Role of satellite RNA of an Indian isolate of cucumber mosaic virus in inducing lethal necrosis of tobacco plants

S K Raj, A Srivastava, G Chandra & B P Singh
Plant Virus Laboratory, National Botanical Research Institute, Lucknow 226 001, India

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Lethal necrosis or systemic stem necrosis followed by death of Nicotiana benthamiana, severe leaf deformations of N. tabacum cv. samsun NN symptoms were induced by experimental inoculations of CMV RNA preparations containing satellite RNA (sat-RNA). Inoculations of RNA preparations without sat-RNA did not induce that severe symptoms on these plants, only late mild mosaic was observed. It is suggested that sat-RNA of CMV isolate has a certain role for enhancing severity of symptoms in tobacco plants. Local and systemic lethal necrosis of N. benthamiana is due to sat-RNA present with genome of CMV isolate. It is the first report of lethal necrosis induced in N. benthamiana by CMV satellite.

Cucumber mosaic virus (CMV) belongs to Cucumovirus group characterized by isometric particles of about 29 nm in diameter, three single-stranded positive sense genomic RNA species, and a fourth subgenomic RNA, which acts as messenger RNA for the coat protein (CP) of about 24 kDa (ref. 1). In addition, some CMV isolates often support replication, encapsidation, and spread of an additional single-stranded RNA species of 330 to 391 nucleotides designated as satellite RNA (sat-RNA) which is known to be involved in modulation of symptoms3. However, some satellite RNAs of CMV increase / exacerbate the pathogenicity in specific hosts5. Lethal necrosis of tomato caused by CMV when a sat-RNA was associated with CMV genome6-8 are the examples of this phenomenon. The yellow chlorosis of tobacco7 and white disease as well as yellow chlorosis of tomato8,9 have also been reported to be associated with satellite RNAs of CMV.

CMV isolates infecting tobacco, petunia, chrysanthemum, carnation and amaranth were isolated and characterized at National Botanical Research Institute, Lucknow on the basis of aphid transmission in non-persistent manner, particle morphology, SDS-PAGE and serological relationships with other CMV strains.10-14 Some isolates of CMV were analyzed earlier15, among them CMV-C and CMV-T showed the presence of sat-RNA in their genome. CMV isolate from amaranth (CMV-A) indicating presence of sat-RNA in its genome induced severe leaf deformation in N. tabacum cv. white burley and Amaranthus hypochondriacus14. Based on earlier evidences that sat-RNA modulates the symptom expression we speculated that severity of symptoms and lethal necrosis in tobacco may be due to sat-RNA associated with CMV-Awar isolate. To prove this hypothesis, inoculations were done on three tobacco cultivars using RNA preparations with or without sat-RNA and symptoms that appeared on experimentally inoculated plants were analyzed.

Amaranth isolate of CMV (CMV-A) containing sat-RNA, isolated earlier14 was used as source of initial inoculum for virus propagation. Virus was purified from inoculated leaves (100 g) of N. tabacum cv. white burley as described16 and suspended in sterile water (1 mL). Virus preparations were observed by transmission electron microscope using uranyl acetate (pH 4.2; 20g/mL) as negative stain. The purified virus preparations were examined by UV spectrum and electron microscopy to assess the presence of CMV particles and by inoculations on Chenopodium amaranticolor (an indicator host of the isolate).

Viral nucleic acid was extracted by disrupting the purified particles of CMV with sodium dodecyl sulphate (SDS; 10 g/L) and an equal volume of phenol/chloroform followed by ethanol precipitation17. Nucleic acid preparations suspended in diethyl pyrocarbonate (DEPC) treated water were treated with DNase and RNase separately at 37°C for 30 min. and infectivity of treated and untreated nucleic acid preparations was checked by inoculating them on C. amaranticolor.
**Table I**—Symptoms appeared on various hosts* after inoculation of purified CMV, CMV-RNA with and without satellite RNA

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Purified particles</th>
<th>RNA 1, 2, 3 &amp; 4 (+) sat-RNA</th>
<th>RNA 1, 2, 3 &amp; 4 (-) sat-RNA</th>
<th>Sat-RNA alone</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N.</em> benthamiana</td>
<td>LN, SN, D or SMSP</td>
<td>LN, SN, D or SMSP</td>
<td>LMM</td>
<td></td>
</tr>
<tr>
<td><em>N.</em> tabacum cv. Samsun NN</td>
<td>LC &amp; SM</td>
<td>LC, BF &amp; SM</td>
<td>LMM</td>
<td></td>
</tr>
<tr>
<td><em>N.</em> tabacum cv. White burley</td>
<td>SM &amp; LD</td>
<td>SM &amp; LD</td>
<td>LMM</td>
<td></td>
</tr>
</tbody>
</table>

SM = Severe mosaic, LD = Leaf deformations, LMM = Late mild mosaic, LC = Leaf crinkle, BF = Blister formations, LN = Lethal necrosis, SN = Stem necrosis, D = Death of plant and SMSP = Severe mosaic in surviving plant and (−) = no symptoms.

* 5 replicates of each plant species were taken for experiments.

For separation of RNA species, electrophoresis was carried out in agarose gel under non-denaturing conditions as described earlier. The gel was stained with ethidium bromide (0.5 μg/mL) and observed under UV light. RNA1 and 2, RNA3, RNA4 (all together) and RNA5 (sat-RNA) were eluted from LMP agarose gel (10 g/L) electrophoresis under protected conditions of RNA and suspended in DEPC treated water. To investigate the role of sat-RNA, inoculations of RNA preparations with and without sat-RNA were done on *N.* tabacum cv. white burley, *N.* tabacum cv. Samsun NN and *N.* benthamiana plant species. Severity of symptoms was judged by visual observations of inoculated plants for 5-6 weeks. The back inoculation tests were also conducted on *C.* amaranticolor to know the presence of virus in symptom expressing plants.

Isolated virus particles (200 μg/mL) which showed UV spectrum characteristic of nucleoprotein (with A260/280 ratio of 1.58). Negatively stained samples of the preparation when observed under electron microscope revealed presence of 28 nm diam (typical of CMV) virus particles. Purified virus preparations were found infectious when inoculated on *C.* amaranticolor.

Nucleic acid extracted from purified particles was also found infectious on *C.* amaranticolor after being treated with DNase but after treatment with RNase infectivity was completely lost which indicated the presence of RNA as infectious entity. Electrophoresis of extracted nucleic acids revealed four distinct bands on agarose gels (15 g/L) which were identified as RNA1 and RNA2, RNA3, RNA4 and RNA5 (sat-RNA) while RNA1 and 2 were very close and did not separate as two distinct bands. Pattern of all RNA species was similar to the one usually observed from CMV. A sat-RNA of about 350 bases nearer to bromophenol blue front was observed (Fig. 1) as reported earlier.

Experimental inoculations of purified preparations and total RNA extracted from particles on *N.* benthamiana plant exhibited lethal necrosis in inoculated leaves after 5-6 days followed by systemic stem necrosis and death of plants after 15-20 days (Fig. 2). In a few surviving plants, lateral emerging branches exhibited severe systemic mosaic symptoms after 25-30 days. RNA preparation without sat-RNA did not produce such severe symptoms, only late mild mosaic was observed on *N.* benthamiana. Results from Table 1 also revealed that *N.* benthamiana. Results from Table 1 also revealed that *N.* benthamiana. Results from Table 1 also revealed that *N.* benthamiana.
saic, leaf deformation and blister formation symptoms after 15-20 days of inoculations with a RNA preparation having sat-RNA, but no lethal necrosis.

CMV genomic RNA (RNA 1, 2, 3 and 4) with sat-RNA could induce only mild mosaic symptoms on *N. tabacum* cv. white burley, *N. tabacum* samsun NN and *N. benthamiana* plants. Satellite RNA alone did not induce any of the symptoms on these plants. However, when the inoculum contained both genomic and sat-RNA together, the symptoms became severe, resulting in lethal necrosis, leaf deformation and stem necrosis or death of the plants (Table 1). These results indicate that sat-RNA is a major cause for enhancement of severity of symptoms in inoculated tobacco plants. Moreover, other Solanaceous species like *Lycopersicon esculentum*, *Solanum melongena* and *Capsicum annuum* did not show local and systemic symptoms when inoculated with a purified CMV preparation (data not included).

Satellite RNAs are the parasites of plant viruses which depend upon their helper viruses for replication and encapsidation. Usually sat-RNAs reduce the level of helper virus accumulation and attenuate disease symptoms induced by helper virus. However, some sat-RNAs of CMV intensify the viral disease symptoms i.e. yellow, white or systemic necrosis in some hosts. Satellite RNA has been reported as causal agent for tomato necrosis, its role in etiology of tomato fruit necrosis in Italy has been observed earlier. Kaper et al. have reported that CMV sat-RNA induced necrosis in tomato plants and suggested that symptom modulation depends upon trilateral interaction among sat-RNA, virus and host plant during infection but it was not clear whether these factors are of host or satellite origin. The cell death (necrosis) and severe yellowing (chlorosis) in tomato induced by cucumber mosaic virus (CMV) supporting particular satellite RNAs have been reported recently. They have also determined whether CMV RNA sequences are needed to induce necrosis or chlorosis and they infected tomato seedlings with potato virus X (PVX) vector expressing either a necrosis or chlorosis inducing sat-RNA of CMV. Infected plants developed necrosis but only all or part of a 335 nucleotide necrogenic sat-RNA was expressed in (—) polarity; i.e., the strand not packaged in virus particles. Plants often respond to virus infection with the development of hypersensitive reaction in which virus is confined or restrict by a localized necrosis but it is unusual that necrosis spreads through out the plant resulting in systemic lethal necrosis. It has been observed that alteration in either pathogen genes or plant genes can affect the extent of cell death.

The observations of earlier workers clearly explain that sat-RNA somehow, enhances severity of CMV symptoms in infected plants. Chandra has observed severe necrosis in *N. tabacum* cv white burley and suggested that the severe necrosis in tobacco is due to sat-RNA present in CMV-T isolate. We have demonstrated that sat-RNA associated with some isolates of CMV genome has a role in enhancing severity of symptoms in *N. tabacum* cv white burley, *N. tabacum* cv samsun NN, leading to death of *N. benthamiana* plants and exclusion of sat-RNA from the RNA preparation resulted in complete abolition of lethal necrotic/severity of symptoms in these plants. However, we could not study the CMV accumulation with and without sat-RNA in these plants species at different intervals of post inoculation. We described the existence of a sat-RNA in an Indian isolate of CMV which causes systemic lethal necrosis of tobacco. Furthermore, the lethal necrosis induced in *N. benthamiana* by sat-RNA associated with CMV amaranth isolate would be a first report.

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References


