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SEQUENTIAL BIOCHEMICAL CHANGES IN ROOTS OF *CALLISTIPHUS CHINENSIS* LINES RESISTANT AND SUSCEPTIBLE TO *MELOIDOGYNE INCOGNITA* RACE 1

by

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Summary. Two lines of china aster, *Callistiphus chinensis*, viz., AST-5 and IIHR-55 (Kamini) developed at the Division of Ornamental Crops, Indian Institute of Horticultural Research, Bangalore, resistant and susceptible, respectively, to the root-knot nematode, *Meloidogyna incognita* race 1, were analysed to record the post-infection sequential biochemical changes in their roots. The results indicate that there were significantly higher contents of total phenols and total proteins, and higher activity of polyphenol oxidase in infested AST-5 roots in the first two weeks after infection which declined in third and fourth week of infection, compared to that of uninfected AST-5 roots and infected and healthy IIHR-55 roots. The activity of phenylalanine ammonia lyase was comparatively higher in the infected AST-5 roots compared to its uninoculated control and IIHR-55 roots. The percentage increase in PAL activity was higher in infected AST-5 roots from 21 days after infection.

China aster, *Callistiphus chinensis* (L.) Ness., is an important annual flower crop grown commercially for cut flowers. Root-knot nematode, *Meloidogyne incognita* is an important limiting factor both in the nursery and in the field (Krishnappa *et al.*, 1980; Khan and Parvatha Reddy, 1991). In order to offer better plant types with resistance to root-knot nematode, several china aster lines developed at the Indian Institute of Horticultural Research, Bangalore, were screened for resistance to *M. incognita* race 1 (Nagesh *et al.*, 1995). Improved lines, AST-5 and IIHR-55 (Kamini), found to be resistant and susceptible, respectively, to *M. incognita* were studied for their biochemical behaviour during infection. A time-course/sequential analysis for post-infective biochemical changes in roots of these two china aster lines was carried out. The roots were analysed for total protein and phenol content and the activities of polyphenol oxidase and phenylalanine ammonia lyase, at weekly intervals for four weeks after inoculation.

Materials and methods

The two lines were raised in seed pans containing an autoclaved soil mixture (2 parts of soil, 1 part of sand, 1 part of compost). Three weeks after plant emergence, freshly hatched second stage juveniles (J_2) of *M. incognita* (Kofoid *et* White) Chitw. race 1 (2 J_2/g soil) were inoculated to one set of seedlings while another set was left uninoculated to serve as control. The seedlings were then harvested or uprooted

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at weekly intervals for four weeks from the date of inoculation. Roots from the uninoculated and inoculated plants were carefully washed, cleared of soil, excised and weighed separately. Fresh root samples of 3 g each were cut into small pieces and homogenised in prechilled pestle and mortar containing four volumes of prechilled extraction buffer (0.05 M Tris-HCl buffer at pH 7.2 containing 12 per cent sucrose, 0.1 per cent cysteine-HCl, 0.1 per cent ascorbic acid and 4 mM $MgCl_2$). The slurry was filtered through Whatman No. 44 filter paper and centrifuged at 10,000 rpm for 20 minutes at 1 °C. The supernatant solutions were subjected to protein analysis following the method of Lowry *et al.* (1951) using Folinphenol reagent. The concentration of protein (mg/g fresh root) was calculated from the standard curve prepared with bovine serum albumen, Cohn-fraction V. Two grams of the root samples were homogenised in 10 ml of 80% ethanol with pestle and mortar, the homogenate was centrifuged at 10,000 rpm for 20 minutes and the supernatant was collected. The residue was washed with 80% ethanol in the same way for four times and the supernatants were pooled. The supernatant was evaporated to dryness and the residue dissolved in 5 ml of distilled water. Aliquots of 0.2 ml were pipetted into test tubes and the volume made up to 3 ml. 0.5 ml of Folin-Ciocalteau reagent was added to each sample in test tubes, followed by the addition of 2 ml of 20% Na_2CO_3 solution and boiled in a water bath for one minute. The final concentrations/contents of phenols were expressed in terms of μg/g fresh root weight derived from the standard curve obtained with catechol (Malik and Singh, 1980).

The estimation of PPO (EC 1.10.3.1 PPO) activity was done according to Malik and Singh (1980). Five grams each of infected and healthy roots of AST-5 and IIHR-55 were homogenised in prechilled 0.1 M phosphate buffer (pH 6.0) in a prechilled pestle and mortar. The catecholase activity of the extract was assayed in a cuvette containing 0.01 M catechol in 0.1 M phosphate buffer (pH 6.0) and crude extract in a final volume of 3 ml. The change in absorbance was recorded in UV-Vis scanning Spectrophotometer (Milton Roy, Inc). The enzyme activity was expressed in terms of rate of increase in absorbance per minute.

The estimation of PAL (EC 4.3.1.5 PAL) activity was done essentially according to the procedure described by Sadasivam *et* Manickam (1992). Enzyme extract was prepared from each root sample by homogenising 1 g of root sample in 5 ml cold 25 mM borate-HCl buffer pH 8.8 containing 5 mM mercaptoethanol (0.4 ml/l), followed by centrifugation at 12,000/g for 20 minutes at 1 °C and the supernatant was collected as enzyme source. 0.5 ml of 0.2 M borate buffer was pipetted into clean test tubes to which 0.2 ml enzyme extract and 1.3 ml distilled water were added. The enzyme activity was initiated by adding 1 ml L-phenylalanine solution and incubated for 30 minutes at 32 °C. The reaction was stopped by adding 1 ml trichloroacetic acid and the absorbance was measured at 290 nm. To compare the PAL activity of different samples, 1 ml of undiluted enzyme of the lines was used. According to Zucker (1965), an increase in optical density of 0.01 is equivalent to the formation of 1 mg of trans-cinnamic acid per ml of reaction mixture. From this relationship, PAL activity was calculated in terms of nanomoles of t-cinnamic acid formed per second per mg of protein.

Results and discussion

The data presented in Tables I and II show that there was post-infection increase in total protein and phenols and activities of PPO and PAL enzymes in the roots of both AST-5 and IIHR-55. However, the increase was higher in all the parameters in the resistant line AST-5, compared to that of the susceptible line, IIHR-55 (Kamini) at all the intervals of observation, except for a marginal increase in PAL activity at the first interval in IIHR-55 roots.

TABLE I - *Sequential changes in protein and total phenol contents in healthy and* Meloidogyne incognita *infested roots of china aster lines.*

Line	Treatment	Total content at weekly intervals							
		Protein (mg/g root)				Phenols (μg/g root)			
		I	II	III	IV	I	II	III	IV
IIHR-55 (Kamini)	Uninoculated	28.0 ±3.0	24.5 ±2.0	23.5 ±3.0	29.5 ±4.0	566 ±22.0	524 ±26.0	428 ±32.0	412 ±11.0
	Inoculated	36.0 ±4.0	32.0 ±3.0	31.5 ±3.0	38.5 ±5.0	748 ±28.0	698 ±19.0	538 ±22.0	544 ±16.0
	% Change	+28.5	+30.6	+34.0	+30.5	+32.1	+33.2	+25.7	+32.0
AST-5	Uninoculated	22.0 ±2.0	26.0 ±1.0	32.5 ±2.0	30.0 ±4.0	628 ±11.0	636 ±26.0	588 ±18.0	558 ±33.0
	Inoculated	31.0 ±4.0	38.0 ±6.0	44.5 ±8.0	40.0 ±6.0	844 ±31.0	828 ±34.0	710 ±25.0	698 ±24.0
	% Change	+40.9	+46.1	+36.9	+33.3	+34.3	+30.1	+20.7	+25.0

TABLE II - *Sequential changes in PPO and PAL enzyme activities in healthy and* M. incognita *infested roots of china aster lines.*

Line	Treatment	Enzyme activity at weekly intervals							
		PPO*				PAL**			
		I	II	III	IV	I	II	III	IV
IIHR-55 (Kamini)	Uninoculated	0.22 ±0.04	0.33 ±0.02	0.26 ±0.02	0.28 ±0.03	0.12 ±0.02	0.19 ±0.03	0.22 ±0.02	0.25 ±0.03
	Inoculated	0.31 ±0.02	0.48 ±0.06	0.36 ±0.05	0.38 ±0.04	0.18 ±0.03	0.27 ±0.03	0.29 ±0.04	0.33 ±0.02
	% Change	+40.1	+45.0	+38.0	+35.7	+50.0	+42.1	+31.8	+32.0
AST-5	Uninoculated	0.18 ±0.01	0.27 ±0.02	0.36 ±0.02	0.24 ±0.02	0.20 ±0.04	0.24 ±0.06	0.26 ±0.03	0.28 ±0.04
	Inoculated	0.38 ±0.04	0.56 ±0.08	0.58 ±0.07	0.38 ±0.04	0.29 ±0.03	0.34 ±0.02	0.46 ±0.06	0.51 ±0.06
	% Change	+111.1	+007.4	+61.1	+58.3	+45.0	+41.6	+76.9	+82.1

* Activity after 5 min calculated as O.D. change at 450 nm per mg of protein per hour; ** Nanokatals cinnamic acid formed per mg protein.

The total contents of proteins and phenols increased at higher rates in the first two weeks of infection compared to the following two weeks. Correspondingly there was a higher rate of increase in PPO activity in the infested roots of both the susceptible and resistant lines over their respective healthy roots. However, there was no corresponding increase in PAL activity

in the first two intervals of observation in roots of AST-5 which almost doubled in third and fourth weeks of infection.

Phenols (the aromatic compounds with hydroxyl groups), PPO (the enzyme which oxidises the phenols to quinones), and PAL (which converts L-phenylalanine to trans-cinnamic acid a precursor for biosynthesis of coumarins, isoflavanoids and lignin) are closely linked biochemical substances which impart resistance to the tissues against several plant pathogens and nematodes (Ebel, 1986; Kaplan, 1987). In the present investigation, it was observed that these compounds increased as the infection advanced, especially in the resistant line, AST-5. The initial increase in phenols and PPO activity in AST-5 would have prevented the nematode penetration and further development. With ageing of roots (third and fourth week after infection) the phenols and PPO activity would have naturally decreased. However, with ageing of the roots, the PAL activity could be high in order to bio-synthesise isoflavanoids that would increasingly activate cellular lignification, thus preventing nematodes from establishing and developing.

Therefore, it is concluded that increased activity of PPO and phenol content in the initial stages of infection followed by increased activity of PAL at later stages of infection contributed to the resistance of AST-5 line to *M. incognita* race 1 compared to the susceptible line, IIHR-55 (Kamini).

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