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Symbiosis with AMF and leaf P_i supply increases water deficit tolerance of woody species from seasonal dry tropical forest

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ABSTRACT

In seasonal dry tropical forests, plants are subjected to severe water deficit, and the arbuscular mycorrhizal fungi (AMF) or inorganic phosphorus supply (P_i) can mitigate the effects of water deficit. This study aimed to assess the physiological performance of *Poincianella pyramidalis* subjected to water deficit in combination with arbuscular mycorrhizal fungi (AMF) and leaf inorganic phosphorus (P_i) supply. The experiment was conducted in a factorial arrangement of 2 water levels (+H_2O and −H_2O), 2 AMF levels (+AMF and −AMF) and 2 P_i levels (+P_i and −P_i). Leaf primary metabolism, dry shoot biomass and leaf mineral nutrients were evaluated. Inoculated AMF plants under well-watered and drought conditions had higher photosynthesis and higher shoot biomass. Under drought, AMF, P_i or AMF + P_i plants showed metabolic improvements in photosynthesis, leaf biochemistry and higher biomass compared to the plants under water deficit without AMF or P_i. After rehydration, those plants submitted to drought with AMF, P_i or AMF + P_i showed a faster recovery of photosynthesis compared to treatment under water deficit without AMF or P_i. However, plants under the drought condition with AMF showed a higher net photosynthesis rate. These findings suggest that AMF, P_i or AMF + P_i increase the drought tolerance in *P. pyramidalis*, and AMF associations under well-watered conditions increase shoot biomass and, under drought, promoted faster recovery of photosynthesis.

1. Introduction

Plants are subjected to severe water deficit in seasonal dry tropical forests, such as Caatinga, covering most of the Brazilian Northeast region (Santos et al., 2014). Additionally, there will be increased evaporation, decreased water availability, and intensified aridity in this region in the next years, directly influencing characteristics and the distribution of vegetation (Magrin et al., 2014). These features will lead to more occurrences of severe drought. Drought is a limiting major abiotic stress that decreases plant development and biomass accumulation (Golldack et al., 2014). Consequently, drought decreases plant water potential, photosynthesis and induces morphological and anatomical changes, as well as changes in organic solutes and antioxidants (Galle et al., 2011; Ivancich et al., 2012; Keunen et al., 2013; Oliveira et al., 2014; Rivas et al., 2013).

In addition to intrinsic mechanisms of tolerance, plants can develop mutualistic associations with different microorganisms present in the rhizosphere to withstand different stresses, including drought (Mendes et al., 2013). Thus, about 80% of land plants form associations with arbuscular mycorrhizal fungi (AMF) (Smith and Read, 2008) nearly 50% of the microbial biomass is found in tropical ecosystems (Olsson et al., 1999). This tolerance is promoted by the increased nutrient and water uptake by fungal hyphae, mainly phosphorus (P). At the same time, there is an increase in root hydraulic conductivity and gas exchange, higher osmotic adjustment, an increase in antioxidant system activity and secondary compound activity (Aroca et al., 2007; Neumann et al., 2009; Ruiz-Sánchez et al., 2010; Pedone-Bonfim et al., 2013; Bompadre et al., 2014).

Indeed, leaf inorganic phosphorus (P_i) content can mitigate water deficit effects on primary metabolism (Santos et al., 2006). Since about 80% of soil phosphorus content is static and unavailable (Suriyagoda et al., 2011), the symbiosis with AMF could increase biomass in woody species (Frosi et al., 2016). Although phosphorus is an essential element used in large amounts by plants, tropical soils generally have it in low concentrations (Miguel et al., 2013). Therefore, the increased plant tolerance to abiotic stress by P has been attributed to a greater stomatal conductance and photosyn-

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thesis (Santos et al., 2006), cell membrane stability and better water use (Faustino et al., 2013).

Thus, AMF inoculation and leaf P_i application may become potential tools for increased tolerance in plants exposed to semi-arid conditions aiming at degraded areas regeneration of seasonal dry tropical forests. Because *Poincianella pyramidalis* is suitable for reforestation of degraded areas and endemic woody species of Caatinga (Maia, 2004), we chose this species for the present work. There are studies on this species in pharmacology (Alviano et al., 2008), genetic diversity (Santos et al., 2012), anatomy and wood density (Silva et al., 2009), ecological succession (Falcão et al., 2015) and on AMF effects under P different levels (Frosi et al., 2016).

Therefore, this study aimed at evaluating *Poincianella pyramidalis* ecophysiological performance under greenhouse conditions and subjected to water deficit in combination with AMF and leaf P_i application. Our hypotheses were: (1) inoculated plants with AMF or leaf P_i supply would grow better under well-watered conditions and show higher stress tolerance when subjected to water deficit; and (2) plants showing combined inoculation of AMF and leaf P_i supply would grow better under well-watered conditions compared to plants with AMF or P_i in isolation, and present higher tolerance and faster recovery of leaf primary metabolism after rehydration when subjected to water deficit.

2. Materials and methods

2.1. Growth conditions and plant material

The experiment was conducted in a greenhouse at the Universidade Federal de Pernambuco (UFPE) (8°08'58"S, 34°56'55"W), under average temperature of 30 ± 2 °C and 50–60% relative humidity, with plants kept in pot capacity (300 mL) until the beginning of the water deficit. The design was completely randomized in a factorial arrangement of 2 water levels [irrigated (+H_2O) and drought (−H_2O)] × 2 symbiosis levels [inoculated (+AMF) and non-inoculated (−AMF)] × 2 leaf phosphorus levels [with leaf inorganic phosphorus (+P_i) and without leaf inorganic phosphorus (−P_i)], totaling eight treatments: +H_2O-AMF-P_i; +H_2O-AMF + P_i; +H_2O + AMF-P_i; +H_2O + AMF + P_i; −H_2O-AMF-P_i; −H_2O-AMF + P_i; −H_2O + AMF-P_i; −H_2O + AMF + P_i; with 8 replicates each and one plant per pot, totaling 64 experimental units. Of these 8 replicates, 4 were designed to evaluate biomass and nutrients (N, P and K) and 4 were intended for relative water content (RWC), gas exchange, chlorophyll fluorescence and biochemical analysis.

Isolated AMF [*Acaulospora longula* Spain & N.C. Schenck (URM FMA 07) and *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler (URM FMA 03)] were provided by the Mycorrhizas Laboratory's Inoculum Bank at the Mycology Department of UFPE. These AMF species were chosen because they belong to two most common genera in the Brazilian semiarid in Northeast region, in Pernambuco State (Silva et al., 2014). Inoculum of each isolate was placed in pots containing 5 kg of sterilized soil with culture of *Panicum miliaceum* L. and *Sorghum bicolor* (L) Moench to increase spore density in three months. Spores were extracted from the soil by wet sieving and centrifugation in water and sucrose (Jenkins, 1964; Gerdemann and Nicolson, 1963) and then measured under a stereomicroscope (40×) to quantify the number of spores per gram of soil used in multiplication process (soil inoculum), to ensure 150 spores of each AMF species per plant.

P. pyramidalis seeds were provided by the Centro de Referência para Recuperação de Áreas Degradadas (CRAD) – UNIVASF/Petrolina-PE. Seeds were sterilized in 1% hypochlorite (v/v) for 5 min, washed with deionized water and put to germinate in trays containing sterilized washed sand, average temperature of 28 ± 2 °C. After 20 days, seedlings were transferred to 100 mL pots with sterilized soil to optimize the colonization due to close contact between spores and roots. In those for inoculation, the soil inoculum content 150 spores from each AMF was applied in the root region, totaling 300 spores per plant. Non-inoculated plants received the same amount of soil inoculum autoclaved. After 30 days under these conditions, plants were transferred to pots with 5 kg with same soil type with phosphorus (P) concentration adjusted to 33 mg dm^{-3} by applying simple superphosphate in soil of all plants of every treatment. This concentration was determined in a prior study (Frosi et al., 2016), in which 33 mg dm^{-3} of P in soil promoted higher gain in plant biomass of this species under well-watered conditions.

The soil used was collected in Instituto Agronômico de Pernambuco (IPA), Pernambuco state, Brazil (7° 35'39"S, 34°54'23"W) and showed the following characteristics: P: 3 mg/dm^3; pH: 5.7 H_2O; Ca: 0.65 $cmol_c/dm^3$; Mg: 0.6 $cmol_c/dm^3$; K: 0:07 $cmol_c/dm^3$, classified as dystrophic yellow latosol. After the experiment, the soil of non-inoculated plants (-AMF) had the following characteristics: P: 29 mg/dm^3; pH: 5.9 H_2O; Ca: 1:15 $cmol_c/dm^3$; Mg: 0.8 $cmol_c/dm^3$; K: 0:07 $cmol_c/dm^3$, while the soil of inoculated plants (+AMF) showed: P: 3 mg/dm^3; pH: 6.4 H_2O; Ca: 0.6 $cmol_c/dm^3$; Mg: 0.6 $cmol_c/dm^3$; K: 0.05 $cmol_c/dm^3$.

Two days before the beginning of the stress, plants intended for leaf P_i supply treatments were sprayed with 20 mL of monoammonium phosphate solution (10 g P_i L^{-1}) and the others were sprayed with 20 mL of an equivalent dose of nitrogen as urea PA solution (2.64 g N L^{-1}) as described in Santos et al. (2006) to compensate for the N added in the Pi treatment. Water deficit was imposed by watering suspension when plants completed 6 months of development. The maximum stress was determined when gas exchange was close to zero, occurring after 12 days of drought. Rehydration was performed soon after measurements of maximum stress day with irrigation to the pot capacity in 4 subsequent days, the time required for the recovery of the relative water content. This investigation is very important to verify whether the plants have the capacity to recovery metabolism under well-hydrated condition after exposure to drought. Under maximum stress, leaf relative water content, soil moisture, gas exchange, chlorophyll *a* fluorescence, organic solutes and photosynthetic pigments were evaluated. After rehydration, the same variables were measured, along with shoot dry biomass and foliar nutrient.

2.2. Mycorrhizal colonization

For quantification of mycorrhizal colonization, four plants of each treatment were sampled. For each plant, 1 g of root were cleared with 10% KOH (w/v) for 48 h at 25 °C, washed with distilled water and stained with 0.1% acid fuchsin (w/v) for 12 h (Koske and Gemma, 1989). One hundred 1 cm pieces of each sample were examined under a microscope at 40× magnification (Giovannetti and Mosse, 1980). Root fragments having some AMF structure (arbuscules, vesicles, hyphae and/or spores) were considered colonized.

2.3. Leaf relative water content

Leaf discs of known size were collected at 6:00 am and immediately weighed on a precision scale (AND H200, Tokyo, JP) to obtain fresh weight (FW) and just after soaking for 24 h in deionized water and weighed again for turgid weight (TW). Subsequently, disks were dried for 48 h in forced ventilation and weighed to obtain dry weight (DW). The leaf relative water content was measured by the formula: Relative water content (%) = [FW-DW/TW-DW] × 100 (Barrs and Weatherley, 1962).