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RESPONSE OF ANTHER CULTURE-DERIVED DIPLOID LINES OF POTATO TO THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

by
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Summary. Evaluation of reproduction of the root-knot nematode *Meloidogyne incognita* race 1 on antherderived diploid potato lines, obtained from hybrids with one root-knot nematode resistant parent, was investigated. Two F_1 hybrids and three anther-derived lines behaved similarly to the resistant parental line, supporting the concept that anther culture technique can be used in potato breeding for studies on resistance to root-knot nematodes. Infection sites, three weeks after inoculation, examined microscopically, exhibited in resistant lines, necrotic tissues, undersized or absence of giant cell formation, with consequent suppression of nematode development. In contrast, on roots of susceptible potato lines, evident swellings were induced at infection sites by the active expansion of multinucleate giant cells associated with feeding, egg-producing females.

Genetic resistance has been indicated as one of the most effective and safe means of control of nematode pests (Sonnino, 1993). However the tetrasomic inheritance of characters and the heterozygosity present in most potato cultivars represent a major drawback in potato breeding for resistance (Sonnino *et al.* 1992). Anther culture of diploid potato lines and production of homozygous diploid genotypes has been proposed as a tool to overcome potato breeding problems and to speed up the process of development of resistant potato germplasm (Sonnino, 1985). In particular, anther culture has been successfully applied to the breeding of potato lines resistant to cyst nematodes (Wenzel and Uhrig, 1981; Uhrig, 1983).

The objective of the study reported here was to ascertain whether it is possible to apply anther culture to potato breeding for resistance to root-knot nematodes. With this in prospect, the ability of *Meloidogyne incognita* (Kofoid *et* White) Chitw. to parasitize and reproduce on anther-derived diploid potato (*Solanum tuberosum* L.) lines obtained from hybrids with one root-knot nematode resistant parent was assessed.

Materials and methods

The parental lines (146 and 102), two F_1 hybrid lines derived from their cross (FI-15 and FI-1) and 7 lines obtained by anther culture of these F_1 hybrids (Sonnino *et al.*, 1989), namely FI-15-5A, FI-15-9, FI-15-10, FI-1-1, FI-1-5A, FI-I-7-C1, and FI-1-7-C2, have been kindly provided by the International Potato Center (CIP), Lima, Peru. The former five anther-derived lines were obtained by direct embryogenesis, from pollen grains inside the anther walls (Fig. 2), while the latter two lines were obtained through organogenesis from a microspore-derived callus (Iwanaga, pers. com.).

Fourteen replications for each line were grown singly in 750 cm^3 clay pots containing sandy loam soil. Each plant received 5 ml aliquots of *M. incognita* race 1 eggs and juveniles suspension, placed in 3-5 cm deep holes in the soil for a total inoculum population density of 10,000 eggs and juveniles per plant. The populations of *M. incognita* used were reared on tomato (*Lycopersicon esculentum* Mill) in glasshouse cultures.

The roots of four plants for each line were harvested at three weeks after inoculation for histological observations, while all the others were collected 60 days after inoculation and their infestation assessed. Galling of each root system was rated according to a 0-5 scale (Table I) (Taylor and Sasser, 1978). Root system were then immersed in phloxine B solution to stain egg-masses which were counted and rated as for the galling index (Daykin and Hussey, 1985).

Root segments were selected from the 21st and the 60th day harvested plant and fixed in FAA, dehydrated in tertiary butyl alcohol series and embedded in paraffin. Sections 12 µm thick were stained with safranin and fast green, mounted in Dammar xylene and examined microscopically (Johansen, 1940).

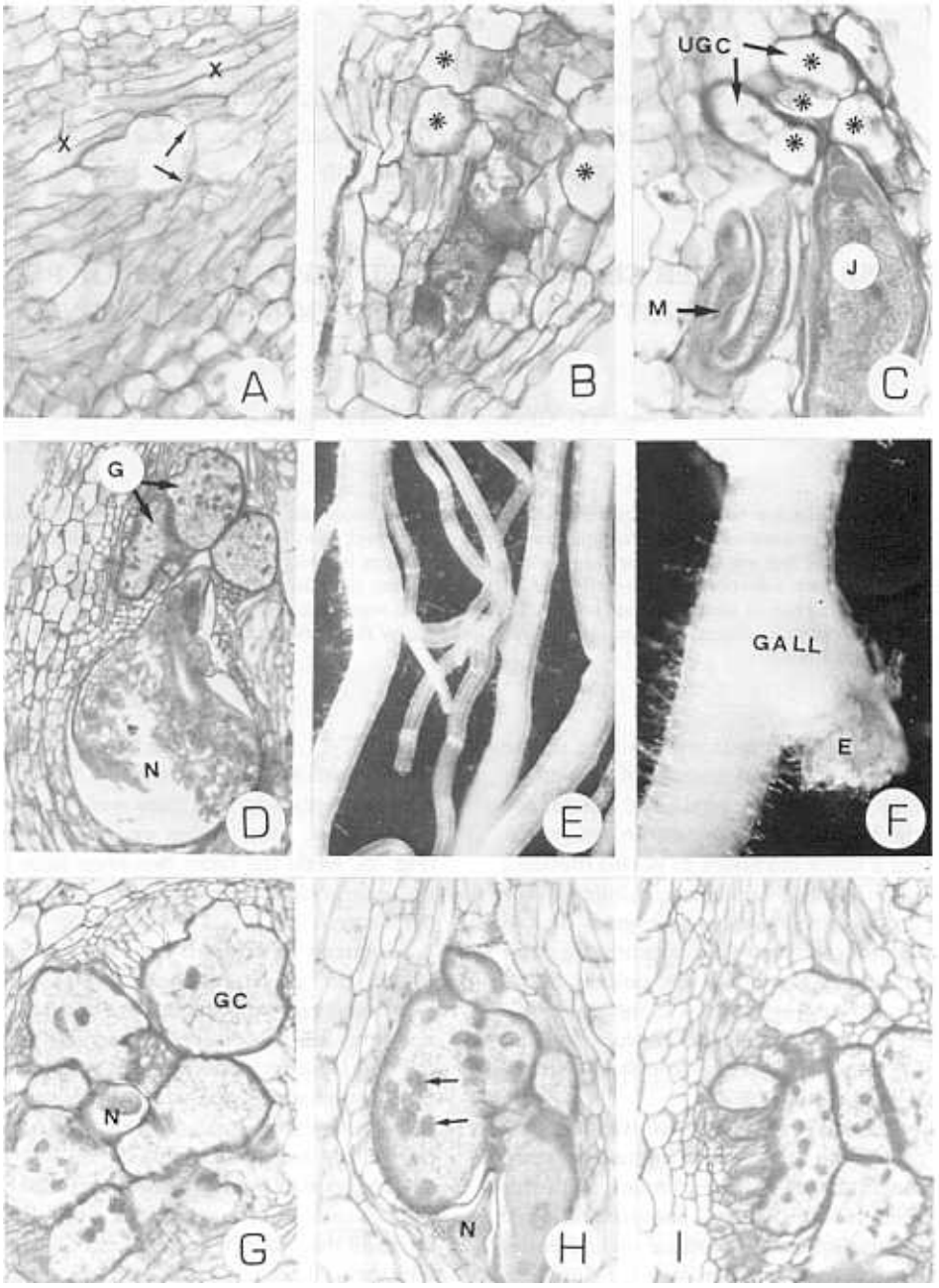

Fig. 1 - Anatomical changes observed on roots of resistant and susceptible potato lines infected by *Meloidogyne incognita* race 1: A. abnormal giant cells produced on line FI-15 by a juvenile *M. incognita* which failed to form functional nurse cells: note non-thickened cell walls (arrowed) adjacent to vascular elements (x); B. undersized giant cells (*) induced by *M. icognita* on the resistant line FI-15-5A observed 21 days after inoculation; C. male (M) and 3rd stage juveniles (J) feeding on an abnormal giant cell (UGC) of the resistant line 146: note the coagulated cytoplasm of these cells in comparison to the granular cytoplasm with numerous hypertrophied nuclei of the functional giant cell (G) showed in fig. 1 D; D, *M. incognita* female (N) feeding on normal giant cells (G) with granular cytoplasm and numerous hypertrophied nuclei, in roots of the susceptible line FI-1-5A; E. F. ungalled roots of the resistant line FI-1-1 in fig. 1E, and large gall on the susceptible line FI-1-5A showing numerous eggs (E) protruding through the root surface, observed 60 days after inoculation; G-I, adult females (N) of *M. incognita* feeding on normal functional giant cells (Fig. 1G = line FI-15-9, Fig. 1H = line 102, Fig. 1 I = line FI-1-7-C1): note in Fig. 1H the numerous (arrowed) hypertrophied nuclei.

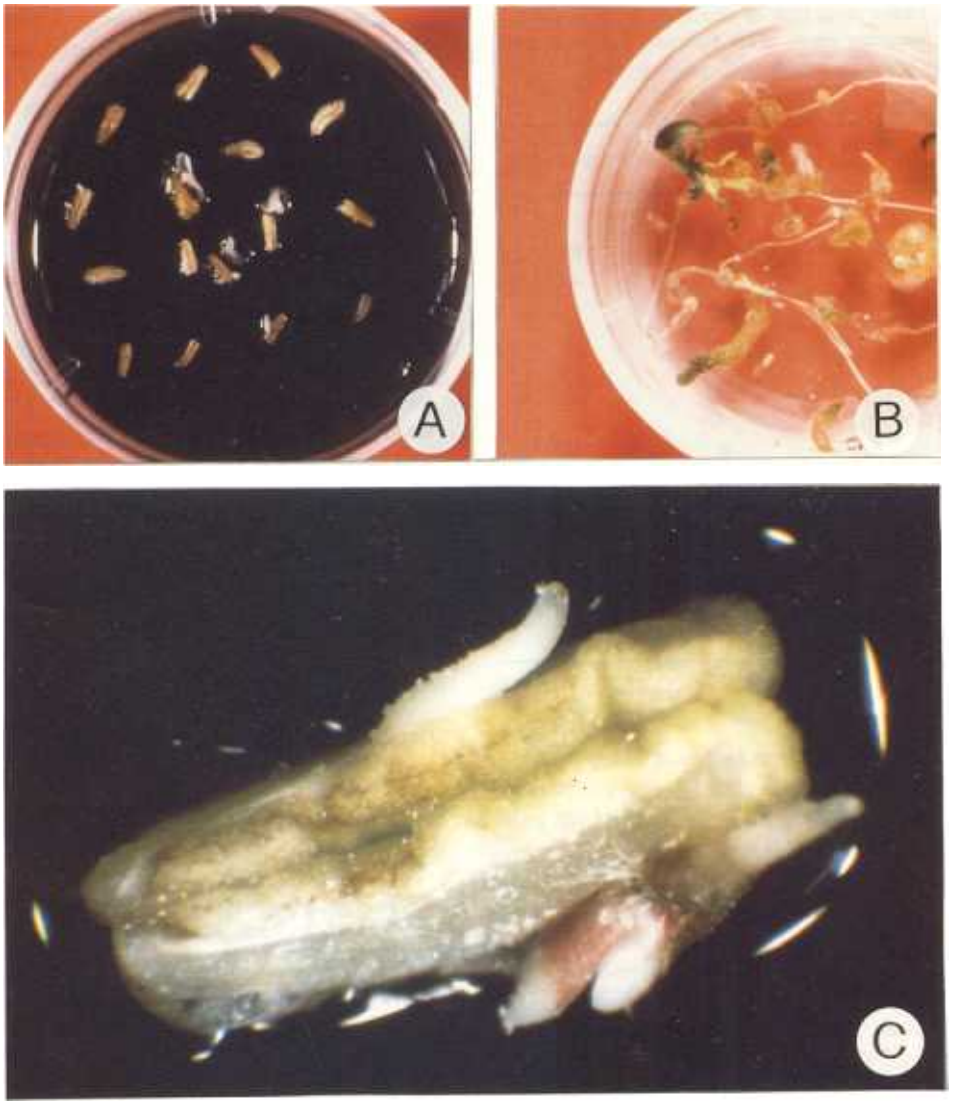

Fig. 2 - Embryoid production: A, embryogenesis from pollen grains inside anther walls; B, embryoids in regeneration medium; C, three weeks old embryoids, protruding from the anther wall.

Results and discussion

Histological observation of root tissues 21 days after inoculation showed that *M. incognita* juveniles penetrated into the roots of all lines tested, but on some of them (for instance the parent line 146, the F_1 hybrids FI-15 and FI-1 and the anther derived lines FI-15-5A, FI-1-1, FI-1-7-C2) they were immobilized and surrounded by necrotic tissues and although they had initiated permanent feeding sites, they failed to form functional modified cells (giant cells) which provide food (Fig. 1A, B, C). Because of the lower amount of food, infact, the nematodes associated with these atypical feeding sites did not develop to adults, while several pre-adult males were observed associated with undersized giant cells which presented coagulated cytoplasm and deeply safranin-stained cell walls (Fig. 1C). Around these atypical hypertrophic cells no hyperplastic formations were observed.

On the remaining lines (FI-15-9, FI-15-10, FI-1-5A, FI-1-7-C1) observed 21 days after inoculation, *M. incognita* invaded potato roots and caused evident swellings at infection sites as a result of active hypertrophic and hyperplas-

TABLE I - *Galling index and type of reaction to* Meloidogyne incognita *on parental lines, their hybrid progenies and lines derived from anther culture of these F_1 hybrids.*

Line	Gall and egg-mass index *	Reaction type
Parental lines		
146	0	R*
102	4	S**
F_1 hybrids		
FI-15	0	R
FI-1	0	R
Anther-derived lines		
FI-15-5A	0	R
FI-15-9		S
FI-15-10	5	S
FI-1-1	0	R
FI-1-5A	5	S
FI-1-7-C1	3	S
FI-1-7-C2	0	R

* = Resistant (Gall index <2)

** = Susceptible (Gall index >2)

 # Scale of evaluation of galling (gall and egg-mass index):
0 galls per plant = score 0; 1-2 galls per plant = score 1; 2-10 galls per plant = score 2; 11-30 galls per plant = score 3; 31-100 galls per plant = score 4; >100 galls per plant = score 5.

tic formation (Fig. 1D). Each adult female, infact, was surrounded by 3-8 large giant cells showing granulated cytoplasm and numerous hypertrophied nuclei and nucleoli (Fig. 1D, G-I).

In contrast with the ungalled roots or those showing only slight swellings (Fig. 1E) of some lines, in other lines, sixty days after inoculation evident egg masses protruded from the galls (Fig. 1F). Histological examination of galled roots revealed the well established permanent feeding sites induced by the nematode feeding (Fig. 1 G-I).

The histological observations have been confirmed by the levels of galling and reproduction (Table I). On the root system of five lines, characterized by high index (3-5) of gall and egg-mass, galls were large in size (3-4 times the normal root diameter) and usually occurred along the root axis. On these lines lateral root formation, which is a typical reaction of galled roots, was induced.

Based on the above results the parental lines 146, the F_1 hybrids FI-15 and FI-1, and the anther derived lines FI-15-5A, FI-1-1 and FI-1-7-C2 can be assumed as resistant, while the remaining lines can be considered as susceptible.

It seems, therefore, that the root-knot nematode resistance present in one of the parental lines (146) has been transmitted to the progenies of a cross with a susceptible line (102) and passed through the process of regeneration from pollen grains of the progenies to be transmitted to the anther culture-derived plants. The occurrence of resistant as well as of susceptible anther culture-derived plants is the effect of the random assortment of chromosomes during meiosis in F_1.

It is particulary interesting to note that the two plants (FI-1-7-C1 and FI-1-7-C2) regenerated through a callus phase, most presumably from the same pollen grain, showed divergent behaviour; the first is susceptible and the second is resistant. For the FI-1-7-C1 a loss of resistance during callus phase can be hypothetised (Fassuliotis and Bhatt, 1982).

In conclusion, the results of the present work tend to support the concept that the anther culture can be used in breeding of potato for resistance to root-knot nematode.

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