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# Resistance to *Rice yellow mottle virus* in rice germplasm in Madagascar

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**Abstract** *Rice yellow mottle virus* (RYMV) is a recent and major threat to rice production in Madagascar. A large scale screening of resistance to RYMV in rice germplasm in Madagascar was conducted by visual symptom scoring and virus-assessment through ELISA tests. The response to virus inoculation of 503 local or introduced rice accessions was assessed. Most of them were susceptible to RYMV. A few cultivars expressed partial resistance at a level similar to the partially resistant *Oryza sativa japonica* cv. Azucena. Only one *O. sativa* cultivar expressed high resistance characterised by a lack of symptoms and an undetectable level of virus. It was a *Malagasy* traditional *indica* cultivar, named Bekarosaka, which originated from the northwest of the country. It was selected by farmers for its field resistance to RYMV. The response of cv. Bekarosaka to four pathotypes of RYMV was similar to that of cv. Gigante, the only other highly resistant *indica* cultivar. The sequence of the middle domain of the eIF(iso)4G, the genetic determinant of *Rymv1* resistance on chromosome 4, of cv. Bekarosaka was similar to that of cv. Gigante. Subsequently, cvs Bekarosaka and Gigante probably carried the same resistance allele *Rymv1-2*. *Rymv1*-2 resistance was efficient against isolates of the major strains of RYMV, but was readily overcome by a pathotype from the northwest of Madagascar.

**Keywords** Participatory plant breeding · Recessive resistance · Resistance-breaking pathotypes

## Introduction

Rice is the staple food of *Malagasy* people. A wide range of cultivars, either native or introduced, is cultivated in the country. *Rice yellow mottle virus* (RYMV) causes the most important rice disease in Africa. The disease was first reported in Kenya in 1966 (Bakker 1974), later in West Africa (Fauquet and Thouvenel 1977) and subsequently in nearly all

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rice-growing countries in Africa (Kouassi et al. 2005). RYMV was first observed in Madagascar in 1989 (Reckhaus and Randrianangaly 1990). The epidemics first developed in the two main rice-growing areas, i.e. the plains surrounding Lake Alaotra and the Marovoay basin, and later spread into all coastal rice-producing regions. Epidemics occur mainly during the rainy season when beetle vector infestations are high. The incidence of infection reached 80% in cultivated lowland rice fields, usually comprising susceptible varieties (Reckhaus and Andriamasintseheno 1997). RYMV is not present in the highlands in the centre of Madagascar where temperatures are moderate.

RYMV is a positive single-stranded RNA virus of the genus *Sobemovirus*. Its genome is 4,450 nt-long and comprises four ORFs (Fargette et al. 2004). RYMV has a high variability at the nucleotide and amino acid levels with a pronounced spatial basis, as six major strains were identified in different regions of Africa (Traoré et al. 2005). The Madagascar isolates are related to the S4 East African strain (Abubakar et al. 2003). RYMV is transmitted by contact during cultural practices and by beetles of the family *chrysomelidae* (Bakker 1974). The main vectors in Madagascar are the beetle species *Dicladispa gestroi* and *Trichispa sericea* of the sub-family *hispinae*, and *Chaectonema pulla* of the sub-family *halticinae*. Transmission by the grasshopper *Oxya* sp. (hyla) is also suspected. Symptoms are characterised by leaf mottle at an early stage of infection that turns into generalised yellowing and overall stunting. Necrosis of the most susceptible cultivars leads to plant death (Fauquet and Thouvenel 1977).

In Madagascar, as in mainland Africa, chemical control of RYMV vectors is not feasible for economic and environmental reasons. Moreover, it is unlikely to be efficient, considering the ubiquity of beetle vectors. Control of the disease relies on host plant resistance. Partial resistance to RYMV, characterised by a delay in symptom expression and virus accumulation, has been reported within the *Oryza sativa* subspecies *japonica* (Ioannidou et al. 2000, 2003). A major QTL of partial resistance was identified on chromosome 12 of the *japonica* cv. Azucena (Albar et al. 1998). A high resistance with a lack of symptoms, an undetectable virus level and no yield losses upon field infection was found in a few cultivars of the African rice *O. glaberrima* and in a single *O. sativa indica* cultivar named Gigante (Thottapilly and Rossel 1993; Ndjiondjop et al. 1999; N'Guessan et al. 2001). The high resistance is controlled by the recessive gene *Rymv1* which maps on chromosome 4 (Albar et al. 2003) and encodes the translation initiation factor eIF(iso)4G (Albar et al. 2006). Three alleles of resistance have been identified so far, one in the *sativa* cv. Gigante (*Rymv1-2*) and two in the *glaberrima* cvs Tog5681 (*Rymv1-3*) and Tog5672 (*Rymv1-4*). They differed from the susceptible *sativa* and *glaberrima* cultivars (*Rymv1-1*) by a point mutation or a small deletion in the middle domain of eIF(iso)4G (Albar et al. 2006).

The high resistance was efficient against representative isolates of the main virus strains (Ndjiondjop et al. 1999). However, resistance of cv. Gigante was overcome by some isolates (Fargette et al. 2002; Traoré et al. 2006; Pinel-Galzi et al. 2007). The Virus Protein genome linked (VPg) encoded by the ORF2a of RYMV was identified as the virulence factor (Hébrard et al. 2006). A single mutation in the VPg was sufficient to break the resistance of cv. Gigante.

Madagascar, with ca. 4,000 cultivars of diverse origins introduced and developed at least since the 10th century, possesses an exceptionally rich rice germplasm (Kochko de 1988; Rabary et al. 1989). Considering the economic importance of rice in Madagascar, the major threat posed by RYMV to its cultivation and the exceptional diversity of the rice germplasm, the response to virus inoculation of 503 rice accessions of native and introduced origins was tested. The overall vulnerability of *Malagasy* germplasm was revealed, partial resistance was found, a new highly resistant *indica* cultivar named Bekarosaka was identified, but a pathotype of RYMV that readily overcame the *Rymv1-2* resistance was encountered in the northwest of Madagascar.

## Materials and methods

### Genetic material

Several thousands of rice accessions have been collected, maintained, improved and characterized at FOFIFA (Centre National de la Recherche Appliquée au Développement Rural, Madagascar). In particular, a collection of 503 accessions combining good agronomic performance and adaptation to rainfed lowland growing conditions was screened for resistance to RYMV. It included both native and introduced accessions. Some of them were introduced from Indonesia and Malaysia by the first *Malagasy* immigrants. Widely cultivated traditional accessions adapted to local environmental conditions in Marovoay region in the northwest, Lake Alaotra in the middle east, Tanandava in the southwest, and the highlands in the centre of the country were tested for their response to RYMV. Most of them belong to the *indica* sub-species or are *indica Malagasy* variants. Cultivars indexed by farmers and assessed as resistant in multi-locational participatory selection trials were also included in the study. In particular, two traditional cultivars, Bekarosaka and Maintimbotsy from the northwest and the northeast of Madagascar, respectively, where severe epidemics developed, were reported by some farmers to be uninfected.

The collection also included locally improved ‘ON’ and ‘T’ lines developed from crosses made at the Tsararano station of FOFIFA between RYMV-susceptible and resistant cultivars. ON lines were derived from crosses between *japonica* upland rice (cvs Ciwini and Moroberekan) and the local *indica* rice referred to as ‘luxury rice’ (cvs Ali-Combo, Boina 1329 and Tsipala). T lines were derived from a successful cross between the local *indica* cv. Miandry bararata (photoperiod sensitive, low yielding and poor grain quality) and the perennial *O. longistaminata* followed by backcrosses with elite *indica* cultivars (Rechkaus and Masajo 1996; Rakotomalala 2003).

Elite cultivars were introduced through a collaborative breeding project with the International Rice Research Institute (IRRI). Since the first occurrence of RYMV epidemics in 1989, cultivars with resistance were introduced from other countries and research institutes: Tox (Tropical *Oryza japonica*×*indica* crosses) from the International Institute of Tropical Agriculture (IITA; Nigeria) and upland rice from EMBRAPA (Brazil). In 1998, a collaborative search for RYMV resistance with INGER–IRRI provided access to several cultivars of *O. glaberrima*: Tog (Tropical *O. glaberrima*) kept at IITA and OG from the West African Centre (WARDA, Bénin; Table 1).

## Screening of 503 rice accessions for resistance to RYMV

The screening was done in three stages, the first two at Tsararano research station in the northwest of Madagascar and the third one in Montpellier in France. At Tsararano, plants were grown in two 1 m-long rows with 20×20 cm spacing, i.e. 10 plants per accession, in an insect-proof greenhouse to avoid contamination. The susceptible *Malagasy* cv. Boina 1329 (Reckhaus and Masajo 1996) was planted every 10 accessions as a control. Infected leaves were collected in several areas in order to prepare a broad-spectrum inoculum for screening tests. The leaves were ground (1g: 10 ml) in 0.1 M phosphate buffer at pH 7.2 mixed with carborundum. Inoculation was done 15 days after transplanting by rubbing leaves with the inoculum. In the first stage, symptom evaluation of the 503 accessions was done 30 days post-inoculation (dpi) using the 1 to 9 standard evaluation system (Konaté et al. 1997; http://www.knowledgebank.irri.org/ses/). Accordingly, the accessions were placed in five classes: highly resistant (HR) for score 1 (no symptoms), resistant (R) for score 3 (sparse dots or streaks), intermediate (I) for

**Table 1** Origin of the rice germplasm tested against RYMV by FOFIFA in Madagascar

| Accessions | Number | Origin | Species or sub-species | Objective |
|---|---|---|---|---|
| Traditional varieties/ landraces | 48 | Prospections in Madagascar | *indica* | Agronomic |
| *Malagasy* ecotypes | 76 | Traditional and ancient introductions | *indica* | Agronomic |
| ON lines | 2 | Breeding in Tsararano station (Madagascar) | *indica*×*japonica* (upland) | RYMV selection |
| T lines | 72 | Breeding in Tsararano station (Madagascar) | *longistaminata*×*sativa* | RYMV selection |
| IRRI lines | 96 | Introduction through IRRI-Madagascar project | hybrids | Agronomic |
| Upland rice | 10 | Introduction from EMBRAPA (Brazil) | *japonica* (upland) | RYMV selection |
| Tox lines | 48 | Introduction from IITA (Nigeria) | *japonica* (upland) | RYMV selection |
| Tog lines | 7 | Introduction from IITA through INGER–IRRI | *glaberrima* | RYMV selection |
| *Oryza glaberrima* (OG) | 144 | Introduction from WARDA through INGER–IRRI | *glaberrima* | RYMV selection |
| Total | 503 | | | |

score 5 (mottling), susceptible (S) for score 7 (yellowing and stunting), and highly susceptible (HS) for score 9 (necrosis and sometimes plant death).

The second stage involved a sub-sample of 233 accessions that included 85 local and 148 introduced accessions and excluded the most susceptible ones (score≥7). Plants were grown and inoculated as previously. The virus content of systemically infected leaves of each accession was assessed by ELISA 60 dpi as described in N'Guessan et al (2000), using an anti-serum prepared against a West African isolate provided by the John Innes Centre, UK. An absorbance≤0.1 was considered as negative. In the third stage, cvs Bekarosaka and Maintimbotsy and 11 ON and T lines resulting from RYMV selection in Madagascar with no or sparse symptoms and/or limited virus content in glasshouse screening tests were further tested in growth chamber conditions at Montpellier. In growth chambers, the experiments were conducted under 13 h illumination at 120 mEM$^{-2}$ of PAR at 30°C and 90% relative humidity. These optimal growth conditions and early inoculation 10 days after planting favour symptom expression and virus multiplication. Subsequently, the growth chambers tests were more severe than the greenhouse tests in Madagascar.

The *Malagasy* accessions were compared to cvs IR64, Azucena and Gigante, three cultivars that showed, respectively, susceptibility, partial and high resistance to RYMV. Ten seeds of each accession were grown in pots in a randomized complete block design with four replications. The experiment was duplicated and two isolates were inoculated. Isolate BF1 from West Africa, used in earlier screening tests, is aggressive towards susceptible and partially resistant cultivars, but avirulent towards the highly resistant cv. Gigante. Isolate Mg15 originated from the northeast of Madagascar and was less aggressive than isolate BF1. A range of pathogenicity was subsequently covered by using both isolates. Symptom intensity was scored. The virus content of systemically infected leaves was assessed by ELISA 30 dpi using an antiserum prepared against a *Malagasy* isolate (N'Guessan et al. 2000). Furthermore, the presence of RYMV in cv. Bekarosaka was tested by RT-PCR following the method implemented earlier (Pinel et al. 2000). In growth chambers, resistance assessment was done 30 dpi because of early symptom expression and virus multiplication, and also because of subsequent poor growth of some rice cultivars.

## Characterisation of the high resistance of cv. Bekarosaka

The specificity of the resistance of cv. Bekarosaka was assessed by its response to inoculation with four pathotypes of RYMV defined by their ability to infect cultivars with three *Rymv1* alleles: BF1 pathotype (1) virulent on *Rymv1-1* only, isolate CI4$_1$ pathotype (1,2) virulent on *Rymv1-1* and *Rymv1-2*, isolate CI4$_2$ pathotype (2) virulent on *Rymv1-2* only, and isolate BF5 pathotype (1,2,3) virulent on *Rymv1-1*, *Rymv1-2* and *Rymv1-3* (Fargette et al. 2002). None of these isolates infected plants with *Rymv1-4*. Symptom intensity and virus content were assessed as above.

Phenotypic and molecular analysis indicated that cv. Bekarosaka was an *indica* cultivar that was clearly distinct from cv. Gigante (Albar et al. 2007). The genetic basis of resistance was studied in two F$_2$ populations developed from the IR64×Bekarosaka, and Gigante×Bekarosaka crosses at IRD, Montpellier. Symptoms were observed after inoculation of 2 week-old plants with the severe isolate BF1. Analysis with RM252 and RM273 microsatellite markers flanking *Rymv1* was carried out as done by Albar et al (2003). The middle domain of the eIF(iso)4G of chromosome 4 of cv. Bekarosaka, known to carry the genetic determinants of the *Rymv1* alleles of resistance (Albar et al. 2006), was sequenced.

The stability of the high resistance of cv. Bekarosaka was tested. Eight isolates representing the six main strains of RYMV in mainland Africa (S1, S2, S3 in West and Central Africa, and S4, S5, S6 in East Africa), and 14 isolates from all rice-growing areas in Madagascar were each inoculated onto 10 plants of cv. Bekarosaka. Symptoms were assessed. Leaves from each of the 10 plants were pooled together and virus content tested by ELISA 15 and 30 dpi as described above. Three isolates that induced generalised symptoms with high virus content in cv. Bekarosaka and two isolates that failed to do so were selected from this experiment for further tests. The rates of infection of these isolates to resistant cvs Bekarosaka and Gigante were assessed by inoculating, depending on the isolate, 10 to 200 plants. Symptom expression and virus content were assessed individually and the rates of infection were subsequently determined.

## Results

### Overall susceptibility of the rice germplasm in Madagascar

A large majority of the 503 accessions tested (83%), whether introduced or native, expressed pronounced symptoms (S≥5) after inoculation (Fig. 1a). Moreover, a significant amount of virus was detected in 88% of the 233 accessions tested by ELISA 60 dpi (Fig. 1b). This shows the overall susceptibility of the rice germplasm to RYMV in Madagascar and underlines the threat posed by the virus. In particular, most accessions without symptoms contained detectable amounts of virus. Of all cultivars tested, most of them selected earlier for resistance, only one *indica* cultivar combined a lack of symptoms and an undetectable level of virus. This cultivar, named Bekarosaka, was noticed as resistant by a farmer within a participatory selection programme in the northwest of Madagascar where RYMV is a major production constraint. Cultivar Bekarosaka was later multiplied at Tsararano agronomic research station of FOFIFA and its high resistance was confirmed in screening tests.

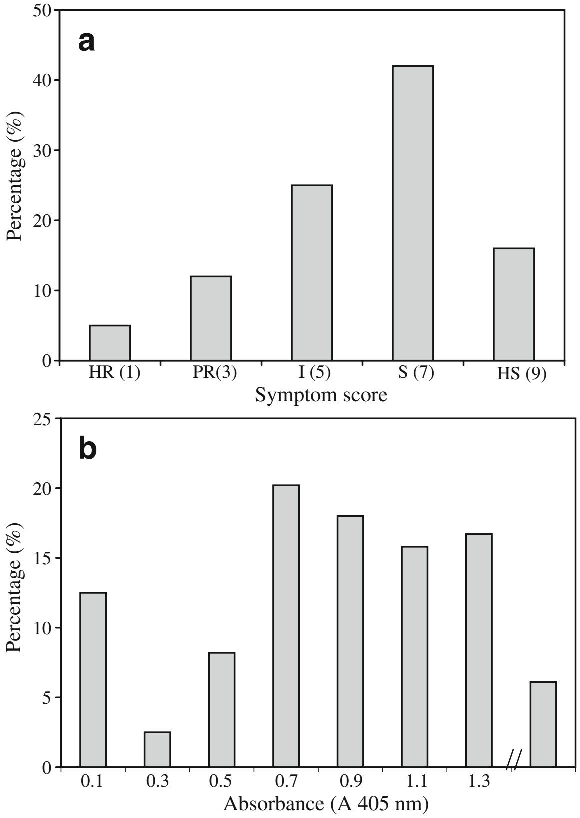

**Fig. 1** **a** Distribution of the response to virus inoculation of 503 rice cultivars in Madagascar assessed 30 dpi by their symptom intensity using the 1–9 standard evaluation system: *HR (1)* Highly resistant, *PR (3)* partially resistant, *I (5)* intermediate, *S (7)* susceptible, *HS (9)* highly susceptible. **b** Distribution of the response to virus inoculation of 233 rice cultivars in Madagascar assessed 60 dpi by their virus content estimated by absorbance (*A405 nm*) in ELISA tests. An absorbance ≤0.1 was considered as negative

Cultivar Bekarosaka and a selection of T and ON accessions combining resistance with other interesting traits were inoculated with isolates BF1 and Mg15 in growth chambers in Montpellier, together with the highly resistant cv. Gigante, the partially resistant cv. Azucena and the susceptible cv. IR64 (Table 2). Cultivar Bekarosaka expressed no symptoms and RYMV was not detected by ELISA or RT-PCR. This response was similar to that of the highly resistant cv. Gigante. With the 12 other accessions, the growth chamber tests were more severe than the greenhouse screening tests in Madagascar as none of them confirmed a high resistance (no symptoms, no detectable virus). However, they had lower symptom expression and virus content than the susceptible control IR64, at least with the less aggressive isolate Mg15. The resistance of introduced cvs Tox 3219-51, Tox 3233-31 and of locally improved ON 332, ON 333 lines was similar to that of the partially resistant cv. Azucena, at least with isolate Mg15. The resistance level of the other accessions and of the traditional cv. Maintimbotsy was significantly lower than cv. Azucena.

### Characterisation of the high resistance of cv. Bekarosaka

The response of cv. Bekarosaka to inoculation with each of the four pathotypes of RYMV was compared to that of cvs IR64 (*Rymv1-1*), Gigante (*Rymv1-2*), Tog5681 (*Rymv1-3*) and Tog5672 (*Rymv1-4*; Table 3). Cultivar Bekarosaka was infected by pathotypes (2), (1, 2) and (1, 2, 3) and not by pathotype (1). Isolates Mg11, Mg16 and Mg35 from Madagascar that readily infected cv. Bekarosaka also infected cv. Gigante, and failed to infect cvs Tog5681 or Tog5672. Conversely, all isolates that infected cv. Gigante also infected cv. Bekarosaka. Altogether, the response of cv. Bekarosaka to the four pathotypes was different from that of cvs IR64, Tog5681 and Tog5672 and was similar to that of cv. Gigante.

**Table 2** Symptom scoring and virus assessment in ELISA test of the response of 16 cultivars (13 *Malagasy* accessions and three control cultivars) to inoculation with RYMV isolates BF1 (West-Africa) and Mg15 (Madagascar)

| Isolate | BF1 | | Mg15 | |
|---|---|---|---|---|
| Cultivar | Score[a] | ELISA[b] | Score | ELISA |
| Bekarosaka | HR (1) | a | HR (1) | a |
| Tox 3219-51 | PR (3) | b c | PR (3) | b |
| Tox 3233-31 | PR (3) | b c | PR (3) | b |
| ON 332 | PR (3) | c | PR (3) | b |
| ON 333 | PR (3) | c | PR (3) | b |
| T 70722 | PR (3) | c | PR (3) | c |
| T 70723 | PR (3) | c | PR (3) | c |
| T 70721 | I (5) | b | PR (3) | c |
| Mahadigny | I (5) | b c | I (5) | c |
| Maintimbotsy | I (5) | c | I (5) | c |
| T 70751 | S (7) | c | I (5) | c |
| T 70752 | S (7) | b | I (5) | c |
| T 70753 | S (7) | b | I (5) | b c |
| Gigante | HR (1) | a | HR (1) | a |
| Azucena | PR (3) | b | PR (3) | b |
| IR64 | S (7) | c | S (7) | c |

[a] *HR (1)* Highly resistant, no symptoms, *PR (3)* partially resistant, sparse dots or streaks, *I (5)* intermediate, mottling, *S (7)* susceptible, yellowing and stunting

[b] Each cultivar was classified by multiple mean comparison after variance analysis of the absorbances 30 dpi and assigned to a reference cultivar: highly resistant (a, cv. Gigante), partially resistant (b, cv. Azucena), susceptible (c, cv. IR64).

The segregation of resistance in the IR64×Bekarosaka population revealed nine resistant and 38 susceptible plants, a distribution consistent with the hypothesis that resistance was recessive and monogenic (1:3 ratio: $\chi^2=0.86$, ddl=1). Segregation analysis of the population with RM252 and RM273 microsatellite markers supported the co-localisation of the resistance with *Rymv1* (data not shown). In addition, the three $F_1$ plants from the Gigante×Bekarosaka crosses and the 30 $F_2$ plants derived from the Gigante×Bekarosaka crosses were all resistant, confirming that the resistance genes of cvs Bekarosaka and Gigante were allelic. Sequencing the middle domain of eIF(iso)4G in chromosome 4 of cv. Bekarosaka revealed that its sequence was identical to that of cv. Gigante (*Rymv1-2*), but different from cv. IR64 (*Rymv1-1*), Tog5681 (*Rymv1-3*) and Tog5672 (*Rymv1-4*) by a lysine in position 309 instead of a glutamic acid. The E309 K substitution in the middle domain of eIF(iso) 4G was earlier shown to confer resistance in cv. Gigante (Albar et al. 2006). Altogether, this indicated that cvs

**Table 3** Response[a] of cultivar Bekarosaka and of cultivars with the four *Rymv1* alleles to inoculation with the four pathotypes of RYMV

| Cultivars | Isolates<br>Pathotype<br>Resistance alleles | BF1<br>(1) | $CI4_1$<br>(1,2) | $CI4_2$<br>(2) | BF5<br>(1,2,3) |
|---|---|---|---|---|---|
| IR64 | *Rymv1-1* | + | + | − | + |
| Gigante | *Rymv1-2* | − | + | + | + |
| Tog5681 | *Rymv1-3* | − | − | − | + |
| Tog5672 | *Rymv1-4* | − | − | − | − |
| Bekarosaka | | − | + | + | + |

[a] + indicates generalised symptoms and high virus content assessed by ELISA 30 dpi, − indicates a lack of symptoms and a non-detectable level of virus in ELISA

Bekarosaka and Gigante probably shared the *Rymv1-2* allele of resistance to RYMV (Fig. 2).

Four types of responses were observed after virus inoculation of cv. Bekarosaka by a range of isolates (Table 4). With most isolates, either from mainland Africa or from Madagascar, neither symptoms nor virus was detected. This result was consistent with earlier outdoor screening and growth chamber tests. With isolates Mg14 and Mg21, a low level of virus was detected; with isolates Mg24 and Ma1, symptoms were further observed in some leaves. However, the symptoms and virus detection remained localised to a few leaves, either inoculated or non-inoculated ones, and the virus did not spread to the rest of the plant. Moreover, inoculation of leaf extracts of these plants failed to infect other Bekarosaka plants. We therefore considered that the resistance of cv. Bekarosaka was not overcome by these isolates. By contrast, three isolates (Mg11, Mg16 and Mg35) that originated from Marovoay in the northwest of Madagascar induced a systemic infection within 2 to 4 weeks with generalized and pronounced symptoms and a high virus titer. These field-isolates were readily transmitted to cvs Bekarosaka and Gigante with transmission rates up to 60%. By contrast, isolates Mg14 and Mg25 did not infect cvs Bekarosaka and Gigante even though a similar number of plants was inoculated (Table 5). The host range of the three isolates Mg11, Mg16 and Mg35 was similar to isolates of pathotype 2: they infected cvs IR64 (*Rymv1-1*) and Gigante (*Rymv1-2*), but not cvs Tog5681 (*Rymv1-3*) and Tog5672 (*Rymv1-4*). However, they differed from isolates of pathotype 2 by their high rate of transmission to *Rymv1-2* resistant plants.

## Discussion

These studies show the overall susceptibility of rice germplasm in Madagascar to RYMV. This was apparent in both native and introduced germplasm even though both groups of cultivars had previously been selected for resistance to the virus. Consequently, there is no genetic barrier to virus spread whenever epidemiological conditions become favourable. This is particularly critical in Madagascar where major epidemics have occurred since 1989 in agro ecologies favourable for spread due to intensive rice cultivation. Several introduced or native cultivars expressed a degree of resistance in glasshouse screening tests. However, the more severe growth chamber tests revealed that their resistance level was at most similar to that of the partially resistant *japonica* cv. Azucena. Some of the Tox and ON lines, derived from crosses between *indica* and upland *japonica*, may have inherited the partial resistance often found in *O. sativa japonica* cultivars (Albar et al. 1998). Several cultivars expressing no symptoms and thus considered so far to be highly resistant had a detectable level of virus. Thus, they are only tolerant to RYMV. Such tolerant

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                       252        262        272        282        292        302
IR64            DVISLIFEKA VFEPTFCPMY AQLCSDLNEK LPSFPSEEPG GKEITFKRVL LNNCQEAFEG
Gigante         .......... .......... .......... .......... .......... ..........
Bekarosaka      .......... .......... .......... .......... .......... ..........
Tog5673         .......... .......... .......... .......... .......... ..........
Tog5681         .......... .......... .......... .......... .......... ..........
Tog5672         .......... .......... .......... .......... .......... ..........

                       312        322        332        342        352     359
IR64            AESLRAEIAK LTGPDQEMER RDKERIVKLR TLGNIRLIGE LLKQKMVPEK IVHHIVQ
Gigante         ......K... .......... .......... .......... .......... .......
Bekarosaka      ......K... .......... .......... .......... .......... .......
Tog5673         D......... .......... .......... .......... .......... .......
Tog5681         D......... .........- --........ .......... .......... .......
Tog5672         D......... ........K. .......... .......... .......... .......
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**Fig. 2** Alignment of amino-acid sequences of the middle domain of eIF(iso)4G (exon 7) of *Rymv1* gene of chromosome 4 in susceptible *O. sativa* cv. IR64 and *O. glaberrima* cv. Tog5673 (allele *Rymv1-1*) and resistant *O. sativa* cv. Gigante (allele *Rymv1-2*), *O. glaberrima* cv. Tog5681 (allele *Rymv1-3*) and *O. glaberrima* cv. Tog5672 (allele *Rymv1-4*; Albar et al. 2006). The middle domain of eIF(iso)4G of *indica* cv. Bekarosaka was sequenced and compared to these resistant and susceptible cultivars. The amino acids that are either mutated or deleted in the resistant cultivars are shaded. A *dash* indicates an indel. Cultivars Bekarosaka and Gigante differed from the other cultivars by a lysine (*K*) at position 309 instead of a glutamic acid (*E*)

**Table 4** Symptom assessment and virus quantification by ELISA of the response of cv. Bekarosaka to inoculation with isolates of the main strains of RYMV in mainland Africa, and of all the rice-growing regions of Madagascar

| Isolate | Strain | Country | Region | Symptoms[a] | ELISA[b] |
|---|---|---|---|---|---|
| CI4 | S1 | Côte d'Ivoire | | 3 | − |
| Ma1 | S1 | Mali | | 3 | +/− |
| CI13 | S2 | Côte d'Ivoire | | 1 | − |
| Ma6 | S2 | Mali | | 1 | − |
| SL4 | S3 | Sierra-Leone | | 1 | − |
| Ke1 | S4 | Kenya | | 1 | − |
| Tz3 | S5 | Tanzania | | 1 | − |
| Tz11 | S6 | Tanzania | | 1 | − |
| Mg19 | S4 | Madagascar | South-West | 1 | − |
| Mg20 | S4 | Madagascar | South-West | 1 | − |
| Mg21 | S4 | Madagascar | Centre | 1 | +/− |
| Mg13 | S4 | Madagascar | North-East | 1 | − |
| Mg14 | S4 | Madagascar | North-East | 1 | +/− |
| Mg15 | S4 | Madagascar | North-East | 1 | − |
| Mg18 | S4 | Madagascar | North-East | 1 | − |
| Mg23 | S4 | Madagascar | South-East | 3 | − |
| Mg24 | S4 | Madagascar | South-East | 3 | +/− |
| Mg25 | S4 | Madagascar | South-East | 3 | − |
| Mg10 | S4 | Madagascar | North-West | 1 | − |
| Mg11 | S4 | Madagascar | North-West | 7 | + |
| Mg16 | S4 | Madagascar | North-West | 7 | + |
| Mg35 | S4 | Madagascar | North-West | 7 | + |

− $A$405 nm≤0.1; +/− 0.1<$A$405 nm≤0.2; + 0.3<$A$405 nm

[a] *1* Absence of symptoms, *3* localised symptoms, *7* generalised symptoms

[b] The absorbances at 30 dpi were considered

cultivars, although of potential interest if yield losses are limited, are of concern epidemiologically as they could serve as virus reservoirs.

Cultivar Bekarosaka consistently expressed high resistance with a lack of symptoms and an undetectable level of virus. Cultivar Bekarosaka was selected by farmers within a field-screening project. This illustrates the scope of a participatory approach to detect interesting cultivars with rare traits of interest (Virk et al. 2003). The response of cv. Bekarosaka to

**Table 5** Transmission rate of field isolates of two pathotypes to the highly resistant cultivars Bekarosaka and Gigante

| Isolate | Pathotype | Cultivar | Nb tested | Nb ELISA[a] | Nb symptoms[b] |
|---|---|---|---|---|---|
| Mg16 | (1,2) | Bekarosaka | 150 | 120 | 38 |
| | | Gigante | 50 | 14 | 9 |
| Mg11 | (1,2) | Bekarosaka | 10 | 3 | 0 |
| | | Gigante | 10 | 5 | 2 |
| Mg35 | (1,2) | Bekarosaka | 10 | 3 | 1 |
| | | Gigante | 10 | 5 | 2 |
| Total | (1,2) | | 240 | 150 (63%) | 52 (22%) |
| Mg25 | (1) | Bekarosaka | 200 | 0 | 0 |
| Mg14 | (1) | Bekarosaka | 10 | 0 | 0 |
| | (1) | Gigante | 10 | 0 | 0 |
| Total | (1) | | 220 | 0 (0%) | 0 (0%) |

[a] Number of plants with positive ELISA values 15 dpi

[b] Number of plants showing generalised symptoms

inoculation with specific resistance-breaking isolates was different from that of cvs Tog5681 (*Rymv1-3*) and Tog5672 (*Rymv1-4*) and similar to that of the highly resistant cv. Gigante (*Rymv1-2*). Sequencing the middle domain of eIF(iso)4G of chromosome 4 of cv. Bekarosaka revealed a lysine at position 309 instead of a glutamic acid. The E309K substitution was earlier found to confer resistance in cv. Gigante (Albar et al. 2006). *Rymv1* is the only gene yet found to express high resistance to RYMV despite intensive screening conducted for decades by national and international research institutes. Bekarosaka and Gigante are the only *indica* cultivars showing high resistance to RYMV and they probably shared the resistance allele *Rymv1-2*. As these two cultivars originated from Madagascar and East Africa, respectively, the resistance may result from gene exchange by natural introgressions followed by farmer selection and movement throughout the sub-continent. Alternatively, the resistance may have been selected independently in several regions under high inoculum pressure of RYMV.

Resistance of cv. Bekarosaka was efficient against representative isolates of the major strains of RYMV in mainland Africa and against isolates of most regions of Madagascar. However, the *Rymv1-2* resistance was broken by some isolates from the northwest of the country more readily than by any other isolate yet tested in Africa (Traoré et al. 2006; Pinel-Galzi et al. 2007). These isolates were similar to isolates of pathotype 2 by their host range, but different by their high rate of infection to *Rymv1-2* resistance and were subsequently considered to belong to a new pathotype. As these isolates originated from the same region as the resistant cv. Bekarosaka, this may reflect adaptation to the *Rymv1-2* resistance. This pathotype, transmitted readily both to susceptible and to highly resistant cultivars, is a major challenge to the durability of cultivars introgressed with the *Rymv1-2* allele in the northwest of Madagascar and possibly elsewhere if it occurs or spreads in the rest of the country.

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