

Molecular Characterization of Papaya Ring Spot Virus Isolates From Different Parts of Tamil Nadu

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ABSTRACT

An attempt has been made to characterize different PRSV isolates which infects papaya plants were collected from different parts of Tamil Nadu. The isolates were of PRSV-P type strain and the Coat Protein gene (CP) varied in size from 840- 860 bp which encodes 280 - 284 amino acids. Sequence alignment results revealed that the seven sequences obtained from different regions shared a homology of about 90% -95% with each other and homology of about 96% with the already reported Indian isolate and 88-90% with the other country isolates collected from NCBI. KE repeats were observed in the N terminus of the CP coding region from the different isolates found out. Phylogenetic analysis revealed that the isolates identified here placed on the same group and reference isolates were grouped in a different progeny. Our study further helped us to identify conserved regions among the seven isolates and we also constructed an RNAi silencing vector targeting Coat protein gene to challenge the Papaya Ring Spot Virus (PRSV) for our future study.

Keywords: Coat protein gene, Papaya Ring Spot Virus (PRSV), FAO

INTRODUCTION

Papaya, *Carica papaya* L., is an important fruit crop in tropical and subtropical regions due to its economic, nutritional, industrial, pharmaceutical and medicinal values, for local

and export markets. *Carica papaya*, a member of the Caricaceae family, probably originated from the Southern part of Mexico and the Northern region of Central America (Badillo, 1993).

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The Food and Agricultural organization (FAO) estimated that about 65235907 ha area harvested by primary fruits in 2016 all around the world yielding 132730 hg/ha producing 870 million metric tons in 2018. Their data reveals papaya is cultivated world wide 13.6 million tonnes in 2018 production in particular. India, is the largest papaya producer of papaya in world with an estimated share of 48 percent output in 2018 (Altendorf, 2019). In India papaya is cultivated in area of around 1,34000 Ha with the production of about 5940000 MT during the year 2016-2017 (National Horticultural Board, 2018).

As reported earlier this fruit is claimed to be an excellent sources of vitamins like A, C and E with exhibiting antioxidant activity (Marfo et al., 1986), additionally it also contains minerals magnesium, potassium and pantothenic acid and folate (Aravind et al., 2013). A part from vitamins Papaya plants can able to produce papain and chymopapain, two vital proteolytic enzymes which are found in the milky white latex secreted from the fruits (Hewitt et al., 2000). One of the major limiting factor faced by farmers during papaya production are the diseases caused by Papaya Ring Spot Virus (PRSV) one of the major viral disease. This PRSV is animated by aphids on very few hosts namely cucurbits and papaya. Based on the host PRSV is classified into two biotypes: Type-P and Type-W where Type W infects cucurbits and papaya, and PRSV-W, infects only cucurbits but not papaya (Purcifull et al., 1984).

Researchers achieved considerable success through classical breeding methods but they were challenged by incorporation of the resistant genes to diseases. Without these resistant genes, yield and productivity will be reduced (Agrios, 1997). Most importantly lack of natural host resistance to PRSV-P in papaya cultivars. Plant genetic engineering has now become one of the most important tools for genetic improvement of rice cultivars with cloned genes conferring resistance to diseases, because it overcomes the incompatibility barriers among crop species by allowing the transfer of foreign genes into crop plants by

transformation (Muthukrishnan et al., 2001). Coat Protein-Mediated Resistance (CPMR) has been successfully used to confer resistance to a wide range of viruses including PRSV (Gonsalves, 1998). Previous studies have indicated that coat protein genes of PRSV strains are very distinct in geographic origin and in pathogenicity (Bateson et al., 1994; & Wang et al., 1994).

Hence, transgenic papaya with CP genes specific to the PRSV-P strains existing in a particular region need to be developed for effective control in that region. The success of CPMR depends on the compatibility and genetic relatedness to the challenging virus of the coat protein genes expressed in the transgenic plants (Hema & Prasad, 2003). Therefore, in a particular region, it is essential to know the nucleotide and amino acid sequences of the PRSV coat protein (CP) gene and to determine how much this differs from those of other PRSV isolates. Knowledge of the genetic variation within isolates is necessary for their efficient control and management. The study of the mechanisms of the CP gene of PRSV viruses will be used to find a CP gene homology DNA sector of PRSV and developing an RNA-mediated resistance transgenic plant stratagem, and a breeding broad-spectrum-virus resistance of papaya line. This is of vital significance in expanding papaya production. With this background, this present study was present to characterize PRSV infected isolates from different parts of Tamil Nadu.

MATERIALS AND METHODS

Cloning and characterization of PRSV CP gene

PRSV infected leaves and fruit samples showing virus infected symptoms such as vein clearing, mottling, malformed leaves, filiformy, ring spots and streaks on fruits, stems and petioles, and stunting (Purcifull et al., 1984) were collected from different places of Coimbatore and Erode districts and the places were mentioned in table (Table 4). Total RNA was extracted from infected papaya leaves using a SV total RNA kit

(Promega, USA) following the instructions from the manufacturer. 1 µg purified RNA was used as a template to synthesis first-strand cDNA using a RevertAid™ kit (Fermentas) as per protocol instructed by the manufacturer. First-strand cDNA (1 µl) was used as template for subsequent PCRs. The thermal profile was 94°C for 5 min (denaturation); 40 cycles of 94°C for 1 min (denaturation), 46°C for 1 min (annealing), and 72°C for 1 min (extension); and 72°C for 5 min. RT-PCR was carried out using the Eppendorf Mastercycler (USA) in a 20 µl final reaction volume containing Phusion enzyme (2u/ µl), 10 pmol of each primer and a template. The *actin* gene was used as an internal control. Primers used in this study are presented in Table 1.

RT-PCR amplification of coat protein gene

The first strand (cDNA) was used as a template for the synthesis of second strand through RT-PCR with the help of gene specific primers (Jain et al., 2004). The purified cDNA from each sample was cloned into the pJET1.2 blunt vector, from Clone JET PCR cloning kit (Fermentas, USA) according to the manufacturer's instructions. 10 µl of the 2x reaction buffer PCR was mixed with 1 µl of the purified DNA, 1 µl of vector, and 1 µl of T4 DNA ligase was added and reaction was set up 20 µl volume by adding nuclease free water. The mixture was incubated for 5 min at 22°C. Transformation was carried out through DH5α competent cells using heat shock. Putative colonies were identified and Plasmid DNA was isolated from the bacterial cultures. Restriction enzyme selection was made based on the restriction map of the vector pJET1.2/blunt and the selected sites for restriction does not occur within our DNA insert. Approximately, 500 ng of each plasmid DNA was digested with restriction endonuclease *XhoI* and *XbaI* in appropriate buffers at 37 °C for 2 h and the restriction products were fractionated on 1.0 % agarose gel. After confirmation by restriction digestion, the remaining plasmid DNA was packed in micro centrifuge tubes, labeled properly and sent for sequencing.

Sequencing of the cloned plasmid products were done using the vector encoded pJET1.2 forward primer (5'- CGACTCACTA TAGGGAGAGCGGC-3') and pJET1.2 reverse primer (5'-AAGAACATCGA TTTTCCATGGCAG-3'). The clones were sequenced using the automated DNA sequencer (1st base, Singapore). The sequences received were searched for primer binding, using the find option. In some instances, primer binding site was not found such sequences are converted to reverse antisense strand and then the primer binding site was matched. Similarly, the reverse primer binding site was identified. After identifying the primer binding sites, sequences flanking the forward and reverse primer binding sites were trimmed. Thus all the sequences for the differentially expressed genes were obtained in this manner.

Blast search for sequence similarity

The BLAST programme (Altschul et al., 1990) was used to identify related sequences available from the GenBank databases. Multiple sequence alignments were made using CLUSTAL X (1.8). Sequence phylograms were constructed using Bio Edit package. The following table shows the list of sequences taken from NCBI for comparative analysis (Table.2).

RESULTS & DISCUSSION

RT-PCR amplification of coat protein gene:

First strand cDNA from total RNA isolated from PRSV infected papaya leaves and fruits were used as template for PCR reaction. PRSV – CP gene of desired size of around 850 bp was amplified (**Figure.1**) and Actin was used as an internal control primers a fragment of 520 bp *actin* gene got amplified. Recombinant colonies were identified by colony PCR. The plasmids isolated from respective colonies (randomly selected) were digested with *Xba I* and *Xho I* resulted in release of the insert DNA of expected size 850 bp.

Gene sequencing and molecular characterization of cloned gene

Plasmids containing PRSV CP gene from five different locations were sequenced and

analysed with each other and compared with the already reported sequences from NCBI. Sequencing results revealed that most of the isolates showed a matrix of around 99 % similarity when compared with each other. A matrix of 96-99% similarity was obtained with already reported Indian isolate collected from NCBI database with the PRSV-CP sequences reported from different locations of Tamil Nadu mentioned here which were collected from NCBI database (Table.3). On the other hand when the isolates were compared with CP gene sequences from China and USA they shared a homology of around respectively according to sequence identity matrix.

Multiple sequence alignment through ClustalW revealed that the Indian isolates were of 95-99% homology with each other when compare with other isolates reported from different countries (Figure.2).

The phylogenetic analysis showed that the Tamil Nadu isolates identified in our experiment were found to be in same cluster (Figure.4). On the other hand the reference isolates which were reported from China and USA were placed in another cluster. The results showed that there is a considerable between sequences from different countries when compared with Indian isolates in general. The alignment of coat protein sequences revealed an average identity of 90-95 % at the nucleotide sequence. The Indian isolates form a close pair with one another but the USA and China isolates forms a distant relation with the isolates reported here.

Analysis of sequence variation in CP genes

Sequencing results showed that the CP genes of the 7 isolates found in this study were approximately 858 bp in nucleotide length and were deposited in Gen Bank except one (Table 1). Three PRSV complete CP sequences originated from China, USA and India were retrieved from Gen Bank and used for comparative analysis along with our reports named as CHN, USA and IND. (Table 1) The sequences referred and reported here were used for amino acid alignment analysis of CP genes. We found a large number of EK (glutamic acid and lysine) repeats. Also we

found conserved WCIEEN and QMKAAA regions, among the seven isolates with the already reported isolates (Figure. 3). These results interprets that the isolates reported here shared a maximum identity with the already reported evidences and shows they are of PRSV originated Coat protein gene.

DISCUSSION

Papaya ring spot virus (PRSV), biotype P (PRSV-P) is one of the most destructive diseases in papaya. This disease has become a major threat to papaya cultivation throughout India by rendering orchards economically unproductive. PRSV infection was reported to occur in every region where papaya is grown irrespective of the agro-climatic conditions, and the disease can result in crop losses of up to 85-90% (Lokhande et al., 1992; & Hussain & Varma, 1994). Coat Protein-Mediated Resistance (CPMR) has been found to be successful in maintaining resistance to PRSV (Lomonosoff, 1995; & Gonsalves, 1998). Studies indicated that coat protein genes of PRSV strains from different places were very different in pathogenicity (Bateson et al., 1994; & Wang et al., 1994). Hence, transgenic papaya expressing CP-genes specific to the PRSV-P strains existing in a particular region need to be developed for effective control in that region.

Coat Protein Mediated Resistance mainly relies on the expression of coat protein genes in the transgenic plants expressing the genes in challenging against the viruses (Bateson et al., 1994; & Hema & Prasad, 2003). Therefore, in a particular region, it is essential to know the nucleotide and amino acid sequences of the PRSV coat protein (CP) gene and to determine how much this differs from those of other PRSV isolates. PRSV- P is prevalent in India (Mali, 1985) but the strains responsible have not been adequately characterized at molecular level. Further, reports of molecular analysis to determine geographical specificity are limited. In the present study, we reported the molecular characterization of the PRSV- P CP gene of few Tamil Nadu isolates and discussed its

distinctiveness and phylogenetic relationship with, other PRSV-P isolates reported from different locations of India and also from China and USA.

As a result of comparative analysis with the already reported sequences from different locations of India, China and USA a homology identity matrix was obtained. This matrix reveals that the isolates reported here shared a homology of around 99% with the each other reported in this study. The data also shows that the CP sequences of PRSV isolates reported in the study shared a homology of 88-91% with the isolates from China and USA (from NCBI) and also they shared 96% with the already reported Indian isolates (from NCBI). From this data we come to know that there is no major difference between the isolates presented in this study. Our reports reveals that that there is no major variations between the isolates of same geographic origin and they show variation between geographical origin. This evidence was supported by Atreya et al. (1990) and we take their study on the CP region as a supporting report which says a substantial sequence variation was obtained between the world wide populations and not on the sequence diversity with the geographic origin (Atreya et al., 1990).

The sequences obtained in the present study also shows that the CP-coding region

lengths to about 858 nucleotides, encoding proteins of maximum around 286. These sequences when translated and aligned for amino acids they contain EK repeats which were thought to be exposed at the outer surfaces of the coat proteins (Shukla & Ward, 1989) and KE repeats boxes, are mostly found in the N terminus of the PRSV CP as reported (Silva et al., 2000). These EK repeats are found in two patterns which is comparable with the four pattern repeats reported by (Echevarria et al., 2016).

Variation in DNA sequences has to be taken into consideration in developing the transgenic plants through CPMR (Nakajima et al., 1993; Taschner et al., 1994; & Savenkov & Valkonen, 2001). Transgenic papaya harboring CP gene of the PRSV mild strain (HA 5- 1) had been found to confer resistance against infection only against the US-isolate (HA) but not against infection with other PRSV-P isolates including Australian, Thai and Taiwan (Chiang et al., 2001). The PRSV – CP isolates reported from Australia shared 96% and 98% nucleotide and amino acid sequence identity respectively in their coat proteins with the US isolates. Variation in the sequences may affect management of viruses. Combating viruses through Coat Protein mediated Resistance was found to be effective and targeting sequences specifically (Tennant et al., 2001).

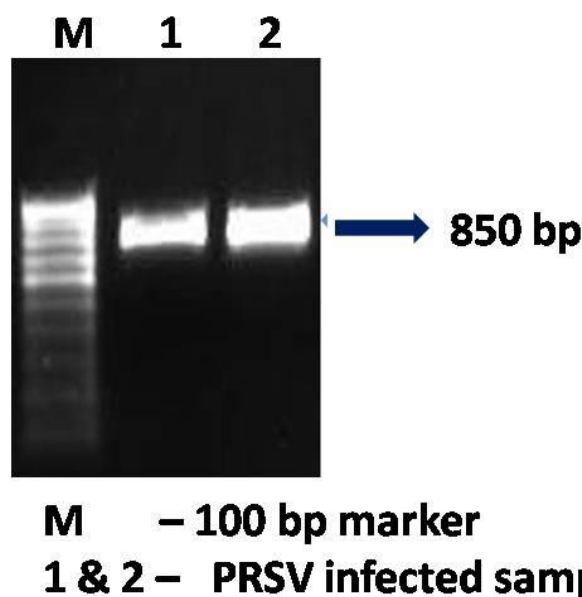


Figure 1: PCR amplification of 850 bp CP gene fragment

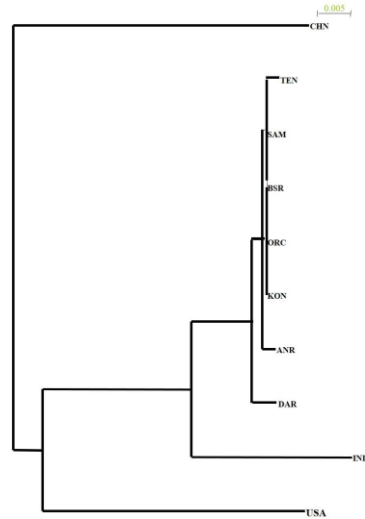


Figure 2: Phylogenetic analysis of PRSV isolates compared with isolates obtained from NCBI

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SAM TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
ORC TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
BSR TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
TEN TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
DAR TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
IND TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
CHN TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
USA TCCAAAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
KON TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 60
ANR TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 58
DAR TCCAAGAAATGAAGCTGTGGATGTGGGTCTCAAATGATAAGCTGAAGGGAGAAAAGAAAAAGAA 58
*****

SAM AAAAGATAAAGAGAGAGAAAAGAAAAAG---AA---AGACAAGAAAGGATGCTGGTGCACGGA 114
ORC AAAAGATAAAGAGAGAGAAAAGAAAAAG---AA---AGACAAGAAAGGATGCTGGTGCACGGA 114
BSR AAAAGATAAAGAGAGAGAAAAGAAAAAG---AA---AGACAAGAAAGGATGCTGGTGCACGGA 114
TEN AAAAGATAAAGAGAGAGAAAAGAAAAAG---AA---AGACAAGAAAGGATGCTGGTGCACGGA 114
DAR AGAGATAAAGAGAGAGAAAAGAAAAAG---AA---AGACAAGAAAGGATGCTGGTGCACGGA 114
IND AAAAGATAAAGAGAGAGAAAAGAAAAAG---AA---AGACAAGAAAGGATGCTGGTGCACGGA 114
CHN -----AAAAGAAAAAGATAAACAAAAGGACAA---AAATAATGATGGTACAGTGCACGGA 111
USA AAAAGAAAAAGAAAAGAAAAGAAAAGAAAAG---AA---AGAAAAGAAAGGATGCTGGTGCACGGA 117
KON GTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCACAGCAAAATGACATCTCAAAACACT 118
ANR GTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCACAGCAAAATGACATCTCAAAACACT 118
*****

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ORC GGTATATGTGTGTCAAC-TAGCACAAAAAAGCTGGAGAGAGAGATAGAGATGTCAATGCTGGGAC 173
BSR GGTATATGTGTGTCAAC-TAGCACAAAAAAGCTGGAGAGAGAGATAGAGATGTCAATGCTGGGAC 173
TEN GGTATATGTGTGTCAAC-TAGCACAAAAAAGCTGGAGAGAGAGATAGAGATGTCAATGCTGGGAC 173
DAR GGTATATGTGTGTCAAC-TAGCACAAAAAAGCTGGAGAGAGAGATAGAGATGTCAATGCTGGGAC 173
IND GGTATATGTGTGTCAAC-TAGCACAAAAAAGCTGGAGAGAGAGATAGAGATGTCAATGCTGGGAC 173
CHN AACGATATGTGTCAAC-TAGTACAAAAAAGCTGGAGAGAGAGATAGAGATGTCAATGCTGGGAC 170
USA AATGATATGTGTGTCAAC-TAGTACAAAAAAGCTGGAGAGAGAGATAGAGATGTCAATGCTGGGAC 176
KON CGTGCACACAAATCGCAGTTTGGAGAAAGTGGTATGAGGGAGTGGGAAATGACATA-TGGCCT 177
ANR CGTGCACACAAATCGCAGTTTGGAGAAAGTGGTATGAGGGAGTGGGAAATGACATA-TGGCCT 177
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ORC TAGTGGTACATTTACAGTTCCAAAGAAATCAAATGATAAAATGATTTTGGCCAG 233
BSR TAGTGGTACATTTACAGTTCCAAAGAAATCAAATGATAAAATGATTTTGGCCAG 233
TEN TAGTGGTACATTTACAGTTCCAAAGAAATCAAATGATAAAATGATTTTGGCCAG 233
DAR TAGTGGTACATTTACAGTTCCAAAGAAATCAAATGATAAAATGATTTTGGCCAG 233
IND TAGTGGTACATTTACAGTTCCAAAGAAATCAAATGATAAAATGATTTTGGCCAG 233
CHN TAGTGGGACTTTTCACTGTTCGGAGGATAAAATCAATTTACTGATAAGATGATTTTACCAG 230
USA CAGTGGAACTTTTCACTGTTCGGAGGATAAAATCAATTTACTGATAAGATGATTTTACCAG 236
KON TAGTGAATA-ATGAAATGCAAGTAAATGTGAAATGGCTTAATGGTTTGGTGTATCGAAAATG 236
ANR TAGTGAATA-ATGAAATGCAAGTAAATGTGAAATGGCTTAATGGTTTGGTGTATCGAAAATG 236
*****

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ORC AAATFAAGGGGAAAAGCTGTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCACAGCAAAAT 293
BSR AAATFAAGGGGAAAAGCTGTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCACAGCAAAAT 293
TEN AAATFAAGGGGAAAAGCTGTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCACAGCAAAAT 293
DAR AAATFAAGGGGAAAAGCTGTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCACAGCAAAAT 293
IND AAATFAAGGGGAAAAGCTGTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCACAGCAAAAT 293
CHN AAATFAAGGGGAAAAGCTGTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCGAAAACAAAT 290
USA AAATFAAGGGGAAAAGCTGTCCCTTAATTTGAAATCACTCTCTCAGTATAATCCGAAAACAAAT 296
KON GTACATCCCCAGACATATCTGGTGTCTGGGTTATGATGGATGGTGGACTCAGGTCGATTT 296
ANR GTACATCCCCAGACATATCTGGTGTCTGGGTTATGATGGATGGTGGACTCAGGTCGATTT 296
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SAM TGACATCTCAAAACACTCGT---GCCACACAAATCGCAGT---TTGAGAAGTGGTATGAGGGAGT 350
ORC TGACATCTCAAAACACTCGT---GCCACACAAATCGCAGT---TTGAGAAGTGGTATGAGGGAGT 350

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BSR TGACATCTCAAACACTCGT--GCCACACAATCGCAGT-TTGAGAAGTGGTATGAGGGAGT 350
TEB TGACATCTCAAACACTCGT--GCCACACAATCGCAGT-TTGAGAAGTGGTATGAGGGAGT 350
DAR TGACATCTCAAACACTCGT--GCCACACAATCGCAGT-TTGAGAAGTGGTATGAGGGAGT 350
IND TGACATCTCAAACACTCGT--GCCACACAATCGCAGT-TTGAGAAGTGGTATGAGGGAGT 350
CHN TGACATCTCAAACACTCGC--GCCACTCAATCTCAAT-TTGAGAAGTGGTACGAGGGAGT 347
USA TGACATTTCAAACACTCGT--GCCACTCAGTCACAA-TTGAGAAGTGGTATGAGGGAGT 353
KOR ACCCAATTAACCATTAAATTGAACATGCAACTCCTTCAATTAAGGCAAAATCATGGCTCACT 356
AMR ACCCAATTAACCATTAAATTGAACATGCAACTCCTTCAATTAAGGCAAAATCATGGCTCACT 356
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BSR GAGGAAATGACTATGGCCTTAGTGATAAATGAAATGCAAGTAATGTTGAAATGGCTTAAATGGT 410
TEB GAGGAAATGACTATGGCCTTAGTGATAAATGAAATGCAAGTAATGTTGAAATGGCTTAAATGGT 410
DAR GAGGAAATGACTATGGCCTTAGTGATAAATGAAATGCAAGTAATGTTGAAATGGCTTAAATGGT 410
IND GAGGAAATGACTATGGCCTTAGTGATAAATGAAATGCAAGTAATGTTGAAATGGCTTAAATGGT 410
CHN GAGGAAATGACTATGGCCTTAGTGATAAATGAAATGCAAGTAATGTTGAAATGGCTTAAATGGT 410
USA GAGGAAATGACTATGGCCTTAGTGATAAATGAAATGCAAGTAATGTTGAAATGGCTTAAATGGT 413
KOR TCAGTAAACGCGGCAGAACATATATCGCGAAGCG-AAAATGCAACTGAGAGATAC-ATGCC 414
AMR TCAGTAAACGCGGCAGAACATATATCGCGAAGCG-AAAATGCAACTGAGAGATAC-ATGCC 414
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AMR GCAAAATCATGGCTCACTTCAGTAAACGCGGCAGAACATATATCGCGAAGCGAAAATGCAAC 590
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ORC TGAGAGATACATGCCGCGGTATGGAAATCAAGAGAAAATTTGACTGACATTAAGCCTCGCTAG 650
BSR TGAGAGATACATGCCGCGGTATGGAAATCAAGAGAAAATTTGACTGACATTAAGCCTCGCTAG 650
TEB TGAGAGATACATGCCGCGGTATGGAAATCAAGAGAAAATTTGACTGACATTAAGCCTCGCTAG 650

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DAR TGAGAGATACATGCCGCGGTATGGAATCAAGAGAAATTTGACTGACATTAGCCTCGCTAG 650
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***
SAM TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
ORC TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
BSR TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
TEN TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
DAR TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
IND TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
CHN CATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 766
USA CATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 772
KOH TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
ANR TATGCAGATGAAGGCAGCTGCACCTGCGGAACACAAATCGCA-GAATGTTTGGAAATGGACG 769
*****
SAM GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
ORC GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
BSR GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
TEN GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
DAR GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
IND GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
CHN GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 826
USA GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 832
KOH GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
ANR GCAGTGTGAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAAGATGTCAATAGAG 829
*****
SAM ACATGCACTCTCTCCTGGG-TATGCGCAAC 858
ORC ACATGCACTCTCTCCTGGG-TATGCGCAAC 859
BSR ACATGCACTCTCTCCTGGG-TATGCGCAAC 858
TEN ACATGCACTCTCTCCTGGG-TATGCGCAAC 858
DAR ACATGCACTCTCTCCTGGG-TATGCGCAAC 858
IND ACATGCACTCTCTCCTGGG-TATGCGCAAC 858
CHN ACATGCACTCTCTCCTGGG-TATGCGCAAC 855
USA ACATGCACTCTCTCCTGGG-TATGCGCAAC 861
KOH ACATGCACTCTCTCCTGGG-TATGCGCAAC 858
ANR ACATGCACTCTCTCCTGGG-TATGCGCAAC 858
*****

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Figure 2: Multiple sequence alignment of PRSV isolates compared with isolates obtained from NCBI

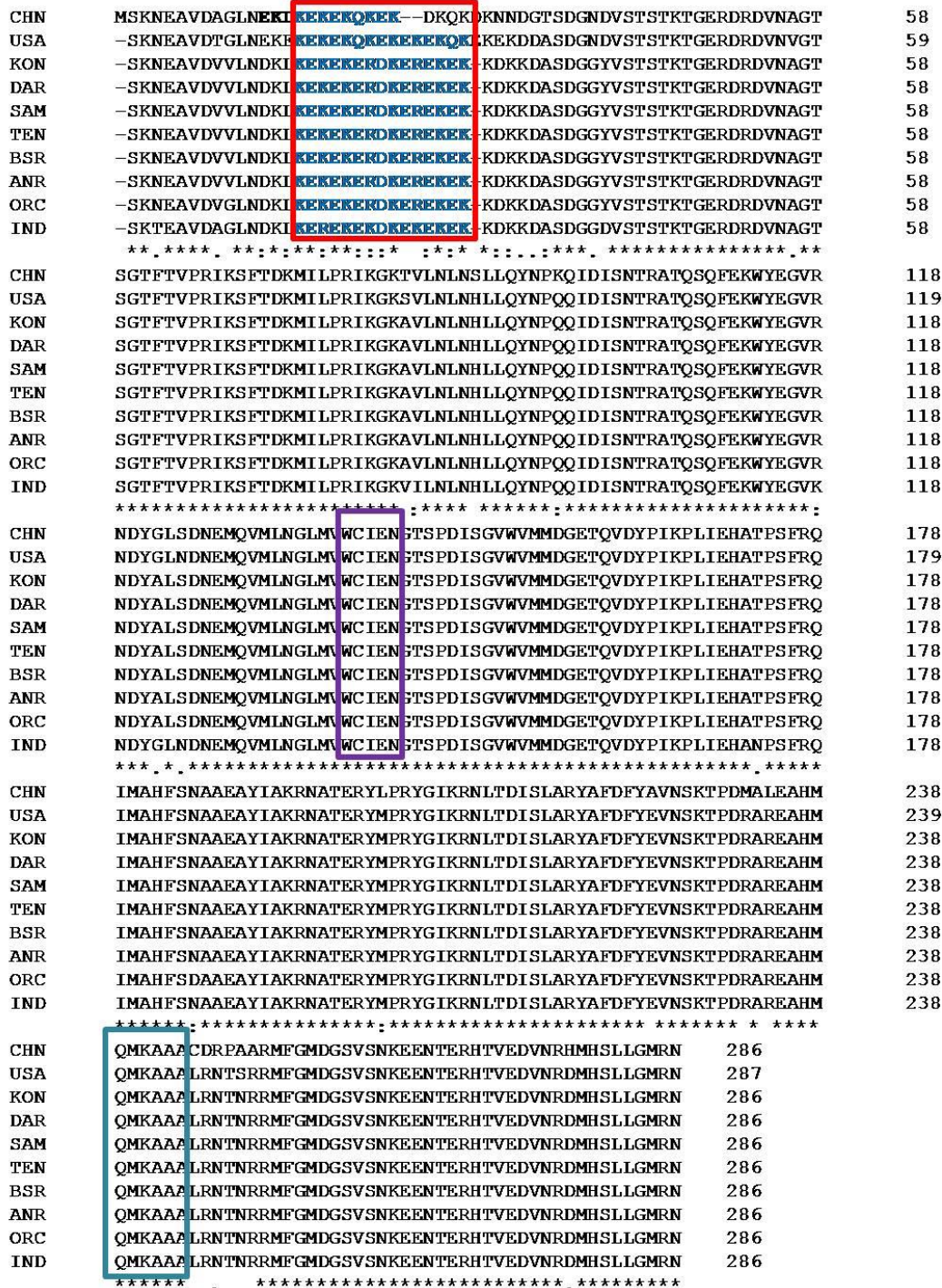


Figure 3: Multiple sequence alignment of amino acid sequences of the PRSV isolates compared with reported isolates obtained from NCBI

Table 1: List of areas where Papaya Ring Spot virus infected samples were collected

Sl No	Places	District
1.	TNAU-Orchard	Coimbatore
2.	Kondaiyampalayam	Coimbatore
3.	Thenamanallur	Coimbatore
4.	Annur	Coimbatore
5.	Dharapuram	Tirupur
6.	Sathyamangalam	Erode
7.	Bhavanisagar	Erode

Table 2: List of Sequences collected from NCBI for comparison

SL. No	Accession no	Country	Reference
1.	AY238880	India	Jain, et al., 2004
2.	AF469065	China	Ye, et al., 1991
3.	D00594	USA	Quemada et al., (1990)
4.	TNAU-Orchard (ORC) HM626464.1	India	Unpublished
5.	Kondaiyampalayam (KON) HM626465.1	India	Unpublished
6.	Thenamanallur (TEN) Not yet submitted	India	Unpublished
7.	Annur HM778170.1	India	Unpublished
8.	Dharapuram (DAR) HM626467.1	India	Unpublished
9.	Sathyamangalam (SAM) HM626466.1	India	Unpublished
10.	Bhavanisagar (BSR) HM778171.1	India	Unpublished

Table 3: Sequence matrix identity among different PRSV-CP sequences

Seq->	KON	ORC	BSR	SAM	TEN	ANR	DAR	IND	USA	CHN
KON	1.000	0.997	1.000	1.000	1.000	0.99	0.994	0.962	0.915	0.900
ORC	-	1.000	0.997	0.997	0.997	0.994	0.991	0.960	0.912	0.898
BSR	-	-	1.000	1.000	1.000	0.997	0.994	0.962	0.915	0.900
SAM	-	-	-	1.000	1.000	0.997	0.994	0.962	0.915	0.900
TEN	-	-	-	-	1.000	0.997	0.994	0.962	0.915	0.900
ANR	-	-	-	-	-	1.000	0.991	0.960	0.918	0.903
DAR	-	-	-	-	-	-	1.000	0.962	0.912	0.897
IND	-	-	-	-	-	-	-	1.000	0.903	0.886
USA	-	-	-	-	-	-	-	-	1.000	0.887
CHN	-	-	-	-	-	-	-	-	-	1.000

CONCLUSION

By applying the concept of this molecular characterization the conserved regions from the different isolates were targeted for silencing the gene of Interest. The report discussed in the present study shows that there is very much less variation between the sequences. The variability in the CP gene of PRSV population paved a way in designing RNA-mediated virus genetic engineering strategy, involving a specially designed primer directing cloning of the PRSV-CP N terminus homology DNA sector. The primer targeting the Coat protein\ gene will be designed to

construct RNAi mediated plant expression vector, which will help a transgene for developing durable virus resistant transgenic papaya.

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