

Micropropagated Banana Infected with *Meloidogyne javanica* Responds to *Glomus intraradices* and Phosphorus

Jorge Pinochet¹ and Carolina Fernández²

Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, Crta. de Cabrils s/n 08348, Cabrils, Barcelona, Spain

María de Carmen Jaizme³ and Pedro Tenoury²

Departamento de Protección Vegetal, Instituto Canario de Investigaciones Agrarias, Apartado 60, 38200, La Laguna, Tenerife, Canary Islands, Spain

Additional index words. vesicular–arbuscular mycorrhizal fungi, *Musa* AAA, root-knot nematode, tolerance

Abstract. The effects of the interaction between the vesicular–arbuscular mycorrhizal fungus *Glomus intraradices* Schenk and Smith and the root-knot nematode *Meloidogyne javanica* (Treb) Chitwood on growth and nutrition of micropropagated ‘Grand Naine’ banana (*Musa* AAA) were studied under greenhouse conditions. Inoculation with *G. intraradices* significantly increased growth of plants in relation to nonmycorrhizal plants and was more effective than P fertilization in promoting plant development. Mycorrhizal colonization did not affect nematode buildup in the roots, although plants with the nematode and mycorrhiza were more galled. *Meloidogyne javanica* had no effect on the percentage of root colonization in mycorrhiza-inoculated plants. No element deficiency was detected by foliar analysis. All elements were within sufficiency levels for banana with exception of N, which was low. Potassium levels were lower in mycorrhizal plants, while Ca and Mg levels were higher with mycorrhiza than without, with or without the nematode. Early inoculation with *G. intraradices* appears to favor growth of banana plants by enhancing plant nutrition.

Meloidogyne spp. root-knot nematodes are present wherever bananas are grown, although they cannot compete with the *Radopholus similis* (Cobb) Thorne, the nematode of major concern in banana production in the humid tropics (Pinochet, 1977). Root-knot nematodes are an important banana pest in dry subtropical conditions where *R. similis* is absent (Gowen and Quéhérvé, 1990). Several species of *Meloidogyne* are widespread in banana plantations in the Canary Islands, Spain (Rodríguez, 1990).

In other Mediterranean environments, such as in Morocco, Cyprus, Crete, Egypt, Lebanon, and Israel, root-knot nematodes can become a limiting factor in banana production in intensive greenhouse operations (Vovlas et al., 1992) or when plantlets are attacked at an

early stage of development in the field (Sikora and Schlosser, 1973).

Vesicular–arbuscular mycorrhizal fungi (VAM) are obligate symbionts that increase nutrient uptake by plants, especially P and minor elements (Gerdemann, 1968; Smith, 1987). This association normally occurs naturally when plantlets are transplanted into the field. Mycorrhizal plants of various species have increased their tolerance when attacked by root-knot nematodes by enhancing plant nutrition or by exerting a suppressive effect over the nematode reproduction (Cooper and Grandison, 1987; Heald et al., 1989; Hussey and Roncadori, 1982; Roncadori and Hussey, 1977; Stroebel et al., 1982).

The high mycorrhizal dependency of Dwarf Cavendish banana cultivars (Jaizme and Azcón, 1995; Rizzardi, 1990), compounded by an increased interest in the use of micropropagated banana cultivars by growers in the Canary Islands, has encouraged studies to determine if early mycorrhizal inoculation of young banana plantlets at a stage when plants are vulnerable to nematode damage would confer some degree of protection/tolerance, as they are established in root-knot-infested soil. The purpose of this investigation was to study the effects of the root-knot nematode *M. javanica* and P fertilization on growth and nutrition in inoculated and noninoculated banana plants with the VAM fungus *Glomus intraradices*, and to determine the influence of these variables on pathogen and symbiont development.

Micropropagated banana *Musa* AAA ‘Grand Naine’ were supplied by Cultivos Vegetales In Vitro de Tenerife (CULTESA), Tenerife, Canary Islands, Spain. Plantlets ≈ 10 ± 1 cm tall were received in 200-cm³ minipots in a 1 peat (Floratorf®; Floraguard GmbH, Germany) : 1 perlite (Iberperlita®; Stavik S.A., Huesca, Spain) substrate (v/v), and were transplanted to 3.2-L pots containing a pasteurized loam textured soil (67% sand, 31% silt, 2% clay; pH 7.1; <3% organic matter; and a cation exchange capacity below 10 meq/100 g soil).

A greenhouse experiment with six treatments lasting 7 months (January to August) was established: 1) control in low P soil (CLP); 2) control in high P soil (CHP); 3) *M. javanica*-inoculated plants in low P soil (Mj LP); 4) *M. javanica*-inoculated plants in high P soil (Mj HP); 5) *G. intraradices*-inoculated plants in low P soil (Gi LP); 6) *G. intraradices*- and *M. javanica*-inoculated plants in low P soil (Gi + Mj LP).

Mycorrhizal treatments were established by inoculation with 10 g of *G. intraradices* soil inoculum during transplant to 3.2-L pots. VAM inoculum consisted of rhizosphere soil from 8-month-old leek (*Allium porrum* L.) pot cultures containing heavily infected root fragments with abundant internal spores. Non-mycorrhizal plants received a filtrate of soil inoculum from leek cultures free from VAM propagules. Plants were kept in a greenhouse until nematode inoculation, 2 months later, in April (simulating nursery inoculated plantlets followed by field exposure to the nematode pathogen).

The nematode inoculum consisted of a population of *M. javanica* isolated from the same banana cultivar originally collected in Las Galletas, Tenerife, Canary Islands. Population was increased on tomato (*Lycopersicon esculentum* Mill. ‘Rutgers Marglobe’) from single-egg-mass cultures. Nematode identification was made by perineal patterns (20 females per population) and confirmed by Random Amplified Polymorphic DNA analysis (Cenis, 1993).

Nematode inoculum was prepared by macerating infected tomato roots in a blender for 15 s at 14,500 rpm in a 0.12% to 0.15% NaOCl solution (Hussey and Barker, 1973). Eggs and juveniles (J₂) were collected using a 25-µm-pore sieve (500 mesh) and rinsed with tap water. Inoculum was adjusted to deliver a suspension of 2500 nematodes per plant through six holes 3 to 4 cm deep and located 4 to 5 cm from the base of the plant. Each treatment was replicated 10 times in a completely randomized design. During the course of the study, ambient greenhouse air fluctuated between 18 and 38 °C. Plants were watered as needed and fertilized weekly with a modified Hoagland’s (Hoagland and Arnon, 1950) nutrient solution low in P (0.10 g KH₂PO₄/L). The plants from the high P treatment received a triple amount of P (0.30 g KH₂PO₄/L).

At harvest, plant growth (number of leaves,

Received for publication 2 Jan. 1996. Accepted for publication 16 Aug. 1996. This work was financed by the Spanish Instituto Nacional de Investigaciones Agrarias, INIA, Grant No. SC94-083. We thank C. Calvet and A. Camprubí for providing mycorrhizal inoculum. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Research Nematologist.

²Graduate Assistant.

³Research Plant Pathologist.

fresh top weight, dry top weight, fresh root weight, shoot diameter, leaf surface, and plant height); nematode reproduction; VAM infection; and N, P, K, Ca, and Mg plant content were assessed.

The percentage of galled root system (Baker, 1985), final nematode population per plant, and the number of nematodes per gram of root were assessed at 150 days after inoculation. Nematodes in soil were recovered by removing soil from pots and placing them in a large pan. Roots were washed free of soil particles in a second pan with a known volume of water. Contents of both pans were mixed and stirred for 1 min. A 250-cm³ subsample of the slurry was obtained and nematodes extracted by differential sieving and sugar flotation (Jenkins, 1964). Nematode extraction from roots was similar to that used for inoculum preparation. Nematodes were concentrated using 150-, 74-, and 25- μ m-pore sieves (100, 200, and 500 mesh, respectively). Root tissue and debris collected on the 150- μ m sieves were discarded.

For assessing mycorrhizal infection, a small root sample (10% in fresh weight) of the whole root system was used to estimate percentage of VAM root infection. Samples were stained with 0.05% trypan blue in lactic acid (Phillips and Hayman, 1970) modified by the procedure described by Koske and Gemma (1989). The percentage of root colonization was determined using the grid line intersect method (Giovanetti and Mosse, 1980). Mycorrhizal root samples, inoculated or noninoculated with *M. javanica*, were excised after clarifying and staining of the root, mounted on millimetric slides, and observed under a light microscope.

Macro- and two microelements (N, P, K, Ca, and Mg) were determined at harvest. Pseudostem and leaves (aerial part) of banana plant were thoroughly washed in mild detergent; rinsed twice in distilled water, avoiding senescent or necrotic tissue; and prepared for foliar analysis. Samples were then dehydrated in a temperature-controlled, fan-ventilated oven at 65 °C \pm 1 during 24 h, ground in a ball mill and digested in wet acid (Jones et al., 1991) using perchloric acid. Analysis for all elements, except N, was made with a Perkin Elmer 1100B atomic absorption spectrophotometer (Perkin Elmer, Richmond, Va.). Phosphorus was determined colorimetrically using vanadomolibdophosphoric acid and absorbances were read at 420 nm in a Technicon AAI Autoanalyzer (Technicon, New York). Two readings were made per sample. Nitrogen content was determined by a Kjeldahl procedure (Rund, 1984).

All data were analyzed by an analysis of variance. Data on nematode reproduction were log₁₀ (x + 1) transformed for analyses. Means were compared by Tukey's multiple range test (P \leq 0.05). Data on percentage of root galling and VAM root colonization were transformed to arcsin for analysis.

Results

Glomus intraradices significantly increased plant growth in relation to nonmycorrhizal

plants, with the exception of plant height in Gi Mj LP (Table 1). Mycorrhizal plants showed better growth response to plants fertilized with a triple dose of P.

Nematode-inoculated 'Grand Naine' high in P (Mj HP) produced significantly more fresh top and dry top weights than Mj LP or CLP. Also, Mj HP differed from CLP for stem diameter and from Mj LP for fresh root weights. Plant height and leaf surface were similar for high and low P treatments with or without nematodes.

At the end of the experiment there were no differences in the final nematode population (63,100 to 84,200) per plant and in the number of nematodes per gram of root (1510 to 2560) between nematode-inoculated treatments, although plants with the nematode plus mycorrhiza were significantly more galled (75%) than those without (43% to 49%). Also, there were no differences in the percentage of mycorrhizal infection between the VAM treatments with and without the nematode (37% to 27%), respectively. The root infection of *G. intraradices* was mainly arbuscular, with few internal vesicles and spores formed within the roots. Mycelium of *G. intraradices* and arbuscules colonizing cells were observed several cell layers apart from where nematode eggs were deposited (egg masses within galled root tissue).

No deficiencies were detected by foliar analysis for any element or treatment. Most

elements were within sufficiency levels for 6- to 9-month-old banana plants, with the exception of N, which was low in all treatments (Table 2). Plants in both high P treatments contained significantly higher P levels than *G. intraradices*-inoculated plants. Potassium levels were also lower in mycorrhizal plants. In contrast, Ca and Mg levels were higher in mycorrhizal plants with or without the nematode in relation to the rest of the treatments.

Discussion

Although P is an essential element, requirements of bananas are not large and deficiency symptoms are rarely seen in the field (Walmsley et al., 1971). Phosphorus appears to be less important to bananas than to the majority of agricultural crops. Bananas are known to demand high amounts of N and especially K (Lahav and Turner, 1983) and tend to suffer from B deficiency, thus the poor response observed by addition of a triple dose P fertilization. Growth response of nematode-free 'Grand Naine' banana plants to high P fertilization was negligible relative to low P treatments. Only in the presence of the nematode pathogen were significant differences recorded for top and root weights in high P plants.

Phosphorus supply was adequate and always within sufficiency levels (Table 2). However, the lower levels of P associated with

Table 1. Growth response of 'Grand Naine' banana to inoculation with *Glomus intraradices* and 2500 *Meloidogyne javanica* per plant and P fertilization 7 and 5 months after vesicular-arbuscular mycorrhizal fungus and nematode exposure, respectively.

Treatment	Growth characteristic					
	Top wt (g)		Stem diam	Plant ht	Leaf surface	Fresh root wt
	Fresh	Dry	(mm)	(cm)	(cm ²)	(g)
Control LP ^a (CLP)	82 a ^b	7.7 a	20.0 a	53.1 a	1000 a	28.1 ab
Control HP (CHP)	89 ab	8.2 ab	21.6 ab	56.1 a	1020 a	27.9 ab
<i>M. javanica</i> LP (Mj LP)	77 a	7.2 a	20.2 ab	53.6 a	940 a	24.6 a
<i>M. javanica</i> HP (Mj HP)	106 b	9.7 b	23.3 b	60.1 ab	1130 a	33.4 b
<i>G. intraradices</i> LP (Gi LP)	144 c	13.5 c	28.1 c	67.7 c	1470 b	45.3 c
<i>G. intraradices</i> + <i>M. javanica</i> LP (Gi + Mj LP)	42 c	13.1 c	29.5 c	66.5 bc	1490 b	45.0 c

^aLP = low phosphorus fertilization (0.10 g KH₂PO₄ L⁻¹); HP = high phosphorus fertilization (0.30 g KH₂PO₄ L⁻¹).

^bData are means of 10 replications. Means in the same columns followed by the same letter do not differ according to Tukey's multiple range test (P \leq 0.05).

Table 2. Mineral constituents of composite leaf samples from 'Grand Naine' banana inoculated with *Meloidogyne javanica* and *Glomus intraradices* at 7 and 5 months after vesicular-arbuscular mycorrhizal fungus and nematode inoculations, respectively.

Treatment	Mineral constituents (% dry wt basis)				
	N	P	K	Ca	Mg
Control LP ^a (CLP)	2.72 a	0.22 ab	5.59 b	1.05 a	0.41 a
Control HP (CHP)	2.70 a	0.24 b	6.13 bc	1.06 a	0.39 a
<i>M. javanica</i> LP (Mj LP)	2.60 a	0.21 ab	6.45 c	1.08 a	0.43 a
<i>M. javanica</i> HP (Mj HP)	2.73 a	0.25 b	5.80 bc	1.14 a	0.44 a
<i>G. intraradices</i> LP (Gi LP)	2.54 a	0.20 a	4.80 a	1.40 b	0.73 b
<i>G. intraradices</i> + <i>M. javanica</i> LP (Gi + Mj LP)	2.69 a	0.20 a	4.50 a	1.61 c	0.74 b
* Interpretative values for foliar analysis in 6- to 9-month-old banana ^b					
Low level	2.5-3.4	0.15-0.19	3.0-3.7	0.5-0.79	0.2-0.24
Sufficiency level	3.5-4.5	0.20-0.40	3.8-5.0	0.8-1.5	0.25-0.50
High level	>4.5	>0.40	>5.0	>1.5	>0.5

^aLP: low phosphorus fertilization (0.10 g KH₂PO₄ L⁻¹); HP: high phosphorus fertilization (0.30 g KH₂PO₄ L⁻¹).

^bInterpretative values for element content for banana by foliar analysis (Jones et al., 1991).

mycorrhizal treatments in comparison to high P treatments may be related to faster growth rates in plants inoculated with the endophyte, thus reducing P concentration due to increased growth (Turner and Barkus, 1981). Similarly, K levels were lower in plants of both mycorrhizal treatments (but within sufficiency levels) and also may have been induced by the same growth dilution effect. Potassium is a key element in banana nutrition where uptake rates are proportionally greater in early vegetative phase (Twyford and Walmsley, 1973).

Mycorrhiza mainly affect the uptake of P (Gerdemann 1968), and several minor elements that are associated to P nutrition (Jones et al., 1991). The growth response observed in mycorrhizal plants in our study seems to implicate other elements besides P. Mycorrhiza-assisted nutrition of Ca and Mg is reflected in the higher values for these microelements in *G. intraradices*-inoculated plants. These higher concentrations indicate that the absorption and transport of these elements were enhanced and not affected by *M. javanica* parasitism and that the plant was capable of maintaining normal levels for Ca and Mg in spite of the nematode reproduction in the roots. Uptake of microelements that were not determined could have also been involved in promoting plant development of mycorrhizal plants.

The percentage of root length colonized by *G. intraradices* on 'Grand Naine' was relatively low (27% in plants with *G. intraradices* alone and 37% on bananas in joint inoculations), although plant growth response to both mycorrhizal treatments was significantly better than to nonmycorrhizal treatments for all growth characteristics. This would suggest that *G. intraradices* is a highly effective symbiont in this mycorrhiza-host association. Besides improving nutrition, *G. intraradices* may have altered the development of root morphology or accelerated plant growth rates accounting for additional plant development (Abbot and Robson, 1984).

Our findings show that early mycorrhizal inoculation of micropropagated banana plantlets is highly beneficial for plant growth and more effective than fertilizing with a nutrient solution high in P. The strong growth response observed with *G. intraradices* has also been reported for several species of endomycorrhizal fungi on micropropagated banana plantlets (Jaizme-Vega and Azcón, 1995; Lin and Chang, 1987; Rizzardi, 1990).

The association between the banana plantlet and the VAM fungus *G. intraradices* also increases host tolerance to *M. javanica*, compensating for the damage caused by the nematode, mainly by enhancing plant nutrition. In the Canary Islands, and in other banana-growing areas of the Mediterranean where chemical control of root-knot nematodes is not

always economically feasible (Vovlas et al., 1993), early mycorrhizal inoculation might represent a new nematode management approach that would be fairly easy to implement at the nursery if adequate amounts of mycorrhizal inocula are prepared for large-scale inoculation during or just after the hardening phase (post vitro), and 2 to 4 months before to final transplant into the field.

Literature Cited

- Abbot, L.K. and A.D. Robson. 1984. The effect of mycorrhizae on plant growth, p. 113-130. In: C.L. Powell and D.J. Bagyaraj (eds.). VA mycorrhiza. CRC Press, Boca Raton, Fla.
- Barker, K.R. 1985. Nematode extraction and bioassays, p. 19-39. In: K.R. Barker, C.C. Carter, and J.N. Sasser (eds.). An advance treatise on *Meloidogyne*. vol. II. Methodology. North Carolina State Univ. Graphics, Raleigh, N.C.
- Cenis, J.L. 1993. Identification of four major *Meloidogyne* spp. by Random Amplified Polymorphic DNA (RAPD-PCR). *Phytopathology* 83:76-78.
- Cooper, K.M. and G.S. Grandison. 1987. Effects of vesicular-arbuscular mycorrhizal fungi on infection of tamarillo (*Cyphomandra betacea*) by *Meloidogyne incognita* in fumigated soil. *Plant Dis.* 71:1101-1106.
- Gerdemann, J.W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* 6:396-418.
- Giovanetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84:489-500.
- Gowen, S. and P. Quénehervé. 1990. Nematode parasites of bananas, plantains and abaca, p. 431-460. In: M. Luc, R.A. Sikora, and J. Bridge (eds.). Plant parasitic nematodes in subtropical and tropical agriculture. CAB Intl. Inst. of Parasitology, Wallingford, U.K.
- Heald, C.M., B.D. Bruton, and R.M. Davis. 1989. Influence of *Glomus intraradices*, and soil phosphorus on *Meloidogyne incognita* infecting *Cucumis melo*. *J. Nematol.* 21:69-73.
- Hoagland, D. and D.I. Arnon. 1950. The water culture method for growing plants without soil. *California Agr. Expt. Sta. Circ.* 347. Univ. of California, Berkeley.
- Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Rptr.* 57:1025-1028.
- Hussey, R.S. and R.W. Roncadori. 1982. Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Dis.* 66:9-14.
- Jaizme, M. and R. Azcón. 1995. Response of some tropical and subtropical cultures to endomycorrhizal fungi. *Mycorrhiza* 5:213-217.
- Jenkins, W.R. 1964. A rapid centrifugal floatation technique for separating nematodes from soil. *Plant Dis. Rptr.* 48:692.
- Jones, J.B., W. Benjamin, and H.A. Mills. 1991. Plant analysis handbook. 1. Methods of plant analysis and interpretation. Micro-Macro Publishing, Athens, Ga.
- Koske, R. E. and J.H. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Res.* 92:486-505.
- Lahav, E. and D.W. Turner. 1983. Fertilizing for high yield banana. IPI Bul. 7. Intl. Potash Inst., Berne, Switzerland.
- Lin, C.H. and D.C.N. Chang. 1987. Effects of three *Glomus* endomycorrhizal fungi on the growth of micropropagated banana plantlets. *Transactions Mycol. Soc. Rep. China.* 2:37-45.
- Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions Brit. Mycol. Soc.* 55:158-161.
- Pinochet, J. 1977. Occurrence and spatial distribution of root-knot nematodes on bananas and plantains in Honduras. *Plant Dis. Rptr.* 62:518-520.
- Rizzardi, V. 1990. Effects of inoculation with vesicular-arbuscular-mycorrhizal fungi on the growth of micropropagated *Musa acuminata* clone 'Grand Naine'. *Revista di Agricoltura Subtropicale e Tropical* 84:473-484.
- Rodríguez, R. 1990. Los nematodos de la platanera (*Musa acuminata* AAA, sub grupo Cavendish Enana) en Canarias (1963-1984). *Caja Insular de Ahorros de Canarias, Las Palmas de Gran Canaria.*
- Roncadori, R.W. and R.S. Hussey. 1977. Interaction of the endomycorrhizal fungus *Gigaspora margarita* and root-knot nematode on cotton. *Phytopathology* 67:1507-1511.
- Rund, R.C. 1984. Fertilizers. In: S. Williams (ed.). Official methods of analysis of the Association of Official Analytic Chemists. Fourteenth edition. AAOAC, Arlington, Va. p. 8-37.
- Sikora, R.A. and E. Schlosser. 1973. Nematodes and fungi associated with root systems of bananas in a state of decline in Lebanon. *Plant Dis. Rptr.* 57:615-618.
- Smith, G.S. 1987. Interactions of nematodes with mycorrhizal fungi, p. 292-300. In: J.A. Veech and D.W. Dickson (eds.). *Vistas on nematology.* Soc. Nematol., Hyattsville, Md.
- Stroebel, N.E., R.H. Hussey, and R.W. Roncadori. 1982. Interactions of vesicular-arbuscular mycorrhizal fungi, *Meloidogyne incognita*, and soil fertility on peach. *Phytopathology* 72:690-694.
- Turner, D.W. and B. Barkus. 1981. Some factors affecting the apparent root transfer coefficient of banana plants (cv. Williams). *Fruits* 36:607-613.
- Twyford, I.T. and D. Walmsley. 1974. The mineral composition of the Robusta banana plant. 1. Methods and plant growth studies. *Plant and Soil* 39:227-243.
- Vovlas, N., A. Avgelis, D. Goumas, and S. Frisullo. 1992. Banana diseases in sucker propagated plantations in Crete, Greece. *FAO Plant Protection Bul.*
- Vovlas, N., M. Di Vito, and G. Grammatikaki. 1993. Growth response of *in vitro* produced banana plantlets to *Meloidogyne javanica* in pots. *Nematropica* 23:203-208.
- Walmsley, D., T.I. Twyford, and I.S. Cornforth. 1971. An evaluation of soil analysis methods for nitrogen, phosphorus and potassium using banana. *Tropical Agr.* 48:141-155.