Genetic diversity studies of tomato spotted wilt virus (TSWV) isolated from Dahlia *via* biotechnological methods

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ABSTRACT

The economic ornamental plant, Dalia (Dahlia variabilis) is affected by many viral diseases among them, tomato spotted wilt virus (TSWV). Detection of TSWV in dahlia cultures was carried out in some nurseries in Qalubia Governorate, Egypt. Although this virus isolate showed some differences in host range and stability characters, it was serologically detected by ELISA and Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) using new design of universal primers and dot blot hybridization with digoxigenin-labeled probes for the universal detection of TSWV targeting a conserved region of the nucleocapsid protein gene on dahlia as well as other transmitted virus isolates from tomato leaves (Lycopersicon esculentum), petunia (Petunia hybrid Valim), Tobacco (N. glutinasa), and Datura (Datura stramoni um.) For our knowledge, this is the first report to isolate and characterize TSWV from naturally infected dahlia plants in Egypt.

Key words: *Tomato spotted wilt virus, dahlia, dot blot hybridization, RT-PCR.*

INTRODUCTION

Tomato spotted wilt virus (TSWV) was first described in 1915 in Australia (Brittlebank, 1919). It is one of the most widespread and economically important plant viruses (Goldbach and Peters, 1994), and infects at least 900 plant species, with a number of natural host species recorded steadily increasing (Peters, 1998). TSWV occurs in countries within the European region as mentioned by Mediterranean Plant Protection Organization (EPPO), Asia, Africa, North America, Central America, Caribbean, South America and Oceania, (EPPO, 1999). In Egypt, the first isolation was from *Physalis peruviama* (Alkhazindar, 1999) and tomato (Abd-El Nazir, 1999). Recently in Egypt, TSWV became an economically important virus infecting major crops and causing losses (Abdelsalam *et al.*, 2005). Earlier reports indicate that this virus is widespread (Hull, 2002). Its incidence in Egypt is low (23.8%) to moderate (30.9%) (EPPO/CABI, 1997), while, high incidence (64.2%) was reported in South Africa OEPP/EPPO, 1999 a,b).

In the recent years, dahlia plantations in Egypt have shown a syndrome suspected to be due to TSWV. However, no detailed studies were reported. In addition, symptoms of TSWV become progressively milder with time and were difficult to identify in rapidly proliferating material (Mertelik and Götzová 1996). This phenomenon makes the use of visual symptoms, as the sole criterion of infection, an unreliable practice.

Previously, TSWV isolates were identified using differential hosts (Norris, 1946) but now development of techniques such as Enzyme Linked Immunoabsorbent

Assay (ELISA), dot blot hybridization with digoxigenin-labeled probes and RT-PCR dramatically improved TSWV identification (Cho *et al.*, 1989; Rice *et al.*, 1990 and Eiras *et al* 2001). Among these methods, ELISA has been widely used employing polyclonal antisera directed to the coat protein encoded by the viral RNA (de Ávila *et al.*, 1990). This technique is virus-specific and in some cases can present high background readings (Rice *et al.*, 1990). With the increasing number of new TSWV isolates (de Ávila *et al.*, 1992), a universal method of detection is therefore urgently needed to avoid the introduction of new TSWV isolates in distinct regions or countries. A few examples in the literature have already illustrated this threat by showing the spread of impatiens necrotic spot virus (INSV) in the Northern Hemisphere (Marchoux *et al.*, 1991) and the accidental introduction of Chrysanthemum stem necrosis virus (CSNV) in The Netherlands (Verhoeven and Roenhorst, 1998).

The present study aims to discover the genetic diversity among TSWV isolated from dahlia plants through infection of different plants within its host range using serological (ELISA) and molecular genetic approaches, i.e. reverse-transcriptase polymerase chain reaction (RT-PCR) and dot blot hybridization in addition to develop a universal detection method for TSWV.

MATERIALS AND METHODS

Virus isolation

Young leaves of dahlia (*Dahlia variabilis*) plants, grown at Qalubia Governorate, suspected to be infected with tomato spotted wilt virus (TSWV) were collected and ground in sterilized mortar with phosphate buffer solution (pH 7.2) and the infectious sap was used to inoculate 2-3 old leaves of tomato plants (cv. Money maker). Inoculated plants were kept under insect-proof cages in the greenhouse.To isolate the virus, single local lesion technique was used. Infectious tomato sap was used to inoculate local lesion host, i.e. *Petunia hybrida* -Valim and *Datura stramonium* plants. A single lesion was used to inoculate healthy tomato plants, which served as a maintenance host. Three to four weeks after inoculation, infected leaves from different hosts were harvested and stored at 80°C for subsequent analysis.

Virus identification and genetic diversity

A-Transmission

1. ***Mechanical transmission:*** Leaves of infected tomato plants were ground in a mortar and pestle with 0.02 M phosphate buffer (pH 7.2) mixed with carborandum. Different plant species (Table 1) were mechanically inoculated then symptoms were observed for 3-4 weeks.
2. ***Seed transmission:*** Seeds collected from mechanically inoculated tomato plants were sown in 30 cm diameter pots and kept under insect proof cages. Observations were recorded 35 days after sowing.
3. ***Dodder transmission:*** Dodder seeds were placed in Petri dishes containing moist filter paper. One week later, young branches were directed towards dahlia leaves exhibiting virus symptoms (Fig.1). When dodder plants were established on dahlia plants, the new branches were directed towards leaf petioles of healthy young petunia and tomato plants. Results were recorded, 30 days after.

B- Host range and symptomatology

Infectious sap was used to inoculate fourteen plant species belonging to eight families (Table1). Ten plants of each species were inoculated, and then kept

under greenhouse conditions for symptoms scoring.

C- Virus stability

Thermal inactivation point (T.I.P), dilution end point (D.E.P) and longevity *in vitro* (L.I.V.) were determined using tomato plants as a donor host and *Nicotiana glutinosa* as a local lesion host.

D- Enzyme linked immunoabsorbent assay (ELISA)

TSWV infections were tested using a commercial kit (ELISA-Plantest-ELISA kit, Sanofi-Pasteur Co., France) according to manufacture instructions.

E- Molecular studies

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Primer design

Two degenerate primers were designed using primer select module, laser gene software ver. 7.0 DNASTAR inc., due to conserved regions of the coat protein gene of TSWV virus with accession numbers showed in Fig. (4): TSWV-F '5-TCC AGC T(C/T)C CAA GAA AAA GAC A-3' and TSWV-R '5-ATT TAT CTA TTG C(C/T)T GCA TTC CA-3'

RNA Isolation

Four isolates of TSWV From tomato, petunia, tobacco, and datura infected leaves derivative from dahlia were used to extract total RNA according to De Vries *et al.* (1982).

RT-PCR

The complementary DNA (cDNA) strands of viral RNA segments were synthesized by mixing 1µg of total viral RNA, 10 pmol of the reverse specific primer and 6µl of RNase free water followed by heating the mixture at 70°C for 2 min and cooling in ice for 2 min, then 4µl of 5X reverse transcriptase buffer, 100 units of reverse transcriptase (MMLuV-RT) (Bioron, Germany), 1 unit of RNase inhibitor (RNAsin, Pharmacia), 1 µl of dNTPs (10 mM each) (Bioron, Germany) and sterile water up to 20 µl were added to the prepared solution, then incubated at 40°C for 1 hr.

PCR was accomplished by adding 5 µl 10X *Taq* DNA polymerase buffer, 1 µl dNTPs (10 mM each) (Bioron, Germany), 2.5 units *Taq* DNA polymerase (Bioron, Germany), 10 pmol of each primer (forward and reverse), 5 µl cDNA and RNase free water up to 50 µl. All PCR reactions were performed in a thermal cycler (GeneAmp PCR System 2400) (Perkin Elmer), by pre-heating at 94°C for 5 min followed by 30 cycles of 1 min at 94°C, 1 min at 48°C and 1 min for extension at 72°C. Finally, the amplified DNA was incubated at 72°C for 7 min to accomplish a final extension. 20 µl of PCR reaction were subjected to electrophoresis in 2% agarose gel containing ethidium bromide (0.01%), subjected to 100 volts for 1hr and then photographed using UVP gel documentation system, UVP corporation-UK.

Dot blot hybridization

Total RNA extracts (1 µg) of each isolate of tomato spotted wilt virus (TSWV) were blotted into nylon membranes (Roche, Germany) using the dot-blot convertible filtration manifold system (Gibco BRL), for 15 min, then fixed by UV irradiation for 5 min. Labeling the probe was performed using DIG-High Prime DNA Labeling and Detection Starter Kit I-Roche (Cat. No. 1 745 832). One µg of TSWV PCR product dissolved in 16 µl of sterile double distilled water, was denatured in 100°C water bath for 10 min, and then quickly chilled on ice. DIG High Prime labeling tube (4 µl) was added to the denatured DNA, mixed and briefly centrifuged, then left overnight in incubator at 37°C. The reaction was stopped by heating to 65°C for 10 min.

Each digoxigenin-labeled probe (5-25 ng/ml) was hybridized for 12hr at hybridization solution (SSC 5X + SDS 0.02% + blocking reagent 0.05% + N-laurylsarcosine 0.1% + blocking solution 1/10v) under low (45°C) and high (65°C) stringency conditions. After washing with SSC 2X at 25°C and twice with SSC 0.1X + SDS 0.1% at 65°C, the anti-DIG-AP conjugate was added (75mU/ml) in blocking solution for 30 min. After treatment with wash buffer (Maleic acid 0.1M + NaCl 0.15M pH 7.5 + Tween 20 0.3%) and detection buffer (NaCl 0.1M + Tris HCl 0.1M + $MgCl_2$50 mM), the membranes were visualized in a solution of nitro blue tetrazolium plus X-phosphate.

RESULTS AND DISCUSSION

This study aimed to isolate, identify and determine different biological and molecular characterization of TSWV isolated from naturally infected dahlia plants in Egypt. TSWV was isolated from naturally infected dahlia plants showing leaf chlorosis, twisting and distortion (Fig 1). Extracted leaf sap was used to inoculate tomato plants and tomato sap was used to inoculate *Petunia hybrida*. Single local lesion technique was used to isolate the virus. Lesions developed on inoculated petunia leaves were used separately to inoculate tomato plants. To isolate the virus in pure form, inoculation from tomato to petunia and vice versa was repeated several times. TSWV - dahlia isolate- was mechanically transmitted from infected dahlia plants to healthy tomato plants. Inoculated tomato plants showed coloristic and necrotic spots, bronzing and striking. Successive leaves tend to curl downwards (Fig. 2 i, j). Symptoms started to appear 14 days after inoculation. These results were similar to those described by (De Angelis *et al.* 1994).

Identification of the isolated virus depended on the mode of transmission, virus stability, symptomatology and host range. Identification was ensured by serological and molecular tests. TSWV was readily transmitted from tomato to different plant species. Symptoms appeared 2 to 7 days after inoculation depending on the plant species as shown later. Tomato seedlings obtained from inoculated plants showed no visible symptoms and DBIA test confirmed these results. This result was similar to that obtained by Smith (1957) while, other authors believe that seed transmission of TSWV did not seem to be high (Brown *et al.*, 1998). Obtained results clearly showed that TSWV was not transmitted to petunia or tomato plants through dodder, where no positive reaction was detected when these plants were serologically tested.

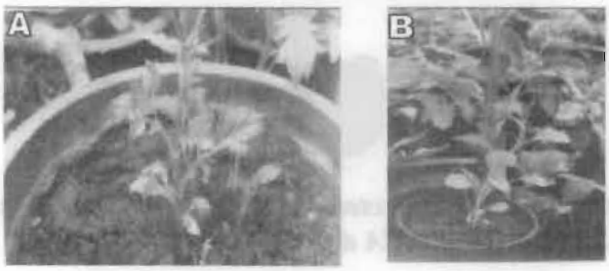

Fig. (1): Naturally infected dahlia with TSWV showing mosaic patterns twisting of leaves (A) and dodder transmission from infected naturally dahlia to petunia plants (B).

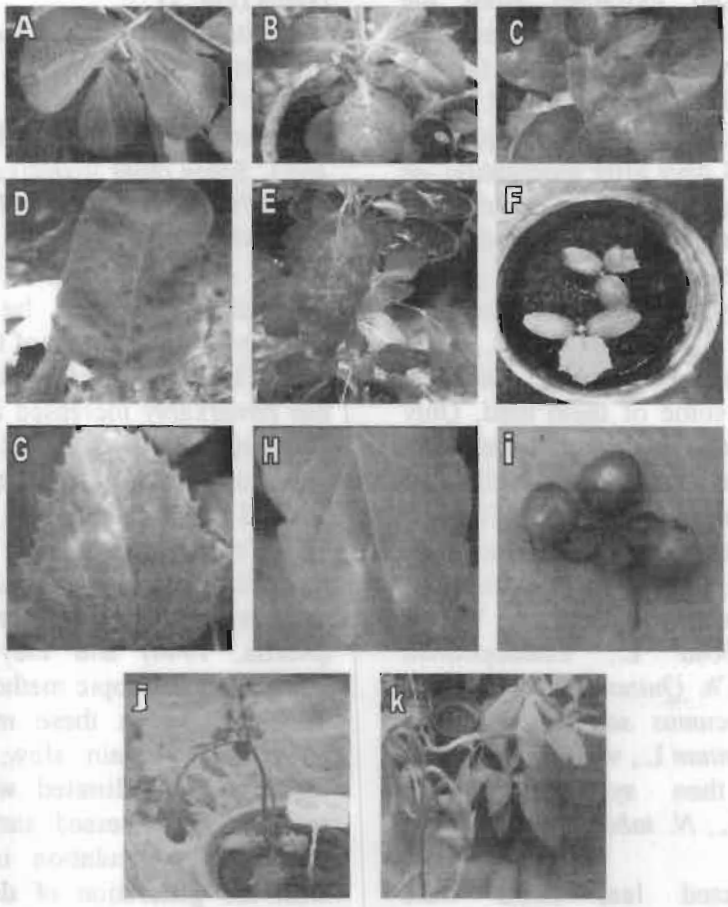

Fig. (2): Symptoms appearing on mechanically inoculated plants. A- Faba plants showing necrotic spots. B- Necrotic local lesions with dark edge and light brown center appeared on petunia leaves. C- N rustica showed large dark local spots a combined with distortion of young non- inoculated leaves. D- Datura metel leaves showed necrotic local lesions. E- Chlorotic lesions developed on Ch. Quinoa leaves. F- Paled lesions appeared on cucumber cotyledons. G- ch amaranticolour showed cholorotic lesions appeared on leaves. H- Concentric large local lesions on N. glutinosa. I- Scattered green and pale red areas developed on some fruits of inoculated plant J and K showed different symptoms on inoculated money maker tomato plants.

Fig. (3): Positive reaction with naturally infected dahlia and inoculated tomato, N. rustica and N. glutinosa plants as example for ELISA detection method.

One hundred thirty plants belonging to fourteen species and eight families were inoculated with sap extracted from the maintenance host (tomato plants) resemble those reported by many investigators (Reddy and Wightman, 1998; De Avila *et. al.* 1992; Parrella *et.al* .2003). Symptoms were appeared on tomato plants 14 days after inoculation -as mentioned before- on the form of thickening of young leaf veins and leaves tending to downwards. In most cases, the latter pattern was accompanied by bronze spots on the older leaves. Excluding the upper leaves, other leaves showed yellowish color and distortion. Plants stunted and some of them died. Only very few plants were able to produce small sized fruits characterized by small yellow or green spots scattered on fruit surface (Fig. 2, i, j). Some plants reacted locally as shown in (Table 1) and (Fig. 2). These include *N. glutinosa* L., *Petunia hybrida* Valim, *Gomphrena golobosa* L., *Chenopodium amaranticolor* L., *Ch. Quinoa* L, *Vinca rosa*, *Vicia faba* L., *Cucumus sativa* L., *Datura metel* L., *D. stramonium* L., while some others reacted locally then systemically like *Nicotiana rustica* L., *N. tabaccum* cv. White Burley.

TSWV infected leaf hosts were serologically detected in all tested host range plant species showed symptoms (Table1). In addition, sap from naturally infected dahlia leaves gave a faint color spot, while that isolated from Tomato, *N. rustica* and *N. glutinosa* gave a dark color spot (Fig. 3). The systemic symptoms appeared on tested hosts showed mosaic, mottle chlorotic and necrotic spots, yellowing, curling downwards of leaves, leaf drop, wilting, stunting and death of some plants (Fig. 2).

Stability of dahlia isolate showed a few differences. TIP (Thermal inactivation point) was 55°C; this result was different from that obtained by Best (1946) who stated that it was 46+1. Some other investigators estimated it as 41- 42°C (Best 1961). DEP was 10 while LIV was 6-8 hours. However; Adam and Kegler (1994) estimated them as 10 and 5- 6 hours. This difference may be attributed to the stability of coat protein of this isolate.

The number of distinct TSWV species has remarkably increased in the last ten years comprising, at present, 12 species (Prins and Goldbach, 1998). Therefore, a sensitive method to detect a broad number of known TSWVs and possible new species in a single assay is urgently needed. Usually most TSWV isolates can be mechanically transmitted (Norris, 1946) and they are detected by electron microscopic methods (Kitajima *et al.*, 1992). Although these methods have been used, they remain slow, time consuming, inaccurate and limited with respect to the number of processed samples. In addition, mechanical inoculation is often associated with the generation of defective interfering mutant RNAs (Resende *et al.*, 1991), which can cause a misinterpretation of TSWV symptoms. Serology using ELISA with antisera directed at the N protein for TSWV diagnosis allows a reliable and sensitive detection method but is highly species-specific (De `Avila *et al.*, 1993).

Table (1): Tomato spotted wilt virus (topovirus) host range, symptoms, ELISA readings of after mechanical inoculation.

Family& species	Observed symptoms	DBIA test
1-Chenopodiaceae:		
Chenopodium amaranticolour L.	Necrotic local lesions	+
Chenopodium quinoa L.	Necrotic local lesions	+
2-Convolvulaceae:		
Convolvulus arvensis L.	No symptoms	-
3-Cururbitaceae:		
Cucumus sativa L.	Chlorotic local lesions	+
4-Fabaceae:		
Vicia faba L. cv. Giza 2	Necortic local lesions:	+
6-Solanaceae:		
Datura metal L.	Necrotic local lesions	+
D. stramonium L.	Necrotic local lesions	
Lycopersicum esculantum	bronze, Necrotic spot,	+
yellowish distortion		
Nicotiona glutinosa L.	Necrotic local lesions	+
N. rustica L.	Necrotic local lesions,	+
distortion of young leaves		
N. tobacum cv. White Burley	Necrotic local lesions,	+
yellowing, top necrosis		
Petunia hybrida Vilm	Necrotic local lesions	+
7-Amaranthaceae:		
Gompherna glubosa L.	Brown local lesions	+
8- Apocynaceae:		
Vinca minor	Local black spots	+

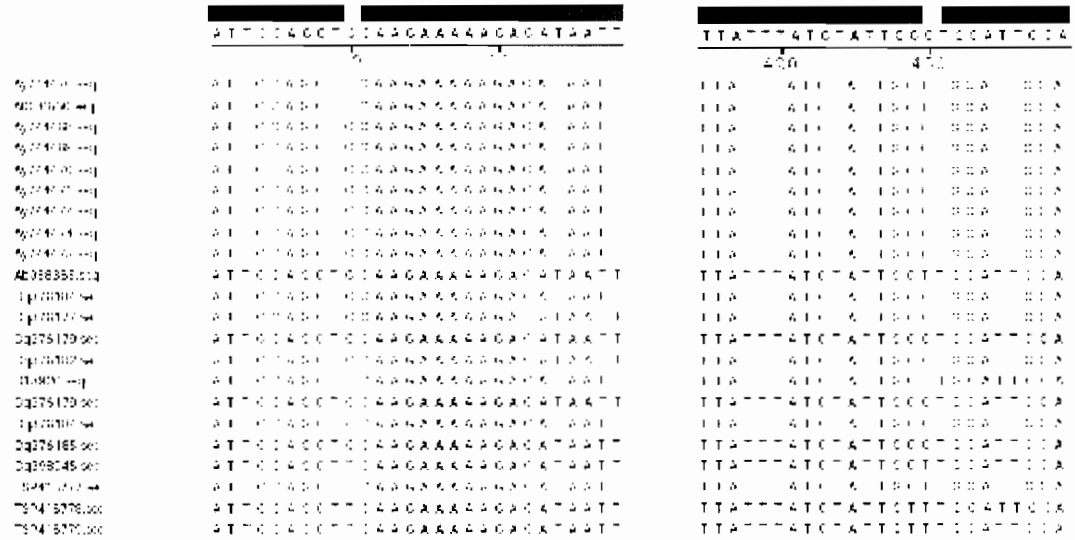

Fig. (4): Design of two primer pairs using primer select module, laser gene software ver. 7.0 DNASTAR inc., due to conserved regions of the TSWV genome virus.

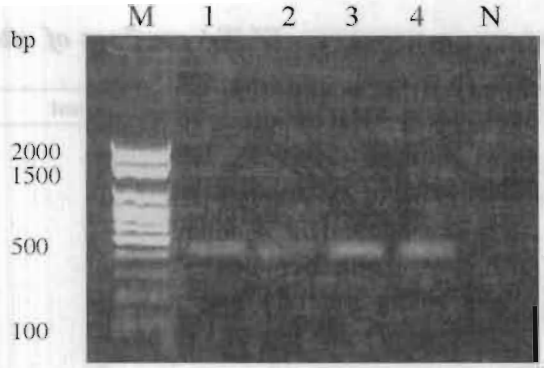

Fig. (5): Results of RT-PCR for total RNAs samples amplified with TSWV primer as example for RT-PCR detection method. M: DNA marker (100 pb DNA ladder) and lanes 1 to 4 corresponding to Lycopersicon esculentum, Petunia hybrid_Valim, N. glutinasa, and Datura stramonium, lanes 5 (N) for healthy plant, respectively.

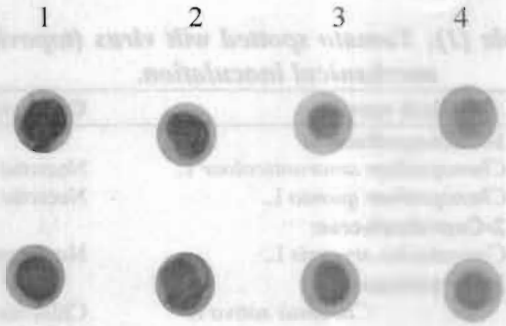

Fig. (6): Dot-blot analysis of TSWV in different plants using a DNA probe labeled with DIG as example for Dot-blot detection method, 1 to 4 corresponding to Lycopersicon esculentum, Petunia hybrid_Valim, N glutinasa, and Datura stramonium respectively.

RT-PCR and dot blot hybridization digoxigenin-labeled probes are the most successfully non-radioactive molecular tools used to detect very low concentrations of nucleic acids belonging to several plant viruses (Sadik *et al.*, 1997) and (Sadik *et al.*, 1999). RT-PCR using the new designed universal degenerate primer was able to detect all TSWV species used in this study at 440 bp (Fig. 5). The dioxigenin-labeled probes derivative from RT-PCR were developed and used for dot blot hybridization. The probe specific for TSWV gave a positive reaction with the homologous species, and some faint reactions with TSWV were observed (Fig. 6). The hybridization with RNA was repeated at least three times.

This study has demonstrated that RT-PCR designed to amplify conserved regions in the coat protein gene of TSWV and hybridization with RNA-specific PCR fragments probes (dot blot) could achieve universal detection of TSWV isolates (Fig. 5).

When RT-PCR with purified viral RNA was used along with the degenerate primers specific for TSWV, all TSWV isolates were detected. So far, more than 77 species within 29 genus and 8 families of plant viruses have been detected by PCR (Eiras *et al.*, 1998), including TSWVs for which PCR has been used showing higher sensitivity than ELISA (Mumford *et al.*, 1996). Aiming at a universal detection method of plant viruses, primers have been designed to anneal to the conserved regions of several viral genomes, as demonstrated for *Cucumber mosaic virus* isolates (Hu *et al.*, 1995), several POTY viruses (Langeveld *et al.*, 1991), and TSWV species (Mumford *et al.*, 1996). Hybridization techniques have been used to detect several plant viruses and have been shown to be more sensitive and more specific than serology (Hahm *et al.*, 1993). Hybridization using

cDNA probes (Ronco *et al.*, 1989) and riboprobes (Huguenot *et al.*, 1990) has also been applied to TSWV detection. Dot blot hybridization with digoxigenin-labeled probes has been used for plant viruses other than TSWV (James *et al.*, 1999). In seeking a broad spectrum detection method, digoxigenin-labeled probes directed at the RNAs and RT-PCR were developed. The hybridization technique and RT-PCR designed for conserved TSWV isolates have been shown to be suitable methods for the universal detection of TSWVs. In addition, the use of non-radioactive probes is highly recommended since they avoid the hazard of manipulating radioactive materials. The variability within the TSWV genus in combination with the intensive exchange of plant material around the world demands an accelerated development of new diagnostic tools for TSWV. The quarantine system plays an important role in indexing plant material to avoid introduction of new TSWV species within a geographic region, as recently shown for INSV (Impatiens necrotic spot virus) in the Northern Hemisphere (Marchoux *et al.*, 1991), and CSNV (Chrysanthemum stem necrosis virus) in the Netherlands (Verhoeven and Roenhorst, 1998). The RT-PCR and hybridization with the TSWV probe, as proposed in this study, were sensitive and broad-spectrum TSWV detection methods especially suited for use in quarantine services.

REFERENCES

Abdelsalam E. T. ; Hala M. Habib ; Hayam S. Abdelkader and Maha M. Al-Khazindar (2005). Molecular Cloning and Expression of Nucleoprotein gene (NPs) of tomato spotted wilt virus in *E.coli* International Journal of Virology 1: 5-5

Abd- El Nazir, N.(1999). Isolation, characterization preservation of some plant viruses. M. Sc. Thesis, faculty of Agriculture, Ain Shames University. P 82.

Adam, G.and Keglar, H.(1994). Tomato spotted wilt virus and related tospoviruses. Archives of phytopathology and plant protection 28: 483- 504.

Alkhazindar, M. (1999). Studies on a Virus Naturally Infecting *Physalis peruviana.* M.Sc. Thesis, Faculty of Science, Cairo University. 225P.

Best, R. J. (1961). On the maintaining the infectivity of a labile plant virus by storage at -69 C. Virol. 14: 440-443.

Best, R. J.(1946). Thermal inactivation of tomato spotted wilt virus. Part 1. Aust. J. Exp. Biol. 24: 21-25.

Brittlebank, C. C. (1919). Tomato diseases. Journal of Agriculture, Victoria. 17: 231-235.

Brown, S.; Todd J.W.; Culbreath A. K.; Baldwin J.; J. Beasley and Pappu H. R. (1998). *Tomato spotted wilt virus* risk index.University of Georgia, Extension Bulletin No. 1165, 12 pp.

Cho, J.J.; Mau, F.R.L.; German, T.L.; Hartmann, R.W.; Yudin, L.S., Gonsalves, D. and Provvidenti, R. (1989). A multidisciplinary aproach to management of TSWV in Hawaii. Plant Disease 73:375-383.

De Angelis; J. D.; Sether, D. M. And Rossignol, P. A. (1994). Transmission of impations necrotic spot virus in peppermint by western thrips(Thysanoptera: Thripidae). J. Econ. Entomol. 87 (1): 197- 201.

De Ávila, A.C.; Huguenot, C.; Resende, R.O.; Kitajima, E.W.; Goldbach, R.W. and Peters, D. (1990). Serological differentiation of twenty isolates of tomato spotted wilt virus. Journal of General Virology 71:2801- 2807.

De Avila, A.C.; Huguenot, C.; Resende, R.D.; Kitajima, R.; Resende, R.O.; Goldbach, R. and Peters, D. (1992).

Characterization of a distinct isolate of tomato spotted wilt virus (TSWV) from *Impatiens sp.* In the Nertherlands. J. Phytopathol. 134: 133-151.

De Ávila, A.C.; De Haan, P.; Kormelink, R.; Resende, R.O.; Kitajima, E.W.; Goldbach, R.W and Peters, D. (1993). Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. Journal of General Virology 74:153-159.

De Vries, S.C.; Springer, J. and Wessels, J.G.H. (1982). Diversity of abundant mRNA sequences and patterns of protein synthesis in etiolated and greened pea **seedlings. Planta 156:129-135.**

Eiras, M.; Resende, R.O. and De Ávila, A.C.(1998). Detecção de vírus de plantas através de Reação em Cadeia daPolimerase. Fitopatologia Brasileira 23:5-17.

Eiras, M.; Resende, R.O.; Missiaggia, A.A. and De Ávila, A.C. (2001). RT-PCR and Dot Blot hybridization for an universal detection of tospoviruses. Fitopatologia Brasileira 26:170-175.

EPPO .(1999). Tomato Spotted wilt Tospovirus EPPO Bulletin 29,465-472.

EPPO/ CABI, (1997). Tomato spotted wilt virus. IN: Quarantine Pests for Europe (2nd. Ed). pp. 1379- 1387. CAB International, Wallingford (GB).

Goldbach, R. W. and Peters, D. (1994). Possible causes of the emergence of Tospoviruses diseases. Seminars in Virology 5:113-120.

Hahm, Y.I.; Choi, K.S.; Kim, Y.S.; Choi, J.K.; Bae, S.W and Hwang, Y.S. (1993). Hybridization detection of potato viruses using digoxigenin-labeled RNA probe. RDAJournal of Agricultural Science 35:239-244.

Hu, J.S.; Li, H.P.; Barry, K.; Wang, M. and Jordan, R. (1995). Comparison of dot blot, ELISA and RT-PCR assays for detection of two cucumber mosaic isolates infecting banana in Hawaii. Plant Disease 79:902-906.

Huguenot, C.; Van Den Dobbelsteen, G.; De Haan, P.; Wagemakers, C.A.M.; Drost, G.A.;Osterhaus, A.D.M.E. and Peters, D. (1990). Detection of tomato spotted wilt virus using monoclonal antibodies and riboprobes. Archives of Virology 110: 47-62.

Hull, R. (2002). Matthews plant Virology. Forth edition. Academic Press. UK. ISBN: 0-12-361160- 1. 1001p.

James, D.; Jelkmann, W. and Upton, C. (1999). Specific detection of Cherry Mottle Leaf Virus using Digoxigeninlabeled cDNA probes and RT-PCR. Plant Disease 83:235-239.

Langeveld, S.A.; Dore, J.M.; Memelink, J.; Derks, A.F.L.M.; Van Der Vlught, C.I.M.; Asjes, C.J. and Bol, J.F. (1991). Identification of potyviruses by polymerase chain reaction with degenerate primers. Journal of General Virology 72:1531-1541.

Kitajima, E.W.; De Ávila, A.C.; Resende, R.O.; Goldbach, R.W. and Peters, D. (1992). Immuno-electron microscopical detection of tomato spotted wilt virus and its nucleocapsids in crude plant extracts. Journal of Virological Methods 38:313-322.

Marchoux, G.; Gebre-Selassie, K. and Villevieille, M. (1991). Detection of tomato spotted wilt virus and transmission by Frankliniella occidentalis in France. Plant Pathology 40:347-351.

Mertelik, J. and B. Götzová (1996). "Epidemiological aspects of tomato spotted wilt virus infection in the Czech Republic." Acta Horticulturae 432: 368-375.

Mumford, R.A.; Barker, I. and Wood, K.R. (1996). An improved method for detection of Tospoviruses using the polymerase chain reaction. Journal of Virological Methods 57:109-115.

Norris, D. (1946). The strain complex and symptom development of tomato spotted

wilt virus. Bull. Aust. Council Sci. Ind. Res. 202.

OEPP /EPPO, (1999)a. Data sheet on quarantine organisms no. 291. Impations necrotic spot tospovirus. Bulletin OEPP / EPPO Bulletin 29: 473- 476.

OEPP /EPPO, (1999) b. Data sheet on quarantine organisms no. 294.Watermelon silver mottle tospovirus. Bulletin OEPP / EPPO Bulletin 29: 489- 492.

Parrella, G.; Gognalons, P.; Gebre Selassie, K.; Vovlas, C. and marchoux, G. (2003). An update of the host range of tomato spotted wilt virus.Journal of Plant Pathology.85(4): 227- 264.

Peters, D. (1998). An updated list of plant species susceptible to tospoviruses. Abstr. Fourth International Symposium on tospovirus and thrips in floral and vegetable crops, 2-6 May, Wageningen 107-110.

Prins, M. and Goldbach, R. (1998). The emerging problem of tospovirus infection and nonconventional methods of control. Trends in Microbiology 6:31-35.

Reddy, D.V.R. and Wightman, J.A. (1998). Thrips transmission and control in advance in Disease Vector Research, ed. K. F. Harris, 8: 203- 220.

Resende, R.O.; De Haan, P.; De Ávila, A.C.; Kitajima, E.W.; Kormelink, R.; Goldbach, R.W. and Peters, D. (1991). Generation of envelope and defective interfering RNA mutants of tomato spotted wilt virus by mechanical passage. Journal of General Virology 72:2375-2383.

Rice, D.J.; German, T.C.; Mau, R.F.L. and Fujimoto, F.M. (1990). Dot blot detection of tomato spotted wilt virus RNA in plant and thrips tissues by cDNA clones. Plant Disease 74:274-276.

Ronco, A.E.; Dal Bó, E.; Ghiringhelli, P.D.; Medrano, C.; Romanovski, V.; Sarachu, A.N and Grau, O. (1989). Cloned cDNA probes for the detection of tomato spotted wilt virus. Phytopathology 79:1309-1313.

Sadik, A.S.; Abdel-Gaffar, M.H.; Salama, M.I. and Allam, E.K. (1997). Evaluation of three different methods for detection of banana bunchy top nanavirus. Proceedings of the 9th Conference of Microbiology, Cairo, March 25-27, 188-202.

Sadik, A.S.; Salama, M.I. and Madkour, M.A. (1999). Purification and Partial characterization of an Egyptian isolate of banana bunchy top virus. Arab J. Biotech. 2 (2):181-192.

Smith, K. M. (1957). A textbook of plant virus disease. Second edition. J.and A. Churchilp Ltd. **London P 574.**

Verhoeven, T.J. and Roenhorst, J.W. (1998). Occurrence of tospoviruses in The Netherlands. Abstracts of the 4thInternational Symposium on Tospoviruses and Thrips in Floral and Vegetable Crops. Wageningen-The Netherlands, pp. 77-80.

الملخص العربي

دراسه التنوع الوراثى لفيروسات تبقع الطماطم المعزولة من نبات الداليا بإستخدام طرق التقنيات الحيوية

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يصاب نبات الداليا المعروف بقيمته الاقتصادية و الجمالية بالعديد من الفيروسات النباتية و من ضمنها فيروس تبقع الطماطم (TSWV) وقد تم ملاحظة هذه الاصابة فى محافظة القليوبية التابعة لجمهورية مصر العربية وبعزل هذا الفيروس لوحظ اختلاف فى درجة ثباته وانتشاره خلال مداة العوائلى. وقد تم تشخيصه باستخدام أكثر من تقنية منها المناعى (ELISA) و الجزيئى عن طريق تفاعل البلمرة المتسلسل والعكسى (RT-PCR) والتهجين الموضعى (Dot Plot) باستخدام مسبار معلم غير مشع. و قد تم اجراء الدراسة علي العديد من النباتات التى تتضمن المدى العوائلى لهذا الفيروس بعد عزله من الداليا . وقد تم تصميم بادئ للجين المشفر للغلاف البروتينى للفيروس روعى فيه ان يكون عام بحيث يستطيع الارتباط باي عزله من هذا الفيروس بغض النظر عن اختلافها عن العزلة الاصلية فى مظهر الاصابة وشدتها . ويعتبر هذا البحث من اوئل البحوث التى تعرضت لتوصيف وتعريف فيروس تبقع الطماطم المنتشر طبيعياً بين نباتات الداليا المصرية .