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Ph. Vaast · R.J. Zasoski · C.S. Bledsoe

Effects of vesicular-arbuscular mycorrhizal inoculation at different soil P availabilities on growth and nutrient uptake of in vitro propagated coffee (*Coffea arabica L.*) plants

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Abstract In a pot experiment, the growth and the nutrient status of in vitro propagated coffee (*Coffea arabica* L.) microcuttings were investigated for 5 months following vesicular-arbuscular mycorrhizal (VAM) inoculation with either *Acaulospora melleae* or *Glomus clarum* at four soil P availabilities. Control plants remained P-deficient even at the highest soil P availability while mycorrhizal plants were P-sufficient at all soil P availabilities. Growth of control plants was only improved at the highest soil P availability. In P-deficient soil, neither of the two VAM species improved plant growth. Plant growth increased by 50% following inoculation with either *A. melleae* or *G. clarum* when P availability went from deficient to low. No further plant growth improvement was induced by either VAM species at intermediate and high soil P levels. Nevertheless, growth of plants inoculated with *G. clarum* was still significantly greater than that of non-mycorrhizal plants at the highest soil P availability. Root colonization by *G. clarum* increased with increasing soil P availability while root colonization by *A. mellea* decreased with soil P level increasing above low P availability. Soil P availability also affected Zn nutrition through its influence on VAM symbiosis. With increasing soil P availability, foliar Zn status increased with *G. clarum* or decreased with *A. mellea* in parallel to root colonization by VAM. This study demonstrates the beneficial effects of VAM inoculation on in vitro propagated Arabica coffee microcuttings, as shown previously for seedlings. This study also demonstrates differences in tolerance to soil P availability between VAM species, most likely resulting from their differing abilities to enhance coffee foliar P status.

Ph. Vaast (✉)
CIRAD-CP, 2477 Avenue du val de Montferrand,
BP 5035, F-34032 Montpellier cedex 1, France
fax: +33-467-61-65-90; e-mail: vaast@cirad.fr

R.J. Zasoski · C.S. Bledsoe
LAWR, Soils and Biogeochemistry Program,
University of California, Davis, CA 95616, USA

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Introduction

Techniques to produce Arabica coffee (*Coffea arabica* L.) plants asexually in vitro, via microcutting and via somatic embryogenesis have been successfully developed during the last 10 years (Sondahl 1991; Teisson et al. 1994). These increasingly used techniques have the following advantages: (1) easy and inexpensive long-term test-tube conservation of wild and selected genotypes, (2) rapid mass production of genotypes free of pests, (3) easy transfer of clean material from one country/continent to another, and (4) favorable environment to initiate genetic manipulation.

In vitro propagated plants are usually susceptible to various environmental stresses following transplanting, such as fungal diseases. To avoid such diseases, axenically propagated plants are generally transplanted to sterile media, wich results in slow vegetative growth due to the poor nutrient composition of the potting medium compared to the in vitro agar medium (Granger et al. 1983). Addition of fertilizers does not greatly improve the nutrient status of propagated plants in their early growing stage because of their relatively small root systems and inability to take up nutrients efficiently.

Vesicular-arbuscular mycorrhizal (VAM) inoculation has been found to improve growth and nutrient uptake of a large variety of in vitro propagated perennial plants (Blal et al. 1992; Granger et al. 1983; Vidal et al. 1992). The goal of this greenhouse experiment was to assess whether VAM inoculation could also be effective with in vitro propagated Arabica coffee, a plant highly VAM-dependent for its P and Zn nutrition in acid soils with strong P-fixing capacity and low Zn availability (Siqueira et al. 1993; Vaast and Zasoski 1992). Two VAM species were tested on microcuttings

of Arabica coffee, cv catuai rojo, at four different soil P availabilities.

Materials and methods

Plant material and treatment

Coffee cuttings shipped in test-tubes from the CATIE research facility, Turrialba, Costa Rica were maintained for several weeks in a growth chamber at 25 °C to allow plant development to a suitable transplantation size.

To initiate rhizogenesis, the bases of microcuttings were washed free of agar with sterile water, rejuvenated by cutting several millimeters, and dipped in a 50 mg/l solution of indolebutyric acid and naphthaleneacetic acid for 24 h in the dark (Bertrand-Desbrunais 1991). Thereafter, microcuttings were grown in autoclaved vermiculite in a growth chamber at 25 °C. The relative humidity was progressively decreased from 100% to 70% over 10 days. Nutrient solution in the vermiculite medium was a 1/4-strength Hoagland solution (Hoagland and Arnon 1950). Roots started to appear after 2 weeks. After 1 month, microcuttings were selected for uniform shoot and root sizes and transplanted into styrofoam pots containing 500 g of an autoclaved soil originating from the Eldorado Forest, Northern California, USA.

Soil preparation and treatment

Prior to potting, the soil was sieved (2 cm) and autoclaved for 1 h. The soil pH was 5.7 (1:1 soil to 0.01 *M* $CaCl_2$), and the soil cation exchange capacity, Ca, K and Mg exchangeable cations, measured by $BaCl_2$ extraction (Gillman 1979), were 3.65, 0.60, 0.10, 0.16 cmol (+) kg^{-1}, respectively. The organic matter content was found to be 1.2% by the Walkley and Black method (Nelson and Sommers 1982).

Subsamples of the autoclaved soil were incubated for 1 month after P addition as KH_2PO_4, at the rates of 0, 40, 80, 160, 240, 320, 400, and 480 mg P kg^{-1}. Four levels of P addition were chosen after measuring the soil P availability following a dilute double acid extraction and colorimetric determination by the molybdenum blue method (Olsen and Sommers 1982). These four levels were selected to cover the range of P availability from deficient (< 11 mg P kg^{-1}), through medium (11–31 mg P kg^{-1}), to high (31–51 mg P kg^{-1}). To compensate for K addition, K_2SO_4 was added to all other treatments at a rate equivalent to the highest KH_2PO_4 fertilization.

Mycorrhizal inoculation

Pure starter cultures of *Glomus clarum* Nicolson & Schenck (BR143A) and *Acaulospora mellea* Spain & Schenck (BR874) were provided by INVAM, Morgantown, W. Va. These two species originated from Brazil, where their high growth-promoting efficiency on Arabica coffee seedlings has been demonstrated (Siqueira et al. 1993). Spores were produced in pot culture on Sudan grass (*Sorghum bicolor* L. Moench) in autoclaved Eldorado soil, extracted by wet sieving, and collected on a 45-mm sieve.

Plants designated to become mycorrhizal were inoculated with a sufficient mass of the sieved soil-spore fraction, mixed with the pot soil during transplanting, to achieve the spore density of 2 spores/g dry soil considered to be adequate for establishment of VAM symbiosis on Arabica coffee (Saggin et al. 1992). Aliquots (5 ml) of VAM inoculum filtrate, passed through a Whatman No. 1 filter paper, were added to the soil in each non-mycorrhizal pot to standardize microflora other than the mycorrhizal fungi.

Experimental design and plant maintenance

The experiment consisted of a 5×4×3 completely randomized factorial design: 5 (replicates) × 4 (P availabilities) × 3 (two VAM species and a control). Plants were grown in a greenhouse for 5 months with temperatures in the range of 22–28 °C during the day and 18–22 °C during the night. Humidity varied between 40 and 70%. Pots were watered every 2–3 days with 20 ml of distilled water, and soil moisture was adjusted to about 80% of field capacity gravimetrically once a week. Twice a month, 20 ml of 1/4-strength Hoagland solution without P was added to the pots during the entire course of the experiment.

Plant analyses

At harvest, shoots were separated from the roots at the root collar and foliar area was recorded using a leaf area meter (Model Li-Cor 3000). Shoots were washed with deionized water, dried at 60 °C for 42 h, weighed and ground in a Wiley mill to pass a 20-mesh screen. Total N was determined directly on a ground subsample with a Carlo Erba NA 1500 gas chromatograph. Concentrations of P, K, Ca, Mg, were determined by ICP (Inductively Coupled Plasma optical Emission Spectrometry) and Zn by AAS (Atomic Absorption Spectrometry) after a nitric-perchloric digestion of the plant tissue (Zasoski and Burau 1977).

Roots were rinsed, blotted dry, and their fresh weight recorded before being cut into 1-cm segments. One root subsample of 0.25–0.50 g was used to assess mycorrhizal infection while the rest was oven dried at 60 °C and weighed after 42 h. Roots were cleared and stained (Phillips and Hayman 1970), and 20 1-cm root segments were randomly examined to estimate the percentage of infected root length (Biermann and Linderman 1981).

Results

Soil P availability

Without P addition (P_0), soil available P was very low (3 mg P kg^{-1}) and the soil was considered P-deficient (< 11 mg P kg^{-1}). With P additions of 80 and 160 mg P kg^{-1}, soil P availabilities were (P_1) 13 and (P_2) 27 mg P kg^{-1} soil, and thus in the intermediate range (11–31 mg P kg^{-1}). Addition of 240 mg P kg^{-1}, resulting in a soil available P value (P_3) of 42 mg P kg^{-1} soil, was selected as the high soil P availability (31–51 mg P kg^{-1}).

Plant growth and mycorrhizal root colonization

Phosphorus effects on plant growth in control and mycorrhizal treatments are presented in Table 1. In P-deficient soil (P_0), all plants grew very little and no significant differences between mycorrhizal and non-mycorrhizal plants were observed for any growth parameter (except for root/shoot ratio with *G. clarum*). When soil P availability increased to 13 mg P kg^{-1} (P_1), plants inoculated with either VAM species grew significantly more than control plants. At the two higher soil P availabilities (P_2 and P_3), only plants inoculated with *G. clarum* grew significantly more than control plants. Root/shoot ratios were significantly lower in mycorrhizal treatments than in control treatments at all P levels (except in the P-deficient soil with *A. mellea*).

Table 1 Effects of soil P availability on growth of coffee microcuttings either non-mycorrhizal (*Control*) or VA mycorrhizal with *Glomus clarum* (*Clarum*), or *Acaulospora mellea* (*Mellea*). Soil P availabilities were: deficient P_0 (3 mg P^{-1}), low P_1 (13 mg P kg^{-1}), intermediate P_2 (27 mg P kg^{-1}), and high P_3 (42 mg P kg^{-1}). Values are means of 5 replicates ± standard error. Means in a column, within a P availability, followed by the same letter(s) do not differ significantly at the 5% level, using the Newman and Keuls test

Available P	VAM treatment	Total dry weight (g/plant)	Root dry weight (g/plant)	Root/shoot ratio
P_0				
	Control	1.17 ± 0.17a	0.44 ± 0.08a	0.52 ± 0.02a
	Mellea	0.89 ± 0.13a	0.27 ± 0.04a	0.50 ± 0.02a
	Clarum	1.01 ± 0.12a	0.30 ± 0.03a	0.44 ± 0.01b
P_1				
	Control	0.75 ± 0.11b	0.31 ± 0.04b	0.64 ± 0.03a
	Mellea	1.82 ± 0.20a	0.60 ± 0.07a	0.49 ± 0.01b
	Clarum	1.94 ± 0.12a	0.51 ± 0.02a	0.36 ± 0.01c
P_2				
	Control	1.27 ± 0.20b	0.46 ± 0.07b	0.57 ± 0.03a
	Mellea	1.52 ± 0.29b	0.49 ± 0.09b	0.43 ± 0.01b
	Clarum	2.36 ± 0.35a	0.75 ± 0.11a	0.47 ± 0.01b
P_3				
	Control	1.82 ± 0.20b	0.64 ± 0.09a	0.50 ± 0.02a
	Mellea	1.53 ± 0.20b	0.44 ± 0.05ab	0.41 ± 0.02b
	Clarum	2.26 ± 0.18a	0.66 ± 0.08a	0.42 ± 0.01b

Within the non-mycorrhizal treatment, only the highest soil P availability enhanced foliar area significantly compared with other P levels (Fig. 1). Within mycorrhizal treatments, soil P availability significantly affected plant growth and VAM colonization. At the two lower soil P availabilities (P_0 and P_1), *A. mellea* infected a higher percentage root length than *G. clarum*, while no difference was observed at higher P availabilities (Fig. 1). Within the *G. clarum* treatment, root colonization increased with increasing soil P availability (Fig. 1), but plant growth (Table 1) and foliar area (Fig. 1) were not significantly affected by soil P levels greater than P_1. Within the *A. mellea* treatment, root colonization (Fig. 1) was significantly reduced at intermediate (P_2) and high P (P_3) availabilities compared with low P availability (P_1) while foliar area (Fig. 1) and plant growth (Table 1) did not differ significantly.

Plant nutrient status

In mycorrhizal plants, foliar P concentration was adequate at all P availabilities while that of control plants was below the P-deficiency level of 0.08% (Fig. 1). Within the *G. clarum* treatment, foliar P concentration was not significantly affected by soil P availability (Fig. 1). In contrast, within the *A. mellea* treatment, foliar P concentration increased significantly at the two highest P availabilities (Fig. 1).

Foliar Zn concentration was not significantly reduced by soil P availability in the control plants (Fig. 1). For plants inoculated with *A. mellea*, foliar Zn concentration (Fig. 1) and content (data not shown) decreased with increasing soil P and were significantly lower at the highest soil P availability. On the other hand, foliar Zn status of the plants inoculated with *G. clarum* tended to increase with increasing soil P levels and was significantly higher at the highest soil P availability (Fig. 1).

Foliar N, K, Mg and Ca concentrations (data not shown) were not significantly influenced by mycorrhizal treatments and soil P levels, and were all within the normal concentration range (Snoeck 1984).

Discussion

Levels of root VAM colonization of in vitro propagated coffee microcuttings were comparable to those observed on coffee seedlings in the greenhouse or nursery after the same period of VAM exposure (Saggin et al. 1992; Siqueira et al. 1993; Vaast and Zasoski 1992). Root colonization by *A. mellea* was diminished by high soil P availability and concomitant enhanced foliar P concentration. Such a suppressive effect of a high plant P status, induced by a high soil P availability, has been documented in several studies (Arias et al. 1991; Habte and Byappanahalli 1994; Kormanick 1985). Contrasting with this suppressive effect observed with *A. mellea*, coffee root colonization by *G. clarum* was stimulated by increasing soil P levels while foliar P status remained unaffected. Therefore, the present results suggest that differences in tolerance between VAM species to increasing soil P availabilities are related to their effect on the foliar P status of their coffee host.

Soil P availability also affected Zn nutrition through its influence on VAM symbiosis. Above low soil P availability (P_1: 13 mg P kg–1), foliar Zn status declined with decreasing root colonization by *A. mellea*, whereas it increased with increasing root colonization by *C. clarum*. Kothari et al. (1991) showed that VAM hyphae have the ability to absorb and translocate Zn to host roots, thereby contributing up to 25% of host-plant Zn acquisition. Reduction in VAM symbiosis has been proposed as an explanation for P-induced Zn deficiency in wheat (Singh et al. 1986) and coffee (Vaast 1995) field trials receiving long-term high P addition. Consequently, this may be particularly relevant to Arabica coffee nutrition in volcanic soils. Heavy P fertilization is recommended on these volcanic soils with high P-fixing capacities and low Zn availability (Wrigley 1988).

Mycorrhizal symbiosis enhanced both root and shoot growth and resulted in significantly lower root/shoot ratios for mycorrhizal plants than for control plants. These results are in agreement with those observed on several in vitro propagated plants (Branzanti et al. 1992; Guillemin et al. 1992), as P-deficient plants lacking VAM symbiosis tend to have a high root/shoot ratio usually associated with nutrient-stressed plants (Pacovsky et al. 1986).

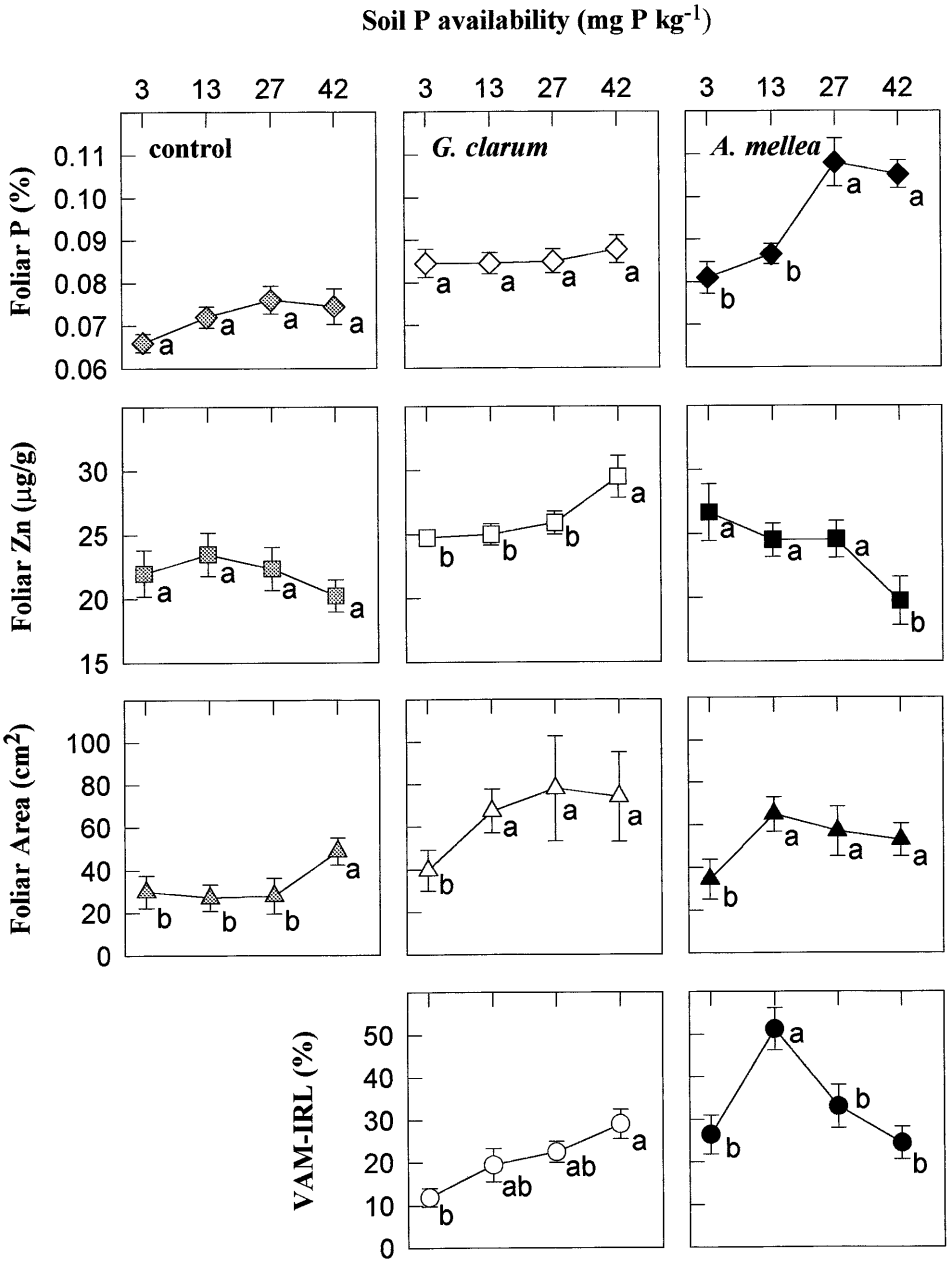

Fig. 1 Effects of increasing soil P availability on foliar P and Zn concentrations, foliar area, and VAM-infected root length (*VAM-IRL*) of in vitro coffee microcuttings non-inoculated (*control*) and VAM-inoculated with either *G. clarum* or *A. mellea*. Values are means of 5 replicates (± sem) and do not differ significantly at the 5% level when followed by the same letter(s), using the Newman and Keuls test

From a practical point of view, an addition of 80 mg P kg^{-1} to increase soil P availability just above soil-deficiency level was sufficient to produce plants, when inoculated with *A. mellea*, with a biomass equivalent to that of non-inoculated plants receiving three times this P addition. Even at the highest soil P availability, non-mycorrhizal seedlings were still significantly smaller than *G. clarum*–inoculated plants.

In conclusion, this study shows that VAM inoculation of in vitro propagated coffee plants produces large plants with improved P status, thus confirming the high VAM-dependency of Arabica coffee observed with seedlings. This study also indicates differences in tolerance to soil P availability between VAM species, most likely resulting from their differing abilities to promote plant P accumulation. This highlights the importance of screening for VAM efficiency under soil chemical conditions similar to those that the host-plant will experience in the field.

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