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FREE AMINO ACIDS AND OXIDATIVE ENZYMES IN INFESTED ROOTS OF TOMATO GENOTYPES RESISTANT AND SUSCEPTIBLE TO *MELOIDOGYNE INCOGNITA*

by

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Summary. The effects of *Meloidogyne incognita* on the free amino acid contents of resistant and susceptible roots of different tomato genotypes were studied. Fourteen free amino acids were commonly found in the roots of all tomato genotypes. Proline, cystine and tyrosine were not dectected in some of the tested tomato populations. The concentrations of the amino acids asparagine, therionin + serine, alanine, valine and leucine appeared to have low value in the resistant genotypes in most instances. On the other hand, the amino acids glutamine, histidine, glycine and arginine occurred in higher concentrations in the resistant genotypes in most instances. The activity rates of the oxidative enzymes peroxidase, polyphenol oxidase, ascorbic acid oxidase and catalase showed significantly higher values in the resistant tomato genotypes of parental cultivars than in susceptible ones.

Tomato cultivars resistant to root-knot nematodes (*Meloidogyne* spp.) have been shown to have higher catalase and peroxidase activity than susceptible ones (Okopnyi and Sadykin, 1978). Ganguly and Dasgupta (1979) reported that peroxidase activity was generally higher in resistant tomato cultivars than in susceptible ones prior to nematode infection and that the increase in peroxidase activity in plants due to nematode inoculation was more conspicuous in resistant cultivars. However, Vacheiskvili *et al.* (1978) detected higher activity of peroxidase and polyphenol oxidase in *Meloidogyne incognita* infected tomato plants than in healthy ones. They also reported that peroxidase and catalase activities decreased following infection with *M. incognita* but to a lesser extent than in the more resistant tomato cultivars.

The objectives of this study were to investigate the free amino acid content and the rate of activity of the oxidative enzymes peroxidase (PO), polyphenol oxidase (PPO), ascorbic acid oxidase (AAO) and catalase in the roots of different tomato (*Lycopersicon esculentum* Mill.) cultivars and isolines, resistant and susceptible to *Meloidogyne incognita* (Kofoid *et* White) Chitw.

Materials and methods

The root-knot nematode *M. incognita* race 1 was isolated from infected tomato roots, authenticated by morphological characters of adult females and the North Carolina Differential Host Test, and then reared on Rutgers tomato plants (Taylor and Sasser, 1978). Two susceptible tomato cultivars (Peto 86 and E 6203) and one resistant cultivar (Rossol VFN) were used as parents in two backcross breeding programmes to obtain two pairs of isolines. Parental seeds were sown in summer, 1986. Rossol VFN was used as the male parent in the two backcross programmes, while cvs Peto 86 and E 6203 were used as the female parents in the first and second programmes, respectively. F_1 generations were backcrossed to their susceptible parent in each programme. From summer 1986 to 1989, four successive backcrossings were made. Seedlings from each backcross generation were tested for resistance to *M. incognita* and the reactions were judged according to Taylor and Sasser (1978). Two isolines of the first backcross programme were designated as isoline 1 and isoline 2, whereas those of the second programme were isoline 3 and isoline 4. Isolines 1 and 3 were resistant while isolines 2 and 4 were susceptible to *M. incognita*.

Seeds of the three parental cultivars were sown and terminal cuttings of the four isolines were planted in 15 cm diam. clay pots filled with autoclaved sandy loam soil. The seedlings were inoculated with 2000 nematode eggs per seedling, 15 days after emergence. Two weeks after inoculation, roots of each plant were removed and processed for amino acid analyses, using an amino acid analyzer (Hamilton, 1962). Oxidative enzymes were determined in the parents, F_1s and four isolines. The pots were arranged in a complete randomized blocks design with three replicates of each treatment. Root samples of inocu-

lated and uninoculated plants of the three parents and two F1s were collected just before inoculation and 14 days after inoculation. Samples of four isolines were taken 14 days after inoculation. Analysis was done according to Hassan (1990) using 0.25 g fresh root tissue from each sample. PO activity was determined according to Chance and Maehly (1955) at 420 nm and PPO activity was measured by following the oxidation of catechol at 450 nm (Maxwell and Bateman, 1967). AAO activity was determined according to Oberbacher and Vines (1963) at 265 nm. Catalase was measured according to Beers and Sizer (1952) at 240 nm. Enzyme activity was expressed as the change in absorbancy per minute. Absolute activity of the enzymes values was expressed as ΔA/min/g fresh weight. Each assay was performed three times.

Results and discussion

Table I gives the amounts of free amino acids in tomato roots of the three parents and four isolines derived from the two backcrosses. Fourteen to 17 free amino acids were commonly found in the analysed samples of the tested tomato genotypes.

The amino acids asparagine, therionine + serine, alanine, valine and leucine had in most instances low magnitudes in the resistant genotypes, as compared with the susceptible ones. On the contrary, the four amino acids glutamine, glycine, histidine and arginine in most instances increased in magnitudes in the resistant genotypes, as compared with susceptible ones. The other amino acids proline, cystine, tyrosine, isoleucine, methionine, phenylalanine and lysine, did not present a consistent trend in relation to the type of reaction of the various tomato genotypes to nematode infection.

The results show that the *M. incognita* susceptible tomatoes (E 6203, Peto 86, isoline 2 and isoline 4) contained greater amounts of some free amino acids than the resistant populations (Rossol VFN, isoline 1 and isoline 3). These amino acids were asparagine, therionine + serine, alanine, valine, leucine and proline. These results largely agree with those reported by Owens and Novotny (1960), Singh *et al.* (1978) and Hassan (1983), who indicated that root-knot nematode gall production was, mainly, associated with such increments of free amino acids. On the contrary, the amino acids histidine, glycine, glutamine and arginine showed an increased rate in resistant tomatoes. However, Singh and Choudhury (1973) found no detectable differences in the number and kinds of free amino acids in resistant and susceptible tomato cultivars, but only in magnitude, which largely agree with our results. In a similar study with *Rotylenchulus reniformis*, Mahmood

TABLE I - *Free amino acid contents (mg/100 g root fresh wt.) in tomato cultivars and isolines in presence of* Meloidogyne incognita, *14 days after nematode inoculation.*

Amino Acids	Rossol VFN (R)*	Peto 86 (S)	E 6203 (S)	Iso. 1 (R)	Iso. 2 (S)	Iso. 3 (R)	Iso. 4 (S)
Asparagine	1.109	3.688	1.944	2.138	3.347	1.313	4.173
Therionine + Serine	5.347	12.488	8.864	8.953	12.806	5.211	14.089
Glutamine	6.271	4.727	3.556	6.035	4.789	8.448	4.112
Proline	–	–	–	0.188	1.039	3.246	5.309
Glycine	0.623	0.561	0.563	0.585	0.580	0.660	0.550
Alanine	1.123	1.418	1.385	0.819	1.092	1.048	1.343
Cystine	–	0.320	–	–	0.226	–	0.405
Valine	0.404	0.817	1.178	0.646	0.789	0.117	0.789
Methionine	0.481	0.307	0.315	0.499	0.390	0.359	0.572
Iso-leucine	0.922	0.691	1.068	0.743	1.114	0.683	1.262
Leucine	0.840	1.063	1.025	0.952	1.327	0.839	1.366
Tyrosine	–	0.289	0.225	–	-	0.152	0.081
Phenylalanine	0.610	0.460	0.459	0.323	0.585	0.511	0.553
Histidine	3.689	0.689	1.220	1.208	0.959	0.962	0.552
Lysine	2.745	1.509	1.103	1.197	1.954	1.169	1.599
Arginine	4.385	1.069	1.069	0.665	0.665	0.693	0.318
Total	28.549	30.096	25.974	24.951	31.662	25.411	37.073

* R = resistant; S = susceptible; Iso. = isoline; - = not found.

TABLE II - *Peroxidase (PO) and polyphenol oxidase (PPO) activity rates (Δ A/min/g fresh wt. X10^{-2}) in roots of resistant and susceptible tomato cultivars, and their F_1 hybrids, as affected by* M. incognita, *0 and 14 days after inoculation.*

Tomato population		Days after inoculation					
		0		14			
				PO		PPO	
		PO	PPO	Inoculated	Uninoculated	Inoculated	Uninoculated
Rossol VFN	(R)*	3.30 abc	0.350 bc	4.45 a	2.32 de	0.108 ef	0.016 f
Peto 86	(S)	1.10 fghi	0.062 ef	2.75 cd	1.08 fghi	0.034 ef	0.020 f
E6203	(S)	0.90 ghi	0.182 e	2.61 d	1.20 efghi	0.110 ef	0.080 ef
Peto 86 X Rossol VFN	(R)	4.20 ab	0.570 ab	2.22 def	2.10 defgh	0.124 ef	0.071 ef
E6203 X Rossol VFN	(R)	3.20 bc	0.126 ef	1.20 efghi	1.75 defghi	0.026 f	0.072 ef

* R = resistant; S = susceptible; means followed by same letter, within each enzyme, do not significantly differ according to Duncan's Multiple Range test at P = 0.05.

(1986) reported that the amino acid content affected the degree of resistance, tolerance or susceptibility of tomato plants to the nematode. The higher concentrations of the amino acid serine in susceptible tomato plants suggests that this amino acid may have a role in the mechanisms of resistance and susceptibility to nematodes. Its importance perhaps arises from its role in the biosynthesis of tryptophans and N10 formyltetrahydrofolate, which are known to be involved in indole acetic acid (IAA) and cytokinin biochemistry, respectively (Slabaugh, 1974). Okopnyi (1980) suggested that the total amino acid content, generally, appeared to decrease in tomatoes with resistance to *M. incognita.*

It was noted that proline, cystine and tyrosine were not found in some of the tested tomato genotypes. Proline was absent in all three parental cultivars while cystine was absent in resistant isolines 1 and 3 and cvs Rossol VFN and E 6203. Tyrosine was absent in cv. Rossol VFN and isolines 1 and 2. Generally, the total free amino acid content was greater in the susceptible isolines 2 and 4 and the susceptible cv. Peto 86 compared with the resistant cv. Rossol VFN and the resistant isolines 1 and 3 (Table I).

Table II indicates significant differences among the PO and PPO rates of activity in the roots of the tested tomato genotypes. At the time of inoculation, the highest rates of PO and PPO activity were detected in the F_1 hybrid (Peto 86 X Rossol VFN) and Rossol VFN, respectively. On the other hand, the two susceptible parents showed a low magnitude of PO and PPO activity. Also, no significant differences in PPO activity were detected among the two susceptible parents and the F_1 hybrid (E 6203 X Rossol VFN). After 14 days from inoculation, some increases in PO and PPO activity were recorded.

Table III shows that the resistant genotypes had higher activity levels for ascorbic acid oxidase (AAO) and catalase than did the susceptible ones. Nematode inoculation slightly raised the levels of AAO and catalase, compared with those of the uninoculated roots within all tested genetic populations.

Table IV lists the means of PO, PPO, AAO and catalase activity rates of the three parental cultivars and the two F1 hybrids in comparison with those of the four isolines, at 14 days after inoculation with nematode. Rossol VFN and isoline 3 gave the highest significant values of all population tested for PO activity. Also, the resistant isoline 3 gave the highest significant value of PPO activity, whereas the rest of tested populations did not differ significantly from one another. The resistant isoline 1 gave the highest value for AAO and catalase. No significant differences were found within resistant and susceptible groups, tested for AAO activity, but only between them. With regard to catalase, the resistant populations possessed relatively higher activity rates than the comparable susceptible populations. Generally, the two resistant isolines maintained significantly higher activity rates for the four enzymes that were studied than did their two corresponding susceptible isolines. This conclusion agrees with Bajaj *et al.* (1986), who stated that highly resistant tomato cultivars to root-knot nematodes had high contents of PPO. Also, Okopnyi and Sadykin (1978) reported that resistant cultivars to root-knot nematodes had high contents of PPO and catalase, which were believed to be involved in the synthesis of tomatine and, consequently, in determining the degree of resistance.

Our results clearly show that the oxidative enzymes had higher rates of activity, at and after the inoculation in tomatoes resistant to nematodes, than did the susceptible

TABLE III - *Ascorbic acid oxidase (AAO) and catalase activity rates (Δ A/min/g fresh wt X10^{-2}) in roots of resistant and susceptible cultivars, and their F_1 hybrids as affected by the inoculation with* M. incognita, *0 and 14 days after the inoculation.*

Tomato population		Days after inoculation					
		0		14			
				AAO		Catalase	
		AAO	Catalase	Inoculated	Uninoculated	Inoculated	Uninoculated
Rossol VFN	(R)*	0.0202 cdef	0.0300 abc	0.052 abc	0.038 cde	0.046 abc	0.037 abc
Peto 86	(S)	0.0059 ef	0.0058 c	0.022 cdef	0.020 cdef	0.022 bc	0.010 c
E6203	(S)	0.0046 ef	0.0060 c	0.038 cde	0.034 cdef	0.033 abc	0.032 abc
Peto 86 X Rossol VFN	(R)	0.0209 cdef	0.0220 bc	0.079 a	0.074 a	0.062 a	0.058 ab
E6203 X Rossol VFN	(R)	0.0151 def	0.0320 abc	0.080 a	0.0695 ab	0.048 abc	0.044 abc

* R = resistant; S = susceptible; means followed by same letter, within each enzyme, do not significantly differ according to Duncan's Multiple Range test at P = 0.05.

TABLE IV - *Activities of peroxidase (PO), polyphenol oxidase (PPO), ascorbic acid oxidase (AAO) and catalase in roots of tomato cultivars, F_1 hybrids and isolines as affected by* M. incognita, *14 days after the inoculation.*

Tomato population		A/min/g fresh wt X 10^{-2}			
		PO	PPO	AAO	Catalase
Rossol VFN	(R)*	4.45 a	0.108 b	0.052 bc	0.046 ab
Peto 86	(S)	2.75 b	0.034 b	0.022 c	0.022 b
E6203	(S)	2.61 b	0.110 b	0.038 c	0.033 ab
Peto 86 X Rossol VFN	(R)	2.22 bc	0.124 b	0.079 ab	0.062 a
E6203 X Rossol VFN	(R)	1.20 cd	0.026 b	0.080 ab	0.048 ab
Isoline 1	(R)	2.80 b	0.053 b	0.095 a	0.068 a
Isoline 2	(S)	0.73 d	0.026 b	0.034 c	0.016 b
Isoline 3	(R)	4.40 a	0.340 a	0.080 ab	0.058 ab
Isoline 4	(S)	0.32 d	0.077 b	0.044 c	0.026 ab

* R = resistant; S = susceptible; means followed by same letter, within each column, do not significantly differ according to Duncan's Multiple Range test at P = 0.05.

plants. These results are in general agreement with those previously reported by other authors (Okopnyi and Sadykin, 1978; Ganguly and Dasgupta, 1979; Haseeb *et al.*, 1988), who demonstrated that the activity of these enzymes increased after nematode inoculation. Such increments in the activity of these oxidative enzymes in tomato roots, following the inoculation with *M. incognita*, suggests certain roles for them in the resistant reactions.

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