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ROLE OF AMINO ACIDS IN PLANT TISSUE RESPONSE TO *HETERODERA ROSTOCHIENSIS*. II. EFFECT OF PROLINE AND HYDROXYPROLINE [1]

by

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In a previous communication (Giebel and Stobiecka, 1974) it is suggested that a decrease in the ratio of protein proline to hydroxyproline in potato roots after their infection with *Heterodera rostochiensis* Woll. may suppress cell hypertrophy and, in this way enhance the resistant response to this nematode.

This paper describes how we tested this hypothesis by introducing into nematode infected potato plants solutions of hydroxyproline, proline and a mixture of the two as well as 2,2′-dipyridyl (an inhibitor of protein-bound hydroxyproline formation) and benzimidazole (a promoter of hydroxyproline synthesis).

Material and methods

Two weeks old seedlings of potato variety « Siglinde » susceptible to *H. rostochiensis*, and those of the varieties « Spekula », « Tunika » and « Scutella » resistant to this nematode, were grown in pots in 100 g quartz sand infested with golden nematode cysts.

After sprouting, aqueous solutions of individual compounds were added to pots with susceptible plants. The following amounts were used daily, for 15 days: (A) L-hydroxyproline, Calbiochem, 10 mg;

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(B) mixture of both L-hydroxyproline, 10 mg, and proline, 10 mg; or (C) benzimidazole, Koch-Light Ltd., 5 mg.

With resistant potato plants solutions of appropriate compounds were added daily as follows: (A) L-proline, Calbiochem, 10 mg; or (B) mixture of both L-hydroxyproline, 10 mg, and L-proline, 10 mg; or (C) 2,2'-dipyridil, Koch-Light, Ltd., 0.5 mg.

After the period mentioned above, root samples were placed in FAA fixative for histological studies. After fixation, roots were dehydrated in an ethanol series, embedded in paraffin and sections 25 µ were cut. Safranin and fast-green were used for staining.

Results

The susceptible potato variety treated with hydroxyproline solution responded to nematode invasion in a similar way to the resistant plant, i.e. giant cells were not formed, and marked necrosis occurred close to the nematode (Fig. 1 A). But in susceptible potato plants tested with the mixture of hydroxyproline and proline no necroses formation was observed after nematode invasion, and giant cells were seen like those in control plants.

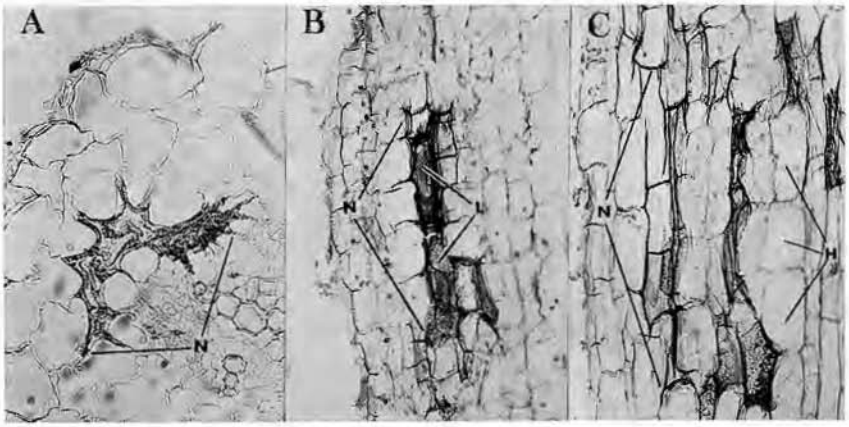

Fig. 1 - Root tissue response to *H. rostochiensis* invasion of susceptible potato treated with: A, L-hydroxyproline (transverse section, x 200); B, and C, benzimidazole (longitudinal sections, x 100).

L - larvae; N - necrose region; H - hypertrophic cell; G - giant cell.

In roots of resistant potato, after the proline addition, some lysis of the cell wall or cell hypertrophy was observed in cells adjacent to the nematode. However, such necrosis were smaller and less evident (Fig. 2 A) when compared with controls. A mixture of these two amino acids did not change the resistant plant response to *H. rostochiensis*.

Susceptible potato plants treated with benzimidazole responded to golden nematode invasion similarly to resistant plants (Fig. 1 B). In no case were typical giant cells observed, although some cell hypertrophy was evident (Fig. 1 C). Nematode larvae were enclosed by necrotic cells.

2,2'-dipyridyl did not change the plant-host tissue response of resistant potato varieties to *H. rostochiensis*. Here, necrosis were always formed around nematodes (Fig. 2 B), with adjacent cells like giant cells (Fig. 2 C). However, controls i.e. resistant potato roots untreated with benzimidazole, occasionally showed a similar response.

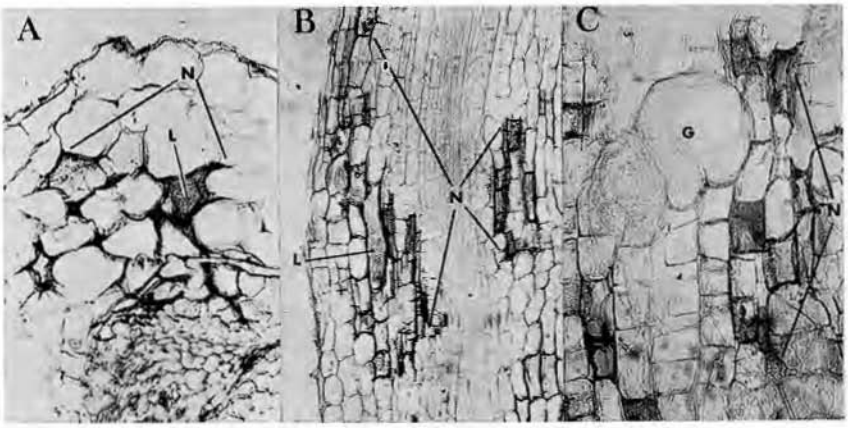

Fig. 2 - Root tissue response to *H. rostochiensis* invasion of resistant potato treated with: A, L-proline (transverse section, x 200); B and C, 2,2'-dipyridyl (longitudinal sections, x 65 and x 160, respectively).

L - larvae; N - necrose region; H - hypertrophic cell; G - giant cell.

Discussion

Our experiments confirmed earlier observations (Giebel and Stobiecka, 1974) that the level of proline (PRO) and hydroxyproline (HYPRO) in cell wall protein can influence the plant tissue response to *H. rostochiensis*. The introduction of HYPRO as well as benzimidazole (an activator of hydroxyproline biosynthesis) into infected susceptible potato varieties, altered the susceptible response of those plants toward a resistant reaction. We explain this as follows.

Plant cell walls contain glycoproteins characterized by a high content of HYPRO (Lamport and Northcote, 1960; Lamport, 1965). Levels of this amino acid can be increased by treating the plant with a benzimidazole solution (Sadava and Chrispeels, 1973). Young, rapidly growing tissues have low levels of wall-bound HYPRO compared with mature tissue (Lamport, 1967). Consequently, the level of HYPRO in the cell wall determines tissue maturation, the cessation of cell growth and wall extensibility. Furthermore, this amino acid is associated with peroxidase activity and lignification of the cell wall (Witmore, 1971).

The effect of hydroxyproline can be reversed by L-proline (Norris, 1967). Therefore, the low ratio of PRO/HYPRO in resistant potato roots infected with *H. rostochiensis* (Giebel and Stobiecka, 1974) together with the results presented in this paper indicate the possible role of HYPRO in the resistant response of plant-host tissues.

However, the high level of HYPRO or a low ratio of PRO/HYPRO are not the only factors for resistance. In our experiment the proline hydroxylation inhibition following application of 2,2′-dipyridyl was not able to shift the reaction of resistant potato root tissues toward a susceptible reaction.

We therefore conclude that in roots of resistant plants there are other resistant agents, e.g. phenolic compounds wich, as suggested by many authors, play a great role in the susceptible - resistant plant response to nematodes. These resistant factors exert an influence on the resistant reaction which is greater than those inducing the hydroxylation of proline. In susceptible plants where no such resistant factors are present the hydroxyproline may plant its role.

SUMMARY

L-hydroxyproline or benzimidazole, as activator of hydroxyproline biosynthesis, reversed susceptible plant response to *Heterodera rostochiensis* Woll. toward a resistant reaction. But, neither L-proline nor 2-2′-dipyridyl, as inhibitors of proline hydroxylation, did altered resistant plant responses. A possible role of the hydroxyproline and proline in resistant-susceptible reactions of the potato to *H. rostochiensis* is discussed.

RIASSUNTO

Il ruolo di alcuni amminoacidi nella reazione di tessuti vegetali ad Heterodera rostochiensis *Woll. II. Effetto di Prolina ed Idrossiprolina.*

La L-Idrossiprolina o benzimidazolo, come attivatore della sintesi dell'Idrossiprolina, ha trasformato la reazione di tessuti vegetali ad *Heterodera rostochiensis* Woll. da suscettibile a resistente. Né la L-Prolina né il 2-2'-dipiridile, come inibitori della Prolina, hanno modificato la reazione di piante resistenti. Viene discusso il possibile ruolo di Prolina ed Idrossiprolina nella reazione di suscettibilità o resistenza di piante di Patata ad *H. rostochiensis*.

RESUMÉ

Le rôle de quelques aminoacides dans la résponse de tissus végétaux à Heterodera rostochiensis *Woll. II. Effet de Proline et Hydroxyproline.*

L-Hydroxyproline ou benzimidazole, comme activateur de la synthèse de l'Hydroxyproline, transforme en résistants la résponse de tissus végétaux susceptibles à *Heterodera rostochiensis* Woll. Ni L-proline ni 2-2'-dipyridyl, inhibiteurs de la proline, n'ont pas modifié la réaction de plantes résistantes. Le rôle possible de la proline et l'hydroxyproline dans les réactions susceptibilité-resistance de la pomme de terre à *H. rostochiensis* est ici discuté.

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