Okra leaf curl virus: A monopartite begomovirus infecting okra crop in Saudi Arabia

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ABSTRACT

Okra leaf curl geminivirus (Family Geminivirdae: Genus Begomovirus) was isolated for the first time in Saudi Arabia, from okra (Abelmoschus esculentus L.) plants grown in the greenhouse of the Agricultural and Veterinary Experimental Station in Hofuf, Al-Hasa, King Faisal Univ. Okra plants were heavily infested with Bemisia tabaci Genn. showing symptoms of severe stunting, leaf crumpling, curling, malformation, vein enation and veinal necrosis. The isolated virus was designated as okra leaf curl geminivirus-Saudi Arabian-isolate (OLCV-SA). Disease incidence ranged from 15-70% and appeared related to the age of plants and infection time. In whiteflies transmission trials, a single whitefly was able to transmit OLCV-SA applying 48hr for both acquisition and inoculation access (AAP and IAP, respectively) periods. Extending both AAP and IAP increased the transmission efficiency from 13.3-93.3% applying an IAP and AAP for 48hr. Purified OLCV-SA formed peak with A_{max} and A_{min} of 258, 240, respectively. An electron micrograph of the purified virus preparation showed both geminate particles (20 x 28 nm) and monomers (15 x 17 nm). Serologic tests including indirect ELISA, tissue blotting immuno-binding assay (TBIA) and dot blot immuno-binding assay (DBIA) detected OLCV-infected okra cultivars in the field and greenhouse grown plants. Indirect ELISA detected OLCV-SA antigens in all infected materials collected from the field and greenhouse trials, while it failed to show any serologic relatedness between OLCV-SA and other geminiviruses. OLCV-PAB detected OLCV-antigen in viruliferous Bemisia tabaci Genn.. On the other hand, DBIA detected positive relatedness between OLCV and ACMV. Distant serologic relationships were observed between OLCV-SA, HLCrV and CLCMV. In contrast, no serologic relatedness between OLCV-SA, SLCV and TYLCV were found.

Key words: *Geminivirdae, geminivirus, begomoviruses, okra leaf curl virus, Bemisia tabaci, tissue blotting immuno-binding assay (TBIA) and dot blotting immuno-binding assay (DBIA).*

INTRODUCTION

Whitefly-transmitted disease agents cause significant losses throughout the world. Although not considered as important as aphids on a worldwide basis, they are responsible for the natural spread of a large number of economically important diseases in the tropical and subtropical areas. Such disease infect vegetables and fiber crops (Brown and Bird, 1992), e.g. in *Cucurbitaceae* (Brown and Nelson, 1989; Abdel-Salam *et al.*, 1997); *Leguminosae* (Fabaceae) (Brown and Bird, 1992); *Malvaceae* (Abdel-Salam *et al.*, 1999b; Mansoor *et al.*, 2001) and *Solanaceae*

(Avgelis *et al.*, 2001; Mendez-Lozano and Rivera-Bustamante, 2001).

Okra leaf curl virus (OLCV) is the name given to a complex of geminiviruses (Family *Geminiviridae*, Genus *Begomovirus*) affecting okra (bamia) (*Abelmoschus esculentus* L.) plants. The virus is characterized by twinned isometric and circular single-stranded DNA genomes. Geminiviruses, belonging to the genus *Begmovirus* have monopartite or bipartite genomes, are transmitted by whiteflies *Bemisia tabaci* Genn. and infect dicotyledonous plants (Givord, 1991; Brunt *et al.*, 1997).

Okra leaf curl virus (OKLV), a persistent geminivirus, transmitted by the whitefly *Bemisia tabaci* causes a severe disease to okra during the summer and autumn in El-Hassa Province, Kingdom of Saudi Arabia, and has become the major factor limiting okra production in the field as well as in greenhouses. Okra plants heavily infested with *B. tabaci* Genn. whiteflies showed symptoms indicative of okra leaf curl virus. Therefore, it was interesting to carry out this study to identify the disease depending on: (i) symptomatology, whiteflies-transmission and seed transmission, (ii) virus-vector relationships, (iii) serologic detection such as indirect-ELISA, tissue blotting immuno-binding assay (TBIA) and dot blot immuno-binding assay (DBIA), and (iv) serologic relationships between the virus and other geminiviruses.

MATERIALS AND METHODS

Source of virus

Samples of okra plants *Abelmoschus esculentus* L. and symptomatic or asymptomatic weeds were collected from different sites in the eastern Province (El-Hassa) in the Kingdom of Saudi Arabia. Okra and weed plants heavily infested with whiteflies exhibited leaf curling, thickening of small leaves, veinal necrosis, upward cupping and/or epinasty. Samples taken from infected plants were detected serologically using TBIA and DBIA assay (see below).

Foliar samples were collected from whitefly-infested weeds surrounding okra plants.

Whitefly transmission

Experimental transmission of OLCV-SA was attempted to test indicator hosts and/or a group of weeds commonly found in close proximity to okra plantations (fields or greenhouses) for confirmation and extension of field survey results. Whitefly-transmission studies used to transmit OLCV-SA from okra to *Malva parviflora* (cheeseweed) and back to okra. Infected okra plants cvs. Viga and Hassawi (Balady) were used to rear colonies of viruliferous *Bemisia tabaci* Genn., while non-viruliferous colonies (see below) were maintained on the same okra cultivar free of OLCV. Tested plants including okra were exposed to viruliferous and/or non-viruliferous whiteflies. For each treatment, ten seedlings were planted under glass cages in a transmission room under fluorescent light at 25 °C. Approximately 50 whiteflies per plant were introduced into the cages and allowed to feed on test plants for 48-27 hr before they were killed with insecticidal soap. All plants were monitored for disease expression for at least 4 weeks. Presence or absence of OLCV-SA in tested plants was determined by DBIA or TBIA and by back transmission to healthy okra using non-viruliferous *B. tabaci*.

Different plant species were used in this experiment such as *Amaranthus retroflexous, Gossypium barbadense, Lycopersicon esculentum, Malva parviflora* (cheeseweed), *Nicotiana tabacum* cv. Havana, *Datura stramonium, Phaseolus vulgaris, Solanum tuberosum, Cucumis sativus and Cucurbita pepo.*

Virus-vector relationships

1) Obtaining virus-free whiteflies culture

Adult viruliferous whiteflies were collected from heavily infested okra plants and reared on non-host sweet potato plants grown in glass-covered cages for 10 days for oviposition. Newly hatched were transferred again onto okra seedlings to assure the obtainment of virus-free insects. This process was repeated four times.

2) Efficiency of insect transmission

Non-viruliferous whiteflies were transferred to OLCV-infected okra plants for a 48 h-acquisition access period (AAP). Whiteflies either singly or in groups of 3, 5, 10, 15 and 20 per plant were transferred onto healthy okra seedlings (30 plants/treatment) kept under glass-cages. Insects were then given a 48 h-inoculation access period (IAP).

The minimum acquisition access period (AAP) required for OLCV-transmission (acqusition-access threshold) was determined by allowing adults of virus-free *B. tabaci* access to OLCV-infected okra plants for 1, 3, 5, 10, 15, 30 min., 1, 3, 5, 24 and 48 hr before being transferred to healthy okra plants for a 48 hr IAP using 10 insects per plant. Twenty plants were used for each treatment. Percentages of infection were recorded after 4 weeks.

To determine the minimum inoculation access periods (IAP), virus-free insects were allowed a 48 hr AAP on OLCV-infected okra plants and IAP of 1, 3, 5, 10, 15, 30 min., 1, 3, 5, 24, and 48 hr on healthy okra plants. Ten insects were used per replicate; ten insects were used per plant. Also, virus-infection was calculated from plants OLCV-SA symptoms after 4-weeks.

Seed Transmission

Okra seeds were collected from infected plants heavily infested with *B. tabaci* Genn. in the field. Seeds were treated with 2% (v/v) sodium hypochlorite for 2 min, rinsed with water several times, then planted in the greenhouse under glass-cages. Seedlings were tested for the virus presence using indirect ELISA.

Purification of OLCV-SA

Test plants (*Nicotiana tabacum* cv. Havana) 3 weeks after whiteflies inoculation access period were used as a source for virus purification according to Czosnek *et al.* (1988). All steps in purification were carried out at 4°C after initial homogenization of tissues.

Electron Microscopy

Purified virus preparations were negatively stained with 2% uranyl acetate and examined in a JEM-100 BS electron microscope in EM-Unit, Fac. of Medicine, King Faisal Univ., Dammam, Saudia Arabia.

Serologic Tests

1) Source of antisera

For okra leaf curl virus (OLCV-SA) detection, several antisera were used. Cotton leaf curl mosaic virus antiserum (CLCMV-AS), hollyhock leaf crumple virus-AS (HLCrV-AS) and tomato yellow leaf curl virus-AS (TYLCV-AS) were presented by Prof. Dr. Abdel-Salam, Plant Pathology Dept., Fac. of Agric., Cairo Univ. Polyclonal antibodies (PAB) antisera for African cassava mosaic virus (ACMV), squash leaf curl virus (SLCV) and okra leaf curl virus (OLCV) were obtained from Agdia, ElKhart, India.

2) Indirect ELISA

Indirect ELISA was run to measure the serologic relatedness between OLCV-SA and other geminviruses as described by Abdel-Salam *et al.* (1998).

3) Tissue blotting immuno-printing assay (TBIA)

The immuno-blotting procedure was done to determine the distribution of OLCV

antigen in infected tissue sections (naturally or artificially infected) as described by Fargette *et al.* (1996) and Abdel-Salam *et al.* (1997). Preparations were treated with equal volumes of TBST buffer containing 0.01 M Tris-HCl, 0.05% Tween-20 and 0.15 M NaCl, pH 8.0 in TBIA test; chromogenic substances were used for color development. Nitroblue tetrazolium (NBT)-5-bromo-4-chloro-3 indolyl phosphate (BCIP) complex was used for purple-color development in positive reaction compared with green color for negative reaction.

4) Dot blot immuno-binding assay (DBIA)

To study serologic relatedness between OLCV-SA and other geminiviruses, DBIA technique was conducted as described by Abdel-Salam *et al.* (1999a) as follows: Infected samples of different geminiviruses were provided by the author. These included African cassava mosaic virus (ACMV), bean dwarf mosaic virus (BDMV), hollyhock leaf crumple virus (HLCrV), tomato yellow leaf curl virus (TYLCV) and squash leaf curl virus (SLCV). Healthy and OLCV-infected plant samples were ground in STEP buffer (0.1M Na_2HPO_4 NaH_2PO_4, pH 8.3, containing 0.02 M of Na_2SO_3 and ethylene diamine tetra cetate (EDTA), and 1.5% Triton X-100. The samples were heated for 10 min, at 50 °C, cooled, then examined serologically. After incubation, 3h/25 °C, membranes were washed three times in TBST buffer containing 0.6% (w/v) Tris-HCl, pH 8.0, 0.019% (w/v) EDTA, 2% (w/v) polyvinylpyrroledone (PVP) and 0.05 (w/v) Tween 20, then incubated (2hr/25 °C) with goat antirabbite-AP conjugate for PAB (diluted 1/500 in TBST). Membranes were washed three times in TBST then stained.

To insure studying the virus relationships with other geminiviruses, OLCV-SA was exposed to ACMV-PAB using DBIA test. The DBIA was also performed to detect OLCV-SA inside its vector *B. tabaci* and in okra plants and other plant species after whitefly inoculation. In DBIA procedure, chromogenic substances were used for color development. This included Fast Red TR/Naphtol AS-MX complex for red color development in positive reactions.

RESULTS AND DISCUSSION

Severe symptoms of okra leaf curl disease were observed in an okra (*Abelmoschus esculentus* L.) crop grown in both the field and under plastic tunnels in the Eastern Province (El-Hassa) of Saudi Arabia, during the spring and early summer. Okra plants showing symptoms indicative of OLCV-SA were observed in both of commercial fields on Viga and Hassawi (Balady) cultivars and in plastic tunnels on South Seed and Klimthon cultivars. Symptomatic plants were severly stunted, and failed to produce normal pods (Fig. 1A); leaves were prematurely senesced (Fig. 1B). OLCV-SA-infected okra in the field on cv.Hassawi (Balady) showing leaf curl, small vein thickening followed by veinal necrosis (Fig. 1C). As the disease develops, affected leaf margins turns inwards and leaves turn downwards (2A&B respectively), as well as vein yellowing developed on the lower surface (Fig. 3). Healthy leaf is depicted as a control (Fig. 4). All okra cultivars currently available are susceptible to the disease. The results were confirmed by ELISA and TBIA procedures.

Such described symptoms are typical to symptoms induced by whitefly-transmitted begomoviruses as have been described on cotton in Egypt (Abdel-Salam *et al.*, 1999b; Bigarré *et al.*, 2001) and Sudan (Idris and Brown, 2000), on okra in Pakistan (Mansoor *et al.*, 2001) and other crops-infecting geminiviruses such as pepper (Samretwanich, 2000), tomato (Avgelis *et al.*, 2001), cotton

(Radhakrishn *et al.*, 2001) and mungbean (Usharani *et al.*, 2001).

Large populations of whiteflies *Bemisia tabaci* Genn. were noticed during the early and moderately stages of the crop. Forty days-old okra plants exhibited upward leaf curling, and stunting. Fifty percent of disease incidence was recorded in both field and plastic tunnels. Nine weeks later, 70% of the plants showed severe symptoms and extremely reduced fruit setting in plant top during summer season (Fig. 1A). In a preliminary experiment, the assumed virus could not be transmitted mechanically, but successful whiteflies transmission was obtained onto healthy okra plants. Similar results were recorded for geminiviruses incidence which ranged from 15-75% with estimated crop losses of over $ 500,000 in Greece and Puerto Rico (Avgelis *et al.*, 2001; Bird *et al.*, 2001).

Host range studies

Date presented in Table (1) indicated that OLCV-SA was *transmitted* to several weeds and plant species: *Amaranthus retroflexous, Malva parviflora* (cheeseweed), (Fig. 5), *Gossypium barbadense* (Egyptian cotton), *Abelmoschus esculentus* (Fig. 6), *Lycopersicon esculentum* and *Nicotiana tabacum* cv. Havana. Whereas, the whiteflies failed to transmit the virus to *Datura stramonium, Phaseolus vulgaris, Solanum tuberosum, Cucumis sativus and Cucurbita pepo*. The obtained results confirmed the whiteflies transmission nature of OLCV. Tested plants exposed to viruliferous whiteflies under greenhouse conditions exhibited symptoms similar but slower in appearance, when compared with naturally infected plants in the field. These results were confirmed serologically using DBIA technique (see below).

OLCV-PAB detected OLCV-antigens in several hosts. The existance of *A. retroflexous* and *Malva parviflora* (cheeseweed) grown around the plastic tunnels or in the fields, plays an important role as reservoir for OLCV. Such results are similar to those hosts reported for geminiviruses i.e., cotton leaf curl mosaic virus (CLCMV) (Ali *et al.*, 1995; Abdel-Salam, 1999b; Sharma and Rishi, 2001), tomato yellow leaf curl (TYLCV) virus (Bedford *et al.*, 1998).

Table (1): Host range of okra leaf curl virus (OLCV-SA) upon testing with viruliferous Bemisia tabaci Genn. and detected with DBIA.

Test plant	Common name	Observed symptoms	Number of infected /tested	% Infectivity	Back inoculation to okra plants	Serologic tests *	
						Indirect ELISA	DBIA
Amaranthaceae:							
Amaranthus retroflexous		IVC	12/20	60	+	+	+
Cucurbitaceae:							
Cucumis sativus	Cucumber	O			-	-	NT
Cucurbita pepo	Squash	O			-	-	NT
Fabaceae:							
Phaseolus vulgaris	Bean	O			-	-	NT
Malvaceae:							
Abelmoschus esculentus	Bamia	Cu, E, VN	17/20	85	+	+	+
Gossybium barbadense	Cotton	Vc, SVG	14/20	70	+	+	+
Malva parviflora	Cheeseweed	Cr, Cu	15/20	75	+	+	+
Solanaceae:							
Datura Stramonium	Datura	O			-	-	NT
Lycopersicon esculentum	Tomato	Cs	12/20	60	+	+	+
Nicotiana tabacum cv.Havana	Tobacco	O			-	-	NT

Cr = crumpled, Cs = chlorotic spots, Cu = cup-shape, E = enation, Ivc= interveinal chlorosis, O = no symptoms, Vc = veinal chlorosis, VN = veinal necrosis, SVG= small vein greening, NT = not tested, - & + ELISA or DBIA = positive and negative results and * = three replicates were tested per plant species.

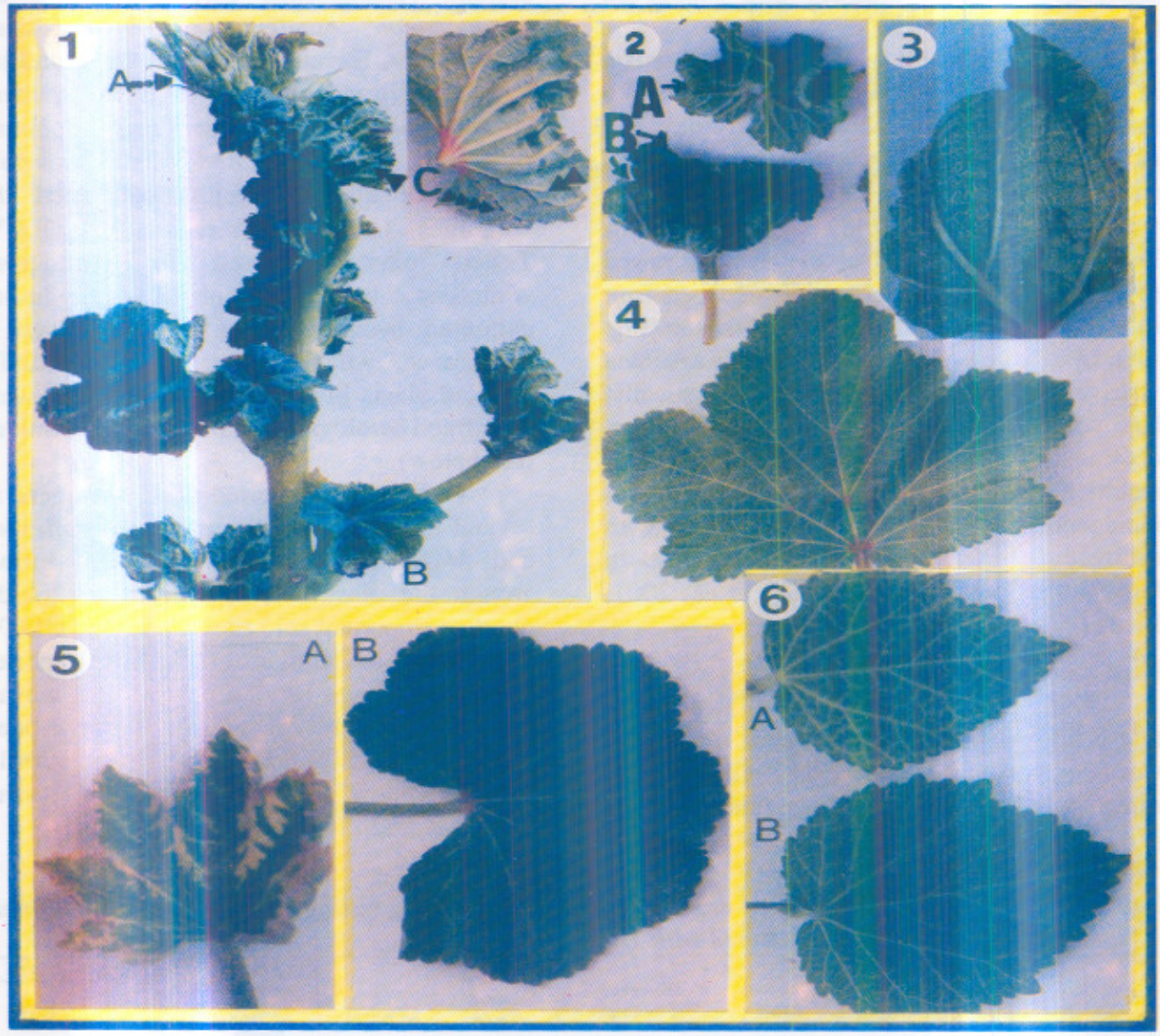

Fig. (1): OLCV-naturally infected okra cv. Viga showing malformed pods (A), leaf prematurely senesced (B) and veinal thickening and veinal necrosis.

Fig. (2): Symptoms of OLCV on okra leaves cv. Viga showing downward (A) and upward (B).

Fig. (3): Vein enation and thickening sypmtoms developed on OLCV-naturally infected okra leaves.

Fig. (4): Healthy leaf of okra.

Fig. (5): Naturally infected reservoir cheeseweed Malva parviflora showing cupping and crumpling sypmtoms (A). Healthy leaf (B).

Fig. (6): Whiteflies inoculated okra plants exhibited vein clearing and chlorosis on infected primary leaf (A). Healthy leaf (B).

Seed transmission

OLCV-SA was not detected in okra seedlings obtained from diseased plants. This results agree with the non-seed *transmission* of other geminiviruses such as tomato mottle virus (TMoV) (Polston *et al.*, 1993) and tomato yellow leaf curl virus (TYLCV) (Fargette *et al.*, 1996).

Purification of OLCV-SA

Yield of OLCV from *N. tabacum* cv. Havana leaves collected 3-weeks after whiteflies inoculation, was about 0.1 mg/250g. The average of the peak A_{max}/A_{min} was 258/240 ratio (see below Fig. 6).

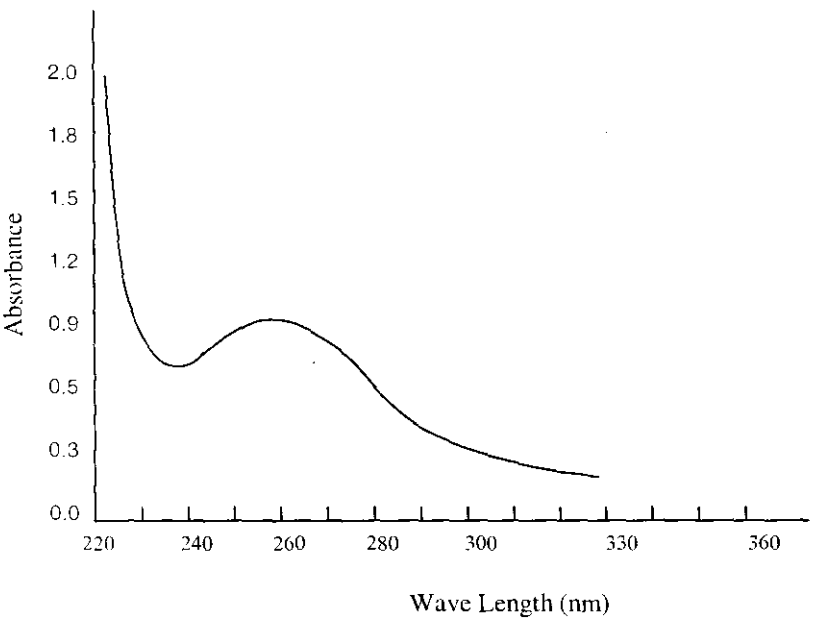

Fig. (6): Ultra violet spectrum of purified OLCV-SA preparation.

Electron microscopy (EM)

Electron microscopy examination of purified OLCV-SA showed both geminate particles (20 x 28 nm) and monomers (15x17nm) (Fig. 7 A & B respectively) that are similar to those examined for geminiviruses i.e., tomato yellow leaf curl virus (TYLCV) (Czosnek *et al.*, 1988), African cassava mosaic virus (Fauquent and Fargette, 1990); OLCV (Brunt *et al.*, 1997). While, OLCV-SA was not detected from leaf dip preparation probably due to the presence of viscous materials in okra (bamia) leaves.

Virus vector relationships

1) Whiteflies efficiency transmission OLCV-SA to test plants

The results summerized in Table (2) showed that a single adult whitefly was able to transmit OLCV-SA at a relative efficiency when applying 48 h and 24 hr for each of AAP and IAP respectively, as determined by subsequent appearance of symptoms. The maximum transmission efficiency (94.4%) was achieved using 20 insects per plant, applying 48hr and 24hr AAP and IAP. These results are resembling those obtained by

Cohen *et al.* (1983); Morales *et al.* (1990) and Mehta *et al.* (1994). Further, efficiency of transmission increased with increasing the number of insects per plant. The obtained results are in agreement with Nateshan *et al.* (1996) working on cotton leaf curl geminivirus and Ghanem *et al.* (2000) working on tomato yellow leaf curl geminivirus.

Table (2): Transmission Efficiency of OLCV-SA by Bemisia tabaci Genn.

No. of insects / plant	No. of infected / tested plant *	Transmission (%)
1	2/18	11.1
3	4/18	22.2
5	5/18	27.8
10	6/18	33.3
15	13/18	72.2
20	17/18	94.4

* Number of plants infected / number of plants tested.

2) AAP and IAP

The results in Table (3) indicate that the minimum AAP required for OLCV-SA transmission (6.6%) was 1hr. Prolonging the AAP from 1hr to 48hr, increased the transmission efficiency from 6.6 to 93.3 %.

Date revealed that the transmission rates have increased as the length of acquisition access was extended, indicating the increasing of viral titre in *B. tabaci* Genn. with longer acquisition access. Also, extending the AAP allows the insects to acquire virus particles from high viscous materials that found in okra leaf.

Longer IAP of 1, 3, 5, 24 and 48hr resulted in higher transmission frequencies up to 80.8%.

The minimum IAP required for OLCV-SA transmission (13.3%) was 30 min.

Bemisia tabaci adults were able to acquire and transmit OLCV-SA after AAP and IAP of 1hr and 30 min, respectively. This result is confirmed by the findings of Fauquent and Fargette (1990); Abdel-Salam *et al.* (1998) and Ghanem *et al.* (2000) who explained that the minimum requirement of AAP is usually higher than that of IAP. Moreover, this result confirms results of Mehta *et al.* (1994) that the latent periods of different plant viruses in their insect vectors may vary from a few hours to serveral weeks.

Table (3): Transmission rates of OLCV-SA by Bemisia tabaci Genn. after different acquisition access (AAPs) and inoculation access periods (IAPs).

AAPs	No. of infected plants / tested plants after 48h IPA	*% Transmission	IAPs	No. of infected plants tested plants after 48h AAP	*% Transmission
1 min	0/15	0.0	1 min	0/15	0.0
3 min	0/15	0.0	3 min	0/15	0.0
5 min	0/15	0.0	5 min	0/15	0.0
10 min	0/15	0.0	10 min	0/15	0.0
15 min	0/15	0.0	15 min	0/15	0.0
30 min	0/15	0.0	30 min	2/15	13.3
1 h	1/15	6.6	1 h	3/15	20
3 h	2/15	13.3	3 h	4/15	26.6
5 h	7/15	46.6	5 h	6/15	40.0
24 h	9/15	60.0	24 h	8/15	53.3
48 h	14/15	93.3	48 h	12/15	80.8

* % Transmission = Number of infected plants / number of tested plants X100.

Detection of OLCV-SA in infected tissues and viruliferous whitefiles

Several serologic procedures including indirect ELISA, TBIA and DBIA were used to detect OLCV-SA.

1) Indirect ELISA

Indirect ELISA detected OLCV-SA in all samples collected from okra and other plants grown in the field and greenhouse experiments (Table 1).

2) Detection of OLCV-SA using TBIA

TBIA was done to detect virus distribution within stem tissue in different okra cultivars grown either in the greenhouse or in the field. Obtained results indicated color formation in diseased plants either in naturally infected cultivars of okra or viruliferous-whiteflies in greenhouse (Fig. 8). No color formation (remained green) was observed in the control healthy okra tissues and non-viruliferous. Similar results have been observed by Fragette *et al.* (1996) working on tomato yellow leaf curl geminivirus (TYLCV) and Abdel-Salam (1999b) working on cotton leaf curl mosaic geminivirus (CLCMV).

3) Detection of OLCV-SA by DBIA

OLCV-PAB antiserum detected OLCV-SA in both naturally infected okra, whiteflies inoculated plants, and cheeseweed *Malva parviflora* (Fig. 9) and viruliferous whiteflies (Fig. 10). The results indicate the efficiency of DBIA for detection of geminiviruses, as well as other viruses (Makkouk *et al.*, 1993; James and Mukerji, 1996; Abdel-Salam, 1999a & 1999b; Ghanem, 2000).

Furthermore, OLCV-PAB differentiated between non-viruliferous *B. tabaci* and viruliferous insects either collected from okra plants in field or greenhouse trails (Fig.10). This confirms the efficiency of DBIA for geminivirus detection as reported by Cancino *et al.* (1995) working on bean golden mosaic geminivirus; Abdel-Salam *et al.* (1998) working on HLCrV and Abdel-Salam (1999b) working on CLCMV.

The aforementioned results indicate that OLCV-SA detection in viruliferous whiteflies and reservoir cheeseweed *Malva parviflora* may help in obtaining invaluable information about OLCV-disease forecast in okra fields, in other alternative hosts, and in virus-vector relationship studies.

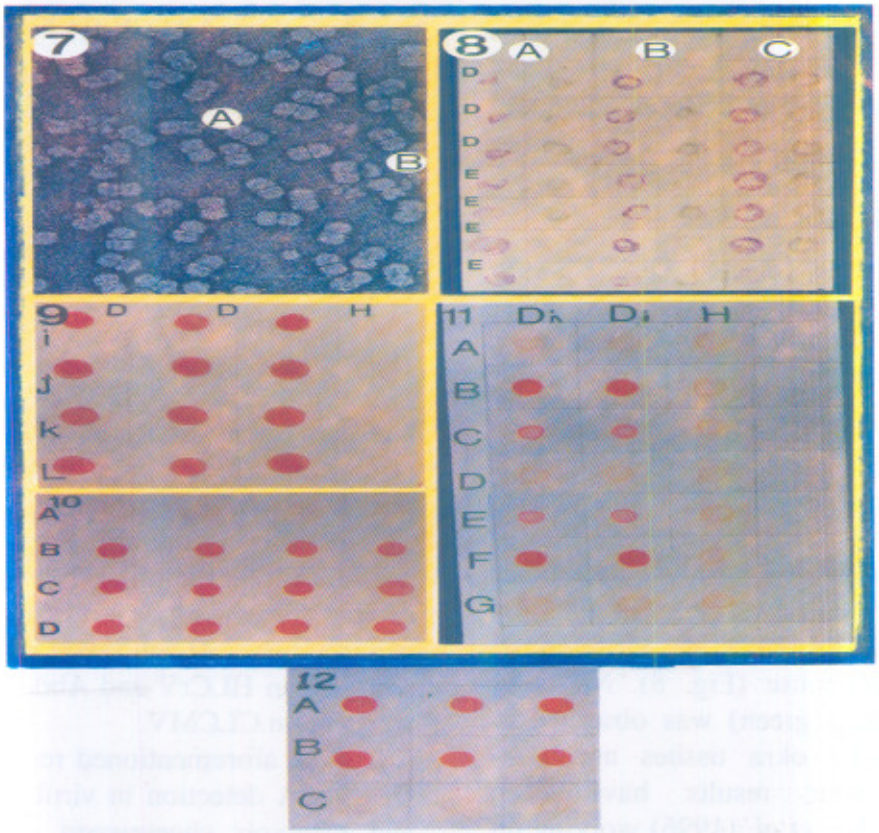

Fig. (7): An electron micrograph of purified virus preparation revealed geminivirus particles 20x28 nm and monomer 15x17nm, scale bar=100nm.

Fig. (8): TBIA detection of OLCV-SA in stems of two okra cultivars: Viga and Hassawi (Balady) growing in the field and whiteflies inoculated plants in greenhouse, respectively.

(A) Stem tip, (B) at 10cm distance from stem tip, (C) at 15cm distance from stem tip, (D)cv. Viga and (E) cv. Hassawi (Balady).

Fig. (9): DBIA detection of OLCV-SA in okra plants and reservoir chesseweed Malva parviflora.

H = Sap from healthy samples, D = Sap from infected samples.I = Naturally infected cv. Viga, j = Naturally infected okra cv. Hassawi, K = Whiteflies inoculated okra cv. Viga, L = Naturally infected reservoir chesseweed.

Fig. (10): Detection of OLCV-SA inside B. tabaci vector using DBIA.

A=Healthy (non-viruliferous) B. tabaci.B=Viruliferous B. tabaci collected from okra cv. Viga grown in the field and showing disease symptoms, C=Viruliferous B. tabaci collected from okra cv. Hassawi grown in the greenhouse.

Fig. (11): Serologic relationships between OLCV-SA and other geminiviruses using OLCV-PAB.

D= Sap from infected plants, H= Sap from healthy plants, A- Healthy okra, B-ACMV, C- HLCrV, D-TYLCV, E- CLCMV, F-OLCV, G- SLCV.

Fig. (12): DBIA showing serologic relationships between OLCV-SA and ACMV using ACMV-PAB.

A. Sap from infected okra cv. Viga (Whitefly inoculation).
B. Sap from infected chesseweed Malva parviflora
C. Sap from healthy okra cv.Viga.

Serologic relationships between OLCV-SA and other geminiviruses

Indirect ELISA failed to detect any serologic relationships between OLCV-SA and other geminiviruses. On the other hand, DBIA illustrated close serological relationships between OLCV-SA and ACMV (Fig. 11). Whilst, distant serological relationships were detected between OLCV-SA and HLCrV and CLCMV. In contrast, no serological relationships were observed between OLCV-SA, SLCV and TYLCV. Moreover, a positive relationship was confirmed between OLCV-SA and ACMV using 1/100 cross absorbed ACMV-PAB (Fig. 12).

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الملخص العربى

فيروس تجعد أوراق الباميا :إحدى الفيروسات التوأمية التي تصيب محصول الباميا في المملكة العربية السعودية

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سجلت هذه الدراسة عزل وتعريف أحد الفيروسات التوأمية المنقولة بالذبابة البيضاء *Bemisia tabaci* Genn. لأول مرة في المنطقة الشرقية الاحساء (الهفوف) بالمملكة العربية السعودية. وقد أعطى هذا الفيروس اسم فيروس تجعد أوراق الباميا (OLCV-SA) نظراً لأعراض التقزم وكرمشة وتجعد الأوراق بالإضافة إلى تضخم العروق الصغيرة وانحناء الاوراق لأعلى أو أسفل. وقد بينت الدراسة تلوث النباتات المظهرة لأعراض المرض بأعداد كبيرة من الذبابة البيضاء الناقلة له. وكانت نسبة تواجد المرض تتراوح بين ١٥-٧٠% ومرتبطة بعمر النبات وقت الإصابة. تم نقل الفيروس المسبب تجريبياً بواسطة الحشرات من نباتات مصابة إلى أخرى سليمة تنحصر في ثلاث عائلات نباتية هى النرجسية والخبازية والباذنجانية. وأثبتت تجارب النقل الحشرى أن فرد واحد من الحشرات الكاملة له القدرة على نقل الفيروس وأن زيادة كل من فترات اكتساب وتلقيح الفيروس أدت إلى زيادة انتقال الفيروس بنسب تراوحت بين ١٣,٣ – ٩٣,٣% عندما كانت فترتى الاكتساب والتلقيح ٤٨ ساعة. استهدف البحث أيضاً تنقية الفيروس من بعض العوائل المشخصة وكان أقصى وأدنى امتصاص لطيف الفيروس المنقى عند ٢٥٨، ٢٨٠ نانوميتر تساوى ١,٥. وعندما فحص الفيروس المنقى بالميكروسكوب الاليكترونى حصلنا على جزيئات الفيروس من نوعين الاولى التوأمية والتى تراوحت أبعادها بين ٢٠×٢٨ نانوميتر والثانية ١٥×١٧ نانوميتر. وقد استخدمت بعض الدراسات السيرولوجية لتعريف الفيروس وهى اختبار الاليزا واختبارى ارتباط رد الفعل المناعى (TBIA) وكذلك اختبار التنقيط (DBIA) على أغشية النتروسيليوز وأكدت الدراسة كفاءة اختبار الارتباط المناعى TBIA في الكشف عن توزيع الفيروس في أجزاء النبات على أبعاد مختلفة. أيضاً أوضح اختبار التنقيط الـ DBIA وجود الفيروس سواء في النباتات المصابة، والنباتات المختبرة للانتقال بواسطة حشرات الذبابة البيضاء بالإضافة إلى كشف الفيروس داخل الحشرة الحاملة للفيروس (Viruliferous). بالإضافة إلى ذلك استخدم اختبار DBIA لتحديد مدى القرابة السيرولوجية لفيروس تجعد أوراق الباميا وبعض الفيروسات التوأمية الأخرى. فقد تبين أن الفيروس محل الدراسة تفاعل مع الأمصال عديدة الكولون لفيروسات توأمية أخرى منقولة بالذبابة البيضاء مثل فيروس موزيك الكسافا الأفريقى (ACMV). بينما أظهر اختبار DBIA العلاقة السيرولوجية المتباعدة بين فيروس تجعد أوراق الباميا وكل من فيروس تكرمش أوراق الخطمية (HLCrV) وفيروس موزيك وتجعد أوراق القطن. كذلك أكدت النتائج عدم وجود علاقة سيرولوجية بين فيروس تجعد أوراق الباميا وفيروسات تجعد أوراق الكوسة (SLCV) وكذلك اصفرار وتجعد أوراق الطماطم (TYLCV). ولتأكيد العلاقة السيرولوجية بين الفيروس والفيروسات الأخرى التوأمية المنقولة بالذبابة البيضاء استخدام الانتسيرم الخاص بفيروس الكسافا الأفريقي (ACMV) للكشف عن فيروس تجعد أوراق الباميا (OLCV) وقد أثبتت النتائج نجاح الانتسيرم المستخدم فى الكشف عن الفيروس مما يثبت علاقتهما السيرولوجية.