Molecular tools to predict resistance-breaking abilities of rice yellow mottle virus isolates

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Introduction

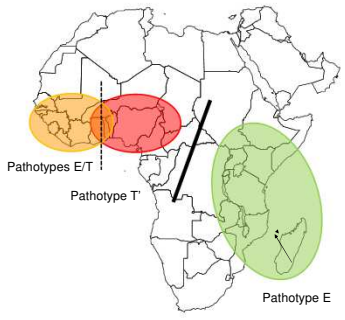


Fig 1 : Distribution of RYMV pathotypes (adapted from Hebrard et al 2018)

Rice yellow mottle virus (RYMV) is the most damageable rice virus in Africa. Previous studies revealed contrasted abilities of RYMV isolates to overcome resistance from African rice (*Oryza glaberrima*) vs. Asian rice (*Oryza sativa*). The codon 49 of the **viral protein genome-linked** (VPg) coding for a threonine T or a glutamic acid E played a major role in the adaptation to *O. glaberrima* and in the resistance-breaking specificities, thus defining **two pathotypes, named T and E**. Later, it has been showed that a subset of pathotype T isolates from West-Central Africa (strain S1ca), are able to adapt to all known highly resistant accessions, from both *O. sativa* and *O. glaberrima* species. These isolates defined **a new pathotype named hypervirulent T'**.

Up to now, **no molecular tool is available** to monitor the RYMV pathotypes, except sequencing to determine both the CP lineage and the VPg polymorphism.

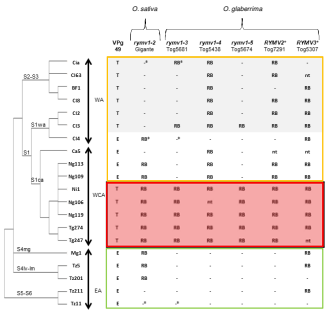


Fig 2 : Resistance-breaking abilities of RYMV isolates according to strain and VPg49 codon
S1wa, S2, S3 (West Africa) : pathotypes E/T
S1ca (West-Central Africa) : pathotypes E/T'
S4, S5, S6 (East Africa) : pathotype E
(adapted from Hébrard et al 2018)

Identification of specific SNPs discriminating pathotypes E/T/T'

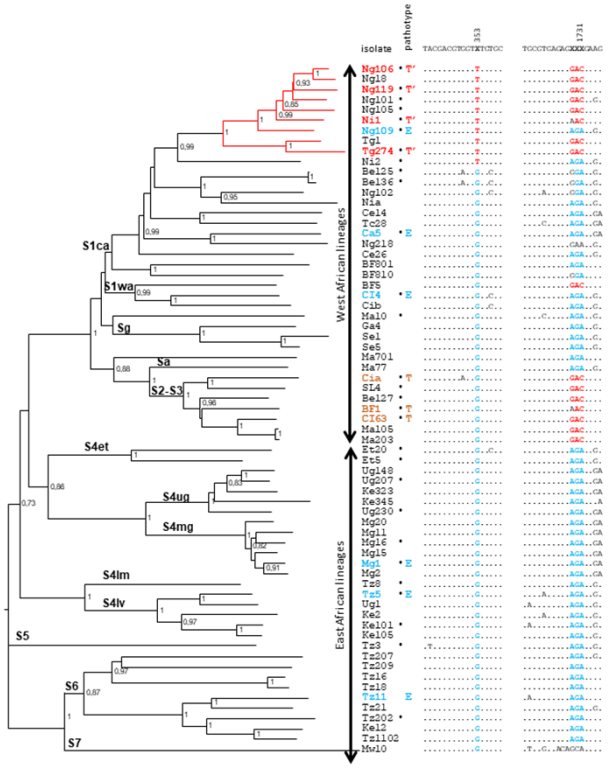
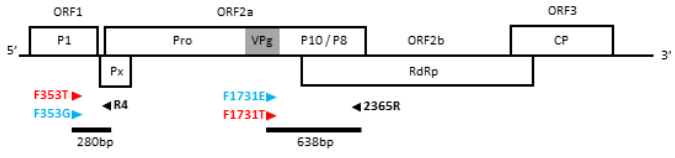


Fig 3: Phylogenetic tree of 67 genomic sequences representative of RYMV genetic diversity in Africa and partial alignements at polymorphic domains chosen to design specific primers.

Alignment of 67 full-length sequences of RYMV isolates representative of all the diversity has been analyzed. Discrimination of pathotypes E vs T/T' is based on the codon 49 of VPg, GAG/ACG at position 1731-1733. To identify the hypervirulent pathotype T', we found two strictly conserved positions in P1 and Cter domains at positions 353 and 2539.

Strain	codon VPg49	P1 353	VPg 1731	VPg 1732	VPg 1733	Cter 2539
S1ca*	T	T	A	C	G	T
	E	G	G	A	G	C
S1wa	T	G	A	C	G	C
	E	G	G	A	G	C
Sg-Sa	E	G	G	A	G	C
S2-S3	T	G	A	C	G	C
S4-S5-S6	E	G	G	A	G	C

Design of specific primer pairs



F353T 5'CCT-GTT-CAT-TAC-GAC-GTG-GTT3'
F353G 5'CCT-GTT-CAT-TAC-GAC-GTG-GTG3'
F1731E 5'ACC-TGG-GTG-CGT-GAG-AGA-GA3'
F1731T 5'ACC-TGG-GTG-CGT-GAG-AGG-AC3'

Fig 4 : Positions and sequence of primers on RYMV genomic organization

Validation of the molecular tools

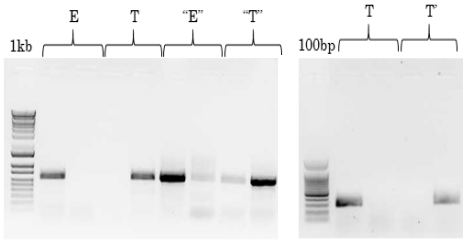


Fig 5. **Left panel.** Types of RT-PCR profiles obtained using F1731E/2365R and F1731T/2365R primer pairs in two independent reactions for each RYMV isolate. **Right panel.** Types of RT-PCR profiles obtained using F353T/R4 and F353G/R4 primer pairs in two independent reactions for each T isolate.

F1731E/2365R reactions	F1731T/2365R reactions	Number of isolates	Inferred pathotypes	Sequence validations
++	-	18	E	17/18
-	++	16	T/T'	16/16
++	(+)	4	"E"	4/4
(+)	++	9	"T/T"	9/9

F353T/R4 reactions	F353G/R4 reactions	Number of isolates	Inferred pathotypes	Phylogenetic validations
+	-	16	T	16/16
-	+	7	T'	7/7
-	-	2	ND	-

In a first step, fourty-seven RYMV isolates representative of the genetic and pathogenic diversity were analyzed with the primer pair designed to discriminate E vs T/T' pathotypes to assess the primers specificity. In a second step, the 22 isolates showing a profile T or "T" were analyzed with specific primers to identify pathotypes T/T'. These results were confirmed by sequencing or using phylogeny data.

Conclusion

We have developped simple molecular tools to discriminate pathotypes E/T/T'. As these pathotypes are characterized by different resistance breaking abilities, these tools will allow to predict RB probability in field at a local scale and to support rice breeders to deploy resistant rice varieties.

Note that all the previous RB experiments have been performed in greenhouse, in controlled conditions and with highly concentrated inocula. Thus, these tools will help to the rice producer to minimize the risk of RB in field according to the virus diversity present at the locale scale, but it will be important to evaluate the resistance durability in field.

Reference : Hébrard E, Pinel-Galzi A, Oludare A, Poulicard N, Aribi J, Fabre S, Issaka S, Mariac C, Dereeper A, Albar L, Silué D, Fargette D. Identification of a Hypervirulent Pathotype of Rice yellow mottle virus: A Threat to Genetic Resistance Deployment in West-Central Africa. Phytopathology. 2018 Feb;108(2):299-307.