12 Integration of Molecular Markers in Rice Improvement: A Case Study on Resistance to *Rice yellow mottle virus*

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Introduction

The development of molecular markers in the 1980s was considered as highly promising for plant breeding (Young and Tanksley, 1989), as they offer to partly replace phenotypic selection (which is often expensive and fastidious) with genotypic selection. A wide range of molecular breeding methods have been described, from marker-assisted selection (MAS) of one major gene to large-scale genotypic selection for multiple traits or genetic background. The main advantages of MAS have been reviewed extensively (Collard and Mackill, 2008; Xu and Crouch, 2008); they generally concern traits for which phenotyping is associated with particular stages of development or environmental conditions, traits with low heritability, recessive characters, or when pyramiding would mask the effect of individual genes. As soon as markers associated with traits of interest are available, selection for major genes is theoretically accessible to any laboratory with basic molecular-biology equipment (Van Damme *et al.*, 2011). However, the impact of molecular markers in breeding appears to be limited in comparison with the number of publications that report genetic linkage between markers and genes of interest. This may be explained by the delay observed in the theoretical development of a methodology and its practical outputs or absence of information on the methodology used (Collard and Mackill, 2008). Van Damme *et al.* (2011) hypothesize that it may result from a lack of access to the information in some developing countries and these authors therefore developed a database that synthesizes all the markers available for MAS in 19 species.

Rice, the staple food of half of the world's population, has two cultivated species, *Oryza sativa* that originated from Asia and is cultivated worldwide, and *Oryza glaberrima*, which is endemic to Africa. *Oryza sativa* is high yielding, but lacks sources of resistance to some strains of rice diseases and insect pests specific to African rice-cropping agro-ecosystems. Conversely, *O. glaberrima* is low yielding, shatters spontaneously and has few panicle branches, but often constitutes a source of resistance to African pests and diseases. Some of its 'rustic' characteristics were successfully transferred into

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the *O. sativa* background by Africa Rice Center (AfricaRice) scientists during the 1990s to develop the set of varieties referred to as NERICA (Jones *et al.*, 1997; Futakuchi and Sié, 2009). Given its small genome, rice was considered as a model species among monocotyledons during the 1990s. Important molecular and genomic tools were developed much earlier for rice than for other crops, and facilitated the identification of molecular markers associated with traits of interest. Jena and Mackill (2008) counted 1488 genes with known chromosomal position in rice databases (www.gramene.org/), including about 100 genes for resistance to biotic stresses. For the most important of these genes, closely linked or allele-specific markers are available. In addition, simple sequence repeat (SSR) markers evenly distributed over the rice genome are available and represent highly efficient tools for MAS (Orjuela *et al.*, 2010; Narshimulu *et al.*, 2011).

So far, MAS has been used in rice mainly to introgress the favourable alleles of genes involved in resistance to diseases and pests. In India and the Philippines, bacterial blight (BB) resistance alleles of genes *Xa4*, *xa5*, *xa13* and *Xa21* have been successfully introgressed into hybrid rice KMR3, PRR78, IR58025B and Pusa 6B (Shanti *et al.*, 2010). Different combinations of favourable alleles of either two or three resistance genes have also been incorporated into elite varieties using MAS, providing high level of resistance to ten highly virulent bacterial isolates (Singh *et al.*, 2001; Leung *et al.*, 2004; Agarcio *et al.*, 2007; Borines *et al.*, 2008; Collard and Mackill, 2008; Perez *et al.*, 2008; Sundaram *et al.*, 2008, 2009; Vera Cruz *et al.*, 2009). Likewise, Chinese elite restorer lines Minghui 63, Shanyou 63 and 6078 were improved for BB resistance using *Xa21* gene (Chen *et al.*, 2000, 2001), while two Indonesian cultivars 'Angke' and 'Conde', containing *Xa4*, were improved using the resistance gene *xa5*. The two BB-resistance genes, *xa13* and *Xa21*, present in IRBB55 were combined with the Basmati quality traits of Pusa Basmati-1 (PB-1), the most popular high-yielding Basmati rice variety (Joseph *et al.*, 2004). Unlike BB, blast and rice gall midge have received less attention with respect to markers and MAS. However, two blast-resistance genes, *Pi-1* and *Pi-2*, were successfully pyramided in the susceptible genotype CO39 and popular rice varieties IR36, IR64, IR72 and Jaya. Meanwhile, *Pi-1*, *Piz-5* and *Pita* were combined into a single genotype (Hittalmani *et al.*, 2000). Chinese varieties Digu, BL-1 and Pi-4, which carry resistance genes *Pi-d(t)*, *Pib* and *Pita2*, respectively, were crossed with G46B to pyramid all these genes in the same background (Chen *et al.*, 2004). Katiyar *et al.* (2001) successfully pyramided two Asian gall-midge-resistance genes *Gm2* and *Gm6t*, using two complementary donors. Kumaravadivel *et al.* (2006) report similar work targeting genes *Gm1* and *Gm4*.

In this chapter, we report on the application of molecular tools for the improvement of resistance to *Rice yellow mottle virus* (RYMV), which is endemic to Africa and can cause up to 80% yield loss in some rice-cropping systems. We specifically focus on the first major gene identified, *RYMV1*.

Rice yellow mottle virus

RYMV was first reported in Kenya (Bakker, 1970), and since the early 1990s, it has been reported in all rice-growing regions of Africa, including Madagascar (Kouassi *et al.*, 2005). The main symptoms are yellowing and mottling, with susceptible varieties showing stunting and sterility (Plate 4). The disease was first observed in lowland rice agro-ecosystems, but it has subsequently been reported in upland agro-ecosystems (Awoderu *et al.*, 1987). Yield losses in affected rice fields ranged from 64% to 100% in Mali (Sy *et al.*, 1994), up to 82% in Sierra Leone (Taylor *et al.*, 1990) and up to 71% in Niger (Issaka *et al.*, 2012). In Central Africa, the virus has been reported in Cameroon and Chad (Traoré *et al.*, 2001); in East Africa, its centre of origin, the disease has been detected in Kenya, Malawi, Rwanda and Tanzania (Thottappilly, 1992; Banwo, 2003; Ndikumana *et al.*, 2011). Madagascar has also been seriously affected, to the point where some farmers have abandoned their fields (Reckhaus and Randrianangaly, 1990).

RYMV is a *Sobemovirus* and its virions are icosahedral particles. The genome is composed of a single-stranded, positive-sense RNA, of about 4451±1 nucleotides (depending on the strain), and contains four open reading frames (ORF1, ORF2a&b, ORF3 and ORF4) (Kouassi *et al.*, 2005). The P1 protein coded by ORF1 is involved in the plant-infection process, virus spread in the plant

(Bonneau *et al.*, 1998) and has been described as a suppressor of virus-induced gene-silencing (VIGS; Voinnet *et al.*, 1999). The polyprotein encoded by ORF2a and ORF2b contains a protease, the genome-linked viral protein (VPg) and an RNA-dependent RNA polymerase, which are involved in virus replication. The coat-protein encoded by ORF3 is implicated in virus encapsidation, cell-to-cell and long-distance movement, and systemic infection (Brugidou *et al.*, 1995).

Both cultivated rice species, *O. glaberrima* and *O. sativa*, can be infected by RYMV. Additionally, the African wild rice species, *O. longistaminata* and *O. barthii*, and several wild grasses (e.g. *Echinochloa colona, Panicum repens, Erasgrotis tenuifilia* and *Dinebra retroflexa*) are hosts of the virus (Bakker, 1971; Konate *et al.*, 1997). Mechanical inoculation is very efficient for transmitting the virus in the laboratory and must also play a role in the field; for example, virus dispersal is facilitated by the wind through contact between plant leaves (Sarra *et al.*, 2004) and by farmers and farming tools during transplanting operations (Abo and Sy, 1998; Abo *et al.*, 2000). Beetles belonging to the family Chrysomelidea can transmit the virus 1–8 days after feeding on an infected plant (Bakker, 1971; Hibino, 1996; Abo and Sy, 1998). Cattle and rodents are also reported as occasional secondary vectors of the virus (Sarra and Peters, 2003). Finally, although RYMV has been detected at high rates (65–100%) in all parts of the seed in several rice genotypes, there is no evidence of seed transmission (Konate *et al.*, 2001). However, rice seedbeds have been identified as primary sources of RYMV infections (N'Guessan *et al.*, 2000; Traoré *et al.*, 2006). Therefore, good cultural practices – including weed elimination, early control of insect populations by chemicals if necessary, as well as adoption of direct seeding or appropriate management of rice seedbeds – are seen as efficient complementary means of reducing the impact of RYMV in rice fields (see also Séré *et al.*, Chapter 17, this volume).

Rice Resistance to RYMV

Studies on resistance to RYMV generally distinguish two types of resistance: partial and high resistance. Partial resistance is expressed as a delay in virus accumulation and symptom expression (Ioannidou *et al.*, 2000). Over time, virus content eventually reaches the same concentration as in susceptible varieties, but plant growth and development are generally much less affected in partially resistant accessions (Albar *et al.*, 1998; Zouzou *et al.*, 2008). High resistance is characterized by the absence of symptoms, irrespective of the rice development stage at inoculation time (Ndjiondjop *et al.*, 1999). The virus is only detected erratically using enzyme-linked immunosorbent assay (ELISA) serological detection method. Viral RNA concentration, estimated by real-time PCR, is approximately 10^6 times lower in resistant plants compared to susceptible ones (Poulicard *et al.*, 2010). High resistance is associated with the failure of virus cell-to-cell movement (Ndjiondjop *et al.*, 2001).

Partial resistance to RYMV and its use in breeding

The delay of symptom development depends greatly on the virus isolate and on the rice variety. Under controlled conditions, using 2-week-old seedlings infected mechanically, the development of highly susceptible lines stops 2–3 weeks after inoculation and plants may die. In field evaluation, using later inoculation (50 days), symptom progression is less severe, but affects more or less severely plant height, time to flowering, panicle exsertion and spikelet sterility (Awoderu, 1991).

Partial resistance is widely distributed in varieties belonging to the tropical *japonica* group of *O. sativa*, such as Azucena or Moroberekan, which are grown in upland conditions. It has not been reported in *indica* varieties cultivated in lowland conditions. Quantitative trait loci (QTLs) analysis using virus accumulation and symptom-expression data, pinpointed seven chromosomic regions involved in the control of partial resistance. Three of them, located on chromosomes 1, 2 and 12, were consistent across environments and responsible for a major part of the phenotypic variations (Albar *et al.*, 1998; Boisnard *et al.*, 2007). From the late 1980s, the International Institute of Tropical Agriculture (IITA, Nigeria) and AfricaRice crossed partially resistant upland varieties with high-yielding susceptible lowland

varieties (ITA212, ITA22, ITA304, ITA230 and ITA306) to improve RYMV resistance (IITA, 1986), but limited positive results were obtained. This is probably related to the strong genetic relationship between plant morphology and resistance (Albar *et al.*, 1998), impairing (to some extent) the full use of partial resistance in breeding for lowland conditions. Partial resistance to RYMV is also present in some *O. glaberrima* accessions with no direct relationship with plant morphology (Thiemélé *et al.*, 2010). This provides a better model to elucidate the genetic basis of partial resistance to RYMV.

High resistance to RYMV, its diversity and genetic basis

High resistance was first described in *O. sativa* accession Gigante originating from East Africa (Ndjiondjop *et al.*, 1999). The genetic basis of resistance in Gigante was identified in the late 1990s: it is recessive and under the control of a single gene, named *RYMV1*. *RYMV1* was mapped on the long arm of chromosome 4 thanks to closely linked SSR markers providing tools for further MAS (Ndjiondjop, 1999; Albar *et al.*, 2003). Positional cloning of *RYMV1* revealed that the gene encodes the translation-initiation factor eIF(iso)4G (Albar *et al.*, 2006) and the resistance is thus associated with a translation-initiation complex, like many other recessive resistances against plant viruses (Robaglia and Caranta, 2006). The resistance allele, *rymv1-2*, is characterized by a single nucleotide polymorphism (SNP), leading to an amino-acid substitution in the conserved central domain of the protein (Table 12.1).

Only one other *O. sativa* accession has been reported that harbours the *rymv1-2* allele: Bekarosaka from Madagascar (Rakotomalala *et al.*, 2008). Conversely, a much larger number of highly resistant *O. glaberrima* accessions have been reported (Thottappilly and Rossel, 1993; Albar *et al.*, 2006; Thiémélé *et al.*, 2010). The survey of a collection of 337 accessions representative of the diversity of *O. glaberrima* identified 29 highly resistant accessions. Analysis of *RYMV1* diversity among these accessions revealed that they did not bear the *rymv1-2* resistance allele. The resistance of 14 of these accessions was due to three new alleles at the *RYMV1* locus, while the remaining 15 accessions did not show any difference from the susceptibility allele, *Rymv1-1*.

The resistance allele *rymv1-3*, found in Tog5681, was characterized by a deletion of three amino acids; the *rymv1-4* allele, found in Tog5672, resulted from one SNP leading to an amino-acid substitution different from that of *rymv1-2*; the *rymv1-5* allele, in Tog5674, presented an amino-acid substitution and a three-amino-acid deletion. The *rymv1-3* and *rymv1-4* alleles were the most frequent with 9 and 4 accessions, respectively, while *rymv1-5* was found in only one accession. The four mutations conferring resistance occurred in the same domain of the eIF(iso)4G factor. Functional analyses involving *rymv1-2* strongly suggested that the mutations impair the interaction between this eIF(iso)4G factor and the VPg of RYMV, a viral protein linked to the 5'-end of the viral RNA, and thus impair the infection cycle of

Table 12.1. Variability of the *RYMV1* gene: alignment of the 299–339 region of the *RYMV1* product (part of the conserved MIF4G domain) from susceptible and resistant *O. sativa* and *O. glaberrima* accessions. (From Thiemélé *et al.*, 2010, with kind permission from Springer Science and Business Media.)

Allele	Phenotype[a]	Species	Amino-acid sequence[b]
Rymv1-1	S	*O. sativa*	AFEGAESLRAEIAKLTGPDQEMERRDKERIVKLRTLGNIRL
rymv1-2	R	*O. sativa*	----------K----------
Rymv1-1	R/S	*O. glaberrima*	------D----------
rymv1-3	R	*O. glaberrima*	------D----------***----------
rymv1-4	R	*O. glaberrima*	------D----------K----------
rymv1-5	R	*O. glaberrima*	------D----------N***----------

[a]S and R refer to susceptible and resistant phenotypes, respectively; R/S indicates the presence of the variant in both susceptible and resistant accessions.

[b]Only amino acids that are different from the first sequence are reported. Deletions are represented by '*'.

RYMV (Hébrard *et al.*, 2010). A set of allele-specific molecular markers was developed to help MAS (Thiémélé *et al.*, 2010).

The detection of 15 highly resistant accessions that did not show any difference from the susceptibility allele *Rymv1-1* in the central conserved domain of *RYMV1* suggested the existence of a different genetic control of their resistance, especially as six of those accessions did not differ from the susceptibility allele in the whole *RYMV1* sequence. Among these accessions, Tog7291 revealed a second recessive resistance gene, *RYMV2*, recently mapped on the long arm of chromosome 1. Characterization of candidate genes is under way and should allow the identification of the gene (unpublished results). Preliminary results also suggest that a third resistance may control the high resistance of some other *O. glaberrima* accessions.

Resistance and Resistance Breakdown

One of the main problems encountered by breeders in the use of major resistance genes is the ability of pathogens to rapidly evolve and overcome such resistance (Lecoq *et al.*, 2004). Resistance-breakdown frequency is dependent on characteristics of the pathogen (including its mutation rate), the nature of resistance genes, and the number and nature of mutations necessary for virulence acquisition, which may vary depending on the virus isolates.

The breakdown of *RYMV1*-mediated resistance and its molecular mechanisms have been studied by comparing the viral sequences from plants showing generalized symptoms and/or high virus content with the sequences of the same isolate propagated on susceptible lines. Both *rymv1-2* and *rymv1-3* have been overcome experimentally by RYMV isolates (Hébrard *et al.*, 2006; Traoré *et al.*, 2006). Resistance breakdown generally involves mutations in the viral protein genome-linked VPg. Such mutations restore the interaction between the eIF(iso)4G factor and VPg (Hébrard *et al.*, 2010). Most *rymv1-2* resistance-breakdown mutations have occurred on amino-acid 48 of the VPg (Pinel-Galzi *et al.*, 2007), while the *rymv1-3* allele was mostly overcome by mutations occurring at positions 41 and/or 52 of the VPg (Traoré *et al.*, 2010). RYMV strains have contrasting abilities to overcome *rymv1-2* and *rymv1-3* resistance alleles according to the presence of a glutamic acid (E) / threonine (T) at codon 49 of the VPg. Isolates with an E at position 49 (E pathotype) of the VPg are only able to break the *rymv1-2* allele-mediated resistance, while isolates with a T at position 49 (T pathotype) overcome with high frequency the *rymv1-3* allele (Poulicard *et al.*, 2012) and at low frequency the *rymv1-2* allele using specific mutational pathway (Traoré *et al.*, 2010). No direct resistance-breaking was observed in using natural isolates collected under virulent conditions (i.e. on susceptible varieties). This may be explained by the fact that resistance genes are not yet deployed on a large enough scale to give sufficient selective value to resistance-breaking viral strains. It could be also a consequence of possible fitness losses of virulent mutants compared to avirulent ones under natural conditions. Competition experiments using co-inoculations of E and T pathotypes under virulent conditions showed a much better adaptation of T pathotype on *O. glaberrima* accessions compared to *O. sativa* (Poulicard *et al.*, 2010).

Introgression of *rymv1-2* Resistance Allele into Elite Cultivars of West Africa

Four varieties (IR64, FKR28, Sahelika and IR47686), widely grown in West Africa but susceptible to RYMV, were selected as recipient parents of the resistance allele *rymv1-2*, the donor parent being Gigante. IR64 is a popular and improved *indica* variety from the Philippines, FKR28 and Sahelika are popular and improved *indica* varieties from Burkina Faso and Mali. IR47686 is a *japonica* variety showing partial resistance to RYMV, and therefore a good candidate for the introgression of the major resistance gene with the final goal of durable resistance to RYMV. These varieties were recommended by the national agricultural research systems (NARS) of Mali, Burkina Faso, Guinea and The Gambia, respectively. The marker RM252 (mapping 1.8 cM downstream from *RYMV1* and polymorphic among the donor and recipient parents) was selected for marker-assisted introgression (Fig. 12.1).

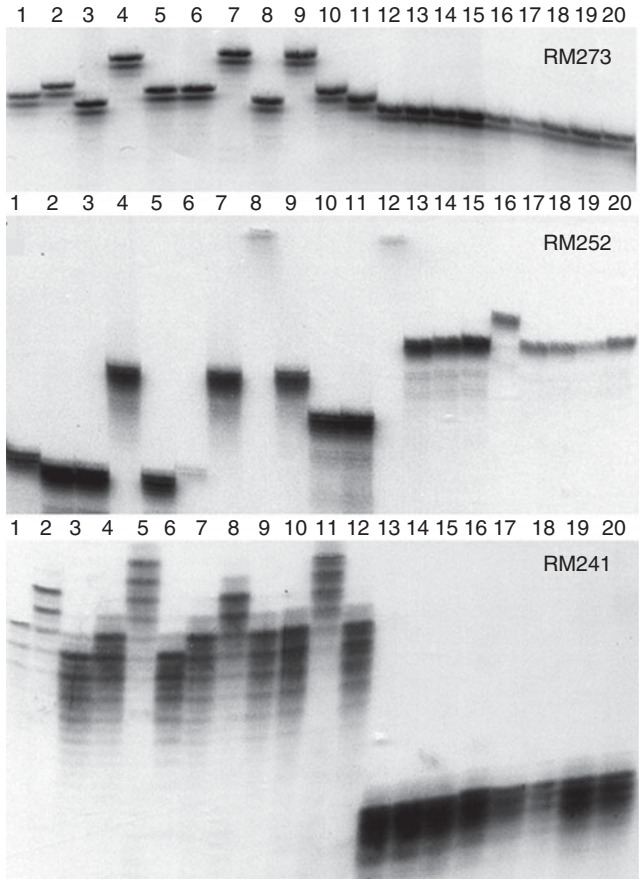

Fig. 12.1. Genetic diversity observed at three SSR loci spanning the *RYMV1* resistance gene in different varieties of rice (*O. sativa* and *O. glaberrima*). *Oryza sativa* subsp. *japonica* (1–6): 1, Haw Mom; 2, Cana Roxa; 3, Carolina Gold; 4, Pate Blanc; 5, Azucena; 6, Nipponbare. *Oryza sativa* subsp. *indica* (7–12): 7, IR64; 8, Gigante; 9, IR5; 10, MTU9; 11, ASD1; 12, Carreon. *Oryza glaberrima* (13–20): 13, Tog5681; 14, Tog5672; 15, Tog5673; 16, IG10; 17, SG329; 18, CG14; 19, CG17; 20, CG20. Resistance alleles of *RYMV1* are present in accessions Gigante, Tog5681 and Tog5672. (From Albar *et al.*, 2003, with kind permission from Springer Science and Business Media.)

DNA extraction and polymerase chain reaction (PCR) were carried out at AfricaRice, Cotonou (Benin), using the protocol described by Ikeda *et al.* (2001) for rice SSR. Amplified products were separated by electrophoresis in 5% polyacrylamide gels using a SequiGEN 38 × 50 cm gel apparatus (BioRad Laboratories) and the banding patterns were visualized using silver staining.

F_1 hybrids obtained by crossing Gigante (RYMV-resistant donor parent) with the four RYMV-susceptible elite lines (recurrent parent) were used to develop BC_1F_1 populations. The BC_1F_1 plants were screened for the presence/absence of the *RYMV1* resistance allele using RM252. Thirty BC_1F_1 plants heterozygous for RM252 were obtained from the four crosses: 3 for IR64/Gigante//IR64, 6 for Sahelika/Gigante//Sahelika, 8 for IR47/Gigante//IR47 and 13 for FKR28/Gigante//FKR28. Twenty-five of those BC_1F_1 plants were used to continue the backcrossing procedure (Table 12.2). BC_2F_1 plants were screened for heterozygous status on RM252 and 35 BC_2F_1 were selected and backcrossed to produce the BC_3F_1. The BC_3F_1 plants

Table 12.2. Workflow summary of the plant material developed for creating RYMV-resistant isolines by marker-assisted selection (no. plants derived at each generation).

		Foreground (RM252)	Foreground (RM252)	Foreground (RM252–RM241–RM73) and background	Foreground + phenotypic	Phenotypic
Recurrent parent	Selection generation	BC_1	BC_2	BC_3	BC_3F_2	BC_3F_5 (NILs)
FKR28		9	4	45	3	5
IR47		8	12	4	5	9
IR64		2	10	80	7	12
Sahelika		6	9	10	3	4
Total		25	35	139	18	30

were screened for RM252. Next, foreground screening was completed with two additional SSR markers – RM241 and RM273 located on opposite sides of RM252 and RYMV1 (Fig. 12.1) for the combinations involving IR64, Sahelika and FKR28; and RM255 and RM451 for the combination of Gigante and IR47. This step confirmed the efficiency of the selection on RM252 and avoided the selection of plants that may have recombined between RM252 and *RYMV1*. One hundred and thirty-nine BC_3F_1 plants heterozygous for the three markers surrounding *RYMV1* were selected for background selection. The return to recurrent parent in genome regions other than the *RYMV1* locus, was surveyed using 69–80 SSR markers well distributed among the chromosomes. The average number of markers per non-carrier chromosome ranged from 5 to 7, depending on the cross combination. Four SSR markers were used for the carrier chromosome. Finally, 24 BC_3F_1 individuals were selected with more than 94% return to the recurrent parent. From these individuals, BC_3F_2 plants were developed and 18 of these, bearing the homozygous *rymv1-2* allele (checked using RM252), were selected. At this stage, selection was also completed by visual observation to keep the individuals as close as possible to the recipient parent. These lines were advanced to F_3 (Plate 5). After a final round of phenotypic selection, 30 BC_3F_3 lines homozygous for *rymv1-2* were selected and used to develop BC_3F_5 lines.

The 30 BC_3F_5 lines were checked for the effectiveness of high resistance to RYMV under controlled conditions at AfricaRice, Cotonou, using artificial infection with virus isolate B27 (S1 strain). Resistance to RYMV was visually scored as described by Konate *et al.* (1997) and virus content was measured by ELISA as described by Sere *et al.* (2007). All BC_3F_5 lines displayed resistant phenotype, similar to that of the donor Gigante (Table 12.3), whereas IR64 and Sahelika were susceptible and IR47 and FKR28 showed intermediate phenotype. The BC_3F_5 lines were also evaluated in field trials, in Guinea and Mali, for general adaptability traits (plant height, phenology, grain weight) and resistance to RYMV. In Guinea, the evaluation was conducted under natural infection in a field often infected by RYMV. In Mali, only 12 IR64 BC_3F_5 lines were evaluated: 2-week-old seedlings of lines and the two parents were first inoculated artificially with a local virus strain, and then transplanted into an often-infected field. For each trial (laid in a randomized complete block design with four replications), a local variety was added as control. In both trials, the BC_3F_5 lines did not show any visual symptoms of RYMV disease. Five BC_3F_5 lines were discarded because of poor agronomic performance. The selected BC_3F_5 lines also showed resistance to RYMV under controlled conditions in Burkina Faso, Côte d'Ivoire and under initial field testing in other countries, including Côte d'Ivoire, Ghana, Nigeria and Sierra Leone.

Conclusion and Perspectives

The fine-mapping and positional cloning of the gene *RYMV1* in the *O. sativa* variety Gigante

Table 12.3. Evaluation of BC_3F_5 progenies for resistance to RYMV.

		Greenhouse evaluation (Benin)	
Near-isogenic line (NIL)/Parental line	Recurrent parent	OD ELISA[a]	Visual symptom
NIL2	FRK28	0.01937 a	R
NIL4	FRK28	0.02661 a	R
NIL5	FRK28	0.02956 a	R
NIL16	Sahelika	0.04689 a	R
NIL36	IR47686	0.01628 a	R
NIL46	IR47686	0.034 a	R
NIL48	IR47686	0.03433 a	R
NIL49	IR47686	0.02772 a	R
NIL54	IR47686	0.03667 a	R
NIL56	IR47686	0.03314 a	R
NIL58	IR47686	0.03589 a	R
NIL59	IR47686	0.022 a	R
NIL127	IR64	0.02022 a	R
NIL129	IR64	0.01633 a	R
NIL130	IR64	0.02872 a	R
NIL132	IR64	0.02367 a	R
NIL133	IR64	0.03467 a	R
NIL135	IR64	0.03544 a	R
NIL145	IR64	0.03433 a	R
NIL147	IR64	0.03489 a	R
NIL157	IR64	0.03422 a	R
Sahelika	Recurrent parent	0.10133b	S
FKR28	Recurrent parent	0.03689 a	PR
IR47	Recurrent parent	0.02678 a	PR
IR64	Recurrent parent	0.09867 b	S
Gigante	Donor	0.008 a	R

[a]OD ELISA, Optical density after enzyme-linked immunosorbent assay; OD values followed by same letter are not significantly different at 5% (Tukey's test); PR, partially resistant; R, resistant; S, susceptible.

have facilitated the introgression of the resistance allele, *rymv1-2*, into four elite cultivars from AfricaRice's NARS partners. Thirty rice lines bearing the *rymv1-2* allele were successfully obtained through MAS and distributed to NARS breeders. The transfer of the high-resistance allele and its functionality (i.e. absence of symptoms or virus in the leaves) in the new genetic backgrounds was confirmed through phenotypic evaluation after inoculation. These lines will be put through Africa-wide Rice Breeding Task Force multi-location evaluation, testing the durability of resistance in various geographical areas (see Kumashiro *et al.*, Chapter 5, this volume).

As *O. glaberrima* shows a greater diversity of *RYMV1* alleles, a similar process of resistance-transferring can be undertaken to diversify the resistance alleles according to the distribution of E and T pathotypes of the virus. For instance, deployment of resistant lines carrying *rymv1-3* is expected to be durable in East Africa, since the E pathotype (the only pathotype present in this region) is unable to break *rymv1-3* resistance. A second resistance gene (*RYMV2*) to RYMV was found in *O. glaberrima* and the existence of a third gene is suspected in the same species. Confirmation of the existence of these genes will pave the way for pyramiding different resistance genes and combining different resistance alleles, to improve the durability of resistance.

AfricaRice's strategy is to build upon the new RYMV-resistant lines by pyramiding with well-characterized and validated resistance genes for other constraints (biotic and abiotic) prevalent in African farmers' fields. The development, by Institut de recherche pour le développement (IRD) and AfricaRice, of fertile interspecific bridge lines bearing favourable genes from *O. glaberrima*

associated with useful traits will enhance the sustainability of this approach and its extension to other rice cultivars. AfricaRice also deems that capacity-building for MAS and more generally for modern plant breeding is of great importance. The Center is planning to establish a genomic platform to allow systematic use of molecular markers in breeding for a wide range of traits. New horizons are also emerging with the availability of the sequence of the *O. glaberrima* genome.

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