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HYDROXYPROLINE-RICH PROTEINS AND PEROXIDASES IN TOMATO ROOTS INFESTED BY ROOT-KNOT NEMATODE

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Hydroxyproline-rich glycoproteins (HRGP) have long been known to be associated with the cell wall of all higher plant tissues (Lamport, 1965). The low level of these proteins is strikingly increased in response to wounding (Chrispeels, 1969; Stuart and Warner, 1980), fungi infection (Hammerschmidt and Kuc', 1982; Touzé and Esquerrè-Tugayè, 1982) and nematode infestation (Giebel and Stobiecka, 1974; Zacheo *et al.*, 1977).

It is suggested that small glycoproteins may play a role in a general defense mechanism of plants against biological stress (Touzé and Esquerrè-Tugayè, 1982). This is of interest as such glycoproteins were associated with the resistant response of plant to pathogens. The role that HRGPs play in defence is not known although there are suggestions that they act as growth regulating materials. Some authors (Whitmore, 1978) have suggested that HRGPs may act as matrices for lignification in a lignin protein complex catalyzed by peroxidase. Ridge and Osborne (1970) proposed that peroxidase could be one of the group of HRGPs in cell walls, the level of which determine wall extensibility and cell growth.

This study was carried out to determine whether the increase of hydroxyproline in plants infested by *Meloidogyne incognita* is associated with parallel changes in peroxydase activity.

Materials and methods

Seedlings of tomato cv. VFN8 (resistant to *M. incognita*) and cv. Roma VF (susceptible) were grown at 27°C and transplanted at 3-4 cm height into 5 cm diameter clay pots containing quartz sterilized sand. The pots were placed in a growth chamber at 27°C or 34°C, 65% Rh, 5000 lux and watered with Hoagland's solution. After a week, the seedlings were inoculated with 60 *M. incognita* second stage juveniles.

Five days after nematode inoculation, root tissues were comminuted in a powder-driven glass homogenizer and HRGP extracted as described by Ridge and Osborne (1970).

Peroxidase activity (PO) was assayed with guaiacol by the method of Evans (1970).

Results and discussion

The hydroxyproline content of four different fractions was used as an estimate of the HRGP. The soluble fraction of buffer-extractable proteins from the tomato roots increased the amount of proline and hydroxyproline during nematode infestation in both resistant and susceptible cultivars. In the resistant VFN8 cultivar the hydroxyproline enhancement was much more (40%) than in the susceptible Roma VF (20%). Our results also suggest that the increased level of hydroxyproline is correlated with the increase of peroxidase activity in the soluble fraction (Table I). In both resistant and susceptible cultivars, the soluble fraction showed high peroxidase activity, but the largest increase in activity (140%) was observed in VFN8 (Tables I, II).

Changes in peroxidase activity and in proline-hydroxyproline content were observed in the microsomial fraction of infested tomato plants. The hydroxyproline content in the infested resistant cultivar increased by 46% (Table I), while there were no differences between infested and unifested plants of the susceptible cultivar (Table II). Peroxidase increased by 45% and 22% respectively in resistant and susceptible cultivars.

In the buffer-Triton fraction a large portion of peroxidase and HRGP was solubilized. The amount of hydroxyproline increased twice in infested compared with uninfested either in susceptible or in resistant plants. Proline content decreased in infested resistant plants. Peroxidase extracted from walls by Triton showed a parallel increase of hydroxyproline contents.

In the covalent bound cell wall proteins (cell wall) of resistant tomato roots, hydroxyproline and peroxidase increased several fold after nematode invasion. Lesser

amounts of proline were detected in wall bound HRGP of resistant infested roots (Table I). This suggests that for most of the HRGP the proline was converted to hydroxyproline. Cell walls in the susceptible cv. were less affected by nematode infestation and showed moderate changes when compared with uninfested (Table II). The hydroxyproline enhancement of the cell wall in the resistant cultivar appeared to occur as a response to the triggering of the resistance mechanism. In this paper we have presented evidence that the enhancement in the HRGP content is due not only to wall-bound proteins but also involves a soluble fraction, non ionically bound proteins and membranes.

Hammerschmidt *et al.* (1984) also found a large increase of hydroxyproline-rich proteins on cucumber infested by *Colletotrichum lagenarium* and suggested it could be associated with lignin deposition. The activity of peroxidase was greatly increased in resistant and less in susceptible cultivars when protein fraction rich in hydroxyproline was enhanced by nematode infestation. The strictly parallel changes in hydroxyproline and peroxidase in all the fraction extracted from tomato roots indicate a structural or functional relationship between the two and the defence mechanism of plants.

We have considered our results in the context of previous work on peroxidase and HRGP. In resistant plants the increased peroxidase activity seems to be more related to lignification process around the necrosis and in the susceptible to the production of secondary wall in the syncytium. Although some authors (Ridge and Osborne, 1970, 1971) speculate that wall peroxidase may facilitate the hydroxylation of proline in wall proteins, it remains an open

TABLE I - *The effect of inoculating resistant tomato seedlings with* Meloidogyne incognita *on proline-hydroxyproline content and peroxidase activity. Each plant was inoculated with 50 juveniles, six days before being removed and analyzed.*

Fraction	Treatment	Aminoacid µg/mg prot		Peroxidase OD/mg prot
		Proline	HO-proline	
Soluble	Uninfested	16.27	1.14	9.5
	Infested	19.93	1.60	23.0
Microsomial	Uninfested	8.33	0.45	2.0
	Infested	10.81	0.66	2.9
Cell-wall Triton-soluble	Uninfested	6.72	0.32	4.5
	Infested	4.72	0.61	13.0
Cell-walls	Uninfested	5.20	1.23	6.1
	Infested	2.60	3.51	19.9

TABLE II - *Effect of nematode infestation on proline-hydroxyproline level and peroxidase activity in susceptible tomato cultivar Roma VF. Seedlings were infested with 50 juveniles of* M. incognita *per plant. Analysis were carried out six days after nematode inoculation.*

Fraction	Treatment	Aminoacid µg/mg prot		Peroxidase OD/mg prot
		Proline	HO-proline	
Soluble	Uninfested	13.44	1.02	20.8
	Infested	15.55	1.24	29.1
Microsomial	Uninfested	10.60	0.67	3.5
	Infested	10.45	0.69	4.3
Cell-wall Triton-soluble	Uninfested	4.03	0.27	5.5
	Infested	7.98	0.47	10.8
Cell-Walls	Uninfested	5.70	5.73	8.2
	Infested	4.94	6.41	10.25

question whether hydroxyproline and peroxidase are casually related. Nevertheless, our results strongly support the views of other authors (Hammerschmidt *et al.*, 1984; Touzè and Esquerré-Tugayé, 1982) who consider HRGP enhancement as a factor in the defence mechanism in plants.

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