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Full Length Research Paper

Host range and some characterization of *Tobacco streak virus* isolated from lettuce in Iran

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Lettuce (*Lactuca sativa*) is one of the most common vegetable planted in the world, so the identification of its viral disease is important because this kind of pathogen causes the loss of quantitative and qualitative characteristic of lettuce. *Tobacco streak virus* (TSV) is an ilarvirus with quasi-isometric particles, 27 - 35 nm in diameter. It has a wide host range and occurs worldwide but not often in epidemic proportions. 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, T_5), which are respectivley collected 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* six days after inoculation showed necrotic local lesions. *Gomphrena globosa* ten days after inoculation developed chlorotic local lesions. Systemic symptoms were produced in *Datura stramonium*. *Phaseolus vulgaris* cv. Red Kidney five days after inoculation developed necrotic local lesions. *Nicotiana tabacum* seven 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 distortion and mosaic. In order to study transmission through seed, 200 seeds of *C. quinoa* (inoculated with TSV) were planted. After seedling and growth, TSV was tested in these plants by DAS-ELISA. The TSV seed infection rates from 2.2%-26% depending on isolate.

Key words: ELISA, transmission trough seeds, *Tobacco streak virus*.

INTRODUCTION

Lettuce (*Lactuca sativa*) is one of the most common vegetable planted in the world, so the identification of its viral disease is important because this kind of pathogen causes the loss of quantitative and qualitative characteristic of lettuce. The *Tobacco streak virus* (TSV) was identified in tobacco (*Nicotiana tabaccum* L.) plants in Brazil in 1940 (Costa, 1945), and it is currently known to infect several cash crops such as cotton (*Gossypium hirsutum* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*N. tabacum* L.), soybean (*Glycin max*), peanut (*Arachis hypogaea* L.), sunflower (*Helianthus annuus* L.) and some weeds (Almedia et al., 2005). TSV was first reported from Australia in 1971 and has subsequently been reported from tobacco, strawberry, dahlia and various weed species, mostly from south-eastern Queensland (Greber 1971, 1979; Greber et al. 1991). Sharman et al. (2008) reported TSV naturally infecting sunflower, cotton, mung bean and chickpea in Australia. Natural field infections with TSV have previously been reported on sunflower, mung bean and cotton from India (Prasada Rao et al. 2000, 2003; Bhat et al. 2001, 2002a, 2002b) and also on cotton from Pakistan and Brazil (Costa and Carvalho 1961; Ahmed et al. 2003). In India, TSV induced sunflower necrosis disease has been responsible for serious economic losses (Bhat et al. 2002b). Kaiser et al. (1991) reported TSV naturally infecting chickpea growing adjacent to plots of inoculated plants in the United States

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of America. Disease symptoms caused by TSV started to appear 15 - 20 days after sowing, when plants exhibited browning buds which later curled downward. It has a wide host range and occurs worldwide but not often in epidemic proportions. It is unstable in plant extracts but can be transmitted mechanically to a number of hosts. It is transmitted through pollen, seed, and by thrips. The TSV was isolated from lettuce in 1982 (http:// www.plant-virology.de/publications.htm).

Yield losses of up to 20% have been reported in lettuce fields in Brazil. Studies have shown that the disease is endemic in specific regions where high populations of the vectors *Thrips tabacci* Linderman and *Frankliniella occidentalis* Pergande (Kaiser et al., 1982) have developed due to the simultaneous presence of a weed (*Ambrosia polystachia* L.) that is a known host of the virus (Costa and Carvalho, 1961). Another vector species (*Microcephalothrips abdominalis* Crawford) has also been reported by Greber et al. (1991). However, the transmission by thrips was firstly demonstrated by Costa and Lima Neto (1976). TSV has a tripartite single-stranded messenger-sense RNA genome. 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. The objective of the present study was to characterize the virus causing mosaic and yellowing on lettuce in Tehran province of Iran, including host range and seed transmission studies.

MATERIALS AND METHODS

Sample collection

Samples were collected during the 2007 and 2008 growing season from lettuce field-grown in Tehran Province. In this region, lettuce is 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 transporttation 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 one hour.

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

Host range studies

Five isolates (T_1, T_2, T_3, T_4, 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), respectivley, 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-roof screen house for observation. Symptoms on both inoculated and upper, uninoculated leaves were recorded. Tests for latent infection conducted by back-inoculation to *C. quinoa* Wild.

Seed transmission

The six *C. quinoa* were inoculated separately at the four-foliate growth stage with five TSV isolates (T_1, T_2, T_3, T_4, T_5). All infections were confirmed by DAS-ELISA. Plants were grown to maturity and seeds were harvested and stored at 4°C. Seed transmission rates were determined by planting up to 200 seeds from infected plants When the seedling reached the 4 leaf stage, tissues were harvested from each plant for determination of TSV infectios by DAS-ELISA.

RESULTS AND DISCUSSION

Host range

Of 29 species in 6 families of plants inoculated, the species in families of Compositae and Cucurbitaceae did not infect. *C. quinoa* six days after inoculation showed necrotic local lesions (Figure 1). *Gomphrena globosa* ten days after inoculation developed chlorotic local lesions. Systemic symptoms were produced in *Datura stramonium* (Figure 2). *Phaseolus vulgaris* cv. Red Kidney five days after inoculation developed necrotic local lesions (Figure 3). *N. tabacum* cv *samsun* seven days after inoculation showed necrotic and chlorotic local lesions (Figure 4). *Nicotiana clevelandii* 15 days after inoculation developed leaf distortion and vein necrosis (Figure 5).

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

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

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

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

Table 1. Reaction of selected indicator plant species to *Tobacco streak virus* isolated from lettuce in Iran.

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	ld, vn
	Petunia hybrid grandiflora	-
	Physalis alkekengi	
Compositae	*Lactuca sativa*	ns
	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	-

id = Leaf distortion; cll = chlorotic local lesion, ns = no symptom, nll = necrotic local lesion, s = systemic sumptom, vn = vein necrosis.

Table 2. Tobacco streak virus (TSV) infection of chenopodium seeds, determined by ELISA.

TSV isolates	Infected seedling	Total seedlings	Infected rate (%)
T_1	1	45	2.2
T2	4	40	10
T3	8	30	26
T4	3	40	7.5
T5	7	45	15.5

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

Lactuca sativa 10-15 days after inoculation developed leaf disortion and mosaic (Figure 6; Table 1).

Seed transmission

The results of ELISA analysis on the seedling derived from seeds of infected *C. quinoa* demonstrated that TSV transmitted through seed with range 2.2 to 26% depending on isolate (Table 2).

Conclusion

The virus analyzed in these studies shows the biological properties similar to those described for TSV (Fulton, 1971). 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 and Carvalho, 1961; Salazar et al., 1982; Kaiser et al., 1982), despite the lack of infection of *Amaranthus paniculatu*s, *C. pepo*, *C. sativus* cv*. Dominus, C. amaranticolor ,* cowpea [*Vigna unguiculata* (L.) Walp.] and *P. sativum* previously mentioned as susceptible species by Costa and Carvalho (1961). In our case, transmission of TSV through seeds ranged from 2.2 to 26% depending on isolate. This the firs report of TSV naturally infecting lettuce in Iran.

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