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Full Length Article

Occurrence of Tomato Spotted Wilt Virus in Lettuce in Cukurova Region of Turkey

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ABSTRACT

Surveys were conducted to detect and determine the incidence of *Tomato spotted wilt virus* (TSWV) in lettuce plants by serological and molecular assays in the winter of 2007 and 2008. A total area of about more than 400.000 plants was monitored in 31 fields in Adana and Mersin provinces. Of these, 336 showing virus-like symptoms were tested using DAS-ELISA for the presence of TSWV infection and ELISA positive lettuce samples were confirmed by RT-PCR using TSWV S RNA gene specific primer pairs. Based on these results, the incidence of TSWV in tested lettuce samples was found to be 1.2%.

Key Words: Thrips; Virus transmission; TSWV RNA S; ELISA; RT-PCR

INTRODUCTION

Lettuce (*Lactuca sativa*), belongs to the family *Compositae*, is an important crop in Cukurova region (especially in Adana & Mersin provinces) located in the eastern part of Mediterranean region of Turkey. In 2004, the total production of lettuce in Turkey was 390,659 tons per year and Cukurova region supplies 25% of total production (Anonymous, 2006).

Tomato spotted wilt virus (TSWV, genus *Tospovirus*, family *Bunyaviridae*) is one of the most widely spread plant viruses and causal agent of economically important yield losses in many crops. It has a broad host range, with more than 900 plant species (Peters, 1998) occurring in ornamental, vegetable (tomato, pepper, lettuce & etc.) and weed hosts. TSWV is transmitted by thrips in a propagative-persistent manner (Wijkamp *et al*., 1993) and western flower thrips (*Frankliniella occidentalis*) was reported as the most important vector (Antignus *et al*., 1997).

TSWV infection is previously reported on tobacco (Azeri, 1981), tomato (Tekinel, 1973; Azeri, 1994) and pepper (Yurtmen, 1998) growing areas in Turkey. To date, there is only one study on TSWV infection in lettuce in Cukurova region that involved symptomatological observation and sap-inoculation tests (Tekinel *et.al*., 1969).

Apart from this data, there is no further information about TSWV incidence in lettuce fields in Cukurova region. The objective of this study was to detect TSWV and determine its incidence in lettuce plants produced in Cukurova region by serological and molecular methods.

MATERIAL AND METHODS

Surveys and sample collection: Surveys were conducted in randomly selected lettuce growing areas in Adana and Mersin provinces of Cukurova region in the winter of 2007 and 2008 (Fig. 1). Samples were collected from symptomatic plants showing general virus symptoms, such as mosaic, deformation and crinkling as well as typical TSWV symptoms such as distorted leaves, vein necrosis, yellowing, brown spotting and wilting. Leaf samples, from each plant put into plastic bags and labeled. The samples were brought to the laboratory by placing on ice and stored at -20ºC until tested.

Serological and molecular tests: Virus detection on lettuce was done by serological and molecular tests and all tests were replicated three times.

DAS-ELISA: Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) tests were carried out as described by Gonsalves and Trujillo (1986) and standart procedure of the antisera's manufacturer (Art. No. 190165, Bioreba, Switzerland).

Polystyrene plates (Nunc Maxisorp) were first coated with TSWV spesific polyclonal antisera diluted in carbonate buffer (pH: 9.6) and incubated for 4 h at 30ºC. After washing the plates with PBST buffer, samples ground in sample extraction buffer (1 g tissue/10 mL buffer) were added to wells in duplicate and incubated at 4ºC overnight. Alkaline phosphatase conjugated antibody diluted in conjugate buffer was coated after washing the plates and incubated for 4 h at 30ºC. Finally, p-nitrophenyl phosphate in diethanolamine substrate buffer; (pH: 9.8) was added and A405 nm values were measured at a microplate reader

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(MEDISPEC ESR-200) after 60-120 min.

Samples were considered TSWV infected if their absorbans values were greater than at least two times that of healthy control (Wang & Gonsalves, 1990). Virus free plants were served as negative control.

Mechanical inoculation: TSWV isolates from infected lettuce plants were maintained in the greenhouse in *Nicotiana rustica* for RT-PCR studies. Four *N. rustica* plants were inoculated per isolate and four uninoculated plants served as control. All plants were investigated for symptom expression for 7-14 days after inoculation. All inoculated plants were tested for TSWV infection by DAS-ELISA.

For sap inoculation, leaf tissues from separate lettuce plants were triturated with mortar and pestle in cold 0.1 M potassium phosphate buffer, pH 7.0, containing 0.01% sodium sulfite (1 g leaf/10 mL buffer) and the homogenate is rubbed immediately onto carborundum dusted leaves of indicator plants in the 3-5 leaf stage using sterile glass rod. After mechanical inoculation the inoculated leaves were sprayed with top water to remove inoculum and to reduce excessive evaporation.

Total RNA extraction: Total RNA was extracted from fresh leaves of TSWV infected *N. rustica* plants according to Astruc *et al*. (1996). Plant materials were homogenized (1 g tissue/2 mL buffer) with mortar and pestle by addition of extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA, 500 mM sodium chloride & 0.1% 2-mercapthoethanol) and centrifuged at low speed in 1.5 mL eppendorf tubes. A total of 50 µL 20% SDS was added on supernatant and incubated at 65ºC for 30 min. Then, 250 µL of 6 M potassium acetate (pH 6.5) was added and tubes transferred on ice for 20 min. After centrifugation at 13,000 rpm for 15 min, nucleic acids were precipitated with ethanol. The pellet was resuspended in 50 µL RNAse-free sterile water.

RT-PCR: RT-PCR reactions were performed in a Techne TC 4000 thermal cycler (Techne, Cambridge, UK) using TSWV-spesific primers CP5- Bam (5'-ACA ACT TTT AGG ATC CTC ATG TCT AAG GTT- 3') complementary to nucleotides 2783-2755 of the S RNA and CP3-Pst (5'-AAC CTG CAG CTG CTT TCA AGC AAG TTC- 3'), corresponding to nucleotides 1972–1995 for the amplication of 811 bp S RNA gene (Antignus *et al*., 1997).

The complementary DNA (cDNA) strands of a portion of S RNA were synthesized by mixing 1 µL of total RNA, 1 µL reverse primer (10 mM) and 10 µL of sterile water followed by heating the mixture at 70ºC for 2 min and cooling for 90 sec. To this mixture, 5 µL of reverse transcriptase buffer (5X), 1 unit of *Moloney murine leukemia virus* reverse transcriptase (MMLV-RT; Fermentas), 1 unit of RNAse inhibitor (RNAsin; Fermentas), 0.3 µL of dNTPs (25 mM) and 10 µL of sterile water were added and incubated at 42ºC for 60 min.

PCR was accomplished by adding 4 µL of *Taq* DNA polymerase buffer (Fermentas), 1 µL of dNTPs (25 mM), 2.5 units of *Taq* polymerase (Fermentas), 10 ng/µL of each primer, 16.8 µL of DNAse free distilled water and 1 µL of the cDNA. PCR program consisted of 2 min at 94ºC, 35 cycles of 30 sec of at 94ºC, 30 sec at 53ºC and 2 min at 72ºC. Finally, the amplified DNA was incubated at 72ºC for 10 min to accomplish a final extension. PCR products were separated by electrophoresis on a 1% agarose gel (Weekes *et al*., 1996; Mumford *et al*., 1996).

RESULTS

The survey and sample collection were carried out in a total of 910 da in 31 mainly lettuce growing fields (4.500 plants/da, a total of ~4.095.000 plants) in 18 villages in Adana (24 fields in 15 villages) and Mersin (7 fields in 3 villages) provinces of Cukurova, Turkey in the winter of 2007 and 2008. More than 400.000 plants (10% of the area surveyed) were monitored in 31 fields (Table I). Of these, 336 showing virus-like symptoms such as mosaic, deformation and crinkling as well as typical TSWV symptoms such as distorted leaves, vein necrosis, yellowing, brown spotting and wilting were tested using DAS-ELISA with polyclonal antisera.

The TSWV infection was detected in four lettuce plants in 2 fields in 2 villages by means of DAS-ELISA, (Günyurdu & B. Dikili) in Adana province, while not found in the samples from Mersin province. The rate of incidence of TSWV in lettuce was found as 1.5% in Adana province, whereas it was 1.2% in overall collected samples both in Adana and Mersin. In ELISA tests, positive samples produced A405nm readings above buffer controls of around 1.5 to 2.0, whereas negative controls gave A405nm readings of 0.096 to 0.141.

A portion of S RNA of TSWV was amplified by RT-PCR with virus specific primers, CP5-Bam and CP3-Pst, by using total RNA extracts from symptomatic plants to confirm the presence of TSWV infection in DAS-ELISA positive samples. The expected fragment size of 811 bp was observed after electrophoresis of PCR products in 1% agarose gel (Fig. 2). The results of RT-PCR were in complete agreement with DAS-ELISA results.

DISCUSSION

Lettuce is one of the most important crops growing in winter and early spring in Cukurova region of Turkey. Our surveys were conducted in lettuce fields close to main pepper and tomato growing areas during the late spring and summer in Adana and Mersin provinces. In our previous study, it has been shown that TSWV is widespread agent and becoming a key disease in the field-and greenhouse-grown peppers and tomatoes in Cukurova region (unpublished data). In present study, TSWV incidence was found to be low throughout lettuce growing areas in the same region. We assume that this is because of the low population of TSWV vectors, *Frankliniella occidentalis* and *Thrips tabaci* L. in winter and early spring time on lettuce in

Table I: Numbers of fields surveyed and samples collected in each district/village

Districts, villages	No. of field surveyed	Total area surveyed (da)	No. of samples collected	No. of samples TSWV positive
Adana Province				
Seyhan	1	60	18	0
Ceyhan	2	39	13	0
Yakapınar	2	83	31	0
Günyurdu	3	200	65	2
B. Dikili	2	25	8	2
Baklalı	1	55	18	0
Alihocalı	1	8	3	0
Herekli	2	20	14	0
Avluk	2	30	10	0
Karakuyu	1	10	5	0
Şambayat	1	10	3	0
Hadırlı	1	10	5	0
Cabbardede	1	50	17	0
Yumurtalık	2	80	26	0
Zeytinli	2	150	57	
Mersin Province				
Yeşiltepe	2	25	14	0
Arpaç	2	20	12	0
Tarsus	3	35	17	0
TOTAL	31	910	336	4

Fig. 1: Map of Adana and Mersin provinces showing villages in which surveys were conducted. Map in the corner indicates the location of Adana and Mersin provinces in Turkey. Adana province: 1.Seyhan, 2. Ceyhan, 3. Yakapinar, 4. Gunyurdu, 5. B. Dikili, 6. Baklali, 7. Alihocali, 8. Herekli, 9. Avluk, 10. Karakuyu, 11. Sambayat, 12. Hadirli, 13. Cabbardede, 14. Yumurtalik, 15. Zeytinli; Mersin province: 16. Yesiltepe, 17. Arpac, 18. Tarsus

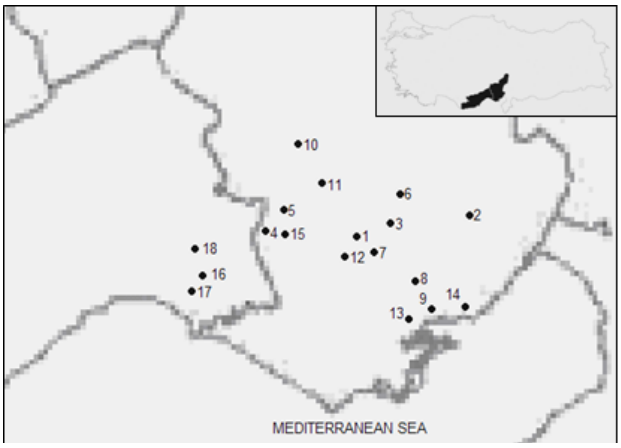
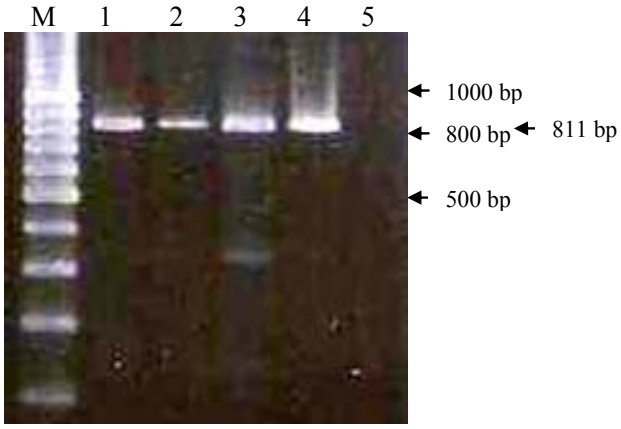


Fig. 2: Detection of TSWV infection in lettuce plants by reverse transcription-polymerase chain reaction (RT-PCR). (Lanes 1, 2, 3 and 4, symptomatic lettuce plants; lane 5, healthy lettuce plant) amplified by RT-PCR with virus specific primers, analyzed by electrophoresis on a 1% agarose gel and stained with ethidium bromide. Lane M, contains marker with sizes in base pair (100 bp DNA marker, Fermentas) indicated to the left of the gel



the region. Our observations indicated that pepper and tomato plantations were highly infected with TSWV in late spring and summer, but winter time there was no high incidences of thrips and TSWV in tomato and pepper as well as lettuce in Cukurova region. The virus becomes more prevalent when thrips populations start to increase due to temperature rise in spring time. These observations suggest that there are possible correlations between mean number of thrips and TSWV incidence in lettuce grown in winter, rainfall, minimum and maximum temperature (Cho *et al.*, 1987).

Atakan and Sarı (2010) reported that low number of *F. occidentalis* and *T. tabaci* were detected on lettuce during spring and winter time (October-March) in the Cukurova region. The same report indicated that, all of the collected thrips species were female and no larval thrips belonging to either thrips species were collected from lettuce fields sampled. Previous studies showed that there were some differences on vector competency between the female and the male populations of *F. occidentalis* and the male population transmitted TSWV at a greater rate than the females (Rotenberg *et al.*, 2009). Van de Wetering *et al.* (1998) reported that ratio of females in the thrips population is negativelly effective on damage of plants and virus transmission and consequently spread of TSWV due to lower mobility and higher consumption rate of females than males. Low thrips poupulation and the presence of mainly female thrips in winter and early spring could be reason of the low TSWV incidence in lettuce plants in Cukurova region.

Wilson (1998) reported that the relative percentage of TSWV infected lettuce plants varied with location on the farm and with season, increasing incidence of infections observed in late summer and early autumn have resulted in 25-65% losses during autumn harvests in southern Tasmania.

Moreno *et al.* (2004) reported that TSWV is one of the most prevalent viruses in lettuce and TSWV and BWYV caused the major virus problems in lettuce fields in the Murcia region in Spain. Their surveys also showed that

virus epidemics were much more common in autumn than in spring and it was related to the lack of virus reservoirs.

In this study, TSWV was detected and its incidence was determined in lettuce plants produced in Cukurova region by serological and molecular methods. Further studies should be initiated to better understand the epidemiology of TSWV in order to develop feasible control recommendations. The population dynamics of thrips in economically important susceptible crops should be monitored during and after the growing season for the region. Distribution of reservoir plants for the virus and insect vectors, efficiency of transmission from these hosts to cultivated crops, determination and characterization of Turkish isolates of TSWV from different locations needs to be investigated in Turkey.

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