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Identification of A Virus From Naturally Infected Garlic Plants

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The isolated virus was identified according to host range, symptomatology, differential hosts, virus stability, modes of transmission and electron microscopy. The virus has narrow host range. Dilution end point was found to be 10-2, thermal inactivation point was determined as 65oC and infectivity was retained for one day only. The virus transmitted mechanically, and by means of *Myzus persicae* and *Aphis fabae*. Green peach aphid was found to be more effective in virus transmission than broadbean aphid. Virus transmission through vegetative propagation reached 100% in both cultivars (Chinese and Balady) with different degrees of symptoms severity. Plant height, leaf area, fresh and dry weight of bulb were decreased significantly with the increasing of severity symptoms on garlic plants of both cultivars. The electron microscope preparations of crude sap extracted from naturally infected garlic plants and negatively stained with 2% PTA showed flexuous rod shaped particles 657-714 nm long.Biological relationships between the virus and insect vectors were studied.

INTRODUCTION

In Egypt garlic is considered one of the main vegetable crops, it is not only used as a food but also is important from the medical point of view. It is grown commercially in most parts of the country as winter crop.* The cultivated area has decreased from 25374 feddans in 1996 to 16632 feddans in 1997 producing 255488 and 159111 ton, respectively. The most common varieties cultivated in Egypt are the Balady and followed by the Chinese.

Garlic is subjected to numerous viruses such as *Garlic mosaic virus* (La, 1973 and Dijh *et al.*, 1993), *Garlic yellow streak virus* (Mohamed and Young, 1981 and Dijh *et al.*, 1993) and *Onion yellow dwarf virus* (El-Kewey and Sidaros, 1996).

Garlic is propagated vegetatively and thus likely to harbour viruses. Virus diseases of garlic are wide spread throughout the world, and losses in crop yields and deterioration of quality due to virus infection. (Mohamed and Young, 1981; Walkey and Antill, 1989; Walkey, 1990; Conci and Nome, 1991; Lot *et al.* 1994,and Tsuneyoshi and Sumi, 1996).

Throughout the world, garlic crop is infected with viruses which cause mosaic symptoms and chlorotic stripes on the leaves, reduce the size of the plants and the crop yield. Description and characterization of different garlic viruses have been confusing and complex on a world basis (Bruna *et al.*, 1992).

Viruses of *Allium* spp. have attracted considerable attention recently, those of garlic and shallot are especially topical internationally, because these crops are of great economic importance and their vegetative propagates are distributed world wide (Bos, 1983 and Walkey, 1990).

Therefore, the present investigation was undertaken to isolate and identify garlic mosaic virus on the

* Ministry of Agric., report of Economic Agric. sector (1997).

basis of host range, physical properties, mode of transmission and electron microscopy.

MATERIALS AND METHODS

A. Solation and identification of the virus

The virus was isolated and identified according to host range, symptomatology, modes of transmission, physical properties and electron microscopic examinations.

1. Host range, symptomatology and differential hosts

Plant species belonging to the following plant families: Chenopodiaceae, Solanaceae, Fabacae, Amaranthaceae, Cucurbitaceae ,Composiae, Cruciferae, Malvaceae, Graminaceae, Euphorbiaceae, Polygonaceae, Portulacaceae, Primulaceae, Liliaceae, and Apocynaeae were inoculated with sap expressed form garlic diseased plants under greenhouse conditions.

Immune hosts were observed at regular intervals. Back inoculations were made from *vicia fabae*, *Pisum sativum*, *Allium porrum*, *Allium cepae*, *Allium kurrat* to *Ch. amaranticolor* in order to check the absence of virus in symptomless plants.

2.Virus stability

Dilution end point, thermal inactivation point and aging *in vitro* of the virus were determined. Crude sap expressed from infected garlic (*Allium sativum*) leaves were used. *Chenopodium amaranticolor* was used as a test plant.

3. Modes of transmission

A- Mechanical transmission

Leaves of garlic (*Allium sativum)* infected with garlic mosaic isolate were collected and homogenized in a morter with a pestle. The infectious sap was pressed through cheesecloth and applied to 10 healthy seedlings of *Raphanus sativus* (Radish) and *Ch.amaranticolor* previously dusted with 600 mesh carborundum, additional 10 seedlings were left as a control.

B. Insect transmission

Individuals of the *Myzus persicae* and *Aphis fabae* collected from growing cabbage and faba bean, respectively were identified by Entomology Department, Faculty of Agriculture, Kafr El-Sheikh. Individuals of *Myzus persicae* and *Aphis fabae* were reared onto healthy Cabbage and faba bean, respectively. New generation of aphids were transferred to other healthy plants of cabbage and faba bean grown in isolated insect proof cages and left for reproduction. Several virus free adults of each of the above mentioned aphids were starved for one hour. The adults were divided into two groups.

The first group was allowed to feed on garlic plants infected with the studied virus isolate for acquisition feeding period of 5 min. There after the aphids were transferred to healthy seedlings of radish and were allowed to feed for 30 min. The second group was permitted to feed on healthy radish plants as a control., then the insects were killed by Azodrin (0.07 %). The inoculated plants were then arranged in insect proof greenhouse at 25-28°C, observed of virus symptoms and percentage of transmission was recorded.

C. Virus transmission through vegetative propagation (bulbs).

An experiment was conducted under filed condition at the farm of the Faculty of Agric, Kafr EL-Sheikh.Twenty bulbs (collected from Kafr El-Sheikh city market) of each

two infected plant cultivars (Chinese and Balady) were divided into cloves and planted in the field. Virus symptoms were observed at regular intervals. Six weeks after planting, data of disease symptoms was evaluated according to mosaic and malforation degrees and the percentage of infection was recorded.

The effect of virus infection on some morphological characters of garlic plants was studied. Plant height, leaf area, fresh and dry weight of bulb were determined. Leaf area was measured by area meter model 3100. Bulbs were dried at 70°C for 24 h in hot air oven.

4. Electron microscopy (Negative staining)

Extracts of infected garlic leaves were prepared as described by Sampson and Taylor (1968). 2% PTA (phosphotungistic acid) stain was mixed (1/1/v) with 1:10 diluted leaves extract. Carbon baked, parlodin coated grids were floated on droplets of stained sap mixture. Five min. later, excess fluid was removed with filter paper. After air drying grids were examined using an electron microscope.

B. Biological relationship between the virus and insect vectors.

1. Acquisition threshold period

Individuals of non-viruliferous green peach aphids (*M. persicae*) were starved for one hour, then were given terminated acquisition feeding periods of 5, 10, 20, 40 and 60 minutes on infected garlic plants. Viruliferous aphids were transferred immediately to healthy seedlings of *Raphanus sativus* for 5 minutes. One insect was used per plant. Inoculated plants were sprayed by Azodrin (6ml./L) and ranging in an insect proof greenhouse, under observation.

2. Inoculation threshold period:

Individuals of *Myzus persicae* were starved for one hour, then were given a limit acquisition feeding period of ten minutes. Viruliferous aphids were transferred to healthy seedlings of *Raphanus sativus*, where they were given terminated inoculation threshold period of 5, 10, 20, 40 and 60 minutes.One insect was used per plant. Aphids were killed by Azodrin after the required period. Inoculated plants were kept under observation in an insect-proof greenhouse.

RESULTS AND DISCUSSION

A Identification of the virus

1. Host range, symptomatology and differential hosts:

Thirty eight plant species and cultivars belonging to fifteen families were mechanically inoculated by the virus isolate .The reaction of the inoculated plants are presented in Table (1).

Results presented in Table (1) indicate that the under investigated virus isolate has a limit host range. The tested plants could be divided into two groups.

I.Susceptible hosts

A. Hosts showing systemic symptoms

Three plant species only showed systemic symptoms. Garlic plants (*Allium sativum*) showed chlorotic spots and dashes developed to mosaic, severe mosaic, yellow striping and malformation on garlic leaves (Fig. 1)

Radish (*Raphanus sativus*) and turnip (*Brassica rapa*) plants showed vein clearing within ten days of inoculation, then developed to mild mottling and mosaic accompanied with slightly marginal malformation

B -Hosts showing local infection

One plants species only (*Chenopodium amaranticolor*) reacted with local chlorotic lesions with narrow red marginal (Fig.2). *Ch. amaranticolor* plants was used as an indicator plant for the isolated virus.

Thirty-five plant species and cultivars of the inoculated plants showed no symptoms. Back inoculation with extracted sap of these hosts on *Ch. amaranticolor* thowed that they are virus free plants. These immune plants are:

11. Immune hosts

Table 1: The reaction of different hosts to infection with the isolated virus.

Tested plants			Infection	Incubation period (day)	Symptoms
Family	Common name	Scientific name			
Amaranthaceae	Amaranth	*Amaranthus retrufflex*	-	-	I
	Globe amaranth	*Gomphrena globosa*	-	-	I
Chenopodiaceae	Gosse foot	*Chenopodium amaranticolor*	-	8 ±1	L.L.
	Pigweed	*Ch. Album*	-	-	I
	Pigweed	*Ch.ambroiodes*	-	-	I
	Pigweed	*Ch.murala*	-	-	I
	Sugar beet	*Beta vulgaris*	-	-	I
Crociferae	Cabbage	*Brassica oleracea*	-	-	I
	Turnip	*B. rapa*	15	9±1	Vc. M
	Roquete	*Eraca sativus*	-	-	I
	Radish	*Raphanous sativus*	25	9±1	Vc. M. Mal
	London rochet	*Sisymbrium irio*	-	-	I
Liliaceae	Egypt leek	*Allium kurrat*	-	-	I
	Leek	*Allium porrum*	-	-	I
	Onion	*Allium cepae*	-	-	I
	Garlic	*Allium sativaum*	100	9±1	Chl.S M. Mal
Cucurbitaceae	Squash	*Cucurbita pepo*	-	-	I
	Cucumber	*Cucumis sativus*	-	-	I
Fabaceae	Faba bean	*Vicia fabae*	-	-	I
	Pea	*Pisium sativum*	-	-	I
	Cow pea	*Vigina sinensis*	-	-	I
Malvaceae	Cotton	*Gossypium barbadence*	-	-	I
	Littlemallow	*Malva parviflora*	-	-	I
Solanaceae	Pepper	*Capsicum annum*	-	-	I
	Stramony	*Datura stramonium*	-	-	I
	Tomato	*Lycopersicon esculentum*	-	-	I
	Tobacco	*Nicotiana glutionsa*	-	-	I
	Tobacco	*N. tabacum*	-	-	I
	Garden petunia	*Petunia hybrida*	-	-	I
	Egg plant	*Solanum melongena*	-	-	I
		Datura metle	-	-	I
Polygonaceae	Dock (sorrel)	*Rumex dentatus*	-	-	I
Portulaceae	Common parslane	*Portulaca oleraacea*	-	-	I
Primulaceae	Scrlet pimpernel	*Anagallis arvensis*	-	-	I
Euphorbiaceae	Castor oil plant	*Ricinus communis*	-	-	I
Apocynaceae	Periwinkle	*Vinca rosae*	-	-	I
Compositae	Annuls owthisle	*Sonchus oleraceus*	-	-	I
Graminaceae	Maize	*Zea mays*	-	-	I

Chl.S=Chlorotic spots, LL= local lesions, Vc = Vein clearing, M = Mosaic, Mal = Malformation, I=Immune, - = No external symptoms.

Amaranthus retrufflex, *Gomphrena globosa*, *Ch. album*, *Ch ambroioides*, *Ch. mural*, *Beta vulgaris*, *Brassica oleracea*, *Eruca sativa*, *Sisymbrium irio*, *Allium kurrat*, *Allium porrm*, *Allium cepae*, *Cucurbita pepo*, *Cucumis sativus*, *Vicia fabae*, *Pisum sativum*, *Vigina sinensis*, *Goossypium barbadence*, *Malva parviflora*, *Capsicum annum*, *Datura stramonium*, *Lycopersicon esculentum*, *Nicotiana glutinosa*, *Petunia hybrida*, *Nicotiana tabacum*, *Solanum melongena*, *Datura metle*, *Rumex dentatus*, *Ricinus communis*, *Vinca rosae*, *Sonchus oleraceus*, *Portulace oleracea*, *Anagallis arvensis*, and *Zea mays*.

The obtained result indicated that the causal virus has narrow host range restricted to one plant species from family *Liliaceae* "garlic" (La, 1973; Bos *et al*. 1978; Mohamed and Young 1981 and EL Kewey and Sidaros 1996) and certain *crociferae* plant species "radish and turnip". On the other hand there is no any literature handled the reaction of radish and turnip as host plants of *Garlic mosaic viruses*. *Chenopodium amaranticolor* showed chlorotic Lesions as a result of infection with the studied virus. This result is in agreement with (La, 1973; Sulyo, 1986; Conci and Nom1991 and El-Kewey and Sidaros1996).

2. Virus stability

Dilution end point, thermal inactivation point and longevity in vitro were determined for the virus isolate. The obtained data show that dilution end point was 10-2, thermal inactivation point was 65 oC and infectivity is retained at room temp. for one day only. These results are in line with those obtained by La (1973), Ahlawat (1974), Mohamed and Young (1981), and Conci and Nom (1991).

3. Modes of transmission
a- Mechanical transmission

The obtained results elucidate the following

1- The isolated virus was found to be readily transmitted by infectious sap extracted from *Allium sativum*.

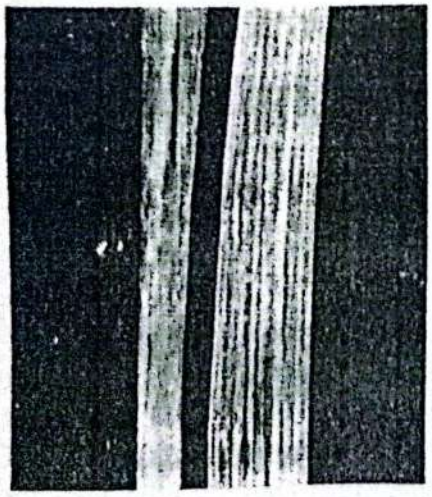

Fig. (1) : Severe mosaic induced by garlic mosaic disease on leaves of Allium sativum.

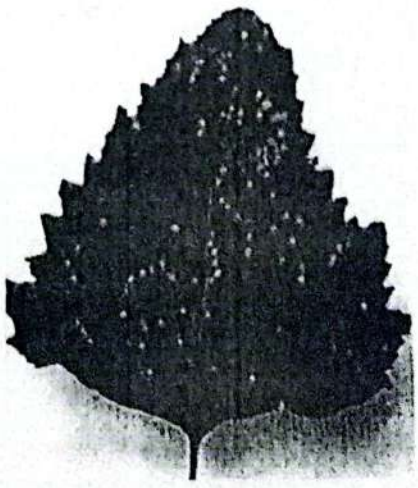

Fig. (2): Chlorotic local lesions, induced by the virus isolate *Chenopodium amaranticolor*.

2-Sap transmission affected by the inoculated host. The virus under study was transmitted to 25% and 15% of *Raphanus sativus* and *Brassica rapa*, respectively. The obtained results showed that the virus under study could be transmitted by sap. This result is in agreement with La (1973), Ahlawat (1974), Sulyo (1986), Graichen and Leistner (1987), Conci *et al.* (1992) and El-Kewey and Sidaros (1996).

b. Insect transmission

Myzus persicae sulz, *Aphis fabae* were used to transmit the virus isolate from infected garlic leaves to radish seedlinges. Aphids were fed on diseased leaves for 5 minutes then they were transferred to healthy seedling. Results show that *Myzus Persicae* could transmit the virus to 20 % of the inoculated plants, whereas *Aphis fabae* could transmit it to 15% of inoculated plants.

Several investigators reported that the causal of mosaic diseased garlic was transmitted by *M. persicae* and *A. gossypi* (Mohamed and Young, 1981; Sulyo, 1986; Sako *et a*L., 1990; Yamashita *et a*L., 1991 and El- Kewey and Sidaros, 1996).

c. Virus transmission through vegetative propagation (bulbs)

Data represented in Tables (2 and 3) showed that the percentage of virus transmission through bulbs reached 100 % in both cultivars. GMV transmission trough garlic cloves was reported by La (1973).

The effect of virus infection on some morphological characters of garlic plants was studied. Plant height, leaf area, fresh and dry weight of bulb were significantly decreased by increasing the severity of symptoms on plants of both Chinese and Balady cultivars (Table 4).

Table (2): Virus transmission through vegetative propagation (bulbs of cv. Balady).

Bulbs	Healthy plants	Severity of symptoms				No. of infected plants	% of infection
		+	++	+++	Severe		
1	-	1	26	15	2	44	100
2	-	2	40	15	1	58	100
3	-	-	45	14	1	60	100
4	-	5	45	10	-	60	100
5	-	-	27	19	-	46	100
6	-	2	30	20	8	60	100
7	-	-	40	15	1	56	100
8	-	4	40	15	1	60	100
9	-	-	32	25	-	57	100
10	-	5	35	20	-	60	100
11	-	-	45	15	-	60	100
12	-	2	40	15	-	57	100
13	-	3	20	30	1	54	100
14	-	6	20	25	9	60	100
15	-	8	25	25	2	60	100
16	-	4	30	25	1	60	100
17	-	1	20	30	5	56	100
18	-	5	25	30	-	60	100
19	-	2	30	25	-	57	100
20	-	3	30	20	-	53	100
Total	-	53	645	408	32	1138	100

+ = Slight mosaic
++ = mild mosaic
+++ = Severe mosaic
Severe = Severe mosaic and malformation

Table (3): Virus transmission through vegetative propagation (bulbs of cv. Chinese)

Bulb	healthy plants	Severity of symptoms				No. of infected plants	% of infection
		+	++	+++	Severe		
1	-	-	-	-	5	5	100
2	-	2	5	7	6	20	100
3	-	2	7	5	6	20	100
4	-	2	11	2	5	20	100
5	-	-	10	2	8	20	100
6	-	1	10	8	1	20	100
7	-	4	2	5	9	20	100
8	-	4	5	8	3	20	100
9	-	3	11	1	5	20	100
10	-	2	10	3	5	20	100
11	-	-	6	5	9	20	100
12	-	4	5	8	3	20	100
13	-	4	4	5	7	20	100
14	-	5	7	5	3	20	100
15	-	3	5	5	7	20	100
16	-	2	10	8	-	20	100
17	-	3	10	7	-	20	100
18	-	-	9	11	-	20	100
19	-	-	15	3	-	18	100
20	-	2	12	6	-	20	100
Total		43	154	104	82	383	100

Table (4): Effect of virus infection on some morphological characters of garlic plants

Garlic cultivar	characters	Severity of symptoms			
		+	++	+++	severe
Balady	Plant height (cm)	107.201 a	101.9 a	80.60 b	68.10 c
	Leaves area/plant (cm^2)	410.25 a	338.06 b	253.76c	72.08 d
	Fresh weight (g)	119.99 a	87.44 b	65.74 c	16.74 d
	Dry weight (g)	91.37 a	62.43 b	41.96 c	7.22 d
Chinese	Plant heig it (cm)	81.10 a	71.70 b	50.5 c	48.90 c
	Leaves area/plant (cm^2)	452.20 a	281.28 b	237.16c	226.05 c
	Fresh weight (g)	138.38 a	100.49 b	58.04 c	43.56 d
	Dry weight (g)	114.39 a	78.67 b	36.18 c	25.77 c

Means Followed by a common letter are not significantly different at 5 % level by DMRT.

Mohamed and Young (1981), Walkey *et al*. (1989), Walkey (1990), Conci and Nome (1991) and Lot *et al*. (1994) and Tsuneyoshi and Sumi (1996), reported that virus diseases of garlic caused losses in crop yields and deterioration of quality.

4. Electron microscopy (morphology of the virus particles)

Flexuous virus particles were consistently found in negatively stained leaf extract preparation from virus infected leaves of *Allium sativum* (Fig. 3). The individual particles measured about (657–714 nm) long. These results are similar in this respect to those reported by Mohamed and Young (1981), Brunt *et al*. (1990) and Choi *et al*. (1992).

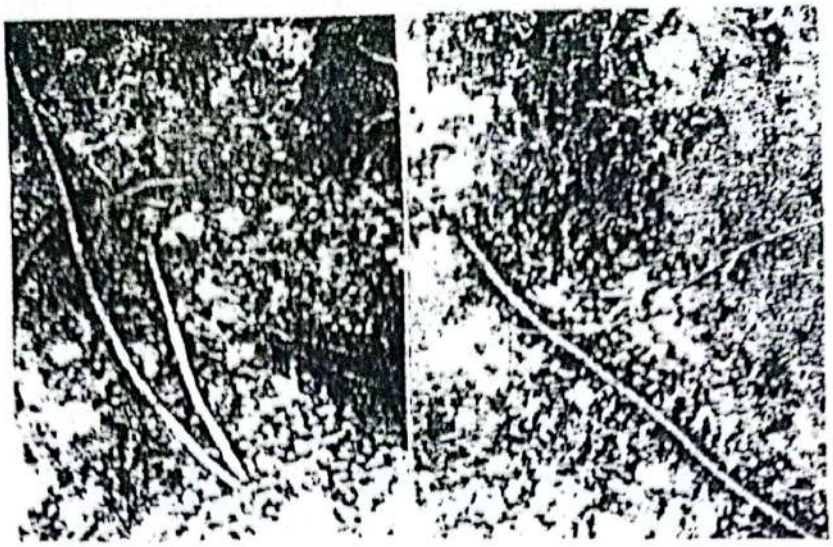

Fig. 3: Virus particles in crude sap of garlic mosaic diseased leaves, negatively stained in 2% phosphotungestic acid (X70, 000)

B. Biological relationship between virus and insect vector.

1 Acquisition threshold period:

The data presented in Table (5) indicate that:

a- The insect could acquired *Garlic mosaic virus* after feeding for 5 minutes on infected plants for both aphids *Myzus persicae* and *Aphis fabae.*

b- The efficiency of *Myzus persicae* was decreased by the increasing of acquisition period and failed to transfer the virus after 20 min. whereas *A. fabae* failed after 5 min.

2. Inoculation threshold period:

Fasted single individuals of both of *Myzus persicae* and *Aphis fabae* were fed on infected garlic plants for 5 minutes and thereafter were transferred to healthy radish seedlings. where they were left to feed for different periods (single adult/plant).

Results demonstrated in table (6) show the followings:

a-The virus was transmitted by both of M. persicae and Aphis fabae.

b- The efficiency of green peach aphids in transmitting the virus isolate decreased by increasing of feeding period and failed to transmit the virus after 10 min. whereas *A. fabae* failed to transmit the virus after 5 minutes.

The periods recorded for acquisition and inoculation are due to that the virus isolate can be transmitted by the tested aphids in non-persistent manner.

Table (5): Determination of the acquisition threshold period of garlic mosaic virus through *Myzus persicae* and *Aphis fabae:*

Aphids	Feeding period (Min.) on the virus source				
	5	10	20	40	60
M.persicae	4/6*	2/6	1/6	0/6	0/6
Aphis fabae	1/6	0/6	0/6	0/6	0/6

* Infected plants/inoculated plants

Table (6): Determination of the inoculation threshold period of the virus isolate using *Myzus persicae* and *Aphis fabae*.

Aphids	Feeding period (min.) on the healthy plants				
	5	10	20	40	60
M. persicae	4/6*	2/6	1/6	0/6	0/6
Aphis fabae	1/6	0/6	0/6	0/6	0/6

* Infected plants/inoculated plants

According to the obtained results which mentioned above, it could be concluded that the causal virus which isolated from mosaic diseased garlic plants, may be *Garlic mosaic virus* which considered as a member of *Carlavirus* group.

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