Tomato yellow leaf curl virus (TYLCV) in Saudi Arabia: Identification, partial characterization and virus-vector relationship

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV), a geminivirus transmitted by the whitefly Bemisia tabaci Genn. is a serious disease in tomato (Lycopersicon esculentum Miller) in all production areas of Kingdom Saudi Arabia under plastic tunnels and field conditions. The virus was isolated and named tomato yellow leaf curl virus-Saudi Arabian isolate (TYLCV-SA) due to its common symptoms in the world. Symptomatic naturally infected tomato plants were severely stunted and exhibited marginal chlorosis, upward or downward, leaf curling, flowers abortion and stem upright as well as stunted plant growth. During autumn, TYLCV causes a serious damage to tomato plants with up to 96.9% yield loss and quality reduction. TYLCV is transmitted by whitefly and mechanically from infected tomato to different species belonging to the families Amarnthaceae, Chenopodiaceae, Cruciferae, Cucurbitaceae, Leguminaceae (Fabaceae), Malvaceae and Solanaceae. In virus-vector studies, the minimum acquisition access period (AAP) and inoculation access period (IAP) were 30 min (10% transmission) and 15 min (10% transmission); respectively. In an insect / plant inoculation test, female B. tabaci are able to transmit TYLCV. The efficiency of transmission was increased by increasing both of AAP and IAP, as well as insect number. Electron micrographs dip preparation technique showed gemini-particles with dimensions of 20×30 nm. Dot-blotting immuno-binding assay (DBIA) proved to be successful tool in detecting TYLCV-SA antigen in naturally and mechanically infected tomato plants as well as viruliferous whiteflies. Infection percentages of TYLCV-SA were 88.46, 85.8, 89.03, 85.94, 89.15 and 96.9%, respectively in the cultivated plastic tunnels in Gizan, Nagran, Hail, Qatif, Riyadh and Al-Hassa. Meanwhile these percentages were 85.65, 93.9, 93.95, 86.71, 87.5, 93.3% respectively, in the cultivated fields in the same regions. Also, TYLCV could be detected by double antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA) and tissue-blotting immuno-binding assay (TBIA) in infected tomato and other host plants. DNA from infected plants was extracted and analyzed by polymerase chain reaction (PCR) using degenerate primers PALIv1978/PARIc496. PCR fragment of the expected size 1.1kb for the common region (CR) in the geminivirus were obtained from infected tomato plants and tobacco (Nicotiana glutinosa) as an indicator host.

Key words: *Geminivirus(es), TYLCV-SA, viruliferous, Non-viruliferous, B. tabaci, DAS-ELISA, DBIA TBIA and whiteflies transmitted-geminiviruses (WFTG).*

INTRODUCTION

In tropical and subtropical climate regions, whitefly *B. tabaci* Gennadius (Homoptera: Aleyrodidae) is an important insect pest. *B. tabaci* provokes direct feeding damages but also causes considerable indirect damage as a vector of numerous geminiviruses such as *Tomato yellow leaf curl virus* [TYLCV] a threatening virus for tomato (Delatte *et al.*, 2003; Delatte *et al.*, 2005; Raj, *et al.*, 2005). TYLCV is the name given to a complex of whitefly transmitted-geminivirus (WTGV) belonging family *Geminiviridae*, genus *Begomovirus* affecting tomato (*Lycopersicon esculentum* Miller) cultures worldwide (Brunt *et al.*,1997 and Czosnek and Laterrot,1997). TYLCV is one of the most devastating whitefly transmitted-geminivirus (WTGV) of cultivated tomato in tropical and subtropical regions, and losses of up to 100% are frequent (Moriones and Navas-Castillo,2000; Idris *et al.*, 2001 and Valverde *et al.*,2001).

TYLCV isolates are monopartite or bipartite begomovirus transmitted by the whitefly, *B. tabaci* (Genn.).. Single insects are able to acquire TYLCV and transmit it to tomato plants in a persistent manner. *B. tabaci* females are more efficient than male insects. AAP and IAP are approximately 10 to 20 min. The rate of transmission increases with increasing both AAP and IAP (Brown and Bird,1992; Ghanem *et al.*,2001; Lapidot *et al.*,2001; Salati *et al.*, 2002 and Goldman and Czosnek,2002). The minimal latent period reported was 21 h but was 24 h for the closely related TYLCV strain from Egypt (Mehta *et al.*, 1994) and 17 h for the more distant virus from Sardinia (Caciagli *et al.*,1995). Recently, Delatte *et al.*(2003) revealed that TYLCV can be acquired by whiteflies from infected fruits and subsequently transmitted to healthy tomato plants. Potential risk of the spread of TYLCV by tomato fruits in natural conditions needs to be further assessed. Several techniques such as enzyme linked immuno-sorabent assay (ELISA), tissue-blotting immuno-binding assay (TBIA) and dot-blotting immuno-binding assay (DBIA) as well as polymerase chain reaction (PCR) provide a sensitive and specific for detection and identification of whitefly transmitted-geminiviruses (WTGV) in the infected plants and their vector whitefly *B. tabaci* (Cohen, *et al.* 1989; Mehta *et al.*, 1994; Fargette *et al.*, 1996; Tsai *et al.*, 2006). The current study was undertaken to: (i) identification, detection and partial characterization the disease and (ii) to study virus-vector relationships.

MATERIALS AND METHODS

Source of the virus and its identification

Virus materials were collected from tomato plants (*Lycopersicon esculentum* Mill.) cv. Nina showing characteristic symptoms of TYLCV with heavy infestation with *B. tabaci* Genn. insects, grown under greenhouse conditions at King Faisal University Agricultural and Veterinary Experimental Station in Hofuf. Infected tomato plants were transported, pruned, transplanted to plastic pots (30 cm-diameter), and maintained as stock plants in the greenhouse at Faculty of Agriculture & Food Sciences, King Faisal University.

Transmission studies

Maintenance of virus culture ,whiteflies and plants

Cultures of the Saudi Arabian isolate of TYLCV were maintained in tomato plants cv.Super Marmand. Adults of whitefly (*B. tabaci* Genn.) were used for transmission studies. Viruliferous whiteflies were reared on

sweetpotato plants grown in insect-proof glass cages at 24-27°C for 5-7 days for ovipositor.

Mechanical transmission and host range studies

The local isolate was tested, through mechanical inoculation, on some host range as mentioned Table (1). Sap was obtained by grinding symptomatic cv.Nina leaves in cold phosphate buffer, pH 8.1 containing 0.2 M Na_2HPO_3, 0.02M Na-EDTA, and 1.5% Triton X-100. Twenty plants of each test plants were inoculated. Control plants were inoculated with the same buffer. Inoculated plants were kept under cages covered with fine muslin in an insect-proof greenhouse for a period of one month. Later on, cages were removed and plants were watched for symptom development until fruit set.

Virus-vector relationships

Transmission tests were done as previously described by Ghanem *et al.* (2003).Three tests were performed for determination of the efficiency of transmission, minimum AAP and IAP.

Efficiency of transmission

Non-viruliferous adults of whiteflies were confined to TYLCV-infected tomato plants for 24 or 48 hr acquisition access period (AAP). Insects either singly or in groups of 3, 5, 10 and 20 per plant were transferred to healthy tomato seedlings (20 plants / treatment) kept in glass cage. Insects were then given 24 hr or 48 hr IAP. The results were examined visually and calculated as well as confirmation by DAS-ELISA test (see below).

Acquisition access period (AAP)

AAP was determined by allowing whitefly *B. tabaci* adults access to TYLCV-infected tomato plants for either 1, 5, 10, 15, 30 min, 1, 3, 5, 24 and 48hr before transferring to healthy tomato seedlings for 48 hr IAP using 20 insects/ per plants. Twenty plants were used for each replicate, and percentages of virus infection were calculated from plants showing TYLCV-symptoms after 3-4 weeks. Results were calculated as mentioned before.

Inoculation access period (IAP)

Non-viruliferous whiteflies were allowed 48 hr AAP on TYLCV-infected tomato plants, then transferred in groups of 20 insects/ per plants, on healthy tomato seedlings for 1, 5, 10, 15, 30 min, 1, 3, 5, 24 and 48 hr infection feeds.

Electron microscopy

Grids were prepared using crude sap extracts of TYLCV-infected plants preparations made from TYLCV-whitefly inoculated tomato plants. Virus preparations were negatively stained with 2% uranyl acetate and examined in a JEM-100 electron microscope in EM-Unit, Fac. of Medicine, King Faisal Univ., Dammam, Saudi Arabia.

Serological tests

Different serological techniques were used for detection the virus in both infected plants or in viruliferous whiteflies through applying DAS-ELISA, Tissue blotting immuno-binding assay (TBIA) and Dot blotting immuno-binding assay (DBIA). TYLCV-Polyclonal antibody (TYLCV- Pab) was obtained from Agdia, ElKhart, India.

Double antibody sandwich enzyme-linked immuno-sorbent assay (DAS-ELISA)

Applying DAS-ELISA to detect TYLCV-SA in infected leaves or in viruliferous whiteflies was essentially similar to those described by Cohen *et al.*(1989) with some modification as follows: leaf extracts were prepared in a micomortar by grinding tissues in 0.4 ml of phosphate-buffered saline (0.02 M, pH 7.4, 0.5 ml Tween-20, 10g

polyvinylpyrrolidone "PVP", 2 g egg albumin) per liter. Also, ten and/or twenty insects whiteflies previously fed on TYLCV-infected plants for 48 hr were prepared in a micromortar by grinding in 0.3 ml of the same buffer. Healthy plants or non-viruliferous whiteflies fed on eggplant were used as a control. Data were expressed and recorded using Multiskan at A_{405nm}.

Tissue and dot blotting immuno-binding assay (TBIA & DBIA)

The TBIA and DBIA techniques were run on nitrocellulose membrane to detect TYLCV-antigen in infected tomato plant tissues and other hosts as described by Abdel-Salam (1999) and Ghanem *et al.* (2003).

Polymerase chain reaction (PCR) detection of TYLCV-SA

PCR was conducted to detect TYLCV-SA as described by Rojas-Maria *et al.*, 1993. TYLCV-geminivirus was maintained in tomato and *Nicotiana glutinosa* plants by whitefly inoculation.

Design of PCR-oligonucleotide primers

Degenerate primers for whitefly-transmitted geminiviruses were designed to anneal to highly conserved nucleotide sequence regions of the open reading frames (ORFs) or the common region of DNA. Primer PAL1v1978 was designed to anneal to the complementary sense strand of the replicative form AL1 sequence encoding the derived amino acid sequence ThrGlyLysTh--rMetTrpAla, which is a conserved, putative NTP-binding site present in viral replication-associated proteins. Primer PAR1c496 was designed to anneal to the viral sense strand of the AR1 ORF sequence encoding for the conserved, derived amino acid sequence ProMetTyrArgLysProArg, which is located near the amino terminus of the coat protein.

RESULTS AND DISCUSSION

Tomato yellow leaf curl virus (TYLCV) is a whitefly-transmitted geminivirus that causes devastating damage and a major constraint to tomato crops in all production areas of the Kingdom Saudi Arabia.

Symptomatology, virus isolation and host range studies

Early diagnosis of TYLCV was essentially based on symptom observation. The name tomato yellow leaf curl virus-SA (TYLCV-SA) was given to this virus due to curling upward and/or downward. Survey of TYLCV-infection was done on tomato plants during 2001-2004 in different provinces in Saudi Arabia (Riyadh, Gizan Nagran, Hail, Qatif, and AL-Hassa) were cultivated in the fields and plastic tunnels. Visual inspection followed by ELISA screening indicated that the naturally infected plants were heavily infested with whitefly *B. tabaci* Genn. Symptoms of TYLCV-infected tomato plants were severe leaf curling with marginal chlorosis, (Fig.1) flowers abortion, stem upright and stunted plant growth (Fig.2a) as well as vein enation (Fig.2b). Such symptoms similar to those characteristic of the disease have been described on cotton in Egypt and Sudan (Abdel-Salam *et al.*, 1999; Idris and Brown, 2000 and Bigarré *et al.*, 2001), on okra in Pakistan (Mansoor *et al.*, 2001) and other crops-infecting geminiviruses such as tomato (Avgelis *et al.*, 2001), cotton (Radhakrishn *et al.*, 2001), mungbean (Usharani *et al.*, 2001) and Pepper (Samretwanich, 2000 and Mendez-Lozano *et al.*, 2003).

Concerning naturally infected tomato with TYLCV, the infection percentages ranged between 21.9-25.54% in the fields compared with 16.3-30.45% in plastic tunnels for early after transplanting, respectively (Table 1). Infection percentages near the end of the

season (late infection) reached 88.46, 85.8, 89.03, 85.94 ,89.15 and 96.9 % respectively in the cultivated plastic tunnels in Gizan, Nagran, Hail, Qatif, Riyadh, and Al-Hassa. Whilst, these percentages were 85.65, 93.9,93.95, 86.71, 87.5, 93.3% respectively, in the cultivated fields in the same regions. Results indicated that the highest infection was recorded when the plants were early infected; whereas infection percentages recorded 96.9%. Such results have previously been reported for TYLCV-infection on tomato in different regions (Pappu *et al.*,2000; Salati *et al.*,2002; Lapidot, *et al.*, 2001; Idris *et al.*,2002; Maruthi *et al.*,2003; Sseruwagi *et al.*,2004; Anfoka *et al.*, 2005 and Fauquet *et al.*,2005).

Table (1): Infection percentages of Tomato yellow leaf curl virus (TYLCV-SA) in different regions on tomato plants cultivated in plastic tunnels and fields.

Tomato cultivated in:	Region	No. of infected plants/ tested		% infection		ELISA detection	
		Early infection	End of the season	Early infection	End of the season	Early infection	End of the season
Plastic tunnels	Gizan	41/146	184/208	28,08	88,46	+	+
	Nagran	31/119	151/176	26,05	85,8	+	+
	Hail	32/126	138/155	25,39	89,03	+	+
	Quatif	40/163	159/185	24,53	85,94	+	+
	Riyadh	53/174	148/166	30,45	89,15	+	+
	Al-Hasa	44/270	218/225	16.3	96.9	+	+
Fields	Gizan	45/205	209/244	21,9	85,65	+	+
	Nagran	39/168	185/197	23,2	93,9	+	+
	Hail	45/169	140/149	23,66	93,95	+	+
	Quatif	33/144	124/143	22,91	86,71	–	–
	Riyadh	35/137	98/112	25,54	87,5	+	+
	Al-Hasa	56/235	168/180	23.9	93.3	+	+

The virus was sap and whitefly transmitted from cv. Nina tomato to tomatoes and other plants as well as back inoculated with high efficiency. Inoculated plants exhibited virus symptoms of interveinal chlorosis, 20 days post-inoculation (Table 2). The isolated TYLCV-SA produced symptoms typical to those of begomoviruses as tomato mottle geminivirus (TMoV) (McGovern *et al.*, 1994) and TYLCV (Czosnek and Laterrot, 1997 and Moriones and Navas-Castillo, 2000).

Results of host range study and the symptoms associated with hosts are summarized in Table (2). The obtained results confirmed the mechanical inoculation (poorly) and whitefly-transmission nature of TYLCV-SA. The host range of TYLCV-SA included members within the families: *Amarnthaceae, Chenopodiaceae, Cruciferae, Cucurbitaceae Euphorbaceae, Leguminaceae (Fabaceae), Malvaceae ,Solanaceae, Vitaceae.* TYLCV-SA induced severe stunting and leaf curl symptoms on all tomato cultivars (AQ 55, Linda, Nina, Marmand and Super Marmand), interveinal chlorosis tissues became yellowed and had associated green vein-banding. Enation symptom frequently formed on the leaves. The virus induced a vein enation with downward (Epinesty) on *Nicotiana glutinosa* and interveinal chlorosis tissues on *Amaranthus retroflexus* L (Figs.3&4), some hosts showed no symptoms. Data indicate that the significant of the effect of these host range have on epidemiology of whitefly-transmitted

giminivirus. Thus, survey for reservoir hosts revealed that few plant species in/or around tomato fields or plastic tunnels play an important role as a reservoir for whitefly which fed on and move to spread WFTG. The results were confirmed serologically by DAS-ELISA as well as DBIA technique. These results and symptoms typical of TYLCV-SA upon mechanical and whitefly inoculation were virtually identical to those described for TYLCV in several countries (Abdel–Salam,1990; Brunt *et al.*, 1997; Jorda *et al.*,2000;Avgelis *et al.*,2001 and Salati *et al.*,2002).

In addition, the present study confirms the success of the mechanical transmissibility of TYLCV-SA similar to some isolates of TYLCV-Egypt (Abdel–Salam, 1990). Holguin-Pena, *et al.*, (2003) confirmed that inoculum prepared from TYLCV-infected tomato plants in California were experimentally transmitted to tomato seedlings and *Datura stramonium* by mechanical inoculation. The present investigation confirms the transmissibility of TYLCV-SA by the whitefly vector to tomato or other plant species similar to all whitefly-transmitted geminiviruses i.e., Bean calico mosaic virus (BCaMV) (Brown *et al.*, 1990); Squash leaf curl virus (SLCV) (Brown *et al.*, 2000); TYLCV (Salati *et al.*, 2002). Sorab, *et al.*, (2003) observed that TYLCV-infected sponge gourd (*Lufa cylindrica*) plants at a rate of 100% in Delhi, India. Also, several investigators have declared the role and efficacy of whitefly vector to increasing spread and epidemiology of geminiviruses either in single or dual infection such as BGMV, TYLCV (Czosnek and Laterrot, 1997; Mendez-Lozano *et al.*, 2002 and Morales and Jones, 2004).

Virus- vector relationships

Transmission efficiency

Results in Table (3) showed that a single whitefly was efficient to transmit TYLCV-SA applying 48 hr for both AAP and IAP. Whilst, a single whitefly failed to transmit the virus applying 24 hr AAP and IAP. The maximum transmission efficiency (95 and 100%) were achieved using 20 insects/plant applying 24 and 48 hr of AAP and IAP. This result is resembling those obtained by Abdel- Salam *et al.* (1998) working on HLCrV; Ghanem *et al.*, (2001) working on TYLCV and Ghanem *et al.*, (2003) working on BDMV.

Acquisition and inoculation of TYLCV-SA

B.tabici Genn. transmitted TYLCV-SA after minimum of 30 and 15 min AAP and IAP ,respectively (Table 4). Increasing both AAP and IAP increased percentages of TYLCV-SA transmission. The maximum transmission efficiency (90 and 95%) were achieved using 20 insects per plant applying 48 hr of AAP and IAP respectively. The percentages of virus transmission increased by increasing the periods of both AAP and IAP (Table 4).Similar results had already been revealed by Metha *et al.* (1994) working on TYLCV; Abdel-Salam *et al.* (1998) working on HLCrV; Soliman-Doaa, (2000) working on BDMV and Salati *et al.*, (2002) working on TYLCV, who mentioned that the minimum requirement of AAP is usually higher than that of IAP. They also, found that the efficiency of transmission increases with prolonging both of AAP and IAP.

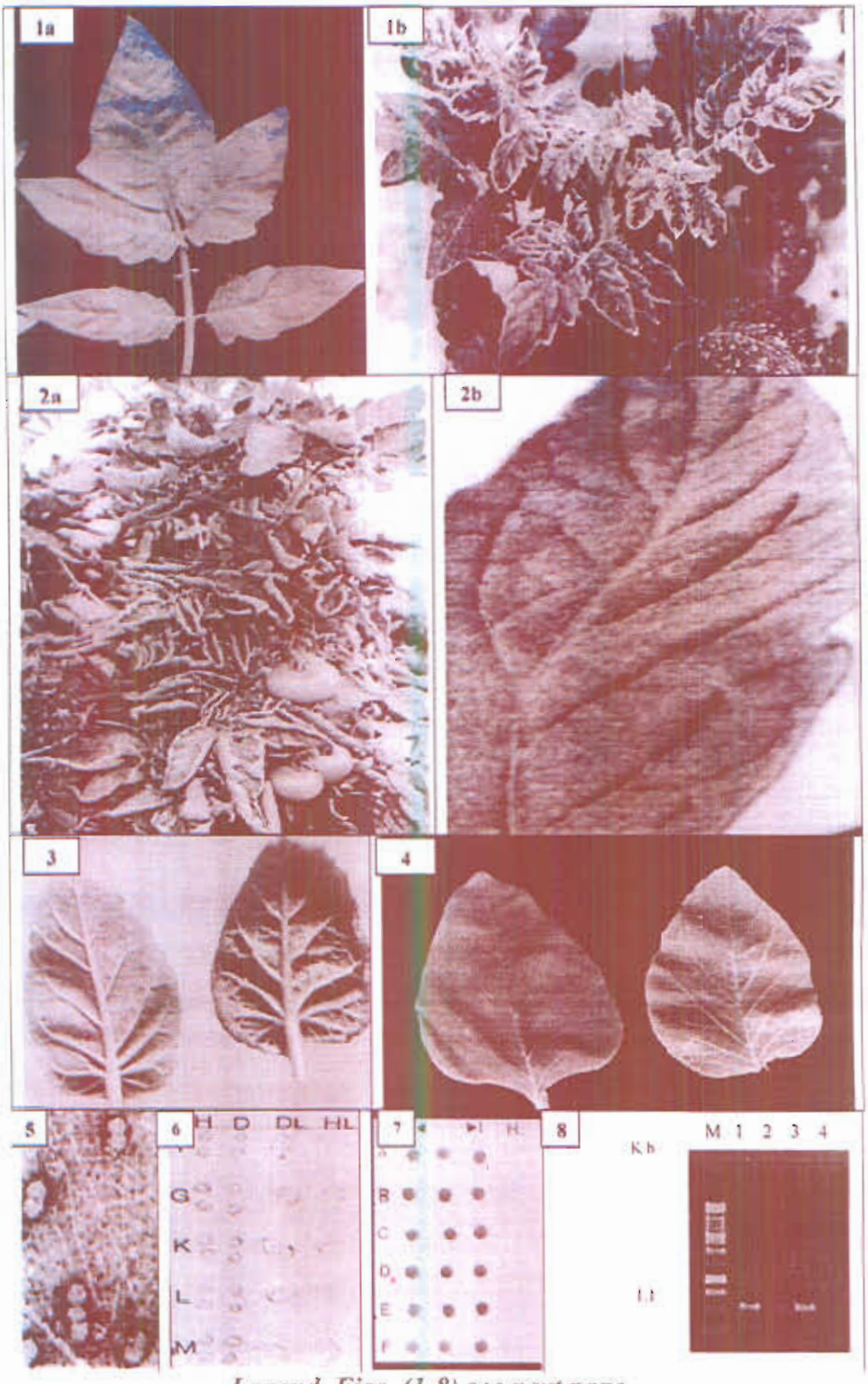
Electron microscopy

Examination of TYLCV-SA of crude sap extracted from infected tomato revealed the presence of geminivirus with dimensions of 20×30 nm (Fig. 5) similar to those reported for geminivirus (Brunt *et al.*, 1997; Soliman-Doaa, 2000 and Ghanem *et al.*, 2003).

Table (2): Experimental host range of TYLCV-SA as determined by mechanical and whitefly–transmission.

Test plant	Mechanical inoculation			Whitefly inoculation		
	Symptoms	Serologic detection		Symptoms	Serologic detection	
		DAS-ELISA	DBIA		DAS-ELISA	DBIA
Family:Amaranthaceae:						
Amaranthus retroflexus L	NS	--	NT	ICh	+	+
Alternanthera achyranthoides L	NS	-	NT	LC	+	+
Family:Chenopodiaceae						
Beta vulgaris L	NS	--	NT	LC	+	NT
Chenopodium amaranticolor L	NT	--	NT	IH, VC	+	NT
Spinacea olcracea L Bloomsdale	NT	--	NT	LC	+	NT
Family:Cruciferae :						
Brassica oleraceae L.	NS	--	NT	LC	+	NT
Raphanus sativus L.	NS	--	NT	LC	+	NT
Family:Cucurbitaceae :						
Cucumis melo L cv. Imperial	NS	--	NT	NS	--	NT
Cucumis sativus L. cv. Beit Alpha	NS	--	NT	VC,MLC	+	NT
Cucurbita pepo L. cv. Regina	NT	--	NT	MLC	+	NT
cv. Desert Queen	NT	--	NT	MLC	+	NT
cv. Scarla	NT	--	NT	MLC	+	NT
Family:Euphorbaceae						
Ricinus communis L	NT	--	NT	IH,D	+	NT
Family:Leguminosae : (Fabaceae)						
Glycine max L. cv. Clark	NS	+	+	Mo	+	NT
Phaseolus vulgaris L. cv. Lolita	IH, SM	+	+	IH, SM	+	+
cv. Strike	LC, MM	+	+	LC, MM	+	+
cv. Merite	LC, SM	+	+	LC, SM	+	+
Pisum sativum L. cv. Little Marvel	NT	--	NT	NT	--	NT
Vicia faba L. cv. Giza- 2	NT	--	NT	NT	+	NT
Vigna unguiculata L. Walp cv. Balady	NT	--	NT	NT	--	NT
Family:Malvaceae :						
Abelmoschus esculentus L. cv. Hasawi	NT	--	NT	LC	+	NT
Althaea rosea Cav.	NT	--	NT	LC	+	NT
Malva pariflora L.	NT	--	NT	IH, LC	+	NT
Family:Solanaceae:						
Capsicum annuum L.cv. Yolo Wonder	LC, IH	+	NT	LC, IH	+	+
Datura stramonium L.	NS	--	NT	ICh	+	NT
Lycopersicon esculentum Mil						
cv.Nina	IH,Chs	+	+	IH, UW	+	+
cv. Linda	IH,Chs	+	+	IH, DW,UW	+	+
cv.AQ 55	IH,Chs	+	+	IH, EP or,UW	+	+
cv. Marmand	IH,Chs	+	+	IH, EP or DW	+	+
cv.Super Marmand	IH	+	+	EP or DW,UW	+	+
Lycopersicon pimpinellifolium L.	NS	+	NT	IH, EP or DW	+	+
Nicotiana benthamiana Domin	NS	+	+	NS	+	+
Nicotiana glutinosa L.	NS	+	NT	IH, EP or DW	+	+
Physalis floridana L.	NT	--	NT	NS	--	NT
Solanum melongena L.	NS	-	NT	LLC	+	NT
Family:Vitaceae						
Vitis vinifera L.	NT	--	NS	LC	+	NT

Chs =chlorotic spots Dw = downward EP = epinasty
ICh = interveinal chlorosis H = interveinal hypertrophy LC = leaf curl
MLC = mild leaf curl Mo= mosaic MM= mild mosaic UW =upward
NS= no symptoms NT = not tested - = negative reaction
+ = positive reaction DAS-ELISA = enzyme linked immuno-sorbent assay



Lagend, Figs. (1-8) see next page.

Fig.(1):Healthy tomato leaves (a), Symptoms of naturally infected tomato plant with TYLCV-SA, showing leaf curling with marginal chlorosis (b).

Fig.(2): Naturally infected tomato plant with TYLCV-SA, showing upward, downward (epinasty), stem upright and stunted plant growth (a), as well as vein enation (b).

Fig.(3): Leaf curl and downward symptoms on Nicotiana glutinosa and after inoculation with viruliferous whiteflies.

Fig.(4):Whiteflies inoculated Amaranthus retroflexus L showed symptoms of interveinal chlorosis.

Fig.(5): Geminivirus particles (20×30nm) were examined from infected tomato leaves using dip preparation and stained with 2% uranyl acetate.

Fig.(6): TBIA test showing the detection of TYLCV-SA in blotted-cut petioles (H=Healthy and D- Infected) and leaves (HL=Healthy and DL=Infected) of naturally infected, (I) whiteflies inoculated tomato cv. Nina (G), mechanically-inoculated cv. Nina (K) test plants N. glutinosa (L) and Amaranthus retroflexus (M).

Fig.(7): DBIA test illustrating the detection of TYLCV-SA: Healthy samples (H), Infected (I) whiteflies inoculated tomato cv. Nina (A), mechanically inoculated tomato cv. Nina (B), naturally infected tomato (C), test plants N. glutinosa (D) and Amaranthus retroflexus (E) and viruliferous whiteflies (F).

Fig.(8): Polymerase chain reaction (PCR) amplification of a = 1.1-Kb DNA fragment with the PCR primer pair for DNA (PAL1v1978 and PAR1c496) from tomato and N. glutinosa plants. Lane M= ladder marker, Lane 1=infected tomato, Lane 2= healthy tomato, Lane 3= infected N. glutinosa and , Lane 4= healthy N. glutinosa.

Table (3): Transmission efficiency of TYLCV-SA by different numbers of B. tabaci.

No. of insects /plants	*24 hr AAP,IAP		*48 hr AAP,IAP	
	No of infected /inoculated plants	**% Transmission	No. of infected / inoculated plants	**% Transmission
1	0 / 20	0	3 / 20	15
3	6 / 20	30	6 / 20	30
5	9 / 20	45	9 / 20	45
10	14/20	70	15/20	75
20	19/20	95	20/20	100

*AAP=Acquisition access period.
* IAP= Inoculation access period.
** % Transmission = No. of infected plants / No. of inoculated plants × 100.

Table (4): Rate of transmission of TYLCV-SA by B. tabaci after different acquisition access periods (AAP) and inoculation access periods (IAP).

AAP	*No. of infected / inoculated plants	**% Transmission	IAP	* No. of infected / inoculated plants	**% Transmission
1 min	0 / 20	0	1 min	0 / 20	0
5 min	0 / 20	0	5 min	0 / 20	0
10 min	0 / 20	0	10 min	0 / 20	0
15 min	0 / 20	0	15 min	1 / 20	10
30 min	2 / 20	10	30 min	3 / 20	15
1 hr	6 / 20	30	1 hr	8 / 20	40
3 hr	8 / 20	40	3 hr	11 / 20	55
5 hr	11 / 20	55	5 hr	12 / 20	60
24 hr	16 / 20	80	24 hr	17 / 20	85
48 hr	18/ 20	90	48 hr	19 / 20	95

* No. of infected / No. of inoculated plant
** No. of infected / No. of inoculated plant × 100

Serological studies

Detection of TYLCV-SA in infected plants and viruliferous whiteflies

DAS-ELISA

ELISA detected TYLCV-SA in infected plants as shown in Table (1). Also, it detected virus antigen in homogenates prepared from 10 or 20 whiteflies previously fed on TYLCV-infected plants for 48 hr, but not from whiteflies fed on eggplant or healthy tomato. Negative results were observed in both whiteflies fed on two different control plants. Obtained result is in accordance with Cohen *et al.* (1989) and Lapidot *et al.* (2001) who detected SLCV and TYLCV respectively, in *B. tabaci* extracts when patches of at least 20 females previously fed for 48 hr or more on infected plants.

Detection by TBIA and DBIA techniques

TBIA technique detected TYLCV-SA antigen in infected cv. Nina tomato leaves. Data indicated that TBIA was able to detect virus in mechanically and whiteflies inoculated plants (Fig.6) and naturally infected tomato as well as test plants a similar to Fargette *et al.* (1996); Abdel-Salam (1999) and Soliman-Doaa, (2000). Also, DBIA detected BDMV antigen in mechanically and whiteflies inoculated tomato plants, and inside single whitefly (Fig.7). The results confirm the success of both mechanical and whiteflies inoculation of TYLCV-SA as reported by Cancino *et al.* (1995) working on BGMV; Soliman-Doaa (2000) and Abdel-Salam *et al.* (2001) working on BDMV. Furthermore, TBIA and DBIA techniques proved to be successful tools in detecting TYLCV-SA inside a single whitefly. Detection of virus inside viruliferous-insects should help obtaining invaluable information about TYLC-disease forecast in tomato fields and greenhouse or plastic tunnels plantations, in other alternative hosts and in virus vector relationships studies. Such result are similar to those observed by Mahmoud *et al.*(1996) and Soliman-Doaa, (2000) who detected MYSV and BDMV, respectively in single insect using DBIA.

Detection of TYLCV- SA by PCR

The most consistent amplification of a DNA fragment was obtained with the primer used PAL1v1978 and PAR1c496. As predicted from the annealing position of these primers with the DNA- of TYLCV, PCR amplified fragments of about 1.1 Kb were obtained from DNA extracts of tomato infected with TYLCV-geminivirus (Fig.8). These fragments are predicted to include part of AL1, AR1 (open reading frame" ORF") and the entire common region. A 1.1-Kb fragment was also amplified from *Nicotiana glutinosa* infected with TYLCV. The results showed that degenerate PCR primers for amplification of portions of the DNA components of whitefly-transmitted geminiviruses were designed from highly conserved regions of the viral genome identified from nucleotide and/or amino acid sequence alignments. Some of these conserved regions may have functional significance (e.g., NTP-binding site of AL1 and the stem-loop of the common region); therefore, these primers should have general application for the amplification of DNA fragments from a wide range of whitefly-transmitted geminiviruses. Such results indicate that PCR technique as an effective diagnostic tool and greatly facilitate studies of geminiviruses epidemiology and etiology. These results are in accordance with those reported by Rojas-Maria *et al.*; (1993); Salati *et al.*; (2002); Simon *et al.*; (2003) and Tsai *et al.*, (2006) that PCR is an extremely sensitive and specific technique for the detection and identification of plant pathogens.

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الملخص العربي

فيروس تجعد واصفرار أوراق الطماطم :أحد الفيروسات التوأمية المنقولة بحشرات الذباب الأبيض التي تصيب نباتات الطماطم في المملكة العربية السعودية

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سجلت هذه الدراسة عزل وتعريف فيروس تجعد واصفرار أوراق الطماطم أحد أخطر الفيروسات التوأمية المنقولة بحشرات الذبابة البيضاء Bemisia tabaci والمصاحب لأعراض تجعد واصفرار بين العروق والتواء حواف الأوراق، وتشوه أوراق نبات الطماطم المنزرعة في الحقول المفتوحة والبيوت المحمية المنزرعة بالمملكة العربية السعودية. وقد تم تسمية العزلة بفيروس تجعد وإصفرار أوراق الطماطم (العزلة السعودية) Tomato yellow leaf curl virus - Saudi Arabia isolate (TYLCV-SA). وقد بينت الدراسة أن الفيروس ينتقل ميكانيكاً و بحشرات الذبابة البيضاء *B. tabcai* وأن حشرة واحدة حاملة للفيروس يمكنها نقله وإحداث الاصابة ، وأوضحت الدراسة أن الفيروس يصيب ثلاثة عشر عائلاً نباتياً تابعة لتسعة عائلات نباتية مختلفة. وأظهرت دراسة فحص الفيروس بالميكروسكوب الإلكتروني جزيئات توأمية أبعادها ٢٠×٣٠ نانوميتر، وتم استخدام المصل المضاد عديد الكولون Polyclonal antibody (TYLCV-PAB) للكشف عن الفيروس بالطرق السيرولوجية DAS-ELISA والارتباط المناعي بواسطة البصمة النسيجية على أغشية النيتروسيليولوز Tissue blotting immuno-binding assay (TBIA) ، والتنقيط Dot-blotting immuno-binding assay (DBIA) ، وقد أوضحت الدراسة كفاءة طريقة TBIA في الكشف عن الفيروس في نباتات الطماطم المصابة بالفيروس ميكانيكياً أو بواسطة الحشرات وكذلك في بعض العوائل المشخصة مثل Nicotiana glutinosa. في حين استخدم اختبار DBIA للكشف عن الفيروس في النباتات المصابة والحشرات الحاملة للفيروس Viruliferous insects. أيضاً تم الكشف عن الفيروس في النباتات المصابة بواسطة اختبار عديد البلمرة المتسلسل Polymerase chain reaction (PCR) بأستخدام البادئات ٤٩٦PARIc/١٩٧٨PALIv وتم الحصول على شظايا الحمض النووي (DNA) أحجمها ١,١ كيلو زوج من القواعد النيتروجينية والتي دلت علي أن هذا الفيروس من الفيروسات التوأمية.