*Nematol. medit.* (1982) *10*: 75-80

*Istituto di Nematologia Agraria, C.N.R. - 70126 Bari, Italy*

# ROLE OF PEROXIDASE AND SUPEROXIDE DISMUTASE ACTIVITY IN RESISTANT AND SUSCEPTIBLE TOMATO CULTIVARS INFESTED BY *MELOIDOGYNE INCOGNITA*

by
G. ZACHEO, T. BLEVE-ZACHEO and F. LAMBERTI [1]

There are many reports about changes of peroxidase activity in the tissues of infested plants. Stahmann and Demorest (1973) correlated an increase in peroxidase with resistance to several fungal, bacterial and viral pathogens; Tomiyama and Stahmann (1964) and Weber *et al.* (1967) reported an increase in peroxidase isoenzymes as a result of fungal and bacterial infection respectively; Sakuma *et al.* (1976) demonstrated different levels of peroxidase activity in incompatible and compatible red clover tissue infected by *Kabatiella caulivora*; also Huang *et al.* (1971) reported changes in peroxidase isoenzymes in galls induced by *Meloidogyne incognita* (Kofoid *et* White) Chitw. in tomato.

Evidence for the involvement of peroxidase in disease resistance is far from conclusive and there are differences in opinion on how the available data should be interpreted (Wheeler, 1975). The different roles attributed to these isoenzymes are partly due to the extremely diversified enzyme populations and the considerable biochemical versatility that they exhibit (Hepler *et al.*, 1972; Srivastava and Van Huystee, 1973).

It has recently been reported that neutrophils released, upon stimulation, myeloperoxidase and $H_2O_2$ into the surrounding medium, being thus capable of destroying adjacent cells. This results in the liberation of a large quantity of $O_2^-$ which could result in the formation

[1] The authors thank Mr. R. Lerario for technical assistance.

Table I. - *Total peroxidase activity of susceptible (S) and resistant (R) cultivars of tomato infested or not by* Meloidogyne incognita, *expressed as* $\Delta$ *O D/mg of proteins.*

| Cultivar | Control | Infested[a] |
|---|---|---|
| ROMA (S) | 2.70 | 2.02 ** |
| BARESANA (S) | 2.85 | 1.74 ** |
| RVE SMD (S) | 2.78 | 1.73 ** |
| EARLY PAK (S) | 2.88 | 2.40 ** |
| VFN 8 (R) | 1.80 | 2.76 * |
| PIERSOL (R) | 2.75 | 3.44 * |
| ROSSOL (R) | 2.43 | 3.31 * |
| BRECH (R) | 1.94 | 2.76 * |

([a]) Statistical significance between uninfested and infested plants was determined according to the Student's *t* test separately for susceptible and resistant cultivars: * and ** significant for P = 0.05 and P = 0.01 respectively.

of hydroxyl radical and singlet oxygen (Badwey and Karnowsky, 1980). Generation of the superoxide radical during the peroxidatic oxidation of NADH and the formation of singlet oxygen from hydrogen peroxide are catalized by catalase and peroxidase respectively (Halliwell, 1977; Piatt, 1977). The deleterious consequences of these radicals could be prevented by a protective action of superoxide dismutase. These enzymes, which are extremely rapid in action, must exercise strict control of the concentration of cellular $O_2^-$ radicals to avoid extensive damage to the various biological structures (Lavelle *et al.*, 1973).

The results of an investigation of the activity of the enzymes peroxidase and superoxide dismutase in resistant and susceptible tomato plants, infested or not by *M. incognita*, are reported in this article.

## *Materials and Methods*

Tomato plants susceptible and resistant to root-knot nematode, *M. incognita* (Zacheo *et al.*, 1977) were transplanted at 6-7 cm height into 10 cm diameter plastic pots containing steam sterilized sandy loam. The pots were placed in a growth chamber (27 °C, 65% RH,

Table II. - *Cyanide-sensitive and cyanide-resistant superoxide dismutase of susceptible and resistant cultivars of tomato infested or not by* M. incognita.

| Cultivar | Control | | Infested (a) | |
|---|---|---|---|---|
| | Total | KCN resistant | Total | KCN resistant |
| ROMA (S) | 23.85 | 6.84 | 29.52** | 11.91** |
| BARESANA (S) | 25.84 | 5.84 | 31.40 ** | 14.40 ** |
| RVE SMD (S) | 27.02 | 7.95 | 31.92 ** | 13.00 ** |
| EARLY PAK (S) | 20.00 | 4.64 | 30.54 ** | 13.79 ** |
| VFN 8 (R) | 25.93 | 6.63 | 15.74 | 4.37 |
| PIERSOL (R) | 34.88 | 9.17 | 25.88 | 8.24 |
| ROSSOL (R) | 22.47 | 5.91 | 19.50 | 6.03 |
| BRECH (R) | 21.32 | 5.15 | 15.90 | 4.46 |

(a) Statistically significant with respect to the control.
** for P = 0.01 according to the Studen's *t* test.

3,000 lux) and after an acclimatization of approximately 15 days, 2,000 active juveniles of *M. incognita* were pipetted into each pot. Twenty days after inoculation the plants were removed, the roots washed thoroughly with tap water, rinsed in distilled water and dried with filter paper.

Root samples (20 g) were homogenized in a Waring blender with two vol 0.1 M potassium phosphate buffer at pH 7.2. The homogenate was centrifuged at 20,000 g for 20 minutes; the supernatant was used for superoxide dismutase (SOD) and peroxidase determination.

Superoxide dismutase was determined by the nitroblue tetrazolium (NBT) method in the absence or presence of 2 mM KCN. The rate of NBT reduction by the xanthine-xanthine-oxidase system was determined at 578 nm, in a cuvet containing 25 M NBT, 0.1 mM xanthin, 0.1 mM EDTA and 50 mM sodium carbonate (Beauchamp and Fridovich, 1971); SOD activity was expressed as units/mg of proteins. Peroxidase was assayed by measuring assorbance at 470 nm, using guaiacol and hydrogen peroxide (Chance and Maehly, 1955), and expressed as $\Delta$ O D/mg of proteins. Proteins were determined by the method of protein-dye binding (Bradford, 1976).

## *Results*

No statistically significant differences were observed in the endogenous content of peroxidase between control plants (non infested) of susceptible and resistant tomato cultivars (Table I), although activity appeared to be lower in cvs. VFN 8 and Brech. However, nematode infestation induced a decrease of peroxidase activity only in the susceptible cultivars and this decrease was greater in cvs. Baresana and RVE SMD.

In resistant plants the nematode attack was always correlated with an increase of peroxidase activity; the highest increase (52%) with respect to non-infested plants was recorded in VFN 8.

Also, no significant differences were found in the SOD content of the root tissues between healthy susceptible and resistant cultivars (Table II). Conversely, after infestation by *M. incognita*, the SOD activity increased in the susceptible cultivars but decreased in the resistant ones. The highest increase occurred in Early Pak with 34% more than the non infested control; the other three cultivars showed an average increase of 18% only. A remarkable decrease, around 40% in VFN 8, slightly lower in Piersol and Brech and even less in Rossol, was noticed in the resistant cultivars.

The enzymatic activity of infested and not infested plants was tested in presence of 2 mM KCN to determine which type of SOD was involved. The results indicated that SOD cyanide resistance was present in both resistant and susceptible cultivars.

This type of SOD has been classified by Fridovich (1974 and 1975) as Mn-SOD and is predominant in the mitochondrial fractions, while the Cu-Zn-SOD is present in the other cellular components. The cyanide resistant Mn-SOD increased greatly in the susceptible cultivars during infestation. In fact, the highest increase of 70% with respect to the non-infested control was detected in cv. Early Pak, while no significant changes were noticed, after nematode penetration, in the resistant cultivars (Table II).

## *Discussion*

The results of our investigations indicate that *M. incognita* infestation of resistant cultivars of tomato induce two fundamental metabolic events, which play a key role in the biological defence mechan-

ism. The first is represented by a large increase of peroxidase, the second by a decrease of superoxide dismutase activity.

The behaviour of these two enzymes, which occur in plants, is in accordance with the data reported for animal tissues (Badwey and Karnovsky, 1980). The root cells of resistant plants might react to the presence of a pathogen by developing high peroxidase activity which could produce a larger quantity of free radicals. These radicals are the basis of the mechanism whereby the pathogen is inactivated by the host cells. Moreover, in resistant cultivars the defence process is facilitated by a decrease of superoxide dismutase, which transforms the superoxides into hydrogen peroxide, the cells readily eliminating this via catalase.

In susceptible cultivars this kind of defence mechanism does not occur because the amount of the superoxides produced by increasing peroxidase activity is inadequate. Moreover, the unbalanced increase of SOD, rapidly transforms the limited amount of radicals into hydrogen peroxide and blocks the defence mechanism of the cell.

We assume that the increase of peroxidase enzymes, or of a particular unknown peroxidase, is capable of generating superoxide radicals through the oxidation of reduced pyridine nucleotides. A similar mechanism has been proposed to account for NADH oxidation by peroxidase and catalase, acting as peroxidase (Halliwel, 1977).

## SUMMARY

Superoxide dismutase (SOD) activities increased in susceptible tomato cultivars after inoculation with *Meloidogyne incognita*; conversely, in resistant cultivars SOD activities decreased after nematode invasion. Peroxidase activity increased in infested resistant plants but showed a decrease in susceptible ones. On the basis of these results it is hypothesized that SOD activity must harmonise with peroxidase activity in the establishment of successful defence mechanisms.

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Accepted for publication on 16 Jaunary 1982.