Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Pakistan Journal of Plant Pathology 2 (1): 1-9, 2003
ISSN 1680-8193

Histopathological Studies of Eggplant Roots as Affected by IAA, *Agrobacterium tumefaciens* and *Meloidogyne incognita* Alone and in Combinations

Mohamed A. Elwakil, [1]Z.A. Mohamed and [2]A.G.El-Sherif
Department of Plant Pathology, [1]Agricultural Botany and
[2]Agricultural Zoology, Faculty of Agriculture, Mansoura University,
El-Mansoura 35516, Egypt

Abstract: The anatomy of eggplant roots as affected by the application of IAA, *Agrobacterium tumefaciens* and *Meloidogyne incognita alone and in combination* was studied. IAA and *A. tumefaciens*, alone and in combination increased the root diameter, cortex thickness, number of cortex layers, stele diameter, number of xylem vessels but decreased the vessel diameter. Inoculation with *M. incognita* led to the formation of multinucleated giant cells surrounded by deformed xylem elements, hypertrophy of the cortex and hyperplasia of the pericycle. More giant cells with higher dimensions were formed and more eggs/egg mass were produced in plants where IAA, *A. tumefaciens* alone or in combination was introduced with *M. incognita*. On the other hand, the presence of IAA, *A. tumefaciens* or their combination extended the life-span of giant cells, providing long-lasting feeding sites for the nematode. The auxin-mediated role of *A. tumefaciens* in development and reproduction of *M. incognita* was discussed.

Key words: *Agrobacterium tumefaciens*, *Meloidogyne incogniat*, IAA, *S. melongena*, histopathology

Introduction

Meloidogyne-induced anatomical alternations were investigated in eggplant roots (Pasha *et al.*, 1987 and Ekanayake *et al.*, 1988) as well as in other plants like tobacco (Mohamed *et al.*, 1993), ipeamarelo (Kunieda-Alonso *et al.*, 1999) and papaya (Sabir, 2001).

The interaction between nematodes and some other plant pathogens have also been studied in eggplant (Hazarika and Roy, 1974; Naqvi and Alam, 1975) and tomato (El-Sherif and Elwakil, 1991). In particular, the nature of the interaction between the root knot nematode and the crown-gall bacterium was investigated (El-Sherif and Elwakil, 1991; Mohamed *et al.*, 1993; Sule *et al.*,1995; Fakhouri *et al.*, 1996; Rubio-Cabetas *et al.*, 2001). This interaction was mostly reported to be synergistic (El-Sherif and Elwakil, 1991; Mohamed *et al.*, 1993). Accordingly *A. tumefaciens* stimulated development and reproduction of *M. incognita* when applied to the opposite split roots of tomato plants. In an attempt to clarify the anatomical basis of such synergism, Mohamed

et al. (1993) investigated the anatomical changes in tobacco roots infected with ***M. incognita*** and/or ***A. tumefaciens***. Their results showed that the bacterium induced proliferation of tracheary elements and formation of lateral root primordia, an effect which was found to results from the application of auxins (Blakely ***et al.***, 1972; Pasqua ***et al.***, 2001). They also found that when the bacterium infection accompanied that of the nematode, more giant cells with higher dimensions and longer life-span were formed. So, it was suggested that the noticeable enhanced development and reproduction of the nematode in the presence of the bacterium (El-Sherif and Elwakil, 1991) is due to induction of more feeding sites (giant cells) probably under the influence of directly or indirectly bacterium-induced auxins, an assumption which remains to be tested.

The present investigation was therefore carried out to compare the effect of IAA application and ***Agrobacterium tumefaciens*** inoculation on root anatomy of eggplant. It is also aimed to elucidate how far IAA application and ***A. tumefaciens*** inoculation affects the anatomical alternations induced by ***M. incognita*** in eggplant roots.

Materials and Methods

Experiments were carried out in the greenhouse of Plant Pathology Dept. and Labs. of the Agriculture Botany Department, Faculty of Agriculture, University of Mansoura, Egypt. Plastic pots 7 cm d containing sterilized sandy clay loam soil, 120 gm/pot were planted with eggplant (***Solanum melongena*** cv. white Balady) seedlings of four-leaf stage at one seedling/pot. Juveniles of ***M. incognita*** extracted from galled tomato roots using modified Baermann technique (Goodey, 1957) were used as inoculum. Treatments were; 1) No organism or IAA (check, CK), 2) Indole-3-acetic acid (IAA), 3) ***Agrobacterium tumefaciens*** (B), 4) ***A. tumefaciens***+IAA (B+IAA), 5) ***Meloidogyne incognita*** (N), 6) ***A. tumefaciens+M. incognita*** (B+N), 7) IAA+***M. incognita*** (IAA+N) and 8) ***A. tumefaciens***+IAA+***M. incognita*** (B+IAA+N).

Ten-day old seedlings were inoculated with the nematode at the level of 2000 newly hatched J_2/seedling. ***A. tumefaciens*** was applied at the rate of 10^6 colony-forming unit/g soil, simultaneously with the nematode addition (in treatments involving the two organisms). IAA solution, at 100 ppm was added twice/week (with total 300 ml/pot). ***A. tumefaciens*** inoculum was prepared as mentioned by El-Sherif and Elwakil (1991).

All treatments were of 5 replicates and arranged in a randomized complete block design (RCBD) in a greenhouse maintained at 26±2°C. Plants were watered when required. After five weeks of transplanting, each plant root system was gently washed and appropriate root samples were taken. The 1 cm of the base of 1st order lateral roots situated on the 2 cm of the lateral root zone towards main root's base constitutes the anatomically-investigated samples. In treatments involving the nematode, samples were galled laterals from a corresponding position. Specimens were immediately fixed in Formalin-Aceto-Alcohol (FAA). After fixation, root samples were dehydrated in ethyl alcohol series, embedded in paraffin wax (O'Brien and McCully, 1981).

Using rotary microtome, transverse sections (15 µ thick) were cut and, after staining with safranin-fast green combination, mounted in Canada balsam. From each treatment, 5 specimens from different plants were examined. From each sectioned specimen, 4 randomly selected sections were examined. Quantitative data were obtained using a calibrated eye-piece micrometer.

Results and Discussion

Inoculation of eggplants with ***Agrobacterium tumefaciens*** and IAA alone and in combination showed an increase in root cross-sectional diameter, cortex thickness, number of cortex layers, stele diameter and number of xylem vessels, whereas vessel diameter showed a decrease (Table 1 and Fig. 1). Combined treatments were found more effective as compared to separate use of IAA or the bacterium. In addition, the bacterium, IAA and their combination enhanced the formation of lateral root primordia (Fig. 1-d).

The similarity of the effects of ***A. tumefaciens*** and IAA on root anatomy would suggest that the effect of the bacterium is mediated through enhancing auxin level in the root. Alternation of endogenous phytohormone levels in pea genotypes by co-culturing with ***Agrobacterium*** strains has been reported by Pavlova ***et al.*** (1998). According to Ullrich and Aloni (2000), ***Agrobacterium*** induces vascularization promoting growth factors; auxins and cytokinins. ***Agrobacterium*** was reported to induce IAA (Barazani and Friedman, 1999). This was attributed to some genes, aux. 1 and 2 in the genetic make-up of the bacterium, which encode enzymes involved in the auxin biosynthesis pathway (Gaudin and Jouanin, 1995). Accordingly the expression of these genes correlates with cell division. Elevated auxin level led to increased DNA and RNA contents of tomato root cells (Mathur and Sharma, 1998) an effect which may induce cell division in root tissues. Enhanced cell division, in addition to auxin-induced cell enlargement (Smith ***et al.***, 1991), may explain the higher dimensions of the tissues of ***Agrobacterium***- and IAA- treated roots compared with control roots (Table 1). In addition, auxins were reported to induce rhizogenesis (Pasqua ***et al.***, 2001).

In the present study, when roots were infected by the nematode either alone or in combination with either the bacterium, IAA or both, the root galls were formed as a result of hypertrophy of the cortical cells, hyperplasia of the pericycle and giant cell formation (Fig. 2). Also nematode invasion caused necrosis of the cortex (Fig. 2a) and malformed xylem elements due to the formation of giant cells within the stele. Giant cells were multinucleated, some of the nuclei coalesced. The protoplasm of some giant cells was found to be partially or completely degenerating (Fig. 2a and b).

The anatomical alternations induced by ***Meloidogyne incognita*** invasion as observed in the present investigation were comparable to those reported in the roots of eggplant (Pasha ***et al.***, 1987; Ekanayake ***et al.***, 1988), tomato (Ekanayake ***et al.***, 1988), tobacco (Mohamed ***et al.***, 1993),

Table 1: Counts and measurements of some anatomical features of eggplant lateral roots as affected by *Agrobacterium tumefaciens* , IAA and their combination (*Agrobacterium tumefaciens*+IAA) compared with untreated control roots

	C.S diameter (µ)		Cortex thickness (µ)		No. of cortex layers		Stele diameter (µ)		No. of xylem vessels		Vessel diameter (µ)	
Treatments	Mean	**	Mean	**	Mean	**	Mean	**	Mean	**	Mean	**
Untreated control	616.2	0	212.9	0	7.5	0	168.7	0	16.3	0	18.6	0
A.tumefaciens	695.0	+12.7	242.8	+14.0	8.0	+6.6	197.0	+16.7	21.8	+33.7	16.4	-11.8
IAA	735.2	+19.3	250.9	+17.8	9.0	+20.0	214.9	+27.3	22.7	+39.2	15.0	-19.3
A.tumefaciens +IAA	790.3	+28.2	269.4	+26.5	9.5	+26.6	238.7	+41.4	25.0	+53.3	14.6	-21.5

** ± % of control.

Table 2: Certain anatomical alternations in eggplant roots as affected by the nematode alone (N) or in combination with bacterium (N+B), auxin (N+IAA) or both (N+B+IAA)

	Treatments							
	N		N+B		N+IAA		N+B+IAA	
Characters	M	**	M	**	M	**	M	**
No. of giant cells	7.0	0	7.8	+11.4	7.9	+12.8	8.1	+15.7
Giant cell length (µ)	72.9	0	93.3	+27.9	102.7	+40.8	112.0	+53.6
Giant cell width (µ)	52.5	0	75.7	+44.1	71.9	+36.9	80.6	+53.5
No. of giant cells having deteriorated protoplasm	4.4	0	2.0	-54.5	1.7	-61.3	1.5	-65.9
No. of eggs/egg mass	36.7	0	48.0	+30.7	52.6	+43.3	59.0	+60.7

** ± % of control (nematode only)

ipeamarelo (Kunieda-Alonso ***et al.***, 1999) and papaya (Sabir, 2001). The work of Pasha ***et al.*** (1987) supported the view that giant cell formation results from hypertrophy and repeated mitosis without cytokinesis. According to Ekanayake ***et al.*** (1988), giant cells were formed in the vascular region with the formation of 4-6 cells or clusters of thick-walled, multinucleated giant cells around the developing nematode. Deformed xylem elements was attributed to developing giant cells between them (Kunieda-Alonso ***et al.***, 1999). Meristematic activity in the cells adjacent to the giant cells was linked with gall formation (Sabir, 2001).

The infection of bacterium as well as IAA application and their combination intensified the anatomical abnormalities induced by ***M. incognita*** when applied in combination. This effect was reflected with an increase of giant cell production and a pronounced increase in the size of giant cells (Table 2). In addition, the number of eggs/egg mass also increased in treatments where the bacterium, IAA or both were accompanied with the nematode. On the other hand, the

The synergistic action between plant pathogens has been well documented. Naqvi and Alam (1975) suggested that the virus is responsible for certain physicochemical changes in the host which favor nematode multiplication. According to Hazarika and Roy (1974) the number of galls on roots as well as the number of egg masses were significantly greater in plants inoculated with nematode and fungus together than in those inoculated with nematode alone. El-Sherif and Elwakil (1991) found that *A. tumefaciens* stimulated development and reproduction of *M. incognita* when applied to the opposite split roots of tomato plants. Anatomically, Mohamed *et al.* (1993) reported that Meloidogyne-induced giant cells as well as their dimensions were enhanced in tobacco roots in the presence of *A. tumefaciens*. In contrast, presence of the bacterium reduced the rate of giant cell's protoplasm deterioration. It was thus suggested that the noticeable enhanced development and reproduction of the nematode in the presence of the bacterium is due to induction of more feeding sites (giant cells) with extended life-span, probably under the influence of directly or indirectly bacterium-induced auxins. This conclusion has been substantiated based on the results of the present investigation because the bacterium and IAA led to comparable effects. In this context, *Agrobacterium* altered endogenous phytohormone levels in pea genotypes (Pavlova *et al.*, 1998) and was reported to specifically secrete IAA (Barazani and Friedman, 1999). According to Gaudin and Jouanin (1995) *Agrobacterium* stimulates auxin biosynthesis due to the presence of genes carried by the bacterium DNA encoding two enzymes involved in the auxin biosynthesis pathway. Auxins led to increased DNA and RNA (Mathur and Sharma, 1998), hence enhanced cell multiplication. Moreover, it is suggested that auxin is needed as a trigger for giant cell initiation (Hutangura *et al.*, 1999). Auxin-induced giant cell initiation means the development of more feeding sites, consequently enhancing development and reproduction of the nematode. It is also possible that maintaining turgor pressure in the cells of the plant co-inoculated with the bacterium and the nematode may lead to sustaining cell division and enlargement of giant cells, thus providing the nematode with suitable feeding sites. Maintaining turgor may be through elevated auxin levels that induce ethylene emission which, in turn, enhance abscisic acid. The enhancement of abscisic acid reduces transpiration and thus protects the host plant from rapid wilting (Ullrich and Aloni, 2000).

Acknowledgment

We thank Prof. Dr. Abdul Ghaffar, Professor (Retd.) Department of Botany, University of Karachi, Karachi-75270, Pakistan, for critical review of the manuscript.

References

Barazani, O. and J. Friedman, 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? J. Chemical Ecol., 25: 2397-2406.

Blakely, L.M., S.J. Rodaway, L.B. Hollen and S.G. Croker, 1972. Control and kinetics of branch root formation in cultured root segments of ***Haplopappus ravenii***. Plant Physiol., 50: 35-42.

Ekanayake, H.M.R., M.di. Vito and N. Vovlus, 1988. Histopathological changes caused by ***Meloidogyne incognita*** on tomato and eggplant roots. Tropical Agriculturist, 144: 89 - 97.

El-Sherif, A.G. and M.A. Elwakil, 1991. Interaction between ***Meloidogyne incognita*** and ***Agrobacterium tumefaciens*** or ***Fusarium oxysporum*** f.sp. Lycopersici on tomato plant using split-root technique. J. Nematol., 23: 239-242.

Fakhouri, W.D., H. Khlaif and W.I. Abu-Gharbieh, 1996. Interaction between ***Meloidogyne javanica*** and ***Agrobacterium tumefaciens*** on tomato plants. Pak. J. Nematol., 14: 49-54.

Gaudin, V. and L. Jouanin, 1995. Expression of ***Agrobacterium rhizogenes*** auxin biosynthesis genes in transgenic tobacco plants. Plant Molecular Biology, 28: 123-136.

Goodey, J.B., 1957. Laboratory methods for work with plant and soil nematodes. Tech. Bull. No. 2. Min. Agri. Fish. Ed. London, pp: 47.

Hazarika, B.P. and A.K. Roy, 1974. Effect of ***Rhizoctonia solani*** on the reproduction of ***Meloidogyne incognita*** on eggplant. Indian J. Nematol., 4: 246-248.

Hutangura, P., U. Mathesius, M.G.K. Jones and B.G. Rolfe, 1999. Auxin induction is a trigger for root gall formation caused by root-knot nematodes in white clover and is associated with the activation of the flavonoid pathway. Australian J. Plant Physiol., 26: 221-231.

Kunieda-Alonso, S., J.M.dos. Santos, A.C. Alfenas and S. Ferraz, 1999. Pathogenicity of ***Meloidogyne javanica*** (Treub) chitwood to ipeamarelo, ***Tabebuia serratifolia*** Nichols. Revista Arvore, 23: 479-485.

Mathur, A. and R.K. Sharma, 1998. Studies on the effect of auxins on the nucleic acid contents of normal and nematode induced root gall tissues of ***Lycopersicon esculentum*** Mill. in culture. J. Phytological Res., 11: 183-185.

Mohamed, Z.A., A.G. El-Sherif and M.A. Elwakil, 1993. Anatomical changes in tobacco roots due to the infection with ***Meloidogyne incognita*** and/or ***Agrobacterium tumefaciens***. J. Agri. Sci. Mansoura Univ., 18: 1387-1394.

Naqvi, S.Q.A. and M.M. Alam, 1975. Influence of brinjal mosaic virus in the population of ***Tylenchorhynchus brassicae*** and ***Rotylenchulus reniformis*** around eggplant roots. Geobios, 2: 120-121.

O'Brien, T.P. and M.E. McCully, 1981. The study of plant structure: Principles and selected methods. Termarcarphipty, Melbourne, Australia.

Pasha, M.J., Z.A. Siddiqui, M.W. Khan and S.I. Qureshi, 1987. Histopathology of eggplant roots infected with root-knot nematode, ***Meloidogyne incognita***. Pak. J. Nematol., 5: 27-34.

Pasqua, G., B. Monacelli, A. Silvestrini and R. Manganaro, 2001. ***In vitro*** root differentiation and essential-oil accumulation in ***Angelica archangelica***. ***In vitro*** Cellular and Developmental Biology-Plant, 37:763-766.

Pavlova, Z.B., N.V. Malyshev, L.V. Kravchenko, V. Chmelev and L.A. Lutova, 1998. Response of pea (***Pisum sativum*** L.) genotypes to ***Agrobacterium*** as a means of probing tbeir endogenous hormone levels. Plant Sci., 133: 167-176.

Rubio-Cabetas, M.J., J.C. Minot, R. Voisin and D. Esmenjaud, 2001. Interaction of root-knot nematode (RKN) and the bacterium ***Agrobacterium tumefaciens*** in roots of ***Prunus cerasifera***: evidence of the protective effect of the Ma RKN resistance genes against expression of crown ga. European J. Plant Pathol., 107: 433-441.

Sabir, N., 2001. Some observations on histopathological changes in the roots of papaya (***Carica papaya*** L.) infested with ***Meloidogyne javanica***. Indian J. Nematol., 31: 177-178.

Smith, M.A.L., M.T. Mc Clelland and R. Timmermann, 1991. Anomalous root structure on woody plants ***in vitro***. J. Environmental Horticulture, 9: 61-64.

Sule, S., J. Lehoczky, G. Jenser, P. Nagy and T.J. Burr, 1995. Infection of grapevine roots by ***Agrobacterium vitis*** and ***Meloidogyne hapla***. J. Phytopathol., 143: 169-171.

Ullrich, C.I. and R. Aloni, 2000. Vascularization is a general requirement for growth of plant and animal tumours. J. Experimental Botany, 51: 1951-1960.