Nematol. medit. (1990), *18*: 161-163

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CHANGES IN PROTEIN CONTENT OF CELLULAR FRACTIONS FROM TOMATO ROOTS ATTACKED BY *MELOIDOGYNE INCOGNITA*

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Summary. Inoculation of the resistant tomato cv Rossol with the root-knot nematode *Meloidogyne incognita* caused an increase of protein content of mitochondrial and washed cell-wall fractions. Dry weight of roots also increased. Susceptible cv Roma VF reacted to nematode invasion by an increased protein content of all the cellular fractions except the cell walls. A decrease in dry weight occurred after inoculation.

The interaction between root-knot nematodes and plants involves the entire metabolism of root as a reaction to the invasion by a parasite (Hussey, 1985). Both compatible and incompatible reactions lead to the neosynthesis of a series of biomolecules and even of whole cellular organelles (Rubenstein and Owens, 1964; Owens and Bottino, 1966; Js Huang, 1985; CS Huang, 1985). Induction of a large amount of enzymes has been reported as particularly important in nematode disease (Webster, 1975).

Variations in the protein content of roots caused by nematode infestation have been tested only in syncytia induced by *Heterodera glycines* and giant cells induced by *Meloidogyne incognita* in soybeans (Gommers and Dropkin, 1977). Therefore, a preliminary study was undertaken to detect variations in the protein content of each cellular fraction obtained by differential centrifugation in inoculated tomato roots resistant and susceptible to the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. Changes in the whole mass have been investigated by measuring dry weights of uninfested and infested roots.

Materials and methods

Seeds of tomato (*Lycopersicon esculentum* Mill.) cvs Roma VF and Rossol, respectively susceptible and resistant to the root-knot nematode *Meloidogyne incognita*, were germinated in sterilized quartz sand. Seven days after germination seedlings were transplanted into 3 cm clay pots containing quartz sand (5 seedlings/pot). Seedlings were allowed to grow for 15 days then half of them was inoculated with 80 second stage juveniles of *M. incognita* race 2 per seedling, and the other half was left uninfested as control. Seven days after inoculation the plants were used for the extraction procedures.

For determination of dry weights seedlings were transplanted immediately after germination. After a growth period of 12 days, seedlings were inoculated as described above. Two days after inoculation roots were excised for the measurements.

Seedlings were grown in a growth chamber at 26 °C, 65% RH, 5000 lux for 12 h per day and watered with Hoagland's solution twice a day.

For preparation of cellular fractions seedlings were washed with distilled water then roots (approx. 50 g for each batch) were separated from the shoots and kept in a ice-bath. Roots were placed in ice-cold 0.05 M potassium phosphate buffer, pH 6.0, and cut into very small pieces to obtain a coarse homogenate. Then they were ground with a Polytron PT-10.35 (Kinematica GmbH- Switzerland) and filtered through four layers of gauze. The homogenate was centrifuged for 10 min at 500 g. The pellet was washed twice; washed supernatants were collected and referred to as 'Washed Fraction', the final pellet was used as 'Cell-wall Fraction'. The supernatant of the first centrifugation was further centrifuged for 15 min at 12,000 g; the pellet of this second centrifugation provided the 'Mitochondrial Fraction'. Finally, the supernatant was spun at 100,000 g for 90 min to obtain the 'Microsomal Fraction'. Ammonium sulphate was added to the last supernatant to 70% saturation. After standing overnight, the residue was collected by centrifugation, redissolved in a minimal volume of 0.05 M phosphate buffer, pH 6.0, and dialysed against 0.05 M phosphate buffer, pH 7.0, containing 0.1 M KCl. The dialysate was centrifuged and the supernatant solution ultrafiltered at 4 °C using a Centricon-10 microconcentrator with a YM ultrafiltration membrane (10,000 molecular weight cut-off). The fraction with retained proteins was designated 'Retained Fraction'; filtered solution rich in

low molecular weight molecules formed the 'Filtered Fraction'.

A volume containing about 100 μg of proteins was used for protein determination of each cellular fractions using Lowry's procedure (Lowry *et al.*, 1951) with pure bovine serum albumin as the standard. Three replicates of each sample were used for each experiment. In filtered fractions, which lack in protein, the content of phenols was determined. Values are expressed as mg per gram fresh weight of roots and as averages of three experiments.

Determination of dry weights of roots was carried out as described in Zacheo and Molinari (1987a).

Results

Table I shows the protein contents of the cellular fractions from resistant (cv Rossol) and susceptible (cv Roma VF) tomato roots, uninfested and infested by *M. incognita*. Nematode inoculation caused a large increase in protein content in the resistant mitochondrial fraction; phenols of filtered cytosolic fraction and proteins of the washed fraction from cell walls also markedly increased. On the contrary, dry weight of resistant roots was higher after nematode attack (Table II). It should be noted that the amount of mitochondrial proteins rises from 0.12 mg/g fresh weight in control roots to 0.23 mg/g in their infested counterparts, whereas the number of mitochondria per mg of protein has been found to range on 2.5 x 10^6 both in uninfested and infested roots (Molinari *et al.*, 1990). Thus, it can be argued that the number of mitochondria per gram of root results almost doubled in resistant cv. after inoculation.

Susceptible roots reacted to nematode invasion by an increase in mainly soluble proteins. Proteins from mitochondrial fraction augmented after inoculation; however, the extent of this increase was rather lower than that occurred in resistant roots (Table I). Microsomal proteins and filtered phenols were slightly higher in infested roots compared with control. There was a decrease in dry weight of roots in susceptible plants after inoculation (Tab. II).

TABLE I - *Protein content from the cellular fractions of resistant (cv. Rossol) and susceptible (cv. Roma VF) tomato roots uninfested and infested by* Meloidogyne incognita. *Values are expressed as mg per g fresh weight of roots and given as averages of 3 experiments.*

Cellular Fractions	Rossol (resistant)			Roma VF (susceptible)		
	Uninfested	Infested	% of control	Uninfested	Infested	% of control
Cell-wall	0.20	0.20	–	0.16	0.14	– 12
Cell-wall «Washed Fr.»	0.19	0.24	+ 26	0.36	0.38	+ 5
Mitochondrial	0.12	0.23	+ 92	0.09	0.13	+ 44
Microsomal	0.19	0.16	– 16	0.18	0.21	+ 17
Cytosol: «Retained Fr.»	0.21	0.20	– 5	0.34	0.48	+ 41
«Filtered Fr.»	0.07	0.10	+ 43	0.09	0.11	+ 22

TABLE II - *Dry weight of resistant (cv. Rossol) and susceptible (cv. Roma VF) tomato roots uninfested and infested by* Meloidogyne incognita. *Values are given as mg per pot (5 seedlings, ± SD, n = 7).*

Tomato cvs	Uninfested	Infested
Rossol (resistant)	3.7 ± 0.6	4.5 ± 0.4
Roma VF (susceptible)	7.3 ± 1.4	5.2 ± 0.7

Discussion

This paper reports evidence of the different involvement of each cellular component in incompatible or compatible *Meloidogyne*-tomato interactions. Respiration of mitochondria isolated from resistant roots has been found to be inhibited after nematode infestation (Zacheo and Molinari, 1987b; Molinari *et al.*, 1990). On the other hand, resistant intact roots have been reported to increase their oxygen uptake after nematode infestation at least with aged seedlings (Zacheo and Molinari, 1987a; Zacheo and Bleve-Zacheo, 1987). Thus, the energy demand by the defence process could be sustained by the proliferation of mitochondria as evident from the data reported in this paper and in Molinari *et al.* (1990).

Cell-wall proteins seem to play a crucial role in resistance of tomato to root-knot nematodes as already suggested in Zacheo *et al.* (1988). Protein content of the other cellular sites such as microsomes and cytosol seem to be unaffected or depressed in infested resistant roots.

The susceptible cultivar exhibited a general increase of protein content in all the cellular components except cell walls. This agrees with the overall increase in protein synthesis observed in the susceptible reaction to root-knot nematode (Owens and Novotny, 1960; Endo and Veech, 1969). Finally, it should be noted that the lowering in dry weight of susceptible roots was monitored only two days after inoculation which might be an early symptom of the delayed development of attacked plants.

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Accepted for publication on 18 June 1990.