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Durability of natural and transgenic resistances in rice to *Rice yellow mottle virus*

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Abstract

A monogenic recessive resistance to *Rice yellow mottle virus* (RYMV) found in the *Oryza sativa indica* cultivar Gigante and in a few *Oryza glaberrima* cultivars provided a higher level of resistance than either a polygenic partial resistance found in some *japonica* cultivars which delayed symptom expression or transgenic resistances which were partial and temporary. This high resistance was overcome by several isolates, but the percentage of such virulent isolates in the fields was low. There was no relationship between the virulence of an isolate towards the high resistance and its aggressiveness in other cultivars. Isolates with either of the two components of pathogenicity – virulence and aggressiveness – were found in each strain and in all regions of Africa, in both wild and cultivated grass species. There was no loss of fitness of resistance-breaking (RB) isolates as they were not counter-selected, impaired or outperformed after serial passages in susceptible cultivars, even in mixture with avirulent quasi-isogenic wild type isolates. Resistance breaking was highly dependent on the amount of virus inoculated and on the mode of transmission. Implications of these results for the durability of the resistances to RYMV and for the development of integrated disease management strategies are discussed.

Introduction

Rice yellow mottle virus (RYMV), of the genus *Sobemovirus* (Tam and Truve, 2000; Fargette et al., 2004), causes a major disease of rice in Africa (Abo et al., 1998; Calvert et al., 2003). Several strains of the virus having different geographic distributions have been described (Abubakar et al., 2003; Traoré et al., 2005). The natural host range is confined to species of the Oryzeae tribes and to a few wild grass species (Bakker, 1974; Konaté et al., 1997). RYMV is transmitted by several species of beetles (Coleoptera), most of which belong to the Chrysomelidae (Bakker, 1974), and it is not seed-transmitted (Konaté et al., 2001). Additional means of biotic transmission by rats and other vertebrates have been described recently (Sarra and Peters, 2003). Abiotic transmission by mechanical means during cultural operations or through soil residues and by irrigation water have also been reported (Abo et al., 2000; Sarra et al., 2004). Infected plants show characteristic mottling and yellowing of the leaves that are associated with stunting, partial emergence of the panicles and sterility. Almost total yield losses have been reported in several cultivars of *Oryza sativa* infected at an early stage of growth (Abo et al., 1998).

Since RYMV management through vector control using insecticides is undesirable on environmental and economical grounds and changes in cultural practices are only partially effective in restricting virus spread, the development of resistant cultivars is an important objective in rice-breeding programmes in Africa. Currently, three types of resistance to RYMV are known: partial natural resistance, high natural resistance, and

resistance obtained through genetic transformation. Partial resistance, associated with tolerance in rice *Oryza japonica* cultivar (cv.) Azucena and a few other *japonica* cultivars, is expressed by a delay in virus accumulation and in symptom expression; a resistance to virus movement is suspected (Ioannidou et al., 2003). Partial resistance is polygenic. A major quantitative trait locus (QTL) was identified on chromosome 12 (Ghesquière et al., 1997; Albar et al., 1998; Pressoir et al., 1998) and was introgressed from cv. Azucena into the *Oryza indica* cv. IR64 (Ahmadi et al., 2001; Ioannidou et al., 2003). High resistance, characterised by a low virus titre and the absence of symptoms, is expressed in the *O. indica* cv. Gigante and in a few cultivars of *Oryza glaberrima* (Ndjiondjop et al., 1999). High resistance involves a single recessive gene *rymv1* located on chromosome 4 (Ndjiondjop et al., 2001; Albar et al., 2003) which was introgressed from cv. Gigante into cv. IR64 (Albar et al., 2003). Transgenic lines have been obtained by inserting the viral polymerase gene into the susceptible *O. indica* cv. Bouaké189. The resistance is due to an RNA-based mechanism associated with post-transcriptional gene silencing (PTGS) (Pinto et al., 1999).

There have been reports of the natural occurrence of resistance-breaking (RB) RYMV isolates (Konaté et al., 1997) and of the emergence of virulent isolates after serial passages in resistant cultivars (Fargette et al., 2002). In the study reported here, the effectiveness and durability of the resistances to RYMV were assessed in relation to the phylogeny, fitness and transmission of RB isolates. Firstly, a comparison was made of the responses to RYMV inoculation of natural, isogenic and transgenic rice lines reported to be resistant. Secondly, the relationships between the pathogenic characteristics of the RYMV isolates and their phylogenic, geographic and host origin were assessed. Thirdly, it was tested whether RB isolates suffered fitness losses. Fourthly, the role of the means and conditions of transmission on the breakdown of resistance was investigated. Implications for resistance durability to RYMV in rice are discussed.

Materials and methods

Resistance of natural, isogenic and transgenic lines

The responses to RYMV infection of natural, isogenic and transgenic rice lines were assessed in cultivars including highly susceptible (*O. sativa indica* cv. IR64), partially resistant (*O. sativa japonica* cv. Azucena) and highly resistant (*O. sativa indica* cv. Gigante, and *O. glaberrima* cv. Tog 5681) genotypes. Isogenic lines 12 and 4 were included in the studies. Isogenic line 12 was obtained by introgressing the major QTL of the partial resistance carried on chromosome 12 from cv. Azucena into cv. IR64 (Ahmadi et al., 2001; Ioannidou et al., 2003). Isogenic line 4 was produced by introgressing the high resistance gene from cv. Gigante into cv. IR64 in the course of the genetic mapping of the gene conferring high resistance from Gigante (Albar et al., 2003). Transgenic lines 6 and 10 (T_L6 and T_L10), reported to be partially resistant and immune, respectively, were also included in the studies (Pinto et al., 1999). These lines were obtained by inserting the viral polymerase gene of an RYMV isolate from Nigeria (S1-ca strain) into the highly susceptible *O. indica* cv. Bouaké189 (Pinto et al., 1999).

All lines were inoculated with the reference isolate Mg1 selected for its pathogenic properties representative of most field isolates. Specific RB isolates were also used to challenge the partial and high resistances of the natural and isogenic lines. Transgenic lines were also inoculated with isolates of the same strain as that used for the transgene (S1-ca) as well as with isolates of other strains originating in west (S1-wa and S2) or east Africa (S4) having higher genetic distances with the transgene (Abubakar et al., 2003). The symptom expression of all lines was followed fortnightly during 2 months and scored according to a 0–3 scale for absence (0), mild (1), intermediate (2) and severe symptoms (3), respectively. Moreover, the virus content of natural and transgenic lines was assessed by ELISA 3 and 6 weeks after inoculation. In all experiments, plants were grown in a glasshouse under controlled conditions (28–32 °C, 13 h of light per 24 h). For each plant, the leaf before the last one produced was tested. Inoculation and ELISA tests were conducted as described in Ioannidou et al. (2000).

The following nomenclature was adopted. Any isolate that induced symptoms in the highly resistant cvs Gigante and Tog 5681 or in the isogenic lines with the high resistance gene *rymv1* was termed virulent, and the others were referred to as avirulent. The aggressiveness of the isolates were

scored 0–3 according to symptom intensity on other cultivars, whether susceptible or partially resistant. Wild isolates were designated field isolates as opposed to evolved isolates obtained after serial inoculations in glasshouse trials. Some isolates used in our experiments which break partial or high resistance (RB isolates) emerged after serial inoculation of wild isolates on cvs Azucena and Gigante, respectively (Fargette et al., 2002). As these differed from their respective wild types by very few substitutions (A. Pinel and D. Fargette, unpublished data), the wild type isolates were referred to as the avirulent quasi-isogenic wild type.

Components of pathogenicity

A sample of 58 virus isolates, collected in fields of all rice-producing regions of Côte d'Ivoire, were each inoculated onto a range of eight cultivars including the highly resistant cv. Gigante, the partially resistant cvs Moroberekan, Lac23, Faro11 and ITA235 and the susceptible cvs Bouaké189, H232 and PNA647F4. Twelve plants of each cultivar were inoculated with each isolate and the experiment was duplicated. The concentration of the inoculum was adjusted by ELISA tests through serial dilutions of sap extract. Disease incidence and symptom intensity were assessed 2, 3 and 4 weeks after inoculation and the symptoms scored 0–3. Relationships between the responses of the cultivars were assessed by principal components analysis (PCA) (Wilkinson, 1992).

Pathogenicity and phylogeny

Relationships between the pathogenicity and the phylogeny of RYMV isolates were studied. The coat protein (CP) gene of a sub-sample of 17 of the 58 isolates, representative of the range of pathogenic responses, was then sequenced as described by Pinel et al. (2000). The CP gene was used as a reliable marker of the phylogenetic relationships between isolates without any assumption of its role as a pathogenic determinant (Abubakar et al., 2003). In order to enlarge the study, additional isolates were included whose virulence and aggressiveness had been assessed in earlier experiments (N'Guessan et al., 2001; Fargette et al., 2002). The coat protein gene of these isolates was sequenced. In all, the pathogenicity and the phylogeny of 41 isolates were compared, including five isolates from four wild gramineaceous host species *Oryza longistaminata*, *Oryza barthii*, *Echinocloa colona* and *Sacciolepis africana*. The phylogenetic relationships among these 41 isolates were reconstructed after alignment of the sequences of the coat protein gene under Clustal W, and using the Kimura-2 distance method with the neighbour-joining agglomeration procedure; bootstrap values at the main nodes are given.

Fitness of resistance-breaking (RB) isolates

Fitness of partial and high RB isolates was assessed directly and indirectly in three ways. Firstly, the RB isolates were inoculated serially five consecutive times to the susceptible cv. IR64. After each passage, the evolved isolates were back-inoculated to cvs Azucena or Gigante, the symptoms assessed and the virus content estimated by ELISA to test whether the isolates retained their ability to break the resistances despite propagation in a susceptible host. Secondly, the RB isolates were mixed with a similar amount of their respective wild type isolates after adjustment by serial dilution and ELISA tests. The resulting mixture was serially inoculated five times onto the susceptible cv. IR64. After each passage, back-inoculations were made onto the resistant cultivars and the virus content was estimated by ELISA. We tested whether the evolved isolates retained their ability to multiply and to induce symptoms in the resistant cultivars after propagation when in competition with their respective quasi-isogenic wild type isolates in a susceptible host. Thirdly, the virus concentration of the RB isolates in the susceptible cultivar cv. IR64, as assessed by ELISA tests, was compared with that of the corresponding quasi-isogenic wild isolates to determine whether they multiplied similarly. For each treatment or passage, four pots each containing five plants were tested.

Transmission of resistance-breaking (RB) isolates

To take into account the diversity of modes and conditions of transmission of RYMV in the fields, we tested the response of cv. Gigante after inoculation of virulent isolates at different concentrations and using different inoculation procedures. For each treatment, four pots each containing five plants were tested. The experiment was replicated

twice. Inoculation was performed using mechanical inoculations to leaves and roots. Different dilutions of the inoculum ranging from 1:10 to 1:100 00 (g ml^{-1}) in leaf extraction buffer were tested. For transmission to leaves, the virus was introduced either by puncture or by rubbing the entire leaf surface with inoculum mixed with carborundum. For transmission to roots, these were rubbed with the virus extract. Symptom expression in cv. Gigante was followed over time and the virus content of the plants was assessed by ELISA 4 weeks after inoculation.

Results

Resistance of natural, isogenic and transgenic rice lines

Table 1 summarises the range of responses to RYMV infection of natural, isogenic and transgenic rice lines. In the susceptible cv. IR64, symptoms developed as early as 1 week after inoculation, and gradually became more pronounced. Cultivar Azucena combined a partial resistance at the early stage of infection with delayed symptom expression and tolerance at later stages manifest by moderate symptoms. The high resistance of cvs Gigante and Tog 5681 was characterised by the complete absence of symptoms at any time after inoculation. By contrast, partial and high RB isolates induced conspicuous symptoms in cvs Azucena and Gigante, respectively. The response of the isogenic lines 12 and 4 to both reference and RB isolates expressed the features of partial and high resistances of cvs Azucena and Gigante, respectively (Table 1).

Transgenic line 10 expressed a partial resistance 1–3 weeks after inoculation comparable to that of isogenic line 12. Transgenic line 6 had a response intermediate between transgenic line 10 and the susceptible cv. IR64 (Table 1). Transgenic lines 6 and 10 each had an ELISA absorbance reading significantly lower than cv. IR64 and similar to cv. Azucena 3 weeks after inoculation. By contrast, 6 weeks after inoculation, their virus content was similar to cv. IR64 and higher than cv. Azucena. At any time, the virus content of the transgenic lines was higher than that of the highly resistant cvs Gigante and Tog 5681 (Figure 1). The resistance was partial after inoculation of homologous isolates Ni1 and Ni3 of the S1-ca strain as well as

Table 1. Symptom severity[1] throughout the course of infection of susceptible, partially or highly resistant cultivars, isogenic lines and transgenic lines after inoculation with reference or resistance-breaking (RB) isolates

Genotype	Resistance level	Isolate[2]	Number of weeks after inoculation					
			1	2	3	4	5	6
IR64 (*O. sativa indica*)	Susceptible	Reference	0	2	2	3	3	3
Transgenic line 6[3]	Susceptible	Reference	1	1	2	3	3	3
Transgenic line 10[3]	Partial	Reference	0	0/1	1	3	3	3
Azucena (*O. sativa japonica*)	Partial	Reference	0	0	1	1	1	2
		RB	0	1	2	3	3	3
Isogenic line 12[4]	Partial	Reference	0	0	1/2	3	3	3
		RB	0	1	2	3	3	3
Gigante (*O. sativa indica*)	High	Reference	0	0	0	0	0	0
		RB	0	0	0/1	1/2	1/2	1/2
Isogenic line 4[5]	High	Reference	0	0	0	0	0	0
		RB	0	0	0/1	1/2	1/2	1/2
Tog 5681 (*O. glaberrima*)	High	Reference	0	0	0	0	0	0
		RB	0	0	0/1	1/2	1/2	1/2

[1]Symptom severity was scored as followed: '0' no symptoms, '1' weak mottle, '2' extensive mottle and yellowing, '3' stunting and generalised necrosis.
[2]For each genotype, the reference isolate Mg1 with pathogenic properties representative of most field isolates was inoculated.
[3]Transgenic lines 6 and 10 were constructed by inserting the viral polymerase gene into the *O. sativa indica* cv. Bouaké189 (Pinto et al., 1999).
[4]Isogenic line 12 was constructed by introgression of the partial resistance QTL12 from cv. Azucena into cv. IR64 (Ahmadi et al., 2001).
[5]Isogenic line 4 was constructed by introgression of the high resistance gene *rymv1* from cv. Gigante into cv. IR64 (Albar, et al., 2003).

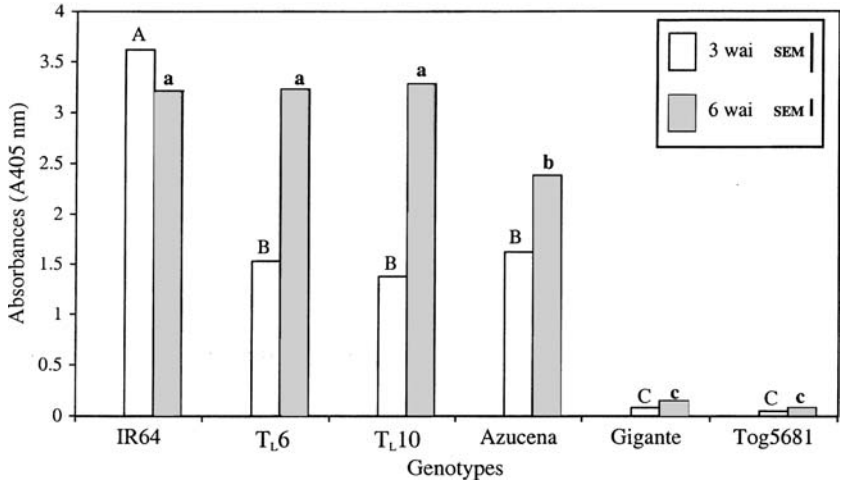

Figure 1. Absorbances in ELISA tests of extracts 3 and 6 weeks (white and grey histograms, respectively) after inoculation (wai) of transgenic lines (T_L6 and T_L10), and of susceptible cv. IR64, partially resistant cv. Azucena and highly resistant cvs Gigante and Tog 5681. The transgenic lines were constructed with the polymerase gene of an isolate of the S1-ca strain. All lines were inoculated with the isolate Mg1 of the distantly related strain S4. Different letters indicate significant differences in responses between genotypes at the 5% level 3 wai (capitals) and 6 wai (lower case); standard errors of the means (SEM) are indicated.

with genetically more distantly related isolates of the heterologous strains CI4 (S1-wa), Ma22 (S2) and Mg1 (S4).

Components of pathogenicity

The response of the highly resistant cv. Gigante differed markedly in two respects from that of susceptible or partially resistant cultivars. Firstly, a low percentage of isolates induced symptoms in cv. Gigante (3 of 58) which contrasted with the high percentage of isolates that induced symptoms in the partially resistant cultivars (30–50%, depending on cultivar) (data not shown). Secondly, there was no relationship between the virulence of an isolate to overcome the high resistance and its aggressiveness towards the other cultivars, whether susceptible or partially resistant. The differential pattern of response was apparent through PCA of the symptom intensity of the 58 isolates on the eight cultivars (Figure 2). The responses to infection of the susceptible and partially resistant cultivars were closely correlated with each other and formed the first axis of the PCA. This explained 52% of the variance and was interpreted as an aggressiveness axis which opposed severe to mild isolates. The response of cv. Gigante was not related to that of any of the other cultivars tested. The second axis of the PCA (14% of the variance) was explained mostly by the response of cv. Gigante and contrasted virulent and avirulent isolates and was designated as a virulence axis. The third axis opposed susceptible (Bouaké189, PNA647) to partially resistant cultivars (Faro 11, H232, Lac 23, 1TA235, Moroberekan) (data not shown).

Pathogenicity and phylogeny

Figure 3 illustrates the lack of relationship between the phylogeny and the pathogenicity, either aggressiveness or virulence, of the isolates. Three strains of RYMV were distinguished on the basis of their CP gene. Two strains grouped isolates from west Africa, from forest and savannah regions (S2 and S1-wa strains, respectively) and one strain grouped isolates from central Africa (S1-ca). Both virulent and avirulent isolates were found in the three strains. Similarly, severe and mild isolates occurred in each strain. This indicated that phytopathological traits of an isolate, be it virulence or aggressiveness, were not inherited from a common ancestor or confined to a specific strain or a particular source region, but occurred naturally in distinct lineages and in different parts of Africa. Furthermore, the full range of responses in pathogenicity was also found with isolates from wild grasses (Figure 3). In particular, one virulent isolate originated in *E. colona* and one in the wild

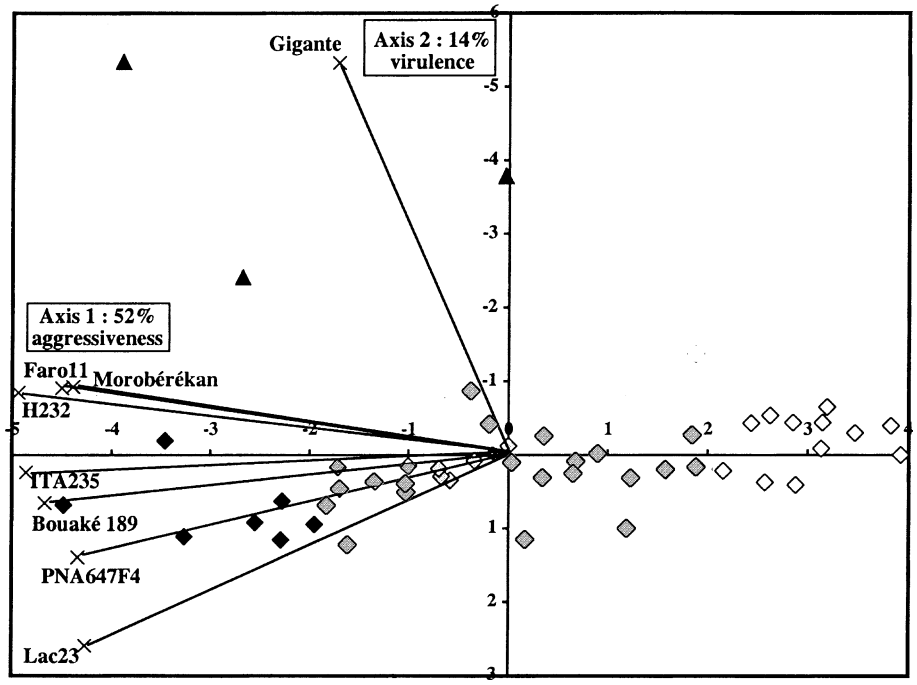

Figure 2. Assessment by principal component analysis of the relationships between the symptom intensity developed by eight rice cultivars 3 weeks after inoculation by each of the 58 RYMV isolates. Black triangles indicate virulent isolates. Diamond symbols refer to mild (white), severe (black) or intermediate (grey) avirulent isolates.

rice *O. barthii*, whereas one avirulent isolate was found in *O. longistaminata* and one in *S. africana*. Thus, virulence and aggressiveness were not confined to isolates from cultivated rice.

Fitness of resistance-breaking (RB) isolates

The occurrence of RB isolates in different lineages, locations and rice species, even though resistant rice cultivars have not yet been deployed in Africa, suggests that RB isolates are not counter-selected in the fields and that RB isolates do not suffer from loss of fitness when maintained on susceptible cultivars. This assumption was tested experimentally and measured in absolute and relative terms. Our results showed that RB isolates retained their ability to break the partial resistance of cv. Azucena or the high resistance of cv. Gigante despite several passages in the susceptible cv. IR64 (Table 2). This indicated that they were not counter-selected in a susceptible host. Moreover, the virus content in the resistant cultivars did not decrease after each passage (data not shown). Ability to break the resistance was maintained when RB isolates were mixed with a similar amount of their respective quasi-isogenic wild type isolates in the passage experiments (Table 2). This further showed that RB isolates were not displaced by the wild type. Last, the virus concentration in the susceptible cv. IR64 was similar after inoculation with either RB or wild isolates (data not shown) which showed that the multiplication of RB isolates was not impaired in a susceptible host. Overall, whatever the method of assessment used, partial or high RB isolates did not show fitness losses.

Transmission of resistance-breaking (RB) isolates

Virus leaf extracts of the virulent isolate were inoculated at different dilutions onto the leaves and the roots of the highly resistant cv. Gigante using various inoculation procedures. The proportion of plants exhibiting the typical mottling symptoms was assessed 6 weeks after inoculation. For each means of inoculation, the proportion of infected plants was greatest at the highest inoculum concentration and decreased

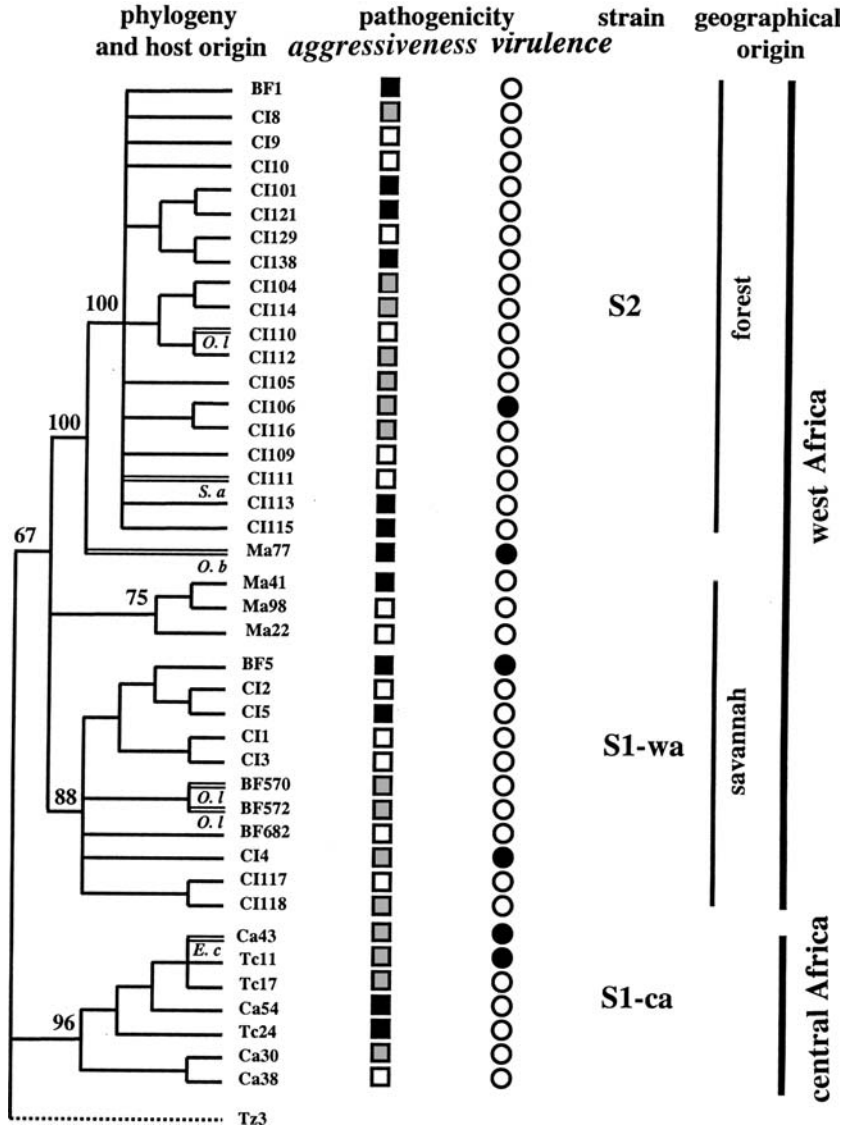

Figure 3. Relationships between the pathogenicity and the phylogeny of 41 RYMV isolates. Left: phylogenetic relationships of the isolates inferred from the CP gene sequences. Single lines indicate that the isolates were from the cultivated rice *Oryza sativa* and double lines indicate that the isolates originated from the wild gramineaceous hosts: *Echinocloa colona* (E. c) *Oryza barthii* (O. b), *Oryza longistaminata* (O. l) and *Sacciolepis africana* (S. a). Centre: aggressiveness and virulence of the isolates; aggressiveness was determined according to the symptom intensity induced in susceptible and partially resistant plants distinguishing mild (white squares), intermediate (grey squares), and severe (black squares) isolates. Virulence was determined from the response to infection of the highly resistant cv. Gigante by distinguishing virulent (black circles) from avirulent (white circles) isolates. Right: strains, geographical and ecological origins of the isolates.

rapidly with inoculum dilution (Table 3). The drop was quite sharp at a 10-fold dilution for leaf inoculation by puncture, at a 100-fold for root infection and at a 1000-fold for whole leaf inoculation. There was a similar decrease in symptom intensity and in virus content with inoculum dilution (data not shown). This indicated that the response of cv. Gigante to inoculation of virulent isolates was dose-dependent and subsequently that inoculum concentration was a key factor influencing the durability of the high resistance.

Table 2. Response of the resistant cvs Azucena and Gigante to inoculation of resistance-breaking (RB) isolates after serial passages on the susceptible cv. IR64

Cultivar	Resistance level	Isolate	Symptom severity on resistant cultivars[1]			
			Number of passages on susceptible cv. IR64			
			1	2	3	4
Azucena	Partial	Severe[2]	3	3	3	3
		Severe + mild[4]	3	2	3	3
Gigante	High	Virulent[3]	2	2	3	3
		Virulent + avirulent[4]	1/2	1	3	3

[1]Symptom severity was scored as followed: '0' no symptoms, '1' weak mottle, '2' extensive mottle and yellowing, '3' stunting and generalised necrosis.
[2]The severe isolate evolved from the mild wild isolate through five successive passages on cv. Azucena.
[3]The virulent isolate evolved from the avirulent wild isolate through six successive passages on cv. Gigante.
[4]The RB isolates were mixed with a similar amount of their respective quasi-isogenic wild type isolates.

Table 3. Percentage of plants of the highly resistant cv. Gigante showing mottle symptoms[1] after inoculation of different dilutions of a virulent isolate according to various inoculation modes

Mode of inoculation	Dilution of the inoculum[2]				
	1	10	100	1000	10,000
Roots[3]	75	85	10	10	10
Leaves[4] (a)	78	70	56	18	13
Leaves[5] (b)	45	10	5	0	0

[1]40 days after inoculation.
[2]The starting inoculum (1 g/10 ml) was diluted serially using a 10-fold dilution factor.
[3]The whole root was rubbed.
[4]The whole leaf was rubbed.
[5]Leaf inoculation was performed by puncture.

Discussion

Although there have been several independent reports of resistance to RYMV in natural, isogenic and transgenic lines (Ndjiondjop et al., 1999; Pinto et al., 1999; Ioannidou et al., 2003), there have been no comprehensive comparative tests of the different sources of resistance using standard protocols. Our results confirmed previous findings on the characteristics of the partial resistance and of the high resistance among genotypes. Isogenic lines 12 and 4 showed the expected response of partial and high resistances, respectively, after inoculation by both reference and RB isolates, confirming that the QTL12 and the *rymv1* genes transferred were the genetic determinants of partial and high resistances found in cvs Azucena and Gigante, respectively.

The transgenic lines showed a less effective, partial and temporary resistance. Expression of resistance through gene silencing is highly dependent on experimental conditions (Agrawal et al., 2003). Moreover, the level of resistance of the transgenic lines may have been underestimated in our experiments as the transformed cv. Bouaké189 was somewhat more susceptible to RYMV infection than our susceptible control cv. IR64 (F. Sorho and D. Fargette, unpublished results). In addition, the P1 protein of RYMV is an inhibitor of gene silencing (Voinet et al., 1999), and differences in PTGS interference among isolates have recently been found (C. Siré and C. Brugidou, personal communication). Altogether, this may account for the discrepancies between our results and earlier reports (Pinto et al., 1999). Although field exposure to the virus may be a less

stringent test than our experimental procedure, our results did not support the claims that the transgenic lines have effective and durable resistance to RYMV whatever the strains and the virus concentration inoculated (Pinto et al., 1999). By contrast, isogenic lines having the high resistance gene *rymv1* introgressed into the highly productive cv. IR64 offered good prospects for the control of RYMV. Subsequently, durability of the natural resistances was assessed in relation to the pathogenicity, phylogeny, fitness and transmission of the virulent isolates.

Only a low proportion of natural RYMV isolates broke the high resistance. The ability to do so (virulence) and to induce pronounced symptoms (aggressiveness) in susceptible or partially resistant plants is not correlated. These results are consistent with earlier studies conducted with a set of isolates belonging to a different strain (Konaté et al., 1997). These results are also in line with earlier work which showed that the ability to break the high resistance of cv. Gigante and the partial resistance of cv. Azucena were not linked (Fargette et al., 2002). Virulent and avirulent isolates were found in all three RYMV strains encountered in west and central Africa and in both cultivated and wild hosts. Similarly, severe and mild isolates were found in each strain and in all regions, in both cultivated and wild grass species. Therefore, both components of RYMV pathogenicity – virulence and aggressiveness – occurred in different virus lineages and were not inherited from a common ancestor or restricted to a particular virus strain, geographic region, rice cultivar or grass species. Similar multiple independent acquisition of virulence by a strain of *Potato virus X* to break the resistance due to the Nb gene in potato has been postulated (Malcuit et al., 2000). In contrast, the Nx determinant had apparently evolved only once (Malcuit et al., 2000).

However, it cannot be inferred only from the presence of RB isolates that the high resistance will not be durable (Harrison, 2002). Studies on resistance durability should integrate information on the evolutionary potential of the RB isolates (Garcia-Arenal and Mc Donald, 2003) and on the overall ecology of the disease (Thresh et al., 2003). Highly resistant cultivars have not yet been deployed, which suggests that the virulent wild isolates were not counter-selected in the susceptible rice cultivars presently grown. Experimentally, the RB isolates did not suffer from fitness losses as there was no loss of ability to break the resistances after serial passages in susceptible cultivars, with or without a mixture of the quasi-isogenic avirulent wild type isolates. Furthermore, the multiplication of RB virus isolates was not impaired in a susceptible host. The RYMV situation contrasts with other viruses where fitness losses of RB isolates have been reported (Goulden et al., 1993; Jenner et al., 2002; Desbiez et al., 2003).

Lack of fitness losses of RB isolates is often interpreted as an indication of the short durability of resistances because they are favoured in resistant plants and not counter-selected in susceptible cultivars and subsequently may spread to all cultivars, whether resistant or not (Leach et al., 2001; Garcia-Arenal and McDonald, 2003). With RYMV, as the RB isolates were found in the absence of selection, it might be expected that their incidence would increase if the resistant cultivars were widely grown. However, assessment of durability should also integrate the general characteristics of the disease ecology (Lecoq et al., 2004; Thresh et al., 2003). In our study, resistance breakdown only occurred when a high inoculum concentration was used, whether transmission was to leaves or roots. Thus, resistance should be deployed within an overall integrated management package, including phytosanitation measures to destroy virus sources such as crop residues, infected weeds and diseased plants within nurseries in order to decrease the inoculum pressure. The durability of the high resistance gene in different genetic backgrounds and in combination with the partial resistance gene should also be tested.

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