EVALUATION OF A COLLECTION OF RICE LANDRACES FROM BURKINA FASO FOR RESISTANCE OR TOLERANCE TO *RICE YELLOW MOTTLE VIRUS*

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SUMMARY

A collection of accessions of Burkina Faso rice germplasm was evaluated for resistance using four *Rice yellow mottle virus* (RYMV) isolates: Ng122, Ng144, B27 and BF1. B27, an isolate from Benin was used first, followed by Ng122 and Ng144 (isolates from Niger), and BF1 an aggressive isolate from Burkina Faso was used last to assess the accessions status against RYMV. Fourteen-day-old plantlets were inoculated and symptoms scored fortnightly from 14 to 56 days post inoculation (dpi). Plant height of all accessions was recorded at 49 dpi with isolates Ng122 and Ng144. The *Oryza sativa* accessions of the collection were highly susceptible except one (BM24), which combined partial resistance and tolerance. Twenty one *O. glaberrima* accessions out of 48 were found resistant to Ng122 and Ng144. When these 21 accessions were subsequently screened with the aggressive RYMV strain BF1, eight of them displayed a delay in the appearance of RYMV symptoms while two showed resistance. The new sources of resistance identified in this study, could be exploited in breeding to control the spread of RYMV in Africa.

Key words: germplasm, disease management, plant viruses, rice, evaluation for resistance.

INTRODUCTION

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

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

Screening for natural resistance to RYMV has been performed on rice varieties of different geographical origins, including the two cultivated rice species, *Oryza sativa* and *Oryza glaberrima* (Fomba, 1988, 1990; Ndjiondjop *et al.,* 1999; Rakotomalala *et al.,* 2008; Thiémélé *et al.,* 2010). Rice cultivar responses to the virus have shown large variability depending upon the genotype and screening

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conditions (environment, climatic conditions, severity of inoculation, and resistance evaluation methods). Responses to RYMV have been distinguished into three types: highly susceptible, partially resistant, and highly resistant. The resistance at the host plant level is characterized by reduced virus multiplication, reduced symptom expression and limited yield loss (Ghesquière *et al.,* 1997). A major gene for resistance against RYMV, *Rymv1*, has been identified in the *O. sativa indica* resistant cultivars Gigante and Bekarosaka, (Ndjiondjop *et al.,* 1999; Rakotomalala *et al.,* 2008). This gene, which encodes a translation initiation factor (eIF(iso)4G), is also responsible for the resistance of the resistant *O. glaberrima* accessions Tog5681, Tog5672, and Tog5674, whose alleles (*Rymv1-3*, *Rymv1-4* and *Rymv1-5*, respectively) are distinct from each other and from that of cv. Gigante (*Rymv1-2*), which was fine-mapped onto chromosome 4 (Albar *et al.,* 2006). Recently, a second major gene *RYMV2* was identified in *O. glaberrima* (Thiémélé *et al.,* 2010).

However, previous attempts to breed for resistance have failed because RYMV mutates to new virulent races that overcome the resistance in the "resistant" cultivars (Traoré *et al.,* 2006). Thus, there is a need to identify and characterize diverse sources of genes for resistance to RYMV, in order to broaden the rice gene pool for a sustainable genetic control of the pathogen. The new approach is to use rice landraces to seek novel genes for resistance or tolerance to RYMV. Despite the endemic status of RYMV in Africa, little screening has been conducted on rice collections at a country level. In Burkina Faso, where both *O. glaberrima* and *O. sativa* are cropped, a collection of rice landraces recently gathered throughout the rice cropping areas of the country was available for screening. The objective of the study was to screen this novel rice collection for resistance or tolerance to RYMV in order to identify new sources of resistance.

MATERIAL AND METHODS

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

Virus multiplication and inoculation. Three RYMV isolates (B27, Ng122 and Ng144) were provided by the AfricaRice Plant Pathology Unit. These were multiplied on the standard susceptible variety, IR64, for two weeks. Isolate BF1 was provided by the Institut de Recherche pour le Développement (IRD), France. It had been multiplied on cv. IR64 and stored at -80°C under liquid nitrogen. Infected leaves were ground in phosphate buffer, pH 7.2 (10 ml g^{-1} of leaf tissue) to produce the inoculum, to which Carborundum (600 mesh) was added. Mechanical inoculation was carried out by rubbing the extracted sap on the upper and lower surfaces of the leaves of two-week-old plants.

Evaluation of resistance to RYMV. Symptom severity was scored on a scale of 1 to 9 according to the IRRI Standard Evaluation System (IRRI, 2002). Highly resistant (HR) plants were scored as 1 for they showed no symptoms; plants scored 3 had green leaves with sparse dots or streaks, thus were considered moderately resistant (MR); plants scored 5 showed a generalized mottling of the leaves, thus were considered moderately susceptible (MS); plants scored 7 were yellow, mottled and stunted, thus were considered susceptible (S), score 9 was for highly susceptible (HS) plants that showed yellowing, mottling, stunting followed by death.

The first evaluation was performed under greenhouse conditions at the AfricaRice Research Station (Cotonou, Benin). Two hundred and ninety six accessions (including 281 *O. sativa* and 15 *O. glaberrima*) were screened with isolate B27. *O. sativa indica* cv. Gigante and *O. glaberrima* cv. Tog5681, both carrying *Rymv1-2* and *Rymv1-3* (recessive resistant alleles), respectively (Albar *et al.,* 2006), were used as resistant controls. The highly susceptible *O. sativa indica* cvs IR64 and Bouaké189 were included as susceptible controls. *O. sativa. japonica* cv. Azucena, was used as a partially resistant control. The experimental design was an augmented design (Federer, 1956) with five blocks. The experiment included two treatments. One treatment was used as a test and inoculated with B27 and the other as a non-inoculated control. The five control cultivars were replicated in the five blocks. Disease symptoms were scored at 21 and 42 days post inoculation (dpi). Plant heights were recorded at 42 dpi. Leaves of resistant and tolerant accessions were collected at 56 dpi for evaluation of virus content by ELISA, as described by Séré *et al.* (2007).

The second evaluation was also performed under greenhouse conditions at the AfricaRice Research Station (Cotonou, Benin). It involved 229 accessions (170 already screened in the previous evaluation and 59 newly added) comprising 181 *O. sativa* and 48 *O. glaberrima*. They were evaluated with isolates Ng122 and Ng144 in an augmented design (Federer, 1956) with five blocks. Four controls, i.e. Tog5681, Gigante, Bouaké189 and IR64 were randomized in each block. Accessions were mechanically inoculated two weeks after sowing. Symptom intensity on the leaves was monitored fortnightly after inoculation until 56 dpi. The area under symptoms progression curve (AUSPC) was calculated as:

$$AUSPC = \sum [(S_i + S_{i+1} - 2)(T_{i+1} - T_i)]/2$$

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

Analysis of the allelic state of resistant and tolerant accessions. The resistant and tolerant accessions identified during the second screening were brought to IRD, Montpellier (France) for an allelic study. These accessions included 21 *O. glaberrima* with a mean disease score ranging from 1 to 3, and five *O. sativa* accessions (BM16, BM24, HB18, HB18B and HB84) with a disease score ranging from 2 to 4.5. The 26 accessions were screened in a glasshouse with the aggressive RYMV isolate BF1 (Ndjiondjop *et al.,* 1999) to identify resistant accessions to be evaluated for the presence of RYMV resistant alleles *Rymv1-3*, *Rymv1-4* and *Rymv1-5* using molecular markers. Ten plants of each accession were sown in a growth chamber under controlled conditions (24-26°C for 12 h in the dark and 28-30°C for 12 h in the light, with 80-90% relative humidity) and mechanically inoculated with BF1 two weeks after sowing. Disease symptoms were monitored from the date of the appearance of symptoms to 42 dpi. Leaf fragments 2 cm in size from accessions displaying a good level of resistance to BF1 were collected 49 dpi for DNA extraction (Edwards *et al.,* 1991). The leaves were ground in liquid nitrogen using a robot Rath MM300 (TissueLyser II, Retsch, Germany) for 30 sec. Aliquots (400 µl) of extraction buffer consisting of 200 µM of Tris/HCl pH 7.5, 250 mM of NaCl, 25 µM of EDTA, and 0.5% of SDS, were added to each tube with ground tissues. This was followed by centrifugation at 4000 rpm for 15 min, and 300 ml of the supernatant were collected in a new labelled tube. Ice-cold isopropanol (300 µl) was added to the supernatant and the sample was centrifuged at 4000 rpm for 15 min, the supernatant was discarded and the DNA was kept in the tube. Ethanol 70% (200 µl) was added to the DNA followed by centrifugation at 4000 rpm for 5 min. After centrifugation, ethanol was eliminated from the tubes, then 400 µl of TE were added to the dried DNA and kept in the refrigerator overnight.

DNA samples from resistant accessions were analyzed by PCR to reveal *RYMV1* resistant alleles. Four different primers (two forward and two reverse) were used in three different PCR reactions to reveal the three different alleles. In reaction 1, primers F2/R5 and F5/R4 would reveal allele *Rymv*1-3 (Tog5681 like); in reaction 2, primers F2/R5 and F4/R3 would reveal allele *Rymv*1-4 (Tog5672 like); in reaction 3, primers F3/R2 and F2/R6 would reveal allele *Rymv*1-5 (Tog5674 like). The sequences of the primers used above are given in Thiémélé *et al.* (2010). The 15 µl reaction mix comprised 15 ng DNA, 200 µM of each desoxyribo-nucleotide 5'-triphosphate (dATP, dGTP, dTTP and dCTP), 1.5 mM $MgCl_2$, 0.02 U μl^{-1} Go*taq* DNA polymerase (Promega, USA), 0.1 mM of each primer, and 1X buffer. The annealing temperature was progressively decreased from 68°C to 62°C during the first five cycles and was then maintained at 62°C, as described by Thiémélé *et al.* (2010). Amplification products were analyzed by electrophoresis in a 1.5% or 2.5% agarose gel.

EVALUATION OF RESISTANCE TO RYMV

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

$$\% \text{ of plant height reduction} = 100 \times (H_o - H_i) / H_o$$

where H_o = height of control and H_i = height of inoculated.

Experiment 2. Two way analysis of variance (ANOVA) was performed to evaluate interaction between virus and accessions, to compare the disease score of the two isolates during the overall period of screening, and to compare the plant heights of inoculated and non-inoculated plants at 49 dpi. The evaluation of interaction virus x accessions, and the comparison of the means of disease scores and plant heights were all performed using GenStat 11th Edition (Payne *et al.,* 2008).

RESULTS

Plant response to viral infections (experiment 1). Symptom scores of the 296 accessions inoculated with isolate B27 ranged between 1 and 9, with most of the accessions scoring between 5 to 7. The plant disease score was highly correlated with plant height, as it decreased when the score increased. Five reaction groups were represented in the collection (Fig. 1). Symptomless accessions

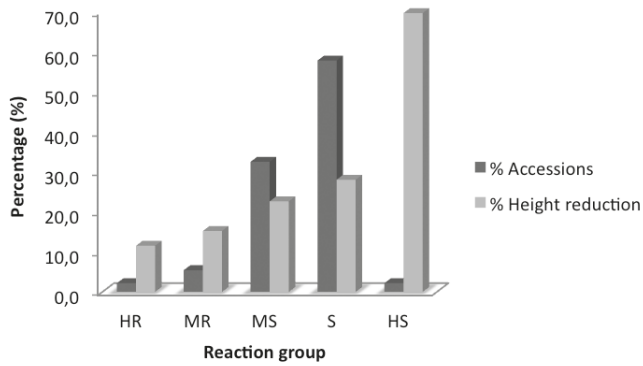

Fig. 1. Distribution of accessions regarding symptom scores and percentages of height reduction in response to the B27 isolate inoculated onto 296 rice accessions from Burkina Faso, at 42 days post inoculation. HR: highly resistant (score 1); MR: moderately resistant (score 3); MS: moderately susceptible (score 5); S: susceptible (score 7) and HS: highly susceptible (score 9).

Table 1. Resistant and tolerant accessions with checks (controls) after inoculation with B27 isolate.

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

*% Plant height reduction; cvs Gigante and Tog5681 are resistant controls, cv. Azucena is a partial resistant control, cvs Bouake189 and IR64 are susceptible controls.

(score 1) represented only 2.2% of the 296 analyzed accessions. The second group included moderately resistant plants showing mild symptoms (score 3), accounting for 5.4% of the collection. In the first two groups, the effect of infection on plant development was mild. More than 90% of the accessions evaluated were susceptible accessions clustered in groups 3 (score 5) and 4 (score 7) with distinct mottling and height reduction. The plants with scores 5 and 7 represented 32.5% and 57.8%, respectively, of individuals in the collection. The last group (score 9) consisted of very susceptible accessions, showing severe symptoms, stunting and, sometime, death. Their proportion was 2.2% of the total collection. The first screening of 296 accessions with B27 demonstrated the widespread susceptibility of most of Burkina Faso rice landraces to RYMV. Only six accessions with score 1 (HR) and 15 with score 3 (MR) were found. Table 1 summarizes the results of ELISA testing at 56 dpi, performed on the six HR and on four MR accessions with a score of 3 at 21 and 42 dpi and the results of the tests performed on controls (cvs Gigante, Tog5681, Azucena and IR64). This table shows that the resistance and susceptibility patterns of the controls were maintained: (i) Gigante and Tog5681 were resistant, with a lower virus content detected by ELISA; (ii) Azucena confirmed its partially resistant status over the period of screening with a score of 3 at 42 dpi but with high virus content and a relatively higher height reduction (39.2%); (iii) the susceptible cv. IR64 had a score of 7 at 42 dpi, and displayed a 50% height reduction at 42 dpi. Of the six HR landrace accessions, five had a low virus content (BM24, BM28, HB88, CC15, and CC29), while one (BM14), even though it showed no symptom on the leaves and little height reduction, had high virus content. Similarly, accession HB88 was symptom-free, had a low virus content and suffered a relatively high decrease in height (21.1%).

Table 2. Effect of the two isolates on overall plant height of the collection at 49 dpi.

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

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

Plant response to viral infection (experiment 2). Screening of the 229 accessions with RYMV isolates Ng122 and Ng144 resulted in different reactions. Significant interaction between viruses and accessions was observed at 28 dpi ($df = 216$, $p = 0.013$), indicating that certain accessions responded differently, depending on the isolate applied. Both viral isolates caused a highly significant reduction in height between infected and control plants ($df = 2$, $p < 0.001$) (Table 2), a differences that emphasized the vulnerability of the collection to the isolates in question.

Over the period of screening only one accession (BM13B) showed no symptom but suffered a height reduction of 29% and 20% caused by isolates Ng122 and Ng144, respectively. Apart from that, the resistance of the other resistant accessions was overcome later either by Ng122 or Ng144. Only six accessions (BM13B, CC2, CC114A, CC123D, HB27A and HB88) presented

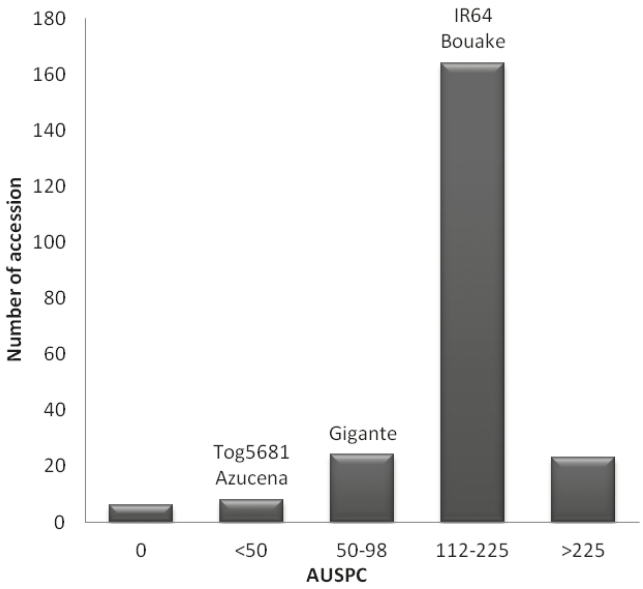

Fig. 2. Distribution of susceptibility, estimated by the area under symptom progress curve (AUSPC) values on a collection of Burkina Faso rice accessions inoculated with the Ng122 isolate of RYMV. The resistance or susceptible level of control varieties is indicated. AUSPC = 0 is for highly resistant accessions with no visible symptoms.

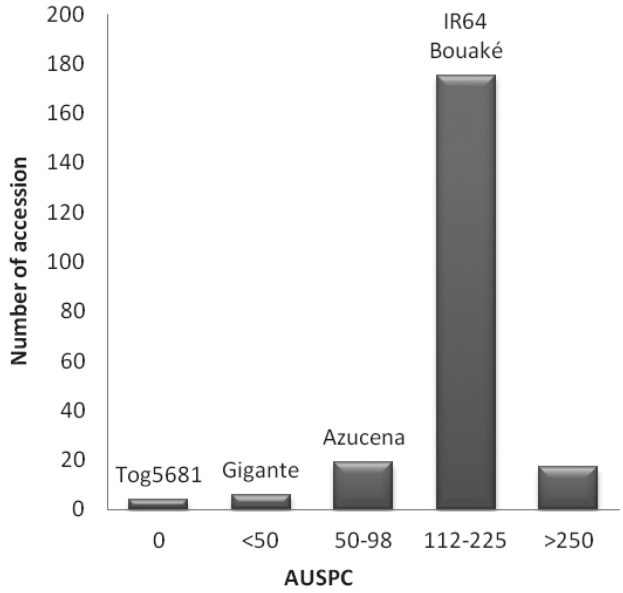

Fig. 3. Distribution of susceptibility, estimated by the area under symptom progress curve (AUSPC) values on a collection of Burkina Faso rice accessions inoculated with the Ng144 isolate of RYMV. The resistance or susceptible level of control varieties is indicated. AUSPC = 0 is for highly resistant accessions with no visible symptoms.

no visible symptoms throughout the period of screening when infected with Ng122. Three accessions (BM13B, BM14 and HB19B), plus Tog5681, showed no visible symptoms when infected with Ng144, which overcame the resistance of CC109A, CC123A and HB88. Also resistant controls (cvs Gigante and Tog5681) were infected. In particular, resistance of cv. Gigante was overcome by both isolates, whereas the resistance of cv. Tog5681 was overcome only by Ng122.

The AUSPC diagrams portray the distribution of resistance and susceptibility of the rice landrace collection to isolates Ng122 and Ng144 (Fig. 2, 3). Fig. 2 shows the matching of the resistance of Tog5681 and Gigante by Ng122, with Gigante showing full symptom expression. Tog5681 and Azucena were clustered in the moderately resistant group, while Gigante was classed as moderately susceptible. IR64 and Bouaké were both classed as susceptible. Similarly, cv. Gigante was ranked as moderately resistant, as it showed symptoms upon infection by Ng144. Azucena was classed as a moderately susceptible while IR64 and Bouaké were classed as susceptible (Fig. 3).

In general, *O. glaberrima* accessions were more resistant than those of *O. sativa.* In fact, 22% of the 48 *O. glaberrima* accessions was resistant to Ng122 and 14% to Ng144, while 67% and 60% of them displayed a moderately resistant pattern when infected by Ng122 and Ng144, respectively. However, 11% and 22% of the *O. glaberrima* accessions was susceptible to Ng122 and Ng144, respectively.

None of the *O. sativa* plants of the collection were symptomless. Nevertheless, 7.4% and 7.5% of them were moderately resistant to Ng122 and Ng144, respectively, and the rest were susceptible (Fig. 4). Fig. 4 portrays the vulnerability of *O. sativa* and the tolerance of *O. glaberrima* to Ng122 and Ng144. This latter isolate showed more aggressiveness to *O. glaberrima* than Ng122, whereas it was not very aggressive on *O. sativa* accessions BM24, HB62, and HB84, which were moderately resistant. However, only BM24 and HB84 disclosed a moderately resistant status while infected by Ng122. Azucena expressed fewer symptoms and relatively less height reduction when infected by Ng122 compared to Ng144. In fact, both isolates, which overcame the high level resistance of cv. Gigante, induced mild symptoms in cv. Azucena.

Allelic test. Accessions susceptible to isolate BF1 were characterized by the early appearance of symptoms, which appeared at 6 dpi in 15 of the 26 accessions. Accession BM13B, which was resistant to both Ng122 and Ng144, displayed symptoms as early as 6 dpi. Later, of the 15 susceptible accessions, six died and six did not bloom confirming the aggressiveness of BF1. The only *O. sativa* (i.e. BM24), which did not show symptoms at 6 dpi, exhibited symptoms two weeks post inoculation. At 21 dpi, only two accessions (HB19B and HB46) were symptomless, although the resistance of some of their individuals was overcome at 42 dpi. The 10 *O. glaberrima* accessions: HB46, HB19B, BM11C, CC2B, CC109A, CC123D, CC2, CC3C, CC123A, and CC2C, which were symptomless at 14 dpi with BF1, were screened in an allelic test. None of the accessions displayed an allelic profile similar to the controls Tog5672 (*Rymv1-4*), Tog5674 (*Rymv1-5*), and Tog5681 (*Rymv1-3*) (Fig. 5).

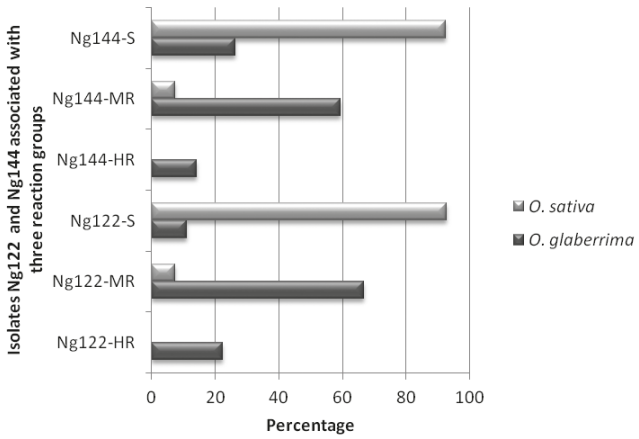

Fig. 4. Comparison of highly resistant (HR), moderately resistant (MR) and susceptible (S) plants in *O. glaberrima* and *O. sativa* species when screened with the two RYMV isolates Ng122 and Ng144. HR plants included accessions with a mean score ranging from 1 to 1.5 during the 56 days of screening; MR included accessions with a mean score ranging from 2 to 3.5 during the 56 days of screening and susceptible plants contained plants with a mean score of > 3.5 during the 56 days of screening.

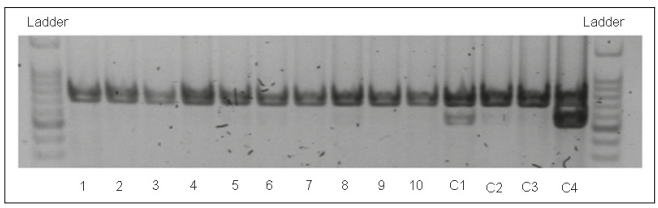

Fig. 5. Gel profiling showing that the resistant accessions do not carry the RYMV resistance allele *Rymv1-3*; lanes 1 to 10, accessions BM11C, CC2, CC2B, CC2C, CC3C, CC109A, CC123A, CC123D, HB19B, and HB; lane C1, AC96; lane C2, Tog5672; lane C3, Tog5674; lane C4, Tog5681.

DISCUSSION

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

A differential behaviour was observed as a result of infections with the two viral isolates used (Ng122 and Ng144) and the differences in their aggressiveness and pathogenicity were highlighted. This can be explained by the viral isolate/host accession interaction, which indicates that the response to viral isolates is dependent on the accessions as well as on the isolates (N'Guessan e*t al.,* 2001). Moreover, virus isolates can differ in their geographical distribution (N'Guessan *et al.,* 2001; Fargette *et al.,* 2002b). Furthermore, RYMV isolates coming from closely related agro-ecological zones can display variability in pathogenicity (Konate *et al.,* 1997). Isolates Ng122 and Ng144, although they originated in the same country, induced differential responses to the same accessions due to virus/host interaction.

Of the 48 *O. glaberrima* accessions, 11% and 22% was susceptible to Ng122 and Ng144, respectively. When Thiémélé *et al.* (2010) screened a collection of *O. glaberrima*, they noted that there were fewer susceptible cultivars among the *O. glaberrima* population. RYMV was first observed in 1966 in Kenya (Bakker, 1974) and *O. glaberrima* originates from Africa (Chang, 1984). Thus, virus and host have co-evolved together for many decades resulting in a moderate resistant status for *O. glaberrima* accessions. Most of *O. glaberrima* accessions showed a delayed emergence of symptoms when screened with isolates Ng122 and Ng144. RYMV isolate BF1 was very aggressive to some *O. glaberrima* accessions and caused their death. Two *O. glaberrima* cultivars (HB19B and HB46) displayed a high level of resistance when infected. However, the two resistant *O. glaberrima* cultivars did not display an allelic status identical to *Rymv1-3*, *Rymv1-4* and *Rymv1-5*.

Amongst the five *O. sativa* accessions, which were tolerant to the isolates Ng122 and Ng144 (BM16, BM24, HB18, HB18B and HB84), only BM24 showed partial resistance to the aggressive isolate BF1. This difference can be explained by two factors: the difference between plant genotypes and the genetic make-up of the isolates. Furthermore, these differential accession reactions to viral infections support the idea that different resistance genes or groups of resistance genes are involved in virus and host interactions (Konate *et al.,* 1997; Albar *et al.,* 1998). Therefore, BM24 appears to be more genetically flexible than the other *O. sativa* cultivars. The resistance in BM24 responds similarly against different isolates of the same virus, meaning that it does not interact with the isolate applied. BM24 displayed a tolerant status against the four isolates applied.

In the present study, cv. Azucena also showed a tolerant status to the infections by the three isolates B27, Ng122 and Ng144. Previous studies (Albar *et al.,* 1998; Ndjiondjop *et al.,* 1999; Ahmadi *et al.,* 2001) reported a partial resistance status of Azucena against the aggressive strain BF1. Therefore, BM24 seems to portray a similar resistance pattern as Azucena. However, morphologically the two cultivars are very different. Azucena is taller (> 1.5 m) and late maturing (> 120 days), whereas BM24 is a shorter plant (around 1 m) and early maturing (< 105 days).

Resistance to RYMV by BM24 accession is characterized by a delay of symptom expression, as well as lower symptom severity, which is very similar to the pattern observed in the partially resistant cv. Azucena.

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

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