

Response of an endangered tree species from Caatinga to mycorrhization and phosphorus fertilization

João Ricardo Gonçalves de Oliveira¹, Eliene Matos e Silva¹, Thaís Teixeira-Rios¹, Nataniel Franklin de Melo² and Adriana Mayumi Yano-Melo^{3,4}

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ABSTRACT

Schinopsis brasiliensis is an endangered tree species found in the Caatinga biome. It presents a characteristic slow development and difficult propagation, although it has been traditionally exploited in the region. Application of arbuscular mycorrhizal fungi (AMF) and phosphorus (P) fertilization may be beneficial to *S. brasiliensis* development at the seedling stage, which at the same time may help species conservation and the recovery of degraded areas in the Caatinga biome. We assessed the response of *S. brasiliensis* to AMF inoculation (*Claroideoglossum etunicatum* and *Acaulospora longula*) and P fertilization (0, 12, 24, and 48 mg dm⁻³ addition of P₂O₅). *S. brasiliensis* responded positively to both AMF inoculation and P fertilization. At low P concentrations, the inoculated plants showed higher leaf area and enhanced vegetative development, nutrient content and biomass production compared with non-inoculated plants. Conversely, increasing levels of P fertilization decreased the level of mycorrhizal colonization, plant responsiveness to inoculation, and spore production in *C. etunicatum*. Thus, P concentrations were able to influence the response of *S. brasiliensis* to mycorrhization and responsiveness to increased mycorrhization with the decrease in P availability. These results showed that mycorrhizal symbiosis plays an essential role in the development of *S. brasiliensis*.

Keywords: arbuscular mycorrhizal fungi, native plants, nutrient concentration, seedling production, semiarid area

Introduction

Caatinga is the only biome exclusive to Brazil. This biome occupies approximately 10% of the entire Brazilian territory (844.453 km²) and is the dominant biome (54%) in the northeast region. The Caatinga is a region rich in habitats and species; however, it is also exposed to anthropogenic pressures and intensive exploitation of the native vegetation. As a consequence, the soil has gradually lost regeneration potential, which has become increasingly apparent over the past few years. However, despite this issue currently affecting approximately 70% of the biome's original area and causing invaluable losses to the biological richness (Tabarelli & Silva 2003), no specific conservation plans are in place to mitigate it.

Not all forest plant species are able to colonize and establish in degraded soils, mainly due to low nutrient acquisition as a consequence of low nutrient availability in these soils (Sugai *et al.* 2010). Native Caatinga plant species are known to present this type of limitation (Andrade

et al. 2009); in addition, many of them are subjected to exploitation due to their multiple traditional uses. This is the case of the tree species *Schinopsis brasiliensis* (Santos *et al.* 2008); this species is considered a secondary succession species of the Caatinga biome (Carvalho *et al.* 2012), listed as an endangered species in Brazil (Oliveira *et al.* 2007).

A possible way to circumvent these difficulties and to conserve Caatinga plant resources is through the use of symbiotic microorganisms, such as arbuscular mycorrhizal fungi (AMF), which plays a key role in ecosystem maintenance (Heijden *et al.* 1998). Mycorrhization increases the absorption area around the root by increasing the area of the surface in contact with the soil, increasing the absorption of mineral nutrients, such as phosphorus, zinc, copper, nitrogen, and potassium. This higher nutrient acquisition directly influences plant growth and tolerance to environmental stresses (Smith & Read 2008), such as drought stress (Zhu *et al.* 2012), typical of the semiarid environment of Caatinga biome.

Approximately 80 species of AMF are known from Caatinga (Goto *et al.* 2010). Some isolates from the region

¹ Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Departamento de Micologia, Rua Nelson Chaves, s/n, Cidade Universitária, CEP 50670-420, Recife, PE, Brazil.

² Embrapa Semiárido, Laboratório de Biotecnologia, Caixa Postal 23, CEP 56302-970, Petrolina, PE, Brazil.

³ Universidade Federal do Vale do São Francisco, Campus de Ciências Agrárias, Rodovia BR 407, Km 12, Lote 543, Projeto de Irrigação Nilo Coelho, s/n, "CI", CEP 56300-990, Petrolina, PE, Brazil.

⁴ Author for correspondence: amymelo17@hotmail.com

have shown tolerance to environmental stress (Teixeira-Rios *et al.* 2013); however, little is known about the contribution of this symbiosis to the survival and development of native plants in semiarid environments (Maia *et al.* 2010).

Native tree species able to interact with AMF are of great importance in the recovery of degraded areas and in reforestation with economically important species because the majority of the areas destined for reforestation are nutrient-poor, which can prevent successful seedling establishment and early development (Sugai *et al.* 2010). The only studies focused on Caatinga native plant species showed that plant development of most plant species could be enhanced by inoculation with AMF or *Rhizobium* spp. and/or phosphorus (P) fertilization. These treatments, alone or in combination, have been shown to enhance shoot and root seedling biomass of *Anadenanthera macrocarpa* (Sugai *et al.* 2010), *Campomanesia cambessedesana*, *Hymenaea courbaril*, *Inga laurina*, and *Sterculia striata* (Lacerda *et al.* 2011).

There is a need to search and implement sustainable strategies for the use of Caatinga natural resources and native flora, particularly for endangered species. Thus, seedlings production technologies could be a suitable alternative for reducing costs and time by growing high quality plants more likely to succeed in degraded soils. Due to the benefits associated with AMF inoculation, mainly in nutrient-poor soils, the goal of the present study was to evaluate the response of *S. brasiliensis* seedlings to AMF inoculation and P fertilization on the initial growth stages and soil nutrient concentrations.

Materials and methods

Experimental conditions and design

The experiment was performed under controlled conditions in a glasshouse located in Petrolina (PE). The soil used (Ultisol) had the following characteristics: pH = 5.8; E.C. (electrical conductivity) = 0.51 dS m⁻¹; P = 6.14 mg dm⁻³; potassium (K) = 0.35 cmol_c dm⁻³; calcium (Ca) = 1.5 cmol_c dm⁻³; magnesium (Mg) = 1 cmol_c dm⁻³; sodium (Na) = 0.03 cmol_c dm⁻³; aluminum (Al) = 0.1 cmol_c dm⁻³; CTC (cation-exchange capacity) = 7.5 cmol_c dm⁻³ and 10.96 g kg⁻¹ organic matter. This soil was not limed. All soil chemical properties were characterized before sterilization and the addition of phosphorus.

The soil was autoclaved 3 times on 3 consecutive days, for 1 h at 121°C and left without treatment for 15 days. P was subsequently added to the soil as single superphosphate (P₂O₅) and thoroughly mixed with the soil. P was added at different quantities depending on the treatment, and the soil-P mixture was placed in bags with a capacity of 2.0 kg. The experiment followed a completely randomized design with a 3 × 4 factorial scheme, with 3 inoculation treatments: Control (non-inoculated); *Claroideoglomus etunicatum* [Becker & Gerd.] C. Walker & A. Schüssler

(UNIVASF06) and *Acaulospora longula* Spain & Schenck (UNIVASF12), 4 levels of added P [0 (no P₂O₅ addition), 12, 24, and 48 mg dm⁻³ P₂O₅], and 10 replicates. The soil without additional P was considered as natural soil fertility, containing 6.15 mg dm⁻³P (hereafter, treatment P6). The remaining treatments were designated as P12, P24, and P48.

Mycorrhizal isolates and *Schinopsis brasiliensis* Engl. Seedlings

C. etunicatum (UNIVASF06) and *A. longula* (UNIVASF12) isolates were obtained from the Univasf inoculum bank and propagated into pots containing previously sterilized sand:soil (1:1 v/v) with sorghum as the host plant (*Sorghum bicolor* [L.] Moench.) maintained in a glasshouse. The spores produced by the inoculum were extracted by wet sieving and decanting (Gerdemann & Nicolson 1963), followed by water and sucrose (40% w/v) centrifugation (Jenkins 1964). The number of spores was quantified in Petri dishes scribed with parallel lines, using a stereomicroscope (40×). The amount of inoculum used was determined based on number of spores contained by the inoculum. The inoculum comprised soil, mycorrhizal mycelium, colonized root fragments, and approximately 200 glomerospores placed below the plant roots.

S. brasiliensis seedlings were germinated from seeds whose tegument had been previously cut in the region opposite to the embryo. The seeds were surface-sterilized with sodium hypochlorite (0.05% active chlorine v/v) for 15 min, rinsed with distilled water and sown in trays containing vermiculite. Seedlings with three leaves were transferred to bags previously filled with inoculated and P-fertilized soil.

Data collection

Height, number of leaves, and stem diameter were measured every two weeks during growth in the glasshouse. After 135 days, we measured the percentage of seedling survival, leaf area, shoot and root fresh and dry weights, mycorrhizal colonization, and number of spores per pot. To determine dry weight, *S. brasiliensis* leaf and root samples were placed in an oven at 65°C until a constant weight was reached. Following weighting, shoot samples were homogenized using a Willey mill. Further, aliquots of 0.5 g of each sample were mineralized by nitric-perchloric acid digestion and used for elemental analysis. Ca, sulfur (S), Mg, iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) were quantified by atomic absorption spectrometry; P and boron (B) were quantified by colorimetry, and K and Na were quantified by flame photometry. Nitrogen (N) content was quantified by digesting a 0.1 g aliquot of each sample in sulfuric acid in the presence of selenium dust, copper sulfate, and potassium. All the analyses were performed according to the Embrapa manual for soil, plant, and fertilizer chemical analyses (Silva 1999).

Mycorrhizal colonization was quantified following the gridline-intersect method as previously described (Giovannetti & Mosse 1980). Prior to quantification, the roots were cleaned with 10% KOH, bleached with 10% H₂O₂, acidified with 1% HCl, and stained with Trypan Blue (0.05%) (Phillips & Hayman 1970). The number of spores was counted using the procedures described above (Gerdemann & Nicolson 1963; Jenkins 1964). Leaf area was estimated using a Li 3100 leaf area meter (LI-Cor Inc. Lincoln, Neb., USA). The increment resulting from the different treatments was calculated according to the following equation, adapted from Weber *et al.* (2004):

$$I (\%) = [(Tr - T) T^{-1}] \times 100$$

where I (%) represents variable increment, Tr is the average value for inoculated plants, and T is the average value for non-inoculated plants.

The mycorrhizal growth response was calculated according to Janos (2007):

$$[(A - B) A^{-1}] \times 100$$

where A is the dry weight of mycorrhizal plants and B is the dry weight of non-mycorrhizal plants (Plenchette *et al.* 1983). This response was categorized according to the terminology adapted from the study by Habte & Manjunath (1991) as very highly responsive (>75%), highly responsive (50%–70%), moderately responsive (25%–50%), marginally responsive (<25%) or non-dependent (non-responsive to mycorrhization).

Statistical analyses

To meet the assumption of homogeneity of variance, spore density data were log (x + 1) transformed, and mycorrhizal colonization data were arcsin ($\sqrt{x}/100$) transformed. A one-way ANOVA, followed by a Tukey's test, was used to test for significant differences among averages at $P \leq 0.05$. A regression was performed for the variables for which significant differences were found among different levels of P fertilization using the SANEST software (Pelotas, Brasil).

Results and discussion

Both AMF inoculation and P addition resulted in an increase in *S. brasiliensis* vegetative development. A maximal plant survival (100%) was obtained in most treatments, except for those inoculated with *C. etunicatum* with P6 and P48, which only reached 90% survival. Data on plant survival following treatments with different P levels in glasshouse assays are scarce; however, Carneiro *et al.* (2004) observed an inverse relationship between survival and increasing P levels in *Cecropia pachystachya* plants in a field experiment, with values of 88% to 25% from the lowest to the highest P addition.

Mycorrhization increased plant development at the lowest P levels tested. Plant height after 45 days, number of leaves after 60 days, and stem diameter after 75 days

differed significantly among P treatments; these results allowed to determine the best inoculation, and P fertilization treatment to achieve the desired positive effects (data not shown). These patterns were confirmed by subsequent measurements obtained up to 135 days after the beginning of the experiment (Fig. 1A-E), indicating that P addition influenced positively non-inoculated plants but had a negative effect on the development of mycorrhizal plants. This pattern was particularly obvious in plants associated with *C. etunicatum*, which were more sensitive to high P levels (P48). Lacerda *et al.* (2011) observed a significant effect of P on stem diameter after 60 days for *Dipterix alata* and after 90 days for *Sterculia striata*, *Jacaranda cuspidifolia*, *Inga laurina*, and *Campomanesia cambessedeanana* but only for plants inoculated with *Glomus clarum*.

The beneficial effects that mycorrhization has on plant development could be quantified by calculating the level of P level required for plants to grow to 60% of their maximum height (Balota *et al.* 2011b). This index exceeded 60% for plants grown without additional P (P6) and for those inoculated with AMF. Plant height did not significantly differ between plants with or without mycorrhizal inoculation, only for the highest P level (P48) (Fig. 1A). These results are similar to those observed by previous researchers regarding the response of Barbados nut (*Jatropha curcas*) plant height (Balota *et al.* 2011b) to 2.3 mg kg⁻¹ P, acerola (*Malpighia emarginata*) (Balota *et al.* 2011a) plant height response to 5.5 mg kg⁻¹ P and the response of *Acacia mearnsii* (Mello *et al.* 2008) to 8 mg L⁻¹P.

Previous research assessing the relation between seed weight and the response to mycorrhizal inoculation of plants at different stages of succession showed that larger seeds have greater energy reserves, hence presenting a reduced reliance on mycorrhization at the early stages of development and growth (Pasqualini *et al.* 2007). Therefore, *S. brasiliensis* seed weight (0.17 ± 0.02 g) may have enhanced plant response to mycorrhization.

P addition positively influenced shoot (SDW) and root (RDW) dry weight in both inoculated and non-inoculated plants (Fig. 1E-F respectively), decelerating plant vegetative development, as indicated by the mycorrhizal growth response. For plants inoculated with *C. etunicatum*, biomass increment (I) decreased from 1700%, 773.33%, 183.33% to 11.54% SDW and from 580%, 369.23%, 12% to -30% RDW from the lowest (P6) to P12, P24 and the highest (P48) P levels tested, respectively. In plants inoculated with *A. longula*, this decrease was less pronounced, from 1360%, 907%, 167% to 47.11% SDW and from 280%, 0.58%, 53.50% to -10% RDW, respectively. Mycorrhizal inoculation, P levels and host plant species are known to influence biomass gain in castor bean (*Ricinus communis*) (Machineski *et al.* 2011) and plantain (*Plantago major*) (Freitas *et al.* 2008).

With the exception of plants inoculated with *C. etunicatum* in the P48 treatment, all the inoculated plants presented significantly higher leaf areas than the respective

non-inoculated controls (Fig. 1D). Leaf area increment of mycorrhizal plants was influenced by both mycorrhizal inoculation and P fertilization. This increment ranged from 2671.6% to 1.3% for *C. etunicatum* inoculated plants and from 2070.7% to 32.8% for those inoculated with *A. longula* from the lowest (P6) to the highest (P48) P levels. Leaf area is directly related to photosynthetic capacity and indicates

mycorrhizal symbiosis efficiency (Balota *et al.* 2011b). Similarly to height, *C. etunicatum* and *A. longula* efficiency was inversely proportional to the level of P added to the soil.

Mycorrhizal colonization and AMF sporulation responded differently to increased P levels (Fig. 2). In both AMF tested, mycorrhizal colonization linearly decreased from the highest to the lowest level of P added to the soil (Fig. 2A). The

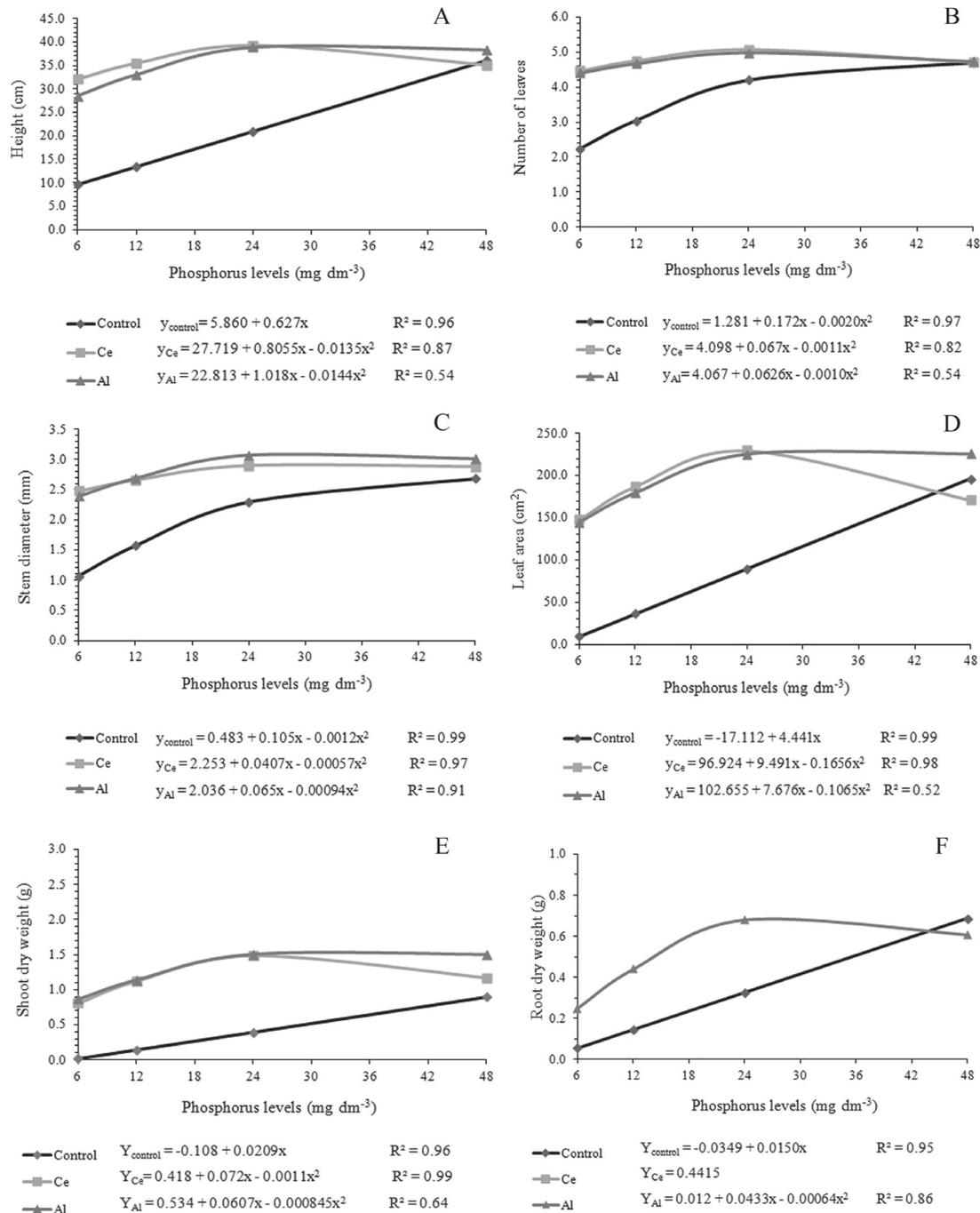


Figure 1. Plant height (A), number of leaves (B), stem diameter (C), leaf area (D), shoot dry weight (E), and root dry weight (F) of *Schinopsis brasiliensis* Engl. individuals inoculated with *Claroideoglomus etunicatum* (Ce) or *Acaulospora longula* (Al), and non-inoculated, grown with different levels of phosphorus (P6, P12, P24, and P48) in a glasshouse for 135 days.

number of spores produced by *A. longula* did not significantly differ among the different P treatments. However, sporulation of *C. etunicatum* peaked after adding 20.18 mg dm⁻³ of P. Similarly to mycorrhizal colonization, the number of spores was higher for *C. etunicatum* than for *A. longula*.

Mello *et al.* (2008) observed that colonization of *A. mearnsii* seedlings by AMF *Glomus clarum* and *C. etunicatum* does not decrease even at high levels of P, although sporulation decreases. Several studies have reported a lack of correlation between mycorrhizal colonization and sporulation (Machineski *et al.* 2011), which is similar to our results, where only the production of *C. etunicatum* spores was observed to respond to high P levels (Fig. 2B). According to Sanders (2004) and Balota *et al.* (2011a), different mycorrhizal isolates have different life strategies, which primarily depends on the plant host.

The decrease in mycorrhizal response with increasing soil P levels observed here showed the beneficial effect that mycorrhization confers when the level of P available is sub-optimal. The response to mycorrhization improved with lower levels of P addition (Fig. 3). Without P addition

(P6), plants were highly responsive to mycorrhization with both *C. etunicatum* (94%) and *A. longula* (92%). Mycorrhizal response gradually decreased with increasing P additions for both *C. etunicatum* (88, 64.4 and 10.3%) and *A. longula* (90, 62.2 and 31.7%). At the highest P level (P48), responsiveness to mycorrhization was marginal with *C. etunicatum* and moderate with *A. longula*. Similar levels of responsiveness were observed in plant species characteristic of Caatinga secondary forest, such as *A. macrocarpa* and *Caesalpinia ferrea* (Sugai *et al.* 2011), and in climax species from other biomes, such as *Luehea grandiflora*, *Senna spectabilis*, *Tibouchina granulosa*, *Cordia trichotoma*, *Leucaena leucocephala*, *Senna macranthera*, *Cedrella fissilis*, and *Myrsine umbellata* (Siqueira & Saggin-Júnior 2001).

In this study, we also quantified macro- (Fig. 4A-F) and micronutrient (Figs. 5A-F) content in *S. brasiliensis* plants. Highest levels of soil P availability (P48) resulted in greater uptake of this element and enhanced uptake of other elements such as N, K, Ca, Mg, B, Cu, Mn, and Zn in non-inoculated plants (Figs. 4A, C-E, 5A-B, and D-E, respectively), accelerating their development (Fig. 1). Efficiency in the acquisition

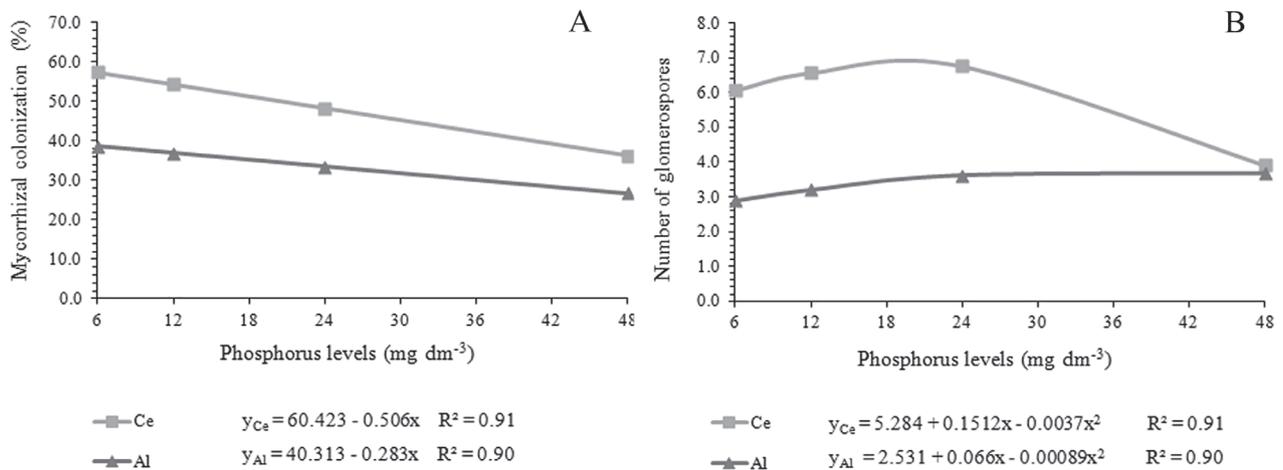


Figure 2. Mycorrhizal colonization (%) (A) and number of glomerospores (B) on the rhizosphere of *Schinopsis brasiliensis* Engl. plants inoculated with *Claroideoglomus etunicatum* (Ce) or *Acaulospora longula* (Al), or non-inoculated, grown with different levels of phosphorus (P6, P12, P24, and P48) in a glasshouse for 135 days.

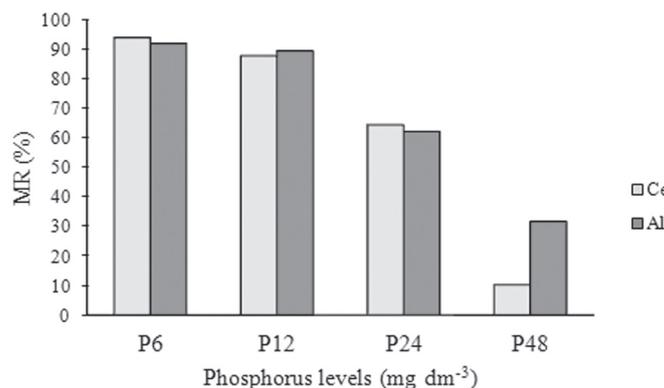


Figure 3. Mycorrhizal response (MR) of *Schinopsis brasiliensis* Engl. plants in association with *Claroideoglomus etunicatum* (Ce) or *Acaulospora longula* (Al) in soils with increasing phosphorus levels (P6, P12, P24, and P48) grown in a glasshouse for 135 days.

of macro- and micronutrients, independently of the amount of P added to the soil, has been also described for 14 out of 31 mycorrhizal tropical tree species analyzed (Carneiro *et al.* 1996), in particular for P, Ca and S.

Plants inoculated with *C. etunicatum* showed a linear decrease in K, B, Cu, Zn, and Na contents with increasing

P levels (Figs. 4C, 5A-B, and E-F, respectively); a similar response was found for N, Mg, Cu, and Zn in plants inoculated with *A. longula* (Figs. 4A, E, 5B, and E, respectively). Plants presenting favorable nutritional status develop mechanisms to reduce the development and/or activity of root AMF to reduce mycorrhization and its associated energy costs

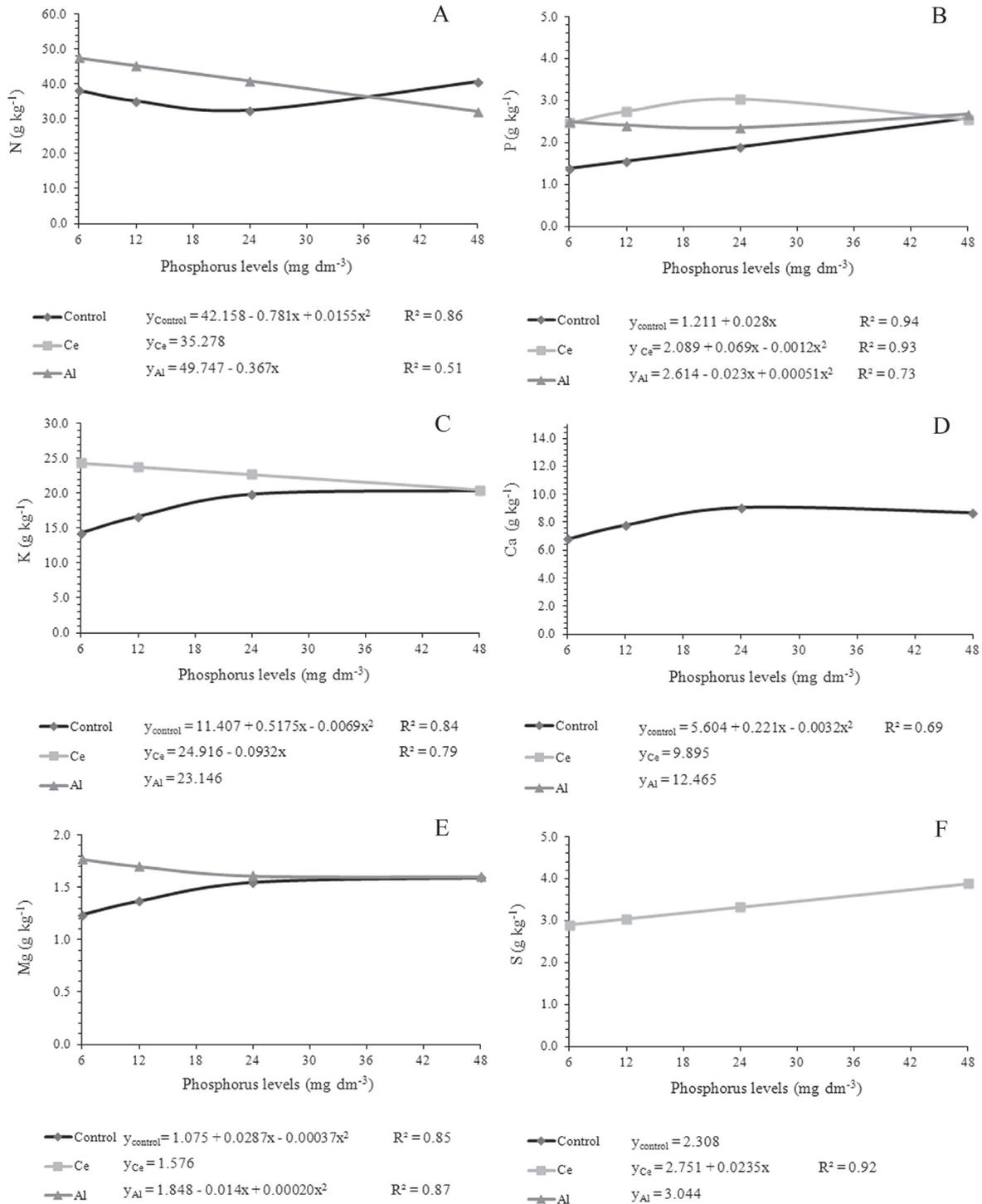


Figure 4. Macronutrient content [N (A), P (B), K (C), Ca (D), Mg (E) and S (F), in g kg⁻¹] in shoots of *Schinopsis brasiliensis* Engl. plants inoculated with *Claroideoglossum etunicatum* (Ce) or *Acaulospora longula* (Al), and non-inoculated, grown with different levels of phosphorus (P6, P12, P24, and P48) in a glasshouse for 135 days.

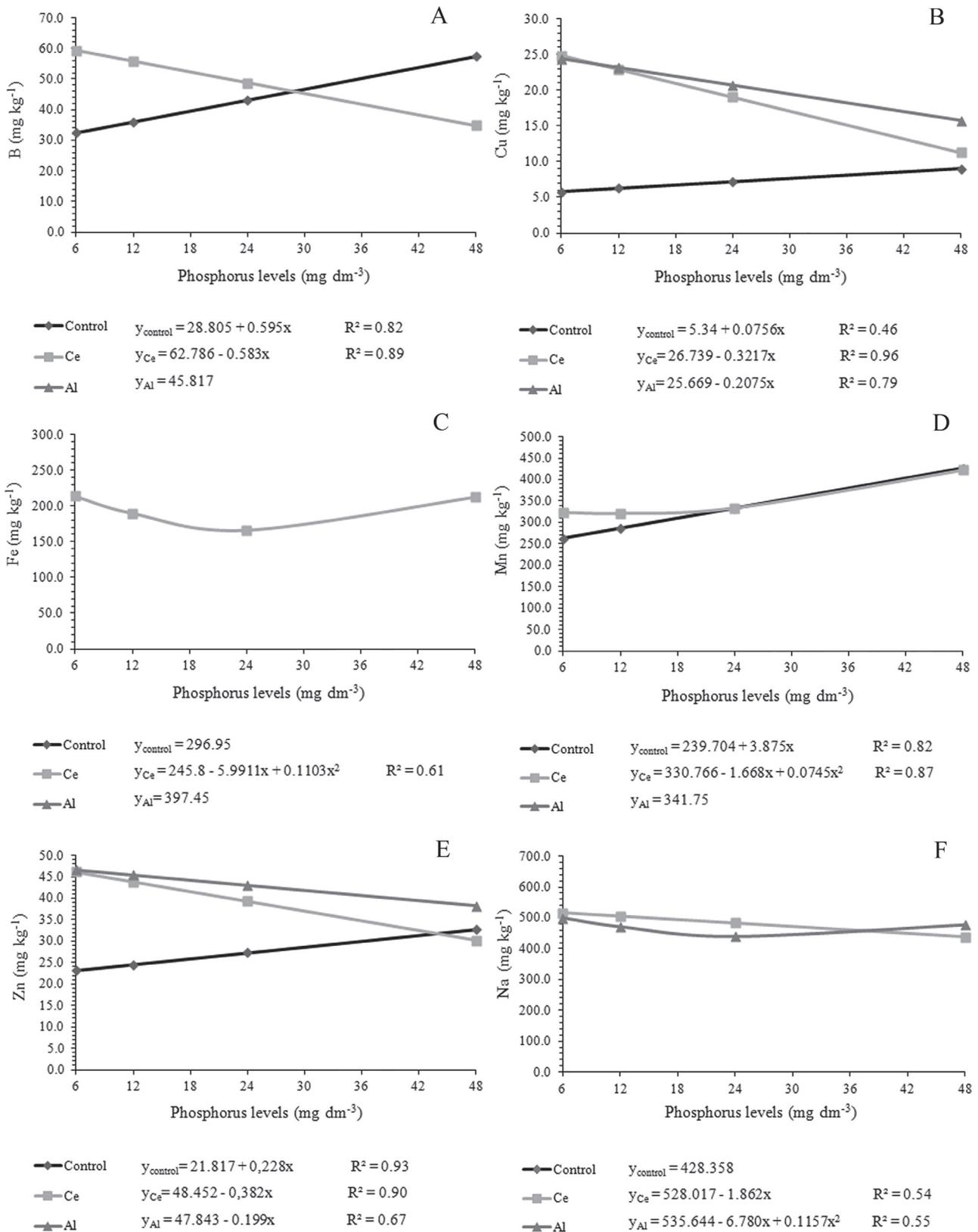


Figure 5. Micronutrient content [B (A), Cu (B), Fe (C), Mn (D) and Zn (E), in mg kg⁻¹] and Na (F; in mg kg⁻¹) in shoots of *Schinopsis brasiliensis* Engl. plants inoculated with *Claroideoglomus etunicatum* (Ce) or *Acaulospora longula* (Al), and non-inoculated, grown with different levels of phosphorus (P6, P12, P24, and P48) in a glasshouse for 135 days.

(Smith & Read 2008). The lower nutrient accumulation observed might be related to a decrease in the need for a symbiotic association due to higher P availability; this is reflected in the decrease of mycorrhizal colonization. This pattern has been also observed in *M. emarginata* plants (Balota *et al.* 2011a).

S and Fe were positively related to higher levels of P addition in plants inoculated with *C. etunicatum* (Figs. 4F, 5C respectively). These elements play important roles in aminoacid, protein, and chlorophyll synthesis and therefore in photosynthetic activity (Dechen & Nachtigall 2007; Hansch & Mendel 2009). Comparing mycorrhizal plants to non-mycorrhizal control individuals for each P level, we observed higher contents of P, Cu, and Zn in inoculated plants up to the P24 treatment (Figs. 4B, 5B, and E, respectively).

Low P availability is characteristic of Brazilian soils; in addition, the use of fertilizers can substantially impact the total costs associated with agricultural practices (Carneiro *et al.* 2009). The present results showed that mycorrhizal inoculation could represent an efficient alternative to obtain *S. brasiliensis* seedlings with an adequate nutrient and developmental status while reducing costs related to the use of P fertilizers, possibly guaranteeing better seedling establishment in the field.

In conclusion, *Schinopsis brasiliensis* plants responded to P fertilization, and showed a linear correlation between the level of P fertilization and both growth and nutrient content. This species was also highly responsive to mycorrhization, benefiting from the inoculation with *Claroideoglossum etunicatum* and *Acaulospora longula* in terms of vegetative development, especially in soils with low P concentrations. Considering that high P levels decreased mycorrhizal colonization and sporulation, particularly for *C. etunicatum* isolates, mycorrhizal inoculation can be recommended in substitution of P fertilization in soils with low P availability.

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