A Study of the Diversity of Burkina Faso Rice Landraces and Identification of Source of Resistance to *Rice yellow mottle virus* (RYMV)

By

Honoré Kam

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in Plant Breeding

School of Agricultural, Earth and Environmental Sciences (SAEES)
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Republic of South Africa

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Collection of rice landraces was by car in the Sourou Province and motorbike in the other provinces of the collection area. Approximately, 4,000 km was covered in search of rice landraces.

Thesis abstract

The main goals of this study were to ascertain farmers' preferred traits in rice landraces and their perception of *Rice yellow mottle virus*, to collect rice landraces across Burkina Faso, investigate their genetic diversity, and to exploit this diversity in a search for varieties resistant and tolerant to RYMV, for their utilisation in rice breeding. Farmers' preferred traits, approaches to crop management, and disease perceptions were assessed using a Participatory Research Appraisal (PRA) approach. In the main rice growing regions of Burkina Faso, 330 rice landraces were collected. The agro-morphological diversity of the germplasms was evaluated in the field with 20 quantitative and 30 qualitative agro-morphological parameters. Thereafter, 22 Simple Sequence Repeat molecular markers were used to assess the genetic diversity and the population structure of the collection. Finally, the rice landraces were screened against four RYMV isolates to assess the susceptibility, tolerance and resistance of the landraces in the collection using visual assessment and Enzyme Linked Immunosorbent Assay.

The PRA identified sweet taste, grain expansion when cooking, easy cooking and yield as paramount selection criteria in rural rice farming communities in Burkina Faso. Drought and disease resistance are characters that farmers wish to have in their varieties. The PRA also highlighted that farmers are conscious of RYMV disease in their fields. However, they are unaware about the epidemiology of the disease.

An agro-morphological study of the phenotypic diversity of the collection confirmed the presence of the two cultivated rice species: *O. glaberrima* and *O. sativa*. There were more *O. sativa* accessions than *O. glaberrima* landraces. There were 48 *O. glaberrima* and 282 *O. sativa* accessions in the collection. Both species were divided into four clusters, reflecting the richness of the collection. The underlying genetic diversity of the collection was confirmed by the use of 22 Simple Sequence Repeat molecular markers. The neutral markers confirmed the existence of two substructures, namely *O. glaberrima* and *O. sativa*, and the presence of admixture varieties. However, a core collection of 52 individuals was developed. This included 13 *O. glaberrima* and 39 *O. sativa* accessions. It reflects the genetic diversity of the sub-clusters present in each species. This core collection contains 89% of the allelic richness of the collection. Its small size will facilitate the maintenance and active use of diversity of germplasm in the core collection. The entire collection was utilised to search for varieties

resistant and tolerant to RYMV disease. The screening of the collection with different RYMV isolates exposed the susceptibility of most of the accessions in the collection. Most of the *O. sativa indica* accessions were highly susceptible. However, ten *O. glaberrima* accessions displayed a delay of symptom expression, and moderate resistance. However, their resistance was overcome later by a particularly virulent RYMV isolate BF1. Remarkably, a single moderately resistant cultivar, BM24, showed that partial resistance and tolerance to RYMV can be found in an *O. sativa* variety. Serological evaluation of this local variety in comparison with the partially resistant variety, Azucena, showed that BM24 and Azucena expressed similar resistance patterns. A genetic profile of both varieties showed that both had an identical allele status at RM101, which is a marker bracketed in the same zone as the QTL12.

DECLARATION

I, Honoré Kam, declare that:

i. The research reported in this thesis, except where otherwise indicated, 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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Signed
…… Date……
Honoré Kam (Candidate)

As the candidate's supervisors, we agree to the submission of this thesis.
Signed
…… .. Date……
Professor Mark D. Laing (Supervisor)

Dr Marie Noëlle Ndjiondjop (Supervisor)
Signed
…… .. Date……

DEDICATION

To the Almighty God who makes all things possible and grants humanity with diversity, a foundation of a marvellous world

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INTRODUCTION

Rice trends in the world

Rice is a major crop and plays an important role in feeding more than half of the world population. It belongs to the category of major cereals of the world and ranks next to wheat in terms of production. Rice is grown in wetland areas throughout the five continents and it is sensitive to water stress (Balasubramanian et al., 2007). Its consumption in the world had reached 432,039,000 tonnes in the year 2008 (IRRI, 2009). It has been at the cornerstone of the green revolution in Asia and contributes significantly towards providing food security in this densely populated region. China and India are the leading rice producing countries in the world. Together they produced 326,624,000 tonnes in 2007, which made up for half of the world's rice production (IRRI, 2009). However, the major rice exporting countries are Thailand, India and Vietnam.

In Africa, rice ranks third after maize and sorghum. It constitutes a substantial part of the diet of the African people. However, Africa produces only 3.6% of the world's paddy rice (IRRI, 2009). As a result, the African countries are not self sufficient in rice production and considerably depend on importing enormous volumes of this cereal. In 2008, more than a quarter of imported world rice was diverted to Africa; therefore, this makes Africa, the second-largest rice importing continent after Asia (IRRI, 2009).

In West Africa, rice consumption is rising, both in the cities, amongst the poorest urban households, as well as amongst smallholder farmers in rural areas. However, rice consumption per capita is higher in the cities in a manner that the annual production of the seventeen countries in West Africa, estimated at 9,677,000 tonnes, does not meet the huge demand. As a result, around 5 million tonnes of rice is imported to cope with the enormous need, notwithstanding the 6 million ha of harvested areas dedicated to rice production (Table 0.1). Therefore, more effort has to be diverted towards improving its yield and filling the gap between the rice supply and demand.

A general view of the West African countries' rice production, consumption and import patterns and systems shows many disparities (Table 0.1). Nigeria and Guinea are leading the

production with more than one million tonnes. Conversely, Nigeria is also the biggest rice importing country in the region. Guinea, Côte d'Ivoire, Senegal, and Sierra-Leone are the bigger consumers of rice in terms of per capita consumption. The group of low producing countries (Benin, Burkina Faso, Cameroon, The Gambia, Mauritania, Niger, and Togo) have their annual production ranging between 22,000 tonnes and 100,000 tonnes. The yields in West African regions are low. For instance, the average yield of 1.62 t ha^{-1} in West Africa (AfricaRice, 2008) is the lowest in the world, whereas Egypt has been consistently achieving 10 t ha^{-1} since 2006 (IRRI, 2009). This necessitates levelling the gap between actual and attainable rice yields in Africa.

Table 0.1: General view of rice production in West Africa (Year 2007)

	Area harvested (ha)	Yield (t ha^{-1})	Production paddy (t)	Import (t)	Per capita consumption (Kg $year^{-1}$)
Benin	33,000	2.12	70,000	160,000	45.70
Burkina Faso	51,000	1.87	123,000	100,000	10.86
Cameroon	20,000	3.10	49,000	300,000	15.75
Chad	80,000	1.36	129,000	5,000	6.87
Cote d'Ivoire	500,000	2.30	677,000	800,000	61.43
The Gambia	16,000	1.38	22,000	95,000	24.44
Ghana	119,392	2.03	242,000	350,000	31.70
Guinea	525,000	1.71	1,402,000	150,000	69.57
Guinea Bissau	65,000	1.95	89,000	39,000	55.20
Liberia	120,000	0.92	155,000	200,000	45.69
Mali	451,000	1.94	955,000	100,000	41.65
Mauritania	17,000	4.53	77,000	36,000	22.30
Niger	27,800	2.75	77,00	160,000	2.31
Nigeria	3,704,000	0.96	4,677,000	1,600,000	25.38
Senegal	95,000	2.78	215,000	700,000	68.81
Sierra Leone	210,000	1.26	650,000	130,000	80.51
Togo	35,000	1.95	68,000	85,000	13.79
West Africa	6,069,192	1.33	9,677,000	5,010,000	18.80

Sources (AfricaRice, 2008; IRRI, 2009)

Rice production in West Africa faces several problems owing to abiotic and biotic stresses. The major constraints are yield instability, drought, problems pertaining to minerals (salinity, acidity, alkalinity, P deficiency and iron toxicity), unavailability of suitable and improved varieties for diverse environments (including low potential areas), weeds, pests and diseases (Nwanze et al., 2001; Adolph and Chancellor, 2006; Balasubramanian et al., 2007). The major diseases which hamper rice production in West Africa are African Gall Midge (AfGM), Bacteria Leaf Blight (BLB), *Rice yellow mottle virus* (RYMV) and blast. Blast in particular is a serious problem in upland rice, while RYMV is a major production constraint in irrigated and rainfed lowland cropping systems (Nwanze et al., 2001). However, RYMV remains the most damaging and widespread pest affecting rice production throughout Africa (Kouassi et al., 2005). First reported in Kenya in 1966, RYMV has since been detected in most rice-growing countries of Africa and Madagascar, but currently remains limited to Africa (Abo et al., 1998). During a survey in 1983 and 1986, 75% of the total cultivated area of rice in the Sahel Region was reported to be affected, along with 40% in the Sudan savannah, 18% in the Guinea savannah, and 7.5% in the tropical rainforest (Awoderu, 1991). The incidence and severity of the disease appear to depend upon the rice cultivars, environment and vegetation zones. The economic impact of RYMV is difficult to evaluate due to the influence of many factors, but average yield losses fluctuate between 25% and 100% (Konate et al., 1997) depending upon the plant age prior to infection, susceptibility of the rice variety and the environmental factors.

RICE IN BURKINA FASO

Burkina Faso is a landlocked country located in West Africa and surrounded by Benin, Côte d' Ivoire, Ghana, Mali, Niger and Togo. In terms of production and cultivated areas, rice ranks fourth after sorghum, millet and maize in Burkina Faso (INSD, 2010). The rice production in the country was 123,000 tonnes in 2007 (IRRI, 2009). Three types of rice cropping systems are encountered: the rainfed upland, lowland, and the irrigated schemes. The rainfed upland contributes to 5% of the whole production and represents 10% of the rice cropping areas. Lowland areas make up for 42% of the entire production and 67% of allocated rice cropping areas. Irrigated rice, with total water management, account for 53% of the total production and covers 23% of the rice cropping areas (Dembélé et al., 2006).

Nonetheless, favourable conditions for lowland and irrigated rice exist: Sourou Valley, Bagré Valley, Banzon Valley, Kou Valley, etc. The potential areas for lowland rainfed rice cultivation are especially widespread in the southern part of the Burkina Faso, where rainfall can exceed 1000 mm per annum. The specific constraints to successful rice yield include drought, heat and cold. Drought is observed in rainfed rice cropping areas owing to irregular weather and rainfall, and also heat and cold induced sterility during hot and cold seasons, respectively.

INVOLVEMENT OF FARMERS IN RICE BREEDING

Earlier, several breeding programmes neglected the farming communities in their breeding processes. Thus, many varieties were developed by breeders, which did not match farmers' preferences. Therefore, the adoption of such varieties was very low and some were even rejected, even though they were considered improved varieties**.** For instance**,** Linares (2002) reported the rejection of American long-grained rice and South Asian rice by traditional Jola's people in Senegal. To avoid such situations, farmers' views on their preferred rice traits should be ascertained through direct discussions with them. They should also be involved in the process of varietal selection. A holistic development approach, which takes into account farmers' views, suggestions and needs must be embraced. Developing acceptable cultivars in West Africa requires breeders to become deeply familiar with the needs and preferences of small-scale farmers. For better agricultural development in Sub-Saharan Africa, Nwanze et al. (2001) recommended the incorporation of indigenous knowledge through high participation of farmers at the grass-roots level. This process ensures that the farmers are owners of their development. In light of the need for farmer's involvement, breeders are using Participatory Research Appraisal (PRA) as a tool to involve communities in the development processes. It is this participatory approach that emphasises local insights and assistance to local people, to help farmers devise their own appraisal, analysis and plans (Efisue, 2006).

RICE LANDRACES – A SOURCE OF IMPORTANT GENES

Local rice varieties, including the native cultivated rice of Africa, *O. glaberrima*, and the Asian rice, *O. sativa*, introduced in Africa several decades ago, are important sources of genetic diversity (Nuijten et al., 2009). In West African farmer fields, the two species are often encountered with the dominance of *O. sativa*. In fact for several years, *O. glaberrima*,

the African rice had been neglected because the Asian rice, *O. sativa*, since the time it was introduced in the African continent, spread rapidly due to its high yield as compared to *O. glaberrima*. Progressively, the exotic rice became the predominant cultivated form throughout the whole continent. Currently, *O. sativa* is grown everywhere in West Africa (Linares, 2002). The replacement of the autochthonal rice by modern varieties has led to concerns that it may jeopardize the conservation of local genetic resources (Barry et al., 2007). Modern varieties do not have the genes to cope with the specific stresses of African ecosystems (Nwanze et al., 2001). In reality, landraces have particular traits, such as their adaptability to local conditions and constraints, which are not easily found in formal varieties. For instance, an *O. glaberrima* line can have multiple resistances to various local constraints because it is adapted to these conditions (Futakuchi et al., 2006).

Although *O. glaberrima* cultivars have low yield, local farmers still favour them, for instance, Baga rice in Guinea, Floating rice in the Niger Valley, 'Ajola', 'Ejonkin' and 'Ecasay' in Senegal (Ghesquiere et al., 1997; Linares, 2002). Although *O. glaberrima* has not been bred as intensively as *O. sativa*, it can carry numerous agronomically important alleles (Sarla and Swamy, 2005). Consequently, in terms of breeding, there is a need to exhaustively exploit *O. glaberrima* for its adaptation to local conditions and its resistance or tolerance to several diseases. Rice landraces have a substantial role to play in countering biotic and abiotic stresses. Hence, the landraces already adapted to agronomical requirements of the West African regions should be further investigated to tackle the spread of RYMV. The diversity of rice landraces including *O. glaberrima* and *O. sativa* should, therefore, be widened and exploited by the establishment and the evaluation of collections. The biotechnological techniques, through new tools of cellular and molecular biology, such as molecular markers and genetic engineering could help identifying important gene pools already harboured in local germplasms.

OBJECTIVES OF THE THESIS

The main objective of this study was to investigate the genetic diversity of the rice landraces of Burkina Faso and to exploit this diversity to search for resistant and tolerant varieties against RYMV for use in breeding. The specific tasks to be achieved through this thesis were:

- to collect rice landraces in the major rice cropping regions of Burkina Faso;
- to investigate rice traits preferred by farmers in their local cultivars and their perception on RYMV;

- to evaluate the phenotypic and molecular diversity within rice landraces of Burkina Faso;
- to screen the rice local germplasm collection for resistance and tolerance to RYMV.

STRUCTURE OF THE THESIS

To document the objectives and tasks proposed in this thesis, seven chapters are included. Chapter 1 carries the review of literature. This chapter gives an overview of rice genetics and diversity, the use of molecular markers in plant breeding and the relevant achievements in the search for resistance against RYMV so far. Chapter 2 deals with the perception of farmers on RYMV and their preferred rice traits investigated through a Participatory Research Appraisal (PRA) in the Cascades Region of Burkina Faso. The collection of the rice landraces, their phenotypic characterisation and their agro-morphological diversity are highlighted in Chapter 3. The evaluation of diversity and the population structure of the collection with molecular markers are covered in Chapter 4. Chapter 5 consists of the screening of the collection with different RYMV isolates to search for resistant and tolerant varieties. Chapter 6 involves phenotypic and genotypic characterisation of an *O. sativa* cultivar from Burkina Faso with partial resistance combined to tolerance against RYMV. The overview of this current thesis research is presented at the end.

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Chapter 1: Literature Review

1.1 Taxonomy, genetics and diversity of rice

Rice is a monocotyledonous cereal plant, which belongs to the *Gramineae* (*Poaceae*) family of the tribe *Oryzeae* and of genus *Oryza*. The rice inflorescence is a panicle that bears single-flowered spikelets. The rice flower is normally self-pollinated and differs from that of the other cereals in having six stamens instead of three. "The extent of natural crossing varies from 0 to 3% with an average of 0.5%, depending on the variety, the season, and the environment" (Poehlman, 1987). Generally, *Oryza* species are diploid (2n = 2X = 24) however, some tetraploid (2n = 4X = 48) species do exist. The genus *Oryza* includes almost 24 species with AA, BB, CC, EE, FF, BBCC, and CCDD genome constitution (Kwon et al., 2006). The species are distributed in the tropical, subtropical, and temperate regions of the world. The two cultivated species *O. sativa* (L.) and *O. glaberrima* (Steud.) belong to the complex of the AA genome. Cheng et al. (2002), in their phylogenetic study through short interspersed elements (SINEs), suggested that the *Oryza* genus with the AA genome must have originated in Africa, rather than in Asia. In their work, they clustered *O. species* in five groups corresponding to *O. longistaminata*, *O. meridionalis*, *O. rufipogon*/*O. sativa*, *O. glameapatula* and *O. glaberrima*/*O barthii*. The complex *O. longistaminata*, *O. rufipogon*/*O. sativa* and *O. glaberrima*/*O barthii* are spread in Africa. The origin and genomic classification of *Orzya* species occurring in Africa are summarised in Table 1.1.

Table 1.1: The origin and genomic classification of *Orzya* species in Africa

Species	N° of chromosome	Genome	Origin
O. sativa (cultivated)	24	AA	Asia
O. glaberrima (cultivated)	24	AA	West Africa
O. stapfii (wild species)	24	AA	West Africa
O. barthii (wild species)	24	AA	West Africa
O. longistaminata (wild)	24	AA	Tropical Africa
O. brachyantha (wild)	48	FF	West and Central Africa
O. eichingeri (wild)	24	CC	West and Central Africa
O. punctata (wild)	48	BBCC	East and Central Africa
O. punctata (wild)	24	BB	East and Central Africa
O. schwein furthiana (wild)	48	BBCC	Tropical Africa

Source: Adapted from Bezançon (1993)

However, genetics and molecular studies substantiate the greater diversity of *O. sativa* the Asian rice over *O. glaberrima* the African rice (Semon et al., 2005; Kwon et al., 2006).

1.1.1 Oryza sativa L.

O. sativa (L.) originated in Asia and was domesticated nearly 10, 000 years ago (Kovach et al., 2007). Wild annual *O. nivara*, which is derived from *O. rufipogon* is considered to be the progenitor of *O. sativa* (Nanda and Virmani, 2000). Cultivated worldwide under a wide range of agro-climatic conditions, *O. sativa* is divided into three subspecies: *japonica, indica* and *javanica*. The *indica* group, which originated in tropical Asia, is confined to the tropics and grown under irrigated areas. The *japonica* subspecies, which originated in temperate and subtropical regions of Asia, grows in temperate climates with long summer days. Subspecies *javanica*, referred today as tropical *japonica*, is equatorial (Second, 1985; Glaszmann, 1987). The subspecies *indica* and *japonica* have been widely introduced in Africa. *Indica* species are grown in the wetland areas, while the *japonica* species are grown in upland areas.

Enzymatic markers as well as molecular markers were used to assess the diversity within *O. sativa* species. Glaszmann (1987) used isozyme markers to assess the genetic structure within 1688 cultivars of *O. sativa* held by the International Rice Research Institute (IRRI). The multivariate analysis identified six varietal groups. The major cultivars were constituted in Groups I and VI corresponding to *O. sativa* subspecies *indica* and *O. sativa* subspecies

japonica. The minor cultivars were grouped under Groups II and V. Group II corresponds to *Aus* ecotype and Group V to high quality aromatic rice such as Basmati rice. Group III and IV correspond to satellite groups. Group III includes short cycle rice, which is photoperiod insensitive and adapted to deep water conditions. Rice samples that have very long cycles, and are also cold tolerant during early stages and photoperiod sensitive are included in Group IV. More recently, 169 SSR markers were used to infer the population structure within *O. sativa* from several countries of origin and five distinct groups were identified. The five groups correspond to *indica*, *aus*, aromatic, temperate *japonica*, and tropical *japonica* rice (Garris et al., 2005). In this study, *Aus* and *indica* were clustered within the traditional *indica* subspecies, while the temperate *japonica*, tropical *japonica* and aromatic subpopulations were grouped within the *japonica* subspecies. The clear division between *indica* and *japonica* is marked by their distinct origin (Vitte et al., 2004).

1.1.2 Oryza glaberrima Steud.

Native to Sub-Saharan Africa, *O. glaberrima* is endemic to the continent and was cultivated in the West African regions, even before the European arrival. The indigenous rice was domesticated in the inland delta of the Upper Niger River (Mali), 2,000 or 3,000 years ago (Portères, 1956). *O. longistaminata* and *O. barthii* are considered to be the wild relatives of the *O. glaberrima* species (Bautista et al., 2001; Kwon et al., 2006). *O. glaberrima* is propagated in all West African countries and seems to be relegated only to the Western African regions. The Inland Niger Delta in Mali is believed to be the primary centre of diversification of *O. glaberrima,* while the regions of Sene-Gambia and Guinea are considered secondary centres of diversification. *O. glaberrima* suits the African environment and possesses early plant vigour and resistance to stresses, for instance, drought, blast, RYMV, nematodes, and insects pests (Sarla and Swamy, 2005; Futakuchi et al., 2006). The species is distinguished by two major agro-ecotypes: a floating photosensitive ecotype grown in deep water, including coastal mangrove areas, and an early upright type cultivated in upland areas or moderately inundated lowlands (Bezançon, 1993; Ghesquiere et al., 1997a). *O. glaberrima* species are typically characterised by a compact panicle with few secondary ramifications, and a short (<5 mm) and truncate ligule (Portères, 1956; Bezançon, 1993). The panicle aspects lend to *O. glaberrima,* a low production status and unattractive grain qualities, in spite of its good nutritive value (Sié et al., 1998).

Compared to the Asian rice, *O. glaberrima* displays a low range of population diversity (Second, 1982; Bezançon, 1993) and has a tendency of lodging and grain shattering. The diversity study of *O. glaberrima* has been seldom addressed. The diversity of a wide collection of *O. glaberrima* with 515 samples (Second, 1982) and 101 samples (Bezançon, 1993) was assessed with isozyme markers. The two authors revealed a weak diversity within *O. glaberrima*. Later, Semon et al. (2005) utilised 93 SSR markers to evaluate the population structure of 198 accessions of *O. glaberrima* from the germplasm collection at West Africa Rice Development Association (WARDA). This work highlighted a subdivision within *O. glaberrima* species that could be linked to the different cropping ecologies: floating, non-floating and upland. Crossing barrier exists between *O. glaberrima* and *O. sativa* species, however, admixed varieties of these two species were acknowledged (Semon et al., 2005; Barry et al., 2007). Although, *O. glaberrima* is less productive and has some weaknesses, its qualities are valuable in inter-specific crossing.

1.1.3 Hybrid rice and interspecific hybridization

Rice is a self-pollinated crop, which produces a single seed per spikelet. Hand fertilisation is the only effective way to control crossing in rice (Efisue, 2006). With an aim to foster genetic improvement, different sets of rice cultivars have been exploited, within and between species to develop new lines with required traits and high yield. Breeders tried the intra-specific breeding strategy. The intra specific *O. sativa* X *O. sativa* hybrid rice technology, started in China in 1964 and now extended in South and Southeast Asia, exploits the phenomenon of heterosis to increase the yield potential of rice varieties. A yield advantage of 15–20% over inbred commercial high-yielding varieties was reported (Xangsayasane et al., 2010). The inter-specific breeding, through the cross of the indigenous African rice with the high yielding *O. sativa*, was attempted to develop a heterotic combination. Unfortunately, offspring infertility was observed owing to incompatibility problems, which led to hybrid mortality and progeny sterility (Jones et al., 1997). To overcome the sterility barrier, biotechnology tools were used (Table 1.2). Embryo rescue was employed to overcome the infertility problem followed by a backcross process to produce progeny with robust fertility. Then, lines were fixed by anther culture techniques to produce true-breeding plants (Nwanze et al., 2001; Jones, 2004). Thus, the NERICAs (New Rice for Africa) were created with the best traits of the Asian and the African rice combined in the progenies. *O. sativa* lent its high yield capacity while *O. glaberrima* transmitted its adaptability to harsh environments.

Table 1.2: Applications of biotechnology techniques to develop hybrid rice

Technique	**Application**
Anther culture	Extraction of high-yielding inbred lines from superior hybrids, purification of male sterile, maintainer and restorer lines
Embryo rescue	Overcoming incompatibility to produce hybrids between wild and cultivated species. Deriving backcross progenies to develop alloplasmic lines for diversification of Cytoplasmic Male Sterility (CMS) sources. Transfer of genes for apomixis from wild species into elite breeding lines.
Protoplast fusion line	Expeditious transfer of CMS into elite breeding, development of hybrids between otherwise sexually incompatible species.
Somatic embryogenesis	Production of artificial seeds for mass of true breeding hybrid varieties
Molecular markers	Tagging genes for wide compatibility, fertility restoration, thermosensitive male sterility, apoximis, and identifying Quantitative Trait Locus (QTL) for heterosis to facilitate marker-based selection; choosing parents based on Restriction Fragment Length Polymorphism (RFLP) diversity to obtain highly heterotic combinations.
Genetic transformation	Transfer or cloned genes governing apoximis for producing true breeding commercial hybrids; exploitation of genetically engineered nuclear male sterility and fertility restoration systems to produce hybrid varieties.

Source: Nanda and Virmani (2000)

Although viable progenies were obtained through inter-specific crossing between *O. sativa* and *O. glaberrima,* the sterility barrier was not completely overcome. Recently, Sattari et al. (2007), using molecular markers, established that it is difficult to achieve 100% pollen fertility.

1.2 Major rice ecosystems in West Africa

In West Africa, rice is cultivated in two major agro-ecosystems: the upland and wetland rice cropping systems. The upland cropping system, also called the dry-land cropping system, depends solely on rainfall. This system is most encountered in the humid forests and Guinea savannah zones, which are well watered with sufficient rainfall. In this cropping system, which accounts for 40% of cultivated rice areas in Western Africa (Nwanze et al., 2001), the fields are not overflowed with water and the soil remains aerobic (not saturated with water) for the most part of the growing season. Upland rice yields range from less than 0.5 t ha^{-1} on subsistence farms to 2 t ha^{-1} in well managed cropping farms (Balasubramanian et al., 2007). Variable rainfall, low temperatures in high altitude areas, poor soils and drought are the chief abiotic constraints in the upland cropping system. The major biotic constraints are weeds and insect pests.

Contrary to upland cropping systems, rice fields in the wetland agro-ecosystems are covered with water during the growing season. Three types of wetland agro-ecosystems are known, depending upon the surface and water regime**:** lowland areas, mangroves with deep water or floating rice cropping system, and the irrigated cropping system (Balasubramanian et al., 2007), which account for 38%, 10% and 12%, respectively of the total rice growing areas in West Africa (Nwanze et al., 2001). Lack of water management characterises lowland and swampland cropping systems. In these two cropping systems, water supplies come from rainwater and stored groundwater. Conversely, in irrigated cropping systems, water movement (entry and drainage) are controlled through dams across rivers, water deviation from rivers, and tubes. In wetland agro-ecosystem, rice yield can reach 6 t. ha^{-1}. However, variable rainfall induces drought and floods due to poor water control. Mineral toxicity (Fe, Al, Mn), salinity, and alkalinity are common in all the wetland cropping systems.

O. glaberrima is better adapted to rainfed and irrigated wetlands as well as rainfed drylands. *O. sativa* subspecies, *indica* and *japonica*, are suitable for rainfed and irrigated wetlands, and to the rainfed upland cropping system, respectively (Balasubramanian et al., 2007). Although the upland cropping system occupies more cropping areas, the lowland potential in terms of production is much higher. The latter is suitable for intensive cropping, with the possibility of growing two crops per year. In the lowland cropping system, the principal biotic constraints include blast disease, African Rice Gall Midge (AfRGM), Bacteria Leaf Blight (BLB), and

the devastating RYMV disease (Koch, 1993, Notteghem, 1993, Jia, 2003; Séré et al., 2005a,b; Nwilene et al., 2006; Djedatin et al., 2011).

1.3 *Rice yellow mottle virus* (RYMV): Taxonomy, Epidemiology and Biology

1.3.1 Taxonomy, biophysics, and structural features

The general features, structure, and genome organisation of RYMV make this virus a member of the genus *Sobemovirus,* wherein the *Southern bean mosaic virus* (SBMV) is the type member. RYMV is a plant virus with an icosahedral shape of about 30 nm in diameter (Hibino, 1996). The RYMV genome is represented by a monopartite, linear, single stranded and positive sense ribonucleic acid (RNA), non-polyadenylated of an average 4450 nucleotides with a molecular mass of 1.4 x 10^6 Da (Qu et al., 2000; Kouassi et al., 2005). The RYMV genome comprises of four Open Reading Frames (ORFs) designated ORF1 to ORF4 (Qu et al., 2000) and three Non-Coding Regions (NCR) (Fargette et al., 2004). The ORF1 encodes a protein of 157 amino acids (17.8 kDa), which plays a key role in the virus spread and infection process (Bonneau et al., 1998). The ORF2 encodes serine protease, a viral genome-linked protein and the RNA-dependent RNA polymerase (Fargette et al., 2004). However, the ORF3 proteins remain unknown thus far. The viral capsid of 239 aa (26 kDa) encoded by ORF4 is almost similar to those belonging to other members of *Sobemovirus* group (Kouassi et al., 2005). The virion capsid contains 180 copies of coat protein (CP) sub-units gathered in a *T*= 3 quasi-equivalent symmetry. The size of the virus is 250 Å across the 2-fold axis, 266 Å across the 3-fold axis and 292 Å across the 5-fold axis**.** The particle is almost spherical, with small protrusions and depressions, which gives it its icosahedral shape (Qu et al., 2000).

RYMV particles contain neither lipids nor carbohydrates but are solely constituted of RNA and protein, which account for 20% and 80%, respectively (Bakker, 1974). The virus framework is stabilised by "divalent cations (Ca^{2+} and Mg^{2+}), pH-dependent protein–protein interactions, and salt bridges between protein and RNA" (Brugidou et al., 2002; Kouassi et al., 2005). Therefore, the virus is recognised to be highly tough and with an estimated survival period of a year in leaf tissue, stored at 4°C in the presence of $CaCl_2$. The virus particles are not damaged by chloroform, butanol, or carbon tetrachloride ether (Bakker, 1974; Brugidou et al., 2002). The virion stability is consolidated by its "3D domain swapping of the βA arms

which regulate the quasi-equivalent interactions and modulate the capsid stability" (Qu et al., 2000). RYMV appears to be more stable than the other sobemoviruses that lack these structures (Opalka et al., 2000; Qu et al., 2000).

1.3.2 Epidemiology and biology

Of the 30 viruses that affect rice in the world, two occur in Africa: The *Rice stripe necrosis virus* (RSNV) and RYMV, which is the most damaging rice virus on the African continent (Abo and Sy, 1998). RYMV originated in East Africa, in Kenya (Bakker, 1974), and then evolved and spread towards the Western regions of the continent (Fargette et al., 2004**).** After its discovery in 1966, RYMV has been observed in rice fields in Tanzania, in the western parts of Africa, and in Madagascar from 1976 to 1989 (Hibino, 1996). The first entry of the virus in West Africa was in Sierra Leone in 1975 (WARDA, 2000). Currently, almost all the Western African countries are threatened by this plague (Kouassi et al., 2005).

RYMV is a rice specific pathogen with various hosts and vectors that facilitate its propagation (Bakker, 1974). The host range is limited to members of the *Gramineae*, represented particularly by *Oryzae* sp. and *Eragrostidae* sp. families (Abo et al., 2003). The wild rice species include, *O. longistaminata* (A. Chev. et Roerh.,), *O. barthii* (A. Chev.,) and the grass species *Echinochloa colona* (L. Link), *Panicum repens* (L.), and *Ischaemum rugosum* (Salisb) (Abo et al., 2003). The rice re-growth constitutes the most known reservoir of RYMV (Konate et al., 1997; Traoré et al., 2009). The insect vectors, chrysomelid beetle and grasshoppers were first reported to be responsible for RYMV transmission (Bakker, 1974). Sarra and Peters (2003) established that the grass rats (*Arvicanthis niloticus*), domestic cows (*Bos* spp.) and donkeys (*Asinus* spp.) are other biotic means in the spread of the virus. The abiotic routes of transmission can be soil, agricultural tools, water irrigation, and wind (through plant-to-plant contact), which plays a tremendous role in the virus propagation (Sarra et al., 2004; Traoré et al., 2009).

Once the rice plant is infected by RYMV, several symptoms occur depending on the age of the infection and the rice genotype involved. The major symptoms include are "yellowing and mottling of leaves, necrosis and stunting of rice plant, reduced tillering, poor panicle exertion leading to incomplete emergence, and sterility of the flowers with possible death of the infected plant" (Hibino, 1996; N'Guessan et al., 2000; Séré et al., 2005b). The virus concentration is high in infected tissues where virus particles are present in the cytoplasm and

in vacuoles (Hibino, 1996; Opalka et al., 1998; Brugidou et al., 2002). Although RYMV was detected in all seed parts including glumella, endosperm and embryo at a rate ranging from 65% to 100%, no seed-borne infection in rice seeds and seeds of wild hosts was found (Konate et al., 2001; Abo et al., 2004; Traore et al., 2006a).

1.3.3 RYMV within the plant

In infected rice plants, several anatomical structures are colonised by the RYMV particles. Opalka et al. (1998), by using Western immunoblotting, Northern blotting, and electron microscopy of thin sections, have sequentially detected the viral RNA in inoculated leaves at three days post-inoculation (dpi). They observed viral RNA in the viral coat protein (CP) at 5 dpi and by 6 dpi both RNA and CP were concentrated and spread to systemically infected leaves. Beyond 6 dpi, high quantities of virion particles were ascertained in xylem parenchyma cells and vessels. Within xylem parenchyma cells, virus particles were concentrated in vacuoles and in the cytoplasm. In xylem vessels, viruses were seen inside pit membranes. Furthermore, some RYMV particles were observed in phloem sieve elements as well as in the vacuoles and the cytoplasm of phloem parenchyma cells. The virus was detected in most cell types including epidermal tissues, mesophyll cells, plasmodesmata, and bundle sheaths. Regarding the important accumulation of RYMV particles in vacuoles, Brugidou et al. (2002) proposed that they may constitute the virus storage compartment.

RYMV can cover long distances from xylem to phloem and reach the vascular parenchyma cells of infected leaves via plasmodesmata, indicating cell-to-cell movement of the virus from the infected to healthy tissues (Opalka et al., 1998). The coat protein of the virus could have a key function in the RYMV dispersion within the plant (Brugidou et al., 1995). Bonneau et al. (1998) further ascertained that the P1 protein encoded by the ORF1 is responsible for the virus replication and scattering. Also, Ca^{2+} displacement from pit membranes of infected cells is suspected to contribute to the disruption of the pit membranes and facilitate systemic virus movement (Opalka et al., 1998).

1.3.4 Diversity of RYMV

RYMV is a very infectious and a variable pathogen. The immunological and serological characterisation of the virus, the analyses of the sequences of the coat protein, and the molecular typing of ORF1 and ORF4, established five serotypes (Konate et al., 1997; N'guessan et al., 2000; Pinel et al., 2000; Fargette et al., 2002; Abubakar et al., 2003). Later,

the sequencing of the full genome of RYMV revealed six major strains or clades (Fargette et al., 2004; Traore et al., 2005). The strains S6, S5 and S4 are widespread in East Africa, which is believed to be the primary centre of diversification of RYMV. S3, S2 and S1 occur in West and Central African zones in the savannah and forest areas. S3 and S2 are typically West African lineages, whereas S1 is split into S1-c and S1-w, shared between Central and West Africa. West Africa appears to be the secondary centre of diversification of RYMV. The sequencing of the genome-linked viral protein (VPg) revealed that the two RYMV lineages are characterised by two different molecular profiles, conferring them different virulence status (Pinel et al., 2000). The isolates from East Africa are called E-pathotypes and break the highly resistant allele (*Rymv*1-2) found in the *O. sativa* cultivars (Pinel-Galzi et al., 2007). The isolates from West Africa are mostly T-pathotypes, which break the resistance in *O. glaberrima* cultivars (Traoré et al., 2010). The diversity of RYMV draws attention to the host specific particularity of RYMV lineages.

1.3.5 Controlling methods

1.3.5.1 Prophylactic methods and cultural practices

The prophylactic methods consist of the management of insect vectors and weed species, which act as virus hosts (Abo et al., 1998). In the field, the infected plants must be gathered and burnt to eliminate any traces of the virus. The seedbeds must be overflowed to prevent rats and other crawling vectors from entering the field. Further, the elimination of host range plants such as *O. longistaminata* is advised (Sarra, 2005). However, these controlling methods may be difficult to practise by small-scale farmers in poor countries, as they require significant investment in machinery and labour. Also, some knowledge transfer and training are imperative for a more effective application of these methods. As this is a difficult plan to execute, considering the farming scale of a region or a country, many efforts are now devoted to breed for resistant cultivars against RYMV.

1.3.5.2 Resistant cultivars

The *O. sativa* subspecies seem to be more vulnerable to rice disease in the African ecosystems. Even if they are resistant, this resistance is unstable. Furthermore, emerging diseases like RYMV disease, threaten its production (Ghesquière et al., 1997b; Fargette et al. 2002). In reality, very few rice cultivars have been shown to be resistant to RYMV (Abo et al., 2005). So far, two types of resistance have been detected in the host plant: the monogenic

high resistance and the partial resistance. A major gene of resistance against RYMV, *Rymv1*, has been identified in the *O. s. indica* resistant varieties - Gigante and Bekarosaka (Ndjiondjop et al., 1999; Rakotomalala et al., 2008). This gene, which encodes a translation initiation factor (eIF(iso)4G), is also responsible for the resistance of the resistant *O. glaberrima* accessions Tog5681, Tog5672 and Tog5674. The alleles of Tog5681, Tog5672 and Tog5674 (*Rymv1-3*, *Rymv1-4* and *Rymv1-5*, respectively) are distinct from each other and from that of Gigante and Bekarosaka (*Rymv1-2*), which was fine-mapped onto chromosome 4 (Albar et al., 2006). Recently, a second major gene *RYMV2* was identified when screening a collection of *O. glaberrima* accessions (Thiémélé et al., 2010).

Azucena, a traditional upland *O. s. japonica* from the Philippines was found to have a partial resistance to RYMV (Ghesquière et al., 1997b; Albar et al., 1998; Pressoir et al., 1998). Responses to RYMV infection in cv. Azucena include partial resistance and tolerance. Partial resistance is expressed only at the early stages of infection and is characterised by delayed and lower virus accumulation in leaves and delayed virus invasion in bundle sheath tissues (Ioannidou et al., 2003). The tolerance in Azucena is marked at the later stages of infection and characterised by low symptom expression, despite high virus accumulation (Ioannidou et al., 2000). Preliminary studies identified only a single Quantitative Trait Locus (QTL) on chromosome 12 that plays a key role in the resistance of Azucena (Ghesquière et al., 1997b). Later, three QTLs on chromosomes 1, 2 and 12 were also suspected to be involved in the partial resistance mechanism of Azucena. QTL1 appears to be involved in resistance to virus accumulation and the expression of symptoms, while QTL2 and QTL12 might be involved in mechanisms contributing to the decrease in virus accumulation and symptom expressions (Albar et al., 1998). The complementary epistasis between QTL12 and QTL7 was evidenced as a genetic factor controlling the virus content and conferring resistance to Azucena (Pressoir et al., 1998; Ahmadi et al., 2001). The QTL12, close to the *indica / japonica* zone of differentiation, is bracketed in an interval of 2.23 Mb containing the marker RM101 (Boisnard et al., 2007). This interval is relatively large and makes it difficult for the tagging and the fine mapping of QTL12 involved in the partial resistance of Azucena. This difficulty hampers the identification and the cloning of its gene(s). The two major QTLs of Azucena were introgressed with backcross and marker assisted introgression techniques in high yielding cultivars. The screening of the introgressed lines demonstrated that the partial resistance was short-lived and consisted of a one-week delay in virus accumulation and symptom expression; over this delay, partial resistance broke down (Ioannidou et al., 2003).

Nevertheless, recent publications (Fargette et al., 2002; Sorho et al., 2005; Traoré et al., 2006b; Traoré et al., 2010) challenged both the highly resistant and the partially resistant cultivars. They demonstrated that the resistance of Gigante and Tog5681 can be overcome and that of Azucena broken. Moreover, the transgenic resistance through encoding RNA-dependent RNA polymerase of RYMV, which was found to have some high resistance (Pinto et al., 1999), was no longer enduring. Furthermore, the transgenic lines obtained from the introgression of QTL on chromosomes 12 and 4 were less effective and transient (Sorho et al., 2005). Therefore, much remains to be done to lessen the incidence of RYMV in farmer fields.

1.4 Molecular markers in plant breeding

In the post-genomic era, DNA markers are useful tools, which assist researches in plant breeding. They may enhance plant genetic improvement through genetic diversity estimation, screening, tagging, and mapping genes of interest. Therefore, they are key tools in ascertaining plant genetic diversity, establishing genetic mapping, and leading marker assisted selection programmes. To date, a wide range of molecular markers have been used in improving plant breeding. The most employed molecular markers are Restriction Fragment Length Polymorphism (RFLP), Microsatellites or Simple Sequence Repeats (SSR), Expressed Sequence Tags (EST), Cleaved Amplified Polymorphic Sequence (CAPS), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLP), Inter Simple Sequence Repeats (ISSR), Diversity Arrays Technology (DArT), and Single Nucleotide Polymorphism (SNP) (Semagn et al., 2006a). Therefore, DNA markers are necessary technologies, which help breeders to select parents, to cross and hybridize in their breeding programmes.

1.4.1 Genetic diversity and molecular characterisation

Genetic diversity refers to the variation of genes within species, that is, the heritable variation within and between organisms. Assessment of genetic diversity is an important step in plant breeding if there is to be improvement by selection. The method of using molecular markers to select parents has the potential to allow simultaneous maintenance of genetic diversity and performance (Lamkey and Lee, 1993). A wide range of molecular markers has been developed to achieve that purpose. The most employed are RAPD, RFLP, AFLP, ISSR, SSR, EST and SNP. They provide reliable estimates of genetic diversity compared to isozymes and morphological markers. They can be used to determine the degree of relation amongst inbred lines and cultivars (through measuring genetic distance), to assess changes in genetic diversity

over time, and to evaluate new germplasm for its potential to increase genetic diversity (Lamkey and Lee, 1993). Furthermore, molecular markers are used for molecular characterisation and genotyping of germplasm, varieties, and plant material.

In sub-Saharan Africa, four marker types were used to characterise rice and wild rice collections. SSR markers were employed to characterise and ascertain the population structure within the collection of *O. glaberrima* accessions (Semon et al., 2005) and to study the diversity of the rice collection from Guinea and Madagascar (Barry et al., 2007; Radanielina et al., 2010). AFLP analysis was used to assess genetic diversity in West African local and formal varieties (Nuijten et al., 2009). The genetic diversity of wild rice populations from Ethiopia was assessed with Inter Simple Sequence Repeat (ISSR) (Girma et al., 2010). RAPD techniques were used to characterise rice landraces from Côte d'Ivoire (Ogunbayo et al., 2007). In fact, Semagn et al. (2007) employed SSR markers to examine the relative contribution of each parent and the extent of genetic differences amongst inter-specific rice populations (NERICA varieties). SSR markers appear to be the most used in rice characterisation as they are widespread in the rice genome. Several hundreds of SSRs were identified and developed (Temnykh et al., 2000; Temnykh et al., 2001; McCouch et al., 2002; Orjuela et al., 2009). Their utilisation is facilitated by their availability, their low cost and their ease of use. Ni et al. (2002) employed 111 SSR to assess the molecular diversity within a diverse collection of *O. sativa* and recommended that a relatively small number of SSRs could be used for the estimation of genetic diversity and the identification of rice cultivars. Moreover, SSR markers detect a significantly high degree of polymorphism in rice and are particularly suitable for assessing genetic diversity amongst closely related cultivars (Jayamani et al., 2007).

1.4.2 Genetic mapping and marker assisted selection

Genetic mapping and Marker Assisted Selection (MAS) of plants have evolved and become more accurate with the breakthrough of molecular markers. DNA markers, through their recombination frequencies, enable one to determine the relative position and genetic distances of genes along a DNA fragment in a genetic map (Semagn et al., 2006b). Thus, genetic maps can be constructed to give an idea of the position of the genes in a chromosome or in a whole genome. Accordingly, DNA markers in genomic regions of interest enable breeders to select on the basis of genotype rather than phenotype, which can be especially helpful if a target trait is time-consuming to score (Young, 1996). They aid in tagging desired genes, enable their

introgression and their screening in MAS and backcross programmes. Therefore, MAS may be profitable in plant breeding programmes by its capacity to be a diagnostic tool for the transfer of desirable genes (foreground selection), and also enabling the identification of individuals with low amounts of the undesirable genome from the donor parent (background selection) in backcross programmes (Babu et al., 2004; Semagn et al., 2006b). Furthermore, DNA markers may assist the incorporation of several genes of interest together into one genome (gene pyramiding).

However, MAS approach seems appropriate in the case of qualitative traits inherited monogenically or oligogenically but the selection process is difficult to implement in case of polygenic characters (quantitative trait) (Semagn et al., 2006b). The manipulation of qualitative genes is feasible, precisely because the number of loci involved and their gene action are well known. They can be easily tagged and followed owing to their mode of expression and their qualitative response. The successful application of MAS requires co-segregation or very tight linkages between markers and genes (1 centimorgan or less), a pair of bracketing markers (flanking markers) very close to the desired gene, and the possibility to screen a large number of individuals in a time and cost effective manner (Lamkey and Lee, 1993; Mohan et al., 1997; Babu et al., 2004; Francia et al., 2005). However, MAS is not easy to achieve with quantitative traits that act additively. Improving polygenic traits through MAS is a complex endeavour. The difficulty in manipulating quantitative traits is relative to their genetic complexity, mainly the number of genes involved in their expression and interaction among genes (epistasis). Because several genes are involved in the expression of a quantitative trait, these genes, in general have smaller individual effects on the phenotype, and the effect of individual genes is not easily identifiable (Babu et al., 2004). Although powerful computer packages have been developed to narrow the confidence interval of detection of small contributed quantitative traits, there is no way of knowing whether the whole quantitative loci involved in the expression of one character has really been taken into account.

Despite the proliferation of QTL mapping experiments in recent years, Babu et al. (2004) identified six salient constraints, which imposed several limitations on efficient utilisation of QTL mapping information in plant breeding through MAS. They are:

- identification of a limited number of major ‘player’ QTLs controlling specific traits;
- the notion that QTL identification is required whenever additional germplasm is used;

- inadequacies or experimental deficiencies in QTL analysis leading to either overestimation or underestimation of the magnitude of effects of QTLs;
- lack of universally valid QTL-marker associations applicable over different sets of breeding materials;
- strong QTL environment interaction; and
- difficulty in precisely evaluating epistatic effect.

In addition, Hospital (2005) also claimed that marker assisted introgression is not always successful. The success of the introgression may depend upon the ability of the target genes to exhibit the expected effects once introgressed in a new genetic background.

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CHAPTER 2: RICE TRAITS PREFERRED BY FARMERS AND THEIR PERCEPTIONS OF RICE YELLOW MOTTLE VIRUS (RYMV) DISEASE IN CASCADES REGION OF BURKINA FASO

Abstract

Participatory Research Appraisal (PRA) was conducted in the Cascades Region of Burkina Faso to determine farmers' selection criteria for rice, their rice cropping management and their perception on *Rice yellow mottle virus* (RYMV) disease. A survey was conducted in ten villages. Group discussions and individual interviews were employed. A total of 212 farmers including 176 women and 36 men were interviewed on their rice cropping management, the rice traits they preferred, and their perception on rice diseases with a focus on RYMV. Taste, yield, grain, and cooking qualities were amongst the most valued traits. The taste was the paramount trait valued by farmers in their selection criteria. Moreover, taste is the reason why farmers still favour their local rice cultivars, although they have some weaknesses like low yield and drought sensitivity. The PRA also revealed that RYMV was the most damaging disease across the villages. Farmers recognised the disease through its symptoms (leaf yellowing, plant stunting, sterility, necrosis and plant death). However, farmers were ignorant of the cause of RYMV disease. Resistant or tolerant varieties against RYMV were identified by farmers, and all belonged to the *O. glaberrima* species. This information is important for future rice breeding programmes in Burkina Faso, undertaken to meet the requirements of small scale farmers, and to avoid rejection of novel varieties.

2.1 Introduction

Burkina Faso is a landlocked country bordered by Ghana and Cote d'Ivoire in the South and by Mali in the North. In the South East, it is surrounded by Benin and Togo, and by Niger in the East and North. Rice is the staple food for many countrymen and women. In Burkina Faso, in term of production it occupies the fourth position in cereal production after sorghum, millet and maize (INSD, 2010). Rice production in 2008/2009 cropping season was estimated at 195,102 tonnes (INSD, 2010). This production was insufficient to meet the demand for rice in the country. During the year 2008/2009, 203,403 tonnes was imported (INSD, 2010) at a cost of millions of dollars. The government of Burkina Faso is trying to increase local rice production and consequently reduce the imports. Providing fertilizer and seed of improved

varieties at low cost to farmers are amongst the measures taken to boost rice production in Burkina Faso. These measures contributed to an increase in rice production to 195,102 t (INSD, 2010), a level of production never reached before in the country, which translates to more than 100% production increase compared to the previous year (INSD, 2010). However, the seeds of improved varieties given to farmers could be rejected, if they do not meet farmers' requirements for cropping and consumption. To avoid such situations, breeders should be aware of farmers' selection criteria by using Participatory Plant Breeding (PPB) programmes. Participation of farmers in PPB can help identify rice cultivars that are acceptable in a given environment (Ceccarelli, 2009). Participatory research is defined as a "process of sequential reflection and action, carried out with and by local people rather than on them" (Cornwall and Jewkes, 1995). The collaboration between farmers, biologists, and plant breeders contributes to bridge differences between these key actors in the concept of selection (Cleveland and Soleri, 2007). Thus farmers should be effectively involved in the creation of genetic diversity, the selection of desired traits, and the testing of progenies. Their involvement should not be limited to the third stage of the process, called Participatory Varietal Selection (PVS), as is often observed (Ceccarelli, 2009). However, involvement of farmers in all the breeding processes is expensive to the breeding programme and time consumable to the farmers. In the PVS processes, farmers assess progenies in the last stages of the breeding process. The advantage of PVS is the "rapid spread and adoption of acceptable varieties" (Dorward et al., 2007). Conversely, the PRA is implemented in the first stages of the research process (Webber, 1995) and farmers are actively involved in the initial stages of the breeding programme and express their selection criteria. In rice breeding in Burkina Faso, the use of PRA approach has not been used previously to address farmer preferences. In the present study, ten villages of the Cascades Region were targeted for a survey on local knowledge and breeding priorities. The objectives were to:

1. ascertain farmers' preferences for rice traits;
2. survey farmers' rice cropping management;
3. capture farmers' perceptions of *Rice Yellow Mottle Virus* (RYMV) disease on rice.

The outcome of this research was meant to provide valuable information to be taken into account in future Burkina Faso rice breeding programmes in order to deliver to farmers, novel but acceptable rice varieties.

2.2 Materials and Methods

2.2.1 Description of the study area

The Cascades Region is located in the South-West of Burkina Faso and is bordered by two neighbouring countries: Côte d'Ivoire and Mali, and by four provinces: Poni, Bougouriba, Houet and Kénédougou (Figure 2.1).

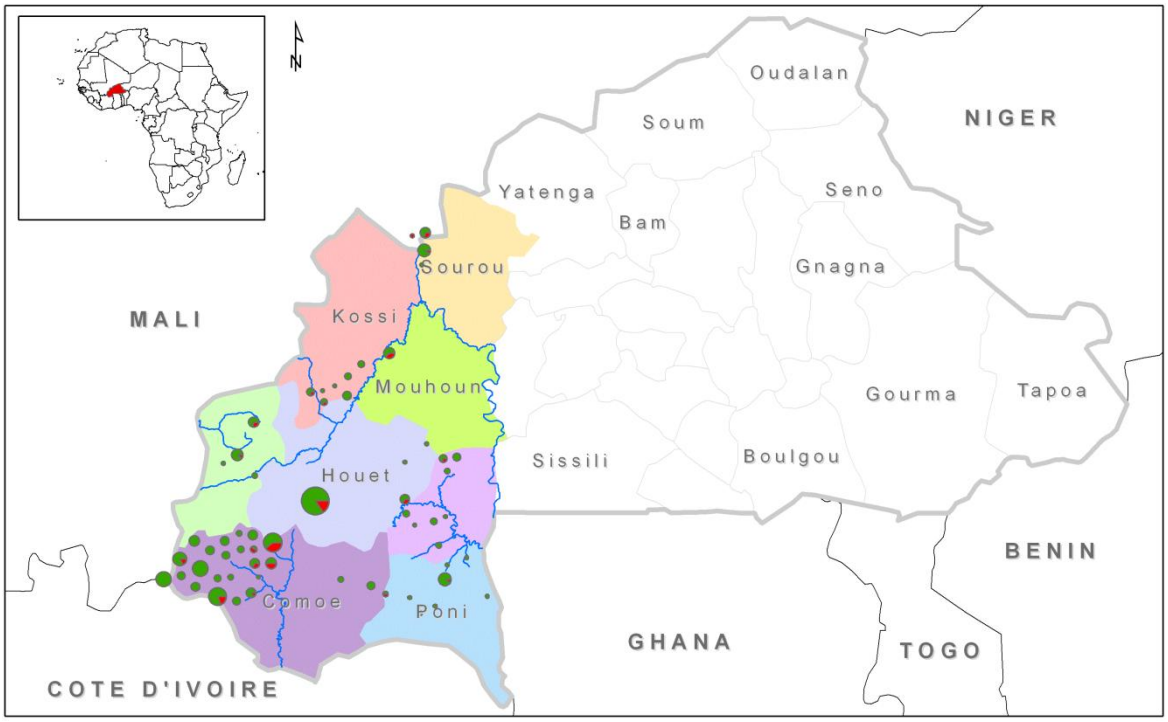

Figure 2.1: Area of collection of rice landraces in Burkina Faso (Cascade Region is highlighted in purple colour

The region includes two provinces: Comoé and Léraba, which are the names of the two principal rivers of the region. The Comoé Province accounts for 85% of the area of the region with an area of 15,826 km^2 (Lankoandé and Sébégo, 2005), while Léraba Province with an area of 2,810 km^2 accounts for 15% of the area of the Cascades Region (Lankoandé and Paré, 2005). The region belongs to the Sudanean transition zone, with an annual rainfall between 1000 mm and 1200 mm. The minimum temperature is nearly 17°C in the cold months (November – December) and the maximum temperature around 36°C in March and April. Rice cropping in the region is facilitated by the presence of rivers Comoé, Léraba and Bougouriba, and the existence of large plains ideal for rainfed lowland and deepwater rice cropping. This region is the granary of local rice in Burkina Faso (Sié, 1991) and is the third largest rice producing region of Burkina Faso averaging 35,635 tonnes (INSD, 2010). Rice is grown mainly by female farmers (often supported by male farmers) in deep water, rainfed

lowland and irrigated schemes with controlled water management. The local rice varieties, including the African rice *O. glaberrima*, and the Asian rice, *O. sativa*, which was introduced in the African continent centuries ago, are grown in traditional basins by low income farmers. To promote and enhance rice production in the region, two sites with complete irrigation management were built at Karfiguela (Comoé Province) and at Nionfila (Léraba Province). The installation of irrigation schemes has boosted the introduction of improved varieties in the region. The modern varieties introduced in the region via the channel of research and agricultural extension were exclusively *O. sativa* species. RYMV disease was first reported in Cascades Region in the irrigated scheme of Karfiguela, in the 1990s (WARDA, 2000).

2.2.2 Sampling procedure and interview techniques

Farmers in the two provinces (Comoé and Léraba) of the region were involved in the Participative Research Appraisal (PRA). The villages were selected on the basis of secondary available information on their long history of rice cropping and on the basis of the presence of several local varieties in the same village. Thus, there were ten villages chosen: six in the Leraba Province (Noussoun, Baguera, Loumana, Kangoura, Kawara, Douna) and four in the Comoé Province (Diarabakoko, Siniena, Tengrela and Kiribina) were selected (Figure 2.2).

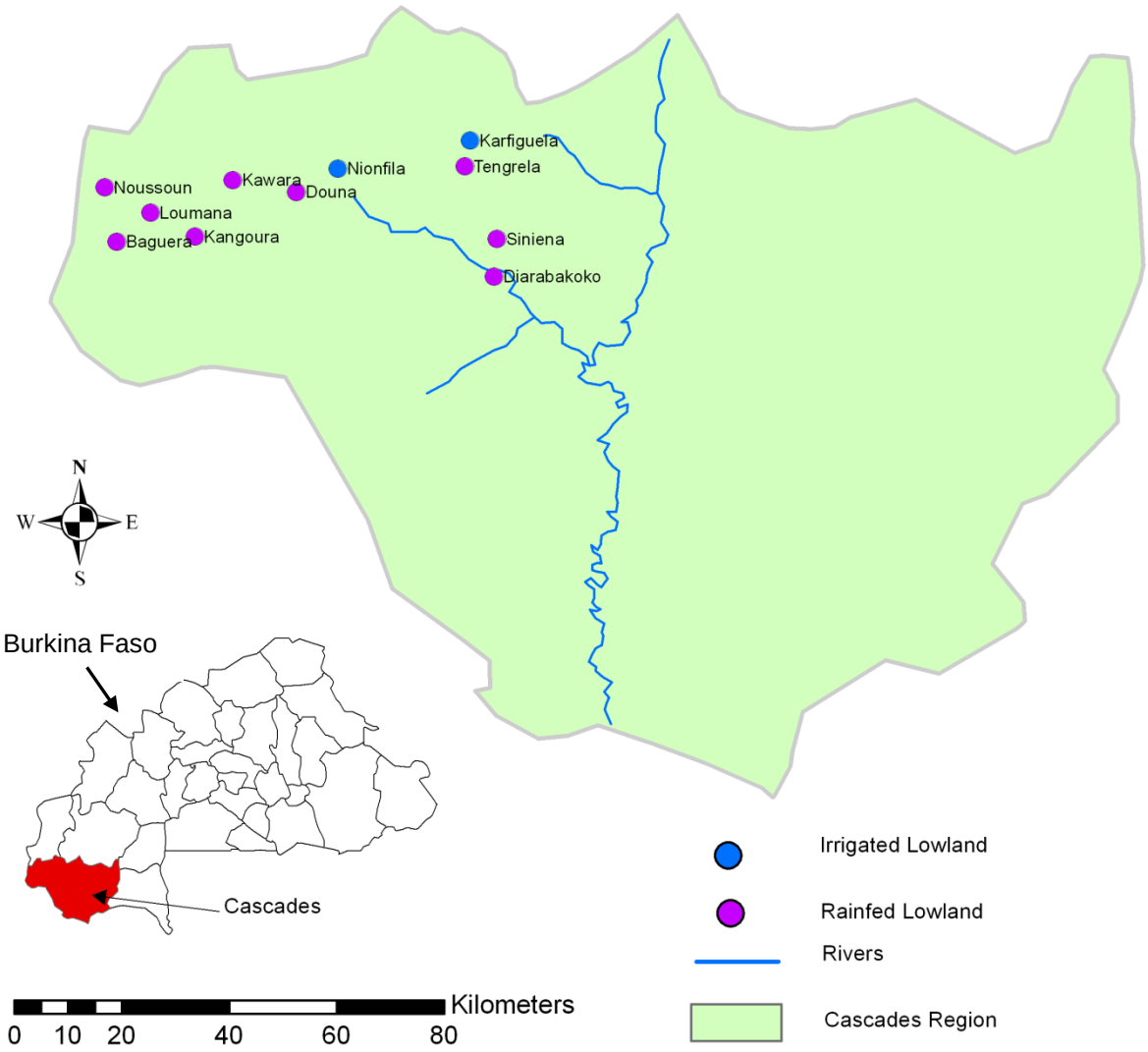

Figure 2.2: Map of the Cascades Region, portraying the selected villages and the two irrigation schemes, Karfiguela and Nionfila

The PRA activities were conducted in joint collaboration with the agricultural extension units of the Cascades Region and the Burkina Faso Agricultural Research Iinstitute, “Institut de l’Environnement et de Recherches Agricoles” (INERA). Agricultural extension officers of each of the relevant departments were contacted and were part of the team conducting the survey process. The PRA team was constituted by a social officer, an extension officer covering the target village, two technicians from INERA and the principal investigator, Honoré KAM. In each village, the team scheduled meetings with farmers. Group discussions and interviews were organised with farmers to obtain general information on rice cropping procedures, and diseases threatening their rice production. Visits to farmer fields, group discussions and interviews were conducted in Malinké, a local language most spoken throughout the Cascades Region. Iteration, open questions, structured questions and the ‘six

helpers' questions (why, who, what, when, where and how) were employed during group discussions. In iteration, the same question was asked more than once in different ways in order to confirm the answer. This technique is recommended in the context where most of the persons in the community are illiterate (Efisue et al., 2008). The open questions were used to allow the farmers to express themselves fully on a given topic. Pictures were portrayed in group so that the community at the village level could identify diseases occurring in their rice field and discuss them fully. The group discussions were recorded on a dictaphone and then transcribed to provide support information on the individual interviews.

After the group discussion, roughly 20 farmers per village were selected randomly for individual interviews. The individual dialogue enabled selected farmers to express their own points of view without any influence from the community. The individual interviews first focused on personal information, identity, origin, and the instruction level of the interviewee. Later, open and semi-structured questions were addressed to farmers on their rice cropping management, their preferred and rejected traits in rice varieties, and their perceptions on pests and diseases threatening their rice production. They were also questioned on their perception of RYMV, through questions and pictures. In villages where the number of individuals was below 20, all the individuals were interviewed. In some villages more than 20 individuals were selected randomly because 200 farmers were targeted. Overall, 212 farmers were interviewed, including 36 males and 176 females (Table 2.1).

Table 2.1: Number of farmers in each village interviewed in the Cascades Region of Burkina Faso, by gender

Village	Female	Male	Total
Baguera	28	0	28
Diarabakoko	30	2	32
Douna	6	5	11
Kangoura	25	0	25
Kawara	11	5	16
Kiribina	17	0	17
Loumana	21	0	21
Noussoun	7	11	18
Siniena	14	10	24
Tingrela	17	0	17
Total	**176**	**36**	**212**

2.3 Data analysis

Data was analysed using the software package SPSS version 17. Frequencies and percentage of responses were computed. Graphs were drawn to illustrate the outputs. Preferred and unfavoured traits, as well as the importance and severity of rice diseases were ranked to highlight farmers' perceptions.

2.4 Results

2.4.1 Rice cropping management practices

Rice farming is gender related in the Cascades Region. Most of the rice farmers encountered were women and accounted for 83% of the sample group. This emphasised the importance of the involvement of women in rice cropping activities in the region. In the dominant ethnic group of the region, among the Gouin people, a woman inherits her mother's rice field and the knowledge of rice cultivation is transmitted from mother to daughter. However, as rice has become an important cash crop, more men are becoming involved in rice cropping, especially in the irrigation schemes. Most of the interviewees were small scale farmers. The rice fields of 72% of them did not exceed 1 ha per household (Table 2.2). For 52%, the rice field represented more than half of their total cropping area. The average age of the interviewed farmers across the ten villages was 43 years and the average size of their household was nine persons. The majority of the interviewees had been cropping rice for 19 years with a minimum of one year and a maximum of 50 years. Two third of the farmers interviewed were illiterate. Ninety six percent of farmers grew rice in the rainy season only, while the remaining 4% grew rice in both the rainy and dry seasons. The majority of farmers cultivated their rice in rainfed lowland areas. The few proportion of farmers growing rice in dry season had access to irrigation schemes with controlled water management. Twelve percent of farmers cropped one variety, 50% cropped two varieties at the same time and the remaining 38% cropped more than two varieties. Local varieties were grown by 41% of the farmers, while 51% grew both local and modern varieties. Seventy four percent of the farmers kept enough seeds to ensure the establishment of their next season's rice cropping. Those lacking seeds bought their seeds from the local market and rarely from a neighbour. In the communities, seeds were obtained as gifts, loans and exchanges.

Different ways of crop establishment were noticed: direct sowing by hand (56%) and transplantation from a nursery (44%). The direct sowing of seeds is judged not to be time

consuming but easy, whereas transplantation is time consuming but has the advantage of reducing weeds competitiveness. The young plants are transplanted into ploughed and flooded fields that have been cleared of weeds. Consequently, the transplanted plants have the time to grow, become vigorous and compete with weeds. The thinning to reduce plant numbers in a hill after sprouting was done by 63% of farmers, while 37% did not thin. Fertilizers (NPK, urea, or manure) were applied by 57% of farmers, while 45% used herbicide in their rice fields. Various herbicides are used by farmers because they find it cheaper than hiring labour for the weeding. The harvest and threshing are done by hands and the harvested grain is stored in different ways: in granary (55%), in bags (16%), in jars (7%). Non-threshed rice panicle is stored in granaries (20%) (frequently in the villages of Baguera, Kangoura and Loumana).

Table 2.2: Rice landholding size per household in ten villages in the Cascades Region, Burkina Faso (ha)

	≤ 0.5 ha	**0.5-1 ha**	**1-1.5 ha**	**1.5-2 ha**	**>2 ha**	**Total**
Baguera	13	10	3	1	1	**28**
Diarabakoko	13	12	4	1	2	**32**
Douna	3	2	2	1	3	**11**
Kangoura	11	8	2	2	2	**25**
Kawara	10	4	0	2	0	**16**
Kiribina	3	13	2	2	0	**20**
Loumana	9	6	3	1	2	**21**
Noussoun	1	8	4	2	3	**18**
Sinièna	9	5	6	2	2	**24**
Tingrela	8	5	3	0	1	**17**
Total	**80**	**73**	**29**	**14**	**16**	**212**

The entire production of rice is self-consumed by 42% of farmers, while 54% sold a part of their production in the local market. A small proportion of farmers (2%), mainly in the irrigation schemes, sold their harvest to the local cooperative. All the rice production of modern varieties from Douna village, both in irrigated schemes and in rainfed lowland fields, was sold to the local cooperative. The modern rice varieties were produced under contract to the local cooperative. The local cooperative provided the seed and the guarantee to purchase the harvest. However, the production of local varieties from Douna village was sold on the Douna local market. The absence of modern varieties and satisfaction with local varieties were the justification of one third of the interviewees who never tested modern varieties. In reality, seeds of modern varieties were mostly introduced to villages through the channel of

agricultural extension. The government, through its Ministry of Agriculture and Non-Governmental Organisations, used the agricultural extension channel to reach farmer associations.

2.4.2 Preferred rice characteristics

Choice of a rice variety in rural areas is determined by some characteristics of the plant and the environment. Thirty nine percent of interviewees preferred to grow local rice varieties because they have mastered its cultivation, and were confident of its adaptation to their environment, and its good taste. Conversely, 47% preferred modern varieties because of their high yield potential, and 14% cropped both because they were happy with both varieties. Taste was the primary quality that farmers liked in their local varieties, followed by the yield. The other important traits making farmers value their traditional rice cultivars were the grain expansion after cooking, the resistance of rice plants to submersion, and the reduced requirements for management. More than 42% of the respondents did not find any bad traits in their local rice varieties. However, low yield and drought sensitivity were mentioned as weaknesses of landraces. Disease sensitivity and the difficulty in de-husking of the grains were also noted by some farmers as the disadvantages of some local varieties (Table 2.3).

Table 2.3: Good and Bad Traits of Landraces in the Cascades Region of Burkina Faso

Good traits in landrace varieties	% of respondents	Bad traits in landrace varieties	% of respondents
Good taste	53.4	No bad traits	42.1
High Yield	52.8	Low yield	12.6
Grain swell when cooked	22.3	Drought sensitive	10.1
Plants tolerate submersion	20.7	Disease sensitive	7.0
Minimal management	15.5	Difficult de-husking	5.7

The ranking of desired traits in rice varieties (Table 2.4) showed that taste of rice was the most important trait. Cooking characteristics (easy cooking and grain expansion after cooking) and disease resistance held significant places in farmers' selection criteria. Likewise, high tillering ability, high yield and post-harvest attributes (easy threshing and easy de-husking) were desirable characteristics taken into account by farmers. The above preferred traits were mentioned by at least 85% of the respondents. Resistance to lodging and to shattering were noted by 78% and 66% of the interviewees, respectively (Table 2.4). Certain

farmers who did not choose resistance to lodging argued that heavy panicles are an indicator of good yield. According to them, lodging is due to heavy panicles. Similarly, some farmers thought that resistance to shattering is positively correlated to difficulty in threshing of the panicles. Therefore, they were satisfied with medium or high shattering varieties that were easily threshed.

Table 2.4: Ranking of preferred rice traits across ten villages in the Cascades Region, Burkina Faso

	High tillering	Resist lodging	Resist disease	Low shattering	Easy threshing	Easy husking	High yield	Good taste	Easy cooking	Grain expansion
Baguera	75	57	75	46	82	86	71	93	89	82
Diaraba	100	94	100	78	84	94	100	97	100	100
Douna	91	91	100	82	91	91	100	100	91	100
Kangoura	100	80	100	64	76	72	96	100	100	100
Kawara	94	56	88	63	88	81	94	94	88	94
Kiribina	100	70	100	90	95	95	95	100	100	100
Loumana	90	81	90	62	90	81	90	100	81	90
Noussoun	83	89	83	39	94	89	72	94	89	83
Sinièna	92	83	96	67	88	79	83	96	96	96
Tingrela	88	82	100	71	65	88	94	94	94	82
Mean %	**92**	**78**	**93**	**66**	**85**	**85**	**89**	**97**	**93**	**93**
Ranking	**3**	**6**	**2**	**7**	**5**	**5**	**4**	**1**	**2**	**2**

Nonetheless, some parameters divided the point of view of the respondents. Medium sized and tall rice plants were preferred by 37% and 39% of the respondents, respectively. Early and medium maturing rice were preferred by 51% and 32% of the respondents, respectively. Long grain and medium grain size were appreciated by 60% and 25% of the respondents, respectively. Non-aromatic rice was preferred by 15% of the farmers (Figure 2.3).

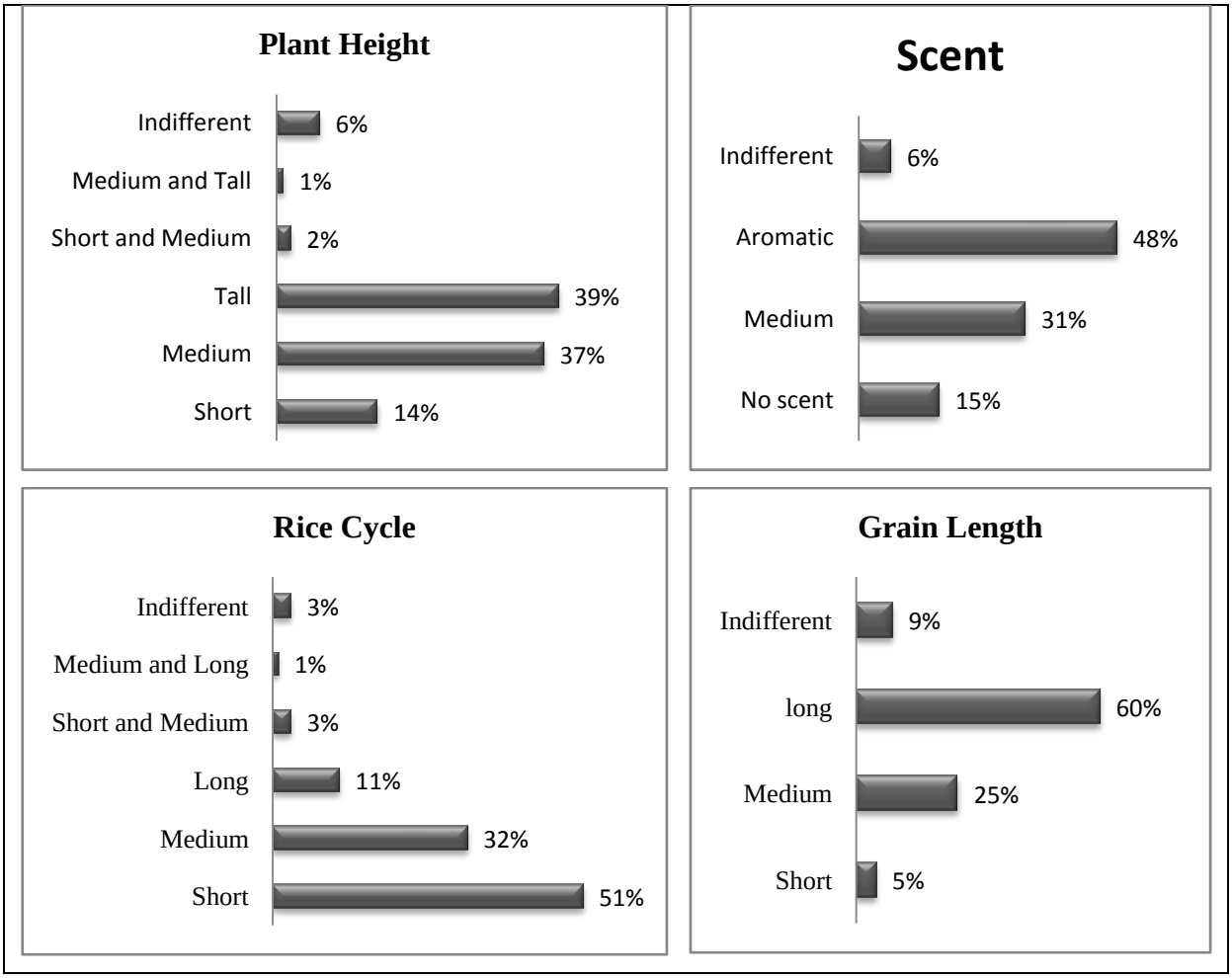

Figure 2.3: Bar charts showing farmers' choice of rice traits in Cascades Region of Burkina Faso

According to farmers, a good rice variety is characterised principally by its high yield and its good taste. In addition, grain quality, expansion of the grains after cooking, white grain and short cooking time are characteristics of good rice. Other attributes like high tillering ability, easy de-husking of the grains and resistance to diseases were noted by respondents to characterise good rice (Table 2.5). Bad grain quality, absence of grain spreading after cooking, difficulty in cooking (need more time when cooking) and red grain (a characteristic of *O. glaberrima)* define bad rice. Disease sensitivity, low tillering ability and the difficulty to de-husk the grain were also cited to characterise bad rice (Table 2.5).

Table 2.5: Traits characterising good and bad rice according to farmers of the Cascades Region of Burkina Faso

Traits characterising good rice	% of respondents	Traits characterising bad rice	% of respondents
High yield	59.4	Not tasty	46.4
Good taste	57	Low yield	41.3
Swell when cooked	28.5	Do not swell when cooked	23.0
Nice grain[a]	18.8	Sticky rice	15.3
Easy cooking	18.8	Disease susceptible	12.8
Strong tillering	18.4	Weak tillering	11.2
Easy de-husking	13.0	De-husking difficulty	10.2
Disease resistance	12.1	Red grain	06.6

[a]The term "nice grain" refers to an integrated evaluation by farmers of a number of traits that have been assessed subjectively and intuitively by the farmers. These include colour, length, breadth, shape, and dustiness.

Grain colour was an important feature for the farmers surveyed. White grain colour was appreciated by 79% while red grain colour was preferred only by 8% of the respondents. The remaining 13% were indifferent about this trait (Table 2.6). Red grain colour is generally used by farmers to characterise *O. glaberrima* varieties while white grain colour appears to be a typical attribute found in *O. sativa* varieties. From the farmers' point of view, white grains refer to attractive grains with a good appearance and good market value. In this context, red grain is a disadvantage of *O. glaberrima*, as it involves the added effort to whiten the grain during the de-husking process. This explains the low popularity of *O. glaberrima* over *O. sativa* in the region where *O. sativa* is dominantly cropped. *O. glaberrima* samples were collected only in four villages of the total villages surveyed: Douna, Kiribina, Noussoun and Sinièna with a maximum of four samples collected at Douna and Sinièna. Sinièna was the only village where the sample number of *O. sativa* did not exceed that of *O. glaberrima*. In this village, *O. glaberrima* is widely grown by elderly women, and many interviewees were indifferent about the grain colour (Table 2.6).

Table 2.6: Preferred grain colour across villages in Cascades Region of Burkina Faso

	Villages										
Grain colour	Baguera	Diaraba	Douna	Kangoura	Kawara	Kiribina	Loumana	Noussoun	Sinièna	Tingrela	**Mean %**
White	82.1%	71.9%	90.9%	84.0%	93.8%	75.0%	81.0%	77.8%	54.2%	100.0%	**79.2%**
Red	10.7%	9.4%	0.0%	12.0%	6.3%	10.0%	0.0%	5.6%	12.5%	0.0%	**7.5%**
Indifferent	7.1%	18.8%	9.1%	4.0%	.0%	15.0%	19.0%	16.7%	33.3%	0.0%	**13.2%**
Total %	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	100.0

2.4.3 Perception of farmers of crop damage caused by biotic and abiotic stresses

Crop losses observed in the field, in descending order of importance, were due to diseases (59%), parasites (30%), drought (17%), animals (14%), flood (8%) and iron toxicity (3%) (Table 2.7). Animal damage occurred mainly in the villages of Diarabakoko, Douna and Tingrela. Elephants were the destroyers in Diarabakoko, while hippopotami were damaging rice fields at Douna and Tingrela. Iron toxicity was a major concern at Kawara and Kiribina.

Table 2.7: Farmers' perception of major threats to their rice production in Cascades Region of Burkina Faso

	Animal	Disease	Drought	Flood	Fe toxicity	Parasites
Baguera	4	75	11	25	0	21
Diarabakoko	59	50	9	0	0	22
Douna	27	55	9	0	0	45
Kangoura	0	88	8	8	0	32
Kawara	0	63	6	6	25	19
Kiribina	5	50	30	5	10	15
Loumana	5	81	19	5	0	48
Noussoun	0	50	11	17	0	61
Sinièna	4	25	42	0	0	17
Tingrela	24	53	24	6	0	35
Overall %	**14**	**59**	**17**	**8**	**3**	**30**

The values represent the % of respondents in each village who mentioned the threat

Farmers were knowledgeable about the threats occurring in their field. They differentiated between threats due to diseases from those caused by parasites. In regard to parasites, they identified the key pests affecting their rice crop, such as insect pests and nematodes. In the case of diseases they ignored the cause and focused on visible symptoms. Nonetheless, they noticed that proliferation of the African Rice Gall Midge (AfRGM) is favoured by an insect which does not directly damage the crop but its larvae. AfRGM, Bacterial Leaf Blight (BLB),

Blast and RYMV were considered as frequent diseases. The neighbouring villages Kangoura, Loumana and Baguera reported frequent outbreaks (≥ 75%) of diseases (Table 2.7). RYMV was the most mentioned disease across villages, followed by blast, AfRGM and BLB. Blast outclassed RYMV in Baguera and Loumana while AfRGM was the more cited disease in Noussoun. However, a high level of occurrence of RYMV was observed in Kiribina (Table 2.8).

Table 2.8: Farmers' perception of frequent pests and diseases threatening their rice production in Cascades Region of Burkina Faso

	AfRGM	Blast	BLB	RYMV
Baguera	14	32	11	29
Diarabakoko	16	28	3	31
Douna	36	18	0	36
Kangoura	20	44	4	44
Kawara	31	0	0	31
Kiribina	30	30	15	50
Loumana	10	29	10	19
Noussoun	50	33	33	39
Sinièna	8	13	4	17
Tingrela	24	12	0	24
Overall %	**22**	**25**	**8**	**32**
Ranking	**3**	**2**	**4**	**1**

The values represent the % of respondents in each village who mentioned the disease

The ranking of the frequent diseases or pests across villages revealed that RYMV was the most threatening disease followed by Blast and AfRGM (Table 2.9). The symptoms observed by respondents to describe RYMV disease were necrosis, stunting, sterility and yellowing of leaves, where rice plants were not suffering from water shortage.

Table 2.9: Ranking from the most damaging to the least damaging pests and disease across the villages in Cascades Region of Burkina Faso

Villages	**AfGM**	**Blast**	**BLB**	**RYMV**
Baguera	3	1	4	1
Diarabakoko	3	2	4	1
Douna	2	3	0	1
Kangoura	3	2	4	1
Kawara	2	0	0	1
Kiribina	3	2	4	1
Loumana	3	1	4	2
Noussoun	1	3	4	2
Sinièna	3	2	4	1
Tingrela	2	3	0	1

The ranking is done per village, 0: means the disease was not mentioned in the village.

Resistant and/or tolerant varieties against RYMV were observed in Douna with the local variety called "Gnou", in Kiribina with the local variety called "Gnonrê" and in Tengrela with the local variety called "Douna". All the three resistant and/or tolerant varieties identified by the farmers belonged to *O. glaberrima* species. RYMV was given local dialect names in Baguera (Djiguikabila), Sinièna (Gbalô), Kiribina (Djoukounou) and in Tingrela (Kounagbê). The discovery of local names and symptoms were signs indicating that the farmers were aware of the existence of this disease. Similarly, local names were also attributed to AfGM and Blast. Farmers had neither preventive methods nor a curative remedy against RYMV epidemics. Some farmers tried using potash, ash, shea butter, and insecticides against RYMV disease but in vain. Fertiliser was also used because some farmers thought that the disease occurred due to soil problems. Yield losses caused by RYMV were estimated by farmers to be between 30% and 100%.

2.5 Discussion

2.5.1 Rice cropping practices

The survey noted that the women dominated rice cropping in the Cascades Region. This is common with rice cropping in most of Africa. Nuijten and Treuren, (2007) reported this trend in The Gambia. Women are the principal actors in rice cropping, grain processing and marketing. Men, on the other hand, are involved in the cropping of maize, sorghum and millet. In some ethnic groups in the Cascades Region, like among Gouin people, a woman can own a rice field inherited from her mother (Lankoandé and Paré, 2005; Lankoandé and

Sébégo, 2005). In almost all the surveyed villages, during the group discussions, women said they grew up seeing their mothers growing rice. As little girls they learnt rice cropping in their mother's rice fields and the tradition is continued through their daughters. The domination of women in rice cropping in the Cascades Region was already found in a Participatory Varietal Selection study at Badini, where 80% of the 570 farmers were women (Sié et al., 2008). Women are key stakeholders in rice cropping in West Africa. Their noticeable contribution in all the processes of rice production and in naming of varieties in The Gambia was documented by Nuijten and Almekinders (2008). The development strategies of rice production in the West African region must take into account gender aspects.

The majority of the respondents were small scale farmers, growing rainfed lowland rice. Water availability limits the option of double rice cropping and also poses a risk during the rainy season. In the absence of rains, rice plants are exposed to drought. Inversely, abundant rainfall causes inundation. Both situations can induce severe damage to rice. Multiple varieties are grown to meet the environment requirements and to minimise risks. Therefore, one farmer can grow a variety adapted to deep water ecology and another variety adapted to lowland ecology in the same season. The rice varieties grown in deep water environments are generally long cycle, while both early maturing and late maturing varieties are grown in lowland ecology. In marginal environments, variety diversification is a risk-reducing strategy, that makes on-farm varietal diversity high (Virk and Witcombe, 2007). It is essential for farmers to have varieties adapted to their marginal agro-systems. Therefore, breeding for a mega variety adapted to a wide range of environments could be hazardous in small scale farming in diverse situations and realities. Varieties that do not require much fertiliser and compete with weeds should be developed for low income farming system.

Nearly 60% of the farmers were willing to crop novel varieties and 45% were already using herbicides. This shows that farmers are not opposed to the introduction of modern varieties and innovation. They are curious and adopt a new variety or technique if they find it advantageous. For example, herbicides are being increasingly used by low income farmers because they are finding them profitable. However, the farmers must be trained in their utilisation in order to avoid causing any harm to themselves and to prevent pollution of the environment. Similarly, farmers were willing to test new rice varieties and see if these varieties meet their requirements. The improved varieties are usually rejected when they do

not satisfy farmers' needs. This was the case of Jola people in Senegal rejecting American rice varieties (Linares, 2002).

2.5.2 Preferred rice traits

The survey revealed the farmers' selection criteria. Farmers valued their local rice varieties because of their taste, their high yields in their environment and the expansion of the grains when cooked. These desired traits were also important for Jola women's choice in the Casamance Region when choosing which rice varieties to grow (Linares, 2002). In the Cascades Region, from a farmer's point of view, taste comes before yield. In West Africa, low income farmers are willing to trade-off yield for taste and grain quality (Linares, 2002; Efisue et al., 2008). However, rice farmers in Uganda and Malawi consider yield as the paramount desired trait (Lamo, 2010; Mzengeza, 2010). The difference is that rice farming is market oriented in Uganda and Malawi, while in West Africa most of the rice production is dedicated to domestic consumption. An important point is that *O. glaberrima* originated in West Africa and is cultivated in most of the West African countries (Semon et al., 2005; Nuijten et al., 2009) and it is particularly appreciated for its taste (Sié et al., 1998). In addition, *O. glaberrima* produces more waxy protein than *O. sativa* varieties (Sano, 1984) and waxy rice is similar to sweet rice. According to Traoré (2005), West African rice consumers prefer aroma, grain elongation and softness after cooking. These attributes are an overriding combination of traits that must be taken into account in breeding rice for West African countries.

Low yield and drought susceptibility were noted as weaknesses of some local varieties. However, the continued survival of traditional varieties in farmers' fields is dependent on the continuing failure of plant breeders to provide better alternatives (Virk and Witcombe, 2007). The improved variety FKR19, although it is high yielding, is not appreciated by farmers. It is characterised as having bad grain quality and short culm length, making it difficult to harvest. Moreover, its husked grains have a bad appearance and the grains break into pieces during the milling process. Most farmers of the Cascades Region like white milled rice with a pleasant appearance, which according to them is qualified as "nice grains". Conversely, red milled rice is seen as "bad rice", although certain farmers liked it. Contrary to the findings in the Cascades Region, in Bohol Region in Philippines, red milled rice is preferred by farmers (Bertuso et al., 2005). However, in Gambia, there is no preference between red and white grain colour (Nuijten and Treuren, 2007). A PVS conducted at Badini in the Cascades Region

(Sié et al., 2008) did not reveal the importance of gastronomic quality or taste. In this study, farmers evaluated known varieties in the field for their agronomic and vegetative performances. The gastronomic attributes were not addressed. In a participatory plant breeding process, the initial PRA should be at the grass-root level to first address the farmers' needs and the traits they use as selection criteria. Then, breeders can build ideotypes based on farmer preferences and try to breed varieties that meet farmers' requirements. PVS must come later to let farmers assess the new varieties developed for their agronomic, vegetative and gastronomic performances, to avoid rejection after release. According to Ceccarelli (2009), PVS is the last stage of involving farmers in a participatory breeding programme. Nonetheless, the agronomic and vegetative characteristics emphasised in the PVS survey are documented in our PRA study.

2.5.3 Perception of RYMV disease

The appraisal amongst communities revealed that RYMV was the most damaging disease across the villages. None of the villages were RYMV free. However, the disease severity and RYMV perception varied across villages. Farmers recognised the disease through its symptoms of yellowing, stunting, sterility, necrosis and plant death. However, they were ignorant of the cause of the disease. Apparently, none of their traditional methods to treat diseased plants succeeded in preventing the damage caused by RYMV. Therefore, the limitation of RYMV progression through prophylactic measures (Traoré et al., 2009) would be tricky without training farmers and agricultural technicians working closely with farmers on the disease epidemiology. However, resistant or tolerant varieties against RYMV were identified by farmers, and all belonged to the *O. glaberrima* species. The previous studies on varieties screening against RYMV has found more resistance in *O. glaberrima* than in *O. sativa* (Ndjiondjop et al., 1999; Albar et al., 2006). Three different alleles of resistance have been identified in *O. glaberrima*, while only one was present in *O. sativa* (Albar et al., 2006). The recent screening of an *O. glaberrima* collection highlighted a second major gene of resistance to RYMV (Thiémélé et al., 2010). Dialogue with farmers could help breeders and pathologists to find landraces that are resistant or tolerant to RYMV. This is what happened in Madagascar, where a new *O. sativa* variety (Bekarossaka) bearing a major RYMV gene of resistance was identified (Rakotomalala et al., 2008). The exploitation of local landraces, mainly *O. glaberrima,* coupled with molecular techniques to transfer the *O. glaberrima* resistance to high yielding *O. sativa* varieties are promising ways to tackle RYMV.

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Chapter 3: Collection and phenotypic characterisation of rice landraces collected in Burkina Faso

Abstract

The collection of rice landraces from farmers' fields has received significant attention as a strategy to conserve the genetic diversity of food security crops in farms, villages, or countries. The existence of genetic variability and diversity for the traits of interest is a prerequisite for the breeding of new varieties that are capable of improved performances. In Burkina Faso, the two rice species, *O. glaberrima* and *O. sativa*, are grown in farmers' fields. To preserve this diversity, a broad collection of rice landraces was acquired in the four main rice growing regions of the country: Boucle du Mouhoun, Cascades, Hauts Bassins and Sud Ouest. Three hundred and thirty rice landraces were collected from farmers' stocks. The collection was evaluated in a field for 20 quantitative and 30 qualitative agro-morphological parameters. Fourteen traits were found to distinguish the entire collection, which was dominated by *O. sativa* relative to *O. glaberrima* varieties. The diversity assessment revealed that *O. sativa* accounted for 86% of the collection and was split into four sub-groups. The 48 samples of *O. glaberrima* could be divided into four groups (Gg1, Gg2, Gg3, and Gg4), based on three quantitative and six qualitative agro-morphological parameters. The application of the Shannon Weaver diversity index, to compare the diversity of the collection on a regional scale, showed that the collection from Boucle du Mouhoun Region was the most diversified, although it accounted for only 15% of the overall collection.

3.1 Introduction

Two rice species are cultivated in the world. The Asian rice, *Oryza sativa* (L.), is the most popular and widespread in the five continents, whereas the African rice, *O. glaberrima* (Steud.), is encountered in some West African rice fields (Nayar, 2010). *O. sativa* was introduced in Africa in the 15th century, while *O. glaberrima* is endemic to West Africa. The primary centre of diversification of *O. glaberrima* is in the inner delta of the River Niger (Republic of Mali), with secondary centres of diversification in the Sene-Gambia regions (Portères, 1950). In Burkina Faso, rice landraces have been systematically collected four times. The first collection took place in 1967 and covered 23 villages in the Cascades Region (Sié et al., 1998). In 1976, the second collection was conducted by Second and Bozza from

the “Office de la Recherche Scientifique et Technique d'Outre-Mer” (ORSTOM) and the “Institut de Recherches Agronomiques Tropicales” (IRAT), respectively, and lasted two weeks (Bezançon, 1993). The third collection, led by the International Institute of Tropical Agriculture (IITA), was carried out in 1978 (Sié, 1991). From November 1983 to February 1984, an extensive collection of rice samples across all the regions of the country was undertaken by the International Board of Plant Genetic Resources (IBPGR). During that time, 527 rice samples were collected, including 475 *O. sativa* and 52 *O. glaberrima* accessions (Sié, 1984). The collections were undertaken to prevent the disappearance of the African rice *O. glaberrima* and to gather rice genetic material for breeding purposes. The agro-morphological characterisation of that collection by Sié (1991), with two quantitative (crop cycle and grain length) and four qualitative parameters (apiculus colour, awn presence, grain colour and pericarp colour) identified 44 and 25 distinct phenotypes in *O. sativa* and in *O. glaberrima*, respectively. The increase in the number of parameters to 12 helped Sié et al. (1999) to highlight three clusters in *O. sativa,* and three subsets in *O. glaberrima*. However, this collection was not properly conserved in the Burkina Faso National Agricultural Research System (INERA) due to a lack of proper seed storage facilities. The components of this collection may still be conserved casually in farmers’ fields. Indeed, most farmers in developing countries like Burkina Faso still breed and conserve crop diversity at the field and village level (Barry et al., 2007a). Crucially, rice diversity at farmer level, if not formally managed by attentive curators, could be lost owing to farmers’ decisions, which are primarily driven by private benefits (Jackson et al., 2010).

Nearly a quarter of a century after the first extensive collection, there is interest again to collect local rice accessions and reassess the extent of its diversity in order to develop strategies for *in situ* conservation. The new rice collection was conducted in 59 villages of the four main rice cropping regions (Cascades, Hauts Bassins, Boucle du Mouhoun, and Sud Ouest) of the country, where rice is mostly cultivated, including landrace varieties. To determine the phenotypic and agro-morphological parameters of the collected samples, it is a prerequisite to evaluate the varieties in the field. Farmers and breeders rely on phenotypic features to characterise and distinguish the varieties they hold. Moreover, the naming of rice varieties by farmers is influenced by the characteristics of the plant (Nuijten and Almekinders, 2008). Furthermore, in breeding programmes, characterisation of varieties, based on multiple phenotypic traits, can be used as a management tool to validate the identity of an accession (Aghaee et al., 2010). Studies on the variations present in rice germplasm collections have

been carried out frequently using characterisation of plant morphological attributes (Sié et al., 1998; Bisne and Sarawgi, 2008; Sanni et al., 2008). The main objectives of this investigation were to evaluate 330 newly collected rice accessions on the basis of multiple agro-morphological traits.

3.2 Material and methods

3.2.1 The collection procedure for the rice samples

Rice collection was conducted in 2008 in 59 villages of the four main rice cropping regions: Boucle du Mouhoun (BM), Cascades (CC), Hauts-Bassins (HB) and Sud-Ouest(SO) of Burkina Faso. The methodology behind the choice of the village was as follows:

1. All villages visited during the IBPGR collection (Sié, 1984) were listed;
2. Villages where more than five varieties were collected during the IBPGR collection were chosen, assuming that they maintained a high level of diversity of rice landraces;
3. Villages that were not visited during IBPGR collection, but listed by regional agricultural officers as having local rice cropping habits, were also added.

In collaboration with the agricultural offices of each region, an agricultural agent of each department of the target villages was contacted. The collection team of each region was composed of an INERA technician, an agricultural agent at the departmental level, and the leader, Honoré Kam. The process typically started with the agricultural agent of the target village informing farmers and summoning a meeting. In each village, an inventory of rice landraces was drawn in the presence of a group of farmers. Farmers were asked to list the rice varieties they had cropped for several years, including those that their ancestors had cultivated that the present generation is continuing to grow. Farmers who owned these antecedent varieties were identified and the collection team went and collected the varieties in their stocks (Figure 3.1). Together, farmers identified the chosen varieties through consensus and information related to each variety (Figure 3.2). The name, the duration, and the cropping system (upland, lowland, deep water) were noted. Then, a seed sample of each variety was collected in a plastic bag. Modern varieties released through the agricultural and research network channels were not collected. Two rice samples were collected in the market place at Douna, a village in the Cascades Region. Data on longitude, latitude and altitude of each village were also collected with a Global Positioning System (eTrex®, Garmin Ltd, USA).

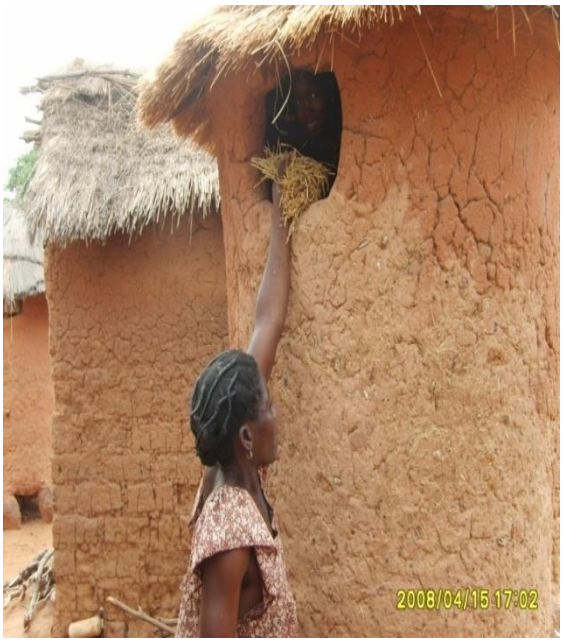


Figure 3.1: Woman collecting a rice sample from a granary in Kangoura (10.5376 W; 5.2790 N) , Burkina Faso

Figure 3.2: Women identifying a rice variety in Baguera (10.5294W; 5.4099N), Burkina Faso

For purposes of easy identification, the samples collected in Boucle du Mouhoun Region, Cascades Region, Hauts-Bassins Region, and Sud-Ouest Region were assigned the prefix BM, CC, HB, and SO, respectively.

3.2.2 Agro-morphological characterisation

Agro-morphological characterisation was implemented to distinguish between *O. sativa* and *O. glaberrima* samples, and to record vegetative data to facilitate comparison between accessions. Subsequently all of the 330 accessions were sown in an experimental field of the AfricaRice at the Benin station (21 m above sea level) using an Augmented Experimental Design (AED) (Federer, 1956). The trial was conducted in the year 2008 in upland condition during the rainy season. The plants were watered on rainless days. Seven checks: CG14, Moroberekan, ITA212, B6144, RAM55, NERICA4, and NERICA14 were used to estimate the field's heterogeneity, and were also used as "references" for the grouping. The ecology, species and morphological characteristics of the checks are summarized in Table 3.1 and Figures 3.3; 3.4; 3.5; and 3.6. The checks were repeated in seven blocks.

Table 3.1: Characteristics of the check varieties used

Check	Species	cropping-system
B6144	*O. sativa* ssp *indica*	Lowland
CG14	*O. glaberrima*	Upland
ITA212	*O. sativa* ssp *indica*	Lowland
NERICA4	Inter-specific	Upland
NERICA14	Inter-specific	Upland
Moroberekan	*O. sativa* ssp *japonica*	Upland
RAM55	*O. glaberrima*	Lowland

Figure 3.3: *O.glaberrima* species, RAM55

Figure 3.4: Interspecific rice, NERICA4

Figure 3.5: *O. s. indica* species, ITA212

Figure 3.6: *O. s. japonica* species, Moroberekan

Plot size for each accession was 4 m x 1 m with three rows of 16 plants each. Each row was spaced by 0.25 m with a border of 0.50 m between plots. After direct sowing, thinning to one plant occurred at 20 Days After Sowing (DAS). Basal application of NPK fertiliser (15-15-15) of 200 kg ha^{-1} was applied during land preparation. At 21 and 42 DAS 30 kg ha^{-1} of urea was applied. Regular weeding was performed as needed. Agro-morphological evaluation was monitored in the field and in the lab, using 20 quantitative and 30 qualitative rice descriptors (Table 3.2), in accordance with the method described by Bioversity International et al. (2007). The measurement of the parameters was monitored on five plants of the inner row of the plot.

Table 3.2: The 20 quantitative and 30 qualitative traits used to evaluate the rice collection in the field

Code	**Quantitative traits measured**
7.2.2.1	Number of days from seedling to first heading
7.2.3.1	Number of days from seedling to main heading
7.2.4.1	Number of days from seedling to maturity
7.3.13	Ligule length (mm)
7.3.18	Leaf blade length (mm)
7.3.19	Leaf blade width (mm)
7.3.20	Flag leaf length (mm)
7.3.21	Flag leaf width (mm)
7.3.25	Culm length (mm)
7.3.26	Culm number
7.3.27	Culm diameter (mm)
7.4.14	Number of basal primary branches on panicle
7.4.17	Number of panicles per plant
7.5.1	Panicle length (mm)
7.5.15	Grain length (mm)
7.5.16	Grain width (mm)
7.5.17	Grain thickness (mm)
7.5.18	100-grain weight (g)
7.5.20	Caryopsis length (mm)
7.5.21	Caryopsis width (mm)
Code	**Qualitative traits measured**
7.3.3	Colour of basal leaf sheath
7.3.8	Leaf blade attitude
7.3.9	Leaf blade pubescence
7.3.9.1	Leaf blade pubescence on blade surface
7.3.10	Leaf margin pubescence

Code	**Qualitative traits measured (cntd)**
7.3.11	Auricle colour
7.3.12	Collar colour
7.3.14.2	Ligule shape
7.3.22	Flag leaf attitude early after anthesis
7.3.23	Culm habit
7.3.32	Lodging resistance
7.3.33	Culm strength
7.3.34	Flag leaf attitude late at maturity
7.3.35	Leaf senescence
7.4.2	Stigma colour
7.4.5	Lemma and palea colour after anthesis
7.4.6	Colour of apiculus
7.4.8	Awn presence
7.4.9	Awn distribution
7.4.10	Awn colour at anthesis
7.4.19	Panicle attitude of main axis
7.4.20	Panicle attitude of branches
7.4.21	Panicle secondary branches
7.4.22	Panicle exertion
7.4.23	Panicle shattering
7.5.3	Awn colour at maturity
7.5.5	Lemma and palea colour at maturity
7.5.8	Colour of apiculus
7.5.10	Sterile lemma length
7.5.23	Pericarp colour of caryopsis

The code in the left of the trait is identical to the one in Bioversity-International et al. (2007)

3.3 Data analysis

The values of the augmented design were adjusted in GenStat 11 (Payne et al., 2008) using an incomplete design model based on the repeated values of the checks in each block. The 50 traits were analysed using Factorial Analysis (FA). Agglomerative Hierarchical Clustering (AHC) (Ward, 1963) was used to construct a dendrogram based on the first four factor scores obtained in FA. Discriminant Analysis (DA) (Jobson, 1992; Huberty, 1994) was performed on the 20 quantitative and 30 qualitative data to obtain the maximum proportion of samples well classed in each group, identified via the AHC analysis. Furthermore, DA analysis was applied on a regional basis to the 20 quantitative and 30 qualitative variables to ascertain the differences between the morphological variations of the accessions. The multivariate analyses (FA, AHC, and DA) were performed in XLSTAT 2010 (www.xlsat.com). An ANOVA was conducted on the 20 quantitative and 30 qualitative variables to highlight the traits differentiating the samples on the regional scale. The phenotypic frequencies of the 14 significant quantitative and qualitative parameters were analysed by the Shannon-Weaver diversity index (H') (Shannon, 1948). Jain et al. (1975) and Sanni et al. (2008) gave *H'* as:

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

Where k is the number of phenotypic classes for a character, and P_i is the proportion of the total number of entries in the i^{th} class.

3.4 Results

3.4.1 Sample collection

The rice collection according to regions was as follow: 48 samples were collected in the Boucle du Mouhoun (BM) Region, 83 in Hauts Bassins (HB) Region, 33 in Sud Ouest (SO) Region, and 166 in the Cascades (CC) Region. In total 330 samples were collected. Half of the rice samples collected were from the CC Region. Amongst the 330 samples collected, 46 were in the form of panicles and the rest were bulk grains. The panicles were collected in the villages of Baguera, Loumana, Kangoura, and Niankorodougou in the CC Region, and in Banzon and Banankoro in the HB Region.

Amongst the 330 samples collected, 229 distinct names of rice varieties were identified. Some names characterised the duration (early or late maturing). For instance, Maloba referred to long cycle rice and Wêrê-wêrê referred to early maturing rice. Names can often describe the

grain shape (Missinni for long grain) and the grain or paddy colour (Malowilé refers to red rice). The term “red rice” can be confusing because it may refer to either grain colour or husk colour. Rice can also bear a name which highlights its particular notable trait such as a high tiller number (Djoutchèmin or Troutchèmin means 100 tillers). Some rice varieties were named after the persons who introduced them in the village, for instance, Mariam, Djénéba, Adama-Sali, Dembélé, etc.

3.4.2 Agro-morphological diversity

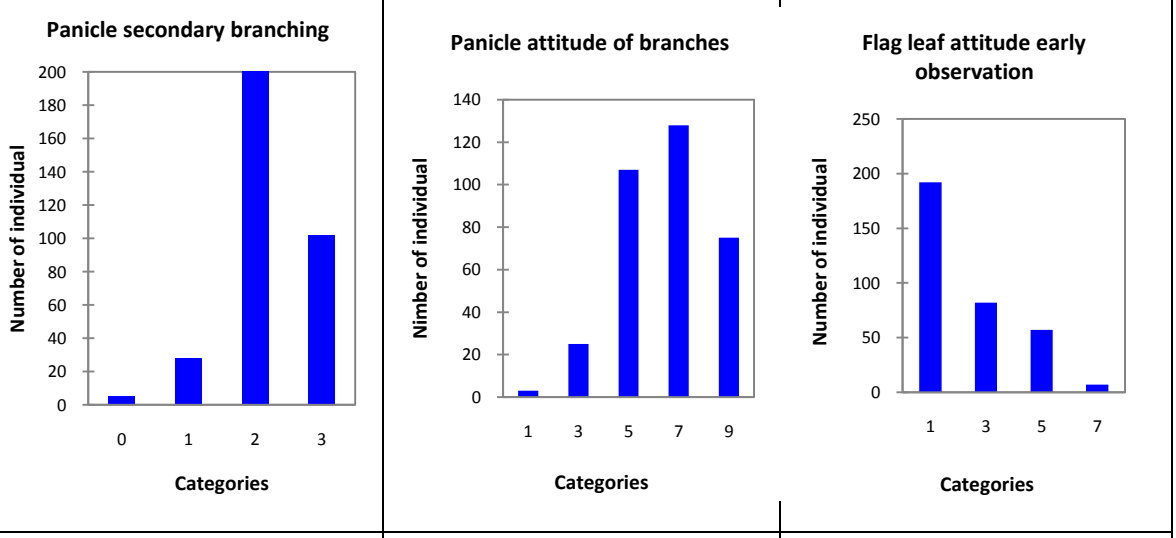
The descriptive statistics on agro-morphological variables highlighted the diversity of accessions in the collection. The minimum, the mean, the maximum, the standard deviation, and the coefficient of variation showed the range of variability of quantitative parameters in the collection (Table 3.3).

Table 3.3: Summary statistics of the 20 quantitative variables showing the diversity of the collection

Code	Quantitative variables	Min	Max	Mean	SD	CV
7.2.2.1	Number of days from seedling to first heading	48	138	86.5	15.5	18
7.2.3.1	Number of days from seedling to main heading	59	156	100.7	17	17
7.2.4.1	Number of days from seedling to maturity	79	178	125.1	19.5	16
7.3.13	Ligule length (mm)	1.8	36	15.3	7	46
7.3.18	Leaf blade length (mm)	209	717	467	85	18
7.3.19	Leaf blade width (mm)	7	20	13	2	19
7.3.20	Flag leaf length (mm)	148	657	347	83	24
7.3.21	Flag leaf width (mm)	9	23	15	3	21
7.3.25	Culm length (mm)	425	1604	962	176	18
7.3.26	Culm number	4.6	70	16.5	5.7	34
7.3.27	Culm diameter (mm)	3.3	10.6	6.2	0.8	12
7.4.14	Number of basal primary branches on panicle	2.4	19.2	12.5	1.8	14
7.4.17	Number of panicle per plant	1.3	40	13	4	31
7.5.1	Panicle length (mm)	211	339	262	27	10
7.5.15	Grain length (mm)	6.1	10.9	8.9	0.9	9.8
7.5.16	Grain width (mm)	2	3.6	2.7	0.3	9.7
7.5.17	Grain thickness (mm)	1.5	2.4	2	0.1	7
7.5.18	100-grains weight (g)	1.2	3.6	2.5	0.4	15
7.5.20	Caryopsis length (mm)	4.2	7.9	6.4	0.7	11
7.5.21	Caryopsis width (mm)	1.8	3.1	2.4	0.2	9.4

Min: minimum, Max: maximum, SD: standard deviation, and CV: Coefficient of Variation

The qualitative parameters emphasised the diversity of the collection (Figure 3.7). However, some categories were dominated by single characteristics. The basal leaf sheath colour of 75% of the accessions was green. The same proportion displayed a white stigma and 69% showed a green apiculus after anthesis to the hard dough stage. Furthermore, 64% of the accessions were awnless, while 72% were low shattering. The collection was constituted of 78% of white grains accessions.



0: absent; 1:~ 1 secondary branch 2: ~ 2-3 secondary branches 3: ~ 3-4 secondary branches	1: compact panicle; 3: semi-compact panicle; 5: open panicle; 7: horizontal branches and 9: drooping branches	1: erect; 3: semi-erect; 5: horizontal 7: descending

Figure 3.7: Characteristics of some categorical parameters

The plan of the two first axes of the factorial analysis highlighted three main groups (Figure 3.8). Group 1 (Grp1) comprised of the *O. glaberrima* check varieties Tog5681, CG14, and RAM55. The samples of this group were believed to be composed of *O. glaberrima* accessions.

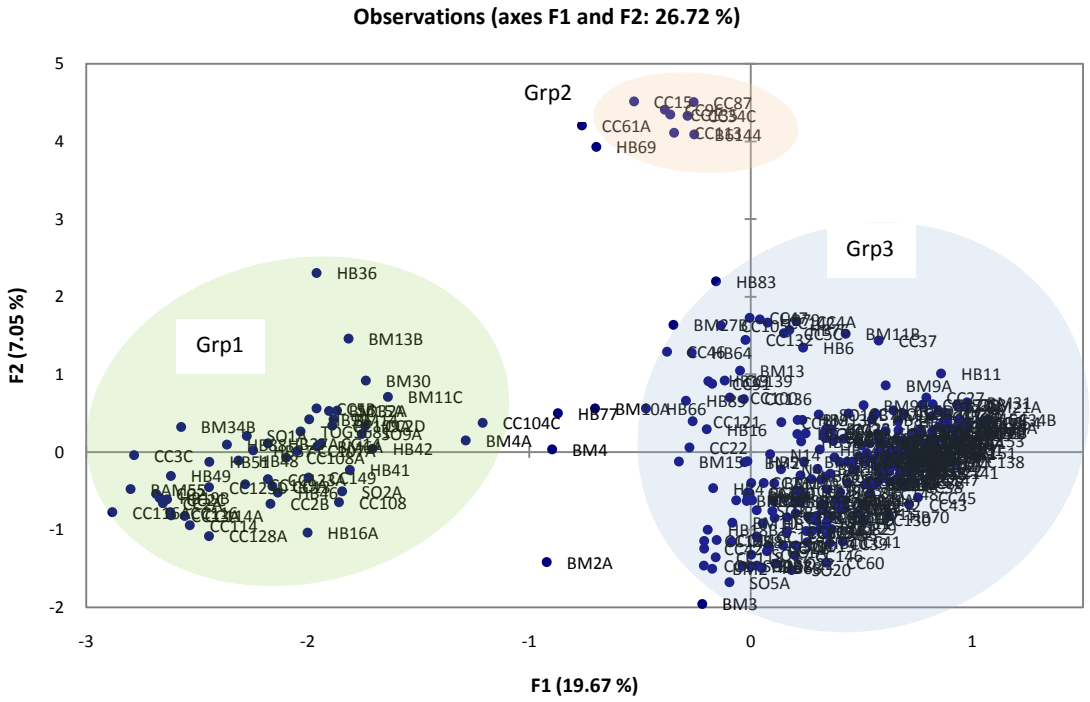
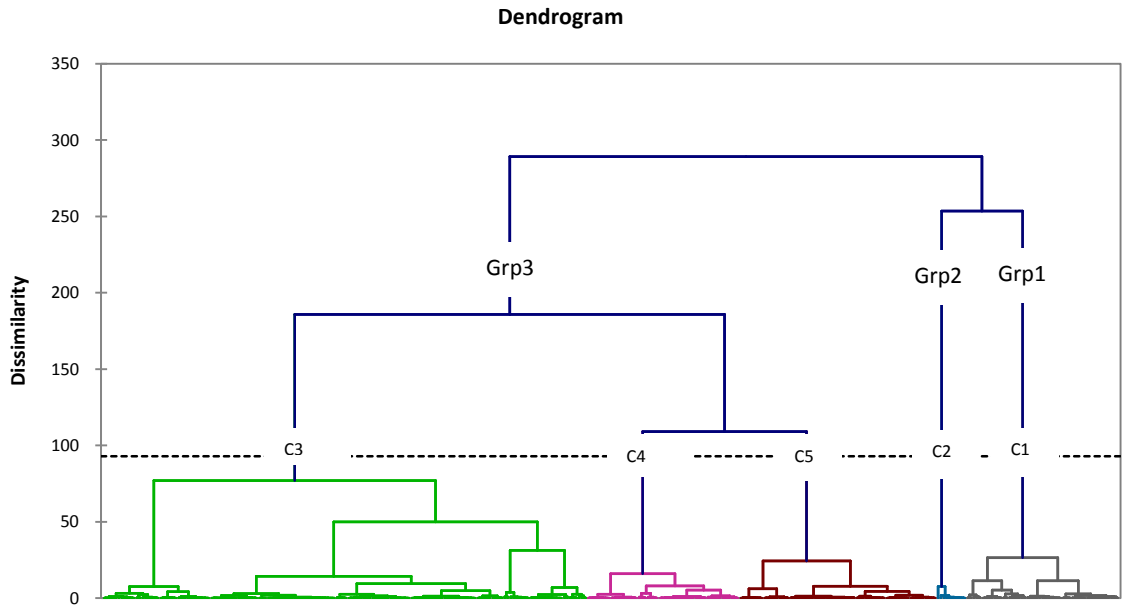


Figure 3.8: Plan of the two first axes of the factorial analysis on 50 agro-morphological traits applied to 330 rice accessions collected in Burkina Faso in 2008

Group 2 (Grp2) included the check variety B6144. As with B6144, the samples in Grp2 had anthocyanin in their leaves and culm. The *O. sativa* checks Moroberekan and ITA212, and the inter-specific checks NERICA4 and NERICA14 were located in Group 3 (Grp3). In order to obtain homogeneous classes, the coordinates of the accessions on the four first axes of FA were used with the help of an Agglomerative Hierarchical Clustering (AHC) to build a dendrogram. Five clusters were identified in the dendrogram (Figure 3.9). Grp3 was split into three clusters (C3, C4, and C5), while Grp1 and Grp2 generated a C1 and C2 cluster, respectively (Figure 3.9). The *O. glaberrima* checks CG14, RAM55, and Tog56581 belonged to C1 with 48 other accessions. B6144 was included in C2 with nine other accessions. ITA212 and Moroberekan constituted C3 with 158 accessions. C4 included 51 accessions, while C5 was composed of 64 accessions and the two inter-specific checks NERICA4 and NERICA14.



Grp1, Grp2 and Grp3 are the three groups identified by Factorial Analysis. C1, C2, C3, C4 and C5 refer to Cluster 1, Cluster 2, Cluster 3, Cluster 4, and Cluster 5 identified by the automatic truncation

Figure 3.9: Dendrogram based on factorial analysis of 50 variables

A scatter diagram obtained through discriminant analysis portraying the five groups highlighted the significant difference between C1 and the four other classes. Axis 2 separated C1 from C2, C3, C4, and C5, while axis 1 separated C2, C3, C4, and C5. The Group C1 is set apart from the other four clusters. Blue, green, brown, light blue and grey dots refer to C1, C2, C3, C4, and C5, respectively. C1 is very different from the four other clusters. (Figure 3.10).

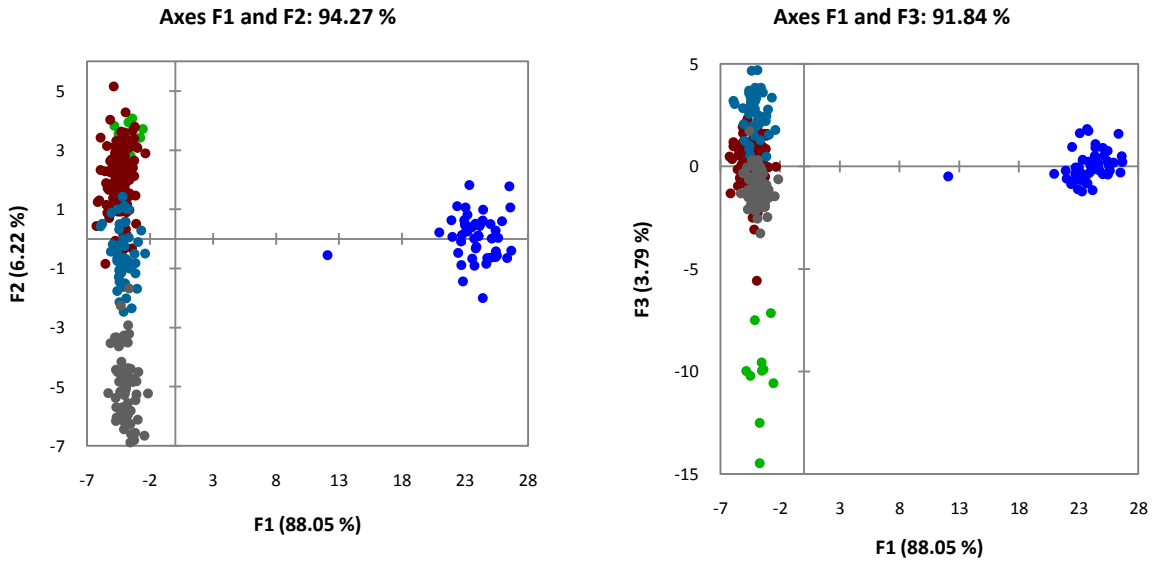


Figure 3.10: Discriminant analysis performed on axes F1/F2 and F1/F3 on the 5 classes obtained through hierarchical clustering analysis.

By avoiding repeats of highly positively correlated variables, the quantitative traits distinguishing the five classes were established and they are: culm length, panicle length, leaf blade length, flag leaf width, ligule length, culm number, number of days to maturity, number of panicle primary branches, grain length and grain width (Table 3.4 and 3.5).

Table 3.4: Major distinguishing quantitative parameters between the five clusters

Traits	C1 ± Sd	C2 ± Sd	C3 ± Sd	C4 ± Sd	C5 ± Sd
Culm length (mm)	950 ± 172	900 ± 125	930 ± 156	**1190 ± 137**	**890 ± 103**
Panicle length (mm)	**297 ± 27**	**238 ± 29**	253 ± 20	270 ± 22	255 ± 19
Leaf blade length (mm)	485 ± 71	**416 ± 7**	438 ± 72	**557 ± 82**	466 ± 82
Flag leaf width (mm)	**19 ± 2**	18 ± 3	**13 ± 2**	16 ± 3	14 ± 2
Ligule length (mm)	**4.4 ± 2.0**	13.6 ± 3.1	17.3 ± 4.9	**19.8 ± 7.3**	15.4 ± 5.0
Culm number	**18.7 ± 5.6**	17.0 ± 4.8	**15.0 ± 3.9**	16.6 ± 5.7	17.6 ± 4.5
Number of days to maturity	**108 ± 12.8**	115 ± 9.4	135 ± 16.3	**138 ± 17.4**	**108 ± 7.9**
Number of panicle primary branches	**13.5 ± 1.9**	**13.5 ± 1.4**	**12.0 ± 1.7**	12.9 ± 1.6	12.3 ± 1.7
Grain length (mm)	**8.3 ± 0.3**	8.3 ± 0.4	8.9 ± 0.8	8.9 ± 0.8	**9.5 ± 1.1**
Grain width (mm)	**3.0 ± 0.1**	2.9 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	**2.5 ± 0.2**

C1, C2, C3, C4 and C5 refer to clusters 1, 2, 3, 4 and 5, and Sd refers to standard deviation.

Table 3.5: correlation matrices of the 20 quantitative variables

	CL	PL	LBL	LBW	FLL	FLW	LL	NPP	FH	MH	cycle	PB	CN	CD	GL	GW	GT	CaL	CaW
CL	1.000																		
PL	0.349	1.000																	
LBL	**0.649**	0.403	1.000																
LBW	0.296	0.424	0.437	1.000															
FLL	**0.508**	0.358	**0.760**	0.330	1.000														
FLW	0.189	0.466	0.413	**0.891**	0.337	1.000													
LL	0.337	-0.207	0.220	-0.355	0.190	-0.456	1.000												
NPP	0.075	0.104	0.057	-0.029	0.067	0.003	-0.053	1.000											
FH	0.438	-0.093	0.172	-0.271	0.050	-0.382	**0.542**	0.059	1.000										
MH	0.434	-0.047	0.178	-0.273	0.067	-0.373	**0.519**	0.052	**0.886**	1.000									
cycle	0.470	-0.007	0.183	-0.245	0.083	-0.357	**0.516**	0.054	**0.858**	**0.934**	1.000								
PB	0.150	0.265	0.282	**0.503**	0.221	0.470	-0.127	-0.107	-0.234	-0.226	-0.203	1.000							
CN	0.071	0.149	0.146	0.188	0.168	0.233	-0.171	**0.570**	-0.120	-0.161	-0.140	0.002	1.000						
CD	0.250	0.112	0.291	0.315	0.225	0.237	0.094	-0.081	0.062	0.069	0.068	0.262	0.016	1.000					
GL	-0.173	-0.226	-0.111	-0.283	0.067	-0.274	0.197	-0.094	-0.104	-0.170	-0.171	-0.143	-0.070	-0.026	1.000				
GW	0.200	0.314	0.165	0.415	0.054	0.453	-0.237	-0.031	-0.096	-0.019	-0.006	0.270	0.071	0.184	-0.481	1.000			
GT	0.234	-0.132	0.067	-0.005	0.003	-0.048	0.247	-0.116	0.179	0.221	0.252	0.043	-0.116	0.272	0.045	0.376	1.000		
CaL	-0.160	-0.195	-0.102	-0.278	0.055	-0.269	0.191	-0.075	-0.094	-0.160	-0.160	-0.144	-0.072	-0.025	**0.977**	-0.487	0.049	1.000	
CaW	0.186	0.321	0.131	0.413	0.026	0.466	-0.286	-0.025	-0.130	-0.071	-0.051	0.263	0.083	0.183	-0.457	**0.962**	0.436	-0.450	1.000
HGW	0.061	-0.088	-0.028	-0.096	0.000	-0.069	0.191	-0.049	0.023	0.010	0.027	-0.009	-0.069	0.157	0.440	0.187	**0.551**	0.465	0.234

CL: Culm length
PL : Number of panicle per plant
LBL: Leaf blade length
LBW: Leaf blade width
FLL: Flag leaf length
FLW: Flag leaf width
LL: Ligule length
NPP: Number of panicle per plant
FH: Number of days from seedling to first heading
MH: Number of days from seedling to main heading
Cycle: Number of days from seedling to maturity
PB: Number of basal primary branches on panicle
CN: Culm number
CD: Culm diameter
GL: Grain length
GW: Grain width
GT: Grain thickness
CaL: Caryopsis length
CaW: Caryopsis width
HGW: 100-grains weight

The qualitative traits: basal leaf colour, culm lodging resistance, flag leaf angle at maturity, panicle attitude of the main axis, panicle secondary branching, and pericarp colour also enabled the differentiation of the five classes (Table 3.6).

Table 3.6: Major distinguishing qualitative parameters amongst the five clusters

Variable	C1	C2	C3	C4	C5
Colour of basal leaf sheath	With anthocyanin (90%)	Strong anthocyanin (100%)	No anthocyanin (83%)	No anthocyanin (100%)	No anthocyanin (95%)
Flag leaf attitude at maturity	Horizontal to descending (96%)	Erect to semi erect (78%)	Erect to semi erect (60%)	Horizontal to descending (96%)	Horizontal to descending (80%)
Panicle attitude of main axis	Upright to semi upright (88%)	Drooping (89%)	Drooping (97%)	Drooping (90%)	Drooping (100%)
Panicle secondary branches	≤ 1 (65%)	2 to 4 (99%)	2 to 4 (100%)	2 to 4 (100%)	2 to 4 (100%)
Pericarp colour	Coloured (96%)	White (78%)	White (88%)	White (96%)	White (100%)
Lodging resistance	Low (87%)	Strong (89%)	Strong (47%)	Low (45%)	Intermediate (45%)

C1, C2, C3, C4 and C5 refer to Clusters 1, 2, 3, 4 and 5. In bracket the percentage of samples showing the trait

The accessions in the C1 cluster (48 accessions) were characterised by long panicle length (300 mm), large flag leaf (19 mm), short ligule (4.4 mm), relatively high number of culms (19), relatively high number of panicle primary branches (14), short grains (8.3 mm), breadth of grain (3.0 mm), and early maturing plants (108 days). Furthermore, 90% displayed anthocyanin in their basal leaf sheath, 96% depicted a horizontal to descending flag leaf at maturity, 88% portrayed upright to semi upright panicles, a maximum of one panicle secondary branch was found in 65% of C1 samples, 96% developed coloured pericarp, and 87% showed low lodging resistance. These are the characteristics of *O. glaberrima* species. The accessions of C1 were grown in lowland and deep water cropping systems. In the C1 cluster, the presence of the *O. glaberrima* checks CG14, RAM55 and Tog5681 confirmed that this was a grouping of *O. glaberrima* accessions.

The Cluster C2 (nine accessions) showed strong anthocyanin presence in their basal leaves, their leaves, and their culm in 100% of the samples, 78% grew with erect to semi-erect flag leaf attitude at maturity, 89% depicted a drooping panicle, 99% of the samples developed two to four panicle secondary branches, 78% had a white pericarp and 89% showed a strong resistance to lodging. They were short plants (900 mm) with an intermediate crop cycle (115

days). The *O. s. indica* variety, B6144, shared those characteristics with the other samples in C2. The accessions of C2 were grown in the lowland cropping system.

The Cluster C3 (158 accessions) grew narrow leaves (13 mm), short leaves (438 mm), short culm length (930 mm), longer crop cycle (135 days), and relatively few panicle primary branches (12). In C3, 83% of the samples were without anthocyanin in their basal leaf sheath, 97% showed drooping panicles, while 100% had two to four panicle secondary branches and 88% had a white pericarp. The check varieties ITA212 and Moroberekan were included in this cluster. The accessions of C3 like those of C2 were grown in lowland cropping systems.

The Cluster C4 (51 accessions) developed longer culm length (1190 mm), long leaf blades (560 mm), long ligules (20 mm), and a long crop cycle (138 days). Anthocyanin was not observed in any of the plants, 96% had a horizontal to descending flag leaf at maturity, and 90% grew drooping panicles. All the accessions developed at least two panicle secondary branches and 96% had a white pericarp. Contrary to C2 and C3, the accessions of C4 were grown in deep water cropping systems.

The Cluster C5 (64 accessions) was characterised by a short culm length (890 mm), early maturing plants (108 days), long grains (9.5 mm), and narrow grain width (2.5 mm). Anthocyanin was not observed on the basal leaf of 95% of the samples, and 80% had a horizontal to descending flag leaf at maturity. Each plant of the group developed at least two panicle secondary branches and 100% had white pericarps. The inter-specific checks Nerica4 and Nerica14 were included in this cluster. Like C2 and C3, the accessions of C5 were grown in lowland cropping systems.

Calculation of the Fisher Distance confirmed the significant differences between each class (p-value < 0.0001). The huge difference between C1 and the other clusters was highlighted. Groups C3, C4, and C5 were closer in distance compared to C1 and C2 (Table 3.7).

Table 3.7: Fisher Distance calculated for the five rice clusters

	C1	C2	C3	C4	C5
C1	0	49.5	128.6	87.9	94.6
C2	49.5	0	27.4	26.8	28.1
C3	128.6	27.4	0	5.1	10.7
C4	87.9	26.8	5.2	0	5.7
C5	94.6	28.1	10.7	5.7	0

P-value < 0.0001 when comparing each cluster with each other

Furthermore, a discriminant analysis performed using both quantitative and qualitative variables confirmed that 99% of the samples were correctly classified. Accessions in clusters C1 and C2 were 100% accurately allotted, while 99%, 96% and 98% of the accessions in clusters C3, C4 and C5, respectively, were accurately allotted.

Given the large distance between C1 and other clusters, and the fact that C1 assembled *O. glaberrima* accessions, the C1 cluster was investigated for intra-cluster differentiation. For this purpose, a dendrogram was built using the coordinate of the accessions on the axes of the factorial analysis. This led to the identification of four sub-clusters (Gg1, Gg2, Gg3, and Gg4) (Figure. 3.11).

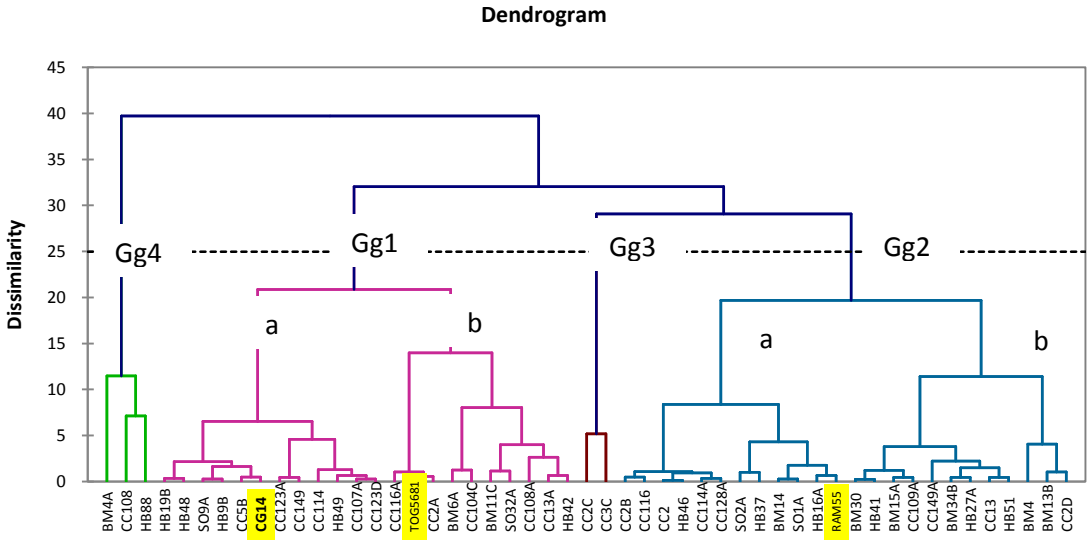


Figure 3.11: Dendrogram of 48 *O. glaberrima* accessions developed from 11 quantitative and 30 qualitative traits

The nine quantitative and qualitative attributes discriminating the four sub-clusters are summarised in Table 3.8.

Table 3.8: Quantitative and qualitative parameters distinguishing between the 48 *O. glaberrima* accessions of the collection

Traits	Gg1 ± Sd	Gg2 ± Sd	Gg3 ± Sd	Gg4 ± Sd
Culm length (mm)	970 ± 121	950 ± 182	1030 ± 86	700 ± 291
Panicle length (mm)	300 ± 22	300 ± 23	330 ± 9	250 ± 41
Number of days to maturity	113 ± 10.4	105 ± 11.9	114 ± 11.3	90 ± 16.3
Leaf blade pubescence on blade surface	Glabrous (100%)	Glabrous (52%)	Hairy on upper (100%)	Hairy on upper (67%)
Auricle colour	Yellowish green (95%)	Yellowish green (70%)	Yellowish green (50%)	Whitish (67%)
Flag leaf attitude at anthesis	Horizontal (70%)	Horizontal (57%)	Horizontal (100%)	Erected to semi-erected (67%)
Panicle secondary branch	1 to 2 (90%)	1 to 2 (83%)	≤ 1 (100%)	1 to 2 (100%)
Lemma and palea colour after anthesis	Brown spot or furrow on green (65%)	Green or yellowish green (52%)	Black (100%)	Yellowish green (67%)
Awn distribution	Tips to upper half (60%)	Tips to upper half (60%)	Whole panicle (100%)	Tips only (67%)

Sd refers to standard deviation. Values in bracket are the proportion of individuals belonging to the major category of the named trait.

The Sub-cluster Gg1, composed of 20 accessions including the checks CG14 and Tog5681, was characterised by glabrous leaves and yellowish green auricles. The Sub-cluster Gg2, containing 23 samples plus the check RAM55, was principally distinguished from Gg1 by green lemma and palea colour after anthesis. The Sub-cluster Gg3 had the longest crop cycle amongst the *O. glaberrima*, and the longest culm and panicle lengths. The two accessions (CC2C and CC3C) in this cluster were pubescent on the upper leaf blade, grew horizontal flag leaf after anthesis, developed a maximum of one secondary branch in their panicle and developed a black lemma and palea after anthesis. The Sub-cluster Gg4 comprised of very early maturing varieties with shorter panicles and culms. The three accessions (BM4A, CC108, and HB88) in this sub-cluster had one to two secondary branches in their panicles and two accessions grew erect flag leaf after anthesis (Table 3.8). The Fisher Distance test confirmed the significant differences between each cluster (p-value < 0.0001). The discriminant analysis performed using both quantitative and qualitative parameters confirmed that the samples were correctly allotted, at 100%.

The agro-morphological characterisation in the field helped to distinguish *O. sativa* and *O. glaberrima*. Figure 3.12 portrays the proportion of *O. glaberrima* and *O. sativa* in the

different collection areas and the predominance of *O. sativa* over *O. glaberrima* in the collected samples.

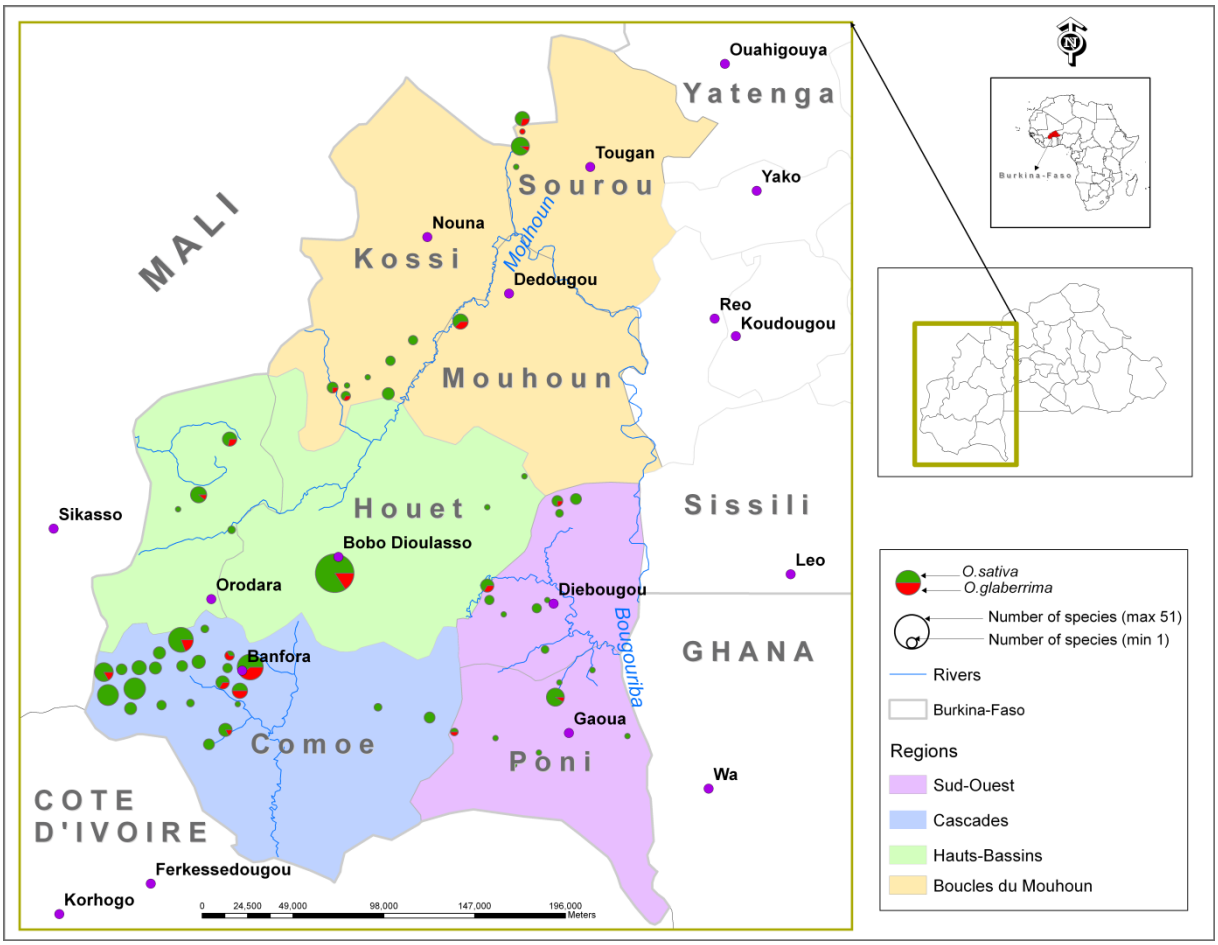


Figure 3.12: Map of collecting zones showing the different proportion of *O. glaberrima* and *O. sativa* in the collected areas in Burkina Faso (source unpublished map, GIS section, AfricaRice)

3.4.3 Geographical pattern of phenotypic diversity

Accessions of CC and SO had long culms and CC samples were late maturing. The samples from BM had shorter leaf blade lengths. Rice plants from SO had longer flag leaves. The samples from CC and HB had more panicles compared to those from BM and SO. SO samples produced more panicle primary branches, which is an important yield parameter. The grains from CC weighed less compared to those of BM. Samples from BM showed differences in culm habit. Samples from BM also had more anthocyanin in their nodes than CC and SO, while SO samples displayed low anthocyanin in their nodes. CC samples shattered less as compared to those of BM and HB (Table 3.9).

Table 3.9: Significant variables on the 50 discriminating traits of the collection on regional basis in Burkina Faso rice collection

Variable \ region	BM	CC	HB	SO
Culm length (mm)	901[a]	996[b]	928[a]	990[b]
Leaf blade length (mm)	432[a]	478[b]	454[a,b]	508[b,c]
Flag leaf length (mm)	328[a]	353[a]	335[a]	387[b]
Number of panicle per plant	11.5[a]	13.1[b]	13.7[b]	12.3[a]
Number of days to first heading	81.1[a]	90.6[b]	83.6[a]	83.4[a]
Number of days to maturity	117.5[a]	131.1[b]	120.4[a]	121.1[a]
Number of panicle primary branches	12.0[a]	12.4[a]	12.5[a,b]	13.1[b]
100 grains weight (g)	2.60[a]	2.41[b]	2.50[a,b]	2.46[a,b]
7.3.23. Culm habit	2.09[a]	1.59[b]	1.54[b]	1.67[b]
7.4.5. Lemma and palea colour at anthesis	8.04[a]	6.57[b]	6.81[b,c]	7.45[a,c]
7.5.5. Lemma and palea colour at maturity	2.23[a]	2.79[b]	2.67[a,b]	2.30[a,b]
7.4.23. Panicle shattering	2.52[a]	1.52[b]	2.17[a]	1.81[a,b]
7.5.13. Sterile lemma colour	1.83[a,b]	1.93[b]	1.77[a]	1.94[b]
7.3.29. Underline node colour on culm	1.8[a]	1.6[b]	1.7[a,b]	1.4[c]

The letters a, b, c and d indicate the significance between variables in a row.

The estimate of the Shannon-Weaver Diversity Index (H') of the 14 variables distinguishing the collection varied from 0.10 to 0.99 according to the region (Table 3.10).

Table 3.10: Estimates of Shannon-Weaver Diversity Index of the 14 most distinguishing characteristics of the rice collection (H' value per region)

Variable	H' BM	H' CC	H' HB	H' SO	Mean
Culm length	**0.71**	0.65	0.62	0.60	0.68
Panicle length	**0.23**	0.12	0.10	0.14	0.14
Leaf blade length	**0.59**	0.41	0.43	0.53	0.47
Flag leaf width	0.30	**0.38**	0.31	0.21	0.34
Ligule length	**0.57**	0.37	0.55	0.27	0.46
Number of days to maturity	**0.92**	**0.92**	**0.92**	0.74	0.97
Grain length	0.59	**0.72**	**0.72**	0.65	0.71
Basal leaf sheath colour	**0.64**	0.55	0.57	0.26	0.56
Culm lodging resistance	**0.99**	0.96	0.98	0.90	0.99
Flag leaf attitude	**0.95**	0.93	0.88	0.79	0.93
Stigma colour	0.34	**0.36**	**0.36**	0.23	0.35
Panicle attitude of main axis	**0.90**	0.79	0.78	0.77	0.81
Panicle secondary branching	0.61	**0.70**	0.65	0.57	0.68
Pericarp colour of the caryopsis	**0.44**	0.35	0.31	0.30	0.37
mean ± SE	**0.63 ± 0.07**	**0.59 ± 0.07**	**0.59 ± 0.07**	**0.50 ± 0.07**	**0.60 ± 0.07**

H' is the Shannon-Weaver Index and SE refers to standard error of the mean

The average phenotypic diversity of the collection was 0.6. BM portrayed the highest index of diversity (0.63), while SO showed the lowest diversity index (0.50). Therefore, the BM Region is the region with the most diversified rice germplasm, although it accounted for only

15% of the collection. Conversely, the SO Region was the region with the least diversified germplasm. Both the CC and HB regions had the same H' (0.59) and occupied the second place of diversification, although 50% and 25% of the collection were collected in those two regions, respectively. The panicle length was the least diversified trait, whereas culm lodging resistance was the most diversified trait across the regions. The discriminant analysis performed on the 50 variables based on the regional scale exhibited the particularity of BM samples (Figure 3.13).

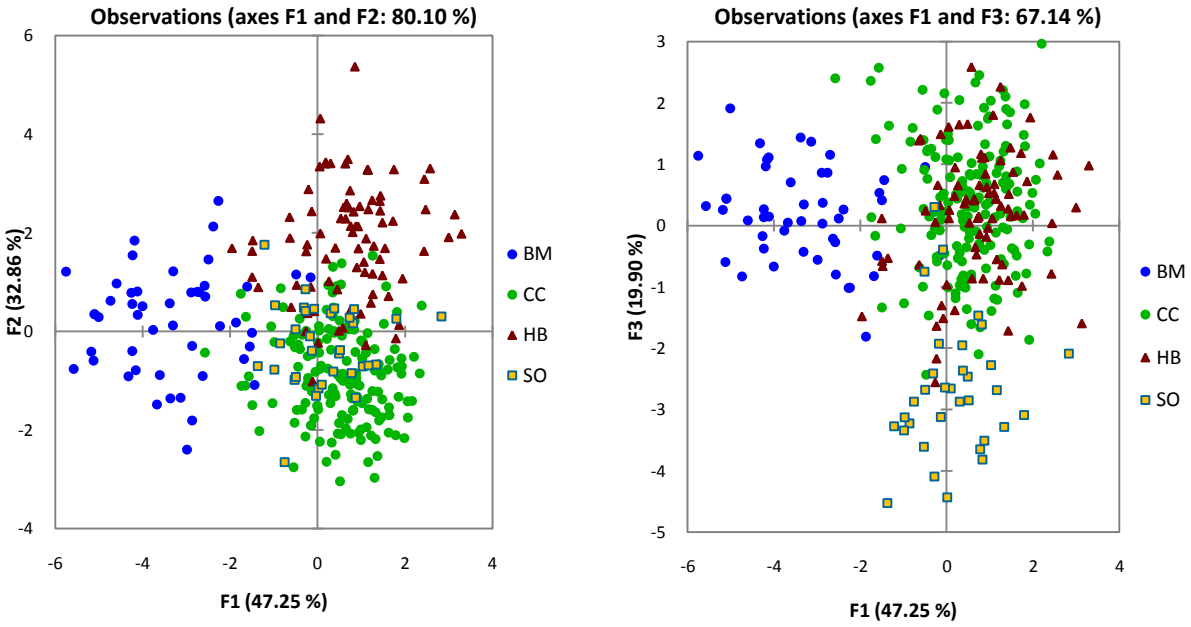


Figure 3.13: Discriminant analysis performed on axes F1/F2 and F1/F3 on the four regions of rice collection in Burkina Faso.

The Fisher's Distance test did not find any significant difference between CC and SO samples, while BM was strongly differentiated from CC, HB and SO (Table 3.11).

Table 3.11: Fisher's Distances and P-values on a regional basis

	BM	CC	HB	SO
BM		< 0.0001	< 0.0001	< 0.0001
CC	2.707		< 0.0001	0.111
HB	2.639	1.948		0.017
SO	1.803	1.209	1.392	

Fisher's Distance estimates appear below the diagonal and the P-values appear above the diagonal

3.5 Discussion

3.5.1 Diversity of the collection

The analysis of the diversity of Burkina Faso rice landraces highlighted the predominance of *O. sativa* relative to *O. glaberrima* accessions. *O. sativa* accounted for 86% of the accessions collected, while *O. glaberrima* represented only 14%. The dominance of *O. sativa* was noted in the previous collection, where 475 of 527 rice samples collected were *O. sativa* landraces (Sié, 1991). Analysis of agro-morphological variables demonstrated important differences between the two species. Almost all *O. glaberrima* accessions belonged to Cluster C1, characterised by short ligules and erect to semi-erect panicles. Three quantitative and six qualitative traits out of the 50 used were able to distinguish the *O. glaberrima* sub-classes. The colour of the lemma and palea after anthesis was one of the contributing criteria to classify the four sub-classes of *O. glaberrima*. This parameter has rarely been taken into account in rice diversity analysis. However, the four groups obtained did not reflect any agronomical differences. None of the *O. glaberrima* sub-classes showed a grouping of accessions related to the cropping systems. Previously, phenotypic differentiation within *O. glaberrima* has been determined by the cropping system and the length of crop cycle (Bezançon, 1993; Sié et al., 1998).

The original clustering gave five clusters, four of which contained *O. sativa* accessions only and one *O. glaberrima*. One of the four *O. sativa* clusters identified in this collection (C2) develops a strong coloration from anthocyanin on their basal leaf sheaths and leaves. This group appears to be similar to *O. glaberrima* but given the absence of short ligules and drooping panicles, they cannot be considered to be *O. glaberrima* plants. *O. glaberrima* and this cluster shared the same average number of panicle primary branches and grain length. Cluster C2 included *O. s. indica* accessions and showed strong presence of anthocyanin, and were grown in lowland cropping systems. The accessions of clusters C3 and C4 portrayed characteristics of traditional *O. s. indica*, with long cropping cycles. The accessions of C3 with a short culm are all lowland accessions, whereas the accessions of C4 with a long culm are deep water accessions. The accessions of C5 had a short height and were early maturing. They must be improved *O. sativa* cultivars introduced decades ago by NGO and government. Early maturing rice cultivars have been introduced into Burkina Faso since 1959 (Dumont, 1966).

The diversity of *O. glaberrima* was hidden when it was pooled with *O. sativa*. But the diversity was revealed when *O. glaberrima* was segregated from *O. sativa* into its own group and analysed alone. *O. glaberrima* and *O. sativa* are two different species and should be evaluated separately. In the characterisation process, some of the *O. glaberrima* accessions developed secondary panicle branches. However, they remained with erect or semi-erect panicles as with all of the *O. glaberrima* accessions. Such *O. glaberrima* cultivars were not identified in rice collection from Guinea (Barry et al., 2007b). Likewise, *O. glaberrima* cultivars with white grains were also found. *O. glaberrima* accessions with white pericarps have not been found in *O. glaberrima* samples from Senegal, Gambia, Guinea Bissau, Guinea Conakry, Sierra Leone, Togo and Ghana (Nuijten et al., 2009). Therefore, the *O. glaberrima* cultivars from Burkina Faso developed certain traits found nowhere else, underlining the richness of this collection. Attention should be paid to the collection for the thorough investigation of particular landrace cultivars in *O. glaberrima* and *O. sativa*.

3.5.2 Regional diversity study

The overall diversity of the collection depicted by the Shannon-Weaver Index (0.6) highlighted the diversity of this Burkina Faso rice collection. This index is higher compared to the ones obtained by Nuijten and Treuren (2007) and Sanni et al. (2008), who studied the genetic diversity of upland rice in Gambia (0.31) and the agro-morphological diversity of the *O. sativa* rice landraces of Côte d'Ivoire (0.47), respectively. More than 50% of the accessions were collected in the CC Region. Rice cultivation abounds in this region due to favourable rainfall and the presence of lowland areas. However, the diversity is less than that of the BM Region. This is understandable because 50% of the rice landraces were collected in a small area in the CC Region, whereas 15% were collected over an extensive area of the BM Region. The movement of varieties is facilitated by short distances between villages. Moreover, as in The Gambia (Nuijten and Treuren, 2007), in the CC Region, women control rice cultivation, rice processing and commercialisation. Through marriage, women bring the favourite varieties of their mother to the village of their husband or share a valuable variety of their mother-in-law with their sisters. Likewise, in Gambia, when women visit relatives, they often bring new rice varieties back to their village (Nuijten and Treuren, 2007). This is supported by the fact that women's names are often assigned to traditional varieties in the CC Region. Consequently, women are more active agents in rice moving from place to place than men.

Varieties tended to have different names across villages (Nuijten and Treuren, 2007). This practice appear to increases rice names diversity at village level, but the genetic diversity at the regional level will not change if the varieties come from within the region. The Baguera and Kangoura villages, where the highest number of varieties (19 varieties each) was found, are located in the CC Region. However, overlapping of varieties does not contribute to increasing diversification. Hence, this could provide an explanation for the fact that, although half of the collection came from the CC Region, the samples of this region were found to be less diversified than those of the BM Region. The BM Region hosts a large share of the total phenotypic diversity and should be considered to be providing informal *in situ* conservation. As the National Agricultural Research Systems of Burkina Faso lack proper infrastructure for long term storage, the identification of a region or an area harbouring the rice diversity of the country would be of great value. The diversity could be conserved in dynamic form in a field and constitute a gene-pool for national and international rice breeders. This requires an active support for the ongoing production of landraces.

There were relatively few landraces and a low diversity in the SO Region. Low diversity in the SO Region could be due to farmers cultivating similar varieties. However, significant differences were not found between landraces from SO and CC. The two regions share the same climatic conditions and are the wettest parts of the country, with rainfall exceeding 1,000 mm per annum. A variety from CC could be easily introduced to SO and vice versa. Moreover, people from CC and SO regions share a strong traditional link. They joke together and there is easy communication between the ethnic groups of the two regions. They still respect the alliance set by their ancestors to live in peace. The adherence to this treaty, sealed many decades ago, facilitates the exchange and the sharing of seeds between these two regions. Consequently, it is not surprising if there is little difference between the landraces of the two zones. In addition, Rice landraces are declining in the SO Region due to the wide-spread adoption of modern varieties. The reports of Projet Riz Pluvial (PRP, 2007) and Projet d'Appui à la filière Riz (PAFR, 2004) documented that in total 7,531.52 ha; 2,674.40 ha; 1,209.74 ha and 566.88 ha of rice areas have been developed in SO, HB, BM, and CC regions, respectively. These projects introduced modern varieties and encouraged farmers to cultivate them, which contributed to the decrease of the traditional varieties. According to Sié (1984), SO Region was ranked second in terms of the number of rice samples collected (after the CC Region) in 1984. In 25 years, the number of rice landraces has decreased drastically.

Local rice is endangered in this region. Hence, specific samples collected in this region must be conserved carefully to avoid the loss of landraces coming from this region.

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Chapter 4: Molecular characterisation of Burkina Faso rice landraces using 22 microsatellite markers and establishment of a core collection

Abstract

Understanding the genetic diversity of rice and its population structure is a key to the sustainable *in situ* and *ex situ* management of genetic resources. In this study, the molecular analysis of 243 rice varieties collected from 59 villages across Burkina Faso was undertaken, using 22 microsatellite markers. The 22 microsatellite markers were found to be polymorphic with an average allele number of 9.3 and a mean PIC per locus of 0.58, ranging from 0.04 (RM338) to 0.84 (RM1). The molecular diversity study helped to identify two subsets in *O. glaberrima* following regional subdivision: the flooded *O. glaberrima* was encountered in the Cascades Region, while lowland *O. glaberrima* was grown in the Boucle du Mouhoun, Hauts Bassins and Sud Ouest regions. The former is characterised by a long culm and a long growth cycle, while the latter is early maturing with a short culm. In *O. sativa* samples, three sub-sets were identified. The sub-set Sg2 has long growth cycle varieties with long culms, and included flooded *O. sativa indica* and included the check ASD1, B6144 and IR64 were clustered in the sub-set Sg1-1, incorporating *O. s. indica* plants with short culms, which are grown in the lowland cropping system. The *O. sativa* group Sg1-2 has different traits, and incorporates the *O. s. japonica* checks (Moroberekan and Nipponbare) and an *O. s. indica* variety, Gambiaka. Using the molecular data and the framework it provided, a core collection of 52 accessions was established that represents the diversity of rice in Burkina Faso.

4.1 Introduction

Africa is the only continent where the two cultivated rice species *O. glaberrima* Steud and *O. sativa* L. are grown. *O. glaberrima* originated in Africa (Chévalier, 1937; Chang, 1984). The primary centre of diversification of *O. glaberrima* is the inner delta of the River Niger, currently in the Republic of Mali. The regions of Sene-Gambia, covering South Senegal (Casamance), The Gambia and Bissau-Guinea, and the Guinea forest between Sierra Leone and western Cote d'Ivoire, are considered the secondary centres of diversification of *O. glaberrima* (Portères, 1950). The Asian rice, *O. sativa*, on the other hand, could have reached the African continent via three possible routes. The first entry might have been Madagascar, and the second through East Africa (Somalia) during the 10th century and the third entry may

have happened during the 15th century through both the East and West coasts, brought in by the Portuguese, Dutch, French and the British sailors (Bezançon, 1993). The native African rice *O. glaberrima* was cultivated many centuries before the first Europeans arrived on the West African coasts (Linares, 2002).

The introduction of modern varieties with high yield can result in the abandonment of landrace varieties, resulting in diminished genetic diversity, which further reduces the potential for genetic improvement. Despite their low yield, landrace varieties harbour important traits that can be used in breeding. Burkina Faso is a West African country bordered by Mali (the primary centre of diversification of *O. glaberrima*) in the North and by Côte d'Ivoire (believed to be one of the secondary centres of diversity for *O. glaberrima*) in the South. Both *O. glaberrima* and *O. sativa* are cropped in Burkina Faso (Sié, 1991). In a previous study, undertaken to preserve local rice varieties, especially *O. glaberrima,* an extensive collection of rice samples was conducted from November 1983 to February 1984 throughout the country (Sié, 1984). Isozyme markers were used for the evaluation of 312 varieties of this collection, including 289 *O. sativa* and 23 *O. glaberrima* accessions. The *O. glaberrima* varieties were found to be the least diverse, whereas in the *O. sativa* subset, the phenol reaction revealed that *O. s. indica* accounted for 95% of the subset and the remaining 5% was *O. s. japonica*. Enzymatic polymorphisms identified a large number of intermediate forms within the *O. s. indica* subspecies. Two *O. sativa* varieties were found to belong to the *Aus* group (Sié, 1991; Sié et al., 1999). This previous rice collection was not properly conserved because the National Agricultural Research System (NARS) of Burkina Faso (INERA) lacks proper seed stores for long term storage. Furthermore, not all of the previous collections by Sié (1984) from Burkina Faso was available within the AfricaRice genebank. Furthermore, there is a possibility that Burkina Faso rice landraces will start to disappear due to the introduction of modern varieties. Therefore, there was an urgent need to initiate the collection and conservation of rice landraces. During January to May, 2008, 330 accessions of rice were collected from farmers' stores across the four main rice cropping regions of the country (Cascades, Hauts Bassins, Boucle du Mouhoun and Sud Ouest).

Information on the genetic diversity within and amongst related crop varieties is essential for rational use of genetic resources in breeding programmes. Molecular markers are useful tools to portray the structure and assess the genetic variability within and amongst different species. Therefore several marker types have been developed to characterise rice populations.

Microsatellites, also called Simple Sequence Repeat (SSR) markers, have proved to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and amongst African rice collections (Semon et al., 2005; Barry et al., 2007). The genotypic approach looks at neutral diversity, while the phenotypic approach reveals functional diversity. Therefore, agro-morphological characterisation complements and illustrates the extent, the organisation and the specificities of genetic diversity revealed by molecular markers. Although abundant genetic resources can provide a broader genetic background for crop breeding, a large germplasm collection with numerous redundant accessions may be an obstruction to its conservation and utilisation by NARS in developing countries. The establishment of relatively small core collections are essential for the sustainable use and conservation of the collection. A core collection is a subset of a large germplasm collection that contains accessions chosen to represent the genetic variability of the given collection (Diwan et al., 1995). Brown (1989) described the core collection concept as a means to enhance the efficiency in the utilisation of germplasm collections. Core collections, with a minimum of entries, represent the diversity of the entire collection as much as possible (Liu et al., 1999). A small set of the collection, representing the diversity of the entire collection, can be manageable *in situ* and *ex situ* by NARS in developing countries like Burkina Faso. In this present study, the molecular diversity of 330 rice accessions was studied. The information generated was used to identify a core collection with no duplication.

4.2 Material and methods

4.2.1 Plant material

The rice plant material was collected in 2008 in 59 villages of the four main rice cropping regions (Boucle du Mouhoun, Cascades, Hauts-Bassins and Sud-Ouest) of Burkina Faso (Figure 4.1). The collection team of each region comprised of an INERA technician, an agricultural agent at the departmental level and the leader, Honoré Kam. The agricultural agent of the concerned village informed farmers about the collection mission and summoned a meeting. An inventory of local rice varieties was drawn up in the presence of a group of farmers. Farmers were asked to list the rice varieties they have been cropping for several years and also those varieties that were cultivated by their ancestors and those that the present generation of farmers were continuing to grow. Farmers who owned the listed varieties were identified and the collection team proceeded to collect the varieties from their stocks. Together, farmers identified the chosen varieties by consensus. They provided information

about each variety, like the name, the growth cycle, and the appropriate rice cropping system. Data on longitude, latitude and altitude of each village were also collected with a GPS (Global Positioning System). Modern varieties released through the agricultural and research networks were not collected. Overall, 330 rice varieties were collected, mostly bulk grains and sometimes in panicles. Agro-morphological evaluation was conducted in the field. Each accession was phenotyped using 19 quantitative and 15 qualitative agro-morphological traits for rice descriptors (Table 4.1), which were extracted from "Descriptors of Wild and Cultivated Rice" (Bioversity-International et al., 2007). The *O. glaberrima* and *O. sativa* species were separated on the basis of ligule length and panicle features. Accessions with long ligules (> 10 mm) and drooping panicles were classified as *O. sativa* species, while accessions with short ligules (<7 mm) and erect panicles were classed as *O. glaberrima* species. Then 48 and 282 accessions, categorised as *O. glaberrima* and *O. sativa* respectively, were analysed for their molecular diversity. Figure 4.1 portrays the distribution of the two species in the collection area. For the purpose of easy identification, the samples collected in Boucle du Mouhoun, Cascades, Hauts-Bassins and Sud-Ouest regions were assigned the prefix BM, CC, HB and SO, respectively.

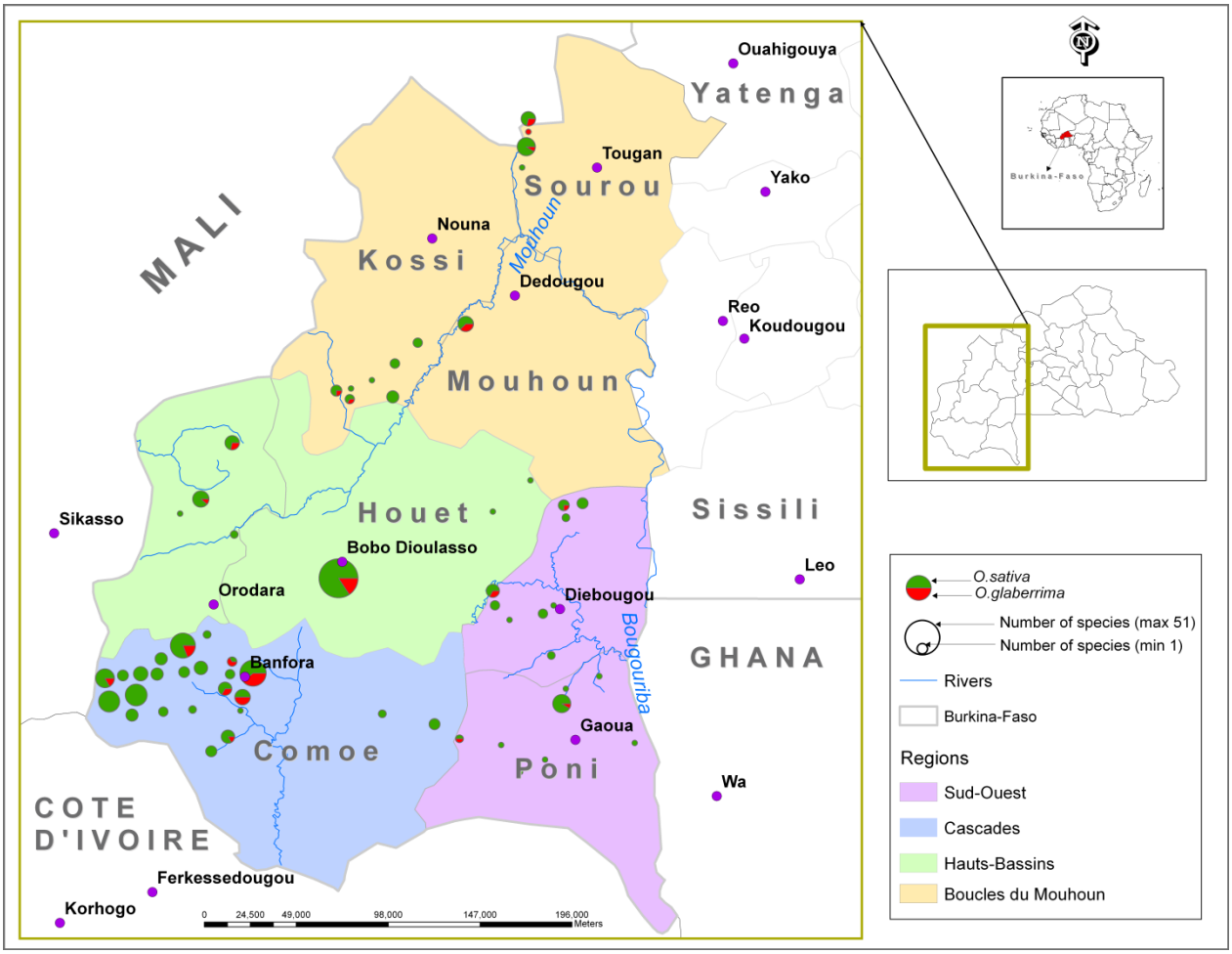

Figure 4.1: Map showing the four regions visited where *O. glaberrima* and *O. sativa* were collected in Burkina Faso (source: unpublished map, AfricaRice, GIS section)

Table 4.1: Nineteen quantitative and 15 qualitative phenotypic traits used to evaluate the collection in the field

Code	**Quantitative traits measured**
7.2.2.1	Number of days from effective seedling to first heading
7.2.3.1	Number of days from effective seedling to main heading
7.2.4.1.	Number of days from effective seedling to maturity
7.3.13	Ligule length (mm)
7.3.18	Leaf blade length (cm)
7.3.19	Leaf blade width (cm)
7.3.20	Flag leaf length (cm)
7.3.21	Flag leaf width (cm)
7.3.25	Culm length
7.3.26	Culm number
7.4.14	Number of basal primary branches on panicle
7.4.17	Number of panicle per plant
7.5.1	Panicle length (cm)
7.5.15	Grain Length (mm)
7.5.16	Grain width (mm)
7.5.17	Grain thickness (mm)
7.5.18	100-grain weight (g)
7.5.20	Caryopsis Length
7.5.21	Caryopsis Width

Code	**Qualitative traits measured**
7.3.8	Leaf blade attitude
7.3.9	Leaf blade pubescence
7.3.9.1	Leaf blade pubescence on blade surface
7.3.22	Flag leaf attitude early observation
7.3.32	Lodging resistance
7.3.34	Flag leaf attitude late observation
7.4.2	Stigma colour
7.4.5	Lemma and palea colour
7.4.9	Awn distribution
7.4.19	Panicle attitude of main axis
7.4.20	Panicle attitude of branches
7.4.21	Panicle secondary branches
7.4.23	Panicle shattering
7.5.10	Sterile lemma length
7.5.23	Caryopsis pericarp colour

The code assigned to each measured trait is in accordance with the "Descriptors of Wild and Cultivated Rice" (Bioversity-International et al., 2007)

4.2.2 DNA extraction

Four seeds from each accession were sown and fresh young leaves of 21 days old of the four plants were harvested and put in a single tube for DNA extraction. DNA was extracted according to the method described by Risterucci et al. (2000). Two centimetres of frozen leaves were powdered in a grinding machine and mixed with 480 µl of extraction buffer (1.4 M NaCl, 100 mM Tris HCl pH 8.0, 20 mM EDTA, 10 mM Na_2SO_3, 1% PEG 6000, 2% MATAB) preheated at 75°C. The extract was then homogenised for 10 s with a vortex and incubated for 30 min at 75°C; after being cooled at room temperature (25°C), a volume of 480 µl of chloroform-isoamyl alcohol (24:1 v/v) was added. The tube was mixed gently before being centrifuged at 4000 rpm for 15 min and the supernatant containing the DNA was transferred to new tubes and precipitated at –20°C after the addition of 270 µl of ice-cold isopropanol. The tubes were centrifuged at 4000 rpm and the supernatant was discarded. A volume of 100 µl ethanol was added and centrifuged at a maximum speed to wash the DNA. Finally, the DNA was suspended in 30 µl of TE buffer (0.7 M NaCl, 50 mM TRIS-HCl,

10 mM EDTA, pH 7.0). DNA concentration was checked with the fluoroscan apparatus (Labsystems, Vantaa, Finland) and the final concentration diluted at 5 ng μl^{-1}.

4.2.3 Genotyping

Twenty-two SSR markers, already used for rice genetic diversity studies (Garris et al., 2005), were chosen. The Polymerase Chain Reaction (PCR) amplification was performed in a 384 well thermocycler (Mastercycler® 384, Eppendorf) on 5 ng of DNA in a 10 µl final volume containing buffer (10 mM Tris-HCl pH 8, 100 mM KCl, 0.05 % w/v gelatin, and 2.0 mM $MgCl_2$), 0.1 µM of reverse primer, 0.08 µM of forward primer, 200 µM of dNTP, 0.1 U of Taq DNA polymerase and 0.1 µM of M13 primer-fluorescent dye IR700 or IR800. The PCR process was: initial denaturation at 94°C for 4 min; 35 cycles of 94°C for 60 s; hybridisation temperature for 60 s; elongation at 72°C for 60 s; and a final elongation step at 72°C for 8 min. The PCR products were mixed with two desmiling reagents migrated in multiplex (two primer pairs on Dye 700 and 800) on polyacrylamide 6.5% gel on a DNA sequencer (Li-cor® DNA analyser Model 4300 (the genotyping platform at Génopole Montpellier Languedoc Roussilon, CIRAD)). Ten Generation Challenge Programme reference set rice varieties (ARC10317, Aswina, Basmati 310 (1), Basmati 310 (2), Dumsikalam, IR64, Khao Yang, Maybelle, Nipponbare and Padi Raoekang) were used as controls for allele size. A size marker was deposited in each side of the gel for allele size detection. The SSR markers were revealed following the protocol of Roy et al. (1996), applied with the automated infrared fluorescence technology of LICOR IR2 sequencers in the genotyping and robotics platform of "Centre de Coopération International en Recherche Agronomique pour le Développement" (CIRAD, Montpellier). For a given locus, the forward primer of the SSR was designed with a 5'-end M13 extension (5'-CACGACGTTGTAAAACGAC-3'). Allele sizes were determined using SAGA (version 3.2) software package, which encodes genes in base pairs. Tog5681 and CG14 (*O. glaberrima*), Moroberekan and Nipponbare (*O. s. japonica*), ASD1, B6144, IR64 and Gambiaka (*O. s.* indica) were used as checks for the classification of the accessions.

4.3 Data analysis

Genotypic data was first assessed for matching genotypes at all loci with GenAlEx 6.2 software (Peakall and Smouse, 2006). The genotypes with identical alleles at all loci were deleted and a matrix of genotypes which differed at least in one locus was considered for further analysis. PowerMarker 3.25 software package (Liu and Muse, 2005) was used to calculate allele number (NA) per locus, Major Allele Frequency (MAF), Heterozygosity (H_o),

Gene diversity (Gd) and Polymorphism Information Content (PIC). The gene diversity, which is referred to as expected heterozygosity (H_e) was calculated as n/(n-1) ($1-\sum p_i^2-H_o/2n$); where n is the number of individuals, p_i the frequency of the i allele and H_o the number of observed heterozygotes (Nei, 1987). The PIC was calculated as $1-\sum p_i^2-\sum_i\sum_{j>i}2p_i^2p_j^2$; where p_i and p_j are the frequencies of the i and j alleles respectively (Botstein et al., 1980).

A Bayesian clustering analysis was undertaken to determine the population structure using the Structure 2.3.3 software package (Pritchard et al., 2000; Hubisz et al., 2009). The posterior probabilities were estimated using 500,000 iterations of Markov Monte Carlo Chain (MCMC) with a length of burn-in period of 50,000 iterations. The optimal population number (*K*) was determined after 20 independent runs, using the admixture model and the option of correlated allele frequencies between populations, as this configuration is considered best according to Falush et al. (2003) in the case of subtle population structures. *K* was tested from *K=1* to *K=12* in order to determine the number of populations (*K*) supported by the data. The method of Evanno et al. (2005) was used to infer population number. The parameter $\Delta K = m(|L''(K)|)/s[L(K)]$ was calculated as the mean of absolute values of the second order change of the likelihood distribution $L''(K)$ divided by the standard deviation of the likelihood $L(K)$. The optimum value of this distribution was interpreted as the true number of populations, *K* (Evanno et al., 2005).

To investigate the genetic relationships between accessions, a genetic dissimilarity matrix was computed in DARwin software 5.0.155 (Perrier and Jacquemoud-Collet, 2006). The dissimilarity between samples was calculated by using simple matching based on the Sokal and Michener (1958) index. The dissimilarity formula is:

$d_{ij} = 1 - \frac{1}{L}\sum_{l=1}^{L}\frac{m_l}{\pi}$ With d_{ij}: dissimilarity between units *i* and *j*, *L*: number of loci, π: ploidy and m_l : number of matching alleles for locus *l*

The dissimilarities were used to perform factorial coordinate analysis for graphical representations on Euclidean planes that conserve, at best, distances between units. The dissimilarities were also employed for the construction of an unweighted neighbour-joining tree (Perrier et al., 2003). The neighbour-joining tree was used to build the core collection by using stepwise techniques that proceed by successive pruning of redundant genotypes. Redundancy means that some genotypes are very close and therefore bring, in part, the same

information on their diversity. This procedure searches for a subset of genotypes minimizing redundancy between genotypes, but limits if possible the loss of diversity.

4.4 Results

4.4.1 Genetic diversity

The genetic diversity analysis was conducted on 243 genotypes including 37 *O. glaberrima* and 206 *O. sativa* accessions, which differed at least in one locus. In total, 204 alleles were found with the 22 SSR markers with a mean of 9.3 alleles per locus. The largest number of alleles was found with RM474 (26 alleles) and the smallest with RM338 (2 alleles). The former distinguished 31 varietal groups, whereas the latter distinguished only three varietal groups. The mean PIC per locus was 0.58 within a range of 0.04 (RM338) to 0.84 (RM1, RM287 and RM514). The heterozygosity rate varied from 0 to 7.7% with a mean value of 2.8% in the overall population (Table 4.2). When comparing SSRs with the different repeat motifs, those with the 2-bp motif had the highest PIC values, particularly the GA repeats (RM1, RM287 and RM514), whereas the 3-bp motif markers (RM338) had the lowest number of alleles and PIC values. Markers with high allele number (Na) values (RM514, RM287, RM1, RM19, RM552, RM154, and RM474) set the samples apart more distinctly in a population, resulting in a higher number of genotypes (varietal groups) and thereby depicting high gene diversity.

The diversity indices of the two species were compared for the 22 SSR loci. The diversity observed in *O. sativa* was higher compared to that of *O. glaberrima*. The mean PIC was 0.53 in *O. sativa* and 0.25 in *O. glaberrima*. Similarly, the Na of *O. sativa* (8.4) was twice as high as that of *O. glaberrima* (4.0). Among the 22 SSR markers, only seven (RM1227; RM215; RM237; RM447; RM474; RM5 and RM514) had more than four alleles in *O. glaberrima* accessions, whereas in *O. sativa* accessions, only 4 loci (RM124; RM338; RM452 and RM484) had less than five alleles. These four loci also showed lower allelic diversity in the overall population. Only two SSRs (RM237 and RM474) had their PIC values higher in *O. glaberrima* than in *O. sativa* (Table 4.2).

Table 4.2: Comparison of the diversity in the overall collection and between the two species (*O. glaberrima* and *O. sativa*) by the 22 SSR markers

		Total (N=243)			*O. sativa* (N=206)			*O. glaberrima* (N=37)		
Marker	**chr**	**Na**	**H**	**PIC**	**Na**	**H**	**PIC**	**Na**	**H**	**PIC**
RM1	1	17	0.050	0.84	16	0.045	0.82	3	0.081	0.08
RM1227	12	9	0.033	0.57	6	0.010	0.44	6	0.162	0.20
RM124	4	3	0.008	0.29	3	0.005	0.33	2	0.028	0.03
RM14643	3	10	0.021	0.47	9	0.015	0.33	3	0.054	0.12
RM154	2	13	0.042	0.78	12	0.050	0.74	2	0.000	0.05
RM19	12	10	0.035	0.79	9	0.041	0.74	1	0.000	0.00
RM215	9	7	0.013	0.58	5	0.000	0.47	5	0.103	0.22
RM237	1	7	0.013	0.53	7	0.005	0.42	5	0.061	0.63
RM271	10	8	0.009	0.62	6	0.010	0.56	3	0.000	0.35
RM287	11	11	0.077	0.84	11	0.059	0.81	4	0.171	0.30
RM316	9	7	0.017	0.33	5	0.010	0.32	4	0.054	0.29
RM338	3	2	0.008	0.04	2	0.005	0.04	2	0.027	0.03
RM431	1	6	0.004	0.62	6	0.005	0.56	2	0.000	0.33
RM447	8	9	0.063	0.58	9	0.050	0.49	5	0.135	0.40
RM452	2	3	0.022	0.34	3	0.010	0.36	2	0.130	0.18
RM474	10	26	0.032	0.79	20	0.026	0.74	15	0.069	0.83
RM484	10	3	0.000	0.34	3	0.000	0.35	2	0.000	0.27
RM5	1	7	0.040	0.70	7	0.026	0.62	5	0.125	0.12
RM510	6	5	0.029	0.32	5	0.010	0.16	3	0.135	0.12
RM514	3	11	0.041	0.84	11	0.035	0.83	8	0.091	0.78
RM538	5	10	0.008	0.72	10	0.005	0.68	2	0.029	0.03
RM552	11	20	0.042	0.83	20	0.035	0.87	3	0.081	0.08
Mean		**9.3**	**0.028**	**0.58**	**8.4**	**0.0207**	**0.53**	**4.0**	**0.070**	**0.25**

Chr: chromosome; Na**:** Allele Number; H: Heterozygosity and PIC: Polymorphism Information Content

4.4.2 Population structure

The inference of population structure using the method of Evanno et al. (2005) in the overall collection strongly supported two populations (*K*=2) as the best number of the group. A clear peak was observed at the value of *K*=2. The first group was designated G1 and the second group G2. G1 was composed of 48 accessions, while G2 had 192 accessions. All the 37 *O. glaberrima* accessions with short ligules (<7 mm) and erect panicles, including the check variety Tog5681, were assigned to G1. However, 11 accessions (BM15, BM18, BM24, BM29, CC31, CC145, SO3, HB5, HB53, HB62, and HB84) falling into G1 are morphologically closer to *O. sativa*. They were considered as atypical accessions. The 192 accessions in G2 had the distinctive features of *O. sativa*: long ligules (> 10 mm) and drooping panicles. In addition to the accessions that were clearly assigned to a single group, where more than 70% of their inferred ancestry was derived from one of the model based group, three accessions in the sample: CC114 (69% G2), HB7 (52% G2), and HB11 (69% G2), were categorised as admixture of G1 and G2, sharing less than 70% of common ancestry

with G2, the *O. sativa* group (Figure 4.2). The check CG14 was an admixture between G1 and G2, sharing 58% of common ancestry with G1, the *O. glaberrima* group.

Figure 4.2: Model based ancestries of 243 rice genotypic accessions estimated from 22 nuclear SSR loci using Structure (Pritchard et al., 2000). Red and green refer to *O. sativa* and *O. glaberrima*, respectively

Each species was then analysed separately to unveil the population structure inside *O. glaberrima* and *O. sativa*. The method of Evanno et al. (2005) revealed three sub-populations in the *O. glaberrima* group and two sub-populations in *O. sativa* group. In *O. glaberrima*, the first group, designated *Glaberrima* Group 1 (Gg1), corresponded to early maturing accessions with a mean cycle of 96 ± 10 days. The check CG14 was included in this group. The second group, named *Glaberrima* Group 2 (Gg2), had an average cycle of 118 ± 6 days, corresponding to late maturing varieties. The third group, designated *Glaberrima* Group 3 (Gg3), was intermediate between Gg1 and Gg2 with a mean cycle of 106 ± 9 days. The check variety Tog5681 was included in this group.

The Gg2 was from the Cascades Region and included lowland and deep water *O. glaberrima* accessions. Two samples from this group, HB42 and HB51, were collected from the Hauts-Bassins Region, even though their origins were from the Cascades Region. HB42 is called "Gnou" and originated in the village of Douna, while HB51 is named "Safati" and came from the village of Diarabakoko. The accessions of Gg3 that originated in the Cascades Region shared the deep water cropping system. The accessions of Gg3, except those from the Cascades Region, were from areas of lowland cropping system. The accessions of Gg1 were from the Hauts-Bassins and Boucle du Mouhoun regions and belonged to lowland cropping system. Accessions with ancestry lower than 70% in any of the three groups were considered as intermediates. The earliest maturing accession, HB88, was an intermediate variety, sharing 42% ancestry and 55% ancestry with Gg1 and Gg3, respectively. In total, nine accessions

were intermediates: BM6A, CC2B, CC107A, CC109A, CC123D, SO9A, HB9B, HB27A and HB88.

The three groups (Gg1, Gg2 and Gg3) of *O. glaberrima* obtained from the Structure analysis were plotted by factorial analysis. Gg2 portrayed consistency in its grouping, while Gg1 and Gg3 were split into two sub-sets. The deep water accessions of Gg3 from the Cascades Region were gathered in a sub-cluster named Gg3-1. The early maturing group Gg1 was split into early and very early maturing groups named Gg1-1 and Gg1-2, respectively (Figure 4.3). The two accessions of Gg1-1 (BM13B and HB46) had more than 100 days to maturity (102 and 104 days respectively), whereas the two accessions of Gg1-2 (HB41 and BM34B) matured in less than 100 days (81 and 96 days, respectively) and were close to the earliest maturing variety, HB88 (79 days).

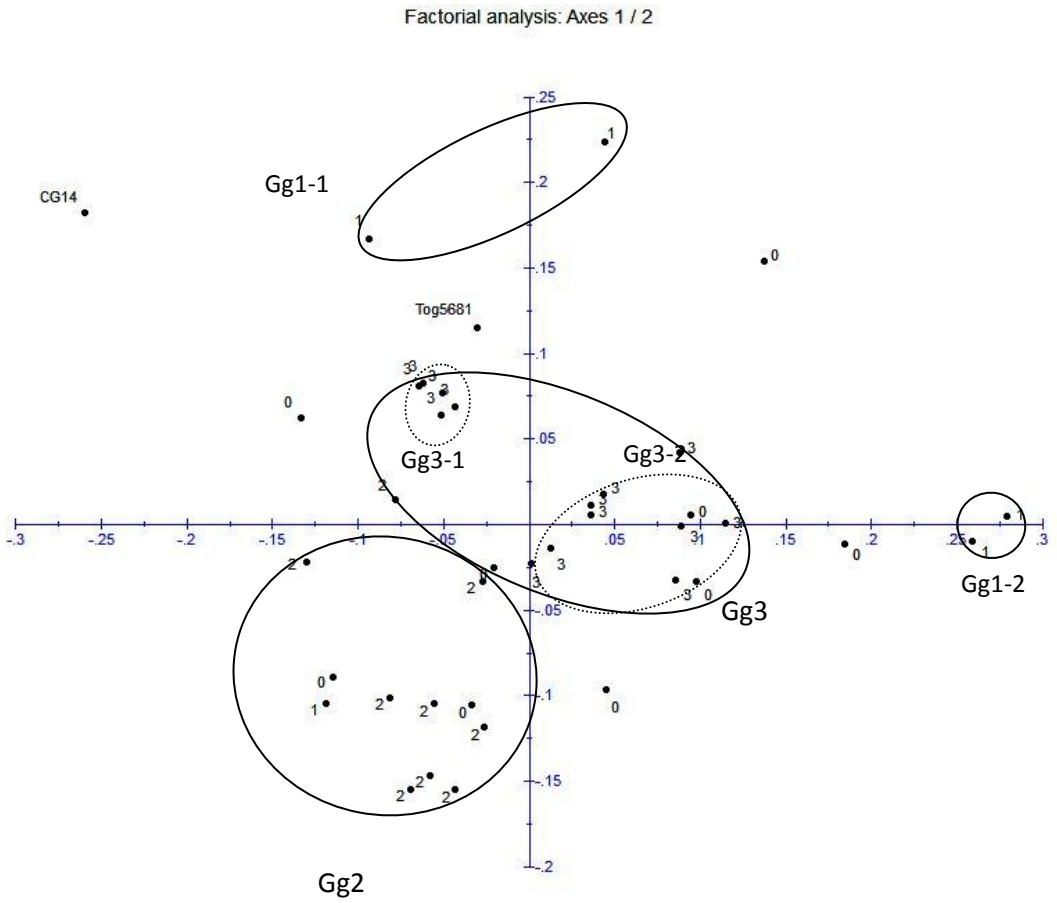

Figure 4.3: Factorial analysis on 39 *O.glaberrima* accessions based on 22 SSRs data projected on axis 1/2

0, 1, 2 and 3 represent intermediate accessions (0); *O. glaberrima* Group 1 (Gg1); *O. glaberrima* Group 2 (Gg2) and *O. glaberrima* Group 3 (Gg3), respectively. CG14 (upland *O. glaberrima*) and TOG5681 (lowland *O. glaberrima*) are check varieties.

Interestingly, the factorial analysis split the *O. glaberrima* accessions that reflected a regional pattern. The first axis seperated *O. glaberrima* collected in Boucle du Mouhoun (BM) Region from that of Hauts-Bassins (HB) Region. The second axis separated *O. glaberrima* collected in the Cascades (CC) Region, corresponding to accessions Gg2 and Gg3-1, from the accessions of the three other regions, corresponding to accessions Gg1 and Gg3-2 (Figure 4.4).

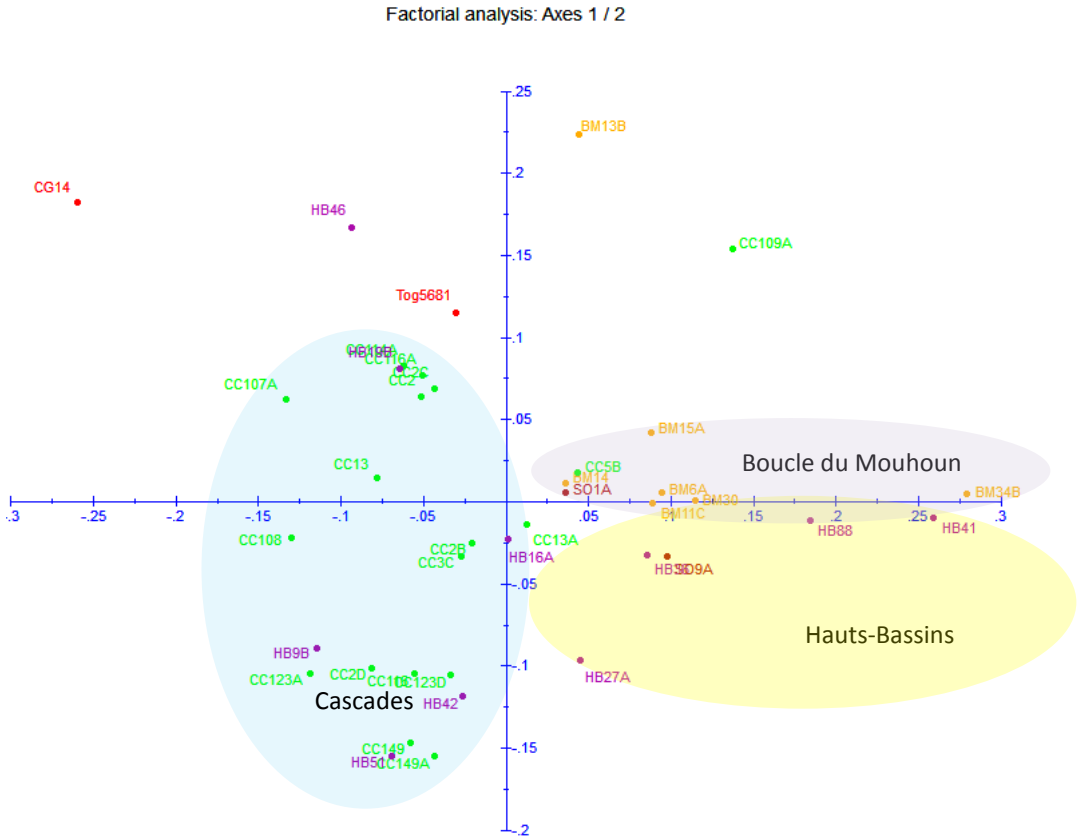

Figure 4.4: Factorial analysis on 39 *O.glaberrima* accessions including checks CG14 and Tog5681 based on 22 SSRs data projected on axis 1/2 showing regional pattern

Samples collected in the Boucle du Mouhoun region (coloured in yellow); samples collected in the Cascades region (coloured in green); samples collected in the Hauts-Bassins region (coloured in purple) and samples collected in the Sud-Ouest region (coloured in brown).

Table 4.3 shows the relative proximity between Gg2 and Gg3-1 in one hand; and between Gg1 and Gg3-2 on the other hand.

Table 4.3: Summary of the main phenotypical traits distinguishing the groups identified in the collection by molecular markers

Group	CL (cm)	PL (cm)	LBL (cm)	cycle (days)	CN	GL (mm)	100 GW (g)
Gg1 ± sd	86.0 ± 15.7	29.2 ± 2.4	46.8 ± 3.1	95.8 ± 10.4	14.9 ± 0.3	8.4 ± 0.3	2.5 ± 0.5
Gg2 ± sd	100.4 ± 13.4	30.2 ± 2.5	51.1 ± 5.2	118.3 ± 5.7	19.4 ± 4.6	8.2 ± 0.3	2.3 ± 0.2
Gg3-1 ± sd	106.4 ± 6.7	32.1 ± 1.5	55.4 ± 4.4	109.6 ± 9.3	20.4 ± 5.4	8.6 ± 0.2	2.4 ± 0.3
Gg3-2 ± sd	95.1 ± 14.8	30.0 ± 2.7	46.5 ± 6.7	104.0 ± 9.3	16.5 ± 3.1	8.4 ± 0.3	2.3 ± 0.2
Sg1-1 ± sd	81.4 ± 17.1	25.3 ± 2.1	40.7 ± 10.6	115.6 ± 9.6	16.4 ± 5.2	9.3 ± 0.7	2.7 ± 0.4
Sg1-2 ± sd	82.4 ± 10.2	26.0 ± 3.1	43.7 ± 8.1	103.5 ± 11.1	13.4 ± 5.4	8.0 ± 1.7	2.0 ± 0.8
Sg2 ± sd	101.3 ± 15.7	25.5 ± 2.1	47.7 ± 7.7	135.6 ± 17.7	16.0 ± 4.6	9.0 ± 0.8	2.5 ± 0.4

CL= culm length; PL= panicle length; LBL= leaf blade length; CN= culm number; GL = grain length; 100 GW = 100 Grain weight (g)

The molecular subdivision was consistent with the phenotypic differentiation between *O. glaberrima*. Indeed, the *O. glaberrima* accessions from the CC Region, including Gg2 and Gg3-1 groups, had longer culms with very weak culm strength. They had very weak resistance to lodging like those from BM, HB and SO regions. The majority of *O. glaberrima* accessions from the HB and BM localities had short cycles of around 100 days, while those from the CC Region had cycles beyond 100 days. The CC accessions had glabrous leaves, while the accessions from HB and BM were hairy on their leaf blade surfaces. The three HB accessions (HB9B, HB42 and HB51) inside the sub-group Gg2 were all glabrous, confirming the provenance of HB41 and HB51 from the CC Region. The accessions from CC had none or a maximum of one secondary branch on their panicles, whereas the ones from the other regions had around two secondary branches per panicle. The *O. glaberrima* from BM, HB and SO had short sterile lemma, while those of CC were a mixture of long sterile lemma (8 accessions) and short sterile lemma (9 accessions). *O. glaberrima* of BM and HB regions were characterised by short flag leaves (210-400 mm) like Gg2 from Cascades, whereas the Gg3-1 from the CC Region had an intermediate flag leaf length (410-600 mm).

An Unrooted Neighbour Joining Tree (Saitou and Nei, 1987) performed on the *O. glaberrima* accessions separated the Deep Water (DW) accessions from Lowland accessions. *O. glaberrima* samples from CC Region were mostly from deep water cultivation, while the *O. glaberrima* from the three other regions were concentrated in lowland cropping systems (Figure 4.5).

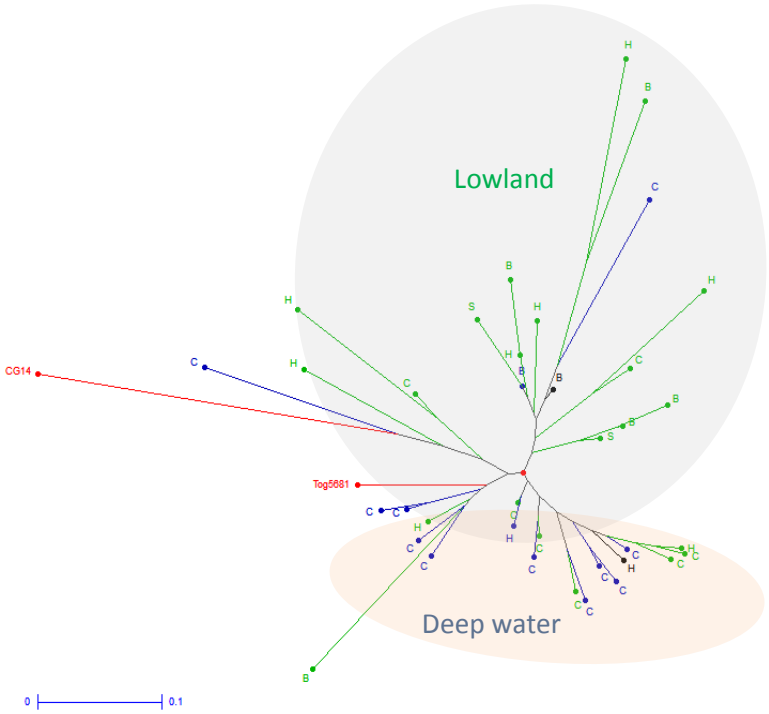

Figure 4.5: Unrooted neighbour-joining tree based on allelic data from 22 SSR loci among 37 *O. glaberrima* accessions from Burkina Faso and two cheks using the simple matching index. Blue lines represent accessions from deep water cultivation and green line accessions from lowland cropping system. Capital letters B, C, H and S stand for Boucle du Mouhoun, Cascades, Hauts-Bassins and Sud-Ouest regions, respectively. CG14 and Tog5681 were used as checks varieties and are highlighted in red colour. *O. glaberrima* samples from the Cascades Region were mostly from deep water cultivation, while the *O. glaberrima* from the three other regions were concentrated in lowland cropping systems.

Contrary to the *O. glaberrima* accessions where three sub-clusters were identified via the Structure output, *O. sativa* spp showed two sub-sets on using the method of Evanno et al. (2005). The two groups were named *Sativa* Group 1 (Sg1) and *Sativa* Group 2 (Sg2). Sg1 was characterised by short culm length (820 ± 160 mm) and early maturing plants (114 ± 12 days), cropped in lowland areas, while Sg2 was characterised by long culm length (1010 ± 160 mm) and late maturing accessions (135 ± 17 days) cultivated in deep water and lowland cropping systems. The check varieties B6144, Moroberekan, Nipponbare and Gambiaka were included in the Sg1 sub-set. Accessions with ancestry lower than 70% in any of the two groups were considered intermediates. Thus, 18 intermediates were identified.

The individuals of the *O. sativa* spp were plotted by factorial analyses, based on their sub-structures determined by Structure analysis. Contrary to the *O. glaberrima* species, no geographical pattern was found in *O. sativa* species. The Sg1 group was split into two sub-sets: Sg1-1 and Sg1-2, whereas Sg2 remained consistent (Figure 4.6). The individuals in the Sg1-1 cluster were cropped in lowland agro-systems, as were the check varieties B6144 and IR64. Moreover, this sub-set was closer to group Sg2. Sg2 contained accessions from the

deep water cropping system as shown by the check variety ASD1, which is from the flooded cropping system. Sg2 and Sg1-2 were *O. s. indica* sub-species, as indicated by the *O. s. indica* checks ASD1, B6144 and IR64. However, the sub-set Sg1-2 was clearly separated from sub-set Sg1-1 and Sg2 (Figure 4.6). Sg1-2 group contained accessions that matured relatively early (104 ± 11 days) compared to Sg1-1 (116 ± 13 days) and Sg2 (136 ± 18 days). Likewise, flag leaf characteristics differentiated Sg1-2 from Sg1-1 and Sg2. Observed during anthesis, Sg1-2 had semi-erect flag leaves inclined at an angle of approximately 45°, while others had erect flag leaves.

The accessions in Sg1-2, including the *O. s. indica* variety (Gambiaka), were closer to the *O. s. japonica* checks Moroberekan and Nipponbare (Figure 4.6). This group contained 11 individuals (considered previously as atypical accessions when analysing the 243 accessions), which showed different features. Two accessions (BM24 and HB62) had the lowest tiller number (5 tillers) of the entire collection. Sg1-2 was divided into weaker culm strength and stronger culm strength plants. Accessions BM15, BM18, CC31, CC145 and HB5 had weaker culm strengths, while accessions BM24, HB62 and HB84 had stronger culm strengths. In addition, the level of panicle shattering separated individuals in Sg1-2. BM15, BM24, CC31, and HB84 were low shattering while BM18, CC145 and HB5 showed high grain shattering at maturity.

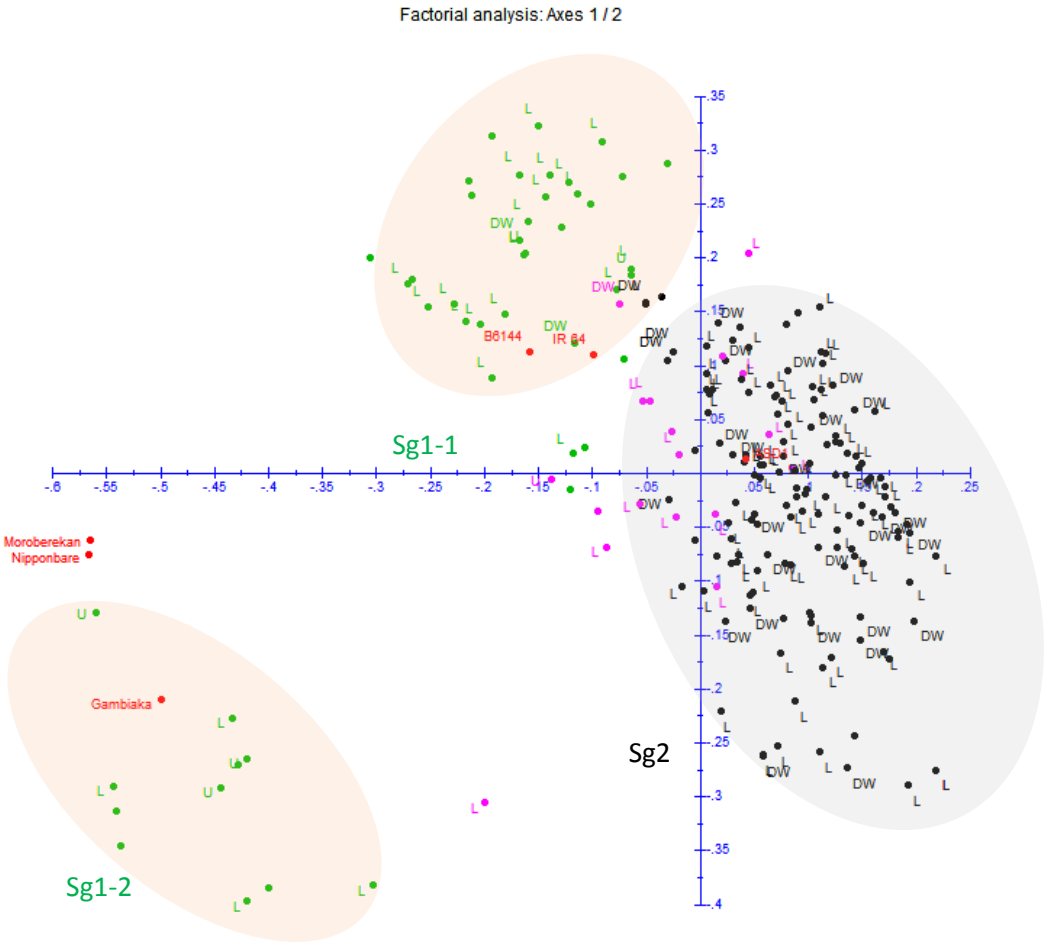

Figure 4.6: Factorial analysis on 206 *O.sativa* accessions from Burkina Faso and 6 checks based on 22 SSRs data projected on axis 1/2

Accessions of Sg2 group shown in black dots; green dots display accessions of Sg1 group split into two sub-sets Sg1-1 and Sg1-2. Pink dots represent intermediate accessions. The cropping systems were taken into account. DW for samples grown in deep water cropping system, L for samples grown in lowland cropping system and U for samples grown in upland cropping system.

The scatter data of the factorial analysis conducted on Axis 1/3 indicated that the accessions HB62 and HB84 were close to the *O. s. japonica* checks Moroberekan and Nipponbare (Figure 4.7).

Differences in grain characteristics were found in the sub-set Gs1-2. The grain length of BM15, BM24, BM29, and CC31 was close to 10 mm while that of BM18, CC145, HB5 and HB53 was close to 6 mm, and the grain length of HB62 and HB84 was intermediate between the two (8 mm). The grain width of BM18, CC31, CC145, and HB53 was close to 2 mm whereas the rest had a grain width closer or higher to 3 mm with HB62 recording a value of 4 mm. Furthermore, BM18, CC145, HB53 and SO3 were also grouped together (Figure 4.7).

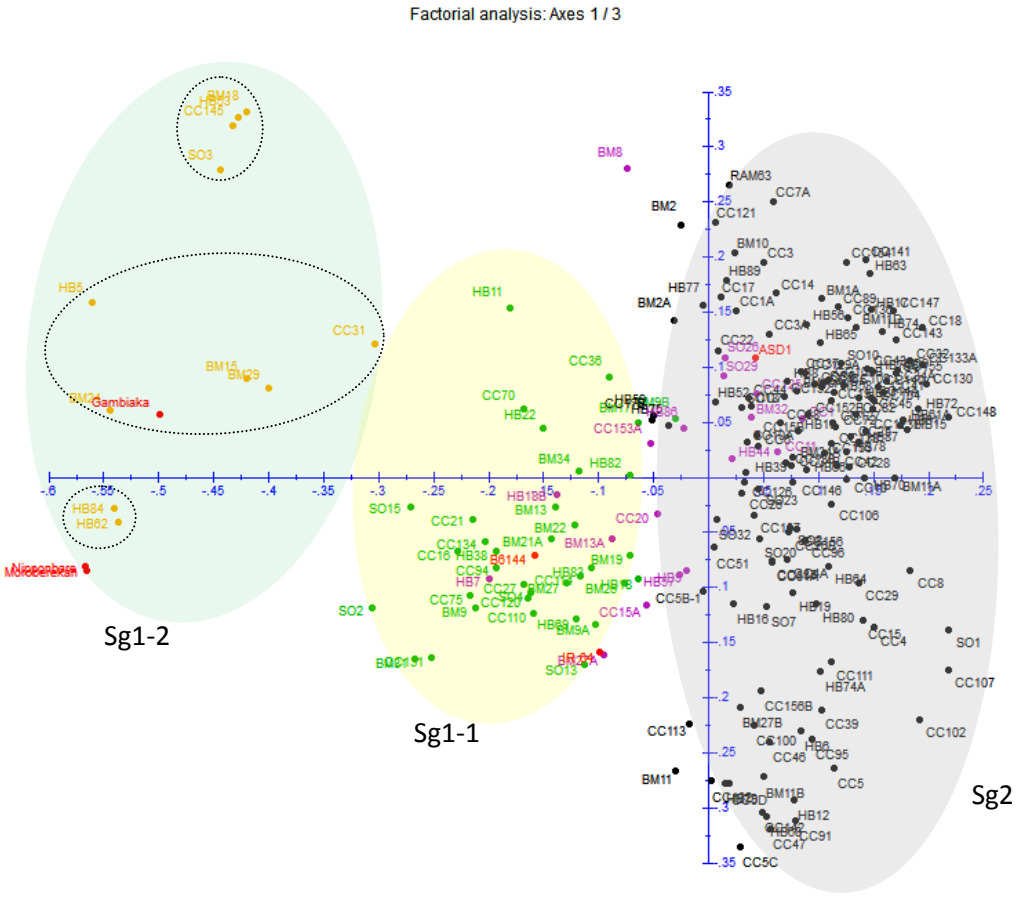

Figure 4.7: Factorial analysis performed on 206 *O.sativa* accessions from Burkina Faso and 6 checks based on 22 SSRs data projected on axis 1/3

In black dots, individuals of *O. sativa* group 2 (Sg2); in green dots, individuals of the sub-set of *O. sativa* group 1-1 (Sg1-1); in yellow, individuals of *O. sativa* group 1-2 (Sg1-2); in purple, intermediate accessions. The check varieties are highlighted in red

Therefore, the phenotypic features of plants from Sg1-2 sub-set were consistent with their division in three grouping levels as shown in Figure 4.7. BM18, CC145, HB53 and SO3 constituted the first level of grouping, BM15, BM24, BM29, CC31 and HB5 constituted the second level of grouping and HB62 and HB84 formed the third level of grouping. HB62 and HB84 are believed to belong to *O. s. japonica* sub-species while the other individuals of Sg1-2 were different from the individuals of that species. However, the Unrooted Neighbour Joining Tree Method split the group Sg1-2 between the *O. s. indica* variety, Gambiaka, and the *O. s. japonica* varieties Moroberekan and Nipponbare. The accessions BM24, HB62, HB84 and the intermediate form HB7 were in the same branch with Moroberekan and Nipponbare, while BM15, BM18, BM29, CC31, CC145, HB5, HB53 and SO3 shared the same branch with the Gambiaka variety. The intermediate accessions HB9, HB18B and CC20 were on one side, whereas on the other side were the intermediate accessions BM13A, BM27A and HB7, which were in-between IR64 and the Gambiaka varieties, and in-between

Moroberekan, Nipponbare, and B6144. The intermediate accessions CC1 SO26, SO29, CC15A and HB57 were in-between *O. sativa* group Sg2 and Sg1-1 (Figure 4.8).

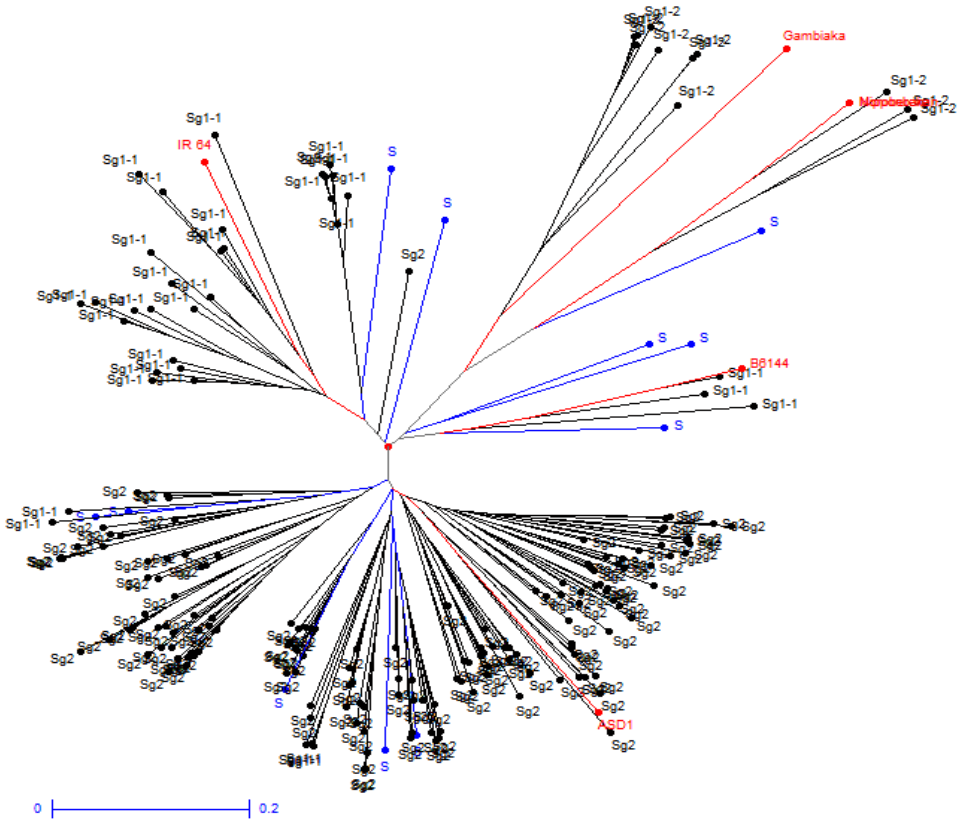

Figure 4.8: Unrooted Neighbour-Joining Tree based on allelic data from 22 SSR loci among 206 *O. sativa* accessions from Burkina Faso rice landraces using the simple matching index.
O. sativa group 2 (Sg2); the two sub-sets of *O. sativa* group 1 (Sg1-1 and Sg1-2); in blue lines, the intermediate accessions (S). The check varieties (ASD1, B6144, Gambiaka, IR64, Moroberekan, and Nipponbare) are highlighted in red

4.4.3 Core collection establishment

A core set with a limited allelic diversity reduction and a higher number of sample reductions was identified separately for the *O. sativa* and *O. glaberrima* accessions, using the software package DARwin. A core set of 13 individuals was obtained for *O. glaberrima,* while 39 individuals were identified for *O. sativa*. The two were pooled together and a unique core of 52 accessions with a total of 182 alleles was established. A novel neighbour-joining tree with the 52 accessions was built. In the core collection, the sub-clusters obtained previously when investigating the *O. glaberrima* and the *O. sativa* collections independently were clearly

represented (Figure 4.9). The alleles present in the core collection accounted for 89% of the alleles of the whole collection. The list of the accessions of the core collection are summarised in Table 4.4.

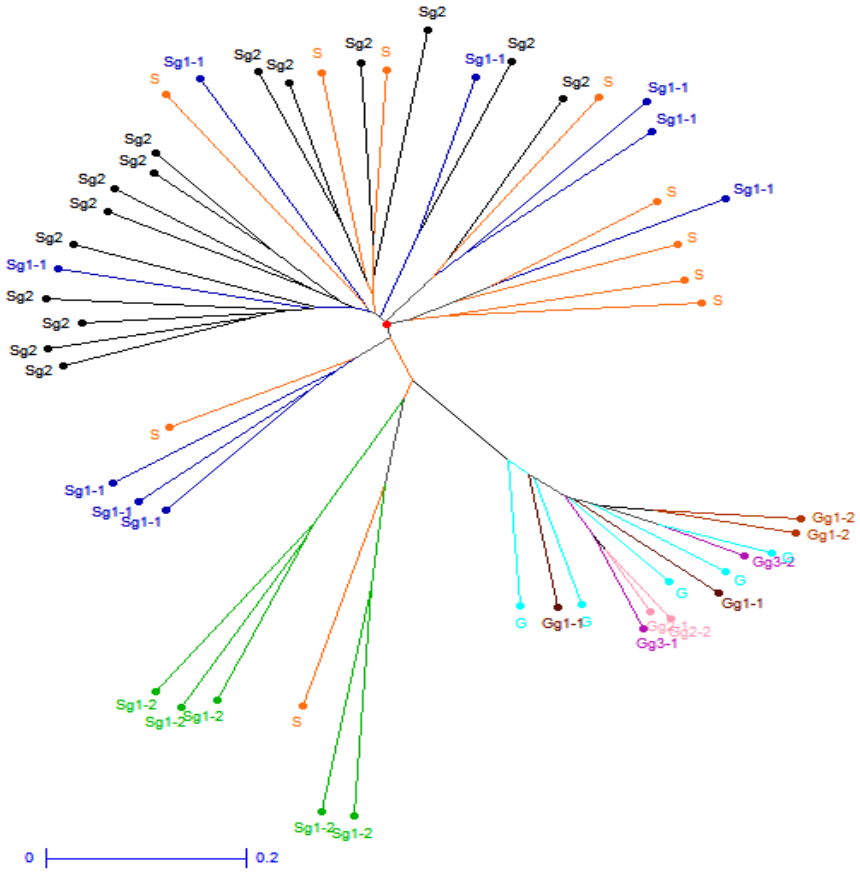

Figure 4.9: Neighbour-Joining Tree of 52 individuals of the core collection including all the groups identified by separately studying *O. glaberrima* and *O. sativa* accession based on 22 SSR data. Intermediate *O. glaberrima* accessions (G); *O. glaberrima* group 1-1 (Gg1-1); *O. glaberrima* group 1-2 (Gg1-2); *O. glaberrima* group 2-1 (Gg2-1). *O. glaberrima* group 2-2 (Gg2-2); *O. glaberrima* group 3-1 (Gg3-1); *O. glaberrima* group 3-2 (Gg3-2); intermediate *O. sativa* accessions (S); *O. sativa* group 1-1 (Sg1-1); *O. sativa* group 1-2 (Sg1-2); *O. sativa* group 2 (Sg2)

Table 4.4: List of the accessions of the core collection and their respective groups

Accession	Local name	Group	Accession	Local name	Group
BM6A	Malotelemani	G	CC35	Gnangnanhou	Sg2
BM13B	Sonksonke	G1-1	CC37	Lassina	Sg2
BM15A	Djoutchèmè	G3-2	CC43	Dembélé	Sg2
BM34B	Lamizana	G1-2	CC51	Gnonkpienkpao	Sg2
CC2	Gnonre	G3-1	CC70	Fitidjio	Sg1-1
CC108	Tchiramahihou	G2-1	CC111	Tiombiè	Sg2
CC109A	Gbarountchiè	G	CC130	Maloba	Sg2
CC114	Lokofouo	Sg1-1	CC131	Kalonani	Sg1-1
CC123A	Kamelekan	G	CC134	Wèrè-wèrè	Sg1-1
CC149A	Malofin	G2-2	CC136	Boriyo	Sg2
HB9B	Banankoro	G	CC139	Woloni	Sg2
HB41	Boewo	G1-2	CC156B	Malowile	Sg2
HB46	Safati	G1-1	SO2	Air-bouake	Sg1-1
HB88	Tchièfagamalo	G	SO3	Mouimiougou	Sg1-2
BM9	Badji -maloba	Sg1-1	SO26	Marnii	S
BM11	Kandeka	Sg2	SO32	Maloba	Sg2
BM13A	Sonksonke	S	HB7	Kankan-malo	S
BM15	Djoutchèmè	Sg1-2	HB9	Banankoro	S
BM24	Kiougayibou	Sg1-2	HB11	Banankoro	Sg1-1
BM27A	Heredougou	S	HB18B	Dorado	S
CC1	Dregbon	S	HB44	Wèrèni	S
CC4A	Toumihiennan	Sg2	HB62	Zia	Sg1-2
CC7	Benkawili	Sg2	HB69	Kolog-noré	Sg1-1
CC11	Trouchèmin	S	HB77	Banfora	Sg2
CC17B	Djongolo	Sg2	HB82	Malblé	Sg1-1
CC31	Beregadougou	Sg1-2	HB86	Boanga	S

Intermediate *O. glaberrima* accessions (G); *O. glaberrima* Group 1-1 (Gg1-1); *O. glaberrima* Group 1-2 (Gg1-2); *O. glaberrima* Group 2-1 (Gg2-1). *O. glaberrima* Group 2-2 (Gg2-2); *O. glaberrima* Group 3-1 (Gg3-1); *O. glaberrima* Group 3-2 (Gg3-2); intermediate *O. sativa* accessions (S); *O. sativa* Group 1-1 (Sg1-1); *O. sativa* Group 1-2 (Sg1-2); *O. sativa* Group 2 (Sg2)

4.5 Discussion

The aim of molecular characterisation of the 330 rice accessions was to study the population structure and the genetic diversity amongst and between *O. glaberrima* and *O. sativa* groups, in order to establish a rice core collection of Burkina Faso landraces, that also reflected their phenotypic traits. The 22 SSR markers used for the diversity analysis were found to be polymorphic. The allele numbers per locus varied from 2 to 26, with a mean allele number of 9.3 per locus. Barry et al. (2007), in their study of diversity of rice in maritime Guinea using 11 SSR markers, found a mean allele number of 12, which was slightly higher than that found

in this study. This could be due to low number of loci used in their studies. However, the main reason could be the diversity richness in Maritime Guinea rice collection compared to that of Burkina Faso rice collection. Guinea is believed to be the second centre of diversity of *O. glaberrima* and a port entry of *O. sativa* in West Africa (Portères, 1950).

The mean allele number found in *O. glaberrima* (4) by Barry et al. (2007) was similar to the results in this study (4), confirming that there is less allelic variability within *O. glaberrima* accessions. However, the structure established three groups (Gg1, Gg2 and Gg3) within the *O. glaberrima* accessions. On a regional basis, the *O. glaberrima* samples can be separated according to their cropping systems: flooded and lowland (Bezançon, 1993). This subdivision was reinforced by phenotypic traits highlighting the differences between the two groups. The Cascades Region harbours many traditional basin areas and this part of the country has rainfall exceeding 1,000 mm per year. Therefore, this region appeared to be conducive for the evolution of *O. glaberrima* with longer growth cycles and long culms adapted to deepwater cultivation. Conversely, lowland *O. glaberrima* with shorter growth cycles and short culms were encountered in the Boucle du Mouhoun, Hauts-Bassins and Sud-Ouest regions.

Contrary to *O. glaberrima*, geographical patterns were not discernible for *O. sativa*. However, analysis of the population structure using molecular markers revealed two subsets (Sg1 and Sg2) and 18 intermediate accessions. The plants from Sg2 with long growth cycles and long culms were cropped in the deep water cropping system. They must be varieties that resist submersion and are adapted to flooded soil like ASD1 variety (Yamauchi and Biswas, 1997). The presence of some lowland rice in that group on factorial analysis on 206 *O. sativa* accessions and six checks suggests that certain accessions could be double cropped in flooded and drained soil. Nevertheless, they would be very sensitive to water shortage. The early maturing *O. sativa* accessions with short culms, grown in lowland areas, were gathered in group Sg1 and split in two sub sets (Sg1-1 and Sg1-2) due to many differences separating individuals close to the checks B6144 and IR64 on one hand and individuals close to the checks Moroberekan, Nipponbare and Gambiaka, on the other hand. Phenotypic traits revealed that the latter (Sg1-2) included plants with dissimilar features. In the Unrooted Neighbour Joining Tree based on allelic data from 22 SSR loci amongst 206 *O. sativa* rice landraces BM24, HB7 (an admixture), HB62 and HB84 were in the same group as Moroberekan and Nipponbare. HB62 and HB84 shared similar characteristics with *O. s. japonica* and they are more probably *O. s. japonica* cultivars. The low representation of *O. s.*

japonica in Burkina Faso was reported earlier by Sié et al. (1999). As in Sié (1991), the two accessions to be most probably *O. s. japonica* (HB62 and HB84) were found in the Hauts-Bassins (HB) Region. In this study, an abundance of intermediate accessions between *O. glaberrima* and *O. sativa* and within the two species is reported. Accessions CC114, HB7 and HB11 are believed to be an interspecific hybrid between of *O. sativa* and *O. glaberrima*. Interspecific varieties between *O. sativa* and *O. glaberrima* were also reported by Barry et al. (2007) and Semon et al. (2005). Admixed varieties are important for breeding purposes. They can help to overcome crossing barriers between species. The *O. glaberrima* parent of upland NERICA varieties, CG14 was found in our study to be an admixture between *O. glaberrima* and *O. sativa*. This could have helped Jones et al. (1997) to overcome the reproductive barrier between the *O. sativa* and *O. glaberrima* and to develop inter-specific progenies.

The organisation of genetic diversity revealed by the genotypic data is in agreement, to some extent, with that based on the phenotypic traits. Genotypic structure took into account the differences between the cropping systems and growth duration in *O. glaberrima* and *O. sativa* species. The phenotypic traits highlighted the differences between groups in detail. Nonetheless, the status of the accessions BM15, BM18, BM24, BM29, CC31, CC145, HB5, HB53, and SO3 was not elucidated enough to put them in any known subspecies. The analysis of the collection structure showed these accessions having in common at least 79% of ancestry with *O. glaberrima* (data not shown). BM24 appears to belong to *O. s. japonica* because it is in the same branch with the two most likely *O. s. japonica* species of the collection and the two *O. s. japonica* checks (Moreberekan and Nipponbare). BM24 is cultivated in the lowlands and its phenotype reflects *O. s. indica* features. The accessions: BM15, BM18, BM29, CC31, CC145, HB5, HB53, and SO3 are clustered with the Gambiaka variety, which is an aromatic rice variety cropped in the lowland cropping system. Accessions: BM18, CC145, HB5, HB53 and SO3 were described by farmers as aromatic rice. Aromatic rices are usually close to *O. s. japonica* (Jayamani et al., 2007; Agrama et al., 2010; Li et al., 2010). BM15 is named "Djoutchèmè". In the rice collection of Maritime Guinea (Barry et al., 2007), a variety named "Djou Kémé" was found to be intermediate between *O. s. indica* and *O. s. japonica.* The two accessions could be the same, given the homonymy and the atypical character of BM15 shown by this molecular study. These atypical accessions are precious resources for rice breeding. The phenotypic and genotypic data were complementary in describing the genetic diversity prevailing in the Burkina Faso rice collection.

The 22 markers used in the diversity analysis of Burkina Faso rice were powerful enough to classify the primary groups. This study showed the efficiency of the Neighbour-Joining Method, as described by Saitou and Nei (1987) and Takahashi and Nei (2000) in the evaluation of genetic diversity. The core collection of 13 *O. glaberrima* and 39 *O. sativa* spp reflects the sub-structures identified in *O. glaberrima* and in *O. sativa* spp, covering the extent of diversity of rice in Burkina Faso. The establishment of seperate *O. glaberrima* and *O. sativa* core collections was followed to avoid reducing the representativeness of *O. glaberrima* in the core collection because *O. sativa* showed more allelic diversity than *O. glaberrima*. This sampling was effective in retaining 89% of the alleles of the entire collection and accounted for 16% of the total collection. This size of the core is acceptable and represents the maximum genetic diversity of the whole collection. In several crop species the sampling percentage of core collections account for 10-30% of the entire resource (Zhang et al., 2009). The genetic diversity present in the entire collection, as well as in the core collection, deserves special attention, to be preserved *in situ* and *ex situ*. This small size of core is manageable by NARS of developing countries and can be easily exploited by breeders and farmers for rice genetic improvement. The accessions excluded from this collection, plus a set of the core collection, will be retained in the AfricaRice genebank for long term storage.

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Chapter 5: Evaluation of a collection of rice landraces from Burkina Faso for resistance or tolerance to *Rice yellow mottle virus* (RYMV)

Abstract

A collection of accessions of Burkina Faso rice germplasm was evaluated for resistance using four Rice yellow mottle virus isolates: Ng122, Ng144, B27 and BF1. B27 is an isolate from Benin was used first, followed by Ng122 and Ng144 (isolates from Niger), and BF1 is an aggressive isolate from Burkina Faso was used last to assess the accessions status against RYMV. Fourteen day old plantlets were inoculated and symptoms scored fortnightly from 14 to 56 days post inoculation (dpi). Plant height was recorded at 49 dpi after all accessions were inoculated with isolates Ng122 and Ng144. All the *O. sativa* accessions were highly susceptible except one (BM24), which combined partial resistance and tolerance. Twenty one *O. glaberrima* accessions out of 49 were found to be resistant to Ng122 and Ng144. When these 21 accessions were subsequently screened with the aggressive RYMV strain BF1, 10 *O. glaberrima* accessions displayed a delay in the appearance of RYMV symptoms. These 10 accessions did not bear the already known *RYMV1* alleles, when an allelic determination test was conducted on them.

5.1 Introduction

Rice is one of the most important cereal crops in the world and especially in many developing countries. In Africa, in terms of consumption, rice is ranked third after maize and sorghum, and constitutes a substantial part of the diet of many African people (FAOSTAT, 2009). However, African countries are not self sufficient in rice production. They depend on huge amounts of imported rice, estimated at 9.6 Million tonnes in 2008 (IRRI, 2009). This makes Africa, the second-largest rice importing region after Asia in the world. Despite the increasing demand for rice, local production of rice remains low because of a series of biotic and abiotic stresses that limit rice productivity in Africa. In Burkina Faso, the major rice pathogens are *Rice yellow mottle virus* (RYMV), *Xanthomonas oryzae* pv *oryzae*, and *Magnaporthe oryzae* Cav. (Balasubramanian et al., 2007). RYMV is by far the most damaging and widespread disease of rice throughout Africa (Kouassi et al., 2005), and severely reduces rice production.

RYMV was first observed in Burkina Faso in 1981 (Salaudeen et al., 2010). Since then, it has become a problematic disease affecting rice in lowland and irrigated cropping systems. RYMV is a member of the genus *sobemovirus*, with a single molecule of positive sense RNA (Opalka et al., 2000). In the field, RYMV is transmitted by insects (beetles, grasshoppers), animals (rats, donkeys, cows) (Bakker, 1974; Sarra, 2005). People also play an important role in its dissemination (Traoré et al., 2009). Susceptible plants are characterised by mottled and yellowing symptoms, stunting, reduced tillering, and the delayed and partial emergence of panicles with reduced grain weight and sterility (Ghesquière et al., 1997). During a survey in 1983 and 1986, 75% of the total cultivated area of rice in the Sahel Region was reported to be affected, together with 40% of the Sudan savannah, 18% of the Guinea savannah, and 7.5% of the tropical rain forest (Awoderu, 1991). The incidence and severity of the disease appeared to depend on the rice varieties, the environment, and the isolate types. The yield losses fluctuate between 10% and 100%, depending upon plant age prior to infection, the rice genotype, and various environmental factors (Konate et al., 1997).

Screening for natural resistance to RYMV has been performed on rice varieties of different geographical origins, including the two cultivated rice species, *O. sativa* and *O. glaberrima* (Fomba, 1988; 1990; Ndjiondjop et al., 1999; Rakotomalala et al., 2008; Thiémélé et al., 2010). Rice cultivar responses to the virus have shown large variability depending upon the genotype and screening conditions (environment, climatic conditions, severity of inoculation, and resistance evaluation methods). Responses to RYMV have been distinguished into three types: highly susceptible, partially resistant, and highly resistant. The resistance at the host plant level is characterised by reduced virus multiplication, reduced symptom expression and limited yield loss (Ghesquière et al., 1997). A major gene for resistance against RYMV, *Rymv1*, has been identified in the *O. s. indica* resistant varieties, Gigante and Bekarosaka (Ndjiondjop et al., 1999; Rakotomalala et al., 2008). This gene, which encodes a translation initiation factor (eIF(iso)4G), is also responsible for the resistance of the resistant *O. glaberrima* accessions Tog5681, Tog5672, and Tog5674, whose alleles (*Rymv1-3*, *Rymv1-4* and *Rymv1-5*, respectively) are distinct from each other and from that of Gigante (*Rymv1-2*), which was fine-mapped onto Chromosome 4 (Albar et al., 2006). Recently, a second major gene *RYMV2* was identified in African cultivated rice, *O. glaberrima* (Thiémélé et al., 2010).
However, prior attempts to breed for resistance using "good sources of resistance" have failed because RYMV mutates to new virulent races that match the resistance in the "resistant" cultivars (Traoré et al., 2006). Thus, there is a need to identify and characterise diverse

sources of genes for resistance to RYMV, in order to broaden the rice gene pool for a sustainable genetic control of the disease. The new approach is to use rice landraces to seek novel genes for resistance or tolerance to RYMV. Despite the endemic status of RYMV in Africa, little screening has been conducted on rice collections at a country level. In Burkina Faso, the two cultivated rice species *O. glaberrima* and *O. sativa* are cropped. A collection of rice landraces undertaken recently throughout the rice cropping areas of the country was available for screening. The objective of the study was to screen the novel rice collection for resistance or tolerance to RYMV and identify their allelic diversity.

5.2 Material and methods

5.2.1 Virus isolates

Four RYMV isolates were used: from Benin (B27), Burkina Faso (BF1) and Niger (Ng122 and Ng144). B27, Ng122 and Ng144 are West African isolates belonging to the same phylogenetic group as the West and Central African strains (Pinel-Galzi et al., 2009). BF1, on the other hand, is an aggressive RYMV S2 strain from Burkina Faso (Ndjiondjop et al., 1999). It was used in previous varietal screening for RYMV resistance evaluation (Ndjiondjop et al., 1999; Rakotomalala et al., 2008; Thiémélé et al., 2010).

5.2.2 Virus multiplication and inoculation

Three RYMV isolates (B27, Ng122 and Ng144) were provided by the AfricaRice Plant Pathology Unit. These were multiplied on the standard susceptible variety, IR64, for two weeks. The BF1 isolate was provided by the « Institut de Recherche Développement (IRD) », France. It had been multiplied on the IR64 variety previously and stored at -80°C under liquid nitrogen. To extract the virus, infected leaves were ground in phosphate buffer, pH 7.2 (10 ml g^{-1} of leaf sample) to produce virus inoculum. An abrasive material, Carborundum (600 mesh), was added to the inoculum. Mechanical inoculation was carried out by rubbing the extracted sap on the upper and lower surfaces of the leaves of two-week-old plants by fingers dipping in the inoculum suspensions. Symptoms' severity was scored on a scale of 1 to 9 according to the IRRI Standard Evaluation System (IRRI, 2002). Highly Resistant (HR) plants were scored as 1 for no symptoms observed; Score 3 for green leaves with sparse dots or streaks, which is considered as Moderately Resistant (MR) plants; Score 5 is for general mottling on the leaves and a 6% to 25% in height reduction and is considered as Moderately Susceptible (MS); Score 7 is for yellowing, mottling and stunting, and is considered as

Susceptible (S). Score 9 is for yellowing, mottling, stunting and plant death, which is considered as Highly Susceptible (HS).

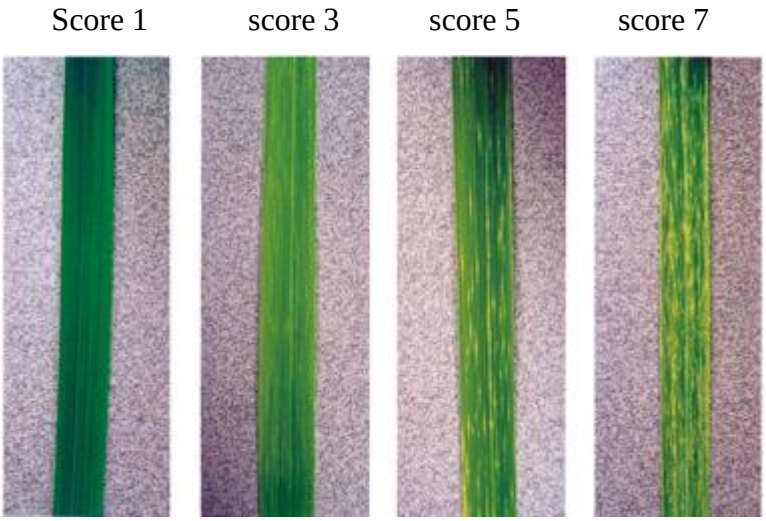


Figure 5.1: Single leaves showing key symptoms rating scale of RYMV disease from 1 to 7. Source: Fargette et al. 2002a

5.2.3 Evaluation of resistance to RYMV

The first evaluation was performed under greenhouse conditions at the AfricaRice research station (Cotonou, Benin) in 2008. Two hundred and ninety six accessions (including 281 *O. sativa* and 15 *O. glaberrima*) were screened with the B27 isolate. In addition, five checks were used: the *O. s. indica* variety Gigante and the *O. glaberrima* variety Tog5681, both carrying *Rymv1-2* and *Rymv1-3* (recessive resistant alleles), respectively (Albar et al., 2006), were used as resistant controls. The highly susceptible *O. s. indica* varieties IR64 and Bouaké189 were also included as susceptible controls in the phenotypic evaluation. Azucena, an *O. s. japonica* variety was used as a partially resistant control. The experimental design was an augmented design with five blocks and two treatments. One treatment was used as a test and inoculated with B27 and the other treatment as a non-inoculated control. The five check varieties were replicated in the five blocks. Disease symptoms were scored at 21 and 42 days post inoculation (dpi). The plant heights were recorded at 42 dpi. Leaves of resistant and tolerant accessions were collected at 56 dpi for the virus content using an enzyme-linked immunosorbent assay (ELISA), as described by Séré et al. (2007).

The second evaluation was performed under greenhouse conditions at the AfricaRice Research Station (Cotonou, Benin) in 2009. It involved 229 accessions (170 accessions already screened in 2008 and 59 newly added accessions) and included 181 *O. sativa* and 48

O. glaberrima samples. They were evaluated with Isolates Ng122 and Ng144 in an augmented randomised incomplete block design, with five blocks. The four checks Tog5681, Gigante, Bouaké189 and IR64 were randomised in each block. Accessions were mechanically inoculated two weeks after sowing. Symptom intensity on leaves was monitored fortnightly after inoculation until 56 dpi. The Area Under Symptoms Progression Curve (AUSPC) was calculated as

$$\text{AUSPC} = \sum_{i=1}^{n} \frac{[(S_i + S_{i+1} - 2)(T_{i+1} - T_i)]}{2}$$

where S_i and $S_{(i+1)}$ correspond to the symptom scores at time T_i and $T_{(i+1)}$ respectively, and n is the total number of observations (Boisnard et al., 2007).

5.2.4 Analysis of the allelic state of resistant and tolerant accessions

The resistant and tolerant accessions identified during the second screening were brought to IRD, Montpellier for an allelic diversity study. Twenty-one *O. glaberrima* accessions with a mean disease score ranging from 1 to 3, and five *O. sativa* accessions (BM16, BM24, HB18, HB18B and HB84) with a disease score ranging from 2 to 4.5 were evaluated for the RYMV resistance alleles *Rymv1-3*, *Rymv1-4* and *Rymv1-5* using molecular markers. Prior to the molecular screening, the 26 accessions were screened in a glasshouse with an aggressive RYMV isolate, BF1 (Ndjiondjop et al., 1999). Ten plants of each accession were sown in a growth chamber under controlled conditions (24-26°C for 12 h in the dark and 28-30°C for 12 h in the light, with 80-90% relative humidity) and mechanically inoculated with BF1 two weeks after sowing. The disease symptoms were monitored from the date of the appearance of symptoms to 42 dpi. Twenty millimetres of the leaves of accessions displaying good level of resistance to BF1 were collected at 49 dpi for DNA extraction, as explained by Edwards et al. (1991). The leaves were ground in liquid nitrogen using a robot Rath MM300 (TissueLyser II, Retsch®) for 30 s. An extraction buffer of 400 µl, including 200 µM of Tris/Hcl at pH 7.5, 250 mM of NaCl, 25 µM of EDTA, and 0.5% of SDS, was added to each tube with ground leaves. This was followed by centrifugation at 4000 rpm for 15 min, and 300 µl of the supernatant was collected in a new labelled tube. Ice-cold isopropanol (300 ul) was added to the supernatant and the whole sample was centrifuged at 4000 rpm for 15 min. After centrifugation, the supernatant was discarded and the DNA was kept in the tube. Ethanol 70% (200 µl) was added to the DNA followed by centrifugation at 4000 rpm for 5 min. After centrifugation, the tubes were stripped of any ethanol content and 400 µl of TE 0.5 was added to the dried DNA and kept in the fridge overnight.

DNA samples from the resistant accessions were analysed using a Polymerase Chain Reaction (PCR) to reveal *RYMV1* resistant alleles. Four different primers (two forward and two reverse) were used in three different PCR reactions to reveal the three different alleles. In Reaction 1, the primers F18/R16/F5/R3 would reveal the *Rymv*1-3 allele (Tog5681 like). In Reaction 2, the primers F17/R15b/F5/R3 would reveal the *Rymv*1-4 allele (Tog5672 like). Reaction 3 used primers R18/F6/F5/R3 to reveal the *Rymv*1-5 allele (Tog5674 like). The 15 µl reaction mix comprised of 15 ng DNA, 200 µM of each desoxyribo-nucleotide 5'-triphosphate (dATP, dGTP, dTTP and dCTP), 1.5 mM $MgCl_2$, 0.02 $\mu.\mu l^{-1}$ Go*taq* DNA polymerase (Promega, Madison, WI, USA), 0.1 µM of each primer, and 1x buffer. The annealing temperature was progressively decreased from 68°C to 62°C during the first five cycles and was then maintained at 62°C, as described by Thiéméle et al. (2010). Amplification products were analysed by electrophoresis in a 1.5% or 2.5% agarose gel.

5.3 Data analysis

Evaluation of resistance to RYMV

Experiment 1

The accessions were allotted into one of the five classes, according to their disease score (1; 3; 5; 7 and 9). The percentage of plant height reduction was calculated using the formula:

$$\%\ \text{of plant height reduction} = 100\ \text{X}\ \frac{\text{height of control} - \text{height of inoculated}}{\text{height of control}}$$

Experiment 2

Two Way Analysis of Variance (ANOVA) was performed to evaluate interaction between virus and accessions, to compare the disease score of the two isolates during the overall period of screening, and to compare the plant heights of inoculated and non-inoculated plants at 49 dpi. The evaluation of interaction virus x accessions, and the comparison of the means of disease scores and plant heights were all performed using GenStat 11th Edition (Payne et al., 2008).

5.4 Results

5.4.1 Plant response to viral infections (Experiment 1)

Symptom scores of the 296 accessions inoculated with B27 isolate ranged between 1 and 9, with most of the accessions scoring between 5 or 7. The plant disease score was highly correlated with the plant height. The plant height decreased when the score increased. Five reaction groups were represented in the collection. The symptomless accessions (Score 1) represented only 2.2% of the 296 accessions of the collection. The second group included moderately resistant plants showing mild symptoms (Score 3), accounting for 5.4% of the collection (Table 5.1). In the first two groups, the effect of infection on plant development was mild. More than 90% of the accessions evaluated were susceptible accessions clustered in Groups 3 (Score 5) and 4 (Score 7) with distinct mottle symptoms and height reduction. The plants with Scores 5 and 7 represented 32.5% and 57.8%, respectively, of individuals in the collection. The last group (Score 9) consisted of very susceptible accessions, showing severe symptoms, stunting and sometimes plant death. Their proportion was 2.2% of the total collection. The first screening of 296 accessions with B27 demonstrated the widespread susceptibility of most of Burkina Faso rice landraces to RYMV. Only six HR (Score 1) and 15 MR (Score 3) accessions were found (Table 5.1).

Table 5.1: The effect of isolate B27 of RYMV on a collection of 296 rice accessions

Status	Score 42 dpi	Number	Percentage (%)	*% Plant height reduction ± std dev
HR	1	6	2.2	11.5 ± 10.8
MR	3	15	5.4	15.2 ± 14.7
MS	5	90	32.5	22.6 ± 11.8
S	7	160	57.8	28.0 ± 13.9
HS	9	6	2.2	69.6 ± 33.8
Total		296	100	

*% Plant height reduction = (Plant height control – plant height inoculated) x 100/ Plant height control

The results of the ELISA test at 56 dpi performed on HR accessions and on four MR accessions with a score of 3 at 21 and 42 dpi and the results of the test performed on check varieties (Gigante, Tog5681, Azucena and IR64) are summarised in Table 5.2.

Table 5.2: Resistant and tolerant accessions with checks (controls) after inoculation with B27 isolate

	Accession	ELISA score	ELISA status	* % Height reduction at 42 dpi	Score at 42 dpi	RYMV status	species
Controls (non-inoculated)	Gigante	0.069	-	3.0	1	R	*O. sativa*
	Tog5681	0.064	-	10.2	1	R	*O. glaberrima*
	Azucena	0.093	+	39.2	3	MR	*O. s. japonica*
	IR64	0.095	+	50.4	7	S	*O. s. indica*
	BM24	0.073	-	16.0	1	R	*O. sativa*
	BM28	0.081	-	-2.2	1	R	*O. sativa*
	HB88	0.070	-	21.1	1	R	*O. glaberrima*
	CC15	0.078	-	22.5	1	R	*O. sativa*
	BM14	0.107	+	3.3	1	R	*O. glaberrima*
	CC29	0.081	-	20.0	1	R	*O. sativa*
	HB51	0.078	-	22.8	3	MR	*O. glaberrima*
	HB19	0.092	+	27.8	3	MR	*O. sativa*
	HB11	0.095	+	9.1	3	MR	*O. sativa*
	CC2	0.083	-	-2.1	3	MR	*O. glaberrima*

*% Plant height reduction = (Plant height control – plant height inoculated) x 100/ Plant height control

Table 5.2 shows that the resistance and susceptibility patterns of the controls were maintained. Gigante and Tog5681 were resistant, with a lower virus content detected by ELISA. Azucena confirmed its partially resistant status over the period of screening with a score of 3 at 42 dpi but with a high virus content and a relatively higher height reduction (39.2%). The susceptible variety IR64 had a score of 7 at 42 dpi, and displayed a 50% height reduction at 42 dpi. In the six resistant landrace accessions, five were confirmed with low virus content (BM24, BM28, HB88, CC15, and CC29), while one, even though it showed little height reduction, had a high virus content (BM14). Similarly, the accession HB88 was symptom-free and showed a low virus content in the ELISA test. It suffered a relatively high height loss (21.1%).

5.4.2 Plant response to viral infection (Experiment 2)

In this experiment, the plant material comprised of 170 accessions that recorded disease scores ranging from 1 to 5 at 21 dpi during Experiment 1, plus 59 new accessions. The number was thus 229 accessions, including 181 *O. sativa* and 48 *O. glaberrima*. The screening of these accessions with RYMV isolates Ng122 and Ng144 resulted in different reactions. The ANOVA table on the disease scores at 14, 28, 42 and 56 dpi showed diverse reactions. Significant interaction between viruses and accessions was observed only at 28 dpi. This interaction showed that certain accessions responded differently, depending on the isolate applied. There was no significant difference between the reactions caused by the

isolates at 14 dpi and 42 dpi. However, a major difference ($P = 0.05$) occurred at 28 and 56 dpi (Table 5.3).

Table 5.3: Summary of the F test of Two Way ANOVA on the overall period of screening

Source of variation	d.f.	*P* at 14 dpi	*P* at 28 dpi	*P* at 42 dpi	*P* at 56 dpi	*P* height at 49 dpi
Virus	1	0.445	0.003	0.591	0.032	0.003
Accession	228	<.001	<.001	<.001	<.001	<.001
Virus.accession	216	0.895	**0.013**	0.681	0.206	0.002

The two isolates caused a highly significant reduction in height between infected and control plants (Table 5.4). A highly significant interaction effect was observed between the two isolates (df = 1; $P < 0.002$) (Table 5.3). This means that there was a differential reaction of resistance expression by the accessions to the different RYMV isolates. The dissimilarity in plant height reduction confirmed that the two isolates are different.

Table 5.4: Summary of the two way ANOVA on height response of non-inoculated and inoculated plants at 49 dpi

Source of variation	d.f.	F. value	P
Virus (RYMV)	2	395.77	<.001
Accession	228	4.03	<.001
Virus x accessions	438	2.37	<.001

The differences in the reduction of plant height observed between infected plants and the checks (Table 5.5) emphasized the vulnerability of the collection to RYMV isolates Ng122 and Ng144.

Table 5.5: Effect of the two isolates on overall plant height of the collection at 49 dpi

Treatment	Plant height
Ng122	88.9a
Ng144	83.8b
Check	121.2c
$Lsd_{0.05}$	2.9

The different letter a, b and c reflect a significant difference between the three treatments

Over the period of screening only one accession (BM13B) was resistant to both isolates but with a height reduction of 29% and 20% with Ng122 and Ng144, respectively. Apart from that, the resistance of the other resistant accessions was overcome either by Ng122 or Ng144. The resistance of the two resistant checks (Gigante and Tog5681) was also overcome. The resistance of Gigante was matched by both isolates, whereas the resistance of Tog5681 was

overcome only by Ng122. Only six accessions (BM13B, CC2, CC114A, CC123D, HB27A and HB88) presented no visible symptoms throughout the period of screening when infected by Ng122. Ng122 overcame the resistance of BM4, CC123A, HB16A and SO2A. Three accessions (BM13B, BM14 and HB19B), plus Tog5681, showed no visible symptoms when infected by Ng144. Ng144 overcame the resistance of CC109A, CC123A and HB88. The AUSPC diagrams portray the distribution of resistance and susceptibility of the rice landrace collection to the two isolates Ng122 and Ng144 (Figure 5.2 & 5.3).

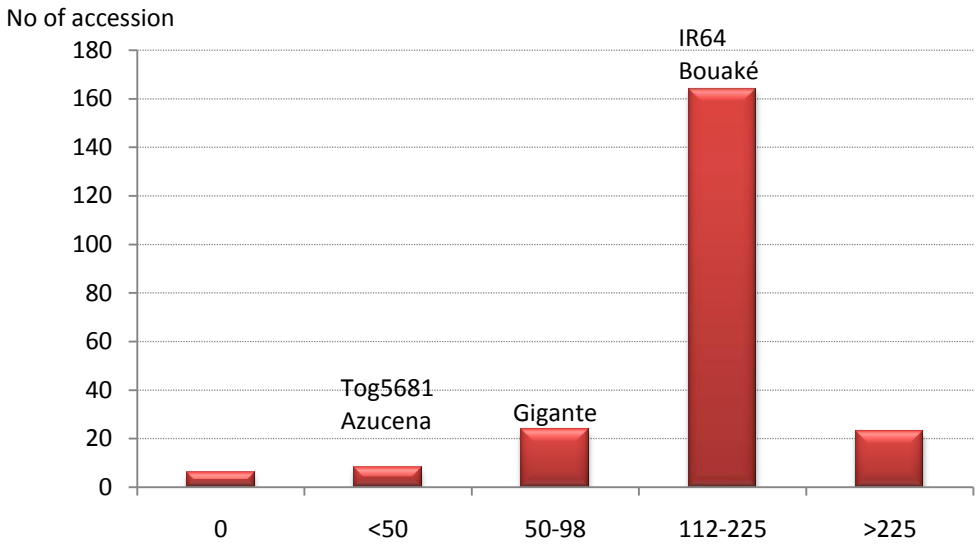


Figure 5.2: Distribution of susceptibility, estimated by the Area Under Symptom Progress Curve (AUSPC) values on a collection of Burkina Faso rice accessions inoculated with the Ng122 isolate of RYMV.

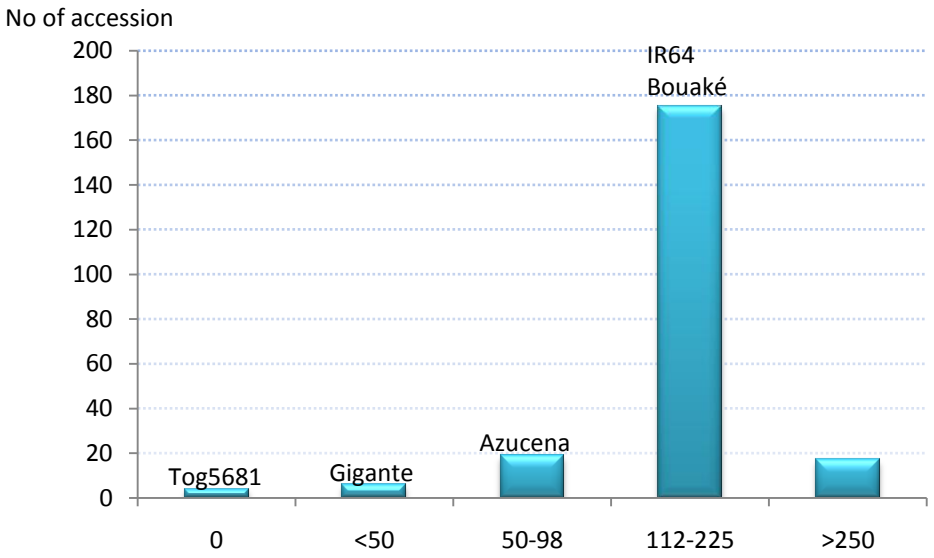


Figure 5.3: Distribution of susceptibility, estimated by the Area Under Symptom Progress Curve (AUSPC) values on a collection of Burkina Faso rice accessions inoculated with the Ng144 isolate of RYMV.

The resistance level of control varieties is indicated. AUSPC = 0 is for highly resistant accessions with no visible symptoms. Tog5681 with AUSPC = 0 is highly resistant with no visible symptoms (Figure 5.3). Figure 5.2 shows the matching of the resistance of Tog5681 and Gigante by Ng122, with Gigante showing full symptom expression. Tog5681 and Azucena were clustered in the moderately resistant group, while Gigante was classed as a moderately susceptible variety. IR64 and Bouaké were classed as susceptible varieties (Figure 5.2). Similarly, the resistance of Gigante was matched by Ng144 (Figure 5.3) causing

symptom expression, and being classed as moderately resistant variety. Azucena was classed as a moderately susceptible variety while IR64 and Bouaké were classed as susceptible varieties (Figure 5.3).

The resistant accessions, which were overcome weeks later, either by Ng122 or Ng144, belonged to the *O. glaberrima* species. The *O. glaberrima* accessions were more resistant than *O. sativa* accessions. Twenty two percent of the 48 *O. glaberrima* accessions were resistant to Ng122, while 14% were resistant to Ng144. Furthermore, 67% and 60% of the 48 *O. glaberrima* displayed a moderately resistant pattern when infected by Ng122 and Ng144, respectively. Nevertheless, some *O. glaberrima* accessions were susceptible: 11% and 22% were susceptible to Ng122 and Ng144, respectively. None of the *O. sativa* plants of the collection were symptomless. However, 7.4% and 7.5% of the *O. sativa* accessions were moderately resistant to Ng122 and Ng144, respectively, whereas 92.6% and 92.5% were susceptible to Ng122 and Ng144, respectively (Figure 5.4).

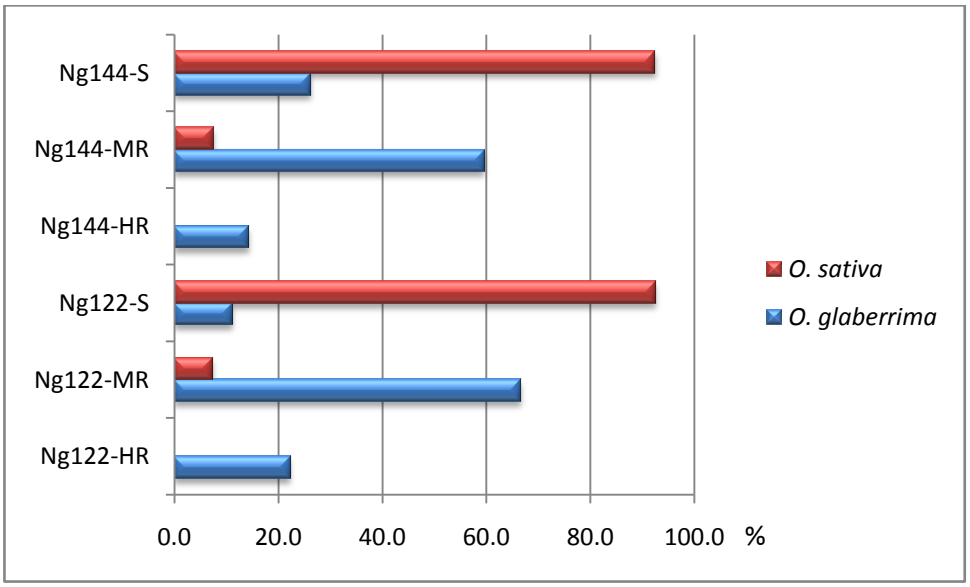


Figure 5.4: Comparison of Highly Resistant (HR), Moderately Resistant (MR) and Susceptible (S) plants in *O. glaberrima* and *O. sativa* species when screened with the two RYMV isolates Ng122 and Ng144.

HR plants included accessions with a mean score ranging from 1 to 1.5 during the 56 days of screening; MR included accessions with a mean score ranging from 2 to 3.5 during the 56 days of screening and susceptible plants contained plants with a mean score of > 3.5 during the 56 days of screening. Figure 5.4 portrays the vulnerability of *O. sativa* and the tolerance of *O. glaberrima* to Ng122 and Ng144. Ng144 showed more aggressiveness in *O. glaberrima*

plants than Ng122. Conversely, Ng144 was not very aggressive on the *O. sativa* accessions BM24, HB62, and HB84, which were moderately resistant. However, only BM24 and HB84 depicted a moderately resistant status while infected by Ng122. Azucena expressed lesser symptoms and relatively less height reduction when infected by Ng122 compared to Ng144. Isolates Ng122 and Ng144, which overcame the high level resistance of Gigante, did not induce increased symptoms on cv Azucena.

Table 5.6 summarises the mean disease score over the period of screening with Ng122 and Ng144; and height reduction at 49 dpi, of the 26 rice landrace accessions compared to the 5 checks (Azucena, Bouaké189, Gigante, IR64, and Tog5681

Table 5.6: Comparison of the 26 resistant and tolerant accessions and the 5 checks varieties at 49 dpi when inoculated with Ng122 and Ng144

Accession	Score Ng122	Score Ng144	% Height reduction Ng122	% Height reduction Ng144	Species
BM11C	3	3	30.1	26.6	*O. glaberrima*
BM13B	1	1	28.9	20.3	*O. glaberrima*
BM14	2.5	1	31.5	10.0	*O. glaberrima*
BM16	2	2.5	49.0	34.3	*O. sativa*
BM24	3.5	2.5	20.0	12.0	*O. sativa*
BM34B	2.5	3	7.1	15.2	*O. glaberrima*
CC2	1	3	28.1	27.3	*O. glaberrima*
CC2B	3	3	33.1	25.0	*O. glaberrima*
CC2C	2.5	3.5	9.6	-1.7	*O. glaberrima*
CC3C	2.5	3	51.1	30.2	*O. glaberrima*
CC108A	3	2	23.3	27.1	*O. glaberrima*
CC109A	2.5	1.5	22.3	23.7	*O. glaberrima*
CC116A	3	3.5	17.0	23.2	*O. glaberrima*
CC123A	1.5	1.5	20.5	25.2	*O. glaberrima*
CC123D	1	2	9.8	25.6	*O. glaberrima*
CC149	2.5	3	8.2	17.3	*O. glaberrima*
SO2A	1.5	2	46.8	20.0	*O. glaberrima*
SO9A	2	3	28.5	23.1	*O. glaberrima*
HB18	4.5	4.5	14.4	26.5	*O. sativa*
HB18B	3	4.5	-6.1	19.1	*O. sativa*
HB19B	1.5	1	11.0	4.4	*O. glaberrima*
HB36	3	2	16.2	7.7	*O. glaberrima*
HB46	3.5	3	24.1	30.3	*O. glaberrima*
HB51	2.5	2	9.9	5.9	*O. glaberrima*
HB84	2.5	2.5	1.0	-6.8	*O. sativa*
HB88	1	1.5	12.3	28.1	*O. glaberrima*
Azucena	**1.5**	**3**	**15.1**	**25.2**	***O. s. japonica***
IR64	**5**	**5**	**19.2**	**37.4**	***O. s. indica***
Gigante	**4**	**3**	**11.8**	**22.4**	***O. s. indica***
Tog5681	**2**	**1**	**7.1**	**-1.4**	***O. glaberrima***
Bouaké189	**5**	**5**	**24.1**	**38.0**	***O. s. indica***

Accessions susceptible to BF1 were characterised by early symptoms. The initial symptoms were observed at 6 dpi in 15 accessions. The accession BM13B, resistant to both Ng122 and Ng144 isolates, displayed symptoms as early as 6 dpi. Later, among the 15 susceptible accessions, six died and six others did not flower confirming the aggressiveness of the BF1 isolate. The only *O. sativa*: BM24 which did not show symptoms at 6 dpi exhibited symptoms two weeks after inoculation. At 21 dpi, only two accessions (HB19B and HB46) were symptomless. All the 26 accessions exhibited symptoms at 42 dpi (Table 5.7). Fourteen accessions flowered and produced seeds.

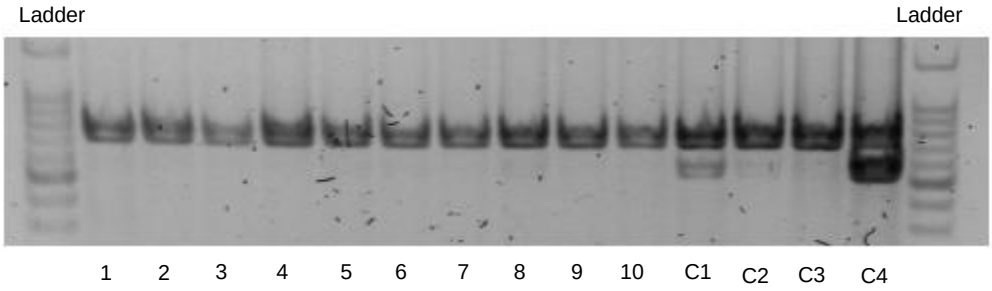
Table 5.7: Summary of the results of the screening of the 26 accessions with BF1

Accessions	1st symptom	Score 14 dpi	Score 21 dpi	Score 42 dpi	Days to heading	Grain number
HB46	42 dpi	1	1	3	83	174
HB19B	42 dpi	1	1	3	78	301
BM11C	21 dpi	1	3	3	96	69
CC2B	21 dpi	1	3	3	88	394
CC109A	21 dpi	1	3	3	82	256
CC123D	21 dpi	1	3	3	85	261
CC2	19 dpi	1	3	3	83	48
CC3C	16 dpi	1	3	3	85	78
BM24	15 dpi	1	3	3	74	338
CC123A	21 dpi	1	3	5	85	40
CC2C	14 dpi	3	3	3	82	180
HB18B	6 dpi	5	5	5	63	8
BM13B	6 dpi	5	5	7	81	27
HB36	6 dpi	5	5	7	sterile	0
CC116A	6 dpi	5	7	7	sterile	0
BM16	6 dpi	7	7	7	sterile	0
HB18	6 dpi	7	7	7	69	126
HB88	6 dpi	7	7	7	sterile	0
CC108A	6 dpi	7	7	9	sterile	0
BM14	6 dpi	7	9	9	sterile	0
SO9A	6 dpi	7	9	9	dead	0
HB51	6 dpi	7	9	9	dead	0
CC107A	6 dpi	7	9	9	dead	0
BM34B	6 dpi	9	9	9	dead	0
CC149	6 dpi	9	9	9	dead	0
SO2A	6 dpi	9	9	9	dead	0

Dpi = 14 days

5.4.3 Test of Allelic state

The 11 accessions: HB46, HB19B, BM11C, CC2B, CC109A, CC123D, CC2, CC3C, BM24, CC123A, and CC2C, which were symptomless at 14 dpi with BF1, were screened in an allelic test. None of the accessions displayed an allelic profile similar to the checks Tog5672 (*Rymv1-4*), Tog5674 (*Rymv1-5*), and Tog5681 (*Rymv1-3*) (Figure 5.5).



1 to 10: accessions; C1: AC96; C2: Tog5672; C3: Tog5674; C4: Tog5681
Figure 5.5: Gel profiling showing that the resistant accessions do not carry the RYMV resistance allele *Rymv1-3*

5.5 Discussion

Rice Yellow Motle Virus (RYMV) is currently the most damaging and widespread disease of lowland rice throughout Africa. So far two major resistance genes displaying several resistance alleles have been identified (Albar et al., 2003; Albar et al., 2006; Thiémélé et al., 2010). However, given the mutation capability of the virus, there is a need for identification and characterisation of new sources of resistance. The evaluation of the collection for resistance to RYMV, performed by inoculation under controlled conditions with isolates of varying aggressiveness, showed that a large majority of the accessions were susceptible to RYMV. The proportion of the susceptible accessions was particularly high among the local varieties belonging to the Asian cultivated rice species *O. sativa*. The susceptibility of *O. s. indica* to RYMV was already reported by several authors (Ghesquière et al., 1997; Ndjiondjop et al., 1999; Rakotomalala et al., 2008). So, in this respect, the Burkina Faso varieties are not different from the *O. sativa* accessions collected in other African countries.

Differential behaviour was observed as a result of the two isolates used and the differences in their aggressiveness and pathogenicity were highlighted. This is explained by isolate and accession interaction, which indicates that the response to isolates is dependent on accessions as well as isolates. This results is supported by the findings of N'Guessan et al. (2001). Moreover, variability was already observed in RYMV isolates through the Open Reading Frame 1 (ORF1) encoding for movement protein and ORF4 encoding for coat protein, confirming that these were two distinct RYMV isolates. This result is matched by the results of Fargette et al. (2002b) and Abubakar et al. (2003). Therefore, virus isolates can differ in their geographical distribution (N'Guessan et al., 2001; Fargette et al., 2002b). Furthermore, RYMV isolates coming from closely related agro-ecological zones can display variability in pathogenicity (Konate et al., 1997). Isolates Ng122 and Ng144, in spite of originating in the

same country, induced differential responses to the same accessions due to virus and host interaction.

Among the 48 *O. glaberrima* accessions, 11% and 22% were susceptible to Ng122 and Ng144, respectively. When Thiémélé et al. (2010) screened a collection of *O. glaberrima*, they noted that there were fewer susceptible cultivars amongst the *O. glaberrima* accessions. RYMV was first observed in 1966 in Kenya (Bakker, 1974) and *O. glaberrima* originates from Africa (Chévalier, 1937; Chang, 1984). They could have co-evolved together for many decades resulting in a moderate resistant status for *O. glaberrima* accessions. Most of *O. glaberrima* accessions delayed the emergence of symptoms when screened with the isolates Ng122 and Ng144. BF1 was very aggressive against some *O. glaberrima* accessions and caused their death. The *O. glaberrima* cultivars did not display a high level of resistance when infected with the aggressive RYMV isolate BF1. Therefore, it is not surprising that any of the resistant *O. glaberrima* cultivars did not display an allelic status identical to *Rymv1-3*, *Rymv1-4* and *Rymv1-5*. So far, Isolate BF1 has not overcome the resistance of Tog5672, Tog5674, and Tog5681 (Thiémélé et al., 2010).

Amongst the five *O. sativa* accessions, which were tolerant to the isolates Ng122 and Ng144 (BM16, BM24, HB18, HB18B and HB84), only BM24 showed partial resistance to the aggressive isolate BF1. This difference can be explained by two factors: the difference between plant genotypes and the genetic make-up of the isolates. Furthermore, these differential accession reactions to viral infections underpin the idea that different resistance genes or groups of resistance genes are involved in virus and host interactions (Konate et al., 1997; Albar et al., 1998). Therefore, BM24 appears to be more genetically flexible than the other *O. sativa* varieties. The resistance in BM24 responds similarly against different isolates of the same virus, meaning it does not interact with the isolate applied. BM24 displayed a tolerant status against the four isolates applied. In the present study, the variety Azucena also showed a tolerant status against the three isolates B27, Ng122 and Ng144. Previous studies (Albar et al., 1998; Ndjiondjop et al., 1999; Ahmadi et al., 2001) reported a partial resistance status of Azucena against the aggressive BF1. Therefore, BM24 seems to portray a similar resistance pattern as Azucena. However, morphologically the two cultivars are very different. Azucena is a taller plant (> 1.5 m) and late maturing (> 120 days), whereas BM24 is a shorter plant (around 1 m) and early maturing (< 105 days). Resistance to RYMV by BM24 accession is characterised by a delay of symptom expression, as well as lower symptom intensity, which

is very similar to the pattern observed in the partially resistant cultivar Azucena. Tolerance that is not associated with partial resistance has been found in irrigated and upland *O. s. japonica* cultivars (Ioannidou et al., 2000). However, *O. sativa* cultivars combining partial resistance and tolerance like Azucena are not widespread (Ioannidou et al., 2000; Ioannidou et al., 2003). Albar et al. (1998) proposed that the component of partial resistance in Azucena is a consequence of the slower plant development and morpho-physiological characteristics of upland rice varieties. Future work on partial resistance to RYMV will be focused on this local cultivar BM24. The next step is to evaluate and compare the resistance of BM24 and Azucena to provide new insights into plant and virus interactions.

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Chapter 6: Phenotypic and genotypic characterisation of an *O. sativa* cultivar from Burkina Faso with partial resistance combined to tolerance against *Rice Yellow Mottle Virus* (RYMV)

Abstract

A local *O. sativa* cultivar collected in Burkina Faso (local name: Kiougayibou, named BM24), which expressed partial resistance, was compared with Azucena. The two cultivars and the varieties Gigante, Tog5681 and IR64 were first involved in a differential screening experiment with three different *Rice yellow mottle virus* isolates (Ng117b, Ng122 and Ng144) from Niger. Secondly, the kinetics, the symptom expression, the virus titre and the allelic status at the RM101 locus of the two varieties were evaluated with ten other accessions, including IR64 and CG14 as checks. Azucena and BM24 portrayed a similar resistance pattern to RYMV. The delay of symptom emergence at an early stage in BM24 was comparable to that of Azucena. Likewise, the virus content in the two accessions at 14 days post inoculation (dpi) was not statistically significant. Moreover, the two accessions exhibited tolerance at a later stage, with BM24 outperforming Azucena. Interestingly, the two accessions depicted an identical allele status at RM101, a marker bracketed in the same zone as QTL12. BM24 appears to be a partially resistant cultivar, with tolerance.

6.1 Introduction

In Africa, two rice species are cultivated: African rice, *O. glaberrima*, and Asian rice, *O. sativa*. These two rice species are threatened by the *Rice yellow mottle virus* (RYMV) disease, which is only found on the African continent (Kouassi et al., 2005). The screenings of cultivars belonging to both species *O. glaberrima* and *O. sativa* (Fomba, 1988; Ndjiondjop et al., 1999; Rakotomalala et al., 2008; Thiémélé et al., 2010) have identified two major genes for resistance: *RYMV1* and *RYMV2*, conferring high levels of resistance. The best resistance is characterised by symptomless plants and blockage of virus movement (Ndjiondjop et al., 2001). *RYMV1* is found in *O. glaberrima* with three different alleles (*Rymv1-3*; *Rymv1-4*; and *Rymv1-5*) and in *O. sativa* with only one allele (*Rymv1-2*) (Albar et al., 2006). So far, *Rymv1-2* has only been detected in Gigante and Bekarosaka, two different cultivars of *O. sativa* var. *indica* (Ndjiondjop et al., 2001; Rakotomalala et al., 2008). The *RYMV2* gene was recently identified in an *O. glaberrima* cultivar, Tog7291 (Thiémélé et al., 2010). Moreover, some *O.*

s. japonica cultivars show partial resistance, associated with tolerance, that is under polygenic control (Ghesquière et al., 1997; Albar et al., 1998; Ioannidou et al., 2000). However, partial resistance was identified in Azucena in several genetic studies (Albar et al., 1998; Pressoir et al., 1998; Ahmadi et al., 2001; Boisnard et al., 2007). Responses to RYMV infection in cultivar Azucena were found to combine partial resistance and tolerance. Partial resistance is expressed only at the early stages of infection and was characterised by delayed and reduced virus accumulation in leaves, and delayed virus invasion in bundle sheath tissues (Ioannidou et al., 2003). The tolerance in Azucena was apparent at the later stages of infection and characterised by reduced symptom expression, despite high virus titre (Ioannidou et al., 2000). Preliminary studies identified only a single Quantitative Trait Locus (QTL) on Chromosome 12 to be implicated in the resistance of Azucena (Ghesquière et al., 1997). Later on, three QTLs on Chromosomes 1, 2 and 12 were suspected to be involved in the partial resistance mechanisms of Azucena. QTL1 appears to be implicated in resistance to virus accumulation and the expression of symptoms, while QTL2 and QTL12 might be involved in mechanisms contributing to the decrease in virus accumulation and symptom expressions (Albar et al., 1998). The complementary epistasis between QTL12 and QTL7 was identified as a genetic factor controlling the virus titre and conferring resistance to Azucena (Pressoir et al., 1998; Ahmadi et al., 2001). The QTL12 close to the *indica / japonica* zone of differentiation is bracketed in an interval of 2.23 Mb containing the marker RM101 (Boisnard et al., 2007). This interval is relatively large due to a lack of recombination and therefore makes the tagging and the fine mapping of the QTL12 implicated in the partial resistance of Azucena difficult. This difficulty hampers the identification and the cloning of the gene(s) involved. Access to the gene content is often the key to identifying factors involved in a phenotypic variation.

The screening of Burkina Faso rice landraces collection with the Isolates B27 and BF1 identified a local cultivar (BM24, local name Kiougayibou) with partial resistance (Chapter 5). BM24 is an early maturing and short height *O. sativa* grown in the rainfed lowland agro-system. The objective of this study was to compare the partial resistance of the local variety BM24 with the resistance of Azucena at the visual symptoms level, the serological level and the molecular level. Characterisation of this new partial resistance to RYMV could provide new insights into the plant / virus interaction and to understanding the expression of partial resistance to RYMV.

6.2 Material and methods

6.2.1 Virus multiplication and inoculation:

Four RYMV isolates were used in this study: from Burkina Faso (BF1) and Niger (Ng117b, Ng122 and Ng144). The three isolates from Niger were from a collection of isolates maintained at the Plant Pathology Unit of AfricaRice, selected for their interaction with different resistance genes and alleles (Séré Yacouba, Personal Communication). BF1 is an aggressive RYMV S2 strain previously employed in the characterisation of the QTL7 and QTL12 in Azucena (Albar et al., 1998; Pressoir et al., 1998; Ahmadi et al., 2001). The isolates Ng117b, Ng122 and Ng144 were multiplied on the standard susceptible variety IR64 during a two week period. The BF1 isolate provided by the "Institut de Recherche Développement" (IRD) - France had already been multiplied in variety IR64 in 2006 and stored at -80°C in liquid nitrogen. Mechanical inoculation was performed with infected leaf samples ground in phosphate buffer, pH 7.2 (10 ml g^{-1} of leaf sample). Carborundum (600 mesh) was added to the extracts as an abrasive agent. Mechanical inoculation was carried out by rubbing the extracted sap on the upper and lower leaf surfaces of two week-old plants by finger-dipping in the inoculum.

6.2.2 Resistance evaluations with the isolates Ng117b, Ng122 and Ng144 (Experiment One)

Experiment One was performed under greenhouse conditions at the AfricaRice Research Station (Cotonou, Benin) in 2009. Resistance of the accession BM24 was evaluated using three isolates (Ng117b, Ng122 and Ng144). The experiment was conducted in the presence of four checks: Gigante and Tog5681 carrying the *Rymv1-2* and the *Rymv1-3* recessive resistant alleles, respectively, the highly susceptible variety IR64, and the partially resistant variety Azucena. The experimental design was a split-plot with three replicates. The main plots were the four treatments (the three isolates Ng117b, Ng122 and Ng144, and a non-inoculated control) and the sub-plots were the different accessions. The elementary plot was an individual plant in a plastic pot of five litres. The disease scores were monitored every week from 14 days post-inoculation (dpi) until 49 dpi and the plant height measured at 49 dpi to estimate the variation in height. The percentage of plant height reduction was calculated using the formula:

$$\%\ \text{of plant height reduction} = 100\ \text{X}\ \frac{\text{height of control} - \text{height of inoculated}}{\text{height of control}}$$

The disease notation used was as described in the International Rice Research Institute (IRRI) Standard Evaluation System (IRRI, 2002) for RYMV symptom severity scale from 1 to 9. Accordingly, accessions were allotted in five classes: Score 1: no symptom observed (Highly Resistant or HR); score 3: green leaves with sparse dots or streaks (Moderately Resistant or MR); score 5: general mottling on the leaves and 6% to 25% of reduction of plant height (Moderately Susceptible or MS); score 7: yellowing and stunting (Susceptible or S) and score 9 for necrosis to plant death (Highly Susceptible or HS).

Analysis of variance was performed on plant heights of the four treatments. The model was defined as $y_{ijk} = \mu + a_i + t_j + r_k + a.t_{ij} + \varepsilon_1 + \varepsilon_2$, where y_{ijk} is the plant height for accession *i* of treatment *j* in replication *k*, μ is the mean effect, a_i is the effect of the accession *i*, t_j the effect of the treatment *j*, r_k the effect of the replication *k*, $a.t_{ij}$ the interaction between accession *i* and treatment *j*, ε_1 is the main plot error term, and ε_2 the subplot error term. As disease scores were measured at different times, a repeated measure model was adopted for the analysis of variance. The model used was as follows: $y_{ijkn} = \mu + a_i + t_j + r_k + T_n + a.t_{ij} + a.T_{in} + t.T_{jn} + a.t.T_{ijn} + \varepsilon_1 + \varepsilon_2$, where y_{ijkn} is the disease reaction (disease score) for accession *i* of treatment *j* in replication *k* at time *n*, μ is the mean effect, a_i is the effect of the accession *i*, t_j the effect of the treatment *j*, r_k the effect of the replication *k*, T_n the effect of the time *n*, $a.t_{ij}$ the interaction between accession *i* and treatment *j*, $a.T_{in}$ the interaction between accession *i* and time *n*, $t.T_{jn}$ the interaction between treatment *j* and time *n*, $a.t.T_{ijn}$ the interaction between accession *i*, and treatment *j*, and time *n*, ε_1 is the main plot error term, and ε_2 the subplot error term.

6.2.3 Evaluation of resistance to isolate BF1 (Experiment Two)

The experiment was performed at "Institut de Recherche Développement", Montpellier, France in a glasshouse under controlled conditions (28 to 32°C, 12 h of light per 24 h and 80-90% relative humidity). The accession BM24 was evaluated with one *O. sativa* accession (HB18B) from a Burkina Faso collection, seven *O. sativa* accession from the "Centre de Coopération International en Recherche Agricole pour le Développement" (CIRAD) Montpellier, France rice collection of the Mini Gene Bank (MiniGB) and three check varieties (IR64, CG14, and Azucena) against the isolate BF1. The seven accessions of the MiniGB were composed of three *O. s. indica* (ASD1, CO18 and PTB9), three *O. s. japonica* (Pagaiyahan, Jumali and Malapkit-Pirurutong) and one accession belonging to the group V of

Glaszmann (ARC13829). In particular, Jumali is an admixture between aromatic and temperate *O. japonica*, while Pagaiyahan is an admixture between tropical *japonica* and *indica* (Garris et al., 2005).

Twenty eight plants of each accession were sown in a tray, with 14 plants in two trays. Each tray included two different accessions allocated randomly. The seed trays were replicated twice in the two treatments (the control non-infected and the infected) and arranged in a completely randomised design. The last leaf of infected plants was mechanically inoculated two weeks after sowing with BF1. Such an aggressive isolate was selected to maximise differences in response to infection between resistant and susceptible cultivars. The following parameters: disease scores (1; 3; 5; 7 and 9), and leaf number were monitored at 4; 7; 11; 14 and 21 dpi while the plant heights were measured at 7, 14, and 21 dpi with aim to compare height differences between accessions at early stages. The Area Under Symptoms Progression Curve (AUSPC) was calculated as:

$$\text{AUSPC} = \sum_{i=1}^{n} \frac{[(S_i + S_{i+1} - 2)(T_{i+1} - T_i)]}{2}$$

to measure disease progress. S_i and $S_{(i+1)}$ correspond to the symptom scores at time T_i and $T_{(i+1)}$, respectively, and n is the total number of observations (Boisnard et al., 2007). The ANOVAs of plant height, number of leaves, and disease score data were implemented and the interaction effects of accession x treatment were primarily considered. A *t* test was used for means comparison between inoculated accessions and their respective control.

At 14 dpi the last leaf of each individual plant was collected to evaluate virus content through Enzyme-Linked Immunosorbent Assay (ELISA). ELISA tests were performed as described in Ndjiondjop et al. (1999). An ELISA response of the inoculated and systemically infected leaves was measured by direct Double Antibody Sandwich (DAS)-ELISA. Plates were coated with 1:1,000 dilution of the polyclonal antiserum in a carbonate buffer (0.015 M Na_2CO_3, 0.034 M $NaHCO_3$, pH 9.6). After incubation for 2 h at 37°C, wells were saturated with 200 µl of 3% skimmed milk in PBS-T buffer (pH 7.4) for 1 h at 37°C. Plates were washed three times with PBS-T buffer after each step. Twenty millimetres of infected leaves were ground in PBS-T buffer at 1:1000 or 1:5,000 dilutions. Samples of 100 µl were incubated for 2 h at 37°C in a microtiter plate, and two replicated wells were used to score Optical Density (OD) values. After 2 h incubation at 4°C, 100 µl of a 1 mg ml^{-1} solution of *p*-nitrophenyl phosphate

in diethanolamine (pH 9.8) was added to each well, and plates were incubated for 3 h at 37°C. The ELISA responses were expressed by scoring the optical densities measured at 405 nm. In all experiments, the non-inoculated variety IR64 and the PBS-T were used as a negative control, whereas the leaves of RYMV infected IR64 was used as positive control.

The leaves collected at 14 dpi were also used for DNA extraction in order to compare allelic profile in locus RM101 (Chromosome 12). The DNA was extracted as described by Edwards et al. (1991). The PCR amplification was performed in a 96 well thermocycler (Tgradient, Biometra) on 5 ng of DNA in a 15 µl final volume of buffer (10 mM Tris-HCl pH 8, 100 mM KCl, 0.05% w/v gelatin, and 2.0 mM $MgCl_2$) containing 0.1 µM of reverse primer RM101, 0.08 µM of forward primer RM101, 200 µM of dNTP, and 0.1 U of Taq DNA polymerase. The PCR program was: initial denaturation at 94°C for 4 min; 35 cycles of 94°C for 60 s, hybridisation temperature 55°C for 60 s, and 72°C for 60 s; and a final elongation step at 72°C for 8 min. The products of amplification were revealed on 2% agarose gel.

6.3 Results

6.3.1 Effects of inoculation with the three different isolates Ng117b, Ng122 and Ng144

6.3.1.1 Effect of inoculation on symptom expression

The analysis of variance of the disease reaction on plant leaves across time highlighted significant interactions between accession and treatment, between time and accession, and between time and treatment (Table 6.1). The accession responses varied according to the isolate applied. The Isolate Ng122 triggered more symptoms expression in Azucena, BM24, IR64, and Tog5681 compared to isolates Ng117b and Ng144. Nonetheless, the Isolate Ng144 induced the highest disease score on Gigante compared to both Ng117b and Ng122 (Figure 6.1). The difference between accessions regarding disease score all over the screening period pinpointed the presence of resistant and susceptible varieties. IR64 showed susceptibility to the three isolates, with mottling and yellowing on leaves, whereas Tog5681 showed a high level of resistance to the three isolates, with inconspicuous symptoms (Figure 6.1).

Table 6.1: ANOVA of data on disease score of five rice accessions subjected to four treatments in a split-plot design with three replications, measured at six growth stages: 14, 21, 28, 35, 42, and 49 dpi

Source of variation	d.f.	s.s.	m.s.	F	P
Replication	2	10.822	5.4111	1.8	
Accession	4	543.04	135.7611	45.05	<.001
Treatment	3	247.94	82.6481	27.43	<.001
Accession x treatment	12	243	20.25	6.72	**<.001**
Error (1)	38	114.51	3.0135	6.11	
Time	5	4.8556	0.9711	1.97	0.106
Time x accession	20	17.089	0.8544	1.73	**0.049**
Time x treatment	15	16.789	1.1193	2.27	**0.013**
Time x accession x treatment	60	32.6	0.5433	1.1	0.327
Error (2)	200	98.667	0.4933		
Total	359	1329.3			

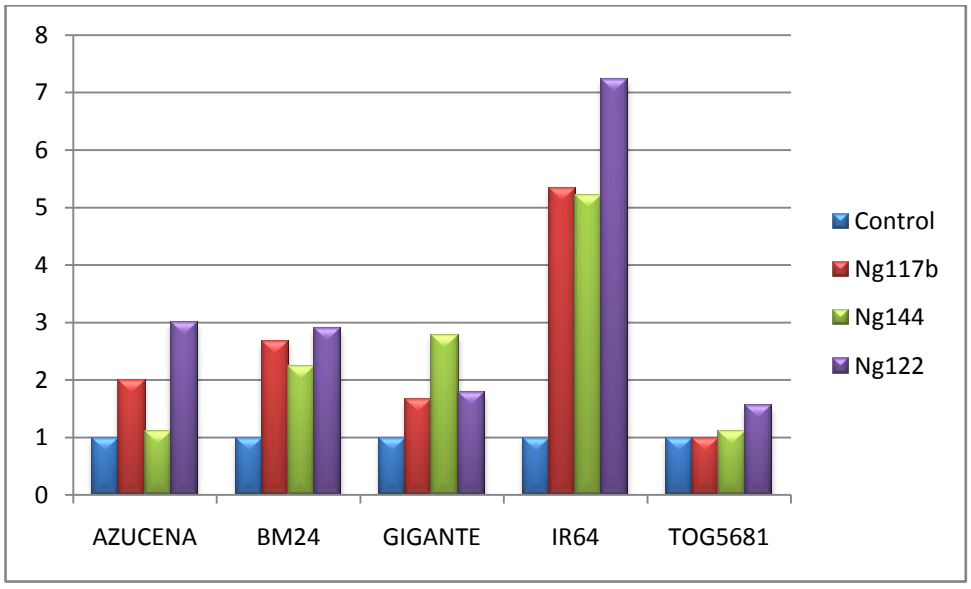

Figure 6.1: Accessions' responses to the infection of the three isolates Ng117b, Ng144, and Ng122 compared to the non-infected control

The comparison of the pathogenicity of the three isolates over time pinpointed a significant difference at 14, 42, and 49 dpi with the isolate Ng122 different from Ng117b and Ng144. On the overall period of screening, Ng122 exceeded Ng117b and Ng144 in symptom expression (Figure 6.2).

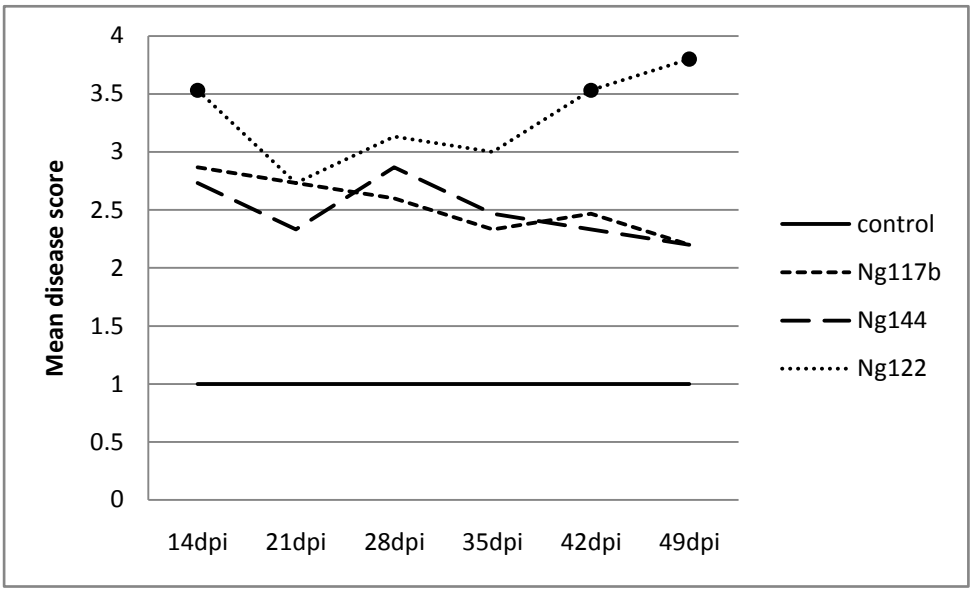

Figure 6.2: Line graph showing that RYMV Isolate Ng122 was more aggressive than Isolates Ng117b and Ng144 over the period of screening. Black dots indicate significant differences in disease expression caused by Isolate Ng122 compared to both Ng117b and Ng144 at the level of 5%. $LSD_{.05} = 0.73$

6.3.1.2 Effect of inoculation on plant height

The analysis of variance performed on the plant height data of the five accessions inoculated separately with the three isolates, and their respective non-inoculated control detected a highly significant interaction between treatment and accession (Table 6.2). The infected accessions exhibited different height reductions. The height differences between the infected plants of the three accessions BM24, Gigante, and Tog5681 relative to their respective controls across the three isolates were not statistically significant. However, Isolates Ng144 and Ng122 induced a significant height reduction to Azucena and IR64, respectively (Table 6.3).

Table 6.2: ANOVA of data on plant height at 49 dpi in a split-plot design with three replicates

Source of variation	d.f.	s.s.	m.s.	F	P
Replication	2	1704.4	852.2	3.11	
Treatment	3	2337.5	779.2	2.85	0.127
Error (1)	6	1641.8	273.6	1.83	
Accession	4	13935.6	3483.9	23.26	<.001
Treatment x accession	12	6043.1	503.6	3.36	**0.003**
Error	32	4793.7	149.8		
Total	59	30456.2			

Table 6.3: Plant Height of the accessions at 49 dpi as affected by the RYMV isolates Ng117b, Ng122, and Ng144

	Plant Height, mm*			
Accessions	Control	Ng117b	Ng144	Ng122
Azucena	990 a	783 a	**700 b**	880 a
BM24	873 a	810 a	887 a	867 a
Gigante	1107 a	1100 a	973 a	973 a
IR64	813 a	697 a	700 a	**217 b**
TOG5681	1010 a	1003 a	1010 a	983 a

*Average of three replications. Mean separation in a row by Least Significance Difference (LSD) test at 5% level. To compare means in a row, $LSD_{.05}$ = 220 mm. Means followed by a common letter are not significantly different at the 5% level.

Compared to the non-inoculated control, Ng122 induced a height reduction of 73%, and Ng117b and Ng144 induced a height reduction of 14%, on IR64. The three isolates also reduced plant height in the partially resistant cultivar, Azucena: 21%, 29% and 11% with Ng117b, Ng144 and Ng122, respectively. Ng144 induced higher height reduction on Azucena than on IR64, while Ng122 caused pronounced symptoms on IR64 but induced limited damage on Azucena. The three isolates induced a plant height reduction of less than 10% in the local variety BM24. The responses of Azucena and BM24 across the three isolates regarding their height were different. Ng144 and Ng122 overcame the resistance of Gigante, causing a 14% reduction in plant height. In contrast Tog5681 did not show any significant reduction in plant height (< 3%) when inoculated with the three isolates (Table 6.3).

6.3.1.3 Combined effect of inoculation on plant height and symptom expression

Based on the importance of symptom expressions and the variation of plant height, the accessions displayed different resistance and susceptibility patterns. Tog5681 expressed a 'High Resistance' to the three isolates, changing to Moderate Resistance occurrence at 42 dpi, with inconspicuous symptom and an insignificant effect on plant height reduction (<3%), caused by the isolate Ng122. Gigante was resistant to the isolate Ng117b with little height reduction (3.3%) but sparse dots appeared on its leaves at 42 dpi, highlighting the occurrence of symptom expression. Ng122 overcame the resistance of Gigante with mild symptoms on leaves at 42 dpi and a height reduction of 14% at 49 dpi. Likewise, Ng144 overcame the resistance of Gigante, with symptom expression at 28 dpi and a height reduction of 14% at 49 dpi. Therefore, Gigante blocked the symptom expression of Ng117b and Ng122 until 42 dpi and of Ng144 to 28 dpi. Gigante and Tog5681 displayed different resistance patterns, thus validating the presence of two different alleles: *Rymv*1-2 and *Rymv*1-3 governing their resistance.

RYMV symptoms on Azucena were green leaves with sparse dots, for the two isolates Ng117b and Ng122, but relatively high reduction in plant height (21% and 11%, respectively). In contrast, mild symptoms were observed on Azucena leaves when inoculated with Ng144, but surprisingly, a significant height reduction of 29% was observed. BM24 combined limited plant height reduction (about 10%) and mild symptoms (green leaves with sparse dots) in response to the three isolates throughout the screening period. Azucena and BM24 showed different resistance patterns to the three isolates. BM24 expressed a partial resistance as good as that of Azucena with mild symptoms and moderate losses in height.

6.3.2 Evaluation of resistance to Isolate BF1 (Experiment Two)

6.3.2.1 Disease reaction on plants

Symptom expression on leaves varied with time and according to the accession involved. At 4 dpi, symptoms were not noticeable in any of the tested lines. At 7 and 14 dpi a significant interaction was detected between accession and disease score (Table 6.4). At 7 dpi all the accessions exhibited symptoms on their leaves except Azucena and BM24. The leaves of BM24 were still symptomless at 11 dpi (Table 6.5). Symptoms were present in all 12 accessions at 14 dpi (Figure 6.3).

Figure 6.3: Leaves of IR64 and BM24 14 days post inoculation, respectively

Azucena and BM24 delayed the symptoms appearance until 11 and 14 dpi, respectively. The interaction effect between accession and disease score disappeared at 21 dpi. At 21 dpi, all the 12 accessions showed susceptibility to BF1. None of the accessions was symptomless and there was a significant difference between inoculated and non-inoculated plants (p <0.001).

Table 6.4: ANOVA of data on disease score in a complete randomised design with 28 replications taken at 7; 11; 14 and 21 dpi

		F. probability (P)			
Source of variation	df	score 7 dpi	score 11 dpi	score 14 dpi	score 21 dpi
rep(treatment)	54	1	1	1	1
accession	11	0.0003	0.00002	0.018	0.82
treatment	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001
treatment*accession	11	0.0003	0.00002	0.018	0.82

Table 6.5: Disease reaction of 12 accessions inoculated with the RYMV isolate BF1 evaluated at 7; 11and 14 dpi

		Difference[a] (score control – score inoculated)		
Accession	score control	7 dpi	11 dpi	14 dpi
ARC	1	1.63***	4.00***	4.00***
ASD1	1	1.56***	2.57***	4.00***
Azucena	1	0.14ns	0.78*	1.57***
BM24	1	0.00ns	0.07ns	1.00**
CG14	1	2.84***	3.57***	3.85***
CO18	1	2.20***	4.00***	4.00***
HB18B	1	0.92**	3.71***	4.00***
IR64	1	2.70***	4.00***	5.35***
Jumali	1	1.77***	3.42***	4.00***
Malagkit	1	0.99**	3.42***	3.00***
PTB	1	1.99***	3.35***	3.50***
Pagaiyan	1	2.41***	3.42***	3.71***

[a]Mean of 28 replications. *** = significant at 0.1% level, ** significant at 1% level, * significant at 5% level, ns = not significant

However, the ANOVA of the plant height data displayed a high significant interaction ($p < 10^{-12}$) between accessions and treatment at 7 and 14 dpi (Table 6.6). The mean comparison of the plant height showed a consistent difference ($p < 0.01$) between the infected accessions and their respective non-inoculated controls at 7 and 14 dpi (Table 6.7). Although Azucena and BM24 expressed no symptoms on their leaves at 7 dpi, the isolate BF1 induced a significant height reduction of 65 mm and 45 mm, respectively. The gap increased at 14 dpi with 107 mm and 67 mm for Azucena and BM24, respectively. A little height reduction (around 10%) at 7 and 14 dpi was observed in Malagkit and BM24 (Table 6.7). However, the disease scores on the 12 accessions clustered Malagkit with susceptible accessions like IR64 and CG14, while Azucena and BM24 portrayed a partial resistance pattern (Figure 6.4). Azucena and BM24 delayed symptom expression for at least 11 days. After 11 dpi Azucena showed more symptom than BM24, although their symptom expressions were similar at 21 dpi (Figure 6.4).

Table 6.6: ANOVA of data on plant height in a complete randomised design with 28 replications measured at 7 and 14 dpi

		F. value (F)		F. probability (P)	
Source of variation	df	height 7	height 14	height 7	height 14
Rep(treatment)	54	1.00	1.34	0.48	0.06
Accession	11	39.40	40.54	1.18E-63	3.54E-65
Treatment	1	447.57	1042.94	1.81E-74	7.23E-133
Treatment x accession	11	7.57	19.21	<2.85E-12	3.75E-33

Table 6.7: Effect of isolate BF1 on plant height of the 12 accessions at 7 dpi and 14 dpi

	Plant height comparison at 7 dpi[a]		Plant height comparison at 14 dpi[a]	
accession	% reduction	difference, mm	% reduction	difference, mm
ARC	7.7	36**	14.7	86***
ASD1	19.8	111***	25.4	181***
Azucena	12.7	65***	15.5	107***
BM24	9.5	45***	10.5	67***
CG14	15.9	81***	23.5	171***
CO18	16.2	79***	33.6	216***
HB18B	10.7	61***	22.1	158***
IR64	14.5	64***	32.5	189***
Jumali	26.3	160***	39.2	316***
Malagkit	10.1	46***	9.6	61***
PTB	14.7	69***	20.4	125***
Pagaiyan	20.6	109***	21.5	146***

[a]Average of 28 replications. *** = significant at 0.1% level, ** significant at 1% level

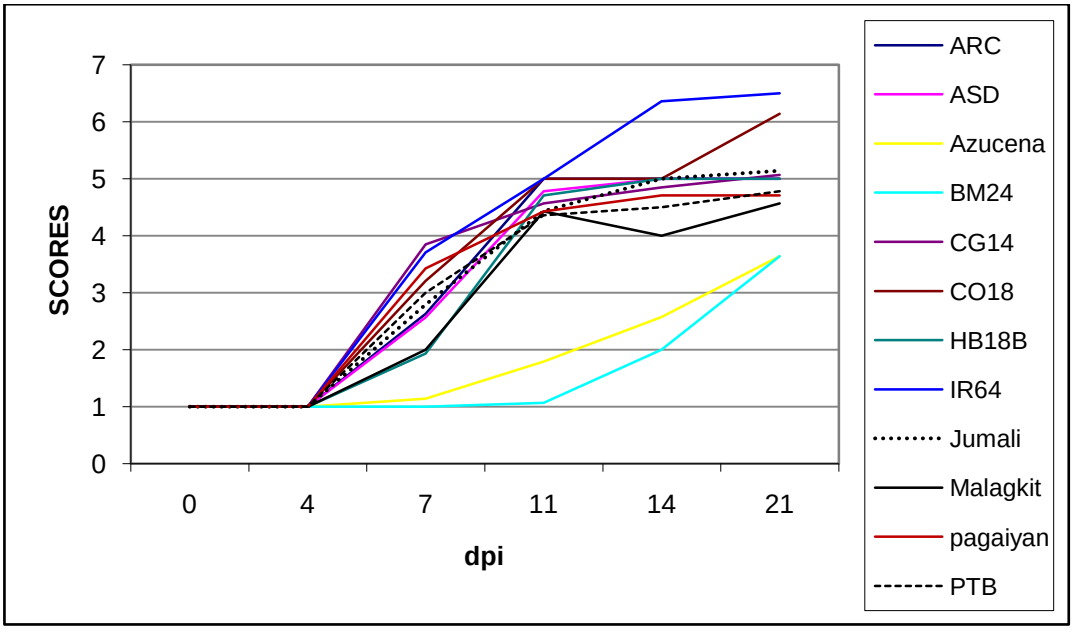

Figure 6.4: RYMV disease progress curve in respect of the 12 accessions from 4 to 21 dpi

The ANOVA of leaf number did not show any significant interaction between treatment and accession. Nonetheless, the main effect leaf number depicted a difference between infected plants and the control non-infected with the increase of the significance level with time (Table 6.8).

Table 6.8: ANOVA of data on leaf number in a complete randomised design with 28 replications counted at 4; 7; 11; 14 and 21 dpi

Source of variation	df	F. probability (P) of leave number at				
		4 dpi	7 dpi	11 dpi	14 dpi	21 dpi
rep(treatment)	54	1	1	1	1	1
accession	11	0.003	0.0005	0.0013	0.008	0.02
treatment	1	**0.011**	**0.003**	**0.001**	**0.00002**	**$< 10^{-07}$**
treatment*accession	11	0.9998	0.9999	0.9994	0.9491	0.9079

The area under symptom progress curve showed that IR64 was the most susceptible variety, with the highest value, while BM24 and Azucena had the lowest values. A remarkable difference was observed with regard to AUSPC values between these two varieties and the ten other varieties (Figure 6.5). Symptom expression evolved rapidly in the susceptible varieties (IR64), while it was delayed in the partial resistant varieties (Azucena and BM24).

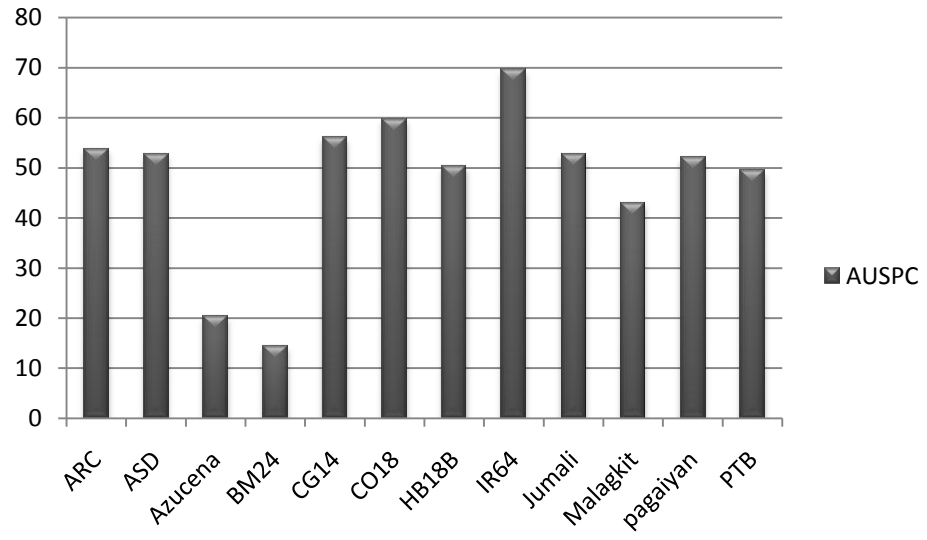

Figure 6.5: Area Under Symptom Progress Curve (AUSPC) values of the 12 rice accessions evaluated over 21 days of screening

6.3.2.2 Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA test, performed at 14 dpi, pinpointed two categories of varieties: IR64, CG14, ARC and HB18B with a high viral content in their leaves, and the other varieties (BM24, PTB, Jumali, Azucena, ASD, CO18, Pagaiyan and Malagkit) with a moderate virus content. The difference of viral content between accessions was highly significant ($p < 0.001$). Viral content was lower in BM24 than Azucena but the difference was not statistically significant. The *O. glaberrima* check CG14 showed the highest virus titre, although its value was statistically similar to that of IR64 (Figure 6.6).

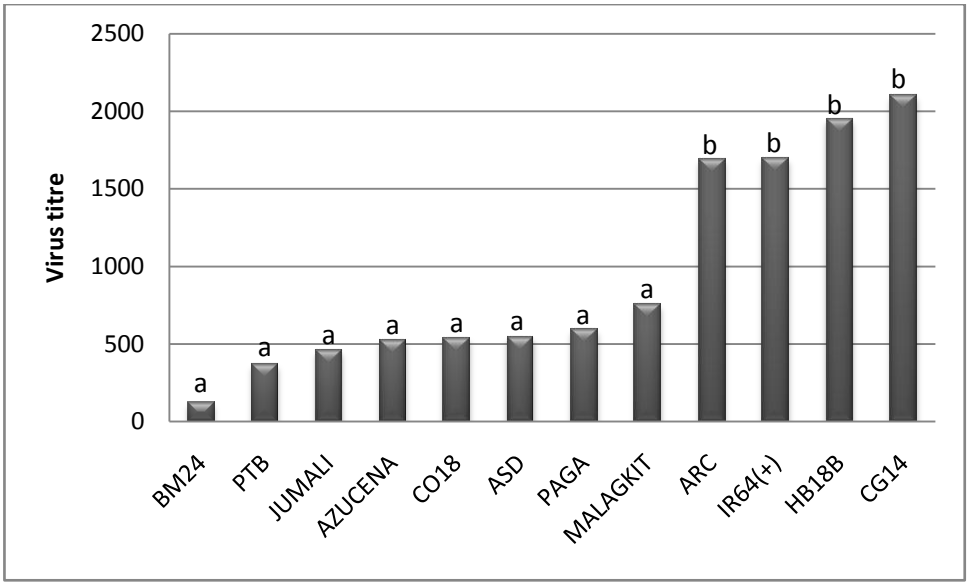

Figure 6.6: Comparison of the virus titre of 12 rice accessions at 14 dpi

Mean separation by Least Significance Difference (LSD) test at 5% level. To compare means, $LSD_{.05}$ = 816. Means followed by a common letter are not significantly different at 5% level

6.3.2.3 Assessment of the RM101 locus.

The assessment of the RM101 locus profile distinguished three groups with different allele sizes (Figure 6.7):

-The first group, with an allele size of 260 bp, included IR64, CG14, HB18B, IR64, Jumali, Malapkit-Pirurutong and Pagaiyahan.

-The second group with an allele size of 300 pb included ARC, ASD1, CO18 and PTB9.

-The third group, with an allele size of 320 bp, included Azucena and BM24.

This distribution suggests that, the BM24 cultivar probably carries the same resistance allele of QTL12 responsible for the partial resistance of Azucena to RYMV.

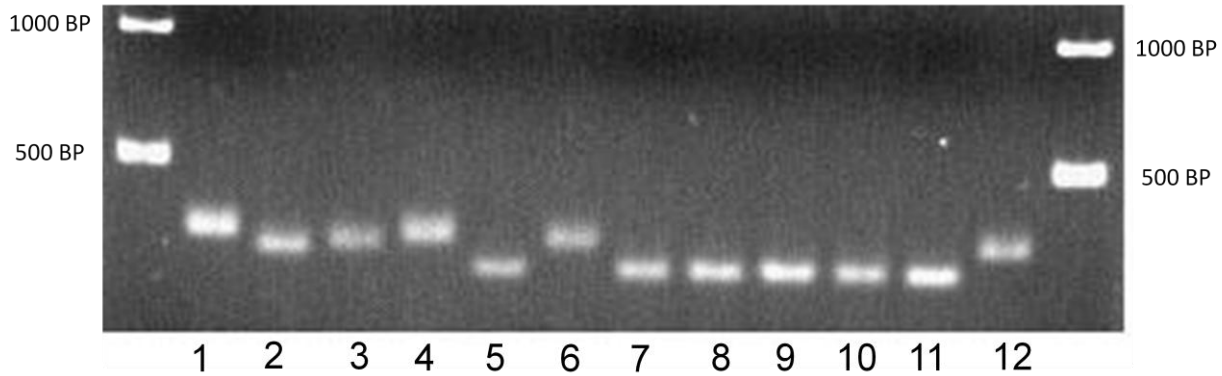

Figure 6.7: Comparison of the allelic status of the 12 accessions at Locus RM101
1-Azucena, 2-ARC, 3-ASD1, 4-BM24, 5-CG14, 6-CO18, 7-HB18B, 8-IR64, 9-Jumali, 10-Malapkit, 11-Pagaiyan, and 12-PTB9

6.4 Discussion

Use of three different RYMV isolates (Ng117b, Ng122, Ng144), confirmed the resistance of local variety BM24 already detected in preliminary experiments. It also demonstrated that this resistance was of the partial type, similar to the one in the well known *O. s. japonica* variety, Azucena. Using the RYMV isolate BF1 confirmed the earlier result and showed that BM24 had a similar genetic makeup to Azucena, at the RM101 locus associated with the QTL12. This is involved in Azucena's partial resistance to RYMV (Ioannidou et al., 2000; Boisnard et al., 2007).

BM24 is cropped in rainfed lowland agro-system and has *O. s. indica* features. A molecular analysis of Burkina Faso collection clustered BM24 between *O. s. indica* and *O. s. japonica* varieties (Chapter 4). Therefore, BM24 could be a good candidate to fine map QTL12, whereas it has been difficult in an IR64 / Azucena background. QTL12 is close to the *indica / japonica* zone of differentiation and a recombination between Azucena and IR64 is rare in this zone (Ghesquière et al., 1997; Boisnard et al., 2007). In addition to its partial resistance status, Azucena combines tolerance at later stages (Ioannidou et al., 2000; Ioannidou et al., 2003). BM24 showed a height reduction of about 10% and mild symptom expressions against three RYMV isolates (Ng117b, Ng122 and Ng144). Partially resistant plants even with mild symptoms are usually stunted by RYMV action. The mechanism developed to delay or to block RYMV spreading into plants affects plant growth. Tog5681 was resistant to the three RYMV isolates but displayed an inconsistent reduction in height. The height difference between inoculated and non-inoculated control plants, combined with a disease score provides a better estimation of resistant status. Azucena and BM24 showed

different resistant features against the four isolates. This could infer the existence of different mechanisms in the two accessions to limit RYMV damages, given that tolerance in RYMV involves complex mechanisms (Ioannidou et al., 2003; Ventelon-Debout et al., 2008). However, the molecular profiling of Azucena and BM24 were similar at the RM101 locus. The partial resistance in Azucena has been shown to be conferred by the combination of QTL12 and QTL7 (Pressoir et al., 1998; Ahmadi et al., 2001; Ioannidou et al., 2003), while the tolerance was associated with the expression of QTL1 (Ioannidou et al., 2000). In this present study the presence of those QTLs was not elucidated in the cultivar BM24. A further investigation should be carried out on this cultivar to map the QTL(s) involved in resistance and tolerance and compare them with those present in Azucena.

Screening for partial resistance with the RYMV isolate BF1 over 21 dpi was sufficient to characterise the 12 rice accessions. BF1 induced very different symptoms at early stages of infection. However, by 21 dpi, all of the 12 inoculated accessions gave symptoms of susceptible plants. Likewise, the ELISA performed at 14 dpi showed a significant differentiation between susceptible and partially resistant plants. Two weeks after inoculation is the optimal period to assess virus titre when assessing for partial resistance (Ghesquière et al., 1997). Cultivars combining partial resistance with tolerance would suffer less from RYMV infection, but would be virus reservoirs (Ioannidou et al., 2000). Although partial resistance is a useful feature, it is not sufficient alone to restrict virus infection and multiplication and should be combined with other genes for resistance (Ioannidou et al., 2003).

The previously resistant varieties Gigante and Tog5681 were shown to be susceptible at later stages when screened by the isolates Ng117b, Ng122, and Ng144. The virulence of novel isolates of RYMV against major genes for RYMV resistance has been identified by Fargette et al. (2002a). Several authors (Sorho et al., 2005; Hébrard et al., 2006; Traoré et al., 2006; Pinel-Galzi et al., 2007; Poulicard et al., 2009; Traoré et al., 2010) established the occurrence of virulent isolates overcoming the major gene *RYMV1*. This is not surprising given the high rate of mutation in RYMV, a virus that evolves rapidly (Fargette et al., 2008b). Moreover, multiple RYMV strains are widespread in Africa, with different traits (Fargette et al., 2002b; Abubakar et al., 2003; Fargette et al., 2004; Pinel-Galzi et al., 2007; Fargette et al., 2008a; Salaudeen et al., 2010). The West African serotypes were confirmed to mostly include isolates with threonine, called the “T-pathotype”. Some few isolates with glutamic acid were

called “E-pathotype”. Ng122 is virulent to the resistance of Tog5681 at 42 dpi. This isolate might have a threonine at Codon 49. According to Traoré et al. (2010) only RYMV isolates with a threonine at Codon 49 of the Viral Protein, Genome-linked (VPg) can break the resistance allele *Rymv1-3* found in Tog5681. The resistance of Gigante was overwhelmed by Ng144, and was also ineffective against Ng122. Ng122 could belong to subset of isolates with virulence matching the recessive resistance alleles R*ymv1-2* and *Rymv1-3* found in Gigante and Tog5681, respectively. The *Rymv1-2* allele is known to be ineffective against isolates with glutamic acid, called “E-pathotypes” (Pinel-Galzi et al., 2007; Poulicard et al., 2009). Very few RYMV isolates from S2/S3 strains widespread in West Africa overcame the resistance of Gigante and Bekarosaka (Pinel-Galzi et al., 2007; Poulicard et al., 2009). In reality, T-pathotypes hardly overcome the resistance of *Rymv1-2*. On the contrary they easily overcome the resistance of *Rymv1-3* (Pinel-Galzi et al., 2007; Poulicard et al., 2009; Traoré et al., 2010). Recently Traoré et al. (2010) established that some T strains (S2/S3) and some T isolates from Niger were found to be able to overcome the resistance of Gigante and Tog5681. Combining partial and major gene of resistance already identified in *O. sativa* could be a best strategy as advised by Van Der Plank (1966). Combining monogenic and polygenic genes of resistance could contribute to building stable resistance (Rubiales and Niks, 2000). Therefore, in the case of RYMV, combining the partial resistance and the high resistance in the same *O. s. indica* variety may be advantageous and could provide stable resistance to a population of virus isolate (Ahmadi et al., 2001; Ioannidou et al., 2003).

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Thesis Overview

Introduction

This research was conducted in Burkina Faso with an aim to exploit local rice germplasm to search for durable resistance against a devastating disease caused by *Rice yellow mottle virus* (RYMV). RYMV disease is mainly threatening irrigated and lowland cropping systems (WARDA, 2000), which constitute the major rice agro-systems in Burkina Faso. Rice landrace collection was conducted using a Participatory Research Appraisal (PRA) in rice growing communities. The diversity and the genetic structure of the germplasm collected were evaluated with agro-morphological parameters and molecular markers. The rice landraces were screened against five RYMV isolates (Ng117b, Ng122, Ng144, B27 and BF1) to assess the susceptibility and resistance status of the collection, in a search for resistant and tolerant varieties.

Major findings and their implications

Farmers' rice landraces were the backbone of this research. Farmers' preferred traits, crop management and disease perceptions were assessed with a community based method: the Participatory Research Appraisal. The PRA found that taste, cooking parameters and yield as paramount selection criteria in rural rice farming communities. Drought and disease resistance are characteristics that farmers wish to have in their varieties. These valuable findings will be of great interest for future breeding programmes, particularly in Burkina Faso and in Africa. In former breeding programmes, farmers' points of view were neglected. However, this collaborative work between farmers and a breeder is an example of reducing the gap between these two partners whose main work and concerns revolve around crops. The PRA confirmed that the farmers are conscious of the existence of RYMV disease in their fields. However, they were unaware of the cause of the disease. Awareness campaigns should be undertaken in communities to thoroughly explain the disease epidemiology. Thereafter, prophylactic methods as suggested by Traoré et al. (2009) could be implemented to limit disease progress.

Taking into consideration farmers' aspirations when breeding new rice varieties has many advantages. The novel varieties developed could be easily introduced in farmers' fields, and their adoption would be accelerated. As a result, the rejection of improved varieties would be

reduced. Farmers brought to our attention that drought and waterlogging can occur in the same season in lowland cropping systems. The lack of rains for periods of the rainy season, mainly in the early stages of plant growth, exposes plants to drought. Subsequent rains often submerge the crop. This is a complicated situation that breeders of lowland rice should try to resolve by developing drought tolerant varieties that can tolerate submersion. Hopefully, the diversity of rice landraces provided by farmers during the collection process will provide a genepool that can help to resolve this dilemma. Three hundred and thirty rice landraces were collected across the four major rice cropping regions of Burkina Faso. The agro-morphological diversity of this collection revealed the presence of the two cultivated rice species: *O. glaberrima* and *O. sativa*. The *O. sativa* varieties outnumbered the *O. glaberrima* varieties. The collection included 48 *O. glaberrima* and 282 *O. sativa* species. Both species were divided into four clusters, reflecting the richness of the collection. The diversity of the collection was confirmed by the use of 22 Simple Sequence Repeat molecular markers. The neutral markers confirmed the existence of two substructures representing the two cultivated rice species *O. glaberrima* and *O. sativa* and the presence of admixture varieties. Therefore, the rice community has pre-breeding material, from Burkina Faso, with phenotypic and molecular information that is vital at the beginning of any breeding programme. Rice plants with specific characteristics can be used by breeders, farmers, plant pathologists, and rice stake-holders around the world. Indeed, the samples deposited at AfricaRice will be available via Standard Material Transfer Agreement (SMTA). Locally, Burkina Faso National Agricultural Research Institute (INERA) now holds a diverse collection of rice germplasm, with which to develop breeding material adapted to the different rice cropping environments across the country. High yielding lines with good taste and good cooking characteristics could be bred to meet farmers' requirements. The collection could be used to tackle biotic and abiotic stresses impeding rice production throughout the country. In addition, a core collection of 52 individuals, including 13 *O. glaberrima* and 39 *O. sativa*, varieties take into account all the sub-clusters present in each species. This core contains 89% of the allelic richness of the collection. Its small size and the fact that it includes the most diverse samples should facilitate and encourage the use of the diversity in rice landraces. This core collection can be used for bridging crossing gaps between *Oryza* species to develop new varieties. Although INERA lacks facilities for long term storage *ex situ* in a convenient genebank, the core can be easily managed *in situ*. Thus, at least the core can be maintained for future generations in a dynamic environment such as farmer fields, taking into account ethnic and social factors. The entire collection was utilised to search for resistant and tolerant varieties against RYMV disease.

The screening of the collection with different RYMV isolates confirmed the susceptibility of most of the samples. Most of the *O. s. indica* plants in the collection were susceptible. The susceptibility to RYMV of *indica* subspecies, the Asian rice (Kouassi et al., 2005), was reconfirmed in this current study. Although two *indica* cultivars, Gigante and Bekarosaka, bear the major resistance allele *Rymv1-2* (Ndjiondjop et al., 1999; Rakotomalala et al., 2008), the susceptibility of *indica* varieties to RYMV remains a major concern in lowland and irrigated areas. However, ten *O. glaberrima* accessions only developed typical RYMV symptoms at a later stage. Moreover, a moderately resistant cultivar, BM24, cumulating partial resistance and tolerance was found in a variety that is close to *O. s. japonica* varieties. Its resistance was comparable to that of Azucena, a partially resistant variety firstly characterised by Ghesquière et al. (1997). The delay of symptom emergence in BM24, the local variety, was similar to that of Azucena. Moreover, the two varieties exhibited tolerance at a later stage, with BM24 outperforming Azucena. BM24 portrayed an identical allele status to Azucena at RM101, a marker bracketed in the same zone with the QTL12. The pest and disease resistance robustness of *O. glaberrima* relative to *O. sativa* (Sarla and Swamy, 2005) was highlighted in this study.

Until now, *O. glaberrima* has displayed genetic richness either in allelic diversity or in gene diversity against RYMV. Three alleles (*Rymv1-3*, *Rymv1-4* and *Rymv1-5*) and a novel RYMV2 gene has been identified in *O. glaberrima* (Albar et al., 2006; Thiémélé et al., 2010). Resistant *O. glaberrima* varieties (HB46 and HB19B) were found in this thesis. They did not carry matching alleles to any of the three alleles *Rymv1-3*, *Rymv1-4* and *Rymv1-5*. Therefore, they could carry RYMV2 or novel alleles that can widen the genepool of resistant or tolerant accessions against RYMV. It should be noted that some RYMV isolates are capable of overcoming the resistance genes, even those with major genes for resistance (Fargette et al., 2002; Traoré et al., 2006). Recently, some West African isolates overcame resistance genes found in both *O. sativa* and *O. glaberrima* cultivars (Traoré et al., 2010). This underscores the idea that breeding for major resistance genes only is not a long term solution to the damage caused by RYMV. The way forward is to combine both major resistance and partial resistance genes to seek to build a durable resistance combination using conventional breeding methods and molecular breeding techniques. Consequently, the genetic basis and mapping of the partial resistance found in BM24 should contribute to a better understanding of the mechanisms of the QTL(s) involved. The interval bracketing the QTL12 present in Azucena is relatively large and makes the tagging and fine mapping of the QTL difficult. This hampers

the identification and the cloning of the responsible gene. BM24 appears to be closer to the *O. s. indica* subspecies than Azucena, as evidenced by its phenotypic characteristics. The crossing of BM24 and IR64 to build a mapping population could provide genetically diverse progenies that would help to narrow the interval and probably identify the gene (s) involved. Then, it could be exploited in Marker Assisted Selection (MAS) or Marker Assisted Recurrent Selection (MARS) programmes.

Conclusion

The thesis activities were centred on the need to develop a collection of Burkina Faso rice landraces. Agro-morphological parameters, coupled with molecular markers, facilitated the assessment of the diversity of this collection. Burkina Faso and other rice communities worldwide, own breeding materials that could be exploited to develop improved rice varieties to boost rice production in Africa and in the world. The increase in rice production in Africa is a prerequisite to enhancing food security. Moreover, the core collection established is of great importance for *in situ* conservation, in a dynamic environment, for future generations. *In situ* conservation the accessions can be maintained in natural environment and facing climate change. The outcomes of the creation of the collection in the search for durable resistance against RYMV revealed very few resistant or tolerant varieties. The resistant and tolerant accessions of the sample group could be used in a process of gene combinations using conventional and molecular breeding tools to attempt to reduce the impact of RYMV on rice production in Africa.

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