

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF TUNISIAN ISOLATES OF POTATO LEAFROLL VIRUS

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SUMMARY

Nine Tunisian isolates of *Potato leafroll virus* (PLRV) were collected from potato plants (cv *Spunta*) with similar secondary leafroll symptoms. These PLRV isolates were readily transmitted by *Myzus persicae* (Sulzer) from potato plants to *Physalis floridana* test plants inducing symptoms of differing severities. In order to investigate the genetic variability between the PLRV isolates, the ORF0 regions of the Tunisian PLRV isolates were sequenced and compared with that of a French isolate (Fr5) that induces mild symptoms and 24 ORF0 PLRV sequences available in Genbank. The Tunisian isolates could be classified in two of the three groupings of the phylogenetic tree obtained. A tentative correlation between symptom expression in *P. floridana* and specific changes in the ORF0 sequences is discussed.

Key words: ORF0 sequence, *Myzus persicae*, *Physalis floridana*, potato, PLRV symptoms.

INTRODUCTION

Potato leafroll virus (PLRV) is the type species of the genus *Polerovirus*, family *Luteoviridae*. It infects potato crops worldwide and can cause significant damage (Robert and Lemaire, 1999). In Tunisia, PLRV is one of the main constraints for potato seed multiplication and production causing 27 to 35% yield losses (Ben Khedher *et al.*, 1998). PLRV is a persistent, circulative and non-propagative aphid-transmitted virus and *Myzus persicae* (Sulzer) is its most efficient and important vector (Harrison, 1984).

Biological variability among PLRV isolates is rather limited. Strains were first identified according to the virulence and severity of symptoms induced in potato and *Physalis floridana* (Harrison, 1984). When infected potato seed is planted, symptoms of secondary infection

consist typically of rolling, reddening or yellowing of leaves and stunting (reviewed by Syller, 1996). The symptom severity in *P. floridana* is not always parallel to that in the potato, which can depend on the potato cultivar, PLRV strain and environmental conditions (Syller, 1985; Rouzé-Jouan *et al.*, 1992a).

PLRV particles contain a monopartite RNA genome of ~6 kb that encodes six open reading frames (ORFs) (reviewed in Miller *et al.*, 1997). The 5'-proximal ORF0 is translated directly from the genomic RNA and encodes a putative 28 kDa protein, P0. There are strong similarities among the nucleotide sequences of geographically different PLRV strains and there is little diversity among their biological properties (Palucha *et al.*, 1994). ORF0 seems to be the least conserved coding region among poleroviruses (Guyader and Giblot-Ducray, 2002).

Little work has been done on trying to correlate PLRV symptoms and ORF0 sequences. Thus, the objective of this study was to sequence the ORF0 regions of the Tunisian PLRV isolates and compare them with that of a French isolate (Fr5) that induces mild symptoms and with 24 ORF0 PLRV sequences available in Genbank. A tentative correlation was observed between symptoms induced in *P. floridana* and changes in the ORF0 sequence.

MATERIALS AND METHODS

Virus isolates and aphid transmission. A collection of nine Tunisian PLRV isolates was obtained by aphid transmission to *P. floridana* from naturally infected potato plants (cv *Spunta*) collected in three Tunisian areas in winter 2002 (Table 1). The collected potato plants showed symptoms characteristic of secondary infection by PLRV that were of similar intensities. Four isolates came from Mahdia (central eastern Tunisia), one from Bizerte (Northern Tunisia) and four from Tunis (north eastern Tunisia) (Table 1). Two PLRV isolates, previously classified as very severe (CU87; Rouzé-Jouan *et al.*, 1992b) and very weak (Fr5; Robert *et al.*, 1990), were used as controls.

Transmission of PLRV was carried out under controlled conditions (Robert *et al.*, 1969) with *Myzus persi-*

Table 1. Comparison of the Tunisian PLRV isolates with regard to symptom expression in *P. floridana* and specific changes of the ORF0 sequences. GenBank accession numbers related to these isolates are included. ELISA and the transmission efficiency of each tested isolate were assessed. Accordingly, only inconsistent differences in aphid transmissibility were detected between the nine Tunisian PLRV isolates and the PLRV concentration in test plant. The severity of symptoms caused by each PLRV isolate was evaluated separately on each plant with the use of a 5-grade scale (N) for *P. floridana*. Underlined names correspond to the newly sequenced isolates.

PLRV isolates	Geographic origin	Severity of symptoms in <i>P. florifana</i> (N)	ELISA OD values	Specific changes (position 556) of the nucleotide ORF0 sequence	GenBank Accession No.
<u>is₅</u>	Mahdia	Very severe (5)	0.307	GAG	AY645677
<u>is₆</u>	Mahdia		0.717		AY645682
<u>is_{SAM}</u>	Bizerte		0.164		AY645685
Cu87	Cuba		0.405		-
<u>is₄</u>	Mahdia	Severe (4)	0.699	ACG	AY645681
<u>is₁₆</u>	Mahdia		0.779		AY645678
<u>is₂₄</u>	Tunis	Mild (3)	0.412	ACG	AY645683
<u>is₂₆</u>	Tunis		1.842		AY645679
<u>is₂₉</u>	Tunis		1.100		AY645684
<u>is₃₀</u>	Tunis		0.555		AY645680
Fr5	France	Very weak (1)	0.833	AAG	-

caec LCSA (a French clone; Bourdin *et al.*, 1998) as an efficient vector. Apterous adults were given a 3 days acquisition aphid period (AAP) on infected potato plants.

Then, aphids were transferred in groups of 3 to healthy *P. floridana* plants for a 3 days inoculation aphid period (IAP). After a 2-3 weeks incubation period, inoculated plants were checked for the typical symptoms (dwarfing, stunting and interveinal chlorosis) of virus infection and by DAS-ELISA (Clark and Adams, 1977). The severity of symptoms caused by each PLRV isolate was evaluated separately on each inoculated *P. floridana* plant with the use of a 5-grade scale. Each isolate was classified as inducing very weak (1), weak (2), mild (3), severe (4) or very severe (5) symptoms (Syller, 1985).

Enzyme-Linked Immunosorbent Assay (ELISA).

Collected potato leaves, tubers and inoculated *P. floridana* test plants were checked for PLRV infection by double antibody sandwich (DAS) ELISA (Clark and Adams, 1977). Leaf extracts were prepared in 3 volumes (w/v) of PBS pH 7.4 containing 0.05% Tween 20 and 2% polyvinylpyrrolidone. The tests were done in wells of microtitre plates (NUNC®, Roskilde, Denmark). They were coated with 0.5 µgml⁻¹ solution of immunoglobuline (Igs) purified from a PLRV-specific polyclonal antiserum. Alkaline phosphatase-conjugated monoclonal Igs were used as a second antibody layer at 1/1000 dilution. After 2 h of incubation with substrate at room temperature, A_{405nm} readings of OD with plate

blank subtracted were recorded using a computer connected to a Precision Microplate Emax Reader (Molecular Devices, Palo Alto, USA). Samples were considered positive when they exceeded twice the mean A_{405nm} mean readings from healthy *P. floridana*.

RT-PCR amplification and sequencing of ORF0. Total RNAs were extracted from infected *P. floridana* leaf samples by using the RNeasy Plant Mini kit (Qiagen, Valencia, CA, USA). Reverse transcription was performed using AMV reverse transcriptase (Promega Co., Madison, WI, USA) and PCR was done using *Taq* DNA polymerase (Promega Co., Madison, WI, USA). Specific PLRV primers were used to amplify a region of 934 nt encompassing the entire ORF0 and the first 748 nt of ORF1 overlapping with ORF0 (Guyader and Giblot-Ducray, 2002). The RT-PCR amplified fragments of nine Tunisian PLRV isolates and the FR5 isolate were sequenced. The forward primer ORFIS2 (5'-GAAATTGCAGCTTTAG-3') and the reverse primer ORFIAS (5'-AGGCGTTCTCTCCACTGTAC-3') were chosen from the sequence of the Scottish PLRV reference isolate (Mayo *et al.*, 1989). Sequencing reactions were carried out directly on purified PCR products (Concert Rapid PCR Purification system, Gibco BRL, Rockville, MD, USA) and analysed on an ABI 310 Automated sequencer (Applied Biosystems, Foster City, CA, USA). Overlapping sequences were assembled with CAPCONTIG (Huang, 1992) and then aligned using CLUSTALW 1.8 (Thompson *et al.*, 1994).

Phylogenetic analysis. The pairwise genetic distances between aligned sequences were calculated by the maximum-likelihood (ML) approach, and a rooted phylogenetic tree was reconstructed by the quartet puzzling method, as implemented in TREEPUZZLE 5.0 (Strimmer and von Haeseler, 1996). The reliability percentages are used to assess the strength of the nodes. The Tamura-Nei model of substitution was used for nucleotide sequences (Tamura and Nei, 1993) and both the transition to transversion ratio (ts:tv) and the shape parameter (α) of the distribution of substitution rate variation among sites (discrete gamma distribution with eight categories) were estimated during the tree reconstruction. Available PLRV sequences used for comparison were retrieved from GenBank (Table 2). P0 sequence for an isolate of the polerovirus *Cereal yellow dwarf virus-RPV* (CYDV-RPV) was used to root the reconstructed phylogenetic tree.

RESULTS

Symptomatology of Tunisian PLRV isolates. According to symptom severity on *P. floridana*, Tunisian PLRV isolates could be classified in 3 groups: very severe (is_5 , is_6 and is_{SAM}), severe (is_4 and is_{16}) and mild

(is_{24} , is_{26} , is_{29} and is_{30}) isolates (Table 1). None of the tested Tunisian isolates induced weak or very weak symptoms on *P. floridana*.

Comparison among ORF0 sequences. The ORF0 sequences of the nine Tunisian and the Fr5 PLRV isolates were aligned with the 24 sequences available in GenBank. The sequenced ORF0 region is 744 nucleotides in length and the deduced P0 protein contains 247 amino acids (12.4% of the total PLRV genome). Nucleotide identities between Tunisian isolates ranged from 91.9% to 100% identity. The comparison of the Tunisian PLRV ORF0 nucleotide sequences with each of the PLRV ORF0 sequences from databases showed that they shared 89.9 to 99% identity. The closest relative to Tunisian PLRV isolates were Polish isolates, which shared ~95.5% identity, followed by French and Dutch isolates with ~95% identity, Zimbabwean and Cuban isolates with ~94.8%, Scottish, Spanish and Brazilian isolates with ~94.4% and Canadian and Australian isolates with ~92.8% (Table 3).

Accordingly, the phylogenetic ML tree reconstructed from all the 34 ORF0 nucleotide sequences and rooted by CYDV allowed the distribution of the isolates in three groups (Fig. 1). Group 1 encompassed three European, one Peruvian and six Tunisian PLRV isolates

Table 2. Geographic origin, symptoms induced in *P. floridana* and references of previously sequenced isolates retrieved from databases.

PLRV isolates	Geographical origin	Symptoms on <i>P. floridana</i>	Reference	GenBank Accession No.
Noir	France	Severe	Guyader and Giblot-Ducray, 2002	AF453390
Fr1	France	Weak	Guyader and Giblot-Ducray, 2002	AF453391
14.2	France	Mild	Rouzé-Jouan <i>et al.</i> , 2001	AF271214
14.1	France	Mild	Guyader and Giblot-Ducray, 2002	AF453405
OP	Spain	Mild	Guyader and Giblot-Ducray, 2002	AF453389
K5	Spain	Mild	Guyader and Giblot-Ducray, 2002	AF453399
Zim13	Zimbabwe	Severe	Guyader and Giblot-Ducray, 2002	AF453388
Br1	Brazil	-	Guyader and Giblot-Ducray, 2002	AF453406
CIP01	Peru	-	Guyader and Giblot-Ducray, 2002	AF453392
Cu87	Cuba	Very severe	Guyader and Giblot-Ducray, 2002	AF27125
PLRV-C	Canada	-	Keese <i>et al.</i> , 1990	D13954
PLRV-N	Holland	-	van der Wilk <i>et al.</i> , 1989	Y07496
PLRV-V	Scotland	Mild	Guyader and Giblot-Ducray, 2002	AF453402
PLRV-S	Scotland	-	Mayo <i>et al.</i> , 1989	D00530
PLRV-A	Australia	-	Keese <i>et al.</i> , 1990	D13953
Au16	Australia	-	Guyader and Giblot-Ducray, 2002	AF453395
Au40b	Australia	-	Guyader and Giblot-Ducray, 2002	AF453396
TYTV2	Australia	-	Guyader and Giblot-Ducray, 2002	AF453397
Au252	Australia	-	Guyader and Giblot-Ducray, 2002	AF453398
PLRV-P	Poland	-	Palucha <i>et al.</i> , 1994	X74789
L18	Poland	-	Guyader and Giblot-Ducray, 2002	AF453400
L7	Poland	-	Guyader and Giblot-Ducray, 2002	AF453401
L13B	Poland	Mild	Guyader and Giblot-Ducray, 2002	AF453403
L13D	Poland	Mild	Guyader and Giblot-Ducray, 2002	AF453404

Table 3. Percentage of nucleotide sequence identity of all isolates compared in the ORF0 region. Tunisian PLRV isolates and the French Fr5, newly sequenced in this work, are in bold.

	CIP01	L13b	L13d	14.1	TYTV2	Au252	Au40b	Au16	PLRVA	PLRVC	PLRVS	PLRVN	PLRVP	L18	L7	Zim13	Fr1
L13b	98.7																
L13d	98.5	99.0															
14.1	98.4	98.8	98.4														
TYTV2	90.2	90.1	89.7	90.2													
Au252	90.6	90.5	90.1	90.7	95.2												
Au40b	89.5	90.2	89.7	89.8	96.5	97.1											
Au16	92.2	92.1	91.7	92.3	95.6	98.2	96.5										
PLRVA	90.6	91.3	90.7	90.9	97.1	96.8	99.4	97.1									
PLRVC	90.4	90.1	90.0	90.2	95.3	95.5	95.8	96.6	96.6								
PLRVS	92.4	92.5	91.9	92.7	96.1	96.1	94.8	97.2	95.6	96.9							
PLRVN	93.2	93.3	92.8	92.8	96.5	96.1	95.5	97.1	96.3	97.3	98.5						
PLRVP	92.8	92.9	92.4	92.6	97.1	96.1	95.5	97.1	96.3	97.6	99.1	99.1					
L18	93.1	92.9	92.7	92.6	96.4	95.1	94.2	96.3	95.0	96.7	97.6	97.9	98.5				
L7	91.7	91.8	91.3	92.1	95.9	95.6	94.3	96.7	95.0	96.4	98.7	98.1	99.0	97.5			
Zim13	93.0	92.7	92.9	92.6	96.3	95.5	94.6	96.6	95.4	96.7	97.8	98.4	98.7	99.0	97.6		
Fr1	93.0	92.7	92.9	92.6	95.9	95.4	94.4	96.5	95.2	96.6	97.8	98.1	98.4	99.0	97.3	99.7	
OP	93.0	92.7	92.6	92.6	96.8	96.4	95.9	97.5	96.6	98.0	98.7	99.0	99.3	98.4	98.3	98.8	98.5
Noir	92.6	92.3	92.2	92.2	96.1	96.1	95.5	97.1	96.3	97.1	98.1	98.7	98.7	97.5	97.6	97.9	97.6
14.2	92.2	92.3	92.2	92.3	95.9	95.9	95.0	97.0	95.8	96.7	98.7	98.7	99.0	97.5	98.3	97.9	97.6
Cu87	92.7	92.5	92.3	92.3	95.9	95.5	95.3	96.6	96.1	96.9	97.9	98.5	98.5	97.3	97.5	97.8	97.5
Br1	92.2	92.3	91.8	91.8	96.1	95.7	95.9	96.5	96.6	97.3	97.8	98.4	98.4	97.3	97.3	97.8	97.5
V	92.3	92.9	92.4	92.8	95.5	95.9	95.5	96.9	96.3	96.7	97.6	98.2	98.2	97.9	97.5	98.1	98.1
K5	91.4	91.1	90.9	90.9	95.4	95.2	94.3	96.0	95.1	96.2	97.2	97.5	98.1	97.3	97.0	97.5	97.5
is₄	99.0	99.1	99.0	98.8	90.3	90.7	90.2	92.3	91.3	90.5	92.5	93.3	92.9	93.3	91.9	93.1	93.1
is₅	92.6	92.3	92.2	92.2	96.4	95.7	95.5	96.8	96.3	97.4	98.1	98.4	99.0	98.1	97.9	98.5	98.2
is_{SAM}	92.6	92.3	92.2	92.2	96.1	95.7	95.5	96.8	96.3	97.1	97.8	98.4	98.4	97.6	97.3	98.4	98.1
is₆	92.6	92.3	92.2	92.2	96.4	95.7	95.5	96.8	96.3	97.4	98.1	98.4	99.0	98.1	97.9	98.5	98.2
Fr₅	91.9	91.8	91.6	91.6	94.7	94.8	94.4	95.9	95.2	96.0	97.3	97.9	97.6	96.6	96.5	96.8	97.1
is₂₄	98.7	99.1	98.7	98.5	90.0	90.4	89.9	91.9	90.9	90.2	92.5	93.4	93.0	92.9	92.3	92.8	92.8
is₂₆	98.7	98.8	98.7	98.5	90.4	90.4	89.9	91.9	90.9	90.2	92.1	93.0	92.6	92.9	91.5	92.8	92.8
is₂₉	98.8	99.0	98.8	98.7	90.6	91.0	90.5	92.5	91.5	90.8	92.7	93.6	93.2	93.5	92.1	93.3	93.3
is₃₀	99.0	99.0	98.8	98.7	90.1	90.5	90.0	92.1	91.0	90.3	92.3	93.1	92.7	93.1	91.6	92.9	92.9
is₁₆	99.1	99.3	99.1	99.0	90.6	91.0	90.5	92.5	91.5	90.8	92.7	93.6	93.2	93.5	92.1	93.3	93.3

	OP	Noir	14.2	Cu87	Br1	PLRVV	K5	is ₄	is ₅	is _{SAM}	is ₆	Fr ₅	is ₂₄	is ₂₆	is ₂₉	is ₃₀
Noir	99.1															
14.2	98.8	98.3														
Cu87	99.0	98.4	98.1													
Br1	98.8	98.3	98.0	98.1												
V	98.7	98.1	97.8	97.6	97.8											
K5	98.3	97.7	97.6	97.5	97.4	97.2										
is ₄	93.1	92.7	92.3	92.9	92.3	92.9	91.5									
is ₅	99.4	98.6	98.3	98.7	98.3	98.1	98.0	92.7								
is _{SAM}	99.1	98.6	98.0	98.1	98.6	98.1	97.7	92.7	98.6							
is ₆	99.4	98.6	98.3	98.7	98.3	98.1	98.0	92.7	100.0	98.6						
Fr ₅	97.8	97.8	97.2	97.0	96.8	97.4	96.8	92.6	97.2	97.2	97.2					
is ₂₄	92.8	92.4	92.4	92.5	92.4	92.6	91.1	99.1	92.4	92.4	92.4	91.8				
is ₂₆	92.8	92.4	92.0	92.5	92.0	92.6	91.1	99.1	92.4	92.4	92.4	91.8	99.1			
is ₂₉	93.4	93.0	92.6	93.1	92.6	93.2	91.7	99.3	93.0	93.0	93.0	92.4	99.5	99.3		
is ₃₀	92.9	92.5	92.1	92.7	92.1	92.7	91.3	99.3	92.5	92.5	92.5	92.0	99.5	99.3	99.7	
is ₁₆	93.4	93.0	92.6	93.1	92.6	93.2	91.7	99.5	93.0	93.0	93.0	92.4	99.3	99.3	99.4	99.4

(is₄, is₁₆, is₂₄, is₂₆, is₂₉ and is₃₀). Group 2 contained Australian and Canadian isolates. Group 3 included the remaining isolates originating from diverse continents, the remaining three Tunisian PLRV isolates (is₅, is₆ and is_{SAM}) and the Fr5 French isolate. Comparison of nucleotide sequences within each group showed that isolates of the group 2 (for which the nucleotide diversity $p = 0.0333$) were more diverse than those in either group 1 and 3 ($p = 0.0095$ and 0.0187 , respectively). It is worth noting that Tunisian isolates do not cluster all together in the phylogenetic tree, but they were distributed in two of the groups, with no correlation between their geographical origin and the branching pattern.

DISCUSSION

This is the first report on the biological and molecular diversity of North African PLRV isolates. In this study, the ORF0 region was chosen because: (i) this protein could be involved in host range (Veidt *et al.*, 1992), virus symptom expression (van der Wilk *et al.*, 1997), virus accumulation and replication (Sadowy *et al.*, 2001); (ii) P0 appeared to be the most variable region in the PLRV genome, thus allowing a better discrimination between isolates; (iii) no studies have been reported about relationship between variability of PLRV symptoms induced on *P. floridana* and the PLRV-ORF0 sequence.

In this study, we distinguished three categories of symptoms induced on *P. floridana* among the nine Tunisian PLRV isolates: very severe (is₅, is₆ and is_{SAM}), severe (is₄ and is₁₆) and mild (is₂₄, is₂₆, is₂₉ and is₃₀). These differences in symptom intensity were comparable with those previously described in the United States (Webb *et al.*, 1951) and Britain (Tamada *et al.*, 1984). Analysis of ORF0 sequence homology showed that European (Polish, French and Dutch) isolates were the closest relative to Tunisian PLRV isolates with ~95% identity. This could be caused by seed exchanges since almost 50% of Tunisian potato seed is imported from Europe [60% from Netherlands and 40% from France (Souibgui, 2000)]. Analysis conducted on ORF0 sequences allowed discrimination between different isolates and distinction between three groups of PLRV isolates, whereas use of the total PLRV genome sequences did not allow this discrimination, showing the high variability level of the ORF0 (Guyader and Giblot-Ducray, 2002). All severe and mild Tunisian isolates clustered with the Peruvian (CIP01) and three European (14.1, L13B and L13D) mild isolates into the first group (Fig. 1). Whereas, the three very severe Tunisian isolates and the Fr5 isolate belonged to the group 3 of the tree. None of the Tunisian isolates clustered with Australian isolates that formed a distinct group. This diversification could be due to the geographical isolation of Australia and the absence of tuber exchanges with other continents.

	20	40	60	
PLRVS :	MIVLTQSGTLLFDQRFKLSKFLFVVIATGFP	LLQOASLIYGYNHEQIYRICRSFLHVLPLLNCKRGRIS :	70	
is4 :	Y.....	70
is16 :	Y.....	70
is24 :	Y.....	70
is26 :	S.....	Y.....	70
is29 :	Y.....	70
is30 :	Y.....	70
CIP01 :	Y.....	70
L13b :	Y.....	70
L13d :	Y.....R.....	70
14.1 :	Y.....	70
is5 :	Y.....	70
is6 :	Y.....	70
isSAM :	Y.....	70
Cu87 :	Y.....	70
Fr5 :	Y.....	70
Fr1 :	Y.....R.....	70
Zim13 :	Y.....R.....	70
L18 :	Y.....	70
PLRVN :	Y.....	70
PLRVP :	Y.....	70
L7 :	Y.....	70
OP :	Y.....	70
Noir :	L.....Y.....G.....	70
14.2 :	Y.....	70
Br1 :	Y.....	70
PLRVV :	Y.....	70
K5 :	Y.....	70
PLRVC :I.....S.....	Y.....	70
Au16 :S.....	Y.....	70
Au252 :S.....H.....	Y.....	70
Au40b :I.....S.....HH.....	Y.....	70
PLRVA :I.....S.....	Y.....	70
TYTV2 :S.....	Y.....	70

Gr 1

Gr 3

Gr 2

	80	100	120	140	
PLRVS :	TSGLQLPRHLHYECLWGLLCGTHPAIQIVGLTIVIKLDDPTTAAAYRSELLRVSSSSYIQNAAGLSNGW	:	140		
is4 :	P.....E.....	K.SV.F.....	140
is16 :	P.....E.....	K.SV.F.....	140
is24 :	I.....	P.....E.....	K.SV.F.....	140
is26 :	P.....E.....	V.....Q.....	K.SV.F.....	140
is29 :	P.....E.....	K.SV.F.....	140
is30 :	P.....E.....	K.SV.Y.....	140
CIP01 :	P.....E.....	K.SV.S.....	140
L13b :	P.....E.....	K.SV.F.....	140
L13d :	P.....E.....L.....	K.SV.F.....	140
14.1 :	P.....E.....	K.S.F.....	140
is5 :	P.....	T.....	140
is6 :	P.....	T.....	140
isSAM :	P.....	140
Cu87 :	P.....	140
Fr5 :	P.....	140
Fr1 :	P.....	140
Zim13 :	P.....	140
L18 :	P.....V.....	140
PLRVN :	P.....	140
PLRVP :	P.....	140
L7 :	V.....	P.....	140
OP :	P.....	140
Noir :	S.....	140
14.2 :	P.....	140
Br1 :	P.....	140
PLRVV :Y.....	PN.....	140
K5 :	P.....	T.....	140
PLRVC :S.....	P.....	140
Au16 :	P.....	140
Au252 :	A.....	P.....	140
Au40b :	P.....	140
PLRVA :	P.....	140
TYTV2 :	P.....	140

Gr 1

Gr 3

Gr 2

	160	180	200		
PLRVS :	GHDMEAFVRNAICLLELRERSIPQSGLRDLMGNYQHLVRSLLDACKVDHFVPLDFQHRSLMLNFARLYNQ :	210			
<u>is4</u> :	T . Y	210	
<u>is16</u> :	T . Y	210	
<u>is24</u> :	T . Y	210	
<u>is26</u> :	T . Y	210	
<u>is29</u> :	T	210	
<u>is30</u> :	T . Y	210	
CIP01 :	T . Y	210	
L13b :	T . Y	210	
L13d :	T . Y	210	
14.1 :	N .	T . Y	210	
<u>is5</u> :	E	210	
<u>is6</u> :	E	210	
<u>isSAM</u> :	E .	F .	210	
Cu87 :	L . N .	H .	E .	210
Fr5 :	R .	S . P	210
Fr1 :	210
Zim13 :	210
L18 :	210
PLRVN :	H	210
PLRVP :	210
L7 :	210
OP :	E	210
Noir :	E	210
14.2 :	L .	210
Br1 :	E	210
PLRVV :	210
K5 :	T .	S .	E . S . L .	210
PLRVC :	E	210
Au16 :	Q	210
Au252 :	K .	Q	210
Au40b :	L .	Q	210
PLRVA :	L .	Q	210
TYTV2 :	Q	210
	220	240			
PLRVS :	LDLQGRAKSFRAITGFPVYVPSDYLEGSFLQKELQE*	247			
<u>is4</u> :	247	
<u>is16</u> :	D	247	
<u>is24</u> :	247	
<u>is26</u> :	247	
<u>is29</u> :	247	
<u>is30</u> :	247	
CIP01 :	247	
L13b :	L	247	
L13d :	247	
14.1 :	L	247	
<u>is5</u> :	247	
<u>is6</u> :	247	
<u>isSAM</u> :	247	
Cu87 :	L	247	
Fr5 :	247	
Fr1 :	247	
Zim13 :	247	
L18 :	D	247	
PLRVN :	247	
PLRVP :	247	
L7 :	247	
OP :	247	
Noir :	247	
14.2 :	S .	R .	248	
Br1 :	247	
PLRVV :	L	247	
K5 :	247	
PLRVC :	FN	247	
Au16 :	247	
Au252 :	247	
Au40b :	I	247	
PLRVA :	I	247	
TYTV2 :	I . Y	247	

Fig. 2. Comparison of the amino acids sequences of one region of the ORF0 from the PLRV isolates sequenced in this work (underlined) and 24 sequences retrieved from GenBank. Grouping of isolates was achieved by shading some amino acids. Three groups could be distinguished: Group 1 contains 3 European isolates (L13b, L13d, 14.1), 1 Peruvian (CIP01) and 6 Tunisian ones (is₄, is₁₆, is₂₄, is₂₆, is₂₉, is₃₀). The second group contains 5 Australian isolates (Au16, Au252, Au40b, PLRVA, TYTV2) and the Canadian one (PLRVC). Group 3 contains 12 European isolates (K5, OP, 14.2, Noir, Fr1, Fr5, PLRVN, PLRVS, PLRVV, PLRVP, L7, L18), the Brazilian Br1 one, the Zimbabwean Zim13 one, the isolate Cu87 from Cuba and 3 Tunisian isolates (is₅, is₆ and is_{SAM}).

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