Comparison of the coat protein genes of two *Papaya ringspot virus* isolates with other isolates from different geographic locations

GOURGOPAL ROY and R.K. JAIN
Advanced Centre for Plant Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012

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*Papaya ringspot virus* (PRSV), currently ascribed to the *Potyvirus* genus of the family *Potyviridae* (3), induces a large array of symptoms in papaya and cucurbit cultivars, such as vein clearing, mottling, malformed leaves, filiformy, ringspots and streaks on fruits, stem and petioles and stunting (7). The virus occurs worldwide and is a major limiting factor for papaya production wherever it is grown (reviewed in (4)). PRSV significantly reduces yields of papaya and cucurbits in India (12) and prevalence of both papaya infecting (Type P) and non-papaya infecting (Type W) pathotypes have been recognised (8).

The virus is readily transmitted mechanically as well as by aphids in a non-persistent manner (7). PRSV particles are flexuous filaments (760-800 nm x 12 nm) and consist of a single polypeptide species of about 36 kDa and a single-stranded positive-sense RNA molecule of 10326 bases. The genome of PRSV is monocistronic and is expressed via a large polypeptide that is subsequently cleaved into functional proteins (13,14).

Both conventional and genetically engineered cross-protection strategies have been successfully applied to manage PRSV in papaya (reviewed in (4)). Durability of both the resistances depends on the variability of coat protein (CP) genes of PRSV population (4,10). Not much progress has been made in this direction in India. Recently, the coat protein (CP) genes of P (Pune) and W (Delhi) isolates from India were cloned and sequenced. Sequence comparison revealed that the CP genes of the two pathotypes shared 87% and 93% identity at nucleotide and amino acid levels, respectively. The nucleotide and amino acid differences were mainly confined to amino terminus (5).

Keeping in view that no information is available on sequence diversity within PRSV population from different locations, nucleotide and amino acid sequences of a part of the CP genes including amino terminal regions of two PRSV isolates from Hyderabad (Andhra Pradesh-AP) and Varanasi (Uttar Pradesh-UP) were determined and compared with three other PRSV isolates from different geographic locations from India whose sequences were already known.

PRSV isolates were collected from Hyderabad (Andhra Pradesh) and Varanasi (Uttar Pradesh) from naturally infected papaya plants showing severe mosaic symptoms. Their identity was confirmed by pathogenecity tests on papaya and were maintained in a glasshouse by mechanical inoculations using 0.01M potassium phosphate buffer (0.033M KH2PO4, 0.067M K2HPO4, pH 7.0).

Total RNA from infected tissue (10 mg) was extracted using RNeasy kit according to manufacturer’s instructions (Qiagen Inc., Chatsworth CA 91311, USA). The resulting RNA preparation was incubated at 70°C for 5 min and snap-cooled on wet ice for 2 min and then used as a template for reverse transcription and polymerase
chain reaction (RT-PCR) (6). The primer pair used to prove the amplification of 3'terminal region comprising of a part of the nuclear inclusion b (Nib) gene and the coat protein (CP) gene were obtained from H.R. Pappu, University of Georgia, USA. The upstream primer sequence, 5' ATG ATA GAG TCA TGG GG 3' and downstream primer sequence, 5' CGC GTT ACT GAA GTG AGC 3' were derived from the Nib region and CP region respectively. The amplification conditions included one cycle of 45 min at 42°C, 40 cycles of 30s at 94°C, 2 min at 46°C and 1 min at 72°C and one cycle of 60 min at 72°C. The amplicon derived from UP isolate was cloned in pUC 19 vector and sequenced while AP isolate was directly sequenced from the amplicon. Sequencing was carried out at Bangalore Genei Pvt. Ltd., Bangalore and Department of Biochemistry, South Campus, University of Delhi, Delhi. Both the sequences were submitted to GenBank under accession no. AF323637 and AF323638. The sequences were compared with three other PRSV isolates from India using Clustal W multiple alignment program (11) (Table 1).

The primers used gave a DNA band of expected size (approximately 800 bp) in both AP and UP isolates. The identity of amplicons was confirmed by sequencing. The sequenced region in both contained a single open reading frame that could potentially encode polyprotein, which included a part of Nib gene and a part of CP gene. The protease cleavage site between glutamine and serine (Q/S) was available in both the isolates. Conserved sequences in the CP gene such as, DAG and WCIE were present in both the isolates.

Comparative sequence analysis of a part of the CP genes including amino terminal regions from AP and UP isolates showed 82% and 87% identity at nucleotide and amino acid levels respectively. Sequence comparisons with three other isolates revealed that PRSV isolates shared 81-90% identity at nucleotide and 87-92% identity at amino acid levels respectively (Table 2). AP isolate shared 92% identity at amino acid levels with an isolate from Karnataka, whereas UP isolate shared 86% identity.

### Table 1. Sources of coat protein (CP) gene sequences of *Papaya ringspot virus* isolates from India used in this study for comparison

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Origin</th>
<th>Pathotype</th>
<th>Amino terminal CP amino acid</th>
<th>GenBank Accession No.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAR</td>
<td>Bangalore</td>
<td>P</td>
<td>165</td>
<td>AF120270</td>
<td>—</td>
</tr>
<tr>
<td>DL</td>
<td>Delhi</td>
<td>W</td>
<td>170</td>
<td>AF063221</td>
<td>Jain et al. (1998)</td>
</tr>
<tr>
<td>AP</td>
<td>Hyderabad</td>
<td>P</td>
<td>171</td>
<td>AF323638</td>
<td>This study</td>
</tr>
<tr>
<td>MAH</td>
<td>Pune</td>
<td>P</td>
<td>170</td>
<td>AF 063220</td>
<td>Jain et al. (1998)</td>
</tr>
<tr>
<td>UP</td>
<td>Varanasi</td>
<td>P</td>
<td>169</td>
<td>AF 323637</td>
<td>This study</td>
</tr>
</tbody>
</table>

Isolates: AP: Andhra Pradesh; UP: Uttar Pradesh; KAR: Karnataka; MAH: Maharashtra; and DL: Delhi

### Table 2. Percentage identities of nucleotide (upper half) and amino acid (lower half) sequences of amino terminal region of coat protein (CP) gene among isolates of *Papaya ringspot virus* from India

<table>
<thead>
<tr>
<th>Isolates</th>
<th>AP</th>
<th>UP</th>
<th>KAR</th>
<th>MAH</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>100</td>
<td>82</td>
<td>90</td>
<td>86</td>
<td>84</td>
</tr>
<tr>
<td>UP</td>
<td>87</td>
<td>100</td>
<td>81</td>
<td>84</td>
<td>83</td>
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<tr>
<td>KAR</td>
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<td>88</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>DL</td>
<td>89</td>
<td>87</td>
<td>87</td>
<td>89</td>
<td>100</td>
</tr>
</tbody>
</table>

Isolates: AP: Andhra Pradesh; UP: Uttar Pradesh; KAR: Karnataka; MAH: Maharashtra; and DL: Delhi
Like other potyviruses and their strains (9), our results confirm that both AP and UP isolates of PRSV possess variable amino termini (data not shown). Divergence in PRSV isolates was thus assessed by comparing amino termini of CP genes. Sequence divergence up to 13% at amino acid level was observed within Indian isolates including the two from this report, suggesting significant differences among the five PRSV isolates. Sequence comparison also revealed that AP isolate originating from Southern India shared a closer relationship (92%) with another isolate (KAR) originating from Southern India as compared to the isolates originating from other regions of India (87-90%), suggesting that the sequence divergence could be attributed to geographical regions. However, large number of isolates will have to be sequenced and compared to arrive at a definite conclusion. By contrast, lower level of divergence (upto 4%) has been observed within CP gene sequences of Australian and US isolates (1).

Realising that the CP gene derived protection against PRSV in papaya is highly sequence specific (reviewed in (4)) and the Indian isolates from different geographical regions are highly divergent within CP gene, transgenic resistance conferred by CP gene in papaya may not be durable in Indian conditions. Recent observation that five nucleotide differences in the challenged virus CP gene from the transgene could break the resistance of Rainbow (transgenic papaya cultivar) supports our view point (2). Hence, attempts will have to be made to align the CP gene sequences from large number of isolates and arrive at a consensus sequence which could be used as transgene to confer protection or look for some other suitable viral gene to confer protection in papaya.

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REFERENCES

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