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Istituto di Nematologia Agraria, C.N.R. — 70126 Bari, Italy
*Istituto del Germoplasma, C.N.R. — 70126 Bari, Italy**

SOME BIOCHEMICAL PROPERTIES OF *PISUM SATIVUM* SUSCEPTIBLE AND RESISTANT TO *HETERODERA GOETTINGIANA*

by
G. ZACHEO, T. BLEVE- ZACHEO, M.T. MELILLO and P. PERRINO*

Summary. Biochemical changes in roots of a germplasm pea accession resistant to *Heterodera goettingiana* were compared with those which occur in roots of a susceptible variety. Increased lipid peroxidation of the host cell membranes was induced by nematode infestation of resistant roots. The increase of lipid peroxidation is believed to be initiated by an $O_2^{\dot{-}}$ generating system (NADPH oxidase) present in the membrane of plant tissue and activated in the resistant accession, but not in the susceptible variety. The lack of $O_2^{\dot{-}}$ activation in susceptible infected tissue may result from the protective activity of enzymes such as superoxide dismutase and catalase. The protective activity of these enzymes was enhanced in susceptible but decreased in resistant plants. The resistant tissue was also associated with increased peroxidase activity. This peroxidase activity can be influenced by catalase destroying the H_2O_2 substrate. Different isozymes were detected in the peroxidase profiles of resistant and susceptible pea plants. An interpretation of these results is proposed in terms of biochemical characterization of pea plants resistant and susceptible to the cyst nematode, *H. goettingiana*.

The cyst nematode *Heterodera goettingiana* Liebscher is a destructive pest of pea (*Pisum sativum* L.) in all regions where the crop is grown. Selection for resistance within cultivars has been unfruitful because nematode-resistant genotypes are rare or nonexistent (Oostenbrink, 1951). Because of the economic importance of pea, some germplasm accessions of *Pisum* were screened in an attempt to identify accessions with resistance to *H. goettingiana* (Di Vito and Perrino, 1978; Zacheo *et al.*, 1981). Among the accessions tested some were defined, arbitrarily, as resistant on the basis of the number of nematode cysts detected in their roots. Recent investigations have identified the early histological and histochemical responses to *H. goettingiana* invasion of susceptible and so- called resistant accessions of pea germplasm (Zacheo *et al.*, 1986; Bleve-Zacheo *et al.*, 1990; Melillo *et al.*, 1990).

These authors reported that *H. goettingiana* juveniles are able to penetrate roots of resistant hosts and to initiate syncytial development in a manner similar to that in susceptible hosts, except that syncytia in resistant hosts degenerated 10 days after nematode penetration. *H. goettingiana* infection of pea roots also induced considerable modification of some enzyme activities in the infected host tissues (Arrigoni *et al.*, 1981; Zacheo *et al.*, 1986). Low levels of superoxide dismutases (SOD) and high levels of NADPH oxidase, resulting in a high concentration of superoxides ($O_2^{\dot{-}}$) in the cell, have been regarded as essential in the development of a biological defence mechanism in pea. The enhancement of $O_2^{\dot{-}}$ generation was interpreted as a biochemical reaction which was activated in resistant plants following pathogen invasion and could be used in characterizing the resistant and susceptible host response. Activation of SOD and catalase, which destroy the superoxide radicals and hydrogen peroxide respectively, was evident in susceptible plants. The net result of the interaction of these two enzymes in infected susceptible plants seems to be the protection of the tissues against the deleterious action of superoxides and other active species of oxygen (Dhindsa *et al.*, 1981; Zacheo and Bleve-Zacheo, 1988). The activities of peroxidase and catalase were also studied in connection with their possible role in the resistance or susceptibility of the plants infected with different pathogens and it seems that heightened peroxidase activity induces resistance (Goodman *et al.*, 1986). In the present paper we report the biochemical characterization of a commercial pea variety Progress 9 susceptible to *H. goettingiana* and a germplasm pea accession MG 103738 which was the most resistant to the nematode among the screened accessions.

Materials and methods

Host and nematode

Seeds of a germplasm pea accession MG 103738 (*P. sativum* sp. *transcaucausicum* Govorov, obtained from Gatersleben collection) collected and multiplied at the Istituto

del Germoplasma, Bari, and a commercial variety of pea, Progress 9, were germinated under sterile conditions. The juveniles of *H. goettingiana* were obtained from cysts collected from pea plants grown in pots of nematode-infested field soil. When root initials appeared the seedlings were transplanted into clay pots containing 10 ml sterilized sand and simultaneously a suspension of 50 *H. goettingiana* second-stage juveniles were added to each pot. Some pots were not inoculated and used as controls. The plants were maintained in growth chambers at 17°C with a 14 h photoperiod at 5,000 lux and relative humidity of 70%. Plants were removed 5 days later and the roots were collected.

Enzyme extraction and assay

Root tissues were homogenized in 100 mM EPPS buffer, pH 8.5, in a Potter homogenizer cooled in ice. The resultant slurry was filtered through 4 layers of cheesecloth and centrifuged at 10,000 g for 20 min and then the supernatant was centrifuged at 131,000 g for 1 h to yield a pellet of microsomal membranes. The pellet was resuspended to form a membrane suspension [2 mg protein/ml in 2 mM EPPS (pH 8.5)] (Mayak *et al.*, 1983) and used for enzymatic assay. From the supernatant a 35% saturated ammonium sulphate precipitate was prepared. The precipitate (fraction a) was resuspended and dialysed against 20 mM phosphate buffer pH 7.5. The supernatant was then saturated in 80% ammonium sulphate and the resultant precipitate (fraction b) was recovered as before. The dialysate was centrifuged at 10,000 g for 10 min and the supernatant was used for enzymatic assay and proteins. The level of superoxide (O_2) in the root tissue was measured in terms of formazan (reduced nitroblue tetrazolium) (Doke, 1983). Superoxide level generated by membranes was assayed by determining the reduction of acetylated cytochrome c (Doke, 1985). Catalase assays were performed with an oxygen monitor equipped with a Clark electrode. Aliquots of tissue homogenate were used to initiate the catalase reaction with 15 mM H_2O_2 in 50 mM, pH 7.0 K-Pi buffer. Reactions were run at 25°C (Olsen and Cook, 1987). The superoxide dismutase activity (SOD) was assayed according to the procedure described by Furusawa *et al.* (1984). Peroxidase activity was assayed with guaiacol as the electron donor. Guaiacol was assayed in 1 ml of 50 mM K-phosphate pH 6.0 containing 6 mM guaiacol and 1.6 mM H_2O_2. The peroxidation of guaiacol was followed at 25°C in a Hitachi - Perkin Elmer 557 spectrophotometer at 470 nm. Proteins were determined according to Lowry (Lowry *et al.*, 1951).

Electrophoretic analysis

Anodic isoperoxidases were separated by electrophoresis on 10% polyacrylamide slab gels (PAGE) in non-denaturating conditions as described by Laemmli (1970) using a separation time of about 4 h at 900 V and 75 mA. After electrophoresis the gels were rinsed with distilled water and then incubated in 100 ml of 20 mM sodium acetate, pH 4.5, containing 3.3 mM 3-3' aminobenzidine and 0.12% H_2O_2. After 2 min the gels were transferred for 1 min to 7% acetic acid to stop the enzymatic reaction; they were then immersed in 10% methanol and photographed. The molecular weights of anodic peroxidases were determined by using apoferritin, alcohol dehydrogenase, bovine serum albumin, ovoalbumin, carbonic anhydrase, cytochrome c and aprotinin (Sigma) as standards. Gels for the standard curve were stained with Coomassie brillant blue R 250. Isoelectric focusing electrophoresis (IEF) was carried out in a horizontal slab gel apparatus using "Ampholine PAGplate", pH 3.5-9.5 (LKB). The run was performed at 1,500 V, 28 mA, 20 W for 2 h at 4°C. Isoperoxidases were stained as described for the SDS non-denaturing system. The pH gradient of the gel was determined by cutting a 1 cm-wide strip of the gel into 0.5 cm segments and equilibrating the sections in deionized water for 30 min prior to pH measurement. The developed bands were quantified with a Beckman DU-70 spectrophotometer equipped with a film scanning device and gel scanning compuset. The optimum wave- length for scanning visualize peroxidase bands was 506 nm. All the values are the means ± standard error of four replications.

Results

When terminal part of pea roots were excised and immersed in nitroblue tetrazolium (NBT) the originally pale yellow solution became bluish. This indicated that NBT was transformed to the reduced form (formazan). The reaction was found to be activated by NADPH. The omission of this substrate or the addition of SOD, a scavenger of $O_2^{\cdot-}$, into the reaction mixture partially prevented the formazan production thus suggesting that NBT reduction could be due to the $O_2^{\cdot-}$ generated by the root tissues. When KCN was added to the assay medium the ability of the roots to produce formazan was enhanced (Table I). The resistant pea accession had more formazan in inoculated roots than in uninoculated (about 30%). In the susceptible Progress 9 nematode infection decreased formazan production by about 25% (Table I). Nitroblue tetrazolium reduction was prevented when boiled roots were used as a source of free radicals. Superoxides were also produced by microsomal membranes extracted from pea roots. Levels of $O_2^{\cdot-}$ in the membranes were determined by acetylated cytochrome c because it is known that this compound is reduced by $O_2^{\cdot-}$ and it is not oxidized by cytochrome c oxidase. The reducing activity of the membranes of both inoculated and uninoculated susceptible pea roots did not change five days after inoculation. The membranes from inoculated resistant accession showed an increased ability to reduce acetylated cytochrome c of about 20 % when compared with the uninoculated control. In both infected and uninfected membranes extracted

from Progress 9 and MG 103738 roots, as reported in Table II, the reducing activity decreased by about 60% when NADPH was omitted in the complete reaction system. The addition of O_2 scavengers (SOD) markedly inhibited the NBT reduction. The reducing activity was not observed when microsomes were heat-denaturated (Table II).

The level of lipid peroxidation (MDA content) showed no changes during the infection of Progress 9 in all the three fractions examined (Table III). The infection of resistant accession with *H. goettingiana* juveniles induced a higher level of lipid peroxidation than in uninfected controls. The maximum MDA content reached (fraction a) represents an increase of more than 34% over the content observed in uninfected control. However a high increase was found in fraction b and in microsomes (Table III).

The changes in the activity of catalase are presented in

TABLE I - *NBT reduction in susceptible (S) and resistant (R) pea roots uninoculated or infected with* Heterodera goettingiana *juveniles. Reduced NBT (*) was expressed as optical density at 580 nm/g dry weight. Complete (**) medium consists of 10 mM phosphate buffer (pH 7.8) 1 mM EDTA, 20 μM NADPH, 0.05% NBT.*

Experimental conditions	NBT reduction			
	Progress 9 (S)		MG 103738 (R)	
	uninfected	infected	uninfected	infected
NBT (*)				
Complete (**)	296 ± 15	223 ± 10	253 ± 12	377 ± 16
− NADPH	207 ± 7	159 ± 11	168 ± 4	250 ± 12
+ 100 μg/ml SOD	194 ± 6	156 ± 7	172 ± 6	221 ± 8
+ 1 mM KCN	503 ± 20	356 ± 18	379 ± 20	527 ± 16

TABLE II - *Effect of nematode infestation on superoxide (SOD) production by microsomal membranes. The reaction medium (*) contained 20 μM cytochrome c acetylate; 10 μM NADPH, microsomal membranes (250 mg/ml), 1 mM KCN in 10 mM EPPS buffer pH 8.5. The changes in NADPH-dependent cytochrome c reducing activity were conducted in the presence or absence of elicitors and inhibitors and expressed as μM cytochrome c min/mg proteins.*

Experimental conditions	NADPH oxidase activity			
	Progress 9 (S)		MG 103738 (R)	
	uninfected	infected	uninfected	infected
medium (*)	7.5 ± 0.2	7.8 ± 0.2	8.0 ± 0.1	10.4 ± 0.3
− NADPH	3.1 ± 0.2	3.2 ± 0.1	3.6 ± 0.2	4.5 ± 0.1
+ SOD (100 μg/ml)	3.0 ± 0.3	2.9 ± 0.2	3.6 ± 0.2	5.0 ± 0.2
control (heat denatured microsomes)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

TABLE III - *Changes in lipid peroxidation content in pea root extract after infestation with* H. goettingiana. *Lipid peroxidation was measured in terms of the levels of MDA (nM/mg proteins).*

Fraction	Lipid peroxidation			
	Progress 9 (S)		MG 103738 (R)	
	uninfected	infected	uninfected	infected
Microsomes	3.8 ± 0.1	3.9 ± 0.2	5.3 ± 0.3	6.8 ± 0.2
Fraction a	1.7 ± 0.2	1.8 ± 0.1	2.2 ± 0.1	3.9 ± 0.1
Fraction b	1.23 ± 0.1	1.27 ± 0.1	1.2 ± 0.2	1.6 ± 0.2

Table IV. The activity of this enzyme increased during nematode infection in the roots of Progress 9 and reached the maximum value in the microsomes. A similar trend was seen in the fractions a and b but the increase was lower. Catalase activity was significantly lower in the resistant pea accession inoculated with nematodes. The decline of the enzyme was higher in microsomes than in fraction a or fraction b.

SOD activity for the susceptible variety Progress 9 and for resistant accession MG 103738 are reported in Table V. In the susceptible variety SOD activity increased after nematode invasion and this was similar in all the three fractions. In MG 103738 accession SOD activity declined by 20% in all the fractions (Table V). SOD activity in the presence of cyanide (which inhibits Cu-Zn SOD activity) indicated that in our experimental conditions the Mn-SOD contributed no more than 15% of the total SOD activity in both peas examined. The changes of Mn-SOD followed the pattern of the total superoxide dismutase activity.

Peroxidase activity in pea root extracts from both Progress 9 and MG 103738 increased in the microsomal fraction five days after nematode infection. The increase over uninoculated controls was 21% with Progress 9 and 38% with MG 103738. The soluble fraction b of both plants had more specific activity than microsomes or fraction a. The changes in peroxidase content in the soluble fraction, following nematode inoculation, distinguished resistant from susceptible plants. In the resistant accession there was an increased activity in both the soluble fractions, whereas it decreased or remained unchanged in the susceptible variety (Table VI). The increase in peroxidase activity in the resistant plant following nematode infection could be due to a general increase of peroxidase isozymes or to a specific subset of peroxidase isozymes produced in response to the attack. To distinguish between these pos-

TABLE IV - *Changes in the activity of catalase on the basis of protein content during nematode infestation. The activity was assayed polarographically by measuring oxygen evolution and expressed as nM 0_2/min/mg proteins.*

Fraction	Catalase activity			
	Progress 9 (S)		MG 103738 (R)	
	uninfected	infected	uninfected	infected
Microsomes	100 ± 5	184 ± 9	114 ± 6	65 ± 1
Fraction a	120 ± 6	198 ± 8	117 ± 5	96 ± 3
Fraction b	150 ± 6	209 ± 7	107 ± 6	85 ± 2

TABLE V - *Superoxide dismutase (SOD) activity in different fractions of susceptible (S) and resistant (R) pea roots. The enzyme activity was assayed by the cytochrome c method and expressed as units mg/proteins.*

Fraction	SOD activity			
	Progress 9 (S)		MG 103738 (R)	
	uninfected	infected	uninfected	infected
Microsomes	3.3 ± 0.1	4.8 ± 0.1	4.3 ± 0.1	3.4 ± 0.1
Fraction a	4.1 ± 0.1	5.1 ± 0.1	1.8 ± 0.1	1.4 ± 0.1
Fraction b	10.3 ± 0.2	14.2 ± 0.1	8.3 ± 0.2	6.6 ± 0.1

TABLE VI - *Activity of peroxidase isozymes from pea var. Progress 9 and accession MG 103738 after inoculation with* H. goettingiana *in microsomes and soluble fractions.*

Fraction	Progress 9 (S)		MG 103738 (R)	
	uninfected	infected	uninfected	infected
Microsomes	95 ± 7.2	120 ± 10	128 ± 4.5	205 ± 6.3
Fraction a	12 ± 0.4	9 ± 0.5	10 ± 0.6	12 ± 0.5
Fraction b	148 ± 9.0	138 ± 6.5	169 ± 8.6	219 ± 10

sibilities the isozyme profile of soluble fraction b was determined by using non denaturating-SDS slab gel and isoelectrofocusing gel electrophoresis. The peroxidase isozymes separated by slab gel electrophoresis were different in resistant or susceptible pea root extracts (Fig. 1). The pattern of Progress 9 presented two slowly migrating bands (Rf 0.16 and 0.22) and three fast migrating bands (Rf 0.43, 0.48, 0.56) which were absent or very weak in MG 103738 (Fig. 1, line 1, 3). This situation was reversed for the isozymes with Rf 0.54, 0.40. No qualitative differences in isozyme patterns were noted between infected and uninfected extracts; however, some quantitative changes were visible in the bands. The molecular weights of the isoperoxidases present in fraction b were calculated to be from 240 to 43 kdalton (Fig. 1). By IEF analysis nine peroxidase isozymes were consistently detected in fraction b of both the plant extracts. These isozymes had pI values from 3.98 to 7.39. Other isozymes, such as those observed

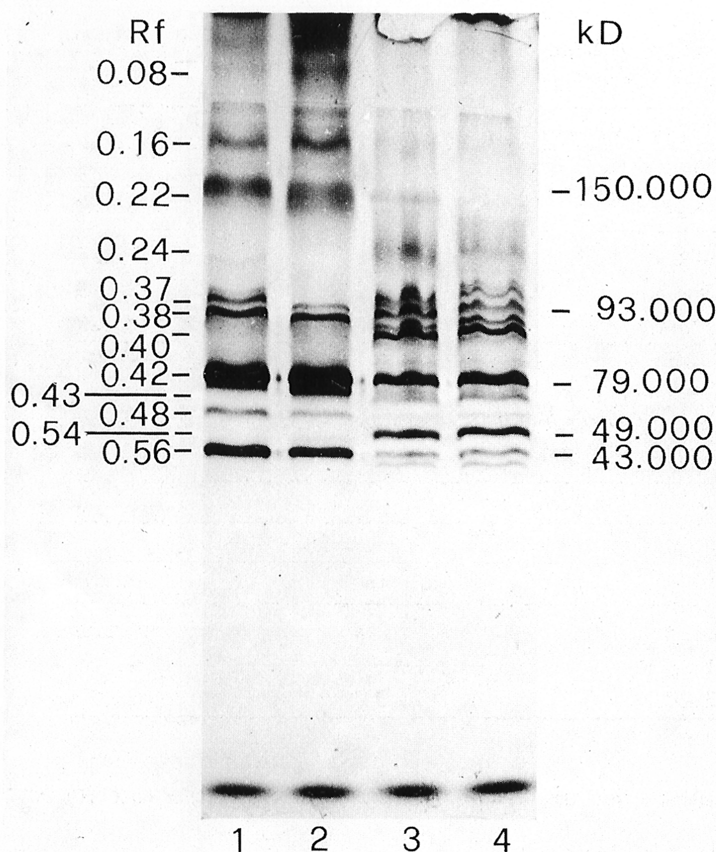

Fig. 1 - Non denaturing SDS-PAGE of soluble peroxidases extracted from pea roots. Line 1 and 2 are Progress 9 (non- inoculated and inoculated respectively). Lines 3 and 4 are MG 103738 (non-inoculated and inoculated respectively).

between pH 7.39 and 7.57 (Fig. 2) and between pH 7.60 and 8.34 were not clearly defined (Fig. 2a). One peroxidase isozyme pI 6.06 appeared to be present only in MG 103738 (Fig. 2, line 2, 3). In the IEF gels a band at pH 7.22 could be observed in the Progress 9 extracts which seemed to be no detectable in the MG 103738. The activity of isoelectric- focused peroxidase was reduced when reaction was performed in presence of 1 mM KCN (Fig. 2b). Peroxidase isozymes lost 80% of their activity after the addition of cyanide and few light bands were stained. Also 1 mM SHAM inhibited peroxidase activity but less than cyanide (Fig. 2c).

Discussion

Large differences in the induction of $O_2^{\overline{\cdot}}$ generation and lipid peroxidation and in enzymatic activities were observed between the cultivated pea variety Progress 9 and the germplasm accession MG 103738. Increased $O_2^{\overline{\cdot}}$ generation and lipid peroxidation are associated phenomena and both are related to the resistance of pea plants to *H. goettingiana*. Morphological studies showed that the response of susceptible and resistant pea plants to cyst nematode is comparable initially, in terms of syncytia formation; subsequently the trophic structure degenerates in resisting tissues (Bleve-Zacheo *et al.*, 1990) These tissues also showed an increased NBT reducing activity. From this it can be concluded that root tissues showing increased NBT reducing activity (i.e., increased $O_2^{\overline{\cdot}}$ generation) will degenerate within a few days after nematode penetration. The biological source of $O_2^{\overline{\cdot}}$ is only partially defined. Some authors (Doke, 1985; Zacheo and Bleve-Zacheo, 1988) suggest that the large amount of superoxides produced in the incompatible combinations, are generated in the membranes by a NADPH-oxidase system. It is interesting that the increase in $O_2^{\overline{\cdot}}$ generation and lipid peroxidation occurs in the microsomal fraction. This suggests that the mechanism of resistance involves damage to the plasma membrane and which results in a hypersensitive reaction and cell death (Doke, 1985) or syncytia degeneration (Bleve-Zacheo *et al.*, 1990). The causes underlying the increased lipid peroxidation and superoxide anions in the resistant pea accession, during nematode infection, are not clear at present.

It has also been suggested that SOD and catalase are involved in the $O_2^{\overline{\cdot}}$ induced lipid peroxidation (Dhindsa *et al.*, 1981). In the resistant accession NBT reducing activity increased and was accompanied by a decrease in SOD and catalase activity and an increased level of lipid peroxidation. SOD and catalase remove $O_2^{\overline{\cdot}}$ and H_2O_2 respectively. An interaction between $O_2^{\overline{\cdot}}$ and H_2O_2 can generate $OH^{\cdot}$ and $^{*}O_2$ and thereby control the level of lipid peroxidation (Fridovich, 1975). Thus a decline in SOD and catalase activities could result in a greater availability of free radicals, increased lipid peroxidation, membrane damage and therefore cell deterioration. The nematode infected tissue of the susceptible variety maintained a lower level of superoxides

and lipid peroxidation at least in part by increased levels of SOD and catalase. This reflects an efficient scavenger system to the site of $O_2^{\dot{-}}$ production in susceptible plants. Thus the increased SOD and catalase activities in response to nematode penetration in a susceptible plant may be preliminary to such a defense system, which minimizes the enzymatic production of oxygen radicals.

Peroxidases are also regarded as detoxifying agents for H_2O_2 and have a precise metabolic function in the defense mechanism. Peroxidase activity in the resistant pea accession MG 103738 showed a greater increase following infection with *H. goettingiana* than did the susceptible variety. Hence it seems that heightened peroxidase activity favours resistance to *H. goettingiana*. In the susceptible variety peroxidases remained on the whole unchanged. The observation that a decrease of soluble peroxidase activity was accompanied by an increase in catalase activity in the nematode infected susceptible variety suggests that these enzymes may play an important role in the initial defense reactions of the plants. The suppression of the peroxidase activity could be a consequence of a lesser availability of H_2O_2 because of the substrate destroying activity of catalase. There is an interaction between H_2O_2, peroxidase, catalase and the degree of disease development in pea plants possessing different susceptibility to nematodes. However, the exact function of peroxidase in the plant defense mechanism remains open to question. Stafford (1974) has written an excellent review on the role and function of peroxidase in lignification. The peroxidases have been readily detected as wall-bound enzymes in lignifying tissues. The induced lignification has been proposed as a mechanism of disease in resistant plants against invasion of pathogens (Goodman *et al.*, 1986) by making host walls more resistant to mechanical penetration and to dissolution by nematode enzymes.

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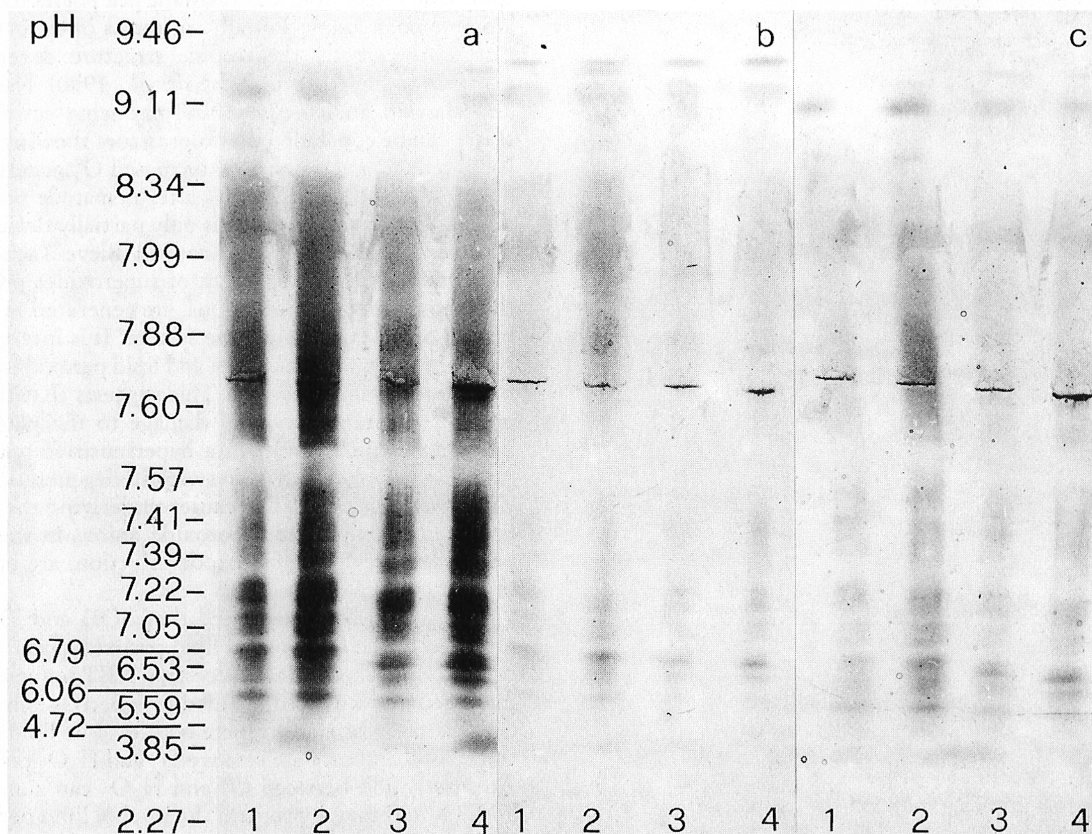

Fig. 2 - The isoelectric focusing profiles of peroxidases from Progress 9 (lines 1, 2) and MG 103738 accession (lines 3, 4) infected and non-infected by *H. goettingiana*. Peroxidase isozymes were stained with 3.3 mM DAB (a) in presence of 1 mM KCN (b) or 1 mM SHAM (c).

Literature cited

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

BLEVE-ZACHEO T., MELILLO M.T. and ZACHEO G., 1990 - Syncytia development in germplasm pea accessions infected with *Heterodera goettingiana. Nematol. medit.*, *18*: 93-102.

DHINDSA R.S., PLUMB-DHINDSA P. and THORPE T.A., 1981 - Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exper. Bot.*, *32*: 93-101.

DI VITO M and PERRINO P., 1978 - Reazione di *Pisum* spp. agli attacchi di *Heterodera goettingiana. Nematol. medit.*, *6*: 113-118.

DOKE N., 1983 - Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissue to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. *Physiol. Plant Pathol.*, *23*: 345-357.

DOKE N., 1985 - NADPH-dependent $O_2^{\cdot-}$ generation in membrane ions fractions isolated from wounded potato tubers inoculated with *Phytophtophora infestans. Physiol. Plant Pathol.*, *27*: 311-322.

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

FURUSAWA I., TANAKA K., THANUTONG P., MIZUGUCHI A., YAKAZI M. and ASADA K., 1984 - Paraquat resistant tobacco calluses with enhanced superoxide dismutase activity. *Plant and Cell Physiol.*, *21*: 227-236.

GOODMAN R.N., KIRALY Z. and WOOD K.R., 1986 - «The Biochemistry and Physiology of Plant Disease». University of Missouri Press, Columbia, USA, 443 pp.

LAEMMLI U.K., 1970 - Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*: 680-685.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L. and RANDALL. R.J., 1951 - Protein measurement with the Folin phenol reagent. *J. Biol. Chem*, *193*: 265-275.

MAYAK S., LEGGE R.L. and THOMPSON J.E., 1983 - Superoxide radical production by microsomal membranes from senescing carnation flowers: an effect on membrane fluidity. *Phytochemistry*, *22*: 1375-1380.

MELILLO M.T., BLEVE-ZACHEO T., ZACHEO G. and PERRINO P., 1990 - Morphology and enzyme histochemistry in germplasm pea roots attacked by *Heterodera goettingiana. Nematol. medit.*, *18*: 83-91.

OLSEN J.A. and COOK C.R., 1987 - Superoxide dismutase in wounded etiolated pea seedlings. *Phytochemistry*, *26*: 71-73.

OOSTENBRINK M., 1951 - Het erwteneystenaaltje, *Heterodera goettingiana* Liebscher, in Nederland. *Tijds Plant.*, *57*: 52-64.

STAFFORD H.A., 1974 - Possible multienzyme complexes regulating the formation of C — C phenolic compounds and lignins in higher plants. *Rec. Advan. Phytochem.*, *8*: 53-79.

ZACHEO G., ARRIGONI-LISO R., BLEVE-ZACHEO T., LAMBERTI F., PERRINO P. and ARRIGONI O., 1981 - Changes of ascorbate free radical reductase in pea roots infested by *Heterodera goettingiana. Nematol. medit.*, *9*: 181-187.

ZACHEO G., BLEVE-ZACHEO T. and PERRINO P., 1986 - Resistance of germplasm pea to *Heterodera goettingiana* based on biochemical and ultrastructural changes. *EUCARPIA*, Proceedings of Symposium, Poland 19-20 June, 144-148.

ZACHEO G. and BLEVE-ZACHEO T., 1988 - Involvement of superoxide dismutases and superoxide radicals in the susceptibility and resistance of tomato plants to infestation by *Meloidogyne incognita. Physiol. and Molec. Plant Pathol.*, *32*: 313-322.

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