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ENZYME ACTIVITY ASSOCIATED WITH RESISTANCE IN POTATO TO THE EARLY STAGES OF *GLOBODERA PALLIDA* INFECTION

by

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Summary. Four potato genotypes of varying sensitivity to the potato cyst nematode, *Globodera pallida*, were studied for the pattern of accumulation of four enzymes, namely, lypoxygenase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase. All the enzymes were found to be proportionately higher in resistant plants after inoculation or wounding. The activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase increased with the increase of days after inoculation and wounding in all potato clones, while that of lypoxygenase decreased. Although the hypersensitive response was suppressed in potatoes against cyst nematode infection, the enzyme requisites for hypersensitivity were present as evident by the enrichment of lypoxygenase, peroxidase and polyphenol oxidase. The total size of the enzyme pool was large in resistant plants, indicating its value as a marker for screening and selection.

Increase in the activity of certain enzymes has been observed in potato tissues infected with nematodes (Giebel, 1974; Kaplan and Davis, 1987). However, there is little information available on the involvement of enzymes in the resistance process relating to the potato cyst nematode species *Globodera pallida* (Stone) Behrens.

Materials and methods

Seed tubers of four potato (*Solanum tuberosum* L.) clones, namely 'Kufri Muthu' (susceptible), P 55/7 (Partially resistant) and two VTn [2] clones 62. 33. 3 and 69-1377/94 (highly resistant) were planted in polyethylene bags containing steam sterilized cyst-free forest soil. Forty days after planting, two actively growing roots were extruded from the bag, cleaned of surface soil particles and placed in a watch-glass containing water with 20,000 juvenile *G. pallida*. Two, four and six days later, sections of each of the roots were cut. Any juveniles present were removed from the sections which were then immediately processed for estimation of enzyme activity.

A second set of bags with the four potato clones was treated as previously but the extruded roots were needle pricked on day 40 to cause small wounds. The roots were then cut on 2, 4 and 6 days after causing the wounds and immediately processed for enzyme activity.

Lypoxygenase activity was measured by the oxidation level of linoleic acid following the method of Lulai *et al.* (1986). Peroxidase activity was estimated following the method of Kahn *et al.* (1981) using the change in absorbance pattern at 485 nm, using guaiacol as the substrate.

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Polyphenol oxidase was measured by the change in the absorbance pattern at 400 nm following the procedure of Vaughn and Duke (1981). Phenylalanine ammonia lyase was estimated following Pendharkar and Nair (1975) as modified by Zucker (1965).

Results and discussion

There was increase in the accumulation of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in the highly resistant VTn^2 clones, with increase of days after inoculation, but they decreased in roots of the susceptible 'Kufri Muthu' (Table I and II). However, lypoxygenase activity decreased with increase of days after inoculation or wounding.

Lypoxygenase activity (Table I) was greater at 2 and 4 days sampling after inoculation in all potato clones. Its activity was very high in highly resistant clones like VTn^2 62. 33. 3 and 69-1377/94. Its activity was uniformly low in the 6 days samples of all potato clones.

Peroxidase activity was also high in 2 and 4 days sampling in all potato clones (Table I). This was the same with both inoculated and uninoculated potatoes. The highly resistant clones (VTn^2 62. 33. 3 and 69-1377/94) had high activity while the partially resistant clone P 55/7 had medium activity, and there was a low level of activity in the susceptible clone 'Kufri Muthu'. Polyphenol oxidase activity also followed a similar pattern (Table II) to those of lypoxygenase and peroxidase. The results also indicate (Table II) that the highly resistant clones produced maximum level of t-cinnamate while the susceptible clone 'Kufri Muthu' and the partially resistant P 55/7 produced lesser quantity of t-cinnamate. Hence, the results indicate an association of phenylalanine ammonia lyase with the degree of resistance.

The pattern of decrease or increase in the three intervals of sampling was similar both

TABLE I - *Lypoxygenase and peroxidase activities in four potato clones of varying sensitivity to potato cyst nematode infection.*

Genotype	Days after inoculation or wounding	Lypoxygenase activity (μ moles 0^2/min/g root)		Peroxidase activity (γ/min/mg root)	
		After inoculation	After wounding	After inoculation	After wounding
'Kufri Muthu'	2 nd day	175 ± 8.20	85 ± 2.40	170 ± 9.80	90 ± 4.50
	4 th day	160 ± 10.10	75 ± 4.50	180 ± 10.20	100 ± 10.40
	6 th day	150 ± 8.60	60 ± 2.60	195 ± 7.30	105 ± 12.60
P 55/7	2 nd day	180 ± 8.40	90 ± 4.80	185 ± 9.10	105 ± 12.00
	4 th day	170 ± 8.20	80 ± 5.50	205 ± 9.60	125 ± 14.50
	6 th day	175 ± 8.20	75 ± 5.40	225 ± 10.50	120 ± 8.80
VTn^2 62. 33. 3	2 nd day	220 ± 10.50	105 ± 8.60	250 ± 10.60	150 ± 8.60
	4 th day	200 ± 10.20	105 ± 8.20	265 ± 12.10	160 ± 15.10
	6 th day	215 ± 10.10	100 ± 10.10	285 ± 12.70	160 ± 20.40
VTn^2 69-1377/94	2 nd day	215 ± 14.20	110 ± 12.40	255 ± 12.50	130 ± 8.90
	4 th day	210 ± 9.40	105 ± 8.60	250 ± 14.10	145 ± 10.50
	6 th day	210 ± 9.80	105 ± 8.50	330 ± 14.00	155 ± 12.30

TABLE II - *Accumulation of polyphenol oxidase and phenylalanine ammonia lyase (t-cinnamate) in the roots of potato clones of varying sensitivity to potato cyst nematode infection.*

Genotype	Days after inoculation or wounding	Polyphenol oxidase (mg/g root)		t-cinnamate (n mole min^{-1} mg^{-1} x 10^2)	
		After inoculation	After wounding	After inoculation	After wounding
'Kufri Muthu'	2 nd day	2.20 ± 1.60	1.75 ± 0.80	120 ± 10.20	50 ± 2.80
	4 th day	2.45 ± 1.20	1.90 ± 0.80	150 ± 8.40	80 ± 3.30
	6 th day	2.50 ± 0.80	1.95 ± 0.70	160 ± 8.80	90 ± 6.20
P 55/7	2 nd day	3.55 ± 2.00	2.15 ± 1.20	120 ± 14.50	100 ± 11.40
	4 th day	4.05 ± 2.20	2.30 ± 1.80	140 ± 12.40	100 ± 11.80
	6 th day	4.00 ± 1.80	2.25 ± 1.20	170 ± 9.40	120 ± 11.20
VTn^2 62. 323. 3	2 nd day	5.50 ± 2.10	2.75 ± 1.40	200 ± 14.20	120 ± 14.50
	4 th day	5.75 ± 2.40	3.10 ± 2.10	260 ± 14.20	120 ± 7.80
	6 th day	6.55 ± 2.80	3.25 ± 2.00	260 ± 14.00	130 ± 8.60
VTn^2 69-1377/94	2 nd day	6.25 ± 3.20	2.05 ± 0.80	240 ± 11.10	120 ± 10.20
	4 th day	6.75 ± 3.00	2.65 ± 0.90	240 ± 12.50	120 ± 11.00
	6 th day	6.50 ± 3.20	2.95 ± 1.40	280 ± 11.80	140 ± 10.50

with the inoculated and wounded conditions. All four enzymes showed the same trend.

Lypoxygenase activity involves selective peroxidation of certain unsaturated fatty acids and increases during pathogenesis (Ocampo *et al.*, 1986; Keppler and Novacky, 1987) and after wounding (Lulai, 1988). The increase of this enzyme activity during *G. pallida* infection indicates an association with host resistance. A similar association could also be inferred between the increase of peroxidase activity and host resistance (Vance *et al.*, 1976; Coffey and Cassidy, 1984; Reuveni and Ferriera, 1985). Peroxidase increase leads to the accumulation of superoxides which also confer effective resistance. This is seen in the overall increase of this enzyme activity in all potato clones after nematode infection and the higher level of increase in resistant clones. Increase of polyphenol oxidase activity has also been considered to confer resistance (Bingefors, 1982; Giebel, 1982) and this is supported by the present study. However, because of the partecipation of this enzyme in many side reactions leading to the intermediate formation of dihydroxy type of phenols and subsequent multistep mixing with other enzymes, resistance is not immediately conferred (Bell, 1981). Phenylalanine ammonia lyase participates in the flavanoid biosynthesis and it is another reliable indicator of resistance (Giebel, 1974). The resistant potato clones accumulated more of this enzyms while in the partially resistant and susceptible clones accumulation was relatively less.

The enzyme lypoxygenase, peroxidase and polyphenol oxidase are reported to impart a hypersensitive response to infection (Doke *et al.*, 1982). Although, this response is seemingly suppressed in potatoes attacked by *G. rostochiensis* (Trudgill, 1991), the presence of these enzymes in sufficient quantities indicates that the preliminary enzymes leading to the response are present which could not express hypersensitivity clearly for some reason. This

study has also shown that the total size of the enzyme pool is higher in resistant clones and this may be taken as a criterion of selection and screening for host resistance, instead of relying on single enzyme accumulation.

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