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CHANGES OF SUPEROXIDEDISMUTASE AND PEROXIDASE ACTIVITIES IN PEA ROOTS INFESTED BY *HETERODERA GOETTINGIANA*

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There are many reports about oxidative enzyme activities in the regulation of metabolic pathways in diseased and injured plant tissue (Frič, 1976), and it is known that plant tissues infected by different pathogens exhibit changes in peroxidase (EC 1.11.1.7). Increased peroxidase activity in various host-parasite interactions was associated with resistance to the disease in the host (Ferhmann and Dimond, 1967; Jennings *et al.*, 1969; Bates and Chant, 1970, Hussey and Krusberg, 1970; Simons and Ross, 1971; Huang *et al.*, 1971; Benedict, 1972; Rathmell and Sequeira, 1980). Lovrekovich *et al.* (1967) reported resistance to wild fire disease induced by infiltrating heat killed cells of *Pseudomonas tabaci* into tobacco leaves, and observed that this was accompanied by a marked increase in peroxidase activity. Increase of peroxidase activity and systemic resistance were also observed in the upper leaves of tobacco plants inoculated with tobacco mosaic virus on the lower leaves (Simons and Ross, 1970). Although peroxidase is known to be involved in disease reactions, its role is far from being conclusive and remains undefined.

Recently the hypothesis was advanced of a biological mechanism of defence in plants dependent on development of cyanide resistant respiration (Arrigoni, 1979), in which the enzymes peroxidase and superoxidedismutase (SOD; E.C. 1.15.1.1) have well defined metabolic functions (Zacheo *et al.*, 1980). As the increase in respiration (Uritani and Akazawa, 1959; Millerd and Scott, 1962; Farkas *et al.*, 1964; Antonelli and Daly, 1966; Daly, 1976; Brenneman and Black,

1979) and the enhanced peroxidase activity has been reported during infection processes in plants, this study was undertaken to establish whether there are also changes in SOD and in which way the activity of the enzymes is correlated during the invasion of pea (*Pisum sativum* L.) roots by the pea cyst nematode, *Heterodera goettingiana* Wollenweber.

Materials and Methods

Seeds from the germplasm pea collection of the Istituto del Germoplasma del CNR, Bari, were soaked overnight and sown in 30 cm plastic pots containing sterilized sandy soil artificially infested with 30 eggs-juveniles of *H. goettingiana*/g of soil. The pots were placed in a growth chamber (16 °C, 65% RH, 3,000 lux) and 25 days later the plants were removed, the roots washed thoroughly in distilled water and dried with filter paper.

A 20 g sample of roots was 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% cystein, 0.1% polyvinylpyrrolidon. The homogenate was centrifuged at 600xg to remove cell debris and nuclei. Mitochondria were precipitated at 7000xg for 20 min, washed twice and resuspended in the same medium, without cystein and pyrrolidon. The microsomal fraction was precipitated from the supernatant at 100,000xg for 2h and resuspended in the same medium as the mitochondria. The mitochondrial fraction was sonicated with a Branson sonifier.

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-oxidase system was determined at 578 nm, in a cuvet containing 25 μM NBT, 0.1 mM xanthine, 0.1 mM EDTA and 50 mM sodium carbonate (Beauchamp and Fridovich, 1971); SOD activity was espressed 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 ΔOD/mg of proteins. Proteins were determined by the method of protein-dye binding (Bradford, 1976).

Results

As indicated elsewhere (Zacheo *et al.*, 1981) three arbitrarily defined resistant and three susceptible pea lines (Table I) were selected among the ten on which penetration of *H. goettingiana* had been tested. With plants defined as resistant, much lower numbers of *H. goettingiana* juveniles were detected in the roots 25 days after inoculation than in roots of plants defined as susceptible (Zacheo *et al.*, 1981).

Remarkable increases were observed in the peroxidase activity of all the cellular components examined — soluble fraction, mitochondria and microsomes — in roots of resistant lines attacked by *H. goettingiana* (Table I). The overall increase (i.e. all fractions) of peroxidase activity ranged from 50% for the resistant line MG 101877 b to 150% for MG 101877 c for nematode inoculated plants compared with the same lines not inoculated.

The largest changes in peroxidase activity were found in the microsomal and soluble fractions while there were only minor changes in the mitochondria (Table I).

Table I - *Peroxidase activity in resistant (R) and susceptible (S) pea lines inoculated with* Heterodera goettingiana.

Lines	Peroxidase as △OD min mg proteins		
	Mitochondria	Microsomes	Soluble Fraction
MG 101877 c (R)	3.74	6.70	7.69
» + Nematodes	7.45	18.00	21.38
MG 101956 a (R)	4.06	11.27	15.39
» + Nematodes	6.43	24.60	24.08
MG 101877 b (R)	0.98	7.94	15.40
» + Nematodes	1.62	11.69	19.61
MG 101956 b (S)	3.48	12.66	12.87
» + Nematodes	2.55	11.12	13.29
MG 101877 a (S)	4.83	16.15	21.41
» + Nematodes	1.63	9.88	16.10
MG 101793 (S)	0.96	15.00	16.79
» + Nematodes	0.88	11.09	10.20

Table II - *Superoxidedismutase activity in resistant (R) and susceptible (S) pea lines inoculated with* Heterodera goettingiana.

Lines	SOD as U/mg of proteins		
	Mitochondria	Microsomes	Soluble Fraction
MG 101877 c (R)	4.92	8.01	3.61
» + Nematodes	4.37	8.45	3.46
MG 101956 a (R)	4.70	10.83	5.14
» + Nematodes	3.20	8.87	4.90
MG 101877 b (R)	7.52	10.22	4.80
» + Nematodes	4.54	7.44	4.00
MG 101956 b (S)	4.27	5.53	4.18
» + Nematodes	6.26	7.92	4.34
MG 101877 a (S)	6.62	3.31	4.85
» + Nematodes	7.20	6.60	4.95
MG 101793 (S)	4.33	8.58	4.92
» + Nematodes	7.02	11.37	5.10

In the susceptible lines, nematode invasion induced no increase of peroxidase activity; for the line MG 101877 a peroxidase activity in infested roots was actually 40% lower than in healthy ones.

Nematode attack induced only slight decrease of SOD activity in roots of resistant lines (Table II). The largest decreases were observed mostly in the microsomes and in the mitochondria.

SOD activity was considerably increased (by 45% with respect to uninfested plants) by nematode invasion of the roots of susceptible plants. Also, in this case, the increases occurred only in the microsomal and mitochondrial fractions (Table II).

A large part of SOD in the mitochondrial fraction was found to be CN -resistant, indicating that the isoenzyme is predominantly a Mn-SOD. However, SOD activity in the microsomes was CN -sensitive due to Cu-Zn-SOD isoenzymes.

Discussion

The results provide evidence, for the first time, of major changes in SOD activity in pea root cells following attack by *H. goettingiana.*

Increases in enzymatic activity occurred in susceptible plants while decreases were noticed in resistant ones.

The indirect correlation between the changes of SOD and peroxidase is of particular interest. Whenever peroxidase activity increased, SOD activity decreased (resistant plants) and, on the contrary, when peroxidase activity decreased or did not change SOD activity increased (susceptible plants).

It is still difficult and premature to interpret the role that these two enzymes have in the biological defence mechanism of plants. Nevertheless several authors attribute a significant role to peroxidase activity in the biochemical processes conferring plant resistance to pathogens (Fehrmann and Dimond, 1967; Simons and Ross, 1971; Benedict, 1972; Rathmell and Sequeira, 1975; Noel and McClure, 1978).

The peroxidase, probably a special peroxidase, would generate by oxidation of NADPH the anionic radical of oxygen, the superoxide $O_2^{\cdot -}$ (Yokota and Yamazaki, 1965) which would then initiate a chain of reactions to overcome the action of the pathogen. Superoxides are only efficiently utilized when SOD remains at low levels. In fact, SOD lowers the concentrations of free radicals, which can be used in the mechanism of defence, by catalizing dismutation of superoxide in hydrogen peroxide and oxygen (Fridovich, 1974 and 1975).

Therefore all the conditions to initiate the defence mechanisms are optimal in the resistant varieties which, following nematode invasion, considerably increase peroxidase activity and maintain SOD activity at low levels. On the contrary, in susceptible plants the increase of SOD activity reduces the availability of the free radicals, utilized in the mechanism of defence, generated by peroxidase. Moreover, in these plants peroxidase activity is not increased (sometimes is decreased) by nematode attack, and thus there is little production of superoxides.

The greater capability of resistant plants to establish defence mechanisms compared to susceptible ones seems to be due to a quicker response to the pathogen. Reynolds *et al.* (1970) reported that rate of penetration of *Meloidogyne incognita* juveniles in the roots of susceptible and resistant alfalfa plants was identical in the first stages of infestation. It is assumed therefore that in the case of peas attacked by *H. goettingiana*, partial resistance is not due to any obstacle to the penetration of the parasite but to the capability of the plant to immediately initiate the biological defence processes which inhibit the trophic action and development of the nematode.

SUMMARY

Marked changes in the activity of two enzymes, peroxidase and superoxidedismutase (SOD), occurred in resistant and susceptible pea plants after invasion by *Heterodera goettingiana* Wollenweber. SOD activity increased in susceptible plants and, conversely, decreased in resistant ones after nematode invasion. Peroxidase activity increased in resistant plants and there was either no change or a decrease in susceptible plants. The largest changes in peroxidase activity were observed in the soluble and microsomal fractions and, for the SOD activity, in the mitochondrial and microsomal fractions. An hypothesis is proposed, on the basis of the results, on the biological mechanism of defence.

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