OnLine Journal of Biological Sciences 2 (9): 582-586, 2002
ISSN 1608-4127

Nucleotide Sequence Analysis for Assessment of Variability of Potato Leafroll Virus and Phylogenetic Comparisons

[1]Kamil Haliloglu and [2]Hidayet Bostan
Department of Field Crops, [2]Department of Plant Protection, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey

Abstract: Relationships were investigated by comparing the nucleotide sequences from 17 PLRV coat protein genes submitted in GenBank. The alignment of 479 positions required few gaps and these positions showed consensus among the sequences. Overall identity was 45.17%. A phylogenetic tree based on the nucleotide sequences was established. There are two major clusters originated from a common branch in the phylogram, but are distinct from each other. Australian isolate was found to be very distinct compared to other isolates in the phylogenetic relationship. In this study nine conserved region and their length ranging from 18 to 58 bp were found. Sequences of conserved regions are prerequisite to design PCR primers for group or isolate based diagnosis of viruses. This study provides preliminary information about relationships of CP genes of PLRV isolates to develop strategy in designing as well as engineering of CP based transgenic resistancy in plants.

Key words: Phylogenetic, nucleotide comparison, conserved regions

Introduction

Potato is one of the world's most important food crops, is propagated vegetatively through tubers. Potato leafroll virus (PLRV) causes one of the destructive diseases of potatoes worldwide (Peters *et al.*, 1981; Schilde-Rentschler and Schmiediche, 1984; Hooker, 1986; Slack, 1995).

PLRV, a member of the luteovirus with isometric particles of ~ 26 nm in diameter and with hexagonal outlines; consist of a positive-sense, single stranded RNA of 6 kDa (Rowhani and Stace-Smith, 1979) and virions of PLRV consist of a major coat protein component of 26 kDa (Smith and Harris, 1990). The virus is transmitted by aphids in a persistent-circulative, non-propagative manner and is not sap transmissible. PLRV is confined to phloem tissue and is low titers in infected plants (Peters *et al.*, 1981; Bagnall, 1988; Lobenstein *et al.*, 1997; Singh and Sing, 1997).

Coat protein (CP)-mediated resistance as reported by Powell-Abel *et al.* (1986) has been successfully applied to wide range of host-virus combinations (Da Camara Machado *et al.*, 1992; Fitch *et al.*, 1992; Stark and Beachy., 1989). Incorporation of the transgenic resistance is considered an effective method for developing PRLV-resistance cultivars. However, the resistance can be highly strain or isolate dependent with specificity determined by the relatedness of the transgene to the CP genes of the challenge viruses (Nakajima *et al.*, 1993; Nelson *et al.*, 1988; Quemada *et al.*, 1990; Quemada *et al.*, 1991). Therefore, the variability in nucleotide sequences among the CP genes of the challenge viruses may have a significant effect on the level and stability of transgenic resistance.

Methods that are capable of both detecting very low virus concentrations and discriminating between different PRLV isolated must be utilized to better understand the epidemiology and relatedness of PRLV isolates. The polymerase chain reaction (PCR) has become method of choice and accepted as more specific and sensitive method than the enzyme linked immunosorbent assay. Using PLRV sequence-specific oligonucleotide primers, reverse transcription linked PCR can amplify specific virus RNA and provide for further analysis by restriction fragment length polymorphism (Robertson *et al.*, 1991). These molecular techniques are very helpful to compare the relatedness of isolates at the nucleotide level. In addition, prerequisite for detection of viruses via PCR is the availability of accurate information of conserved and variable regions of nucleotide sequences of target virus isolates.

In this study, the variability among the CP genes of published PLRV sequences from GenBank was analyzed. In addition, phylogenetic relationships based on the actual nucleotide sequences of published PLRV sequences from GenBank were analyzed.

Materials and Methods

This study was carried out in Research Laboratories of Ataturk University, Faculty of Agriculture, Department of Field Crops in years between 2001-2002.

Comparative analysis and the construction of phylogenetic trees: The published PLRV sequences (Table 1) from GenBank were

Table 1: Information about seventeen isolates published in GenBank and used in this study

Accession	Definition	Locus	Authors
A07941	DNA for coat protein	1000	Mayo, M.A. and B. Reavy.
A07943	DNA for coat protein	2154	Mayo, M.A. and B. Reavy.
AF271215	isolate CU87 coat protein and ORF IV genes	2154	Rouze-Jouan, J., L.Terradot, F. Pasquer, S. Tanguy and D. Giblot Ducray-Bourdin.
F296280	coat protein mRNA	627	Seo, H.W., S.E. Oh, J.Y. Yi, Y.I. Hahm and H.M. Cho.
AY007727	oat protein (ORFIII) gene	627	Terradot, L., V. Tran and D. Giblot Ducray-Bourdin.
D13753	23K ORF and 17K ORF	693	Kawchuk, L.M., R.R. Martin, D.M. Rochon and J. McPherson.
D13953	(Australian solate) genomic RNA	5882	Keese, P., R.R. Martin, L.M. Kawchuk, P.M. Waterhouse and W.L. Gerlach.
D13954	(Canadian isolate) genomic RNA	5883	Keese, P., R.R. Martin, L.M. Kawchuk, P.M. Waterhouse and W.L. Gerlach.
I19653	Sequence 1 from patent US 551053	3901	Mitsky, T.A., C.L. Hemenway and N.E. Tumer.
NC_001747	complete genome, Scotland olate	5987	Mayo, M.A., D.J. Robinson, C.A. Jolly and L. Hyman.
S77421	CP, Cuban Isolate. Genomic RNA	627	Lopez, L., R. Muller, E. Balmori, G. de la Riva, N. Ramirez, V. Doreste, Lopez, M., S. Perez, P. Oramas and G. Selman-Housein.
U74377	CP and 17k protein genes	663	Joung,Y.H.,
X13906	CP	627	Prill, B., E. Maiss, U. Timpe and R. Casper.
X74789	RNA sequence, Poland isolate	5882	Palucha, A., E. Sadowy, A. Kujawa, M. Juszczuk, W. Zagorski and D. Hulanicka.
X77321	(V) gene for coat protein	2154	Jolly,C.A. and M.A. Mayo.
X77322	PLRV-11 gene for coat protein	2154	Jolly,C.A. and M.A. Mayo.
Y07496	genomic RNA, Netherlands	5882	van der Wilk, F., M.J. Huisman, B.J. Cornelissen, H. Huttinga and R. Goldbach.

Table 3: Percent similarity and divergence among seventeen PLRV isolates based on nucleotide sequences

Percent divergence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1		38.0	38.0	17.0	17.0	18.0	93.0	98.0	93.0	96.0	17.0	17.0	98.0	38.0	38.0	24.0	17.0	1	Y07496 Netherland
2	2		99.0	100.0	100.0	99.0	37.0	38.0	18.0	37.0	100.0	100.0	38.0	99.0	99.0	98.0	100.0	2	A07943
3	2	2.8		100.0	100.0	99.0	37.0	38.0	17.0	36.0	100.0	100.0	38.0	99.0	99.0	97.0	100.0	3	AF271215
4	2	1.6	3.1		100.0	99.0	16.0	17.0	18.0	14.0	100.0	100.0	17.0	100.0	100.0	97.0	100.0	4	AF296280 Korea
5	2	2.1	2.9	2.4		99.0	16.0	17.0	18.0	14.0	100.0	100.0	17.0	100.0	100.0	97.0	100.0	5	AY007727
6	1	0.9	2.2	1.3	1.5		18.0	18.0	18.0	16.0	99.0	99.0	18.0	99.0	99.0	98.0	99.0	6	D13753
7	7	5.1	5.4	3.9	3.8	3.3		93.0	88.0	92.0	17.0	17.0	94.0	37.0	37.0	23.0	17.0	7	D13953 Australia
8	2	2.4	2.9	1.3	1.5	0.0	7.1		93.0	96.0	17.0	18.0	98.0	38.0	38.0	24.0	17.0	8	D13954 Canada
9	3	3.4	4.7	3.9	4.1	3.2	9.0	3.5		91.0	18.0	18.0	94.0	18.0	18.0	20.0	18.0	9	I19653 USA
10	2	0	2.8	1.6	2.1	0.9	7.2	2.4	2.7		14.0	15.0	97.0	36.0	36.0	22.0	14.0	10	NC_001747 Scotland
11	2	1.3	2.4	1.6	1.8	0.6	3.3	0.6	3.6	1.3		100.0	17.0	100.0	100.0	97.0	100.0	11	S77421
12	1	1.4	1.8	1.6	1.5	0.6	3.1	0.6	3.2	1.4	1.0		18.0	100.0	100.0	97.0	100.0	12	U74377
13	2	2.1	2.3	1.6	1.8	0.9	6.8	2.1	2.5	1.6	1.3	0.9		38.0	38.0	24.0	17.0	13	X74789 Poland
14	2	1.9	2.4	2.4	2.3	1.4	4.9	2.1	4.3	1.9	1.6	1.4	1.7		100.0	97.0	100.0	14	X77321
15	2	1.6	2.1	1.5	1.3	0.5	4.6	1.8	3.4	1.6	0.8	0.5	1.4	1.3		9.07	100.0	15	X77322
16	2	0	3.1	1.6	2.1	0.9	4.9	1.6	3.6	0.0	1.3	1.4	1.9	1.9	1.5		97.0	16	A07941
17	2	1.8	1.9	2.1	1.9	1.1	3.1	1.1	4.1	1.8	1.5	1.1	1.5	1.9	1.0	1.8		17	X13906

aligned to identify the conserved nucleotide sequences of the available PLRV sequences in GenBank. Further investigation was made to find relationship among them. Multiple sequence alignment of nucleotide sequences of PLRV coat protein gene regions was accomplished using the alignment program CLUSTALW designed by Higgins and Sharp (1988, 1989) in the BIOEDIT software (version 5.0.6) (Hall, 1999). Parameters of CLUSTALW for multiple alignment were as follow; gap penalty was 15, gap length penalty was 6.66, delay divergence sequences was 30 % and DNA transition weight was 0.5.

A phylogenetic tree based on the nucleotide sequences was established by observed divergency method and bootstrapping of nucleotide sequences of published 17 PRLV isolates.

Search for conserved regions in an alignment: Parameters taken into consideration to find conserved regions were as follow; minimum segment length (actual for each sequence) was 15, maximum average entropy was 0.2, maximum entropy per position was 0.2, gaps were limited to 2 per segment and contiguous gaps were limited to 1 in any segment.

Results and Discussion

Relationships were investigated by comparing the coat protein nucleotide sequences from published 17 PLRV coat protein genes (Table 1). Multiple alignment consisted of 5986 positions, of which the first 3697 5' positions and positions between 4176 and 5986 contained a large number of gaps and no positions which were common (consensus) among the sequences. The alignment of the remaining 479 positions required few gaps and these positions showed consensus among the sequences (Table 2). Overall identity was 45.17%. Percent similarity and divergence (Table 3) was generated by Megalign program (DNASTAR Inc. Madison, WI).

Comparisons of the nucleotide sequences of the CP genes of the reported seventeen PRLV isolates were summarized in Table 2. The sequences of A07943, AF271215, AF290280 (Korean), AY007727, D13753, S77421, U74789, X13906, X77321, X77322, and A07941 shared high nucleotide similarities of 91.1 to 99.9%.

Table 4: Conserved regions in an alignment of 17 PLRV isolates

Regions	Positions	Consensus	Segment length
1	3763-3787	-CCCTTCGCAGGCGCGCTAACAGAGT-	25
2	3795-3812	-GTGGTTATGGTCACGGCC-	18
3	3872-3929	-TGGAGTTCCCCGAGGACGAGGCTCAAGCG AGACATTCGTGTTTACAAAGGACAACCTC-	58
4	3940-3964	-CCCAAGGAAGTTTCACCTTCGGGCC-	25
5	3970-3985	-TATCAGACTGTCCGGC-	16
6	3996-4033	-GGAATACTCAAGGCCTACCATGAGTATAAG ATCACAAG-	38
7	4035-4073	-ATCTTACTTCAGTTCGTCAG CGAGGCCTCTTCCACCTCC-	39
8	4080-4108	-TCCATCGCTTATGAGTTGGACCCCCATTG-	29
9	4114-4139	-TATCATCCCTCCAGTCCTACGTCAAC-	26

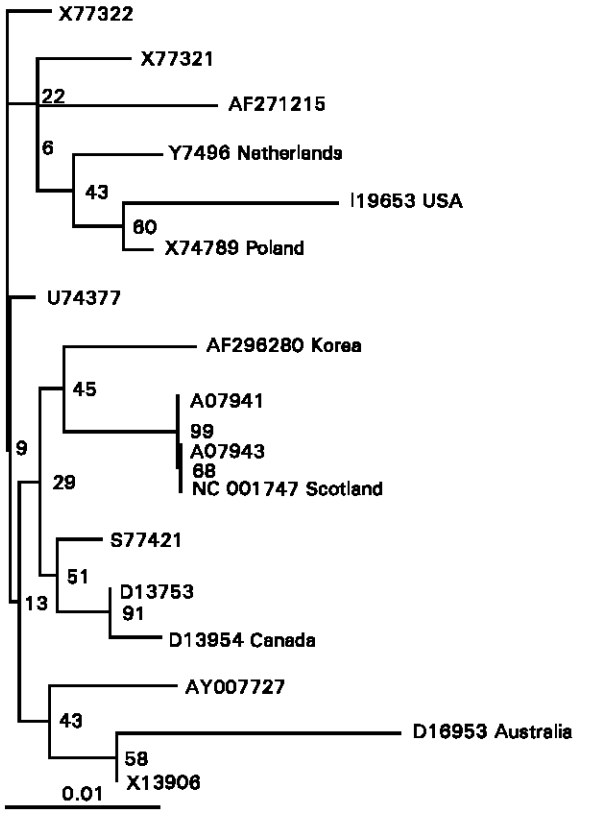

Fig. 1: Phylogenetic tree based on divergence method of seventeen potato leaf roll virus (PLRV) isolates. Numbers indicating the percentage of 100 bootstrap replicates are shown at the appropriate nodes. The scale for genetic distance is indicated in the lower left corner

However, they had lower nucleotide similarities of 14.3 to 88.1% with PRLV isolates reported from Y07496 (Netherlands), Australia, Canada, I19653, Scotland and Poland.

A phylogenetic tree based on the nucleotide sequences was established by observed divergency method and bootstrapping of nucleotide sequences of published 17 PRLV isolates (Fig. 1). The numbers at the forks indicate the number of times the group consisting of the strains which are to the center of that fork occurred among the trees. There are two major clusters originated

from a common branch in the phylogram, but are distinct from each other. First cluster includes X77321, AF271215, Y07496 (the Netherlands), I96653 USA and X74789, Poland isolates confirming that they have a common lineage. In addition, U74377, AF296280 (Korea), A07541, A07943, NC001747 (Scotland), S77421, D13753 and D13954 (Canada) isolates fall into same sub-group of second cluster. Meantime, AY007727, D13953 (Australia) and X13906 isolates were sub-grouped in the second cluster. Australian isolate was found to be very distinct compared to other isolated in the phylogenetic relationship.

Comparisons among sequences of PLRV isolates from around the world (Keese *et al.*, 1990; Palucha *et al.*, 1994) have shown that isolates from Canada, Poland, Scotland and the Netherlands differed in nucleotide sequence over the whole genome by about 2%. An Australian isolate differed from all these isolates by about 7%. In a comparative study of Scottish isolates, Jolly (1994) found that the sequences of the P3 and P5 genes of Scottish isolates were as divergent (*c.* 1%) from each other as any was from the overseas isolates except that from Australia (Keese *et al.*, 1990). Similarly, isolates of PLRV obtained from infected potato plants in the Peruvian highlands were no more divergent from each other or from any other isolates than were the Scottish isolates from each other (Mayo, unpublished data).

It can be very informative to locate regions of several sequences, which are well conserved to design primers for detection of viruses or isolates by using PCR. We found nine conserved region and their length were ranging from 18 to 58 bp. Their positions, consensus sequences and segment lengths were presented in Table 4. The use of PCR technologies to detect different patogens of plants has become a powerful tool in disease diagnosis. Depending on the choice of primers, PCR can provide both narrow and broad specifities for various isolates or strains of pathogens (Hadidi *et al.*, 1995; Henson and French, 1993).

Sequence variability may have important implications in use of the CP gene for transgenic resistance. Several lines of evidence from different host-systems suggest that such resistance can be highly sequence specific (Gonsalves, 1998; Nakajima *et al.*, 1993; Nelson *et al.*, 1988; Sanders *et al.*, 1992). This study provides preliminary information about relationships of CP genes of PLRV isolates to develop strategy in designing as well as engineering of CP based transgenic resistancy in plants.

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