Histopathological changes induced by *Nacobbus aberrans* in resistant and susceptible potato roots

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SUMMARY

Histopathological responses to *Nacobbus aberrans* infection in roots of the susceptible *Solanum tuberosum* cv. Revolución, the clone of *S. sparsipilum* 7601217.7, and the resistant hybrid B-25 from *S. tuberosum* ssp. *andigena* were compared. In the compatible response, *N. aberrans* was able to initiate and maintain the formation of a syncytium and develop to maturity. The syncytia were characterized by dense cytoplasm, hypertrophied nuclei and nucleoli, partial cell wall dissolution, hyperplasia, and accumulation of starch grains in the cells surrounding the syncytial zone. In the roots of the " resistant " (incompatible) hybrid B-25, development was inhibited by the formation of necrotic cells surrounding the invading nematode. The clone 7601217.7 showed the presence of physical barriers that delayed nematode invasion; development of the syncytium and of the nematodes were, however, almost the same as observed in the cultivar Revolución.

RÉSUMÉ

Modifications histopathologiques causées par Nacobbus aberrans *aux racines de pomme de terre résistante et sensible*

Les réactions histopathologiques à l'infestation des racines par *Nacobbus aberrans* ont été comparées chez le cv sensible de *Solanum tuberosum* Revolución, le clone 7601217.7 de *S. sparsipilum* et l'hybride résistant B-25 provenant de *S. tuberosum* ssp. *andigena.* Lors des réactions de compatibilité, *N. aberrans* provoque la formation d'un syncitium et peut se développer jusqu'à maturité. Le syncitium est caractérisé par un cytoplasme dense, des noyaux et des nucléoles hypertrophiés, une dissolution partielle des membranes cellulaires, une hyperplasie, et l'accumulation de grains d'amidon dans les cellules entourant la zone du syncitium. Dans les racines de l'hybride « résistant » (non-compatible) B-25, le développement est inhibé par la formation de cellules nécrotiques entourant le nématode venant de pénétrer. Chez le clone 7601217.7 il existe des barrières physiques qui retardent l'invasion par le nématode; toutefois, le développement du syncitium, et celui du nématode, sont presque identiques à ce qui est observé chez le cultivar Révolución.

A compatible response of the plant to a sedentary endoparasitic nematode results in the formation of the feeding site that is essential for the establishment and development of the nematode (Dropkin, 1969; Endo, 1971; Jones & Payne, 1977). In resistant plants different mechanisms are involved in the host parasite relationship. The nematode cannot develop or larvae may enter roots in low numbers (e.g. potato clones E 2 and N 4 resistant to race 1 of *Meloidogyne incognita;* Canto-Saenz & Brodie, 1987) or, as in the most common resistant reaction, larvae may enter the roots but the cells adjacent to the invasion site become necrotic (e.g. the hypersensitive response to *M. incognita* in soybean *Glycines max* cv. Centennial) and encapsulate the larvae (Kaplan & Van Gundy, 1979). Potato germplasm has been mainly evaluated for resistance to cyst-nematodes (Turner & Stone, 1984; Rice, Leadbeater & Stone, 1985; Dallaert & Hoekstra, 1987), and few information is available on mechanisms of resistance to the false root-knot nematode *Nacobbus aberrans* (Thorne) (Quimi, 1981).

The present investigation has been carried out to show the succession of plant cell responses associated with resistance to *N. aberrans* in potato roots and to compare them with cellular changes produced in susceptible cultivars.

Materials and methods

Plants were provided by the International Potato Center (CIP), Lima, Peru. The following varieties were studied : the susceptible variety *Solanum tuberosum,* L. cv. Revolución, the clone of *Solanum sparsipilum* (Britt.)

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Juz. *et* Buk. 7601217.7, and, the resistant hybrid B-25 from *Solanum tuberosum* ssp. *andigena* (Juz. *et* Buk.).

Nacobbus aberrans from Puno, Peru, was maintained in the greenhouse on the susceptible cv. Revolución. About one month after planting in infested soil, 0.5-1.0 cm potato roots were collected and homogenized in a blender for 8 sec. With the use of a stereoscopic microscope, immature females were identified and collected with an eyebrow mounted on a steel needle.

Infestation experiments were set up in the following way : sprouted tuber pieces from each variety to be tested were placed on 2 % water agar in 100 × 15 mm Petri dishes and maintained in the dark in an environmental growth chamber at 25 °C. The water agar contained streptomycin (0.005 %) and 200 U penicillin/ml to prevent the growth of bacteria. Inoculations were carried out by placing four immature females on individual 3 day-old root-tips in the agar. After inoculation, the tubers were placed back in the growth chamber.

Root pieces were collected 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and 35 days after inoculation. For each time interval four separate infected roots were examined. The root pieces were fixed for at least 24 h in FAA, washed in 50 % ethanol, dehydrated in an ethanol-TBA (tertiary butyl alcohol) series, and finally embedded in tissue prep (melting point : 56 ± 0.5 °C). Sections 12-13 µm (thick were prepared and stained with Johansen's quadruple stain technique (Johansen, 1940), mounted in Permount and observed under a compound microscope.

Results

Histopathology in the susceptible cultivar Revolución

Nematode penetration occurred within 24 h after inoculation. Three or four necrotic cells in the day-1 samples were observed at the point of entry. The nematodes were then observed in the cortical parenchyma. After 2 days the immature females were located parallel to the vascular cylinder, but in samples from the third and fourth day no changes were observed in the nematode's morphology or in the host cells. Syncytia started to develop five days after inoculation, and increased cellular activity was observed in the vicinity of the nematode's head. The cytoplasm was more granular and more dense in appearance than that of cells not in the syncytial zone. Ten days after inoculation the nematodes had increased in size and were embedded in the cortical parenchyma with their labial region oriented to the edge of the vascular cylinder. Small cavities had formed inside the cortex. The cells at the feeding site were enlarged with irregular shapes and the syncytial nuclei were usually hypertrophied (about 2.5 fold), containing prominent nucleoli (Fig. 1 A). During expansion of the syncytium adjacent phloem cells were displaced or incorporated into the syncytium (Fig. 1 A). Cellular proliferation was accompained by cell wall dissolution. In some cells two or three nuclei (Fig. 1 B) were observed. During subsequent observations syncytia had the same principal characteristics, however, they had now increased in size and extended not only around the nematode's head, but also both vertically and longitudinally in the root. Large accumulation of starch grains in the cells surrounding syncytia was observed (Fig. 1 C). The development of the feeding site caused asymmetry in the root structure (Fig. 1 D).

Histopathology in the clone of *S. sparsipilum* 7601217.7

The reaction to the nematode infection was similar to that observed in the cv. Revolución, but a significant delay in the rate of entry in this clone was the result of a physical barrier that retards nematode invasion.

Thereafter, the nematode's establishment and development were almost the same as in the cv. Revolución. The syncytia were smaller, but production of starch grains in the cells surrounding the feeding site was greater (Fig. 2 C-D) and the cell cytoplasm was more granular (Fig. 2 B).

Histopathology in the resistant hybrid *S. tuberosum* ssp. *andigena* clone B-25

The nematode readily invaded roots of this resistant hybrid (Fig. 3 A). The modifications induced by immature females on the susceptible cultivar were not observed in this hybrid, and there was no development of a feeding site. Generally, no cellular changes became apparent until the fourth day following inoculation. On the fifth day plant response was characterized by a disorganization of cell structures (cell membranes and cytoplasm) expressed in the infected area by an increased affinity of hypersensitive cells for stains (stained red by safranin). Necrosis was initiated near the nematode's head, extended through the tissue and surrounded the female body (Fig. 3. B-D). The cytoplasm of cells contiguous with the necrotic layer appeared unaffected. The earliest hypersensitive reaction was found close to immature females at the second day after inoculation, before the nematode reached the vascular cylinder.

Discussion

Syncytium development was apparent 5 days after inoculation in the susceptible cultivar, and continued until maturation of the female nematode and showed the same cellular features described previously in pepper (Castillo & Marban-Mendoza, 1984), sugar beet (Inserra *et al.*, 1983, 1984), and tomato roots (Jones & Payne, 1977*a*, *b*). In contrast to soil infestations, no lateral root

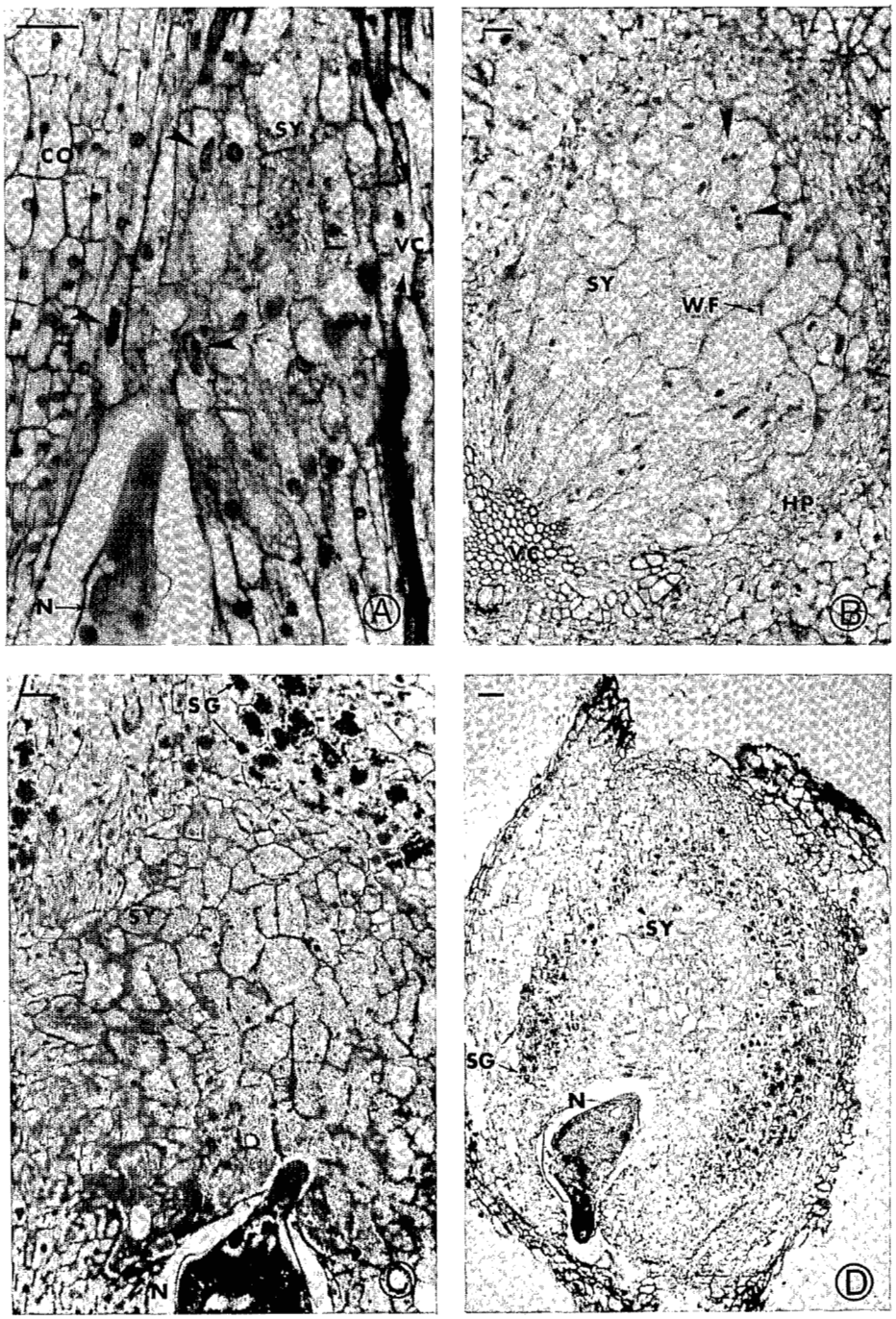

Fig. 1. Histopathological changes induced by *N. aberrans* in the susceptible potato cultivar Revolución — A, C & D : longitudinal sections; B : transverse section — A : Ten days after inoculation with an immature female nematode (N), the syncytium (SY), adjacent to the vascular cylinder (VC), is evident with dense cytoplasm and hypertrophied nuclei (arrow heads) and nucleoli; cortical parenchyma (CO); B : Cross section of the syncytium (SY) ten days after inoculation, with hyperplasia (HP) of tissue surrounding the syncytial zone. Cells with two or three nuclei (arrow heads), and wall fragments (WF) resulting from cell wall dissolution are present; C : The syncytium (SY) 30 days after inoculation with accumulation of starch grains (SG) around it; D : Galling, 30 days after inoculation, syncytium (SY), starch grains (SG), nematode (N) *(Scale bars represent : A-C = 100 µm; D = 200 µm).*

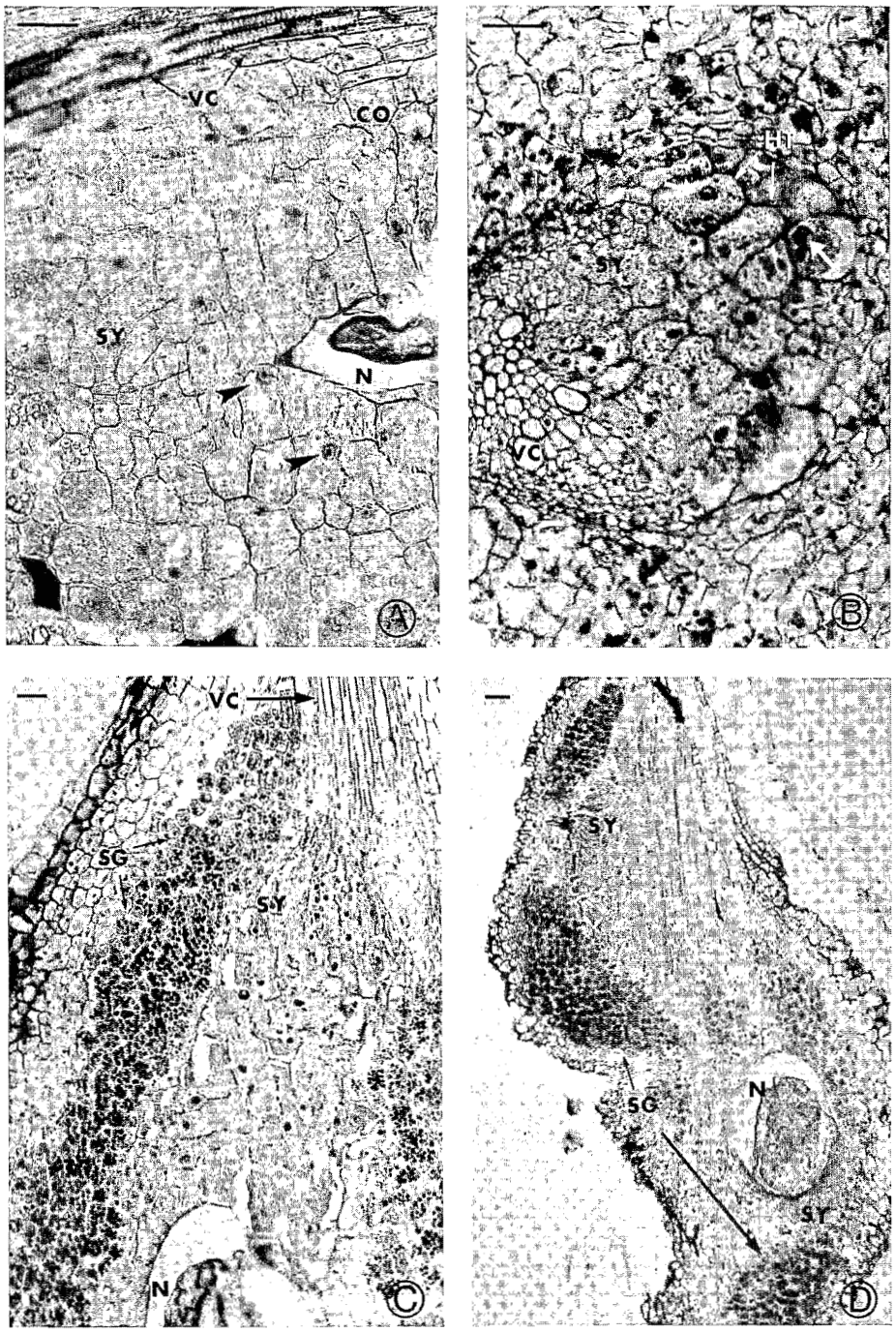

Fig. 2. Histopathological alterations induced by *N. aberrans* in *S. sparsipilum* clone 7601217.7 — A, C, & D : longitudinal sections; B : transverse section — A : 20 days after inoculation; B : 15 days after inoculation — A : Syncytium (SY) with enlarged nuclei (arrow heads) has developed in the cortical parenchyma (CO), nematode (N), vascular cylinder (VC); B : Hypertrophied cells (HT) with granulated cytoplasm and enlarged nuclei (arrow heads); C & D : 35 days after inoculation. Starch grains (SG), are aggregated in the cells surrounding the syncytium (SY); vascular cylinder (VC); D : The syncytia are not connected in the root infected with two females *(Scale bars represent : A-C = 100 µm; D = 200 µm).*

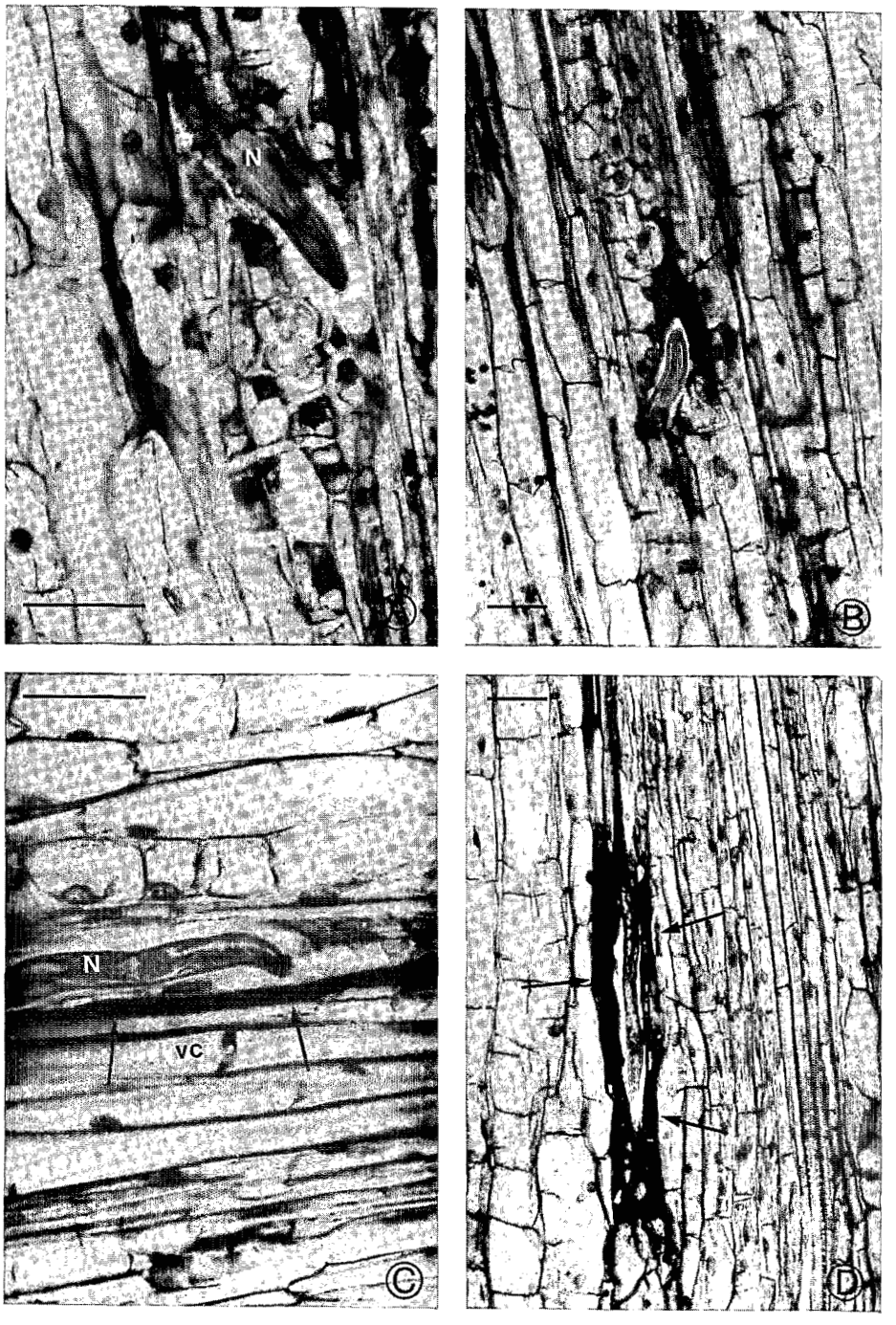

Fig. 3. Necrotic response induced by *N. aberrans* in roots of the resistant hybrid, *S. tuberosum* ssp. *andigena*, clone B-25 — A-D : longitudinal sections — A : Three days after inoculation with immature females (N); B : Five days after inoculation, necrotic cells (arrow) surround the nematode head; C : Ten days after inoculation, the necrotic cells (arrows) form a barrier along the vascular cylinder (VC); D : *N. aberrans* immature female surrounded by necrotic cells (arrows) ten days after inoculation *(Scale bars represent : A-D = 100 µm).*

formation on the galls was observed in the agar culture system. In *S. sparsipilum* 7601217.7 the development of the syncytium was delayed and, although this may be related to a weaker susceptibility of this cultivar, it was anyway insufficient to prevent or limit the formation of the nematode feeding site. An intense hypersensitivity response in the resistant host *S. tuberosum* ssp. *andigena* clone B-25 produced necrosis around the invading nematodes. This reaction may be induced by a component of the nematode's cuticle or secretions or excretions produced by the nematode (Rice, Leadbeater & Stone, 1985) that may or may not be associated with the initiation of the syncytia. As a consequence, the nematodes were not able to establish feeding sites and did not exibit further development during the course of this study.

The resistance mechanism of plants to infection by sedentary endoparasites is not yet understood. It is presumed that host-cell changes are initiated and controlled by salivary secretion produced in the nematode's esophageal glands (McClure & Mende, 1987; Rice, Leadbeater & Stone, 1985). In resistant hosts these materials may elicit a response incompatible to parasitism, possibly a toxification mechanism, which involves a phenolic glucoside and a glucosidase. As a product of hydrolysis of the glucoside a toxic phenolic aglycone may induce host cell death and thus cause starvation of the nematodes (Veech & Endo, 1970).

The hypersensitivity reaction of potato roots is good evidence for the early recognition process of resistance to *N. aberrans*. The divergent resistant responses observed showed important variations in the genetics of the cultivar tested. A more thorough evaluation of the available potato germplasm responses to *N. aberrans* and other plant-parasitic nematodes of economic importance is needed to understand the nature of the incompatible interaction, its biochemistry and the gene(s) involved in resistance. In addition, biochemical tests to assess the sources of resistance at molecular level would be useful in large and fast screening assays for resistance.

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