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RESEARCH PAPERS

Serological and molecular characterization of Syrian *Tomato spotted wilt virus* isolates

Faiz ISMAEIL[1], Amin Amer HAJ KASSEM[2], Salah AL-CHAABI[1], Ahmed ABDULKADER[3] and Mouhamed ALKHALAF[4]

[1] General Commission for Scientific Agricultural Research (GCSAR), Administration of Plant Protection Research, Douma, P. O. Pox 113, Damascus, Syria
[2] Plant Protection Division, Aleppo University, Aleppo, Syria
[3] General Commission for Scientific Agricultural Research (GCSAR), Division of Biotechnology, Douma, P. O. Pox 113, Damascus, Syria
[4] Aleppo Scientific Agricultural Research Center, Aleppo, Syria

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 *Solanacae*, 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

Corresponding author: F. Ismaeil
E-mail: faizismail@mail.sy

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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).

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

Serogroups	Reacted isolates	Crop	Origin	MAbs names					
				MAb-2	MAb-4	MAb-5	MAb-6	MAb-7	Cocktail
1	6	Pepper	Damascus countryside	+	+	+	+	-	+
1	4	Tomato	Damascus countryside	+	+	+	+	-	+
2	4	Pepper	Dara'a	+	+	+	+	+	+
1	10	Tomato	Dara'a	+	+	+	+	-	+
1	8	Pepper	Dara'a	+	+	+	+	-	+
1	1	Pepper	Idleb	+	+	+	+	-	+
1	1	Pepper	Hama	+	+	+	+	-	+

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		SY-TSWV-621		SY-TSWV-311		SY-TSWV-303		SY-TSWV-238		SY-TSWV-874	
	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
SY-TSWV-624	-	-										
SY-TSWV-621	99.03	99.03	-	-								
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	-	-

[a] **nt,** nucleotide; **aa,** amino acid.

Molecular characterization of six TSWV isolates

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

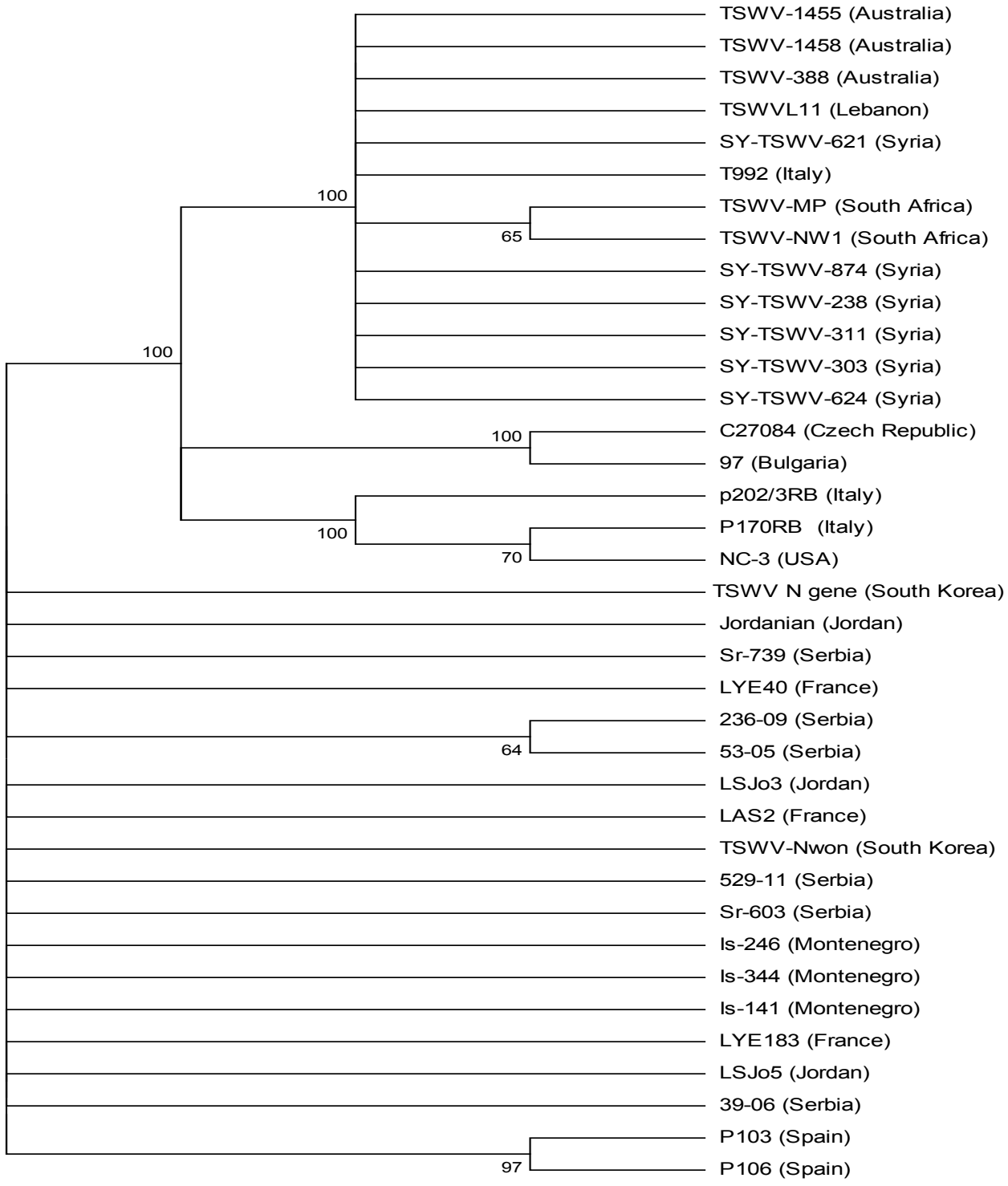

Figure 1. Unrooted phylogenetic tree of six Syrian *Tomato spotted wilt virus* isolates and other representative sequences of the virus nucleocapside protein (NP) gene obtained from GenBank.

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SY-TSWV874(JN561615)  QGKDLEFEEDQNLVAFNFKTFCLENLDQIKKMSIISCLTFLKNRQSIMKVIKQSDFTFGK 60
SY-TSWV624(JN561616)  QGKDLEFEEDQNLVAFNFKTFCLENLDQIKKMSVISCLTFLKNRQSIMKVIKQSDFTFGK 60
SY-TSWV621(JN561617)  QGKDLEFEEDQNLVAFNFKTFCLENLDQIKKMSVISCLTFLKNRQSIMKVIKQSDFTFGK 60
SY-TSWV311(JN561618)  QGKDLEFEEDQNLVAFNFKTFCLENLDQIKKMSVISCLTFLKNRQSIMKVIKQSDFTFGK 60
SY-TSWV303 (JN561619) QGKDLEFEEDQNLVAFNFKTFCLENLDQIKKMSVISCLTFLKNRQSIMKVIKQSDFTFGK 60
SY-TSWV238(JN561620)  QGKDLEFEEDQNLVAFNFKTFCLENLDQIKKMSVISCLTFLKNRQSIMKVIKQSDFTFGK 60
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SY-TSWV874(JN561615)  ITIKKTSDRIGATDMTFRRLDSLIRVRLVEETGNSENLNTIKSKIASHPLIQAYGLPLDD 120
SY-TSWV624(JN561616)  ITIKKTSDRIGATDMTFRRLDSLIRVRLVEETGNSENLNTIKSKIASHPLIQAYGLPLDD 120
SY-TSWV621(JN561617)  ITIKKTSDRIGATDMTFRRLDSLIRVRLVEETGNSENLNTIKSKIASHPLIQAYGLPLDD 120
SY-TSWV311(JN561618)  ITIKKTSDRIGATDMTFRRLDSLIRVRLVEETGNSENLNTIKSKIASHPLIQAYGLPLDD 120
SY-TSWV303 (JN561619) ITIKKTSDRIGATDMTFRRLDSLIRVRLVEETGNSENLNTIKSKIASHPLIQAYGLPLDD 120
SY-TSWV238(JN561620)  ITIKKTSDRIGATDMTFRRLDSLIRVRLVEETGNSENLNTIKSKIASHPLIQAYGLPLDD 120
                      ************************************************************

SY-TSWV874(JN561615)  AKSVRLAIMLGGSLPLIASVDSFEMISVVLAIYQDAKYKDLGIDPKKYDTREALGKVCTV 180
SY-TSWV624(JN561616)  AKSVRLAIMLGGSLPLIASVDSFEMISVVLAIYQDAKGKDLEIDPKKYDTREALGKVCTV 180
SY-TSWV621(JN561617)  AKSVRLAIMLGGSLPLIASVDSFEMISVVLAIYQDAKYKDLGIDPKKYDTREALGKVCTV 180
SY-TSWV311(JN561618)  AKSVRLAIMLGGSLPLIASVDSFEMISVVLAIYQDSQCKDLGIDPKKYDTREALGKVCTV 180
SY-TSWV303 (JN561619) AKSVRLAIMLGGSLPLIASVDSFEMISVVLAIYQDSCGKDLEIDPKKYDTREALGKVCTV 180
SY-TSWV238(JN561620)  AKSVRLAIMLGGSLPLIASVDSFEMISVVLAIYQDSQGKDLEIDPKKYDTREALGKVCTV 180
                      ********************************   *** ******************

SY-TSWV874(JN561615)  LTHNAFEMNEDQVKKGKEYAAILSSS 206
SY-TSWV624(JN561616)  LKSKAFEMNEDQVKKGKEYAAILSSS 206
SY-TSWV621(JN561617)  LKSKAFEMNEDQVKKGKEYAAILSSS 206
SY-TSWV311(JN561618)  LKSKAFEMNEDQVKKGKEYAAILSSS 206
SY-TSWV303 (JN561619) LKSKAFEMNEDQVKKGKEYAAILSSS 206
SY-TSWV238(JN561620)  LKEKAFEMNEDQVKKGKEYAAILSSS 206
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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

Abdelkader H.S., G.M. Allam, T.A. Moustafa and M.H. El-Hammady, 2004. Characterization and molecular detection of *Tomato spotted wilt virus* (Tospovirus) infecting tomato in Egypt. *Egyptian Journal of Virology* 1, 103–120.

Abou-Jawdah Y., C. El Mohtar, H. Sobh and M.K. Nakhla, 2006. First report of *Tomato spotted wilt virus* on tomatoes in Lebanon. *Plant Diseases* 90, 376.

Adam G., D.E. Lesemann and H.J. Vetten, 1991. Monoclonal antibodies against *Tomato spotted wilt virus*: characterization and application. *Annals of Applied Biology* 118, 87–104.

Adam G., D. Peters and R.W. Goldbach, 1996. Serological comparison of tospovirus isolates using polyclonal and monoclaonal antibodies. *Acta Horticulturae* 431, 135–158.

Adkins S. and E.N. Rosskopf, 2002. Key West nightshade, a new experimental host for plant viruses. *Plant Disease* 86, 1310–1314.

Anfoka G.H., M. Abhary and M.R. Stevens, 2006. Occurrence of *Tomato spotted wilt virus* (TSWV) in Jordan. *Bulletin EPPO/OEPP Bulletin* 36, 517–522.

Aramburu J. and M. Marti, 2003. The occurrence in north-east Spain of a variant of *Tomato spotted wilt virus* (TSWV) that breaks resistance in tomato (*Lycopersicon esculentum*) containing the *Sw-5* gene. *Plant Pathology* 52, 407.

Bandla M.D., D.M. Westcot, K.D. Chenault, D.E. Ullman, T.L. German and J.L. Sherwood, 1994. Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of *Tomato spotted wilt tospovirus* to identify viruliferous thrips. *Phytopathology* 84, 1427–1431.

Ben Moussa A., M. Makni and M. Marrakchi, 2000. Identification of the principal viruses infecting tomato crop in Tunisia. *Bulletin OEPP/EPPO Bulletin* 30, 293–296.

Best R.J., 1968. Tomato Spotted Wilt Virus. In: *Advances in Virus Research* (K.M. Smith, M.A. Lauffer, ed.), Academic Press New York, NY, USA, vol. 13, 65–145.

Boiteux L.S. and L.D. Giordano, 1993. Genetic-basis of resistance against 2 tospovirus species in tomato (*Lycopersicon esculentum*). *Euphytica* 71, 151–154.

Chatzivassiliou E.K., R. Weekes, J. Morris, K. Wood, I. Barker and N.I. Katis, 2000. *Tomato spotted wilt virus* (TSWV) in Greece: its incidence following the expansion of *Frankliniella occidentalis*, and characterization of isolates collected from various hosts. *Annals of Applied Biology* 137 (2), 127–134.

Ciuffo M., M.M. Finetti-Sialer, D. Gallitelli and M. Turina, 2005. First report in Italy of a resistance-breaking strain of *Tomato spotted wilt virus* infecting tomato cultivars carrying the *Sw-5* resistance gene. *Plant Pathology* 54, 564.

Clark M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay

for the detection of plant viruses. *Journal of General Virology* 34, 475–483.

De Avila A.C., C. Huguenot, R.O. De Resende, E.W. Kitajima and R.W. Goldbach, 1990. Serological differentiation of 20 isolates of *Tomato spotted wilt virus*. *Journal of General Virology* 71 (12), 2801–2807.

EPPO, 1999. EPPO PQR database (Plant Quarantine data Retrieval system). EPPO, Paris, France.

Finetti-Sialer M.M., C. Lanave, M. Padula, C. Vovlas and D. Gallitelli, 2002. Occurrence of two distinct Tomato spotted wilt virus subgroups in southern Italy. *Journal of Plant Pathology* 84, 145–152.

Huguenot C., G. Dobbelsteen, P. Van Den Haan, C.A.M. De Wagemakers, G.A. Drost, A.D.M.E. Osterhaus and D. Peters, 1990. Detection of *Tomato spotted wilt virus* using monoclonal antibodies and riboprobes. *Archives of Virology* 110, 47–62.

Ismaeil F., A.A. Haj Kasem and S. Al-Chaabi, 2010. Current status of *Tomato spotted wilt virus* on tomato and pepper crops in Syria and serological characterization of some its isolates. *Arab Journal of Plant Protection* 28 (2), 120–126.

Jahn M., I. Paran, K. Hoffmann, E.R. Radwanski, K.D. Livingstone, R.C. Grube, E. Aftergoot, M. Lapidot and J. Moyer, 2000. Genetic mapping of the *Tsw* locus for resistance to the Tospovirus *Tomato spotted wilt virus* in Capsicum spp. and its relationship to the *Sw-5* gene for resistance to the same pathogen in tomato. *Molecular Plant Microbe Interaction* 13, 673–682.

Jain R.K., S.S. Pappu, H.R. Pappu, A.K. Culbreath and J.W. Todd, 1998. Molecular diagnosis of *Tomato spotted wilt tospovirus* infection of peanut and other field and greenhouse crops. *Plant Disease* 82, 900–904.

Margaria P., M. Ciuffo and M. Turina, 2004. Resistance breaking strain of *Tomato spotted wilt virus* (*Tospovirus*; *Bunyaviridae*) on resistant pepper cultivars in Almeria, Spain. *Plant Pathology* 53, 795.

Mumford R.A., I. Barker and K.R. Wood, 1994. The detection of *Tomato spotted wilt virus* using the polymerase chain reaction. *Journal of Virological Methods* 46, 303–311.

Murphy F.A., C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P. Martelli, M.A. Mayo and M.D. Summers, 1995. Virus Taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses. *Archives of Virology*, Supplement 10, 398–400.

Nolasco G., C. De Blas, V. Torres and F. Ponz, 1993. A method combining immunocapture and PCR amplification in a microliter plate for the detection of plant viruses and subviral pathogens. *Journal of Virological Methods* 45, 201–218.

Nono-Womdim R., B. Moury, D. Ansan, P. Gognalons, K. Gebre-Selassie, A. Palloix and G. Marchoux, 1996. Natural and induced variability of *Tomato spotted wilt virus*. *Acta Horticulturae* 431,186–192.

Pappu H.R., R.A.C. Jones and R.K. Jain, 2009. Global status of *tospovirus* epidemics in diverse cropping systems: Successes achieved and challenges ahead. *Virus Research* 141, 219–236.

Parrella G., P. Gognalons, K. Gebre-Selassiè, C. Vovlas and G. Marchoux, 2003. An update of the host range of *Tomato Spotted Wilt Virus*. *Journal of Plant Pathology* 85, 227–264.

Roggero P., P. Oliara, G. Dellavalle, V. Lisa, F. Malavasi and G. Adam, 1996a. A general Tospovirus assay using monoclonbal antibodies against *Tomato spotted wilt virus* glycoprotiens. *Acta Horticulturae* 431, 167–175.

Roggero P., P. Ogliara, E. Ramasso, A. Arzone, L. Tavella and A. Alma, 1996b. Detection by TAS-ELISA of *Tomato spotted wilt virus* nucleocapsid and G1 glycoprotein in *Franklineilla occidentalis*. *Acta Horticulturae* 431, 333–340.

Roggero P., V. Masenga and L. Tavella, 2002. Field isolates of *Tomato spotted wilt virus* overcoming resistance in pepper and their spread to other hosts in Italy. *Plant Disease* 86, 950–954.

Sherwood J.L., M.R. Sanborn, G.C. Keyser and L.D. Myers, 1989. Use of monoclonal antibodies in detection of Tomato spotted wilt virus. *Phytopathology* 79, 61–64.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28, 2731–2739.

Thompson J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins, 1997. The clustal_x windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876–4882.

Tsompana M., J. Abad, M. Purugganan and J.W. Moyer, 2005. The molecular population genetics of the *Tomato spotted wilt virus* (TSWV) genome. *Molecular Ecology* 14, 53–66.

Turina M., L. Tavella and M. Ciuffo, 2012. *Tospoviruses* in the Mediterranean area. *Advances in Virus Research* 84, 403–437.

Wang M. and D. Gonsalves, 1990. ELISA detection of various *Tomato spotted wilt virus* isolates using specific antisera to structural protein of the virus. *Plant Disease* 75, 154–158.

Zhang Z., S. Schwartz, L. Wagner, and W. Miller, 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7 (1-2), 203–214.

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