MARKER ASSISTED SELECTION FOR RESISTANCE TO RICE YELLOW MOTTLE VIRUS IN FARMERS'PREFERRED RICE VARIETIES IN BURKINA FASO

By

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DECLARATION

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

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ABSTRACT

Rice yellow mottle disease (RYMD), caused by *Rice yellow mottle virus* (RYMV), is a very damaging disease of rice in Sub-Saharan Africa. A participatory rural appraisal was conducted in Burkina Faso to assess farmers' awareness of rice production constraints with emphasis on rice yellow mottle disease (RYMD) and its management. RYMD was mentioned by farmers as the most important rice disease. Management practices included replacement of rice varieties and spray of pesticides. Farmers' choice for rice varieties was based on grain yield and taste as major criteria. Thirty four (34) farmers' rice varieties and 91 varieties from agricultural research institutions in Burkina Faso and Ghana were screened for resistance to RYMV. Partial resistance was found in 29.6% of the varieties, while all other varieieties were susceptible to the virus apart from the high resistance control. Well characterized non-resistance breaking (nRB) isolates of the virus was critical in identifying resistance sources. RYMV1 high resistance gene in Gigante and Bekarosaka (bearing *rymv1-2* allele) was introgressed into both susceptible and partial resistant farmers' preferred varieties. Interspecific crosses involving *Oryza glaberrima* cultivar Tog5681 (bearing *rymv1-3* allele of RYMV1 resistance gene) were successful but introgression of the resistance gene to RYMV failed. Recombinant lines were readily genotyped for the presence of the resistance gene at both homozygous and heterozygote states using SNP-markers. Genotypic characterization of recombinant lines was confirmed by assessment of their phenotypes through virus inoculation. Field evaluation of recombinant lines revealed high (77.29%) broad sense heritability estimates for grain yield. Path coefficient analysis indicated that grain yield was highly and positively correlated with plant panicle number (r=0.80), tiller number (r=0.76) and 1000-grain weight (r=0.61) but negatively to above-ground total biomass (r=-0.28). Per plant panicle number had the highest direct and positive effect on grain yield

(0.94). Major indirect effects on grain yields were exerted by tiller number (0.76), number of days for first flowering (0.72) and above- ground total biomass (0.58). Most recombinant lines performed better than their parents with up to 27.5% highest increase compared to mid-parents. Best performing recombinant lines resulted from crosses involving high resistance donor Gigante or partial resistance donor Digang. From these recombinant lines, several high yielding lines are likely to be developed for release in the near future. By taking into account farmers' preferences, adequate varietal screening process and marker-assisted selection, it can be expected that the new rice varieties to be developed will make great impact in rice production in West Africa.

Keywords: rice, recombinant lines, rice yellow mottle virus, resistance, marker-assisted selection, farmers' preferred varieties.

DEDICATION

To Almighty God,

To Neila & Grace my daughters and Yollande my lovely spouse, for their patience;

To the Grand Family TRAORE and related;

To my late mother and my old father who kept praying for the success of this stud.May God give him longer life to benefit from this study.

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*Finally, my prayer goes to **late Dr Charles THE** who was part of my supervisor team from the beginning; at the early stage he contributed significantly to clear the pathway of this study; may his soul rests in peace eternally in heaven,*

Amen!

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AfricaRice	:	Ex WARDA
AGTB		Above Ground Total Biomass
ANOVA	:	Analyse of variance
BC(n)F1	:	n^{th} Backcross generation between F1 and recurrent parent
BC(n)F1S(n)	:	n^{th} self of n^{th} Backcross generation between F1 and recurrent parent
cDNA	:	Complementary Deoxyribonucleic Acid
CP	:	Coat Protein
CRD	:	Complete Randomized Design
CSIR-CRI	:	Consil for Scientific and Industrial Research-Crop Research Institute
CV	:	Coefficient of variation
cv.	:	cultivar
ELISA	:	Enzyme-linked immunosorbent assays
DAS	:	Double antibody sandwich
DFF	:	Days To First Flowering
DNA	:	Deoxyribonucleic Acid
Dpg	:	Days post germination
Dpi	:	Days post inoculation
E	:	Glutamic acid residue
eIF(iso)4G	:	Eukaryotic translation initiation factor (isoform) 4G
EMS	:	Error mean square;
F(n)	:	n^{th} breeding generation
F1	:	First breeding generation
FAO	:	Food and Agriculture Organization of United Nation
FAOSTAT	:	Food and Agriculture Organization of United Nation, statistic department
FKR	:	Fara Koba Rice
FKR(n)N	:	FKR(number) NERICA
FLL		Flag Leaf Length
GA	:	Genetic advance

GCV	:	Genotypic coefficients of variation
GMS	:	Genotypic mean square
GW		Thousand Grain Weight
H^2 or h_b^2	:	Broad sense heritability
HIV	:	Human immunodeficiency virus
HR	:	High Resistance
IRRI	:	International Rice Research Institute
IITA	:	International Institute Of Tropical Agriculture
INERA	:	'Institut de l'Environnement et de Recherches Agricoles'
K	:	Lysine residue
Kbr	:	Kamboinse riz
LSD	:	Least significant difference
m^2	:	Metre square
MAS	:	Marker Assisted Selection
mm	:	Millimetre
MMLV-RT	:	Moloney Murine Leukemia Virus-Retro transcriptase
NERICA	:	New Rice for Africa
NPK	:	Nitrogen – Phosphorous – Potassium
nRB	:	Non resistance breaking
NS	:	No symptom
nt	:	Nucleotide
ORF	:	Open Reading Frame
PCV	:	Phenotypic coefficients of variation
PH		Plant Height
PL		Panicle Length
PPPN		Per-Plant-Panicle Number
PPTN		Per-Plant-Tiller Number
PR	:	Partial Resistance
PRA	:	Participatory Rural Appraisal
QTL	:	Quantitative Trait Loci
RB	:	Resistance breaking

RFLP	:	Restriction fragment length polymorphisms
RILs	:	Recombinant inbred Lines
RIPs	:	Recombinant inbred line Populations
RNA	:	Ribonucleic Acid
rr	:	Recessive resistant homozygous alleles
RR	:	Dominant susceptible homozygous alleles
rR	:	Heterozygous alleles
RT-PCR	:	Reverse Transcription-Polymerase Chain Reaction
RYMV	:	Rice yellow mottle virus
RYMV1	:	Rice yellow mottle virus resistance gene 1
RYMV2	:	Rice yellow mottle virus resistance gene 2
RYMD	:	Rice yellow mottle virus disease
S	:	Susceptible
SNDR	:	National Strategic plan for Rice Development
SNP	:	Single Nucleotide polymorphism
SPY		Single Plant Grain Yield
SSA	:	Sub-Saharan Africa
SSD	:	Single Seed Decent
SSR	:	Single Sequence Repeats
T	:	Threonine residue
t/ha	:	Ton per hectare
Tob	:	Tropical *oryza barthii*
Tog	:	Tropical *oryza glaberrima*
VPg	:	Viral genome-linked protein
WARDA	:	West African Rice Development Association

CHAPTER 1

1. GENERAL INTRODUCTION

Rice is one of the most important food crops in the world. It is the main staple food for more than half of the world's population (Barker *et al.*, 2007; Ray *et al.*, 2013). Rice is mostly consumed directly as cooked meals but it is also processed into various industrial products (rice cakes, rice bran oil, and wine). Rice straw is used for livestock feed, bedding for livestock and straw for mushroom production. In the early times, rice straw was also used for thatching roofs in Asia, and to make ropes, mats, paper, baskets, and bags. Nowadays rice straw is mainly used for animal feed or as organic fertilizer (Janick, 2002).

In 2011, the overall rice production was estimated at 718,345,380 tons (FAOSTAT, 2013). This makes rice the second most produced cereal in the world after maize (875,098,631 tons) and more than wheat (674,884,372 tons). Following the green revolution, rice production was boosted in several Asian countries (Conway, 2012) and Asia remains the continent where most rice is grown, accounting for about 90% of the whole rice production. The leading rice producing countries are China (204,285,000 tons) and India (152,600,000 tons), which together represent about 55% of the total Asian rice production (Wasim, 2002).

In Sub-Saharan Africa, rice is also an important staple and strategic food crop for several reasons. Milled rice consumption *per capita* has risen steadily from 14 kg in 1970 to 22 kg in 1980 and more than 39 kg in 2009 (Diagne, 2011). This rapid increase has been attributed mainly to changes in food preferences in favour of rice in both rural and urban areas, high population growth rates and rapid urbanization. The relative rate of growth in demand for rice in Africa and

particularly in Sub-Saharan Africa has been faster than in other regions of the world (Somado *et al.*, 2008).

Rice production in West Africa in 2012 was estimated at 11.94 million tons, which represented 45.8% of the whole African production. Nigeria, Guinea, Mali and Sierra Leone were the biggest producing countries with annual production of 4.8 million tons, 1.92 million tons, 1.91 million tons, and 1.15 million tons respectively. Yields varied between 1.2 and 2.8 t/ha in most countries. However, significantly higher yields (about 5 t/ha) were recorded in Mauritania and Senegal.

Most African countries, especially in West Africa, have worked to boost rice production following the 2008 food crisis (Seck *et al.*, 2012). As a result, rice production increased significantly by 36% from 18,375 million tons in 2007-2008 to 25,018 tons in 2011-2012. However, in most West African countries, this increment was insufficient to meet the rice demands. Rice imports for countries like Mali, Guinea and Sierra Leone ranged from 5 to10% of the local consumption. In Côte d'Ivoire, Senegal, Gambia and Niger, rice imports accounted for more than half of the local consumption. Up to date, West Africa imports about 35% of its local production to meet the demand. These imports represent large quantities of rice, as Nigeria alone imported more than 1.6 million tons which represents quarter of its local production (FAOSTAT, 2013).

Rice is a major food crop in Burkina Faso, ranking fourth after sorghum, millet and maize among cereals. Like other countries in Africa, rice consumption in Burkina Faso is subject to rapid changes mainly in urban populations but also in rural areas. Per capita rice consumption estimated at 14.8 kg in 1992 and has increased to 21 kg in 2008 (SNDR, 2011).

Rice is grown everywhere in Burkina Faso by smallholder farmers. The overall rice production in the country reached 300,000 t in 2012. Cropping systems include rainfed upland rice, lowland rice and irrigated rice. Lowland rice is the most widespread cropping system and covers almost half of the total rice cultivation area. Upland rice (17% of total rice area) has been promoted in different parts of the country, especially in the south-western cotton belt where rice is used as a rotation crop with maize. Irrigated rice (34%) was introduced in the 1960s (Illy, 1997; Wopereis *et al.*, 1999; Segda *et al.*, 2005; SNDR, 2011). Irrigated rice in Burkina Faso is the most productive cropping system due to better water management which allows growing two crops per year with averagely good yields of 3 to 7 t/ha compared to 1 to 2 t/ha for other systems. Total irrigated area under rice cultivation represents only 5% of the overall irrigable land estimated at 233,500 ha. Therefore, there is a great potential to increase rice production in the country and a strategic plan was adopted in 2011. Under the plan, the increase in rice production should result in substantial increases in both cultivated acreages and productivity (SNDR, 2011).

High productivity depends primarily on quality seeds (Conway, 2012). More than 60 improved rice varieties have been released in the country but only a few of them are currently grown by farmers (Chapter 3; SNDR, 2011). Most varieties were developed without considering farmers and consumers' preferences. The national rice research programme included that aspect in its breeding schemes through participatory varietal selection. Farmers' expectations are being considered more and more in the development of new rice varieties (Kam, 2011). Efforts have also been made to collect rice landraces to be used as genetic sources of farmers' preferred traits and other desirable traits.

Because rice is widely adapted to different environments, its production on the African continent faces several problems due to abiotic and biotic stresses (Abo *et al.*, 1998). Major abiotic constraints include iron toxicity, phosphorus and zinc deficiencies, acid or alkaline soils, drought, cold and poor soils (Balasubramenian *et al.* 2007). The main biotic stresses are weeds, nematodes, birds, stem borers, and diseases. The effect of each constraint usually varies depending on locations, years, seasons of the year, and varieties.

The most common rice diseases in Africa are caused by *Xanthomonas sp* responsible for bacterial blight, rice blast fungal diseases caused by *Magnaporthe grisea* and rice yellow mottle disease caused by *Rice yellow mottle virus* (RYMV)(Mew, 1991). RYMV is an emergent and highly damaging rice pathogen which is confined to the African continent including Madagascar (Fargette *et al.*, 2006).

Attempts made to control rice yellow mottle disease have been directed mainly to breeding for resistance to RYMV. Several screening programmes were conducted to identify sources of resistance to the virus (Awoderu, 1991; Thottappilly and Rossel, 1993; Thiemele *et al.*, 2010). A few resistant sources have been identified and are being used in different breeding programmes. These resistant sources have not been stable in the field and have succumbed to the virulent strains of the virus (Traore *et al.*, 2006a).

Rice is among the most promising food crops for feeding the rapid growing population of the world, particularly in Africa. It is projected that to meet the demand for food by 2050, the overall food crop production should double. Without changing the overall rice cropping system, rice production is expected to increase by only 42% (Tilman *et al.*, 2011). Unless yields are boosted further, most rice producing countries in Sub-Saharan Africa will experience significant

decreases in the per capita rice harvests (Ray *et al.*, 2013). Despite the great potential of arable land available, a sustainable increase in rice production in the region should result from increasing yields, rather than clearing more land (Foley *et al.*, 2011, Conway, 2012, Tscharntke *et al.*, 2012).

Substantial increase in rice yields through genetic control of RYMV can be achieved by a combination of several approaches. Firstly, additional resistance sources to the virus need to be identified within rice germplasm including landraces and wild rice species (Thiemele *et al.*, 2010, Kam, 2011). Therafter there is a need to develop new rice varieties that are more resilient to climate change. Such varieties can benefit from useful genes coming from poorly exploited sources including African rice species *Oryza glaberrima* Steud (Sarla and Mallikarjuna, 2005). Secondly, the proper identification of suitable resistance genes to RYMV requires a good knowledge of virus-host interactions. Thirdly, molecular tools such as marker assisted selection have to play a major role in plant breeding along with conventional techniques. Lastly, to meet its ultimate goal of widespread utilization of improved varieties, plant breeding must take into account the preferences of end users, of which farmers are at the forefront. Failure to fulfill this requirement usually leads to poor adoption or even rejection of newly developed high yielding varieties due to the lack of some traits not considered in the breeding process (Larc, 1995; Linares, 2002). Participatory plant breeding is now being used to include preferences of farmers and other stakeholders (consumers, processors, extension agents, vendors, industry, etc.) at all major stages of the breeding and selection process (Sperling *et al.*, 2001).

This research is aimed at contributing to food security and improving livelihoods of small-scale rice farmers in Burkina Faso by generating improved higher yielding and farmer-preferred

varieties that are more resistant to RYMV. To achieve the above goal, the specific objectives of the study were:

- to appraise farmers' awareness regarding constrains to rice cultivation with focus on *Rice yellow mottle virus* (RYMV) disease;
- to assess farmers for their preference for rice varieties and evaluate impact of RYMV on their cultivated rice varieties;
- to identify new sources of resistance to RYMV and confirm efficacy of the existing sources of resistance;
- to implement marker assisted selection to introgress resistance genes to RYMV into farmers' varieties;
- to determine relationships between secondary traits and grain yield in recombinant lines.

These specific objectives aim at providing responses to the following assumptions:

- Farmers have preferences for rice varieties for various reasons and are aware of rice diseases, especially RYMV and its damage;
- Farmers use indigenous methods to control rice diseases;
- Resistant rice genotypes to RYMV are available within collected rice germplasm and can be identified by proper screening;
- Molecular approach using DNA markers is effective in improving farmers' preferred rice varieties for resistance to RYMV;
- Breeding gain for yield and resistance to RYMV is achievable with recombinant inbred line population involving farmers' preferred varieties.
- Yield components have direct and indirect effects on grain yield

CHAPTER 2

2. LITTERATURE REVIEW

2.1. Rice origin and domestication

Rice is a grain crop which belongs to the family *Poaceae* (*Gramineae*) and the genus *Oryza*. The *Oryza* genus includes two cultivated (*Oryza sativa* L. and *O. glaberrima* Steud.) as well as several wild rice species. Wild rice also refers to a small group of aquatic grasses of the genus *Zizania*. Both *Oryza* and *Zizania* are members of the tribe *Oryzeae* (Lee, 2002) but they are not sexually compatible. However, successful introgressions of Zizania genes into *O. sativa* by repeated pollination have been achieved (Wang *et al.*, 2005). Unlike species of the genus *Oryza*, *Zizania* species have very limited in geographical distribution and lower contribution as a food crop.

In addition to the two cultivated rice species, the genus *Oryza* also includes 21 wild species (Aggarwal *et al.*, 1997). The main differences in botanical morphology between *O. sativa* and *O. glaberrima* are the ligule size and the glume pubescence. Most of *O. glaberrima* varieties have fewer hairs, short ligules, and fewer or no branches. They also have red-hulled grains on a shattering panicle. Another distinctive criterion between the two cultivated rice species is that *O. glaberrima* is strictly annual, whereas *O. sativa* is potentially perennial (Sacks *et al.*, 2003; Sarla & Mallikarjuna, 2005).

According to Khush (1997), the genus *Oryza* probably originated from Gondwanaland about 130 million years ago, before the domestication of the two cultivated rice species (Figure 2.1). *Oryza sativa* is composed of two major varietal groups, namely *O. sativa indica* and *O. sativa japonica*, sometimes referred to as subspecies. Rice domestication has been long debated, especially that of

O. sativa species with respect to questions on the right area from where cultivated rice originated, the types of *O. rufipogon* which served as direct progenitors and how indica and japonica types evolved (Huang *et al.*, 2012).

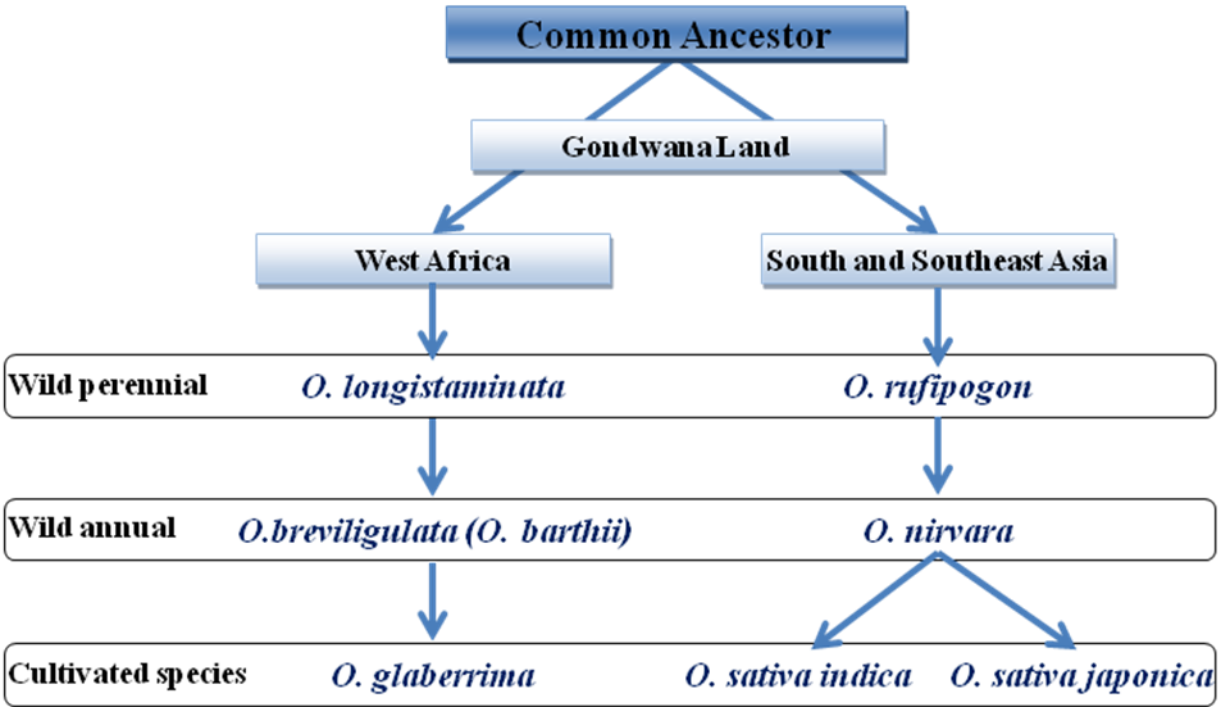

Figure 2.1. Pathways of the domestication of the two cultivated rice (Khush, 1997)

Based on genomic data, it has been clearly shown that *O. sativa* was domesticated in South East Asia from its ancestor *Oryza rufipogon* (Huang *et al.*, 2012). However, indica and japonica groups were domesticated independently from different gene pools within *O. rufipogon*. Recently, new insights in *O. sativa* domestication indicated that japonica rice was first domesticated from a specific population of *O. rufipogon* in the region of the Pearl River in southern China (Huang *et al.*, 2012). *Oryza sativa indica* rice was subsequently developed from crosses between japonica rice and local wild rice.

Oryza glaberrima was domesticated in the Niger River delta about 2,000–3,000 years ago

(Porteres, 1950). Its ancestors were the wild perennial *O. longistaminata* and the annual wild ancestor *Oryza barthii* (formerly known as *Oryza brevilugata*). Due to its center of domestication, *O. glaberrima* is sometimes referred to as 'African rice' as opposed to *O. sativa* (Kush, 1997). The African rice is cultivated only in West Africa where it is mainly used for ritual practices and traditional medicine (Linares, 2002). By contrast, the Asian rice *O. sativa* is cultivated worldwide because of its high yield potential and better adaptation to rice intensification. Presently, *O. glaberrima* is being replaced by *O. sativa* species in West Africa, contributing to the progressive disappearance of cultivated *O. glaberrima* genotypes (Linares, 2002).

2.2. Rice genetic diversity

The genomes of *Oryza* and *Zizania*, both members of the tribe *Oryzeae*, have been mapped. The basic number of chromosomes of the rice species of the genus *Oryza* is 12 while that of *Zizania* is 15. Comparison of *Oryza and Zizania* genomes indicated that 12 of the 15 Zizania chromosomes correspond to the 12 chromosomes of Oryza. The additional three chromosomes in Zizania are likely duplications of three Oryza chromosomes (Kennard *et al.*, 1999).

Oryza species have been grouped into nine types of diploid (2n =2x = 24) genomes and various combinations among them at the tetraploid level (2n =4x= 48) (Aggarwal *et al.*, 1999; Ge *et al.*, 1999). The two cultivated rice species and their wild ancestors are included into the type AA genome group (Table 2.1). Phylogenetic analysis of alcohol dehydrogenase genes (*Adh1* and *Adh2*) in rice indicated that type AA genome species diverged recently and radiated rapidly within the rice genus (Ge *et al.*, 1999). The middle domain of eukaryotic initiation factor 4G (eIF4G) is involved in the genetic differentiation of *Oryza sativa* and *O. glaberrima.* At position

303 of the protein sequence, amino acid residues were Alanine (A) in *O. sativa* and Aspartic acid (D) in *O. glaberrima* (Albar *et al.*, 2003; 2006). Other known diploid genomes are types BB, CC, EE, FF and GG. The tetraploid genomes are consisting of allotetraploid combinations of two distinct diploid genomes. Combinations found so far include BBCC, CCDD, HHJJ, and HHKK genome types. Among all species, only *O. punctata* contains both diploid and tetraploid genome types.

Genomic organization of *Oryza* species confirmed, to some extent, the previous grouping of species members into four complexes based on morphological characters (Cheng *et al.*, 2002).

Table 2. 1 Genomic classification and distribution of *Oryza* species (Adapted from Khush, 1997)

Species	**2n Chromosomes**	**Genome**
***Oryza sativa* complex**		
Oryza sativa L.	24	AA
O. nivara Sharma et Shastry	24	AA
O. rufipogon Griff.	24	AA
O. breviligulata A. Chev. et Roehr.	24	AA
O. glaberrima Steud.	24	AA
O. longistaminata A. Chev. et Roehr.	24	AA
O. meridionalis Ng	24	AA
O. glumaepatula Steud.	24	AA
***Oryza officinalis* complex**		
O. punctata Kotschy ex Steud.	24	BB
O. punctata Kotschy ex Steud	48	BBCC
O. minuta J. S. Pesl. ex C.B. Presl.	48	BBCC
O. officinalis Wall ex Watt	24	CC
O. rhizomatis Vaughan	24	CC

O. eichingeri A. Peter	24	CC
O. latifolia Desv.	48	CCDD
O. alta Swallen	48	CCDD
O. grandiglumis (Doell) Prod.	48	CCDD
O. australiensis Domin.	24	EE
O. brachyantha A. Chev. et Roehr.	24	FF
***O. meyeriana* complex**		
O. granulata Nees et Arn. ex Watt	24	GG
O. meyeriana (Zoll. et Mor. ex Steud.) Baill.	24	GG
***Oryza ridleyi* complex**		
O. longiglumis Jansen	48	HHJJ
O. ridleyi Hook. f.	48	HHJJ
O. schlechteri Pilger	48	HHKK

Thus, type AA genome contains cultivated rice species and their ancestors form the *O. sativa* complex. *Oryza officinalis* complex includes diploid genomes BB, CC, EE and FF and tetraploid genomes BBCC and CCDD. *Oryza meyeriana* complex contains the diploid genome GG only. *Oryza ridleyi* complex includes tetraploid genomes HHJJ and HHKK.

Genome types HH, JJ and KK have not been reported in any *Oryza* species although they were involved in tetraploid types in the *O. ridleyi* complex. This suggests that diploid species with HH, JJ, or KK genomes are either extinct or are yet to be discovered (Ge *et al.*, 1999).

2.3. Rice yellow mottle disease

2.3.1. Symptoms and geographical distribution

Rice stripe necrosis and rice yellow mottle are the two major rice virus diseases reported in Africa. Rice stripe necrosis disease was first reported in Côte d'Ivoire (Fauquet and Thouvenel, 1983) and subsequently in Colombia (Morales *et al.*, 1999) and Brazil (Maciel *et al.*, 2006). Rice

yellow mottle is the most widespread viral disease of rice in Africa, south of the Sahara (Abo *et al.*, 1998). The disease has not been reported in any other continent. First symptoms were observed in 1966 in Kenya (Bakker, 1970; Bakker, 1974) and consisted of yellowing or mottling of the leaves of infected plants (Figure 2.2). Additional symptoms are stunting, partial emergence of panicles and sterility. Early infection of susceptible cultivars often leads to plant death. Rice yellow mottle symptoms in the field appear as yellow patches which often coalesce when conditions are favourable for disease spread (Figure 2.3). From the mid-1970s, several rice growing countries in West, Central and East Africa have been affected by the disease (Abo *et al.*, 1998; Fauquet and Thouvenel, 1977; Fomba, 1988; John *et al.*, 1984; John *et al.*, 1985; Raymundo and Buddenhagen, 1976; Traore *et al.*, 2001). Recently, the disease was reported in Rwanda, Central African Republic and Democratic Republic of Congo (Ndikumana *et al.*, 2011; Longue-Sokpe *et al.*, 2013).

Figure 2.2. Typical symptoms of rice yellow mottle disease on susceptible rice variety BG90-2 and its healthy control (right)

Figure 2. 3. Severe infestation of rice field by rice yellow mottle disease (Photo: Traore Oumar)

To date, rice yellow mottle has been found in almost all major rice growing countries in Sub-Saharan Africa including Madagascar. It has been estimated that the disease emerged about 200 years ago in East Africa (Fargette *et al.*, 2008). However, it started to be a serious problem for rice cropping after the introduction of exotic and highly susceptible *O. sativa* varieties from Asia (Abo *et al.*, 1998; Reckhaus and Adamou, 1986). Also the increase in rice cultivation due to the availability of water for sequential plantings throughout the year favoured its increase (Bakker, 1974; Thresh, 1989).

Rice yellow mottle is responsible for serious yield losses in all rice growing systems. However, irrigated rice is most affected because of more favourable conditions for disease spread (Traore *et al.*, 2009). Yield losses vary from 25 to 100% depending on the rice cultivars grown and how

early the infection sets in, and the rice cultivation system (Awoderu, 1991; Konate *et al.*, 1997; Taylor *et al.*, 1990). Nearly total yield losses have been reported in several rice cultivars infected at an early growth stage (Abo *et al.*, 1998).

2.3.2. Rice yellow mottle virus

2.3.2.1. Taxonomy and genome organization

Rice yellow mottle disease is caused by *Rice yellow mottle virus* (acronym: RYMV), member of the genus *Sobemovirus* (Hull and Fargette, 2005). It is an isometric virus measuring 28 ± 3 nm in diameter. RYMV has a single-stranded positive RNA genome of about 4450 nucleotides. The genome organisation of sobemoviruses has been recently updated with the identification of a new open reading frame (ORF) (Ling *et al.*, 2013). Therefore, RYMV genome is organized into five open reading frames (Figure 2.4). ORF1, located at the 5' end of the genome, encodes protein P1 involved in virus movement and gene silencing suppression or activation (Sire *et al.*, 2008, Lacombe *et al.*, 2010). ORF2a encodes a serine protease and a viral protein genome-linked (VPg). VPg is involved in virulence and determines the ability to overcome resistance genes (Hebrard *et al.*, 2006; Pinel-Galzi *et al.*, 2007). It is also involved in the adaptation of RYMV to Asian or African rice species (Thiemele *et al.*, 2010). ORF2b, which is translated through a -1 ribosomal frame shift mechanism as a fusion protein, encodes the RNA-dependent-RNA polymerase. ORF3 is expressed through a subgenomic RNA and encodes the coat protein (CP) involved in virus spread within the plant (Brugidou *et al.*, 1995). The 3' end of the full viral RNA is deprived of the poly (A) tail found in most viral RNA genomes and the 5' end is covalently linked to the VPg (Hull, 1988; Hull and Fargette, 2005). The functional role of ORFx is not fully known but it is suggested to control the establishment of systemic infection (Ling *et al.*, 2013).

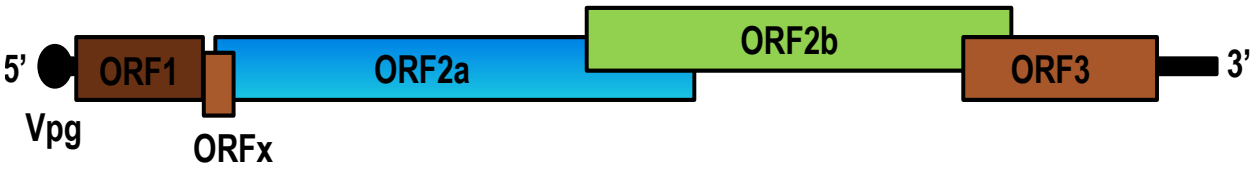

Figure 2. 4. Organization of *Rice yellow mottle virus* genome; five open reading frames (ORF) are mapped on the ~4450 bases RNA genome.
ORFs and corresponding proteins are as follows: ORF1 (P1), **ORFx**, ORF2a (Protease and VPg), ORF2b (RdRp) and ORF3 (coat protein) (Ling *et al.*, 2013**)**

2.3.2.2. Biochemical properties, transmission and susceptible hosts

RYMV is a highly infectious and stable virus with the thermal inactivation point of 70°C, the dilution end-point of more than 10^{-6} and the longevity *in vitro* of 100 days at 20°C (Bakker, 1975; Fauquet and Thouvenel, 1977). RYMV was found infectious in infected dry leaves after one year of storage over $CaCl_2$ (Bakker, 1974).

RYMV is easily transmitted by mechanical inoculation in the laboratory. Vectors involved in its transmission in the field include mainly beetles and also mammals such as cows (*Bos spp*.), rats (*Arvicanthis niloticus*, and donkeys (*Asinus spp.*) (Sarra and Peters, 2003). RYMV has been transmitted through abiotic factors including wind (Sarra *et al.*, 2004), and contact between plants (Traore *et al.*, 2008b). During some cultural practices, the virus was transmitted through contaminated hands and transplantation of rice seedlings into contaminated soil (Traore *et al.*, 2008b). Transplanting contaminated seedlings from nurseries into the field contributed to a rapid spread of the virus (Traore *et al.*, 2006b). RYMV is not seed-transmitted in rice or its wild hosts (Fauquet and Thouvenel, 1977; Konate *et al.*, 2001; Abo *et al.*, 2004; Allarangaye *et al.*, 2006). Non-seed transmission of RYMV, despite clear detection in all seed parts, including the embryo, has been attributed to virus inactivation during seed maturation and desiccation. Such virus

inactivation has been found in cow dung frequently used as manure in rice fields, indicating that this organic fertilizer is not a source for virus infection in the field (Sarra, 1998).

RYMV is transmitted to a narrow host range limited to members of the family *Poaceae*. Apart from rice, natural hosts include wild rice *O. longistaminata*, *O. barthii*, *Ischaemum rugosum* Salisb., *Echinocloa colona* (L.) Link, *Echinochloa crus-pavonis* (Kunth) Schult., *Eragrostis atrovirens* (Desf.) Trin. ex Steud and *Panicum repens* L. (Awoderu, 1991; Konate *et al.*, 1997). Several graminaceous species members of the tribes *Chloridae* and *Eragrostidae* were identified as experimental hosts (Awoderu, 1991; Allarangaye *et al.*, 2007).

2.3.2.3. Serological and molecular diversity

Yellow mottle symptoms induced by RYMV are most of the time typical, but they can be confounded with other disorders such as those caused by iron deficiency or mite feeding damage. Moreover, symptomless infections by RYMV have been found in some host species (Bakker, 1974). Serological tools have long been used to ascertain RYMV infections, as the virus is highly immunogenic and does not have serological relationships with any other plant virus (Calvert *et al.*, 2003; Traore *et al.*, 2008a). Additionally, RT-PCR has gained high popularity in the virus diagnosis due to its sensitivity, possibility for downstream tests on amplification products and increasing affordability of the technique all over the world.

Both serological and molecular techniques have been used to assess RYMV diversity. The virus serological variation was first demonstrated by cross reactivity studies with polyclonal antibodies in double diffusion gel assays (Fauquet and Thouvenel, 1987; Mansoor and Baillis, 1994; Sere *et al.* 2005). More accurate data were obtained later, when monoclonal antibodies and sequences of virus genes became available (Traore *et al.*, 2009). Five serotypes were distinguished and named

Ser1 to Ser5. Sequence analyses of the coat protein gene confirmed the grouping of virufs isolates into serotypes. Accordingly, virus strains identified using this molecular tool was referred to as S1 to S5 (Fargette *et al.*, 2002). However, this molecular typing was found more accurate than serological typing, as it allowed the identification of an additional strain S6 that was serologically indistinguishable from strain S5 (Traore *et al.*, 2005).

RYMV diversity was found to be dependent of the ecology and geographical area from where isolates were collected (Konate *et al.*, 1997, Traore et al, 2009). A close relationship was found between pairwise geographic and genetic distances calculated on the full virus genome or on individual genes (Fargette *et al.*, 2004, 2006).

The emergence, diversification and dispersion of RYMV were studied thanks to the availability of full or partial virus genome sequences at continental level (Abubakar *et al.*, 2003; Fargette *et al.*, 2004; Traore *et al.*, 2005). The highest number of virus strains, including the most divergent ones, was found in the eastern region of Tanzania, suggesting that this region is the centre of origin of RYMV (Traore *et al.*, 2005; Fargette *et al.*, 2006). Although the route of dispersal remains unravelled, the RYMV is thought to have spread in other regions of the continent from an ancestor which first emerged and diversified in East Tanzania. A second centre of diversification was identified in the inner delta of the Niger in the north of Mali.

2.4. Virus host interactions

2.4.1. Rice genes involved in resistance to RYMV

Most rice varieties grown worldwide including Africa are of *O. sativa* species, of which, the vast majority are highly susceptible to RYMV. In such varieties, virus infection is systemic (Bakker, 1974) and virions multiply in all organs including roots, stems, leaves, flowers and

seeds. However, virus concentrations may vary depending on the plant parts. Higher concentrations were found in xylem parenchyma cells and sieve elements (Opalka *et al.*, 1998).

A lot of research work was carried out in order to identify sources of resistance to RYMV and to understand the genetic mechanisms that govern such resistance (Attere and Fatokun, 1983; Okioma and Sarkarung, 1983; John *et al.*, 1985; Fomba, 1988; Thottappilly and Rossel, 1993; Paul *et al.*, 1995; Albar *et al.*, 1998; Coulibaly *et al.*, 1999; Ioannidou *et al.*, 2000; Thiemele *et al.*, 2010). Hundreds of rice accessions have been assessed and results indicated the occurrence of two types of resistance which are partial resistance and high resistance. Partial resistance, associated with low virus titres at early stages of infection and delay in symptom expression, were predominantly found in upland *O. sativa japonica* cultivars such as FKR33, Lac23, Moroberekan and Azucena. By contrast, temperate *O. sativa japonica* and most *O. sativa indica* cultivars were susceptible. Partial resistance was found to be polygenic and markers targeting eight regions of the rice genome were used to map QTLs (Albar *et al.*, 1998). A major QTL was identified on chromosome 12 acting in epistasis with other QTLs on chromosome 7 (Ahmadi *et al.*, 2001; Pressoir *et al.*, 1998).

The high resistance was found only in a few African rice *Oryza glaberrima* cultivars (Thottappilly and Rossel, 1993) and only two *O. sativa* cultivars, namely Gigante and Bekarosaka (Ndjiondjop *et al.*, 1999; Rakotomalala *et al.*, 2008). This high resistance is under the control of the *RYMV1* gene (Albar *et al.*, 2006). *RYMV1* gene encodes an eukaryotic translation initiation factor eIF(iso)4G. Two important amino-acid positions (E309 and E321) involved in resistance to RYMV are shown on the three-dimensional model of the gene (Figure 2.5). They both represent substitutions of a glutamic acid residue (E) by a lysine residue (K) at

position 309 (E309K) and 321 (E321K), respectively. The two mutations were found in highly resistant varieties, the wild type residue E being found in susceptible varieties.

At least four alleles of RYMV1 gene have been identified in resistant rice accessions: rymv1-2, *rymv1-3*, rymv1-4 and rymv1-5 in Gigante or Bekarosaka, Tog5681, Tog5672, and Tog5674, respectively (Table 2.2). Allele rymv1-1 was found in susceptible rice varieties of the two cultivated *Oryza* species. Rymv1-2 identified only in *O. sativa indica* varieties whereas all other alleles were found in *O. glaberrima* varieties. The molecular bases of resistance alleles, rymv1-2 and rymv1-4 were found to be single mutations E309K and E321K, respectively. Allele *rymv1-3* was characterized by a tripeptide deletion at positions 322-324 while allele rymv1-5 was based on the mutation K312N associated with a tripeptide deletion at positions 313-315.

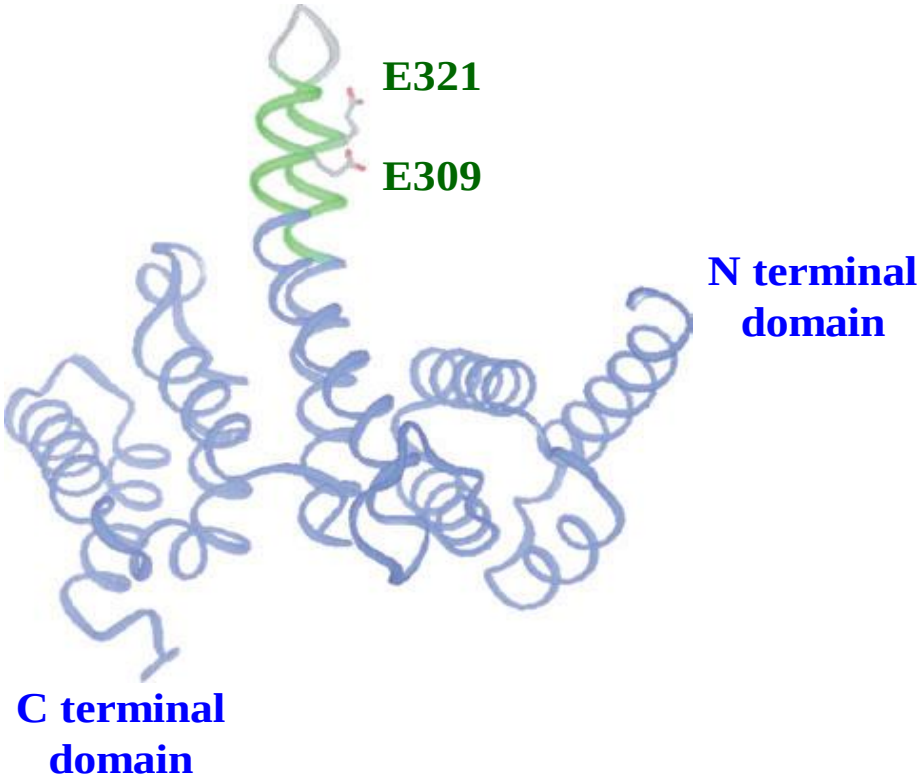

Figure 2. 5. Three-dimensional model of the central domain of *RYMV1* gene (Albar *et al.*, 2006)

Table 2. 2. Alignment of the 301-325 region of *RYMV1* gene product from susceptible and resistant cultivated *Oryza* species (adapted from Thiemele *et al.*, 2010)

Alleles	Rice species[a]	Amino Acid residue position																								
		301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325
rymv1-1	*O. s* (S)	E	G	A	E	S	L	R	A	E	I	A	K	L	T	G	P	D	Q	E	M	E	R	R	D	K
rymv1-2	*O. s* (R)	E	G	A	E	S	L	R	A	K	I	A	K	L	T	G	P	D	Q	E	M	E	R	R	D	K
rymv1-1	*O. g* (S)	E	G	D	E	S	L	R	A	E	I	A	K	L	T	G	P	D	Q	E	M	E	R	R	D	K
rymv1-3	*O. g* (R)	E	G	D	E	S	L	R	A	E	I	A	K	L	T	G	P	D	Q	E	M	E	*	*	*	K
rymv1-4	*O. g* (R)	E	G	D	E	S	L	R	A	E	I	A	K	L	T	G	P	D	Q	E	M	K	R	R	D	K
rymv1-5	*O. g* (R)	E	G	D	E	S	L	R	A	E	I	A	N	*	*	*	P	D	Q	E	M	K	R	R	D	K

[a] *Oryza* species are *O. sativa* (*O.s*) and *O. glaberrima* (*O.g*) with susceptible (S) and resistant (R) phenotypes. Polymorphic sites involved in alleles differentiation, are shaded.

Recently, a second major recessive resistance gene to *Rice yellow mottle virus* (*RYMV2*) has been reported in the African cultivated rice species Tog7291 (Thiemele *et al.*, 2010). A single mutation affecting the *CPR5* gene was associated with *RYMV2* resistance. This mutation was characterized by a 1-base deletion leading to a truncated and probably non-functional protein (Orjuela *et al.*, 2013). CPR5 gene is involved in pathogen defence responses *in Arabidopsis thaliana* (Yoshida *et al.*, 2002).

Partial and high resistances to RYMV were found associated with failure in cell-to-cell movement (Ndjiondjop *et al.*, 2001). The virus was able to multiply equally in protoplasts of susceptible rice cultivars as well as in those of partially or highly resistant ones. Rice cultivar discrepancies in susceptibility to the virus were evident *in planta* where virus movement was blocked or not.

2.4.2. RYMV genes involved is resistance-breaking in rice

RYMV pathogenic diversity has been assessed mainly on the aspects of isolates capabilities to break down resistances in rice. Biological and molecular characterization of RYMV isolates of different origins led to the identification of resistance breaking strains. In West and Central Africa, such stains represented about 40% of the virus isolates and were able to overcome high resistance in Gigante (allele rymv1-2) and Tog5681 (allele *rymv1-3*) (Traore *et al.*, 2006a). Of course, partial resistance in rice cultivars was also overcome (Fargette *et al.*, 2002). Studies on interactions between RYMV and other resistance alleles are in progress. To date, RYMV diversity is such that all known resistances against the virus conferred by RYMV1 and *RYMV2* genes are overcome by some isolates (Koala, 2012).

The molecular basis of breaking resistance conferred by *RYMV1* gene has been studied in detail for the alleles, *rymv1-2* and *rymv1-3* (Galzi-Pinel *et al.*, 2007; Traore *et al.*, 2010; Poulicard *et al.*, 2012). The VPg coded by ORF2a is the protein interacting with the host factors to determine the RYMV ability to overcome resistances (Hebrard et al, 2006; 2010). Interaction between VPg and eIf(iso)4G is illustrated in Figure 2.6. Several mutations occurring at codon 48 of the Vpg have been attributed to the ability for the virus to overcome resistance conferred by allele *rymv1-2* (Table 2.3). Break down of allele *rymv1-3* was associated mainly with mutations that occur at codon 41 and 52.

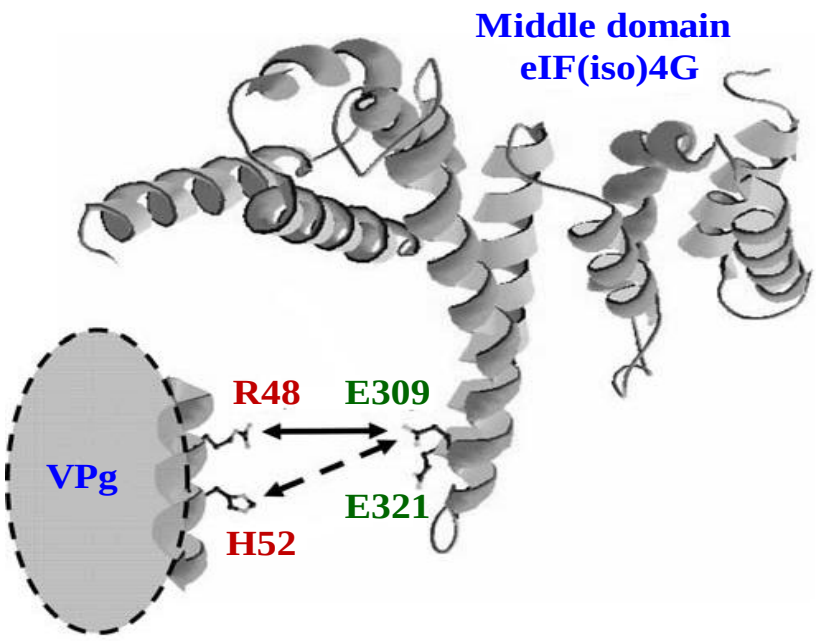

Figure 2. 6. Model showing RYMV-*RYMV1* gene interaction through the viral protein genome- linked (VPg) and the translation initiation factor eIF(iso)4G (Hebrard *et al.*, 2008)

Table 2.3. Alignment of the (41-52) region out of the 79 amino acids RYMV VPg (Traore *et al.*, 2010)

Isolate phenotype[a]	**VPg codon position**											
	41	42	43	44	45	46	47	48	49	50	51	52
nRB	S	N	T	W	V	R	E	R	E	R	Y	H
rymv1-2 RB	S	N	T	W	V	R	E	E	E	R	Y	H
rymv1-2 RB	S	N	T	W	V	R	E	G	E	R	Y	H
rymv1-2 RB	S	N	T	W	V	R	E	I	E	R	Y	H
rymv1-2 RB	S	N	T	W	V	R	E	V	E	R	Y	H
rymv1-2 RB	S	N	T	W	V	R	E	R	E	R	Y	Y
rymv1-3 RB	A	N	T	W	V	R	E	R	T	R	Y	Y
(*rymv1-2* + *rymv1-3*) RB	P	N	T	W	V	R	E	R	T	R	Y	Y
(*rymv1-2* + *rymv1-3*) RB	S	N	T	W	V	R	E	W	T	R	Y	H

[a] phenotype include non-resistance breaking (nRB), allele 2 resistance breaking (*rymv1-2* RB), allele 3 resistance breaking (*rymv1-3* RB) and both alleles 2 and 3 resistance breaking (*rymv1-2+rymv1-3*) RB

It was found that the emergence of resistance breaking isolates depended on the identity of the amino acid residue at codon 49 (Poulicard *et al.*, 2012). Codon 49 is a polymorphic position with either a glutamic acid (E) residue or a threonine (T) residue. On the one hand, when codon 49 has glutamic acid, resistance breaking isolates could emerge on *O. saliva*. On the other hand, virus isolates with a threonine residue were most adapted to *O. glaberrima* background. A subset of "threonine isolates" were able to adapt to *O. sativa*, leading to double allele resistance breaking ability. Altogether, host-virus interaction suggested that prior knowledge of the structure of the viral populations in a given region (resistance-breaking types, adaptation to *O. sativa* or *O. glaberrima*) is necessary for any judicious deployment of resistant rice varieties (Traore *et al.*, 2009).

2.5. Control of rice yellow mottle disease

Two major control strategies were envisaged soon after the first outbreaks of rice yellow mottle disease. Firstly, phytosanitary measures were advised mostly on the basis of what was applied to other similar plant virus diseases (Abo *et al.*, 1998; Fauquet and Thouvenel, 1987). They include protection of seedbeds using nets, disinfections of the tools used at replanting and weeding, destruction of reservoir host and rice ratoons and other residues.

Substantial progress has been made to understand the RYMV epidemiology but most of them did not result in recommendations for disease control at the farmer's level. Rice seedlings infection at the nursery level and further transplantation into the field served as the main primary sources of infection (Traore *et al.*, 2006b). Several sources previously thought to be involved in the spread of the virus including seeds and straw from infected rice or dung from cows fed on

infected rice were ruled out of the process (Sarra, 2005; Allarangaye *et al.*, 2006; Traore, 2012; Traore *et al.*, 2009).

Genetic control of rice yellow mottle disease was considered as the most effective way to combat the disease (Leung *et al.*, 2003). Partially resistant rice varieties were recommended in replacement of susceptible ones (Abo *et al.*, 1998; Sy and Sere, 2001). After the identification of highly resistant sources bearing *RYMV1* gene, new rice varieties have been developed by ingression of *rymv1-2* allele (Jaw *et al.*, 2012). The occurrence of resistance breaking isolates of RYMV undermined the sole usage of genetic control against rice yellow mottle disease.

Attempts were made to develop transgenic lines that could be useful for rice yellow mottle disease control. They were all based on the concept of ‘’pathogen-derived’’ resistance which was successfully used in some pathosystems (Tai *et al.*, 1999; Mathew *et al.*, 2002). Transgenic plants expressing RNA-dependent RNA polymerase of RYMV were produced (Pinto *et al.*, 1999). However, the level of the resistance in the transgenic plants was similar to that of partial natural resistance. Like natural resistance, it could also be overcome by some isolates (Sorho *et al.*, 2005). Transgenic rice plants expressing RYMV coat protein were also produced (Kouassi *et al.*, 2006). In this case, most of transgenic plants accumulated more virus than non-transgenic controls while only partial resistance was found in other transgenic plants. These results indicated that coat protein gene was not suitable for genetic engineering of rice for resistance to RYMV.

No single management means could be used solely to control RYMV efficiently. Chemical control of the vectors was not considered as economically feasible and not even envisaged in most areas to avoid pollution of water resources concomitantly used for livestock and farmers’

domestic needs. New strategies involved judicious combinations of prophylactic measures and genetic control have been put forward in an integrated management approach for RYMV. Such approach is being advocated strongly in the frame of sustainable agriculture under the double green revolution concept (Conway, 2012).

2.6. Breeding for rice improvement

2.6.1. Major objectives in rice breeding

The rice green revolution has been one of most important milestones for rice genetic improvement. The major objectives were to develop high yielding varieties that could be grown in several countries where rice was the main staple food crop. Thus, the semi-dwarf rice IR8 was developed to efficiently use nitrogen without easily lodging (Guimares, 2009).

A great number of traits were targeted afterwards. More than 600 genes have been identified from the 12 chromosomes (Jiang *et al.*, 2012). Some important genes are presented in Table 2.4. Only a few genes controlling traits such as aroma, fertility, grain weight, nitrogen use efficiency and seed shattering were reported. By contrast, several genes were found for traits related to yield (plant architecture, flowering date and panicle size), and biotic and abiotic stresses. The great number of genes that control biotic and abiotic stresses indicated that these constraints are of major concern (Jiang *et al.*, 2012).

The perfect condition of varietal development is breeding to meet the diverse needs of overall rice production for high yield, superior quality, multiple resistances and high nutrient-use efficiency. Such design should follow five different steps: (1) the population structure that can make maximum use of the solar energy in given ecological conditions; (2) the plant architecture to realize the population structure; (3) the traits to make up the plant architecture and to achieve

high quality, resistances to multiple biotic and abiotic stresses, and high nutrient use efficiency; (4) the genes to produce the traits; and (5) the breeding strategy to assemble the genes. The rice genome is being actively screened to elucidate the functions of the genes and identify new useful genes for better varietal improvement (Zhang *et al.*, 2008; Delseny *et al.*, 2013)

Table 2. 4. Some important genes characterized in rice (Adapted from Delseny *et al.*, 2013)

Controlled traits	**Names of the genes**
Architecture	*MOC1, DWARF10, DWARF27, D3, OsTB1, HTD1, OsSPL14*
Flowering date	*OsG1, Hd1, Hd3a, RFT1, RCN1, Ehd1, Ehd2, SE5, PHYB, ETR2, Hd6, Ghd7, OsMADS50, OsMADS51, RFL, OsMADS56, OsMADS14, OsLFL1*
Panicle size	*RCN1, RCN2, LAX1, Gn1a, Ghd7, APO1, LOG, RFL, LRK1, EP3, Ghd8, SPA, FZP, ASP1*
Grain weight	*GS3, GW2, GW5, GIF1, RISBZ1, RPBF*
Shattering	*Sh4, qSH1*
Fertility	*S5, SaM, SaF, S1*
Aroma	*BADH2*
Biotic Stresses : Resistance to pyricularia, Xanthomonas, brownleafhopper, RYMV	*Pib, Pi-ta, Pi-k, Pi9, Pi21, Pi36, Pi37, Pikm, Pi5-1, Pi5-2, Pid3, Pb1, Pi-d2 Xa1, Xa3, xa5, xa13, Xa21, Xa27 OsH1-LOX, OsLox1, Bph14, Rymv1*
Abiotic Stresses : drought salinity, cold, submergence	*OsSKIPa, DSM1, DSM2, OsCIPK12, OsGH3.13 SKC1, OsNAC6, OsKAT1, OsCIPK15 SNAC1, OsbZIP23, DST, AP59, OsSIK1, OsNAC10 OsCIPK03, qLTG3-1, Ctb1 OsMYB3R-2, MYBS3 Sub1A, SNORKEL1, SNORKEL2*
Nitrogen use efficiency	*GS1.1, GS1.2, GlnA, GOGAT, OsAAT1, OsAAT2*

2.6.2. Conventional rice breeding

Conventional breeding of rice through Mendelian genetics took advantage of the identification of genes for major diseases and insects, agronomic traits and abiotic stresses. The major breakthroughs in conventional breeding of rice have been the development of high-yielding, semi-dwarf genotypes from different sources mainly from China and Japan (Rutger and Mackill, 2001). Another major achievement was the development of high-yielding varieties with broad adaptation for irrigated areas due to the insensitivity to photoperiod. Several photoperiod-associated genes were identified, some of which were used to improve semi-dwarf and photosensitive varieties. The first major farmers and industry-oriented traits used in rice selection have been the glabrous-hull characteristic controlled by the *gl*-gene (Delseny *et al.*, 2013). The gene also confers the glabrous characteristic to the leaves, hence making hand harvesting and threshing easier. Another farmer- oriented trait was the purple leaf conferred by the *pl* gene. Purple leaf rice varieties have been adopted by farmers in some areas to facilitate the removal of green weeds, particularly in direct-seeded systems (Kinoshita and Maekawa, 1986). In order to exploit full potential of rice varieties, compatible genotypes were used to develop hybrid rice. Hybrid rice produced 15-22% higher yield than the best available inbred cultivars (Nanda and Virmani, 2000); this increase has gone to over 55% yield increase with newer hybrids (Akram *et al.*, 2007; Chen *et al.*, 2007; Xangsayasane *et al.*, 2010; Berger *et al.*, 2012).

Grain quality was also a major focus in conventional rice breeding for instance waxy gene (*wx*) is expressed in varieties that have low amylose content in endosperm starch. As a result, waxy rice varieties are preferred for pastries and ceremonial foods (Kobayashi and Nishimura, 2007). As part of the improvement of grain quality, aromatic rice varieties have been developed mainly

in USA and Asian countries (Kibria *et al.*, 2008). Recently, some breeding programmes in West Africa showed interest in such varieties (Asante, 2009).

Conventional breeding of rice for resistance to pest and diseases contributed a lot to reduction of in yield. Breeding for resistance to diseases and insects in rice has been considered as one of the most successful examples of the use of major genes in crops species (Rutger and Mackill, 2001). Rice varieties resistant to rice blast caused by *Magnaporthe grisea* and bacterial blight caused by *Xanthomonas campestris* have been produced (Ogawa and Khush, 1989). In some cases, gene pyramiding has been performed to achieve more durable and broader resistance (Huang *et al.*, 1997). Two major virus diseases (rice tungro and rice yellow mottle diseases) have attracted much attention from breeders. Several near-isogenic lines carrying resistance genes from diverse donors including traditional varieties and wild rice (*O. rufipogon*) have been produced (Azzam *et al.* 2002). In the case of rice yellow mottle disease, development of resistance varieties mainly targeted the high resistance and recessive RYMV1-gene. Allele *rymv1-2* from Gigante was introduced in susceptible rice IR64 and other varieties do develop resistant near isogenic lines (Jaw *et al.*, 2012). Introgression of allele *rymv1-3* from resistant *O. glaberrima* sources into high yielding *O. sativa* backgrounds by conventional breeding usually failed because of genetic barriers (Jones *et al.*, 1997b).

2.6.3. Biotechnological and molecular approach in rice breeding

Despite the barriers, natural gene flows among *O. glaberrima*, *O. sativa* and *O. longistaminata* have been reported, indicating the possible use of these species for rice improvement. Incompatibility barriers are now being overcome through different techniques such as embryo rescue and double haploid breeding. These techniques were successfully used to develop the so-

called Nerica (New rice for Africa) varieties (Jones *et al.*, 1997b; Li *et al.*, 1997; Sie *et al.*, 2010).

Advances in biotechnology, genomic research, and molecular marker applications and their integration with conventional plant breeding have revolutionized crop improvement practices. For example, marker assisted backcrossing can halve the number of backcrosses necessary to incorporate a gene of interest into a preferred genetic background (reviewed by Dudley, 1993). Marker assisted backcrossing is especially attractive for recessive genes, such as *RYMV1*. There is no need to identify heterozygous individuals in the backcross generations by traditional methods using several selfing steps. The heterozygous individuals carry the gene of interest are needed for the production of the next backcross. The traditional genotyping procedure is time consuming and involves the detection of segregation in progeny produced by selfing individuals from each backcross generation. Marker assisted selection was used for rice improvement in relation to resistance to RYMV. RFLP and microsatellites markers were used to test efficiency of introgression of partial resistance into *O. sativa* cultivars (Ahmadi *et al.*, 2001). Recently, markers specific to the different alleles of the *RYMV1* gene were developed for marker-assisted selection (Thiemele *et al.*, 2010). They are located inside RYMV1 gene and differed from previously used markers such as RM241, RM273 and RM252 which were outside the gene (Albar *et al.*, 2003; Sow, 2012).

CHAPTER 3

3. IMPACT OF RICE YELLOW MOTTLE DISEASE ON FARMERS' PREFERRED RICE VARIETIES IN BURKINA FASO

3.1. Introduction

Rice (*Oryza sativa* L. and *O. glaberrima* Steud.), as a major food crop in Sub-Saharan Africa (SSA), plays a key role for food security in this region. The overall paddy rice production in the region was estimated at 18.5 million tons (FAOSTAT 2011), which covers only half of the consumption needs. Rice demand has more than tripled from 1.9 to 5.8 million tons over the past two decades in SSA countries (Ogunbayo *et al.*, 2005; 2007). Following the recent food crisis (Seck *et al.* 2012), several West African countries adopted strategies for increasing rice production. These included large scale use of improved seeds, better technical assistance to rice farmers and increase of rice cultivation.

Since the early 1990s, rice production has been severely affected by rice yellow mottle disease caused by *Rice yellow mottle virus* (RYMV) (Kouassi *et al.* 2005). The disease is widespread in most rice growing countries in Africa including Madagascar. RYMV is non-seed transmissible in rice and wild host species (Konate *et al.* 2001; Allarangaye *et al.* 2006). However, it is known as a highly infectious and very stable virus transmitted by several means including wind, insects, mammals and man (Bakker 1970; Sarra *et al.* 2004; Traore *et al.* 2005). Significant yield losses, induced by RYMV (25-100%), have been reported, although most studies were done under experimental conditions (Rechkaus and Adamou 1986; Fomba 1988; Taylor *et al.*, 1990; Konate *et al.* 1997; Kouassi *et al.* 2005). Rice genotype and age of plants during infection have been shown to be major factors influencing yield reduction.

Most cultivated rice varieties belong to *O. sativa* species and have been reported to be highly susceptible (Rakotomalala *et al.* 2008). Attempts to control rice yellow mottle disease have been mainly directed to breeding for resistance to RYMV (Thiemele *et al.* 2010). A few sources of resistance to be used in breeding programs have been identified (Thottappilly and Rossel 1993; Ndjiondjop *et al.* 1999; Rokotomala *et al.* 2008; Thiemele *et al.* 2010). However, the durability of such resistance has been questioned, as resistance-breaking isolates of the virus have been shown to occur frequently (Sorho *et al.* 2005; Traore *et al.* 2006a). Consequently, Traore *et al.* (2009) concluded that integrated management strategies should be adopted for durable control of the disease. The use of insecticides was found to effectively control populations of some insect vectors (Abo *et al.* 1998). However, chemical control of rice yellow mottle is economically unfeasible and difficult to effectively use due to the large number vector species (Calvert *et al.* 2003). Consequently, sustainable management of the disease should rely mainly on genetic control combined with effective phytosanitary and good cropping measures.

Farmers' involvement in the process of disease control can be of great importance in the success of management practices as exemplified by the wide application of farmers' field schools in recent years (Roling *et al.* 1994). The development of integrated pest management technologies for rice farmers in Asia has been relatively successful (Adesina *et al.* 1994). This success was attributed to extensive creation of farm-level awareness of pest and diseases and management strategies. By contrast, only a few similar studies have been conducted in Africa and this has probably limited the success of IPM implementation for sustainable rice production. In a previous study focused on traditional rice varieties, farmers' perception of rice yellow mottle disease was appraised in a limited area of South West region of Burkina Faso (Kam, 2011). The present study was conducted in other rice growing areas of the country to assess farmers'

awareness, perception and management practices of rice yellow mottle disease and the impact of RYMV on their preferred varieties.

3.2. Materials and methods

3.2.1. Survey areas

In this study, surveys were conducted in two locations including Banzon (11°19'0.00"N; 4°47'60.00"W) and Mogtedo (12°17'03.84"N; 0°50'14.00"W), representative of the wet and the dry savannah zones, respectively. In both locations, rice is grown under irrigation and rice yellow mottle disease occurs endemically. Banzon has a full irrigation system for growing rice all year round using water from a permanent river. At Mogtedo, irrigation water is mostly from reservoirs fed by rainwater and located up stream. Availability of water for irrigation is therefore dependent on rainfall and there is more lowland and rainfed rice than in Banzon.

3.2.2. Sampling procedures and data collection

Surveys were conducted using two complementary approaches: (i) informal discussions with farmers in the field and (ii) questionnaires (Figure 3.1). All interactions with farmers were done in local languages to ensure effective understanding. Over 200 farmers (100 per locality) were interviewed (appendice 1). Farmers were randomly selected regardless the gender around their cultivation perimeters. Data were collected on farmers' awareness, perception and control of rice yellow mottle disease. Data on farmer's criteria for preference of rice varieties were also

Figure 3.1. Interaction with farmers during interviews and informal discussions

3.2.3. Yield loss and disease incidence assessment in farmers' field

Yield losses due to the virus were assessed in three rice varieties in Banzon locality during 2010 and 2011 main rice growing seasons spanning from June to October. This activity was conducted from 100% flowering to grain filling stage. For each rice variety, a total of 50 diseased plants and 50 symptomless plants were randomly selected in five distinct 500 m²-blocs which were at least 50 meters apart. All plants were tagged with two different color labels (Figure 3.2). At maturity, rice panicles from individual plants were harvested and dry seeds (11% humidity) weighed. Yield losses were computed by comparing yield means from healthy and diseased plants.

Figure 3.2. Single plant yield comparison: (A) Healthy and diseased plant tagged with white and black sachet respectively; panicles harvested from healthy (B) and from diseased (C) plants.

Disease incidence was assessed in the whole perimeter regardless of rice varieties in five blocs which were 100 meters apart. In each bloc, 1000 plants were randomly examined and diseased plants were counted.

3.2.4. Data analysis

Data on farmer's interviews were analyzed using SPHINX-PLUS© software version 4.5. Significance of differences in mean yield losses between rice varieties were tested by analysis of

variance (ANOVA) using STATISTICA software. Data on disease incidence were also analyzed by ANOVA after angular transformation (Zar 1999).

3.3. Results

3.3.1. Features of rice production

Rice production in both Banzon and Mogtedo was dominated by male farmers (87.2%). There were more female farmers in Mogtedo (22.3%) than in Banzon (3.0%). Most farmers appeared to have long experience in rice cultivation since 87.2% had been producing rice for more than five years. Rice production was largely dominated by smallholders in both locations. About 98.5% of farmers cultivated fields ranged from 0.5 ha to less than 5 ha. Larger field sizes (5 to more than 10 ha) were held by only a few farmers. Most farmers (58% and 94% at Banzon and Mogtedo, respectively) experienced low yields of 1-2 t/ha. Accordingly, 42% of Banzon farmers and only 6% of Mogtedo farmers recorded higher yields of 3-5 t/ha. All farmers were also involved in the production of other crops including cereals, dry legumes and vegetables.

3.3.2. Rice varieties grown and farmers' preferred varieties

A total of 13 rice varieties were inventoried in the two study areas which shared only six varieties (Figure 3.3). Most farmers indicated simultaneous cultivation of several varieties. The proportion of farmers using one, two or three varieties were 17.7%, 31% and 51.3%, respectively. Varieties common to the two localities included FKR14, FKR19, FKR28 and three interspecific *O. sativa* x *O. glaberrima* derived NERICA varieties (FKR56N, FKR60N and FKR62N) (Sie *et al.* 2007). The majority of farmers in Banzon (70.8%) cited four varieties including FKR18, FKR19, FKR60N and FKR62N as the common varieties grown, FKR18 being the most common (20.4%). However, FKR62N was quoted as the best yielding among NERICA varieties. At Mogtedo, FKR19, FKR56N, FKR60N and FKR62N were the most frequently

grown varieties (83.5% of farmers). However, FKR19 clearly was favoured over the NERICA varieties as indicated by 42% of farmers.

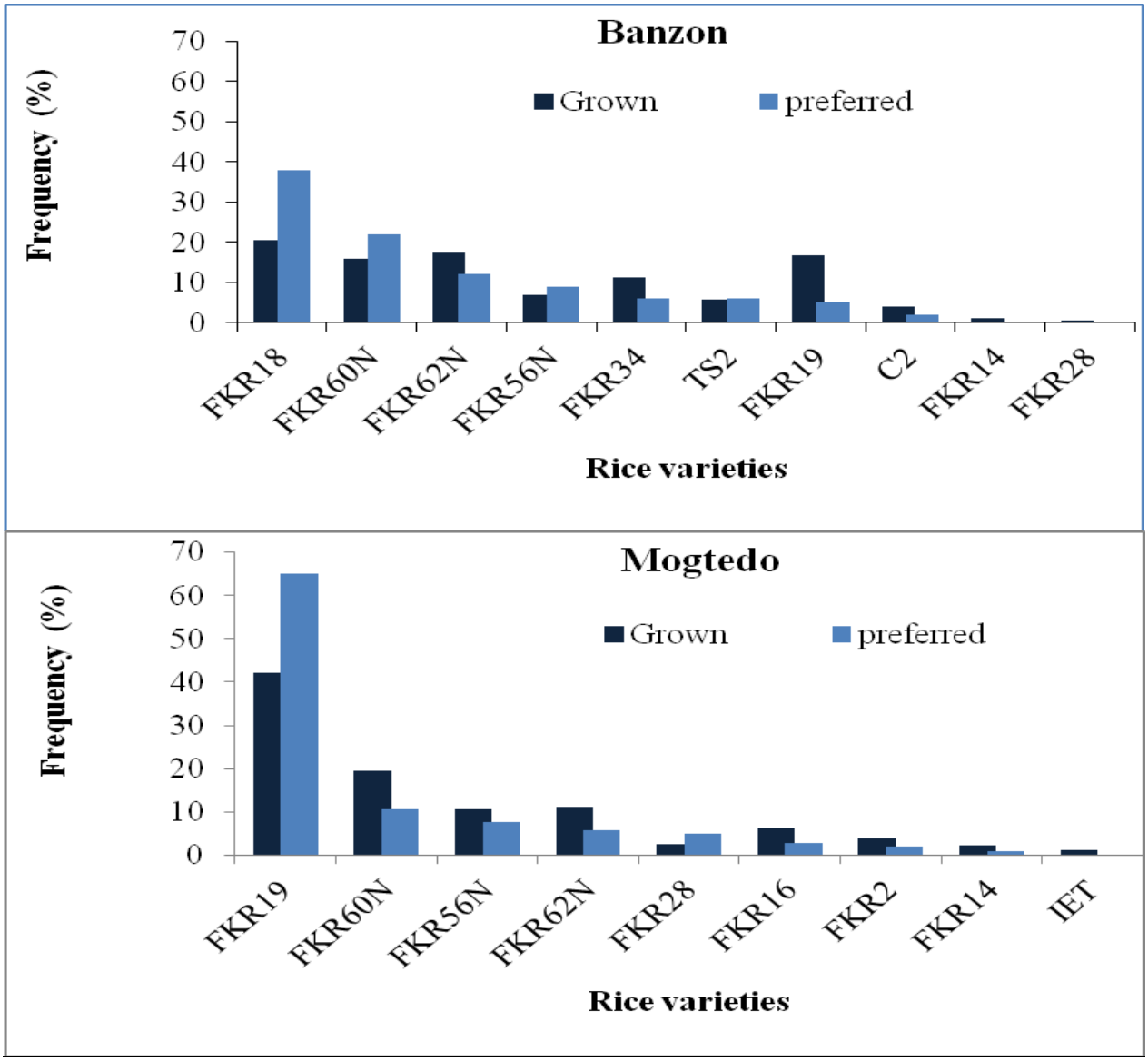

Figure 3.3. Farmers' grown and f preferred rice varieties in Banzon (A) and Mogtedo (B).

Farmers' preferred rice varieties in Banzon somewhat matched the top grown varieties except for FKR19. The three NERICA varieties were part of the most preferred varieties, as 43% of farmers preferred them. Yet, most farmers (38%) preferred FKR18, indicating a clear-cut choice for this variety. In Mogtedo, FKR19 was by far the most preferred variety chosen by 65% of farmers.

NERICA varieties were also among Mogtedo farmers' top choices but to a lesser extent compared to Banzon. Hence, the proportion of farmers who preferred these varieties as a whole was only 24.3%. Altogether, farmer's choice for rice varieties strongly depended on varieties they usually grew. This was apparent in both Banzon and Mogtedo ($P<0.001$). Definitely the five top farmers' preferred rice varieties in both study areas included FKR18, FKR19, FKR56N, FKR60N and FKR62N followed by TS2, FKR28, FKR16, FKR14, FKR34 and C2.

The underlying criteria driving farmers' preference for rice varieties varied in the different the localities surveyed. In Banzon and Mogtedo, high yielding and high market value varieties were among the important criteria considered (Figure 3.4). Taste was the first criterion mentioned by farmers at Banzon. Other characteristics were disease resistance and availability of good seeds.

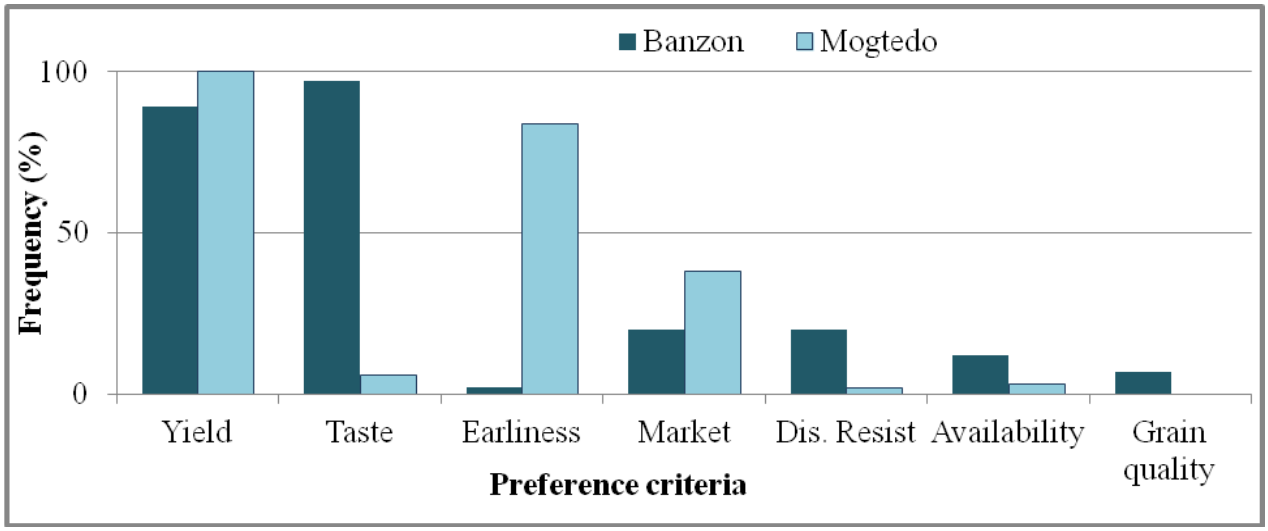

Figure 3. 4. Farmers' preferential criteria for rice varieties in Banzon and Mogtedo

Grain quality, resistance to pests and plant height and length of the growing period were considered to be of secondary importance. By contrast, farmers in Mogtedo stated yield as their foremost criterion. Although high market value varieties were among their primary criteria,

length of the growing period and plant height were also important. All other factors including pest and disease resistance were minor.

3.3.3. Major constraints to rice production

Prominent constraints to rice production mentioned by farmers at Banzon and Mogtedo were water shortaage and diseases (Figure 3.5). Lack of access to fertilizers and their high cost were considered as moderate constraints, particularly at Banzon. Other constraints including availability of quality seeds, lack of technical assistance and damage by insect pests, weeds, birds, and grazing mammals were referred to as less important. Water shortage and diseases were the most important constraints in Mogtedo. Moreover, constraints in this locality were of greater importance than in Banzon.

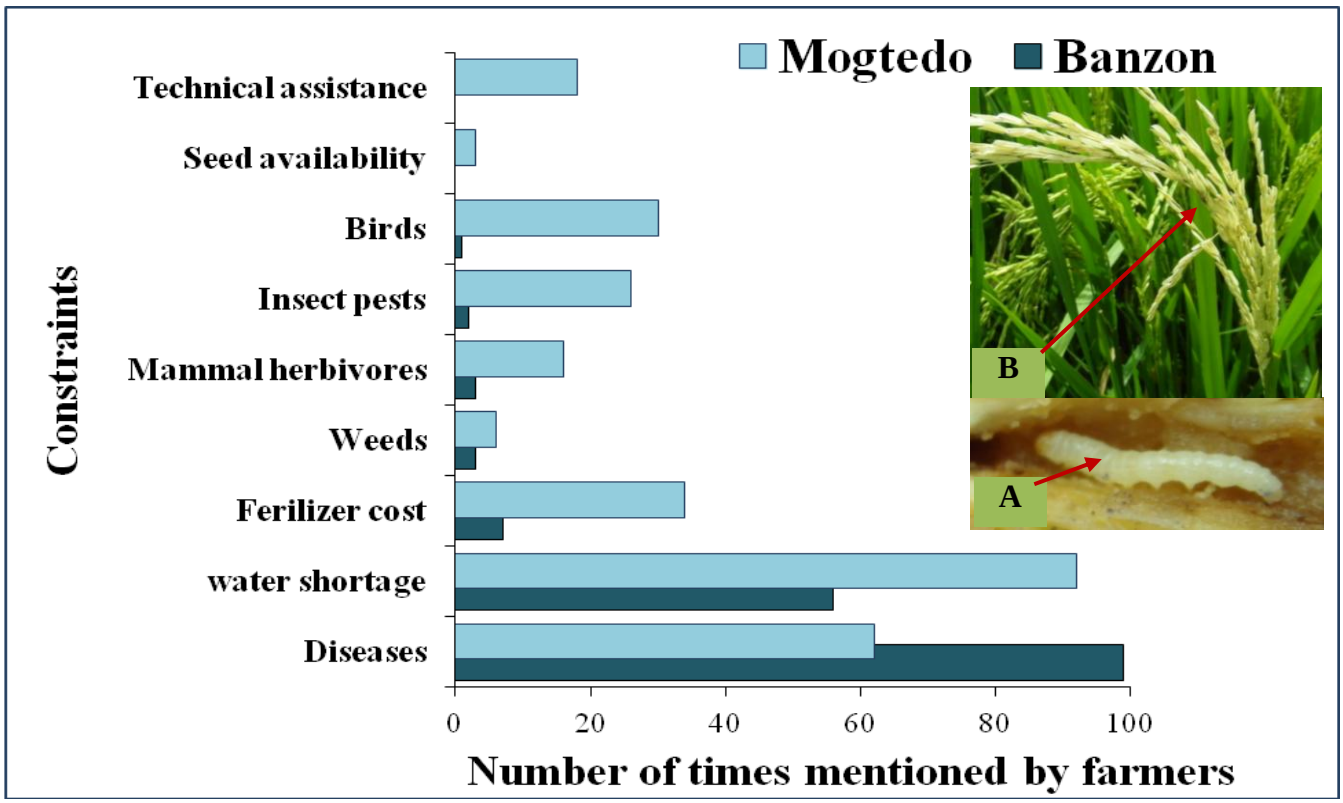

Figure 3. 5. Main constraints to rice production mentioned by farmers at Banzon and Mogtedo
(A) Rice stem borer in rice stalk, showed by a farmer at Banzon and (B) dead panicle caused by rice stem borer

Rice yellow mottle disease was the most important disease cited by farmers at both Banzon and Mogtedo (Figure 3.6). Secondary diseases and pests included African gall midge (*Orseolia oryzivora* Harris & Gagne [Diptera: *Cecidomyiidae*]) and rice blast. Iron toxicity was also mentioned, particularly at Banzon, even though it is an abiotic stress. Some of the farmers were unaware of the occurrence of diseases. They recognized symptoms but attributed them to different factors such as soil infertility, soil degradation or by ash coming from burned rice residues when cleaning the fields.

3.3.4. Rice yellow mottle disease and its management

Farmers used expressive terms to refer to most important diseases they observed. Hence, rice yellow mottle was referred to as "rice HIV" because like Human immunodeficiency virus, once a rice plant was infected, it remained so until death or harvest (Figure 3.5).

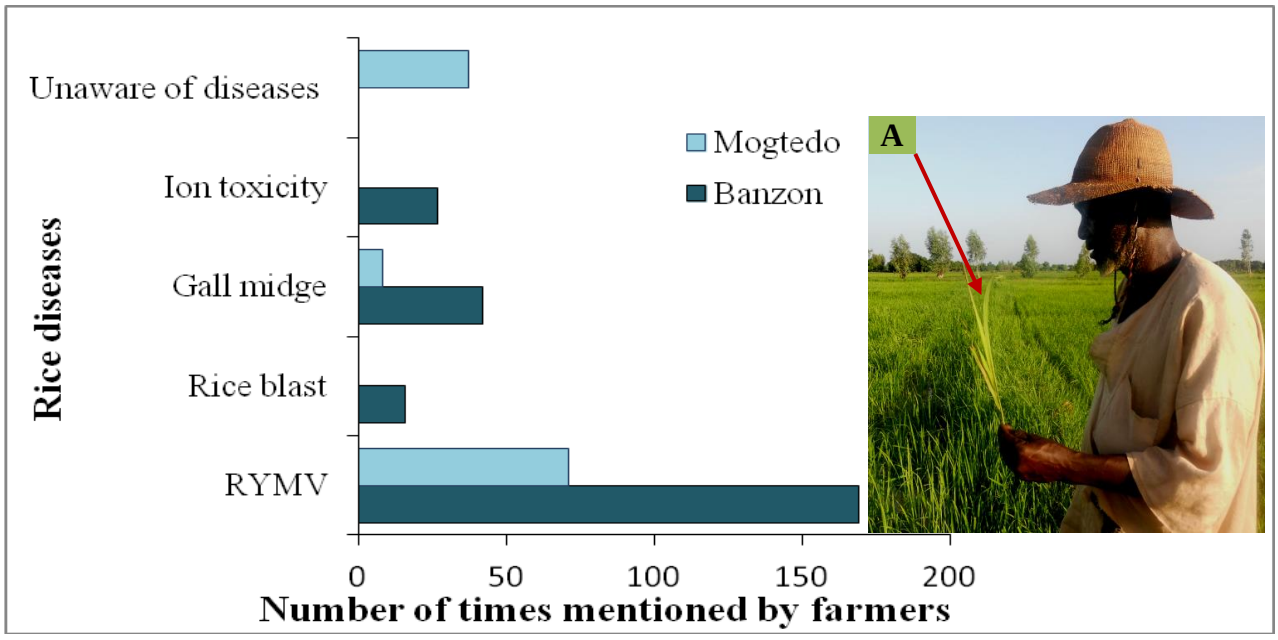

Figure 3. 6. Farmers' awareness of rice diseases in Banzon and Mogtedo; (A) a farmer in Mogtedo able to identify rice yellow mottle disease symptoms on rice leaves

In several instances, farmers did not give any particular name, but stated symptoms which might have been caused by RYMV. Such symptoms included "yellowing of the leaves", "plant stunting" "panicle sterility", cited alone or in combinations. Gall midge was referred to as "dead heart" or "antenna" to describe the characteristic onion shoots galls.

Up to 85% of farmers stated that they adopted at least one control measure against rice yellow mottle disease. Replacement of rice varieties and use of pesticides were some of the common control measures. Farmers indicated that no recommendations were given to them for the use of pesticides, yet they systematically adopted this measure blindly as a first solution. Shifting varieties was more widespread at Banzon where farmers benefited more from technical assistance while pesticides application in rice fields was practiced more at Mogtedo (Figure 3.6). Indigenous control methods included cropping practices, spray of of pesticides and abandon of the fields for one or two years. Cropping practices comprised disease avoidance through delay of sowing and transplanting dates, weeding rice fields as well as clearing levees and ditches around fields.

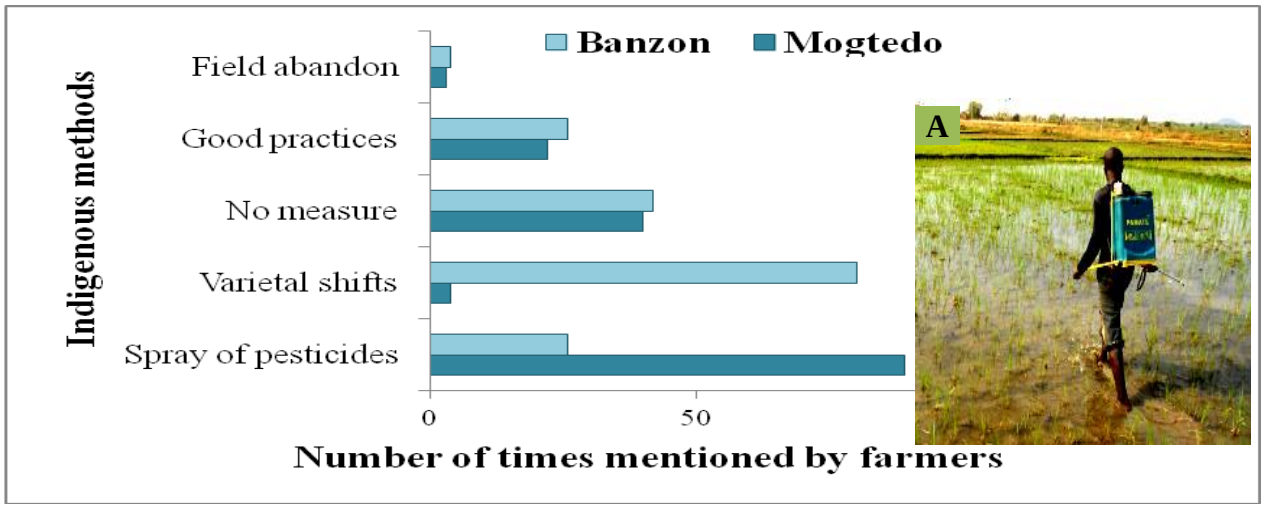

Figure 3.7. Rice yellow mottle control methods used by farmers in Banzon and Mogtedo; (A) a farmer spraying pesticide on rice field in Mogtedo

Most farmers indicated that varietal shifts and other cultural practices were recommended by extension agents although these recommendations were not usually effective because they continued to experience yield losses due to diseases.

3.3.5. Yield loss assessment and disease incidence

Yield loss due to rice yellow mottle disease was assessed for three rice varieties including FKR56N, FKR62N and TS2 (Table 1). Rice yellow mottle disease had a high impact on grain yield reduction. Average yield losses of approximately 75%, 79% and 84% were recorded in rice varieties TS2, FKR62N and FKR56N, respectively. Consequently, the overall yield loss induced by rice yellow mottle disease was estimated at 79.33% in average.

Analysis of variance of per plant grain yield indicated significant effects of disease ($F=1832.1$; $df=1, 600$; $P<0.0001$) and rice variety ($F=25.95$; $df=2, 600$; $P<0.0001$). There were also disease-variety ($F=12.61$; $df=2, 600$; $P<0.001$) and disease-year ($F=6.35$; $df=1, 600$; $P=0.012$) interactions, indicating that the effect of disease on yield depended on varieties and year of production. In the first year, diseased plants of rice varieties TS2 and FKR62 yielded twice as much as those of FKR56N. By contrast, higher yields were found in TS2 only during the second year.

Table 3.1. Effect of rice yellow mottle disease on grain yield in three rice varieties

Variety	Year 1			Year 2		
	Healthy	Diseased	Yield loss (%)	Healthy	Diseased	Yield loss (%)
FKR56N	38.94a[a]	4.40a	88.70a	35.83a	7.30a	79.63a
FKR62N	47.33b	10.10b	78.66a	47.23b	7.43ab	80.14a
TS2	38.18a	8.03b	78.97a	35.59a	10.50c	70.50a

[a]Figures in the table represent means of per plant paddy rice yields in grams (n=50). Means within the same column followed by the same letter(s) are not significantly different (P=0.05), according to Fisher's LSD.

The levels of rice yellow mottle disease infections in Banzon were moderate. Disease incidence was evaluated at 29.54% in 2010 and 26.98% in 2011. The difference in disease incidence was not significant (F= 0.19; df=1; P=0.67), indicating consistent epidemic levels over the two years. Taking into account the average yield loss assessed earlier, observed disease incidences would give extrapolated yield loss of 22.33% per year in Banzon perimeter.

3.4. Discussion

The two study areas had contrasting ecologies and rice growing systems. Rice cultivation in both areas was at subsistence levels. Most farmers held small fields, and also most (76%) had low yields of 1-2 t/ha, which is consistent with the results reported for the whole West Africa (Saito *et al.* 2012). Almost all farmers practiced mixed cropping. The strategy of growing several crops has been considered as a way to improve resilience (Lin 2011). However it may prevent farmers from focusing sufficiently on adequate production of one particular crop, which could benefit rice intensification. Altogether, features of rice cultivation observed here reflected a more general situation common to SSA which also applies to other major crops like maize, sorghum, and cassava (Fermont *et al.*, 2009). For rice in particular, Adesina *et al.* (1994) reported that rice farming in the Ivory Coast was dominated by small scale farmers with minimal external fertilizer input and very little technical assistance from extension agents. Similar characteristics were found in the present study, as the vast majority of farmers (98.5%) held small plots of less than 5 ha and technical assistance was stated as a constraint, particularly at Mogtedo. However, efforts are being made to provide more support to farmers by providing them with improved quality rice seeds and fertilizers along with closer technical assistance.

At both Banzon and Mogtedo, several rice varieties were grown by farmers on a regular basis, irrespective of recommendations from extension agents. By growing several varieties, farmers

adopted the same resilience strategy for rice as for other crops. As such, based on their own empirical knowledge, one particular farmer would grow at least two rice varieties, one early maturing though usually low yielding and one late maturing. This strategy was clearly exemplified by the choice of FKR18 and FKR19 as top varieties by farmers at Banzon and Motgedo,. FKR18 was considered by farmers as high yielding with good taste at Banzon but needed more water than FKR19. As for Mogtedo farmers, whose main concern was water availability, FKR19 was their top variety because of its better resistance to drought as well as its capability to be grown under irrigated and rainfed conditions.

NERICA varieties were among top farmers' preferred rice varieties. Indeed, successful promotion strategies were put in place by AfricaRice through participatory variety selection work involving female and male farmers after the development of these varieties (Seck *et al.* 2012). The national research team also provided strong advertising locally. In the present study, women only represented a small proportion of farmers despite the promotion of several women farmers' organizations in Mogtedo. However, they may have played a significant role in the adoption of varieties through the appreciation of post-harvest processing properties. Possibly, the utmost reason for choosing NERICA varieties was their good yields as indicated by farmers who experienced 3-5 t/ha. These data are consistent with the potential yields of 5 to 7 t/ha (Sie *et al.*, 2007) as on-farm yields were usually found to be lower than potential yields (Becker *et al.*, 2003). Results on yield loss assessment (Table 1) confirmed the higher yield of FKR62N as quoted by farmers during the survey. Although NERICA varieties were highly preferred by farmers, they were not the top preferred, neither at Banzon nor at Mogtedo. According to farmers, FKR18, the top preferred variety at Banzon was one of the recommended rice varieties about 30 years ago. Later on, technical assistance dissuaded farmers from growing it because of

its high susceptibility to rice blast despite the high yield potential of 5-6 t/ha. NERICA varieties which exhibited moderate resistance to this disease (Sie *et al.*, 2007) were deployed but farmers kept growing FKR18 primarily for its good taste. A similar behaviour was observed in 2002 in Mali where rice variety BG90-2 was grown by some farmers for their own consumption because of its good taste while varieties recommended for rice yellow mottle disease management purpose were grown for market (Traore O., unpublished data). The choice of FKR 19 as top preferred variety at Mogtedo did not follow the same rationale. The water shortage constraint and need for getting good yields made FKR19 the best choice in that locality.

Rice diseases were reported by farmers as very important constraints. The opposite opinion was reported eighteen years ago in Ivory Coast where only a small proportion of farmers (9%) mentioned diseases among major constraints in rice (Adesina *et al.* 1994). Farmers' unawareness of diseases was one of the main reasons for the low level of importance they attached to such constraints. Hence, farmers usually mistook diseases for soil infertility symptoms. Recently, farmers and extension agents have been involved in routine trainings with the goal of improving rice production. These trainings allowed several farmers to become familiar with diseases and pests including rice yellow mottle, rice blast, African gall midge, particularly at Banzon and other main irrigated rice areas. This confirmed results obtained in other rice cultivation areas in Burkina Faso (Kam, 2011). Sow (2012) also reported that farmers in Niger mentioned rice yellow mottle disease and bacterial leaf blight as major diseases in irrigated rice systems. Lack of knowledge on rice diseases was perceivable especially at Mogtedo, which may have lowered the proportion of farmers considering diseases as constraints.

Farmers recognized RYMD as the most important rice disease but control measures applied were not effective. Due the complexity of disease epidemiology, Traore *et al.* (2009) argue that an

integrated disease management approach combining the deployment of resistant cultivars with prophylactic measures should be implemented. Although economically unfeasible (Calvert *et al.* 2003) and environmentally-unfriendly, the use of pesticides by farmers possibly had some effect on insect vectors. Effectiveness of varietal shift would have been significant if farmers' varieties were resistant to the disease. Unfortunately, all rice varieties, including NERICA varieties were severely affected by the disease, indicating their susceptibility.

Yield loss assessment due to RYMD on three rice varieties indicated the high yield reduction of the disease whatever the variety. Average per plant yield was estimated at 79.33% with an extrapolated overall yield loss of 22.3% based on disease incidence in the fields. Such moderate levels of yield loss were less than losses ranging from 58-82% reported during severe epidemics (Reckhaus and Adamou 1986; Taylor *et al.* 1990). This was consistent with relatively low disease incidence observed in the fields and indicated that the survey was done in low epidemic years.

CHAPTER 4

4. SCREENING OF RICE ACCESSIONS FOR RESISTANCE TO RICE YELLOW MOTTLE VIRUS IN BURKINA FASO

4.1. Introduction

The necessity for disease management in rice has come to the foreground of crop production since the green revolution. Several damaging outbreaks occurred with all the main virus diseases of rice including rice yellow mottle, hoja blanca, rice grassy stunt and rice ragged stunt (Thresh 1989). Rice yellow mottle disease is endemic to Africa where it is confined. It is induced by *Rice yellow mottle virus* (RYMV) which is considered as the most damaging rice pathogen on the continent. Yield losses often vary from 25 to 100% (Abo *et al.*, 1998). RYMV is easily transmitted mechanically but field dissemination is done by a number of vectors among which beetles are likely the most important.

The main control methods of rice yellow mottle disease include the use of resistant genotypes and application of insecticides to control the vector of RYMV. The use of pesticides in modern agriculture has contributed to improved world food supply through the achievement of better plant growth and yield. However, pesticides and particularly insecticides are often hazardous and their indiscriminate use for controlling pests in crops has been associated with several drawbacks such as resurgence of resistant insect populations, poisoning of farmers and environmental pollution (Hashmi and Khan, 2011). Pesticides, therefore, need to be used in a more responsible manner in order to preserve the environment (Conway, 2012).

Host plant resistance to biotic stresses can play a pivotal role in crop protection (Bonman *et al.*, 1992; Leung *et al.*, 2003). Use of resistant varieties has been considered as an attractive and effective means to control diseases. It requires no additional cost other than that of seeds of

resistant genotypes and it is environmentally safe (Mew, 1991). Moreover, unlike other disease management technologies, resistant varieties can easily be adopted by farmers and widely disseminated. These considerations are particularly applicable to the context of rice growing systems in Africa where almost all farmers are smallholders.

Sources of resistance to pests and diseases need to be identified and evaluated for their efficiency. A lot of research on rice has been devoted to screening rice germplasm and wild rice species for resistance to biotic and abiotic constraints. Many reports by international institute of tropical agriculture (IITA) and by AfricaRice (formerly West African Rice Development Association, WARDA) have identified sources of resistance to RYMV within rice germplasm. Many accessions including *O. sativa*, *O. glaberrima* and wild species *O. longistaminata* and *O. barthii* were screened at IITA and at AfricaRice using either mechanical inoculation of the virus or direct field exposure (Thottappilly and Rossel, 1993; Ng *et al.*, 1988; Attere and Fatokun, 1983; Awoderu, 1991; Raymundo and Konteh, 1980; Okioma and Sarkarung, 1983). Several national research institutions have also screened local accessions for resistance to RYMV (Fomba, 1988; Coulibaly *et al.*, 1999; Zouzou *et al.*, 2008; Rakotomalala *et al.*, 2008; Moga *et al.*, 2012; Sow, 2012, Kam, 2011; Jaw, 2010).

Resistance to RYMV has been found in several accessions (Table 4.1). Consistency in varietal reaction between authors has been observed in a few cases such as the high resistance in Gigante, Bekarosaka, Tog5672, Tog5674, Tog5681 and Tog7291. By contrast, conflicting reactions were observed in several cases. For instance, accessions such as rice cultivars Moroberekan and OS6 were found highly resistant or even immune in some studies (Awoderu, 1991, Zouzou *et al.*, 2008) but only partially resistant in others (Thottappilly and Rossel, 1993). Coulibaly *et al.* (1999) reported OS6 as a susceptible accession. More strikingly, the rice cultivar Moroberekan

showed different reactions when it was grown under irrigated versus rainfed conditions (Zouzou *et al.*, 2008).

.**Table 4. 1.** Some reactions of rice accessions to rice yellow mottle virus

Resistance level	Accession	Reference
Immune	ExDoko, Tob5689, Tob5701, Tob7382, Tog5379, Tog5674, Tog5681, Tog7235, Tog7291 Tol12, Tol268	Thottappilly *et al.*, 1993
	TOG 5672	Coulibaly *et al.*, 1999
High resistance	ITA235, ITA257, IDSA6, FAROX299, IAC164, Itame Nembeika,Azi, Toubabou, Gnonkonsoka, Moroberekan, 0S6	Awoderu, 1991; Zouzou *et al.*, 2008
	IRAT156, ITA 315, IR50, IR56, IRAT170, ITA128, IRAT161, IRAT104, ITA305, ITA303, BPT1235, W1263, GEB24, PY2, Kalinga2, Kannagi, IR9830-26-3-3	Awoderu, 1991
	Gigante, Bekarosaka, Tog5681, Tog7235, Tog7291, Tog5675, Tog5674, Tog7226, Tog7238, VL6, VL123	Coulibaly *et al.*, 1999; Ndjiondjop *et al.*, 1999 ; Rakotomalala *et al.*, 2008 Thiemele *et al.*, 2010,
Partial resistance	MRC603-303. Ratna, Tnau175, TKM9, MTU15, KAU I675. Kaohsiung-Senyu, IR29, IR46, PVRI, UPR254-21-1,IR9802-31-2,IITA, FR77068-2, IR 19473-461-2-3-3-2	Awoderu, 1991
	OS-6, Moroberekan, LAC23, CT19, IRAT110. ITA-235, ITA257, ITA303, ITA305, ITA307, ITA313, ITA315	Thottappilly *et al.*, 1993
	IRAT104, Moroberekan, FKR33	Coulibaly *et al.*, 1999

Inconsistancies in reactions to RYMV across accessions likely reflect the fact that RYMV isolates differed. Therefore, accessions reported as resistant in a given area were susceptible elsewhere. RYMV isolates are known to display a high diversity according to their geographical and ecological origins (Traore *et al.* 2005; Traore *et al.* 2006a; Nguessan *et al.*, 2000). In West Africa alone, three major RYMV strains, S1, S2 and Sa, were found based on the coat protein variability. Another layer of complexity is that each strain exhibits different pathogenic features. The occurrence of resistance-breaking isolates that are able to overcome all known resistant genes (Koala, 2012) is a serious threat for the durability of resistances in fields. Crosses between a few *O. glaberrima* accessions have indicated the existence of additional potential resistance genes (Ahmadi N. and Singh B., 1995; Paul *et al.*, 2003).

The objective of this study was to evaluate the reaction of rice accessions collected from Burkina Faso and Ghana to all the major RYMV strains occurring in West Africa.

4.2. Material and methods

4.2.1. Study area

This study was conducted at Kamboinse research station of the Institute of Environment and Agricultural Research (INERA, Burkina Faso), at 12˚28'N latitude, 1˚32'W longitude. Local weather conditions were characterized by 600-900 mm annual rainfall, 75-90% relative humidity, and temperature between 25-33°C.

4.2.2. Germplasm collection

Rice varieties were collected from national research systems including INERA (Burkina Faso) and CSIR-crops research institute of Kumasi/Ghana. Farmer's landraces were also collected mainly from lowland rice cultivation areas in different localities of the western region of Burkina Faso and from the Volta region of Ghana. Germplasm collected from INERA included a subset of ten top farmers'

preferred rice varieties identified from a participatory rural appraisal study in Banzon and Mogtedo rice growing areas.(Chapter 3) The rice accessions were stored in a cold room at 10-15°C.

4.2.3. Sources of inoculum

All virus isolates used in the experiments originated from West African countries. They were part of INERA plant virus collection maintained at the Laboratory of Plant Virology and Biotechnology. In a first experiment, a viral mixture (Virus mixture-1) was made of all non-resistance breaking isolates (nRB) presented in Table 4.2. Leaf samples infected by corresponding isolates were mixed at equal weights. Of the 11 nRB isolates, six were of strain S1 and the remaining isolates belonged to strain S2. In a second experiment, another mixture (Virus mixture-2) made of nine resistance breaking (RB) isolates of RYMV strains S1 (4 isolates), S2 (4 isolates) and Sa (1 isolate). A third experiment involved 20 RYMV field isolates collected from main rice cultivation areas in Burkina Faso, distinct from those used in the two previous experiments. These isolates were all used singly to screen 23 rice accessions including resistant check varieties.

4.2.4. Inoculation

4.2.4.1. Virus propagation

All selected virus isolates (Table 4.2) were first multiplied in susceptible rice cultivar BG90-2 using mechanical inoculation. Inoculations were done in an insect-proof greenhouse. Infected leaf samples were ground with sterile pestles and mortars in inoculation buffer (0.05 M potassium phosphate buffer, pH 7.0). To ease the grinding process, 1 g of leaf sample was homogenized in 10 ml of buffer containing a pinch of acid-washed sterile sand. Then, carborundum (600 mesh) was added to the extracts which were subsequently rubbed onto the leaves of 21 days post-germination rice seedlings. Leaves from plants infected with each isolate

that showed clear visible symptoms were harvested two weeks post-inoculation and used as source of inoculum.

Susceptible variety BG90-2 and other rice varieties with known resistance phenotypes were used as controls (Table 4.3). All rice accessions were screened in the greenhouse by mechanically inoculating the virus to five plants of each accession. Virus inoculation was done 21 days post germination (dpg). Symptoms development was monitored for up to 45 days post-inoculation (dpi).

Table 4. 2. RYMV isolates selected from INERA RYMV collection used for screening rice accessions

RYMV isolates	**Origin**	**Strain**[a]	**Pathogenicity**[b]		
			Gigante	**Tog5681**	**Pathotype**
854-1	Burkina Faso	S1	-	+	RB-rymv1-3
854-2	Burkina Faso	S1	+	-	RB-rymv1-2
854-3	Burkina Faso	S1	-	-	nRB
854-4	Burkina Faso	S2	-	-	nRB
854-5	Burkina Faso	S2	+	+	RB-rymv1-1/rymv1-3
466-1	Mali	S1	-	-	nRB
466-2	Mali	S1	-	-	nRB
466-3	Mali	S2	-	+	RB-rymv1-3
466-4	Mali	S2	-	-	nRB
466-5	Mali	Sa	+	-	RB-rymv1-2

562-1	Niger	S1	+	+	RB-rymv1-2/rymv1-3
562-2	Niger	S1	-	-	nRB
562-3	Niger	S1	+	-	RB-rymv1-2
562-4	Niger	S1	-	-	nRB
562-5	Niger	S1	-	-	nRB
288-1	Ghana	S2	-	-	nRB
288-2	Ghana	S2	-	-	nRB
288-3	Ghana	S2	-	-	nRB
288-4	Ghana	S2	+	-	RB-rymv1-2
288-5	Ghana	S2	+	-	RB-rymv1-2

[a]Virus strains were determined based on the variability of the coat protein gene (Traore *et al.*, 2010).
[b]Virus isolates were assigned to pathotypes depending on their ability to overcome (+)allele rymv1-2 in Gigante (RB-rymv1-2) or Tog5681 (RB-rymv1-3) or simultaneously both alleles (RB-rymv1-2/ rymv1-3). Isolates not able to overcome (-) any *RYMV1* resistance allele as well as *RYMV2* gene were included in pathotype nRB.

4.2.4.2. Screening of rice accessions

Leaves of inoculated plants were collected at 14 dpi for leaf virus content assessment. Leaf virus content was assessed in leaf extracts by double antibody sandwich Enzyme-linked immunosorbent assays (DAS-ELISA) using a broad spectrum polyclonal antibody (Traore *et al.*, 2008a). All leaf extracts were tested in triplicate.

Table 4. 3. Characteristics of rice varieties used as susceptible and resistant checks

Rice varieties	Species	Gene	Allele	Phenotype
BG90-2	*O. sativa*	RYMV1	*rymv1*	Susceptible
Azucena	*O. sativa japonica*	RYMV1	*rymv1*	Partial resistance
Gigante	*O. sativa indica*	RYMV1	*rymv2*	High resistance
Bekarosaka	*O. sativa indica*	RYMV1	*rymv2*	High resistance
Tog5681	*O. glaberrima*	RYMV1	*rymv3*	High resistance
Tog5672	*O. glaberrima*	RYMV1, RYMV2	*rymv4*	High resistance
Tog5674	*O. glaberrima*	RYMV1	*rymv5*	High resistance
Tog7291	*O. glaberrima*	RYMV2	-	High resistance

4.2.5. Data analysis

Data was analyzed using Statistica software ver.6 (StatSoft France, 2001). One-way analysis of variance (ANOVA) was used to test differences in the mean number of days for symptom appearance between accessions. Data from each accession was compared to the control BG90-2 using Dunnett's test (cf article from Sayes *et al.*, 2006). ANOVA was also used to test for significant differences between leaf virus contents in rice accessions.

4.3. Results

4.3.1. Germplasm collection

In total, 125 rice accessions were collected from 16 locations in Burkina Faso and Ghana (Figure 4.1). Accessions were predominantly from research institutes (46 accessions from INERA including the eight checks and 45 accessions from CSIR-CRI). Most of these accessions were released after varietal improvement which did not consider rice mottle disease management. Thus, apart from varieties used as checks, the accessions had never been screened for resistance

to the RYMV disease. Out of 34 accessions collected from farmers in both countries, 21 were landraces that belonged to *O. glaberrima* species and 13 were of *O. sativa* species.

4.3.2. Reactions of rice accessions to mixtures of RYMV isolates

The reactions of rice accessions to the RYMV isolates are summarized in Table 4.4. Days to symptom appearance varied among the accessions inoculated with virus mixture-1. Symptoms on the leaves of the susceptible control BG90-2 were observed as early as 10 dpi and all inoculated plants showed symptoms at 13 dpi. Partially resistant control Azucena showed symptoms between 15 and 17 dpi. No symptoms were observed in highly resistant rice accessions until 45 dpi when the experiment was terminated.

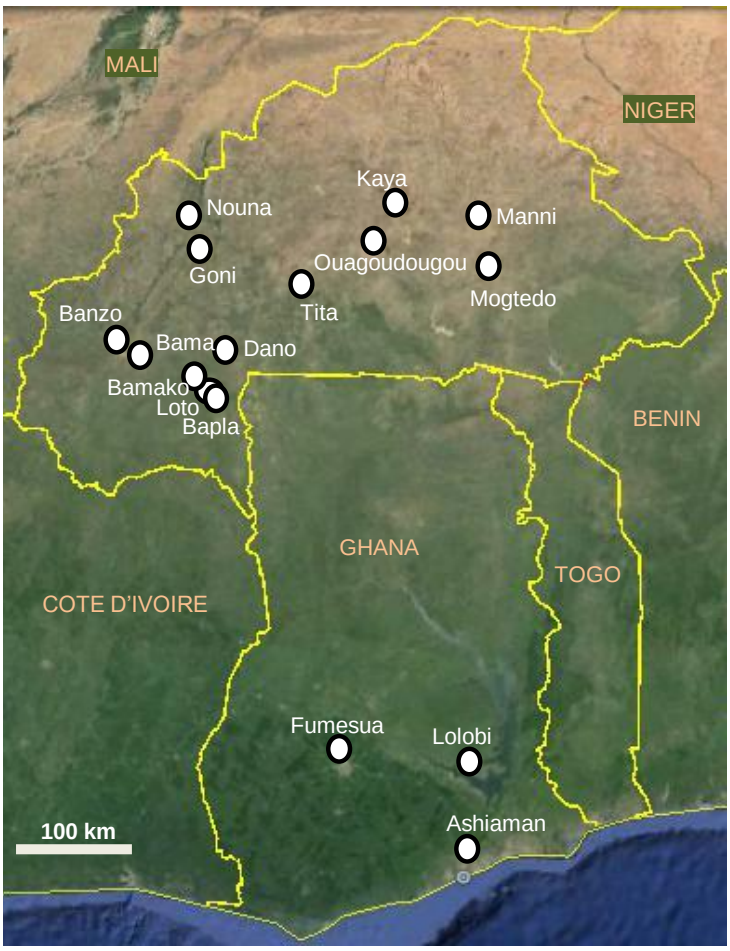

Figure 4. 1. Map of Burkina Faso and Ghana showing rice accessions collection sites

Analysis of variance of the number of days for symptom appearance indicated a significant rice accession effect ($F=45.38$; $P<0.001$, $df=118$), which confirmed differences in reactions among the rice accessions. Post-hoc analysis using Dunnett's test and taking BG90-2 as control group indicated that, apart from accessions used as checks, all accessions could be grouped in two categories. Accessions which did not differ significantly from BG90-2 were susceptible to RYMV. They represented the largest group (65.8%). They were assigned to the susceptible (S) group. Varieties preferred by most farmers' belonged to this group. The second group (29.6%) included accessions which showed symptoms significantly later than BG90-2. Accessions in this category belonged to the partially resistant (PR) phenotype. Only two farmers' preferred varieties (TS2 and FKR28) exhibited the PR phenotype.

Reactions of rice accessions after inoculation with RYMV mixture-2 resulted in the expression of symptoms in BG90-2 earlier than with mixture-1. Symptoms appeared in some plants after 7 dpi and all plants were symptomatic at 10 dpi. By contrast, inoculated plants of the partially resistant accession Azucena showed symptoms between 14 and 18 dpi. Inoculated plants of all highly resistant checks, apart from Tog5672, were symptomatic at 17 dpi.

Symptoms were visible on plants of highly resistant accessions Bekarosaka, Gigante and Tog5681 between 13 and 17 dpi. By contrast, in Tog5674 and Tog7291, inoculated plants showed symptoms between 8 and 9 dpi. Differences in reactions of rice accessions following inoculation with RYMV isolates mixture 2 were found significant in one-way ANOVA ($F=42.03$; $P<0.001$; $df=123$). As with virus mixture 1, Dunnett's post-hoc test resulted in three distinct groupings of accessions. Susceptible accessions formed the largest group (81.6%) while partially resistant accessions and highly resistant ones represented only 17.6% and 0.8%, respectively.

Table 4. 4. Reactions of rice accessions to inoculation of two mixtures of RYMV isolates

N°	Rice accession[a]	Number of days for symptom appearance[b]			
		Virus mixture-1		Virus mixture-2	
1	TS2	17.6 ± 1.5	(PR)	17.2 ± 4.1	(PR)
2	FKR2	7.8 ± 1.1	(S)	8.2 ± 1.6	(S)
3	FKR14	9 ± 0	(S)	7.2 ± 0.4	(S)
4	FKR16	9 ± 0	(S)	6.6 ± 0.5	(S)
5	FKR18	9 ± 0	(S)	7 ± 0	(S)
6	FKR19	11.6 ± 0.5	(S)	7.4 ± 0.9	(S)
7	FKR28	17.2 ± 2.9	(PR)	10.4 ± 0.5	(S)
8	FKR62N	9 ± 0	(S)	7 ± 0	(S)
9	FKR56N	9 ± 0	(S)	7 ± 0	(S)
10	FKR60N	10 ± 0	(S)	7.4 ± 0.9	(S)
11	Adaisi	9 ± 0	(S)	10.2 ± 1.8	(S)
12	Alcame-Femelle	9 ± 0	(S)	7 ± 0	(S)
13	Alcame-Male	9 ± 0	(S)	7.8 ± 1.1	(S)
14	Aromatic	15.2 ± 0.8	(PR)	15.8 ± 1.1	(PR)
15	Aromatic-short	16 ± 1	(PR)	18.2 ± 3.5	(PR)
16	Azucena	16 ± 1	(PR)	16.6 ± 1.7	(PR)
17	Basmati370	9.2 ± 0.4	(S)	8.2 ± 1.6	(S)
18	Beauty	18 ± 3	(PR)	19.6 ± 0.5	(PR)
19	Bekarosaka	NS	(HR)	14.2 ± 1.1	(PR)
20	BG90-2	11.6 ± 1.3	(S)	8.2 ± 1.6	(S)
21	Boning kari	9 ± 0	(S)	8.6 ± 0.5	(S)
22	Bouake189	9.8 ± 0.8	(S)	7.6 ± 0.5	(S)
23	CG14	16.4 ± 1.3	(PR)	12.6 ± 4.3	(PR)
24	Chinoire maalo	10.2 ± 1.3	(S)	6 ± 0	(S)
25	Chinois	10.2 ± 1.3	(S)	8.4 ± 0.9	(S)
26	CRI38 NERICA 5	17.8 ± 2.7	(PR)	7.2 ± 0.4	(S)
27	Digang	16 ± 2.5	(PR)	17 ± 1.7	(PR)

28	Dissi	16.2 ± 1.3	(PR)	6.4 ± 0.9	(S)
29	Djineve	10.2 ± 1.1	(S)	9.4 ± 2.2	(S)
30	”Fao”	8.6 ± 0.5	(S)	8.2 ± 1.1	(S)
31	FKR1	15.2 ± 1.8	(PR)	7 ± 0	(S)
32	FKR21	17.2 ± 1.6	(PR)	17.4 ± 2.2	(PR)
33	FKR29	21.2 ± 2.5	(PR)	16 ± 1.4	(PR)
34	FKR33	14.4 ± 0.9	(PR)	18.4 ± 0.9	(PR)
35	FKR35	10.2 ± 1.1	(S)	9.4 ± 2.2	(S)
36	FKR37	15.2 ± 1.3	(PR)	9.4 ± 0.5	(S)
37	FKR39	10 ± 0	(S)	9.4 ± 1.3	(S)
38	FKR41	14.4 ± 3.6	(PR)	15 ± 0	(PR)
39	FKR42	9 ± 0	(S)	7.4 ± 0.5	(S)
40	FKR43	21.4 ± 5	(PR)	15 ± 0	(PR)
41	FKR45N	9.8 ± 1.8	(S)	8.6 ± 0.5	(S)
42	FKR47N	21.8 ± 1.8	(PR)	10.6 ± 1.3	(S)
43	FKR48	9 ± 0	(S)	7.2 ± 0.4	(S)
44	FKR49	19 ± 0	(PR)	10.4 ± 0.5	(S)
45	FKR50	10 ± 0	(S)	7.4 ± 0.5	(S)
46	FKR58N	13 ± 0.7	(S)	8.8 ± 0.4	(S)
47	GH 4008	9 ± 0	(S)	9.2 ± 0.8	(S)
48	GH1520	21 ± 5	(PR)	21.4 ± 3.6	(PR)
49	GH1571	8.8 ± 0.4	(S)	7.4 ± 0.5	(S)
50	GH1577	19 ± 0	(PR)	16.4 ± 0.5	(PR)
51	GH1584	7.2 ± 0.4	(S)	8 ± 0	(S)
52	GH1584 bis	9 ± 0	(S)	8.2 ± 0.4	(S)
53	GH1585	7.6 ± 0.5	(S)	8.4 ± 0.5	(S)
54	GH1589	7 ± 0	(S)	6.2 ± 0.4	(S)
55	GH1796	9 ± 0	(S)	8 ± 0	(S)
56	GH1801	10.2 ± 1.3	(S)	8 ± 0	(S)
57	GH1835	8.4 ± 0.5	(S)	8.8 ± 0.4	(S)

58	GH4008	10 ± 0	(S)	9.4 ± 0.5	(S)
59	Gigante	NS	(HR)	15.2 ± 1.8	(PR)
60	GR18	8.4 ± 1.3	(S)	7.2 ± 0.4	(S)
61	IDSA85	15.2 ± 1.1	(PR)	16.6 ± 2.6	(PR)
62	IET6279	9.8 ± 0.4	(S)	8.8 ± 1.6	(S)
63	IR5	9 ± 0	(S)	7.2 ± 1.1	(S)
64	IR64	8.6 ± 0.5	(S)	6.6 ± 0.5	(S)
65	IR67908-5-1	9.8 ± 0.4	(S)	9.4 ± 1.3	(S)
66	IR70445-146-3-3	8.8 ± 1.1	(S)	9.4 ± 1.3	(S)
67	IR70445-229-4-1	9 ± 0	(S)	10.6 ± 0.9	(S)
68	IR71137-184-3-2-3-3	11 ± 1.2	(S)	10 ± 0	(S)
69	IR71138-49-2-2-1-2	14.6 ± 1.3	(PR)	10.2 ± 0.4	(S)
70	IR72870-120-1-2-2	9.2 ± 0.4	(S)	8.2 ± 1.6	(S)
71	ITA320	9.8 ± 0.4	(S)	10.6 ± 0.5	(S)
72	ITA324	9 ± 0	(S)	10.4 ± 0.5	(S)
73	Jasmine85	9.8 ± 0.4	(S)	10 ± 0	(S)
74	KRC-Baika	10.2 ± 0.4	(S)	9.4 ± 1.3	(S)
75	Kumazuce	16.4 ± 0.5	(PR)	7.8 ± 1.1	(S)
76	Maalobo	11.2 ± 0.8	(S)	6.4 ± 0.5	(S)
77	Maalo-gwai	21.8 ± 4	(PR)	6 ± 0	(S)
78	Maaloteliman	17.2 ± 2	(PR)	7.6 ± 0.9	(S)
79	Maalowouleen	9.6 ± 0.5	(S)	6.4 ± 0.9	(S)
80	Malina	9 ± 0	(S)	8.2 ± 1.1	(S)
81	Maloba	9 ± 0	(S)	6.8 ± 1.1	(S)
82	Maloboo	9 ± 0	(S)	6.8 ± 1.1	(S)
83	Marobou	10.4 ± 1.9	(S)	7.6 ± 0.9	(S)
84	Marshall	9.2 ± 0.4	(S)	7 ± 0	(S)
85	Moobou	9.8 ± 0.8	(S)	7.6 ± 0.9	(S)
86	Moui	9 ± 0	(S)	6 ± 0	(S)
87	Mouikwin1	9 ± 0	(S)	7.6 ± 0.9	(S)

88	Mouikwin2	9 ± 0	(S)	7.6 ± 0.9	(S)
89	Mouikwin3	8.4 ± 0.5	(S)	7.6 ± 0.9	(S)
90	Mouikwin4	17.6 ± 1.7	(PR)	7.2 ± 1.1	(S)
91	Mouikwin5	9 ± 0	(S)	7.6 ± 0.9	(S)
92	Mouiplaa	9 ± 0	(S)	7.2 ± 1.1	(S)
93	N28K	19.6 ± 0.5	(PR)	10.8 ± 0.4	(S)
94	Napone	8.4 ± 0.5	(S)	8 ± 0	(S)
95	NERICA1	16 ± 1.7	(PR)	10 ± 0	(S)
96	Nerica16	9 ± 0	(S)	8.8 ± 1.1	(S)
97	NERICA2	12.8 ± 1.6	(S)	9 ± 0	(S)
98	Nerica23	19.8 ± 0.4	(PR)	9.2 ± 0.4	(S)
99	Nerica24	19 ± 2.2	(PR)	13.8 ± 1.6	(PR)
100	Nerica28	16.8 ± 2.2	(PR)	7.4 ± 0.5	(S)
101	NERICA3	12 ± 0	(S)	7.2 ± 0.4	(S)
102	NERICA4	16 ± 0	(PR)	8.4 ± 0.5	(S)
103	Nerica54	9 ± 0	(S)	9 ± 0	(S)
104	Nerica7	15.4 ± 0.5	(PR)	16.6 ± 3.1	(PR)
105	Nerica9	19 ± 1.2	(PR)	14.8 ± 0.4	(PR)
106	Nerica-pluvial	14.6 ± 0.5	(PR)	13.8 ± 1.6	(PR)
107	Orodara	9.2 ± 0.4	(S)	6 ± 0	(S)
108	P38	13.2 ± 1.5	(S)	8.4 ± 0.9	(S)
109	Paroyente	8 ± 0	(S)	8 ± 0	(S)
110	Perfum-rice	10.2 ± 0.8	(S)	8 ± 0	(S)
111	Rox-cv	9.2 ± 0.4	(S)	9 ± 1.4	(S)
112	Sikamoo	9 ± 0	(S)	8.6 ± 2.2	(S)
113	Soomalo	10.2 ± 1.1	(S)	6.4 ± 0.9	(S)
114	TanghinI	9 ± 0	(S)	7 ± 0	(S)
115	TanghinII	9 ± 0	(S)	7.8 ± 0.4	(S)
116	Tiefagamalo	9 ± 0	(S)	7.2 ± 0.8	(S)
117	Tog5672	NS	(HR)	NS	(HR)

118	Tog5674	NS	(HR)	8.6 ± 0.5	(S)
119	Tog5681	NS	(HR)	16.6 ± 0.5	(PR)
120	Tog7291	NS	(HR)	8 ± 0	(S)
121	Tox728-1	9.2 ± 0.4	(S)	8.6 ± 1.2	(S)
122	Viwonor short	19 ± 2	(PR)	10.2 ± 1.8	(S)
123	Viwonor tall	13.8 ± 0.8	(S)	7.6 ± 1.3	(S)
124	Wita7	9.8 ± 0.4	(S)	7.6 ± 1.3	(S)
125	Woussou	9 ± 0	(S)	7 ± 0	(S)

[a]Farmer's ten top preferred rice accessions are in boldface
[b]mean number of DSA (days for symptom appearance) post inoculation ± standard deviation (n=5) with virus mixture 1 and mixture 2 (see Material and methods); no symptom (NS) was observed in some cases;
[c] Reaction phenotypes (indicated in parentheses) were attributed to accessions after one-way ANOVA of the number of days for symptom appearance followed by Dunnett's test ($P < 0.05$), taking BG90-2 as control group: S, susceptible; PR, partially resistant; HR, highly resistant.

As shown in Figure 4.2, the proportion of resistant accessions identified after inoculation with virus mixture-1 was significantly less (χ^2 =7.43; P=0.006) when mixture 2 was used. Up to 14.4% of accessions identified as partially resistant following inoculation with virus mixture-1 were susceptible after inoculation with mixture-2.

4.3.3. Virus accumulation in inoculated plants

Assessment of the levels of virus multiplication in plants, expressed as absorbances, indicated that rice accessions could be grouped based on the leaf virus content. Following inoculation with virus mixture 1, three groups of accessions were distinguished (Figure 4.3A). The first group consisted of all accessions identified as highly resistant (HR) when assessing the time for symptom appearance. No virus could be detected in these accessions because they reacted as the healthy control leaf extract giving a background reaction only. A second group included the susceptible check BG90-2 and accessions of the S phenotype. As indicated by the high absorbance values, accessions of the second group supported high virus multiplication. The third

group included accessions of the PR-phenotype and Azucena. In this group, ELISA reactions indicated relatively low virus titres. There was a large variation in reactions of PR pathotypes as indicated by the high standard deviation value.

Assessment of virus titre in leaf extracts infected by virus mixture-2 resulted in a different pattern (Figure 4.3B). High virus titre was found in Tog5672 as well as in another group of accessions including BG90-2, Tog7291, Tog5674 and all S-phenotype accessions. Lower virus titre was obtained from PR-phenotype accessions as well as Tog5681, Gigante, Bekarosaka and Azucena.

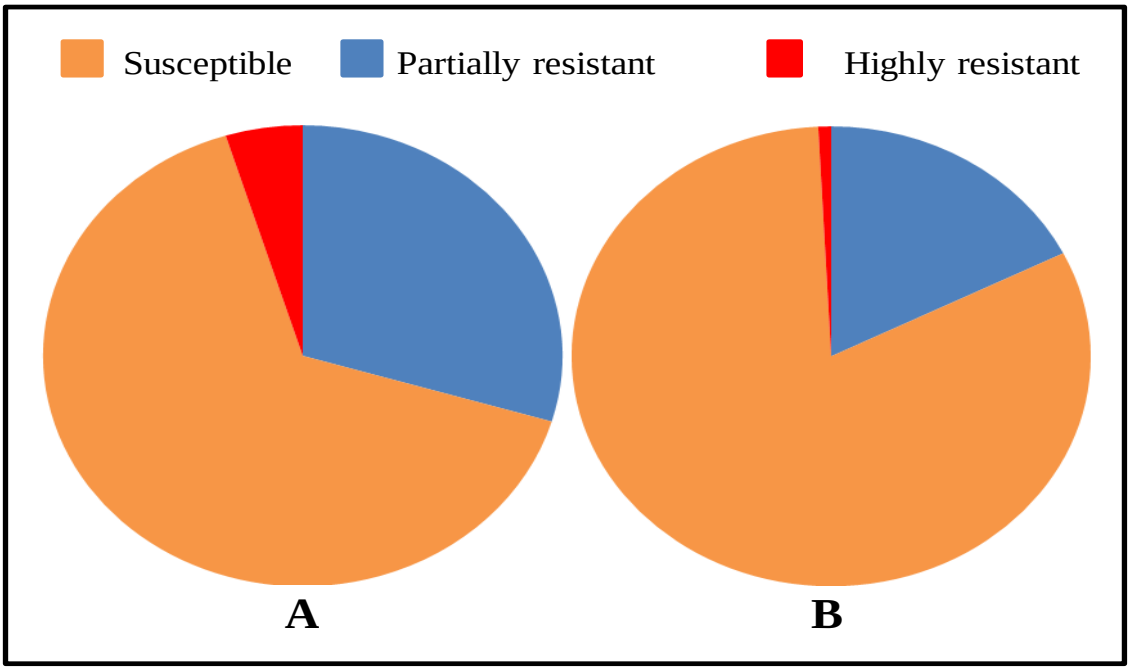

Figure 4. 2. Proportions of susceptible, partially resistant and highly resistant rice accessions identified after inoculation of RYMV isolates mixture 1 (A) and mixture 2 (B)

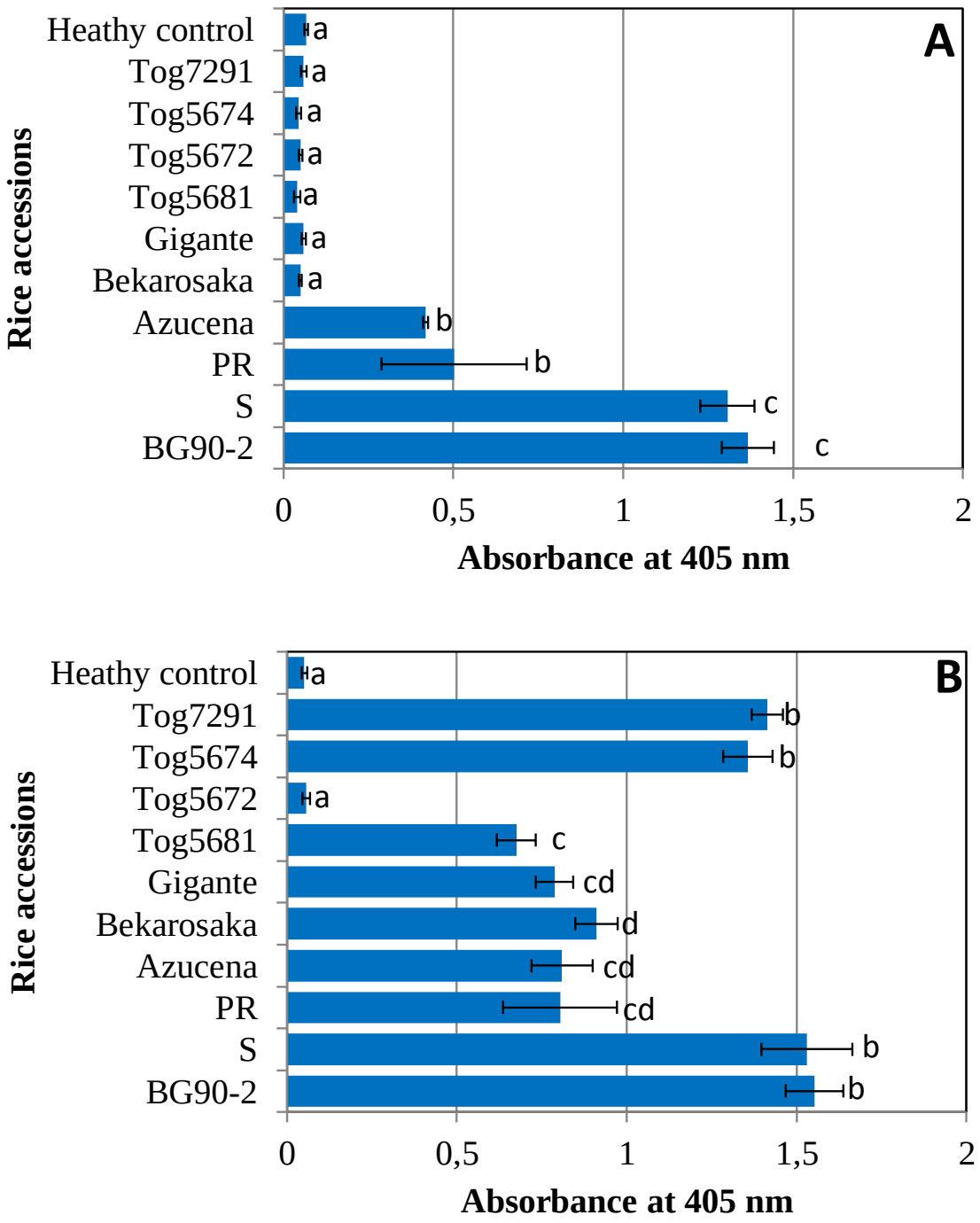

Figure 4. 3. Mean of virus titres in leaves of rice accessions inoculated with mixture 1 (A) and mixture 2 (B) of RYMV isolates (see material and methods).

Data from susceptible (S) and partially resistant (PR) accessions were pooled, respectively. Means associated with the same letter(s) did not differ significantly according to Fisher's LSD test at P=0.05. Error bars indicate standard deviation of the mean.

4.3.4. Reactions of rice accessions to field isolates

Following inoculation with individual field RYMV isolates that had not been characterized, the susceptible check BG90-2 developed symptoms with all virus isolates (Table 4.5). Partially resistant check Azucena displayed PR-phenotype with almost all virus isolates.Only isolate VII was able to overcome its partial resistance. Similarly, two accessions (Gh1577 and FKR33) showed the PR phenotype with almost all isolates but were susceptible to isolate III. The highly resistant check Gigante remained symptomless after inoculation with six of the 10 virus isolates, therefore displaying a high resistant (HR) phenotype. However, it developed symptoms similarly to BG90-2 to four isolates, indicating a S phenotype. Consequently, the six isolates which could not overcome resistance in Gigante were non-resistance breaking isolates. Alternatively, the four other isolates which induced symptoms on Gigante were resistant breaking isolates. Half of the 20 rice accessions tested showed the PR phenotype, regardless of the isolate used. The remaining accessions displayed the S phenotype in most cases, particularly with virus isolates that were able to overcome resistance in Gigante. With non-resistance breaking isolates, all accessions except CG14 showed resistance (PR phenotype).

Table 4. 5. Reactions of 20 rice accessions to inoculation with 10 RYMV isolates[a]

Rice accessions	RYMV isolates									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
Digang	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
TS2	.	.	.	.	.	.	.	.	.	.
CG14	.	S	S	S	.	S	S	S	.	S
GH1577	.	.	S	.	.	.	.	.	.	.
FKR21	.	.	.	.	.	.	.	.	.	.
Aromatic short	.	.	.	.	.	.	.	.	.	.
FKR28	.	.	S	S	.	.	.	.	.	.
FKR29	.	.	.	.	.	.	.	.	.	.
Beauty	.	.	.	.	.	.	.	.	.	.
Dissi	.	S	S	S	.	.	.	.	.	.
FKR49	.	.	S	S	.	.	.	.	.	.
FKR33	.	.	S	.	.	.	.	.	.	.
FKR43	.	.	S	S	.	.	.	.	.	.
FKR47N	.	.	.	.	.	.	.	.	.	.
IDSA 85	.	.	.	.	.	.	.	.	.	.
Maalo-teliman	.	.	S	S	.	.	.	.	.	.
Moui kwin4	.	S	S	S	.	.	.	.	.	.
Viwonor short	.	.	S	S	.	.	S	.	.	.
NERICA 1	.	.	.	.	.	.	.	.	.	.
CRI38 NERICA 5	.	.	.	.	.	.	.	.	.	.
BG90-2	S	S	S	S	S	S	S	S	S	S
Azucena	.	.	.	.	.	.	S	.	.	.
Gigante	HR	S	S	S	HR	HR	S	HR	HR	HR

[a]For each RYMV isolate, reaction phenotypes were attributed to rice accessions after one-way ANOVA of the number of days for symptom appearance followed by Dunnett's test ($P < 0.05$), taking susceptible (S) variety BG90-2 as control group. Azucena and Gigante were used as partially resistant (PR) and highly resistant (HR) checks. Dots represent the PR phenotype expressed by Digang.

4.4. Discussion

Part of the rice germplasm (16.8%) collected during the surveys consisted of accessions of the African rice *O. glaberrima* held by farmers. This indicates that some farmers continue to grow *O. glaberrima* varieties despite the fact that most rice varieties grown in West Africa belong to *O. sativa* species. The African rice has a low yield potential compared to its Asian counterpart, but it is used by some communities for food, rituals and herbal medicine (Linares, 2002; Van Andel, 2010). Cultivation of *O. glaberrima* by smallholder farmers may also be due its better adaptation to stresses caused by pests, diseases and abiotic constraints (Jones *et al.*, 1997a). Although rice accessions in this study were collected in locations distinct from previous collection surveys (Sie, 1998; Kam, 2011), duplications likely occured. The use of molecular markers for germplasm diversity studies may provide useful information for cleaning up the duplicated rice accessions from the collection (Wong *et al.*, 2009; Some, 2012)

Screening of the collected rice accessions for resistance to RYMV indicated that virus-host interactions strongly depended on the virus isolates. Up to 45.9% of rice accessions expressed the PR phenotype with virus mixture 1. They were found to be susceptible when mixture 2 was used. Consequently, virus mixture 1, composed of non-resistance breaking isolates, was more effective in the identification of resistance in rice accessions. Virus mixture 2 was able to overcome resistance in highly resistant accessions used as controls. However, some of these accessions displayed partial resistance even though the high resistance was no longer effective. These results suggest that the mechanisms for overcoming partial and high resistance are distinct. Previous studies clearly indicated that high resistance and partial resistance have different genetic bases (Ndjiondjop *et al.*, 1999; Ahmadi *et al.*, 2001). Therefore, the ability of virus mixture 2 to overcome the partial resistance in some of the accessions PR to mixture 1 was not

unexpected. Possibly, mixture 2 also included virulent isolates distinct from those which overcame the high resistance conferred by the RYMV1 gene. This was apparent in the breakdown of resistance conferred by RYMV2 gene in Tog7291.

Altogether, screening rice accessions for resistance to RYMV indicated that most rice accessions were susceptible to RYMV, which is consistent with previous studies (Calvert *et al.*, 2003;Coulibaly *et al.*, 1999; Calvert *et al.*, 2003; Zouzou *et al.*, 2008; Sow, 2012). No new highly resistance source was identified in collected rice accessions including *O. glaberrima* species from which such resistance are more frequent. Additional high resistance genes are yet to be searched in rice, particularly the African rice (Ahmadi N. and Singh B., 1995; Paul *et al.*, 2003). Therefore, screening rice germplasm for resistance to disease, particularly RYMV, needs to be continued in order to identify suitable resistance sources. Efforts are continuously to collect and preserve rice germplasm at both national and international levels. More than 200,000 rice accessions are reported in 40 national and international rice gene banks (Chen *et al.*, 2007; Berger *et al.*, 2012). Most accessions in these collections have not been screened for disease resistance. The present study contributed to the characterization of national rice collections to identify partial resistant accessions which can be used in breeding programmes for rice yellow mottle disease management.

Conflicting results attributed to the effect of environment have been frequently reported in screening experiments conducted for the identification of resistance sources to RYMV (Kouassi *et al.*, 2005; Zouzou *et al.*, 2008). Indeed, the environmental conditions may have some effects on the virus-host interactions but our results suggest that most screening experiments failed to take into account the virus dimension adequately. The use of virus mixture 1 and mixture 2 composed of nRB and RB isolates, respectively, led to inconsistent identification of PR-

phenotype rice accessions. This result was confirmed when field isolates of the virus were used for screening. Moreover, isolates which did not overcome RYMV1 resistance gene in Gigante gave inconsistent virus-host interactions in CG14 (Table 4.5).

Overall, screening for resistance to RYMV should be based on a good knowledge of the virus diversity. The identification of sources of resistance to the virus requires the use of well characterized nRB isolates. Although virus mixture 1 and individual nRB isolates led to similar results in the identification of PR-phenotype accessions, inoculum consisting of a mixture of virus isolates may drive to synergic effects in overcoming some potential sources of partial resistance to RYMV. Indeed the biological effects of interactions between RYMV isolates are poorly known. In mixed infections of rice plants, S2 isolates dominated over S1 isolates for virus accumulation but there was no evidence of interaction in the virus accumulation between either types of isolates and S4 isolates (N'Guessan *et al.*, 2001).

CHAPTER 5

5. MARKER ASSISTED INTROGRESSION OF RYMV1 RESISTANCE GENE INTO FARMERS' PREFERRED RICE VARIETIES

5.1. Introduction

Rice plays an important role as a staple food crop in Africa, especially in West Africa (Diagne, 2011). Several authors indicated that rice is one of the four top crops that will be feeding the world population by 2050 and efforts must be made to increase its productivity (Seck *et al.*, 2012; Alexandratos *et al.*, 2012; Ray *et al.*, 2013). This goal can be achieved by breeding rice to develop new high yielding and adapted rice varieties. Rice breeders have been interested in developing high yielding varieties which also combine desirable agronomic traits such as earliness and grain quality. Constraints such as pests and diseases have not been systematically taken into account in most breeding strategies.

Rice yellow mottle disease is one of the most damaging rice diseases in Africa. It is caused by *Rice yellow mottle virus* (RYMV) which causes yield losses of 25-100% (Abo *et al.*, 1998; Kouassi *et al.*, 2005). In order to develop resistant rice varieties against RYMV, sources of resistance were identified by screening rice germplasm (Thottappilly and Rossel, 1993; Awoderu, 1991; Coulibaly *et al.*, 1999). Screening rice germplasm for resistance to RYMV has been an ongoing process (See Chapter 4) (Thiemélé *et al.*, 2010, Kam, 2011; Mogga *et al.*, 2012). Partially resistant varieties were identified and recommended to farmers, during severe disease epidemics (Coulibaly *et al.*, 2001). Highly resistant sources were later identified in *Oryza sativa* cv. Gigante (Ndjiondjop *et al.*, 1999) and Bekarosaka (Rakotomalala *et al.*, 2008). High resistance was also found in a few *O. glaberrima* varieties among which cv. Tog5681 is the most

studied (Thottappilly and Rossel, 1993; Konaté *et al.*, 1997; Ndjiondjop *et al.*, 1999; Thiemele *et al.*, 2010).

The identified sources of high resistance were poor yielding or poorly adapted, so they have only been used in breeding for resistance to RYMV (Ndjiondjop *et al*., 1999). Studies were conducted to ascertain genetic basis of resistance to the virus. Partial resistance was found to be polygenic (Albar *et al.*, 1998; Ioannidou *et al.*, 2000) and high resistance was monogenic and recessive, and involved two genes, namely *RYMV1* and *RYMV 2* (Ndjiondjop *et al.*, 1999; Thiemele *et al.*, 2010). Further studies on *RYMV1* gene led to the identification of five alleles (Albar *et al.*, 2006). Several breeding programmes are currently developing or testing *RYMV1*-mediated resistant rice varieties (Seck *et al.*, 2012). Marker-assisted selection (MAS) is being used as a more efficient breeding strategy. Recently, microsatellite markers were used to develop near-isogenic resistant lines (Kam, 2011; Jaw *et al.*, 2012). PCR-based single nucleotide polymorphism markers have been also used to tag specific resistance alleles (Thiemele *et al.*, 2010; Sow, 2012).

The stability of RYMV resistance has been questioned since several resistance-breaking isolates of the virus were found in most rice growing areas (Traore *et al.*, 2006a; Amoncho *et al.*, 2009; Ochola and Tusiime, 2011; Issaka *et al.*, 2012). Some authors suggest that genetic control should be included in a broader disease management strategy, taking into account prophylactic measures (Traore *et al.*, 2009). In such a strategy, partial resistance may be used to efficiently control the disease. Moreover, as reported in other viral pathosystems, combining partial and high resistance to the virus may result in more resistance stability (Palloix *et al.*, 2009).

In this chapter, recombinant inbred line populations were developed using different hybridization schemes involving partial resistance and RYMV1-mediated high resistance donors and

susceptible farmers' rice varieties. RYMV1-mediated resistance alleles were tagged using specific SNP markers and inbred lines were assessed for resistance to RYMV.

5.2. Materials and methods

5.2.1. Research time frame and study areas

The development of recombinant inbred line populations (RIPs) was carried out from March 2011 to October 2012. Thereafter, genotyping and phenotyping of recombinant inbred lines (RILs) and backcrossed inbred lines (BILs) for resistance to RYMV were performed from October 2012 to December 2012. All experiments were carried out in greenhouse and laboratory facilities of INERA Kamboinse research station (12˚28'N latitude, 1˚32'W longitude). Local weather conditions were characterized by 600-900 mm annual rainfall, 75-90% relative humidity and temperature between 25-39°C.

5.2.2. Parental lines

In total, 14 rice varieties were used as parental lines (Table 5.1). Half of them were farmers' varieties while the rest consisted of RYMV resistant genotypes possessing resistance alleles, *rymv1-2* (Gigante and Bekarosaka) and *rymv1-3* (Tog5681). Most farmers' varieties were among the top preferred ones (Chapter 3) and were also shown to be susceptible to RYMV (see Chapter 4). Kumazuce was included in the experiment because it was preferred by farmers in some areas of Ghana. Partial resistance donors included varieties CG14, GH1520, Nerica28, Digang and Azucena which showed partial resistance consistently with virus mixture 1 and mixture 2 (chapter 4). All parental lines were chosen based on a prior assessment of crossing compatibility.

5.2.3. Breeding nursery establishment

Parental seeds were sown in a nursery three times at intervals of 14 days to ensure synchronization of flowering times. Over 100 seeds from each parental line were cleaned using

0.08% sodium hypochlorite and pre-germinated into ‘’Petri dishes’’ using sterile water containing 0.01 % of fungicide (ThiramTM: dithiocarbamate).

Table 5. 1. Rice genotypes used for the development of recombinant and backcross inbred lines

Genotypes	Source	Species[a]	Phenotype[b]
Farmers' rice varieties			
FKR16	INERA/BURKINA FASO	*Oryza sativa*	Susceptible
FKR19	INERA/BURKINA FASO	*O. sativa*	Susceptible
FKR56N	INERA/BURKINA FASO	Interspecific	Susceptible
FKR60N	INERA/BURKINA FASO	Interspecific	Susceptible
FKR62N	INERA/BURKINA FASO	Interspecific	Susceptible
Nerica28	INERA/BURKINA FASO	*O. sativa*	PR
Kumazuce	SRI-CRI/GHANA	*O. sativa*	PR
Resistance donors			
CG14	SRI-CRI/GHANA	*O. glaberrima*	PR
Gh1520	SRI-CRI/GHANA	*O. glaberrima*	PR
Digang	SRI-CRI/GHANA	*O. sativa*	PR
Azucena	INERA/BURKINA FASO	*O. sativa*	PR
Gigante	INERA/BURKINA FASO	*O. sativa*	HR (*rymv1-2*)
Bekarosaka	INERA/BURKINA FASO	*O. sativa*	HR(*rymv1-2*)
Tog5681	INERA/BURKINA FASO	*O. glaberrima*	HR(*rymv1-3*

[a]Interspecific lines resulted for *O. sativa* x *O. glaberrima* crosses.
[b]Rice line phenotypes are related to their reactions to RYMV as determined in Chapter 4. PR and HR indicate partial and high resistance, respectively.

Pre-germinated seeds were planted in nurseries (average density of 1000 seeds/m^2) for 3 weeks. Subsequently, plantlets were transplanted singly into 20 litre buckets filled with clay soil. Fertilizer was applied at a rate of 200 kg of NPK (15:15:15) per hectare. Nitrogen fertilizer (urea: 46% N) was applied in two dressings, at maximum tillering and at panicle initiation, stages.

5.2.4. Rice emasculation and cross-pollination for F1 seeds production

An electric vacuum emasculator was adapted from that of Cornell University (Figure 5.1). This device was used to remove anthers from rice flowers. Selected partially emerged (50-60%) panicles on female plants were first disinfected using 0.5% chlorometric sodium hypochlorite prior to emasculation. Panicle leaf sheaths were folded down and upper and the lower florets were cut off with scissors. Tips of florets were clipped off and anthers were removed by suction using the vacuum emasculator. Emasculated panicles were covered with paper bags and tagged to prevent undesired pollination. Pollen was collected from male plants and poured into emasculated florets within 24 hours after emasculation. Pollinated panicles were immediately enveloped in paper bags to prevent out crosses and provide protection against bad weather, pests and contaminations by pathogens. Progeny seeds were harvested when they lost their green colour, usually about 25 to 30 days after pollination. All crosses were reciprocal, donor parent and recurrent parent representes male and female respectively.

5.2.5. Development of recombinant inbred populations

Pre-germinated F1 seeds were planted in plastic buckets and fertilizers were applied as indicated above. RIPs were developed as indicated in Figure 5.2. At flowering stage, emerging panicles from F1 plants where bagged in order to produce F2 seeds by selfing. To develop BC1F1 seeds, F1 plants were crossed to recurrent parents. Backcrosses were performed in both directions whereby F1 plants were used alternatively as female or male parents (Figure 5.3). Harvested F2 and BC1F1seeds were planted and selfed to yield F3 and BC1F2 seeds respectively while BC1F1 plants were backcrossed to generate BC2F1 seeds. Finally, F3, BC1F2 and BC2F1 seeds were planted and selfed to generate targeted recombinant inbred line populations (RIPs) including F4, BC1F3 and BC2F2 seeds, respectively.

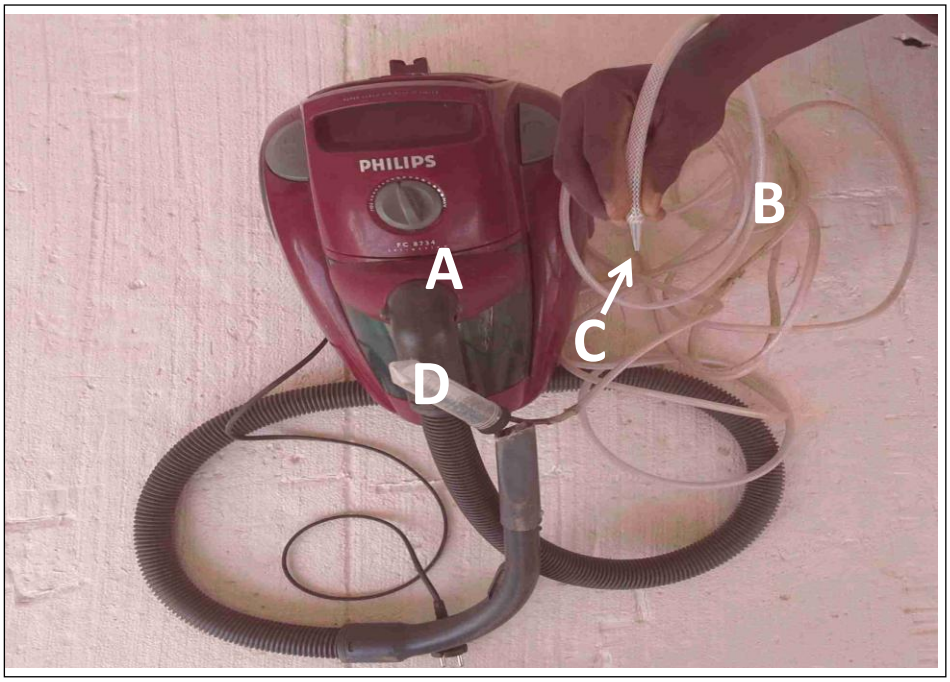

Figure 5. 1. Device used for rice emasculation.

A= Electric house cleaning vacuum with pressure selector; B= pipe; C: anther sucking tip; D: pollen collection tube.

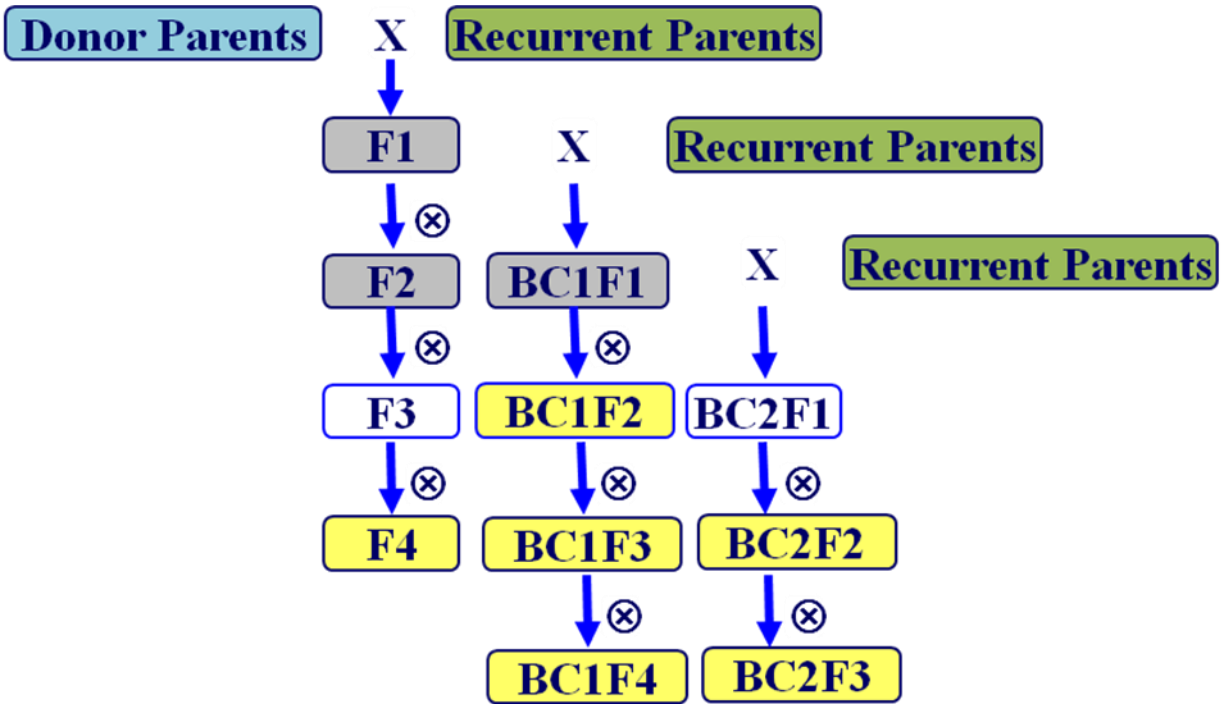

Figure 5. 2. Breeding scheme for the development of recombinant inbred line populations

5.2.6. Marker assisted foreground selection for RYMV1 resistance gene

5.2.6.1. Extraction of total RNA from rice leaves

Total RNA was extracted using Qiagen® Rneasy kit (Qiagen, France) as recommended by the manufacturer. Briefly, 100 mg of finely ground leaves were mixed with 450 µl of lysis buffer (RLT buffer) containing 2% (v/v) β-mercaptoethanol. After filtration, total RNA was precipitated by addition of 225 µl absolute ethanol. RNA extract was washed once with 700 µl of RW1 buffer and twice with 500 µl of RPE buffer. Clean total RNA was eluted from spin column by addition of 30 µl of nuclease free H_2O and centrifugation at 10,000 rpm for 1 min. Eluted RNA was used immediately in RT-PCR reactions or stored at -70°C for further use

5.2.6.2. Reverse Transcription PCR

Reverse transcription (RT) PCR were done in two steps using oligonucleotide primers P2 and R4 (Table 5.2) specific to *rymv1-2* and *rymv1-3* alleles respectively (Figure 5.3). RT reactions were done in total volumes of 25 µl containing 9 µl RNA, 1µl of 100 µM reverse primer, 2 µl of 5 mM dNTPs, 200 U of RNase inhibitor, 5 µl of (5x) RT buffer and 1 µl of MMLV-RT. cDNA synthesis was performed in a PTC100 thermocycler at 42°C for 60 min. PCR reactions were done in 20 µl reaction volumes using AccuPower PCR Premix kit from Bioneer®. A reaction mix containing 2.5 µl of cDNA template, 17.5 µl of nuclease-free water and 4 picomoles of each primer (forward and reverse) was added to the lyophilised PCR premix tube. Primer combinations for PCR reactions are indicated in figure 5.3 (Thiemele *et al.*, 2010).The set of primers P1, P2, Pi and Pg (Table 5.2) was used to detect rymv1-2 allele in RIPs (Figure 5.3A). Combination of primers P2 and Pg specifically detects *rymv1-2* resistance allele with an expected PCR product of 127 nucleotides (nt). P1-Pi combination yields an expected product of 187 nt specific to the susceptible allele. P1-P2 combination (269 nt) detects the entire central region of the resistance gene and was used as internal PCR control. The set of primers F1, F5, R1

and R4 (Table 5.2) was used to detect *rymv1-3* allele (Figure 5.3B). Specifically, R4-F5 primer combination amplifies a fragment of 540 nt in *rymv-3* mediated resistant lines. Primers F1-R1 amplifies a 725 nt fragment used as internal PCR control. Cycling conditions for primers set P1, P2, Pi and Pg were as follows: 94°C, 3 min; 30 cycles of 94°C, 30 sec; 61°C, 30 sec; 72°C, 1 min; and 72°C, 10 min. For the set of primers F1, F5, R1 and R4, cycling conditions were: 94°C, 3 min; 30 cycles of 94°C, 30 sec; 58°C, 45 sec; 72°C, 1 min; and 72°C, 10 min. PCR products were electrophoresed in 2% agarose gels containing 0.05% ethidium bromide and visualized under UV trans-illumination.

Table 5. 2. Primers used for detecting alleles of RYMV1 gene within RIPs

Primer	**Sequence (5' → 3')**
P1	GAGCCCACCTTCTGTCCGATG
P2	AGTAGCTCACCAATTAGACGGA
Pi	CAGGGCCAGTCAATTTTGCTATTTC
Pg	GTGCTGAGAGCCTAAGGGCTA
F1	CACGTCGGCGGCGCATCCAAG
R1	CGAACACGCTCGCGCACCTCA
F5	CCCTGACCAAGAGATGGAGAAAG
R4	CCTCGGTACAACCAAGAGAC

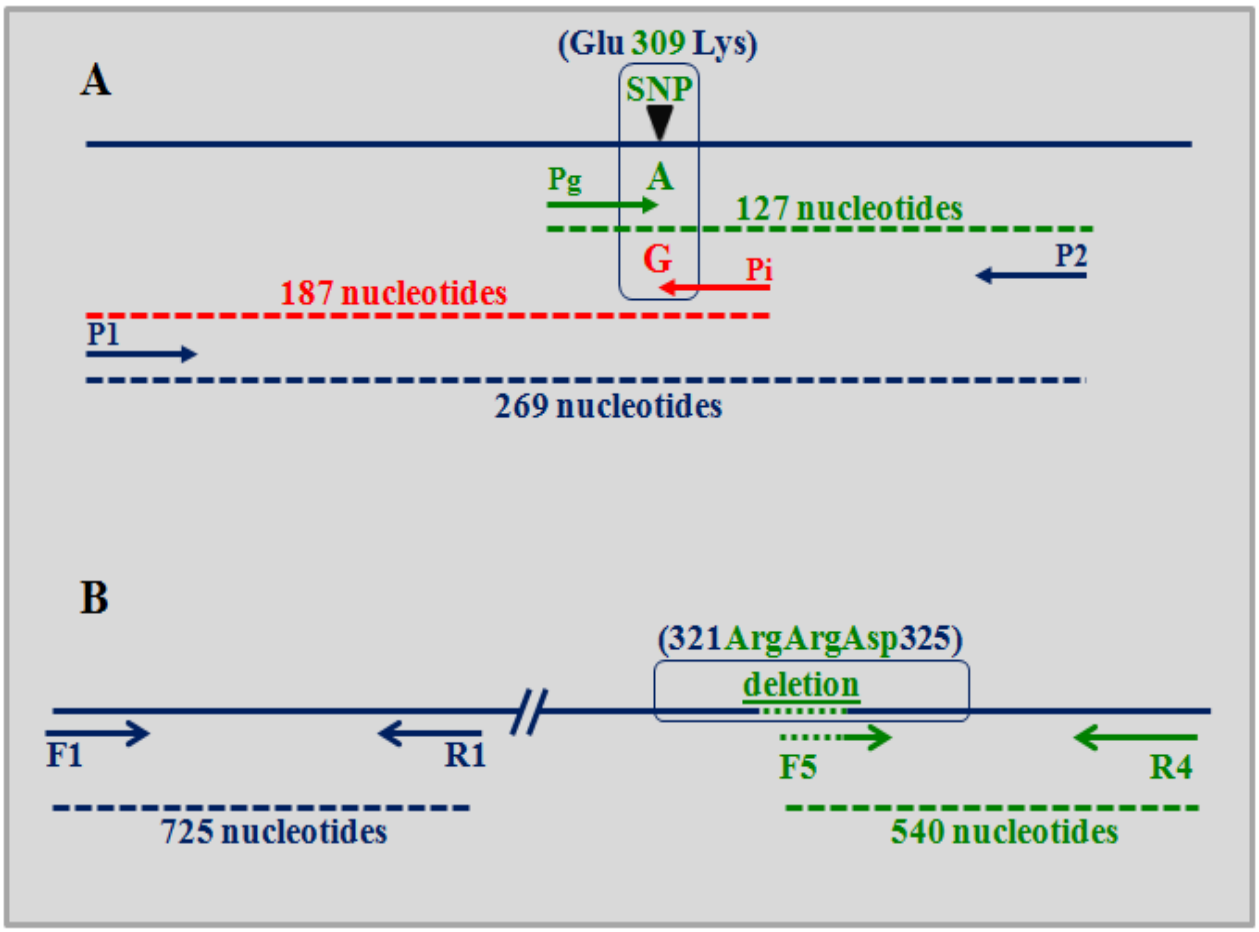

Figure 5. 3. PCR-based RYMV1 allele specific amplification strategy using SNP markers.
Primers, polymorphic sites and sizes of PCR products are indicated for rymv1-2 (A) and rymv1-3 (B) alleles (Albar *et al.*, 2006; Thiemele *et al.*, 2010).

5.2.7. Phenotyping RIPs for resistance to RYMV

RIPs included progenies from F3, F4, BC1F2, BC1F3 and BC2F2 families. Parental varieties and different rice cultivars were used as checks. A total of 93 recombinant inbred populations were planted in buckets arranged in a complete randomized design (CRD).

For each population, 20 pre-germinated seeds were directly sown into 20 litre plastic buckets in three replications. Plants were inoculated as indicated in chapter 4, using virus inoculums 1 and 2 composed of non-resistance-breaking (nRB) and resistance-breaking (RB) isolates, respectively (Table 5.3).

5.2.8. Data analysis

Data were analyzed using Statistica software ver.6 (StatSoft France, 2001). One-way analysis of variance was used to test differences in the mean number of days for symptom appearance between rice genotypes. Data from each genotype was compared to the control BG90-2 using Dunnett's test (Sayes *et al.*, 2006).

Table 5. 3. Selected RYMV isolates used for screening rice accessions

RYMV isolates	Origin	Strains[a]	Pathogenicity[b]			
			BG90-2	Gigante	Tog5681	Pathotype
Virus inoculum 1						
854-3	Burkina Faso	S1	+	-	-	nRB
466-4	Mali	S2	+	-	-	nRB
562-2	Niger	S1	+	-	-	nRB
288-1	Ghana	S2	+	-	-	nRB
Virus inoculum 2						
288-4	Ghana	S2	+	+	-	RB-*rymv1-2*
466-5	Mali	Sa	+	+	-	RB-*rymv1-2*
854-5	Burkina Faso	S2	+	+	+	RB-*rymv1-2*/*rymv1-3*
562-1	Niger	S1	+	+	+	RB-*rymv1-2*/*rymv1-3*

[a]Virus strains were determined based on the variability of the coat protein gene (Traore *et al.*, 2010);
[b]Virus isolates were assigned to pathotypes depending on their ability to overcome (+) singly allele rymv1-2 or simultaneously both alleles (RB-rymv1-2/ rymv1-3). Isolates not able to overcome (-) any *RYMV1* resistance allele as well as *RYMV2* gene were included in pathotype nRB.

5.3. Results

5.3.1. Recombinant inbred populations

To develop recombinant inbred line populations, parental lines were used to make 16 crosses (Table 5.3). The number of pollinated florets for each cross ranged between 30 and 255. Intraspecific *O. sativa* x *O. sativa* crosses were most successful (23-56.6%). However, such intraspecific cross between Azucena and Bekarosaka gave a very low success rate (2%) in spite of the higher number of pollinated florets. Low rates of successful crosses were also found between *O. sativa* x NERICA crosses (4.45% in average) as well as all interspecific *O. sativa* x *O. glaberrima* crosses (1.72% in average). All crosses were advanced to form six breeding families composed of 79 recombinant inbred line populations labelled from KBR1 to KBR79.

Table 5. 4. Development of recombinant inbred line populations for resistance to RYMV

Crosses	**Cross type**[a]	**Pollinated florets**	**F1 seeds**[b]	**RIPs composition**
Gigante x FKR16	*indica* x *indica*	30	17 (56.7)	BC1F1, BC1F3
Gigante x FKR19	*indica* x *indica*	30	15 (50.0)	BC1F1
Gigante x Digang	*indica* x *indica*	30	16 (53.3)	BC1F2, BC1F3
Azucena x Gigante	*japonica* x *indica*	30	7 (23.3)	F3
Azucena x Bekarosaka	*japonica* x *indica*	150	3 (2.0)	F3
FKR19 x Digang	*indica* x *indica*	30	12 (40.0)	F3
Kumazuce x Digang	*indica* x *indica*	30	17 (56.7)	F3
FKR56N x Gigante	*nerica* x *sativa*	180	8 (4.4)	F3
FKR60N x Gigante	*nerica* x *sativa*	180	7 (3.9)	F3
Gigante x FKR62N	*sativa* x *nerica*	180	12 (6.7)	BC1F3, BC2F1
Digang x Nerica28	*sativa* x *nerica*	180	5 (2.8)	F3

FKR56N xTog5681	*nerica* x *glaberrima*	255	4	(1.6)	BC1F3
Gigante x Tog5681	*sativa* x *glaberrima*	225	3	(1.3)	F3
GH1520 x Gigante	*glaberrima* x *sativa*	180	4	(2.2)	BC2F1, BC2F2
CG14 x Digang	*glaberrima* x *sativa*	150	3	(2.0)	BC1F1, BC1F2
CG14 x Gigante	*glaberrima* x *sativa*	135	2	(1.5)	F1

[a]Rice genotype belonged to *Oryza glaberrima* and *O. sativa* species, the latter being subdived into *indica* and *japonica* subspecies
[b]Number of F1 seeds indicating successful crosses which percentages are indicated in parentheses.

5.3.2. Molecular screening for RYMV1 gene identification

Crosses involving *rymv1-2* allele bearing genotypes (Gigante and Bekarosaka) were screened using primers P1, P2, P1 and Pg. Combinations of the three expected RT-PCR amplification fragments (127 bp, 187 bp and 269 bp) determined three allelic patterns (Figure 5.4A). Resistant genotypes Gigante and Bekarosaka showed an allelic pattern (rr) involving the presence of 127 bp and 269 bp fragments. Allelic pattern (RR) resulting from simultaneous amplifications of 187 bp and 269 bp fragments was found in the susceptible genotype FKR16. Recombinant lines from crosses between Gigante or Bekarosaka and other parental genotypes showed both allelic patterns rr and RR. A third allelic pattern (rR) determined by the presence of all three amplification fragments was also found in some recombinant lines.

Primers R1, F1, R4, and F5 were used to screen recombinant populations resulting from crosses which involved Tog5681 as donor of *rymv1-3* resistance allele. The expected amplification fragment of 725 bp was found in Tog5681 as well as FKR16, CG14 and all recombinant lines. In contrast, the second expected fragment (540 bp) was found only in Tog5681. This result indicated that allele rymv1-3 was detected only in resistance donor Tog5681.

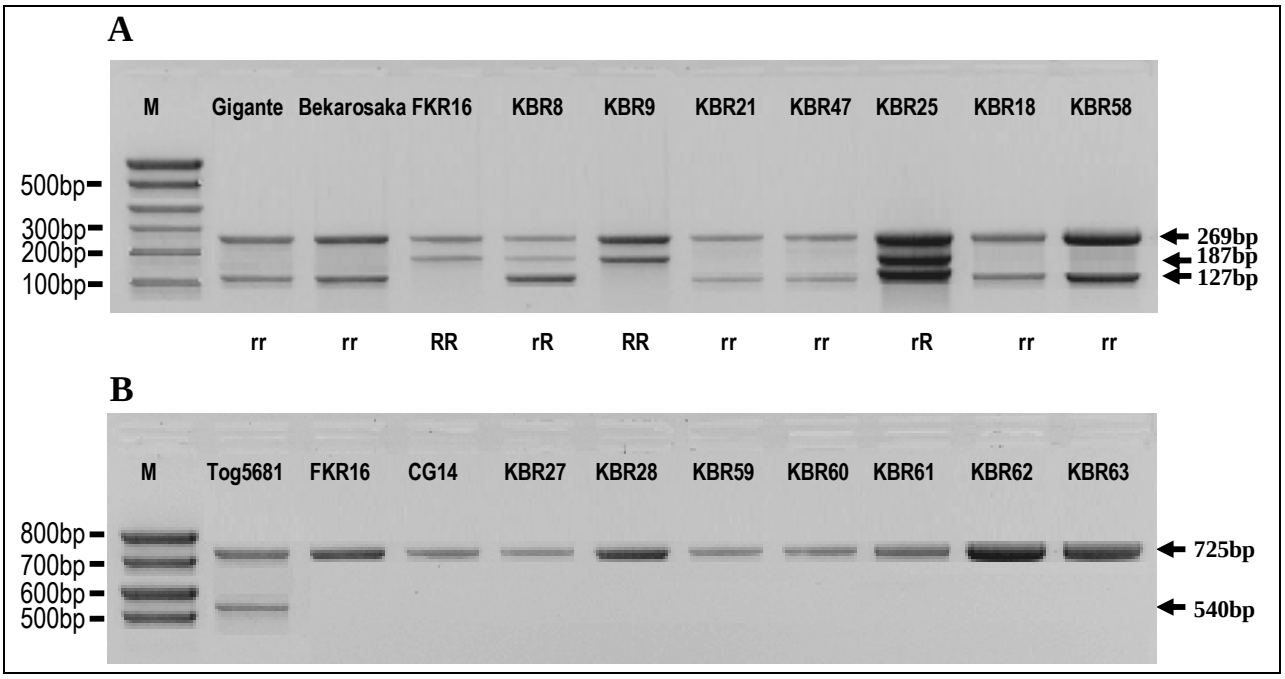

Figure 5. 4. Electrophoregrams showing RT-PCR amplification profiles using *rymv1-2*- and *rymv1-3*-allele specific primers.

(A) Amplification profiles in Gigante, Bekarosaka, FKR16 and their progenies. Allelic patterns (rr, RR, rR) corresponding to amplification profiles are indicated. (B) Amplification profiles in Tog5681, susceptible genotypes FKR16 and CG14 and progenies from crosses involving Tog5681. Sizes of marker (M) fragments are indicated in base pairs (bp).

Results of the molecular screening of all 79 Kbr recombinant subfamilies are summarized in Table 5.4. Only nine subfamilies were derived from crosses involving Tog5681. The absence of *rymv1-3* allele in these lines indicated their genotype predicted susceptibility to RYMV. However, two of these subfamilies (Kbr21 and Kbr22) which were derived from a cross between Tog5681 and Gigante were homozygous for resistant allele *rymv1-2*. Consequently, these subfamilies were predicted as resistant to RYMV. In addition to Kbr21 and Kbr22, 72 subfamilies were derived from crosses involving *rymv1-2* allele from Gigante or Bekarosaka. *Rymv1-2* allelic pattern rr (homozygous genotype with predicted resistance to RYMV) was found in more than half (58.3%) of the subfamilies. Notably, rr allelic pattern was found in all

subfamilies derived from the following crosses: Gigante x FKR16, Gigante x Digang, Gigante x Azucena and Bekarosaka x Azucena.

Table 5. 5. Allelic pattern of recombinant subfamilies from crosses involving donors of *rymv1-2* and *rymv1-3* resistance alleles

Genotypes	**Parents / Crosses**	**Breeding families**	**Allelic pattern**[a]		**Predicted phenotype**
			rymv1-3	***rymv1-2***	
FKR16	Susceptible control	Line	nt	RR	Susceptible
Bekarosaka	*rymv1-2* donor	Line	nt	rr	Resistant
Gigante	*rymv1-2* donor	Line	nt	rr	Resistant
Tog5681	*rymv1-3* donor	Line	+	nt	Resistant
Kbr1	Gigante x FKR16	BC1F1	nt	rr	Resistant
Kbr2	Gigante x FKR16	BC1F1	nt	rr	Resistant
Kbr3	Gigante x FKR16	BC1F1	nt	rr	Resistant
Kbr4	Gigante x FKR16	BC1F1	nt	rr	Resistant
Kbr5	Gigante x FKR16	BC1F1	nt	rr	Resistant
Kbr6	Gigante x FKR16	BC1F1	nt	rr	Resistant
Kbr55	Gigante x FKR16	BC1F3	nt	rr	Resistant
Kbr56	Gigante x FKR16	BC1F3	nt	rr	Resistant
Kbr57	Gigante x FKR16	BC1F3	nt	rr	Resistant
Kbr58	Gigante x FKR16	BC1F3	nt	rr	Resistant
Kbr7	Gigante x FKR62N	BC1S2	nt	rr	Resistant
Kbr8	Gigante x FKR62N	BC1S2	nt	rR	Susceptible
Kbr9	Gigante x FKR62N	BC2F1	nt	RR	Susceptible
Kbr47	Gigante x FKR62N	BC1F3	nt	rr	Resistant
Kbr48	Gigante x FKR62N	BC1F3	nt	rr	Resistant
Kbr49	Gigante x FKR62N	BC1F3	nt	rr	Resistant

Kbr50	Gigante x FKR62N	BC1F3	nt	rr	Resistant
Kbr51	Gigante x FKR62N	BC1F3	nt	rr	Resistant
Kbr52	Gigante x FKR62N	BC1F3	nt	rr	Resistant
Kbr10	Gigante x FKR19	BC1F1	nt	rR	Susceptible
Kbr11	Gigante x FKR19	BC1F1	nt	rr	Resistant
Kbr12	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr13	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr14	Gigante x Digang	BC1F2	nt	rr	Resistant
Kbr40	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr41	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr42	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr43	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr44	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr45	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr46	Gigante x Digang	BC1F3	nt	rr	Resistant
Kbr25	FKR56N x Gigante	F2:3	nt	rR	Susceptible
Kbr26	FKR56N x Gigante	F2:3	nt	rR	Susceptible
Kbr66	FKR56N x Gigante	F2:3	nt	rR	Susceptible
Kbr67	FKR56N x Gigante	F2:3	nt	RR	Susceptible
Kbr68	FKR56N x Gigante	F2:3	nt	rR	Susceptible
Kbr69	FKR56N x Gigante	F2:3	nt	RR	Susceptible
Kbr70	FKR56N x Gigante	F2:3	nt	RR	Susceptible
Kbr71	FKR56N x Gigante	F2:3	nt	rR	Susceptible
Kbr72	FKR56N x Gigante	F2:3	nt	RR	Susceptible
Kbr73	FKR56N x Gigante	F2:3	nt	RR	Susceptible
Kbr15	GH1520 x Gigante	BC2F1	nt	rr	Resistant
Kbr16	GH1520 x Gigante	BC2F1	nt	rr	Resistant

Kbr17	GH1520 x Gigante	BC2F1	nt	rR	Susceptible
Kbr18	GH1520 x Gigante	BC2F1	nt	rr	Resistant
Kbr19	GH1520 x Gigante	BC2F1	nt	rr	Resistant
Kbr20	GH1520 x Gigante	BC2F1	nt	rr	Resistant
Kbr53	GH1520 x Gigante	BC2F2	nt	rr	Resistant
Kbr54	GH1520 x Gigante	BC2F2	nt	rr	Resistant
Kbr29	FKR60N x Gigante	F2:3	nt	rR	Susceptible
Kbr30	FKR60N x Gigante	F2:3	nt	rR	Susceptible
Kbr64	FKR60N x Gigante	F2:3	nt	RR	Susceptible
Kbr27	FKR56N x Tog5681	BC1F3	(-)	nt	Susceptible
Kbr28	FKR56N x Tog5681	BC1F3	(-)	nt	Susceptible
Kbr59	FKR56N x Tog5681	BC1F3	(-)	nt	Susceptible
Kbr60	FKR56N x Tog5681	BC1F3	(-)	nt	Susceptible
Kbr61	FKR56N x Tog5681	BC1F3	(-)	nt	Susceptible
Kbr62	FKR56N x Tog5681	BC1F3	(-)	nt	Susceptible
Kbr63	FKR56N x Tog5681	BC1F3	(-)	nt	Susceptible
Kbr23	Azucena x Gigante	F2:3	nt	rr	Resistant
Kbr24	Azucena x Gigante	F2:3	nt	rr	Resistant
Kbr31	Azucena x Bekarosaka	F2:3	nt	rr	Resistant
Kbr32	Azucena x Bekarosaka	F2:3	nt	rr	Resistant
Kbr76	Azucena x Bekarosaka	F2:4	nt	rr	Resistant
Kbr21	Gigante x Tog5681	F2:3	(-)	rr	Resistant
Kbr22	Gigante x Tog5681	F2:3	(-)	rr	Resistant

[a] Rice genotypes were screened for the presence (+) or absence (-) of *rymv1-3* resistance allele; nt= not tested; detection of *rymv1-2* allele in rice genotypes determined allelic patterns RR (susceptible homozygote), rr (resistant homozygote) and rR (susceptible heterozygote).

Heterozygous rR pattern was found in 13.9% of the recombinant subfamilies which were predicted as susceptible. Susceptibility phenotype was also expected from the remaining 27.8% of the recombinant subfamilies belonging to the RR allelic pattern.

5.3.3. Reactions of rice recombinant inbred populations (RIPs) to RYMV inoculation

5.3.3.1. Latency period

From the Kbr recombinant subfamilies, 79 recombinant inbred line populations (RIPs) were generated and were exposed to both non-RB isolates in virus inoculum 1 and RB isolates in inoculum 2. A wide range of variation in the time required for disease symptom appearance were recorded (Table 5.5). The average time for symptom development in plants of the susceptible control BG90-2 was 11.7 days post-inoculation (dpi). First symptoms in RIPs were observed as early as 7 dpi, particularly in RIPs from cross between FKR56N and Tog5681. By contrast, several RIPs developed symptoms after 20 dpi. A highly significant genotype effect was found ($F=390.2$, $df=50$; $P<0.001$) in one-way ANOVA when inoculum 1 was used. Dunnett's test using BG90-2 as control group indicated that 21.52% (17/79) of RIPs reacted similarly to the susceptible control and were assigned the S-phenotype. More than half of the RIPs (45/79) did not develop any symptom up to 45 dpi when the experiments ended. Such RIPs were derived from crosses involving Gigante or Bekarosaka. They were classified as highly resistant (HR-phenotype) as their reactions were similar to those of the resistant progenitors. All other RIPs (17/79) which showed symptoms later than BG90-2 were referred to as partially resistant (PR-phenotype).

When inoculum 2 was used, symptoms were observed in individual genotypes within all HR-phenotype RIPs previously identified with inoculum 1. However, in all of the RIPs, symptoms appeared 15 to 27 dpi, which was significantly longer than in the control group BG90-2 (F=

421.5, df=98; P<0.001). Consequently, they were attributed to the PR-phenotype. Altogether, 79.7% of the RIPs were partially resistant and 20.3% were susceptible.

RIPs were subdivided into two major groups according to their phenotypes in the first group composed of 53 (out of the 79 RIPs) all recombinant lines within the same population exhibited the same phenotype (either S or PR or HR). In this group, proportions of phenotypes S, PR and HR were 24.5%, 17% and 58.5%, respectively with inoculum1. Using inoculum 2, only PR (71.7%) and S (28.3%) phenotypes were found. The second group (26/79) consisted of populations in which recombinant lines belonged to different phenotypes (Table 5.5).

Table 5. 6. Reactions of rice accessions to inoculation of two mixtures of RYMV isolates

Rice accessions	**Parents/ Families**	**Line/cross**	**Duration for symptom appearance (days)[a]**			
			Virus inoculum 1		**Virus inoculum 2**	
FKR16	Parent	Line	10.65±0.67	(S)	9±0	(S)
FKR19	Parent	Line	7.89±0.32	(S)	7.74±0.45	(S)
FKR56N	Parent	Line	9.5±0.51	(S)	9.75±0.44	(S)
FKR60N	Parent	Line	9.65±0.61	(S)	8.71±0.46	(S)
FKR62N	Parent	Line	8.5±0.76	(S)	7.74±0.45	(S)
Kumazuce	Parent	Line	19±0	(PR)	17.6±0.51	(S)
Digang	Parent	Line	20.3±2.03	(PR)	18.5±1.32	(PR)
Nerica 28	Parent	Line	17.92±2.75	(PR)	18.33±2.71	(PR)
GH1520	Parent	Line	17.3±1.66	(PR)	18.19±2.06	(PR)
CG14	Parent	Line	19.3±2.39	(PR)	13.9±0.31	(S)
Azucena	Parent	Line	17.29±2.05	(PR)	16.95±2.03	(PR)

Bekarosaka	Parent	Line	NS	(HR)	11.44±0.51	(S)
Gigante	Parent	Line	NS	(HR)	13.77±0.43	(PR)
Tog5681	Parent	Line	NS	(HR)	0±0	(HR)
BG90-2	Control	Line	9.64±0.49	(S)	9.19±0.93	(S)
Kbr1	BC1F2	Gigante x FKR16	NS	(HR)	23.25±0.44	(PR)
Kbr2	BC1F2	Gigante x FKR16	NS	(HR)	21.7±0.47	(PR)
Kbr3	BC1F2	Gigante x FKR16	NS	(HR)	25±0	(PR)*
Kbr4	BC1F2	Gigante x FKR16	NS	(HR)	22.5±1	(PR)*
Kbr5	BC1F2	Gigante x FKR16	NS	(HR)	24.5±0.51	(PR)
Kbr6	BC1F2	Gigante x FKR16	NS	(HR)	23±0	(PR)
Kbr55	BC1F4	Gigante x FKR16	NS	(HR)	23.85±1.05	(PR)
Kbr56	BC1F4	Gigante x FKR16	NS	(HR)	23.54±0.51	(PR)
Kbr57	BC1F4	Gigante x FKR16	NS	(HR)	23.92±1.05	(PR)
Kbr58	BC1F4	Gigante x FKR16	NS	(HR)	22.54±0.51	(PR)
Kbr7	BC1F4	Gigante x FKR62N	NS	(HR)	26.6±0.5	(PR)
Kbr8	BC1F4	Gigante x FKR62N	21.08±1.97	(PR)*	29±0	(PR)*
Kbr9	BC2F2	Gigante x FKR62N	8.27±0.7	(S)	9±0.94	(S)
Kbr47	BC1F4	Gigante x FKR62N	NS	(HR)	23.77±0.86	(PR)
Kbr48	BC1F4	Gigante x FKR62N	NS	(HR)	22.62±0.5	(PR)
Kbr49	BC1F4	Gigante x FKR62N	NS	(HR)	22.65±0.49	(PR)
Kbr50	BC1F4	Gigante x FKR62N	NS	(HR)	16.47±0.83	(PR)
Kbr51	BC1F4	Gigante x FKR62N	NS	(HR)	22.3±1.43	(PR)
Kbr52	BC1F4	Gigante x FKR62N	NS	(HR)	24.73±0.78	(PR)

Kbr10	BC1F2	Gigante x FKR19	19.38±2.06	(PR)*	15.14±1.04	(PR)
Kbr11	BC1F2	Gigante x FKR19	NS	(HR)	22±0	(PR)*
Kbr12	BC1F4	Gigante x Digang	NS	(HR)	23.41±0.5	(PR)
Kbr13	BC1F4	Gigante x Digang	NS	(HR)	27.41±0.5	(PR)
Kbr14	BC1F3	Gigante x Digang	NS	(HR)	23.4±1.47	(PR)
Kbr40	BC1F4	Gigante x Digang	NS	(HR)	24.35±0.49	(PR)
Kbr41	BC1F4	Gigante x Digang	NS	(HR)	25.69±0.47	(PR)
Kbr42	BC1F4	Gigante x Digang	NS	(HR)	22.09±0.79	(PR)
Kbr43	BC1F4	Gigante x Digang	NS	(HR)	23±0	(PR)
Kbr44	BC1F4	Gigante x Digang	NS	(HR)	25.19±1.13	(PR)
Kbr45	BC1F4	Gigante x Digang	NS	(HR)	27±0	(PR)*
Kbr46	BC1F4	Gigante x Digang	NS	(HR)	22.42±0.5	(PR)
Kbr25	F4	FKR56N x Gigante	10.64±0.76	(S)*	21.44±2.53	(PR)*
Kbr26	F4	FKR56N x Gigante	9.3±0.66	(S)*	21.42±2.83	(PR)*
Kbr66	F4	FKR56N x Gigante	21.77±0.99	(PR)*	22.35±0.49	(PR)*
Kbr67	F4	FKR56N x Gigante	18±1.38	(PR)*	22.96±0.82	(PR)*
Kbr68	F4	FKR56N x Gigante	19.56±1.47	(PR)*	22.85±0.73	(PR)*
Kbr69	F4	FKR56N x Gigante	9.73±0.46	(S)	11±0	(S)
Kbr70	F4	FKR56N x Gigante	11.33±0.97	(S)	9.67±0.48	(S)
Kbr71	F4	FKR56N x Gigante	11±0.98	(S)*	11.25±0.85	(S)*
Kbr72	F4	FKR56N x Gigante	11.43±0.51	(S)	10.43±0.9	(S)
Kbr73	F4	FKR56N x Gigante	20.56±1.24	(PR)	16.06±0.93	(PR)
Kbr15	BC2F2	GH1520 x Gigante	NS	(HR)	23.5±0.51	(PR)

Kbr16	BC2F2	GH1520 x Gigante	NS	(HR)	24.19±1.36	(PR)
Kbr17	BC2F2	GH1520 x Gigante	16.29±2.47	(PR)*	20.48±5.08	(PR)
Kbr18	BC2F2	GH1520 x Gigante	NS	(HR)	26±0	(PR)*
Kbr19	BC2F2	GH1520 x Gigante	NS	(HR)	27.58±0.5	(PR)
Kbr20	BC2F2	GH1520 x Gigante	NS	(HR)	23±0	(PR)*
Kbr53	BC2F3	GH1520 x Gigante	NS	(HR)	23.12±0.65	(PR)
Kbr54	BC2F3	GH1520 x Gigante	NS	(HR)	23.96±1.22	(PR)
Kbr29	F4	FKR60N x Gigante	20±0.73	(PR)*	24.23±1.37	(PR)
Kbr30	F4	FKR60N x Gigante	12.5±0.98	(S)*	20.5±2.95	(PR)
Kbr64	F4	FKR60N x Gigante	9±0	(S)	21.95±1.16	(PR)
Kbr27	BC1F4	FKR56N x Tog5681	11.71±0.46	(S)	21.5±2.14	(PR)
Kbr28	BC1F4	FKR56N x Tog5681	16.88±2.39	(PR)	22.85±0.83	(PR)
Kbr59	BC1F4	FKR56N x Tog5681	10.77±0.43	(S)	10.6±0.5	(S)
Kbr60	BC1F4	FKR56N x Tog5681	8.84±0.37	(S)	8.25±0.9	(S)
Kbr61	BC1F4	FKR56N x Tog5681	8.7±0.63	(S)	8.57±0.51	(S)
Kbr62	BC1F4	FKR56N x Tog5681	12.73±0.46	(S)	9.56±0.51	(S)
Kbr63	BC1F4	FKR56N x Tog5681	9.88±0.33	(S)	13.6±0.5	(S)
Kbr33	BC1F2	CG14 x Digang	20.96±1.15	(PR)	27±0	(PR)*
Kbr34	BC1F2	CG14 x Digang	21.08±1.9	(PR)	24.69±0.74	(PR)
Kbr35	BC1F2	CG14 x Digang	21.62±0.8	(PR)	22.54±0.51	(PR)
Kbr36	BC1F2	CG14 x Digang	18.85±0.73	PR)	23±0	(PR)
Kbr37	BC1F2	CG14 x Digang	20.08±0.84	(PR)	23.69±0.47	(PR)
Kbr38	BC1F2	CG14 x Digang	NS	(HR)	23.67±0.49	(PR)*

Kbr39	BC1F3	CG14 x Digang	9.6±0.5	(S)	23±0	(PR)
Kbr80	F4	FKR19 x Digang	11.52±0.51	(S)	10.53±0.51	(S)
Kbr81	F4	Kumazuce x Digang	NS	(HR)	9.47±0.51	(S)
Kbr82	F4	Kumazuce x Digang	NS	(HR)	10.79±1.58	(S)
Kbr83	F4	Kumazuce x Digang	21.42±0.5	(PR)	13±0.8	(S)
Kbr84	F4	Digang x Nerica28	20.92±0.89	(PR)	13.3±1.69	(S)
Kbr85	F4	Digang x Nerica28	17.96±0.8	(PR)	13.24±0.89	(S)
Kbr23	F4	Azucena x Gigante	NS	(HR)	23.4±0.51	(PR)*
Kbr24	F4	Azucena x Gigante	NS	(HR)	26±0	(PR)*
Kbr31	F4	Azucena x Bekarosaka	NS	(HR)	23.43±0.53	(PR)*
Kbr32	F4	Azucena x Bekarosaka	NS	(HR)	23±0	(PR)*
Kbr76	F4	Azucena x Bekarosaka	NS	(HR)	23±0	(PR)*
Kbr21	F4	Gigante x Tog5681	NS	(HR)	27±0	(PR)*
Kbr22	F4	Gigante x Tog5681	NS	(HR)	27±0	(PR)*

[a]Mean number of days for symptom appearance after virus inoculation ± standard deviation (n=20) with virus inoculum 1 and inoculum 2 (see Material and methods); no symptom (NS) was observed in highly resistant (HR) genotypes; Reaction phenotypes (indicated in parentheses) were attributed to accessions after one-way ANOVA of the number of days for symptom appearance followed by Dunnett's test ($P < 0.05$), taking BG90-2 as control group: S, susceptible; PR, partially resistant. Stars (*) indicate the presence of additional phenotypes.

5.3.3.2. Disease incidence

Kbr populations in which recombinant lines belonged to different phenotypes were studied in more detail. Proportions of recombinant lines (n=20) which showed disease symptoms within each of the 26 populations (disease incidence) were determined. Kbr populations derived from crosses between Gigante and susceptible recurrent parents FKR62N, FKR56N, FKR60N, FKR16 and FKR19 were more resistant to the virus (Figure 5.5A). Recombinant lines in eight populations (Kbr8, Kbr10, Kbr25, Kbr26, Kbr29, Kbr66, Kbr67 and Kbr68) showed only PR and HR phenotypes. Recombinant lines of the S phenotype were found in Kbr30 and Kbr71 populations derived from FKR60N x Gigante and FKR56N x Gigante crosses. Altogether, HR and PR phenotypes represented 94.1% and S-phenotype were only 5.8% of the recombinant lines when inoculum 1 was used as virus source. When virus inoculum 2 was used, the proportion of resistant recombinant lines dropped to 72.1% while that of S-phenotype lines increased to 27.9%. Interestingly, half of the resistant lines belonged to the HR phenotype despite the use of resistant breaking isolates in inoculum 2. Such lines were found in all crosses except FKR60N x Gigante. Crosses where HR phenotype lines were found at high rates (60-90%) were those involving FKR16, FKR19 and FKR62N. Crosses involving high resistance rymv1-2 donors (Gigante and Bekarosaka) and partially resistant genotypes yielded higher proportions of resistant progenies. Using inoculum 1, 100% of progenies in all populations except Kbr17 fell into the HR phenotype (Figure 5.6A).

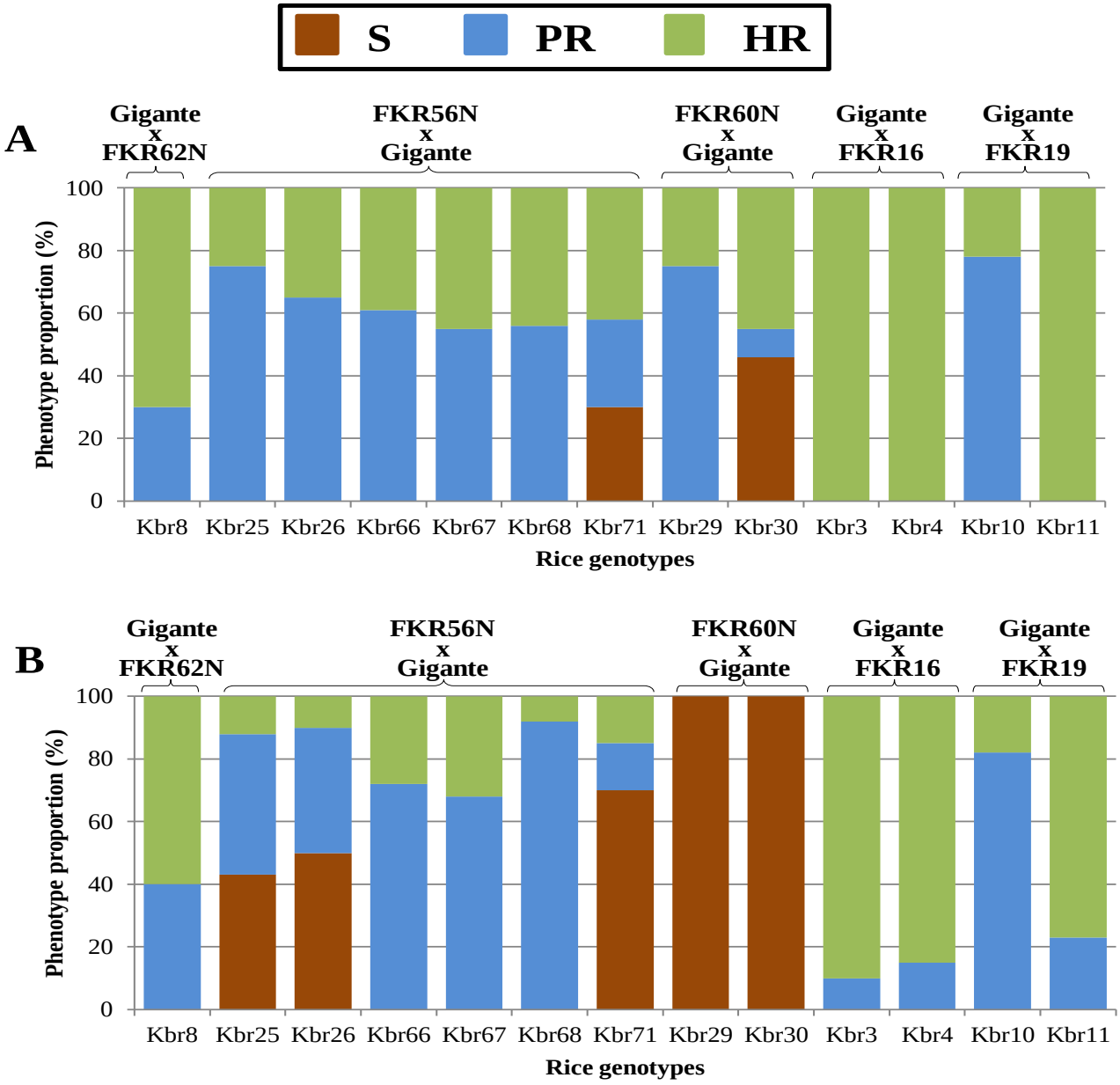

Figure 5. 5. Reaction of RIPs to non-resistance breaking (A) and resistance breaking (B) RYMV isolates. RIPs were developed from crosses between rymv1-2 resistance allele donor and five susceptible recurrent parents (FKR16, FKR19, FKR56N, FKR60N and FKR62N). RIPs were classified as susceptible (S), partially resistant (PR) and highly resistant (PR) according to their reaction to RYMV.

Half of Kbr17 lines belonged to HR phenotype and the other half to PR phenotype. When progenies were screened with virus inoculum 2, all Kbr17 lines became partially resistant. In all other progenies, 15 to 58% of recombinant lines that were highly resistant to inoculum 1 were PR phenotype when exposed to inoculum 2 (Figure 5.6B).

Crosses between partially resistant genotypes Digang and CG14 resulted in two populations (Kbr33 and Kbr38) in which two phenotypes were found. Surprisingly, HR phenotype was found in high proportions (over 70%) when recombinant lines were challenged with inoculum 1. HR phenotype was also found although to a lesser extent, when recombinant lines were screened with inoculum 2 (Figure 5.7A).

Crosses involving both donors of high resistance alleles, rymv1-2 (Gigante) and rymv1-3 (Tog5681) resulted in progenies belonging to the HR phenotype when exposed to inoculum 1(Figure 5.7B). Only a small proportion (12-15%) of these progenies, were partially resistant when inoculum 2 was used.

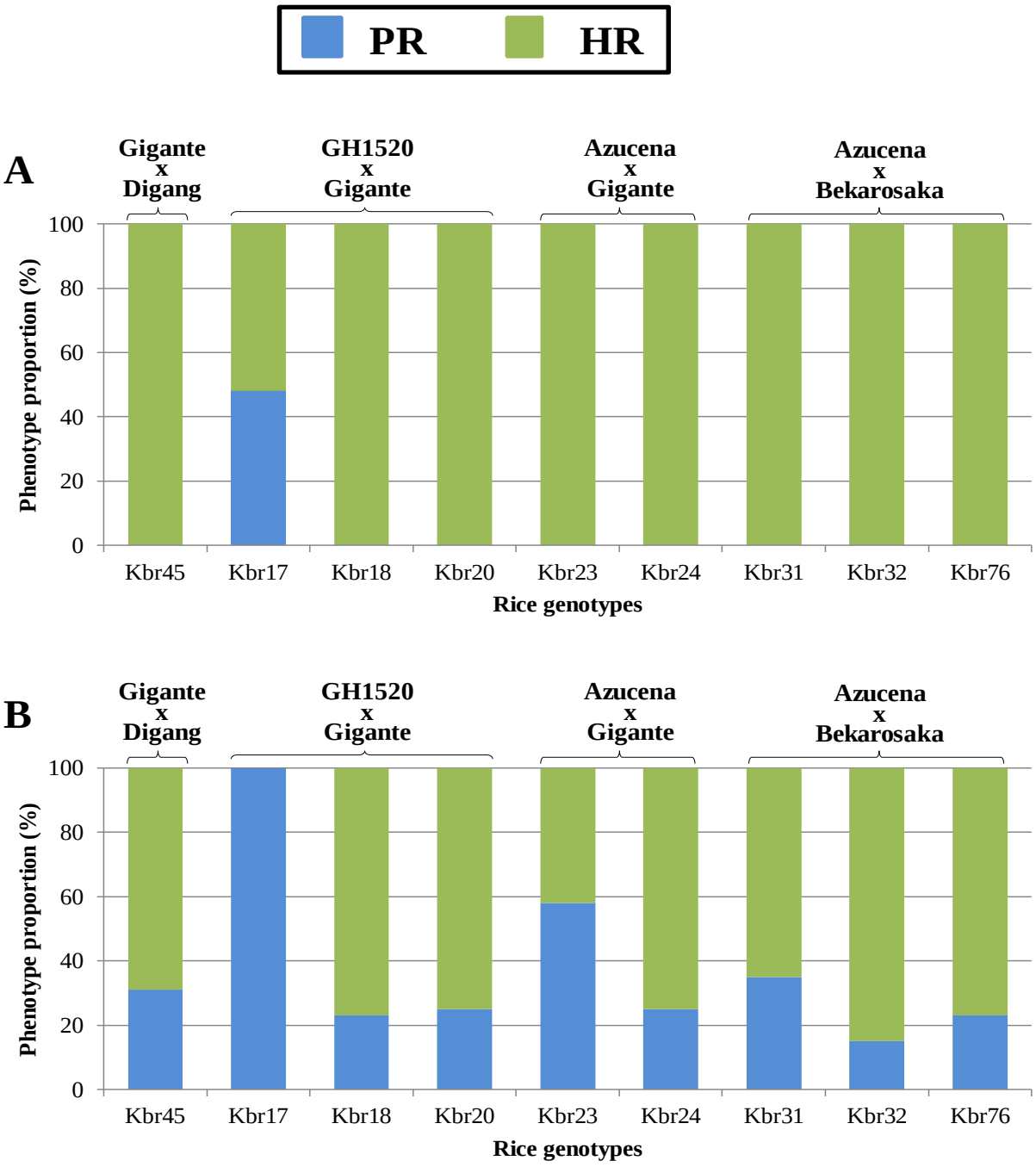

Figure 5. 6. Reaction of RIPs to non-resistance breaking (A) and resistance breaking (B) RYMV isolates.

RIPs were developed from crosses between rymv1-2 resistance allele donors (Gigante and Bekarosaka) and three partially resistant recurrent parents (Digang, GH1520, and Azucena). RIPs were classified as partially resistant (PR) and highly resistant (HR) according to their reaction to RYMV.

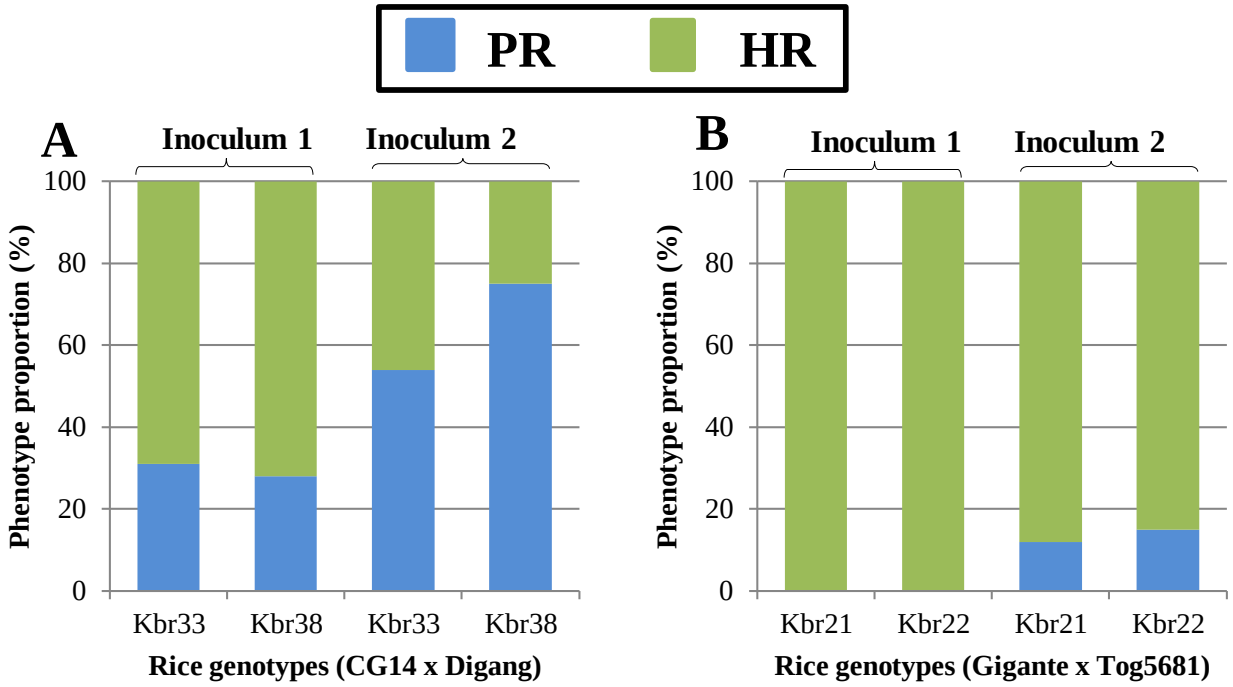

Figure 5. 7. Reaction of RIPs to non-resistance breaking (Inoculum 1) and resistance breaking (Inoculum 2) RYMV isolates.

RIPs were developed from crosses between partially resistant genotypes CG14 and Digang (A) and between highly resistant genotypes Gigante and Tog5681 (B). RIPs were classified as partially resistant (PR) and highly resistant (HR) according to their reaction to RYMV.

5.4. Discussion

Recombinant inbred line populations were generated by crossing several farmers' rice varieties susceptible to RYMV with partial and high resistance donors. There were clear differences in the number of viable F1 seeds produced, which indicated that genotypes were not always fully cross compatible. Crosses were most successful (40-57%) when all parental genotypes belonged to *O. sativa indica* subspecies. Indica x japonica crosses were moderately successful in the Azucena x Gigante cross (23%) and worse in the Azucena x Bekarosaka cross (2%). Our results are consistent with previous studies on F1 hybrids sterility from both intrasubspecific *indica* x *indica* and intersubspecific *indica* x *japonica* crosses (Stebbins, 1958; Ikehashi, 1982; Oka, 1988; Harushima *et al.*, 2003; Najeeb *et al.*, 2013). Hybrid sterility in *indica* x *japonica* crosses has

been attributed to the interaction of several genes which leads to varying degrees of fertility in F1 hybrids, from fully fertile to almost completely sterile (Liu *et al.*, 1996; Zhang *et al.*, 1997; Asante *et al.*, 2006).

Interspecific crosses *O.sativa* x *O.glaberrima* also yielded low proportions of F1 hybrid seeds. Most previous studies indicated that such crosses resulted in 100% spikelet sterility in F1 hybrids (Sano, 1990; Ghesquiere *et al.*, 1997; Jones *et al.*, 1997b; Huer and Miezan, 2003; Geravito *et al.*, 2010). In this study, F1 hybrids were produced with four distinct, *O.sativa* x *O.glaberrima* crosses, indicating that they were not developed by chance. However, proportions of successful crosses were very low (1.3 to 2.2%). Possibly, interspecific crosses were successful because clipped florets were filled in with fresh pollen during the pollination procedure used in this study, or some selfing occurred although rare, *O.sativa* x *O.glaberrima* hybrids were found in the field (Barry *et al.*, 2007). Semon *et al.* (2004) also indicated that many rice varieties grown in Africa were admixtures between *O. sativa* and *O.glaberrima.* Natural occurrence of *O.sativa* x *O.glaberrima* hybrids seemed to be favoured by the fact that farmers grew varieties of both rice species in neighbouring fields. Some farmers even intercropped the two rice species within the same field.

Resistance alleles *rymv1-2* and *rymv1-3* were detected in recombinant inbred lines using PCR base SNP-markers. Allele identification was done unambiguously so that homozygous as well as heterozygous recombinant lines could be detected. SSR marker RM252 was most often used to tag RYMV1 resistance gene (Albar *et al.*, 2003; Jaw *et al.*, 2012; Sow, 2012). SNP markers used in this study are more than adequate for marker assisted selection (MAS) because they are located within the target gene and also allow the identification of specific alleles of the gene (Thiemele *et al.*, 2010). Although recombinant lines were developed from interspecific *O.sativa*

x *O.glaberrima* crosses, RYMV1 resistance from Tog5681 was not introgressed into *sativa* genetic background. This was determined only by using MAS. The purpose of using *O. glaberrima* in rice breeding is to transfer into *O. sativa* desirable traits such as resistance to pests and diseases and resilience to abiotic stresses (Jones *et al.*, 1997a; Sarla and Mallikarjuna, 2005). Crosses aimed at introgressing *rymv1-3* resistance allele into *O. sativa* background failed to do so (Ndjiondjop *et al.*, 1999). The reasons for this failure remain unknown. Possibly, RYMV resistance gene may be tightly linked to sterility genes (Levings, 1990; Garavito *et al.*, 2010; Ott *et al.*, 2013).

Latency period for disease symptom expression was used to determine RILs' phenotypes resulting from their reactions to RYMV inoculation (Albar *et al.* 1998; chapter 4). MAS-predicted phenotypes were confirmed by virus inoculation. All rr rice genotypes were found to be highly resistant when non- resistance breaking isolates were used. Although progenies from Gigante x Tog5681 crosses lacked the *rymv1-3* allele, they were also found to be highly resistant because of the inheritance of *rymv1-2* resistance allele from Gigante. They are likely to have inherited some partial resistance from their *O.glaberrima* (Tog5681) parent as well because resistance in most of them could not be broken even by RB isolates in inoculum 2 (Figure 5.7B).

Molecular and biological screening of RILs confirmed successful introgression of resistance genes into farmers' preferred susceptible rice genotypes. Introgression of partial and high resistance was evidenced by the reaction of RILs in the screening tests. Although *rymv1-3* resistance failed to be introgressed, resistant inbred lines were obtained by transferring *rymv1-2* resistance allele from both Gigante and Bekarosaka. Interestingly, resistance that was not broken down by RB isolates in inoculum 2 was achieved in several RILs. In particular, the combination of high and partial resistance yielded several recombinant lines which can be used in short term

breeding programmes for resistance to RYMV. Field testing of these recombinant lines for yield and stability of resistance is a step towards this goal. Progenies with superior resistance were even found in crosses involving only partial resistant parents, indicating additive effects of PR genes. These results fully agree with the view that pyramiding resistance genes to plant pathogens, especially viruses, is an effective way to ensure durable resistance (Parlevliet, 2002; Moullet *et al.*, 2009; Shi *et al.*, 2009).

CHAPTER 6

6. EVALUATION OF RICE RECOMBINANT INBRED LINES FOR YIELD AND YIELD COMPONENTS IN THREE ENVIRONMENTS

6.1. Introduction

Rice yellow mottle virus disease caused by *Rice yellow mottle virus* (RYMV) is considered as the most devastating rice disease in Sub-Saharan Africa (Kouassi *et al.*, 2005). The disease occurs erratically and epidemics are not predictable even at field level. Therefore, plots with high disease incidence (sometimes referred to as 'disease hotspots') in one season may be free of disease during the next season and vice-versa. Typical of many plant viruses, breeding for resistance to RYMV has been considered by several authors as the most convenient means to control the disease (Mew, 1991; Leung *et al.*, 2003).

Rice recombinant inbred line populations (RIPs) were developed and screened for resistance (Chapter 5). These RIPs were evaluated in the field to determine their productivity. Agronomic value of a rice variety depends on many traits (Huang *et al.* 1991) and the most essential characteristics include high yielding ability, resistance to pests and diseases, tolerance to undesirable environmental factors and good grain quality. Increasing grain yield of rice, given the complexity of the environment, is one of the key objectives for breeding rice (Ashura, 1998; Swaminathan, 1999).

The approaches for breeding high yielding rice varieties largely depend on the estimation of genetic variability, heritability, genetic advance and correlations between grain yield and yield components. Useful yield components to be used for yield improvements should be highly heritable traits. Heritability (h^2) is one of the popular indexes, between the phenotypic and breeding value and direct effect on selection (Falconer, 1989). It indicates to what extent

progenies resemble their parents. Broad sense heritability measures the fraction of total variation which is heritable (genotypic). Narrow sense heritability quantifies the portion of phenotypic variation that is additive by nature. Heritability of 45% and 31% was determined in rice for panicle number and for panicle weight, respectively (Gravois and McNew, 1993). Several studies reported high narrow-sense heritability for grain weight, moderate for per-panicle-spikelets number and low for per-plant-panicle number (Surek and Korkut, 1998; Surek and Beser, 2005). Highly realized heritability ranging from 63% to 90% was reported for grain weight in rice (Mustafa and Elsheikh, 2007). Kato (1997) estimated 16% of realized heritability for per-plant-panicle number in rice and 20 to 33% for per-panicle-spikelets number.

Grain yield is a complex character which involves several components such as number of panicles per plant or unit area, plant height, number of fertile tillers, number of spikelets per panicle, panicle length, percentage of filled grains, grain filling period, weight of 1000 grains, and other factors (Halil and Necmi, 2005; Surek and Beser, 2005; Mustafa and Elsheikh, 2007; Ukaoma *et al.*, 2013). Therefore, selecting directly for yield may be misleading (Mustafa and Elsheikh, 2007). Knowledge of inter-relationships of yield components among each other and their contribution to yield is useful in selecting high yielding varieties. Simple correlation analyses relating grain yield to each component may not provide complete understanding of the contribution of components to yield (Dewey and Lu, (1959). A statistical technique referred to as path coefficient analysis is more adequate for this purpose (Surek and Beser, 2003; Azarpour, 2013). It partitions the correlation coefficients into its direct and indirect effects, so that the contribution of each component to yield can be estimated. Many studies using path analysis have shown direct effects of various yield components including harvest index, biomass yield (Ibrahim *et al.*, 1990; Kumar and Hunshal, 1998), and 1000 grain weight (Yagdi, 2009) on

wheat grain yield. Recently, Sadeghi, (2011) reported high direct influence of productive tillers number on rice grain yield. Other yield components such as filled grains per panicle, panicles per plant, grains per panicle, plant height and days to flowering were also reported to have positive impact in rice grain yield (Mustafa and Elsheikh, 2007; Kole *et al.*, 2008; Hairmansis *et al.*, 2010; Akinwale *et al.*, 2011).

The present study was carried out to estimate heritability, genetic variation and direct and indirect contributions for grain yield of some yield components in RIPs evaluated for resistance to rice yellow mottle virus disease.

6.2. Materials and methods

6.2.1. Plant materials and field experiments

Field experiments were carried out in three locations including Kamboinse (12˚28'N latitude, 1˚32'W longitude) and two different locations at Banzon (11°19'0.00"N; 4°47'60.00"W). The two locations in the Banzon irrigated rice scheme were 5 km apart from each other. Kamboinse is located in the dry savannah zone characterized by 600 to 900 mm annual rainfall. Banzon is located in the moist savannah zone characterized by annual rain falls ranging from 900 mm to 1100 mm.

Experiments involved 100 rice genotypes comprising 13 parental lines and eight check varieties and 79 recombinant inbred line populations (RIPs). The 79 RIPs belonged to six advanced breeding families including F4 (26), BC1F2 (14), BC1F3 (2), BC1F4 (28), BC2F2 (7) and BC2F3 (2).

The experimental design was an alpha lattice of 100 entries laidout in 10 x10 with 2 replications and in one location. Rice genotypes were first sown in nurseries and thereafter 21 days-old

seedlings of each genotype were transplanted each 25 cm in rows separated by 30 cm. Fertilizer was applied at a rate of 200 kg of NPK (15:15:15) per hectare. Nitrogen fertilizer (urea: 46% N) was applied in two dressings, at maximum tillering and at panicle initiation stages.

6.2.2. Data collection

Apart from data on days to first flowering (DFF), measurements were taken at rice physiological maturity stage. Measured parameters were number of days to first flowering, plant height (PH), per-plant-tiller number (PPTN), per-plant-panicle number (PPPN), panicle length (PL), flag leaf length (FLL), above ground total biomass (AGTB), single plant grain yield (SPY), and thousand grain weight (TGW).

Measurements of parameters were done as follow (Sarker *et al.*, 2013) :

DFF: numbers of days required for the plant to show the first panicle emergence or blooming counted from the date of sowing.

PH: measured (cm) from ground level to the tip of the tallest panicle.

PPTN: total numbers of stalks of each single plant bearing panicle or not.

PPPN: total numbers of productive panicles were counted from each single plant in each plot.

PL: measured (cm) from the basal node to the tip of any single well developed panicle of each single plant in each plot.

FLL: measured (cm) from the basal node to the tip of any single well developed flag leaf of each single plant in each plot.

AGTB: each entire single plant including mature panicles in each plot was mown from the bottom, dried for 1 week and weighted (g).

SPY: total grain weight (g) per plant was taken after cleaning.

TGW: 100 garins were randomly counted out of the total seeds of each single plant and weighted (g); TGW was calculated from average weights of 100 seeds lots.

6.2.3 Data analysis

Analysis of variance (ANOVA) was done following Singh and Chaudhary (1985) with the mean data of all the replications. To test the differences between genotypes, Duncan's new Range Test (DMRT) was performed following the method of Steel and Torrie (1997).

6.2.3.1. Computation of variance components and estimation of genotypic and phenotypic variances

Estimation of genotypic and phenotypic variances according to the formula given by Johnson *et al.*, (1955):

Genotypic variance (σ^2_g) = (GMS-EMS) / r

Where: GMS = Genotypic mean square; EMS = Error mean square; and r = Number of replications.

Phenotypic variance (σ^2_p) = σ^2_g + EMS

Where: σ^2_g = Genotypic variance and EMS = Error mean square.

Estimation of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) (Burton, 1952; Singh and Chaudhary, 1985):

Genotypic coefficients of variation (GCV) = $\frac{\sqrt{\sigma_g^2}}{\bar{x}} x100$

Where: σ^2_g = Genotypic variance and x = Population mean.

Phenotypic coefficients of variation (PCV) = $\frac{\sqrt{\sigma_p^2}}{\bar{x}} x100$

Where: σ^2_p = Phenotypic variance and x = Population mean.

Estimation of heritability in broad sense by the formula suggested by Johnson *et al.* (1955) and Hanson *et al.* (1960):

Heritability (h^2_b) = (σ^2_g /σ^2_p) x 100

Where: σ^2_g = Genotypic variance and σ^2_p = Phenotypic variance.

Estimation of genetic advance was done following formula given by Johnson *et al.* (1955) and Allard (1960).

Genetic advance (GA) = h^2_b. K.σ_p

Where k = 1.76 at 10% selection intensity.

Genetic advance noted GA (%) was calculated by the formula of Comstock and Robinson (1952) as follows:

Genetic advance in percentage of mean, GA (%) = $\frac{GA}{\bar{x}} x100$

Where GA= Genetic advance and x = Population mean.

The genotypic and phenotypic correlation coefficients between yield and different yield contributing characters were estimated as:

Genotypic correlation = $r_{g(xy)} = \frac{Cov(g)1.2}{\sqrt{\sigma^2(g)1.\sigma^2(g)2}}$

Where: $Cov_{g(xy)}$ = Genotypic covariance between the variables X and Y; $\sigma^2_{(g)1}$ = Genotypic variance of the variable X1 and $\sigma^2_{(g)2}$ = Genotypic variance of the variable X2.

Similarly, phenotypic correlation $r_{p(xy)} = \frac{Cov(ph)1.2}{\sqrt{\sigma^2(ph)1.\sigma^2(ph)2}}$

Where $Cov_{ph(xy)}$ = phenotypic covariance between the variables X and Y; $\sigma^2_{(ph)1}$ = phenotypic variance of the variable X1 and $\sigma^2_{(ph)2}$ = phenotypic variance of the variable X2.

6.2.3.2. Estimation of Path coefficients

Path coefficient analysis was done according to the procedure employed by Dewey and Lu (1959) also quoted in Singh and Chaudhary (1985), using phenotypic correlation coefficient values. In path analysis, correlation coefficients between yield and yield contributing characters were partitioned into direct and indirect effects of yield contributing characters on grain yield per hectare. In order to estimate direct and indirect effects of the correlated characters, i.e. 1, 2, 3…and 10 on yield y, a set of simultaneous equations is required to be formulated as shown below:

$r_{1.y} = P_{1.y} + r_{1.2} P_{2.y} + r_{1.3} P_{3.y} + r_{1.4} P_{4.y} + r_{1.5} P_{5.y} + r_{1.6} P_{6.y} + r_{1.7} P_{7.y} + r_{1.8} P_{8.y} + r_{1.9} P_{9.y} + r_{1.10} P_{10.y}$

$r_{2.y} = r_{1.2} P_{1.y} + P_{2.y} + r_{2.3} P_{3.y} + r_{2.4} P_{4.y} + r_{2.5} P_{5.y} + r_{2.6} P_{6.y} + r_{2.7} P_{7.y} + r_{2.8} P_{8.y} + r_{2.9} P_{9.y} + r_{2.10} P_{10.y}$

$r_{3.y} = r_{1.3} P_{1.y} + r_{2.3} P_{2.y} + P_{3.y} + r_{3.4} P_{4.y} + r_{3.5} P_{5.y} + r_{3.6} P_{6.y} + r_{3.7} P_{7.y} + r_{3.8} P_{8.y} + r_{3.9} P_{9.y} + r_{3.10} P_{10.y}$

$r_{4.y} = r_{1.4} P_{1.y} + r_{2.4} P_{2.y} + r_{3.4} P_{3.y} + P_{4.y} + r_{4.5} P_{5.y} + r_{4.6} P_{6.y} + r_{4.7} P_{7.y} + r_{4.8} P_{8.y} + r_{4.9} P_{9.y} + r_{4.10} P_{10.y}$

$r_{5.y} = r_{1.5} P_{1.y} + r_{2.5} P_{2.y} + r_{3.5} P_{3.y} + r_{4.5} P_{4.y} + P_{5.y} + r_{5.6} P_{6.y} + r_{5.7} P_{7.y} + r_{5.8} P_{8.y} + r_{5.9} P_{9.y} + r_{5.10} P_{10.y}$

$r_{6.y} = r_{1.6} P_{1.y} + r_{2.6} P_{2.y} + r_{3.6} P_{3.y} + r_{4.6} P_{4.y} + r_{5.6} P_{5.y} + P_{6.y} + r_{6.7} P_{7.y} + r_{6.8} P_{8.y} + r_{6.9} P_{9.y} + r_{6.10} P_{10.y}$

$r_{7.y} = r_{1.7} P_{1.y} + r_{2.7} P_{2.y} + r_{3.7} P_{3.y} + r_{4.7} P_{4.y} + r_{5.7} P_{5.y} + r_{6.7} P_{6.y} + P_{7.y} + r_{7.8} P_{8.y} + r_{7.9} P_{9.y} + r_{7.10} P_{10.y}$

$r_{8.y} = r_{1.8} P_{1.y} + r_{2.8} P_{2.y} + r_{3.8} P_{3.y} + r_{4.8} P_{4.y} + r_{5.8} P_{5.y} + r_{6.8} P_{6.y} + r_{7.8} P_{7.y} + P_{8.y} + r_{8.9} P_{9.y} + r_{8.10} P_{10.y}$

$r_{9.y} = r_{1.9}P_{1.y} + r_{2.9}P_{2.y} + r_{3.9}P_{3.y} + r_{4.9}P_{4.y} + r_{5.9}P_{5.y} + r_{6.9}P_{6.y} + r_{7.9}P_{7.y} + r_{8.9}P_{8.y} + P_{9.y} + r_{9.10}P_{10.y}$

Where:

r_{1y} = Genotypic correlation coefficients between y and i^h character (y = Grain yield = **GY**)

P_{iy} = Path coefficient due to i^{th} character (i = 1, 2, 3… 10)

1 = Days to First Flowering **(DFF)**
2 = Plant height **(PH)**
3 = Per-Plant-Tillers-Number **(PPTN)**
4 = Per-Plant-Panicles-Number **(PPPN)**
5 = Panicle Length **(PL)**
6 = Flag Leaf Length **(FLL)**
7 = Upper Ground Total Biomass **(AGTB)**
8 = Single Plant Grain Yield **(SPY)**
9 = Thousand grains weight **(TGW)**

Partitioning of total correlation is done as indicated below taking as example number days to first flowering (**DFF)** and grain yield (y = **SPY)** i.e., r_{1y}:

$P_{1.y}$ = Direct effect of **DFF** on **SPY**

$r_{1.2} P_{2.y}$ = Indirect effect of **DFF** on **SPY** via **PH**

$r_{1.3} P_{3.y}$ = Indirect effect of **DFF** on **SPY** via **PPTN**

$r_{1.4} P_{4.y}$ = Indirect effect of **DFF** on **SPY** via **PPPN**

$r_{1.5} P_{5.y}$ = Indirect effect of **DFF** on **SPY** via **PL**

$r_{1.6} P_{6.y}$ = Indirect effect of **DFF** on **SPY** via **FLL**

$r_{1.7} P_{7.y}$ = Indirect effect of **DFF** on **SPY** via **AGTB**

$r_{1.8} P_{8.y}$ = Indirect effect of **DFF** on **SPY** via **SPY**

$r_{1.9} P_{9.y}$ = Indirect effect of **DFF** on **SPY** via **TGW**

Where:

$P_{1.y}$, $P_{2.y}$, $P_{3.y}$ …$P_{10.y}$ = Path coefficient of the independent variables 1, 2, 3… 10 on the dependent variable y, respectively.

$r_{1.y}$, $r_{2.y}$, $r_{3.y}$… $r_{10.y}$ = Correlation coefficient of 1, 2, 3 …10 with y, respectively. After calculating the direct and indirect effect of the characters, residual effect (R) was calculated by using the formula given below (Singh and Chaudhary, 1985): $P^2_{RY} = 1- (r_{1.y}P_{1.y} + r_{2.y}P_{2.y} +\ldots + r_{10.y}P_{13.y})$

Where, $P^2_{RY} = R^2$ and hence residual effect, $R = (P^2_{RY})^{½}$; $P_{1.y}$ = Direct effect of the i^{th} character on yield y and $r_{1.y}$ = Correlation of the i^{th} character with yield y.

6.3. Results

6.3.1. Analysis of variance and heritability

Significant differences among genotypes and locations (P=0.001) for all grain yield related characters (Table 6.1) were observed. On average, rice genotypes in RIPs showed first flowers at 93 days (DFF) after germination. In some families, DFF was as low as 60 days but reached 128 days in other families. Mean values for plant height, number of tillers per plants and total above ground biomass were 125 cm (90-180 cm), 17.5 (6-31 tillers) and 117.9 g (64.2-157.5), respectively. Per plant panicle number and single plant grain yield were 13.7 (5-27 panicles) and 29.4 g (14.5-48.5 g), respectively.

Genotypic and phenotypic variances, coefficients of variation and broad sense heritability for characters, were calculated (Table 6.2). Genotypic coefficients of variation ranged from 13.50 to 85.95 and phenotypic coefficients of variation ranged from 18.1 to 90.95 among various parameters under consideration. Total number of tillers (PPTN) and number of fertile panicles (PPPN) showed the highest genotypic as well as phenotypic coefficients of variation. Both genotypic and phenotypic coefficients of variation were lowest for panicle length (PL) and 1000-grain weight (TGW). All characters showed high heritability estimates and ranged from 55.26 to 95.34%). Genetic advance in response to selection was highest for per plant panicle number and total tiller number. Most characters including DFF, PH, PL, FLL and AGTB showed low genetic advance estimates ranging from 0.36% to 0.76%.

Genotype effect reflected the performance of recombinant lines for grain yield (Table 6.3). Grain yield was especially higher in four recombinant families compared to mid-parents. These families resulted from the following crosses: Gigante x FKR16 (27.5 % increase over mid-parents), Gigante x FKR62N (27.1% increase), Digang x Gigante (22.1%) and FKR19 x Digang

(19.1%). Some crosses resulted in decrease in grain yield. The biggest decrease in yield was observed in Digang x CG14 cross (17% decrease over mid-parents) and in Gigante x Tog5681 cross (10.7% decrease). Most often, decrease in yield over mid-parents was due to the lower performance of recombinant lines over the male parents.

Table 6. 1. Analysis of variance yield and its components of 79 RIPs and their parents evaluated in three locations and two replications in fixed genotypes, random environments and random blocks model

Source Of Variation	DF	Mean Square [a]								
		DFF	PPPN	PPTN	PH	PL	FLL	AGTB	TGW	SPY
Means		93	13.65	17.53	125.57	25.91	33.94	117.98	26.34	29.4
Location	2	1548.71	1371.55***	2636.77***	103223.43***	177.26***	12256.28***	177280.22***	72.23***	477.65
Replications (Locations)	3	3430.33***	264.77***	40.78	1475.18***	39.63	1200.58***	25764.53***	35.1	1459.03***
Genotype	99	12918.06***	629.13***	1052.32***	12873.43***	58.14***	424.22***	15141.88***	214.57***	1872.91***
Locations*Genotypes	198	275.44	122.43***	173.03***	2291.78***	40.84***	303.66***	10498.2***	7.47	676.98***
Error	599	336.78	26.49	32.59	145.28	10.46	50.33	1625.01	7.27	190.75
R-Square		0.33	0.32	0.39	0.76	0.17	0.37	0.28	0.28	0.18
CV (%)		19.73	37.71	32.56	9.6	12.48	20.9	34.17	10.24	46.98

[a]DFF: days to first panicle flowering; PH: plant height; PPTN: number of tillers per plant; PPPN: number of panicles per plant; PL: panicle length; FLL: flag leaf length; AGTB: above ground total biomass; TGW: 1000 grain weight; SPY: single plant grain yield. ***: Significant effects at P=0.001.

Table 6. 2. Mean squares, heritability (broad sense) and co-efficient of variability estimates for grain yield components in rice

Characters	MS	CV%	Mean	σ_g^2	σ_p^2	σ_e^2	Coefficient of variation		H^2 (%)	GA%
							Genotypic	Phenotypic		
SPY	1172.82***	43.12	29.4	506.07	666.74	160.67	76.52	87.83	77.29	2.67
PPPN	290.82***	34.72	13.65	134.19	156.64	22.45	84.89	91.71	85.67	6.77
PPTN	481.34***	29.74	17.53	227.07	254.26	27.19	85.95	90.95	89.3	5.45
DFF	4909.3***	19.17	93	2295.8	2613.51	317.71	51.52	54.97	87.84	0.61
PH	4967.46***	8.67	125.57	2424.46	2543	118.54	39.21	40.16	95.34	0.36
PL	34.02***	12.08	25.91	12.11	21.91	9.8	13.43	18.07	55.26	0.45
FLL	213.81***	19.85	33.94	84.22	129.6	45.38	27.04	33.54	64.98	0.76
AGTB	10530.73***	30.65	117.98	4611.8	5918.92	1307.12	57.56	65.21	77.92	0.51
TGW	129.54***	8.93	26.34	62	67.54	5.53	29.89	31.2	91.81	1.28

[a]DFF: days to first panicle flowering; PH: plant height; PPTN: number of tillers per plant; PPPN: number of panicles per plant; PL: panicle length; FLL: flag leaf length; AGTB: above ground total biomass; TGW: 1000 grain weight; SPY: single plant grain yield, GA: genetic advance; CV: coefficient of variation. ***: Significant effects at P=0.001.

Table 6. 3. Parents and offspring mean performance for grain yield

Families of RIPs	Average yield per plant (g)[a]				% increase or decrease over		
	OF	FP	MP	MiP	FP	MP	MiP
Gigante x FKR16	33.94±14.56	25.07±8.59	28.17±13.25	26.62±10.92	35.4	20.5	27.5
Gigante x FKR19	27.12±13.16	25.07±8.59	25.3±17.44	25.18±0.00	8.2	7.2	7.7
FKR56N x Gigante	29.04±12.88	29.97±10.83	25.07±8.59	27.52±9.71	-3.1	15.8	5.5
Gigante x FKR60N	24.23±10.18	25.07±8.59	24.9±9.09	24.98±0.00	-3.4	-2.7	-3
Gigante x FKR62N	30.42±16.11	25.07±8.59	22.8±11.11	23.93±9.85	21.3	33.4	27.1
Digang x Gigante	30.38±16.91	24.7±8.81	25.07±8.59	24.88±8.70	23.0	21.2	22.1
GH1520 x Gigante	32.76±12.96	38.97±15.44	25.07±8.59	32.02±12.02	-15.9	30.7	2.3
Azucena x Gigante	19.98±19.90	14.27±7.14	25.07±8.59	19.67±7.86	40.0	-20.3	1.6
Azucena x Bekarosaka	23.08±19.28	14.27±7.14	34.67±20.01	24.47±13.57	61.7	-33.4	-5.7
Gigante x Tog5681	29.28±14.34	25.07±8.59	40.53±16.76	32.8±12.67	16.8	-27.8	-10.7
FKR56N x Tog5681	31.8±15.75	29.97±10.83	40.53±16.76	32.8±12.67	6.1	-21.5	-3.0
Kumazuce x Digang	24.01±10.58	24.6±9.45	24.7±8.81	24.65±9.13	-2.4	-2.8	-2.6
FKR19 x Digang	29.77±14.39	25.3±17.44	24.7±8.81	25±13.13	17.7	20.5	19.1
Digang x CG14	30.4±12.53	24.7±8.81	48.53±21.1	36.62±14.95	23.1	-37.4	-17.0

[a]OF: offsprings; FP: female parent; MP: Male parent; MiP: Mid-parent

6.3.2. Correlation among characters

The genotypic and phenotypic coefficients of correlation among grain yield and its components are presented in Table 6.4. Four components (PPPN, PPTN, TGW, and AGTB) were highly correlated genotypically to grain yield (SPY). Although AGTB was highly correlated to SPY, the correlation coefficient was negative, indicating that the more biomass produced, the less grain yield is obtained. No significant correlation was found between SPY and other characters. PPPN was correlated to most characters, DFF and FLL being the only ones with which it was correlated.

Few significant correlations were observed at the phenotypic level. Only PPPN showed significant correlation with grain yield. AGTB was highly correlated with three other components including PPPN, PPTN and DFF. No significant correlations were found between PH and any other character. At genotypic and phenotypic levels, only PPPN was correlated with grain yield. At both levels, PPTN was consistently correlated with PPPN and PL. In several cases, correlation coefficients were only significant either at genotypic or phenotypic levels.

6.3.3. Phenotypic and genotypic path coefficient analysis

Path coefficients were computed for the estimation of the contribution of individual components (dependant variables) to grain yield (independent variable). Direct positive effects on grain yield were found with PPPN, DFF, PH, PL and TGW (Table 6.5). The highest direct effect resulted from the number of panicles per plant (+ 0.944), which alone exceeded the sum of all other direct effects. Negative direct effects resulted from per plant tiller number (-0.183), flag leaf length (-0.116) and above ground total biomass (-0.0007). Indirect genotypic effects on grain yield were low. The highest positive effect was due to 1000 grain weight and the number of fertile panicles (+0.137).

Table 6. 4. Genotypic (lower diagonal) and phenotypic (upper diagonal) correlation of grain yield components

Character[a]	SPY	PPPN	PPTN	DFF	PH	PL	FLL	TGW	AGTB
SPY		0.2485**	0.18482	0.00304	-0.00306	-0.16262	-0.01737	0.28765	-0.01385
PPPN	0.8031***		0.80308***	0.76098***	0.01677	-0.03978	-0.19655	-0.05107	0.61041***
PPTN	0.7610***	0.9672***		0.96719***	0.02479	-0.3429***	-0.27409**	-0.11212	0.56445***
DFF	-0.0328	0.0286	0.0313		-0.00838	-0.34711***	-0.26487**	-0.11034	0.54848***
PH	-0.0398	-0.3429***	-0.3471***	-0.1304		0.01302	-0.12709	-0.0968	-0.11134
PL	-0.1965	-0.2741**	-0.2649**	-0.1497	0.1855		0.18555	0.3539***	-0.143
FLL	-0.0511	-0.1121	-0.1103	0.0443	0.3539***	0.4449***		0.44487***	-0.17779
TGW	0.6104***	0.5645***	0.5485***	0.2096*	-0.1430	-0.1778	0.1054		0.10543
AGTB	-0.2751**	-0.3793***	-0.4088***	-0.1123	0.2657**	0.2004*	0.0247	-0.3158***	

[a]DFF: days to first panicle flowering; PH: plant height; PPTN: number of tillers per plant; PPPN: number of panicles per plant; PL: panicle length; FLL: flag leaf length; AGTB: above ground total biomass; TGW: 1000 grain weight; SPY: single plant grain yield. Significant effects at P=0.05 (*); P=0.01 (**) and P=0.001 (***) are indicated.

Table 6. 5. Genotypic direct (bold face shaded and diagonal) and indirect effects of various components on rice grain yield

Characters[a]	PPPN	PPTN	DFF	PH	PL	FLL	TGW	AGTB
PPPN	**0.943572**	0.757767	0.718042	0.015824	-0.037538	-0.185459	0.006962	0.575962
PPTN	-0.177644	**-0.183671**	-0.177645	-0.004553	0.062981	0.050342	0.003171	-0.103674
DFF	0.000116	-0.000039	**0.004677**	-0.000039	-0.001623	-0.001239	0.000002	0.002565
PH	-0.097861	-0.099064	0.003715	**0.285393**	0.003715	-0.036271	-0.000135	-0.031776
PL	-0.015616	-0.01509	-0.007241	0.010571	**0.056973**	0.010571	0.000602	-0.008147
FLL	0.013049	0.012842	0.011266	-0.041188	-0.051776	**-0.116385**	0.006026	0.020692
TGW	0.137033	0.133154	-0.02703	-0.034716	-0.043161	0.025595	**0.24277**	0.025595
AGTB	0.000274	0.000295	-0.000048	-0.000192	-0.000145	-0.000018	0.000228	**-0.000722**

[a]DFF: days to first panicle flowering; PH: plant height; PPTN: number of tillers per plant; PPPN: number of panicles per plant; PL: panicle length; FLL: flag leaf length; AGTB: above ground total biomass; TGW: 1000 grain weight; SPY: single plant grain yield compared to genotypic ones. Such phenotypic effects were most evident in the number of fertile panicles per plant compared to three other components, number of tillers per plant (+0.758), the number of days for first flowering (+0.718) and the total biomass above ground (+0.576).

The most important negative indirect effect on grain yield observed with the number of tillers per plant and the number of fertile panicles (-0.177). Phenotypic indirect effects were higher compared to genotypic ones. Such phenotypic effects were most evident in the number of fertile panicles per plant compared to three other components, number of tillers per plant (+0.758), the number of days for first flowering (+0.718) and the total biomass above ground (+0.576).

6.4. Discussion

Recombinant inbred rice lines with resistance to rice yellow mottle virus disease were evaluated in the field for grain yield. The high level of differences observed in yield components considered in the study is consistent with the nature of rice genotypes evaluated. All genotypes belonged to segregating recombinant families. The extensive variability of rice genotypes for grain yield suggests that promising high yielding rice lines with resistance/tolerance to RYMV can be selected. The highest performances were recorded in progenies from crosses involving Gigante, which is a donor of high resistance gene to the disease. One parent of each set of progenies was farmers' preferred varieties. Therefore, future varieties developed from the progenies will likely be adopted by farmers. Estimates of broad sense heritability for grain yield (77.29%) were consistent with estimates from several previous studies. Khan *et al.* (2009) found an estimate of more than 50% heritability for yield in rice. Similarly, high heritability estimates of 76.18% and 99% for rice grain yield were also reported by Rahman *et al.* (2012) and Sathya and Jebara (2013), respectively. These heritability estimates are helpful in making selection for yield in rice on the basis of phenotypic performance.

High genotypic correlations between grain yield and its components have been reported in rice (Rahman *et al.*, 2012) and other cereal crops (Debebe *et al.*, 2013). This was also confirmed in the present study. The high genotypic positive correlations between grain yield

and three components (PPPN, PPTN and TGW) suggest that selection directed at any of these components may directly affect grain yield. Negative correlations between plant height and grain yield per plant at both genotypic and phenotypic levels are in agreement with the findings of Saif-ur-Rasheed *et al.* (2002). However, a positive correlation between the two factors was observed by Sharma and Reddy (1991). In this study, discrepancies between genotypic and phenotypic correlations were clearly found. Such discrepancies have been observed in other studies (Saif-ur-Rasheed *et al.*, 2002; Debebe *et al.*, 2013; Sarker *et al.*, 2013). On the one hand, correlations found only at the genotypic level suggest possible environmental effects (Aktar *et al.*, 2011) , however, correlations at the phenotypic level only may be misleading for breeders as selection based on such correlations could result in unstable performance.

Direct and positive effect (0.943) of panicle number per plant appeared to be the most important component with the biggest influence on grain yield. This component was also reported elsewhere to have positive and direct effect on rice grain yield although at a lower magnitude (Karad *et al.*, 2008; Mugemangango and Vinod, 2011; Haider *et al.*, 2012). The positive effect of panicle number per plant and highly significant positive correlation at genotypic and phenotypic levels revealed by the present study indicates that PPPN is an important yield related trait that can be used for selection. Among other components that had positive and direct effects on grain yield, 1000- grain weight, per plant tiller number, plant height and panicle lenght were also reported by several authors (Khan *et al.*, 2009; Aktar *et al.*, 2011; Seyoum *et al.*, 2012). The magnitude of the direct effects on grain yield found by these authors was quite low for all of the components under consideration, which is in agreement with the results obtained in the present study. Major indirect effects on grain yield were found in number of tillers, days to first flowering and the above ground biomass and most of these components had low positive or even negative direct effects. Therefore, such

components should be used in combinations in order to achieved indirect selection for grain yield in rice.

Path coefficient analysis provided an insight into the inter-relationship of various characters with grain yield. Considering grain yield as the artifact of all the causal characters PH, PPTN, PPPN, PL, FLL, TGW, DFF, AGTDM, the correlation coefficients of these causal factors with grain yield are partitioned into direct and indirect effects. As yield is influenced by many factors, selection based on correlation may be misleading because it measures only the mutual association between two variables, whereas path coefficient analysis specifically measures the relative importance of different yield components. To find out the direct and indirect effects and to measure the relative importance of causal factors, path coefficient analysis is useful, which permits critical examination of the specific forces acting to produce a given correlation plant for more reliable selection for high yielding genotypes.

CHAPTER 7

7. GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1. General discussion

7.1.1. Farmers' preferences in the breeding strategy

As a contribution to a better productivity of rice through the control of rice yellow virus disease (caused by Rice yellow mottle virus, RYMV), this thesis used an approach combining several aspects of field crop research. Attention was first focused on farmers as the main stakeholders in rice production. Most farmers in Burkina Faso were smallholder farmers and 98.5% of them held fields of less than 5 ha. This is consistent with reports from many other African countries (Nakano *et al.* 2011; GRiSP, 2013). Rice yellow mottle virus disease appears to be known by the majority of farmers but the success of disease management procedures is questionable. Replacement of varieties and insecticide applications used as the most common methods of control are likely to be ineffective in a sustainable production system. Farmers' varieties are susceptible or partially resistant to RYMV (Chapter 4). The indiscriminate use of insecticides is not desirable (Hashmi and Khan, 2011). Insecticides are hazardous and environmentally unfriendly. They may prevent disease spread to some extent but it is not known whether insects are the major factor for virus dissemination in the field (Calvert et al., 2003; Traore et al., 2009). Given that rice cultivation is dominated by smallholder farmers, rice yellow mottle disease management through genetic control is probably the best alternative for control (Bonman et al., 1992; Mew, 1991; Leung et al., 2003).

The participatory rural appraisal approach used in this study identified farmers' indigenous knowledge and perceptions on RYMV and measures for control. This is important as a

starting point for more effective farmers' capacity building from extensive farm-level awareness of pest and diseases and their management strategies. Farmers' involvement in the process of disease control through farmers' field schools and integrated pest management technologies has led to the adoption of successful management practices (Adesina et al. 1994; Roling et al. 1994). Moreover, farmers' preferences for rice varieties were also determined. If such varieties were to be improved for resistance to RYMV, it is expected that their wide adoption will not be a major problem (Debebe et al., 2005).

7.1.2. Screening of rice germplasm for resistance to rice yellow mottle virus disease

Most screenings of rice germplasm for resistance to RYMV in the past did not consider the virus diversity as a critical factor. In this study, both non-resistance breaking (nRB) and resistance breaking (RB) isolates of the virus were used to screen rice varieties. The diversity in varietal reaction and inconsistency in results from nRB and RB isolates clearly demonstrated that screening experiments for resistance of rice to RYMV should be done with well characterized virus isolates. At least, the pathogenic properties (nRB or RB) of virus isolates should be well-known beforehand. Failure to use nRB isolates in the screening experiments could affect the identification of stable sources of resistance (at both partial and high level). Only two resistance genes (RYMV1 and RYMV2) have been reported for RYMV (Ndjiondjop et al., 1999; Thiemele et al., 2010). New resistance genes are likely to be found, especially in the African rice *Oryza glaberrima* (Paul et al., 2003). Concomitant use of nRB and RB isolates may help identify additional resistance genes that cannot be broken down by RB isolates. Screening rice germplasm for resistance in the greenhouse was more efficient than running the experiment in field conditions for two main reasons:

(i) field virus isolates characteristics are unknown, which may lead to different conclusions if experiments are done in different environments (Awoderu, 1991; Thottappilly and Rossel, 1993; Coulibaly et al. (1999; Zouzou et al., 2008);

(ii) due to the erratic nature of rice yellow mottle virus disease, inoculum pressure may be very low or greatly variable between locations. Locations sometimes referred to as 'disease hotpots' may not be equally affected by the disease from one season to another. Therefore, even if multi-location trials are conducted, this does not always guarantee adequate disease pressure necessary for the screening.

Many breeders argue that greenhouse screening is inappropriate because of the higher virus contents in the inoculum compared to field transmission by vectors or other means. This should not be a major concern since resistant varieties found in the greenhouse will likely be resistant also in the field. What could be missed is field resistance in case of antibiosis to RYMV vectors. Antibiosis was reported in several rice varieties against the brown planthopper *Nilaparvata lugens* which vectors rice ragged stunt and rice grassy stunt viruses (Kenmore *et al.*, 1984). Antibiosis against the green leafhopper (*Nephotettix virescens*), vector of rice tungro virus disease, was also reported (Park et al., 2013). Antibiosis against RYMV vectors has not been reported yet.

7.1.3. Development of high yielding quality rice with resistance to RYMV

High resistance conditioned by RYMV1 in rice cultivars Gigante and Bekarosaka was transferred into farmers' preferred varieties. Both cultivars are homozygous for the *rymv1-2* allele of the resistance gene (Albar et al., 2006; Rakotomalala et al., 2008). Of the 79 recombinant inbred populations developed from crosses of these varieties and farmer preferred varieties, 57% showed high resistance to RYMV. Most populations resulted from crosses between Gigante or Bekarosaka and partially resistant rice varieties. The high resistance was broken down by RB isolates but most inbred lines exhibited partial resistance

characterized by a significant delay in symptom expression compared to the susceptible cultivar BG90-2. Therefore, combining both high and partial resistance resulted in a more stable disease resistance in the progenies even when they were challenged with RB virus isolates. Gene pyramiding for resistance to plant pathogens, especially viruses, has been reported to be an effective way to ensure durable resistance (Parlevliet, 2002; Moullet et al., 2009; Shi et al., 2007). Therefore, both high and partial resistance could be introgressed into susceptible farmers' preferred rice varieties. Partial resistance is controlled by several genes (polygenic) (Albar et al., 1998) therefore; resistance genes may not be completely the same in all partially resistant rice varieties. This was apparent in differential virus-host interactions in partially resistant cultivars Azucena (Ioannidou et al., 2000) and Digang (Chapter 4). Thus, bringing resistance from several partially resistant rice donors may be more beneficial in breeding for resistance to RYMV.

Although crosses involving resistance donor Tog5681 bearing *rymv1-3* allele were successful, no resistance was transferred to susceptible recurrent parents (Chapter 5). Progenies did show heterosis but only marker-assisted selection (MAS) was able to track the resistance gene. This exemplified the power of MAS as a key tool for modern plant breeding (Thiemele et al., 2010; Kam, 2011; Jaw et al., 2012). Efforts need to be made to identify suitable molecular markers for partial resistance in order to efficiently combine both high and partial resistance in rice.

Field evaluation of recombinant inbred populations indicated tremendous variability for grain yield, suggesting the possibility for selecting high yielding rice varieties with resistance/tolerance to RYMV. Estimates of broad sense heritability for grain yield were high (77.29%) and consistent with estimates from several previous studies (Khan et al., 2009; Rahman et al., 2012; Sathya and Jebara, 2013). High genotypic correlations were found

between yield and three of its components: number of panicles or tillers per plant and 1000-grain weight. These highly correlated yield related components will be useful for the next selection steps within recombinant inbred populations developed during this study.

7.2. General Conclusion

Five farmers' most preferred rice varieties were identified in both Banzon and Mogtedo. Results revealed that farmers grow rice varieties according to their preferences. Breeding efforts for rice improvement should therefore take into consideration farmers preferred attributes.

Rice yellow mottle disease (RYMD) appeared to be reconized as a major constraint by most rice farmers but the success of disease management procedures remain uncertain. Inconsistency in rice genotype reactions to RYMV isolates suggested that well characterized virus isolates is crutial in screening for resistance to RYMV. At least, the pathogenic properties regarding the ability of virus isolates to overcome existing resistance genes should be defined.

The genetic basis of resistance of newly identified sources of partial resistance seemed to be different from the control cv Azucena. High resistance conditioned by RYMV1 in rice cultivars Gigante and Bekarosaka was succssessfully transferred into some farmers' preferred rice varieties. This high resistance was lower and not active against resistant breaking (RB) isolates but most inbred lines exhibited partial resistance reaction characterized by a significant delay in symptom expression compared to the Partial Resistance control Azucena. Combining both high and partial resistance resulted in a more stable disease resistance in the progenies when they were challenged with RB virus isolates (Moullet et al., 2009; Shi et al., 2007). Progenies from crosses between PR genotypes were found to express high resistance

phenotype. Ioannidou et al. (2000) indicated that combining resistance from several partial resistant rice donors may be more beneficial in breeding for durable resistance to RYMV.

Field evaluation of recombinant inbred populations indicated high significant variability for grain yield, suggesting the possibility for selecting high yielding rice varieties with resistance/tolerance to RYMV. At least 600 randomly selected F2 seeds from all crosses were advanced by single seed decent (SSD) method. F5 seeds were generated from SSD to be used in future rice selection programmes for grain yield and resistance to RYMV.

7.3. Recommendations

1. Further characterization of viral populations' structure in each rice cultivation area should be undertaken for an effective breeding strategy for resistance to RYMV.
2. Further characterization of resistance using associated mapping populations developed from newly identified partial resistant lines could provide interesting contribution in exploiting partial resistance to control RYMV.
3. Efforts need to be made to identify suitable molecular markers for partial resistance in order to efficiently combine both high and partial resistance to RYMV in rice.
4. Genetic engineering chould be explored for more durable control of the virus.

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Appendice 1

Appraisal for farmers' preference for rice varieties and their awareness of RYMV

January 2011 - WACCI

Farmers/Structure interview

1. Area/village/Names

2. Sex
○ 1. Male ○ 2. female

3. Type d'amenagement
☐ 1. perimetre irrigue ☐ 2. hors perimetre
☐ 3. bafond amenage ☐ 4. bafond non amenage
Vous pouvez cocher plusieurs cases (2 au maximum).

4. Superficie exploitee
○ 1. <0.5ha ○ 2. 0.5-1ha ○ 3. 1-2ha ○ 4. 2-5ha
○ 5. 5-10ha ○ 6. >10ha

5. Depuis quand produisez-vous du riz?
○ 1. 1an ○ 2. 2ans ○ 3. 3ans ○ 4. 4ans
○ 5. 5ans ○ 6. >5ans

6. Quelle autre cultures produisez-vous?
☐ 1. sorgho ☐ 2. mil ☐ 3. mais
☐ 4. fonio ☐ 5. niebe ☐ 6. arachide
☐ 7. sesame ☐ 8. Pois-de-terre ☐ 9. maraichage
☐ 10. fruitiers
Vous pouvez cocher plusieurs cases.

7. Pour quelles raisons avez vous opte pour le riz?
☐ 1. Facilite d'acces au terrain ☐ 2. Facilite de produire
☐ 3. rentabilite ☐ 4. heritage
☐ 5. autres
Vous pouvez cocher plusieurs cases.

8. Quelles sont les varietes que vous cultivez?

9. Laquelle des varietes preferez vous? Pourquoi?

10. Quels niveau de rendements obtenez vous?
○ 1. <1T ○ 2. 1-2T ○ 3. 2-3T ○ 4. 3-4T ○ 5. >4T

11. Source d'approvisionnement en semence?
☐ 1. Production precedente ☐ 2. Autres producteurs
☐ 3. ZAT ☐ 4. semencier
☐ 5. INERA ☐ 6. ONG/Projet
☐ 7. autres
Vous pouvez cocher plusieurs cases.

12. Criteres de choix des varietes
☐ 1. disponible ☐ 2. cycle
☐ 3. rendement ☐ 4. taille
☐ 5. resistance ravageurs ☐ 6. resistance maladies
☐ 7. gout ☐ 8. taille-grain
☐ 9. en fonction du marche ☐ 10. autres
Vous pouvez cocher plusieurs cases.

13. Contraintes de production rencontrees?
☐ 1. Disponibilite de semence ☐ 2. Insuffisance d'eau
☐ 3. enherbement ☐ 4. exigence de fertilisants
☐ 5. maladies ☐ 6. insectes
☐ 7. oiseaux ☐ 8. animaux
☐ 9. encadrement technique ☐ 10. autres
Vous pouvez cocher plusieurs cases.

14. Quel type de maladies connaisez vous sur le riz?

15. Avez vous deja vu un champ devaste par une maladie?
○ 1. oui ○ 2. non

16. Que faites vous devant une situation de degats causes par maladies?
☐ 1. Rien ☐ 2. traitement chimques
☐ 3. lutte culturale ☐ 4. changement de varietes
☐ 5. abandon du champ ☐ 6. autres
Vous pouvez cocher plusieurs cases.

17. Quelle serait la variete de riz de votre reve?
☐ 1. cycle court ☐ 2. grande taille
☐ 3. petite taille ☐ 4. feuillue
☐ 5. longues graines ☐ 6. grosses graines
☐ 7. doux ☐ 8. suculent
☐ 9. haut rendement ☐ 10. autres
Vous pouvez cocher plusieurs cases.

18. Que pouvez vous conseiller a quelqu'un qui veut creer une noouvelle variete de riz pour vous?

19. Un dernier mot sur vos attentes en matiere de soutien a la riziculture?