Serological and molecular characterization of Syrian Tomato spotted wilt virus isolates

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Summary. Thirty four Syrian isolates of Tomato spotted wilt virus (TSWV) collected from tomato and pepper were tested against five specific monoclonal antibodies using TAS-ELISA. The isolates were in two serogroups. Fourteen tomato and sixteen pepper isolates were similar in their reaction with MAb-2, MAb-4, MAb-5 and MAb-6, but did not react with MAb-7 (Serogroup 1). Meanwhile, four isolates collected from pepper reacted with all the MAbs used (Serogroup 2). The expected 620 bp DNA fragment was obtained by RT-PCR from six samples using a specific primer pair designed to amplify the nucleocapsid protein (NP) gene of TSWV. The PCR products were sequenced and a phylogenetic tree was constructed. Sequence analysis revealed that the Syrian TSWV isolates were very similar at the nucleotide (97.74 to 99.84% identity) and amino acid (96.17 to 99.03% identity) sequences levels. The phylogenetic tree showed high similarity of Syrian TSWV isolates with many other representative isolates from different countries.

Key words: monoclonal antibodies, RT-PCR, Serogroups.

Introduction

Tomato spotted wilt virus (TSWV, genus Tospovirus, family Bunyaviridae) is the type member of the genus Tospovirus (Murphy et al., 1995). This virus causes serious damage to the production of most crops of the family Solanaceae, and ranks first in importance on tomato and pepper in the Mediterranean area (Turina et al., 2012). TSWV has become an increasingly important limiting factor contributing to economic losses in many vegetable crops and some ornamental plants worldwide (Pappu et al., 2009). This virus has one of the widest host ranges of any plant viruses, including more than 1,000 plant species belonging to about 90 families (Parrella et al., 2003).

TSWV has a worldwide distribution (EPPO, 1999), and has been reported in some Arab countries: Tunisia (Ben Moussa et al., 2000), Egypt (Abdelkader et al., 2004), Jordan (Anfoka et al., 2006), Lebanon (Abou-Jawdah et al., 2006). In Syria, TSWV has been recorded on tomato and pepper (Ismaeil et al., 2010). Different strains of the virus with varied severity of symptoms produced on host plants have been characterized (Best, 1968). The Sw-5 gene was found to be responsible for TSWV resistance in tomato (Boiteux and Giordano, 1993) and Tsw gene in pepper (Jahn et al., 2000). Resistance-breaking strains of TSWV for Sw-5 and Tsw genes were reported from open fields in Spain (Aramburu and Marti, 2003; Margaria et al., 2004) and in Italy (Roggero et al., 2002; Ciuffo et al., 2005). Polyclonal antibodies (PAbs) and monoclonal antibodies (MAbs) were used to detect TSWV (Huguenot et al., 1990, Wang and Gonsalves, 1990) and to
characterize isolates in plant extracts or in the thrips vectors (Sherwood et al., 1989, Adam et al., 1991, Bandla et al., 1994) and to detect isolates of different viruses belonging to genus Tospovirus (Adam et al., 1996, Roggero et al., 1996a). Molecular methods were developed, using PCR and IC-PCR to detect this virus and to characterize isolates (Nolasco et al., 1993, Mumford et al., 1994, Jain et al., 1998).

During 2007 and 2008, a survey was conducted in Syria aiming to investigate the status of TSWV on tomato and pepper using DAS-ELISA (Clark and Adams, 1977). The overall incidence of the virus in both host crops was 19.6%, 11.1% in tomato and 41.2% in pepper (Ismaeil et al., 2010). The aim of the present study was to characterize some Syrian TSWV isolates using serological and molecular approaches.

Materials and methods

Virus sources

Thirty four isolates of TSWV (14 from tomato and 20 from pepper) were collected during the spring (April and May) of 2007 and 2008, from different geographical regions in four governorates in Syria. These included Dara’a (22 isolates), Damascus countryside (ten isolates) and Hama and Idleb (one isolate for each).

Serotyping

Five different specific MAbs (MAb-2, MAb-4, MAb-5, MAb-6 and MAb-7) and one cocktail were used for serotyping the 34 TSWV isolates by TAS-ELISA (Roggero et al., 1996b). MAbs were produced against an Italian isolate of TSWV, provided by Dr Donato Boscia and Dr Oriana Botere, Faculty of Agriculture, Bari University, Italy.

RNA isolation

Total RNA was isolated from lyophilized leaves and fruits of pepper plants infected by TSWV, using TRIzol Reagent (Invitrogen) following the manufacturer’s protocol. RNA obtained was ready to use in RT-PCR.

RT-PCR

Three isolates from Dara’a (SY-TSWV-624, SY-TSWV-238 and SY-TSWV-874) and three from Damascus countryside (SY-TSWV-303, SY-TSWV-624 and SY-TSWV-874) were analyzed by Reverse Transcription Polymerase Chain Reaction (RT-PCR). One step RT-PCR was performed using the Access RT-PCR System (Promega Corporation). The primers 722 and 723 (Adkins and Rosskopf, 2002) were used to amplify a fragment of 620 bp of the virus nucleocapsid protein (NP) gene. Fifty ng of RNA were used as template and the following thermal cycling scheme was applied in an Eppendorf thermocycler: 45°C for 45 min; then 94°C for 2 min; and 35 cycles of: 94°C for 1 min, 56°C for 45 s and 72°C for 1 min. A final extension cycle at 72°C for 5 min ended the run. PCR products were resolved in 1% agarose gels and visualized after ethidium bromide staining with a UV transilluminator. A 100 bp DNA MW Ladder (Promega) was used.

Sequencing

PCR products of the six Syrian TSWV isolates were sequenced at LGC Genomics GmbH, Germany, and the bioinformatic programs Clustal X (Thompson et al., 1997) and BLASTN 2.2.25+ (Zhang et al., 2000) were used to analyze the data obtained. A phylogenetic tree was constructed using the neighbour-joining method with MEGA 5 (Tamura et al., 2011), and the major clades were supported by bootstrap values greater than 60. NP sequences of the six Syrian TSWV isolates were deposited in GenBank under accession numbers JN561615 to JN561620.

Results and discussion

Serotyping of Syrian TSWV isolates

Fourteen tomato isolates of TSWV collected from two different governorates (Damascus countryside and Dara’a), and sixteen pepper isolates collected from four different governorates (Damascus countryside, Dara’a, Hama and Idleb) reacted similarly with the five MAbs used. They reacted with MAB-2, MAB-4, MAB-5 and MAB-6, but did not react with MAB-7. Meanwhile, four pepper isolates collected from Dara’a reacted with all of the MAbs used. According to these data the Syrian TSWV isolates could be designated into two serogroups (Serogroups 1 and 2) which varied in their frequency and distribution (Table 1).
Six TSWV isolates were analyzed by one-step RT-PCR using the specific primer pair (722/723), and the expected 620 bp DNA fragment of the NP gene was obtained. Amplified PCR products were sequenced, and sequencing analysis revealed that these isolates were very similar to each other, with nucleotide identity ranging from 97.74 to 99.84% (Table 2). The identity at amino acid level ranged from 96.17 to 99.03% (Table 2). Sequencing analysis showed that the TSWV isolates had very close similarity with other isolates of the virus, with nucleotide sequence identity 97 and 99%. The phylogenetic tree differentiated two clades. The first contained the Syrian isolates and isolates from Australia, Lebanon, Europe, USA and South Africa. The second clade contained isolates from Europe, Jordan and South Korea (Figure 1).

The results of this study have demonstrated that serological variation was observed only between Syrian TSWV isolates collected from pepper in Dara’a governorate. The distribution of this virus in the Dara’a governorate in southern Syria was near to the Jordanian borders in the Al-Yarmouk valley, Irbid and the Jordan valley, where the occurrence of TSWV was previously reported on tomato and pepper (Anfoka et al., 2006). Similar results were obtained in the Netherlands, where three different serogroups of TSWV isolates were identified using

### Table 1. Reaction of specific MAbs with 34 Syrian Tomato spotted wilt virus isolates.

<table>
<thead>
<tr>
<th>Serogroups</th>
<th>Reacted isolates</th>
<th>Crop</th>
<th>Origin</th>
<th>Mabs names</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAb-2</td>
<td>MAb-4</td>
<td>MAb-5</td>
<td>MAb-6</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>Pepper</td>
<td>Damascus countryside</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>Tomato</td>
<td>Damascus countryside</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Pepper</td>
<td>Dara’a</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>Tomato</td>
<td>Dara’a</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>Pepper</td>
<td>Dara’a</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Pepper</td>
<td>Idleb</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Pepper</td>
<td>Hama</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 2. Sequence identity (%) of the nucleocapside protein (NP) gene of six Tomato spotted wilt virus isolates from Syria at nucleotide and amino acid levels.

| Isolate       | SY-TSWV-624 nt | SY-TSWV-624 aa | SY-TSWV-621 nt | SY-TSWV-621 aa | SY-TSWV-311 nt | SY-TSWV-311 aa | SY-TSWV-303 nt | SY-TSWV-303 aa | SY-TSWV-238 nt | SY-TSWV-238 aa | SY-TSWV-874 nt | SY-TSWV-874 aa |
|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| SY-TSWV-624   | -              | -              | -              | -              |                |                |                |                |                |                |                |                |
| SY-TSWV-621   | 99.03          | 99.03          | 98.87          | 98.06          | 99.20          | 98.54          | -              | -              |                |                |                |                |
| SY-TSWV-311   | 98.87          | 98.06          | 99.20          | 98.54          |                |                |                |                |                |                |                |                |
| SY-TSWV-303   | 99.20          | 99.03          | 98.87          | 98.06          | 99.55          | 99.03          | -              | -              |                |                |                |                |
| SY-TSWV-238   | 99.03          | 98.54          | 98.87          | 97.57          | 99.20          | 98.54          | 99.84          | 99.51          |                |                |                |                |
| SY-TSWV-874   | 97.90          | 97.09          | 98.54          | 98.06          | 98.06          | 96.60          | 97.74          | 96.17          | 97.90          | 96.17          |                |                |

* nt, nucleotide; aa, amino acid.
Figure 1. Unrooted phylogenetic tree of six Syrian *Tomato spotted wilt virus* isolates and other representative sequences of the virus nucleocapside protein (NP) gene obtained from GenBank.
Figure 2. Amino acids sequence alignment of the nucleocapsid protein (NP) gene of six *Tomato spotted wilt virus* isolates from Syria, showing variability among them.
a wide spectrum of MAbs (De Avila et al., 1990). Another study confirmed the existence of five epitopes on (N) protein related to TSWV isolates collected from different plant hosts (Chatzivassiliou et al., 2000). In contrast, a study characterizing many isolates of TSWV collected from France, Belgium and Italy (Nono-Womdim et al., 1996) did not detect any differences in serological reactions using many MAbs.

Several studies have demonstrated high sensitivity and accuracy of RT-PCR for detecting TSWV in different plant species, host tissues and in vectors (Mumford et al., 1994, Jain et al., 1998, Tsompana et al., 2005). Results of sequencing analysis of the isolates SY-TSWV-238 and SY-TSWV-303 originating, respectively, from Dara’a and Damascus countryside governorates, agreed with serotyping results. These two isolates reacted positively with four MAbs and did not react with the fifth MAb and belonged to the same serogroup (Serogroup 2). They had very high homology at the nucleotide sequence level of the NP gene and at amino acids sequence level except for one amino acid (Figure 2). Meanwhile, isolate SY-TSWV-624, which originated from Dara’a governorate, reacted with all the MAbs used and belonged to Serogroup 1. This isolate was different in four nucleotides and two amino acids from isolate SY-TSWV303, and in five nucleotides and three amino acids from isolate SY-TSWV-238. This suggests that isolate SY-TSWV-624 is serologically different from the two other isolates, and belonged to a different serogroup. The isolate SY-TSWV-624 also had some differences at the molecular level, as confirmed by analysis of the nucleotide and amino acid sequences of the three isolates. In Apulia, southern Italy, many TSWV isolates were divided into two different sub-groups depending on RT-PCR results. The first was TSWV-A which contained isolates which had the ability to overcome the resistance gene in tomato plants. The second sub-group was TSWV-D which contained isolates that did not have the ability to overcome this resistance gene (Finetti-Sialer et al., 2002). Results of nucleotides sequence analysis of the NP gene of Syrian TSWV isolates demonstrated high similarity with most known isolates of this virus from other countries. This indicates that further analyses should be carried out of other sites of the virus genome in order to obtain more genomic information about the Syrian TSWV isolates.

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Literature cited


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