Phytopathol. Mediterr. (2011) 50, 359–367

Coat protein sequence comparison of south Indian isolates of *Papaya ringspot virus* with other Indian subcontinent isolates

M. SRINIVASULU[1], D.V.R. SAI GOPAL[2]

[1] Department of Virology, College of Biological and Earth Sciences, Sri Venkateswara University, Tirupati, A. P., India
[2] Department of Virology, College of Biological and Earth Sciences, Sri Venkateswara University, Tirupati - 517502, A.P., India

Summary. *Papaya ringspot virus* (PRSV) is an economically important pathogen causing the most devastating disease in papaya worldwide. Isolates of PRSV originating from different geographical regions in south India were collected and maintained on natural host papaya. The 3' terminal region of about 1.7 kb comprising a part of the nuclear inclusion b (NIb) gene, complete coat protein (CP) gene and the 3' untranslated region (3'UTR) of seven *Papaya ringspot virus* (PRSV) isolates from south India were cloned and sequenced. The CP nucleotide and deduced amino acid sequences of seven isolates were compared to each other and to sequences of 22 other PRSV isolates from different geographical locations in the Indian subcontinent. The sequence comparisons revealed greater sequence divergence (up to 18.4% and 15% at nucleotide and amino acid levels, respectively) within Indian PRSV populations. All south Indian isolates were clearly separated from isolates of other geographical regions and formed a major group in phylogenetic trees, and the clustering pattern of isolates did not correlate well with their geographical origins.

Key words: 3' UTR, genetic diversity.

Introduction

The papaya and watermelon biotypes of *Papaya ringspot virus* (PRSV-P&W) cause major diseases in papaya and cucurbits, respectively, in tropical and subtropical regions including the Indian subcontinent, resulting in significant yield losses (Varma, 1988; Gonsalves, 1998). The disease has become a major threat to papaya cultivation throughout India by rendering orchards economically unproductive. *Papaya ringspot virus* was first reported in western India in 1958 (Capoor and Varma, 1958). Since then it has spread to different geographical locations of India irrespective of the agro-climatic conditions, and causes crop losses of up to 85–90% (Hussain and Varma, 1994). *Papaya ringspot virus* is a member of the genus *Potyvirus* of the family *Potyviridae* (van Regenmortel, 2000), and is non-persistently transmitted by numerous species of aphids in nature. The virus is morphologically characterized as flexuous filamentous rods of dimensions 780–800×12 nm (Gonsalves and Ishii, 1980). The virions each contain a positive sense, single stranded RNA genome of approximately 10.3 kb length, surrounded by a coat protein (CP) consisting of a single protein subunit of molecular weight 36 kDa (Yeh *et al*., 1992).

Papaya ringspot virus is difficult, if not impos-

Corresponding author: D.V.R. Sai Gopal
Fax: + 91 0877 2220031
E-mail: dvrsaigopal@rediffmail.com

* The nucleotide sequences reported in this paper have been assigned the accession numbers-DQ666638-41 and AY839863-65 by GenBank.

sible, to control by conventional methods (Gonsalves, 1998). Due to lack of natural host resistance to PRSV in papaya cultivars, breeding for resistance has shown only limited success. However, papaya genetically transformed with a CP gene of the virus has proven to be resistant to the virus (Lines *et al.,* 2002; Bau *et al.,* 2003). Resistance conferred by the CP gene in transgenic papaya is homology-dependent and RNA-mediated via post-transcriptional gene silencing (Meyer, 1996; Bau *et al.,* 2003). Since the success of the CP mediated resistance (CPMR) depends on the relatedness of the transgene with the challenging virus at nucleotide level, the selection and utilization of a CP gene of a local isolate might be a prerequisite to obtain effective PRSV resistance in transgenic papaya plants for a specific geographic region, as long as genetic variation among strains in that region is not a limiting factor. Hence, it is essential to have the sequence data of CP gene, and to determine the level of variability among PRSV isolates occurring in the Indian subcontinent, to choose the appropriate transgene for developing durable, virus resistant transgenic papaya using CPMR. The genome sequence comparative studies of isolates will also provide data about the complexity in PRSV populations in the country, and help to trace strain phylogeny for better understanding of the evolution and molecular epidemiology of PRSV.

The CP gene of several north Indian isolates and a few south Indian isolates has been sequenced and compared with sequences of other PRSV isolates published from different countries (Jain *et al.,* 1998; Hema and Prasad, 2004; Jain *et al.,* 2004). However, data on the evolution and molecular epidemiology of PRSV are limited, as variability analysis has been conducted mainly on north Indian isolates and a few south Indian isolates. Moreover, 3' UTR sequence data of PRSV isolates is known only for two north Indian isolates. In countries like India, where the geographical and climatic conditions are highly divergent, more sequence data need to be generated from isolates from different regions of the country to provide the complete PRSV population profile, and allow improved assessment of the sequence divergence within the PRSV population.

The present paper reports the 3' terminal nucleotide sequence of the RNA from seven different geographical PRSV isolates in south India and sequence comparison analysis to ascertain the genetic diversity among PRSV isolates in the Indian sub-continent.

Materials and methods

Virus isolates and maintenance

Virus isolates used in this study were collected from commercial papaya orchards in South Indian states. Leaves from papaya plants showing symptoms such as vein clearing, mild mottle, leaf blistering, leaf distortion and filiformy were collected from Kodur of the Rayalaseema region (AP-Ra), Kovvur of the Costal Andhra region (AP-Ko), Hyderabad of the Telangana region (AP-Te) in Andhra Pradesh state; Hospet (KA-Ho) and Gulbarga (KA-Gu) in Karnataka state; Tiruvallur (TA-Ti) in Tamil Nadu state and Calicut (KE-Ca) in Kerala state of south India. The identity of the virus was confirmed by pathogenicity tests on papaya and *Cucurbita pepo* L. cv. Patipan (squash). The presence of PRSV in the different isolates was also verified by direct antigen coating ELISA (DAC-ELISA) using polyclonal antiserum of PRSV-W and Papaya leaf distortion mosaic virus (PLDMV). After confirming their identities, isolates were maintained on their natural host papaya in an insect-proof glasshouse by mechanical inoculation.

RNA extraction and RT-PCR

The total RNA from 100 mg of fresh healthy and PRSV-infected papaya leaf tissue was isolated using RNeasy plant mini kit according to the manufacturer's instructions (Qiagen). The resulting total RNA (2 μL aliquot of 20 μL volume) was incubated with oligo (dT) primer at 70°C for 5 min and snap-chilled on ice for 2 min. cDNA was synthesized using M-MuLV reverse transcriptase (Fermentas, Glen Burnie, MD, USA) at 37°C for 1 h. The sequence and position of oligonucleotide primers used to amplify the 3' terminal region encoding C-terminal region of NIb, complete CP and 3' UTR (size ~1.7kb) are listed in Table 1. The genome of all isolates except that of AP-Ko was amplified using a primer pair PRSV-F_1/PRSV-R_1. For the genome of isolate AP-Ko, the forward primer PRSV-F_1 was replaced with PRSV-F_2. Two to three μL of cDNA was amplified in a 50 μL reaction volume containing 2.5 units of *Taq* DNA polymerase

(Fermentas), 10 pmol of forward (PRSV-F_1/F_2) and reverse primer (PRSV-R_1), 2.25 mM $MgCl_2$ and 0.2 mM each of the dNTPs. PCR amplification conditions included an initial denaturation cycle of 2 min at 94°C followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 45°C and extension for 1 min at 72°C with a final extension of 30 min at 72°C.

Cloning and nucleotide sequencing

The PCR-amplified fragments were purified using QIAquick PCR purification kit (Qiagen) and then cloned into the pGEM-T easy cloning vector (Promega, Madison, WI, USA) according to the manufacturer's instructions. The resulting recombinant plasmids were transformed into *Escherichia coli* strain $DH_5\alpha$ cells. The selected clones with expected size DNA inserts were sequenced using T7 and SP6 universal primers and a gene specific primer PRSV-R_i at MWG Biotech Pvt. Ltd., Bangalore, India.

Sequence analysis

The Sequences of CP and 3' UTR were aligned and compared with sequences of 22 other PRSV isolates from different geographical locations in the Indian subcontinent (Table 2). Multiple sequence alignments were carried out for the nucleotide and deduced amino acid sequences using CLUSTAL X software V.1.81 (Thompson *et al.,* 1997) and the generated alignments were used to determine the percentage of homologies between the sequences using Bioedit software. Neighbor joining phylogenetic trees were generated using CLUSTAL X software tool. In order to calculate the confidence limits placed in construction of phylogenetic trees, bootstrapping analysis was carried out using 1000 replicates and 111 random odd seed number, resulting in a boot strapped NJ tree. To view the deduced phylogenetic trees, TREE VIEW software V.1.6.6 was used and the trees were rooted by choosing *Moroccan watermelon mosaic virus* (MWMV) (Accession No. EF211959) from GenBank as the outgroup.

Results

Symptomatology and confirmation of PRSV infection in isolates

All isolates induced symptoms typical of PRSV infection on papaya. TA-Ti, KA-Gu, AP-Ra, AP-Te and KE-Ca induced mild mosaic symptoms whereas isolates AP-Ko and KA-Ho caused mild mosaic and slight leaf distortion symptoms on squash cv. Patipan. All isolates were strongly reacted in DAC-ELISA with PRSV-W polyclonal antibodies but not with antibodies of PLDMV, thus confirming the identity of PRSV in all collected isolates.

Amplification, cloning and sequencing of the 3' terminal region of PRSV isolates

The 3' terminal region of the genome of all isolates except APKo was amplified with a primer pair PRSV-F_1/PRSV-R_1 on the cDNA by PCR, which yielded a product of approximately 1.7 kbp fragment, and no such amplification was observed on cDNA of healthy papaya tissue. The isolate AP-Ko failed to give an amplification of DNA with the above primer combination and when the forward primer PRSV-F_1 was replaced

Table 1. Description of the oligonucleotide primers used in this study.

Primer	Sequence[a, b]	Orientation	Position[c]
PRSV- F_1	5' ATCACAATGTATTACGC 3'	Sense	8615-8631
PRSV-F_2	5' GGVAAYAAYAGYGGBCAACC 3'	Sense	8562-8581
PRSV– R_1	5' CTCTCATTCTAAGAGGCTC 3'	Antisense	10311-10329
[b]PRSV – R_i	5' CCGCGTTACTRAAGTGAGCCAT 3'	Antisense	9798-9819

[a] The positions of the primers are indicated according to the PRSV genomic sequence in the GenBank (Acc. No. NC_001785).
[b] IUPAC Code for mixed bases: B, C/G/T; R, A/G; V, A/C/G; Y, C/T.
[c] Internal primer used for sequencing.

Table 2. Sources of CP gene and 3'- UTR sequences of PRSV isolates from Indian subcontinent used in this study for comparison.

Isolate	Pathotype	Origin	3'-UTR bases	CP amino acids	GenBank Accession No.	Reference
AP-Ko	P	Kovvur (Andhra Pradesh)	209	286	DQ666638	This study
AP-Ra	P	Rly Kodur (Andhra Pradesh)	209	280	AY839863	This study
AP-Te	P	Hyderabad (Andhra Pradesh)	209	286	AY839864	This study
KA-Gu	P	Gulbarga (Karnataka)	209	286	DQ666639	This study
KA-Ho	P	Hospet (Karnataka)	209	279	AY839865	This study
KE-Ca	P	Calicut (Kerala)	209	286	DQ666640	This study
TA-Ti	P	Tiruvallur (Tamil Nadu)	209	286	DQ666641	This study
KA-Dh	P	Dharwad (Karnataka)	NA[a]	285	AY458618	Jain *et al*., 2004a
TN-Co	P	Coimbatore (Tamil Nadu)	NA	286	EF104919	Unpublished
TN-Tr	P	Trichy (Tamil Nadu)	NA	286	DQ077175	Unpublished
KA-Ba	P	Bangalore (Karnataka)	NA	285	AY238884	Jain *et al*., 2004a
CG-Ra	P	Raipur (Chattisgarh)	NA	284	AY491011	Jain *et al*., 2004a
MP-In	P	Indore (Madhya Pradesh)	NA	285	DQ650651	Unpublished
CG-Bi	P	Bilaspur (Chattisgarh)	NA	287	DQ354071	Unpublished
JK-a	P	Ranchi (Jharkhand)	NA	284	AY458619	Jain *et al*., 2004a
BH	P	Bihar	NA	284	EF210196	Unpublished
HAR	P	Haryana	NA	284	DQ088670	Unpublished
DL-P	P	Delhi	NA	282	AY238883	Jain *et al*., 2004a
DL-W	W	Delhi	209	285	AF063221	Jain *et al*., 1998
UP-Lu	P	Lucknow (Uttar Pradesh)	NA	284	AY458620	Jain *et al*., 2004a
UP-Va	P	Varanasi (Uttar Pradesh)	NA	285	AY238882	Jain *et al*., 2004a
HP-So	P	Solan (Himachal Pradesh)	NA	284	AY458617	Jain *et al*., 2004a
SIk	P	Sikkim	NA	284	DQ354072	Jain *et al*., 2004a
WB-Ba	P	Bardhaman (West Bengal)	NA	284	AY238885	Jain *et al*., 2004a
Pu-S	P	Pune (Maharashtra)	NA	285	AY238881	Jain *et al*., 2004a
Pu-M	P	Pune (Maharashtra)	209	285	AF063220	Jain *et al*., 1998
INU-01	W	India	NA	287	AF506845	Bateson *et al.*,2002
BD	P	Bangladesh	NA	287	AY423557	Jain *et al*., 2004b
SL	P	Sri Lanka	NA	278	U14741	Bateson *et al.*,1994

[a] NA, Sequence of first eight amino acids were not available.

with PRSV-F_2, the PCR resulted in the production of approximately 1.76 kbp fragment. From the sequence of the PCR product, it was revealed that there was non-complimentary between 3' region of PRSV-F_1 primer and template DNA because of two nucleotide changes at the extreme 3' end of the primer position, thus causing failure of PCR reaction. Ligation of amplicons into pGEM-T easy vector resulted in the recombinant clones (pGEM-APKo, pGEM-APRa, pGEM-APTe, pGEM-KAHo, pGEM-KAGu, pGEM-TATi, and pGEM-KECa). Two clones for each isolate were sequenced. There was no sequence difference between the two clones of each isolate. The sequence data revealed that

the 3' terminal region of all isolates contained a single ORF encoding a polyprotein that included a C-terminal part of the NIb protein and complete CP. The ORF was followed by an UTR of 209 nucleotides excluding the poly (A) tail in all isolates.

The comparison of coat protein sequences indicated the presence of conserved motifs commonly found in potyviral coat proteins including (i) 'WCIEN' box in the core region, (ii) QMKAAA in the c-terminal region and (iii) an RQ and AFDF motif (Shukla *et al.,* 1994). The DAG motif potentially associated with the aphid transmissibility (Atreya *et al.,* 1990) at the N-terminus of CP was also conserved in all seven isolates. Like all the known PRSV CP sequences, a stretch of glutamic acid and lysine repeats (EK region) was found after the DAG motif in the N-terminus of the CP of all isolates. The boxes with consensus KEKEK, previously reported in American, Australian and Mexican isolates (Silva-Rosales *et al.,* 2000) were not so evident among Indian isolates due to the variability in arrangement and number of EK repeats. As reported previously (Bateson *et al.,* 1994; Wang and Yeh, 1997; Silva-Rosales *et al.,* 2000), amino acid sequence comparisons revealed a highly variable N-terminal region. The core and C terminal regions were more conserved. The variability in the N-terminal region was most evident in the first 35–40 amino acids that contained a stretch of 'EK' (glutamic acid and lysine) repeats starting at the third amino acid after the DAG motif. The differences in length of CP were mainly confined to the N-terminus. The heterogeneity in CP length resulted from differences in the number of EK repeats at N terminal region.

The putative cleavage site 'Q/S' at the N-terminus of the CP as predicted by the Quemada *et al.,* (1990) was present in all isolates. Another putative cleavage site 'E/S' was also present 20 amino acids upstream from the 'Q/S' cleavage site. Both putative cleavage sites 'VYHQ/S' and 'V(Y or F) HE/S' fitted the consensus cleavage sequence V(Y or F)(H or E)(Q or E)/(S or G) (Yeh *et al.,* 1992) recognized by the NIa proteinase of PRSV.

Variability and phylogenetic analysis of the PRSV CP and 3' UTR

The CP nucleotide and derived amino acid sequences of seven south Indian PRSV isolates used in this study were compared to each other and to sequences of 22 other PRSV isolates from different geographical locations in the Indian subcontinent (Table 2). The sequence identities at nucleotide and amino acid levels within the seven isolates used in the present study were 91.4% (between KA-Ho and KE-Ca) to 98.7% (between KA-Gu and TA-Ti) at the nucleotide level and 91.2% (between AP-Te and AP-Ra) to 98.6% (between KA-Gu and TA-Ti) at the amino acid level. The isolates shared maximum nucleotide sequence identities of 91.4 to 99.3% and maximum amino acid identities of 91.9 to 98.9%, with other south Indian isolates (TN-Tr, TN-Co, KA-Ba and KA-Dh), but they all had lower percentage identities with isolates from central (MP-In, CG-Bi and CG-Ra) (84.6–86.6%); eastern (SIK and JK-Ra) (83.2–85.5%); northern (BH, HAR, UP-Lu, UP-Va, HP-So and DL-W) (83.9 to 88.2%) and western (PU-S and PU-M) (85.1–86.9%) India. The north Indian isolate DL-P and eastern Indian isolate WB-Ba are exceptional, as they shared nucleotide sequence identities of 91.8–100% and 88.9–94.2%, respectively with all south Indian isolates compared. The percentage nucleotide sequence identities between the Bangladesh isolate (BD) and isolates of present study ranged from 82.2% (with isolate AP-Ra) to 84.6% (with isolate KE-Ce). With the Sri Lankan isolate (SL), they had nucleotide identities of 81.6% (AP-Ra) to 83.8% (KE-Ce).

Among all south Indian isolates, the nucleotide identity patterns were almost similar with those of amino acid sequences, but these identity patterns were not exactly reflected with amino acid sequences of isolates from other regions. The seven south Indian isolates shared more amino acid sequence identities than those of nucleotide sequences with isolates from central (89.5–92.6%), eastern (88–91.9%), northern (88.1–96.8%) and western (91.6–93.7%) India; Sri Lanka (87.4–89.1%) and Bangladesh (88.8–91.2%). The 3' UTR sequences of isolates within south India were more identical (percent identities between each other ranging from 93.6 to 99.5% with the mean of 96.3%) compared to isolates from northern (91.2–93.6%) or western India (91.7–93.6%).

The CP nucleotide and amino acid distances of all 29 isolates were used to generate phylogenetic trees and included one isolate of the related potyvirus, *Moroccan watermelon mosaic virus* (MWMV), as the out group reference. A phylogenetic tree,

generated using CP nucleotide sequences of all Indian sub-continent isolates produced two major and distinctly branched groups. One of the major groups (I) consisted of all south Indian isolates while the second main group (II) contained rest of the isolates from northern, eastern, western India, Sri Lanka and Bangladesh. Two isolates from north India (DL-P and DL-W) and one from eastern India (WB-Ba) were exceptional. DL-P and WB-Ba were clustered along with KA-Ba in the south Indian major group while DL-W existed as a sister isolate to all south Indian isolates. The two major groups further divided into two clusters (a and b). Though all south Indian isolates were clearly separated from isolates of other geographical regions and formed into a major group, the clustering of isolates in both major groups did not correlate well with their geographical origins. For instance, WB-Ba, though originating from eastern India, showed a comparatively high degree of nucleotide sequence identity with those of south Indian isolates, forming one cluster. In contrast, the UP-Va and UP-Lu isolates despite originating from same region were grouped in different clusters (Figure 1a).

The clustering pattern of the dendrogram, constructed based on amino acid distances, was very similar to that of nucleotide distances with a few exceptions. The Sri Lankan isolate (SL) did not group with any of the four clusters and came out of two major groups as separate and distinct. The isolate WB-Ba came out from cluster Ib and grouped in cluster IIb. The isolate AP-Te moved out from the cluster Ib in the south Indian major group (Figure 1b). The phylogenetic tree derived from the 3' UTRs of the PRSV isolates branched into two clusters. The north Indian isolate DL-W shared maximum sequence identity with isolate Pu-M, which originated from western India, and these formed together into one cluster whereas all isolates originated from south India grouped as another cluster, indicating closer relationships among them (Figure 1c).

Discussion

In this study, the nucleotide sequences of the 3' part of the genome (about 1.7 kb), encompassing the coding region for c-terminal NIb, complete CP and 3' UTR of seven geographically distinct PRSV isolates were determined, and variability in CP and 3' UTR of isolates from the in Indian subcontinent were analyzed. This clearly revealed sequence divergence within PRSV population in Indian subcontinent. The mean nucleotide sequence divergence between isolates was 11.8% with a maximum of 18.4% (AP-Ra/SL), while the mean divergence at the amino acid level was 8.1% with a maximum of 15% (SIK/SL). Considering pair wise divergence between sequences of south Indian isolates only, the mean of the percentage pair wise divergence was found to be less at both nucleotide (5.2% with a maximum of 8.6% between KA-Ho/KE-Ca and KA-Ho/KA-Dh) and amino acid (5.1% with a maximum of 8.8% between AP-Ra/AP-Te) levels compared to those of other regions of the country or further afield in the Indian subcontinent.

The mean nucleotide and amino acid sequence divergence within the PRSV population in Vietnam (6.4% and 4.1), Thailand (2.6% and 2.6%), Australia (1.5% and 2.3%) and worldwide (9.2% and 5.3%) was found to be less than those of Indian subcontinent PRSV populations. This observation is consistent with the recent reports (Hema and Prasad, 2004; Jain *et al.,* 2004). As opined in an earlier report (Jain *et al.,* 2004), the higher sequence divergence within the PRSV population of the Indian sub-continent could be attributed to the wide range of cropping systems and cultivation practices followed in different geographical regions. This might have resulted in different levels of selection pressure on the virus. Compared to known PRSV isolates from different regions in the Indian subcontinent, the south Indian isolates exhibited less sequence divergence in their CP genes and were more closely related to each other than to the isolates from other regions. The reason for this low level of variation among south Indian isolates might either be the recent occurrence of PRSV in this region or low levels of selection pressure on the virus in south India. Recent occurrence is the most likely explanation, which is consistent with the recent report of Jyoti Sharma *et al.* (2005).

With few exceptions, the south Indian isolates clearly separated from isolates of other regions and created a separate branch in phylogenetic trees. In contrast to other north Indian isolates, DL-W and DL-P showed close relationships with all south Indian isolates. As seen in dendrograms in terms

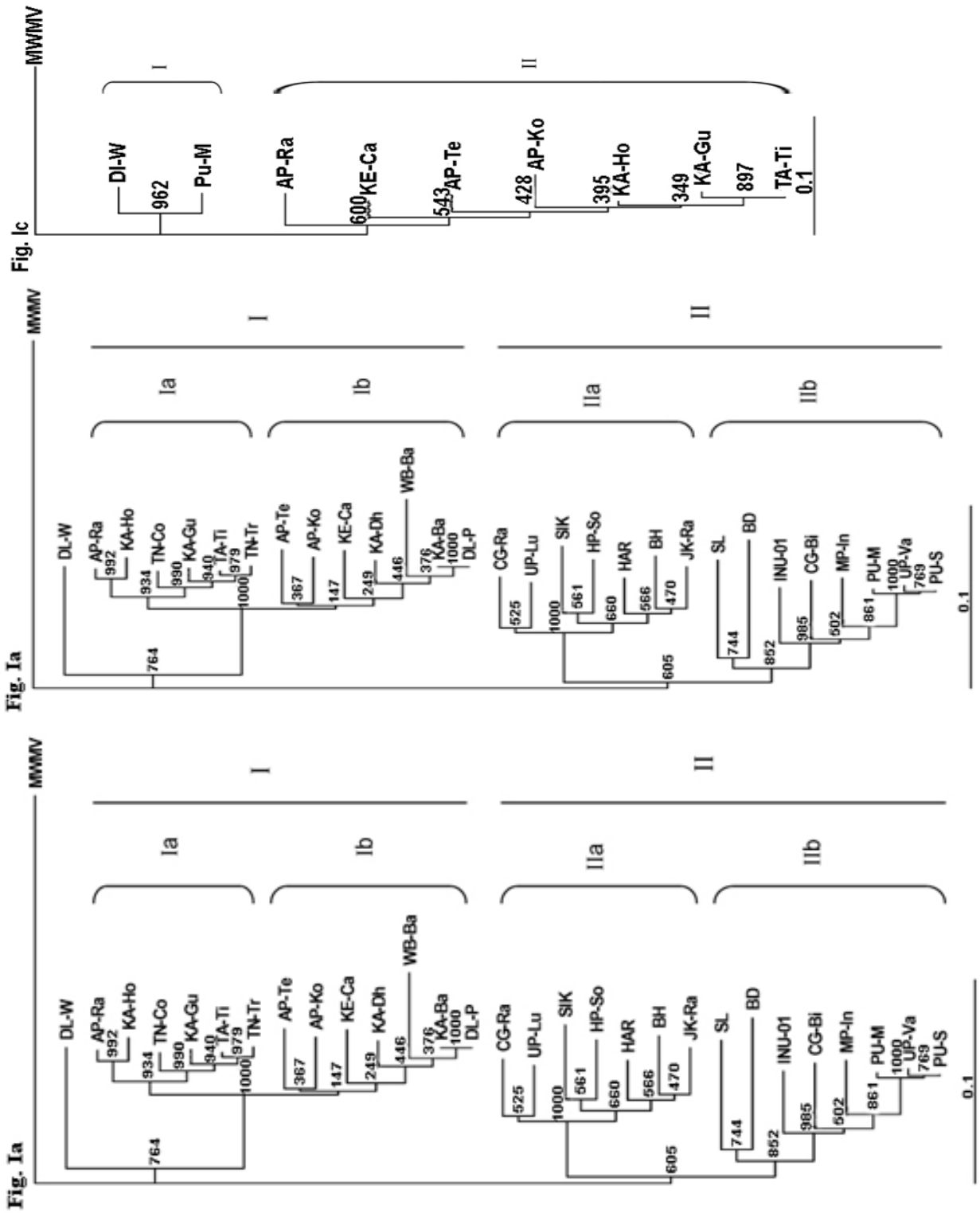

Figure 1. Bootstrapped neighbor joining tree showing the relationships between the nucleotide (a) and deduced amino acid (b) sequences of coat protein gene and 3′ UTR (c) of PRSV isolates from different geographical locations in the Indian sub-continent. Numbers at nodes represent bootstrap values with 1000 replications. Value on the scale bar represents 0.1 substitutions per site. The tree was rooted on the MWMV sequence.

of both nucleotide and amino acid sequence variability, DL-P clustered in one of the two south Indian isolate clusters, and DL-W existed as sister isolate. These relationships suggest that DL-P and all south Indian isolates could have evolved from DL-W. Furthermore, SL isolate showed high divergence with all south Indian isolates in terms of both nucleotide and amino acid sequences, suggesting that Sri Lanka is unlikely to be the source of infection for south India despite their geographical proximity.

Though the phylogenetic trees produced two major lineages, separating south Indian isolates from isolates of other regions, the clustering pattern in both main lineages did not correlate well with their geographical origins, and isolates thus appeared to be a single mixed population with some defined sub-populations. The phylogenetic relationships among isolates suggest that there has been considerable mixing and movement of isolates within and among all regions.

The present study agrees with recent reports (Hema and Prasad, 2004; Jain *et al.,* 2004; Jyoti Sharma *et al.,* 2005), revealing that the PRSV isolates from the Indian sub-continent are most diverse, and among south Indian isolates the divergence was found to be relatively low most probably due to the recent occurrence of the virus. This coincides with the rapid and recent increase in papaya cultivation in south India. The close relationship among south Indian isolates was also noticed from the dendrogram constructed based on available sequences of 3' UTR. However, sequences of CP and 3' UTR of more isolates are needed to ascertain the complete population profile and to draw strong conclusions on sequence divergence within the PRSV population in the Indian sub-continent.

Sequence variability has important implications for the use of CP genes to develop transgenic plants by CPMR (Savenkove and Valkonen, 2001). Evidence already exists for several virus-host systems to suggest that such resistance can be highly sequence specific (Sanders *et al.,* 1992; Lomonossoff, 1995; Tennant *et al.,* 2001). The selection of the transgene would be vital step to develop long-lasting virus resistant transgenic papaya. It has been suggested that when designing transgenes for potyvirus resistance, it is essential to select regions of at least 90% identity between strains to obtain a wide resistance (Moreno *et al.,* 1998). RNA mediated resistance to potyviruses has been reported with sequence identity of 88% or greater (Mueller *et al.,* 1995), while Jones *et al.*, (1998) showed that 89% identity of the Nib gene was the minimum sequence identity for the specificity required to trigger gene silencing in the pea seed-borne mosaic potyvirus. Based on the data on genetic diversity among south Indian isolates of PRSV, the CP gene of TA-Ti isolate seems to be an ideal choice to develop transgenic papaya resistant to isolates specific to south India, using CPMR. This isolate shares maximum sequence identities both at nucleotide and amino acid levels with most of the south Indian isolates.

Acknowledgements

The authors are grateful to Dr. J.A.M. Rezende, Departmento de Entomologia e Zoologia Agricola, Universidade de Sao Paulo, Brazil and Dr. Tetsuo Maoka, Plant pathology Lab, NARC for Hokkaido, Japan for providing antiserum of PRSV-W, and PLdMV, respectively. This research was funded by University Grants Commission, Government of India [31-254/2005 (SR), Dt. 31/03/2006]

Literature cited

Atreya C.D., B. Raccah and T.P. Pirone, 1990. A point mutation in the coat protein abolishes aphid transmissibility of a potyvirus. *Virology* 178, 161–165.

Bateson M.F., J. Henderson, W. Chaleeprom, A.J. Gibbs and J.L. Dale, 1994. *Papaya ringspot potyvirus*. Isolate variability and the origin of PRSV type P (Australia). *Journal General Virology* 75, 3547–3553.

Bau H.J., Y.H. Cheng, T.A. Yu, J.S. Yang and S.D. Yeh, 2003. Broad spectrum resistance to different geographic strains of *Papaya ringspot virus* in coat protein gene transgenic papaya. *Phytopathology* 93, 112–120.

Capoor S.P. and P.M. Varma, 1958. A mosaic disease of papaya in Bombay. *Indian Journal of Agriculture Science* 29, 225–233.

Gonsalves D., 1998. Control of *Papaya ringspot virus* in papaya: a case study. *Annual Review of Phytopathology* 36, 415–437.

Gonsalves D. and M. Ishii, 1980. Purification and serology of *Papaya ringspot virus*. *Phytopahology* 73, 1028–1032.

Hema M.V. and D.T. Prasad, 2004. Comparison of the coat protein of a south Indian strain of PRSV with other strains from different geographical locations. *Journal of Plant Pathology* 86(1), 35–42.

Hussain S. and A. Varma, 1994. Occurrence of *Papaya ringspot virus* from Amritsar (Punjab) India. *Journal of Phytopathology Research* 7, 77–78.

Jain R.K., J. Sharma, A.S. Sivakumar, P.K. Sharma, A.S. Byadgi, A.K.Verma and A. Varma, 2004. Variability in the coat protein gene of *Papaya ringspot virus* isolates from multiple locations in India. *Archives of Virology* 149, 2435–2442.

Jain R.K., H.R. Pappu, S.S. Pappu, A. Varma and R.D. Ram, 1998. Molecular characterization of papaya ringspot potyvirus isolates from India. *Annals of Applied Biology* 132, 413–425.

Jones A.L., I.E. Johansen, S.J. Bean, I. Bach and A.J. Maule, 1998. Specificity of resistance to pea seed-borne mosaic potyvirus in transgenic peas expressing the viral replicase (NIb) gene. *Journal of General Virology* 79, 3129–3137.

Jyoti Sharma, R.K. Jain, M. Ramaiah, A. Varma, 2005. Natural spread of *Papaya ringspot virus* to new areas: occurrence in Coimbatore, Tamil Nadu. *Indian Phytopathology* 58 (2), 245–249.

Lines R, D. Persley, J. Dale, R. Drew and M.F. Bateson, 2002. Genetically engineered immunity to *Papaya ringspot virus* in Australian papaya cultivars. *Molecular Breeding* 10, 119–129.

Lomonossoff G.P., 1995. Pathogen-derived resistance to plant viruses. *Annual Review of Phytopathology* 33, 323–343.

Meyer P., 1996. Homology-dependent gene silencing in plants. *Annual Review of Plant Physiology* 47, 23–48.

Moreno M., J.J. Bernal, I. Jimenez and E. Rodriguze-Cerezo, 1998. Resistance in plants transformed with the P1 or P3 gene of tobacco vein mottling potyvirus. *Journal of General Virology* 79, 2819–2827.

Mueller E., J. Gilbert, G. Davenport, G. Brigneti and D.C. Baulcombe, 1995. Homology-dependent resistance: transgenic virus resistance in plants related to homology-dependent gene silencing. *The Plant Journal* 7, 1001–1013.

Quemada H., B. L'Hostis, D. Gonsalves, I.M Reardon, R. Heinrikson, E.L. Heigert, L.C. Sieu and J.L. Slightom, 1990. The nucleotide sequences of the 3' terminal regions of *Papaya ringspot virus* strains W and P. *Journal of General Virology* 71, 203–210.

Sanders P.R., B. Sammons, W. Kaniewski, L. Haley, J. Layton, B.J. Lavallee, X. Delannay and N.E. Tumer, 1992. Field resistance of transgenic tomatoes expressing the tobacco mosaic virus or tomato mosaic virus coat protein genes. *Phytopahology* 82, 683–690.

Savenkove E.I. and J.P. Valkonen, 2001. Coat protein gene mediated resistance to potato virus-A in transgenic plants is suppressed following infection with another potyvirus. *Journal of General Virology* 82, 2275–2278.

Shukla D.D., M.J. Frenkel, N.M. McKern and C.W. Ward, 1994. New developments in the detection and classification of potyviruses. In: *Virology in the Tropics* (Narayan Rishi, K.L. Ahuja, B.P. Singh, ed.), Malhotra Publishing House, New Delhi, 571–581.

Silva-Rosales L., N. Becerra-Leor, S. Ruiz-Castro, D. Teliz-Ortiz and J.C. Noa-Carrazana, 2000. Coat protein sequence comparisons of three Mexican isolates of *Papaya ringspot virus* with other geographical isolates reveal a close relationship to American and Australian isolates. *Archives of Virology* 145, 835–843.

Tennant P.F., M.M. Fitch, R.M. Manshardt, J.L. Slightom and D. Gonsalves, 2001. *Papaya ringspot virus* resistance of transgenic rainbow and sunup is affected by gene dosage, plant development, and coat protein homology. *European Journal of Plant Pathology* 107, 645–665.

Thompson J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins, 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24, 4876–4882.

Varma A., 1988. The economic impact of filamentous plant viruses – The Indian sub-continent. In: *The Plant Viruses* (R.G. Milne, ed.), Plenum Press, New York, NY, USA, 371–376.

van Regenmortel M.H.V., C.M. Fauquet, D.H.L. Bishop, E. Carstens, S. Lemon, J.Maniloff , M.A. Mayo, D. McGeoch, C.R. Pringle and R.B. Wickner (ed.), 2000. *Virus Taxonomy*. Seventh report of the International committee on taxonomy of viruses. Academic press, New York, NY, USA, 709 pp.

Wang C.H. and S.D. Yeh, 1997. Divergence and conservation of the genomic RNAs of Taiwan and Hawaii strains of *papaya ringspot potyvirus*. *Archives of Virology* 142, 271–285.

Yeh S.D., F.J. Jan, C.H. Chiang, P.J. Doing, M.C. Chen, P.H. Chung and H.J. Bau, 1992. Complete nucleotide sequence and genetic organization of *Papaya ringspot virus* RNA. *Journal of General Virology* 73, 2531–2541.

Accepted for publication May 11, 2011