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Mycorrhizal fungi enhancement of growth and gas exchange of micropropagated guava plantlets (*Psidium guajava* L.) during *ex vitro* acclimatization and plant establishment

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Abstract The effect of mycorrhizal fungi on growth, nutrient uptake and gas exchange of micropropagated guava (*Psidium guajava* L.) plantlets was determined during acclimatization and plant establishment. Guava plantlets were asexually propagated through tissue culture and grown in a glasshouse for 18 weeks. Half of the plantlets were inoculated with a mixed endomycorrhiza isolate from Mexico, ZAC-19, containing *Glomus diaphanum, G. albidum* and *G. claroides*. Plantlets were fertilized with modified Long Ashton nutrient solution that supplied 11 μg P ml^{-1}. Gas exchange measurements were taken at 2, 4, 8, and 18 weeks after inoculation using a portable photosynthesis system. All micropropagated guava plantlets survived transplant shock. After 6 weeks, mycorrhizal plantlets had greater shoot growth rates and leaf production than non-mycorrhizal plantlets. This also corresponded with increased photosynthetic rates and stomatal conductance of mycorrhizal plants. By 18 weeks, mycorrhizal plantlets had greater shoot length, leaf area, leaf, stem, and root dry mass. However, gas exchange was comparable among treatments, in part because the container size was restricting growth of the larger mycorrhizal plantlets. Non-mycorrhizal plantlets had greater leaf area ratios and specific leaf areas than mycorrhizal plantlets. Increased leaf tissue mineral levels of P, Mg, Cu, and Mo also occurred with mycorrhizal plantlets. Roots of inoculated guava plantlets were heavily colonized with arbuscules, vesicles and endospores. Guava plantlets were highly mycotrophic with a mycorrhizal dependency index of 103%.

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Introduction

Guava is cultivated worldwide in every country with tropical and subtropical climates. It has become an important fruit tree by virtue of its commercial and nutritional value, including high levels of vitamin C (Samson 1986). The species is native to tropical America from Mexico to Peru. Major world producers are India, Mexico and Pakistan (Adsule and Kadam 1995).

Guava plants can be propagated by sexual or asexual methods. Seeds have traditionally been used to multiply plants, but natural cross-pollination is common and considerable seedling variability occurs. Vegetative propagation is obtained by rooting stem and root cuttings, and suckers developed from the roots near the base of the trunk, air layering, grafting, and budding onto seedling rootstock (Menzel 1985; Mata and Rodríguez 1990; Jaiswal and Amin 1992). Micropropagation protocols have advantages over traditional clonal propagation methods, including high multiplication rates and production of disease-free plants (Amin and Jaiswal 1988; Ghaffoor and Alderson 1994; Gowda and Muralikrishna 1994; Sidiqqui and Farooq 1995). However, limited information is available on the performance of guava plantlets after micropropagation. Slow growth rates and up to 30% plantlet losses can occur during transplanting and acclimatization of guava (Amin and Jaiswal 1988; Loh and Rao 1989; Mohamed-Yasseen et al. 1995).

Recently, early mycorrhizal inoculation and colonization of tissue-cultured plantlets of other plant species has been reported to reduce transplant shock during acclimatization, thus increasing plant survival and establishment rates. Micropropagated plantlets that were inoculated with mycorrhiza had enhanced plant quality, nutrient uptake, and plant growth (Azcón-

Aguilar et al. 1994; Lovato et al. 1994a, b; Rapparini et al. 1994). Mycorrhiza enhancement of rooting and root function have been reported in several species as well as increased pest resistance, enhanced crop production, and tolerance to environmental stress (Guillemin et al. 1994; Sbrana et al. 1994; Uosukkainen and Vestberg 1994; Wen and Chang 1994). The benefits of mycorrhiza for micropropagated plantlets have been reported in high value crops such as grapes, oil palm, apple, plum, pineapple, avocado, strawberry, raspberry, cherry, ash, pear, *Hortensia* spp, and Rhododendron (Varma and Schüepp 1995; Fortuna et al. 1996; Lovato et al. 1996; Azcón-Aguilar and Barea 1997).

Little is known, however, about the effect of mycorrhizal fungi on gas exchange of plantlets (Lovato et al. 1996). Erratic stomatal control and low photosynthetic rates are important problems encountered during transplanting and acclimatization of micropropagated plantlets (Kozai 1991; Preece and Sutter 1991; Santamaria et al. 1993; Varma and Schüepp 1995; Hartmann et al. 1997). The effect of mycorrhiza on the growth, development and gas exchange of guava plantlets after micropropagation has not been reported. The objective of this research was to determine whether vesicular arbuscular mycorrhiza could enhance transplanting success and plantlet acclimatization as indicated by gas exchange, plant growth, and nutrient uptake.

Materials and methods

Explant material and micropropagation

Guava plantlets (*Psidium guajava* L.) cv. Media China were micropropagated following the procedure of Mohamed-Yasseen et al. (1995). Seeds from physiologically mature fruits were harvested and cleaned with running tap water and detergent for 1 h. Under aseptic conditions, seeds were surface disinfected with 70% ethanol for 5 min. Seeds were then placed in a bleach solution (6% $NaClO{\cdot}5H_2O$) plus Tween-20 (0.2%) for 20 min, and rinsed 5 times with sterilized distilled water. Seeds were aseptically germinated in a Murashige and Skoog (1962) basal medium supplemented with sucrose (3%) and Difco-Bacto agar (0.6%) at a pH of 5.7. After germination, micro-shoots of the seedlings were dissected and transferred to a shoot-proliferation medium containing 6-benzyl aminopurine (4.4 μmol). Regenerated shoots were later transferred to a rooting medium supplemented with indole-3-butyric acid (9.8 μmM). *In vitro* cultures were grown in a laboratory room with 100 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) (Phillips, cool white slimline bulbs, 215 W) at plant level and a photoperiod of 16 h. Temperature was maintained at 29 ± 2 °C.

Plant acclimatization and culture conditions

Uniform plantlets were selected from *in vitro* cultures and washed with tap water to remove remnants of agar from the root system. Plantlets were transplanted into Jiffy-7 pellets (Hummert Intern., Earth City, Miss.), covered with plastic cups and grown for 8 days under the same light and temperature conditions provided during micropropagation. Afterwards, plantlets were transferred to a glasshouse and placed on a bench with an intermittent mist system for 6 days. Glasshouse conditions were a maximum PPFD of 1100 μmol m^{-2} s^{-1} at plant level, with an average day/night temperature of 35/30 °C. Plantlets were retransplanted into individual plastic pots (7.5 × 8.5 × 5.0 cm) containing a sterilized sand: mineral soil mixture (1 : 1 v/v) with low P fertility (1.7 μg g^{-1} bicarbonate extractable P). At this step, half of the plantlets were inoculated by banding 200 g of a pasteurized sand: mineral soil carrier containing root fragments, hyphae and approximately 4,000 spores of a ZAC-19 mycorrhiza isolate collected in Zacatecas, Mexico. The isolate contained *Glomus diaphanum* (Morton and Walker), *G. albidum* (Walker and Rhodes) and *G. claroides* (Schenck and Smith). ZAC-19 was collected from a non-irrigated, low nutrient and low soil organic matter soil used for commercial bean production in the State of Zacatecas, Mexico, with an annual precipitation of 450 mm. The field soil was sandy-loam with a pH of 5.4, 16 ppm P and 1.1% organic matter (Contreras and Ferrera-Cerrato 1989). Plantlets were fertilized twice weekly with 100 ml of Long Ashton nutrient solution, LANS (Hewitt 1966) with pH adjusted to 5.6 and modified to supply 11 μg P ml^{-1}. Plantlets were irrigated with water as needed.

Plant growth measurements

Total shoot length (cm) and number of leaves were monitored every 2 weeks following *ex vitro* transplanting and mycorrhizal inoculation. After 18 weeks of growth in pots, plants were harvested and evaluated for leaf area, leaf, shoot, root and total plant dry mass. Leaf area ratio [LAR = leaf area (cm^2)/plant dry mass (g)], specific leaf area [SLA = leaf area (cm^2)/leaf dry mass (g)], and root to shoot ratio were also determined. Relative growth rate (RGR) of shoots was also calculated by using the formula: $RGR = ln\ TSL_2 - ln\ TSL_1 / t_2 - t_1$, where ln = natural logarithm, TSL_2 = total shoot length at final measurement, TSL_1 = total shoot length at initial measurement, $t_2 - t_1$ = days between measurements.

The mycorrhizal plant dependency index (RMDI) was estimated according to the formula: $RMDI = 100\ (VAM\ dm - non\text{-}VAM\ dm)/(non\text{-}VAM\ dm)$, (Plenchete et al. 1983), where $VAM\ dm$ = dry mass of mycorrhizal plantlets, $non\text{-}VAM\ dm$ = dry mass of non-mycorrhizal plantlets.

Gas exchange measurements

Gas exchange measurements were made 2, 4, 8, and 18 weeks after inoculation with mycorrhiza to monitor the physiological response of the guava plantlets during acclimatization and plant establishment. Measurements were made between 9 : 00 and 12 : 00 on the first pair of fully expanded leaves from the shoot apex of 6 plantlets per treatment ($n = 12$). Before measurements, the plantlets were acclimatized for a 20-min period under a 1 000-W metal halide lamp (PPFD was 1 000 μmol m^{-2} s^{-1} at plant height), filtered through a 5-cm non-circulating water bath enclosed in a Plexiglas box. Gas exchange was measured using a LI-6200 Portable Photosynthesis System (LI-COR Inc., Lincoln, Neb.). Gas exchange measurements included net photosynthesis (A) and stomatal conductance (g_s).

Leaf elemental analysis

Mineral status of the plants was obtained by conducting leaf tissue elemental analysis on an inductively coupled plasma atomic emission spectrophotometer (Fison Horticulture Inc., Warik, N.Y.) using 8 plants per treatment ($n = 8$). Non-senescent leaves were harvested.

Stomata density assessment

Stomatal density was determined by counting the number of stomata per unit of leaf area on leaf replicas made with a colorless cellulose acetate (commercial clear nail polish) (Sampson 1961) on abaxial leaf surfaces. To determine differences in stomatal frequency, samples were taken from acclimatized and non-acclimatized leaves, and leaves of different chronological age [leaves that developed during the *in vitro* conditions and leaves that developed *ex vitro* (during the acclimatization stage)] ($n = 25$). Leaf replicas were mounted on glass slides and observed under a light microscope (Nikon Alphaphot YS) at a magnification of $\times 400$.

Determination of mycorrhizal colonization

Subsamples of non-suberized roots were collected, cleared with 2.5% KOH, and stained with 0.05% trypan blue (Koske and Gemma 1989). Roots were then divided into 1-cm pieces and mounted lengthwise on a microscope slide. Fifteen slides per treatment containing 10 root pieces per slide were examined by making 3 microscopic observations (top, middle, and bottom) per 1-cm root piece at $\times 400$ magnification ($n = 450$). The total mycorrhizal colonization, arbuscules, vesicles, spores, internal hyphae in the root cortex, and entry points were recorded.

Experimental design, treatments, and statistical data analysis

The experiment was arranged in a completely randomized design. Treatments included the presence and absence of mycorrhiza (colonized and non-inoculated plantlets). Each plantlet was a single replicate with 23 replicates per treatment. All data were analyzed using analysis of variance (ANOVA) (SAS Institute Inc., Cary, N.C. 1995) and a *T*-test ($\alpha = 0.05\%$).

Results

Between 2 and 4 weeks after transplanting, there was an increase in number of stomata from 103 to 146 per mm^2 of both mycorrhizal and non-mycorrhizal guava plantlets during acclimatization. The number of stomata per mm^2 of acclimatized plantlets 4 weeks after transplanting was similar to non-micropropagated, greenhouse-grown plants. Through week 4, cumulative shoot growth and leaf production was low and comparable between mycorrhiza treatments (Fig. 1). However, by week 6, mycorrhizal guava had greater shoot growth and leaf production than non-mycorrhizal plantlets. Mycorrhizal plantlets were well colonized by the ZAC-19 isolate by week 6 (data not reported).

Table 1 Effects of mycorrhiza on relative growth rate (RGR cm day^{-1}) of shoots of micropropagated guava plantlets (*Psidium guajava* L.) during acclimatization and plant establishment. Means in each column followed by different letters are significantly different ($P \leq 0.05$) as indicated by *T*-test; $n = 23$

Mycorrhiza	RGR (weeks after inoculation)				
	6	8	10	12	18
Yes	0.026 a	0.031 a	0.038 a	0.034 a	0.018 b
No	0.014 b	0.024 b	0.024 b	0.027 b	0.028 a

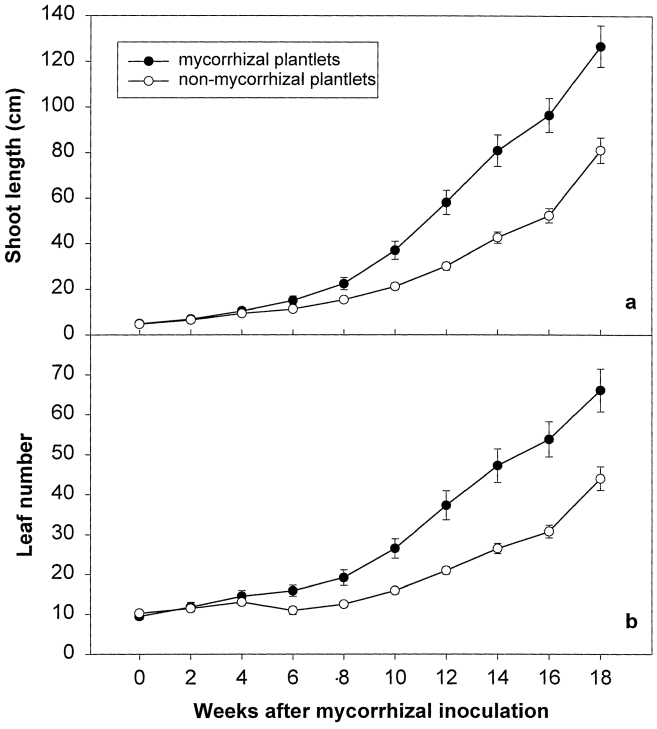

Fig. 1 Effects of mycorrhiza on shoot length (a) and leaf number (b) of micropropagated guava plantlets (*Psidium guajava* L.) during acclimatization and plant establishment 18 weeks after inoculation; $n = 23 \pm \text{SE}$

RGR of shoots was also higher with mycorrhizal than non-mycorrhizal plantlets 6 through 12 weeks after transplanting (Table 1).

The growth-promoting effect produced by the presence of mycorrhiza resulted in the development of stronger and more vigorous shoots than in non-mycorrhizal plantlets (Table 2; Fig. 1). In addition to this effect, mycorrhizal plants also had an increased number of fully expanded leaves, greater leaf area and leaf DM (Fig. 1, Table 2). Mycorrhizal plantlets had 1.5 and 1.8 times more leaves and leaf area, respectively, than non-mycorrhizal plantlets (Fig. 1, Table 2). At weeks 14 and 16 (data not reported), the RGR of shoots was comparable between mycorrhizal and non-mycorrhizal plants, but by week 18 the RGR was greater for non-mycorrhizal than mycorrhizal plants. However, the cumulative growth of mycorrhizal plants was always greater than non-mycorrhizal plants (Fig. 1, Table 2). At the end of the study, mycorrhizal plantlets had 1.6 times higher shoot length than non-mycorrhizal plants (Fig. 1). The reduction of RGR of colonized plants was due in part to the container size restricting growth of the large-sized mycorrhizal plants. Despite the reduction in RGR at 18 weeks after inoculation, mycorrhizal plants had significantly greater overall growth than non-mycorrhizal plants. Mycorrhizal symbiosis enhanced DM accumulation in the roots and overall shoot system of guava plantlets. The DM accumulation of the leaf, stem, shoot, root,

Table 2 Effects of mycorrhiza on dry mass partitioning, leaf area, root : shoot ratio (g g^{-1}), leaf area ratio [(LAR) cm^2 leaf g^{-1} plant dry mass], and specific leaf area [(SLA) cm^2 leaf g^{-1} leaf dry mass] of micropropagated guava plantlets (*Psidium guajava* L.) 18 weeks after *ex vitro* inoculation and transplanting. Means in each column followed by different letters are significantly different ($P \leq 0$ 0.05) as indicated by *T*-test

Mycorrhiza	Dry mass					Leaf area (cm^2)[b]	Root : shoot ratio[b]	LAR (cm^2 g^{-1})[b]	SLA (cm^2 g^{-1})[a]
	Leaf (g)[a]	Stem (g)[a]	Shoot (g)[a]	Root (g)[b]	Plant (g)[b]				
Yes	15.7 a	9.8 a	25.2 a	15.8 a	40.9 a	1977.0 a	0.64 a	49.7 b	128.9 b
No	7.4 b	5.2 b	12.7 b	8.0 b	20.2 b	1106.6 b	0.65 a	56.7 a	153.1 a

Table 3 Effects of mycorrhiza on element concentrations in leaf tissue of micropropagated guava plantlets (*Psidium guajava* L.) 18 weeks after *ex vitro* inoculation and transplanting. Means in each column followed by different letters are significantly different ($P \leq 0.05$) as indicated by *T*-test; $n = 8$

Mycorrhiza	N (%)	P (%)	K (%)	Mg (%)	Cu (μg g^{-1})	Mn (μg g^{-1})	Mo (μg g^{-1})
Yes	1.20 b	0.101 a	1.51 b	0.19 a	1.56 a	114.75 b	2.46 a
No	1.48 a	0.088 b	1.77 a	0.16 b	1.19 b	129.13 a	1.24 b

and total plant of mycorrhizal plants were 2.1, 1.9, 2.0, 2.0 and 2.0 times higher than those of non-mycorrhizal plants, respectively (Table 2). Values of root to shoot ratio were comparable between mycorrhizal and non-mycorrhizal plantlets (Table 2). Mycorrhizal plants had lower LAR and SLA than controls (Table 2). The RMDI was high for inoculated than uninoculated plantlets, with a value of 103%.

Mycorrhiza altered nutrient uptake of micropropagated guava plantlets. Mycorrhizal plantlets had significantly greater leaf elemental concentration of P, Mg, Cu, and Mo, but lower N, K, and Mn than non-mycorrhizal plants (Table 3).

Extensive mycorrhizal structures were observed in root systems of inoculated plants, while none were observed in non-inoculated plantlets. Arbuscules, vesicles, entry points, spores, and total colonization and hyphae formed in mycorrhizal plant roots were 50%, 60%, 20%, and 94%, respectively.

Leaf gas exchange data taken during the experiment support what was observed for plant growth variables. By 4 weeks, both stomatal conductance (g_s) and net photosynthesis (A) were greater for mycorrhizal than non-mycorrhizal plants (Fig. 2). At 8 weeks, g_s and A were 54% and 33%, respectively, higher for mycorrhizal than non-mycorrhizal plants. However, by 18 weeks, when the experiment was terminated, there was no difference in gas exchange between treatments. The shoot RGR closely mirrored gas exchange measurements.

Discussion

This study demonstrates the benefits of early inoculation with mycorrhiza during *ex vitro* acclimatization and establishment of micropropagated guava plantlets. This is one of the first reports to characterize gas exchange and plant growth during acclimatization and establishment of micropropagated, mycorrhizal plantlets. Problems with erratic stomatal control and low

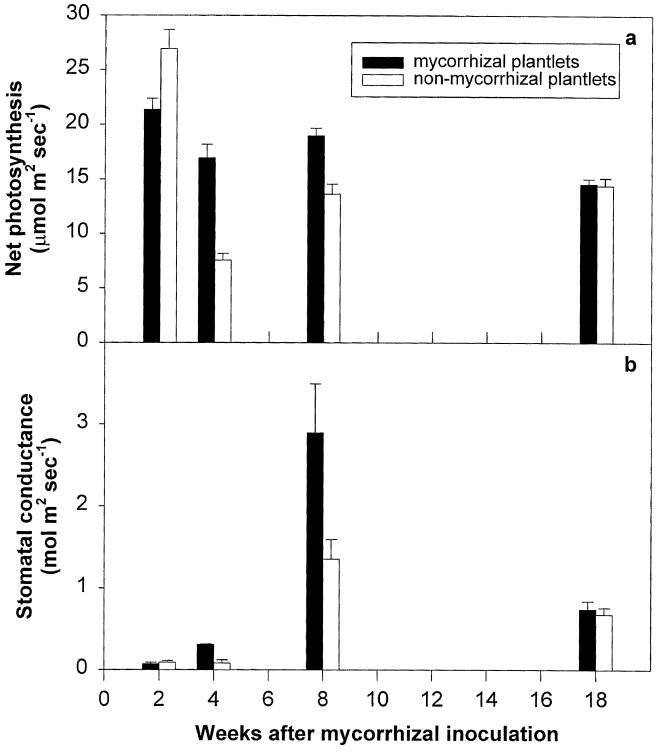

Fig. 2 Effects of mycorrhiza on (a) net photosynthesis and (b) stomatal conductance of acclimatizing micropropagated guava (*Psidium guajava* L.) plantlets 2–18 weeks after inoculation; $n = 12 \pm$ SE

photosynthetic rates can limit successful acclimatization and establishment of micropropagated plantlets (Kozai 1991; Preece and Sutter 1991; Varma and Schüepp 1995; Hartmann et al. 1997). Stomata of micropropagated plants responded to ABA, CO_2, light and changing water potential, but failed to close fully (Santamaria et al. 1993). In our study, mycorrhiza enhanced gas exchange, uptake of selected nutrients, and growth of guava plantlets. Mycorrhiza can also enhance growth of plantlets of selected species (Brazanti et al. 1992; Azcón-Aguilar and Barea 1997), and cause earlier resumption of shoot apical growth (Fortuna et al. 1992, 1996; Azcón-Aguilar et al. 1994). Improvement in rooting and root function have also been observed in several micropropagated species (Schellenbaum et al. 1991), along with greater tolerance of environmental stress (Varma and Schüepp 1995).

After transplanting *ex vitro* and acclimatizing in the greenhouse, guava plantlets had slow initial growth during the first 6 weeks with only marginal shoot growth and leaf production (leaf number). Increases in the number of leaf stomata also were observed in leaves developed after micropropagation compared with those initially produced under *in vitro* conditions; this is part of the acclimatization process (Preece and Sutter 1991). Mycorrhizal colonization was also occurring and was well established by week 6, which enabled higher growth by mycorrhizal plants.

Within 8 weeks, non-mycorrhizal guava plantlets were actively growing. However, plantlets colonized with the ZAC-19 isolate had higher overall plant growth, achieving higher and more constant growth rates throughout the study. By week 4, mycorrhizal plantlets had higher photosynthetic rates (A) and stomatal conductance (g_s), which can be associated with the higher CO_2 influx into the mesophyll tissues, as indicated by higher g_s. Higher photosynthetic rates have been reported with non-tissue culture-produced mycorrhizal plants (Druge and Schonbeck 1993; Mathur and Vyas 1995; Phavaphutanon 1996). The comparable gas exchange between treatments and reduction in the RGR of mycorrhizal plants at the end of the experiment were in part consequences of the restriction of root growth of the larger mycorrhizal plants.

At the end of the experiment, higher total shoot length of mycorrhizal plantlets was accompanied by a higher number of expanded leaves and leaf area compared with that of non-mycorrhizal plants, which is consistent with data obtained in other micropropagated species. Granger et al. (1983), Vidal et al. (1992), Lovato et al. (1994a, b), and Rapparini et al. (1996) reported increases in plant height of 1.5 to 3.0 times utilizing mycorrhiza with micropropagated apple, avocado, grapevine, common ash, and pear plantlets, respectively.

Stem, root and leaf mass were higher in mycorrhizal guava plantlets as a consequence of the better nutritional status and higher photosynthetic rates during acclimatization and active growth of mycorrhizal guava plantlets. Mycorrhizal colonized plants may have lower root: shoot ratios in response to a greater increment of shoot mass relative to root mass (Azcón-Aguilar and Barea 1997). However, mycorrhizal and non-mycorrhizal guava plantlets had comparable values. The LAR, which relates the leaf area to whole plant dry mass (Hunt 1982) was higher with non-mycorrhizal plants; this allowed for growth to be sustained, albeit at a reduced level. However, mycorrhizal plants had a lower LAR, indicating a more efficient photosynthesizing surface, since the leaf area to plant dry mass was reduced, i.e. less photosynthesizing surface per total plant mass (Hunt 1982). This corresponds with the lower SLA (thicker leaves) of mycorrhizal guava plants, indicating higher photosynthetic leaf mesophyll with mycorrhizal plants. Chlorophyll was not evaluated in this guava study, but higher levels of chlorophyll and a higher A value were observed in a study of mycorrhizal chile ancho plantlets during acclimatization (Estrada-Luna 1999).

Leaf mineral content of guava trees primarily depends on the age of leaves and the cultivar (Kumar and Pandey 1979). However, the mycorrhizal dependency of guava trees on P uptake was illustrated in this experiment. The low supplementary rate of P added during fertilization (11 μg P ml^{-1}) allowed mycorrhizal plantlets to have significantly greater P uptake (0.101 vs. 0.088%) than non-mycorrhizal plantlets. The P levels of both mycorrhizal and non-mycorrhizal plantlets were in the 0.09–0.20% range reported for healthy field-grown guava trees (Kumar and Pandey 1979). However, the fact that large differences in P concentration did not occur does not necessarily mean that P was not involved in growth enhancement. Sometimes leaves are very conservative in their P concentration. When more P becomes available, they produce a greater leaf area and thus maintain similar tissue concentration levels (Smith and Read 1997). Furthermore, there was greater total plant acquisition of P, since the plant biomass was greater with mycorrhizal than non-mycorrhizal plantlets.

Net photosynthesis of mycorrhizal plants can increase as a result of improved plant nutritional status (Koch and Johnson 1984; Fitter 1988). Plants with an optimal P concentration should be more vigorous with higher A and g_s than plants with limiting P (Radin and Eidenbock 1986). Low P can also reduce the concentration and activity of rubisco, and slow the regeneration of intermediate substances in the Calvin cycle (Lauer et al. 1989). However, an improved A value of mycorrhizal plants can also occur independent of P status (Bethlenfalvay et al. 1987; Davies et al. 1992, 1993).

In a study of seedling chile ancho plants, mycorrhizal enhancement of growth and gas exchange was in part due to greater uptake of P and greater extraradical hyphae development (Aguilera-Gomez et al. 1999).

Mycorrhiza in increasing lateral root formation, can indirectly increase cytokinin production, which itself enhances photosynthesis (Davies et al. 1996). Mycorrhizal chile ancho plantlets had lower levels of ABA (Estrada-Luna 1999). The content of abscisic acid tends to be lower in the xylem sap of mycorrhizal plants, which in part explains the higher g_s frequently reported in mycorrhizal plants (Duan et al. 1996). It also could partially account for the higher g_s and A values we observed until the container size limited growth of the larger mycorrhizal guava plantlets. Mycorrhizal fungi explore the soil volume in a manner analogous to increasing root density. Extraradical hyphae bridge gaps between the soil and roots as well as binding soil particles to each other and to roots. This can be beneficial for enhancing nutrient uptake and minimizing water loss due to diurnal fluctuations in soil water and subsequent soil shrinkage and gaps in the soil-root interface and between soil particles.

Mycorrhizal symbiosis can enhance not only P acquisition in some plant species but other nutrients such as Cu, NH_4, and Zn that are present in low concentrations in the soil or have poor mobility rates (Galli et al. 1994; Marschner and Dell 1994; Marschner 1995; Azcón-Aguilar and Barea 1997). Mycorrhizal guava plantlets had significantly higher Mg, Cu, and Mo. Besides the factor of higher P levels of mycorrhizal plantlets, increased levels of Mg and Cu can also contribute to higher photosynthetic rates. Magnesium is an essential component of chlorophyll and a cofactor of most enzymes that activate phosphorylation processes, while Cu is involved in the electron transport system and is a component of the chloroplast protein plastocyanin (Mills and Jones 1996).

Non-mycorrhizal guava plantlets had higher leaf tissue N, K, and Mn concentrations than colonized plants. The range of leaf N concentration with guava was slightly deficient for mycorrhizal plantlets and adequate for the non-mycorrhizal plantlets based on an N sufficiency range of 1.25–1.6% (Reuter and Robinson 1986; Mills and Jones 1996). This decrease in leaf N concentration was probably because of a dilution effect of increased dry mass of mycorrhizal plants (Johnson et al. 1980). Moreover, the lower N concentration did not appear to affect the gas exchange capacity of the colonized guava plantlets. Despite the differences in leaf concentration of K, both mycorrhizal and non-mycorrhizal plantlets were K-adequate because their values were within the range of 1.3–1.75% (Kumar and Pandey 1979; Reuter and Robinson 1986; Mills and Jones 1996). Likewise, both mycorrhizal and non-mycorrhizal guava plantlets were Mn-sufficient (Mills and Jones 1996).

Field-grown guava plants can be colonized by different mycorrhizal fungus species such as *Sclerocystis coremioides, Acaulospora* spp, *Scutellospora calospora*, and *Glomus mosseae* (Janos 1980; Kumuran and Azizah 1995). In our research, inoculated plantlets were extensively colonized by the ZAC-19 mixed isolate of *G. diaphanum, G. albidum* and *G. claroides*. In a study of acclimatization of chile ancho pepper plantlets (Estrada-Luna 1999), the ZAC-19 isolate was more aggressive than two isolates of *G. intraradices*, and rapidly developed internal hyphae to produce high levels of colonization (29%) within a 10-day period. Zac-19 was an effective isolate for enhancing P uptake and growth of seedling chile ancho pepper (*Capsicum annuum*) plants under P-stress conditions, including the development of greater extraradical hyphae (Davies et al. 1994) and under drought stress (Davies et al. unpublished data). The total root colonization of the guava plantlets was 94%, which compares with reports on mycorrhizal guava seedlings observed by Kumuran and Azizah (1995). With guava plantlets, arbuscules and vesicles in cortical cells were found at relatively high frequencies, demonstrating that the fungal colonization remained very active throughout the study (Smith and Gianinazzi-Pearson 1988; Azcón-Aguilar and Barea 1997). Guava plants are highly dependent on mycorrhizal colonization as shown by the high value of RMDI obtained over this study (103%), which agrees with Kumuran and Azizah (1995).

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