TOMATO CHLOROTIC SPOT VIRUS (TOSPOVIRUS) IN DIEFFENBACHIA AND BOUVARDIA *

ABSTRACT

Virus diseases characterized by vein-banding, necrotic lines, chlorotic spots and rings in Dieffenbachia amoena leaves and mild mosaic in Bouvardia sp. were observed in growing regions of São Paulo State, Brazil. From both infected plants, tospoviruses serologically related to Tomato chlorotic spot virus (TCSV) and Groundnut ringspot virus were isolated and identified. The tospoviruses infected amaranthaceous, balsaminaceous, chenopodiaceous and solanaceous plants. Their 3'-terminal 434 nucleotides, corresponding to part of the N-gene and untranslated region, were sequenced and identified as isolates of TCSV. This is the first report of a TCSV in araceous and rubiaceous plants.

INTRODUCTION

The plant-infecting members of the Bunyaviridae are classified within the Tospovirus genus. Until the early 1990, TSWV (Tomato spotted wilt virus) was the only recognized virus in what is now the genus Tospovirus, but later serological and molecular studies revealed the existence of seven more definitive species and five putative species (Elliot et al., 2000).

During the last decade tospoviruses have been spreading due to various factors including the rapid global displacement of people with their domestic
plants, the increasing invasion of primary uninhabited forests thereby it permitting the release of new viruses. Added to this is the growing range of the vector’s hosts and the number of virus reservoir species, which now totals 1050, and includes weeds, fruit trees, vegetables and ornamentals (German et al., 1992).

On cultivated ornamental species, the tospoviruses TSWV and INSV (Impatiens necrotic spot virus) are placed among the world’s most important viral pathogens (Daughtry et al., 1997). It is worth mentioning that besides INSV the other tospoviruses originally from ornamentals are IYSV (Iris yellow spot virus) (Cortês et al., 1998) and CSNV (Chrysanthemum stem necrosis virus) (Duarte et al., 1995).

Our work deals with the identification of Tospovirus species isolated from Dieffenbachia amoena and Bouvardia sp. grown in ornamental growing areas. We also discuss biological, cytopathological and serological aspects.

MATERIAL AND METHODS

Virus source and host range.

The tospoviruses were isolated from leaves of naturally infected Dieffenbachia amoena, sample A68 (Araceae), showing chlorotic spots, rings and vein-banding as well as necrotic lines, and from naturally infected Bouvardia sp. (Rubiaceae) displaying mild mosaic, both collected in different growing regions of São Paulo State.

Testing for susceptibility through mechanical transmission was carried out to 12 plant species or cultivars in the Amaranthaceae, Balsaminaceae, Chenopodiaceae and Solanaceae. Dieffenbachia sp. (Araceae) and Coffea arabica were also inoculated with tospovirus from D. amoena and Bouvardia sp., respectively.

Serology.

The tospoviruses were serologically compared to other tospovirus species by dot-blot (Fuentes et al., 1993), using polyclonal antisera against the nucleoprotein (N) of CSNV, GRSV (Groundnut ringspot virus), INSV, IYSV, TCSV and TSWV.

Transmission electron microscopy.

For ultrastructural observations, leaf tissues of experimentally infected N. clevelandii (first transfer from infected D. amoena) 14 days after inoculation with tospovirus-A68, as well as of experimentally Bouvardia tospovirus-infected ‘White Burley’, were processed for thin sectioning as described previously (Duarte et al., 1999).

Total nucleic acid extraction, RT-PCR and sequencing.

Nucleic acids were extracted from healthy and virus-infected N. clevelandii (first transfer) leaves and also from healthy and virus-infected D. stramonium (first transfer) leaves using acid guanidium thiocyanate-phenol-chloroform procedure (Chomczynski & Sacchi, 1987). cDNA was synthesized using an oligonucleotide BR-60, and followed by a PCR in combination with primer BR-65 to amplify the region comprised between part of intergenic region and 300 bases downstream from the N protein AUG start codon (Resende et al., 1996). The amplified products were purified with
"CONCERT Rapid Gel Extraction System" (GIBCO BRL) according to the manufacturer's instructions. The amplicon was sequenced using the ABI Prism Big Dye Terminator System (PE Applied Systems).

**Sequence alignments.**

The putative amino acid sequence of the PCR products was deduced by using the DNA sequence translation program implemented in the "DNA>proteins" (www.expasy.ch/tools/translate). The sequences were submitted to the Basic Local Alignment Search Tool (BLAST 2.0) (www.ncbi.nlm.nih.gov/BLAST). The nucleotide and putative amino acid sequences of the nucleocapsid encoding gene of tospoviruses were aligned with other species and isolates of tospovirus by using the program CLUSTAL X (Thompson et al., 1997) and the percentage of identity between them was determined by using the program GeneDoc (Nicholas et al., 1997).

**RESULTS**

**Host range and serological relationship of tospoviruses.**

A comparative host range between tospoviruses isolated from *D. amoena* (tospovirus-A68) and *Bouvardia* sp., resulting from first inoculation, is shown on Table 1. The experimental host range of tospovirus-A68 was broader than tospovirus isolated from *Bouvardia. Dieffenbachia* sp. and *C. arabica* were not experimentally infected by tospoviruses.

Both viruses reacted serologically with TCSV and GRSV antisera in dot-blots.

**Ultrastructure.**

The *D. amoena* (A68) was infected by 2 viruses: the former with pleomorphic, tospovirus-like particles, and the latter with *Potyviridae*-like particles which did not serologically react with *Dasheen mosaic Potyvirus* antisera (data not shown). Leaf-dip preparations from *Bouvardia* sp. presented only pleomorphic particles.

In experimentally infected *N. clevelandii* leaf tissues, enveloped particles clustered within membrane-bounded cistercmae in the cytoplasm of the mesophyll cells were observed; the viroplasm was associated with aggregates of non-enveloped nucleocapsids, which appear as electron-dense bodies in amorphous medium-density material (Fig. 1). Loosely arranged filamentous material was also observed in association with such viroplasm (Fig. 1) and a few structures consisting of paired parallel membranes were observed in cytoplasm (Fig. 2).

In *Bouvardia* tospovirus-infected cell of *N. tabacum* 'White Burley' few enveloped particles clustered within membrane bounded cistercmae were noted, but viroplasm with aggregates of non-enveloped nucleocapsids were present in large numbers (data not shown).

**Sequencing and sequences analysis.**

As dot-blot assays showed positive reactions to TCSV and GRSV antisera from both samples, an RT-PCR with specific tospovirus primers was performed. The primers amplified a fragment corresponding to 434 nucleotides of the 3'
terminal of S RNA, covering the intergenic region and part of the N gene. The GenBank accession number of the TCSV-A68 is AF 454913.

The higher homology percentages between nucleotide sequences of the present tospoviruses and those from 18 Tospovirus species and isolates were obtained from TCSV isolates – especially that of TCSV-tomato from Viçosa (MG). Because of high amino acid and nucleotide homology with TCSV, both D. amoena and Bouvardia sp. tospovirus were identified as TCSV isolates.

**DISCUSSION**

In the present work we determined the partial nucleotide sequences of TCSV-A68 and TCSV-Bouvardia S RNAs, originally isolated from dieffenbachia and bouvardia, respectively, along with their biological and serological properties.

The symptoms found on sample A68 were generally very similar to those induced by DsMV on Dieffenbachia sp., e.g., foliar chlorotic veinbanding (Rivas et al., 1998). But a striking difference appeared as necrotic lines surrounding foliar bands and chlorotic spots on Dieffenbachia-A68 kept at 4°C. The most common virus in dieffenbachia is Dasheen mosaic Potyvirus, although it is also reported as a TSWV host (Kaminska & Korbin, 1994), while Bouvardia has been described harboring INSV (Vaira et al., 1993).

Biologically, the TCSV isolates A68 and Bouvardia turned out to be distinctly in regard to host range and symptom aggressivity, and TCSV-A68 induced more drastic symptoms.

Mechanical transmission of both tospovirus to their original host species failed. Such failure has been reported for some viruses which can only be transmitted to the original hosts through their vectors, including, among tospoviruses, the lack of mechanical transmission of TSWV isolated from Dieffenbachia sp. to indicator species (Kaminska & Korbin, 1994).

Among tospoviruses, the mechanical serial transmission induces phenotypic changes, e.g., the accumulation of defective particles in cytoplasm and symptom attenuation in host plants (Resende et al., 1991). Due to the tendency in generate the so-called defective interfering, we decide to present a host range from the former inoculation and from the original host whose symptoms represent those induced by normal tospoviruses. In the case of defective forms, only viroplasms with nucleocapsid aggregates, and not enveloped particles, could be seen (Resende et al., 1991). To show the absence of defective forms in the first inoculation hosts, foliar thin sections of systemically infected N. clevelandii and 'White Burley' were observed in the electron microscope. In ultra-thin sections from leaf tissues infected by TCSV-A68 and TCSV-Bouvardia, typical structures associated with tospovirus infections could be observed, such as the viroplasm associated with nucleocapsid aggregates and singly enveloped particles clustered within endoplasmic reticulum membranes (Kikkert et al., 1999). Besides, in TCSV-A68 infected cells a
structure similar to paired parallel membranes, which is characterized as membrane cisternae that contain G1, G2 and N proteins and that are probably derived from Golgi stacks (Kikkert et al., 1999; Kitajima et al., 1992), was rarely found. This fact could be attributed to an advanced stage of the viral infection in *N. clevelandii* and ‘White Burley’ since a previous study showed that such structures are rarely found 30-40 h after inoculation (Kikkert et al., 1999).

Fibrous material, constituted by NSs, could be observed in loose arrays like those found in GRSV e TSWV infections and different from paracrystalline arrays induced by INSV (Kitajima et al., 1992; Vaira et al., 1993). In this aspect, the array of the fibrilar protein NSs from TCSV-A68 is similar to that from TSWV or GRSV.

N protein was used as a criteria to establish species, since it is highly preserved among members of the same serogroup (TCSV and GRSV, for example) and among different species present an amino acid sequence homology lower than 90% (Satyanarayana et al., 1998). Thus, the percentage of amino acid homology between the TCSV here under study and those stored in GenBank varied from 100% to 92%. Homology values between 90% and 100% in the N gene are expected for isolates within a same tospovirus species, including isolates from distinct geographic regions and hosts (Satyanarayana et al., 1998). It is noteworthy that the knowledge of the N gene diversity not only provides taxonomic and epidemiological information but is also useful to developmental programs aiming at resistant plants to tospovirus (German et al., 1992).

Infections by TCSV seem to occur only in South America. In Brazil, it was reported on vegetables, tomato, sweet pepper, lettuce, egg-plant, endive (Colaricció et al., 2001) and on the ornamental *Eustoma grandiflorum* (Alexandre et al., 1999) and alstroemeria (Duarte et al., 1999). In Argentina it was isolated from sweet pepper (Dewey et al., 1996). Then, this is the first report of TCSV in *Dieffenbachia* and *Bouvardia*.
TABELA 1 – Reactions of host mechanically inoculated with tospovirus from *Dieffenbachia amoena* and *Bouvardia sp.*

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Dieffenbachia amoena</th>
<th>Bouvardia sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Local</td>
<td>Systemic</td>
</tr>
<tr>
<td><strong>Amaranthaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gomphrena globosa</em></td>
<td>RS</td>
<td>S*</td>
</tr>
<tr>
<td><strong>Balsaminaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Impatiens walleriana</em></td>
<td>L</td>
<td>NR, CL, N</td>
</tr>
<tr>
<td><strong>Chenopodiaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chenopodium amaranticolor</em></td>
<td>CS</td>
<td>S</td>
</tr>
<tr>
<td><em>C. murale</em></td>
<td>NR, CS, NPP</td>
<td>S</td>
</tr>
<tr>
<td><strong>Solanaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>NR</td>
<td>B, LF, M</td>
</tr>
<tr>
<td><em>D. stramonium</em></td>
<td>NR, CNR, CS</td>
<td>FL, B, M, CS, NS</td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em></td>
<td>CS</td>
<td>FL, VC, BS</td>
</tr>
<tr>
<td><em>N. clevelandii</em></td>
<td>NL</td>
<td>C, LR, St</td>
</tr>
<tr>
<td><em>N. glutinosa</em></td>
<td>NR, CS</td>
<td>E, C, D, St, NSt</td>
</tr>
<tr>
<td><em>N. rustica</em></td>
<td>CS</td>
<td>B, C, LD, M</td>
</tr>
<tr>
<td><em>N. tabacum</em> ‘Samsun’*</td>
<td>NR, NL</td>
<td>CR, B, LD, NL, M</td>
</tr>
<tr>
<td><em>N. tabacum</em> ‘White Burley’</td>
<td>CR, NL</td>
<td>B, Cr, M</td>
</tr>
<tr>
<td><em>Petunia hybrida</em></td>
<td>NR, NPP</td>
<td>S</td>
</tr>
</tbody>
</table>

Legend: —: non inoculated; B: blistering; BS: bronzing spots; C: chlorosis; CNR: concentric necrotic rings; Cr: crinkle; CR: chlorotic rings; CS: chlorotic spots; D: death; E: eplasty; FL: filiform leaf; L: latent; LD: leaf deformation; LR: leaf roll; M: mosaic; N: necrosis; NL: necrotic line-pattern; NPP: necrotic pin-points; NR: necrotic rings; NS: necrotic spots; NSt: necrotic streak; RS: red spots; S: symptomless; St: stunt; VC: vein clearing

* No virus recovered
FIGURES 1 and 2 – Electron micrographs of ultra thin sections from experimentally infected tissues. **Fig. 1** - Cisternae of endoplasmic reticulum from palisade parenchyma cells of *Nicotiana clevelandii* containing pleomorphic particles (arrow head). Note nucleocapsid (Nu) - associated viroplasm within a region plenty of filamentous structures (F). **Fig 2** - Inclusion apparently formed by filamentous or membranous close together structures (I). Bars = 200 nm
REFERENCES


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