FIRST REPORT OF TOBACCO CURLY SHOOT VIRUS (TBCSV) AND ITS ASSOCIATED SATELLITES FROM WATERMELON IN CHINA

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SUMMARY

Tobacco curly shoot virus (TbCSV), a monopartite begomovirus (genus Begomovirus, family Geminiviridae), is a serious threat to many crops and weeds in China and India. In this study, a begomovirus disease complex was identified from a watermelon sample displaying severe leaf curling and yellowing symptoms. The genome of this TbCSV isolate Y3560 contained 2,744 nucleotides (nt) and shared 97.9% identity with the TbCSV YN4519 isolate. The complete nucleotide sequence of the betasatellite DNA molecule associated to isolate Y3560 contained 1,342 nt and shared 91.0% sequence identity with the tomato yellow leaf curl China virus betasatellite isolate TYLCCNB-Y319. The nucleotide sequence of the alphasatellite contained 1,372 nt and shared the highest levels of identity (94.0%) with tobacco curly shoot virus associated DNA1 (TbCSA). This is the first report of a monopartite begomovirus and associated satellites from watermelon.

Keywords: Begomovirus, betasatellite, alphasatellite, watermelon.

The genus Begomovirus includes the majority of geminiviruses that are transmitted by whitefly Bemisia tabaci and are widespread in tropical, sub-tropical and increasingly warm temperate regions (Varma and Malathi, 2003; Nawaz-ul-Rehman and Fauquet, 2009). In the New World, begomoviruses have genomes consisting of two components (DNA-A and DNA-B). However, the Old World begomoviruses only have a single component that is homologous to the DNA-A component of bipartite begomoviruses (Harrison and Robinson, 1999). These monopartite begomoviruses are frequently associated with betasatellites (formerly DNAβ) and alphasatellites (formerly DNA1), both of which are approximately a half of the DNA-A genome in size (Briddron et al., 2002; Briddron et al., 2003; Xie et al., 2010). Recently, it has become clear that some betasatellites affect the replication of their helper begomoviruses and contain a \( \beta C1 \) gene coding for a homonymous protein which has important roles in symptom induction, suppression of transcriptional and posttranscriptional gene silencing, and can affect jasmonic acid responsive genes (Zhou, 2013). Alphasatellites have also been mostly identified in plants infected with begomovirus-betasatellite complexes, however, their exact role (if any) in pathogenesis is yet to be elucidated (Zhou, 2013).

Watermelon is an important cash crop worldwide with over 81million metric tons produced annually, that is subjected to many yield-affecting adversities including diseases (Compton et al., 2004). At present, five bipartite begomoviruses have been known to infect watermelon plants: watermelon chlorotic stunt virus (WmCSV) (Jones et al., 1988), tomato leaf curl New Delhi virus (ToLCNDV) (Mansoor et al., 2000), squash leaf curl virus (SLCV) (Abudy et al., 2010), cucurbit leaf crumple virus (CuLCrV) (Guzman et al., 2000), and melon chlorotic mosaic virus (MeCMV) (Romay et al., 2010). However, there are no reports of any monopartite begomovirus isolated from watermelon plants.

In spring of 2014, begomovirus-centered surveys were conducted in several districts of Yunnan province, China. Three watermelon plants (Y3560, Y3561 and Y3562) showing upward leaf curling and yellowing symptoms were collected from watermelon fields located in Mangshi, a western state of Yunnan province (Fig. 1).

Fig. 1. Upward curling and yellowing of leaves observed in watermelon plants in Mangshi, Yunnan province during 2014.
Total DNA was extracted from symptomatic watermelon leaves using the CTAB method and submitted to PCR-based detection of DNA-A, beta- and alphasatellites using three previously reported primer sets to amplify partial DNA-A, betasatellite and alphasatellite, respectively (Doyle and Doyle, 1987; Briddon et al., 2002; Bull et al., 2003; Rojas et al., 2005). Amplification products of the expected size were cloned and sequenced. Rolling circle amplification (RCA) was also carried out using a TempliPhiTM kit (GE Healthcare) to obtain enriched product. Amplification products of the expected size were cloned and sequenced. Sequence comparison using BLASTn showed that the sequences of amplicons generated with PA/PB primer set shared 98% identity with the CP gene of TbCSV-Y132 (data not shown), while Y3560 beta had the highest similarity with TYLCCNB-Y319 (91.0%). Clones Y3560-19 and Y3560-27 were identical to each other and shared 94.0% identity with TbCSA-Y99. However, no product of either begomovirus or satellite DNAs was obtained from the samples of Y3561 and Y3562, suggesting that observed symptoms were not caused by begomoviral infection.

The virus infecting sample Y3560 was further cloned and sequenced to generate the complete genome of TbCSV, which consisted of 2,744 nucleotides (KU198364). Comparisons with other sequences of TbCSV available in the databases showed the DNA sequences of Y3560 shared the highest levels of sequence identity (97.9%) with isolate YN4519 (KU94095) (Table 1). Similar to other TbCSV isolates, genomic DNA of isolate Y3560 encoded seven open reading frames (ORFs), including \( \alpha V1 \) (295-1065) and \( \alpha V2 \) (135-491) in the viral sense strand, \( \beta C1 \) (1514-2599), \( \beta C2 \) (1207-1611), \( \beta C3 \) (1062-1466), \( \beta C4 \) (2149-2448) and \( \beta C5 \) (273-440) in the complementary strand. Although the \( \beta C5/C5 \) ORF is only conserved in some bipartite and monopartite begomoviruses, an increasing number of begomoviruses have been annotated to contain an \( \alpha C5/C5 \) ORF. Recently, research on the bipartite begomovirus mungbean yellow mosaic India virus (MYMIV) demonstrated that \( \alpha C5 \) is a pathogenicity determinant and a strong RNA silencing suppressor that employs novel mechanisms to suppress antiviral defenses (Li et al., 2015). Phylogenetic analysis of all TbCSV isolates from GenBank database along with the selected begomoviruses showed that the isolate TbCSV-Y3560 from watermelon grouped with isolate TbCSV-WSF1, and was closely related to other TbCSV isolates reported in China (Fig. 2a).

Clones with the anticipated insert size were selected for sequencing in commercial facilities of Life Technologies, Shanghai, China. The nucleotide sequences were assembled with DNAStar version 7.1 (DNAStar Inc., USA) and open reading frames (ORFs) were identified by DNA-MAN version 5.22 (Lynnon Biosoft, Canada). An initial sequence identity was compared by BLAST in the NCBI database (www.ncbi.nlm.nih.gov). A further comparison and multiple alignments were performed by the Clustal W method of MegAlign (DNAStar version 7.1). Phylogenetic trees were constructed using the neighbour-joining method with 1000 bootstrap replications available in MEGA5 version 5.05 (Tamura et al., 2011). Recombination analysis was performed by recombination detection programs including the RDP, GENECOV, Bootscan, MaxChi, Chimera, SiScan and 3Seq methods in the RDP version 4.46 (Martin et al., 2010).

Table 1. Percent sequence identities of DNA-A of the isolate Y3560 (KU198364) with isolates of tobacco curly shoot virus (TbCSV) infecting different hosts.

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>TCSV isolate</th>
<th>Host</th>
<th>Country</th>
<th>DNA-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>KU934095</td>
<td>YN4519</td>
<td>Tomato</td>
<td>China</td>
<td>97.9%</td>
</tr>
<tr>
<td>AJ475986</td>
<td>Y41</td>
<td>Tomato</td>
<td>China</td>
<td>95.4%</td>
</tr>
<tr>
<td>GU199584</td>
<td>YN18</td>
<td>Mirabilis</td>
<td>China</td>
<td>96.0%</td>
</tr>
<tr>
<td>AF240675</td>
<td>Y1</td>
<td>Tobacco</td>
<td>China</td>
<td>95.1%</td>
</tr>
<tr>
<td>AJ20318</td>
<td>Y33</td>
<td>Tobacco</td>
<td>China</td>
<td>92.8%</td>
</tr>
<tr>
<td>HG003650</td>
<td>Y'18</td>
<td>Ageratum</td>
<td>China</td>
<td>94.8%</td>
</tr>
<tr>
<td>AJ971266</td>
<td>Y262</td>
<td>Ageratum</td>
<td>China</td>
<td>92.8%</td>
</tr>
<tr>
<td>GU199583</td>
<td>YN20</td>
<td>Alternanthera</td>
<td>China</td>
<td>94.6%</td>
</tr>
<tr>
<td>JQ733577</td>
<td>FB-01</td>
<td>Common bean</td>
<td>India</td>
<td>92.6%</td>
</tr>
<tr>
<td>GU001879</td>
<td>SC18</td>
<td>Pepper</td>
<td>China</td>
<td>92.9%</td>
</tr>
<tr>
<td>JX457341</td>
<td>TC366</td>
<td>Tomato</td>
<td>India</td>
<td>92.2%</td>
</tr>
<tr>
<td>JX457342</td>
<td>TCbl</td>
<td>Tomato</td>
<td>India</td>
<td>92.0%</td>
</tr>
<tr>
<td>JN387045</td>
<td>To-Ag-1</td>
<td>Tomato</td>
<td>India</td>
<td>91.3%</td>
</tr>
<tr>
<td>HQ407395</td>
<td>WSF1</td>
<td>Sunflower</td>
<td>India</td>
<td>91.4%</td>
</tr>
</tbody>
</table>
No. KU198366. They consisted of 1,372 nucleotides and had the typical organization of alphasatellites (Briddon et al., 2004), containing a single gene in the viral strand (Rep; 71-1018), an A-rich sequence (1052-1220; with 53.2% adenine content) and a predicted hairpin structure with the loop sequence TAGTATTAC typical of alphasatellites.

Comparison of the sequence of Y3560 DNA1 to other alphasatellites in the GenBank showed that it shared the highest levels of identity (94.0%) with tobacco curly shoot virus associated DNA1, isolate Y99 (TbCSA-Y99), indicating it is an isolate of TbCSA. Phylogenetic analysis of the alphasatellite with selected sequences from the databases showed the present alphasatellite isolate belongs to TbCSA and was also closely related to TYLCCNA cluster (Fig. 2c).

No recombination events were detected by RDPv4.46 in any of the three molecules sequenced from sample Y3560.

This is the first identification of TbCSV/TYLCCNB/TbCSA complex in watermelon and also the first identification of a monopartite begomovirus in watermelon. TbCSV is a monopartite begomovirus that was firstly identified from tobacco in China in 2002 (Xie et al., 2002) and subsequently it was reported in India (Shilpi et al., 2015). The host range of the virus extends to tomato, pepper, Alternanthera philoxeroides, Mirabilis jalapa, ageratum, sunflower and common bean. These reports indicate that TbCSV is a potential serious begomovirus threatening crop production. The cognate betasatellite of TbCSV is TbCSB, which was previously found in a small proportion of field samples from southern China (Li et al., 2005). In our study, however, TYLCCNB was also associated with TbCSV and was detected in a single sample of watermelon showing leaf curl and yellowing symptoms.

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REFERENCES


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