The Biological Properties of a Distinct Tospovirus and Sequence Analysis of Its S RNA

Sheng-Zhi Pang, Jerry L. Slightom, and Dennis Gonsalves

First and third authors: Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456; second author: Molecular Biology Research, Unit 7242, The Upjohn Company, Kalamazoo, MI 49007.

This work was partially supported by grants from California Plant Company and from the Cornell Biotechnology Program, which is sponsored by the New York State Science and Technology Foundation, a consortium of industry and the National Science Foundation.

Accepted for publication 20 February 1993.

ABSTRACT


A tospovirus isolate from Brazil, designated TSWV-B, was first identified as a unique isolate based on our observation that transgenic plants expressing the N gene of the lettuce strain of tomato spotted wilt virus (TSWV-BL) were susceptible to TSWV-B but showed resistance to both TSWV (L type) and impatiens necrotic spot virus (INSV). TSWV-B was serologically distinct from TSWV and INSV. TSWV-B generally incited symptoms resembling those caused by other TSWV isolates, except TSWV-B systemically infected Petunia hybrid, which is a local-lesion host of TSWV. Unlike the cucurbit isolate TSWV-W, TSWV-B did not infect Cucumis sativus and only occasionally induced systemic infections on C. melo. The complete nucleotide sequence of the S RNA of TSWV-B was determined with cDNA clones to be 3,049 nucleotides long. The genome organization of this S RNA was similar to those of TSWV and INSV. The alignment of the S RNA nucleotide and deduced amino acid sequences with the homologous sequences of TSWV (isolates CNPH1, L3, and BL) and INSV revealed that TSWV-B was related more closely to all the TSWV isolates than to INSV. There was a higher degree of identity among the TSWV isolates than with TSWV-B. Thus, TSWV-B appears to be a distinct tospovirus; however, a precise classification requires additional biological and molecular information on this isolate as well as comparison to other tospovirus isolates.

Tospoviruses have a wide host range and cause significant economic losses to vegetables and ornamental plants worldwide (2, 4, 15–17, 25). Based on serological affinities and nucleotide sequence identity, tomato spotted wilt virus (TSWV, formerly L type) and impatiens necrotic spot virus (INSV, formerly TSWV-L) have recently been classified as two separate viruses within the Tospovirus genus (17). Several other TSWV-like viruses (1, 5, 8, 18, 22, 23, 34, 38) have been reported as serologically distinct from TSWV and INSV and may represent additional virus species of the Tospovirus genus (17, 27).

The tospovirus genome consists of three single-stranded RNAs designated S RNA (2,900 nucleotides), M RNA (~5,000 nucleotides), and L RNA (8,900 nucleotides) (10, 11, 13, 37). From the S RNA, a 52-kDa nonstructural (NS) protein is translated from the large ORF (open-reading frame) on the viral RNA strand, and the 29-kDa nucleocapsid (N) protein is translated from the small ORF on the viral complementary RNA strand (12, 26, 29). This ambisense-coding arrangement is also a characteristic of the INSV M RNA, which encodes 58- and 78-kDa membrane-associated glycoproteins in one ORF and a 35-kDa NS protein in the other ORF (27). The TSWV L RNA encodes a large 200-kDa protein, presumably for the viral transcriptase (9).

Transgenic Nicotiana tabacum L. plants expressing the N gene of TSWV are resistant to homologous isolates (19, 28). Subsequent study in our laboratory (33) demonstrated that transgenic tobacco plants expressing the N gene of TSWV-BL displayed a broad spectrum of resistance not only to heterologous isolates of TSWV but also to a begomovirus isolate of INSV. However, these transgenic plants showed no resistance to a Brazilian isolate (TSWV-B), first described by Wang and Gonsalves (38), although the plants that accumulated high levels of the N protein did display a delay in symptom expression (33). ELISA (enzyme-linked immunosorbent assay) with polyclonal antibodies against the N protein of TSWV and INSV showed that the TSWV-B N protein is not serologically related to either TSWV or INSV (33). TSWV-B biologically differs from a cucurbit isolate (TSWV-W; [22, 39]) because it usually does not induce systemic infection on cucurbits (33). Here we report the biological properties of TSWV-B, the nucleotide and deduced amino acid sequences of its S RNA, and sequence comparisons among tospovirus S RNAs.

MATERIALS AND METHODS

Host-range tests. TSWV-B was isolated originally from Lyco-peresico esculentum Mill. and was first described by Wang and Gonsalves (38). TSWV-B was subsequently transferred to and maintained in Nicotiana benthamiana Domin. To characterize its biological properties, plants from several species representing four families were used for a host-reaction test. Inoculum was

© 1993 The American Phytopathological Society

728 PHYTOPATHOLOGY
prepared by grinding leaf tissue (0.5 g) from *N. benthamiana*
infected with TSWV-B in 15 ml of cold inorganic buffer (0.033
M KH2PO4, 0.067 M K2HPO4, and 0.01 M Na2SO4). The inoculum
extracts immediately were rubbed onto corundum-impregnated leaves
of test plants, and the inoculated leaves were subsequently rinsed
with water. Inoculated plants were grown in the greenhouse and
observed daily for at least 3 wk. In cases of ambiguous results,
extracts from the test plants were checked on systemic hosts,
*N. benthamiana* and *N. tabacum*, for the presence of the virus.
In addition, because we did not have antibodies to detect this virus
by ELISA, PCR (polymerase chain reaction) was used to
analyze the inoculated plants for the virus infection. Total plant
RNA was isolated according to the method of Napoli et al. (32).
The isolated total RNA was subjected to first-strand cDNA
synthesis and subsequent PCR-based amplification as described
by Pang et al. (33). The synthetic oligonucleotides used for both
first-strand cDNA synthesis and PCR amplification were 5'-TAC-
TTACTTTACACCTTGGTTGATCAGCTCACTAC and 5'-TCCGTAGAC
CTCGAATGTGAGCACATCATATTGGG, corresponding to the 5' (nucleotide positions 2,879-2,899 of the TSWV-B S RNA) and the 3' (nucleotide positions 2,122-2,145 of the TSWV-B S RNA) ends of the TSWV-B
N gene, respectively.

**Viral RNA purification.** TSWV-B nucleocapsids were purified from *N. benthamiana* plants infected with TSWV-B as described
by Law and Moyer (25). The purified nucleocapsids were resuspended in a solution of 0.04% bentonite, proteinase K at 10 μg/ml,
0.1 M ammonium carbonate, 0.1% (w/v) sodium diethyl-
ithiocarbamate, 1 mM EDTA, and 1% (w/v) sodium dodecyl
sulfate (SDS), were incubated at 65°C for 5 min, and were extracted immediately with H2O-saturated phenol, followed by another extraction with chloroform/isooamylic alcohol (24:1). The single-
stranded (ss) viral RNA were precipitated in 2.5 volumes of ethanol in the presence of 0.3 M sodium acetate and were dissolved in distilled H2O. Double-stranded (ds) viral RNAs also were iso-
lated from the infected leaf tissue by a combination of methods
(24,30), which has been successfully used for purifying isolated dsRNA from tissues infected with grapevine leafroll virus (21). These two methods were chosen for viral RNA purification because we were unable to purify the virus particle from the infected tissue by the method of Gonsalves and Trujillo (20).

**cDNA synthesis and cloning.** To construct cDNA libraries specific to the TSWV-B S RNA, both viral ssRNAs and dsRNAs were separated on 0.7% agarose gels, and the S ssRNA (conjugated with the S RNA of TSWV-BL) and the S dsRNA (determined by its molecular weight) were removed from gels. The gel slices were incubated at ~20°C, crushed, loaded into a 5-ml syringe with glass wool at the bottom, and centrifuged for 5 min at 3,000 rpm. The RNA solutions collected were precipitated with 2.5
volumes of ethanol in the presence of 0.3 M sodium acetate. The isolated S ssRNA and dsRNA were denatured by meral-
mercury treatment and subjected to a cDNA-synthesis procedure,
as described in the Protocols and Applications Guide provided
by Promega (Madison, WI). The cDNA synthesis was primed by random primers, and the cDNA fragments were cloned via an
EcoRI adapter into the EcoRI-digested δ ZapII according to the manufacturer's instructions (Stratagene, San Diego, CA).
Two libraries were constructed, one from the S ssRNA and the other from the S dsRNA. Both cDNA libraries were screened with the cDNA probes prepared by reverse transcription of gel-
purified S RNA. Positive clones were further characterized by restriction enzyme site mapping and by Southern DNA analysis.
Hybridizations were performed essentially according to the manu-
facturer's protocol (GeneScreen Plus, E. I. Du Pont De Nemours
& Co., Inc., Boston, MA).

The 3' terminus of the TSWV-B S RNA was isolated from the
dsRNA library by screening with an end-labeled synthetic oligomer (5'-TGTGTTAGCGATTGCTAGCGAAG) complementary
to the 746-763 nucleotide positions of the TSWV-B S RNA. The
3' extremity of the S RNA was obtained with the 5' race
system from GIBCO BRL (Gaithersburg, MD). Both ssRNA of
TSWV-B and total RNAs isolated from tobacco plants infected
with TSWV-B were used to synthesize first-strand cDNA with an
oligonucleotide (5'-CTGTGATGGTACAGTGGAAAG) complement-
tary to the 746-763 nucleotide positions of the TSWV-B S
RNA. The 3' end of the first-strand cDNA was tagged with dCTP with terminal deoxynucleotidy transferase. Tailed cDNA was amplified by PCR with an anchor primer that anneals to the
homopolymeric tail and an oligonucleotide (5'-TTATATTTCTTCTCTTTCTTAAATTGGCC), corresponding to the 5' (nucleotide positions 2,879-2,899 of the TSWV-B S RNA) and the 3' (nucleotide positions 2,122-2,145 of the TSWV-B S RNA) ends of the TSWV-B
N gene, respectively.

**RESULTS AND DISCUSSION.**

**Properties of TSWV-B.** Host reactions to TSWV-B are sum-
marized in Table 1. TSWV-B caused systemic infections on all
the test plants in Amaranthaceae and Solanaceae. Symptoms
usually included local necrotic lesions, systemic mottling, leaf
distortions, and plant stunting. TSWV-B induced only local lesions
on Chenopodium quinoa Willd. Test plants in Cucurbitaceae responded differently to inoculations of TSWV-B. On *Cucumis sativus* L., neither local lesions nor systemic symptoms developed;
however, five of 32 *Cucumis melo f* uxer ex Naudin plants showed symptoms of systemic mottling and leaf distortions.
Uninoculated leaves of all test plants also were analyzed for the prevalence of the virus by back inoculation on *N. benthamiana* and *N. tabacum* and by PCR amplification with the synthetic primers specific to the TSWV-B N gene. Results from these tests were consistent with visual symptom observations (data not shown).

Comparisons of the symptomatology of TSWV-B and two other tospoviruses (Table 1; [39]) showed that TSWV-B differed from
TSWV-NY, in that TSWV-B systemically infected *Petunia hyb-
rida* Hort. vilm.-andr., which is a local-lesion host of TSWV-
NY. INSV incited only local lesions on *L. esculentum* N. tabacum, and *P. hybida* (Table 1; [7,25]), and TSWV-B infected all of
them systemically. Unlike TSWV-W, which primarily infects cu-
curbits (22,23,39), TSWV-B did not systemically infect *C. sat-
vus* and only occasionally induced systemic infections on *C.
metaliferus*. Viral RNAs and structural proteins also were an-
alyzed on agarose and polyacrylamide gels, respectively, and they
were similar in size to the corresponding counterparts of TSWV
and INSV (data not shown).

**Nucleotide sequence analysis of TSWV-B cDNA clones.** Both
viral ssRNAs and dsRNAs were isolated from the infected leaf
tissue of *N. benthamiana* and separated on agarose gels. The
TSWV-B S RNA was gel-purified and cloned into cDNA with
the random primers. Two cDNA libraries were constructed: one
from the S ssRNA purified from TSWV-B nucleocapsids and the other from the S dsRNA. Positive clones were identified from
both cDNA libraries by the cDNA probes prepared from the
test plants infected with TSWV-B S RNA. Three overlapping clones containing the largest inserts (L1, L2, and L30) were selected from the cDNA library constructed from the S ssRNA for nucleotide sequence analysis.

**Nucleotide sequence analysis of the inserts in clones L1, L2, and L30 revealed** 1,994+, 2,368-, and 1,576-bp inserts, respectively. The comparison with the TSWV-CNP1H S RNA (12) indicated that the assembled sequence of 2,842 bp represented 93% of the S RNA genome, excluding both 5' and 3' ends of the S RNA (Fig. 1). Together these three clones contained the NS, ORF, the complete intergenic region (629 nucleotides), and 738 nucleo-
tides of the N gene (only 39 nucleotides at the N-terminus of

Vol. 83, No. 7, 1993 729
TABLE I. Host reactions of three distinct tospoviruses

<table>
<thead>
<tr>
<th>Host plants</th>
<th>Reaction to TSWV-B</th>
<th>Reaction to TSWV-NY</th>
<th>Reaction to INSV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Local</td>
<td>Systemic</td>
<td>Local</td>
</tr>
<tr>
<td>Amaranthaceae</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Gomphrena globosa</td>
<td>+</td>
<td>NS, M, LD</td>
<td>+</td>
</tr>
<tr>
<td>Chenopodiaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chenopodium quinoa</td>
<td>+</td>
<td>NS, M</td>
<td>+</td>
</tr>
<tr>
<td>Solanaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Datura stramonium</td>
<td>+</td>
<td>NS, LD</td>
<td>+</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>+</td>
<td>NS, M, LD, S</td>
<td>+</td>
</tr>
<tr>
<td>Nicotiana benthamiana</td>
<td>+</td>
<td>NS, M, LD, S</td>
<td>+</td>
</tr>
<tr>
<td>N. tabacum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. glauca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. rustica</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petunia hybrida</td>
<td>+</td>
<td>NS, M, LD, S</td>
<td>+</td>
</tr>
<tr>
<td>Cucurbitaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumis metulisferus</td>
<td>+</td>
<td>M, LD</td>
<td>+</td>
</tr>
<tr>
<td>C. sativus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reactions to tomato spotted wilt virus (TSWV-ny) and impatients necrotic spot virus (INSV) were reported by Yeh et al (39) and de Avila et al (7), respectively. Abbreviations for symptoms: +, local lesions; –, no symptoms; CS, chlorotic spots; LD, leaf distortions; M, mosaic; NS, necrotic spots; S, stunting; VC, vein clearing; VN, vein necrosis; W, wilting; ( ), erratic systemic infection; [ ], sporadic systemic infection (only five of 32 C. metulisferus plants were systemically infected).

The N protein was not represented in the cDNA clones. The 3′ region of the S RNA was isolated from the original cDNA library constructed from the S dsRNA with an end-labeled synthetic oligomer corresponding to the 3′ end of cDNA clone L22 (Fig. 1). Five clones that hybridized with the synthetic oligomer were sequenced with the primer. Among the five clones, only two, designated S6 and S7, contained the N-terminal nucleotide sequence of the N gene. In addition, clone S7 also contained the extreme consensus 3′ end of the TSWV-B S RNA (Figs. 1 and 2). The 5′ end of the S RNA was obtained with the 5′ RACE system and a T-vector pT7Blue Cloning system (Novagen, Madison, WI), as described previously. Eight independent clones were sequenced with an oligomer primer (5′-GTTCTCAGATTGCTAGT) close to the 5′ region of the S RNA (nucleotide positions 40–57 of the TSWV-B S RNA). Six contained the 5′ extreme end of the S RNA, and the 5′-terminal nucleotide sequence from these six clones was identical. Thus, the complete nucleotide sequence of the TSWV-B S RNA was 3,049-nucleotides long, as shown in Figure 2.

Sequencing of TSWV-B S RNA. Sequence analysis revealed that the complete nucleotide sequence of the TSWV-B S RNA was 3,049-nucleotides long, 134 nucleotides longer than the S RNA of TSWV-CNPH1. This difference was mainly attributed to the elongated intergenic region of the TSWV-B S RNA. The 5′ and 3′ extreme ends (Fig. 2, 15 nucleotides in italic lowercase letters) of the TSWV-B S RNA were conserved (almost identical to those of TSWV-CNPH1 and INSV S RNAs) and complementary, except for a mismatch at nucleotide position 11. A similar mismatch was also observed in the S RNAs of TSWV-CNPH1 (11,12) and in animal viruses (3,14) in the family Bunyaviridae but at nucleotide position nine. The significance of this discrepancy is unknown. Nevertheless, conservation of the terminal sequences among tospoviruses and of the mismatch within the family Bunyaviridae indicate their possible roles in replication and encapsidation (3,14).

The sequence analysis of TSWV-B S RNA revealed two ORFs with an ambisense arrangement (Fig. 2), similar to other tospoviruses. The large ORF (1,604 nucleotides) was localized on the viral RNA strand originating at nucleotide 88 and terminating at nucleotide 1,491 (Fig. 2). This ORF encoded a protein of 467 amino acids with a predicted molecular mass of 52.6 kDa, presumably for the NS protein. The deduced amino acid sequence of this ORF contained four potential glycosylation sites, three of which (the last three from the N-terminus) were located at the same positions as those of TSWV-CNPH1 and -L3. The small ORF (777 nucleotides), located on the viral complementary strand, was defined by an initiation codon at nucleotide 2,898 and by a termination codon at nucleotide 2,122 (Fig. 2). This ORF encoded an N protein of 258 amino acids with a predicted molecular mass of 28.7 kDa. Unlike the N proteins of TSWV and INSV, which do not contain an N-glycosylation site, the deduced amino acid sequence of TSWV-B N protein contained one potential N-glycosylation site at amino acid residue 68 (Fig. 2).

The two ORFs were separated by an intergenic region of 629 nucleotides that was, because of several insertions, 126 and 44 nucleotides longer than its counterparts in TSWV-CNPH1 and -L3, respectively. The complex and stable hairpin structures (data not shown) that potentially could be generated by internally base-pairing U-rich stretches with A-rich stretches of the intergenic region seem to be similar to those produced in TSWV-CNPH1 and -L3, in both structure and stability (as indicated by minimal free-energy values; data not shown). It has been suggested that this internally base-paired structure acts as a transcription termination signal (3).

Comparative analyses of the S RNA sequences of tospoviruses. Pairwise comparisons of the entire TSWV-B S RNA with the published S RNA regions of tospoviruses (Table 2) showed that it shares its greatest nucleotide sequence identities with TSWV isolates CHNII, L3, and BL (75.8–76.4%, overall; 77.5–78.0%, N gene; 72.4–76.4%, intergenic region; and 79.0–80.0%, NS gene), which are, nevertheless, much lower than those found among the TSWV isolates (94.8–96.4%, overall; 96.8–97.3%, N gene; 89.2–95.9%, intergenic region; and 93.6%, NS gene). A similar pairwise comparison between the homologous S RNA sequences of TSWV-B and INSV revealed a much lower degree of shared identity (63%). Consistent with our results from ELISA (33) and Western analysis (data not shown), the amino acid sequences of the N and NS genes from TSWV isolates CNPH1, L3, and BL were more closely related to each other (96.9–98.5%, N gene, and 89.4%, NS gene) than to TSWV-B (79.1–79.9%, N gene, and 78.3–82.0%,
Fig. 2. The complete nucleotide sequence of the cDNA copy of tomato spotted wilt virus (TSWV)-B S RNA and the deduced amino acid sequences of the nonstructural and nucleocapsid proteins. The cDNA sequence of the TSWV-B S RNA was determined from both DNA strands by the primer-directed dideoxy chain termination reactions with double-stranded cDNA templates. The first nucleotide residue is numbered at the 5' end of the S RNA, and the amino acid residues are numbered from the first methionine of proteins. Potential N-linked glycosylation sites are bold and underlined, and the 15 nucleotides that represent the conserved 5' and 3' terminal sequences of the S RNA are shown in italic lowercase letters.
TABLE 2. Pairwise comparisons of aligned nucleotide (nt) and deduced amino acid (aa) sequences of the S RNA of tospoviruses

<table>
<thead>
<tr>
<th>Comparison</th>
<th>52-kDa protein gene (NS gene)</th>
<th>Intergenic nt</th>
<th>29-kDa protein gene (N gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>overall nt (%)</td>
<td>nt (%)</td>
<td>60.7</td>
</tr>
<tr>
<td>B/CNP1</td>
<td>76.4</td>
<td>80.0</td>
<td>78.3</td>
</tr>
<tr>
<td>B/L3</td>
<td>75.8</td>
<td>79.0</td>
<td>82.0</td>
</tr>
<tr>
<td>B/BL</td>
<td>76.3</td>
<td>66.7</td>
<td>81.4</td>
</tr>
<tr>
<td>B/INSV</td>
<td>63.0</td>
<td>63.0</td>
<td>89.4</td>
</tr>
<tr>
<td>CNPH1/L3</td>
<td>94.8</td>
<td>95.6</td>
<td>89.4</td>
</tr>
<tr>
<td>CNPH1/BL</td>
<td>96.4</td>
<td>95.6</td>
<td>95.9</td>
</tr>
<tr>
<td>L3/BL</td>
<td>95.1</td>
<td>95.1</td>
<td>95.9</td>
</tr>
<tr>
<td>INSV/INSV</td>
<td>62.7</td>
<td>62.7</td>
<td>62.7</td>
</tr>
<tr>
<td>INSV/L3</td>
<td>60.9</td>
<td>60.9</td>
<td>60.9</td>
</tr>
</tbody>
</table>

*The partial or complete S RNA sequences of isolates of tomato spotted wilt virus (TSWV)-CNP1H (2,916 kb), L3 (2,837 kb), -BL (2,037 kb), and impatiens necrotic spot virus (INSV, 1,144 kb) were published by de Haan et al (12), Maiss et al (29), Pang et al (33), and Law et al (26), respectively, and were used for pairwise comparisons with the S RNA sequence of TSWV-B (3,049 kb) or with each other.

Percent identity was calculated by comparing nucleotide or predicted amino acid sequences with the BESTFIT program of the GCG sequence-analysis software package.

NS, gene). In contrast, a much lower degree of amino acid identity was observed between the N proteins of TSWV-B and INSV (55.3%), with a value similar to those found between the N proteins of INSV and TSWV isolates (53.9-55.1%). Furthermore, the N gene of INSV was longer, encoding 262 amino acids, than those of the TSWV isolates and TSWV-B, which encoded 258 amino acids. The amino acid sequence alignment suggested that the four additional residues in the INSV N protein were the result of two insertions: a three amino acid insertion (residues 83 to 85) and an amino acid insertion at residue 110 (data not shown).

Our data show that the N gene of TSWV-B is much more distantly related to the N gene of INSV (~63%) than to that of TSWV (~78%) isolates (Table 2). Should TSWV-B be considered as a new tospovirus? After this manuscript had been submitted for publication, we learned from the dissertation of de Avila (6) that the N genes of two tospovirus isolates from South Africa (SA-05) and Brazil (BR-03) have been sequenced, and our analyses show 94.4 and 81.5% identities with that of TSWV-B, respectively. Thus, data accumulated on isolates of tospoviruses should allow the classification of TSWV-B in the near future.

In summary, the entire S RNA of TSWV-B was sequenced from cDNA clones. The genomic size and organization of its S RNA is similar to those of other tospoviruses. The comparative analyses (Table 2) suggest that TSWV-B is more closely related to the TSWV isolates than to INSV but is much less similar to the TSWV isolates than the TSWV isolates are to each other. To our surprise, transgenic plants expressing the N gene of TSWV-B (L type) are resistant to INSV but not to TSWV-B (33). Although our ELISA results show that TSWV-B does not react with antibodies against the N protein of either TSWV-BL or INSV (33), weak cross-reactions to antibodies against the N protein of both TSWV-BL and INSV were clearly observed in Western analysis (data not shown). Thus, it appears that TSWV-B is a distinct tospovirus; however, a precise classification requires additional comparisons of biological and molecular information of this isolate with other tospovirus isolates.

LITERATURE CITED