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MITOCHONDRIAL PEROXIDASE AND SUPEROXIDE DISMUTASE ACTIVITIES DURING THE INFECTION BY *MELOIDOGYNE INCOGNITA* OF SUSCEPTIBLE AND RESISTANT TOMATO PLANTS

by
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It has been reported that attacks of *Heterodera goettingiana* and of *Meloidogyne incognita* induce changes in the peroxidase (PO) and superoxide dismutase (SOD) activities in pea and tomato roots. Such alterations involve various cellular components such as mitochondria, microsomes and soluble fractions (Zacheo *et al.*, 1980; Arrigoni *et al.*, 1981).

Changes induced by the nematode in the roots of susceptible lines differ from those occurring in the resistant ones (Zacheo *et al.*, 1980). The PO activity increases in the second and decreases in the first ones, while the SOD activity increases in the susceptible and decreases in the resistant lines.

The aim of the present study was to ascertain when such changes in the activity of the two enzymes are initiated, their duration and whether the activities are synchronized.

Materials and Methods

Seedlings of tomato cultivars Roma VF and VFN8, susceptible and resistant respectively to *M. incognita*, were transplanted at 6-7 cm height into 10 cm diameter plastic pots containing steam sterilized sandy loam. Approximately 2,000 active juveniles of *M. incognita* were pipetted into four holes spaced equidistantly around the plants in

each pot. The pots were then placed in a growth chamber, at 27°C, 65% RH and 3,000 lux for 15 hrs each day, and kept for 27 days. At 3-day intervals, after inoculation of the nematodes, the plants of each cultivar were removed, the roots washed thoroughly in distilled water and dried with filter paper.

Root samples of 20 g were homogenized in a Waring blendor for 8 sec in a medium containing 50 mM Tris-HCl, 0.3 M mannitol, 1 mM EDTA, 10 mM $MgCl_2$, 0.1% bovine serum albumin, 0.05% cysteine and 0.1% polyvinylpyrrolidone. The homogenate was centrifuged at 600 g for 10 min, to remove cell debris and nuclei. Mitochondria were precipitated at 7,000 g for 20 min, washed twice and resuspended in the same medium, without cysteine and pyrrolidone. The mitochondrial fraction was sonicated with a Branson sonifier (mod. w 18SD) for one minute at 20 sec intervals at 50 Watt and 0°C.

Peroxidase was assayed by measuring absorbance at 470 nm using guaiacol and hydrogen peroxide (Chance and Maehly, 1955), in the absence or presence of 2 mM salycilic hydroxamic acid (SHAM) and expressed as ΔOD/mg of proteins.

Superoxide dismutase was determined by the nitroblue tetrazolium (NBT) method. The rate of NBT reduction was determined at 578 nm in a cuvet containing 25 μM NBT, 0.1 mM xanthine, 3.3×10^{-9}M xanthine oxidase, 0.1 mM EDTA and 50 mM sodium carbonate. SOD activity was expressed as units/mg of proteins (Beauchamp and Fridovich, 1971). Proteins were determined by the method of protein-dye binding (Bradford, 1976).

Results

The data on peroxidase activity in mitochondria isolated from tomato roots at 3-day intervals from infestation until 27th day are reported in Fig. 1.

The mitochondria obtained from the nematode-infested roots of the resistant cv VFN8 showed the highest peroxidase activity 10-12 days after inoculation. The peroxidase activity was, in the mitochondria of the resistant nematode-infested plants, 5 times higher than in the healthy ones, but began to decrease and to reach normal levels, similar to those of the healthy plants, about 24 days after inoculation.

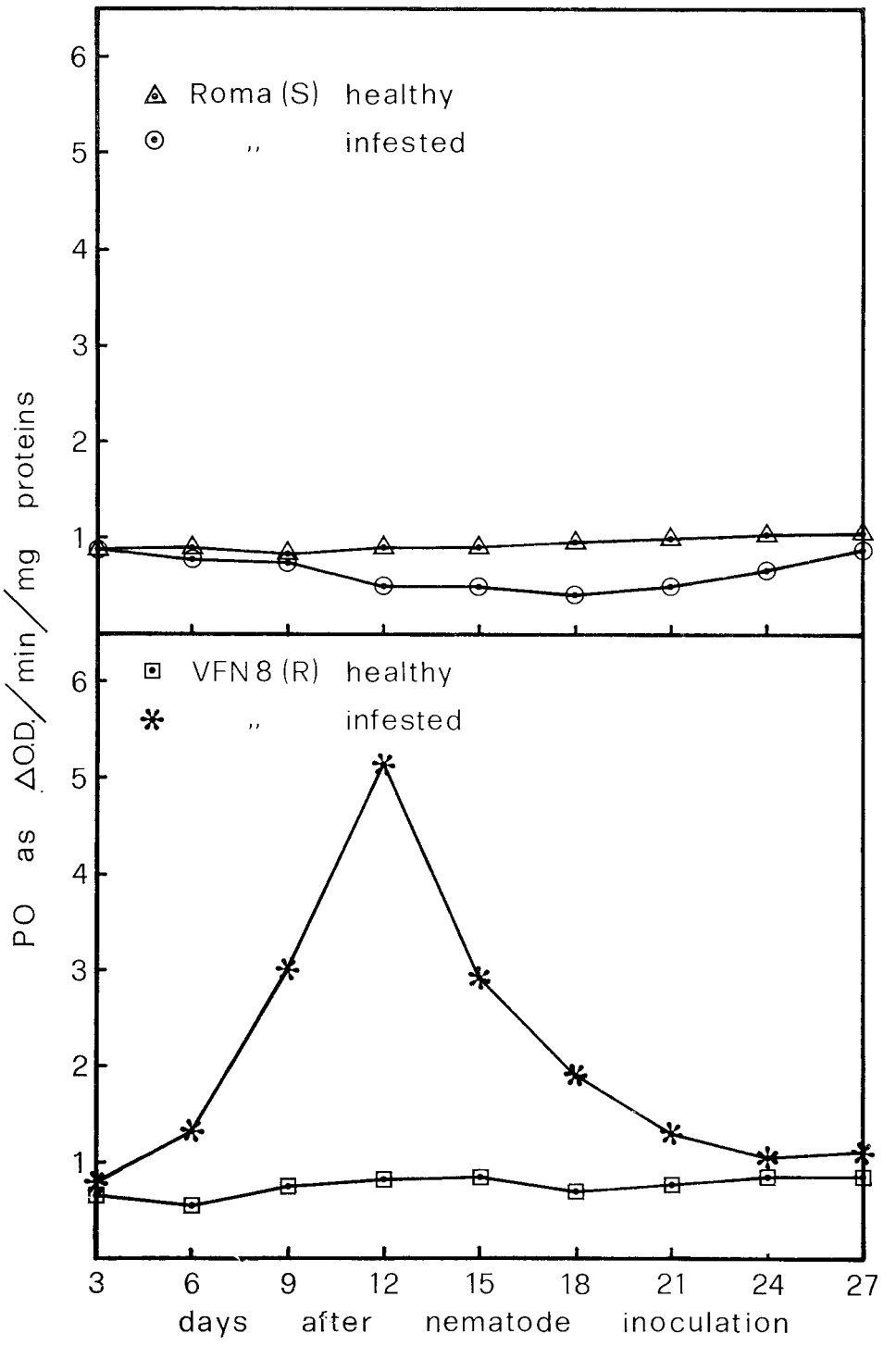

Fig. 1 - Time course of mitochondrial peroxidase in susceptible (S) and resistant (R) tomato roots after inoculation with *Meloidogyne incognita*.

No remarkable differences were observed in the activity of this enzyme between infested or uninfested roots of the susceptible cultivar Roma VF.

Mitochondrial peroxidase in tomato plants is strongly inhibited by SHAM (Table I); however, no differences were detected in the response to SHAM between the resistant and the susceptible cultivars infested or uninfested by the nematodes. Since SHAM also inhibits myeloperoxidase activity in polymorphonuclear leukocytes (Arrigoni *et al.*, 1981) (myeloperoxidase is known to be involved in animal biological defence mechanisms) this compound could be a useful tool in investigating the role of peroxidases in the defence mechanism of both plants and animals (Rich *et al.*, 1978).

During the process of infestation, SOD activity decreased sharply in the isolated mitochondria of the resistant cultivar, from the third day after infestation, to become lowest (45% less with respect to the healthy plants) at nine days after inoculation (Fig. 2); the enzyme activity then increased and rapidly reached normal levels. The pathway of the mitochondrial SOD was completely different in the susceptible cultivar. The enzyme activity did not in fact undergo any change during the first seven days after inoculation, and then increased to reach the highest level (+107% with respect to non-infested control) about the 20th day and decreased sharply thereafter.

Table I - *Effect of SHAM on the peroxidase activity in mitochondria of roots of susceptible (S) and resistant (R) tomato cultivars inoculated with* Meloidogyne incognita, *expressed as ΔOD/mg proteins. Table shows data at 12th day after infestation.*

Cultivars	Treatment		% inhibition
	Untreated	2mM SHAM	
Roma VF (S)	0.600	0.085	86
» + nematodes	0.610	0.100	83
VFN8 (R)	0.550	0.110	80
» + nematodes	3.710	0.595	84

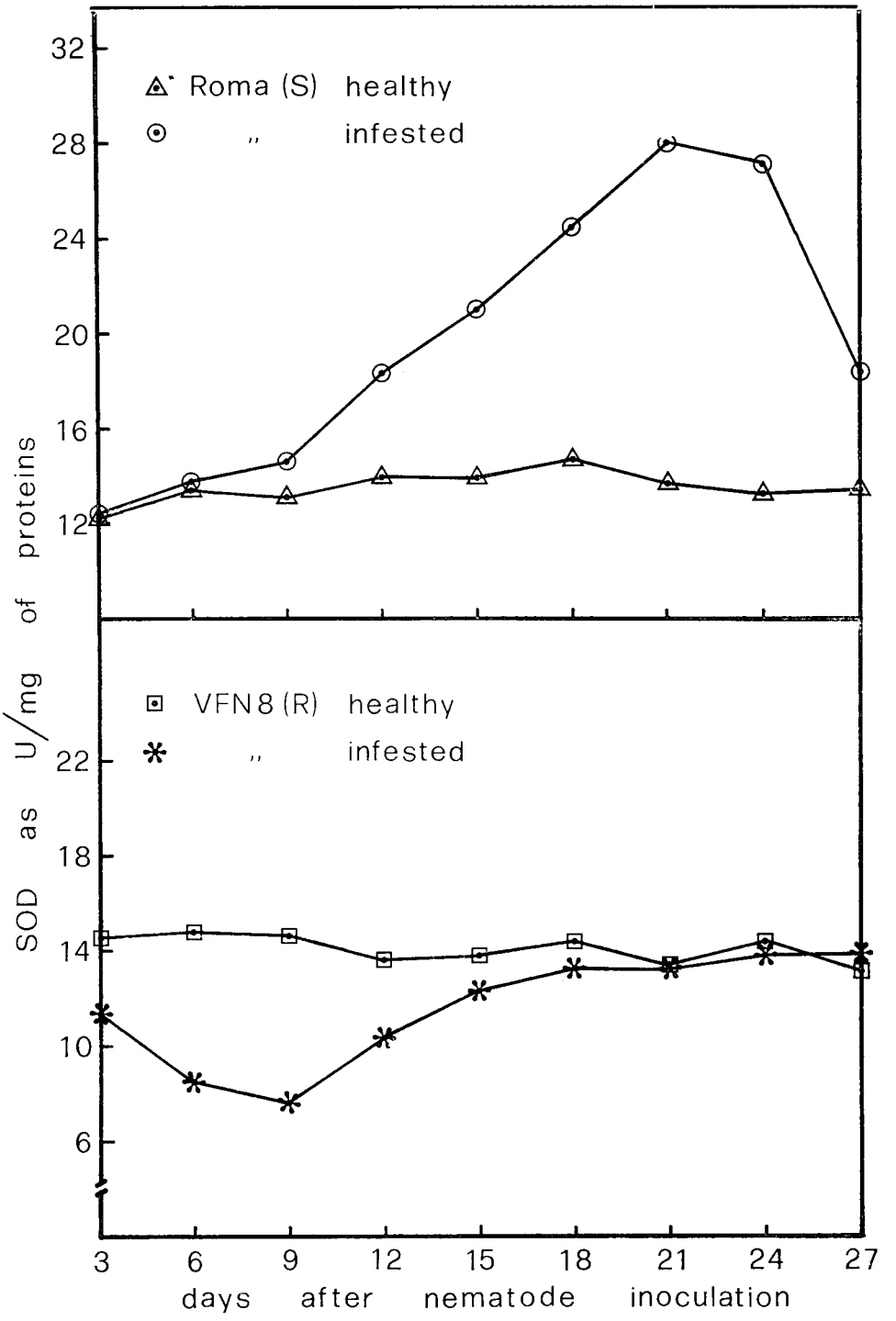

Fig. 2 - Time course of mitochondrial SOD in susceptible (S) and resistant (R) tomato roots after inoculation with *Meloidogyne incognita*.

Discussion

These results concerning the PO and the SOD activity confirm that resistant cultivars of tomato respond to attack by *M. incognita* as do resistant lines of pea to attack by *H. goettingiana.*

The identical response of different plant species to the attacks of different nematodes would lead to the conclusion that the changes in the activity of these two enzymes is a generalized response of plants towards the nematode.

The increase of PO and the decrease of SOD during the process of nematode infestation of resistant cultivars are substantially concomitant and are evident within the first 10-12 days after inoculation.

How these changes could act as a biological defence mechanism of plants is not clear at present. According to the biological defence mechanism proposed by Arrigoni (1979), PO would generate the anionic radicals of oxygen: the superoxide. It is known that the superoxide in the presence of H_2O_2 and traces of Fe^{3+} originates the hydroxyl radical, $OH^{\cdot}$, which is the most powerful oxidant known in chemistry (Rowley and Halliwell, 1982). To this radical are attributed the damaging effects of $O_2^{\cdot-}$ generating systems (Fridovich, 1974, 1975; Klebanoff, 1967; Piatt *et al.*, 1977, Rosen and Klebanoff, 1977).

The increase of the PO activity in resistant cultivars would increase production of superoxides and their derivates by which the cells counteract the action of the pathogen. The presence of a large quantity of free radicals of oxygen would produce direct inactivation of the pathogen, similar to what happens in phagocytosis (Badwey and Karnovsky, 1980) where the microbicidal and cytotoxic activity is attributed to myeloperoxidase (Klebanoff and Hamon, 1972; Klebanoff and Clark, 1978).

Recent data indicate that *M. incognita* juveniles are very sensitive to the radical action (Zacheo *et al.*, 1982). Free radicals, besides inactivating the nematode, could also cause necrosis of the cells surrounding the pathogen (Bleve-Zacheo *et al.*, 1982; Paulson and Webster, 1972).

If the role of PO is to generate free radicals, as proposed by Klebanoff and Hamon (1972), Klebanoff and Clark (1978), Piatt *et al.* (1977), and Yokota and Yamazaki (1965), the utility of the decrease of SOD activity in resistant cultivars would clearly be explained. In fact, the decrease of SOD activity, which normally eliminates the

superoxides by dismutation to H_2O_2,would increase the quantity of free radicals available for the mechanism of defence.

However, no large quantity of free radicals should be generated after infestation in the susceptible cultivars because PO activity does not increase. This should render such plants, in which also a remarkable increase of SOD activity occurs, more vulnerable to the nematode.

SUMMARY

The mitochondrial peroxidase (PO) and superoxide dismutase (SOD) activities were determined, in roots of susceptible and resistant tomato plants, during a period of 27 day following infection with *Meloidogyne incognita*. Peroxidase activity increased in the resistant cultivar up to a level five times the level in healthy plants, as measured 10 days after inoculation; thereafter it decreased to normal levels within a few days. No changes in PO activity occurred in susceptible cultivars. Superoxide dismutase decreased by 45% and increased by 107%, compared with noninfested plants, in the resistant and in the susceptible cultivars, respectively. The behaviour of these two enzymes accords with the pathway of the biological defence mechanism recently proposed by Arrigoni (1979).

LITERATURE CITED

ARRIGONI O., 1979 - A biological defence mechanism in plants. In: Root-knot Nematodes (*Meloidogyne* Species) Systematics, Biology and Control (Lamberti F. and Taylor C. E., Eds.) Academic Press, New York, pp. 457-467.

ARRIGONI O., ZACHEO G., BLEVE-ZACHEO T., ARRIGONI-LISO R. and LAMBERTI F., 1981 - Changes of superoxidedismutase and peroxidase activities in pea roots infested by *Heterodera goettingiana*. *Nematol. medit., 9*: 189-195.

ARRIGONI O., LISO R., CALABRESE G., DE SANTIS A., FUMARULO R., and GIORDANO D., 1981. Acido salicilidrossamico: un potente inibitore della mieloperossidasi. *Accad. Pugliese Sci., 39*: 1-7.

BADWEY J. A. and KARNOVSKY M. L., 1980 - Active oxygen species and the function of phagocytic leukocytes. *Ann. Rev. Biochem., 49*: 695-726.

BEAUCHAMP C. and FRIDOVICH I., 1971 - Superoxide dismutase improved assay applicable to acrylamide gels. *Anal. Biochem., 44*: 276-287.

BLEVE-ZACHEO T., ZACHEO G., MELILLO M. T. and LAMBERTI F., 1982 - Ultrastructural aspects of the hypersensitive reaction in tomato root cells resistant to *Meloidogyne incognita*. *Nematol. medit., 10*: 81-90.

BRADFORD M. M., 1976 - A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem., 72*: 248-254.

CHANCE B. and MAEHLY A. C., 1955 - Assay of catalase and peroxidases. In: Methods in Enzymology. (Colowick S. P. and Kaplan N. O., Eds.) Vol. 2, Acad. Press, New York, pp. 769-773.

FRIDOVICH I, 1974 - Superoxide Dismutase. In: Molecular Mechanisms of Oxygen Activation (Hayaishi O., Ed.) Acad. Press, New York, pp. 453-477.

FRIDOVICH I., 1975 - Superoxide Dismutase. *Ann. Rev. Biochem., 44*: 147-159.

KLEBANOFF S. J., 1967 - Iodination of bacteria: a bactericidal mechanism. *J. Exptl. Med., 126*: 1063-1078.

KLEBANOFF S. J. and HAMON C. B., 1972 - Role of myeloperoxidase-mediated antimicrobial systems in intact leukocytes. *J. Reticuloendothel. Soc., 12*: 170-196.

KLEBANOFF S. J. and CLARK R. A., 1978 - The Neutrophil: Function and Clinical Disorders. North-Holland, Amsterdam, pp. 409.

PAULSON R. E. and WEBSTER J. M., 1972 - Ultrastructure of the hypersensitive reaction in roots of tomato, *Lycopersicon esculentum* L., to infection by root-knot nematode, *Meloidogyne incognita. Physiol. Plant. Pathol., 2*: 227-234.

PIATT J. F., CHEEMA A. S. and O'BRIEN P. J., 1977 - Peroxidase catalyzed singlet oxygen formation from hydrogen peroxide. *FEBS Lett., 74*: 251-254.

RICH P. R., WIEGAND N. K., BLUM H., MOORE A. L., and BONNER W. D., Jr., 1978 - Studies on the mechanism of inhibition of redoxenzymes by substituted hydroxamic acids. *Biochim. Biophys. Acta, 525*: 325-337.

ROSEN H. and KLEBANOFF S. J., 1977 - Formation of singlet oxygen by the myeloperoxidase-mediated antimicrobial system. *J. Biol. Chem., 252*: 4803-4810.

ROWLEY D. A. and HALLIWELL B., 1982 - Superoxide-dependent formation of hydroxyl radicals in the presence of thiol compounds. *FEBS Lett., 138*: 33-36.

YOKOTA K. and YAMAZAKI I., 1965 - Reaction of peroxidase with reduced nicotinamide-adenine dinucleotide and reduced nicotinamideadenine dinucleotide phosphate. *Biochim. Biophys. Acta, 105*: 301-312.

ZACHEO G., BLEVE-ZACHEO T., ARRIGONI-LISO R. and ARRIGONI O., 1980 - Mitochondrial superoxide dismutase and peroxidase activities in susceptible and resistant tomato plants infected by *Meloidogyne incognita*. XV Int. Nematology Symposium, Bari, Italy, 24-30 Aug. 1980, pp. 20-21.

ZACHEO G., BLEVE-ZACHEO T. and LAMBERTI F., 1982 - Heat-induced susceptibility on tomato cultivars resistant to *Meloidogyne incognita*. XVI Int. Nematology Symposium, St. Andrews, Scotland, 30 Aug.-3 Sept. 1982, p. 77.

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