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Biological, Biochemical, Serological, Molecular and Tissue Cultural Studies on an Egyptian Isolate of *Tomato Spotted Wilt Virus* Infecting Chrysanthemum Plants

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Tomato spotted wilt virus (TSWV) has been isolated and identified from naturally infected chrysanthemum plants, collected from the experimental farm of the Fac. of Agric., Cairo Univ. by indirect-enzyme linked immunosorbent assay (Indirect-ELISA) and dot blotting immunobinding assay (DBIA) using an induced antiserum for TSWV. The symptoms consisted of dark coloured leaf necrosis, necrotic line patterns, necrotic local lesion and tip necrosis on leaves, stunting, wilting and flower distortion. All the tested chrysanthemum cultivars were found to be susceptible when mechanically inoculated under greenhouse conditions. Wide variations of symptoms were found between Shakira, Feling, Grancl, Zambla and Maxx cultivars. Cv. Zambla was found to be more susceptible than any other cultivar tested in the present study, thus it was used for the production of virus-free plants using tissue culture technique. TSWV was purified from infected *Gompherina globosa* plants. The UV absorption spectrum had A_{max} at 260nm, A_{min} at 245nm and $A_{260/280}$ ratio of 1.2. Coat protein subunit of TSWV had a value of 29 KDa. The IgG fraction of the prepared antiserum had A_{max} and A_{min} at 280 and 242 nm, respectively. Immunocapture-reverse transcription-polymerase chain reaction (IC- RT-PCR) was used to amplify 760 bp cDNA fragments from infected chrysanthemum leaves using the primers (SLS90–47) and (JLS90-46) specific to TSWV. The dilution end point of TSWV in infected tissue extracts was 1/1280, 1/2560 and 10^{-5} for indirect ELISA, direct ELISA and IC- RT- PCR, respectively. IC- RT-PCR proved to be more sensitive than indirect and direct ELISA. In this study, meristem-tips were excised from infected chrysanthemum plants. Thermotherapy and/or chemotherapy were used to eliminate TSWV. Results demonstrated that application of thermotherapy at 38°C for 28 days followed by 10 to 20 mg/l Virazole *in vitro* gave a survival rate of 53 and 77%, respectively. The presence of virus in the produced plants was evaluated by ELISA technique. *In vitro* thermotherapy combined with chemotherapy eliminated TSWV from infected chrysanthemum and reduced the risk of introducing this pathogen through vegetative propagation.

Keywords: Chemotherapy, chrysanthemum, DBIA, indirect-ELISA, meristem tip, immunocapture RT-PCR, thermotherapy, tissue culture and *Tomato spotted wilt virus*.

Chrysanthemum (*Chrysanthemum marifolium* Ramat), belonging to family Asteraceae, is one of the most important cut flower crops grown worldwide on a commercial scale and has an economical importance as ornamental, culinary, insecticidal, environmental and medicinal uses. Four kinds of viruses and two viroids have been reported as major pathogens on chrysanthemum worldwide (Bong *et al.,* 2006). These are *Tomato aspermy virus* (TAV), *Chrysanthemum virus B* (CVB), *Tomato spotted wilt virus* (TSWV), *Impatients necrotic spot virus* (INSV), *Chrysanthemum stunt viroid* (CSVd) and *Chrysanthemum chlorotic mottle viroid* (CChMVD). TSWV has been reported as an epidemic virus on chrysanthemum in many countries, *i.e.* USA (Hausbecak *et al.,* 1992), Canada (Allen and Broadbent, 1986), Japan (Tsuda *et al.,* 1994) and in European countries including, Portugal (Louro, 1996) and the Netherland (Verhoeven *et al.,* 1996). TSWV causes symptoms of chlorotic spots, foliar necrosis, leaf bronzing, chlorotic rings, line patterns, internal necrosis, stem cankers and stunting (Hausbeck *et al.,* 1992; Moriones *et al.,* 1998 and Gera *et al.,* 2008). Commercial flower production is also affected by losses in flower weight and petal number as well as the reducing number of flowers (Bong *et al.,* 2006). The virus is transmitted mechanically and by several species of thrips in a persistent manner (Akademiai, 2008 and Orosz *et al.,* 2008). TSWV is further distinguished from other plant viruses by its quasi-spherical enveloped virions, and tripartite RNA genome. The virion envelope contains two virus-coded glycoproteins and surrounds three ssRNA molecules which complex with N proteins to form circular nucleocapsids.

The success of tissue culture propagation of chrysanthemum plants is greatly technique for influenced by the nature of culture medium used MS medium (Murgshige and Skoog, 1962). Studies on meristem tip culture, thermotherapy and chemotherapy give satisfactory results in virus elimination (Minas, 2007 and Ram *et al.,* 2009).

The present study focused on partial characterization of TSWV isolated from chrysanthemum plants. Furthermore, biological, biochemical, serological and molecular aspects of this virus isolate was studied. Tissue culture techniques were utilizing antivirus compounds for the production of virus-free chrysanthemum plants was considered.

Materials and Methods

Source of the virus isolate:

Samples from naturally infected chrysanthemum (*Chrysanthemum marifolium* Ramat) plants exhibiting characteristic TSWV were collected from the experimental farm of Fac. of Agric., Cairo Univ. The observed symptoms included dark coloured leaf necrosis, necrotic line patterns, necrotic local lesions on leaves, necrotic streaks on the stem, tip necrosis, wilting and flower distortion. Dot blotting immunobinding assay (DBIA) and indirect ELISA technique were conducted to test the collected samples using an induced antiserum for TSWV (El-Shazly *et al.,* 2006).

Isolation and propagation of TSWV:

Naturally infected chrysanthemum leaf tissues, which reacted positively with DBIA and indirect-ELISA, were used for virus isolation and identification. About 2g of naturally infected leaf tissues were ground in 0.01M sodium phosphate buffer, pH 7.0, containing 0.1% sodium sulfite according to Bezerra *et al.* (1999), then inoculated onto carborandum dusted leaves of the local lesion host *Chenopodium amaranticolor* Coste and Reyn plants (Kuhn, 1964) for biological purification of the virus isolates, then mechanically transmitted to *Gomphrena globosa* L. which served as a source and for virus propagation.

Cultivars susceptibility:

Twelve chrysanthemum seedlings from each of five cultivars namely; Shakira, Feling, Grancl, Zambla and Maxx, obtained from Floramax Company for flowers and plants at Kafr-Hakim, Giza, were examined for reaction to TSWV. Sixty chrysanthemum seedlings grown in clay pots (18-cm-diameter) containing sterilized soil, kept in an insect-proof greenhouse, then examined using indirect ELISA before inoculation with sap extracted from TSWV infected plants. Upon plant emergence, ten plants from each cultivar were mechanically inoculated. Two plants from each cultivar were kept as a control check and inoculated with buffer only. Four weeks later, seedlings were examined for symptoms expression and then the percentages of infection were recorded by visual inspection and/or indirect ELISA according to Converse and Martin (1990).

Purification of the virus isolate:

The virus was partially purified from infected *Gomphrena globosa* as described by El-Shazly *et al.* (2006) with some modifications. Differential centrifugation was used instead of 8% (w/v) of polyethylene glycol (PEG, 6000 MW) and 1% (w/v) NaCl.

Molecular weight determination:

The molecular weight of the virus (TSWV) coat proteins (NP) was estimated by a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS - PAGE) according to Laemmli (1970) using three dilutions of purified virus of 1/10, 1/20 and 1/40, in 0.01 M potassium phosphate buffer and stained with Coomassie Brilliant Blue (CBB). Low molecular weight size markers ranging from 14.2 to 66 KDa (Promega, Madison, WI) were included for molecular weight determination.

Extraction and purification of immunoglobulins (IgG):

Gammaglobulins were purified from the induced antiserum prepared by El-Shazly *et al.* (2006) using the caprylic acid method adopted by Steinbuch and Audran (1969).

Preparation of IgG-enzyme conjugate:

Alkaline phosphatase (AP) was conjugated to rabbit IgG adjusted to 1mg/ml (A_{280nm}) according to the technique described by Clark and Adams (1977). Different dilutions of purified gammaglobulins and IgG enzyme conjugate (e.g. 8.0μl/ml, 4.0μl/ml, 2.0μl/ml, 1.0μl/ml and 0.5μl/ml) were examined using direct ELISA to measure the best dilution for both IgG and IgG conjugate with AP for antigen detection.

Serological detection of TSWV

Dot blotting immunobinding assay (DBIA) (Lin and Hus, 1990), three enzyme linked immunosorbent assay (DAS - ELISA) (Clark and Adams 1977, and indirect ELISA (Converse and Martin, 1990) were used for virus detection experiments.

Determination of antigen dilution end point

The dilution end point of TSWV was determined using direct and indirect ELISA. TSWV infected tissue extract was diluted with carbonate buffer pH 9.6 for indirect ELISA and/or with phosphate buffer pH 7.2 for direct ELISA, for the dilutions of 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280 and 1/2560, respectively. Secondary antibody, goat antirabbit conjugated with alkaline phosphatase (Sigma, A 4503) for indirect ELISA was added to the wells at dilution of 1/7000 in conjugate buffer, whereas the induced conjugate for direct ELISA was added to wells at dilutions of 1/1000 in conjugate buffer. Control of healthy and infected *G. globosa* sap was used.

Immunocapture RT-PCR (IC-RT-PCR)

Immunocapture RT-PCR was performed on the plant sap extracts as described by Pang *et al.* (1992). Two primers were used as the following

1.5µl of 10µM of the complementary primer (JLS90-47, 5'-AGCATTCCATGG TTAACACACTAAGCAAGCAC-3') and nuclease-free water to a final volume of 15µl. While, PCR amplification was performed in a final volume of 25µl of 2.5µl of cDNA, 2.5µl of 2.5mM of dNTPs, 2.5µl of 10X buffer, 2.5µl of 25mM MgCl2, 1µl of each forward primer (JLS90-46, 5'-AGCTAACCATGGTTAAGCTC ACTAAGGAAAGC-3') and reverse primer (JLS90-47) at 10µM, 0.2µl Taq DNA polymerase, and water. Mixtures were incubated for 2 min at 94°C, followed by 30 cycles of 30sec at 94°C, 30sec at 68°C, and 1min at 72°C, with a final incubation of 7min at 72°C, and then the reaction was held at 4°C. 5µl aliquots were separated by electrophoresis in 1% agarose gel in 0.5X TBE buffer using 100bp DNA ladder (Promega). The gel was visualized with UV illumination using Gel Documentation System (Gel Doc 2000, BioRad).

Production of proliferation clumps:

Shoot tips (1cm-long) of infected chrysanthemum plants (cv Zambla) were collected and short segments of each petiole were left attached to the stem covering the auxiliary bud. Shoots were sterilized with 2.5% sodium hypochlorite and cultured on Murgshige and Skoog (1962) medium supplemented with 0.5 and 1.5 mg/l Indole butyric acid (IBA) for starting and shooting, respectively. 0.4 mg/l Naphtalene acetic acid (NAA) for rooting and 3% sucrose (Min *et al.*, 2004). Shoots were subcultured every one month at 24±1°C for 16 h. at 5000 Lux photoperiod. Only active proliferating shoots were selected and transferred to fresh medium. The growing plantlets became ready for therapeutic treatments

Meristem tip culture:

Apical meristem tip (apical domes with 1-2 leaf primordia) were excised either from *in vivo* or highly proliferating materials in sterilized conditions and were then transferred to glass tubes containing 10 ml of the solid medium. Tubes were maintained in a culture room at 24±1°C in dark conditions for 3 days, then they were kept under standard illuminated conditions (Ram *et al.*, 2009).

Thermotherapy treatment:

Heat treatment was applied to eliminate TSWV from the infected plant material propagated *in vitro*. The highly proliferated shoots were placed in a growth chamber at 37°C and 38±1°C for 28 days at 16-h photoperiod under 5000 Lux. After the variable periods of heat treatment, the apical part of the axis, about 1-2 mm in length, was transferred to a fresh medium, then grown at 25°C.

Chemotherapy:

Antiviral substances:

Ribavirin (1-ß-D-ribofuransyl-1,2,4-triazole-3-carboxyamide "Virazole" and thiouracil (sigma)) were used at different concentrations, *i.e.* 10, 20, 30 mg/l, in order to determine the efficiency and appropriate concentration to eliminate TSWV .

Combined treatment:

Thermotherapy and chemotherapy combination was carried out by subjecting *in vitro* micro propagated shoots to 38°C for 21 days and transferred on medium containing 20mg/l Virazole. After 21 days, shoots were transferred onto antiviral free medium.

Plant regeneration:

The produced shoots at the end of each treatment were transferred onto multiplication MS medium and then placed onto rooting MS medium for root formation.

TSWV indexing:

The percentages of TSWV-free plants were indexed using ELISA test.

Results and Discussion

Symptomatology:

Naturally infected chrysanthemum plants grown in the Faculty of Agriculture, Cairo University, showing two types of symptoms. Firstly, necrotic local lesions followed by shot holes on the leaves. Secondly, the leaves often droop on the plants creating a wilt-like appearance, other symptoms include die-back of the growing tips, necrotic line patterns, stunting, dark streaking of the terminal stem and flower distortion (Fig. 1-A, B). Such collective symptoms have previously been described for TSWV infection on chrysanthemum (Hausbeck *et al.*, 1992; Sherman *et al.*, 1998; Balukiewicz *et al.*, 2005 and Bong *et al.*, 2006).

Virus isolation and propagation:

Artificially inoculated *C. amaranticolor* with sap from infected chrysanthemum plants gave positive reaction with induced antiserum specific to TSWV using indirect ELISA and dot blot technique (Fig. 6).It produced chlorotic and necrotic local lesion whereas, systemically chlorotic ring spot were induced on *G. globosa* which used as a source for virus replication. The isolated virus was identified on the basis of symptomatology, serology, molecular weight of coat protein and molecular detection.
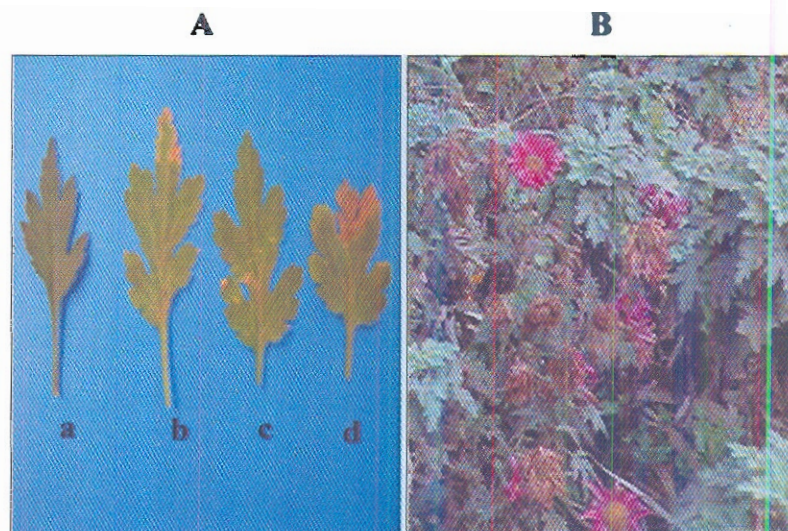
Fig. 1-A. Symptoms of TSWV naturally infected chrysanthemum plant in the field. (a), healthy chrysanthemum leaf; (b), tip necrosis. (c), necrotic local lesion; (d), dark coloured leaf necrosis.

Fig. 1-B. Severe symptoms of distortion on flowers of TSWV - infected chrysanthemum plants.

Cultivar susceptibility:

Data in Table (1) indicate that all the five tested chrysanthemum cvs. under greenhouse conditions proved to be susceptible to TSWV according to indirect ELISA test. The percentages of infection in different chrysanthemum cvs. were 70% for cv. Zambla and 30% for both cvs. Shining and Feling. Symptoms developed on mechanically infected chrysanthemum were identical to those observed on naturally infected plants. There was a wide variation of symptoms among cultivars as shown in Table (1) and Fig. (2). This result is in agreement with several investigators reporting that TSWV symptoms vary depending on the host plant, time of year, environmental conditions and cultivars (German *et al.*, 1992; Mumford *et al.*, 1996 and Balukiewicz *et al.*, 2005).

Virus purification:

The absorption spectrum of the purified TSWV was typical for nucleoprotein as determined by a «Spectrometer 2000» spectrophotometer with A_{max} at 260 and A_{min} at 245nm (Fig. 3). Furthermore, the 260/230 ratio of 1.20 is typical to several isolates of TSWV (Le, 1970 and Gibbs, 1985).

Molecular size determination of the N-protein:

Purified TSWV NP material, where denaturated with SDS and analyzed by PAGE, revealed one major polypeptide band (Fig. 4). The molecular weight of the N protein was estimated to be 29 KDa at dilution of 1/10, 1/20 and 1/40 partially purified. These results agree with those recorded by many researchers (Adam *et al.*, 1993; Feldhoff *et al.*, 1997; Kritzman *et al.*, 2000 and Balukiewicz *et al.*, 2005).

Table 1. Percentage of infection of different chrysanthemum cultivars upon mechanical inoculation with TSWV by visual inspection and indirect – ELISA

Chrysanthemum cultivar	Symptom	Ratio*	Infection (%)
Zambla	Dark coloured leaf necrosis, tip necrosis and stem necrosis	7/10	70
Shakira	Necrotic line pattern and vine necrosis	5/10	50
Maxx	Necrotic local lesion and stem necrosis	4/10	40
Shining	Necrotic local lesion followed by tip necrosis	3/10	30
Feling	Necrotic local lesion	3/10	30

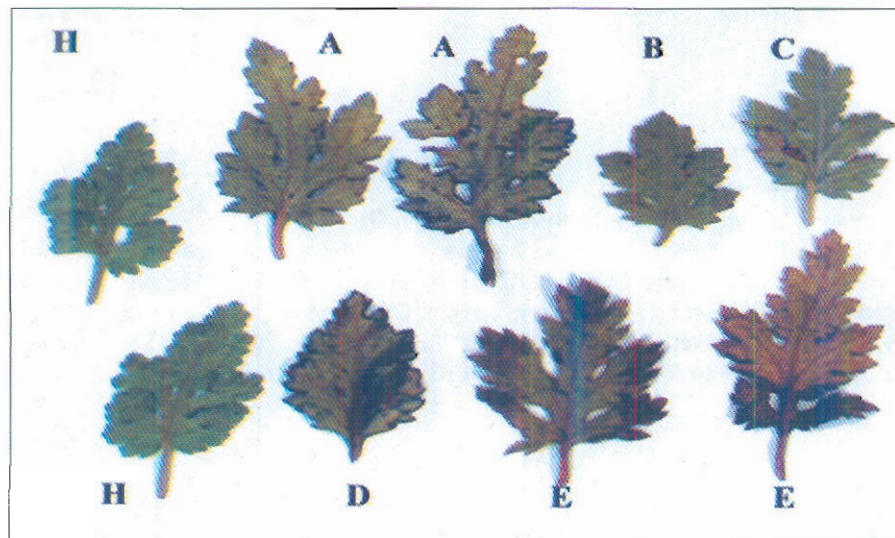* No. of infected plant / No. of tested ones.

Fig. 2. Symptoms of TSWV on artificially infected chrysanthemum cvs. Upon mechanical inoculation showing different types of necrotic local lesions. (A): cv. Maxx, (B): cv. Feling, (C): cv. Shining, (D): cv. Shakira, (E): cv. Zambla and (H): Healthy leaf.
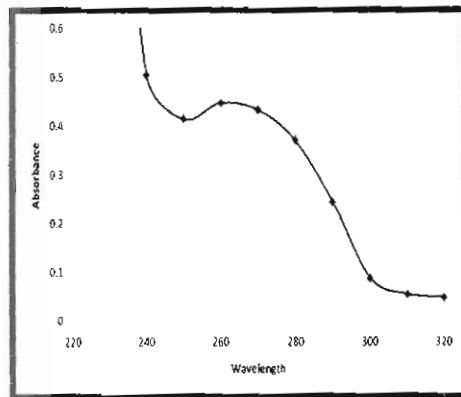
Fig. 3. Ultra-violet absorption spectrum of the partially purified TSWV preparation.
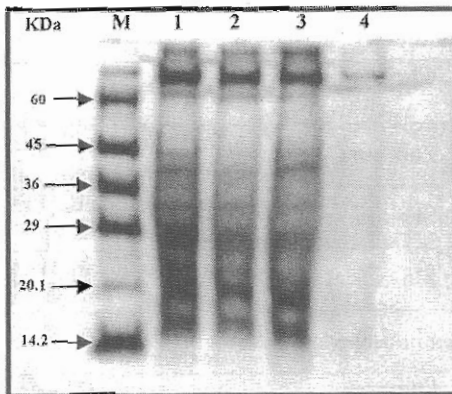
Fig. 4. SDS-PAGE showing similarities in coat protein subunit molecular weight of TSWV at different dilutions, (1): 1/10; (2): 1/20; (3): 1/40 of partially purified virus. Lane (4): buffer. (M): molecular weight marker (Sigma).

Extraction and purification of immunoglobulin (IgG):

Purification of IgG was done using caprylic acid method as described before. It has an A_{max} and A_{min} at 280 and 242nm, respectively (Fig. 5). The obtained UV spectrum indicate that IgG nature as a protein. On the other hand, positive reaction was obtained when purified IgG and IgG conjugated AP was tested for TSWV using direct ELISA at dilution of 1/1000 in both IgG and IgG conjugated AP.

Serological detection of TSWV:

TSWV was serologically reactive to the induced TSWV-antiserum using DBIA technique (Fig. 6). In addition, the induced antiserum detected the presence of TSWV in both naturally infected chrysanthemums in the field and in all chrysanthemum cvs. in the greenhouse using indirect ELISA test. Such results are confirmed by several authors applying serological tests for TSWV identification (Hsu and Lawson, 1991; Tamito *et al.*, 2003; Gera *et al.*, 2008 and Orosz *et al.*, 2008).

Sensitivity of indirect ELISA and IC-RT-PCR for the detection of TSWV:

The sensitivity of direct, indirect ELISA and IC-RT-PCR was studied by determining the dilution end point of the antigen. The obtained data indicated that, the dilution end point of TSWV in infected chrysanthemum extract was 1/1280 as determined by indirect ELISA using the produced antiserum against TSWV (Table 2), whereas, the dilution end point of TSWV in infected chrysanthemum extract was 1/2560 using direct ELISA at dilution of 1/1000 in both IgG and IgG
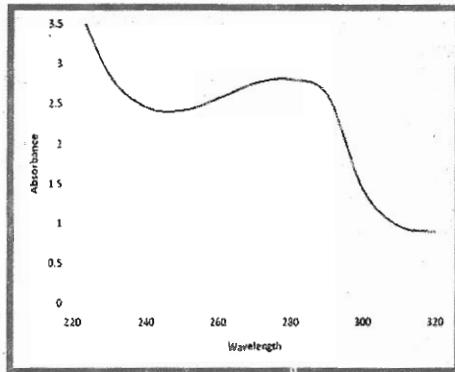
Fig. 5. Ultra-violet absorption spectrum of the IgG fraction of TSWV antiserum after separation with caprylic acid.
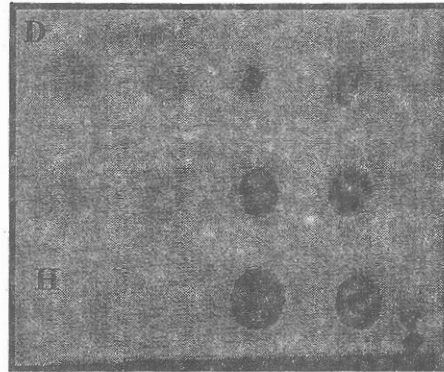
Fig. 6. Detection of TSWV by dot blot immunobinding assay (DBIA) on nitrocellulose membrane. D: diseased and H: Healthy plant.

Table 2. Determination of TSWV dilution end point by indirect ELISA using the induced antiserum

Dilution of tissue extract	Indirect – ELISA reading (A_{405} values) in: *	
	Infected plant	Healthy plant
1/5	1.203	0.240
1/10	1.139	0.237
1/20	0.993	0.196
1/40	0.699	0.190
1/80	0.626	0.175
1/160	0.606	0.173
1/320	0.595	0.170
1/640	0.576	0.150
1/1280	0.239	0.149
1/2560	0.237	0.146

* Reading after one hour of incubation with the substrate. Reading greater than twice the A_{405} value of healthy control was considered positive.

conjugate (Table 3). Results in Table (2 and 3) indicated that the higher of ELISA values for antigen dilution using direct ELISA than indirect ELISA values for the same antigen dilution revert to the sensitivity of direct ELISA than indirect ELISA technique. On the other hand, the IC-RT-PCR successfully amplified DNA of the extracted size from TSWV infected chrysanthemum plant but not for the healthy control plant. Gel electrophoretic analysis of IC-RT-PCR product obtained from TSWV infected chrysanthemum leaves showed a fragment with the expected size for amplification (760 Pb) as described by Pang et al. (1992), Balukiewicz et al. (2005) and Bong et al. (2006). This fragment observed in sap dilutions 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} (Fig. 7). No PCR product was obtained from uninfected control samples (Lane 7).

Table 3. **Determination of TSWV dilution end point by direct ELISA using the produced IgG and IgG Conjugate**

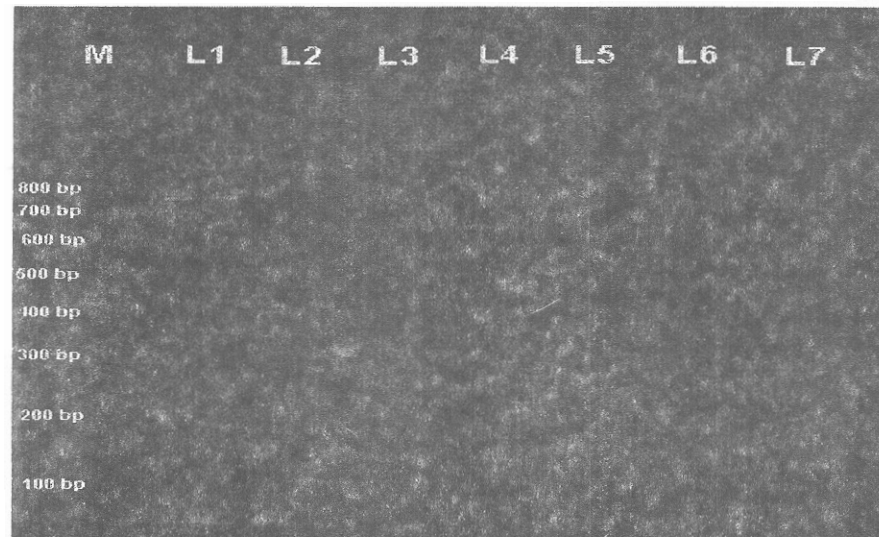| Dilution of tissue extract	Direct – ELISA reading (A_{405} values) in: *	
	Infected plant	Healthy plant
1/5	2.003	0.360
1/10	1.818	0.354
1/20	1.761	0.337
1/40	1.500	0.321
1/80	1.111	0.284
1/160	0.771	0.283
1/320	0.677	0.276
1/640	0.637	0.258
1/1280	0.576	0.255
1/2560	0.505	0.254

* As described in footnote of Table (2).

Fig. 7. **Agarose gel electrophoresis analysis of IC/RT-PCR amplified products. M: 100 bp DNA ladder (Promega), from L1 to L6: serial dilutions (10^0-10^{-5}) of antigen (infectious crude sap) extracted, and L7: healthy plant (-ve control).**

Results in Tables (2 and 3) and Fig. (5) indicate that IC-RT-PCR is more sensitive than direct and indirect ELISA and would be used for detection of a small quantity of viral DNA in plant tissues.

Shoot survival and virus elimination

According to the literature, meristem culture is considered to be the reference tool for virus disease eradication (Faccidi and Marrani, 1998) and (Ram *et al.*, 2009). The application of meristem tip culture to eradicate viral infection is based on the meristem immunity towards virus (Kumar *et al.*, 2009). Data presented in Table (4) showed that meristem tip culture gave 20% shoot survival and produced 10% (5 plants out of 50 tested) free of TSWV. The low percentage of TSWV eradication confirms previous results obtained by Nehra and Kartha, (1994) who observed positive ELISA results of plants regenerated after meristem culture. Concerning thermotherapy, the percentage of TSWV-free plants were 20 and 30% in case of exposure to 37°C and 38°C/4 weeks, respectively. This result had already been reported by Horvath *et al.* (2006) and Diaz-Barrita *et al.* (2008) as they mentioned that the heat therapy can be considered as a good method to eliminate certain viruses that are difficult to eradicate by other means such as meristem culture.

Table 4. Effect of meristem tip culture, thermotherapy and chemotherapy on virus elimination and shoot survival

Treatment *	Shoot survival (%)	Virus elimination (%)
Meristem tip	20	10
Thermotherapy		
37°C/4weeks	100	20
38°C/4weeks	93	30
Chemotherapy		
Virazole : 10 mg/l	89	6
20 mg/l	79	19
30 mg/l	28	30
Thiouracil: 10 mg/l	85	3
20 mg/l	60	10
30 mg/l	25	18.5
Combined treatment		
38°C/21days	77	53
+ 20mg/l Virazole		

* 20 plantlets / treatment.

Chemotherapy is an alternative *in vitro* technique traditionally used for virus eradication (Sharma *et al.,* 2007). Addition of Virazole or thiouracil at concentrations of 10, 20 and 30 mg/l increased the percentages of TSWV-free chrysanthemum plantlets to 6, 19, 30 and 3, 10 and 18.5%, respectively. Increasing concentrations of chemical substances decreased shoot survival percentages. In combination between thermotherapy (38°C/21 days) and Virazole at a concentration of 20mg/l produced 53% of TSWV-free plantlets out of 77% survived shoots. Such results are similar to those reported for the combination of different therapeutic operations and tissue culture technique that have been shown to be a more difficult method than the use of thermotherapy or meristem tip alone as reported for chrysanthemum and sour cherries (Wang *et al.*, 2008).

In conclusion, meristem tip and chemotherapy are not efficient techniques for TSWV eradication from chrysanthemum. whereas combination of chemotherapy and thermotherapy seemed to be more successful in the context of germplasm preservation and international diffusion. However, the thermotherapy is time consuming and the survival plantlets of the treated plantlets is high. Therefore, combination of different therapeutic operations and tissue culture proved to be an attractive alternative method for virus eradication.

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دراسات بيولوجية ، بيوكيميائية ، سيرولوجية ، جزيئية
وزراعة الانسجة على عزلة مصرية لفيروس الذبول المنقط
في الطماطم التي تصيب نباتات الأراولا

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تم عزل فيروس الذبول المنقط في الطماطم من نباتات الأراولا المصابة
طبيعياً بالفيروس والمزروعة في مشاتل الزينة بالمزرعة التجريبية بكلية الزراعة
– جامعة القاهرة. وقد تم تعريف الفيروس باستخدام الطرق السيرولوجية
المختلفة مثل طريقة الإليزا غير المباشرة واختبار الارتباط المناعي على أغشية
النيتروسليلوز باستخدام الانتيسيرم المنتج والمتخصص للفيروس. والأعراض
الظاهرية لتلك الفيروس تتمثل في بقع صفراء وبقع ميتة على الأوراق قد تأخذ
مظهر الطرز الخطي داكنة اللون قد تمتد إلى الساق بالإضافة إلى موت القمة
الطرفية أو حواف الأوراق وتقزم النباتات وذبولها وتشوه الأزهار. وقد أختبرت
حساسية خمسة أصناف من الأراولا للإصابة بالفيروس و تبين من اختبار النقل
الميكانيكي حساسية جميع الأصناف المختبرة للفيروس وخاصة صنف زامبلا
الذي استخدم في هذه الدراسة لإنتاج نباتات خالية من الإصابة
الفيروسية عن طريق زراعة الانسجة. وقد تم تنقية الفيروس من
نباتات *Gomphrena globosa* المصابة ميكانيكيا ووجد أن جزيئات الفيروس
الناتجة لها أعلى إمتصاص وأدنى إمتصاص عند طول موجى A_{260} و A_{245}
نانوميتر على التوالي ، ووجد أن نسبة الامتصاص عند ٢٨٠/٢٦٠ نانوميتر
تساوي ١،٢. كذلك وجد أن وحدات الغلاف البروتيني للفيروس تساوي ٢٩ كيلو
دالتون. وقد تم فصل الأمينوجلوبيولين من الانتيسيرم المنتج وقد أعطى أعلى
امتصاص وأدنى امتصاص عند طول موجي ٢٨٠ ، ٢٤٢ نانوميتر على التوالي
وبينت دراسة البيولوجيا الجزيئية باستخدام تفاعل عديد البلمرة المتسلسل المعتمد
على اتحاد الفيروس المختبر بالأجسام المضادة المناعية المنممصة
(IC- RT- PCR) على بلمرة 760 bp باستخدام بادئات متخصصة للفيروس
(JLS90-46) و (JLS90-47). وجد أن نقطة التخفيف النهائية لعصير
الأوراق المصابة بفيروس الذبول المنقط للطماطم هي ١٢٨٠/١ ، ٢٥٦٠/١ ،
٥٠٠٠/١ باستخدام طـريقة الإليـزا غيـر المباشـرة – الإليزا المباشرة
وIC-RT- PCR ، مما يوضح حساسية اختبار IC-RT- PCR بالمقارنة بكل
من طريقة الإليزا المباشرة وغير المباشرة. وفي هذه الدراسة تم استخدام كلا من
العلاج الحراري والعلاج الكيميائي للأجزاء النباتية (القمة النامية) المأخوذة من
نباتات أراولا مصابة صنف (زامبلا) لإنتاج نباتات خالية من الإصابة الفيروسية
باستخدام زراعة الانسجة وقد وجد أن استخدام المعاملات الحرارية عند ٣٨°م
لمدة ٢٨ يوم فقط أو بالإضافة إلى استخدام مضادات الفيروس مثل الفيرازول
بتركيز ١٠-٢٠ مليجرام/لتر يمكن الحصول على نباتات سليمة بنسبة تتراوح ما
بين ٥٣-٧٧%.