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The Influence of Gibberellic Acid (GA) and Indole Acetic Acid (IAA) on The Production of Local Lesions on *Nicotiana sylvestris* Plants Infected With *Tomato Mosaic Tobamovirus* (ToMV)

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Tomato mosaic disease caused by*Tomato mosaic virus* (ToMV) is widespread wherever tomato is grown. ToMV affects plants and yields. Virus was isolated and identified on the base of symptomatology on tomato plants, host range, diagnostic hosts, physical properties, mode of transmission and ELISA detection. ToMV had a longevity *in vitro* (LIV) of 90 days at room temperature, dilution end point (DEP) of 10^{-6} and thermal inactivation point (TIP) of 90C. ToMV was easily transmitted by sap. The obtained results revealed that ToMV was not transmitted by any insects used in this study. The identification of ToMV was confirmed serologically using ELISA technique. The effectiveness of gibberellic acid (GA) and indole acetic acid (IAA) against*Tomato mosaic virus* (ToMV) *in vitro* and *in vivo* has been evaluated. GA and IAA reduced *in vitro* and *in vivo* the infectivity of ToMV to a certain extent, expressed as the number of local lesions induced by ToMV on *Nicotiana sylvestris*. This reduction increased gradually by increasing GA and IAA concentrations. ToMV inhibition percentages induced by GA and IAA varied according to the time of treatment (1, 2, 3 and 4 hours). High percentages of inhibition were recorded for *in vitro* treatment. ToMV inhibition of pre-inoculation treatment was higher than that of post-inoculation treatment. IAA was more effective than GA.

INTRODUCTION

Virus diseases are the major limiting factor of tomato production and cause its deterioration (Hollings and Huttinga, 1976; Zitter and Isai, 1981 and Agranovsky & Anisimoff, 1986). Tobamoviruses contain more than a dozen rod-shaped viruses that cause serious losses in their hosts by damaging the leaves, flowers and fruits and by causing stunting of the plant (Agrios, 1997). *Tomato mosaic virus* (ToMV) is widespread wherever tomato is grown. Symptoms of ToMV in tomato occur as a pale-and dark-green mosaic on the young leaves which became malformed, and stunting of the plants. The most damaging symptom is necrosis in leaves, along the stems and petioles and on the fruits (Singh, 1983 and Sutic *et al.*, 1999).

Many investigators have studied the effect of gibberellic acid and indole acetic acid on different plant viruses (Mukherjee and Raychaudhuri, 1966; Raychaudhuri and Mishra, 1974; Datta *et al.*, 1980; Shukla and Joshi, 1981; Rao *et al.*, 1983; Hecht, 1984; Rajasegar and Jeyarajan, 1984; Sharma and Varma, 1986; Pizarro, 1989; Cheema *et al.*, 1991 and Mougheith & Gendiah, 1991). Dubey (1983) found that the growth of potato plants infected with *Potato virus X* was increased by treating them with 100 ppm of GA. Zherebchuk (1984) revealed that treatment of potato plants with GA before PVX-infection favored plant growth and increasing resistance to infection.

This investigation was carried out to study the effect of gibberellic acid (GA) and indole acetic acid (IAA)

on *Tomato mosaic tobamovirus* (ToMV) infectivity *in vitro* and *in vivo*.

MATERIAS AND METHODS

Chemicals were kindly obtained from Prof. Dr. A.A. El-Shewy, Professor of Plant Physiology, Agricultural Botany Department, Faculty of Agriculture, Fayoum, and Cairo University. Different concentrations of GA and IAA were prepared separately in distilled water: 100, 200, 400 and 500 ppm (w/v). All experiments were repeated twice. Five replicates were used for each treatment.

Virus isolation

Tomato plants (*Lycopersicon esculentum*) cv. Cassel Rock showing typical symptoms of ToMV infection were collected and enfolded in wet paper until used. Inoculum was prepared by grinding (1:1, w/v) infected tomato leaf tissues in phosphate buffer solution (pH 7.0) with sterilized pestle and mortar, then pressing the wet pulp through two layers of cheesecloth. The obtained crude sap was used in mechanical inoculation of one month old *Nicotiana sylvestris* plants. Mechanical inoculation was performed by the method described by Rawlins and Tompkins (1936). Plants were kept in an insect proof green-house. Inoculated plants were checked daily for the appearance of local lesion symptoms. When symptoms appeared on the above mentioned indicator plant, back inoculation onto *Nicotiana sylvestris* and *Lycopersicon esculentum* cv. Cassel Rock was carried out to insure ToMV presence. Virus was propagated into *Lycopersicon esculentum* cv. Cassel Rock using single local lesion obtained from *Nicotiana sylvestris* leaves.

Identification of virus isolate

Ten seedlings of the following plants were inoculated with sap obtained from virus-infected tomato leaves: *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana sylvestris*, *Gomphrena globosa*, *Datura metel*, *Nictiana glutinosa*, Lycopersicon *esculentum*, *Cucumis sativus*, *Vicia faba*, *Capsicum annum*, *Pisum sativum*, *Gossypium barbadense*, *Cucurbita pepo* and *Lactuca sativa*

Physical properties

Thermal inactivation point(TIP), longivity *in vitro*(LIV) and dilution end point (DEP) of the isolated virus were determined as described by Noordam (1973).

Insect transmission

Myzus persicae, *Aphis faba e.* and *Aphis craccivora* were used to transmit the virus isolate from infected tomato leaves cv. Cassel Rockto healthy seedlings of the same cultivar. Insect colonies were initiated by rearing individual insects on healthy seedlings of cabbage (*Brassica oleracea* L.) grown in insect proof cages. For transmission test, insects were fastened for a bout one hour in petri dishes and then given an acquisition feeding for about 10 min. on the virus infected tomato plants (source of the virus) then transferred to healthy tomato plants for an inoculation feeding of about 10 min. Insects were later killed by spraying with 1% Malathion and plants were maintained in an insect proof green-house. Ten aphids were used per plant and ten healthy plants were also used for this experiment. The same procedure was used for the control except that virus-free aphids were used.

Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) method described by

Clark and Adams (1977) was used for rapid serological detection of *Tomato mosaic virus* (ToMV).

Effect of GA and IAA on ToMV infectivity

For testing the effect of different concentrations of GA and IAA (100, 200, 400 and 500 ppm w/v) for different time intervals (1, 2, 3, and 4 hours) on ToMV infectivity *in vitro*, 1 ml of the expressed sap containing virus was added to 1 ml of each of GA and IAA concentrations (100, 200, 400 and 500 ppm), mixed well and allowed to stand for 1, 2, 3 and 4 hours. Distilled water was used as a control. Virus-GA and IAA mixtures and the control were inoculated into one month old *Nicotiana sylvestris* at previously mentioned. The developed local lesions were counted and the percentages of inhibition were calculated from the following formula according to Taha and Mousa (2000).

% Inhibition = (control -treatment) x100/ control

Effect of GA and IAA on ToMV infectivity

1. Pre-inoculation treatment

1 ml of each GA and IAA concentrations was rubbed on leaves of *Nicotiana sylvestris*, then they mechanically inoculated with ToMV infected sap (1ml/plant) at different intervals: 1, 2, 3, and 4 hours respectively. Distilled water was used as a control.

2. Post-inoculation treatment

The former steps in pre-inoculation were applied except that, virus infected sap was applied first followed by GA and IAA treatments.

RESULTS AND DISCUSSION

Virus isolation

Samples collected from naturally infected tomato plants were tested and the virus was obtained from a single local lesion produced on *Nicotiana sylvestris* test plant. To insure the purity of the isolated virus, three cycles of consecutive serial transfer of single local lesion developed on *Nicotiana sylvestris* were carried out. Virus was maintained on tomato seedlings which were used as a source of virus during subsequent studies. Virus was easily transmitted by sap. *Nicotiana sylvestris* was used as a local lesion diagnostic host because it reacts by ToMV with local lesions and by *Tobacco mosaic virus* (TMV) with systemic infection (Sherwood and Fulton, 1983 and Sutic *et al.*, 1999).

Virus identification

According to symptomatology on tomato plants, diagnostic hosts, physical properties, insect transmission, and ELISA, the isolated virus was identified as *Tomato mosaic virus* (ToMV).

1. Symptoms

Symptoms of ToMV in tomato occur as mosaic on the young leaves, leaf malformation and stunting of the plants.

2. Diagnostic hosts

The reaction of certain host range to the isolated virus can be summarized in Table (1).

Data obtained in Table (1) agreed with that obtained by Osman (1980); Matthews (1993) and Sutic *et al.* (1999).

3. Physical properties

ToMV was found to be inactivated at temperature of 90 C, dilution end point of 10^{-6} and after 90 days at room temperature. Similar results were obtained by Hollings and Huttinga (1976); Agranovsky and Anisimoff (1986); El-Sanusi *et al.*

(1991); Brunt (1996); and Sutic *et al.* (1999).

4. Insect transmission

The obtained results indicated that ToMV could not be transmitted by any insects used in this study. These results agreed with that obtained by Osman (1980) and Brunt (1996).

5. Enzyme linked immunosorbent assay (ELISA)

Results showed the possibility of using ELISA as a tool for rapid detection of ToMV (Briand *et al.*, 1982; Dekker *et al.*, 1987; Dore *et al.*, 1987 and Takahashi *et al.*, 1989).

Effect of GA and IAA on ToMV infectivity

The results obtained from Table (2) and Fig. (1) show that the inhibitory effect of GA against ToMV infectivity increased gradually by increasing GA concentrations from 100 to 500 ppm. The highest effect of GA against ToMV infectivity was at 500 ppm and after 4 hours (percentage of inhibition was 65.51 %).

Data obtained from Table (3) and Fig. (2) show that the inhibitory effect of IAA against ToMV infectivity increased gradually by increasing IAA concentrations from 100 to 500 ppm. The highest effect of GA against ToMV infectivity was at 500 ppm and after 4 hours (percentage of inhibition was 68.96 %). IAA was more effective in reducing the local lesions produced by ToMV on *Nicotiana sylvestris* than GA.

Table (1): Host range and diagnostic hosts of ToMV under green-house conditions.

Diagnostic host	Family	Reaction
Gomphrena globosa L.	Amaranthaceae	Necrotic local lesions
Chenopodium amaranticolor Coste&Reyn	Chenopodiaceae	Necrotic local lesions
C. quinoa L.	Chenopodiaceae	Necrotic local lesions
Lactuca sativa L.	Compositae	-
Cucumis sativus *L. cv. Madina*	Cucurbitaceae	-
Cucurbita pepo L. cv. Eskandarany	Cucurbitaceae	-
Vicia faba L. cv. Giza 402	Faba ceae	-
Pisum sativum L. cv. Little Marvel	Faba ceae	-
Gossypium barbadense L. cv. Giza 83	Malvaceae	-
Nicotiana sylvestris L.	Solanaceae	Necrotic local lesions
Datura metel L.	Solanaceae	Necrotic local lesions
Nictiana glutinosa L.	Solanaceae	Necrotic local lesions
Lycopersicon esculentum Mill cv. Cassel Rock	Solanaceae	Mosaic
Capsicum annum L. cv. California Wonder	Solanaceae	Mild mosaic

(-) No reaction

Table (2): Effect of different concentrations of Gibberellic acid on local lesions number produced by ToMV on *Nicotiana sylvestris* at different intervals

Time intervals	Mean number of local lesions								
	Control	GA concentrations(ppm)				% Inhibition			
		100	200	400	500	100	200	400	500
One hour	30	26	22	19	16	13.33	26.67	36.67	46.67
Two hours	29	23	19	16	13	20.68	34.48	44.82	55.17
Three hours	31	19	16	13	11	38.70	48.38	58.06	64.51
Four hours	29	17	14	12	10	41.37	51.72	58.62	65.51

Table (3): Effect of different concentrations of Indole acetic acid on local lesions number produced by ToMV on *Nicotiana sylvestris* at different intervals.

Time intervals	Mean number of local lesions								
	Control	IAA concentrations(ppm)				% Inhibition			
		100	200	400	500	100	200	400	500
One hour	30	25	21	18	14	16.67	30.00	40.00	53.33
Two hours	29	21	18	15	12	27.58	37.93	48.27	58.62
Three hours	31	20	15	12	10	35.48	51.61	61.29	67.74
Four hours	29	15	13	11	9	48.27	55.17	62.02	68.96

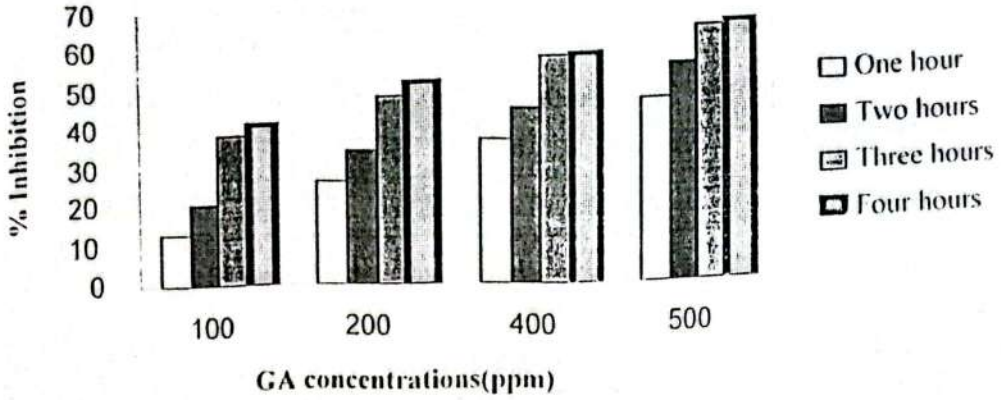

Fig. (1): % of inhibition produced by gibberellic acid on ToMV infected sap

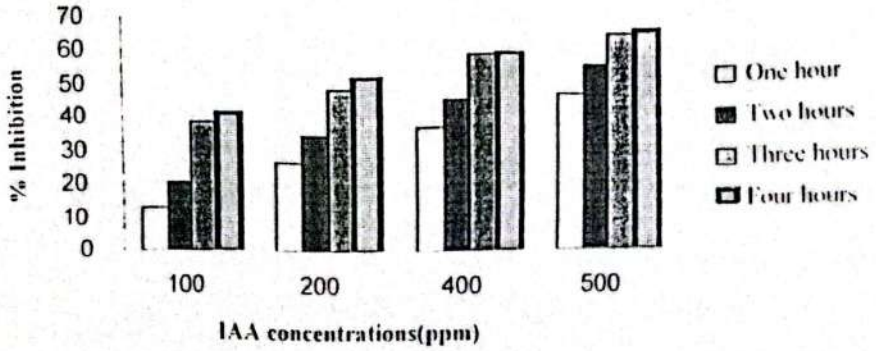

Fig. (2): % of inhibition produced by indole acetic acid on ToMV infected sap

Effect of GA and IAA on ToMV infectivity

Data obtained from Table (4) and Fig. (3) indicate that better inhibitory effect of GA was obtained by pre-inoculation treatment than post-inoculation one. Effect of GA increased gradually by increasing GA

concentrations. In pre-inoculation treatment, the percentages of inhibition at 500 ppm were 43.33, 51.72, 61.29 and 62.06 % after 1, 2, 3 and 4 hours respectively. While in post-inoculation treatment, the percentages of inhibition at 500 ppm were 36.67, 48.27, 58.06 and 58.62 % after 1, 2, 3 and 4 hours respectively.

Similar results were obtained in Table (5) and Fig. (4) using IAA. In pre-inoculation treatment the percentages of inhibition at 500 ppm were 50, 51.72, 67.74 and 68.96 % after 1, 2, 3 and 4 hours respectively. While in post-inoculation treatment the percentages of inhibition at 500 ppm were 46.67, 48.27, 64.51 and 65.51 % after 1, 2, 3 and 4 hours respectively. IAA was more effective than GA.

Table (4): Effect of different concentrations of Gibberellic acid on local lesions number produced by ToMV on *Nicotiana sylvestris* at different intervals.

Time intervals	Mean number of local lesions																
	Control	Pre-inoculation								Post- inoculation							
		GA concentrations(				% Inhibition				GA concentrations(				% Inhibition			
		100	200	400	500	100	200	400	500	100	200	400	500	100	200	400	500
One hour	30	27	23	20	17	10	23.33	33.33	43.33	28	24	21	19	6.67	20	30	36.67
Two hours	29	24	20	17	14	17.24	31.03	41.37	51.72	26	22	19	15	10.34	24.13	34.48	48.27
Three hours	31	22	17	14	12	29.03	45.16	54.83	61.29	23	18	15	13	25.8	41.93	51.61	58.06
Four hours	29	19	15	12	11	34.48	48.27	58.62	62.06	20	16	13	12	31.03	44.82	55.17	58.62

Table (5): Effect of different concentrations of Indole acetic acid on local lesions number produced by ToMV on Nicotiana *sylvestris* at different intervals

Time intervals	Mean number of local lesions																
	Control	Pre-inoculation								Post- inoculation							
		IAA concentrations(ppm				% Inhibition				IAA concentrations(ppm				% Inhibition			
		100	200	400	500	100	200	400	500	100	200	400	500	100	200	400	500
One hour	30	27	23	17	15	10	23.33	43.33	50	26	22	19	16	13.33	26.67	36.67	46.67
Two hours	29	24	21	15	14	16.67	27.58	48.27	51.72	23	19	16	15	20.68	34.48	44.82	48.27
Three hours	31	22	17	12	10	30	45.16	61.29	67.74	20	16	13	11	35.48	48.38	58.06	64.51
Four hours	29	18	14	10	9	37.93	51.72	65.51	68.96	18	14	11	10	37.93	51.72	62.06	65.51

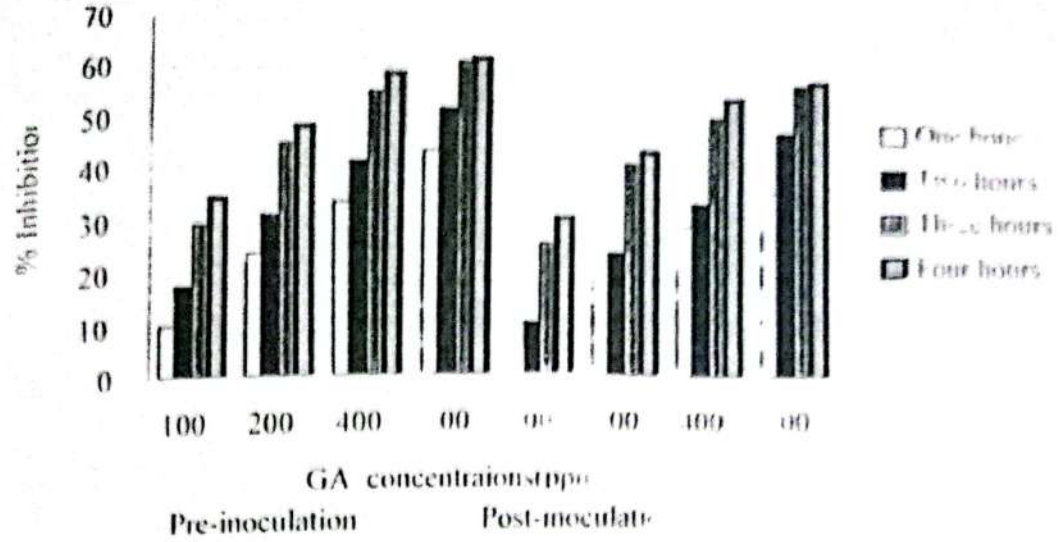

Fig. (3): % of inhibition produced by gibberellic acid on ToMV infection

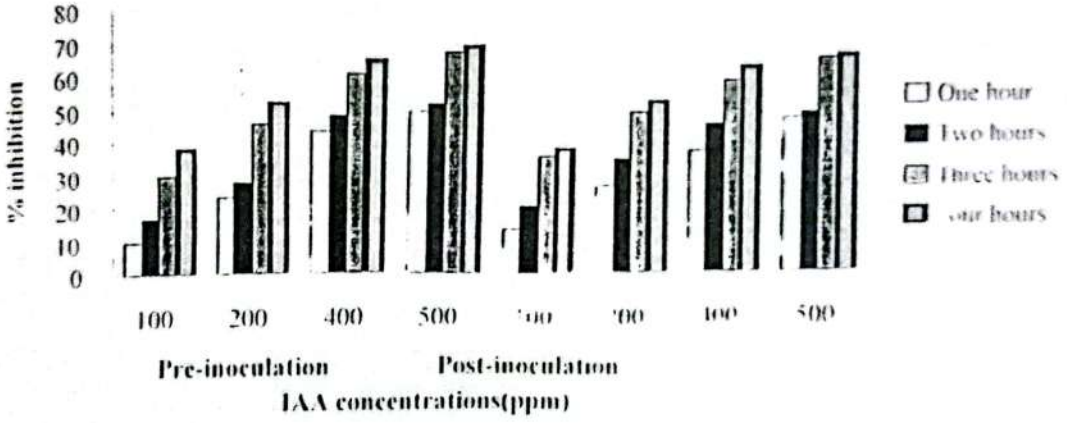

Fig. (4): % of inhibition produced by indole acetic acid on ToMV infection

These findings are compatible with that of Shukla and Joshi (1981) they found that maximum inhibition (70%) of the infectivity of sugarcane mosaic virus was obtained with coconut milk and gibberellic acid. Application before inoculation was better than that after inoculation. Rao *et al.* (1983) revealed that treatment of *Rice tungro virus* (RTV)-infected rice plants with GA at 2ug/ml negated the effects RTV infection. Hecht (1984) found that GA was most effective against PVY in potato plants, followed by piperonyl butoxide and abscisic acid, then ethrel, kinetin, phytin acid and (least effective) ribavirin. Rajasegar and Jeyarajan (1984) reported that, the percentage of tungro virus-infected rice plants after root dipping in growth regulators gradually decreased as the IAA and GA concentrations increased. IAA was more effective than GA. Sharma and Varma (1986) revealed that, IAA, GA, IBA and 2-thiouracil were effective in reducing the infectivity of *Cowpea Banding mosaic virus*. The inhibitory

effect of post-inoculation sprays was not as high as that of sprays before inoculation. IAA and 2-thiouracil were more effective than other tested chemicals. Pizarro (1989) found that leaf sheaths of infected rice plants by RTV lengthened 2 weeks after application of gibberellic acid, and became free of disease symptoms. Cheema *et al.* (1991) found that application of GA, IAA and other chemicals inhibited the infectivity of *Cucumber mosaic virus*. Mougheith and Gendiah (1991) stated that, spraying vines affected by a fan leaf-like disease with 50 ppm GA3 or 125 ppm tetracycline controlled the disease.

The inhibitory effect of GA and IAA on the virus infectivity can be interpreted in terms of changes in host cell metabolism and induced formation of some inhibitors, or interfere with the protein synthesis of the virus, or may alter the gene transcription(mRNA) of the virus, or may be related to cell elongation rather than to antiviral activity because gibberellic acid and indole acetic acid have biological activity in stimulation of cell elongation or cell division or both(Devlin, 1975; Lepold and Kriedmann, 1975; Noggle and Fritz, 1983).

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