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DIVISÃO 2 - PROCESSOS E PROPRIEDADES DO SOLO

Comissão 2.1 - Biologia do solo

MYCORRHIZAL EFFECTIVENESS ON PHYSIC NUT AS INFLUENCED BY PHOSPHATE FERTILIZATION LEVELS⁽¹⁾

Elcio Liborio Balota⁽²⁾, Oswaldo Machineski⁽³⁾ & Alexandra Scherer⁽⁴⁾

SUMMARY

In recent years, physic nut (*Jatropha curcas* L.) has attracted attention because of its potential for biofuel production. Although it is adapted to low-fertility soils, physic nut requires soil acidity corrections and addition of a considerable amount of fertilizer for high productivity. The objective of this research was to evaluate the effectiveness of arbuscular mycorrhizal fungi (AMF) (control without AMF inoculation, *Gigaspora margarita* inoculation or *Glomus clarum* inoculation) on increasing growth and yield of physic nut seedlings under different rates of P fertilization (0, 25, 50, 100, 200, and 400 mg kg⁻¹ P soil) in greenhouse. The experiment was arranged in a completely randomized, block in a factorial scheme design with four replications. The physic nut plants were harvested 180 days after the beginning of the experiment. Mycorrhizal inoculation increased physic nut growth, plant P concentration and root P uptake efficiency at low soil P concentrations. The P use quotient of the plants decreased as the amount of P applied increased, and the P use efficiency index increased at low P levels and decreased at high P levels. Mycorrhizal root colonization and AMF sporulation were negatively affected by P addition. The highest mycorrhizal efficiency was observed when the soil contained between 7.8 and 25 mg kg⁻¹ of P. The physic nut plants responded strongly to P application, independent of mycorrhizal inoculation.

Index terms: *Jatropha curcas* L., mycorrhizal inoculation, phosphorus efficiency, phosphate nutrition.

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RESUMO: *EFICIÊNCIA MICORRÍZICA NO PINHÃO-MANSO SOB DIFERENTES DOSES DE ADUBAÇÃO FOSFATADA*

Nos últimos anos, a cultura do pinhão-mansó (*Jatropha curcas* L.) tem despertado grande interesse pelo seu alto potencial na produção de matéria prima para a produção de biocombustíveis. Apesar de sua adaptabilidade às condições de baixa fertilidade do solo, as plantas requerem a correção da acidez do solo e a adição de considerável quantidade de fertilizantes para alta produção de frutos. O objetivo deste trabalho foi avaliar a efetividade de fungos micorrízicos arbusculares em mudas de pinhão-mansó em diferentes doses de P no solo. O experimento foi conduzido em casa de vegetação, utilizando solo arenoso (LVd) autoclavado como substrato em vasos com capacidade de 4 kg. Foram avaliados em um delineamento completamente casualizado – em um esquema fatorial, com quatro repetições – os tratamentos de fungos micorrízicos (controle, *Gigaspora margarita* ou *Glomus clarum*) e a adição de P (0, 25, 50, 100, 200 e 400 mg kg⁻¹ de solo). O experimento foi conduzido por 180 dias. A inoculação micorrízica proporcionou aumento no crescimento das plantas, nos teores de P e na eficiência de absorção de P pelas raízes nas baixas doses de P. O pinhão-mansó apresentou decréscimo no quociente de utilização de P com o aumento da adição de P. O índice de eficiência de utilização de P aumentou nas menores doses e diminuiu nas altas doses de adição deste nutriente. A colonização micorrízica e a esporulação foram influenciadas negativamente pela adição de P. A mais alta eficiência micorrízica ocorreu entre as concentrações de 7,8 e 25 mg kg⁻¹ de P no solo. O pinhão-mansó respondeu positivamente à adição de P, independentemente da presença micorrízica.

Termos de indexação: *Jatropha curcas* L., inoculação de micorrizas, eficiência de utilização de fósforo, adubação fosfatada.

INTRODUCTION

In recent years, physic nut (*Jatropha curcas* L.) has attracted attention because of its potential to be used for biofuel production. Dry physic nuts have approximately 28 % oil, and plants can produce more than 6,000 kg ha⁻¹ of seeds or over 2,000 kg ha⁻¹ of oil (Saturino et al., 2005; Dias et al., 2007; Laviola & Dias, 2008).

The physic nut plant is found in the tropical and subtropical regions of Brazil, and is considered a rustic crop that can grow under diverse soil and climate conditions and thrive in low-fertility soils (Saturino et al., 2005; Dias et al., 2007). However, the plants depend on the neutralization of soil acidity and fertilization to achieve high yield levels. As interest in the plant as a biofuel source has increased, the demand for information related to the nutritional demand of the plant is increasingly required.

Phosphorus is one of the major limiting nutrients for crop production, particularly in the tropics, and is found at extremely low levels in the soil solution. In soil solutions, the P concentration is nearly a thousand times lower than the amounts of other soil nutrients. Low P levels in acidic soils are caused by the reactivity of the element with Fe and Al, leading to P precipitation and reducing

the nutrient content (Novais et al., 2007). Up to 90 % of P in fertilizers can be precipitated by Fe, Al and Ca complexes in the soil. Furthermore, it has been estimated that if the current P fertilizer application rates are maintained worldwide, the available supply of P rocks could be depleted by the end of the 21st century.

The symbiotic relationship between plant roots and arbuscular mycorrhizal fungi (AMF) is partially responsible for nutrient uptake efficiency in plants. AMF form a symbiosis with the roots of more than 90 % of terrestrial plants and significantly contribute to plant nutrition and growth. This symbiosis is characterized by the formation of spores, hyphae and diverse structures in plant roots, including hyphae, vesicles and arbuscules. The extensive extraradical hyphae network effectively increases the absorptive area of roots and enhances nutrient uptake efficiency, especially for nutrients with low mobility and low concentrations in the soil solution (Smith & Read, 1997; Moreira & Siqueira, 2006). The mycorrhizal association may be responsible for up to 80 % of P uptake in plants (Marschener & Dell, 1994) and plays an important role in tropical regions where P availability is low. AMF have the potential to decrease dependence on P fertilizers, which are considered to be vitally important for the establishment of most crops (Marschener & Dell, 1994; Novais et al., 2007).

The plant response to mycorrhizal inoculation can vary widely, depending on the AMF species and soil conditions, namely on the level of available P. Increases in the P concentration significantly influence plant growth and development and the efficiency of the mycorrhizal symbiosis, as observed in papaya (Trindade et al., 2001), Brazilian native wood species (Siqueira & Saggin-Júnior, 2001), citrus (Nogueira & Cardoso, 2006), and mangaba (Cardoso Filho et al., 2008). Since P availability affects the development and functioning of the mycorrhizal symbiosis, it is of fundamental to assess the soil P level that induces the greatest physic nut plant response to mycorrhizal inoculation.

Efficient AMF species are those capable of surviving, colonizing plant roots, producing a large volume of mycelium and establishing an efficient mutualistic relationship with plants under specific soil fertility conditions (Moreira & Siqueira, 2006). At a given level of soil fertility, the effectiveness of a mycorrhizal symbiosis can be calculated by comparing the biomass yields with and without mycorrhizal inoculation.

The mycorrhizal symbiosis also affects nutrient uptake efficiency, which can be defined as the ability of a plant to take up a nutrient and use it for biomass production. There are several ways to calculate nutrient uptake efficiency. The nutrient use efficiency may be calculated based on the biomass yield by a plant per unit of accumulated nutrient (Steenbjerg & Jakobsen, 1963; Siddiqi & Glass, 1981). The agronomic efficiency method defines uptake efficiency as plant production per unit of nutrient applied. In addition, the nutrient root uptake efficiency method calculates the efficiency of nutrient uptake per unit of root weight (Elliott & Läuchli, 1985).

The objective of this study was to evaluate the effectiveness of arbuscular mycorrhizal fungi in increasing growth and yield of physic nut seedlings at different P levels.

MATERIALS AND METHODS

Pots in greenhouse were filled with 4 kg of sandy soil (Oxisol), classified as dystrophic Red Latosol, with the following chemical characteristics: pH in $\text{CaCl}_2 = 4.1$; P in Mehlich-1 solution = 2.3 mg kg^{-1} ; and 12.9 g dm^{-3} of organic C. Dolomitic limestone was added to the soil to raise the cation base saturation level to 70 %, and the pots were incubated for 60 days at field-level moisture. After incubation, the soil pH was 6.4 and the P concentration 7.8 mg kg^{-1} . The substrate was autoclaved twice at 121°C for one hour. After 20 days, the substrate was fertilized with P in the form of fine triple superphosphate.

The experiment was arranged in a completely randomized block design in a factorial scheme with four replications. Three AMF treatments (control without AMF inoculation, inoculation with *Gigaspora margarita* or with *Glomus clarum*) and six P rates (0, 25, 50, 100, 200, and 400 mg kg^{-1} soil, labeled as P0, P25, P50, P100, P200, and P400, respectively) were evaluated, in four replications per treatment. After the application of 25, 50, 100, 200, and 400 mg kg^{-1} P, soil P concentrations (Mehlich-1) were 15, 25, 48, 101, and 221 mg kg^{-1} , respectively.

Physic nut seeds were germinated in sterilized vermiculite, and when the seedlings had two leaves, the seedlings were transplanted to the pots. After 20 days, two of the three seedlings per pot were removed. Approximately 120 AMF spores were placed 5 cm below the seedling roots. The mycorrhizal inoculum, grown on the host plant *Brachiaria decumbens*, was obtained from the IAPAR Collection. After 180 days, the following characteristics were evaluated: total biomass dry matter, P concentration in shoots and roots, mycorrhizal root colonization and sporulation. All soil and plant analyses were performed according to IAPAR protocols (Pavan et al., 1992).

Mycorrhizal root colonization was evaluated after clarifying and staining roots. Roots were clarified in 10 % KOH, acidified with 1 % HCl, washed in running water and stained with 0.05 % trypan blue in lactoglycerol solution (Phillips & Hayman, 1970). Then, root colonization (vesicles, arbuscules and hyphae) was evaluated by the gridline intersect method (Giovannetti & Mosse, 1980). AMF spores were extracted from 50 cm^3 substrate, with three replications per treatment, and separated by wet sieving (sieves from 710 to $53 \mu\text{m}$) (Gerdemann & Nicolson, 1963). Spores were separated from fine organic detritus by centrifugation in 50 % sucrose and counted under a stereoscopic microscope.

Mycorrhizal efficiency (ME) was calculated from dry shoot and root variables. ME is defined as the dry matter mass of a mycorrhizal plant minus the dry matter mass of a non-mycorrhizal plant divided by the dry matter mass of a non-mycorrhizal plant multiplied by 100. The P utilization quotient (PUQ) was calculated as the total plant dry matter (TPDM) divided by the total P accumulation (TPA) (Steenbjerg & Jakobsen, 1963). Siddiqi & Glass (1981) suggested that the nutrient use efficiency method, which is based on the product of the biomass yield and the inverse of the nutrient concentration, is a more appropriate measure of uptake efficiency. So the P use efficiency index (PUEI) is calculated as the total plant dry matter divided by the total P accumulation (TPDM^2/TPA). The P root uptake efficiency (PRUE) is calculated as P accumulation in the shoot divided by the dry root mass.

Analysis of variance (ANOVA) was performed with the factors AMF inoculation and P application

rate. The treatment effects were statistically evaluated by mean comparisons by the Tukey test ($p \leq 0.05$). Data for each AMF species were subjected to regression analysis with increasing P rates. A significant equation, as determined by the F test ($p \leq 0.05$), was chosen due to its significance and higher coefficient of determination (R^2). Prior to analyses, the percentages of mycorrhizal root colonization were transformed with an arcsin $\sqrt{x}/100$ function, and spore numbers with a $\log(x + 1)$ function. All statistical analyses were performed using version 9 of the SAS statistical package (SAS, 2002).

RESULTS AND DISCUSSION

Interaction effects were observed between AMF and P doses in mycorrhizal and non-mycorrhizal plants. At low P levels, the mycorrhizal plants were more developed and had higher P concentrations than the non-mycorrhizal plants. In general, the beneficial effects of mycorrhizal inoculation decreased as P levels increased.

Total dry matter

At low P levels, the mycorrhizal plants produced more total dry matter (TDM) (shoot + root) than the non-mycorrhizal plants. Data on the effects of P application to mycorrhizal and non-mycorrhizal plants were fitted with a second-degree polynomial regression model (Figure 1). AMF inoculation significantly increased TDM; increases of 522, 213 and 210 % were recorded in the treatments P0, P25 and P50, respectively. No significant differences were observed between AMF species.

The mycorrhizal had higher growth rates than the non-mycorrhizal plants, mainly at low P levels.

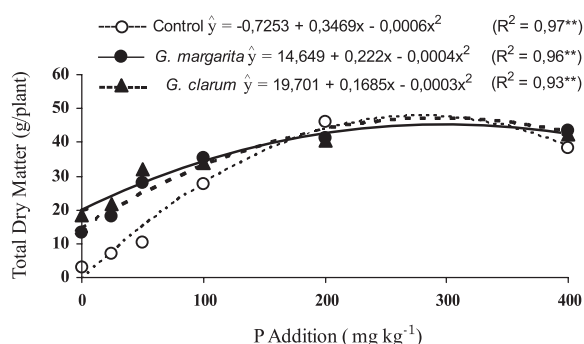


Figure 1. Total dry matter (shoot and root) of physic nut plants inoculated with arbuscular mycorrhizal fungi at different P levels. **: significant at 1 % level, by F test.

The level of mycorrhizal efficiency to benefit plant development can be determined by calculating the P rate required to produce 60 % of the maximum total dry matter. Plants inoculated with *G. margarita* grew to 60 % of the maximum plant height at a rate of 50 mg kg⁻¹ P. In non-mycorrhizal plants, 100 mg kg⁻¹ P was required to obtain the same response. This high mycorrhizal efficiency at low P levels is in agreement with other results obtained in the same experiment. At low soil P levels, AMF inoculation had a positive effect on physic nut plant development, number of leaves, total leaf area and area per leaf (Balota et al., 2011).

In both mycorrhizal and non-mycorrhizal plants, the addition of P increased TDM at low P levels. The values recorded in this study were higher than reported by Carvalho (2008), who stated that mycorrhizal inoculation increased physic nut TDM by 113 %. In the present study, mycorrhizal benefits were greater at lower P levels, and decreased with increasing P rates. According to Carvalho (2008), mycorrhizal benefits were greater at high P levels, while no differences were observed between mycorrhizal and non-mycorrhizal plants at low P levels. Our study was evaluated after 180 days, while the said study of Carvalho (2008) lasted only 78 days.

A number of authors reported that high P levels hampered the growth of mycorrhizal plants on several species, including papaya (Trindade et al., 2000, 2001), trema, fedegoso (Paron et al., 1997) and the mangaba tree (Cardoso Filho et al., 2008). The high P levels can inhibit symbioses and affect growth. However, this effect is variable in different plant species, because it depends on the plant nutrient requirement. While negative effects were observed on mycorrhizal passion fruit plants at 16 mg kg⁻¹ of available P (Anjos et al., 2005), 50 and 80 mg kg⁻¹ P were required to induce negative changes in mycorrhizal mangaba trees (Cardoso Filho et al., 2008) and papaya (Trindade et al., 2000), respectively. The results show that mycorrhizal physic nut plants were less sensitive to P levels than these other plant species, as little more than 100 mg kg⁻¹ P was required to induce significant changes in its development.

These growth-inhibiting effects are generally explained by the energy demand of maintaining a symbiosis. The carbohydrates required by the fungi are supplied by the plant. Graham (2000) estimated that up to 20 % of the carbohydrates produced by a plant can be allocated to fungi.

Phosphorus concentration in shoots and roots

The P concentrations in the shoots and roots of physic nut plants increased with P application. A second-degree polynomial model was used to describe

these changes in the non-mycorrhizal plants, and a linear model was used for the mycorrhizal plants. At low P levels, the mycorrhizal plants had higher P shoot and root concentrations than the non-mycorrhizal plants. AMF significantly increased P concentrations in the shoots, with increases of 158, 280, 187 and 183 % in the treatments P0, P25, P50 and P100, respectively (Figure 2a). Similarly, P root concentration increases of 96, 156, 231 and 170 % were observed in the roots in the treatments P0, P25, P50 and P100, respectively (Figure 2b). No significant differences in the P concentrations of shoots and roots were observed between the plants inoculated with the two AMF species. The P concentration data recorded in this study were similar to those of Carvalho (2008), who observed that mycorrhizal inoculation increased the P concentrations in physic nut shoots and roots by 110 and 136 %, respectively.

At low P levels, AMF contributed to P uptake. However, compared to non-mycorrhizal plants, this contribution decreased with increasing P rates. Nevertheless, up to the rate P100, the P concentration was approximately twice as high in the mycorrhizal plants. At higher P levels (e.g., P400), the P concentrations in the mycorrhizal and non-mycorrhizal plants were similar. Similar results were observed in citrus seedlings by Nogueira & Cardoso (2006). At high soil P levels, the symbiosis did not benefit the plant ability to take up P because the root system can absorb P independent of mycorrhizae.

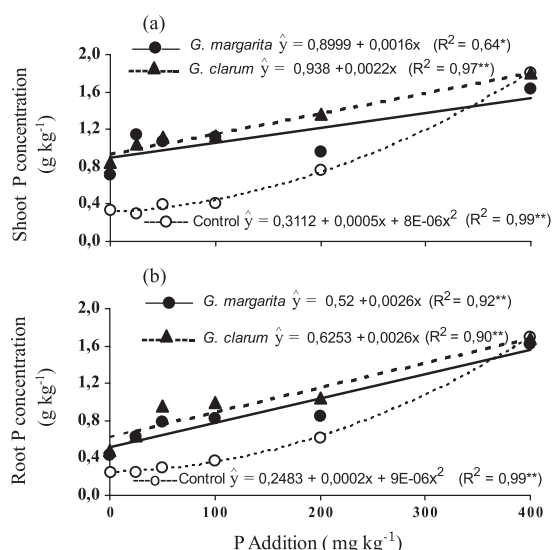


Figure 2. Shoot (a) and root (b) P concentration of physic nut plants inoculated with arbuscular mycorrhizal fungi at different P levels. * and ** significant at 5 and 1 % levels, respectively, by F test.

The results indicate the strong effects of AMF inoculation and high P demand of the physic nut plant, which, in turn, emphasize the importance of fertilizer application in soils with low P availability. Plants with coarsely branched roots with few or no root hairs, such as physic nut (Carvalho, 2008), are expected to be more dependent on (responsive to) mycorrhizae than plants with finely branched or fibrous roots (Siqueira & Saggin-Júnior, 2001; Zangaro et al., 2005).

Increased P uptake by mycorrhizal plants has been attributed to the increased soil exploration area that results from the production of external mycorrhizal mycelium, mycorrhizae ability to absorb nutrients more efficiently and the high affinity (lower K_m) of mycorrhizae for P uptake in soils with low P concentrations (Smith & Read, 1997). In addition, mycorrhizal hyphae absorb P more efficiently and can transport it over longer distances than the plant root system (Smith & Read, 1997; Sylvia, 1998). These characteristics are important because mycorrhizal plants access the same available P forms as non-mycorrhizal plants (Bolan, 1991). However, some studies have demonstrated that mycorrhizal plants obtain P from otherwise unavailable inorganic and organic sources (Koide & Kabir, 2000; Feng et al., 2003).

Phosphorus use quotient

In both mycorrhizal and non-mycorrhizal plants, the P use quotient (PUQ) indicated that the relationship between plant dry matter and P concentration followed a linear model, according to the P rate (Figure 3a). The non-mycorrhizal plants decrease markedly as P rates increased, while the decrease in the mycorrhizal plants was subtler. These results are in agreement with those of Baon et al. (1993), who studied eight barley cultivars for which the PUQ of mycorrhizal plants was lower than of non-mycorrhizal plants and in both treatments, the PUQ decreased as P rates increased.

Phosphorus use efficiency index

In the mycorrhizal and non-mycorrhizal plants, the P use efficiency index (PUEI), which describes the relationship between plant dry matter and P concentration, followed a quadratic model, adjusted for P addition, according to the P rate (Figure 3b). At low P levels (P0), the PUEI of the mycorrhizal was higher than of the non-mycorrhizal plants, while at higher P levels (P50 and P100), the PUEI of the non-mycorrhizal plants was approximately twice as high as in the mycorrhizal plants.

The PUEI of plants inoculated with *G. margarita* or *G. clarum* was similar. These results are in agreement with those for seedlings of *Dalbergia*

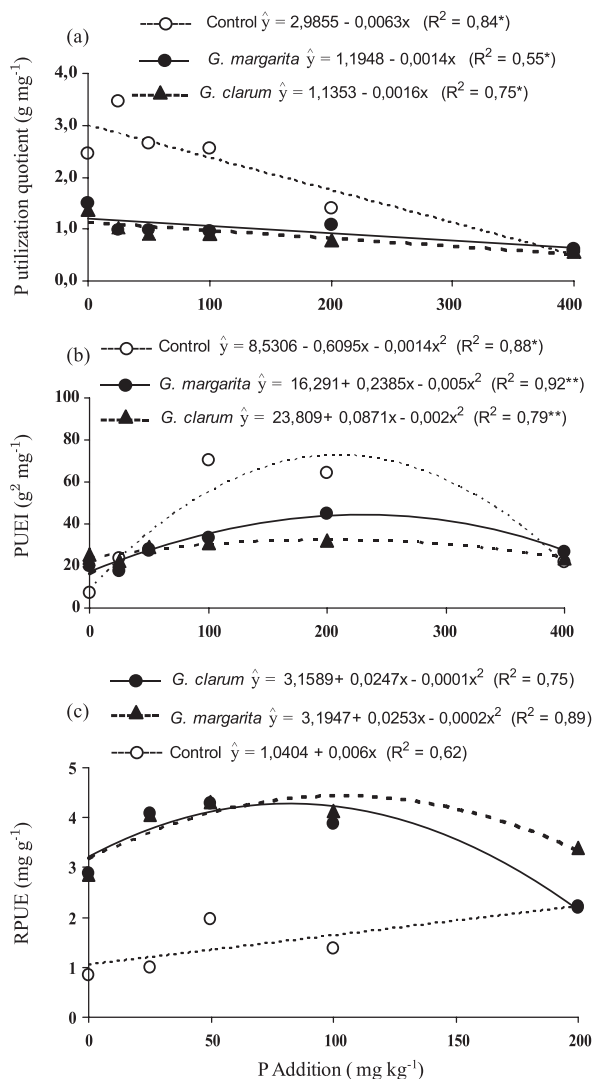


Figure 3. P use quotient (a), P use efficiency index (PUEI) (b) and root P uptake efficiency (RPUE) (c) of physic nut plants inoculated with arbuscular mycorrhizal fungi at different P levels. *: significant at 5 % by F test. The RPUE (c) was adjusted from P0 to P200 rates.

nigra, for which no differences in PUEI were observed for various AMF species (Chaves & Borges, 2005). However, the PUEI for coffee seedlings inoculated with *G. margarita* was higher than for seedlings inoculated with other AMF species (Tristão et al., 2006).

The initial increases of PUEI in physic nut plants at lower P levels and the subsequent decreases at higher P levels were also reported by Araújo et al. (1994) for tomato and by Trindade et al. (2000) for papaya. However, Araújo et al. (1994) stated that the PUEI of non-mycorrhizal tomato plants decreased with P rates and the PUEI of mycorrhizal

was higher than of non-mycorrhizal plants only at intermediate P levels (30 mg kg⁻¹). For papaya, the PUEI of mycorrhizal and non-mycorrhizal plants increased with P rates of up to 80 mg kg⁻¹ P and decreased above this rate, and the PUEI of mycorrhizal was higher than of non-mycorrhizal plants (Trindade et al., 2000). However, the effect of mycorrhizal inoculation on PUEI may depend on the plant and AMF species. For example, compared to non-mycorrhizal plants, mycorrhizal inoculation increased the PUEI of *Eucalyptus grandis* by 12 % and decreased the PUEI of *Sesbania virgata* by 28 % (Rodrigues et al., 2003). The PUEI values of chrysanthemum varied based on the AMF species. Compared to non-mycorrhizal plants, inoculation with *G. macrocarpum* or *G. leptotichum* increased PUEI values by 194 %, while inoculation with *G. margarita* reduced the index by 45 % (Silveira & Lima, 1996). Compared to the PUEI for non-mycorrhizal plants, the lower index of inoculated physic nut plants observed in this study could be attributed to higher P concentrations in the inoculated plant tissues and a higher C demand due to the mycorrhizal symbiosis.

Calculating P uptake efficiency by several methods with the same experimental data led to different results. Decreases in P utilization quotient (PUQ) values with increasing P rates could indicate that the plants grown at the lowest P levels used P most efficiently for biomass production. However, plants grown at lower P concentrations were minimally limited by P availability. Researchers have suggested that the apparent efficiency of P use in biomass production can result from a P dilution effect (Jarrel & Beverley, 1981; Israel & Rufty, 1988; Gourley et al., 1994). In addition, the PUQ is inversely related to nutrient concentrations that normally increase with P addition.

PUEI values had a similar relationship with biomass. Maximum PUEI and plant growth were observed in the P200 treatment, and the values decreased at higher P levels. Decreases in PUEI at higher P levels resulted from increased P accumulation without corresponding increases in plant growth, which could indicate a luxury level of P accumulation. The excessive accumulation of P without plant growth at high P levels may indicate that the use of P for dry matter production is not closely related with the regulation of P uptake by the root system (Israel & Rufty, 1988).

The PUEI of mycorrhizal plants was lower than that of non-mycorrhizal plants because in non-mycorrhizal plants the P concentrations increased by approximately 50 % per unit of applied P and the amount of biomass increased by 100 % per unit of P over mycorrhizal plants. However, the P concentrations in mycorrhizal plants in the P0 treatment (0.68 g kg⁻¹) were only observed in non-

mycorrhizal plants at P levels higher than those in the P200 treatment. Increases in the P concentration above 0.68 g kg^{-1} in non-mycorrhizal plants (P400) did not increase plant growth, while the biomass production of mycorrhizal plants increased as P concentrations increased to 1.21 g kg^{-1} (P200). These results suggest that increases in the amount of total dry matter for rates above P25 could have been due to other mycorrhizal effects. However, Carvalho (2008) observed significant increases in physic nut biomass production at shoot P concentrations of up to 2.5 g kg^{-1} in both non-mycorrhizal and mycorrhizal plants.

PUEI can help determine deficient, optimal and luxury P levels and decrease the dilution effects, allowing a better comparison of plant responses to nutrient addition (Israel & Rufty, 1988; Siddiqi & Glass, 1981). However, according to Araújo (1996), this index does not completely eliminate the effects of P dilution in plants.

Root phosphorus uptake efficiency

The root P uptake efficiency (RPUE), which is based on P concentrations in dry shoots, indicates the ability of plants to absorb and transport P to shoots. RPUE values for the non-mycorrhizal plants were fitted with a linear model while the mycorrhizal plants followed a second-degree polynomial regression model, adjusted for P addition (Figure 3c). However the RPUE was adjusted according to the P rates up to P200 due to the atypical behavior of RPUE at P400 in mycorrhizal plants, which was observed below that rate due to a great increase of P accumulation (78 %), compared to P200, without corresponding increases in plant growth (Balota et al., 2011), which could indicate a luxury level of P accumulation. AMF inoculation increased RPUE values by 238, 307, 117, and 198 % in the P0, P25, P50 and P100 treatments, respectively. No significant differences were observed between the mycorrhizal and non-mycorrhizal plants in the P200 and P400 treatments. These beneficial results are in agreement with those observed in *Dalbergia nigra* (Chaves & Borges, 2005) and papaya (Trindade et al., 2000).

Variations in the RPUE indicate a varying capacity of the plant roots of each treatment to absorb P from the soil solution and transport it to the shoots. The higher values for the mycorrhizal plants could be attributed to the external mycelium ability to increase the surface area of absorption and to stimulate P transport in the host plant (Smith & Read, 1997). High RPUE values at low P levels are important because absorption efficiency is related to a plant ability to absorb sufficient amounts of P without establishing an extensive root net, an important condition for plant growth at low P levels.

Mycorrhizal root colonization and sporulation

The values of mycorrhizal root colonization and AMF sporulation were negatively influenced by P addition and followed a linear model, according to the P rate (Figure 4). The amount of mycorrhizal root colonization was considered to be low (10 to 29 %) compared to other studies with physic nut (Carvalho, 2008). Mycorrhizal sporulation was high in the P0 treatment (more than 200 spores) and low in the P400 treatment (approximately 3 *G. margarita* spores and 13 *G. clarum* spores). In general, sporulation decreased as P rates increased. No differences in mycorrhizal root colonization and sporulation were observed between the two AMF species.

Previous studies indicated that mycorrhizal root colonization and sporulation decrease with increasing P availability. However, this effect and its intensity are determined by the host plant, AMF species and soil conditions (Paron et al., 1997; Siqueira et al., 1998; Melloni et al., 2000; Trindade et al., 2000; Costa et al., 2005; Nogueira & Cardoso, 2006; Cardoso Filho et al., 2008). The addition of P decreased root colonization in the mangaba tree (Costa et al., 2005; Cardoso Filho et al., 2008). Cardoso Filho et al. (2008) observed that mangaba root colonization by *G. margarita* or *G. etunicatum* continuously decreased with increasing P levels, but when native AMF species were used, P levels

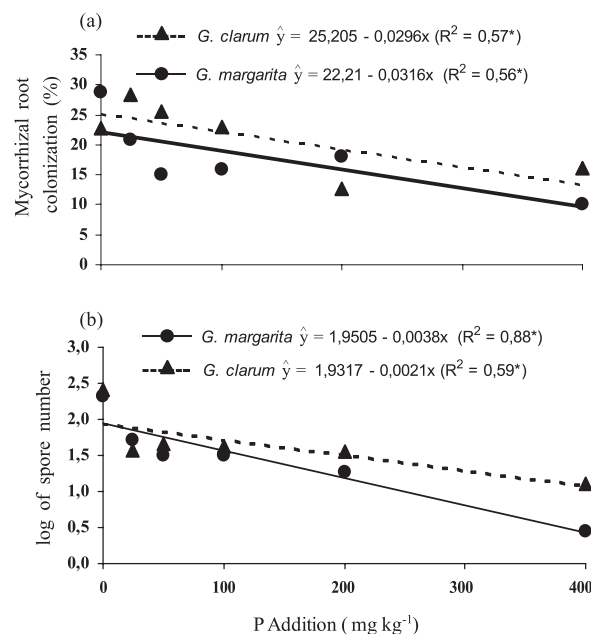


Figure 4. Mycorrhizal root colonization (a) and log of spore number (b) in 50 cm^3 soil cultivated in physic nut plants inoculated with arbuscular mycorrhizal fungi at different P levels. *: significant at 5 % levels by F test.

did not affect mycorrhizal colonization. Decreased colonization with increasing P levels was also observed in papaya inoculated with *G. margarita* and *G. clarum*, while colonization increased in plants inoculated with *A. scobiculata* and other indigenous species up to an intermediate P level (about P60) and decreased at higher levels (Trindade et al., 2000). P addition induced variable root colonization effects in native woody species; minor effects were observed in fedegoso (*Senna macranthera*) tree, but colonization was high (75 %) in trema (*Trema micrantha*), and decreased at rates above P220 (Paron et al., 1997).

Similar effects were observed in citrus seedlings, where root colonization was high (60 %) at up to 200 mg kg⁻¹ P and an accentuated decrease (8 %) at 1,000 mg kg⁻¹ P (Nogueira & Cardoso, 2006). Although this colonization level was considered to be low, it hampered plant growth. Similar results were observed under field conditions. Root colonization and spore numbers decreased with P addition in inoculated coffee seedlings transplanted into a nutrient-poor soil (Siqueira et al., 1998). However, the effects of P addition may vary based on the fungal species. For example, P fertilization negatively affected the sporulation of *Gigaspora* and *Scutellospora* but increased that of *Glomus intraradices* (Kahiluoto et al., 2001).

The mechanism by which soil P regulates mycorrhizal symbiosis is not yet clearly understood. However, several hypotheses have been proposed to explain the effects of P concentration on mycorrhizal colonization: increased lecithin in the roots may block the mycorrhizal infection; alteration of the permeability of the root cell membranes may decrease the quantity of exudates and consequently decrease germination and mycelium growth; and root sucrose increases may decrease root colonization because of changes in photosynthesis (Moreira & Siqueira, 2006).

The reduction in root colonization and sporulation caused by P addition (above P25) observed in this study indicated that the species used were sensitive to low soil P level (15 mg kg⁻¹). Although no positive effect was observed on dry matter production, AMF increased plant P concentrations after the addition of up to 48 mg kg⁻¹ (P100). AMF species with high levels of colonization at high P levels may become parasitic as a result of the great plant demand for carbohydrates (Graham et al., 1996; Moreira & Siqueira, 2006).

Mycorrhizal effectiveness

At a given level of soil fertility, mycorrhizal effectiveness (ME) indicates the advantage in variables related to dry shoots and roots in mycorrhizal than in non-mycorrhizal plants. At low P levels, the ME values were relatively high, with

maxima of 486 % and 310 % for shoot dry matter (SDM) and root dry matter (RDM), respectively. For P rates above those in the P200 treatment, ME decreased to nearly zero (Figure 5). However, ME could be highly variable as a result of the underlying variables. Decreases in ME with increasing soil P levels indicate the mycorrhizal beneficial effect at low P levels.

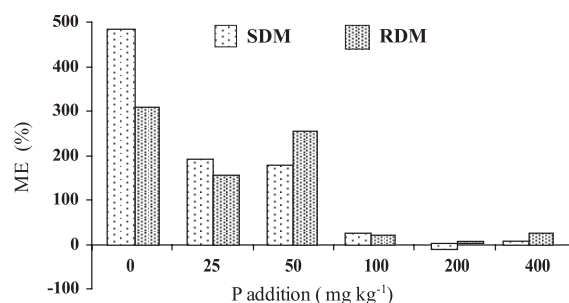


Figure 5. Mycorrhizal effectiveness based on shoot (SDM) and root (RDM) dry matter. Mycorrhizal effectiveness (ME) = [(dry matter of mycorrhizal plant) – (dry matter of non-mycorrhizal plant)] / (dry matter of non-mycorrhizal plant) × 100.

CONCLUSIONS

1. Mycorrhizal inoculation of the physic nut plants increased growth, P concentration and root P uptake efficiency at low soil P concentrations.
2. As P rates increased, the P use quotient decreased. In turn, the P use efficiency index increased at low P levels and decreased at high P levels.
3. Mycorrhizal root colonization and AMF sporulation were negatively influenced by P rates.
4. The highest mycorrhizal efficiency was observed when the soil contained between 7.8 and 25 mg kg⁻¹ P in soil.
5. The physic nut plants responded strongly to P addition independent of mycorrhizal inoculation.

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