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INTERACTIONS BETWEEN RESISTANT TOMATO CVS AND *MELOIDOGYNE* SPP. *IN VITRO*

by
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Summary. Single avirulent populations of *Meloidogyne incognita* and *M. hapla* and a virulent population of *M. javanica* were used to inoculate the resistant tomato cvs VFN8, Rossol, Small-Fry and Cold-Set either in root culture *in vitro* or in pots in a glasshouse. Incompatible reactions were observed *in vitro* and in pots when all the resistant cvs, except Cold-Set *in vitro*, were inoculated with *M. incognita*. *M. hapla* was able to develop on VFN8 and Rossol in pots but was unable to do it in root cultures, whereas Small-Fry was susceptible in both situations. The virulent population of *M. javanica* heavily infested all the resistant cvs used either *in vitro* or in pots. The unexpected incompatible reaction of the *Mi*-bearing cultured roots to *M. hapla* is discussed and the difference of *Mi*-gene with respect to the resistant factor present in Small-Fry is also suggested.

Resistance to *Meloidogyne* spp. in tomato is conferred by the dominant *Mi*-gene which was introduced into *Lycopersicon esculentum* (L.) Mill from the wild species *L. peruvianum* (L.) Mill (Smith, 1944; Gilbert and McGuire, 1956). However, *Mi*-gene shows several limitations in that it is not effective with *M. hapla* (Hadisoeganda and Sasser, 1982), with non-selected, or selected on *Mi*-bearing plants, virulent populations of root-knot nematodes (Roberts *et al.*, 1990) and at temperatures above 28 °C (Ammati *et al.*, 1986).

Two other dominant and possibly one recessive resistance genes (R-genes) were identified by classical genetic studies and designated as $LMiR_1$, $LMiR_2$, and $LMir_3$, respectively, in the commercially available tomato cvs Nematex, Small-Fry and Cold-Set (Sidhu and Webster, 1973). $LMiR_1$, gene was found to be the same or an allele of the *Mi* locus, whereas $LMiR_2$ was different but closely linked to $LMiR_1$ (Sidhu and Webster, 1975).

Recently, additional sources of resistance have been characterized from wild tomato genotypes: *Mi2*, which confers heat-stable resistance to *M. incognita* (Cap *et al.*, 1993) and *Mi3*, which confers resistance against nematode strains that can infect plants carrying *Mi* (Yaghoobi *et al.*, 1995). No specific R-gene has so far been characterized as effective against *M. hapla* although some accessions of *L. peruvianum* and *L. glandulosum* show some resistance (Ammati *et al.*, 1985).

Mi-mediated resistant response is rapid and results in a hypersensitive reaction (HR) occurring about 12 hours after inoculation of roots with the nematode juveniles (Paulson and Webster, 1972; Trudgill, 1991). Such an incompatible interaction has been extensively studied from a genetic (Roberts, 1995) and molecular (Williamson *et al.*, 1994; Molinari, 1996) point of view but there is no report on the monitoring of incompatible *Meloidogyne*-tomato interactions in axenic cultures. Therefore, a study of such an interaction *in vitro* was undertaken using tomato cvs carrying the well-characterized R-genes *Mi* (cv. VFN8), $LMiR_2$ (cv. Small-Fry) and $LMir_3$ (cv. Cold-Set) inoculated with single avirulent

populations of *M. incognita* (Kofoid *et* White) Chitw. and *M. hapla* Chitw. and a virulent population of *M. javanica* (Treub) Chitw. The cultivar Rossol, in which another partially dominant R-gene was found (Fatunla and Salu, 1977) in addition to the *Mi*-gene (Netscher, 1978), was also tested.

Materials and methods

The avirulent populations of *Meloidogyne incognita* and *M. hapla* used to inoculate resistant tomato cultivars were from susceptible tomato cultures grown in a glasshouse; the virulent population of *M. javanica* was from Tunisia and was from Prof. M. M. B'Chir of the Institut National Agronomique De Tunisie (INAT, Tunis). Resistant tomato cultivars used were VNF8 and Small fry (Petoseed Co., Inc., California), Rossol (Peto Italiana s.r.l., Parma) and Cold-Set (Stokes, New York).

Inoculation in pots. Roots of the susceptible tomato cv. Roma VF, infested by *M. incognita*, *M. hapla* and the virulent population of *M. javanica*, were weighed, finely chopped and each separately mixed with 1 kg of soil (Di Vito *et al.*, 1985). Three samples of 1 g per batch of roots were previously collected and processed by the sodium hypochlorite method (Hussey and Barker, 1973) to estimate the number of eggs per g of roots. The infested soil was then diluted to give an inoculum of 40 eggs per g and filled in pots into which seedlings of resistant tomato and the susceptible tomato Roma VF, used as a control, were transplanted. Pots were maintained at 26-28 °C in a glasshouse for approx. 50 days after which the root systems were washed, examined for the presence of nematodes and photographed.

Axenic cultures of nematodes. Tomato seeds were surface sterilized by immersion for 20 min in 1% sodium hypochlorite and 2 drops/100 ml of Tween 20, under vacuum, and then rinsed with sterile distilled water. Then they were transferred to 1% water Gelrite® (Sigma Co.) plates using a sterile technique and put in a growth chamber at 28 °C, in the dark. After the seed had germinated, 2 cm lengths of the primary root tips were excised and transferred to Petri dishes containing root growth medium. One litre of this medium contained 3.2 g of Gambor's Basal Medium with Minimal Organics (Sigma Co.), 20 g sucrose, 20 g Gelrite®, pH 5.7. This mixture was autoclaved for 40 min, cooled to approx. 45 °C and a complex of vitamins consisting of 0.05 mg/l biotin, 0.5 mg/l folic acid, 2.0 mg/l glycin, 100 mg/l myo-inositol, 5.0 mg/l nicotinic acid, 0.5 mg/l pyridoxine and 0.5 mg/l thiamine was added. The vitamin solution was previously sterilized by filtering through 0.22 µm filters (Whatman type). When root growth was clearly visible (after 4-5 days), uncontaminated plates were inoculated with 3-5 egg masses of the *Meloidogyne* species that had been sterilized as follows: egg masses were hand-picked from roots and directly put into a 1:10 dilution of a sterilizing solution consisting of 5 mg/100 ml amphotericin B and 1 mg/ml streptomycin sulphate (Sigma, Co.). They were then thoroughly rinsed with sterile water and stirred into the amphotericin-streptomycin solution for 1 h. After a rinse in sterile water, they were treated with 1% chlorhexidine diacetate (Sigma, Co.) for 15 min and again rinsed twice with sterile water. Using a sterile technique, egg masses were finally transferred to a minimal volume of sterile water and placed in root culture Petri dishes. The plates were sealed and transferred to a growth chamber at 28 °C, in the dark. Hatching of the juveniles was visually monitored by a light microscope and only plates with a large number of living pre-parasitic second-stage juveniles were used for testing *Meloidogyne*-tomato interactions.

The same batch of egg masses was used to inoculate cultured roots of each resistant tomato cultivar together with a susceptible control (cv. Roma VF). A susceptible interaction was clearly visible by the heavy galling of the roots 40-45

days after inoculation, as that shown in Fig. 1A. Most of the galls contained well-developed egg masses and females (Fig. 1B). Where there was a strong incompatible reaction by the roots to the invading juveniles, large necrotic areas were apparent mainly on the secondary roots and the overall growth of the roots was strongly inhibited very soon after inoculation (Fig. 1C). Limited root growth was monitored as many as 30-40 days after inoculation when most of the plates were photographed.

Results

The avirulent population of *M. incognita* tested showed, *in vitro*, an incompatible interaction with the *Mi*-bearing tomato cvs VFN8 (Fig. 2C), Rossol (2D) and Small Fry (2A), but it was able to develop on cv. Cold-Set (not shown). Fig. 2B shows that VFN8 and Small-Fry (respectively, a-b) were likewise resistant when inoculated in pots. Full resistant reaction was observed with Rossol and few galls developed on Cold-Set (not shown). Surprisingly, reaction of cvs Rossol and VFN8 to *M. hapla* attack *in vitro* was incompatible and there were no apparent differences from those in which *M. incognita* was used as the inoculum (Fig. 3A-B). On the contrary, *M. hapla* was able to develop on Small Fry (Fig. 3D) and Cold-Set (not shown) *in vitro*. In pots, VFN8 and Small-Fry showed a lower degree of susceptibility to *M. hapla* than the susceptible control (Fig. 3C). Rossol and Cold-Set were fully susceptible (not shown).

The virulent population of *M. javanica* tested was able to develop *in vitro* on both Rossol (Fig. 4A) and VFN8 (4B) and heavily infested all the resistant tomato cultivars tested in pots (4C). Galls from the plates of resistant tomato roots infested by the virulent *M. javanica* are the only galls so far obtained from *Mi*-bearing cultured roots and, therefore, they were collected when they were fully developed and saved for further analysis.

Discussion

Resistant tomato roots retained their capability to react hypersensitively to avirulent populations of *Meloidogyne* when excised and inoculated *in vitro* but showed normally developed galls with mature female and egg masses when attacked by virulent biotypes. The *Mi*-bearing cvs VFN8 and Rossol had previously been found to have different degrees of susceptibility to *M. hapla* but never showed hypersensitivity to this species (Hadisoeganda and Sasser, 1982; Ammati *et al.*, 1985). Surprisingly, roots of VFN8 showed a strong HR to *M. hapla in vitro* and also Rossol suppressed the development of the nematodes. The intrinsic inability of such cultured roots to develop galls in such modified conditions has been negated by the successful development of the virulent *M. javanica* on them. Also, the supposed avirulence of the *M. hapla* population used on *Mi* can be excluded by considering that the egg masses used to inoculate the plates came from the same infested roots used to inoculate seedlings. Therefore, it seems that the virulence of *M. hapla* with respect to the *Mi*-bearing tomato cultivars tested is completely eliminated *in vitro*.

This opposite reaction of excised cultured roots, compared with that of the intact plant, to the same population of *M. hapla* is intriguing and suggests that avirulence to *Mi*-gene in *M. hapla* is maintained and virulence is not the result of permanent loss or mutation into nonfunctional alleles of avirulence genes thus evading recognition and triggering of HR. The possibility that some additional resistance factor, other than *Mi* and specific to *M. hapla*, may be induced *in vitro*, seems unlikely. Thus, the commonly observed virulence of *M. hapla* to *Mi* might be due to some specific factors which are able to overcome or avoid the triggering of plant reactions caused by recognition, but, evidently, these factors are hampered *in vitro* for reasons unknown at present.

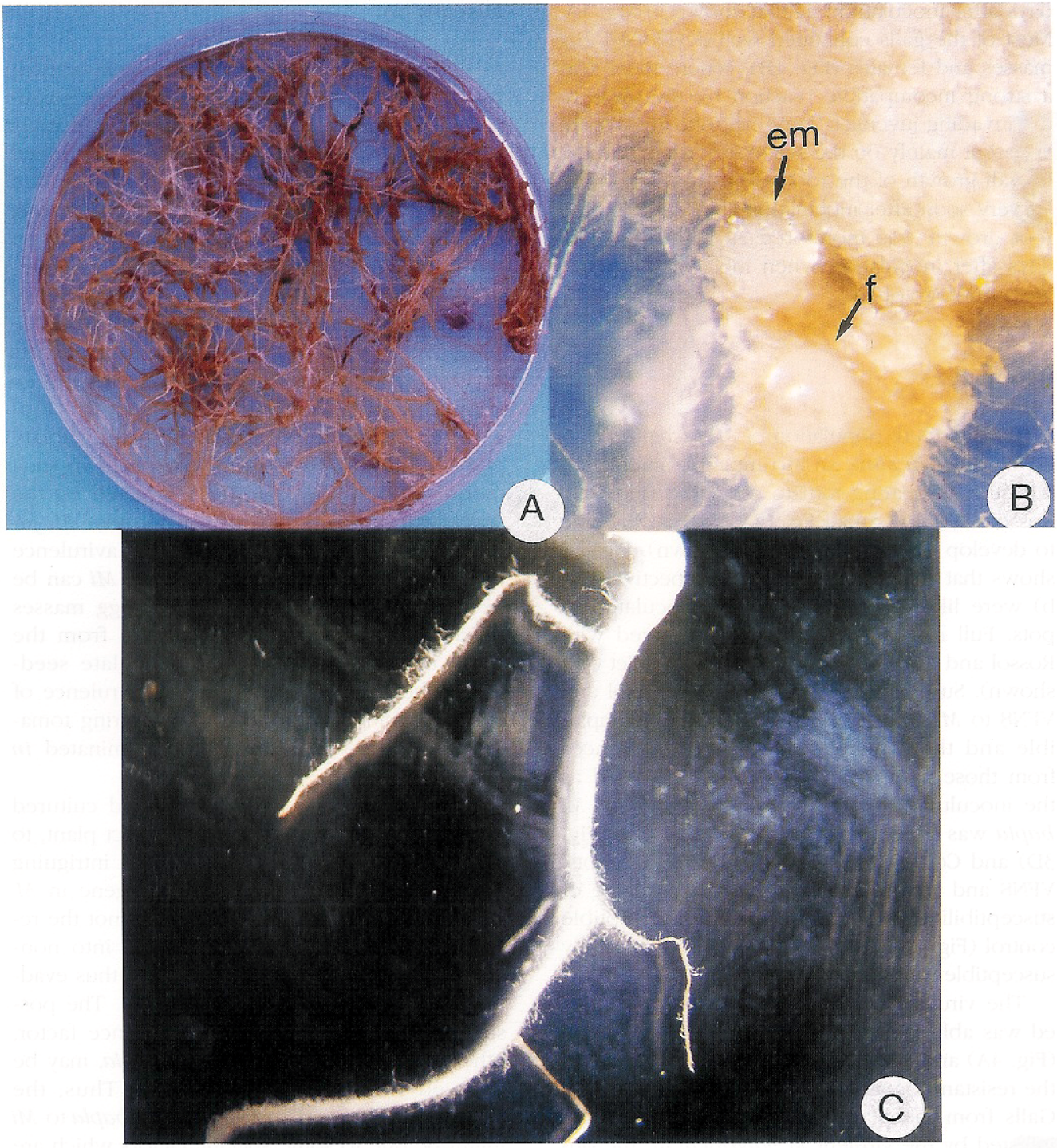

Fig. 1 - *Meloidogyne*-tomato interactions *in vitro*: A, a typical compatible interaction about 50 days after inoculation; B, part of plate A at x40 magnification: mature female (f) and its egg mass (em); C, roots of cv. VFN8 infested by *M. hapla*: note necrosis of secondary roots and the poor growth of the root system.

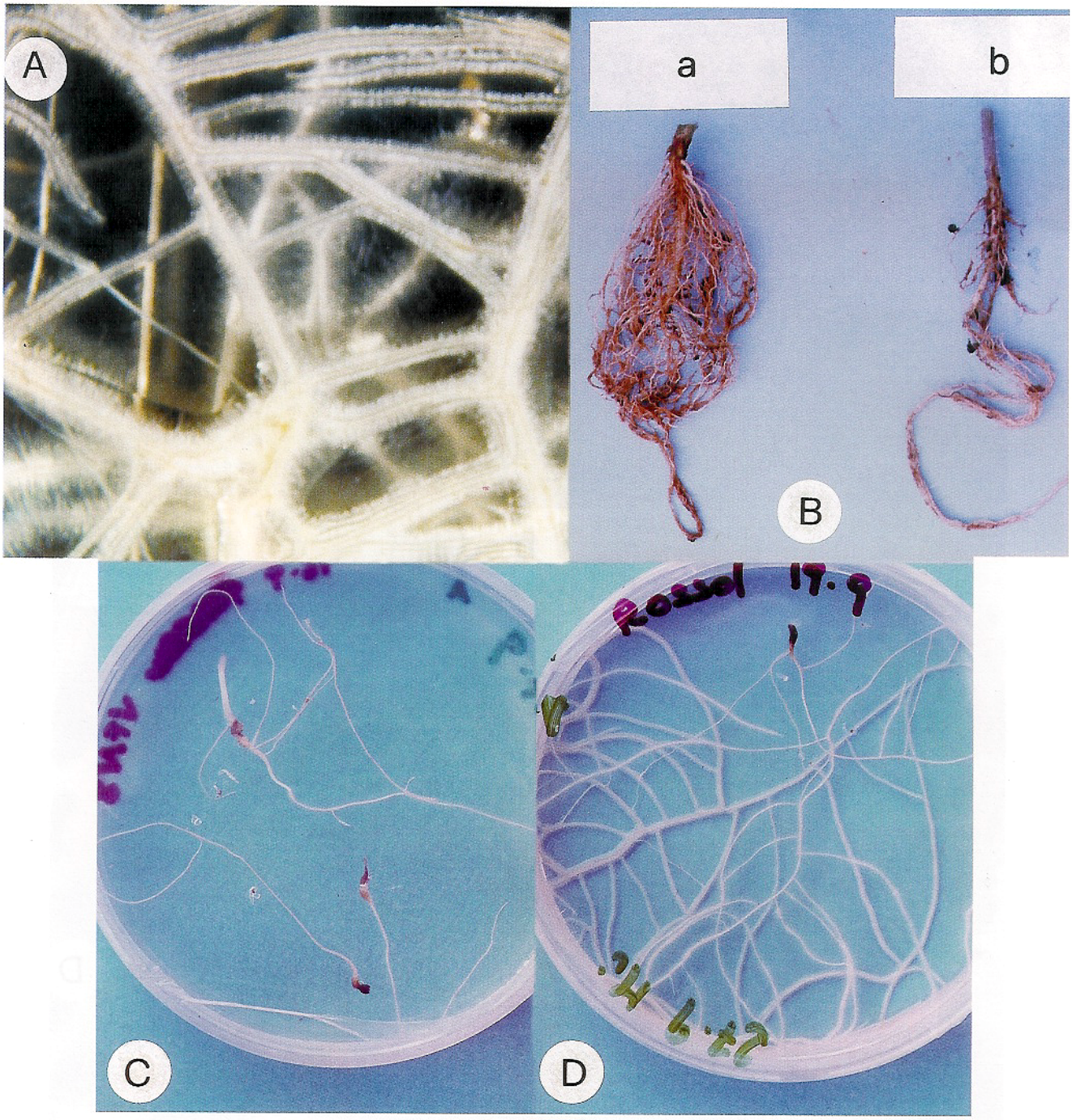

Fig. 2 - *M. incognita*-resistant tomato interactions: C, cultured roots of cv. VFN8 40 days after inoculation with egg masses of *M. incognita*; D, the same as C with cv. Rossol; A, x40 magnification of a plate with *M. incognita*-Small-Fry tomato roots interaction; B, tomato roots 50 days after inoculation with *M. incognita* infested soil: a, VFN8 - b, Small-Fry.

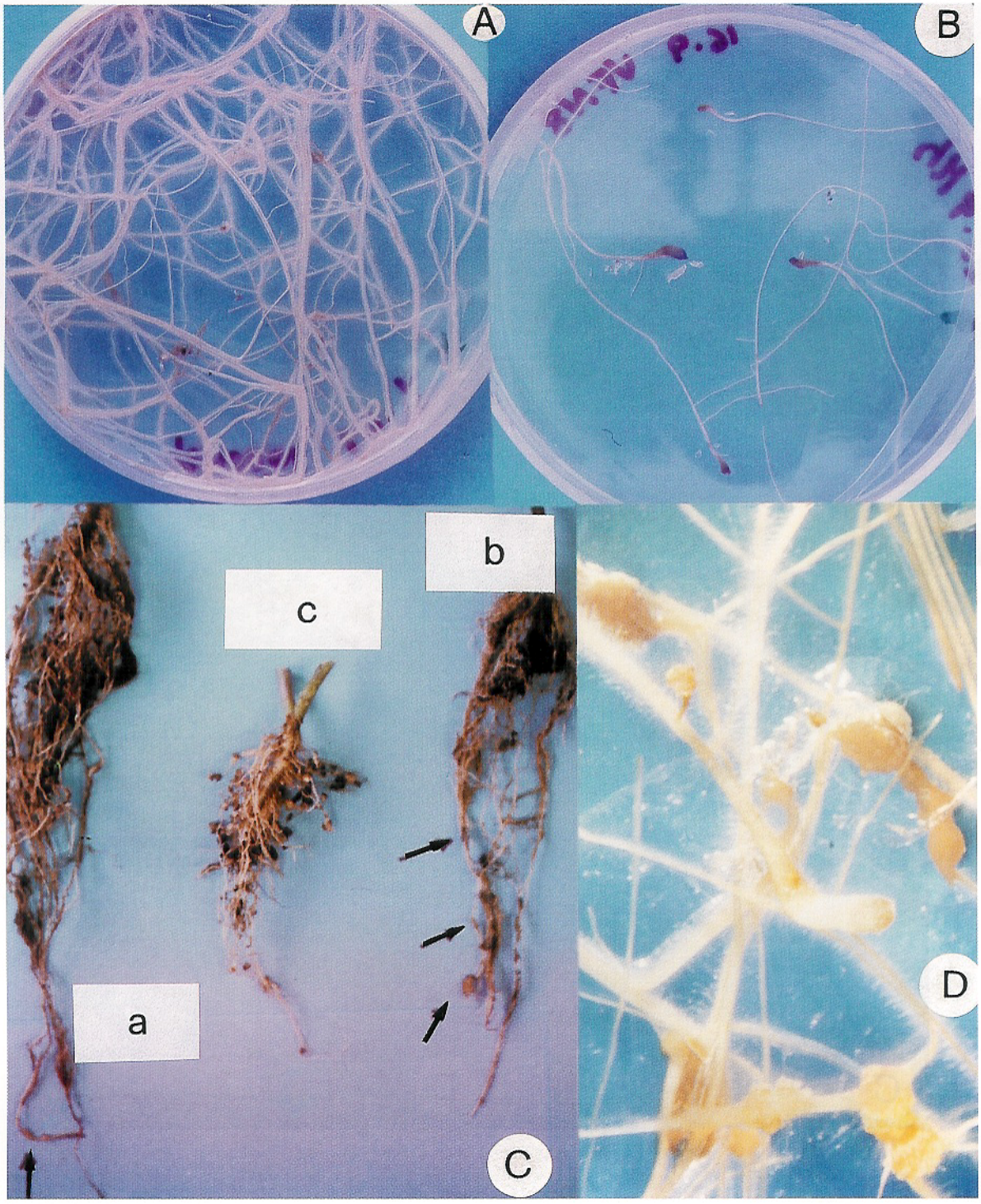

Fig. 3 - *M. hapla*-resistant tomato interactions: A, cultured roots of Rossol 40 days after inoculation with egg masses of *M. hapla*; B, the same as A with VFN8; D, x40 magnification of a plate with *M. hapla*-Small-Fry tomato roots interaction: several galls are visible; C, tomato roots 50 days after inoculation with *M. hapla* infested soil: a, VFN8 - b, Small-Fry - c, Roma VF (control); arrows indicate galling of the roots.

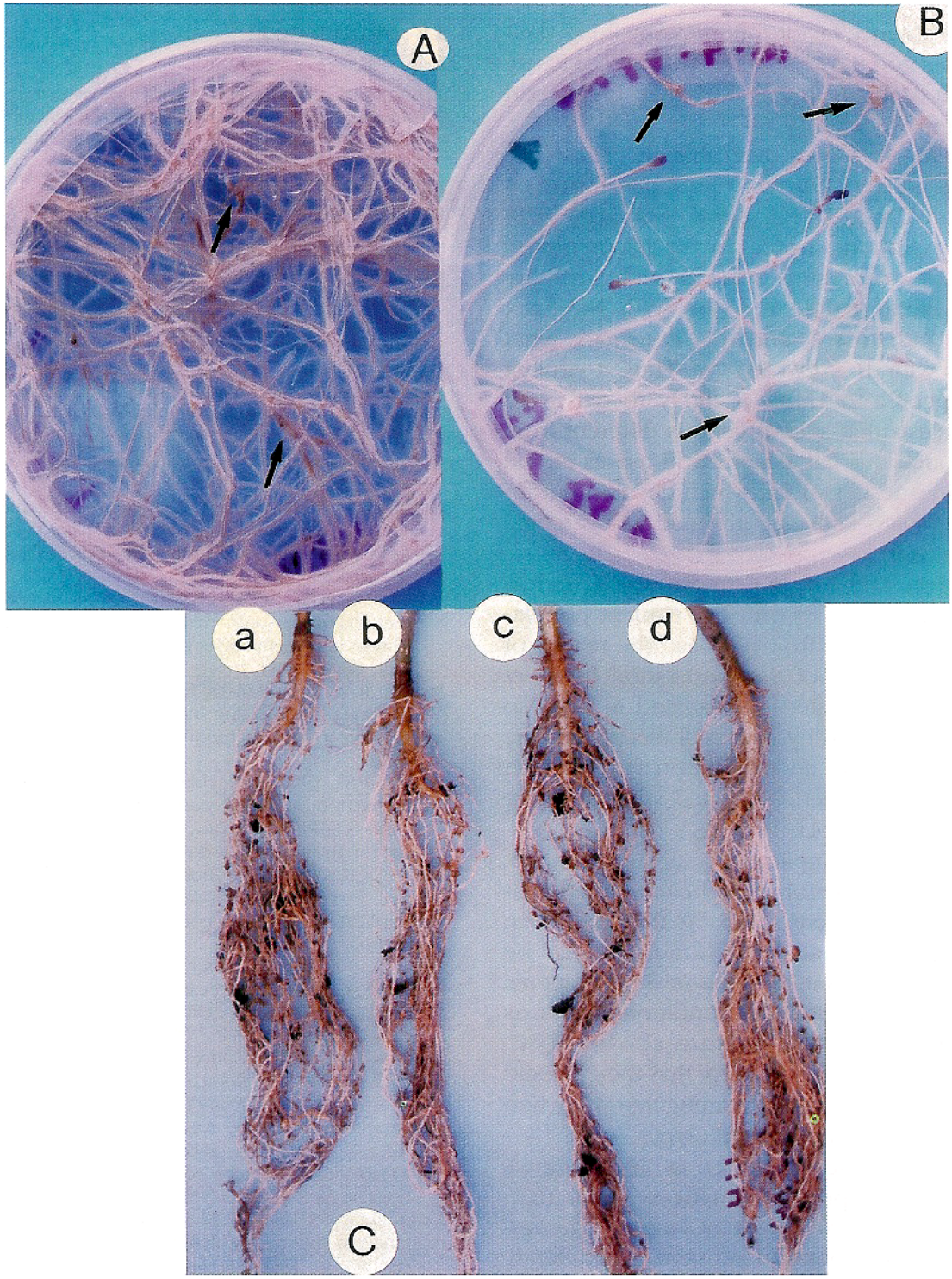

Fig. 4 - Virulent *M. javanica*-resistant tomato interactions: A, cultured roots of Rossol 30 days after inoculation with egg masses of virulent *M. javanica*, arrows indicate galls; B, the same as A with cv. VFN8, arrows indicate galls; C, heavily infested tomato roots 50 days after inoculation with virulent *M. javanica* infested soil: a, Small-Fry - b, VFN8 - c, Roma VF (control) - d. Rossol.

The resistance factor found in Small-Fry (heterozygous for the dominant $LMiR_2$) is closely linked to *Mi*-locus and is 5.65 map units apart (Sidhu and Webster, 1975).

Meloidogyne hapla overcame the resistance by this R-gene *in vitro* and fully developed on the cultured roots of Small-Fry. Netscher (1978) found a virulent population of *M. incognita* which had a compatible reaction with Small-Fry and an incompatible reaction with Nematex ($LMiR_1$) and Rossol, and this is exactly what happens with *M. hapla in vitro*. Thus, a virulent factor specific to $LMiR_2$ is likely to exist and thus suggests that such a gene is different from *Mi*, although this has no practical use as all the populations tested so far, which develop on *Mi*-bearing tomato cultivars, are able to overcome $LMiR_2$ as well.

No successful resistant factor was found in pot tests and *in vitro* with the virulent population of *M. javanica* from Tunisia. The reason of the aggressiveness of this population to every resistant cultivar tested *in vitro* and in pots, so far, is being studied by means of electrophoretic isozyme profiles of antioxidant enzymes, which have previously been correlated with virulence (Molinari and Miacola, 1997).

Our findings complicate an already complex picture of the gene-for-gene *Meloidogyne*-tomato interaction (Roberts, 1995) as they introduce the suggestion that the interaction may be markedly influenced by environmental factors. As there are biochemical conditions, such as those realized at temperature above 28 °C, which inactivate *Mi*, it is likely that there should be other conditions which strengthen *Mi* action against normally virulent pathotypes.

Finally, avirulence genes in *Meloidogyne* may encode important functions other than avirulence and virulence may result in the appearance of biochemical properties apparently unrelated with avirulence, enabling the nematode to overcome plant resistance. Our future research will focus on such putative biochemical properties.

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