http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (9): 707-711, 2009
ISSN 1028-8880

Tobacco streak virus Isolated from Lettuce

[1]F.S. Abtahi and [2]M. Khodai Motlagh
[1]Department of Plant Protection, Collage of Agriculture, University of Tehran,
Karaj, Islamic Republic of Iran
[2]Faculty of Agriculture, University of Arak, Islamic Republic of Iran

Abstract: *Tobacco streak virus* (TSV) is an ilarvirus with a worldwide distribution. This virus infects many plants and causes significant yield losses. In this study, 300 samples of lettuce were collected from lettuce fields in Tehran Province. Infected plants show symptoms such as: mosaic, vein clearing, vein necrosis, yellowing and leaf distortion. DAS-ELISA (Double Antibody Sandwich-ELISA) was used with a polyclonal antiserum against TSV. Five isolates (T_1, T_2, T_3, T_4 and T_5), which are collected, respectively from Mohammad Abad (Karaj), Malek Abad (Karaj), Hashtgerd (Karaj), Tarand Balla (Varamin) and Deh mah sin (Pishva) were inoculated on 29 species of Cucurbitaceae, Amaranthaceae, Solanacea, Compositae, Leguminosae and Chenopodiacea. *Chenopodium quinoa* 6 days after inoculation showed necrotic local lesions. *Gomphrena globosa* 10 days after inoculation developed chlorotic local lesions. Systemic symptoms were produced in *Datura stramonium*. *Phaseolus vulgaris* cv. Red Kidney 5 days after inoculation developed necrotic local lesions. *Nicotiana tabacum* 7 days after inoculation showed necrotic and chlorotic local lesions. *Nicotiana clevelandii* 15 days after inoculation developed leaf distortion and vein necrosis. *Lactuca sativa* 10-15 days after inoculation developed leaf istortion and mosaic. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed using one primer pairs designed by DSMZ. An approximately 710 bp fragment was amplified with a specific primer.

Key words: ELISA, RT-PCR, TSV

INTRODUCTION

Tobacco streak virus (TSV), a ilarvirus with a world wide distribution, was first discovered in tobacco (*Nicotiana tabaccum*), in Brazil in 1940 (Costa, 1945). Over the 30 years, TSV was reported to cause economical important diseases on cotton, tomato, tobacco, soybean, peanut, sunflower and some weeds in Brazil (Gracia and Feldman, 1974). That was also found on strawberry, dahlia, various weeds species, sunflower, cotton, mung bean, chickpea and tobacco in Astralia (Fagbenle and Ford, 1970; Greber *et al*., 1991; Sharman *et al*., 2008). In India, TSV induced necrosis disease has caused serious economic losses (Bhat *et al*., 2001, 2002a-c). Kaiser *et al*. (1991) reported TSV naturally infecting chickpea growing adjacent to plots of inoculated plants in the United State. The TSV was isolated from lettuce in 1992 (McDaniel *et al*., 1992). TSV belongs to the genuse ilarvirus in the Bromoviridae. Particles are quasi- isometric and of three major size 27, 30 and 35 nm in diameter. The genome is segmented, tripartite (segmented are distribute among 3 particle types of different size) and consist of four segments of linear, positive-sense, single-stranded RNA. The encapsidate nucleic acid is mainly of genomic origin, but virions may also contain subgenomic RNA. The RNA-1 is partially sequence and sequenced region in 294 nucleotides long. RNA-2 is fully sequenced, complete sequence is 2770 nucleotides long and has the accession No. RNA-4 is subgenomic has been sequenced, but only an estimate is provided, complete sequence is 881 nucleotides long. The RNAs 1 and 2 encode proteins involved in viral RNA replication, whereas RNA-3 encodes a protein required for cell-to-cell movement. The viral Coat Protein (CP) is expressed by a subgenomic RNA, designated RNA 4, collinear with the 3' end of RNA 3 (Bol, 1999). Studies on ilarviruses revealed that in addition to functioning as a structural protein, the CP is also involved in many steps of virus replication (Prasada Rao *et al*., 2003). In this study, we present some biological and molecular characteristics of an Iranian isolate of TSV from lettuce, as well as a phylogenetic analysis of the CP gene compared with other isolates of TSV.

Corresponding Author: Faeze Sadat Abtahi, Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran Tel: +98-913-333-945 Fax:+98-331-2627392

MATERIALS AND METHODS

Sample collection: Samples were collected during the 2007 and 2008 growing season from Lettuce (*Lactuca sativa*) field-grown in Tehran Province. In this region, Lettuce are planted during early April and harvested from June to July. Virus infections become visible after the setting of the first leafs. Infected plants show symptoms such as: mosaic, yellowing, leaf distortion and yield reduction. Young leaves from some symptomatic plants were collected at random. All samples were kept in ice chests for transportation to the laboratory. Each plant sample was kept separately in a plastic bag at 4°C until analyzed.

Virus identification: DAS-ELISA (Double Antibody Sandwich-ELISA) as described by Clark and Adams (1977) was used with a polyclonal antiserum against TSV (DSMZ-AS0913). A 200 µL aliquot of IgG was added to coat each well of plates. Each step of ELISA was followed by a 4 h incubation at 37°C or a 12 h incubation at 4°C. This was followed by three washes with a washing buffer. Ten milliliters of sample buffer, pH 7.4, was added to 1 g tissue samples that had been ground in liquid nitrogen and 200 µL of this extracted was added to each well. The reaction was read using a colorimeter at 405 nm after adding conjugate incubation with substrate for about 1 h.

Host range studies: Five isolates (T_1, T_2, T_3, T_4 and T_5), from infected lettuce plants in the host range studied, which is collected from Mohammad Abad (Karaj), Malek Abad (Karaj), Hashtgerd (Karaj), Tarand balla (Varamin) and Deh mah sin (Pishva), respectively, were maintained in *Chenopodium quinoa* by sap inoculation. For plant assays, 29 species from 6 families were inoculated with the virus isolates. Sap prepared from leaves which were in 0.01 M sodium phosphate buffer, pH 7, was rubbed onto leaves dusted with carborundum powder. The leaves were then rinsed with water and plants were maintained in an insect-proof screen house for observation, symptoms on both inoculated and upper, uninoculated leaves were recorded. Tests for latent infection were conducted by back-inoculation to *C. quinoa* Wild.

Reverse transcription polymerase chain reaction: Viral preparations were treated with proteinase K and RNA was extracted using an RNAeasy clean-up protocol (Qiagen, Hilden, Germany) comprising chromatography on silica columns with on-column DNAse I digestion. To generate the first sequence information, the coat protein gene was amplified by using the Superscript™ III One-Step RT-PCR System with Platinum® Taq DNA polymerase. One microliter of RNA were submitted to reverse transcription in a final volume of 50 µL, using 1 µL (100 pmol μL^{-1}) of primer TSV CP RNA3 express1 (5'-AGG TAG CAG GAG ATA TAA CAA TGA ATA CTT TGA TCC AAG G-3'), 1 µL (100 pmol μL^{-1}) of primer TSV CP RNA3 express 2 (5' -TCG ACT CTA GAA ACT AGT CTT GAT TCA CCA GAA AAT CTT C-3') 1 µL of RT Platinum Taq HiFi enzyme mix, 25 µL of reaction mix buffer and 21 µL of deionized H_2O_3. The reaction mixture was heated to 50°C for 30 min, 95°C for 3 min, submitted to 30 cycles at 95°C for 1 min, 60°C for 1 min, 68°C for 2 min and finally heated to 68°C for 10 min. PCR products were controlled by electrophoresis on 1% agarose gel.

RESULTS

Host range: The virus was isolated from infected lettuce species from the families Cucurbitaceae, Amaranthaceae, Solanacea, Compositae, Leguminosae and Chenopodiacea but it did not infect the numbers of Compositae and Cucurbitaceae that were tested. *Chenopodium quinoa* 6 days after inoculation showed necrotic local lesions (Fig. 1). *Gomphrena globosa* 10 days after inoculation developed chlorotic local lesions. Systemic symptoms were produced in *Datura stramonium* (Fig. 2). *Phaseolus*

Fig. 1: Symptoms of necrotic local lesions of *Tobacco streak virus* isolated from lettuce in Iran on *Chenopodium quinoa*

Fig. 2: Systemic symptoms of *Tobacco streak virus* isolated from lettuce in Iran on *Datura stramonium*

Fig. 3: Symptoms necrotic local lesions of *Tobacco streak virus* isolated from lettuce in Iran on *Phaseolus vulgaris* cv. Red Kidney

Fig. 4: Necrotic and chlorotic local lesions symptoms of *Tobacco streak virus* isolated from lettuce in Iran on *Nicotiana tabacum* cv. samsun

Fig. 5: Systemic leaf distortion and vein necrosis symptoms induced by *Tobacco streak virus* isolated from lettuce in Iran on *Nicotiana clevelandii*

vulgaris cv. Red Kidney five days after inoculation developed necrotic local lesions (Fig. 3). *Nicotiana tabacum* cv. *samsun* 7 days after inoculation showed necrotic and chlorotic local lesions and it gave also systemic necrotic lines patterns after several weeks of

Fig. 6: Symptoms of leaf deformation induced by *Tobacco streak virus* isolated from lettuce in Iran on *Lactuca sativa*

Table 1: Reaction of selected indicator plant species to *Tobacco streak virus*

Families	Test plants	Symptoms in leaves
Cucurbitaceae	*C. pepo* cv. Khoy	-
	C. sativus cv. Dominus	-
	Gomphrena globosa	cll
Amaranthaceae	*Amaranthus paniculatus*	-
	Chenopodium quinoa	nll
Chenopodiaceae	*C. amaranticolor*	-
	Spinacia oleraceae cv. Keshtzar	-
Leguminoseae	*P.vulgaris* cv. Red Kidney	nll
	P. vulgaris cv. Bountiful	-
	Vigna unguiculata	-
	Vicia faba	-
	Pisum sativum	-
Solanaceae	*Dautura stramonium*	S
	Nicotiana tabacum var. *samsun*	nll
	N. glutinosa	-
	N. benthamiana	-
	N. rustica	-
	N. cocker	-
	N. clevelandi	Id, vn
	Petunia hybrid grandiflora	-
	Physalis alkekengi	
Compositae	*Lactuca sativa*	s
	L. sativa cv. trocodera	-
	L. sativa cv. montillia	-
	L. sativa cv *salinas*	-
	Zinnia elegans	-
	Dahlia pinnata	-
Papilionaceae	*Cicer arietinum*	-
Umbeliferaceae	*Beta vulgaris* L. subsp. *esculenta* (Salisib). Gurke var. *altissima*	-

cll: Chlorotic local lesion, ns: No symptom, nll: Necrotic local lesion, S: Systemic symptom, Vn: Vein necrosis and id: Leaf distortion

inoculations (Fig. 4). *Nicotiana clevelandii* 15 days after inoculation developed leaf distortion and vein necrosis (Fig. 5) and *Lactuca sativa* 10-15 days after inoculation developed leaf distortion and mosaic (Fig. 6, Table 1).

RT-PCR: RT-PCR was carried out using the primers TSV CP RNA3 express1/TSV CP RNA3 express 2 (described previously) which resulted in a fragment of 710 nts (Fig. 7).

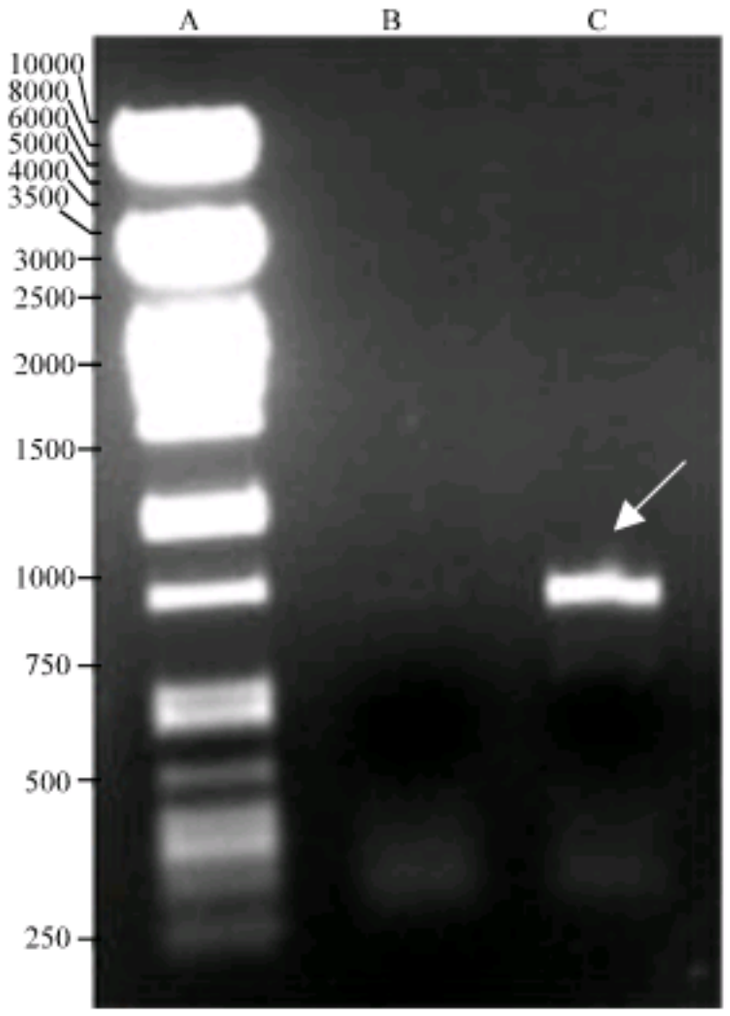

Fig. 7: One percent agarose gel electrophoresis analysis of RT-PCR products amplified with TSV CP RNA3 express1/ TSV CP RNA3 express 2 primer pair. Lane A: Ladder, Lane B: Healthy extract, Lane C: TSVLIR

DISCUSSION

The virus analyzed in these studies shows the biological and molecular properties similar to those described for TSV (Gracia and Feldman, 1974). Therefore, the isolate of TSV is one of the agents which causes the loss of lettuce yield that occurred in Tehran Province.The host range data are in agreement with several reports for TSV (Costa, 1945; Kaiser *et al*., 1982, 1991; Krishnareddy *et al*., 2003; Regenmortel *et al*., 1997) despite the lack of infection of *Amaranthus paniculatus*, *C. pepo*, *C. sativus* cv. Dominus, *C. amaranticolor*, cowpea [*Vigna unguiculata* (L.) Walp.] and *P. sativum* previously mentioned as susceptible species by Costa (1945). The PCR primers designed for using in this study will be helpful as a primary diagnostic tool for TSV isolated from lettuce. Molecular characteristics such as full-length genomic RNA sequences will also be required in order to define different pathological characteristics among the TSV isolates.

REFERENCES

Bhat, A.I., A. Kumar, R.K. Jain, R.S. Chander and M. Ramiah, 2001. Development of serological based assays for the diagnosis of sunflower necrosis disease. Ann. Plant Prot. Sci., 9: 292-296.

Bhat, A.I., R.K. Jain, V. Chaudhary, M. Krishna Reddy, M. Ramiah, S.N. Chattannavar and A. Varma, 2002a. Sequence conservation in the coat protein gene of *Tobacco streak virus* isolates causing necrosis in cotton, mungbean, sunflower and sunn-hemp in India. Ind. J. Biotechnol., 1: 350-356.

Bhat, A.I., R.K. Jain, A. Kumar, M. Ramiah and A. Varma, 2002b. Serological and coat protein sequence studies suggest that necrosis diseases on sunflower in India is caused by a strain of *Tobacco streak virus*. Arch. Virol., 147: 651-658.

Bhat, A.I., R.K. Jain and M. Ramiah, 2002c. Detection of *Tobacco streak virus* from sunflower and other crops by reverse transcription polymerase chain reaction. Ind. Phytopathol., 55: 216-218.

Bol, J.F., 1999. *Alfalfa mosaic virus* and ilarviruses: Involvement of coat protein in multiple steps of the replication cycle. J. Gen. Virol., 80: 1089-1102.

Clark, M.F. and S.A.N. Adams, 1977. Characteristics of micro plates method of enzyme-linked-immunosorbent assay for detection of plant viruses. J. Gen. Virol., 34: 475-483.

Costa, A.S., 1945. The relationship between American tobacco streak and Brazilian necrose branca or couve. Phytopathology, 35: 1029-1030.

Fagbenle, H.H. and R.E. Ford, 1970. *Tobacco streak virus* isolated from soybeans, *Glycine max*. Phytopathology, 60: 814-820.

Gracia, O. and J.M. Feldman, 1974. *Tobacco streak virus* in pepper. J. Phytopathol., 80: 313-323.

Greber, R.S., M.J. Klose and D.S. Teakle, 1991. High incidence of *Tobacco streak virus* in tobacco and its transmission by *Microcephalothrips abdominalis* and pollen from *Ageratum houstonianum*. Plant Dis., 75: 450-452.

Kaiser, W.J., S.D. Wyatt and G.R. Pesho, 1982. Natural hosts and vectors of *Tobacco streak virus* in Eastern Washington. Phytopathology, 72: 1508-1512.

Kaiser, W.J., S.D. Wyatt and R.E. Klein, 1991. Epidemiology and seed transmission of two *Tobacco streak virus* pathotypes associated with seed increases of legume germ plasm in Eastern Washington. Plant Dis., 75: 258-264.

Krishnareddy, M., D. Lakshmi Raman, S. Jalali and D.K. Samuel, 2003. Outbreak of *Tobacco streak virus* causing necrosis of cucumber (*Cucumis sativus*) and gherkin (*Cucumis anguria*) in India. Plant Dis., 87: 1264-1270.

McDaniel, L.L., R.N. Raid, C.L. Elliot, J.H. Tsai and R.N. Nagata, 1992. Purification and serological characterization of *Tobacco streak virus* isolate infecting field-grown escarole and lettuce. Plant Dis., 76: 966-971.

Prasada Rao, R., A.S. Reddy, S.V. Reddy, K. Thirumala Devi, S. Chander Rao, V. Manoj Kumar, K. Subramaniam, T. Yellamanda Reddy, S.N. Nigam and D.V.R. Reddy, 2003. The host range of *Tobacco streak virus* in India and transmission by thrips. Ann. Applied Biol., 142: 365-368.

Regenmortel, M.H.V., C.M. Fauquet, D.H.L. Bishop, M.A. Mayo, J. Maniloff and C.H. Calisher, 1997. Guidelines to the demarcation of virus species. Arch. Virol., 142: 1505-1518.

Sharman, M., J.E. Thomas and D.M. Persley, 2008. First report of *Tobacco streak virus* in sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), chickpea (*Cicer arietinum*) and mung bean (*Vigna radiata*) in Australia. Aust. Plant Dis. Notes, 3: 27-29.