Phytopathol. Mediterr. (2009) 48, 262–268

Physiological changes in leaves of mungbean plants infected with *Meloidogyne javanica*

NAEEM AHMED, M. WASEEM ABBASI, S. SHAHID SHAUKAT and M. JAVED ZAKI

Department of Botany, University of Karachi, Karachi-75270, Pakistan

Summary. Sequential changes induced by the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood in mung bean (*Vigna radiata* [L.] Wilczek cv. MN95) were studied. Physiological and biochemical changes were recorded 15, 30 and 45 days after nematode inoculation. The changes noted varied with the length of exposure to the nematode. Chlorophyll and carotenoid contents decreased in nematode-infected plants. Total phenols increased in the leaves compared with the controls for up to 30 days after inoculation. Protein content declined significantly at 30 days after exposure to the nematodes. Amylase activity was enhanced in both the leaves and the stems as compared with the controls. The results suggested that plants responded to the nematode by adopting biochemical strategies to withstand the adverse effects of infection.

Key words: root-knot nematode, chlorophyll, protein, phenols, amylase activity.

Introduction

Plant parasitic nematodes are highly destructive plant pathogens causing worldwide losses exceeding $ 125 billion annually (Chitwood, 2003). The primary symptom of root-knot nematode infection is the formation of typical root galls on the roots of susceptible host plants. Nutrient and water uptake are substantially reduced because of the damaged root system, resulting in weak and low-yielding plants (Abad *et al.,* 2003).

Root-knot nematodes cause measurable changes in the morphology and physiology of the host (Williamson and Gleason, 2003). Root damage from the nematode results in stunted and chlorotic plants. In tomato and bean leaves, root-knot infection reduces the photosynthetic rates (Loveys and Bird, 1973; Melakeberhan *et al*., 1984).

Corresponding author: S.S. Shaukat
E-mail: shahid_shaukat2007@yahoo.co.uk

Mohanty and Pradhan (1989) reported that protein content decreased and most amino acids and amides increased after inoculation in susceptible as well as resistant cultivars. Phenolic compounds have also been associated with nematode injury, leading to the browning of plant tissues. In addition, a marked increase in pre-existing phenols as well as the synthesis of small amounts of phenols in infected roots has been found to result from nematode infection (Kosuge, 1969; Sharma *et al*., 1990).

The study examined the physiological and biochemical changes caused by the root-knot nematode *Meloidogyne javanica* in mung bean (*Vigna radiata* [L.] Wilczek) at various post-inoculation times.

Materials and methods

Plant material

Seeds of mungbean cv. MN95 susceptible to *M. javanica* were surface-sterilized with 0.85% sodium hypochlorite, washed three times with

sterile $MgSO_4$ (0.1 M) and dried under a laminar flow hood. The seeds were used for the greenhouse experiments.

Preparation of root-knot nematode inoculum

Roots of egg-plants infected with *M. javanica* (Treub) Chitwood were collected from the University of Karachi garden. Identification of the root-knot nematode species was based on the shape of the perennial pattern (Taylor and Netscher, 1974). Eggs of *M. javanica* were extracted using a 1% sodium hypochlorite solution and collected using the modified technique of McClure *et al.* (1973). The egg suspension was poured on a cotton-wool filter and incubated at 25±2°C to obtain freshly hatched juveniles. Only juveniles collected within 72 h were used as inoculum.

Greenhouse experiments

Seeds of mung bean were sown in 8.1 cm diameter plastic pots containing 300 g sterilized sandy loam (six seeds per pot). One week after germination, two seedlings of equal height were maintained in the pots while the others were removed. After 15 days about 2000 freshly hatched second-stage juveniles were introduced into four holes made around the roots of the plants. Control plants did not receive nematode inoculum. The pots were placed in a completely randomized design in the greenhouse. Pots were watered daily. Biochemical tests were performed 15, 30 and 45 days after nematode inoculation.

Estimation of chlorophylls and carotenoids

The leaves of the mung bean plants were extracted with 80% acetone. The leaf extract was centrifuged three times for about 5 min and the supernatant was collected. Chlorophylls and carotenoid contents were estimated in accordance with Maclachlan and Zalik (1963). The absorbance of the supernatant was recorded at 645 and 663 nm for chlorophyll a and b contents, respectively, and at 480 and 510 nm for carotenoid contents, on a UV-mini 1240 spectrophotometer (Shimadzu, Kyoto, Japan). The amount of chlorophylls and carotenoids was expressed as mg g^{-1} fresh weight.

Estimation of total proteins and nucleic acids

Fresh leaves (0.5 g) were immersed in hot 80% ethanol to kill the tissue quickly. After 5 min, the ethanol was removed and the leaf tissue was crushed in a mortar with 10 ml of 5% trichloroacetic acid (TCA) and centrifuged for 5 min. The tissue was washed separately in 5 ml of absolute ethanol, an ethanol-chloroform mixture (3:1, v:v), and finally in an ethanol-ether mixture (3:1 v:v). The washed residue was then incubated in 5 ml of 0.5 N NaOH for 16 h at 37°C. The sediment was removed by centrifuging and was washed once with 5 ml of 0.5 N NaOH. The extract and wash were combined and 0.5 N NaOH was added to make up 10 ml. Two ml of extract were used for protein determination. Total proteins were estimated using the method of Bradford (1976), and expressed as mg g^{-1} fresh weight. The remaining 8 ml of extract was acidified to pH 1 with 15% PCA, held at 4°C for 40 min, and then centrifuged. The supernatant was collected as an RNA fraction. The DNA-protein sediment was suspended again with 2 ml of 1 N PCA, held at 4°C for 20 min, centrifuged, and the supernatant added to the previously obtained RNA fraction. The DNA-protein sediment was suspended with 3 ml of 0.5 N PCA, heated to 70°C for 15 min, centrifuged and the supernatant recovered as a DNA fraction, after which 0.5 N PCA was added to obtain 5 ml of final DNA. The nucleic acid (RNA and DNA) contents were estimated by the method of Nieman and Poulsen (1963), and expressed as mg g^{-1} fresh weight.

Estimation of total phenols

Total phenols were estimated using the method described by Swain and Hillis (1959). The leaves were immersed in 2 N HCl and the tissues extracted using 10 ml of 2 N HCl. The crushed material was placed in a test-tube and boiled for half an hour in a water bath. It was then filtered and the filtrate was placed over anhydrous $CaCl_2$ at room temperature until dryness. Pure ethanol (0.5 ml) was added to the dried extract. After 5 min, 0.1 ml of extract was taken, to which 0.2 ml of Folin-Ciocalteu reagent (1:9, v:v) and 4.8 ml of distilled water were added. Tubes were shaken in an electric shaker for 10 min and saturated with a $NaHCO_3$ solution. Tubes were then shaken again and incubated at 25°C for 30 min. Absorbance was recorded at 660 nm. The amount of total phenols was expressed as mg g^{-1} fresh weight.

Amylase activity

Amylase activity was assayed by agar-gel dif-

fusion as described by Clum (1967) with minor modifications. Five grams of leaves and stem were first extracted separately in 15 ml ice-cold 0.2 N acetate buffer (pH 5.3) and then centrifuged for 15 min at 4°C. Acetate buffer (0.2 N) was added to the supernatant to make up 24.5 ml, and then 0.5 ml of a chloramphenicol solution (5000 ppm) was added to get a final concentration of 10 ppm chloramphenicol to control the growth of micro-organisms without seriously affecting the metabolism of the tissue or tissue extract (Sabota *et al*., 1968). Four Whatman No. 1 filter paper discs (9 mm diameter) were evenly placed on agar-gel dishes and 10 μl of amylase extract was poured on each disc. The dishes were then placed at 21±1°C for 2, 3 or 4 days. After that, the discs were removed and the dishes flooded with I_2KI solution. The diameter of the clear zones was measured twice at right angles.

Analysis of data

Data sets were subjected to analysis of variance (ANOVA) or to factorial analysis of variance (FANOVA) depending upon the experimental design. As a follow up of ANOVA or FANOVA, Duncan's multiple range test and least significant difference (LSD) were computed.

Results

Infection effect on plant growth

Meloidogyne javanica significantly affected the growth of mung bean plants. Shoot length was significantly reduced ($P<0.001$) in infected plants, as was shoot fresh weight, but root fresh weight showed a significant increase ($P<0.01$) (Table 1).

Chlorophyll and carotenoid

Nematode infection reduced chlorophyll a, b and total chlorophyll (a+b) contents in the leaves. Chlorophyll a and total chlorophyll (a+b) showed a significant reduction ($P<0.001$) in the plants 15, 30 and 45 days after nematode infection, while the chlorophyll a/b ratio significantly decreased($P<0.001$) in infected plants compared with the uninoculated controls. Chlorophyll content reached its lowest level 30 days after nematode infection, at which time the carotenoid contents was also significantly ($P<0.05$) lower (Table 2).

Total proteins

Total proteins showed no significant changes in the leaves of mung bean plants infected with the root-knot nematodes, apart from a slight decline detected 30 days after infection (Table 3).

Nucleic acid

The RNA contents of the leaf extract of infected mung bean plants went down. The maximum reduction (29.8%) occurred 45 days after inoculation (Table 3). The DNA contents was also lower in the leaf extracts of mung bean plants 15 and 30 days after inoculation with *M. javanica*. The maximum reduction (39.6%) occurred at 15 days, but an increase of 28.6% compared with the uninfected control was detected 45 days after nematode infection (Table 3).

Total phenols

The amount of total phenols in the leaves of plants 15 days after inoculation was 11% greater than that in the leaves of uninoculated control plants. At each measuring date after nematode inoculation, the amount of total phenols increased in both infected and uninfected plants, but the differences were not significant (Table 3).

Amylase activity

Amylase activity increased significantly ($P<0.01$) in both the leaves and the stems of infected plants, and it was higher than in the uninfected plants (Table 4).

Table 1. Effect of *Meloidogyne javanica* on the growth of mungbean.

Treatment	Root weight (g)	Shoot weight (g)	Shoot length (cm)	No. of knots in root system
Control	0.26	3.74	22.7	0
Infected	0.72	2.57	19.4	73
$LSD_{0.05}$	0.27	0.55	0.75	16.96

Table 2. Sequential changes in the chlorophylls and carotenoid (mg g^{-1} fresh weight) of leaves of mungbean after inoculation with *Meloidogyne javanica*.

Time (d)	Chlorophyll a (mg g^{-1})		Chlorophyll b (mg g^{-1})		Chlorophyll a+b (mg g^{-1})		Chlorophyll a/b (mg g^{-1})		Carotenoids (mg g^{-1})	
	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected
15	0.700±0.001	0.658±0.017	0.484±0.001	0.479±0.001	1.184±0.002	1.137±0.018	1.446±0.002	1.374±0.019	0.321±0.003	0.321±0.006
30	0.537±0.005	0.483±0.002	0.378±0.001	0.373±0.001	0.915±0.006	0.856±0.002	1.421±0.004	1.295±0.006	0.285±0.003	0.277±0.001
45	0.534±0.003	0.512±0.007	0.371±0.001	0.370±0.000	0.906±0.004	0.881±0.007	1.439±0.003	1.384±0.011	0.282±0.002	0.279±0.001
$LSD_{0.05}$ (Treatment)	0.014		0.002		0.015		0.017		0.003	
$LSD_{0.05}$ (Time)	0.018		0.002		0.018		0.02		0.004	

Table 3. Sequential changes in the proteins, nucleic acids and total phenol (mg g^{-1} fresh weight) of leaves of mungbean after inoculation with *Meloidogyne javanica*.

Time (d)	Protein (mg g^{-1})		RNA (mg g^{-1})		DNA (mg g^{-1})		Total phenols (mg g^{-1})	
	Control	Infected	Control	Infected	Control	Infected	Control	Infected
15	5.24±0.00	5.24±0.16	0.362±0.009	0.306±0.018	0.096±0.007	0.058±0.004	0.646±0.015	0.700±0.012
30	5.39±0.08	4.61±0.19	0.270±0.004	0.247±0.003	0.062±0.006	0.048±0.001	1.030±0.029	1.175±0.012
45	4.68±0.25	4.59±0.2	0.336±0.018	0.236±0.030	0.090±0.004	0.126±0.007	1.063±0.014	1.075±0.039
$LSD_{0.05}$(Treatment)	0.3		0.03		0.009		0.04	
$LSD_{0.05}$(Time)	0.37		0.037		0.012		0.049	

Table 4. Amylase activity of mungbean plant 45 days after inoculation with *Meloidogyne javanica* in terms of clear zone diameter (cm).

Incubation time (d)	Leaves		Stem	
	Control	Infected	Control	Infected
2	1.3±0.03	1.5±0.09	1.1±0.07	1.5±0.07
3	1.9±0.03	2.3±0.03	1.9±0.1	2.4±0.09
4	2.8±0.03	2.9±0.03	2.6±0.03	3.0±0.09
$LSD_{0.05}$(Treatment)	0.1		0.14	
$LSD_{0.05}$(Time)	0.1		0.18	

Discussion

Plant growth was severely affected by nematode infection in terms of shoot length and shoot weight, while root weight increased. Levels of chlorophyll and carotenoids generally went up, while total phenols increased at the early stages of nematode infection, but declined at later stages. There was no effect on the total protein contents of infected plants, while the nucleic acid content varied with time. Amylase activity increased in infected plants. Root-knot nematodes cause giant cells to form in the roots, and this disrupts the root vascular system, reducing the uptake of water and nutrients and their transport from the roots to the shoots (Abad, 2003). The plant response to nematode parasitism thus causes morphological and physiological changes that affect photosynthetic processes (Melakeberhan *et al*., 1986; Hussey and Williamson, 1998).

In contrast to the reduction in the length and weight of shoots, root weight increased in infected plants, possibly due to the formation of giant cells in root galling. Giant cells provide a nutrient sink on which the nematode feeds. As a result the plant is no longer able to provide nutrients to its upper part. This limitation of nutrient elements in the plant is probably the first effect that the nematode has on the physiology and metabolism of its host. These effects increase with the duration of infection (Melakeberhan *et al*., 1987). A reduction in total chlorophyll has also been reported in French bean and rice infected *with M. javanica* (Melakeberhan *et al*., 1986; Swain and Parasad, 1988). Leaf pigment composition is sensitive to plant stress and nematode infection causes either a loss of photosynthetic pigments (e.g. chlorophylls) or higher levels of photoprotective pigments, such as zeaxanthin or β-carotene (Demming-Adams and Adams, 1992). Various forms of abiotic and biotic stresses damage plant leaf tissue and the chloroplasts (Karpinski *et al*., 2003). The chlorophyll released from damaged chloroplasts has to be degraded rapidly to avoid cellular damage owing to its high reactivity (Takamiya *et al*., 2000). Failure to degrade the chlorophyll may cause an accumulation of reactive oxygen species (ROS) that can easily damage the cellular organelles (Foyer *et al*., 1994; Wojtaszek, 1997). That is why chlorophyll must be degraded rapidly following pathogen attack (Kariola *et al*., 2005).

In our experiments, more chlorophyll a was degraded than chlorophyll b. This may have happened because chlorophyll a was degraded before chlorophyll b. This is in agreement with suggestions that the chlorophyll catabolic pathway, and specifically the enzyme chlorophyllase, is involved in modulating the plant defense response by affecting damage-derived photodynamic free chlorophyll levels, leading to a rise in ROS (Kariola *et al*., 2005).

The protein content underwent a non-significant reduction in the leaves of mung bean after inoculation with the nematode. Similarly, Oka *et al*., (1997) found that, at an early infection stage, tomato plants susceptible to *M. javanica* did not change the soluble protein composition of their leaves as compared with uninfected plants. Therefore, no pathogenesis-related (PR) proteins (chitinase, glucanase, or P-14) were produced in the leaf apoplast. However, the leaves of potato plants showed a great number of PR proteins after being infected with the potato cyst nematode, *Globodera* species (Hammond-Kosack *et al*., 1989; Rahimi *et al*., 1996). Lower levels of proteins during the later stages of infection suggest that developing nematodes continuously withdraw large amounts of nutrients from the giant cells (Dorhout *et al*., 1993). These cells are major sinks for amino acids, which are imported into the roots via the vascular system, as reported for the syncytia of the cyst nematode (Hoth *et al*., 2005). A lack of amino acids in the giant cells meant that less protein was synthesised.

The results presented here warrant the conclusion that the plant-nematode interaction altered the nucleic acid metabolism, DNA and RNA. Decreased levels of RNA due to ribonuclease activity during the early and late phases of *M. incognita* infection have been reported in tomato plants (Premachandran and Dasgupta, 1983). The initial increase in ribonuclease activity during the early phase of infection plays a vital role in disease development. Apparently, this increase causes the synthesis of macromolecules that favour the activity of the parasite (Chakaravorty and Shaw, 1977). At a later phase, the ribonuclease is involved in the post-transcriptional processing of RNA molecules, and this occurs only in plants susceptible to the growth and multiplication of the nematode.

Total phenol levels increased during the early stages of infection but later declined. Phenols may serve as defence compounds against pathogens (Kosuge, 1969). Early increases in phenol caused by pathogen invasion trigger the transcription of messenger RNA

that codes for phenylalanine ammonia lyase (PAL); increasing amounts of PAL in the plant brings about the synthesis of phenolic compounds (Taiz and Zeiger, 2002). The resistance of tomato to *M. incognita* has been attributed to high concentrations of phenols in the leaves and roots (Bajaj and Mahajan, 1977). Lower levels of phenols during the later stages is linked to the oxidation of phenols by polyphenoloxidase (PPO). PPO is widely distributed in plants and catalyses the hydroxylation of monophenols to O-diphenols and their oxidation to O-diquinones (Mayer and Harel, 1979). The quinones so formed are highly reactive molecules that spontaneously aggregate into large structures of various types of molecules, including proteins, lipids, nucleic acids and carbohydrates (Melo *et al*., 2006). The quinones alkylate proteins, mainly by covalently linking to amino acids susceptible to alkylation, thereby reducing the bioavailability of such proteins (Felton *et al*., 1992).

Nematode-infected plants had more amylase activity in the stems and leaves than the uninfected control plants. Amylase breaks down starch and long-chain carbohydrates into simple sugars. The expression of α-amylase in the first leaves of mung bean seedlings was also induced by wounding (Koizuka *et al*., 2004). The giant cells caused by root-knot nematodes also act as a sink for sugars, which are transported through the phloem (Dorhout *et al*., 1993). This suggests that an increase of amylase activity in the shoots of infected plants contributes to ensuring a continuous supply of sugars through the phloem to the giant cells.

It is concluded that root-knot nematodes bring about great physiological changes in infected plants, which seem to employ physiological and biochemical strategies either to avoid or to tolerate the adverse effects of nematode infection.

Literature cited

Abad P., B. Favery, M. Rosso and P. Castagnone-Sereno, 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Molecular Plant Pathology* 4, 217– 224.

Bajaj K.L. and R. Mahajan, 1977. Phenolic compounds in tomato susceptible and resistant to *Meloidogyne incognita* (Kofoid & White) Chitwood. *Nematologia Mediterranea* 5, 329–333.

Bradford M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248–254.

Chakravorty A.K. and M. Shaw, 1977. The role of RNA in host-parasite specificity. *Annual Review of Phytopathology* 15, 135–151.

Chitwood D.J., 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture-Agricultural Research Service. *Pest Management Science* 59, 748–753.

Clum H.H., 1967. Formation of amylase in disks of bean hypocotyls. *Plant Physiology* 42, 568–572.

Demmig-Adams B. and W.W. Adams, 1992. Carotenoid composition in sun and shade leaves of plants with different life forms. *Plant Cell and Environment* 15, 411–419.

Dorhout R., F.J. Gommers and C. Kollöffel, 1993. Phloem transport of carboxyfluorescein through tomato roots infected with *Meloidogyne incognita*. *Physiological and Molecular Plant Pathology* 43, 1–10.

Felton G.W., K. Donato, R.J. Del Vecchio and S.S. Duffey, 1992. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore. *Journal of Insect Physiology* 38, 277–285.

Foyer C.H., M. Lelandais and K.J. Kunert, 1994. Photo-oxidative stress in plants. *Physiologia Plantarum* 92, 696–717.

Hammond-Kosack K., H.J. Atkinson and D.J. Bowles, 1989. Local and systemic changes in gene expression in potato plants following root infection with cyst nematode *Globodera rostochiensis*. *Physiological and Molecular Plant Pathology* 37, 339 –354.

Hoth S., A. Schneidereit, C. Lauterbach, J. Scholz-Starke and N. Sauer, 2005. Nematode infection triggers the *de novo* formation of unloading phloem that allows macromolecular trafficking of green fluorescent protein into syncytia. *Plant Physiology* 138, 383–392.

Hussey R.S. and V.M. Williamson, 1998. Physiological and molecular aspects of nematode parasitism. In: *Plant and Nematode Interactions* (K.R. Barker, G.A. Pederson, G.L. Windham, ed.), American Society of Agronomy, Madison, WI, USA, 87–108.

Kariola T., G. Brader, J. Li and E. Tapio Palva, 2005. Chlorophyllase 1, a damage control enzyme, affects the balance between defense pathways in plants. *Plant Cell* 17, 282–294.

Karpinski S., H. Gabrys, A. Mateo, B. Karpinska and P.M. Mullineaux, 2003. Light perception in plant disease defense signalling. *Current Opinion in Plant Biology* 6, 390–396.

Koizuka N., Y. Tanaka and Y. Morohashi, 1995. Expression of α-amylase in response to wounding in mung bean. *Planta* 195, 530–534.

Kosuge T., 1969. The role of phenols in host response to infection. *Annual Review of Phytopathology* 7, 195–222.

Loveys R.R. and A.F. Bird, 1973. The influence of nematodes on photosynthesis in tomato plants. *Physiological Plant Pathology* 3, 525–529.

Maclachlan S. and S. Zalik, 1963. Plasted structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of barely. *Canadian Journal of Botany* 41, 1053–1062.

Mayer A.M. and E. Harel, 1979. Polyphenol oxidases in plants. *Phytochemistry* 18, 193–215.

McClure M.A., T.H. Kruk and I. Misaghi, 1973. A method for obtaining quantities of clean *Meloidogyne* eggs. *Journal of Nematology* 5, 230.

Melakeberhan H., J.M. Webster and R.C. Brooke, 1984. Improved techniques for measuring the CO_2 exchange rate of *Meloidogyne* nematode bean plants. *Nematologica* 30, 213–221.

Melakeberhan H., R.C. Brook and J.M. Webster, 1986. Relationship between physiological response of French beans of different age to *Meloidogyne incognita* and subsequent yield loss. *Plant Pathology* 35, 203–213.

Melakeberhan H., J.M. Webster, R.C. Brooke, J.M. D'Auria and M. Cackette, 1987. Effect of *Meloidogyne incognita* on plant nutrient concentration and its influence on the physiology of beans. *Journal of Nematology* 19, 324–330.

Melo G.A., M.M. Shimizu and P. Mazzafera, 2006. Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. *Phytochemistry* 67, 277–285.

Mohanty K.C. and A.K. Pradhan, 1989. Quantitative estimation of free aminoacids and amides in resistant and susceptible greengram varieties inoculated with root-knot nematode, *Meloidogyne incognita*. *Indian Journal of Nematology* 19, 74–76.

Nieman R.H. and L.L. Poulsen, 1963. Spectrophotometric estimation of nucleic acid of plant leaves. *Plant Physiology* 38, 31–35.

Oka Y., I. Chet and Y. Spigel, 1997. Are pathogenesis-related proteins induced by *Meloidogyne javanica* or *Heterodera avenae* invasion? *Journal of Nematology* 29, 501–508.

Premachandran D. and D.R. Dasgupta, 1983. Changes in nucleic acids and ribonuclease in tomato plants during infection by the root-knot nematode *Meloidogyne incognita*. *Revue de Nématologie* 6, 33–38.

Rahimi S., R.N. Perry and D.J. Wright, 1996. Identification of pathogenesis-related proteins induced in leaves of potato plants infected with potato cyst nematodes, *Globodera* species. *Physiological and Molecular Plant Pathology* 49, 49–59.

Sabota A.E., C.J. Leaver and J.L. Key, 1968. A detailed evaluation of possible contribution of bacteria to radioactive precursor incorporation into nucleic acids of plant tissues. *Plant Physiology* 43, 907–913.

Sharma J.L., P.C. Trevidi, M.K. Sharma and B. Jiagi, 1990. Alteration in prolin and phenol content of *Meloidogyne incognita* infected bringal cultivars. *Pakistan Journal of Nematology* 8, 33–38.

Swain B. and J.S**.** Prasad, 1988. Chlorophyll content in rice as influenced by the root-knot nematode, *Meloidogyne graminicola* infection. *Current Science* 57, 895–896.

Swain T. and W.E. Hillis, 1959. The phenolic constituents of *Prunus domestica*. *Journal of the Science Food and Agriculture* 10, 63.

Taiz L. and E. Zeiger, 2002. *Plant Physiology*. 3rd ed., Sinaur Associates Inc, Sunderland, MA, USA, 290 pp.

Takamiya K.I., T. Tsuchiya and H. Ohta, 2000. Degradation pathway(s) of chlorophyll: what has gene cloning revealed? *Trends in Plant Science* 5, 426–431.

Taylor D.P. and C. Netscher, 1974. An improved technique for preparing perennial pattern of *Meloidogyne* spp. *Nematologica* 20, 268.

Williamson V.M. and C.A. Gleason. 2003. Plant-nematode interactions. *Current Opinion in Plant Biology* 6, 327–333.

Wojtaszek P., 1997. Oxidative burst: an early plant response to pathogen infection. *Biochemical Journal* 322,

Accepted for publication: June 22, 2009