite three backcrosses. In contrast, Ahmadi et al. (2001) introgressed Azucena's QTLs for partial resistance into susceptible IR64, and their BC_3 progenies carried 95% of the recurrent parent genome. They estimated a length of less than 20 cM of the donor chromosome segment surrounding the introgressed QTLs. Similarly, 97% or more of a recurrent parent genome was recovered in wheat after just two backcrosses (Randhawa et al., 2009). However, other reports pointed out the possibility of retaining (drag) a high proportion (nearly half) of the donor genome even after 11 backcrosses in tomato cultivars (Young and Tanksley, 1989) or up to 14 cM in barley lines (Bjørnstad et al., 2002), after seven backcrosses. Such situation results from linkage drag and consequently a larger number of backcrosses may be needed to decrease the donor's genome (Semagn et al., 2006).

MABC was successfully used in transferring RYMV resistance from Gigante into some West African elite rice varieties. The expression of the RYMV resistance was successfully tested both in farmers' fields in Mali and Guinea, and also in a screenhouse under controlled conditions. The combination of field and screenhouse screenings reinforced the molecular selection process and provided information on the functionality of the transferred gene in its new genetic backgrounds. Presently the five best progenies, one per crossing combination, are being tested in farmers' fields

in seven African countries for adaptability and resistance to RYMV. Meanwhile, further backcrosses to increase the recurrent parents' genome are being conducted

6.7 References

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Appendix 6.1: Pictures of some of the elite varieties and their progenies introgressed with Gigante's allele *rymv1-2*

Susceptible IR64

Susceptible Sahelika

Partially resistant IR47

Resistant line L130, derived from IR64

Resistant line L16 derived from Sahelika

Resistant line L54 derived from IR47

Appendix 6.2: Summary of 36 most resistant BC_3F_4 lines screened with virus isolate B27

Code Lines	DI(%)	rPHgt(%)	rTilN(%)	Status VS	VC (%)	Status ELISA	Score Blast
L2	50	20.5	56.3	MR	0.02	R	1
L4	22.2	17	30.4	R	0.03	R	1
L5	11.1	12.1	20	R	0.03	R	1
L6	13.9	47	28.6	R	0.05	R	1
L16	33.3	5.2	25	MR	0.05	R	1
L24	44.4	18	12.5	MR	0.06	R	1
L30	38.9	11.5	50	MR	0.04	R	1
L31	33.3	17.7	12.5	MR	0.06	R	1
L36	50	26	37.5	MR	0.02	R	1
L39	38.9	32.6	70.8	MR	0.04	R	1
L42	38.9	17.8	50	MR	0.06	R	1
L43	33.3	21.4	66.7	MR	0.04	R	1
L46	50	64.4	75	MR	0.03	R	1
L48	33.3	9.8	41.7	MR	0.03	R	1
L49	50	7.6	43.8	MR	0.03	R	1
L52	44.4	6.1	25	MR	0.06	R	1
L54	33.3	16.4	50	MR	0.03	R	1
L56	44.4	7.9	25	MR	0.03	R	1
L58	44.4	28.9	25	MR	0.04	R	1
L59	33.3	11.8	37.5	MR	0.02	R	1
L127	44.4	14	52.8	MR	0.02	R	1
L129	33.3	23.1	9.6	MR	0.02	R	1
L130	38.9	31.4	40	MR	0.03	R	1
L132	44.4	10.4	41.7	MR	0.02	R	1
L133	22.2	47.4	50	R	0.03	R	1
L135	27.8	11	35.6	R	0.04	R	1
L139	22.2	40.6	29.2	R	0.02	R	1
L141	27.8	6.3	38.2	R	0.05	R	1
L145	22.2	8.1	25	R	0.03	R	1
L147	44.4	26.8	53.6	MR	0.03	R	1
L154	11.1	10.5	50	R	0.02	R	1
L155	50	15.5	40	MR	0.06	R	1
L157	33.3	28.2	57.3	MR	0.03	R	1
L160	22.2	22.2	34.5	R	0.06	R	1
L163	27.8	6.3	25	R	0.04	R	1
L165	27.8	11.6	42.9	R	0.05	R	1
L17	88.9	25.7	59.4	S	-	-	1
Gigante	22.2	21.4	33.3	R	0.04	R	3
IR64	77.8	34.5	41.7	S	-	-	7
IR47	44.4	19.9	99	MR	2.69	S	5
Sahelika	75.6	31.5	63.4	S	-	-	5
FRK28	66.7	23.8	63.4	S	-	-	5
Grand Mean	**43****	**26.3****	**42.5****	**-**	**0.058****	**-**	
5%LSD	**4.1**	**13.4**	**6.7**	**-**	**0.030**	**-**	
SE	**1.5**	**4.8**	**2.5**	**-**	**0.017**	**-**	

DI: Disease incidence, rPhgt: reduction of plant height, rTilN: reduction of tiller number. Status VS: status for visual score, R : resistant, MR : moderately resistant, **: Significant at 1% level ($P \leq 0.01$); ns = not significant

Thesis Overview

Introduction

This section provides an overview of the research done relative to the original objectives. In addition, major findings, as well as their implications for rice breeding are noted, and finally suggestions for future research are presented.

The objectives of this pre-breeding and breeding study were to:

- Generate formal data on farmers' perception of rice varieties, and their views on the main constraints on rice production, and on RYMV, in the main rice growing area of Niger;
- Build the first exhaustive collection of rice varieties in Niger, representing the genetic variability of the rice species in the country
- Study the extent, partitioning and genetic structure of the collection
- Establish the phenotypic variability and potential of the collection for future breeding programs
- Screen the collection for resistance to RYMV and to find new sources of resistance
- Convert some elite West African rice cultivars using MAS for resistance to RYMV

Summary of the major findings and their implications

The participatory rural appraisal revealed that:

- Despite the availability of high yielding, modern Asian rice varieties, a number of farmers are still growing *O. glaberrima* and other landraces for several reasons including ecological adaptability and resistance to flood.
- The main reasons for farmers to adopt a new rice variety in the irrigated cropping system were found to be grain quality, high market price, disease resistance and advice from their local association, and above all, yield. The variety IR1529-680-3 and its derivative "Waihidjo" are the most preferred varieties.
- In the lowland cropping system apart from yield, good taste, flood tolerance, heritage and tolerance to birds are among the preferred traits of farmers. Degaulle/ D5237 is the post popular cultivar.
- Susceptibility to disease, birds, drought, flood bad grain quality, as well as just "testing novelty" are among the reasons that lead farmers to abandon rice varieties.

- The diseases RYMV and BLB, along with hippopotamuses, birds, flood, drought and seed degeneration are the main constraints on production.
- Farmers are aware of the RYMV and preventive methods are being implemented

New rice breeding lines could be easily brought to farmers through PVS relying on local farmers' unions. However, those new lines should be carefully tested for resistance to RYMV and BLB, and for grain quality, taste and milling quality. Failing to do so will result in the same trend as the variety WITA 8, which is tolerant to RYMV, but has been abandoned due to poor milling quality. Moreover, the fact that yield losses due to RYMV did not significantly drop below 56-68% (Reckhaus and Adamou, 1986; Reckhaus and Adamou, 1989) over 20 years, despite the amount of knowledge and insights developed on the virus' epidemiology, its interaction with rice and management (Kouassi et al., 2005; Sarra, 2005; Nwilene et al., 2009; Traoré et al., 2009; Hébrard et al., 2010; Traoré et al., 2010) provide evidence that appropriate measures remain to be taken in term of plant breeding and capacity building.

The main findings from Chapter Three and Four include:

- The first exhaustive collection of rice species from Niger, including both cultivated and wild species for ex- situ conservation was created;
- The establishment of the agro-morphological characteristics as well as the phenotypic diversity of the collection;
- The establishment of the genetic structure and diversity of the collection;
- Few varieties were released for the lowland ecology, despite the importance of dedicated surfaces;
- The gene pools of *O. glaberrima* and *O. barthii* have not been exploited yet in breeding new rice varieties.

Oryza glaberrima and *O. barthii* accessions from the collection have been stored in the genebank of AfricaRice, and will shortly be available to the rice science community under SMTA. Information generated from those landraces will be valuable for breeding programs. In addition, they are sources of new genes which will be useful in future breeding programs (Thomson et al., 2003; McCouch et al., 2007; Chen et al., 2009), as crosses between genetically distant accessions tend to produce higher genetic gain (Friedt et al., 2007). Meanwhile the government's efforts to

overcome the chronic cycle of food insecurity in Niger, via the development and promotion of irrigation, coupled with climate change are contributing to further genetic erosion of the remaining landraces.

Major findings in Chapter Five and Six include:

- The presence of variable phenotypic reactions to RYMV, ranging from highly susceptibility to high resistant, within the rice collection from Niger;
- New *O. glaberrima* accessions bearing known RYMV resistance alleles *rymv1-3* and *rymv1-4* have been collected;
- The new *O. glaberrima* accessions bearing a new resistance gene to RYMV;
- The *rymv1-2* resistance allele from Gigante was introgressed into five elite rice cultivars of West Africa.

The presence of a wide range of phenotypic responses to RYMV infection in the rice collection offers interesting prospects for breeding. Both vertical resistance, based on major genes, and horizontal resistance, based on the addition of several quantitative traits loci (QTLs) with small effects, could be exploited to develop varieties with comprehensive resistance to the virus. Furthermore, with the development of MAS using highly precise genetic tools, good combination of both kinds of resistance could be achieved, without any risk of horizontal resistance erosion, as postulated by Robinson (1997). Indeed the genome of the parent to be improved can be recovered to over 93% in four backcrosses in rice (Yi et al., 2009), and up to three QTLs for earliness and grain yield have been successfully introgressed into maize (Bouchez et al., 2002).

This study presented however certain limitations.

Conclusion and recommendations

This study provided a pre-breeding framework for rice in Niger, as well as a short-term breeding solution for resistance to RYMV in West Africa. Information was captured on farmers' perception of constraints to their production, as well as their preferences in rice varieties. However, only a limited number (153) of farmers have been approach during this study. For a broad involvement of farmers in farmer-oriented-breeding, a larger sample of farmers, covering all the agrosystems and socio-economical areas of rice workers and rice value-chain workers would be required.

The raw genetic material was gathered and *ex-situ* conservation proposed as a temporary answer to the genetic erosion of landraces as well as the preservation of wild rice species in Niger. As with the PRA's survey, a more precise sampling strategy should be adopted, to ensure a more comprehensive capture of the diversity of *Oryza* species in Niger. New accessions with various levels (moderate and high) of resistance to RYMV were identified and five popular rice varieties converted for resistance to RYMV.

The emergence of resistance-breaking RYMV isolates in farmers field should be monitored and new varieties with different allelic forms of RYMV1 should be created and deployed with caution. For practical reasons, only one late (BC_3F_4) stage background selection for the recurrent parent's alleles was conducted during the MAS process. Such an approach should be avoided, for a better conservation of the full genetic potential of the recurrent parent.

However, to benefit from these findings, a more rigorous involvement of farmers in variety release should be implemented in Niger. Similarly, inspections should be conducted during seed production process at the seed farm of Saguia (ONAHA), to ensure that good quality seed is delivered to farmers' unions, in order to restore their trust in this system. Farmers, as well as agricultural extension officers, should be trained in seed production and disease management.

For the short and medium-term, popular rice varieties IR1529-680-3 and Degaulle should be improved for resistance to RYMV and flood-tolerance, using marker-assisted selection protocols developed in others studies (Albar et al., 2003; Neeraja et al., 2007; Thiémélé et al., 2010). Meanwhile, a functional rice breeding program, taking into account the main constraints to production, and farmers' preferred traits as well as the genetic and phenotypic data generated in this study, should be established by the Institut National de la Recherche Agronomique du Niger (INRAN). Crossing of genetically distant parents and the use of the *O. glaberrima* and wild species gene pools to develop interspecific lines would be advantages. Further collection of wild rice species and landraces should also be conducted to improve this collection. Finally, more studies on farmers' involvement in rice genetic resource conservation should be performed, to develop a comprehensive *in-situ* conservation plan for African rice to ensure its survival as a vital source of genetic material for rice breeders.

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