

# **Characterization of Brazilian tobacco Potato virus Y (PVY) isolates: identification of a new group in the PVY species**



#### Frédéric BOULARD<sup>1,3</sup>, Bérenger JANZAC<sup>2,3</sup>, Emmanuel JACQUOT<sup>3a</sup> and Laurent GLAIS<sup>1,3</sup>

<sup>1</sup> Fédération Nationale des Producteurs de Plants de Pomme de Terre (FN3PT), 43-45 rue de Naples, 75008 Paris, France; <sup>2</sup> Imperial Tobacco Group, SEITA, Institut du Tabac, Domaine de la Tour, F-24100 Bergerac, France; <sup>3</sup> INRA, UMR1349 IGEPP, 35653 Le Rheu, France; <sup>a</sup> present address: INRA-Cirad-Montpellier SupAgro, UMR BGPI, 34398 Montpellier, France

Potato virus Y (PVY, genus Potyvirus, family Potiviridae) is known to be one of the most variable RNA plant viruses. The variability and distribution of PVY populations have been extensively studied in Europe and North-America while such type of data is still scarce for PVY isolates from South America. A survey performed in 2007 in Brazilian tobacco fields has previously shown that most of PVY isolates were unconventional (PVY<sup>U</sup>), because they are not detected by two commercial PVY<sup>N</sup> and PVY<sup>O/C</sup> monoclonal antibodies, and they are not able to infect PVY-susceptible potato cultivar (cv. Bintje) and pepper (cv. Yolo Wonder). Here, we present new analyses performed on PVY<sup>U</sup> isolates to improve knowledge on their features.

## **Antigenic properties**

### A) Serotyping

 Brazilian PVY tobacco isolates were serologically characterized with 4 monoclonal antibodies (Mab)



 Only two Brazilian PVY isolates were assigned to the N (isolate 07/1061) and the O (isolate 07/1054)

used as references in PVY serotyping procedure:

- 1 Mab anti-PVY (FN3PT/INRA)
- 2 Mabs anti-PVY<sup>N</sup> (FN3PT/INRA and Bioreba)
- 1 Mab anti-PVY<sup>O/C</sup> (Neogen)
- In addition, these isolates were analysed with 18 Mabs from a new library of antibodies raised against PVY<sup>N</sup> or PVY<sup>O</sup> isolates.

**Figure 1.** Schematic representation of the clustering of Brazilian tobacco PVY isolates (07/10..) based on serological patterns obtained with 18 monoclonal antibodies. N605, 06B14: referent PVY<sup>N</sup> isolates; O139, Oirl: referent PVY<sup>O</sup> isolates.

serotypes.

- Most of the Brazilian PVY isolates were not detected by the 4 reference monoclonal antibodies.
- However, 2 out of the 18 Mabs of the recently developped library are able to detect PVY<sup>U</sup> Brazilian isolates.

#### B) Coat protein amino acid sequences diversity

AA position in C	P sequence	• • •		••••	• • • • •	11.		20		30		•••	40
Serotype-N -{	N605 07/1061	GND	T I D	AGGS	TKK	DAK	QE(	QGS I	QPNLI	- · · · · ·	KDVN	IVG	TSGT
Serotype-O -{	0139 07/1054	A A			N	. T . 	Р. Р.	.s	. S . F . S . F	.G.D		A - A -	
Referent PVY <sup>c</sup> isolate	SON41	A		EN	۱s		Ρ.		. <b>R</b> . F	G . D		A .	
Unconventional serotype	07/1024 07/1065 07/1059 07/1057 07/1037 07/1046 07/1044 07/1003 07/1013 07/1050 07/1055 07/1008 07/1034								. QSF . QSF . QSF . QSF . SGA . SGA . SGA . SGA . SGA . SGA . SGA . SGA	A			
		' I	$\checkmark$		~~ ~	~	$\checkmark$	<b>└</b>					

- Particular features observed in the N-terminal region of the Brazilian PVY CP protein could explain their unconventional serological properties.
- As observed in PVY<sup>O/C</sup> N-terminal CP region, PVY<sup>U</sup> isolates displayed an Alanine residue at the first position.
- Three of PVY<sup>U</sup> isolates (07/1024, 07/1065, 07/1059) displayed a coat protein-N-ter sequence closer to PVY<sup>O</sup> and/or PVY<sup>C</sup> isolates (residues  $S_{11}$ ,  $P_{17}$ ,  $P_{26}$  and  $A_{36}$ ).
- Most of the analysed PVY<sup>U</sup> isolates have a deletion of one amino acid (AA<sub>22</sub>) and presented 10 specific residues in their coat protein sequence.

#### 

First amino acid characteristic of N type (G) or O/C type (A)

**Figure 2.** Amino acid alignment of the N-terminal part (AA 1 to 40) of the coat protein (CP) of referent and Brazilian PVY isolates. Sequences of N605 (X97895), O139 (U09509) and SON41 (AJ439544) correspond to referent N, O and C isolate, respectively.  $\bigstar$ : specific amino acid to some Brazilian PVY<sup>U</sup> isolates. Amino acid in the black box correspond to deleted residue in some PVY<sup>U</sup> isolates. Amino acid in the black box correspond to deleted residue in some PVY<sup>U</sup> isolates. Amino acid in the red boxes correspond to specific PVY<sup>O</sup> and /or PVY<sup>C</sup> amino acid present in CP sequence of three PVY<sup>U</sup> isolates.

### Virulence and aggressiveness on potato

- 12 Brazilian PVY isolates were mechanically inoculated to PVY-susceptible potato cultivars (Bintje and Béa) and to varieties carrying hypersensitive resistance genes to PVY<sup>o</sup> (Desirée (Ny<sub>tbr</sub>)), PVY<sup>c</sup> (Eersteling (Nc)) or to PVY<sup>z</sup> isolates (Maris Bard (Nz)).
- 6 out of the 12 Brazilian PVY isolates were non infectious on potato.
- As does PVY<sup>o</sup> isolates, 07/1050, 07/1059, 07/1065 and 07/1054 isolates induced an HR reaction in cvs. Désirée and Maris Bard.
- As does PVY<sup>z</sup> isolates, 07/1061 isolate induced an HR reaction in cv. Maris Bard only, and is able to induce tuber necrosis symptomes in cv. Béa.

<b>PVY isolate</b>		Tobacco				
	Désirée (Ny <sub>tbr</sub> )	Eersteling (Nc)	Maris Bard (Ny <sub>tbr</sub> , Nc, Nz )	Bintje	Béa	Xanthi
N605	S	HR	~	S	S	VN
0139	HR	HR	~	S	S	
07/1003	~	~	~	~	~	
07/1034	~	~	~	~	~	
07/1037	~	~	~	~	~	- Mo
07/1044	~	~	~	~	~	
07/1055	~	~	~	~	~	
07/1057	~	~	~	~	~	
07/1046	HR	~	~	~	~	VN
07/1065	HR	~	HR	~	~	
07/1059	HR	~	HR	~	~	
07/1050	HR	HR	HR	HR	~	- Mo
07/1054	HR	S	S	S	~	
07/1061	S	~	HR	S	S +PTNRD	

**Table 1.** Biological properties of 12 Brasilian PVY isolates on potato and tobacco. Five potato cultivars (Désirée, Eersteling, Maris Bard, Bintje and Béa,) and the tobacco cv. Xanthi (two plants per isolate/host combination) were used as host indicators in the experiement. ~ : asymptomatic plant ; HR: hypersensitive resistance symptom ; S: systemic viral symptom; Mo: mosaic; VN: vein necrosis; PTNRD: Potato Tuber Necrotic Ringspot Disease. The red boxes corresponds to the proportion of systemic infection for each isolate/host combination.

### - Molecular properties

- The genome of 07/1034 isolate seemed to result from recombination event(s) between a PVY<sup>N</sup> isolate (HC-Pro, VPg regions) and one isolate sharing an amino acid sequence neither N-or O/C-type.
- The full length genome of 07/1046 seemed to be closer to PVY<sup>0</sup>/PVY<sup>c</sup> referent PVY isolates.



**Figure 3.** Percentage of amino acid identity in HC-Pro, CI, VPg and CP regions (illustrated in grey) of the PVY genome between Brazilian PVY<sup>U</sup> isolates (07/1034 and 07/1046), and referent PVY<sup>N</sup> 605, PVY<sup>O</sup>139 and PVY<sup>C</sup> SON41 isolates were used to calculate the presented percentage.

## **Conclusions and Prospects**

- PVY<sup>U</sup> isolates from tobacco in Brazil are not detected with referent Mabs and not infectious on some potato cultivars.
- The genome of PVY<sup>U</sup> isolates display specific features in their coat protein sequence (e.g E<sub>9</sub> and/or deletion of residue 22).
- Two monoclonal antibodies recently developped allowed the detection of some PVY<sup>U</sup> isolates (i.e isolates encoding a CP without residue 22).
- The whole sequence of PVY unconventional genomes and the virulence in different host plants (tobacco, ...) would be available soon.

