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Biological and ultrastructural studies of zucchini yellow mosaic virus in squash plants

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Abstract

Zucchini yellow mosaic virus (ZYMV) was isolated from squash plants showing virus like symptoms collected from open fields of Sohag Governorate. The host range of the isolated virus was studied. Twenty six plant species and varieties belonging to nine families were mechanically inoculated with the studied zucchini yellow mosaic virus. *Lupinus sp., Vigna unguiculata, Arachis hypogaea, Convolvulus arvensis, Gossypium sp* were found to be infected systemically with the studied virus. Whereas necrotic local lesions were detected on leaves of *Cheopodium album* and *Datura metale* as a result of infection with the studied Zucchini yellow mosaic virus. Dilution end point, thermal inactivation point and longevity in vitro of the studied virus were found to be 10-3, 60 ^{o}C and 24 hours, respectively. Ultrathin sections of squash leaves infected with ZYMV revealed various ultrastructure alterations. The chloroplast, mitochondria, and nucleus were clearly affected by viral infection.

Keywords:
Zucchini, mosaic virus, squash

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INTRODUCTION

Zucchini yellow mosaic virus (ZYMV) is a member of family *Potyviridae* and is considered the most economically important virus attacking cucurbit plants under field conditions (Abd El-Aziz, 2020). One of the most commercially important viruses of cucurbit crops is zucchini yellow mosaic potyvirus (ZYMV), which was discovered in Italy in 1973, reported in 1981, and found in all continents within a decade. It is effectively aphid-transmitted and seed-borne in zucchini squash, which may have contributed to its fast expansion globally. ZYMV isolates exhibit biological heterogeneity in terms of host range, symptomatology, and aphid transmissibility. Recent research has also demonstrated serological and molecular diversity. (Desbiez & Lecoq, 1997). ZYMV is a member of the potyvirus genus (Murphy *et al.*, 1995). 750 nm long flexuous filamentous particles (Lisa *et al.*, 1981), comprised of approximately 9600 nucleotides of single-stranded RNA (Balint *et al.*, 1990). ZYMV is found in practically every country where cucurbits are planted, in temperate, subtropical, and tropical climates. It has been found in cucurbit farms or greenhouses in various European and Asian nations, Africa, and the Middle East, North and South America, and Oceania. The virus is extremely harmful in both highly automated production regions and more traditional agro-ecosystems. (Desbiez & Lecoq, 1997). Therefore, the purpose of this research was focused on biological, characterization of ZYMV isolate from Sohag Governorate. Moreover, the effect of ZYMV infection on ultra-structure of squash leaf cells was also studied.

MATERIALS AND METHODS

Sample collection

Zucchini yellow mosaic Potyvirus was identified from squash plants exhibiting virus-like symptoms (yellow mosaic, leaf curl and deformation, and fruit abnormalities) obtained from open fields in Sohag Governorate. Figure (1) showed the samples were stored at -20˚C until use.

Fig. (1): Naturally infected squash plant exhibited severe mosaic and blisters, leaves deformation which used as a source of the virus.

Host range

Twenty seven plant species and varieties belonging to different families were used. These plants are presented in table (1). These plants' seeds were planted in clay loam soil in pots (20 cm diameter). The pots were placed in an insect proof greenhouse. The plants were watered when needed.

Virus inoculation

Leaves were homogenised in 0.02 M phosphatase buffer pH=7.2 (1 g/4 ml) from systemically infected squash plants. The homogenate was filtered through a double layer of cheese cloth, and the filtrate was used as viral inoculum. At the 3-4 leaf stage, the inoculum was gently rubbed on the top leaf surface of test and host plants that had previously been dusted with carborandum (600 mesh). as described by Rawlins and Tompkins (1936). The inoculum was gently rubbed with forefinger. After inoculation the leaves were washed immediately with tap water.
The plants were monitored daily for symptoms development.

Virus stability

Dilution end point, thermal inactivation point and longevity *in vitro*
of zucchini yellow mosaic Potyvirus isolate were determined. Crude sap from squash leaves infected with the virus was used to determine the virus stability. Thermal inactivation point of the virus isolate was carried out by exposing the infectious sap in test tubes (3 ml of sap/ tube) to certain temperature degrees (50, 55, 56, 57, 58, 59 and 60ºC) for 10 minutes using thermostatically controlled water bath. Tubes were then rapidly cooled by dipping in cold water. The treated saps were used to inoculate leaves of squash plants.

The dilution end point of the virus isolate was determined by preparing dilutions of infectious sap with sterile distilled water. Dilutions prepared up to 10^{-7}. Each dilution was used for inoculation of leaves of squash plants.

Longevity *in vitro* of the virus was determined. Three ml of infectious crude sap in Stoppard tubes were kept at room temperature (24-26 ºC) for 9 days. Every day the sap in in one tube was tested on leaves of squash plants.

Ultrastructural study

Thin sections of symptomatic leaf tissue of squash plants inoculated with infectious crude sap were prepared and stained with uranyl acetate and lead citrate. The prepared thin sections were examined with transmission electron microscopy (McDowell and Trump, 1976).

RESULTS AND DISCUSSION

Characterization of zucchini yellow mosaic virus

1- Host range, symptomology and differential hosts

Twenty six plant species and varieties belonging to different families were mechanically inoculated with the studied zucchini yellow mosaic virus. The host range and the response of different plants are recorded in Table (1).

The tested plants could be divided according to their reaction into the following groups:

a-Susceptable hosts

The host range of zucchini yellow mosaic is presented in Table (1). The studied virus infected 8 plant species out of 26 tested plant species and varieties belonging to 9 families (*i.e. Fabaceae , Chenopodiaceae, Poaceae, Asteraceae, Pedaliaceae, Malvaceae, Solanaceae, Cucurbitaceae* and *Convolvulaceae*)

The susceptable host plants can be divided into the following groups (table 1).

Hosts showing systemic symptoms

Lupinus sp., Vigna unguiculata, Arachis hypogaea, Convolvulus arvensis, Cucurbita pepo, Gossypium sp were found to be infected systemically with the studied virus. These plant species exhibited mosaic mosaic, chlorosis vein banding and malformation symptoms (Figure 2). Previously, similar findings about these hosts' responses to ZYMV were described. (Al-Shahwan, 1990 .; Lisa *et al.*, 1981 .; Lisa and Lecoq, 1984 .; Provvidenti, 1984 .; Stobbs *et al.*, 1990 and Wong, 1992).

Hosts showing local lesions symptoms

As shown in Figure (3) necrotic local lesions were detected on leaves of *Cheopodium album* and *Datura metale* as a result of infection with the studied Zucchini yellow mosaic virus. Similar results were obtained by Al-Ani *et al*. (2011).

b- Non infected hosts

As shown in Table (1) eighteen plant species belonging to different families were found to be resistant to the stied virus, since no symptoms were observed after the mechanical infection.

2- Virus stability in sap

As shown in tables (2, 3 and 4) dilution end point, thermal inactivation point and longevity *in vitro* of the studied virus were found to be 10^{-3}, 60 ºC and 24 hours, respectively. Similar results were obtained by El- Baz (2004); Mochizuki and Ohki (2012) and Abdel-Wahed (2012).

Ultrastructural study

As shown in Figure (4) ZYMV infection caused various ultrastructure changes in squash leaf cells. ZYMV infection clearly had a negative effect on chloroplasts, mitochondria, and the nucleus. Several viral infections have been related to the formation of an aberrant membrane system within mitochondria. (Francki, 1987 and Khalifa *et al.*, 2015).

Table (1): Plant species' responses to the Zucchini yellow mosaic virus in greenhouse conditions

Family	English name	Scientific name	Symptoms		Incubation period (days)
			Local	Systemic	
Fabaceae	Faba bean	*Viciafaba*	No	No	-
	Green beans	*Phaseolus vulgaris*	No	No	-
	Alfalfa	*Medicago sativa*	No	No	-
	Clover	*Trifolium alexandrinum*	No	No	-
	Lupine	*sp Lupinus*	No	M	4-5
	green beans (cowpea)	*Vigna unguiculata*	No	M	8-9
	Peas	*Pisum sativum*	No	No	-
	Fenugreek	*Trigonella foenum-graecum*	No	No	-
	Peanut	*Arachis hypogaea*	No	M	3-5
Convolvulaceae	Bindweed(lablab)	*Convolvulus arvensis*	No	M	3-7
Cucurbitaceae	Egyptian cucumber	*Cucumis Melo var. flexuosus*	No	No	-
	Pumpkin	*Cucurbita pepo*	No	M	5-6
	Cucumber	*Cucumissativus*	No	No	-
	Watermelon	*Citrulluslanatus*	No	No	-
	Muskmelon	*Cucumismelo*	No	No	-
Solanaceae	Tomato	*Solanum lycopersicum*	No	No	-
	Pepper	*Capsicum annuum*	No	No	-
	Datura	*Datura metel*	NLL	No	7
Malvaceae	Cotton	*Gossypium sp.*	No	M	10-13
Pedaliaceae	Sesame	*Sesamumindicum*	No	No	-
Compositae (Asteraceae)	Sunflower	*Helianthusannuus*	No	No	-
Poaceae	Maize (Corn)	Zea mays	No	No	-
	Sorghum	*Sorghum bicolor*	No	No	-
	Pearlmillet	*Pennisetumglaucum*	No	No	-
Chenopodiaceae	Suger beet	*Beta vulgaris*L	No	No	-
	Goosefoot	*Chenopodium album*	NLL	No	7

M= Mosaic NLL= Necrotic local lesions No= No symptoms

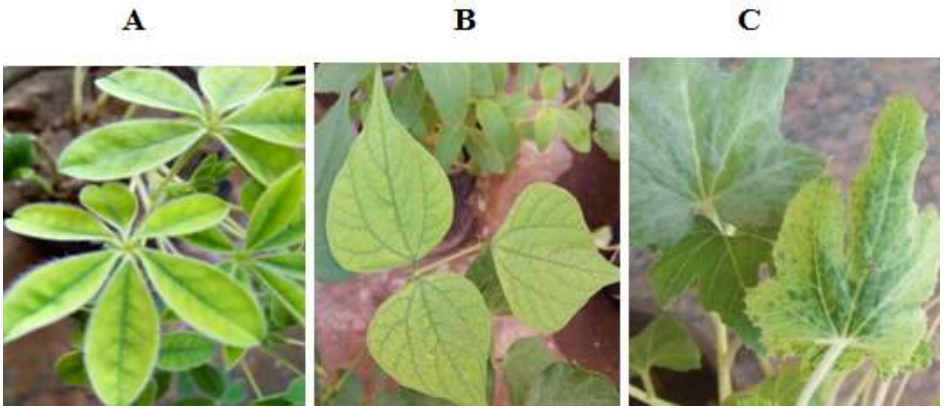

Figure (2) *Lupinus* sp. (A), *Convolvulus arvensis* (B) and *Cucurbitapepo*(C) infected with Zucchini yellow mosaic virus showing mosaic, chlorosis vein banding and malformation.

Figure (3): Necrotic local lesions on *Cheopodium album* (A) and *Datura metale* (B) inoculated with Zucchini yellow mosaic virus.

Table (2) Dilution end point of Zucchini yellow mosaic virus.

Dilutions of the crude sap					
Undiluted	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
+	+	+	-	-	-

Table (3) Thermal inactivation point of Zucchini yellow mosaic virus.

Temperature (ºC)							
40	**50**	**55**	**56**	**57**	**58**	**59**	**60**
+	+	+	+	+	+	+	-

Table (4) Longevity in vitro of Zucchini yellow mosaic virus.

Incubation period at room temperature (hours)									
0	**24**	**48**	**72**	**96**	**120**	**144**	**168**	**192**	**216**
+	+	-	-	-	-	-	-	-	-

+= Infectious - = Non infectious

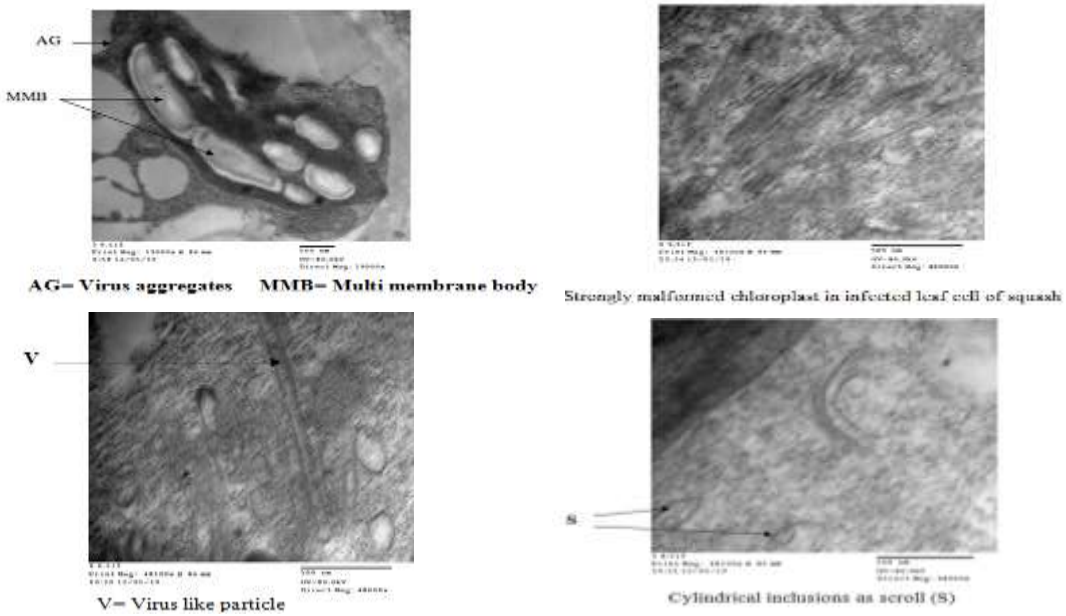

Figure (4) Ultrathin sections of squash leaves infected with ZYMV-EG display a variety of ultrastructure alterations.

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الملخص العربي

عزل وتوصيف فيروس موزايك الزوكيني الاصفر في نباتات الكوسة النامية بمحافظة سوهاج

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تم عزل فيروس موزايك الزوكيني الاصفر من نباتات كوسة مصابة طبيعيا ويظهر عليها اعراض الاصابة الفيروسية والتي تم جمعها من الحقول بمحافظة سوهاج . لدراسة المدي العوائلي للفيروس تم اجراء عدوي صناعيه لستة وعشرون نوع نباتي ينتمي لتسعة عائلات نباتية مختلفه. وقد تبين ان نباتات الترمس واللوبيا والفول السوداني والقرع واللبلاب اظهرت اصابة جهازية . بينما وجد ان نباتات الداتورا والزربيح اظهرت اصابة موضعية . بدراسة درجة التخفيف النهائي لعصير النباتات المصابة وجد 10^{-3} كما تبين ان درجة التثبيط الحراري للفيروس هي 60 درجة مئوية . وقد وجد ايضا ان مدة بقاء الفيروس في العصير المعدي علي درجة حرارة الغرفه هي 24 ساعة . بددراسة التركيب الدقيق لنباتات الكوسة المصابة بالفيروس وجد ان الفيروس يؤثر علي خلايا الاوراق المصابة حيث ادت الاصابة الي تحورات في المكونات الخلوية ومنها تحلل الكلوروبلاستيدات وتشوهات في الميتوكوندريا وتكون زوائد في الاغشية البلازميه كما لوحظ تجمعات فيروسية وتواجد جزيئات فيروسية في السيتوبلازم .