LOCALIZATION OF SOME ENZYMES IN ROOTS OF SUSCEPTIBLE AND RESISTANT POTATOES INFECTED WITH HETERODERA ROSTOCHIENSIS

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The localization of the active peroxidase, tyrosinase and β-glucosidase in roots of potatoes both susceptible and resistant to Heterodera rostochiensis was tested.

In the necrotic cells in roots of resistant potato a high activity of all the three above mentioned enzymes was observed. Giant cells formed in roots of susceptible potato contained an active tyrosinase, but did not show the presence of active peroxidase and β-glucosidase. β-glucosidase was seen only in the cells lying close to the nematodes.

The role of the enzymes tested in resistant — susceptible reactions of the potato to H. rostochiensis is discussed.

In our earlier work (Giebel & Wilski, 1970) we proposed the hypothesis that the phenolic compounds present in roots of potatoes susceptible or resistant to Heterodera rostochiensis Woll. play a very important role in the reaction of plants to the invasion of nematodes. These compounds may influence the activity of the indoleacetic acid (IAA) and, on the other hand, they may be precursors of lignin.

But the influence of phenolics on IAA activity is strictly connected with the presence of some enzymes in potato roots. For example, peroxidase through phenolic compounds may decompose IAA: tyrosinase may act as an oxidating agent of phenols altering their activity as cofactors of peroxidase; β-glucosidase may release free active phenols from complexes and these phenols may in turn modify the activity of IAA-oxidase or peroxidase. As was stated earlier β-glucosidase plays an important role in potato resistance to H. rostochiensis (Giebel, Pieglat & Wilski, 1966; Wilski & Giebel, 1966; Giebel & Wilski, 1970).

The aim of this work was to investigate the localization of these three above mentioned enzymes in potato roots. As it is probable that the reaction of plant tissues to the presence of nematodes (i.e. giant cell formation in roots of susceptible potatoes or necroses in the resistant ones) depends on the activity of IAA in cells lying near to the nematodes, the localization of enzymes was investigated in these cells alone.

MATERIAL AND METHODS

Tubers of three potato varieties: ‘Pierwiosnek’ (susceptible), ‘Spekula’ and ‘Sagitta’ (both resistant) were planted in pots filled with soil heavily infested with H. rostochiensis. Two weeks after sprouting the plants were lifted from pots and hand sections from fresh roots were treated with adequate substrate solutions.
The localization of the active peroxidase was detected by using the mixture containing 1 ml of saturated NH₄Cl solution, 1 ml of 5% EDTA, 9 ml of saturated benzidine solution and 1 drop of 3% H₂O₂ (Pearse, 1960). The same mixture but without H₂O₂ and the mixture with the addition of KCN served as control. Tissues stained blue indicated the presence of peroxidase.

For tyrosinase (DOPA-oxidase) a substrate containing 0.0056 M dihydroxyphenylalanine (DOPA) in phosphate buffer pH 7.4 was used. Foot sections were incubated in this substrate for 4 hours at 37°C. Tissues stained black indicated the localization of tyrosinase (Nemeč, 1962). A substrate with the addition of KCN was used as control.

β-glucosidase was detected as shown by Cohen et al. (1962). Root sections were incubated for 4 hours at 37°C in the solution of 6-bromo-naphthyl-β-D-glucopyranoside in citrate-phosphate buffer pH 5.4, washed with water and transferred to a solution of Diazo-Blue B. Tissues stained blue indicated the presence of β-glucosidase. Root sections treated with Diazo-Blue B but not incubated in the substrate served as control.

RESULTS

Peroxidase. On transverse root sections of both susceptible and resistant potatoes the greatest peroxidase activity was observed in the pericycle. Other parts of the stele showed low enzyme activity, only. A high activity of peroxidase was observed in parenchyma cell walls. In general a higher peroxidase activity could be observed in resistant by the intensity of blue colouration in the tissues than in susceptible potatoes as indicated.

In roots of the susceptible potato the hypertrophic cells of the parenchyma lying close to the nematode did not show the presence of the active peroxidase but it was detected in tissues with normal cells situated a little further from the nematode. Such tissues formed a kind of “barrier” in the shape of a ring or a semi-ring, there by separating the larva from the stele. The typical giant cells did not show reaction to peroxidase, and as they enlarged towards the stele, they inactivated this enzyme in the pericycle (Fig. 1).

The necrotic cells formed in roots of resistant potatoes after nematode invasion showed high peroxidase activity. In one or two cell layers adjacent to the necrotic cells, no enzyme activity was seen but it was high in deeper parenchymatous cell layers (Fig. 2). High enzyme activity was also observed in cells surrounding those larvae which penetrated into the stele.

Tyrosinase. DOPA-oxidase occurred in the epidermis and in the stele of roots of both susceptible and resistant potatoes, but its activity was higher in roots of resistant potatoes. All the necrotic cells showed the presence of highly active tyrosinase. A rather highly active enzyme was observed also in the giant cell of the susceptible potato (Fig. 3). DOPA-oxidase was also observed in sections of nematodes which entered the roots.

β-glucosidase. This enzyme was found to be present in cell walls of both kinds of potato especially in the vascular bundles. In roots of the susceptible potato, high