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Responses of some tropical and subtropical cultures to endomycorrhizal fungi

Abstract This paper reports the effects under greenhouse and field conditions of four arbuscular-mycorrhizal (AM) fungi on the growth and nutrition of some tropical and subtropical crops of economic importance in the Canary Islands (Spain) whose mycorrhizal dependency was unknown. Avocado (*Persea americana* Mill.), papaya (*Carica papaya* L.), pineapple (*Ananas comosus* (Merr.) L.) and banana (*Musa acuminata* Colla) were highly responsive to the mycorrhizal condition when the AM inoculum was *Glomus* sp. endophytes. Under our experimental conditions, *Glomus fasciculatum* was the most effective fungus at improving plantlet growth and nutrition. *Acaulospora* sp. was ineffective and *Scutellospora* was only effective with banana. The data clearly show the advantage of mycorrhization during the first phase of development of the plant system.

Key words Arbuscular mycorrhiza · Avocado Banana · Papaya · Pineapple

Introduction

Arbuscular mycorrhiza is the most common type of mycorrhizal association and it is formed by nearly all plants of agricultural importance. The symbiotic status is very important for plant mineral nutrition and plant health, but the degree of mycorrhizal dependence differs with the plant species (Hayman 1986; Nemec 1986).

The effects of arbuscular-mycorrhizal (AM) fungi result from an increased phosphate uptake by the host plant, but the benefits derived from the root colonization differ between plant species (Gerdemann 1975). Observations have shown that a wide range of plants depend on mycorrhizae for maximal growth in soil at a given level of fertility. The different capacities of plants to absorb P from low-P soils can be the major factor involved in the mycorrhizal interdependency relationship (Baylis 1975; Hall 1975, 1978). The connections between AM fungi and host plant are nonspecific and this interaction is highly compatible at both the structural and physiological levels. Efficient associations are those which successfully cope with physiological and biochemical processes (Smith and Gianinazzi-Pearson 1988). Since variation occurs in the symbiotic response, measured in terms of plant growth and nutrition, selection of efficient soil fungal isolates is required. Plant-fungal compatibility seems to be related to plant, fungal and environmental factors, as recently reviewed by Koide and Schreiner (1992).

Work on the application of AM fungi in tropical and subtropical crops of ecological and economic importance for the Canary Islands, such as avocado, pineapple, papaya, and banana, is limited. The interest in determining the mycotrophic behaviour of these plant species arose from the morphological characteristics of the root systems: relatively unbranched, thin and, in general, sparse. The most efficient moment for the application of mycorrhizal fungi is during the first developmental phase of these plants.

The normal techniques for the multiplication of papaya and avocado in our conditions are based on germination of their seeds. In the case of the pineapple, vegetative multiplication is normally achieved through sucker production, a characteristic of this species, although multiplication is also possible using in vitro culture. Finally, although banana is traditionally multi-

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plied by vegetative reproduction in the orchard, micropropagation of this species is increasingly used.

The aims of the present research were (1) to determine the advantages obtained from inoculation of different endophytes to the above-mentioned tropical and subtropical species during the first phases of development under controlled conditions using sterile substrate, (2) to select the most effective mycorrhizal fungus for improving plant growth and development, and (3) to verify continuing symbiosis under standard nursery conditions.

Materials and methods

The plant material used in these experiments belonged to the following species and cultivars: avocado (*Persea americana* Mill.), West Indian rootstock; papaya (*Carica papaya* L.) cv. Sunrise; pineapple [*Ananas comosus* (Merr.) L.] cv. Smooth Cayenne; banana (*Musa acuminata* Colla AAA subgroup Cavendish) cv. Johnson II, a local variety from the Centro de Investigación y Tecnología Agrarias (CITA) collection.

The avocado and papaya plants were obtained by germination in sterile conditions of their respective seeds, using a quartz sand substrate for the avocado and a mixture of soil and peat (1/1, v/v) for the papaya. The pineapple and banana plants were produced from in vitro culture systems in the CITA Ornamental Plants Department.

The soil used in the experiments was collected from Granada, southeast Spain, where the crops are cultivated. It was calcareous with a silty-loam texture, pH 8, 2.5% organic matter, 26.7 ppm P_2O_5 (Olsen), 0.15% N and 34.24 mg/100 g K. The soil was sieved (5 mm), diluted with quartz sand (5/2, v/v), and steam sterilized (100° C for 1 h on 3 consecutive days). After transplantation, every pot was reinoculated with a soil filtrate containing its own microbial population (30 ml/pot), except propagules of the Endogonaceae, which were retained on the filter paper (Whatman No. 1).

The mycorrhizae-forming fungi used were from the Microbiology Department of the Consejo Superior de Investigaciones Cientificas (CSIC) at Granada, and the plant species were inoculated as follows: Avocado, *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe; *G. fasciculatum* (Thaxter) Gerd. and Trappe; *Scutellospora heterogama* (Nicol. and Gerd.) Walker and Sanders, and *Acaulospora laevis* Gerd. and Trappe. In pineapple the same species of fungi were inoculated as in avocado, except *S. heterogama*. *Acaulospora laevis* was not inoculated in the case of the banana plants, and in papaya only *G. mosseae* and *G. fasciculatum* were inoculated. All the endophytes were previously multiplied under greenhouse conditions on alfalfa roots (*Medicago sativa* L. cv. Aragon).

The avocado and papaya plants were inoculated and transferred to pots after the seed bed phase. Micropropagated pineapple and banana were inoculated in the post-vitro phase. The pots used in all the treatments were 2 l capacity. The mycorrhizal inoculum inoculum consisted of rhizosphere soil with spores and mycelium and mycorrhizal roots fragments of alfalfa infected with the different AM fungi studied. Thoroughly homogenized inoculum (5 g/pot) was applied to the planting hole.

The plants were grown in controlled environmental conditions at a temperature of 19–25° C and 70–90% relative humidity. During the experiment, the pots were watered from below and fertilized every 2 weeks using Hewitt's nutrient solution without phosphate (Hewitt 1952).

Six months after the start of the experiment, to attempt to establish the conditions nearest to those normal for plant production, plants were transplanted to pots of a larger capacity (3 l) which contained the same test soil used in the earlier phase but nonsterilized. The natural soil possesses a large population of native fungi in which *Glomus* spp. was the dominant species. The trial was terminated 5 months later, at which time a series of parameters was measured for each of the studied hosts: fresh weight, dry weight and nutrient content of aerial parts, fresh weight of the root, plant height, stem diameter, and the number of suckers.

The degree of internal colonization produced by each AM endophyte in the roots of the plants was rated by observation with a light microscope. The roots were clarified and stained according to the method of Phillips and Hayman (1970). The extent of colonization was estimated in terms of percentage of mycorrhizal root length. After segmenting the roots into approximately 1-cm pieces, they were placed on a slide for microscopic observation (Brundrett et al. 1985). In all experiments, five replications were set up for each host and for each AM fungus studied, as well as five uninoculated controls.

The summarized data in Tables 1–4 represent the means of the data obtained in different experiments. The data were statistically analyzed using analysis of variance and by Duncan's multiple range test ($P \leqslant 0.0.5$).

Results

The effects of inoculation with AM fungi on the plants studied were estimated at the end of two phases of growth under different conditions in the presence of populations of different endophytes. All the species

Table 1 Dry weights, nutrient contents and mycorrhizal root colonization of avocado inoculated with four different arbuscular mycorrhizal (AM) fungi. Mean values without a letter in common differ significantly ($P \leq 0.05$) according to Duncan's multiple range test

Fungus	Shoot dry wt. (g)	Nutrient content (mg/plant) N	P	K	Colonization (%)
Control	21.8 b	357.9 a	30.2 a	143.4 b	0.0
Glomus mosseae	29.7 a b	328.9 a	23.7 a	141.8 b	21.1
Glomus fasciculatum	38.5 a	454.9 a	41.0 a	315.9 a	53.1
Scutellospora heterogama	20.2 b	340.5 a	28.5 a	157.1 b	—
Acaulospora laevis	25.2 b	326.9 a	21.1 a	150.5 b	—

Table 2 Dry weights, nutrient contents and mycorrhizal root colonization of pineapple inoculated with three different AM fungi. Mean values without a letter in common differ significantly ($P \leq 0.05$) according to Duncan's multiple range test

Fungus	Shoot dry wt. (g)	Nutrient content (mg/plant) N	P	K	Colonization (%)
Control	6.7 b	74.0 b	12.2 b	245.3 b	59.5
Glomus mosseae	21.6 a	235.2 a	25.1 a b	727.5 a	30.0
Glomus fasciculatum	19.7 a	180.2 a	37.5 a	780.0 a	65.7
Acaulospora laevis	11.6 b	96.3 b	13.4 b	435.5 b	15.5

Table 3 Dry weights, nutrient contents and mycorrhizal root colonization of banana inoculated with three different AM fungi. Mean values without a letter in common differ significantly ($P \leq 0.05$) according to Duncan's multiple range test

Fungus	Shoot dry wt. (g)	Nutrient content (mg/plant) N	P	K	Colonization (%)
Control	3.9 b	49.8 b	5.8 b	85.6 c	24.0
Glomus mosseae	8.4 a	100.5 b	14.9 a b	268.7 a b	27.5
Glomus fasciculatum	10.1 a	170.2 a	18.9 a	369.7 a	38.0
Scutellospora heterogama	5.6 a	80.2 b	9.4 a b	172.2 b c	10.0

Table 4 Dry weights, nutrient contents and mycorrhizal root colonization of papaya inoculated with two different AM fungi. Mean values without a letter in common differ significantly ($P \leq 0.05$) according to Duncan's multiple range test

Fungus	Shoot dry wt. (g)	Nutrient content (mg/plant) N	P	K	Colonization (%)
Control	3.47 b	41.5 a	5.5 b	57.1 b	43.5
Glomus mosseae	6.43 a	53.3 a	8.5 a	99.5 a	61.0
Glomus fasciculatum	5.77 a	43.1 a	7.8 a	95.9 a	58.0

studied benefitted from symbiosis with the AM fungi, although to different degrees.

Avocado

Glomus fasciculatum was the most effective and infective endophyte for avocado, increasing the growth to 176% that of the control (Table 1). The lack of root colonization produced by the native endophytes present in the transplant soil was surprising, considering the relatively high percentage caused by *G. fasciculatum* (53%). Avocado developed a highly functional compatibility with this fungus.

The higher root infection level noted under these experimental conditions indicated increased compatibility with this fungus. However, the N and P nutrition of plants colonized by *G. fasciculatum* did not significantly differ from that of control plants. The K content of the plants colonized by *G. fasciculatum* showed a notable increment compared with the control treatment.

Pineapple

In pineapple (Table 2), mycorrhizal colonization by *G. mosseae* or *G. fasciculatum* was highly effective, improving both growth (322% and 294%, respectively) and nutrient content. The root length of plants colonized by native endophytes reached 59.5%, which was double that of *G. mosseae* and similar to that found with *G. fasciculatum*. Interactions between native and introduced fungi were observed.

Banana

In banana (Table 3), all endophytes studied produced improvements in plant development and nutritional state. Particularly significant was the increment produced by inoculation with *G. fasciculatum* (259% on shoot biomass and 324%, 326% and 463% on N, P and K contents, respectively). Although the indigenous endophyte infection was acceptable (24%), it was low compared with the other treatments.

Papaya

With papaya, only *G. mosseae* and *G. fasciculatum* were used for the studies of mycorrhizal effectivity. Both fungi improved the growth and nutrition of papaya when compared to native endophytes (Table 4). AM infection by introduced endophytes increased that of native ones.

Discussion

It is evident that inoculation with mycorrhizal fungi stimulates the growth and nutrition of avocado, papaya, pineapple, and banana in the studied growth phases, i.e. the results indicate the mycotrophic conditions of these tropical and subtropical plant species. It is well known that mycorrhizal fungi improve P transport from the soil solution to cortical root cells (Mosse 1973), and the effects of arbuscular mycorrhizae on plants are the result of this ability to increase P uptake by roots. According to Gerdemann (1975), the benefits derived from AM infection differ among plant species. The ability of plants to absorb P from low-P soils, root morphology and development, and plant growth rates appear to be the major factors involved (Baylis 1970; Mosse 1973; Hall 1975).

Although the advantages resulting from inoculation with specific AM fungal isolates are not the same for each plant species, in every case the inoculation is of benefit to the plant, and this is useful from an agronomic point of view. Recently, Habte and Manjunath (1991) established experimentally five distinct mycorrhizal dependency categories.

The crops which benefitted most from mycorrhization were pineapple and banana, followed by papaya. Avocado showed the least capacity to benefit from mycorrhizal symbiosis in the trial conditions. Although variation was apparent in the effectiveness and infectiveness of the different endophytes inoculated onto each of the hosts, in general *G. fasciculatum* and *G.*

mosseae were the most effective fungi among those tested for these host plants. This situation has already been described in other studies of the mycorrhiza-plant relationship, expressed in the concept of "functional compatibility" (Gianinazzi-Pearson 1984) and in the absence of any correlation between the parameters infectivity and effectivity (Sieverding 1991).

The native fungi in general show a high capacity for colonization of host roots but poor efficiency; an exception was the avocado, where the roots were not colonized by the native endophytes at the time of transplantation. Relative comparisons of effectiveness and infectivity of inoculated versus indigenous endophytes cannot be made, since mycorrhizal development differed by several weeks between the control and inoculated treatments. This circumstance can be explained by a low susceptibility of the plants to mycorrhization at this stage of development.

These results support those from the few trials already undertaken. For the avocado, Menge et al. (1978) and Dharmaraj and Irulappan (1982) observed that inoculation with *G. fasciculatum* produced an improvement in the development of this species. In the case of papaya, the data obtained in the present study revealed a significant benefit from inoculation with AM-producing fungi at the time of transplanting and during the first phase of development. Ramirez et al. (1975) observed that the effects of different species of *Gigaspora* on growth are positive only when fertilizers are applied. This seems to confirm the dependence of this crop on mycorrhizae. Casagrande et al. (1986) evaluated the effects of various AM fungi on the development of this host and determined that a certain level of dependence does exist. This fact was confirmed in the present study, despite the fact that our control plants were also mycorrhizal.

In the case of pineapple, the results obtained are especially convincing since the data describes both vegetative development and the nutritional content of the plants, and confirm the increased efficiency of inoculation compared with natural mycorrhization. Inoculation in this case must be carried out during the first phase of the development of plantlets in the post vitro stage. Recently Guillemin et al. (1991) also described positive effects of two species of *Glomus* on the development of pineapple produced in vitro. Thus, it is possible to conclude that pineapple obtains a greater benefit from mycorrhizae than avocado, with all three endophytes studied, in spite of the high degree of mycorrhizal colonization by indigenous AM endophytes of the pineapple controls. In previous studies, we showed that the high level of dependence of this crop is due to symbiosis (Jaizme-Vega and Azcón 1991).

In banana, inoculation with AM fungi also showed a positive growth response, with consequent nutritional improvement. There was a considerable benefit to the plant from the mycorrhization in this early developmental phase. Other investigators have arrived at a similar conclusion; for example, Lin and Fox (1987), Lin and Chang (1987), and Knight (1988), in their studies on the mycorrhization of this host under controlled conditions, showed a positive effect of the AM fungi on the P and N concentrations in the plant and an improvement of vegetative development. The nature of the AM endophytes was determinant for the effect, i.e. native versus the AM fungi inoculated.

In conclusion, our results provide evidence that growth of the tropical crops studied improved when they were inoculated with selected AM fungi during the first phase of growth. In spite of the high capacity for colonization shown by the native endophytes, these had a low effectivity. These data indicate that mycorrhization and fungal selection at the first phase of development of the plant production system confer the greatest benefit on these plant species.

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