Symbiotic compatibility between arbuscular mycorrhizal fungi (autoctone or exotic) and three native species of the Caatinga in different phosphorus levels

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ABSTRACT. The aim of this study was to evaluate three plant species native to Caatinga (Aspidosperma pyrifolium, Ziziphus joazeiro and Pseudobombax simplicifolium) in response to inoculation with two arbuscular mycorrhizal fungi (autoctone: Claroideoglomus etunicatum or exotic: Acaulospora longula) and phosphate fertilization. The experiment was conducted in a greenhouse with a completely randomized design involving a factorial arrangement of three inoculation treatments (control, C. etunicatum or A. longula) and 4 levels of single superphosphate (0 - no added P₂O₅, 12, 24 and 48 mg dm⁻³) in 10 repetitions. Under the conditions of this study, the inoculum of C. etunicatum, autoctone to the semi-arid region, showed great compatibility and efficiency in promoting plant growth and nutrient intake when compared to the exotic inoculum of A. longula. Plants of A. pyrifolium were not responsive to mycorrhization and phosphate fertilization. In soil with low phosphorus content, mycorrhized plants of Z. joazeiro and P. simplicifolium presented better vegetative development with high shoot dry weight. Mycorrhized plants of Z. joazeiro had a proportional increase in the levels of macro- and micronutrients at the phosphorus doses applied. Mycorrhizal symbiosis may have a fundamental role in the development of these species from the Caatinga.

Keywords: Semi-arid, mycorrhiza, endemic species.

Introduction
The Brazilian semi-arid region covers an estimated area of 982,000 km² distributed in eight northeastern states, in addition to northern Minas Gerais (Silva et al., 2015). This region is home to a unique biome in the world, the Caatinga, which presents rich plant diversity despite years of intense extrativism for obtaining wood and opening of grazing areas in addition to other agricultural activities. The biome is considered to have high environmental vulnerability (Castelletti, Silva, Tabarelli, & Santos, 2003), which combined with...
low water availability, high average temperature and soil nutrient deficiency, characteristics of semi-arid regions (Martínez-García, Armas, Miranda, Padilla, & Pugnaire 2011), place even greater strains on plant development.

Arbuscular mycorrhizal fungi (AMF) are root symbionts of plants that add a new dimension to the soil/plant/microorganism system, directly and indirectly influencing soil nutrient cycling, thus being an important component of ecological plant succession and increasingly important for agriculture, agro-forestry, recovery of degraded areas and conservation of natural ecosystems (Covacevich, Echeverría, & Pagano, 2012).

Certain species of AMF feature great specificity with plant communities (Pagano et al., 2011) playing, according to standard plant succession, an important role in the composition and stability of these communities (Stürmer, Klauberg Filho, Hering de Queiroz, & Mendonça, 2006; Martínez-García & Pugnaire, 2011). Works with native species of AMF in other semi-arid regions have demonstrated great efficiency in their implementation, with advantages that can be extended to the time of transplanting, due to the maintenance of the biological properties of the soil (Barea et al., 2011) including the initial phase of revegetation (Alguacil, Torres, Torrecillas, Díaz, & Roldán, 2011).

Although it has been observed that native plants of the Caatinga present a mycorrhizal responsiveness modulated by the adverse conditions of the environment, such as low nutrient availability, including phosphorus (Sousa, Maia, Menezes, Sampaio, & Garrido, 2008) there are, as yet, few works that list the benefits of this symbiosis on survival and development of native plants in semi-arid regions (Maia, Silva, Yano-Melo, & Goto, 2010) and/or demonstrate the functional role of indigenous isolates from the biome in the development of native plants.

Considering the high diversity of AMF observed in the Caatinga (Goto, Silva, Yano-Melo, & Maia, 2010), optimization of phosphorus supply provided by mycorrhization (Smith & Smith, 2012) and the benefits in promoting the growth of native plants of other semi-arid regions subjected to inoculation with native AMF (Alguacil et al., 2011), this study aimed to test the efficiency of native and exotic mycorrhizal isolates of the Caatinga biome in the development and accumulation of nutrients of three native species (Aspidosperma pyrifolium Mart., Ziziphus joazeiro Mart. and Pseudobombax simplicifolium A. Robyns) in the biome’s own soil, supplemented or not with single superphosphate.

Material and methods

Procedure and experimental design

The experiment was developed under controlled conditions in a greenhouse, using soil (Acrisol) with the following physical and chemical attributes: 672.9, 250.1 and 76.94 g kg⁻¹ of sand, silt and clay, respectively, pH 5.8, electrical conductivity (E.C.) 0.51 dS m⁻¹, phosphorus (P) 6.14 mg dm⁻³, potassium (K) 0.35 cmol dm⁻³, calcium (Ca) 1.5 cmol dm⁻³, magnesium (Mg) 1 cmol dm⁻³, sodium (Na) 0.03 cmol dm⁻³, aluminium (Al) 0.1 cmol dm⁻³, iron (Fe) 34.1 mg dm⁻³; cationic exchange capacity (CEC) 7.5 cmol dm⁻³ and 10.96 g kg⁻¹ of organic matter (O.M.).

The soil was autoclaved three times for one hour at 121°C on three consecutive days, remaining at rest for 15 days. After this period, P in the form of single superphosphate (P₂O₅) was homogenized into the soil according to each treatment of phosphate fertilization and packaged in bags with a capacity of up to 2.5 kg. The experiment was conducted in a completely randomized design with a 3 × 4 factorial arrangement, of which 3 are inoculation treatments (control - not inoculated and inoculated with *Claroideoglomus etunicatum* (Becker & Gerd.) C. Walker & A. Schüssler or with *Acaulospora longula* Spain & Shenck) and 4 levels of phosphorus (0 – no P₂O₅ added, 12, 24 and 48 mg dm⁻³ of P₂O₅), in 10 repetitions. The soil without addition of P represents the natural fertility of the soil (6.14 mg dm⁻³) and in this way the treatments were designated as: P6, P12, P24 and P48.

Preparation of mycorrhizal isolates and seedlings

Isolates of *C. etunicatum* (UNIVASF06) and of *A. longula* (UNIVASF12) were propagated in the greenhouse, in pots containing a previously disinfected sand:soil (1:1 v/v) substrate and having sorghum [*Sorghum bicolor* (L.) Moench.] as host plant for three months. The inoculum produced was evaluated on the basis of the number of glomerospores using the techniques of decanting and wet sieving (Gerdemann & Nicolson, 1963), followed by centrifugation in water and sucrose (40% p/v) (Jenkins, 1964), and counting held in plates (grooved) under a stereomicroscope (40×). Upon counting the glomerospores, it was possible to determine the rate to be used as soil-inoculum, which contained about 200 glomerospores, in addition to fragments of colonized root and mycorrhizal mycelium to be deposited in the vicinity of the plant’s roots.

The seedlings of *A. pyrifolium*, *Z. joazeiro* and *P. simplicifolium* were obtained from the germination of

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seeds collected in an caatinga area of the Embrapa Semi-arid field station, located in Petrolina, State of Pernambuco, with a geographical coordinate of 09º04'14.8"S, 40º19'04.2" W, and 378 m (altitude). The seeds underwent a fumigation process with sodium hypochlorite (0.05% active chlorine) for 15 minutes followed by washings with distilled water, and seeds of Z. joazeiro were also passed through scarification, prior to distribution in trays containing autoclaved vermiculite. Seedlings of the three species with three definitive leaves were then transferred to previously prepared bags with soil in increasing doses of P in their respective inoculation treatment.

**Experimental evaluations**

The plants were monitored during the entire development phase in the greenhouse. After 120, 135 and 180 days of inoculation, respectively in P. simplicifolium, Z. joazeiro and A. pirifolium, the following parameters were measured: leaf area, shoot dry weight and root dry weight, nutrient content in the aerial part, mycorrhizal colonization and number of glomerospores. For determination of dry weight, samples of leaves and roots were placed in an oven (65°C) until constant weight was reached. After weighing, samples from the shoot were pounded in a Willye-type mill and portions of 0.5 g of sample were mineralized by nitric-perchloride digestion for subsequent determination of the levels of Ca, sulfur (S), Mg, Fe, zinc (Zn), copper (Cu) and manganese (Mn), by atomic absorption spectrophotometry, P and boron (B) by colorimetry, K and Na by emission flame photometry, in addition to nitrogen (N) by digestion of 0.1 g of the sample in sulfuric acid in the presence of a mixture of selenium, copper sulfate and potassium. All analyses followed the methodology described by Silva (1999). Mycorrhizal colonization was estimated by the intersection method of the quadrants (Giovannetti & Mosse, 1980), after diaphanization of the roots with 10% KOH and clearing with 10% H2O2, acidification in 1% HCl, and staining with Trypan Blue solution (0.05%) in lactoglycerol (Phillips & Hayman, 1970, modified). The number of glomerospores was evaluated by counting, using the techniques of Gerdemann and Nicolson (1963) and Jenkins (1964) for recovery of spores from the soil. The leaf area was estimated using the Li 3100 device (LI-Cor Inc. Lincoln, Neb., USA) and for determination of the resulting increase of the treatments an adapted formula of Weber et al. (2004) was used:

\[
I(\%) = \frac{(\text{Tr} - \text{T}) \times 100}{\text{T}}
\]

where:
- I (%) = increment;
- Tr = average value for the inoculated treatment;
- T = average value of the uninoculated treatment.

Following the concepts established by Janos (2007) as to the responsiveness of plants to mycorrhization, this was determined by the formula:

\[
\text{MR (\%)} = (\text{A-B}) \times A^{-1} \times 100
\]

where:
- A = dry weight of mycorrhized plants;
- B = dry weight of unmycorrhized plants; and
- categorized in terms adapted from the work of Habte & Manjunath (1991) as one of the following: excessively responsive plants (>75%), highly responsive (50-70%), moderately responsive (25-50%), marginally responsive (<25%) or independent (no response to mycorrhization).

**Statistical analyses**

To satisfy the homogeneity of variance, the data of the number of glomerospores were transformed into log (x + 1) and those of mycorrhizal colonization on arcsine (\(\sqrt{x/100}\)). The data were subjected to analysis of variance and averages compared by the Tukey test (p ≤ 0.05) and the significant variables for doses of P were subjected to regression analysis, which were performed using the SANEST program.

**Results and discussion**

The vegetative growth of plants of A. pirifolium was not influenced by the phosphate fertilization and/or inoculation with AMF. On the other hand, the growth of seedlings of P. simplicifolium and Z. joazeiro presented was influenced by mycorrhizal inoculation and phosphate fertilization, respectively. P. simplicifolium seedlings inoculated with C. etunicatum (Ce) had a greater number of leaves in relation to the control given only the natural fertility of the soil (P6), while for dry weight, there was an increase both in P6 and P12 using the same mycorrhizal inoculum.

For the plants of Z. joazeiro there was an interaction between mycorrhization and phosphate fertilization (Figure 1A-F), with pronounced development due to mycorrhization under smaller doses of P (6 and 12 mg dm\(^{-3}\)). The leaf area of mycorrhized plants was greater than that observed in...
uninoculated plants, increasing linearly with the doses of P (Figure 1D). Similarly, the shoot dry weight in plants associated with Ce had a 1515% increment in P6 and 448.12% in P12, whereas in plants inoculated with A. longula (Al) the increments were 1298.8% in P6 and 342.08% in P12 (Figure 1E). In relatively smaller proportion, the root dry weight (Figure 1F) also reflected the development of plants, with increment values of 305% in P6 and 136.18% in P12 when inoculated with Ce, and 177.67% in P6 and 86.72% in P12 when inoculated with Al.

**Figure 1.** Height (A), number of leaves (B), stem diameter (C), leaf area (D), shoot dry weight (E) and root dry weight (F) of Ziziphus joazeiro Mart., inoculated or not with Claroideoglomus etunicatum (Ce) and Acaulospora longula (Al), in soil with increasing doses of phosphorus, after 135 days in the greenhouse.
The benefits of mycorrhization for *Z. joazeiro* were more evident when the plants were grown in soils with low availability of P. Similar results were reported to tomato (*Lycopersicon esculentum* Mill.) (Schroeder & Janos, 2004) and peanut (*Arachis hypogea* L. cv. Runner IAC 886) (Hippler & Moreira, 2013). In addition, in other semi-arid regions of the world, such as India and Africa, plants of *Acacia nilotica* Delile var. cupressiformis Stewart and *Albizia lebbek* Benth. (Sharma, Bhatia, & Adholeya, 2001), and fruit trees of *Ziziphus mauritiana* Lam. and *Tamarindus indica* L. (Guissou, 2009), respectively, showed similar results, suggesting that AMF has played an important role for improving the growth of plants in limited availability of P. This relationship could result as much from a decrease in mycorrhizal colonization with increasing levels of P (Smith & Read, 2008) as from a smaller fungal contribution to the translocation of nutrients to the plant due to excessive drain of carbohydrates by the extrarradicular mycelium (Sena, Labate, & Cardoso, 2004).

Mycorrhizal colonization and production of glomerospores reduced linearly with the addition of P in the plant species studied (Figure 2).

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**Figure 2.** Mycorrhizal colonization and number of glomerospores in *Ziziphus joazeiro* Mart., *Pseudobombax simplicifolium* A. Robyns and *Aspidosperma pyrifolium* Mart., inoculated or not with *Claroideoglomus etunicatum* (Ce) and *Acaulospora longula* (Al) in soil with increasing doses of phosphorus, after 135, 120 and 180 days, respectively, in the greenhouse.
At the lowest concentration of P in the soil, the percentage of mycorrhizal colonization was observed to be low, especially in plants of *A. pirifolium* (9.35% *Ce* and 10% *Al*), showing similar values to those found by Souza, Maia, Sales, and Trufem (2003) under field conditions for the same plant species (7.2 to 18.6%). On the other hand, in plants of *Z. joazeiro* and *P. simplicifolium*, the root colonization by inoculum of *Ce* (54.54 and 50.27%, respectively) and *Al* (57.78 and 16.25%, respectively) reached percentages which provided developing plants with an accumulation of dry weight (Figure 1 and Table 1) and nutrient content (Figures 4 and 5).

According to Martínez-García & Pugnaire (2011), the AMF may exhibit specificity and/or preference for a particular plant host and this relationship can be mediated by the physical and chemical properties of the soil. For plants of *A. pirifolium*, no effect of the mycorrhizal isolates applied could be observed while increasing doses of P were seen to reduce mycorrhizal colonization and the production of glomerospores. In associations consisting of simbiotically cooperative isolates, high doses of P can trigger direction of photosynthates to other fungal activities, such as the increased production of vesicular structures in *G. aggregatum* (=Rhizoglomus aggregatum) and glomerospores in *G. custos* (=Rhizoglomus custos) (Kiers et al., 2011), in addition to the external mycelium of *G. etunicatum* (= *C. etunicatum*) (Melloni, Nogueira, Freire, & Cardoso, 2000).

**Table 1.** Number of leaves and shoot dry weight of plants of *Pseudobombax simplicifolium* A. Robyns (Imbiruçú), inoculated or not with *Claroideoglomus etunicatum* (*Ce*) and *Acaulospora longula* (*Al*) in soil with increasing doses of phosphorus (P6, P12, P24 and P48), after 120 days in the greenhouse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phosphorus levels</th>
<th>Number of leaves</th>
<th>Shoot dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P 6</td>
<td>P 12</td>
<td>P 24</td>
</tr>
<tr>
<td>Control</td>
<td>3.457 b</td>
<td>3.745 a</td>
<td>3.915 b</td>
</tr>
<tr>
<td>Ce</td>
<td>4.412 a</td>
<td>4.162 a</td>
<td>4.052 a</td>
</tr>
<tr>
<td>Al</td>
<td>4.080 ab</td>
<td>4.080 a</td>
<td>3.830 a</td>
</tr>
<tr>
<td>CV% = 12.22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter in the column do not differ by Tukey's test (P<0.05).

Plants of *Z. joazeiro* and *P. simplicifolium* were not shown to be responsive to the highest dose of P applied (P48), independent of the mycorrhizal inoculum used (Figure 3).

However, at smaller doses of P, it was noted that the responsiveness of the plants to mycorrhization varied with the inoculum used. In soil with natural fertility (P6) and P12, plants of *Z. joazeiro* mycorrhized by *Ce* and *Al* went from overly responsive to marginally responsive and non-responsive, respectively at P24. Plants of *P. simplicifolium* were categorized as highly responsive to mycorrhization up until the dose of P24, especially when the inoculum used was *Ce*, but when mycorrhized by *Al* the species presented moderate responsiveness at P6 and marginal responsiveness at P12.

In soils of semi-arid regions, nutrient deficiency is common and the climatic factors may contribute to making this environment severe and unfavorable to the growth of plants, making AMF highly important to the plant community (Martínez-García et al., 2011). Considering the results of responsiveness of plants of *Z. joazeiro* and *P. simplicifolium*, AMF possibly play an important role in the development of these species, especially at the natural fertility of soil of the Caatinga. Considering the adaptation of *Z. joazeiro* to the hydric deficiency and high evaporation of the Brazilian semi-arid region, studies must be conducted to verify whether...
AMF contribute to drought-tolerance and by what mechanisms this might occur, in order to optimize the development of this species and reduce extractivist pressure.

Macro and micronutrient content were determined only for plants of *Z. joazeiro* as the shoot dry weight production in the other species studied was insufficient for analysis (Figures 4 and 5). There was similarity between the treatments only in the S content (Figure 4E), which showed a linear increase with the addition of P to the soil. According to Malavolta (2006), there is an intrinsic relationship between S and P, it being likely that increased P availability in soil is related to the response observed to the content of S obtained by plants of *Z. joazeiro*. Working with a large number of tree species, Carneiro et al. (1996) were able to observe the same relationship in S content, principally in the species less responsive to the combined addition of P and mycorrhizae.

Figure 4. Macronutrient content (P, K, Ca, Mg, and S in g kg⁻¹) in the aerial part of plants of *Ziziphus joazeiro* Mart., inoculated or not with *Claroideoglomus etunicatum* (Ce) or *Acaulospora longula* (Al), in soil with increasing doses of phosphorus, after 135 days in the greenhouse.
Figure 5. Micronutrient content (Cu, Fe, Mn and Zn in mg kg\(^{-1}\)) and Na (mg kg\(^{-1}\)) in the aerial part of plants of *Ziziphus joazeiro* Mart., inoculated or not with *Claroideoglomus etunicatum* (Ce) or *Acaulospora longula* (Al), in soil with increasing doses of phosphorus, after 135 days in the greenhouse.

Some studies have indicated an interaction between content of Zn and shoot dry weight in 60% of the inoculated treatments (Pouyu-Rojas, Siqueira, & Santos, 2006). In plants of *Z. joazeiro* mycorrhized by *Ce*, this relationship has not yet been established, having instead a decrease in this micronutrient (Figure 5D), but not affecting the shoot dry weight (Figure 1E) and root dry weight (Figure 1F) of the plant, particularly at higher doses of P added to soil.

The other macro- and micronutrients showed specificity in relation to the dose of P and/or inoculum of AMF for accumulation, with the
addition of P exerting a differential effect on the isolates of AMF. Non-inoculated plants of Z. joazeiro showed a linear increase in the levels of Ca, Mg, Cu and Mn with the addition of P to the soil (Figure 4C and D, 5A and C). For levels of K, Zn and Fe, the quadratic model was observed with maximum absorption at 44.22; 31.25 and 28.6 mg dm⁻³ of P₂O₅, respectively (Figure 4B, 5B, and 5D), in addition to P and Na, respectively, with minimal absorption at 29.73 and 20.51 mg dm⁻³ of P₂O₅ (Figure 4A and 5E).

In plants mycorrhized by Ce, the macro- and micronutrients showed quadratic behavior with the addition of P, except for the content of Zn which decreased linearly with the increase in P dosage, a fact probably related to precipitation in zinc phosphate (Mengel & Kirkby, 2001). It is estimated that the maximum absorption for contents of P, K, Ca and Mg would be, respectively, at 39.46; 44.36; 34.5 and 39.26 mg dm⁻³ of P₂O₅ and for the micronutrients Cu, Fe and Mn at 31.15; 31.46 and 38.31 mg dm⁻³ of P₂O₅.

On the other hand, it is estimated that plants mycorrhized by Al have maximum absorption of Zn at 24.36 mg dm⁻³ of P₂O₅, showing a linear increase only in Fe content with added P. It is estimated that the concentrations of P, Ca, Mg, Mn, Cu and Na in plants mycorrhized by Al would have the minimum absorption in 14.53; 22.52; 25.30; 21.81; 27.44 and 28.62 mg dm⁻³ of P₂O₅, respectively.

Pouyu-Rojas et al. (2006) verified that although P is a key element for the nutritional status of plants, differences in compatibility between the symbionts could not be neglected in their work. The application of an inoculum of G. etunicatum (= C. etunicatum), isolated from a coffee agroecosystem, promoted benefits in the development of 13 out of 16 tropical tree species, however there was no nutritional benefit to any species studied (Pouyu-Rojas et al., 2006). On the other hand, the Ce inoculum applied in plants from Z. joazeiro promoted further development and supply of nutrients in the aerial part, confirming the efficiency attributed to isolates of the species used and also the compatibility between the symbionts (Munkvold, Kjoller, Vestberg, Rosendahl, & Jakobsen, 2004). It should be noted that the source of the isolate may influence efficiency, because the isolated from C. etunicatum (Univasf 06) used in this experiment, coming from soil cultivated with banana trees in the semi-arid region (Yano-Melo, Trufém, & Maia, 2003), was more effective than the exotic isolate of A. longula (Univasf 12), which came from an area of Atlantic forest. In the same way as noted by Barea et al. (2011), the use of AMF isolates native to the region where it is intended to apply them, proves to be an interesting strategy for greater efficiency in promoting development and nutritional intake of plants.

**Conclusion**

The inoculum of Claroideoglomus etunicatum, autoctone to the semi-arid region, proved to be more compatible and efficient in promoting plant growth and nutrient intake when compared to the exotic inoculum of Acaulospora longula.

Plants of A. pyrifolium are not responsive to phosphate fertilization nor mycorrhization for its vegetative development, but in plants of Z. joazeiro and P. simplicifolium, AMF possibly play an important role in the development of these species, especially at the natural fertility of soil of the Caatinga.

Mycorrhizal colonization and glomerospore production, independent of the host and inoculum used, reduces linearly with the addition of phosphorus to the soil.

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