Resistance of selected Oryza glaberrima landraces and their intra-specific breeding lines to Beninese Rice yellow mottle virus isolates

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**A R T I C L E   I N F O**

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O. sativa
Landraces
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Resistance/susceptibility to RYMV

**A B S T R A C T**

*Rice yellow mottle virus* (RYMV) is a widespread and damaging pathogen, endemic to Africa and affecting rice in lowland, irrigated and upland ecologies. Yield losses of 10–100% have been recorded, depending on rice genotypes, time of infection, cropping system, vector abundance and isolate pathogenicity. Control measures for this disease are based mainly on cultural practices and the use of varietal resistance. Specifically, *Oryza glaberrima*, the cultivated African rice species, has provided several of the known resistance alleles/genes. In this study, 160 *O. glaberrima* landraces, 55 of their intra-specific progenies and two controls - Tog5681 (resistant) and IR64 (susceptible) - were screened against five RYMV isolates collected from Northern Benin. Disease severity was measured on plants at 21 and 42 days after inoculation. Two cultivars - IRGC104019 and IRGC96787 (Tog5644) - from Tanzania and Nigeria respectively - and 16 intra-specific lines were identified as highly resistant to all five isolates. The genotyping of IRGC104019 and IRGC96787 revealed that they share allele *rymv1*-3, identified in Tog5681 (the resistant control) and that confers resistance, bringing the number of *O. glaberrima* accessions known to date to harbor this allele to 11. The importance of this finding and the level of resistance displayed by these two accessions strengthen the hypothesis that the African rice collection is a potential RYMV resistance donor. It is, therefore, necessary to further screen it for valorizing its usefulness in breeding programs.

**1. Introduction**

Despite efforts at increasing production, rice yields in Africa range from 1 to 3 t ha⁻¹ and remain far below the potentials (Traoré et al., 2006). Current production cannot meet the demand of the growing population. Several factors militate against optimum rice production in most African rice-growing countries, including the susceptibility of cultivars to diseases. *Rice yellow mottle virus* (RYMV) is a major constraint in several countries (Séré et al., 2013). The disease is endemic to and widespread the African continent, causes severe damage to rice plants (Abo et al., 1998), reduces grain yield by 10–100%, and severe attacks can lead to plant death (Calvert et al., 2003; Kouassi et al., 2005). The disease has been widely observed in lowland, irrigated and upland ecologies in most rice-producing countries.

RYMV belongs to the Sobemovirus genus, that includes the type species of the *Southern bean mosaic virus* which infects both dicotyledonous and monocotyledonous plants (ICTV, 2018). Symptoms of RYMV include yellowing, mottling, necrosis, stunting of rice plants, incomplete emergence of panicles, and plant death. In general, RYMV is highly infectious and stable in different environments. It also infects all wild rice species (*Oryza* spp.) particularly the perennial wild species *O. longistaminata* as well as several weeds that serve as inoculum reservoir in the Soudano-Sahelian zone of West Africa where it is endemic.

For a long time, breeders had access to only one resistance gene (RYMV1) that has four known resistance alleles which confer a high resistance level - *rymv1*-2 available in Asian cultivated rice species, *O. sativa*, while *rymv1*-3, *rymv1*-4 and *rymv1*-5 are available in African cultivated rice species, *O. glaberrima* (Ndjiondjop et al., 1999; Albar et al., 2006; Thiemele et al., 2010). A few years ago, *RYMV2* was discovered in Tog7291, an *O. glaberrima* accession (Thiemele et al., 2010) and a third resistance gene, *RYMV3* was recently found in Tog5307 (Pidon et al., 2017).

Previous molecular analyses showed that the two resistance alleles of the *RYMV1* gene (i.e *rymv1*-3 carried by Tog5681 and *rymv1*-4 carried by Tog5672) are characterized by a short deletion (RRD322-324) and substitution (E321K) of amino acids in the same central region of the MIF4G
domain of the protein (Albar et al., 2006). The modeling of this domain shows that these mutations are very close and located on the surface of the protein. The second resistance gene (RYMV2) is governed by a recessive allele and codes for CPR5-1, a probable component of the nucleo- 

clear pore complex involved in the regulation of defense mechanisms (Orjuela et al., 2013). The third gene (RYMV3), with a NLR as a very 

convincing candidate gene, is probably involved in the virus recognition 

mechanism (Pidon et al., 2017).

Specific molecular markers for the different alleles of RYMV1 gene were 

developed for accelerating the cultivars’ resistance improvement using 

Marker-Assisted Selection (MAS). These markers were used to introgress 

rymv1-2 into popular RYMV-susceptible elite varieties, such as 

Bouake 189, a very popular cultivar in Côte d’Ivoire which subsequently 

became resistant to the virus (Bouet et al., 2013). However, the virus 

is able to evolve and some isolates are known to have overcome resistance 

(Fargette et al., 2002; Hebrard et al., 2006, 2018; Pinel-Galzi et al., 

2016), thus making the identification of new resistance sources and 

genes critical in programs for sustainable resistance breeding. As 

O. glaberrima species appeared to be an interesting source of resistance to 

RYMV (Thiéméle et al., 2010), screening its landraces for resistance is 

continuously being conducted at AfricaRice and this study reports on the 

screening of 160 individuals and 55 intra-specific lines. In addition, 

resistance durability may be increased by combining different resistance 

genes/alleles in a single genetic background. Intra-specific lines were 

thus developed as suggested by Ghessièrê et al. (1997) through crosses 

between the resistant accessions Tog5681, Tog5672 and Tog7291 in 

order to develop varieties with improved high yields and RYMV resis- 
tance as these varieties would combine several of these genes/alleles. 

Fifty-five of these derived progenies were selected based on their 

agro-morphological characters (Agnoun et al., 2012) and evaluated for 

their resistance to the virus.

2. Materials and methods

2.1. Plant materials and virus strains

The study evaluated 160 rice accessions from an O. glaberrima core 
sample of AfricaRice’s GeneBank. These materials cover the wide 

phenotypic and eco-geographic diversity of an O. glaberrima collection 

originating from 20 African countries. Also screened were 55 intra-

specific O. glaberrima lines (F7 generation), developed from two 

different crosses, 11 from the cross Tog5681/Tog5672 and 44 from 

Tog5681/Tog7291. All three parental lines of these crosses are resistant 

to the disease. Indeed, Tog5681 carries rymv1-3, the most widespread 

allele (Albar et al., 2006) while Tog7291 carries RYMV2 gene (Thiémele 
et al., 2010). Tog5672 harbors rymv1-4 and is suspected to also carry 

RYMV3 (Thiémele et al., 2010; Pidon et al., 2017). This means that 

three resistance genes or alleles will potentially segregate in those derived 

progenies. IR64 (susceptible O. sativa) and Tog5681 (resistant O. 
glaberrima) served as controls. Plant materials were screened using five 

RYMV isolates - RBBe24 and RBBe25 from Malanville (N1’52.279’ 

E003 23.112), RBe73, RBe76, and RBe77 from Tangueta (N10’39.315’ 

E001 15.033). Both localities are in the north of Benin and are very close 
to the border with Niger. All these isolates were diagnosed as belonging to 

the S1 serotype based on Triple Antibody Sandwich (TAS) ELISA with 

monoclonal antibodies (Oludare et al., 2016). To this end, micro-titre 

plates were coated with polyclonal antibody anti-RYMV. Thereafter, 
saps from leaves ground in PBST x 1 buffer (pH 7.2 (1/10 w/v ratio) were 

used as antigen. Six monoclonal antibodies (Mabs) A, B, D, E, G and M 

were used as secondary antibodies and bound Mabs were detected with 
goat anti-mouse globulin/alkaline phosphate conjugate. After incubating 

the samples with p-nitrophenyl phosphate for 1 and then 3 h at room 
temperature or after overnight incubation at 4 °C, absorbance at 405 nm 

was recorded using a spectrophotometer.

2.2. Establishment of the experimental trials

The experiment was established in 2012 in the experimental screen- 

house of the AfricaRice research station at Abomey-Calavi, Benin. Rice 

plants (one plant/pot) were grown in 1 L pots filled with soil collected on 

the station. The pots were arranged in a randomized complete 
admixed block design and each block, replicated three times for each 

of the five RYMV isolates, comprised 20 O. glaberrima genotypes and the 
two controls (Tog5681 and IR64). Fertilizers were applied at the rate of 

1 g of NPK/pot at sowing and 0.2 g of urea/pot one month later. Soil 

moisture was frequently checked and hand weeding was carried out 

throughout the experiment.

In a second experiment, only the selected resistant genotypes iden- 
tified in the first experiment were again tested to confirm their resistance 

status. Ten pots each containing 5 plants (total of 50 plants for each 

resistant accession) were sown and tested as described below. For each 

accession, a water control set consisting of the same number of plants was 

also tested.

Fully developed leaves of IR64 infected with each of the five isolates and 

conserved at −20 °C were used to infect new sets of IR64 plants to get 

fresh inoculum. Plant leaves that developed visible and severe symptoms 

were collected and ground in phosphate buffer (0.01 M pH 7.0) at a ratio 
of 1:10 (w/v). The virus content of the inocula was checked with the ACP 

ELISA test as described by Afolabi et al. (2009). Thereafter, 0.5 g 
carborundum (600-mesh) was added to the solution and the resulting 
mixture was used to mechanically inoculate rice plants by gently rubbing 
it on leaves (2–3 leaves/plant) of 21-day old plants.

2.3. Data collection

The Standard Evaluation System (IRRI, 2002) was used to score disease 

symptoms at 21 and 42 DAI. Disease scoring was done by using a scale 
(Table 1) in which the rating “0” was assigned to plants with no 

visible symptoms and “9” to those that showed full infection. Ratings 1, 

3, 5, and 7 correspond to the other plant infection levels. The average 

disease severity per plant was then calculated as follows:

\[
\text{Disease Severity (DS) = } \left( \frac{(n1*1) + (n3*3) + (n5*5) + (n7*7) + (n9*9)}{100} \right) \text{ where, n1, n3, n5, n7 and n9 represent the number of leaves/plant scored 1, 3, 5 and 7 respectively.}
\]

A mean disease severity for each accession was then calculated by 
averaging the means of all plants assessed.

2.4. Statistical analysis

Data were analyzed with Excel and JMP Pro12.01 software. The av- 

erages of the three replicated plants/isolate were subjected to ANOVA 
test for means comparison using Newman-Keuls test at 5% level of 

probability for all isolates.

Table 1

<table>
<thead>
<tr>
<th>Scale</th>
<th>Symptoms</th>
<th>Code</th>
<th>Nature of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Leaves green with no symptom observed</td>
<td>HR</td>
<td>Immune</td>
</tr>
<tr>
<td>1</td>
<td>Leaves pale or green with no symptom observed</td>
<td>HR</td>
<td>Highly resistant</td>
</tr>
<tr>
<td>2</td>
<td>Leaves greens with sparse dots or streaks</td>
<td>R</td>
<td>Resistant</td>
</tr>
<tr>
<td>3</td>
<td>Leaves pale or green with mottling</td>
<td>MR</td>
<td>Moderate resistant</td>
</tr>
<tr>
<td>4</td>
<td>Leaves pale yellow or yellow with mottling</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>5</td>
<td>Leaves turned yellow or orange with often plant dead</td>
<td>HS</td>
<td>Highly susceptible</td>
</tr>
</tbody>
</table>
2.5. Genotyping

Genotyping of highly resistant accessions for RYMV1 and RYMV2 resistance genes was conducted as follows: PCR amplification was performed on 1 mm-diameter discs of dried leaves (with two discs/10 μl reaction) using the Terra PCR Direct Polymerase Mix (Clontech) as described in Orjuela et al. (2013). A CAPS marker was used to detect the mutation of the RYMV2 allele in Tog7291 as described in Orjuela et al. (2013). The central domain of RYMV1 gene, containing the mutations known to be involved in resistance, was amplified using the primers 5’-CCTTGGTCAGCTAGAAGAGGCA-3’ and 5’-CCTCGGTACAACCAAGAAGC-3’ and the amplification product was sent to Beckman Coulter Genomics for sequencing with the primer 5’-CTCTTCAAGTCGGACA CCA-3’.

3. Results

3.1. Severity of RYMV disease on O. glaberrima accessions and intraspecific lines infected with RYMV

As disease severity (DS) at both 21 and 42 DAI were highly correlated ($r = 0.8561$), only data for 42 DAI are presented because symptom development was maximum at this period and differences between isolates were best expressed.

The results confirmed the susceptible and resistant status of the controls, thus indicating that rice accessions could be properly screened for resistance to RYMV. DS on the susceptible control, IR64, ranged from 41.93 (isolate RBe76) to 100% (isolate RBe24) with the latter isolate being the most aggressive (Figs. 1 and 2). Conversely, DS on the resistant controls, thus indicating that rice accessions could be properly screened for resistance to RYMV. DS on the susceptible control, IR64, ranged from 41.93 (isolate RBe76) to 100% (isolate RBe24) with the latter isolate being the most aggressive (Figs. 1 and 2). Conversely, DS on the resistant

The highly resistant accessions IRGC104019 and IRGC96787 (Tog5644) were checked for the presence of the RYMV1 resistance alleles previously shown to be present in Gigante, Tog5681, Tog5672 and Tog5674 and the RYMV2 allele identified in Tog7291 (Albar et al., 2006; Thiémélé et al., 2010). None of the accessions had RYMV2. The central domain of the RYMV1 gene was sequenced in the new immune genotypes IRGC96787 and IRGC104019 and the sequences obtained were compared to the known alleles at this locus (Fig. 3). The accessions IRGC96787 and IRGC104019 showed the deletion that characterizes the rymv1-3 resistance allele in Tog5681, suggesting that the high resistance of these two accessions is conferred by this allele.

4. Discussion

Despite the eco-geographic diversity and the presence of two serogroups (S1 and S2) of RYMV in Benin, variability of RYMV population is quite low (Oludare et al., 2016). While both serotypes were found in all rice-growing areas, S1 serotype, to which the five isolates tested in this study belong, is the most frequent in Northern Benin where these isolates originate from. Isolates RBe24 and RBe25, collected from an irrigated scheme in Malanville, were more pathogenic than the other three (RBe73, RBe76 and RBe77) that originated from lowland ecologies
Table 2
Analysis of variance performed on the disease severity data collected at 21 and 42 DAL.

<table>
<thead>
<tr>
<th>Source</th>
<th>DAl</th>
<th>P-value 21 DAL</th>
<th>P-value 42 DAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates</td>
<td>4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Genotypes</td>
<td>117</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blocks</td>
<td>10</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Isolates * Genotypes</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Genotypes * Block</td>
<td>50</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Isolates * Genotypes * Block</td>
<td>40</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Legend: Highly significant (P < 0.001). ANOVA test performed on *O. glaberrima* accessions and intra-specific breeding lines.

**Fig. 2.** Distribution of intra-specific *O. glaberrima* lines derived from crosses between resistant lines (Tog5681, Tog5672 and Tog7291) and arranged by disease severity (DS) values measured for all five RYMV isolates at 42 DAL Legend: Five classes of DS were defined and range from 0 to 100. The position of the two controls (Tog5681 and IR64) were reported for each RYMV Isolate (RBe73, RBe76, RBe77, RBe24 and RBe25) according to their DS values.

in Tanguinéa. The higher pathogenicity of RBe24 and RBe25 (with RBe24 being more pathogenic than RBe25) might be due to intensive rice cultivation in Malanville. As this area is also very close to the Republic of Niger, which is a hotspot of the disease, migration into Benin of highly virulent isolates cannot be ruled out. The hypothesis that pathogenicity increases as one moves from Southern Benin to Niger was drawn and there is thus a need to verify this assumption by testing more isolates originating from this area. Indeed, previous studies (Reckhaus and Amadou, 1986; Séré et al., 2005) reported highly pathogenic isolates devastating rice production in Niger.

The current study tested an *O. glaberrima* collection representing the diversity of the species. The centers of origin of *O. glaberrima* are in West Africa. However, introductions and adoption of some accessions from within and outside the continent explain that some of the accessions used in this study do not originate from West Africa but, for instance, from Tanzania. The existing rice accessions in the genebank were collected at different times in several African countries. Collection was initiated in 1974 by the Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM) (now known as Institut de Recherche pour le Développement or IRD) with the aim of preserving the species’ genetic diversity (Besancon and Second, 1984).

The *O. glaberrima* landraces screened in this study exhibited great variability for resistance to the disease. Artificial inoculation resulted in greater infections than what is obtained naturally in the field. Most of the 160 *O. glaberrima* landraces tested were susceptible to all the five RYMV isolates and only three of them (1.9%) were resistant. The ACP ELISA Test (data not shown) revealed differences in the virus content of each isolate and zero or low virus content was detected beyond 42 DAL in the newly identified resistant accessions. This strengthens the hypothesis that *O. glaberrima* is a potential source for high resistance to the disease (Futauchi and Sie, 2009; Thiemélé et al., 2010). Moreover, two levels of RYMV resistance were reported by Ndjonjio et al. (2001) - high resistance which results in an absence of symptoms, and partial resistance which delays their onset. The variability of the response observed among the moderately resistant accessions confirms that *O. glaberrima* may also be an interesting source of partial resistance as previously suggested by Thiemélé et al. (2010).

Specifically, this study identified two novel high disease resistance sources, namely IRGC104019 from Tanzania and IRGC96787 (Tog5644) from Nigeria that resisted all five isolates. They did not exhibit any detectable symptoms and had features comparable to those of the resistant control Tog5681. The partial sequencing of the RYMV1 gene in the two novel resistance sources showed that they harbor the *rymv1-3* allele (Fig. 3), the most widespread *O. glaberrima* resistance allele of this gene (Albar et al., 2006; Thiemélé et al., 2010). RYMV1 is a recessive gene located on chromosome 4 and codes protein eIF(iso)4G, a factor of the host interacting with the virus (Hebrard et al., 2010).

IRGC96851, another genotype from Nigeria, was resistant to all isolates except RBe24 and did not express any visible symptom. Further investigations are needed to identify the genes/alleles involved in its resistance.

A second group of *O. glaberrima* material was developed with the objective of pyramiding known resistance genes/alleles in progenies - RYMV1 (alleles *rymv1-3* and *rymv1-4* carried, respectively, by Tog5681 and Tog5672); RYMV2 carried by Tog7291; and RYMV3 carried by Tog5672 (Albar et al., 2006; Thiemélé et al., 2010; Pidon et al., 2017). This material consisted of 55 lines that had already been selected by breeders for interesting agronomic characteristics. Among this material,
16 genotypes (29.1%) exhibited high resistance to all five isolates tested with no visible symptoms developed. Seven of them were derived from the cross Tog5681/Tog5672 while nine came from Tog5681/Tog7291.

The presence of two resistance RYMV1 alleles (rymv1-3 and rymv1-4) in the Tog5681/Tog5672 cross should confer resistance to all the derived lines but some infected lines were observed in the progenies. Moreover, an unbiased population derived from the Tog5681/Tog7291 cross involving rymv1-3 and RYMV2 should result in more than nine resistant lines. Selection for agronomic traits may have modified the segregation ratio or resistance-breaking may have occurred in some resistant lines. Resistance-breaking might specially concern lines with the rymv1-4 allele or lines with RYMV2 as these alleles and genes are known to be more frequently overcome than rymv1-3 or RYMV3 (Pinel-Galzi et al., 2016; Hebrard et al., 2018). Regarding the plant’s response to all virus attacks and the serological results obtained, the significant interaction (P < 0.001) between the main factors - isolates and genotypes - (Table 2) suggests that the effects of isolates (expressed by disease severity) depended on the resistance/susceptibility status of the genotypes and showed that some accessions were more resistant or susceptible than others.

Further molecular analysis is, however, needed to identify the number and nature of the resistance alleles in the 16 resistant lines identified. Lines combining the rymv1-3 allele, RYMV2 and RYMV3 would constitute an interesting material for evaluating the durability of resistance as this combination has so far not been identified in wild genotypes. This perspective supports the initiation of an intra-specific plant breeding approach using these genetic crosses for developing lines with added values. In addition, the development of interspecific O. sativa/O. glaberrima lines with RYMV resistance inherited from O. glaberrima is a good perspective for valorizing the African rice species (Ghesquière et al., 1997). The resulting progenies will also combine O. glaberrima’s adaptability and to tolerance to many local stresses with the high yielding potential of the Asian species O. sativa - this was the key to the success of NERICA varieties. Therefore, the use of phenotypic characteristics and molecular tools, such as MAS, could be one option for unravelling the secrets of the offspring derived from such crosses.

Acknowledgments

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