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Induced Resistance in Tomato Plants by IAA against *Fusarium oxysporum lycopersici*

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Abstract

The phytohormone IAA (indol-3-acetic acid) was tested *in vitro* on growth of tomato wilt pathogen *Fusarium oxysporum lycopersici*. The hormone reduced spore germination, mycelial dry weight and protein content. Such reduction was matched with the elevation in the hormone concentration. The *in vivo* application of IAA to soil of the uninoculated plants (controls) improved growth and yielded longer shoot and root, particularly at low concentrations. Moreover, the hormone could prevent completely any chance for disease incidence by soil pathogens. Presence of IAA in soil of inoculated plants not only reduced the infection rate but also increased plant growth, causing that they appeared healthy and normal. Disease suppression in tomato plants, exerted by application of IAA, was achieved through either increasing plant growth, exerting a direct harmful effect on the target pathogen and/or inducing resistance in host tissue. The induced resistance was correlated with induction of certain secondary metabolites which may have a role in increasing tolerance in tomato plants to the pathogen.

Key words: IAA, wilt, phytopathogens, secondary metabolites, host

Introduction

Fungal disease management is an essential requirement in crop production. Chemical control is adequate, but it represents threats to the environment. Accordingly, it is necessary to develop environmentally friendly methods for disease control. Plant extracts, plant growth hormones or other natural products are recently substituted the chemical control (Bekheit, 2002).

Plant hormones, indol-3-acetic acid (IAA) included, are well known to be bioactive growth regulators controlling stem elongation, flowering and seed germination. Yet, they have been established to induce a concomitant resistance against phytopathogen attack through regulation of defense response mechanisms in plants (Mayda *et al*., 2000). Among these mechanisms is biosynthesis of certain secondary metabolites such as silicon-containing compounds, amino acids, phytoalexins and others. IAA induces some metabolites which stimulate resistance in *Phaseoulus vulgaris*, against *Collitotrichum vulgaris* (Huges and Dickerson, 1990). However, these secondary metabolites are sometimes developed spontanously when plants coevolved with pathogens, in a trial for resistance (Agrios, 1997). Tomato plants produce some phenolics and benzoic acid derivatives under *F. oxysporum lycopersici* stress (Vidhyasekaran, 2001).

On the other hand, the role of IAA in controlling diseases is not restricted to induce resistance in host cells, but it may extend to the pathogen itself. The inhibitory activity of IAA was detected for several phytopathogenic fungi like *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis*, *Hilmenthosporium sativum* and *Phytophthora capisici* (Lu *et al*., 2000).

The objective of this study aims at the *in vitro* effects of IAA on *Fusarium oxysporum lycopersici*, which cause tomato root wilt, and the possibility of the *in vivo* application to control the disease. Induced resistance in the infected tissues pre and post hormonal application, in the light of dynamic changes in secondary metabolites, was also discussed.

Experimental

Materials and Methods

Pathogen. *Fusarium oxysporum f. sp. lycopersici* was isolated from wilted tomato seedling and maintained on Dox's agar medium.

Plant material. A susceptible strain of tomato (*Lycopersicum esculentum* Mill Marmand cv.) was provided by Agricultural Research Center, Giza, Egypt.

Hormone. Indol-3-acetic acid (IAA, Sigma-12886) was used at concentrations 10, 25, 50 and 75 µg ml^{-1}.

***In vitro* assay**

1. Spore germination. Fresh spore suspension of the pathogen was mixed with IAA at the desired concentrations, placed into depression glass slides (0.2 ml), in triplicates, and covered with a sterile edge-greased cover glass; control was without hormone. The slides were incubated at 28°C for suitable period, at the end of which the germinated spores were counted and the percentage germination was calculated.

2. Mycelial growth. Flasks (250 ml) contained 50 ml Czapek Dox's medium and supplied with the used concentrations of IAA, were inoculated with 5 mm diameter agar disc of a 7-day old culture of the pathogen. The flasks were incubated at 28°C for 7 days and the mycelia produced were harvested and dried at 60°C till constant weight. Three replicates were used for each treatment and control was without hormone.

3. Protein content. The protein content (mg g^{-1} d.wt.) of the fungal mats was detected according to Lowry *et al.* (1951).

***In vivo* assay**

1. Cultivation, inoculation and treatment of tomato plants. Cultivation and inoculation of tomato plants were carried out according to Fuchs and Sacristan (1996). Six 14-days old tomato seedlings were cultivated, under green house conditions, in 25 cm diameter pots containing 2 kg of natural (non sterilized) sandy clay soil. Four sets of plants were used in triplicates. Two sets were inoculated with 2 ml of the pathogen spore suspension (10^6 ml^{-1}) at the upper 10 cm of the soil, whereas the other two were left without inoculation (healthy). The following treatments were established: 1. uninoculated untreated controls; 2. uninoculated plants (controls) treated with different concentrations of IAA; 3. inoculated plants (diseased) and 4. inoculated plants treated with different concentrations of IAA.

IAA was applied to the soil 2 days after inoculation. All the plants were placed under natural light conditions at 20–25°C and irrigated regularly by equal volume of water. The experiment was terminated after 8 weeks and it was carried out twice. The results of both were averaged.

2. Phytopathological analysis. Disease symptoms was assessed 30-days post inoculation and/or treatment with IAA using a scale of five classes: 0 = no symptoms, 1 = slight and few lesions on the main root and epinasty of older leaves, 2 = roots yellowed and moderate lesions cover the root, yellowing of lower leaves and stunting of the plant, 3 = leaves wilted and the root system affected and 4 = plants severely stunted, the root system was completely destroyed and marginal necrosis of the remaining leaves. Disease index (DI) was calculated according to Leath *et al*. (1989) using the formula: $DI = (1n_1 + 2n_2 + 3n_3 + 4n_4)\ 100/4N_t$ where $n_1 \sim n_4$ is the number of plants in indicated class and N_t is the total number of plants tested.

3. Plant growth and chlorophyll content. At the end of the experiment the fresh and dry weight (g/plant) and shoot and root length (cm) of the plants under different treatments were estimated. The chlorophyll content (mg g^{-1} fresh wt) was determined according to Vernon and Seely (1966).

4. Phytochemical analysis. Ethanol extract of the plants at different treatments were qualitatively analysed for their composition of secondary metabolites. The best concentration of IAA which achieved effective disease suppression and maximal plant growth, determined from the previous experiments, was chosen for phytochemical analysis. A gas chromatography [GC Mass spectrometer detector (MSD)] Device, Model 5973 Network G (6890 N-Aglient, U.S.A.) was used for analysis.

Statistical analysis. The data shown in Table I were means ± standard errors. Data in Table III were means and L.S.D. at 1% confidence limits was calculated.

Results

***In vitro* assay**

Spore germination and mycelial growth. Growth criteria of tomato wilt pathogen *F. oxysporum lycopersici* were markedly inhibited by IAA at all tested concentrations; such inhibition was concentration dependent (Table I). Complete suppression for either spore germination or mycelial dry weight was reached at 50 and 75 µg ml^{-1} IAA, respectively.

Mycelial protein. Presence of different concentrations of IAA, in growth medium of the target pathogen exerted a reduction in mycelial protein which was matched with the increase in hormone concentration (Table I).

***In vivo* assay**

Phytopathological analysis. The *in vivo* studies revealed that, few uninoculated plants showed minor disease symptoms (11% infection, class 1). Application of IAA completely prevented disease development at 25 µg ml^{-1} and above (Table II). Inoculation of tomato plants with *F. oxysporum lycopersici* resulted in heavily infection of all the plants (100% infection, class 2, 3, 4). A gradual reduction in disease incidence was observed as a response to application of different concentrations of IAA. The infection rate was reduced to 22% at 25 µg ml^{-1}, whereas at 50 and 75 µg ml^{-1} it was reduced to 17 and 11%, respectively.

Table I
Effect of different concentrations of IAA (µg ml^{-1}) on growth criteria of *Fusarium oxysporum lycopersici*

IAA concentration (µg ml^{-1})	**Spore germination (%)**	**Dry weight (g)**	**Protein (mg g^{-1} D.wt.)**
0.0 (Control)	72.4 ± 3.24	1.450 ± 0.150	1.005 ± 0.095
10	51.7 ± 2.16	0.545 ± 0.043	0.401 ± 0.025
25	9.3 ± 0.218	0.253 ± 0.015	0.317 ± 0.018
50	0.0	0.101 ± 0.009	0.183 ± 0.011
75	0.0	0.0	–

Data are mean ± standard error.

Table II
Effect of different treatments with IAA (µg ml^{-1}) on disease incidence by *F. oxysporum lycopersici* on tomato plants

Plants	IAA (µg ml^{-1})	Class					Disease index	Infection rate (%)
		0	1	2	3	4		
Uninoculated untreated plants	0.0 (control)	16	2	0	0	0	3	11
Uninoculated treated plants	10	17	1	0	0	0	1	6
	25	18	0	0	0	0	0	0
	50	18	0	0	0	0	0	0
	75	18	0	0	0	0	0	0
Inoculated plants	0.0 (diseased)	0	0	1	4	13	92	100
Inoculated treated plants	10	11	4	2	1	0	15	39
	25	14	3	1	0	0	7	22
	50	15	3	0	0	0	4	17
	75	16	2	9	0	0	3	11

Table III
Fresh and dry weight (g/plant) shoot and root length (cm) and chlorophyll content (mg g^{-1} fresh wt.) of tomato plants as affected by different treatments with IAA (µg ml^{-1})

Plants	IAA (µg ml^{-1})	Fresh wt. (g/plant)	Dry wt. (g/plant)	Shoot length (cm)	Root length (cm)	Chlorophyll content (mg g^{-1} fresh wt.)
Uninoculated untreated plants	0.0 (Control)	28.7	7.1	22.3	9.1	18.7
Uninoculated treated plants	10	30.1	7.4	25.4	9.2	18.9
	25	32.0	7.6	27.5	9.5	18.8
	50	28.5	6.8	20.8	8.5	18.5
	75	26.1	6.1	19.5	7.8	18.3
Inoculated untreated plants	0.0 (diseased)	22.1	5.4	18.1	7.6	13.6
Inoculated treated plants	10	24.8	6.2	19.9	8.6	16.4
	25	27.1	6.9	21.6	9.0	17.8
	50	25.8	6.0	20.1	8.3	16.0
	75	24.2	5.5	19.8	7.9	15.1
L.S.D. at 1%		1.3	0.3	1.2	0.21	0.94

Plant growth and chlorophyll content. Low concentrations of IAA (10 and 25 µg ml^{-1}) slightly increased fresh and dry weight and root and shoot length of the uninoculated controls (Table III). Such increment was more evident with shoot length particularly at 25 µg ml^{-1} where it increased by 1.2 – fold of the untreated controls. At 50 and 75 µg ml^{-1}, IAA exerted a significant reduction in growth parameters

Table IV
Phytochemical characterization for secondary metabolites of tomato plants after treatment with 25 µg ml^{-1}IAA

Library of compounds							
Uninoculated untreated plants (control)	S	Uninoculated treated plants	S	Inoculated untreated plants (diseased)	S	Inoculated treated plants	S
Dimethylamine	56	Acetic acid	86	Acetic acid	91	Pentadecane	95
Cyclobutanol	52	Neophytadiene	91	Dimethylamine	43	Cycloheptasiloxane	93
Acetic acid	72	Benzene dicarboxylic acid	93	Butaned iamine	72	Methylglycine	43
Neophytadiene	92	Hexadecanoic acid	99	Propylene glycol	64	Dimethyl phthalate	78
Benzene dicarboxylic acid	53	Dibutyl phthalate	95	Indol	80	Hexadecane	95
Hexadecanoic acid	98	Butanol	64	Propenamide	38	Methanone	97
Dibutyl phthalate	95	Butanoic acid	58	Furanone	74	Benzene methanol	90
Benzenaldehyde	97	Oxime methoxyphenyl	83	Phenol	49	Benzene acetic acid	90
Benzen propanol	90	Ethyl oleate	98	Pyrrolidinone	93	Pyrralidinone	22
Benzene acetic acid	87	N-octylphthalate	92	Salicylic acid	98	Propenone	43
Tributyrin	64			Methyl salicylate	92	Benzenedicarboxylic acid	60
				Pyridine	98	Amino benzoic acid	59
				Neophytadiene	99	Cyclotrisiloxane	38
				Hexadecanoic acid	90	Benzoic acid	95
				Vinylcyclodecane	90		
				Phytol	91		
				Linoleic acid	99		
				Benzenedicarboxylic acid	93		

S: Means similarity % of the compound.

especially with root and shoot length. No statistical difference was observed between chlorophyll contents of controls before and after IAA application.

With regard to the infected plants, all growth criteria were markedly inhibited under pathogen stress. Amendment of different concentrations of IAA improved growth of the infected plants and a progressive increase in fresh and dry weight and shoot and root length was detected; such increase reached the maximum at 25 µg ml^{-1}and the plants seemed normal and healthy. At 50 and 75 µg ml^{-1}, IAA also improved growth of the infected plants but to a less extent and the plants appeared weaker than the healthy controls (Table III). The chlorophyll content of infected plants was obviously reduced, compared to the healthy ones. Incorporation of IAA at different concentrations resulted in elevation of chlorophyll content that reached 1.31– fold at 25 µg ml^{-1}.

Phytochemical analysis. Although 25 µg ml^{-1} IAA was less effective than 75 µg ml^{-1} in reducing disease incidence in inoculated plants, yet plant growth and chlorophyll content were higher at the former than the latter. Therefore, tomato plants treated with 25 µg ml^{-1} IAA was chosen for phytochemical analysis.

Qualitative analysis of endogenous secondary metabolites, revealed that acetic acid, neophytadiene, benzene dicarboxylic acid, hexadecanoic acid and dibutylphthalate were common in both IAA-treated and untreated controls (Table IV). Dimethylamine, cyclobutanol, benzenaldelyde, benzenapropanol, benzeneacetic acid and tributyrin, were detected in untreated controls, whereas butanol, butanoic acid, oxime methoxyphenyl, ethyl oleate and N-octyl phthalate were detected in the treated ones.

Concerning the inoculated tomato plants, in addition to acetic acid, dimethylamine, hexadecanoic acid, neophytadiene and benzene dicarboxylic acid which were originally present in the untreated control, propylene glycol, pyrrolidinone, phenol, methylsalicylic acid, salicylic acid, indole, pyridine, furanone, linoleic acid and vinylcyclodecane were traced in the diseased tissues under pathogen stress (Table IV).

With regard to the infected plants treated with IAA, cyclotri-and cycloheptasiloxane, methylglycine, benzoic acid and amino benzoic acid were detected in the tissues. Hexa-and pentadecane, dimethyl phthalate, methanone, benzene methanol, benzene acetic acid, pyrrolidinone, and propenone were also detected.

Discussion

Plant hormones can influence disease-induced resistance in plants which may provide new strategies for crop protection (Bodnaryk, 1994).

The *in vitro* application of IAA exerted a general inhibition in both spore germination and mycelial growth of tomato wilt pathogen *Fusarium oxysporum lycopersici*. Such effect was reflected on reduction of the protein content. The present results were in line with the observation that 30 µM IAA completely suppressed the growth of *Glomus mossae* and *G. fistulosum* (Gryndler *et al*., 1998).

The *in vivo* studies revealed that the appearance of minor pathogenic symptoms on uninoculated tomato plants (controls) could be attributed to the fact that the pathogen might be originally present in the used natural (non sterilized) soil. However, application of IAA to the soil could completely protect the plants from infection. Furthermore, IAA increased growth of healthy plants and yielded longer shoot and root, particularly at lower concentrations. Higher concentrations retarded such effect. Similarly, Abd El-Samad (1998) reported that low concentrations of IAA induce stem and root elongation, but Salisbury and Ross (2002) referred their reduction, at higher concentrations, to stimulation of ethylene production by the hormone.

Moreover, under pathogen stress, IAA greatly suppressed disease incidence and rebalanced chlorophyll content of the inoculated plants. This result led to activation of photosynthesis, stimulation of plant growth and thus increasing its resistance to pathogen attack. On the contrary, IAA seemed to be supportive supplement for development of club root disease of *Arabidopsis thaliana* caused by *Plasmodiophora brassicae* (Siemens *et al.*, 2002).

In this study, disease suppression and increased resistance in tomato against wilt pathogen, after application of IAA, could be related to more than one factor. One of these factors might be due to the improved plant growth. In this connection Long *et al.* (1990) attributed resistance of spinach to wilt disease, caused by *F. oxysporum spinaciae,* to the increase in plant growth. The second factor might be due to the direct toxic effect of IAA on phytopathogens (Lu *et al*., 2000). The third factor was the antagonistic soil microflora which may be flourished by IAA and produce effective antifungal metabolites able to protect plants from infection by the pathogen.

Another factor which may influence resistance in tomato plants was the induction of certain secondary metabolites that act as defensive mechanisms against the disease. However, there was no significant difference between the composition of secondary metabolites of IAA-treated controls and the untreated ones. This observation suggested that the protective role of IAA on healthy plants was due to increasing growth rather than inducing metabolic changes.

It is noteworthy that indole was detected in the infected tissues and although it has unknown phytotoxic effects, yet it has been implicated in plant diseases (Rudolph, 1976). Furthermore, phenol was elicited in diseased tomato plants under *F. oxysporum lycopersici* stress, in a trial for resistance. Phenolics can reduce pectinases activity, produced by this pathogen, which facilitates its invasion to host cells (Vidhyasekaran, 2001). Salicylic acid and methylsalicylate were also detected in diseased tomato plants. Thomma *et al*. (1998) reported that endogenous salicylic acid, which level increases on pathogen infection, activates certain antimicrobial proteins in *Arabidopsis thaliana* against *Peronospora parasitica*. More recently, salicylic acid was found to increase resistance in cotton plants against phytopathogens (Abd-El Aziz, 2002).

Nevertheless, accumulation of these defensive metabolites in the present infected tomato could not confer resistance and all the inoculated plants failed completely to resist the pathogen and became diseased. These findings might be attributed to one or more of the following probabilities: the pathogen may be able to detoxify these metabolites into less toxic compounds, it may be non sensitive or it can adapt quickly to these metabolites, the metabolites are not produced at a sufficient level and/or they may not be responsible for disease resistance in this study. Moreover, the produced phenol are not only fungitoxic but also phytotoxic and it may be accumulated at a level that retard plant growth, and enhance susceptiblity to infection (Vidhyasekaran, 2001).

Detection of pyridine in the diseased tomato plants is noteworthy. This compound is essential for synthesis of fusaric acid by *F. oxysporum lycopersici* and other species. Fusaric acid is considered as vivotoxin, it induces wilting, epinasty, necrosis of leaves and it is responsible for the intensification of disease severity. Also, fusaric acid inhibits glycolate oxidase which is responsible for photosynthesis; reduction of photosynthesis hastens wilting (Vidhyasekaran, 2001).

Surprisingly, application of IAA to soil of inoculated plants induced biosynthesis of certain metabolites which possess the ability to stimulate resistance against the target pathogen and thus reduced disease

development. Among these metabolites are the cyclic ether, cyclotri- and cycloheptasiloxane. These compounds contained silicon which was found to suppress haustoria formation by pathogenic fungi and therefore hinder its invasion to host cells and prevent infection (Vidhyasekaran, 2001). On the other hand, IAA stimulated accumulation of methyl glycine. Ogura *et al*. (2001) confirmed that exogenous application of phytohormones induced accumulation of free amino acids in plant cells. The relationship between amino acids and inducing resistance in tomato plants against *F. solani* have been reported (Abo-Ellil *et al.*, 1998). Moreover, the methyl radicle of methyl glycine may alter the nature of pectin in the middle lamella of host cells, a response which induce resistance against phytopathogens (Vidhyasekaran, 2001). In addition, benzoic acid and amino benzoic acid were detected in infected IAA-treated plants. These compounds may also confer resistance. Ebrahimzadeh and Rahnama (1998) elucidated that amino benzoic acid and its derivatives prevented rhizomorph formation and growth of the phytopathogen *Armilaria mellea.*

Finally, it could be concluded that the *in vivo* application of IAA to soil not only improves growth of tomato plants but also protects them against infection by soil pathogens. Furthermore, IAA can reduce disease development in infected plants, therefore it can be exploided as antifungal agent in management of tomato wilt disease; low concentrations are preferred and recommended.

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