Rice yellow mottle virus diversification impact on the genetic control of RYMV

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Abstract

Rice yellow mottle is the most important virus disease of rice in Africa. The disease is a major constraint due to its wide geographical distribution and the extent of yield losses it induces. The role of the causal agent *Rice yellow mottle virus* (RYMV) in virus–host interactions was studied in relation to the current knowledge of rice genetics. Most cultivated rice varieties are susceptible to the virus, but a monogenic and recessive high resistance has been found in two varieties of *Oryza sativa* and a few varieties of *O. glaberrima*. The high resistance *RYMV1* encodes an eukaryotic translation initiation factor eIF(iso)4G and at least five alleles have been identified. However, studies on virus diversity indicated the occurrence of virus pathotypes capable of overcoming the high resistance gene at rates up to 40%. Such pathotypes could also be generated experimentally. At molecular level, RYMV pathogenicity was associated with mutations in the viral protein genome-linked (VPg), which interacts with the eIF(iso)4G factor. Patterns of the breakdown of alleles *rymv1-2* in *O. sativa* and *rymv1-3* in *O. glaberrima* cv. Tog5681 differed greatly and were modulated by virus adaptation features found in the VPg. The specificity of virus–host interactions between RYMV and rice suggests that the deployment of resistant varieties should take into account a good knowledge of virus populations.

Introduction

Rice yellow mottle virus (RYMV) was observed for the first time in Kenya in the late 1960s (Bakker, 1970). The disease is now present in all or almost all African rice-producing countries (Kouassi *et al.*, 2005). It has never been reported outside of Africa. Since the beginning of the 1990s, RYMV has become a major constraint to rice production, mainly in west Africa, with losses that can reach 25–100% depending on the rice varieties (Abo *et al.*, 1998).

Cultivated rice belongs to two species *Oryza sativa* and *O. glaberrima.* Screening of many varieties of these two species as well as wild species of rice led to the identification of resistance sources. Types of resistance have been characterized in few rice varieties. There is, on the one hand, a partial resistance marked by a delay in the appearance of symptoms and in virus accumulation (Ioanidou *et al.*, 2000; Fargette *et al.*, 2002). It has polygenic determinism and has been identified in varieties of *O. sativa* subsp. *japonica*, e.g. Azucena. The second, high resistance is characterized by an absence of symptoms, very low virus accumulation and a blockage of virus movement (Ndjiondjop *et al.*, 1999). It is monogenic and recessive. Progress in rice genetics has revealed that the high resistance gene *RYMV1* has at least five alleles: *rymv1-1*, present in varieties susceptible to the virus; *rymv1-2* identified in Gigante and Bekarosaka varieties of *O. sativa* subsp. *indica* (Ndjiondjop *et al.*, 1999; Rakotomalala *et al.*, 2008); all the other alleles have been identified in *O. glaberrima*: Tog5681 (*rymv1-3*), Tog5672 (*rymv1-4*) and Tog5674 (*rymv1-5*). The gene *RYMV1* codes protein eIF(iso)4G that is the factor of the host interacting with the virus (Albar *et al.*, 2006). There are ongoing efforts to introgress the resistance conferred by the *RYMV1* gene into susceptible but yielding varieties.

RYMV has a great molecular diversity (Traoré *et al.*, 2005). There are more than six strains with distinct geographical distributions. Strains S1-AO and S1-AC are present in savannahs of west and central Africa, respectively. In west Africa, there is also a Sahelian strain (Sa) and a forest strain (S2/S3). Three other strains (S4, S5 and S6) have been identified in east Africa and Madagascar. Virus pathogenic diversity studies revealed the presence of RYMV isolates capable of avoiding the resistance conferred by the *RYMV1* gene (Konate *et al.*, 1997; Fargette *et al.*, 2002; Traoré *et al.*, 2006). The high occurrence of such isolates (40%) is an important parameter that can compromise the sustainability of resistance. The severity factor has been identified as being the virus protein linked to RYMV genome (VPg) (Hébrard *et al.*, 2008).

During this work, biological and molecular aspects of the interactions between rice and RYMV were studied for resistance alleles *rymv1-2* and *rymv1-3*. Results obtained show that resistance to RYMV can be sustainable if resistant varieties are deployed by taking into account the virus diversity in the deployment zone.

Material and methods

Rice varieties, inoculum sources and plant inoculations

The two rice varieties having the resistance allele *rymv1-2* (Gigante and Bekarosaka) and the variety Tog5681 having the *rymv1-3* allele were used. The experiments were conducted in insect-free screen houses at 28°C and 60% relative humidity. Representative virus isolates of the RYMV molecular diversity from different countries of the entire continent were used. For each virus source, the virus inoculum was prepared by crushing infected

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leaves in phosphate buffer 0.1*M* pH 7.2 (with 10 ml of buffer per 1 g of leaves). Carborundum 600-grid was added to the extract, which was then used to rub the leaves of young rice plants (aged 14 days). Appearance of symptoms was monitored every week for 6 months.

Determining VPg sequences and directed mutagenesis

The viral protein genome-linked was amplified by reverse transcription polymerase chain reaction (RT-PCR) as described by Pinel-Galzi *et al.* (2007). Nucleotide sequences were then determined by direct sequencing of amplification products. Sequence collection and analysis were carried out using bioinformatics software package Lasergene DNAStar. Mutations associated with resistance avoidance were identified. To validate the role of each of these mutations in the virus pathogenicity, corresponding changes were introduced in an infectious clone through direct mutagenesis (Hébrard *et al.*, 2008; Brugidou *et al.*, 1995). The infectious clone thus modified was then inoculated to rice plants and symptoms monitored as previously.

Results and discussion

Mutations associated with* rymv1-2 *allele avoidance

In total, 10 isolates out of 114 were able to avoid the resistance in the highly resistant rice variety Gigante. The VPg sequences (41 sequences) were determined in the plants that presented symptoms and were compared with those of the original isolates. Five nonsynonym mutations were located in a short region of 15 amino acids (aa) out of the 79 aa that make the VPg (Table 1). The implication of these mutations in avoiding the resistant allele *rymv1-2* was demonstrated by directed mutagenesis (Pinel-Galzi *et al.*, 2007). Candidate mutations were separately introduced in an infectious clone of RYMV which could not avoid resistance (Brugidou *et al.*, 1995). All candidate mutations introduced in the infectious clone allowed the avoidance of allele *rymv1-2* in Gigante or Bekarosaka.

The most frequent mutation was that of position 48. At this position, the arginine residue (R) was replaced by six other possible amino acids: tryptophan (W), glycine (G), glutamic acid (E), valine (V) or threonine (T). Monitoring the appearance of these mutations over time has shown a major scenario bringing into play R, G and E residues. In a first phase, the R residue of the avirulent isolate was replaced by the G residue (making the isolate virulent). In a second phase, the G residue was replaced by an E residue that, when fixed, was never replaced. Thus, the E residue appeared as more adapted to *rymv1-2* allele avoidance.

Table 1. Mutations of the viral protein genome-linked (VPg) involved in *rymv1-2* allele avoidance

RYMV isolate	Strain	Sequences after avoidance	Amino acid positions in the VPg†				
			38	42	43	48	52
Control	S1	1	R	N	T	R	H
Mli 203	S2	1	.	.	.	W	.
Mli202	Sa	1	Y	.	.	G	.
Mli 206	Sa	1	.	.	.	G	.
Mli 145	Sa	1	.	.	.	.	Y
CI4	S1	1	Q	.	.	E	.
CI4	S1	2	.	.	.	G	
CI4	S1	3	.	.	.	I	
CI4	S1	4	.	.	.	V	
BF5	S1	1	.	.		.	Y
Mg16	S4	1	.	Y	A	E	Y
Mg16	S4	2	.	.	.	G	
Mg16	S4	3	.	.	.	T	
Tz225	S4	1	.	.	.	E	.
Tz230	S4	1	.	.	.	G	.
Tz209	S6	1	.	.	.	E	.

† The dots indicate the presence of the same amino acids as the control isolate not able to avoid the resistance allele *rymv1-2*.

Mutations associated with* rymv1-3 *allele avoidance

In the case of allele *rymv1-3*, sequences of avoidance isolates were obtained by inoculating variety Tog5681. Analyzing mutations that occurred allowed identification of two nonsynonym mutation positions involved in resistance avoidance. These were positions 41 and 52. In position 41, the serine residue (S) present in the avirulent isolate was replaced by an alanine residue (A) or by a proline residue (P). In position 52, the histidine residue (H) was replaced by a tyrosine residue (Y). The individual introduction of mutations 41A and 41P in the infectious clone or their respective combination with mutation 52Y allowed us to reproduce the symptoms of RYMV in Tog5681. This confirmed that all three mutations were responsible for the capacity of RYMV isolates

to avoid the resistance allele *rymv1-3*. Position 52 appeared to be very important in the resistance avoidance, since mutation 52Y was associated with avoidance of allele *rymv1-2* as well as to that of allele *rymv1-3*.

Role of the polymorphism E/T in position 49 of the VPg

The capacity of RYMV isolates to undergo mutations leading to avoidance of alleles *rymv1-2* and *rymv1-3* has been found to be closely associated with polymorphism in position 49 of the protein sequence of VPg. In all RYMV isolates, two amino acids can be observed in this position. This is a glutamic acid residue (E) or a threonine residue (T). Isolates capable of avoiding *rymv1-2* had the entire residue 49E. None of these isolates were able to infect Tog5681, showing the inability to avoid *rymv1-3* when isolates have residue E in position 49 of the VPg. Avoidance of *rymv1-3* was achieved exclusively by isolates having residue 49T. A few isolates with residue 49T capable of avoiding *rymv1-3* were capable of avoiding *rymv1-2*, although the majority were not able to do so. Codon 49 of the VPg is under possible high selection, a situation that shows that mutations occurring at the level of this codon give a selective advantage to RYMV isolates. Pathogenicity contrast observed in isolates with residue 49E and those with residue 49T shows an adaptation of the 49E isolates to the genetic pool *O. sativa*. Conversely, isolates with residue 49T are adapted to genetic pool *O. glaberrima*. This adaptation to genetic pool modulates the propensity of RYMV to develop virulence capacities allowing them to avoid resistances.

RYMV adaptation to the gene pool *O. sativa* or to that of *O. glaberrima* indicates that the sustainability of the resistance to the virus depends closely to the resistance allele deployed and to the virus populations. Thus, the deployment of allele *rymv1-3* (gene pool *O. glaberrima*) in regions where the virus populations comprise isolates with residue 49E would result to resistance sustainability. Conversely, the deployment of allele *rymv1-2* (gene pool *O. sativa*) in a region dominated by virus isolates with residue 49E would favor the emergence of virulent variants and the nonsustainability of the resistance.

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