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GABRIELLA FROSI ALBUQUERQUE FIGUEIRÔA FARIA

Aspectos ecofisiológicos de espécies lenhosas nativas da Caatinga associadas a fungos micorrízicos arbusculares sob condições de seca e salinidade

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Orientador: **Prof. Dr. Mauro Guida Santos**

Co-orientadora: **Profª. Drª Leonor Costa Maia**

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"ASPECTOS ECOFISIOLÓGICOS DE ESPÉCIES LENHOSAS NATIVAS DA CAATINGA ASSOCIADAS A FUNGOS MICORRÍZICOS ARBUSCULARES SOB CONDIÇÕES DE SECA E SALINIDADE"

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BANCA EXAMINADORA

Prof° Dr. Mauro Guida dos Santos (Orientador) - UFPE

Prof° Dr. Pedro Sansberro (Universidad Nacional Del Nordeste)

Dr. José Ribamar Costa Ferreira Neto - UFPE

Prof[a] Dr[a]. Jarcilene Silva de Almeida Cortez - UFPE

Dr[a]. Indra Elena Costa Escobar – UFPE

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RESUMO

A seca e salinidade são os principais estresses abióticos causadores de perda de produtividade nas plantas, comuns em áreas áridas e semiáridas, como o Nordeste brasileiro. Duas ferramentas que podem mitigar os efeitos deletérios desses estresses nas plantas são os fungos micorrízicos arbusculares (FMA) e suprimento de fósforo inorgânico foliar (P_i), aumentando as chances de recuperação dessas áreas. O presente estudo analisou a simbiose com FMA de duas espécies lenhosas nativas da região, *Poincianella pyramidalis* e *Cnidoscolus quercifolius*, em diferentes níveis de fósforo no solo, além de aspectos ecofisiológicos de uma dessas espécies associada com FMA e aplicação de P_i foliar sob seca e salinidade em casa de vegetação. Foram avaliadas a colonização micorrízica, trocas gasosas, solutos orgânicos, pigmentos fotossintéticos, biomassa e nutrientes. Ambas espécies realizaram associação com FMA e foram beneficiadas no crescimento. Para *P. pyramidallis* e *C. quercifolius*, os níveis de 33 e 15 mg dm^{-3} de fósforo no solo, respectivamente, promoveram maior crescimento nas plantas micorrizadas. *P. pyramidalis* foi selecionada para os demais experimentos devido aos ganhos mais representativos. Nos experimentos de seca e salinidade, essa espécie na condição hidratada foi mais beneficiada com FMA isolado, com maior fotossíntese e biomassa. Quando submetidas à seca, plantas com FMA, P_i ou ambos não tiveram reduções da biomassa aérea e acumularam prolina; plantas com FMA isolado tiveram maiores concentrações de açúcares e clorofilas. Após reidratação, plantas com FMA, P_i ou ambos tiveram recuperação das trocas gasosas, enquanto plantas sem FMA e P_i apresentaram recuperação parcial comparado ao Controle. Entretanto, plantas com FMA isolado submetidas à seca tiveram fotossíntese superior ao Controle após reidratação. Sob condição salina, plantas com FMA, P_i ou FMA+P_i foram beneficiadas, apresentando menores reduções nas trocas gasosas e sem redução na biomassa da parte aérea. As plantas com FMA isolado sob salinidade tiveram menor acúmulo de Na^+ e Cl^- nas folhas e raízes, enquanto plantas com P_i e FMA+P_i tiveram maiores concentrações de Cl^- nas folhas. Já plantas sem FMA e P_i na condição salina apresentaram maior concentração de Cl^- na raiz. Portanto, a aplicação de P_i, FMA ou ambos promoveram maior tolerância de *P. pyramidalis* à seca e salinidade. Entretanto, a resposta superior foi observada nas plantas com FMA isolado.

Palavras chaves*:* Semiárido. Simbiose. Estresse abiótico. Fósforo. Tolerância.

ABSTRACT

Drought and salinity are the main abiotic stresses that cause loss in plant productivity, common in arid and semiarid areas, such the Brazilian Northeast. Two tools that can to mitigate the deleterious effects of these stresses are arbuscular mycorrhizal fungi (AMF) and leaf inorganic phosphorus supply (P_i), increasing the chances of recovery of these areas. The present study analyzed the symbiosis with AMF of two native woody species in the region, *Poincianella pyramidalis* and *Cnidoscolus quercifolius*, at different phosphorus levels in the soil, in addition to ecophysiological aspects of one these species associated with AFM and leaf P_i in a greenhouse. Were evaluated the mycorrhizal colonization, gas exchange, organic solutes, photosynthetic pigments, biomass and nutrients. Both species perform associations with AMF and were benefited in growth. For *P. pyramidalis* and *C. quercifolius*, the phosphorus levels of 33 e 15 mg dm^{-3}, respectively, promoted the higher growth in mycorrhizal plants. *P. pyramidalis* was selected for the other experiments by presenting more representative gains. In drought and salt experiments, this species under well-watered condition was more benefited with AMF isolated, with higher photosynthesis and biomass. When submitted to drought, plants with AMF, P_i or both did not have reductions in shoot biomass and had a proline accumulation; plants with AMF isolated had higher concentrations of sugars and chlorophyll. After rehydration, plants with AMF, P_i or both had a recovery in gas exchange, while plants without AMF and P_i showed a partial recovery compared to Control plants. However, plants with AMF isolated submitted to drought had a higher photosynthesis compared to Control after rehydration. Under saline condition, plants with AMF, P_i or both were benefited, showed lower reductions in gas exchange and no reduction in shoot biomass. Plants with AMF isolated under salt had less accumulation of Na^+ and Cl^- in leaves and roots, while plants with P_i and AMF+P_i had higher accumulation of Cl^- in leaves. Plants without AMF and P_i showed a higher concentration of Cl^- in roots. Therefore, the leaf P_i supply, AMF or both promoted a higher tolerance in *P. pyramidalis* to drought and salt. However, the superior response was observed in plants with isolated AMF.

Keywords*:* Semiarid. Symbiosis. abiotic stress. Phosphorus. Tolerance.

SUMÁRIO

1. APRESENTAÇÃO

A Caatinga é uma Floresta Tropical Sazonalmente Seca brasileira, sendo boa parte de sua localização no semiárido nordestino, com características fitogeográficas específicas (MORO et al. 2016). Diante da forte pressão antrópica que essa região vem sofrendo nos últimos anos, consequência da atividade exploratória não sustentável da floresta como única ou principal fonte de renda da população local (AB'SÁBER, 2003), houve um aumento das áreas degradadas nesse ecossistema. A recuperação de uma área Florestal Tropical Sazonalmente Seca tem como principal entrave o regime hídrico irregular e escasso, normalmente com períodos de seca por mais de 5 meses no ano (SANTOS et al. 2014; MORO et al. 2016); além da salinidade, provocada principalmente pelas altas taxas de evapotranspiração do solo, baixa lixiviação devido às chuvas escassas e irrigação das terras utilizando água salinizada (RENGASAMY, 2006).

A seca é o principal estresse abiótico que reduz o acúmulo de biomassa na planta (GOLLDACK et al. 2014). Essa redução é consequência da diminuição do potencial hídrico do solo que restringe a disponibilidade de água para planta, induzindo o fechamento estomático que promove menores taxas fotossintéticas devido à limitação na captação e difusão de CO_2 atmosférico no mesofilo (CHAVES et al. 2009; FLEXAS et al. 2012). Já a salinidade é um dos maiores problemas ambientais, principalmente em áreas áridas e semiáridas (PORCEL et al. 2012; POLLE; CHEN, 2015). Globalmente, cerca de 1128 Mha de terras são afetadas pela condição salina (WICKE et al. 2011), e cerca de 30% das áreas irrigadas no Nordeste brasileiro são acometidas por essa condição (AGUIAR NETO et al. 2007).

Tanto a seca como salinidade podem promover alterações fisiológicas FROSI et al. 2013; OLIVEIRA et al. 2014; RIVAS et al. 2013), anatômicas (IVANCICH et al. 2012), morfológicas (RIVAS et al. 2013) e moleculares nas plantas (MUNNS; TESTER, 2008; POLLE; CHEN, 2015; PORCEL et al. 2012). Para tolerar essas condições adversas, as plantas possuem mecanismos intrínsecos, como redução da condutância estomática e área foliar, ajustamento osmótico, acúmulo de moléculas osmoprotetoras como prolina e açúcares, aumento da atividade do sistema antioxidante, homeostase iônica e plasticidade morfológica e anatômica, levando à proteção do maquinário fotossintético para manutenção do crescimento (POLLE; CHEN, 2015; RUIZ-LOZANO et al. 2012).

Além dos mecanismos intrínsecos de tolerância, as plantas podem realizar associações mutualísticas para tolerar as condições estressantes do ambiente (MENDES et al. 2013).

Cerca de 80% das plantas terrestres realizam associação com os fungos micorrízicos arbusculares (FMA) (SMITH; READ, 2008), visando obter melhorias em para seu desenvolvimento. O ganho de tolerância a estresses abióticos como seca e salinidade pelas plantas associadas aos FMAs são relacionados ao aumento de captação de água e nutrientes, principalmente o fósforo, pelas hifas fúngicas, aumento na condutividade hidráulica da raiz e trocas gasosas (BÁRZANA et al. 2014; RUIZ-SÁNCHEZ et al. 2010), maior ajustamento osmótico (AROCA et al. 2007), aumento na atividade do sistema antioxidante (BOMPADRE et al. 2014) e compostos secundários (PEDONE-BONFIM et al. 2013), equilíbrio iônico, menor acúmulo de sódio e cloro e manutenção da razão Na^+/K^+ (GIRI et al. 2007), além da regulação de genes envolvidos na tolerância à seca e salinidade (EVELIN et al. 2009; PORCEL et al. 2012).

Outra ferramenta mitigadora dos efeitos negativos da seca e salinidade é o suprimento de fósforo inorgânico foliar (P_i). A manutenção do P_i pode diminuir os efeitos do déficit hídrico e salinidade no metabolismo, já que cerca de 80% do fósforo encontrado no solo é estático e indisponível (SURIYAGODA et al. 2011). O fósforo é um elemento essencial para a planta, contudo, solos tropicais geralmente possuem uma baixa concentração desse nutriente (MIGUEL et al. 2013). Tem sido atribuída à aplicação de P_i um maior controle da condutância estomática (NAEEM; KHAN, 2009), aumento da fotossíntese e biomassa (OLIVEIRA et al. 2014; SANTOS et al. 2004, 2006; SINGH et al. 2013), aumento da estabilidade da membrana celular e melhoria na eficiência do uso da água (FAUSTINO et al. 2013).

Diante do exposto, o presente estudo avaliou a simbiose com FMA em duas espécies lenhosas nativas da Caatinga, *Poincianella pyramidalis* (Tul.) L.P.Queiroz (Fabaceae) e *Cnidoscolus quercifolius* Pohl ex Baill (Euforbiaceae), utilizadas em área de reflorestamento, além de aspectos ecofisiológicos de uma dessas espécies, *Poincianella pyramidalis*, associadas com FMA e aplicação de P_i foliar submetida à seca e salinidade em casa de vegetação. Nossa hipótese geral é que uma espécie nativa lenhosa da caatinga quando micorrizada e suprida com fósforo via foliar simultaneamente, apresentará maior tolerância aos principais fatores estressantes do semiárido nordestino, salinidade e/ou deficiência hídrica.

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Espécies vegetais

2.1.1 *Poincianella pyramidalis* (Tul.) L.P.Queiroz

A espécie *Poincianella pyramidalis* pertence à família Fabaceae, uma das famílias mais representativas da Caatinga, tendo três subfamílias: Faboideae, Mimosidae e Caesalpinoideae, sendo esta última a segunda maior dentre elas e mais bem estudada na Caatinga (HERENDEN, 2000; LEITE; MACHADO, 2009), onde *P. pyramidalis* está inserida (GIULIETTI et al 2004). A família Fabaceae compreende cerca de 727 gêneros e 19.327 espécies de distribuição cosmopolita (LEWIS, 2005). Na Caatinga, ocorrem 77 gêneros e 293 espécies, constituindo cerca de um terço da riqueza do ecossistema (QUEIROZ, 2006). O gênero *Poincianella* (antigamente denominado de *Caesalpinia*) possui cerca de 140 espécies que apresentam um alto grau de plasticidade fenotípica, especialmente das folhas (GASSON et al. 2009; LEWIS, 2005).

Essa espécie ocorre na região Norte, no estado do Amazonas, sendo também amplamente distribuída no Nordeste semiárido brasileiro, nos estados de Alagoas, Bahia, Ceará, Maranhão, Rio Grande do Norte, Paraíba, Pernambuco, Piauí e Sergipe, tendo como domínios fitogeográficos a Amazônia e Caatinga (LEWIS, 2016; QUEIROZ, 2009; SILVA; SANTOS; CUTLER, 2009). É considerada uma das espécies mais representativas da Caatinga, sendo chamada popularmente de catingueira (SILVA; SANTOS; CUTLER, 2009).

P. pyramidalis apresenta uma grande faixa de tolerância ambiental, apresentando um porte arbustivo quando a disponibilidade hídrica é restrita e porte arbóreo quando a disponibilidade hídrica é maior, com porte médio de 4-6 m de altura, podendo atingir até 12 m. Sua madeira é branco-amarelada com elevada densidade, contendo grandes quantidades de celulose e lignina, sendo indicada para recomposição florestal mista de áreas degradadas (MAIA, 2004). Essa espécie se adapta facilmente a diferentes tipos de solos, apresentando uma rápida germinação após o início das chuvas. Suas sementes possuem comportamento ortodoxo (ANTUNES et al. 2010), podendo permanecer nos solos por um grande período de tempo até que uma manifestação de chuva ocorra, ocasionando o início do processo germinativo (MAIA, 2004). Ela investe na caducifolia durante a estação seca, estratégia relacionada à economia de água por transpiração, e na estação chuvosa apresenta um rápido rebrotamento de suas folhas. Essas folhas são bipinadas, apresentando uma coloração rosada quando jovens

e esverdeadas quando maduras e as flores são racimos curtos de coloração amarela. Os frutos são vagens achatadas de coloração castanho-claro quando maduros, as sementes também são achatadas de coloração castanho-claro quando maduras e a raiz é do tipo pivotante (MAIA, 2004).

Essa espécie apresenta múltiplas utilidades, como potencial madeireiro, restauração florestal, aplicações industriais, com suas folhas constituindo fonte de forragem para o gado, podendo ser utilizadas, assim como as flores e cascas, no uso medicinal (tratamento de diversas infecções, atividade antioxidante, farmacológica e antifúngica) (ALVIANO et al., 2008; BAHIA et al., 2005; BRAGA, 1976; MAIA, 2004; CRUZ et al. 2007). A *P. pyramidalis* também é estuda sobre outros aspectos, como diversidade genética (SANTOS et al. 2012); anatomia e densidade de madeira (SILVA et al. 2009), plasticidade fenotípica em gradientes de sucessão ecológica em área semiárida (FALCÃO et al. 2015) e associação com fungos micorrízicos arbusculares (FROSI et al. 2016).

2.1.2 *Cnidoscolus quercifolius* Pohl

A espécie *Cnidoscolus quercifolius* pertence à família Euphorbiaceae, que está representada nas regiões tropicais e temperadas de todo o planeta por um total de 8.000 espécies, distribuídas em 317 gêneros. Estes estão agrupados em 49 tribos e cinco subfamílias, segundo o sistema de classificação proposto por Webster (1994). No Brasil, levantamentos florísticos revelam que a família Euphorbiaceae é uma das mais ricas em número de espécies, aproximadamente 1.000, segundo Carneiro et al. (2002), distribuídas em cerca de 80 gêneros (BARROSO et al. 1991). Muitas dessas espécies são endêmicas da Caatinga, cerca de 17, segundo Sampaio et al. (2002). O gênero *Cnidoscolus* compreende cerca de 50-75 espécies, distribuídas exclusivamente na América tropical e concentradas principalmente no México e Nordeste brasileiro (MELO; SALES, 2008; WEBSTER, 1994).

Cnidoscolus quercifolius é nativa e endêmica do Brasil, conhecida popularmente como faveleira, ocorrendo na Região Sudeste, no estado de Minas Gerais, e tipicamente encontrada no semiárido nordestino, nos estados da Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, Alagoas. (CORDEIRO; SECCO, 2016; LORENZI, 1998). Essa espécie é encontrada nas Caatingas hiperxerófilas, vegetando em áreas que apresentam precipitação pluviométrica abaixo dos 600-700 mm anuais em solo seco e pedregoso, onde outras espécies normalmente não conseguem se estabelecer (DUQUE, 1980). A principal

característica dessa espécie é a presença de tricomas agudos e urticantes que podem recobrir totalmente ou parcialmente a superfície da planta, chegando a alcançar mais de 1 cm de comprimento, e toda a planta contém látex abundante. Tem altura variável entre 4 a 12 m, apresentando porte arbustivo em de baixa fertilidade, enquanto se desenvolve em árvores em solos mais férteis. Seu tronco é curto e ramificado desde a base, aproximadamente cilíndrico, com casca fina e lenticelada. As folhas são alternas simples, membranáceas, de bordas profundamente lobadas e terminadas em pequenos espinhos. Sua inflorescência é do tipo cimeira, onde se desenvolve primeiramente a flor central, e seu fruto é uma cápsula tricoco esquisocárpica. Possui raízes tuberculadas e sementes de testa dura, lisa com albúmem rico em óleo comestível (LIMA, 1989; LORENZI, 2000).

As folhas maduras e a casca de *C. quercifolius* são ricas em proteína, conferindo um bom potencial alimentício e forrageiro, constituindo-se em fonte de alimento aos animais, principalmente no período seco. Detém uma grande potencial para o desenvolvimento da região em virtude dos seus múltiplos usos, como atividades farmacológicas (SILVA; AGUIAR, 2004).

C. quercifolius tem sido descrita como alternativa para recuperação de áreas degradadas. É considerada uma espécie bioindicadora de áreas antropizadas, constituindo um dos principais elementos de margens de áreas impactadas. É uma das primeiras espécies a se estabelecer em áreas recém-degradadas e a desaparecer quando as áreas estão regeneradas (VIEIRA et al. 2007). A sua alta disseminação e completa adaptação às condições climáticas adversas contribuem para o equilíbrio do ecossistema e diminuem a degradação ambiental (PASSOS, 1993).

2.2. Seca

Estresse pode ser definido em um sentido geral como uma pressão excessiva de algum fator adverso que apresenta a tendência de inibir o normal funcionamento dos sistemas (NIU et al.1995). No universo vegetal, a produtividade das plantas pode ser limitada ou co-limitada por uma ampla gama de fatores, incluindo a luz, água, nutrientes, temperatura e eventos de perturbações, bem como fatores edáficos, como um solo de baixa qualidade para o crescimento vegetal. Desses fatores, temperatura e água são geralmente apontados como os principais que influenciam a produtividade dos ecossistemas, ditando os principais biomas do mundo. Isso não sugere que outros fatores não são importantes; contudo, estas limitações

adicionais são geralmente consideradas relevantes em escala muito menor do que fatores climáticos (JONES, 2014).

O aumento das pressões sobre a agricultura, incluindo uma população crescente e aumento do cultivo de culturas para uma bioeconomia em expansão (biocombustíveis e produtos industriais), pode elevar a demanda por água (FOLEY et al. 2011; GODFRAY et al. 2010; PELLETIER; TYEDMERS, 2010). Além disso, é amplamente aceito que as mudanças climáticas irão agravar a escassez de água e aumentar a severidade da seca em grandes áreas da superfície terrestre (IPCC, 2013). As mudanças climáticas estão dirigindo as flutuações tanto na frequência quanto intensidade de precipitação pluviométrica, um fator que coloca em risco tanto os ecossistemas naturais (WILLIAMS et al. 2013) e como gerenciados (HATFIELD et al. 2011). Grandes áreas de florestas em todas as regiões do planeta estão enfrentando desafios a partir da disponibilidade de água reduzida (ALLEN et al. 2010).

A seca é um problema que afeta boa parte dos solos em todo mundo, principalmente os da região semiárida e árida. No Brasil, esta condição é percebida, sobretudo na região Nordeste, onde aproximadamente 54% de toda a área está situada no semiárido. A baixa disponibilidade de água no solo é induzida pelo déficit no balanço hídrico no solo, causado pela falta de precipitação de chuvas e/ou excesso da evapotranspiração. A evapotranspiração refere-se à perda de umidade dos solos, quer através da transpiração pelas plantas (água extraída pelas plantas e perdida através dos estômatos) ou por evaporação direta das superfícies úmidas (solos nus, lagos, rios ou água armazenada no topo das folhas) (SENEVIRATNE, 2012).

2.2.1 Respostas e mecanismos de tolerância à seca

As plantas apresentam diferentes mecanismos para suportar a condição de seca. Esses mecanismos podem ser divididos em escape, retardo e tolerância (LEVITT, 1972). No primeiro, as plantas adotam uma estratégia de “fuga” apresentando rápido desenvolvimento fenológico e alto grau de plasticidade, sendo capazes de completar seu ciclo de vida antes que o déficit hídrico torne-se severo o bastante para provocar dano fisiológico. O retardo da desidratação corresponde à manutenção do turgor e volume celular, tanto pela absorção de água por um sistema radicular abundante quanto pela redução da perda por transpiração através do fechamento estomático ou por vias não estomáticas como a cutícula. Por fim, a tolerância à seca é um mecanismo que permite à planta manter o metabolismo, mesmo com a redução do potencial hídrico dos tecidos, devido principalmente ao acúmulo de solutos

compatíveis ou osmólitos e à capacidade antioxidante (VERSLUES et al. 2006). No semiárido, há a predominância de comportamento decíduo no período de estiagem, e, no curto período de chuva as plantas desenvolvem o brotamento e a floração (SOUZA et al. 2010).

Os vários níveis de respostas do vegetal são modulados pela intensidade, duração e taxa de progressão da seca imposta. A seca pode promover alterações a nível molecular (GOLLDACK et al. 2014), na morfologia e anatomia (IVANCICH et al. 2012), fisiologia (GALLE et al. 2011), bioquímica e sistema antioxidante da planta (OLIVEIRA et al. 2014; RIVAS et al. 2013). A manutenção apropriada do status hídrico da planta sob déficit hídrico é essencial para o crescimento continuado. Este processo pode ser alcançado pela regulação estomática e acumulação de solutos compatíveis (ASHRAF; FOOLAD, 2007), redução da área foliar, senescência e caducifolia (SANTOS; CARLESSO, 1998). As plantas, quando são submetidas a algum tipo de estresse, como a seca, passam por uma verdadeira reprogramação para atingir um novo estado energético e equilíbrio em seu desenvolvimento para lidar com a condição adversa, onde os produtos de genes relacionados ao estresse funcionam tanto nas respostas iniciais da planta como no estabelecimento da tolerância.

Sob condição de seca, a disponibilidade de água no solo e a pressão de vapor atmosférico são os dois fatores dominantes. Uma diminuição no potencial hídrico do solo ou um aumento no déficit de pressão de vapor atmosférico induz uma cascata hidráulica que provoca uma redução do potencial hídrico da planta, levando a uma diminuição da condutância estomática através de mecanismos físicos precisos (PEAK; MOTT, 2011). Eventos bioquímicos também estão envolvidos com o fechamento estomático, e este pode ocorrer mesmo sem nenhuma alteração no potencial hídrico foliar (GOLLAN et al. 1986). Acredita-se que as raízes atuam como sensores do déficit de água no solo, que é detectado pelas células-guarda dos estômatos mesmo antes de qualquer déficit hídrico ser observado nas folhas (SALAH; TARDIEU, 1997) por meio de sinais (ácido abscísico - ABA) enviados à parte aérea da planta.

Em condições de restrição hídrica, as variáveis de trocas gasosas podem apresentar alterações de forma distinta de acordo com a espécie, tanto por limitações difusivas, restringindo a disponibilidade de dióxido de carbono para assimilação, quanto por limitações metabólicas, pelo aumento do efeito fotoinibitório (GLAZ et al. 2004). Em nível foliar, estômatos, mesofilo e respostas bioquímicas, juntamente com adaptações estruturais ao déficit hídrico interagem para melhorar a capacidade da planta para sobreviver à seca. Os estômatos são os reguladores primários da hidrologia da folha, e representam um importante meio pelo qual as plantas regulam o uso da água em resposta ao estresse (NIINEMENTS; KEENAN,

2014). Como consequência do fechamento estomático, diversos estudos demonstraram uma redução na concentração de CO_2 nas cavidades sub-estomáticas, indicando que a redução da condutância estomática para conservar a água desempenha um papel importante na redução da fotossíntese (CHAVES et al. 2009; FLEXAS; MENDRANO, 2002; GRASSI; MAGNANI, 2005). Além das limitações de difusão pelos estômatos, a concentração de CO_2 no local da sua fixação nos cloroplastos é dependente de resistências dentro do mesofilo (FLEXAS et al. 2012), e alguns estudos têm demonstrado reforçada limitação de difusão de cavidades sub-estomática aos cloroplastos (redução da condutância de difusão mesofílica) em folhas sob escassez de água (CORNIC; MASSACCI, 2004) e uma diminuição das reações bioquímicas e fotoquímicas em resposta à seca (TEZARA et al. 1999).

Além do fechamento estomático, mecanismos de proteção contra o excesso de poder redutor é uma importante estratégia da planta sob déficit hídrico. Esses mecanismos fotoprotetores competem com energia absorvida para a parte fotoquímica, levando a uma diminuição da eficiência quântica do fostossistema II (PSII) (GENTY et al. 1989). Tal proteção pode ser pela regulação da dissipação térmica nos complexos coletores de luz, ciclo das xantofilas e luteínas (BAKER, 2008; DEMMING –ADAMS; ADAMS, 1996; MATSUBARA et al. 2001). A fotorrespiração também pode estar envolvida na proteção da maquinaria fotossintética, aumentando em diversas espécies submetidas à seca (CHAVES et al. 2003). Esse processo produz o peróxido de hidrogênio (H_2O_2), uma espécie reativa de oxigênio (EROs) que está envolvida na sinalização e aclimatação das plantas sob estresse (NOCTOR et al. 2002; POLLE; CHEN, 2015).

Quanto à bioquímica foliar, o amido transitório é o primeiro produto da fotossíntese nas plantas. Ele serve como estoque de carboidratos, que suporta o metabolismo e crescimento durante o período na noite, onde a fotossíntese não é realizada. Em muitas espécies, o amido é produzido no cloroplasto, e ao mesmo tempo, a sacarose é produzida no citosol. O particionamento desses dois produtos é regulado por estímulos tanto externos como internos (ZEEMAN, SMITH; SMITH, 2004). O metabolismo desse amido é influenciado por fatores ambientais, tais como comprimento do dia, temperatura e disponibilidade de nutrientes (MARTINDALE; LEEGOOD, 1997; STRAND et al. 1997). O padrão de acúmulo desse amido varia bastante. Algumas espécies acumulam pouco ou até mesmo nenhum amido; já em outras, o acúmulo e degradação mudam drasticamente através do desenvolvimento da folha (SMITH, ZEEMAN; SMITH, 2005). O consumo de amido pode ser uma das respostas iniciais à seca, permitindo a manutenção do suprimento do carbono em uma condição de

reduzida fixação de CO_2 atmosférico (KAKANI et al. 2011). A redução no teor de amido em plantas submetidas à seca também pode ser consequência da menor síntese de amido nessa condição (PINHEIRO; CHAVES, 2011).

Os carboidratos são a principal fonte de energia das mudanças metabólicas que ocorrem durante o estresse ambiental (SIVACI, 2006), embora a acumulação de aminoácidos livres também seja comum. Os açúcares solúveis (sacarose, glicose e frutose) e os aminoácidos livres totais apresentam alterações sob seca, e podem atuar como moléculas sinalizadoras de estresse e interagir com hormônios como parte da rede de sinalização (ROLLAND et al. 2006; SANTOS; PIMENTEL, 2009). Tanto os açúcares como os aminoácidos desempenham papel importante no aumento da tolerância ao déficit hídrico por serem osmoprotetores, sinalizadores e eliminadores de espécies reativas de oxigênio (FILEK et al. 2015; LABANOWSKA et al. 2013). Muitos pesquisadores têm investigado a relação entre a assimilação de CO_2 e de produtos finais em várias espécies (QUICK et al. 1992; WINGLER et al. 2006), mostrando o papel dos montantes de açúcares solúveis da folha no mecanismo de *feedback* regulador da fotossíntese. Além disso, a síntese de aminoácidos livres também pode agir como um mecanismo de *feedback* secundário na regulação fotossintética (PAUL; PELLNY, 2003).

A prolina é um tipo de aminoácido e um dos principais osmoprotetores da célula, sintetizada por muitas espécies vegetais em resposta a diferentes estresses, incluindo a seca (YAMADA et al., 2005). Seu acúmulo nas células ajuda na manutenção do *status* osmótico celular aliviando os efeitos do referido estresse abiótico (RHODES; HANSON, 1993). Ela tem papel no sequestro de radicais livres, como a hidroxila (um dos radicais livres celulares mais energéticos) e estabilização de estruturas subcelulares (CHEN; DICKMAN, 2005). Em plantas superiores, a prolina é sintetizada a partir do ácido glutâmico pela ação de duas enzimas, P5CS e P5CR (PORCEL et al. 2012).

A atividade proteolítica levando a um menor conteúdo de proteínas a nível foliar em resposta a senescência provocada pela seca é bem documentado. Entretanto, pouco se conhece sobre a relação entre a seca e essa atividade nas células (DUNGEY; DAVIES, 1982; PIERRE; SAUVORÉ, 1990). Sob condições de déficit hídrico pode haver uma diminuição na quantidade de proteínas devido ao aumento das enzimas proteolíticas e/ou diminuição da sua síntese (LECHINOSKI et al. 2007). Contudo, algumas proteínas podem ser sintetizadas sob condição de estresse como as deidrinas e proteínas de choque térmico (HSP), que têm função na estabilização de outras proteínas citoplasmáticas e membranares (HOEKSTRA;

GOLOVINA; BUITINK, 2001; YORDANOV; VELIKOVA; TSONEV, 2000), atuando também na recuperação dos danos causados pelos estresses térmico, osmótico e de desidratação (XIONG; SCUMAKER; ZHU, 2002). Outras proteínas que podem ter sua síntese ou atividade aumentada são as aquaporinas, proteínas intrínsecas de membrana que facilitam e regulam o movimento passivo da água e outras moléculas ao longo de um gradiente de potencial hídrico (MAUREL et al. 2008).

O conteúdo de clorofila foliar é frequentemente associado à capacidade fotossintética de uma espécie por estar relacionada à captação de luz e transferência de energia. No geral, acredita-se que uma planta que possua altos teores de clorofila apresentará maiores taxas fotossintéticas. Entretanto, nem sempre essa relação existe, pois a etapa bioquímica da fotossíntese pode limitar o processo (CHAPPELLE; KIM, 1992; PORRA et al., 1989). As pesquisas relacionadas aos pigmentos fotossintéticos têm sido desenvolvidas durante décadas e continuam atualmente em estudos relacionados à ecologia e fisiologia da produção vegetal (CHEN et al. 2008; NINEMETS, 2007).

Trabalhos com espécies encontradas na Caatinga têm sido realizados para verificar a tolerância das mesmas em relação à seca. Frosi et al. (2013) e Rivas et al. (2013) trabalharam com espécies exóticas, *Calotropis procera* e *Moringa oleifera*, respectivamente, sob condições de seca em casa de vegetação. Outro estudo, realizado por Oliveira et al. (2014a), investigou uma espécie nativa (*Anadenanthera colubrina* - angico) e invasora (*Prosopis juliflora* - algaroba) sob seca. em casa de vegetação. Outros trabalhos, como o de Oliveira et al. (2014b), estudou a espécie nativa *Cnidoscolus quercifolius* (faveleira) em campo, avaliando os respostas das plantas nas estações chuvosas e secas.

2.3. Salinidade

O termo salinidade se refere à existência de níveis de sais solúveis no solo que possam prejudicar significativamente o rendimento das plantas cultivadas (MUNNS; TESTER, 2008). As regiões áridas e semiáridas, onde a agricultura é viável pela irrigação, engloba uma grande porção das terras do planeta, sendo regiões muito afetadas pela salinidade. Um acúmulo na concentração de sais no solo depende de vários fatores como a evaporação (levando um aumento da concentração de sais) e a quantidade de precipitação de chuvas (levando a uma diminuição na concentração de sais) (MAHAJAN; TUTEJA, 2005). Ao mesmo tempo, uma irrigação e fertilização do solo inadequada são os maiores problemas que levam à perda de terras aráveis no planeta (PITMAN; LÄUCHLI, 2002). Algumas das razões a respeito da

degradação dos solos via antropogênica é a utilização de águas de irrigação, que possuem resíduos (fertilizantes), ou águas subterrâneas com elevadas concentrações de íons minerais, por exemplo, águas de poços. A água subterrânea salinizada é muitas vezes o resultado de desmatamento que promove a mobilização de sal para a superfície (SALAMA et al. 1999). Em áreas secas, o sal é transportado para a superfície por evapotranspiração, onde pode ser acumulado em concentrações tóxicas. Este cenário é denominado de salinização secundária e resulta em perdas de terras aráveis anuais estimadas em cerca de 16.000 km^2 (GHASSEMI et al. 1995). De acordo com estimativas mais recentes, 1128 Mha de terras no globo são afetadas pela salinidade (WICKE et al. 2011) e cerca de 30% da região nordestina brasileira é afetada por tal condição (AGUIAR NETO et al. 2007).

Solos que são ditos salinizados incluem aqueles que apresentam elevadas concentrações de NaCl, Na_2SO_4, Na_2CO_3, $MgSO_4$, $CaSO_4$ ou $CaCO_3$. Entretanto, de uma forma geral, os solos salinizados são descritos como aqueles que apresentam elevadas concentrações de Na^+ (POLLE; CHEN, 2015), e uma condutividade elétrica entre 2 e 4 dS cm^{-1}, equivalente a uma solução de 100 mM de NaCl (US SALINITY LABORATORY, 1954). Elevadas concentrações de Na^+, em particular, que são depositados no solo, podem alterar a sua textura básica, resultando em uma diminuição da porosidade e consequente redução da aeração e condutância hidráulica desse solo (PORCEL et al. 2012). A salinidade prejudica o estabelecimento, crescimento e desenvolvimento das plantas, promovendo reduções na produtividade (EVELIN et al. 2009), afetando aspectos da fisiologia da planta, estressando-a principalmente de duas maneiras (MUNNS; TESTER, 2008). Primeiramente, elevadas deposições de sais no solo geram uma zona de baixo potencial osmótico, dificultando a absorção de água e nutrientes pelas raízes, gerando uma seca fisiológica na planta (PORCEL et al. 2012; RUIZ-LOZANO et al. 2012), dificultando a absorção de água e nutrientes pelas raízes. Além de dificultar a absorção, a salinidade também afeta o transporte de nutrientes para a parte aérea, provocando um desequilíbrio nutricional (EVELIN et al. 2009). Em um segundo momento, o efeito tóxico dos íons Na^+ e Cl^- inibem a síntese de proteínas, causam danos celulares e na estrutura de enzimas e afetam negativamente o processo fotossintético e respiratório (RUIZ-LOZANO et al. 2012).

A existência de habitats que são afetados pelas altas concentrações de sais promoveu adaptações evolutivas pelas plantas pra suportarem essa condição. As espécies vegetais podem ser divididas entre (I) glicófitas, que toleram uma concentração moderada de salinidade, e (II) halófitas, que são plantas que possuem a habilidade de completar seu ciclo

de vida em ambientes com concentrações acima de 200 mM de NaCl, sob condições semelhantes às que poderiam ser encontradas no ambiente natural (FLOWERS et al. 1986).

2.3.1 Respostas e mecanismos de tolerância à salinidade

Quando a planta é exposta à condição salina, determinados processos são danificados, tais como síntese de proteínas, metabolismo de lipídios e fotossíntese. Uma das respostas iniciais da planta é a redução da expansão da superfície foliar (PARIDA; DAS 2005). A diminuição do crescimento foliar depois de um aumento da salinidade no solo é devido ao efeito osmótico do sal ao redor das raízes. Um aumento repentino na salinidade do solo faz com que as células foliares percam água, mas esta perda de turgor celular é transitória. Em poucas horas, as células recuperam seu volume e turgor original devido ao ajuste osmótico, entretanto, a taxa de elongação celular é reduzida (CRAMER, 2002; PASSIOURA; MUNNS, 2000). Quando a salinidade se estende por dias, as reduções na elongação e divisão celular levam ao aparecimento mais lento de novas folhas, sendo estas de menor área foliar e mais espessas (MUNSS; TESTER, 2008). Além disso, a pressão osmótica pode levar à alterações na composição das paredes celulares (JANZ et al. 2012). O crescimento da raiz é geralmente menos afetado do que o crescimento foliar, e a taxa de elongação radicular é recuperada rapidamente após uma exposição ao NaCl ou outro estresse osmótico (MUNNS, 2002).

Os efeitos da salinidade na fotossíntese vão desde a restrição da difusão do CO_2 para dentro do cloroplasto via limitação na abertura estomática mediada por hormônios produzidos via aérea/radicular e no transporte de CO_2 no mesofilo. Além disso, também são observadas alterações na fotoquímica foliar e metabolismo do carbono. Esses efeitos variam de acordo com a intensidade, duração do estresse e espécie vegetal, bem como a idade foliar, onde folhas mais velhas acumulam mais conteúdos de sais (FLEXAS et al. 2004; GALMÉS et al. 2007; MUNNS, 2002). De todas as respostas da planta à salinidade, a mais dramática é a redução da abertura estomática. As respostas estomáticas são induzidas pelo efeito osmótico nas raízes, sendo primeiramente afetada pelas alterações transitórias de água, e logo depois, pela síntese local do ABA (MUNNS; TESTER, 2008). As relações causa efeito entre fotossíntese e taxa de crescimento podem ser difíceis de serem estabelecidas. É sempre difícil saber se a taxa fotossintética reduzida é a causa da redução do crescimento ou o resultado. Com o aparecimento do estresse salino, uma fotossíntese reduzida não é certamente a causa da diminuição da taxa de crescimento, devido à taxa de expansão celular ser um dos principais processos envolvidos no crescimento (FRICKE et al. 2004).

Algumas plantas são hábeis em prevenir a entrada de sal, excluir os sais da planta a nível celular ou minimizar a sua concentração no citoplasma pela compartimentalização nos vacúolos, evitando seus efeitos negativos na fotossíntese e outros processos chaves do metabolismo pelas alterações iônicas (MUNNS; TESTER, 2008). Alternativamente à compartimentalização no vacúolo, os sais podem ser transportados para a parede celular, o que, por sua vez, pode resultar na desidratação da célula (MUHLING; LAUCHLI, 2002). De acordo com Willadino et al. (2010), a nível de planta inteira, a tolerância à salinidade depende de cinco pontos específicos, sendo eles: (1) seletividade iônica no processo de absorção pelas células radiculares; (2) carregamento do xilema com K^+ em maior proporção do que Na^+; (3) remoção do sal do xilema na parte superior das raízes, caule, pecíolo ou bainhas foliares; (4) retranslocação de Na^+ e Cl^- no floema evitando a translocação desses íons para a parte aérea; e (5) excreção de sais através de glândulas, presentes apenas nas halófitas. Quando esses processos não existem ou são insuficientes ocorrem danos nas plantas. Já foi mostrado que elevadas concentrações dos íons, principalmente Na^+ acima de 100 mM, inibem severamente muitas enzimas, incluindo aquelas envolvidas na fotossíntese (MUNNS; LÄUCHLI, 2006). Quando o Na^+ ou os sais são elevados no solo, a planta tende a absorver mais Na^+, resultando na diminuição da absorção do K^+. O Na^+ compete com o K^+ pelos seus sítios de ligação, que estão envolvidos em várias funções celulares essenciais. Entretanto, essas funções não podem ser realizadas pela ligação do Na^+. Uma elevada razão Na^+/K^+, geralmente promovida pela salinidade, causa desequilíbrio iônico no citoplasma e consequentemente prejuízos em vias metabólicas (GIRI et al. 2007). O K^+ possui um papel chave no metabolismo da planta, pois está envolvido na regulação do movimento estomático e síntese de proteínas, onde elevadas concentrações são requeridas para ligação do tRNA aos ribossomos (BLAHA et al. 2000), além de ser cofator para ativação de várias enzimas.

Em plantas que são submetidas à salinização, é comum o aumento de solutos orgânicos primários, os quais estão relacionados ao metabolismo dos aminoácidos e nitrogênio ou carboidratos. Esses solutos compatíveis têm um papel importante no ajustamento osmótico, proteção de membrana e proteínas, atuando no sequestro de espécies reativas de oxigênio e do excesso de íons acumulados (CHAVES et al. 2009; MUNNS; TESTER, 2008). Variações na concentração de carboidratos solúveis totais (MUNNS; TESTER, 2008), glicina betaína e prolina (ASHRAF; FOOLAD, 2007), clorofilas (SANTOS, 2004) e proteínas solúveis (LUNDE et al. 2007) são exemplos de alterações bioquímicas observadas em espécies cultivadas sob salinidade. O acúmulo dessas moléculas atua na redução do potencial osmótico

das plantas, constituindo um importante mecanismo de proteção contra os efeitos negativos do estresse, além de atuarem como moléculas sinalizadoras (KEUNEN et al. 2013; THANNA; NAWAR, 1994).

O acúmulo do aminoácido prolina é um dos mais frequentemente reportados como modificações induzidas pela salinidade nas plantas, podendo apresentar valores 100 vezes mais elevados comparados a plantas não salinizadas (EVELIN et al. 2009; VERBRUGGEN; HERMANS, 2008). Em condição salina, a prolina atua na célula como um osmólito protetor e não tóxico para manter um balanço osmótico sob baixos potenciais hídricos (ASHRAF; FOOLAD, 2007; STEWART; LEE, 1974), atuando também como reserva energética e de nitrogênio pra utilização durante o estresse salino (GOAS et al. 1982). Além disso, a prolina pode atuar na estabilização das estruturas das proteínas, favorecer a estabilização do pH citosólico, auxiliar no equilíbrio redox das células estressadas e no sequestro de espécies reativas de oxigênio (VERBRUGGEN; HERMANS, 2008).

Em termos de pigmentos fotossintéticos, a salinidade causa redução nas concentrações de clorofilas (SHENG et al. 2008) devido a supressão de atividades específicas que são responsáveis pela sua síntese, ou devido à alteração do ácido aminolevulínico (ALA) o qual é o precursor da protoclorofila (que é convertido em clorofila quando exposto à luz) (MURKUTE et al. 2006). A redução de nutrientes minerais, como Mg e P, necessários para a biossíntese das clorofilas também prejudica a síntese desses pigmentos (EL-DESOUKY; ATAWIA, 1998). Variações na concentração de clorofilas também podem estar relacionadas pela sua própria degradação através da enzima clorofilase (SANTOS, 2004). A redução de parâmetros fotoquímicos em plantas sob estresse podem estar atrelados à degradação das clorofilas (OLIVEIRA et al. 2014), entretanto, elevadas concentrações de sais na planta podem destruir os centros de reação do fotossistema II e prejudicar a taxa de transporte de elétrons no aparato fotossintético (EVELIN et al. 2009).

Alguns estudos têm sido realizados com espécies encontradas na Caatinga, voltados para a questão da salinidade. Bessa et al. (2017) investigou a tolerância à salinidade de seis espécies lenhosas nativas: *Myracrodruon urundeuva* (urundeúva), *Handroanthus impetiginosus* (ipê-roxo), *Bauhinia unguculata* (pata-de-vaca), *Erythrina velutina* (suinã), *Mimosa caesalpiniifolia* (sabiá) e *Luetzelburgia auriculata* (pau-mocó); já Campos et al. (2012) estudou a espécie exótica *Jatropha curcas* (pinhão manso) submetida à condição salina, ambos os estudos em casa de vegetação. Outros estudos têm sido realizados com o objetivo de investigar o impacto da salinidade na germinação e produção de mudas de espécies da

Caatinga. Dantas et al. (2014) avaliou a germinação de *Anadenanthera macrocarpa* (angico vermelho), *Myracrodruon urundeuva* (urundeúva), *Aspidosperma pyrifolium* (pereiro) e *Erythrina velutina* (suinã) com agricultura biossalina, enquanto Santos et al. (2016) avaliou a germinação das espécies *Poincianella pyramidalis* (catingueira) e *Anadenanthera colubrina* (angico) sob diferentes concentrações de sais.

2.4 Fungos Micorrízicos Arbusculares (FMA)

2.4.1 Colonização

De toda vasta diversidade encontrada no universo das micorrizas, a simbiose com os fungos micorrízicos arbusculares (FMA) é o tipo mais amplamente encontrado no mundo, ocorrendo em 80-90% das plantas terrestres e envolve como fungo simbiótico os pertencentes ao filo Glomeromycota. Esse é um filo ancestral que co-evoluiu com as plantas por mais de 450 milhões de anos (BONFANTE; DESIRÒ, 2015; ÖPIK et al. 2013; SMITH; READ, 2008).

O estabelecimento da simbiose das plantas com os FMAs requer um alto grau de coordenação entre as duas partes, sendo baseado em um diálogo molecular finamente regulado (HAUSE et al. 2007). Os propágulos dos FMAs são esporos assexuados que germinam independentemente da planta hospedeira, desenvolvendo um micélio assimbiótico, que não pode crescer por mais do que poucos dias sem uma raiz hospedeira, pelo fato de serem organismos biotróficos obrigatórios, característica do filo Glomeromycota (BONFANTE; DESIRÒ, 2015; PORCEL et al. 2012). A comunicação entre hospedeiro e FMA se inicia na rizosfera, com a produção e exudação de moléculas sinalizadoras pelas plantas hospedeiras, que são reconhecidas pelos FMAs e estimulam a germinação dos esporos e crescimento das hifas. Entre esses sinais encontram-se as estrigolactonas, consideradas essenciais para a simbiose micorrízica (LÓPEZ-RÁEZ et al. 2011). Elas são derivadas dos carotenoides, pertencendo à classe dos apocarotenóides, assim como o ácido absícico (ABA) (OHMIYA, 2009), sendo classificadas como uma nova classe de fitorreguladores (KOHLEN et al., 2012). Além das estrigolactonas, outros fitorreguladores como ácido jasmônico, ácido salicílico, auxinas e giberelinas estão envolvidos na formação, regulação e funcionamento dos FMAs (GUTJAHR; PASZKOWSKI, 2009; GUTJAHR, 2014). O próprio ABA também é fundamental no processo de colonização, sendo necessário para completar a formação dos

arbúsculos nas raízes, estruturas característica dos FMAs (LÓPEZ-RÁEZ et al. 2011). Quando o FMA percebe os exudados do hospedeiro, a hifa extraradial inicia o processo de ramificação, provavelmente para aumentar as chances de contato com a superfície radicular. Esse contato leva à diferenciação de uma estrutura denominada hifopódio, que adere na parede celular das células da raiz e produz a primeira estrutura intraradial. Esse contato induz a agregação do citoplasma das células corticais e a formação do aparato de pré-penetração (APP). Assim, o fungo inicia a invasão intracelular da raiz, seguindo a rota formada pelo APP da superfície para dentro das células corticais, onde a hifa se ramifica e forma os arbúsculos (BONFANTE; DESIRÒ, 2015). Além da sinalização emitida pelo hospedeiro, os FMAs, após a germinação, também emitem sinais que são percebidos pelas raízes das plantas antes mesmo do contato físico das hifas e raiz. Esses fatores são referidos como fatores Myc, que promovem várias repostas nas plantas, como indução de genes envolvidos na transdução de sinais, estimulação do desenvolvimento de raízes laterais, acúmulo de amido e oscilações de picos de cálcio nas células epidérmicas (BONFANTE; DESIRÒ, 2015). Essas oscilações são necessárias para a formação do APP e subsequente colonização pelos FMAs (BONFANTE; GENRE, 2010).

2.4.2 Benefícios da simbiose micorrízica

Quando a simbiose micorrízica é estabelecida, ela é caracterizada por uma troca de substâncias bidirecional, onde a planta fornece fotossintatos para os fungos, enquanto o FMA promove um aumento da absorção de água e nutrientes do solo, predominantemente o fosfato (SCHWEIGER; MÜLLER, 2015). A troca de fotoassimilados e nutrientes minerais entre os parceiros simbióticos ocorre em estruturas altamente ramificadas em formatos de pequenas árvores, denominadas arbúsculos, localizadas dentro das células corticais das raízes, através de diferentes transportadores (PARNINSKE, 2008; CASIERI et al. 2013). Cerca de 10 a 20% do carbono da produtividade primária líquida das plantas são alocados para os FMAs (GENRE; BONFANTE, 2010).

As consequências ecológicas da interação das plantas com os FMAs na questão nutricional, crescimento, competição, tolerância a diferentes estresses, bem como estruturação do solo são reportados (PORCEL et al. 2012). Os principais efeitos da simbiose micorrízica podem ser sumarizados como aumento do fluxo de íons móveis na planta, aumento na qualidade do solo e diversidade da comunidade vegetal, melhorias no enraizamento e

estabelecimento das plantas, aumento da ciclagem de nutrientes, ganhos no crescimento e na tolerância das plantas a estresses bióticos a abióticos (POZO; AZCÓN-AGUILAR, 2007; SMITH; READ, 2008).

Dentre os estresses abióticos, muitos estudos tem demonstrado que essa simbiose confere tolerância à seca (LAZCANO et al. 2014; MIRANSARI, 2010), calor (COMPANT et al. 2010), salinidade (EVELIN et al. 2009; RUIZ-LOZANO et al. 2012) ou estresse osmótico (RUIZ-LOZANO, 2003). De uma forma geral, os FMAs aliviam os efeitos do déficit hídrico pelo aumento na absorção de água e transporte através das hifas fúngicas para a planta hospedeira (AUGÉ et al. 2007), aumento na absorção de nutrientes (MICHALIS et al., 2013), aumento na condutividade hidráulica da raiz (BÁRZANA et al. 2014) e das trocas gasosas (HABIBZADEH et al. 2013; RUIZ-SÁNCHEZ et al. 2010), alterações nas propriedades de retenção de água no solo (AUGÉ, 2001), aumentos no ajustamento osmótico (AROCA et al. 2007) e atividade antioxidante (BOMPADRE et al. 2014).

O crescimento e produtividade das plantas são intimamente associados ao nível do déficit hídrico no qual as plantas foram expostas, induzindo uma diminuição do potencial hídrico foliar e abertura estomática, afetado negativamente a disponibilidade de CO_2. Os FMAs podem alterar o comportamento estomático, reduzindo a perda na produtividade vegetal (AUGÉ et al. 2015)

Além da seca, muitos estudos tem investigado o papel dos FMAs na proteção das plantas contra o estresse salino, e têm demonstrado que a simbiose promove ganhos na captação de nutrientes, acúmulo de compostos osmorreguladores e aumentos nas taxas fotossintéticas e eficiência do uso da água, sugerindo que os FMAs resultam em uma combinação de efeitos bioquímicos, fisiológicos e moleculares (PORCEL et al. 2012). A colonização das plantas com os FMAs podem reverter o efeito da salinidade quanto à nutrição do Na^+ e K^+. As micorrizas podem aumentar a absorção do K^+ sob condição salina, enquanto previne a translocação do Na^+ para a parte aérea (ZUCCARINI; OKUROWSKA, 2008). Estudos realizados por Hammer et al. (2011) indicam que os FMAs podem selecionar elementos como o K^+ e Ca^{2+}, que atuam como equivalentes osmóticos, evitando a absorção do Na^+ e Cl^-. Aumentos nos conteúdos de açúcares solúveis também são correlacionados positivamente com a micorrização das plantas hospedeiras, como reportado por Thomson et al. (1990).

Plantas micorrizadas podem apresentar um aumento de carboidratos tanto sob déficit hídrico quanto salinidade (EVELIN et al. 2009; PORCEL; RUIZ LOZANO, 2004). Esse aumento pode estar relacionado ao efeito dreno de carbono que o fungo promove na planta

(AUGÉ, 2000). Além disso, Sheng et al. (2011) sugerem que o aumento nos níveis de açúcares em plantas micorrizadas podem ser resultado do aumento na capacidade fotossintética dessas plantas e que esses açúcares contribuem para o ajustamento osmótico.

A prolina tem sido encontrada em maiores concentrações em plantas micorrizadas quando comparada com plantas não micorrizadas sob salinidade e/ou seca (SHARIF et al. 2007; TAALAT; SHAWKY, 2011). Entretanto, essas respostas de plantas associadas aos FMAs quanto à acumulação de solutos nas plantas são complexas.

Em termos nutricionais, os FMAs contribuem principalmente com a aquisição do fósforo, um nutriente de baixa mobilidade no solo, pelas hifas extraradiais. O micélio estende a superfície efetiva de absorção da planta para além da zona empobrecida de nutrientes que se desenvolve em torno da raiz causada pelo próprio processo de absorção direto pelo sistema radicular. É estimado que as hifas externas são responsáveis por até 80% da absorção de fósforo necessário para as plantas (MATAMOROS et al. 1999). O fósforo entra nas hifas extraradiais pela alta afinidade de transportadores simportes $P:H^+$ (BENEDETTO et al. 2005), sendo rapidamente condensado em grânulos de polifosfatos (Poli-P) (EZAWA et al. 2004). Nas hifas intraradiais, o Poli-P é hidrolisado por fosfatases para deixar o fósforo disponível para a planta, e finalmente transportado pelo compartimento apoplástico peri-arbuscular, provavelmente pelo controle dos FMAs, e removido desse compartimento pelos transportadores de fósforo da planta (JAVOT et al. 2007). A aplicação dos FMAs também pode melhorar a absorção do nitrogênio para as plantas hospedeiras, sendo reportados elevadas concentrações de nitrogênio na parte aérea em plantas micorrizadas (GIRI; MUKERJI, 2004). O micélio externo capta o nitrogênio disponível do solo na forma de nitrato, o qual é assimilado pela via de nitrato redutase nos arbúsculos (KALDORF et al. 1998).

Diante de processos de degradação e desertificação, comuns em regiões semiáridas, como no nordeste brasileiro, os distúrbios das comunidades vegetais são acompanhados ou precedidos pela perda das propriedades físico-químicas e biológicas do solo, como estrutura e disponibilidade de nutrientes, conteúdo de matéria orgânica e atividade microbiana. Dessa forma, em solos degradados e contaminados, que são pobres em nutrientes e com baixa disponibilidade hídrica, o manejo dos FMAs são de grande importância (JEFFRIES; BAREA, 2001). Além disso, esses benefícios promovidos pelos FMAs podem ser fundamentais diante das mudanças climáticas iminentes no planeta, particularmente a respeito da escassez de água e o processo de revegetação das áreas de ecossistemas degradados.

Estudos têm avaliado os benefícios dos FMAs em plantas nativas da Caatinga em diferentes condições. Existem trabalhos voltados para a análise de plantas nativas micorrizadas sob diferentes níveis de fósforo no solo, como o de Oliveira et al. (2015) avaliando plantas de *Schinopsis brasiliensis* (baraúna); Sugai et al. (2010) estudando *Anadenanthera macrocarpa* (angico vermelho); Pagano et al. (2007) avaliando *Anadenanthera peregrina* (angico branco) e *Enterolobium contortisiliquum* (tamboril); Pedone-Bonfim et al. (2013) estudando *Anadenanthera colubrina* (angico). Também existem estudos sobre espécies de caatinga micorrizadas submetidas a concentrações elevadas de metais pesados (LINS et al. 2007); levantamento de diversidade micorrizica (SILVA et al. 2014); estresse salino (SILVA; AMORIM, 2009)

2.5 Fósforo

O fósforo (P) é um dos 17 nutrientes essenciais necessários para o desenvolvimento da planta, representando cerca de 0,2% do peso seco da mesma (LIU et al. 2015). Em geral, cerca de 80% do total de P no solo encontra-se imóvel e indisponível para as plantas, nas formas de Ca-P, Fe-P, Mg-P, Al-P e fósforo orgânico (SURIYAGODA et al. 2011). Ele está envolvido no metabolismo das plantas, atuando nos processos de transferência de energia da célula, na respiração e na fotossíntese, como componente estrutural dos ácidos nucleicos, assim como de muitas coenzimas, fosfoproteínas e fosfolipídeos (MALAVOLTA, 1980). Embora a necessidade de P requerida seja menor que as quantidades de potássio (K) e de nitrogênio (N), sua aplicação nas culturas ocorrem em doses iguais ou superiores à esses dois elementos. Isso se deve à elevada taxa de fixação do P, principalmente em solos tropicais, fazendo com que a maior parte não possa ser utilizada pelas plantas (VIEIRA, 2006). Em geral, solos tropicais possuem uma baixa concentração de fosfato, sendo esperado que solos fosfatados sejam exauridos nas próximas décadas (MIGUEL et al. 2013).

O P pode ser facilmente absorvido pelas plantas nas formas de ortofosfato (fósforo inorgânico - P_i; $H_2PO_4^-$; HPO_4^{2-}), que ocorrem na solução do solo em baixas concentrações. A maioria dos solos, até mesmo os férteis, são deficientes em P_i devido à rápida absorção pelas raízes comparado a sua reposição na solução do solo (SURIYAGODA et al. 2011). Estudos recentes tem domonstrado que a deficiência de P nas plantas prejudica processos metabólicos e bioquímicos, como fotossíntese e respiração, principalmente na via glicolítica, e reduz a atividade de enzimas chaves envolvidas no metabolismo do carbono e nitrogênio (BURMAN

et al. 2009; SANTOS et al. 2004). Além disso, a deficiência do P pode causar uma arquitetura aberrante do sistema radicular das plantas em termos de número e densidade de raízes laterais, afetando o transporte do P_i (LIN et al. 2014). Consequentemente, o P é considerado um dos elementos mais importantes para o crescimento das plantas. A deficiência do fósforo pode levar à reduções na fotossíntese, que pode ser atribuída ao papel regulatório do P na via de assimilação de CO_2 pela redução do conteúdo e atividade da Rubisco e capacidade de regeneração da RubP (FLEISHER et al. 2012).

A seca e a salinidade reduzem a disponibilidade hídrica do solo significativamente e a disponibilidade do P (SURIYAGODA et al. 2014). Essas duas situações inevitáveis para a planta, principalmente em ambientes semiáridos, induzem um desarranjo metabólico resultando em perda de produtividade (SINGH et al. 2006). Dessa forma, a fertilização do P não alivia somente a escassez do nutriente no solo, mas também aumenta a tolerância das plantas aos estresses (CORTINA et al. 2013). A habilidade do P em aumentar a tolerância das plantas aos estresses abióticos tem sido atribuídos ao aumento no controle da condutância estomática (NAEEM; KHAN, 2009), ganhos na fotossíntese (OLIVEIRA et al., 2014; SANTOS et al. 2004; 2006; SINGH et al. 2013), aumento na estabilidade da membrana celular e tonoplasto e maior eficiência do uso da água (SAWWAN et al. 2000; FAUSTINO et al. 2013) e aumento na condutividade hidráulica do xilema (JONES et al. 2005). Outros estudos indicam que o fornecimento de P pode afetar a o particionamento da matéria seca da planta, pois elas passam a investir relativamente menos carbono e nitrogênio nas raízes e mais nas folhas, que podem levar ao aumento do crescimento relativo e assimilação de CO_2 por unidade de área foliar, bem como o conteúdo de carboidratos, como sacarose, frutose e amido (BURMAN et al. 2009; SANTOS et al. 2004).

Uma alternativa para minimizar a perda de P devido à sua fixação pela fertilização do solo é a adubação foliar. Esse processo possibilita a diminuição do uso de adubos fosfatados, gerando ganhos econômicos e ambientais, pois estes são produzidos a partir de reservas minerais de caráter não renovável. A fertilização foliar é definida como a aplicação de nutrientes solúveis na parte aérea das plantas, visando complementar a nutrição nos períodos de grande consumo de nutrientes e favorecer o equilíbrio nutricional (BRAKEMEIER, 1999). Essa prática tem o objetivo de corrigir deficiências e complementar a fertilização do solo, resultando em economia na utilização dos fertilizantes (WINTER et al. 1963). A absorção de nutrientes via foliar se dá em três passos. Primeiro, após a deposição do elemento na superfície da folha, os nutrientes atravessam a cutícula e as paredes celulares; depois esses

nutrientes são absorvidos na superfície dos plasmalemas e por último passam pela membrana citoplasmática e entram no citoplasma e vacúolo (MALAVOLTA; VITTI; OLIVEIRA; 1997). Na planta o P é um elemento móvel, facilmente redistribuído entre os órgãos, das folhas velhas para as novas, para os frutos e sementes, característica importante para a adubação foliar (SILVA, 2006).

Estudos com adubação foliar em plantas de Caatinga são restritos. Em termos de fósforo, pode-se citar o trabalho de Oliveira et al. (2014), onde foi avaliada a adubação foliar em uma planta nativa (*Anadenanthera colubrina* - angico) e outra exótica (*Prosopis julifrola* – algaroba) da Caatinga, submetidas à seca.

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4. MANUSCRITO I (Increase in biomass of two woody species from a seasonal dry tropical forest in association with AMF with different phosphorus levels)

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(ANEXO A)

Increase in biomass of two woody species from a seasonal dry tropical forest in association with AMF with different phosphorus levels

G. Frosi[a], V. A. Barros[a], M.T. Oliveira[a], U.M.T. Cavalcante[b]; L.C. Maia[b]; M.G. Santos[a]*

[a]Departamento de Botânica, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brasil

[b]Departamento de Micologia, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brasil

*Corresponding author: Tel.: +55 81 21268844; Fax: +55 81 21267803.

E-mail address: mauro.gsantos@ufpe.br (M.G. Santos)

Abbreviation: *A*, net photosynthetic rate; *E*, transpiration rate; AMF, arbuscular mycorrhizal fungi; g_s, stomatal conductance; P, phosphorus; PPFD, photosynthetic photon flux density; RDW, root dry weight; RWC, relative water content; SDW, shoot dry weight; TDW, total dry weight; VPD, vapor pressure deficit; WUE, water use efficiency

Abstract

The study aimed at assessing whether there is association between arbuscular mycorrhizal fungi (AMF) and two woody species from a seasonal dry tropical forest, *Poincianella pyramidalis* and *Cnidoscolus quercifolius*, besides the occurrence of an increase in biomass under different availability of phosphorus (P) in the soil. The experimental design was completely randomized, with a factorial design with two mycorrhizal levels [inoculated (+AMF) and control (-AMF)] × six phosphorus levels (3, 9, 15, 21, 27, 33 mg dm^{-3}). The presence of mycorrhizal structures, mycorrhizal growth, mycorrhizal efficiency, leaf relative water content (RWC) and gas exchange were evaluated. +AMF plants of both species had mycorrhizal structures, while -AMF showed no structures. In *P. pyramidalis*, +AMF plants had benefits with increasing phosphorus level, while -AMF plants had increases biomass only at the P9 level if compared to the P3-AMF level. The total leaf area was correlated with total dry weight (TDW) in +AMF and -AMF plants. However, +AMF plants had more responses in RWC. In *C. quercifolius*, +AMF and -AMF plants did not differ in RWC and showed reductions in gas exchange with increased phosphorus level. However, these reductions were lower in +AMF plants, besides having a better performance over -AMF plants. The growth and mycorrhizal efficiency were higher at the P15 level, and relationship between total leaf area and TDW were significant only in +AMF plants. Thus, both species perform association with AMF and show increases in growth. The concentrations of phosphorus in the soil for *P. pyramidalis* (33 mg dm^{-3}) and *C. quercifolius* (15 mg dm^{-3}) are indicated for increased effectiveness of mycorrhization, promote increases in gas exchange and growth in +AMF plants.

Keywords: gas exchange; mycorrhizal efficiency; *C. quercifolius*; *P. pyramidalis*

1. Introduction

Under natural conditions plants grow in association with various soil microorganisms, besides the intrinsic adjustment mechanisms (Ruiz-Lozano et al., 2012) considered as important strategies to withstand adverse conditions (Pagano et al., 2011). AMF may account for almost 50% of the microbial biomass in tropical ecosystems (Olsson et al., 1999), establishing a symbiotic association with the roots of 80-90% of terrestrial plants (Gadkar et al., 2001; Smith and Read, 2008; Van der Heijden et al., 1998), including angiosperms, gymnosperms, pteridophytes and briophytes (Smith and Read, 1997). Mycorrhizal association was recorded from the first land plants. Among the benefits provided to the host, the nutritional status improvement, especially in the capture of nutrients with low mobility in soil such as P; the increased biomass, water absorption through aquaporins, soil structuring, higher CO_2 assimilation rate, increased growth and increased water use efficiency can be emphasized (Bolandnazar et al., 2007; Drüge and Schönbeck, 1992; Neumann et al., 2009; Querejeta et al., 2007; Ruiz-Lozano and Aroca, 2010; Smith and Read, 2008).

A critical criteria in the association between plant and AMF is the availability of nutrients in the soil (Zangaro et al., 2000). Root colonization usually occurs in soils with low availability of nutrients (Rillig, 2004) and varies among host and AMF species, since establishing a symbiotic relationship requires a fine adjustment (Hause et al., 2007). The presence of mycorrhizal colonization in plants, associated with the increased growth promoted by inoculation, indicates the degree of mycotrophy. It can be high or low (Siqueira and Saggin-Junior, 2001) and such responses may vary. For the tree species, benefits provided by the mycorrhizal colonization on growth and nutrition occur in the restricted limits of P content (Anjos et al., 2005; Costa et al., 2005; Oliveira et al., 2015; Peng et al., 1993; Samarão et al., 2011; Seine et al., 2004).

The nutrient availability is limited in seasonal dry tropical forests, encouraging mycorrhizal establishment. It can be favored by adding phosphorus, which promotes increases in the total number of hyphae (Bago et al., 2000; Nielsen et al., 2002). The association of plants with microorganisms has been identified as important survival strategy in semiarid environments (Pagano et al., 2011). However, little is known about the contribution of mycorrhizal mutualistic relationship in the development of native species in these regions (Maia, 2010).

In this scenario, the present study aims at evaluating the occurrence of association with AMF in two native, endemic and widespread woody species *Poincianella pyramidalis* and *Cnidoscolus quercifolius* from a seasonal tropical dry forest in Brazil (Maia, 2004). For such, the following questions needed to be answered: (1) Do *P. pyramidalis* and *C. quercifolius* associate with AMF? (2) Are both species under study physiologically favored by the increased availability of phosphorus nutrients? (3) Are both species favored by the mycorrhizal association? Therefore, inoculated and non-inoculated seedlings were exposed to different levels of phosphorus under greenhouse conditions.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted in a greenhouse (8°08'58'' S; 34°56'55'' W), with a mean temperature of 30 °C and a relative air humidity of 60%. The plants were kept under pot capacity (300 mL). The design of the study was completely randomized, with a factorial design with two mycorrhizal levels [inoculated (+AMF) and control (-AMF)] × six phosphorus levels (3, 9, 15, 21, 27, 33 mg dm^{-3}). Each treatment had six replicates, totaling 72 experimental units. These concentration intervals were defined from the results reported in the

literature, with positive responses from the AMF for the growth of tree species with lower levels of phosphorus (Costa et al., 2005; Oliveira et al., 2015).

The soil used in the experiment was collected in northeastern of Brazil (07º 33' 38" S; 35º 00' 09" W), due to its low phosphorus content (P: 3 mg/dm^3; pH: 5.7 H_2O; Ca: 0.65 $cmol_c/dm^3$; Mg: 0.6 $cmol_c/dm^3$; Na: 0.04 $cmol_c/dm^3$; K: 0.07 $cmol_c/dm^3$; Al: 0.55 $cmol_c/dm^3$; H: 1.75 $cmol_c/dm^3$; S: 1.4 $cmol_c/dm^3$; CEC: 3.7 $cmol_c/dm^3$). It is classified as Yellow Podzolic. The soil was entirely sterilized by autoclaving at 121°C for 2 h in two alternate days, and dried in a forced ventilation oven at 70°C for 24 h.

AMF isolates [*Acaulospora longula* Spain & NC Schenck (URM AMF 07), and *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler (URM AMF 03)] were provided by the Inoculum Bank of the Laboratory of Mycorrhizas at the Department of Mycology of UFPE. Isolates of both AMFs were placed together in pots with 5 kg of autoclaved soil with *Panicum miliaceum L.* and *Sorghum bicolor* (L) Moench in pot cultures to increase spore density for three month. The number of spores was determined by wet sieving in 50 g of soil, followed by centrifugation at 700 x *g* for 3 min in sucrose (40% w/v) (Jenkins, 1964). The quantification of the spores was performed with a stereoscopic microscope with a 40x magnification on plates with concentric channels (Gerdemann and Nicolson, 1963).

The seeds of *Poincianella pyramidalis* (Tul.) LP Queiroz and *Cnidoscolus quercifolius* Pohl ex Baill were provided by the Reference Center for the Recovery of Degraded Areas (CRAD) - UNIVASF/Petrolina-PE. The seeds were sterilized in 1% hypochlorite (v/v) for 5 min, rinsed in deionized water and placed to germinate in trays containing sterilized washed sand. After 20 days, the plants were transferred to 100 mL pots with autoclaved soil. The plants destined for inoculation received 150 spores of each AMF at the root region, totaling 300 spores per plant. Control plants received the same amount of inoculum soil without AMF

propagules. After 30 days under these conditions, the plants were transferred to pots containing 2 kg of autoclaved soil with its phosphorus level determined by the application of simple superphosphate (P_2O_5), where the first level had no P addition (soil concentration was 3 mg dm^{-3}) followed by five levels with addition: 9, 15, 21, 27, 33 mg dm^{-3}, representing the levels P3 (soil concentration), P9, P15, P21, P27 and P33, respectively. When plants completed four months of development, mycorrhizal structures in roots, biometrics, total leaf area, biomass, leaf relative water content, soil water status and gas exchange were evaluated.

2.2. Mycorrhizal structures

The verification of root colonization was conducted by observing fungal structures. For this, the roots were clarified with KOH (10%, w/v) for 24 h, washed with distilled water and stained with trypan blue in lactoglycerol (0.05%, w/v) for 12 h (Phillips and Hayman, 1970). The evaluation was performed using the glass plate method with 1 cm root fragments from all individuals with a microscope with a 40x magnification (Giovannetti and Mosse, 1980).

2.3. Growth, mycorrhizal increase and mycorrhizal efficiency

The biometric data on the number of leaves, height (cm) and diameter (mm) was taken on the last day of the experiment. The number of leaves were counted, the height was obtained with a millimeter ruler and the diameter was measured with a digital caliper. Subsequently, leaves were scanned and the total leaf area was calculated using the Image Pro software. Afterwards, shoots and roots were placed in a forced ventilation oven at 70°C for 5 days. The material was weighed on precision scales (AND H200, Tokyo, JP), thus obtaining total dry weight. Using biometrics (number of leaves, height, diameter and total leaf area) and dry biomass data [shoots (SDW), roots (RDW) and total biomass (TDW)], the mycorrhizal

136 increase (100[(X-Y)]/Y) and the mycorrhizal efficiency (100[(X-Y)]/X) were calculated,
137 where X is mycorrhizal plants and Y is control plants (Weber et al., 2004).
138
139 *2.4. Leaf relative water content (RWC) and soil water status*
140 Leaf disks with a known size were collected at 06:00 and immediately weighed on a
141 precision scale to obtain the fresh weight (FW). Then, the leaf disks were soaked for 24 h in
142 deionized water and reweighed to obtain the swelling weight (SW). Subsequently, the disks
143 were dried in a forced ventilation oven for 48 h and reweighed to obtain the dry weight (DW).
144 The leaf relative water content (RWC) was calculated by the following formula, according to
145 Barrs and Weatherley (1962): RWC (%)=[FW-DW/SW-DW]x100. Soil moisture was
146 obtained in all replicates used for the evaluation of gas exchange using the meter Falker HFM
147 2030 (v/v), the soil moisture measured was 12.2% in the pots for the two species.
148
149 *2.5. Gas exchange*
150 Gas exchange was measured in expanded and non-senescing leaves through an infrared
151 gas analyzer (IRGA, ADC, model LCi-Pro; Hoddesdon, UK), obtaining stomatal conductance
152 (g_s), net photosynthetic rate (A) and transpiration rate (E). The water use efficiency (WUE)
153 was calculated by the ratio A/E. Measurements were taken from 09:00 to 10:00 with a 1,000
154 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD),
155 ranging from 2.3 to 2.7 kPa, which corresponded to ambient conditions.
156
157 *2.6. Statistical analysis*
158 RWC data, g_s, A, E and WUE were subjected to a linear regression analysis with R^2
159 adjusted at a 5% significance level. The lines of the regressions between inoculated (+AMF)
160 and control (-AMF) treatments were compared with an analysis of covariance. Linear

161 correlations were made between leaf area and TDW. For *P. pyramidalis*, a Pearson correlation
162 was performed because the data did not show a normal distribution, while for *C. quercifolius*,
163 a Spearman correlation was used because data had a normal distribution. Increase data and
164 mycorrhizal efficiency data were transformed to √arcsine(x) and analyzed by ANOVA one
165 way, with phosphorus levels used as an independent variable. When necessary, the means
166 were compared using the Student Newman Keuls test at a 5% significance level. Analyses
167 were performed with the software BioEstat 5.0. Normality and homogeneity were tested for
168 all analyses. Except the correlation of *P. pyramidalis* plants with leaf area and TDW, the data
169 showed a normal distribution.
170
171 **3. Results**
172 *3.1. Mycorrhizal structures*
173 The presence of hyphae, external mycelium and vesicles were observed in the two plant
174 species only in inoculated plants (+AMF), and were absent in non-inoculated plant roots.
175 Arbuscules were observed only in +AMF *P. pyramidalis* plants at the P33 level (**Fig. 1**).
176
177 *3.2. Growth, mycorrhizal increase and mycorrhizal efficiency*
178 The biomass of -AMF *P. pyramidalis* plants for SDW, RDW, TDW was higher at the P9
179 level if compared to the P3-AMF level (**supplementary material 1**). An increase in the
180 number of leaves was observed in +AMF *P. pyramidalis* plants at P9, P15, P27 and P33
181 levels, while increases in the height of plants occurred at P3 and P21 levels. There were
182 increases in diameter in +AMF plants at P21, P27 and P33 levels. For total leaf area, the
183 highest increase occurred at the P33+AMF level (**Table 1**). Regarding mycorrhizal efficiency,
184 increases in SDW were obtained at P27 and P33 levels in +AMF plants. Regarding

underground parts (RDW) and total biomass (TDW), the highest values were observed at P33+AMF. (**Table 1**).

In *C. quercifolius* plants, the greatest increase in the number of leaves occurred at P9+AMF level. For height and leaf area, the highest percentage was obtained at P15+AMF. In diameter, increases were observed at P9+AMF and P27+AMF. Regarding mycorrhizal efficiency, higher percentages of SDW, RDW and TDW occurred at P15+AMF. (**Table 1**).

3.3. Leaf relative water content (RWC)

The mycorrhizal treated *P. pyramidalis* in low phosphorus (P3) had 25% lower RWC compared to -AMF plants, while the increase in the level of phosphorus promoted a RWC increase in +AMF plants and a reduction in -AMF plants (**Fig. 2a**). At the P33 level, +AMF plants showed a 32% increase compared to the lower P3+AMF level, while -AMF plants had 13.6% reductions with the increase in phosphorus when compared to P3-AMF. The regression lines between +AMF and -AMF plants differed as to slope ($P<0.0001$; $t=4.55$). *C. quercifolius* did not differ between mycorrhizal and phosphorus levels in RWC, with an average water content of 86% (**Fig. 2b**).

3.4. Gas exchange

In *P. pyramidalis*, +AMF plants showed increases in gas exchange variables with the increase in the level of phosphorus in the soil. -AMF plants had reductions, except for WUE. In +AMF plants, the P33 level promoted increases of 52, 71, 55 and 17% in g_s, *A*, *E* and WUE compared to P3+AMF plants; -AMF plants showed 97, 18 and 62% reductions in g_s, *A* and *E*, and 123% increases in WUE compared to P3-AMF (**Fig. 3a, c, e, g**). The regression lines between +AMF and -AMF plants differed as to slope for g_s ($P<0.0001$, $t=7.17$); *A* ($P<0.0001$, $t=5.31$); *E* ($P<0.0001$, $t=6.12$) and WUE ($P<0.0001$, $t=-4.08$).

In *C. quercifolius*, mycorrhizal treatments increased in g_s, *A* and *E*. However, the increase in the level of phosphorus in the soil caused reductions in these variables, except WUE in +AMF plants. +AMF plants at the P33 level showed reductions of 14.5, 7.3 and 27.2% in g_s, *A* and *E*, and 27% gains in WUE compared to the P3+AMF level, while -AMF plants showed reductions of 39.6, 19.5, and 17.5% in g_s, *A* and WUE in P33-AMF plants; for *E*, the regression was not significant (**Fig. 3b, d, f, h**). The regression lines between +AMF and -AMF plants differed as to slope for g_s ($P<0.05$, $t=2.18$), *E* ($P<0.01$, $t=-3.34$) and WUE ($P<0.0001$, $t=7.25$), and in the intercept for *A* ($P<0.0001$, $t=11.82$).

3.5 Relationship of total leaf area and total biomass

The increase in total leaf area promoted significant increases in TDW in *P. pyramidalis* with both mycorrhizal levels (-AMF and +AMF). However, the greatest response was observed in +AMF plants(**Fig. 4a**). In *C. quecifolius*, the increase in total leaf area only showed increases in TDW in +AMF plants (**Fig. 4b**).

4. Discussion

The main focus of this study was on evaluating whether two native woody species of a seasonal tropical dry forest in Brazil performed a mycorrhizal association were associated with AMF fungi, whether both species were physiologically favored by the increased availability of phosphorus nutrients and whether both species had advantage with this association. For this, mycorrhizal structures, mycorrhizal increase (number of leaves, height, diameter and total leaf area) and efficiency (shoot and root biomass), RWC and gas exchange were measured.

+AMF *P. pyramidalis* plants were favored regarding the variables evaluated with the increased phosphorus levels, while -AMF plants had reduction in such variables. *C.*

235 *quercifolius* had reduced rates of gas exchange with increased availability of P in both
236 mycorrhizal levels. However, +AMF plants had higher values of gas exchange considering
237 the evaluated variables if compared to -AMF plants. Generally, the contribution of AMFs
238 regarding water, nutrients and biomass increases may be important when there is low
239 availability of nutrients in the soil, especially P, while fungi benefit from photoassimilates
240 produced by the host plant (Neumann et al., 2009; Smith and Read, 2008; Parniske, 2008; Xie
241 et al., 2014).

242 The presence of arbuscules indicates mycorrhization. These structures were present in
243 +AMF *P. pyramidalis* plants at the P33 level. The exchange of nutrients between AMF and
244 the host plant occurs in fungal structures such as hyphae, and especially arbuscules -
245 structures inside root cells (Nara and He, 2007; Parniske, 2008). This species, showed greater
246 mycorrhizal increase in total leaf area, mycorrhizal efficiency in the biomass, RWC and gas
247 exchange rates in +AMF plants. The increased biomass, evidenced by higher values of
248 mycorrhizal efficiency for SDW, RDW and TDW, is associated with higher photosynthetic
249 rates (**Table 1; Fig. 3c**). According to Balota et al. (2011), total leaf area and photosynthetic
250 capacity are closely related. A larger leaf surface improves light interception, promoting
251 increases in photosynthesis and growth. A relationship between total leaf area and TDW was
252 also observed for both control and inoculated plants. However, the increase in photosynthetic
253 area and biomass allocation was enhanced in the presence of AMFs (**Fig. 4a**). This is another
254 indicative of the relationship among leaf area, photosynthetic rates and biomass production. In
255 addition to total leaf area, +AMF *P. pyramidalis* plants, especially at the P33 level, may have
256 benefited from greater water absorption as indicated by the higher RWC. The highest rates of
257 *E* at that level, with little changes in WUE, suggest that instead of economy, a greater
258 absorption of water by fungal hyphae may have occurred (**Fig. 2a, Fig. 3e, g**). At the P3 level,
259 mycorrhizal plants had gas exchange rates similar to control plants, but with RWC reduction.

260 The water is absorbed by the external mycelium and moves through the hyphae favoring the
261 apoplastic stream in the root system (Bárzana et al., 2012). This response may be studied in
262 future works to assess the activity of aquaporins in plants inoculated with AMF.

263 Different responses were found for *Anadenanthera peregrina*, a pioneer legume. When
264 inoculated with *Glomus* sp. and *Acaulospora* sp., there were no differences in terms of growth
265 at the tested phosphorus levels (32.5, 65 and 136 mg dm^{-3}) (Pagano et al., 2007). On the other
266 hand, *Schinopsis brasilienses* plants inoculated with *C. etunicatum* showed behavior opposite
267 to that observed for *P. pyramidalis*, whose inoculated plants had lower growth at the highest P
268 level (48 mg dm^{-3}), while control plants were benefited by it (Oliveira et al., 2015). These
269 results emphasize the importance of species-dependent responses. The symbiotic efficiency
270 between symbiotic pairs and a better understanding of the gains for both parties should be
271 investigated.

272 *C. quercifolius* had different behavior when compared to *P. pyramidalis* plants. In this
273 species, arbuscules were not observed in roots of +AMF plants at all phosphorus levels. This
274 absence may indicate that roots were at different stages of colonization, besides having a short
275 life span, i.e., 7 to 12 days (Gadkar et al., 2001). The mycorrhizal increase in total leaf area,
276 especially at the P15 level in +AMF plants, was not reflected in higher gas exchange rates at
277 this level (**Table 1; Fig 3d**). However, the relationship between the leaf area and TDW of
278 these plants indicates higher biomass with the increase in leaf surface (**Fig. 4b**). The reduction
279 observed for *A* at the P15 level (3.1%) when compared to the P3 level was offset by this
280 photosynthetic surface increase, showing a trade-off between leaf area and photosynthesis and
281 thus promoting dry biomass increase indicated by increases in the mycorrhizal efficiency in
282 SDW, RDW and TDW at this phosphorus level. The growth reduction of these plants from
283 the P21 level may be related to the symbiotic association cost to the host, in addition to this
284 nutrient's toxicity at higher concentrations (Abbott and Robson, 1986). The RWC was similar

285 for the two symbiotic levels at all phosphorus levels. However, there was a greater reduction
286 of *E* in +AMF plants at the P15 level, resulting in a greater WUE in comparison with -AMF
287 plants (**Fig. 2b; Fig. 3 f, h**). Thus, there was greater water economy in these plants rather than
288 increased absorption.

289 Studies on native plants from Brazil, typical from a seasonal dry tropical forest (caatinga)
290 one of the five Brazilian ecosystems, located in northeastern Brazil, showed that the
291 development of various species might be benefited from the association between AMF and/or
292 phosphorus fertilization (Oliveira et al., 2015). These treatments, alone or in combination,
293 promoted increase of biomass production in *Hymenaea courbaril*, *Inga laurina* and *Sterculia*
294 *striata* plants (Lacerda et al., 2011). In our case, *P. pyramidalis* plants showed increase when
295 using phosphate fertilization in the presence of AMF, being emphasized the total leaf area,
296 biomass allocation, RWC and gas exchange. Control plants had reduction in RWC and gas
297 exchange. However, these plants had increase in biomass at the P9 level, whose values
298 obtained were similar to those of +AMF plants at the P3 level. For *C. quercifolius*, +AMF
299 plants increased with the use of phosphate fertilization up to the P15 level. This was mainly
300 indicated by a greater increase in total leaf area and mycorrhizal efficiency in dry biomass,
301 without increase in the absence of AMF. This is corroborated by the relationship between leaf
302 area and TDW. There was no greater biomass allocation to a larger leaf area in control plants,
303 while +AMF plants had moderate and significant relationship, showing the importance of
304 AMF for the development of benefits for this species.

305 Determining the optimal P dose to optimize production and plant response under a
306 specific condition is important considering that such a dose is different for each plant species
307 and fungus. In addition, a combined assessment of physiological data is critical in
308 determining the best level of fertilization for an effective mycorrhizal association. Through
309 this study, it is possible to point to total leaf area, biomass allocation and photosynthetic rates

as the most responsive attributes. Considering all the attributes evaluated, *P. pyramidalis* and *C. quercifolius* plants responded differently regarding AMF and tested phosphorus levels. However, because they are seasonal dry tropical forest species, the increase in the speed of biomass increment may determine their survival under limited water availability conditions.

5. Conclusion

It was found that the two species analyzed perform association with AMFs. *Poincianella pyramidalis* had benefits regarding the evaluated variables with the increase of P level in +AMF plants, while -AMF plants had biomass increases at the P9 level only. However, these values were similar at the lowest P level for mycorrhizal plants. *Cnidoscolus quercifolius* had decreases in the variables evaluated at the two mycorrhizal levels, with an increase in P level. However, +AMF plants had a better performance in relation to -AMF plants. Thus, both species were favored with the mycorrhizal association. Phosphorus concentrations in soil of 33 and 15 mg dm^{-3} may be recommended to *P. pyramidalis* and *C. quercifolius*, respectively, in association with mycorrhizal fungi for a greater effectiveness of mycorrhization, increase in gas exchange and growth.

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436

Figure legends

Fig. 1 Mycorrhizal structures in roots of young *Poincianella pyramidalis* (a, c, e) and *Cnidoscolus quercifolius* (b, d, f) plants subjected to six phosphorus levels (3, 9, 15, 21, 27 e 33 mg dm^{-3}) under greenhouse conditions. 1- Arbuscules, 2- Hyphae, 3- Vesicles.

Fig. 2 Relative water content (RWC) in the leaves of young *P. pyramidalis* and *C. quercifolius* plants in two mycorrhizal (-AMF and +AMF) and at six phosphorus levels (3, 9, 15, 21, 27 and 33 mg dm^{-3}) under greenhouse conditions. Measurements refer to the last day of experiment (n=3). *P<0.05, **P<0.01, ***P<0.001

Fig. 3 Gas exchange in the leaves of young *P. pyramidalis* and *C. quercifolius* plants in two mycorrhizal (-AMF and +AMF) and at six phosphorus levels (3, 9, 15, 21, 27 and 33 mg dm^{-3}) under greenhouse conditions. Measurements refer to the last day of experiment. The variables are: a- stomatal conductance (g_s); b – net photosynthetic rate (A); c – transpiration rate (E); d – water use efficiency (WUE) (n=6). *P<0.05, **P<0.01, ***P<0.001

Fig. 4 Correlations between total leaf area and total dry weight (TDW) of young *P. pyramidalis* (a) and *C. quercifolius* (b) plants in two mycorrhizal (-AMF and +AMF) and at six phosphorus levels (3, 9, 15, 21, 27 and 33 mg dm^{-3}) under greenhouse conditions. Measurements refer to the last day of experiment (n=6). *P<0.05, **P<0.01, ***P<0.001

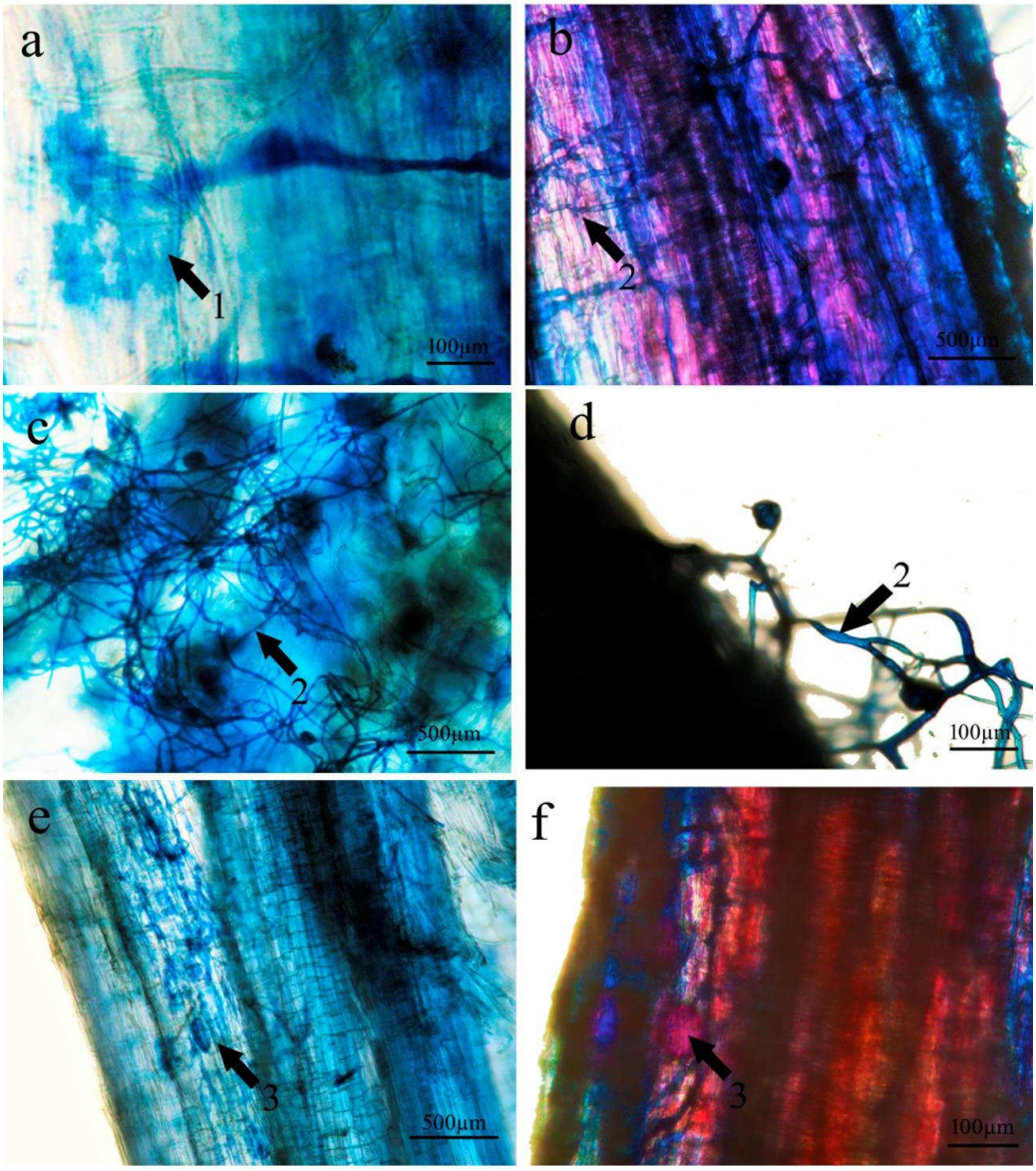

Fig. 1

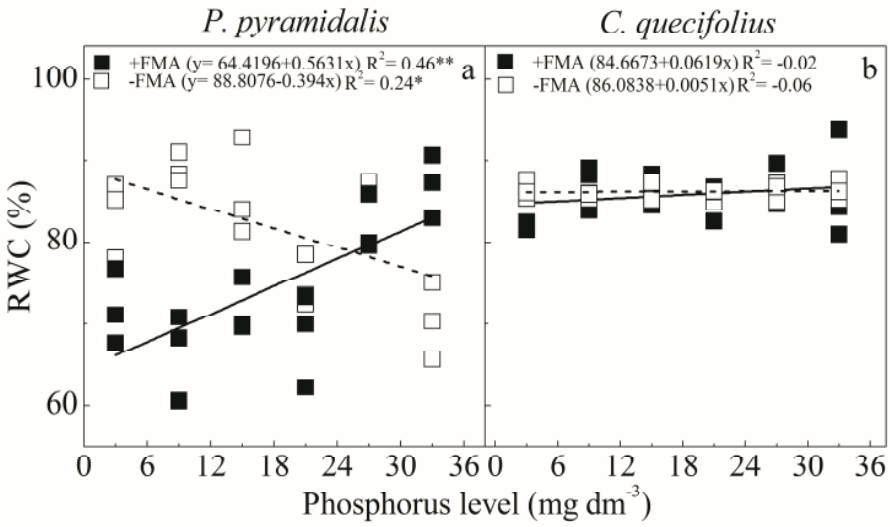
P. pyramidalis
C. quecifolius
+FMA (y= 64.4196+0.5631x) R2= 0.46**
-FMA (y= 88.8076-0.394x) R2= 0.24*
+FMA (84.6673+0.0619x) R2= -0.02
-FMA (86.0838+0.0051x) R2= -0.06
a
b
RWC (%)
100
80
60
0
6
12
18
24
30
36
Phosphorus level (mg dm-3)

Fig. 2

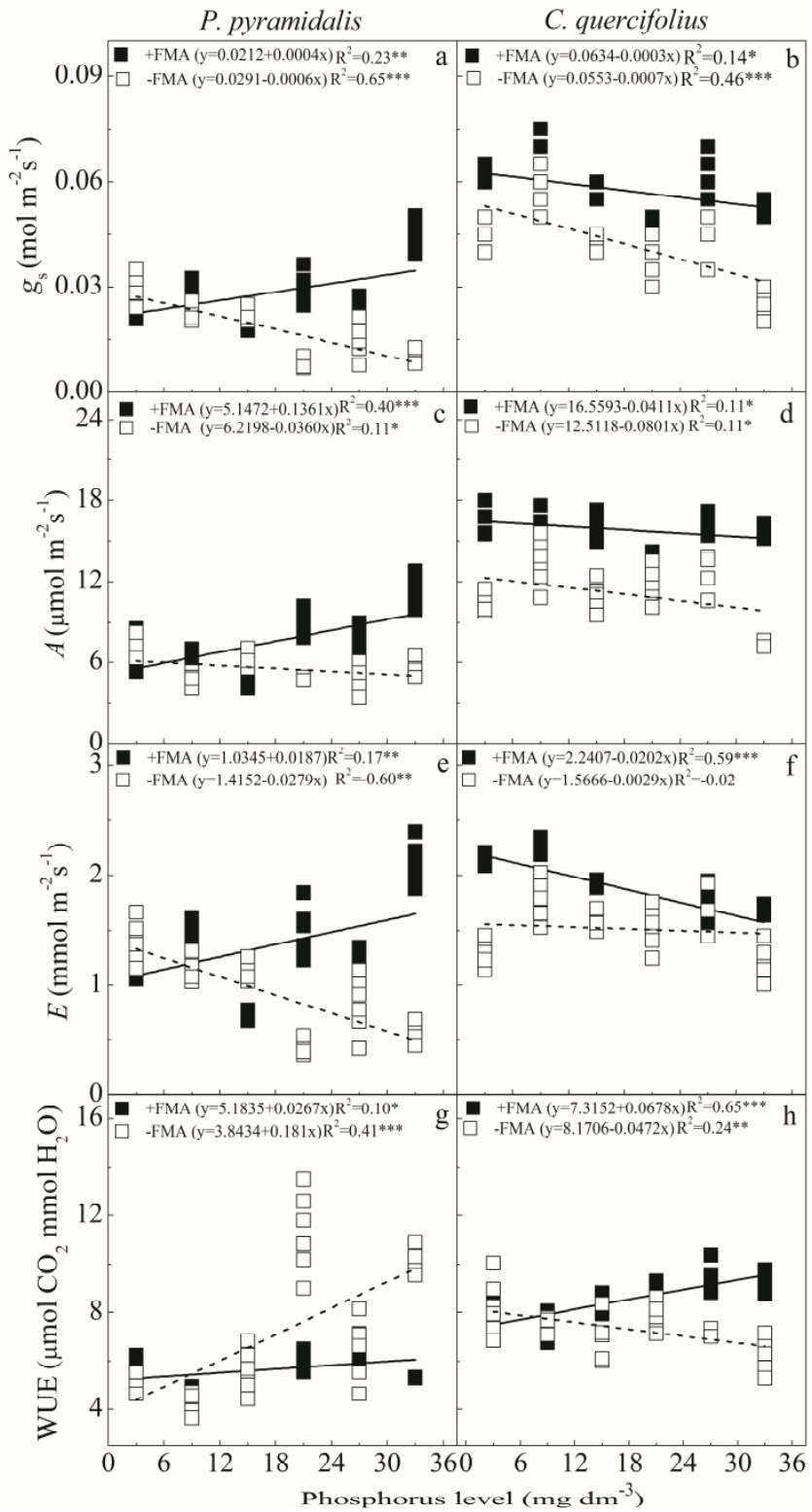
P. pyramidalis
C. quercifolius
+FMA (y=0.0212+0.0004x) R2=0.23** a
-FMA (y=0.0291-0.0006x) R2=0.65***
+FMA (y=0.0634-0.0003x) R2=0.14* b
-FMA (y=0.0553-0.0007x) R2=0.46***
gs (mol m-2 s-1)
+FMA (y=5.1472+0.1361x)R2=0.40*** c
-FMA (y=6.2198-0.0360x)R2=0.11*
+FMA (y=16.5593-0.0411x) R2=0.11* d
-FMA (y=12.5118-0.0801x) R2=0.11*
A (μmol m-2 s-1)
+FMA (y=1.0345+0.0187)R2=0.17** e
-FMA (y=-1.4152-0.0279x) R2=-0.60**
+FMA (y=2.2407-0.0202x)R2=0.59*** f
-FMA (y=-1.5666-0.0029x)R2=-0.02
E (mmol m-2 s-1)
+FMA (y=5.1835+0.0267x)R2=0.10* g
-FMA (y=3.8434+0.181x)R2=0.41***
+FMA (y=7.3152+0.0678x)R2=0.65*** h
-FMA (y=8.1706-0.0472x) R2=0.24**
WUE (μmol CO2 mmol H2O)
Phosphorus level (mg dm-3)

Fig. 3

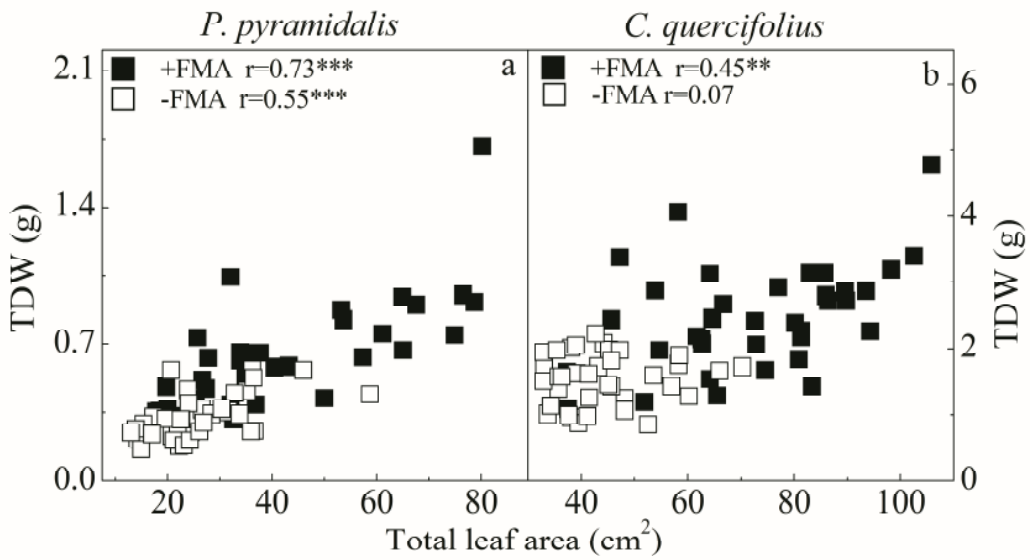
P. pyramidalis
C. quercifolius
+FMA r=0.73***
-FMA r=0.55***
+FMA r=0.45**
-FMA r=0.07
a
b
TDW (g)
TDW (g)
Total leaf area (cm2)
2.1
1.4
0.7
0.0
6
4
2
0
20
40
60
80
40
60
80
100

Fig. 4

Table 1. Mycorrhizal increase and mycorrhizal efficiency in the young *P. pyramidalis* and *C. quercifolius* plants in two symbiosis (-AMF and +AMF) and six phosphorus levels (P3, P9, P15,P 21, P27 and P33 mg dm^{-3}) under greenhouse conditions. Where: shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW). Values represent the mean of replicates (n=6±S.E).

Treatment	Mycorrhizal Increase (%)				Mycorrhizal Efficiency (%)		
	Leaf Number	Height	Diameter	Leaf Area	SDW	RDW	TDW
P. pyramidalis							
P3	17.0 ± 3.3^{C}	25.9 ± 2.3^{A}	23.5 ± 1.3^{C}	10.1 ± 1.3^{E}	43.2 ± 2.25^{B}	53.3 ± 2.73^{C}	46.2 ± 2.55^{C}
P9	33.3 ± 6.1^{AB}	12.1 ± 1.6^{B}	8.7 ± 1.5^{D}	-16.1 ± 1.1^{F}	16.7 ± 0.70^{D}	34.8 ± 1.00^{E}	23.1 ± 0.98^{E}
P15	51.1 ± 6.5^{A}	14.3 ± 1.8^{B}	35.3 ± 2.7^{B}	125.9 ± 6.9^{B}	43.5 ± 2.76^{B}	43.5 ± 1.51^{D}	43.5 ± 1.51^{C}
P21	30.9 ± 2.8^{BC}	20.8 ± 2.9^{A}	46.7 ± 2.8^{A}	21.5 ± 1.2^{D}	35.7 ± 0.75^{C}	20.0 ± 1.63^{F}	31.6 ± 1.04^{D}
P27	37.5 ± 4.9^{AB}	9.3 ± 0.7^{B}	53.3 ± 4.5^{A}	75.7 ± 3.2^{C}	55.9 ± 2.04^{A}	68.4 ± 1.96^{B}	60.4 ± 1.36^{B}
P33	40.0 ± 4.0^{AB}	12.1 ± 1.6^{B}	47.1 ± 4.3^{A}	172.7 ± 7.7^{A}	60.0 ± 1.87^{A}	86.8 ± 2.21^{A}	69.9 ± 1.58^{A}
C. quercifolius							
P3	6.9 ± 1.1^{E}	37.5 ± 1.1^{C}	14.8 ± 0.8^{B}	27.2 ± 0.8^{E}	-25.0 ± 1.64^{E}	2.4 ± 0.50^{E}	-5.7 ± 0.94^{D}
P9	78.9 ± 1.2^{A}	32.2 ± 1.7^{C}	26.1 ± 1.0^{A}	113.4 ± 2.2^{B}	22.2 ± 0.70^{D}	21.4 ± 0.61^{D}	21.6 ± 0.64^{C}
P15	52.6 ± 3.9^{B}	76.9 ± 2.6^{A}	8.7 ± 0.5^{C}	138.8 ± 3.3^{A}	52.9 ± 2.39^{A}	73.5 ± 2.20^{A}	67.5 ± 1.90^{A}
P21	32.6 ± 1.5^{C}	46.7 ± 1.7^{B}	17.4 ± 0.8^{B}	14.1 ± 0.8^{F}	36.1 ± 1.80^{C}	43.5 ± 1.36^{B}	40.9 ± 1.62^{B}
P27	39.5 ± 0.8^{C}	32.1 ± 1.5^{C}	25.0 ± 0.9^{A}	75.4 ± 2.1^{C}	21.3 ± 1.02^{D}	49.6 ± 1.28^{B}	39.2 ± 1.33^{B}
P33	25.0 ± 1.1^{D}	51.8 ± 1.5^{B}	6.4 ± 0.4^{D}	33.5 ± 0.9^{D}	44.5 ± 1.13^{B}	32.8 ± 1.00^{C}	38.0 ± 1.51^{B}

Different letters indicate statistically significant differences by Newman-Keul test between treatments (columns) within the same species.

Supplementary 1 Attributes of biometrics and biomass in the young *P. pyramidalis* and *C. quercifolius* plants in two symbiosis (-AMF and +AMF) and six phosphorus levels (3, 9, 15, 21, 27 and 33 mg dm^{-3}) under greenhouse conditions. Where: shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW). Values represent the mean of replicates (n=6±S.E).

Treatment	Biometric				Biomass		
	Leaf Number	Height (cm)	Diameter (mm)	Leaf Area (cm^2)	SDW (g)	RDW (g)	TDW (g)
P. pyramidalis							
P3-FMA	4.7±0.4^{B}	5.4±0.2^{B}	1.7±0.1^{B}	27.8±2.2^{DEF}	0.21±0.02^{DEF}	0.07±0.01^{EF}	0.28±0.03^{DE}
P9-FMA	4.5±0.5^{B}	6.6±0.4^{B}	2.3±0.1^{B}	40.9±4.0^{C}	0.35±0.02^{BCD}	0.15±0.02^{BCD}	0.50±0.02^{BC}
P15-FMA	4.5±0.3^{B}	7.0±0.5^{B}	1.7±0.2^{B}	25.1±2.6^{DEFG}	0.26±0.03^{CDEF}	0.13±0.01^{DE}	0.39±0.04^{CD}
P21-FMA	4.2±0.2^{B}	5.3±0.2^{B}	1.5±0.1^{B}	17.7±1.4^{G}	0.18±0.01^{EF}	0.08±0.00^{EF}	0.26±0.02^{DE}
P27-FMA	4.0±0.3^{B}	6.4±0.3^{B}	1.5±0.1^{B}	18.9±1.9^{FG}	0.15±0.01^{F}	0.06±0.00^{F}	0.21±0.01^{E}
P33-FMA	4.5±0.4^{B}	6.6±0.3 ^{B}	1.7±0.1^{B}	27.1±1.8^{DEFG}	0.26±0.01^{CDEF}	0.05±0.00^{F}	0.31±0.02^{DE}
P3+FMA	5.5±0.2^{A}	6.8±0.4^{A}	2.1±0.1^{A}	30.6±3.1^{DE}	0.37±0.03^{BCD}	0.15±0.01^{BCD}	0.52±0.03^{BC}
P9+FMA	6.0±0.3^{A}	7.4±0.4^{A}	2.5±0.2^{A}	34.3±0.8^{CD}	0.42±0.05^{BC}	0.23±0.04^{BC}	0.65±0.09^{B}
P15+FMA	6.8±0.4^{A}	8.0±0.5^{A}	2.3±0.1^{A}	56.7±2.3^{B}	0.46±0.04^{B}	0.23±0.02^{BC}	0.69±0.07^{B}
P21+FMA	5.5±0.3^{A}	6.4±0.5^{A}	2.2±0.1^{A}	21.5±1.5^{EFG}	0.28±0.03^{CDEF}	0.10±0.01^{CDE}	0.38±0.05^{CD}
P27-FMA	5.5±0.3^{A}	7.0±0.3^{A}	2.3±0.2^{A}	33.2±2.7^{CD}	0.34±0.04^{BCDE}	0.19±0.02^{BCD}	0.53±0.06^{BC}
P33+FMA	6.3±0.3^{A}	7.4±0.3^{A}	2.5±0.2^{A}	73.9±3.6^{A}	0.65±0.08^{A}	0.38±0.05^{A}	1.03±0.14^{A}
C. quercifolius							
P3-FMA	4.3±0.4^{BC}	3.2±0.2^{B}	4.7±0.2^{B}	40.5±1.8^{DE}	0.65±0.06^{BCD}	1.20±0.10^{BC}	1.85±0.08^{BCD}
P9-FMA	3.8±0.2^{C}	3.1±0.0^{B}	4.6±0.2^{B}	35.8±1.4^{E}	0.63±0.04^{BCD}	1.14±0.09^{BC}	1.77±0.13^{BCD}
P15-FMA	3.8±0.4^{C}	2.6±0.2^{B}	4.6±0.3^{B}	39.7±2.0^{DE}	0.48±0.06^{D}	0.63±0.06^{D}	1.11±0.11^{D}
P21-FMA	4.3±0.2^{BC}	3.0±0.3^{B}	4.6±0.3^{B}	49.1±3.2^{CD}	0.69±0.02^{BCD}	1.13±0.08^{BC}	1.82±0.09^{BCD}
P27-FMA	4.3±0.5^{BC}	2.8±0.2^{B}	4.0±0.3^{B}	41.1±2.1^{DE}	0.59±0.02^{BCD}	0.65±0.08^{D}	1.24±0.09^{D}
P33-FMA	4.8±0.3^{BC}	2.7±0.1^{B}	4.7±0.2^{B}	59.9±2.8^{C}	0.56±0.05^{BCD}	0.86±0.08^{CD}	1.42±0.13^{CD}
P3+FMA	4.6±0.5^{BC}	4.4±0.2^{A}	5.4±0.4^{A}	51.5±4.8^{C}	0.52±0.05^{CD}	1.23±0.13^{BC}	1.75±0.22^{BCD}
P9+FMA	6.8±0.6^{A}	4.1±0.3^{A}	5.8±0.4^{A}	76.4±4.5^{B}	0.81±0.08^{B}	1.45±0.21^{B}	2.26±0.26^{B}
P15+FMA	5.8±0.4^{AB}	4.6±0.3^{A}	5.0±0.3^{A}	94.8±3.7^{A}	1.02±0.06^{A}	2.40±0.26^{A}	3.42±0.28^{A}
P21+FMA	5.7±0.3^{AB}	4.4±0.4^{A}	5.4±0.3^{A}	56.0±3.5^{C}	1.08±0.08^{A}	2.00±0.15^{A}	3.08±0.23^{A}
P27-FMA	6.0±0.4^{AB}	3.7±0.1^{A}	5.0±0.6^{A}	72.1±5.3^{B}	0.75±0.05^{BC}	1.29±0.15^{BC}	2.04±0.19^{BC}
P33+FMA	6.0±0.4^{AB}	4.1±0.3^{A}	5.0±0.2^{A}	80.0±2.4^{B}	1.01±0.07^{A}	1.28±0.09^{BC}	2.29±0.15^{B}

Different letters indicate statistically significant differences by Newman-Keul test between treatments (columns) within the same species.

5. MANUSCRITO II (Symbiosis with AMF and leaf P_i supply increases water deficit tolerance of woody species from seasonal dry tropical forest)

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(ANEXO B)

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

Gabriella Frosi[a], Vanessa A. Barros[a], Marciel T. Oliveira[a], Mariana Santos[a], Diego G. Ramos[a], Leonor C. Maia[b], Mauro G. Santos[a,*]

[1]Departamento de Botânica, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brazil

[2]Departamento de Micologia, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brazil

*Corresponding author: Mauro G. Santos

Tel.: +55 81 21268844; Fax: +55 81 21267803.

E-mail address: mauro.gsantos@ufpe.br

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 (Pi) supply. The experiment was conducted in a factorial arrangement of 2 water levels (+H2O and −H2O), 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.

Keywords: Caatinga; Chlorophyll *a* fluorescence; Gas exchange; Mycorrhiza; Phosphorus; Plant biochemistry

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 with stand 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 bythe 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 hasbeen attributed to a greater stomatal conductance and photosynthesis (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 semiarid 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

89 *2.1. Growth conditions and plant material*

90

91 The experiment was conducted in a greenhouse at the Universidade Federal de
92 Pernambuco (UFPE) (8°08'58"S, 34°56'55"W), under average temperature of 30 ± 2 °C and
93 50-60 % relative humidity, with plants kept in pot capacity (300 mL) until the beginning of
94 the water deficit. The design was completely randomized in a factorial arrangement of 2 water
95 levels [irrigated (+H_2O) and drought (-H_2O)] x 2 symbiosis levels [inoculated (+AMF) and
96 non-inoculated (-AMF)] x 2 leaf phosphorus levels [with leaf inorganic phosphorus (+P_i) and
97 without leaf inorganic phosphorus (-P_i)], totaling eight treatments: +H_2O-AMF-P_i; +H_2O-
98 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; -
99 H_2O+AMF+P_i; with 8 replicates each and one plant per pot, totaling 64 experimental units.
100 Out of these 8 replicates, 4 were designed to evaluate biomass and nutrients (N, P and K) and
101 4 intended for relative water content (RWC), gas exchange, chlorophyll fluorescence and
102 biochemical analysis.

103 Isolated AMF [*Acaulospora longula* Spain & N.C. Schenck (URM FMA 07) and
104 *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler (URM FMA
105 03)] were provided by the Mycorrhizas Laboratory's Inoculum Bank at the Mycology
106 Department of UFPE. These AMF species were chosen because they belong to two most
107 common genera in the Brazilian semiarid in Northeast region, in Pernambuco State (Silva et
108 al., 2014). Inoculum of each isolate was placed in pots containing 5 kg of sterilized soil with
109 culture of *Panicum miliaceum* L. and *Sorghum bicolor* (L) Moench to increase spores density
110 in three months. Spores were extracted from the soil by wet sieving and centrifugation in
111 water and sucrose (Jenkins, 1964; Gerdemann and Nicolson, 1963) and then measured under
112 stereomicroscope (40x), to quantify the number of spores per gram of soil used in
113 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 a close contact between spores and roots. In those for inoculation, the soil inoculum content 150 spores from each AMF was applied in 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 treatments were sprayed with 20 mL of equivalent dose of nitrogen as urea PA solution (2.64 g N L^{-1}) as described in Santos et al. (2006) to compensate the N added in the Pi treatment. Water deficit was imposed by watering suspension when plants completed 6 months

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, time required for the recovery of the relative water content. This investigation is very important to verify if the plants have the capacity to recovery your metabolism under well-hydrated condition after exposure to drought. . In 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 were sampled of each treatment. For each plant, 1g 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 pieces of 1 cm of each sample were examined under a microscope at 40x 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 soaked for 24 h in deionized water and weighed again for turgid weight (TW). Subsequently, disks were dried in 48 h forced ventilation and weighed to obtain dry weight (DW). The leaf relative

164 water content was measured by the formula: Relative water content (%) = [FW-DW/TW-DW]
165 x 100 (Barrs and Weatherley, 1962).
166
167 *2.4. Gas exchange, vapour pressure deficit and soil moisture*
168
169 Gas exchange was measured in third expanded leaf pair with an infrared gas analyzer
170 (IRGA, ADC, LCi-Pro model, Hoddesdon, UK), obtaining stomatal conductance, net
171 photosynthetic rate and transpiration rate. Measurements were performed between 9:00-11:00
172 am and photosynthetic photon flux density was determined by global radiation incident at
173 measurement time, 800 μmol $m^{-2}s^{-1}$, with the air flow through the sample chamber of 400
174 μmol $m^{-2}s^{-1}$ and CO_2 concentration of 400 ppm, due local atmospheric concentration. The
175 vapor pressure deficit during experiment was calculated by the formula e_s-e_a, where e_s is the
176 saturated vapor pressure and e_a is the ambient vapor pressure (Campbell and Norman, 1998),
177 obtained using temperature and relative humidity and measured with a digital thermo
178 hygrometer (Termo-Higro SH 122, J Prolab. São José dos Pinhais, BR). Soil moisture content
179 was obtained using a soil moisture meter (v/v) (HFM 2030 Falker, Porto Alegre, BR).
180
181 *2.5. Chlorophyll a fluorescence*
182
183 Chlorophyll *a* fluorescence was obtained with portable fluorometer Fluor Pen FP100
184 (Photon Systems Instruments, Brno, Czech Republic) in third expanded leaf pair at the
185 moment of gas exchange. Leaves were adapted to dark for 30 min in order to determine
186 minimal fluorescence of adapted leaf to dark (F_o). Maximum fluorescence of adapted leaf to
187 dark (F_m) was calculated after saturating pulse of ~5000 μmol m^{-2} s^{-1}. Variable fluorescence
188 dark adapted leaf (F_v) is calculated as F_v=F_m-F_o. Fluorescence emission at steady state (F') and

189 maximum fluorescence (F_m') were determined for adapted leaves to light subjected to stable
190 photosynthesis. Data were used to calculate the maximum efficiency of PSII, operating
191 efficiency of PSII, photochemical quenching, electron transport rate and non-photochemical
192 quenching (Baker, 2008).
193
194 *2.6. Organic solutes*
195
196 Leaves were collected (~ 3g) at 3:00 pm during the maximum stress and at the last day of
197 rehydration, immediately frozen in liquid nitrogen and stored at -20 °C. Starch content, total
198 soluble sugars, sucrose, fructose, total free aminoacids, proline and total soluble proteins were
199 determined. All readings were performed under double beam spectrophotometer (Geneses
200 10S UV-Vis, Thermo Scientific, Waltham, USA). For starch, total soluble sugars, sucrose,
201 fructose and total free amino acids, 50 mg fresh weight leaf was used in ethanolic extraction.
202 The total soluble sugars were measured according to Dubois et al. (1956), using D (+) glucose
203 as a standard and 490 nm absorbance. Sucrose was measured according to Handel (1968),
204 using sucrose as standard and 660 nm absorbance. Fructose was measured according to
205 Foreman et al. (1973) using fructose as standard and 410 nm absorbance. Total free amino
206 acids were analyzed according to Moore and Stein (1948) using solution of 1 mM glycine,
207 glutamic acid, phenylalanine and arginine as standard and 570 nm absorbance. Insoluble
208 fraction of the total soluble sugars extracting of leaves was used to determine the starch
209 content. The pellet was hydrolyzed for 1 h with 10 units of amyloglucosidase and resulting
210 sugars were analyzed (Dubois et al., 1956). To determine proline 50 mg of leaf fresh mass
211 was used and the proline content measured by the acidic ninhydrin method (Bates, 1973),
212 using proline as standard and 520 nm absorbance. For the total soluble proteins determination,
213 200 mg of leaf fresh mass were macerated in 100 mM potassium phosphate buffer at pH 6.5

214 and measured according to Bradford (1976) using 0.1% bovine serum albumin (v/v) as
215 standard and 595 nm absorbance.
216
217 *2.7 Photosynthetic pigments*
218
219 For chlorophyll *a* chlorophyll *b* and carotenoids, 50 mg of leaf fresh material was
220 macerated in 2 mL of acetone (80%) with calcium carbonate ($CaCO_3$) to prevent the
221 chlorophyllase activity. Samples were filtered and read at 470.0; 648.8 and 663.2 nm
222 absorbance. Additionally, a 710 nm nonspecific absorbance was read to correct color,
223 turbidity and contaminating compounds, since pigments are not read in this wavelength. Final
224 pigment concentrations were calculated as described by Lichtenthaler and Buschmann (2001).
225 *2.8. Shoot dry biomass and leaf mineral nutrients*
226
227 Shoot parts were placed under forced ventilation at 70 °C to constant weight and weighed
228 on a precision scale (AND H200, Tokyo, JP). For nitrogen (N), phosphorus (P) and potassium
229 (K^+) quantification, 250 mg of leaf dry material were used. The material was digested in
230 sulfuric acid solution (H_2SO_4) in digester block at 350 °C to obtain sample extract (Thomas et
231 al., 1967). Total N content was determined by titration with HCl extract after boric acid and a
232 colorimetric indicator addition (Thomas et al., 1967). The P content was
233 spectrophotometrically determined (Spectrophotometer 600S, FEMTO, São Paulo, BR)
234 (Murphy and Riley, 1962) using a P concentration curve. The K^+ content was determined by
235 flame photometry (DM-62, Digimed, São Paulo, BR) using K^+ solution as standard at 5 mg L^{-1}
236 (Silva, 2009). The N/P ratio was calculated from N and P results.
237
238 *2.9. Statistical analysis*

239

240 Data (means ± SE, n = 4) were subjected to factorial ANOVA meeting all prerequisites
241 (normality and homogeneity). The independent variables were AMF, P_i and H_2O. Data
242 expressed in percentage (colonization, relative water content and soil moisture) were
243 transformed into √arcosine (x) for statistical analysis. Significant differences were compared
244 using the Student Newman Keuls test ($P < 0.05$). Data were analyzed with Statistica 8.0
245 software (StatSoft. Inc, Tulsa, Oklahoma, USA). In order to minimize the type I error
246 probability for multiple comparisons resulting from simultaneous analysis of attributes during
247 the experiment, the Benjanmini and Hochberg (1995) procedure was used.

248 A Principal Component Analysis (PCA) was performed to check for possible clusters, to
249 eliminate redundancies and define the most important variables in group separation under
250 maximum stress and rehydration. For rehydration PCA mycorrhizal colonization were
251 removed because it is specific for +AMF plants. Data were transformed (ranging) for
252 standardization due to different scale magnitudes and importance level of each variable was
253 determined by the eigenvectors values (McGarigal et al., 2000), with substantial correlation
254 values shown for each attribute in relation to principal components (PC). The importance
255 level of each PC was determined by the Broken-stick method, where eigenvalues that have
256 exceeded the expected were kept for interpretation. Analyses were performed using the
257 Fitopac 2.1.2.85 program (Shepherd, 2010).

258 The statistical summary of differences in variables between treatments in maximum stress
259 and rehydration with Benjanmini and Hochberg (1995) procedure were described in
260 supplementary material (Supplemental Table S1 and S2).

261

262 **3. Results**

263

264 *3.1. Mycorrhizal colonization*

265

266 No mycorrhizal colonization was observed in the non-inoculated treatments (-AMF). In
267 inoculated treatments (+AMF), the highest percentage of colonization occurred in –
268 H_2O+AMF+P_i (31.8±0.95^A) and +H_2O+AMF-P_i (29.8±1.70^A), followed by -H_2O+AMF-P_i
269 (23.8±1.11^B) and +H_2O+AMF+P_i (21.0±0.70^C). Hyphae and vesicles were observed in these
270 plants.

271

272 *3.2. Leaf relative water content*

273

274 At maximum stress, RWC reduced by about 27% in –H_2O-AMF-P_i, -H_2O-AMF+P_i, -
275 H_2O+AMF-P_i and by 11% in -H_2O+AMF+P_i and +H_2O+AMF+P_i compared to +H_2O-AMF-P_i
276 treatments (Table 1).

277 After rehydration, relative water content was recovered in plants under drought and was
278 5% higher in -H_2O+AMF+P_i and +H_2O+AMF+P_i treatments compared to +H_2O-AMF-P_i
279 (Table 2).

280

281 *3.3. Gas exchange*

282

283 The photosynthetic photon lux density was 800 μmol $m^{-2}s^{-1}$ and vapour pressure deficit
284 was 1.43 kPa in maximum stress (12th day) and 1.84 kPa in the last rehydration day (16th
285 day). The soil moisture content decreased in drought treatments from the second day of the
286 experiment, with values of 1.8% in these treatments and 11% in well-watered ones under
287 maximum stress. After rehydration, there was no difference among treatments, being 10.5%
288 in all treatments.

289 Throughout the experiment, gas exchange was generally higher in AMF plants both under
290 drought and well-watered conditions (Table 1). In drought treatments, stomatal conductance
291 was close to zero at maximum stress and 11% lower in +H_2O-AMF+P_i, while there was 35
292 and 16% increase in treatments with AMF (+H_2O+AMF-P_i and +H_2O+AMF+P_i),
293 respectively, compared to +H_2O-AMF-P_i (Table 1). For net photosynthetic rate, values were
294 higher in well-watered treatments with AMF, P_i or both compared to +H_2O-AMF-P_i at
295 maximum stress day, while drought treatments showed reductions. However, the reductions
296 were lower in –H_2O+AMF-P_i and higher in in –H_2O-AMF-P_i in comparison with +H_2O-
297 AMF-P_i (Table 1). The transpiration rate was higher in all well-watered treatments, with
298 higher values in +H_2O+AMF-P_i and +H_2O+AMF+P_i and reductions in all drought treatments
299 compared to +H_2O-AMF-P_i. However, the reductions were lower in–H_2O+AMF-P_i and higher
300 in–H_2O-AMF-P_i and -H_2O-AMF+P_i (Table 1).
301 After rehydration, stomatal conductance was partially recovered, with higher values in –
302 H_2O+AMF-P_i and –H_2O+AMF+P_i among drought treatments compared to +H_2O-AMF-P_i,
303 while –H_2O-AMF+P_i did not differ from -H_2O-AMF-P_i (Table 2). The net photosynthetic rate
304 was recovered in all drought treatments, except for –H_2O-AMF-P_i, compared to +H_2O-AMF-
305 P_i, while –H_2O+AMF-P_i showed 24% higher values. In well-watered treatments, the net
306 photosynthetic rate was 42% higher in plants with AMF and AMF+P_i compared to +H_2O-
307 AMF-P_i (Table 2). In transpiration rate, –H_2O-AMF-P_i showed 22% reduction compared to
308 +H_2O-AMF-P_i, while –H_2O+AMF-P_i showed higher values, not differing of well-watered
309 treatments with AMF and AMF+P_i (Table 2).
310
311 *3.4 Chlorophyll a fluorescence*
312

313 At maximum stress, maximum efficiency of PSII, operating efficiency of PSII and electron
314 transport rate decreased in all treatments, except for $+H_2O+AMF-P_i$ in maximum efficiency of
315 PSII compared to $+H_2O-AMF-P_i$. In maximum efficiency of PSII, the higher reduction was
316 observed in drought treatments with Pi, $-H_2O+AMF+P_i$ and $-H_2O-AMF+P_i$, and 38%
317 reduction in $+H_2O+AMF+P_i$ and $-H_2O+AMF-P_i$ compared to $+H_2O-AMF-P_i$. The operating
318 efficiency of PSII, similarly to maximum efficiency of PSII, had higher reductions in -
319 $H_2O+AMF+P_i$ and $-H_2O-AMF+P_i$ treatments, followed by other drought treatments and
320 $+H_2O-AMF+P_i$ compared to $+H_2O-AMF-P_i$. The electron transport rate was more decreased
321 in $-H_2O-AMF+P_i$ and $-H_2O+AMF+P_i$, as maximum efficiency of PSII and operating
322 efficiency of PSII, besides $-H_2O-AMF-P_i$, followed by $+H_2O-AMF+P_i$ and $-H_2O+AMF-P_i$
323 compared to $+H_2O-AMF-P_i$. The photochemical quenching showed 13% reduction in all
324 treatments compared to $+H_2O-AMF-P_i$, except in $-H_2O-AMF-P_i$ and $-H_2O+AMF-P_i$. In non-
325 photochemical quenching all treatments had increase compared to $+H_2O-AMF-P_i$, except in
326 $+H_2O-AMF+P_i$ (Table 1).

327 After rehydration, all treatments showed reduction in maximum efficiency of PSII values
328 compared to $+H_2O-AMF-P_i$, except in $+H_2O+AMF-P_i$. The operating efficiency of PSII
329 showed greater increases in-$H_2O+AMF+P_i$, followed by $+H_2O-AMF+P_i$, $-H_2O-AMF-P_i$ and -
330 $H_2O+AMF-P_i$ compared to $+H_2O-AMF-P_i$. The electron transport rate was 15% higher in
331 $+H_2O-AMF+P_i$, $-H_2O-AMF-P_i$ and $-H_2O+AMF-P_i$ compared to $+H_2O-AMF-P_i$ and showed
332 reduction of 15 and 35% in $+H_2O+AMF+P_i$ and $-H_2O+AMF+P_i$, respectively. The
333 photochemical quenching increased in $+H_2O-AMF+P_i$ and $-H_2O-AMF-P_i$ as electron transport
334 rate, in addition to $-H_2O+AMF+P_i$, with a reduction of 13% in $-H_2O-AMF+P_i$ compared to
335 $+H_2O-AMF-P_i$. The non-photochemical quenching was about 32% lower in all treatments
336 compared to $+H_2O-AMF-P_i$, except in $-H_2O+AMF+P_i$ (Table 2).
337

338 *3.5. Organic solutes*

339

340 At maximum stress, starch decrease in all treatments, with greater reductions in
341 $+H_2O$+AMF-P_i, -H_2O-AMF+P_i, -H_2O+AMF-P_i and –H_2O+AMF+P_i compared to +H_2O-
342 AMF-P_i (Fig. 1A). The total soluble sugars was higher in treatments under drought inoculated
343 with AMF, –H_2O+AMF-P_i and –H_2O+AMF+P_i, followed by +H_2O-AMF+P_i compared to
344 +H_2O-AMF-P_i (Fig. 1C). Sucrose was 182% higher in +H_2O+AMF+P_i and -H_2O+AMF-P_i,
345 followed by –H_2O+AMF+P_i, and showed reduction in treatments with isolated P_i supply,
346 +H_2O-AMF+P_i and –H_2O-AMF+P_i, compared to +H_2O-AMF-P_i (Fig. 1E). Fructose was
347 111% higher in –H_2O+AMF-P_i, followed by +H_2O+AMF+P_i and–H_2O-AMF-P_i, while
348 reduction was observed in –H_2O-AMF+P_i and –H_2O+AMF+P_i compared to +H_2O-AMF-P_i
349 (Fig. 1G).

350 With respect to nitrogenous compounds at maximum stress, the total free amino acids was
351 170% higher in –H_2O+AMF-P_i and 46% in +H_2O+AMF+P_i, while +H_2O-AMF+P_i,–H_2O-
352 AMF+P_i and +H_2O+AMF-P_i treatments showed 15% reduction compared to +H_2O-AMF-P_i
353 (Fig. 2A). Proline increased in all drought treatments and +H_2O+AMF+P_i, with higher values
354 in –H_2O-AMF+P_i and –H_2O+AMF-P_i and decrease in +H_2O+AMF-P_i compared to +H_2O-
355 AMF-P_i (Fig. 2C). The total soluble proteins were 59% higher in -H_2O-AMF+P_i and 28%
356 lower in +H_2O-AMF+P_i and +H_2O+AMF-P_i compared to +H_2O-AMF-P_i (Fig. 2F).

357 After rehydration, starch was 42% higher in –H_2O+AMF-P_i and decreased in other
358 treatments compared to +H_2O-AMF-P_i (Fig. 1B), while the total soluble sugars were 17%
359 lower in –H_2O-AMF+P_i and –H_2O+AMF-P_i (Fig. 1D). Sucrose was 21% lower in –
360 H_2O+AMF+P_i and higher in well-watered treatments and -H_2O-AMF-P_i compared to +H_2O-
361 AMF-P_i (Fig. 1F), while fructose was 32% lower in -H_2O-AMF+P_i and –H_2O+AMF+P_i
362 treatments (Fig. 1H). The total free amino acid was higher in all treatments compared to

363 $+H_2O$-AMF-P_i (Fig. 2B). Proline showed a reduction of 34% in -H_2O-AMF-P_i and -H_2O-
364 AMF+P_i treatments and 69% in +H_2O+AMF-P_i, while +H_2O-AMF+P_i and +H_2O+AMF+P_i
365 showed increases of 39 and 79%, respectively, compared to +H_2O-AMF-P_i (Fig. 2D). The
366 total soluble proteins were 13% higher in +H_2O+AMF-P_i and –H_2O+AMF-P_i and 22% lower
367 in –H_2O-AMF-P_i and 38% lower in +H_2O-AMF+P_i and –H_2O-AMF+P_i compared to +H_2O-
368 AMF-P_i (Fig. 2F).
369
370 *3.6. Photosynthetic pigments*
371
372 At maximum stress, both chlorophyll *a* and *b* were higher in –H_2O+AMF-P_i. In chlorophyll
373 *a*, there were increases of 194% in –H_2O+AMF-P_i, 162% in +H_2O+AMF+P_i and 58% in
374 +H_2O+AMF-P_i, -H_2O-AMF-P_i and –H_2O-AMF+P_i treatments compared to +H_2O-AMF-P_i
375 (Fig. 3A). The chlorophyll *b* was 148% higher in –H_2O+AMF-P_i and 63% in +H_2O+AMF-P_i,
376 +H_2O+AMF+P_i, -H_2O-AMF-P_i and –H_2O-AMF+P_i treatments compared to +H_2O-AMF-P_i
377 (Fig. 3C). The carotenoids concentration was 150% higher in –H_2O-AMF+P_i and –
378 H_2O+AMF-P_i, and 80% in +H_2O+AMF+P_i and –H_2O-AMF-P_i compared to +H_2O-AMF-P_i
379 (Fig. 3E).
380 After rehydration, chlorophyll *a* and *b* were higher in all treatments, with higher values in -
381 H_2O-AMF-P_i. Only +H_2O-AMF+P_i had reductions in chlorophyll *a* and *b* of 23 and 31%,
382 respectively, compared to +H_2O-AMF-P_i (Fig. 3B and D). The carotenoids were higher in
383 drought treatments, except –H_2O+AMF+P_i, and +H_2O+AMF+P_i, compared to +H_2O-AMF-P_i
384 (Fig. 3F).
385
386 *3.7. Shoot dry biomass and leaf mineral nutrients*
387

388 Shoot dry biomass was 39% higher only +H_2O+AMF-P_i and was 33% lower in -H_2O-
389 AMF-P_i compared to +H_2O-AMF-P_i, while other treatments not differ (Table 3).
390 The P in shoots was higher in well-watered treatments that received leaf P_i supply and in -
391 H_2O+AMF+P_i, providing lower N/P ratios in shoots compared to +H_2O-AMF-P_i. The -H_2O-
392 AMF-P_i, -H_2O+AMF-P_i and +H_2O+AMF-P_i treatments showed 16% reduction of P, resulting
393 in higher N/P ratios, compared to +H_2O-AMF-P_i. The K^+ in shoots was lower in all treatments
394 if compared to +H_2O-AMF-P_i (Table 3).
395
396 *3.8. Principal component analysis (PCA)*
397
398 At maximum stress, ordination with all variables accounted for 66.32% of total data
399 variation. The most important attributes in the group distinction had eigenvectors values >
400 0.22 with their respective coefficients of substantial correlation discriminated. Treatments
401 were separated as water regime in PC1 and as AMF and P_i presence on PC2. For PC1 the
402 most relevant attributes and their respective correlation values were proline (0.89), relative
403 water content (-0.88), non-photochemical quenching (0.86), stomatal conductance (-0.85), net
404 photosynthetic rate (-0.84), carotenoids (0.78), transpiration rate (-0.76), maximum efficiency
405 of PSII (-0.71), starch (-0.66) and total soluble proteins (0.65). For PC2, the most relevant
406 attributes and their respective correlation values were fructose (0.89), sucrose (0.81), total free
407 amino acids (0.79), chlorophyll *a* (0.72), chlorophyll *b* (0.62), photochemical quenching
408 (0.58), electron transport rate (0.52) and maximum efficiency of PSII (0.50) (Fig. 4A).
409 The separation of well-watered and drought treatments was evident in the ordination,
410 plants under drought showed decrease in relative water content, gas exchange, chlorophyll
411 fluorescence parameters, starch and increased proline. The -H_2O-AMF+P_i and -
412 H_2O+AMF+P_i treatments showed higher non-photochemical quenching rates and fructose

reduction, while the fructose was accumulated in -H_2O-AMF-P_i forming a distinct cluster the latter two (Fig. 4A). However, -H_2O+AMF+P_i showed less reduction in relative water content compared to the other drought treatments, showed no increases in carotenoids and had higher reductions in maximum efficiency of PSII, while -H_2O-AMF+P_i had higher total soluble proteins levels, differentiating these two treatments in the ordination. The -H_2O+AMF-P_i separation from the other treatments was due to higher chlorophyll *a*, chlorophyll *b*, fructose, sucrose and total free amino acids levels and lower maximum efficiency of PSII reductions. Among well-watered treatments, +H_2O+AMF-P_i and +H_2O+AMF+P_i plants had higher stomatal conductance, net photosynthetic rate, transpiration rate, non-photochemical quenching, chlorophyll *a* and chlorophyll *b* than +H_2O-AMF-P_i. The +H_2O+AMF+P_i separation in ordination was due to higher sucrose, fructose, total free amino acids and chlorophyll *a* concentrations, while +H_2O-AMF+P_i and +H_2O+AMF-P_i plants had reduction in fructose and total free amino acids. However, these two last treatments were separated due to +H_2O+AMF-P_i present higher maximum efficiency of PSII, sucrose, chlorophyll *a* and chlorophyll *b* levels and lower electron transport rate values.

In rehydration, all variables accounted for 38.60% of total data variation. The more relevant attributes in group distinction had eigenvectors values > 0.26, with their coefficients of substantial correlation discriminated. Treatments were separated as water regime in PC1, with larger separation for -H_2O-AMF-P_i, while remaining drought treatments were grouped near to well-watered treatments. In PC2, the treatments were separated due to P_i and AMF presence. For PC1, the most relevant attributes and their correlation values were chlorophyll *b* (-0.81), carotenoids (-0.80), chlorophyll *a* (-0.74), shoot dry biomass (0.72), stomatal conductance (0.64), maximum efficiency of (-0.61) and net photosynthetic rate (0.56). For PC2, the most relevant attributes and their respective correlation values were transpiration rate(0.76), maximum efficiency of (-0.68), electron transport rate (-0.64), total soluble

438 proteins (0.62), total free amino acids (0.61) and relative water content (0.54) (Fig. 4B). In
439 ordination, $+H_2O$+AMF-P_i formed a distinct group of remaining well-watered treatments for
440 presenting higher shoot dry biomass and total soluble proteins. Moreover, $+H_2O$+AMF-P_i and
441 $+H_2O$+AMF+P_i plants had increase in net photosynthetic rate, while $+H_2O$-AMF+P_i showed
442 no gains and had reduction in stomatal conductance with higher electron transport rate values,
443 forming a distinct group. Furthermore, $+H_2O$+AMF+P_i showed a higher relative water content
444 and total free amino acids concentration, with reductions in maximum efficiency of PSII and
445 electron transport rate, forming a distinct group. Among drought treatments, $-H_2O$-AMF-P_i
446 presented reduction in shoot dry biomass, lower stomatal conductance and net photosynthetic
447 rate and higher chlorophyll concentration, forming a more distant group, while $-H_2O$-AMF+P_i
448 showed higher stomatal conductance reduction, but recovered net photosynthetic rate, forming
449 a distinct group. The $-H_2O$+AMF-P_i plants showed higher *A* among drought treatments,
450 forming another distinct group, while $-H_2O$+AMF+P_i had higher relative water content and
451 reductions in maximum efficiency of PSII and electron transport rate, as $+H_2O$+AMF+P_i.
452 However, $-H_2O$+AMF+P_i had lower total free amino acids than $+H_2O$+AMF+P_i, forming
453 other distinct group (Fig. 4B).
454
455 **4. Discussion**
456
457 The first hypothesis (plants inoculated with AMF or leaf P_i supply will grow better under
458 well-watered conditions and when subjected to water deficit will have higher tolerance to
459 stress) was partially corroborated. *P. pyramidalis* benefited more in terms of growth gains
460 when in the isolated presence of AMF under well-watered conditions promoted by increased
461 photosynthesis, while the leaf P_i supply in isolation not promote gains in growth. Under the
462 drought condition, plants that received P_i or AMF showed photosynthetic rates recovery after

463 rehydration, indicating higher tolerance, as initially expected. On the other hand, the second
464 hypothesis was not corroborated (plants with AMF and leaf P_i supply will grow better under
465 well-watered conditions and will present higher tolerance and faster recovery of leaf primary
466 metabolism after rehydration when subjected to water deficit) because plants inoculated with
467 AMF and leaf P_i supply did not show higher shoot dry biomass under well-watered conditions
468 compared to those with AMF or P_i in isolation.
469
470 *4.1. Plant-AMF relationship*
471
472 Recent studies have shown that relationship between AMF and host plant may not always
473 be species specific (Öpik et al., 2009) and that factors such as soil properties can determine
474 the AMF-plant interactions (Landis et al., 2004). The effects of mycorrhizal symbiosis depend
475 on a soil fungus-host combination (Costa et al., 2001).
476 Higher gas exchange in inoculated plants with AMF under well-watered and drought
477 conditions and after rehydration was observed in this study and also found by Liu et al.
478 (2015b). Mycorrhizal symbiosis can promote changes in water movement of host plants,
479 changes in sap flow rate and root hydraulic conductivity (Bárzana et al., 2012), contributing to
480 higher photosynthesis.
481 The -H_2O+AMF-P_i treatment in maximum stress had the highest proline accumulation, one
482 of the most reported changes induced by water deficit (Ruiz-Lozano et al., 2012). This amino
483 acid acts in stabilizing subcellular structures, cellular redox buffering, and free radicals
484 scavenger, especially hydroxyl (Chen and Dickman, 2005). Furthermore, at maximum stress
485 had increased total soluble sugars, sucrose, fructose and total free amino acids. The sugars
486 accumulation could act as a drain of reactive oxygen species and stress signaling molecules
487 (Keunen et al., 2013; Sperdouli and Moustakas, 2012). Plants associated with AMF may also

exhibit higher expression of sugar transporter encoding genes (Boldt et al., 2011). The total free amino acids accumulation may also combat the free radicals in combination with sugars, mitigating its deleterious effects and acting in the protein synthesis stabilization (Dolatabadian et al., 2008). After rehydration, -H_2O+AMF-P_i had higher starch accumulation, which may indicate that these plants were able to supply their metabolic energy needs through the total soluble sugars and even accumulating energy as starch for night respiration, supported by higher photosynthetic rates in this period compared to the other treatments subjected to drought stress and +H_2O-AMF-P_i (Fig. 4B).

The highest shoot biomass in +H_2O+AMF-P_i indicates that AMF promoted gains in growth correlated to higher photosynthetic rates, similar to the results found by Frosi et al. (2016) when working on the same species under well-watered conditions in the greenhouse. In addition to the higher photosynthetic rates, the growth gains in plants associated with AMF have been partly attributed to the higher nutrient uptake, especially P, by mycorrhiza (Beltrano et al., 2013; Plenchette and Duponnis, 2005); such a fact was not observed in this study. Thus, plants under well-watered conditions were benefited with biomass gains in the presence of AMF. In drought, AMF promoted higher tolerance and fast metabolism recovery with higher shoot biomass compared to -H_2O-AMF-P_i treatment, indicating mitigation of negative effects of water deficit, as shown by other studies on plants colonized by AMF subjected to different abiotic stresses, including drought, with increased growth and photosynthetic metabolism (Liu et al., 2015a; Wu et al., 2010).

4.2 Leaf P_i supply

In plants with only leaf P_i supply, the photosynthetic rate was higher in $+H_2O$-AMF+P_i than that in $+H_2O$-AMF-P_i on the 12th day of experiment (maximum stress in drought plants), whereas $-H_2O$-AMF+P_i had lower reductions than $-H_2O$-AMF-P_i in same period, and faster recovery in net photosynthetic rate after rehydration. This higher CO_2 assimilation may be related to increased production of ATP and NADPH, Calvin cycle enzymes activity and phosphate sugar regeneration due to higher P_i supply (Rao and Terry, 1995). The $-H_2O$-AMF+P_i plants presented reduction in photochemical activity with increased non-photochemical quenching and carotenoids at maximum stress. Oliveira et al. (2014) working on a woody species from semiarid region also found reduction in photochemical activity in drought treatment with leaf P_i supply combined with chlorophyll degradation. This higher carotenoids amount in $-H_2O$-AMF+P_i may have acted on excess energy dissipation and photoprotection, as well as increased NPQ. This photosynthetic machinery protection may have contributed to *A* recovery after rehydration, although maximum efficiency of PSII and photochemical quenching did not fully recovered. In addition, the $-H_2O$-AMF+P_i treatment at maximum stress had reduction of starch, fructose, sucrose and total free amino acids and increase of proline and total soluble proteins, indicating that P_i may have favored sugar allocation to proline and protein metabolism. It is reported that P application could regulate the synthesis or degradation of starch under water deficit in order to avoid shortages (Liu et al., 2015b).

When plants were well-watered and received leaf P_i supply, $+H_2O$-AMF+P_i did not promoted increase in shoot biomass compared with $+H_2O$-AMF-P_i. On the other hand, $-H_2O$-AMF+P_i plants had higher shoot dry biomass under drought than to $-H_2O$-AMF-P_i, where P_i was proved to be beneficial under this condition. Leaf P_i supply promoted fast CO_2 assimilation recovery and higher shoot dry biomass under drought compared to $-H_2O$-AMF-P_i treatment, indicating relief in the negative effects of water deficit as shown in previous

537 studies on herbaceous species such *Phaseolus vulgaris* (Santos et al., 2004) or on woody
538 species (Oliveira et al., 2014).
539
540 4.3. *Plant AMF+P_i relationship*
541
542 In plants with AMF and leaf P_i combination, the +H_2O+AMF+P_i had gas exchange similar
543 to +H_2O+AMF-P_i treatment trough the measurement periods. Therefore, +H_2O+AMF+P_i
544 showed higher organic solutes accumulation, including sucrose, fructose, total free amino
545 acids, proline on the 12th day (maximum stress) among well-watered treatments, being
546 highlighted in the PCA (Fig. 4A), and also maintaining high total free amino acids and proline
547 concentrations after rehydration. This accumulation indicates that leaf primary metabolism of
548 these plants was benefited by AMF and P_i presence, which could be favoring carbon leaf
549 metabolism and nitrogen compounds, maybe acting as energy reserve. On the other hand, this
550 reserve was not converted into higher shoot biomass among well-watered treatments (Table
551 3).
552 Under drought, -H_2O+AMF+P_i had higher reduction in *A* and *E* similar to -H_2O-AMF-P_i
553 and -H_2O-AMF+P_i treatments at maximum stress, with photochemical reduction similar to -
554 H_2O-AMF+P_i. Furthermore, -H_2O+AMF+P_i had a higher reduction in photosynthetic
555 pigments, with photosynthetic rate recovery after rehydration, similar to -H_2O-AMF+P_i.
556 Results similar to our study were found on a woody species of semiarid region with only leaf
557 P_i supply under drought that showed reduction in photochemical activity combined with
558 chlorophyll degradation (Oliveira et al., 2014). Regarding organic solutes at maximum stress,
559 plants of -H_2O+AMF+P_i decreased starch combined to increase in total soluble sugars, similar
560 to -H_2O+AMF-P_i treatment. These sugars would act as signaling molecules and reactive
561 oxygen species drain (Keunen et al., 2013; Sperdouli and Moustakas, 2012), protecting the

562 photosynthetic machinery, remaining high after rehydration. Furthermore, the shoot dry
563 biomass values in -H_2O+AMF+P_i were higher than -H_2O-AMF-P_i treatment, being similar to
564 other drought treatments and indicating a relief from negative effects of water deficit. In this
565 way, plants with AMF and P_i combination showed no gains on growth under well-watered
566 condition compared to +H_2O-AMF-P_i. Under drought, these plants did not have higher
567 tolerance and faster metabolic recovery after rehydration compared to the other treatments
568 with AMF and P_i in isolation as initially expected.

569 In conclusion, *P. pyramidalis* was more benefited by AMF presence under well-watered
570 and drought condition. Under well-watered condition, the plants had increases in
571 photosynthetic rates and shoot biomass. Under drought condition, the isolated AMF presence
572 promoted the highest tolerance to drought, with fastest recovery of leaf metabolism after
573 rehydration compared with other drought treatments. The inoculation with AMF it is more
574 suitable for use in recovery to degraded areas. For this practice, the seedlings must be
575 inoculated and transferred when they reach the ideal size for the field, being able then to more
576 efficiently tolerate adverse environmental conditions.

577

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586

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767

Figure legends

Fig. 1. A and B – starch; C and D – soluble sugars; E and F – sucrose; (*E*), G and H - fructose of young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply (+P_i and –P_i) and two water levels (+H_2O and –H_2O) under greenhouse conditions in maximum stress (12th day) and rehydration (16th day). Different letters indicate statistically significant differences by Student Newman Keuls test ($P < 0.05$) between treatments (columns) (n=4±SE).

Fig. 2. A and B – free amino acids; C and D – proline; E and F – total soluble proteins of young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply (+P_i and –P_i) and two water levels (+H_2O and –H_2O) under greenhouse conditions in maximum stress (12th day) and rehydration (16th day). Different letters indicate statistically significant differences by Student Newman Keuls test ($P < 0.05$) between treatments (columns) (n=4±SE).

Fig. 3. A and B – chlorophyll *a*; C and D – chlorophyll *b*; E and F – carotenoids of young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply (+P_i and –P_i) and two water levels (+H_2O and –H_2O) under greenhouse conditions in maximum stress (12th day) and rehydration (16th day). Different letters indicate statistically significant differences by Student Newman Keuls test ($P < 0.05$) between treatments (columns) (n=4±SE).

Fig. 4. Principal component analysis (PCA) based on the whole dataset of the study of young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply (+P_i and –P_i) and two water levels (+H_2O and –H_2O) under greenhouse conditions. A- maximum stress, B- rehydration (n = 4).

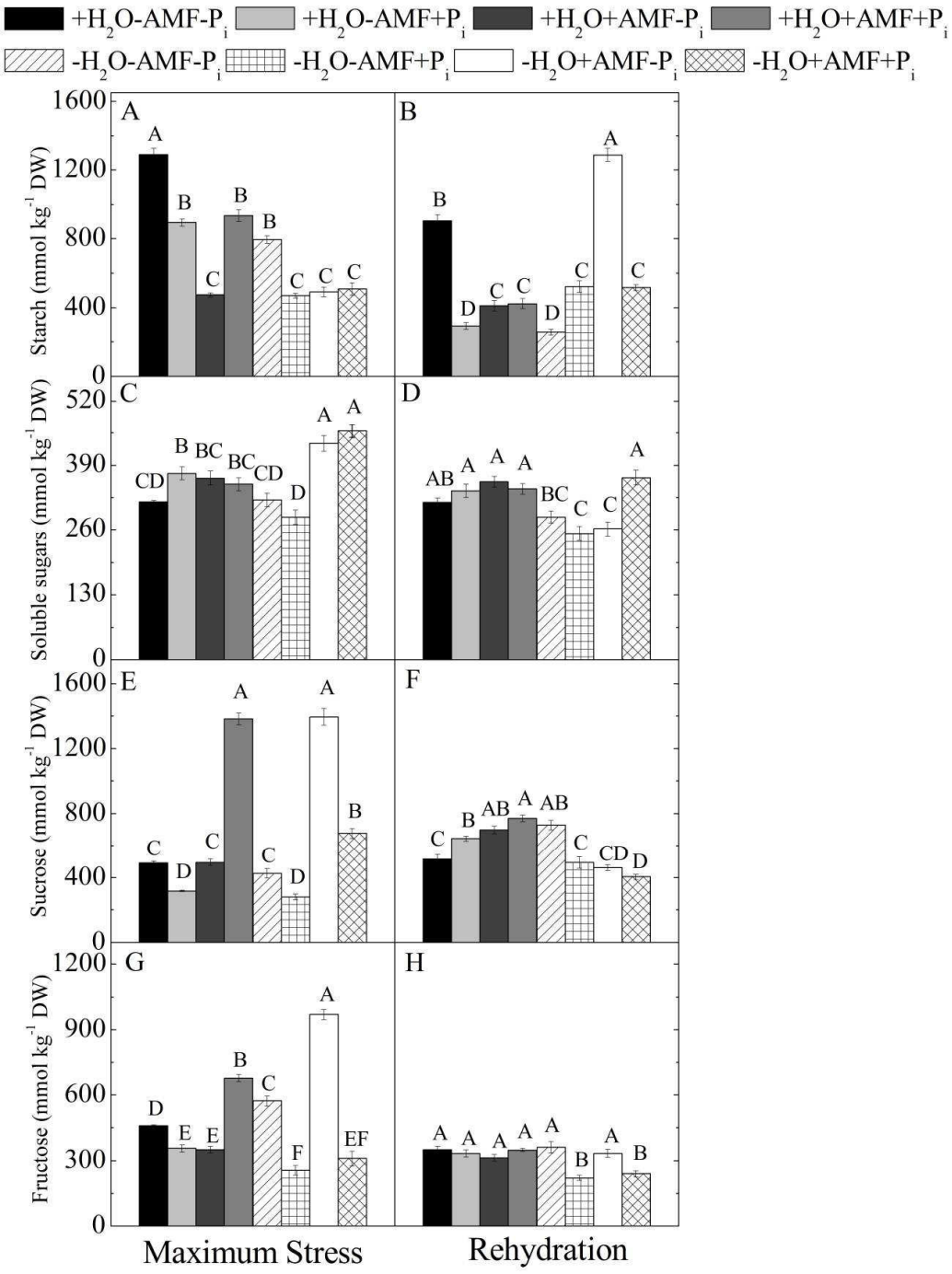
+H2O-AMF-Pi
+H2O-AMF+Pi
+H2O+AMF-Pi
+H2O+AMF+Pi
-H2O-AMF-Pi
-H2O-AMF+Pi
-H2O+AMF-Pi
-H2O+AMF+Pi
Starch (mmol kg-1 DW)
Soluble sugars (mmol kg-1 DW)
Sucrose (mmol kg-1 DW)
Fructose (mmol kg-1 DW)
Maximum Stress
Rehydration

Fig. 1

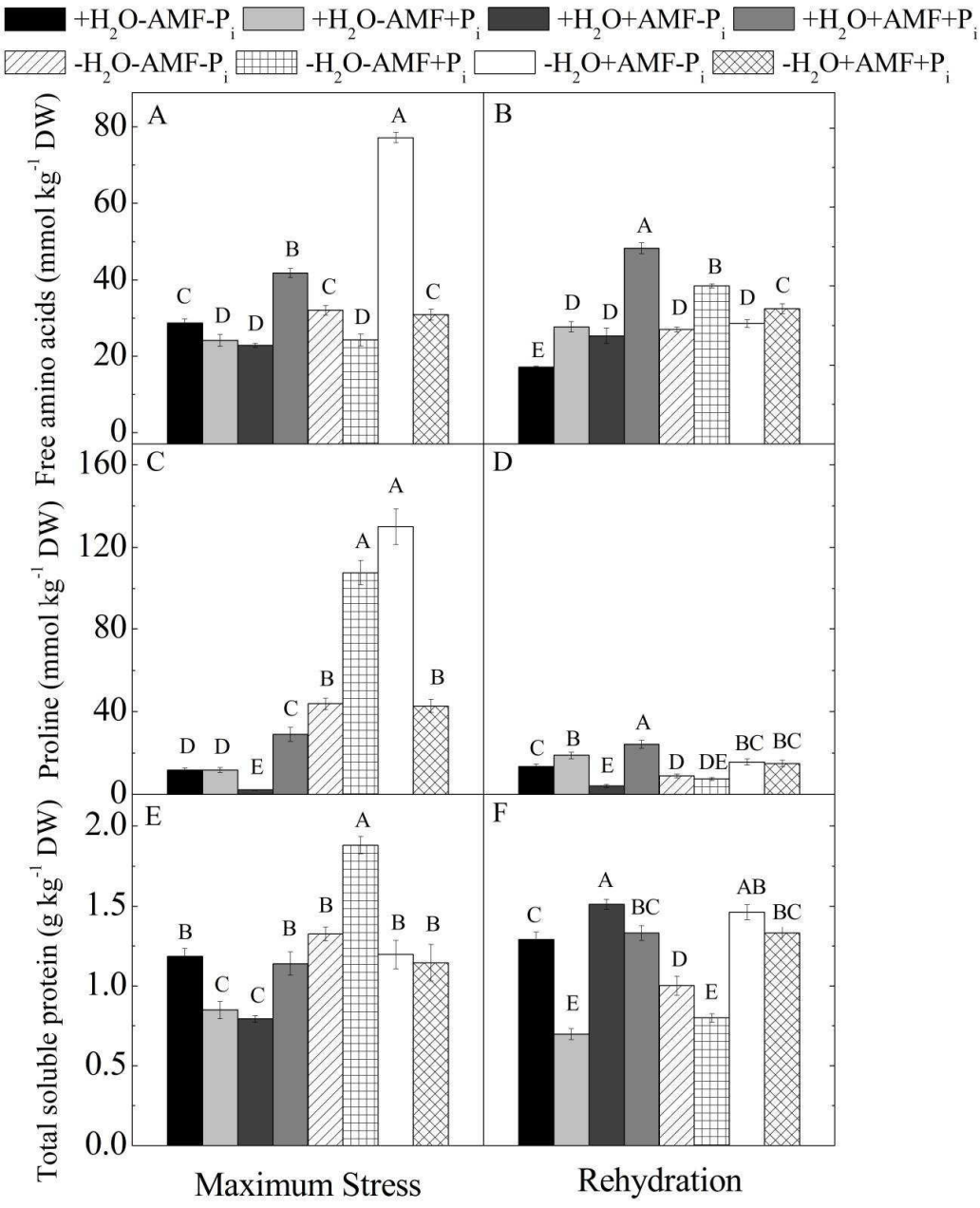
+H2O-AMF-Pi
+H2O-AMF+Pi
+H2O+AMF-Pi
+H2O+AMF+Pi
-H2O-AMF-Pi
-H2O-AMF+Pi
-H2O+AMF-Pi
-H2O+AMF+Pi
Free amino acids (mmol kg-1 DW)
Proline (mmol kg-1 DW)
Total soluble protein (g kg-1 DW)
Maximum Stress
Rehydration

Fig. 2

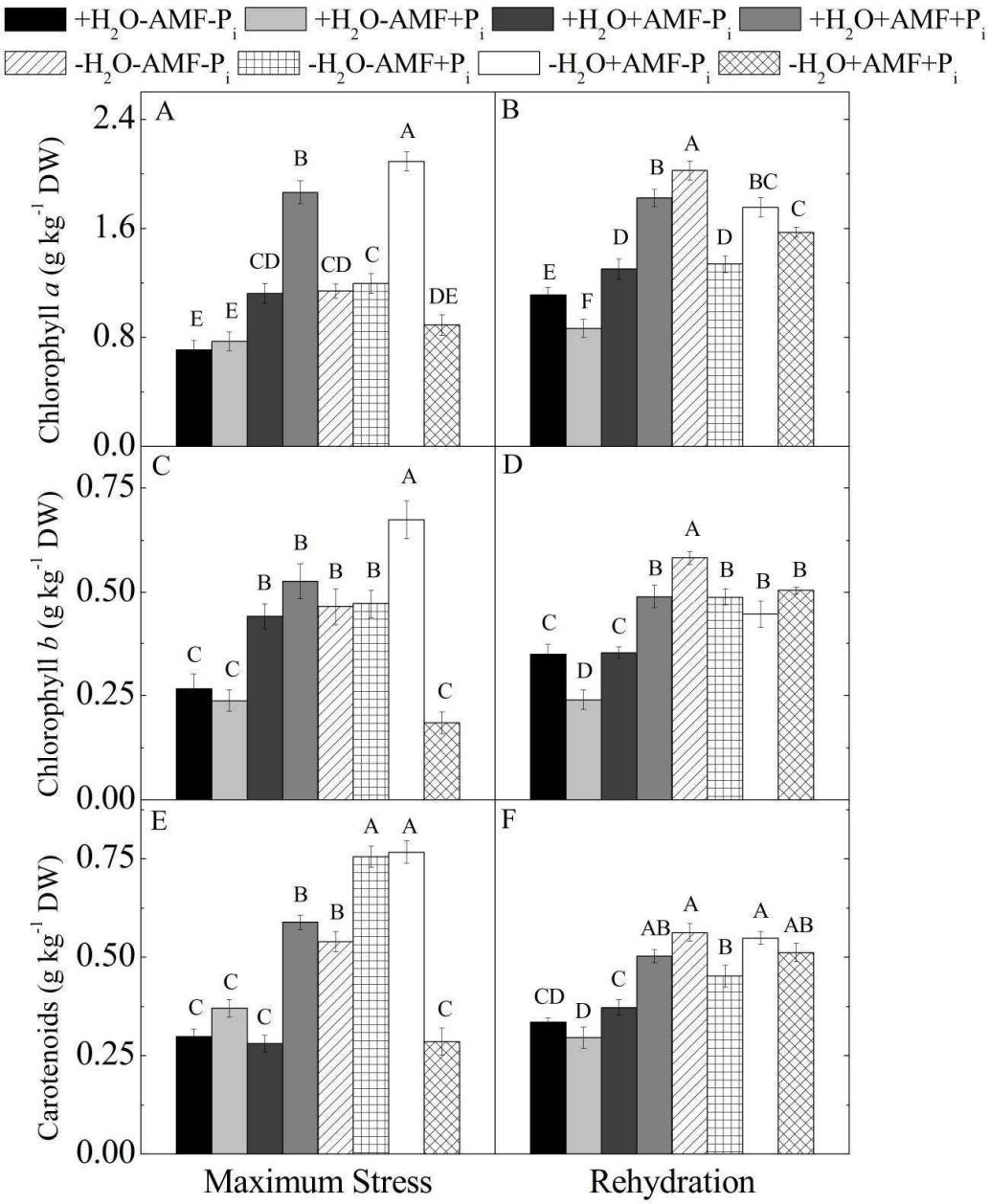
+H2O-AMF-Pi
+H2O-AMF+Pi
+H2O+AMF-Pi
+H2O+AMF+Pi
-H2O-AMF-Pi
-H2O-AMF+Pi
-H2O+AMF-Pi
-H2O+AMF+Pi
Chlorophyll a (g kg-1 DW)
Chlorophyll b (g kg-1 DW)
Carotenoids (g kg-1 DW)
Maximum Stress
Rehydration
A
B
C
D
E
F

Fig. 3

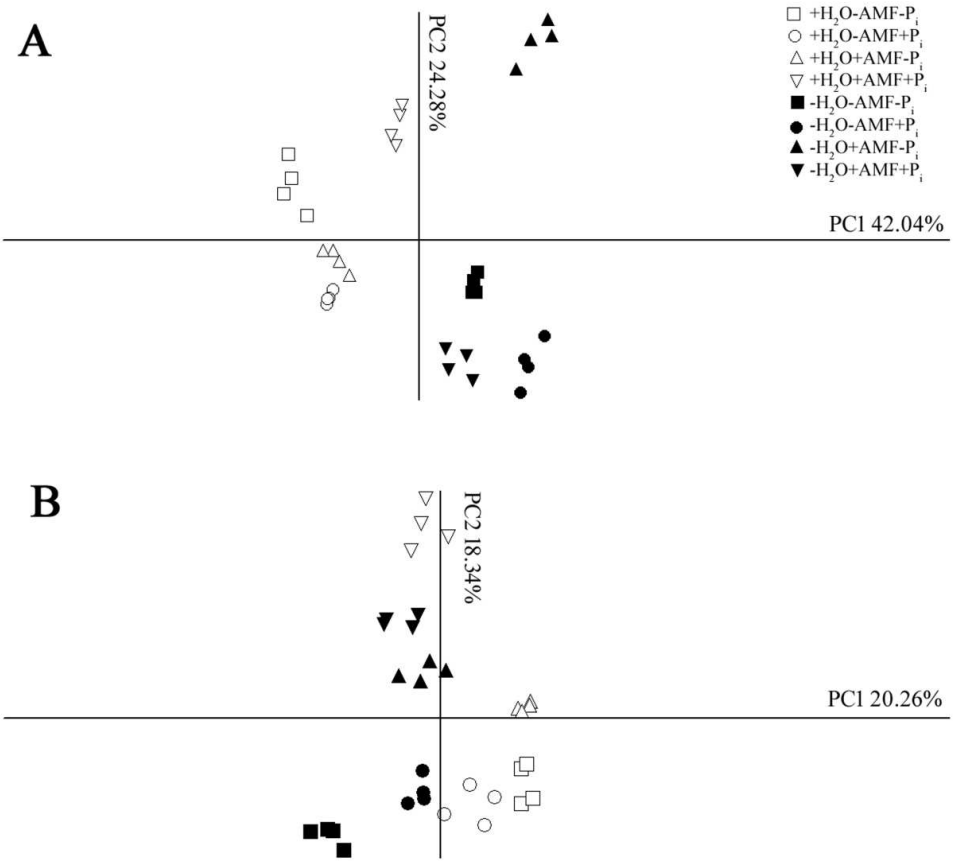
A
PC2 24.28%
PC1 42.04%
+H2O-AMF-Pi
+H2O-AMF+Pi
+H2O+AMF-Pi
+H2O+AMF+Pi
-H2O-AMF-Pi
-H2O-AMF+Pi
-H2O+AMF-Pi
-H2O+AMF+Pi
B
PC2 18.34%
PC1 20.26%

Fig. 4

Table 1

Attributes evaluated (RWC - relative water content; g_s - stomatal conductance; A - net photosynthetic rate; E - transpiration rate; F_v'/F_m' - maximum efficiency of PSII; F_q'/F_m'- operating efficiency of PSII; ETR - electron transport rate; qP - photochemical quenching; NPQ - non-photochemical quenching) in maximum stress day (12th day of experiment) in young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply ($+P_i$ and $-P_i$) and two water levels ($+H_2O$ and $-H_2O$) under greenhouse conditions.

Attributes	**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**
RWC (%)	92.1 ± 1.37^{A}	91.4 ± 2.62^{A}	88.8 ± 3.17^{AB}	81.7 ± 3.94^{B}	64.5 ± 2.37^{C}	58.1 ± 2.97^{C}	67.0 ± 2.95^{C}	80.0 ± 1.22^{B}
g_s (mol $m^{-2}s^{-1}$)	0.19 ± 0.01^{C}	0.17 ± 0.01^{D}	0.25 ± 0.01^{A}	0.22 ± 0.01^{B}	0.02 ± 0.00^{E}	0.01 ± 0.00^{E}	0.03 ± 0.00^{E}	0.02 ± 0.00^{E}
A (µmol $m^{-2}s^{-1}$)	7.83 ± 0.69^{B}	9.34 ± 0.26^{A}	10.96 ± 0.66^{A}	10.08 ± 0.14^{A}	0.40 ± 0.02^{E}	0.87 ± 0.08^{D}	1.74 ± 0.09^{C}	1.01 ± 0.04^{D}
E (mmol $m^{-2}s^{-1}$)	2.07 ± 0.11^{C}	2.67 ± 0.04^{B}	3.34 ± 0.06^{A}	3.52 ± 0.11^{A}	0.21 ± 0.01^{F}	0.30 ± 0.01^{F}	0.76 ± 0.05^{D}	0.50 ± 0.01^{E}
F_v'/F_m'	0.90 ± 0.03^{A}	0.48 ± 0.03^{C}	0.84 ± 0.03^{A}	0.56 ± 0.01^{B}	0.34 ± 0.01^{D}	0.30 ± 0.00^{E}	0.53 ± 0.01^{B}	0.21 ± 0.01^{F}
F_q'/F_m'	0.38 ± 0.02^{A}	0.21 ± 0.02^{B}	0.17 ± 0.01^{C}	0.17 ± 0.01^{C}	0.21 ± 0.01^{B}	0.12 ± 0.01^{D}	0.21 ± 0.01^{B}	0.14 ± 0.01^{CD}
ETR	99.7 ± 3.73^{A}	72.0 ± 2.25^{B}	57.4 ± 2.58^{C}	58.8 ± 2.19^{C}	48.8 ± 2.24^{D}	42.5 ± 1.42^{D}	71.3 ± 1.63^{B}	48.3 ± 1.95^{D}
qP	0.54 ± 0.01^{A}	0.41 ± 0.01^{C}	0.45 ± 0.02^{C}	0.47 ± 0.02^{BC}	0.50 ± 0.01^{AB}	0.44 ± 0.02^{BC}	0.53 ± 0.01^{A}	0.46 ± 0.02^{BC}
NPQ	0.27 ± 0.01^{D}	0.56 ± 0.06^{D}	1.51 ± 0.13^{C}	1.26 ± 0.04^{C}	2.34 ± 0.16^{B}	3.03 ± 0.02^{A}	2.42 ± 0.07^{B}	3.31 ± 0.14^{A}

Different letters indicate statistically significant differences by Student Newman Keuls test at a 5 % significance level between treatments (lines) (n=4±SE)

Table 2

Attributes evaluated (RWC - relative water content; g_s - stomatal conductance; A - net photosynthetic rate; E - transpiration rate; F_v'/F_m' - maximum efficiency of PSII; F_q'/F_m'- operating efficiency of PSII; ETR - electron transport rate; qP - photochemical quenching; NPQ - non-photochemical quenching) in last rehydration day (16th day of experiment) in young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply (+P_i and –P_i) and two water levels (+H_2O and –H_2O) under greenhouse conditions.

Attributes	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**
RWC (%)	89.2±2.38^{B}	89.3±1.99^{B}	87.1±3.74^{B}	93.6±0.57^{A}	87.6±0.25^{B}	86.4±1.69^{B}	89.7±1.28^{B}	95.0±1.54^{A}
g_s (mol $m^{-2}s^{-1}$)	0.37±0.01^{A}	0.23±0.01^{D}	0.32±0.01^{B}	0.35±0.01^{AB}	0.17±0.01^{E}	0.15±0.01^{E}	0.27±0.01^{C}	0.25±0.01^{CD}
A (µmol $m^{-2}s^{-1}$)	9.16±0.14^{C}	9.89±0.23^{C}	13.57±0.29^{A}	13.03±0.38^{A}	5.58±0.32^{D}	9.79±0.42^{C}	11.32±0.21^{B}	9.78±0.24^{C}
E (mmol $m^{-2}s^{-1}$)	2.78±0.13^{B}	2.98±0.08^{B}	3.67±0.15^{A}	3.85±0.10^{A}	2.16±0.10^{B}	2.53±0.19^{B}	3.82±0.11^{A}	3.25±0.23^{A}
F_v'/F_m'	0.86±0.01^{A}	0.82±0.01^{B}	0.88±0.02^{A}	0.67±0.01^{E}	0.77±0.01^{C}	0.81±0.00^{B}	0.72±0.01^{D}	0.65±0.01^{E}
F_q'/F_m'	0.24±0.01^{DE}	0.30±0.00^{B}	0.24±0.01^{E}	0.22±0.01^{E}	0.29±0.00^{BC}	0.26±0.01^{CD}	0.28±0.01^{BC}	0.34±0.01^{A}
ETR	84.6±2.04^{B}	102.8±1.19^{A}	87.7±4.22^{B}	71.5±1.50^{C}	97.2±2.95^{A}	85.7±1.46^{B}	99.3±4.19^{A}	54.8±1.33^{D}
qP	0.45±0.01^{B}	0.52±0.02^{A}	0.47±0.01^{B}	0.46±0.01^{B}	0.56±0.01^{A}	0.39±0.01^{C}	0.43±0.01^{B}	0.53±0.01^{A}
NPQ	1.39±0.02^{A}	0.64±0.02^{D}	0.94±0.05^{B}	0.66±0.01^{D}	0.43±0.01^{E}	0.83±0.01^{C}	0.64±0.02^{D}	1.35±0.04^{A}

Different letters indicate statistically significant differences by Student Newman Keuls test at a 5 % significance level between treatments (lines) (n=4±SE)

Table 3

Shoot dry biomass and leaf nutrients (N - nitrogen; P - phosphorus; K^+ - potassium; N/P - ratio nitrogen/phosphorus) of young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply ($+P_i$ and $-P_i$) and two water levels ($+H_2O$ and $-H_2O$) under greenhouse conditions in last rehydration day (16th day of experiment).

		Nutrients (g kg^{-1})			
Treatments	**Biomass (g)**	**N**	**P**	**K^+**	**N/P**
$+H_2O$-AMF-P_i	1.19 ± 0.10^{B}	56.7 ± 3.4^{A}	10.8 ± 0.2^{CD}	19.2 ± 0.5^{A}	5.3 ± 0.7^{BC}
$+H_2O$-AMF+P_i	1.10 ± 0.08^{B}	60.4 ± 2.9^{A}	16.3 ± 0.6^{A}	12.0 ± 0.6^{BCD}	3.7 ± 0.1^{D}
$+H_2O$+AMF-P_i	1.65 ± 0.08^{A}	55.2 ± 2.6^{A}	9.5 ± 0.8^{DE}	11.5 ± 0.5^{BCD}	5.8 ± 0.4^{AB}
$+H_2O$+AMF+P_i	1.17 ± 0.09^{B}	56.8 ± 2.5^{A}	15.0 ± 0.2^{A}	10.5 ± 0.4^{D}	3.8 ± 0.2^{D}
$-H_2O$-AMF-P_i	0.80 ± 0.03^{C}	54.1 ± 3.0^{A}	9.0 ± 0.3^{E}	13.4 ± 0.5^{B}	6.0 ± 0.3^{AB}
$-H_2O$-AMF+P_i	1.33 ± 0.08^{B}	59.5 ± 1.8^{A}	11.7 ± 0.3^{C}	11.3 ± 0.4^{CD}	5.1 ± 0.0^{BC}
$-H_2O$+AMF-P_i	1.28 ± 0.10^{B}	57.6 ± 0.9^{A}	8.1 ± 0.0^{E}	11.5 ± 0.6^{BCD}	7.1 ± 0.8^{A}
$-H_2O$+AMF+P_i	1.12 ± 0.09^{B}	59.0 ± 1.4^{A}	13.4 ± 0.6^{B}	12.9 ± 0.4^{BC}	4.4 ± 0.1^{CD}

*Different letters indicate statistically significant differences by Student Newman Keuls test at a 5 % significance level between treatments (columns) (n=4±SE).

Table S1

Statistical summary of effects in attributes (RWC - relative water content; g_s - stomatal conductance; *A* - net photosynthetic rate; *E* - transpiration rate; F_v'/F_m' - maximum efficiency of PSII; F_q'/F_m'- operating efficiency of PSII; ETR - electron transport rate; qP - photochemical quenching; NPQ - non-photochemical quenching; SS - soluble sugars; FAA- free amino acids; TSP- total soluble proteins; Chl *a* - chlorophyll *a*; Chl *b* - chlorophyll *b*; Car - carotenoids) of young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply ($+P_i$ and $–P_i$) and two water levels ($+H_2O$ and $–H_2O$) under greenhouse conditions. The values represent the statistical differences in maximum stress day (12th day of experiment).

Attributes	Effect						
	Water	**AMF**	**P_i**	**Water vs AMF**	**Water vs P_i**	**AMF vs P_i**	**Water vs AMF vs P_i**
RWC	**108.130*****§	0.830	0.060	**20.630*****§	3.260	1.540	**9.540****§
g_s	**2323.103*****§	**77.564*****§	**18.692*****§	**39.000*****§	**9.256****§	0.641	0.641
A	**3085.087*****§	**146.704*****§	4.123	**51.654*****§	0.552	**84.524*****§	**37.577*****§
E	**2984.815*****§	**251.837*****§	**11.383****§	**59.419*****§	**27.982*****§	**18.168*****§	0.162
F_v'/F_m'	**881.590*****§	**5.40***§	**488.69*****§	0.970	**11.350****§	**19.000*****§	**95.510*****§
F_q'/F_m'	**61.730*****§	**52.260*****§	**127.69*****§	**70.550*****§	1.030	**37.390*****§	**16.820*****§
ETR	**134.319*****§	**16.833*****§	**70.496*****§	**160.107*****§	0.212	3.509	**47.838*****§
qP	2.216	0.017	**31.438*****§	**4.884***§	0.084	**9.458****§	**15.731*****§
NPQ	**999.579*****§	**252.322*****§	**45.034*****§	**208.620*****§	0.025	**25.138*****§	**31.287*****§
Starch	**308.608*****§	**190.58*****§	**10.252****§	**45.731*****§	**24.719*****§	**251.709*****§	**45.955*****§
SS	**6.306***§	**72.406*****§	0.9	**50.648*****§	2.198	0.066	**12.265****§
Sucrose	0.84	**949.18*****§	**9.73****§	**19.58*****§	**308.36*****§	**64.08*****§	**329.97*****§
Fructose	**20.016*****§	**125.879*****§	**162.384*****§	**16.186*****§	**414.899*****§	2.362	**172.643*****§
FAA	**173.932*****§	**318.95*****§	**123.951*****§	**125.952*****§	**369.329*****§	**18.211*****§	**302.066*****§
Proline	**1359.2*****§	**9.01****§	**117.54*****§	**19.59*****§	**163.58*****§	**7.66***§	**449.22*****§

TSP	**67.755*****§	**25.285*****§	**7.222***§	**15.79*****§	**6.671***§	0.156	**45.349*****§
Chl *a*	**17.597*****§	**111.965*****§	2.843	**17.851*****§	**91.688*****§	**8.178****§	**90.539*****§
Chl *b*	**9.95****§	**14.298*****§	**17.205*****§	**27.733*****§	**27.891*****§	**13.843****§	**35.687*****§
Car	**130.788*****§	0.322	2.653	**38.927*****§	**83.517*****§	**42.36*****§	**173.573*****§

Boldface denotes significant results. *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$. §Denotes P-values significant at $\alpha = 0.05$ when corrected for 19 comparisons according to the Benjamini-Hochberg procedure.

Table S2

Statistical summary of effects in attributes (RWC - relative water content; g_s - stomatal conductance; *A* - net photosynthetic rate; *E* - transpiration rate; F_v'/F_m' - maximum efficiency of PSII; F_q'/F_m'- operating efficiency of PSII; ETR - electron transport rate; qP - photochemical quenching; NPQ - non-photochemical quenching; SS - soluble sugars; FAA- free amino acids; TSP- total soluble proteins; Chl *a* - chlorophyll *a*; Chl *b* - chlorophyll *b*; Car - carotenoids; N- nitrogen; P - phosphorus; K - potassium; N/P - ratio nitrogen/phosphorus) of young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply ($+P_i$ and $-P_i$) and two water levels ($+H_2O$ and $-H_2O$) under greenhouse conditions. The values represent the statistical differences in last rehydration day (16th day of experiment).

Attributes	Effect						
	Water	**AMF**	**P_i**	**Water vs AMF**	**Water vs P_i**	**AMF vs P_i**	**Water vs AMF vs P_i**
RWC	0.010	**5.240***§	3.810	2.290	0.230	**5.330***§	0.000
g_s	**202.238*****§	**77.234*****§	**26.145*****§	**15.957*****§	3.677	**29.515*****§	**29.515*****§
A	**123.423*****§	**258.897*****§	**11.949****§	**4.851***	**9.014****§	**72.526*****§	**29.639*****§
E	**14.0190****§	**102.112*****§	0.189	2.328	2.109	**5.451***§	**5.003***
F_v'/F_m'	**61.070*****§	**93.93*****§	**58.690*****§	**5.61***	**42.41*****§	**61.36*****§	2.560
F_q'/F_m'	**58.131*****§	1.215	**8.763****§	**43.484*****§	0.351	0.090	**44.734*****§
ETR	1.697	**58.086*****§	**52.077*****§	0.007	**60.500*****§	**81.423*****§	0.040
qP	0.000	0.720	0.030	3.010	**19.250*****§	**33.830*****§	**120.180*****§
NPQ	**36.450*****§	**24.670*****§	**4.470***	**231.650*****§	**864.160*****§	**72.000*****§	**8.470****§
Starch	**45.333*****§	**65.638*****§	**182.409*****§	**288.832*****§	1.288	**25.362*****§	**410.953*****§
SS	**28.496*****§	**14.359*****§	**4.922***	1.517	3.037	**7.879****§	**24.259*****§
Sucrose	**58.392*****§	0.449	1.703	**90.214*****§	**49.696*****§	2.88	**11.308*****§
Fructose	**16.723*****§	0.489	**21.67*****§	0.071	**29.699*****§	**4.601***	0.027

FAA	**5.319***	**51.225*****§	**205.52*****§	**95.114*****§	**28.224*****§	2.1	**34.068*****§
Proline	**14.0045****§	**7.8123***§	**39.6483*****§	**24.8245*****§	**56.2203*****§	**17.7073*****§	**14.3934*****§
TSP	3.905	**238.169*****§	**85.234*****§	1.373	**13.628****§	**16.386*****§	**8.095****§
Chl *a*	**78.988*****§	**38.698*****§	**11.126****§	**44.088*****§	**41.242*****§	**50.644*****§	2.191
Chl *b*	**88.941*****§	**4.469***	0.016	**35.521*****§	1.024	**40.383*****§	2.204
Car	**90.364*****§	**23.58*****§	0.896	**10.991****§	**15.881*****§	**16.479*****§	2.529
Biomass (shoot)	**6.062***§	**11.400****§	0.576	1.049	**15.670*****§	**20.660*****§	1.632
N (shoot)	0.027	0.096	3.061	1.364	0.058	0.760	0.082
P (shoot)	**52.835*****§	2.025	**216.009*****§	**6.555***§	**5.886***§	3.998	3.968
K (shoot)	**8.588****§	**48.576*****§	**43.193*****§	**43.480*****§	**29.548*****§	**50.680*****§	3.671
N/P (shoot)	**14.875*****§	0.910	**51.894*****§	0.020	0.190	**4.332***	1.815
Root colonization	**4.422***	**7953.517*****§	0.198	**4.422***	**52.685*****§	0.198	**52.685*****§

Boldface denotes significant results. **P* < 0.05; ***P* < 0.01; *** *P* <0.001. §Denotes *P*-values significant at $\alpha = 0.05$ when corrected for 26 comparisons according to the Benjamini-Hochberg procedure.

6. MANUSCRITO III (Plants with AMF symbiosis and foliar P_i supply alleviate salt stress effects and increase the biomass in woody species of semiarid environment)

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(ANEXO C)

Plants with AMF symbiosis and foliar P_i supply alleviate salt stress effects and increase the biomass in woody species of semiarid environment

Gabriella Frosi[a], Vanessa Andrade Barros[a], Marciel Teixeira Oliveira[a], Mariana Santos[a], Diego Gomes Ramos[a], Leonor Costa Maia[b], Mauro Guida Santos[a,*]

[a]Departamento de Botânica, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brazil

[b]Departamento de Micologia, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brazil

*Corresponding author: Tel.: +55 81 21268844; Fax: +55 81 21267803.

E-mail address: mauro.gsantos@ufpe.br (M.G. Santos)

Abbreviations: *A*, net photosynthetic rate; AMF, arbuscular mycorrhizal fungi; Car, carotenoids; Chl, chlorophyll; *E*, transpiration rate; EC, electrical conductivity; ETR, electron transport rate; FAA, free amino acids; F_m, maximal fluorescence from dark-adapted; F_m', maximum fluorescence from light-adapted; F', fluorescence emission from light-adapted; F_v/F_m, maximum quantum efficiency of PSII photochemistry; F_o, minimal fluorescence from dark-adapted; F_q'/F_m', PSII operating efficiency; F_v'/F_m', maximum efficiency of PSII; g_s, stomatal conductance; NPQ, non-photochemical quenching; PCA, principal component analysis; PPFD, photosynthetic photon flux density; qP, photochemical quenching; RWC, relative water content; SM, soil moisture; SS, soluble sugars; TSP, total soluble protein; VPD, vapor pressure deficit

Abstract

Salinity may limit plant growth especially in arid and semiarid regions Arbuscular mycorrhizal fungi (AMF) and the supply of inorganic phosphorus (P_i) could alleviate the negative effects of such stress on the leaf metabolism. The aim of this study was to evaluate the ecophysiological performance of *Poincianella pyramidalis* (Fabaceae) in a greenhouse under salinity conditions in combination with AMF and leaf P_i supply. The experiment was conducted in a factorial design with 2 levels of salinity (+NaCl and -NaCl), 2 levels of AMF (+AMF and -AMF) and 2 levels of leaf P_i supply (+P_i and -P_i). The variables gas exchange, leaf primary metabolism, dry biomass and nutrients were measured. Plants with AMF under non-saline conditions showed a higher photosynthesis and a lower decrease under salinity, a higher accumulation of dry matter in shoots under both conditions and a lower accumulation of Na^+ and Cl^- in leaves and roots under salinity. Plants with leaf P_i increased the biomass and photosynthetic pigments under both conditions, and accumulated more Cl^- in shoots under salinity. When combined, AMF*P_i increased the photosynthesis under non-saline conditions and decreased it under salinity. Plants under salinity without AMF and P_i had greater decreases in gas exchange and a greater accumulation of Cl^- in roots. Therefore, *P. pyramidalis* plants ameliorated improved their metabolism under both growth conditions in the presence of AMF, P_i or a combination of these both. However, the greatest increases in growth and tolerance to salinity occurred in the isolated presence of AMF.

Keywords: Caatinga; Chlorophyll fluorescence; Gas exchange; Leaf biochemistry; Mycorrhiza; Phosphorus

1. Introduction

Salinization of soils is one of the main environmental problems in various parts of the world. It is increasing especially in arid and semiarid environments (Evelin et al., 2009; Munns and Tester, 2008; Porcel et al., 2012). According to recent estimates, 1,128 Mha of land throughout the world are affected by salinity. In northeastern Brazil, approximately 30% of all irrigated areas are affected by salinity (Aguiar Neto et al., 2007; Wicke et al., 2011). High salt concentrations in the soil affect plants physiologically, anatomically and molecularly level, and may change the basic soil texture, resulting in a decreased soil porosity and, consequently, reducing aeration and water conductance (Munns and Tester, 2008; Polle and Chen, 2015; Porcel et al., 2012). In order to tolerate this condition, plants have intrinsic mechanisms such as osmotic adjustment, accumulation of osmoprotective molecules (such as proline and sugars), an increased activity of the antioxidant system, homeostasis, and morphological and anatomical plasticity. This leads to the protection of the photosynthetic machinery in order to maintain growth (Polle and Chen, 2015; Ruiz-Lozano et al., 2012).

Arbuscular mycorrhizal fungi (AMF) are an important integral component of the natural ecosystem, and are known to exist in saline environments (Giri et al., 2007). Many researchers have reported that AMF may increase the plant's capacity to handle stress (Jahromi et al., 2008; Sheng et al., 2008; Yano-Melo et al., 2003) through gains in the absorption of nutrients (Cantrell and Linderman, 2001), ionic balance (Giri et al., 2007), protecting the activity of enzymes and facilitating the absorption of water (Ruiz-Lozano et al., 2012), thus promoting less accumulation of sodium and chlorine, stabilizing the Na^+/K^+ ratio and regulating genes involved in tolerance to salinity (Evelin et al., 2009; Porcel et al., 2012).

Salinity promotes the reduction of the soil osmotic potential, making it difficult for roots to absorb water and leading to water stress conditions (Munns and Tester, 2008). Several studies indicate that the use of inorganic phosphorus (P_i) may improve plant growth under low water

availability conditions (Campbell and Sage, 2006; Garg et al., 2004; Santos et al., 2004). A greater control of stomatal conductance, increased photosynthesis and biomass (Oliveira et al., 2014; Santos et al., 2004, 2006; Singh et al., 2013), increased cell membrane stability and an improved water use efficiency (Faustino et al., 2013) have been attributed to the application of P_i.

In this scenario, the supply of leaf P_i and AMF may be tools for a greater tolerance in plants under salinity conditions aiming to regenerate areas in seasonally dry tropical forests. Among native woody species, *Poincianella pyramidalis* is widely distributed in the Brazilian northeastern semiarid. It is used for the recovery of degraded areas and may be an interesting model species for studying mechanisms of tolerance to abiotic factors such as drought and salinity. Recent studies on this species focused on the ecophysiological aspects of successional gradients (Falcão et al., 2015) and on the physiological performance in association with AMF under hydrated (Frosi et al., 2016) and drought conditions (Frosi et al., unpublished data).

We believe that AMF and the supply of leaf P_i may be tools that mitigate the deleterious effects of salinity, as in plants under water stress (Frosi et al., unpublished data). Thus, this study aimed to evaluate the ecophysiological performance (gas exchange, leaf primary metabolism, nutritional status and growth) of *P. pyramidalis* plants subjected to salinity associated with AMF and supply of leaf P_i. Our hypotheses are: (1) AMF or the supply of leaf P_i promotes a higher growth and tolerance in young plants when subjected to salinity, (2) the combination of AMF and the application of leaf P_i promotes a higher growth and a greater tolerance under salinity conditions in young plants compared to plants with only AMF or P_i.

2. Materials and methods

2.1. Growth conditions and plant material

The experiment was conducted in a greenhouse in northeastern Brazil (8°08'58" S, 34°56'55" W), atan average temperature of 34±2 °C, 40-60% of relative humidity and natural daylight (maximum light flux: 1,400 μmol $m^{-2}s^{-1}$). Plants were kept at an ideal hydration (pot capacity - 300 mL) until the beginning of the salinity stress. The experiment was completely randomized in a factorial design with 2 salinity levels [no salinity (-NaCl) and saline (+NaCl)] x 2 symbiosis levels [inoculated (+AMF) and non-inoculated (-AMF)] x 2 leaf phosphorus levels [with leaf inorganic phosphorus (+P_i) and without leaf inorganic phosphorus (-P_i)], totaling eight treatments: Control, P_i, AMF, AMF*P_i, Salt, Salt*P_i, Salt*AMF and Salt*AMF*P_i, with 8 replicates each and one plant per pot, totaling 64 experimental units. From these 8 replicates, 4 were selected to evaluate biomass and nutrients and 4 were intended studying for the other variables.

Isolated AMF [*Acaulospora longula* Spain & N.C. Schenck (URM AMF 07) and *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler (URM AMF 03)] were provided by the Mycorrhizas Laboratory's Inoculum Bank at the Mycology Department of UFPE. These AMF species were chosen because they are widely distributed in the Brazilian northeastern semiarid (Silva et al. 2014). The inoculum of each isolate (spores and pieces of roots) was placed in pots containing 5 kg of sterilized soil with *Panicum miliaceum* L. and *Sorghum bicolor* (L) Moench cultures to increase the spore density within three months. The soil inoculum consisted of spores and roots fragments. Spores were extracted from the soil by wet sieving and centrifuged with water and sucrose (Gerdemann and Nicolson, 1963; Jenkins, 1964). Then they were measured using a stereomicroscope (40x) to quantify the number of spores per gram.

P. pyramidalis (Tul.) LP Queiroz (Fabaceae) 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 placed in

trays containing sterilized washed sand to germinate. After 20 days, seedlings were transferred to 100 mL pots with sterilized soil. Seedlings destined for inoculation received soil inoculum with 150 spores from each AMF in roots, totaling 300 spores per plant. Non-inoculated plants received the same amount of autoclaved rhizosphere soil. After 30 days under these conditions, the plants were transferred to pots containing 5 kg of same soil type with a phosphorus (P) concentration adjusted to 33 mg dm^{-3} by applying simple superphosphate (P_2O_5) in all treatments for standardization. This concentration was determined in a prior study (Frosi et al., 2016), in which 33 mg dm^{-3} of P in the soil promoted higher gains in plant biomass in this species under well-watered conditions.

The soil used was collected in the Pernambuco state, Brazilian Northeast (7° 35'39 "S, 34°54'23"W). It was classified as dystrophic yellow Latosol and its biochemical characteristics are shown in the supplementary material 1.

Salinity stress was imposed by saline irrigation with a NaCl 100 mM solution (300 mL) around 8:00 am every day when plants completed 6 months of development. This concentration is sufficient to achieve a soil electrical conductivity above 2 mS cm^{-1}, characterizing the soil as saline (US Salinity Laboratory, 1964). Two days before the beginning of the stress, plants intended for leaf P_i supply treatments were sprayed with a monoammonium phosphate solution in leaves (10 g P_i L^{-1}), and the others plants received an equivalent dose of nitrogen as urea PA (2.64 g N l^{-1}), according to Santos et al. (2006). Leaf relative water content (RWC), soil moisture (SM), soil electrical conductivity (EC), gas exchange and chlorophyll *a* fluorescence were evaluated when saline condition was established. Plants were harvested on the last day of the experiment (maximum stress) for performing an analysis of mycorrhizal colonization, organic solutes, photosynthetic pigments, dry biomass and leaf and root nutrients. The maximum stress was determined when gas exchange was close to zero, which occurred 11 days after beginning of the saline irrigation.

149 *2.2. Mycorrhizal colonization*

150 For the quantification of mycorrhizal colonization, fine roots from four plants per
151 treatment were used. They were clarified with 10% KOH (w/v) for 48 h at 25 °C, washed
152 with distilled water and colored with 0.1% acid fuchsin (w/v) for 12 h (Koske and Gemma,
153 1989). One hundred 1 cm pieces from each sample were examined under a microscope at a
154 40x magnification (Giovannetti and Mosse, 1980). Roots that presented some AMF
155 structures: arbuscules, vesicles, hyphae and spores were considered colonized.

156 *2.3. Leaf relative water content (RWC) and soil electrical conductivity (EC)*

157 Leaf discs with a known size were collected at 06:00 am and immediately weighed on a
158 precision scale (AND H200, Tokyo, JP) to obtain fresh weight (FW). Then, they were
159 immediately soaked for 24 h in deionized water and weighed again to determine turgid weight
160 (TW). Subsequently, discs were dried for 48 h in a forced-air ventilation oven and weighed to
161 obtain dry weight (DW). The leaf relative water content (RWC) was obtained by using the
162 formula RWC (%) = [FW-DW/TW-DW] x100 (Barrs and Weatherley, 1962).
163 Soil samples were collected, weighed (10 g) and homogenized in 25 ml of deionized water.
164 This solution was stirred three times for 2 min, totaling 10 min. The solution was kept still for
165 40 min, and the supernatant was collected to measure the electrical conductivity using a
166 conductivity meter (Model CD-4306, Lutron Electronic Enterprise Co., Taipei, Taiwan,
167 China).

168 *2.4. Dry biomass*

169 Shoot parts were separated and placed under forced ventilation at 70°C until constant
170 weight. They were weighed on a precision scale (AND H200, Tokyo, JP). We measured the

171 dry weight of the root system, but decided not to discuss it here because there was a great
172 variation, probably due to loss upon removing the plants from the pots.

173 *2.5. Gas exchange*

174 Gas exchange was measured in the third expanded leaf pair with an infrared gas analyzer
175 (IRGA, ADC, LCi-Pro model, Hoddesdon, UK). Stomatal conductance (g_s), net
176 photosynthetic rate (A) and transpiration rate (E) were obtained. The maximum stress was
177 determined when g_s and A were close to zero, which occurred after 11 days under salinity.
178 Measurements were performed between 09:00 and 11:00 am. The photosynthetic photon flux
179 density (PPFD) was determined by the measuring the global radiation incident at the
180 measurement time, with an air flow through the sample chamber of 400 μmol $m^{-2}s^{-1}$ and a
181 CO_2 concentration of 400 μmol $m^{-2}s^{-1}$. The vapor pressure deficit (VPD) during the
182 experiment was calculated by the formula e_s-e_a, where e_s is saturated vapor pressure and e_a is
183 ambient vapor pressure (Campbell and Norman, 1998). It was obtained by temperature and
184 relative humidity, and measured with a digital thermal hygrometer (Termo-Higro SH 122, J
185 Prolab. São José dos Pinhais, BR). Soil moisture (SM) was obtained with a soil moisture
186 meter (v/v) (HFM 2030 Falker, Porto Alegre, BR).

187 *2.6. Chlorophyll a fluorescence*

188 Chlorophyll *a* fluorescence was obtained with a Fluor Pen FP100 portable fluorimeter
189 (Photon Systems Instruments, Brno, Czech Republic) in the third expanded leaf pair at the
190 moment of gas exchange. Leaves were adapted to dark for 30 min in order to determine the
191 minimal fluorescence of leaves adapted to dark (F_o). The maximum fluorescence of leaves
192 adapted to dark (F_m) was calculated after the incidence of a saturating pulse of ~5,000 μmol
193 m^{-2} s^{-1}. The variable fluorescence of leaves adapted to dark (F_v) was calculated by F_v=F_m-F_o.
194 Fluorescence emission at steady state (F') and maximum fluorescence (F_m') were determined

for leaves adapted to light and subjected to stable photosynthesis. Data were used to calculate the maximum quantum efficiency of PSII (F_v/F_m), maximum efficiency of PSII (F_v'/F_m'), operating efficiency of PSII (F_q'/F_m'), photochemical quenching (qP), electron transport rate (ETR) and non-photochemical quenching (NPQ) (Baker, 2008).

2.7. Organic solutes and photosynthetic pigments

Leaves were collected (~3 g) at 03:00 pm during maximum stress. They were immediately frozen in liquid nitrogen and stored at -20 °C. Starch content, soluble sugars (SS), sucrose, fructose, total free amino acids (FAA), proline and total soluble proteins (TSP) were determined. All readings were performed with a double beam spectrophotometer (Geneses 10S UV-Vis, Thermo Scientific, Waltham, USA). For starch, SS, sucrose, fructose and FAA, 50 mg of fresh-weight leaves were used in an ethanol extraction. The SS was measured according to Dubois et al. (1956) using D (+) glucose as a standard and a 490 nm absorbance. Sucrose was measured according to Handel (1968), using sucrose as a standard and a 660 nm absorbance. Fructose was measured using fructose as a standard and a 410 nm absorbance according to Foreman et al. (1973). FAA were analyzed using a solution of 1 mM of glycine, glutamic acid, phenylalanine and arginine as a standard and a 570 nm absorbance according to Moore and Stein (1948). The insoluble fraction of the SS extract was used to determine the starch content. The pellet was hydrolyzed for 1 h with 10 units of amyloglucosidase, and the resulting sugars were analyzed (Dubois et al., 1956). For proline, 50 mg of leaf fresh mass were used, and the content was measured using the acidic ninhydrin method (Bates, 1973); it used proline as a standard and a 520 nm absorbance. For the TSP, 100 mg of leaf fresh mass were macerated in 100 mM potassium phosphate buffer (pH 6.5). It was measured using 0.1% of bovine serum albumin (w/v) as a standard and a 595 nm absorbance according to Bradford

(1976). The concentrations of organic solutes were expressed as dry weight by converting the fresh weight, used in the extraction to dry weight.

For chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car), 50 mg of leaf fresh mass were macerated in 2 mL of acetone (80%) with calcium carbonate ($CaCO_3$) to prevent the activity of chorophyllase. Samples were filtered and read at the absorbances 470.0, 648.8 and 663.2 nm. Additionally, a 710 nm nonspecific absorbance was used to correct color, turbidity and contaminating compounds, since pigments are not read at this wavelength. Final pigment concentrations were calculated as described by Lichtenthaler and Buschmann (2001). The concentrations of organic solutes were expressed as dry weight by converting fresh weight, used in the extraction to dry weight.

2.8. Nutrients

For the quantification of nitrogen (N), phosphorus (P) potassium (K^+) and sodium (Na^+), 250 mg of leaf and root dry matter were used. The material was digested in a sulfuric acid solution (H_2SO_4) in a digester block at 350 °C to obtain a sample extract (Thomas et al., 1967). Total N content was determined by titration with HCl extract after the addition of boric acid and a colorimetric indicator (Thomas et al., 1967). The P content was spectrophotometrically determined (Spectrophotometer 600S, FEMTO, São Paulo, BR) (Murphy and Riley, 1962) using a P concentration curve. K^+ and Na^+ contents were determined by flame photometry (DM-62, Digimed, São Paulo, BR) using 5 ppm of K^+ solution as a standard (Silva, 2009). For chloride (Cl^-), 500 mg of leaf and root dry matter were used. The extracts were obtained using the Mohr method, and the content was determined by titrating the extract with silver nitrate ($AgNO_3$) after the addition of potassium

chromate (K_2CrO_4) (Lacroix et al., 1970). The Na^+/K^+ ratio was calculated for shoots and roots using Na^+ and K^+ data.

2.9. Statistical analysis

We performed a factorial ANOVA with AMF, P_i and Salt as independent variables, respecting all prerequisites (normality and homogeneity). Data expressed in percentage (colonization, RWC and SM) were transformed into arcsine $\sqrt{x}$ for statistical analysis. Significant differences were compared using the Student Newman Keuls test ($P < 0.05$). Data were analyzed with the software Statistica 8.0 (StatSoft. Inc, Tulsa, Oklahoma, USA). In order to reduce the probability of type I error for multiple comparisons resulting from simultaneous analyses of attributes during the experiment, the Benjamini and Hochberg (1995) procedure was used with an α of 0.05 The statistical summary of differences in variables between experiment treatments using the Benjanmini and Hochberg (1995) procedure is described in the supplementary material (Supplementary material 2 and 3).

A principal component analysis (PCA) was performed to verify possible clusters, eliminate redundancies and define the most important variables during the separation of groups under maximum stress. For PCA, mycorrhizal colonization (exclusive of inoculated plants) and Na^+/K^+ ratio (co-dependent relation of nutrients) were discarded. Data were transformed (ranging) for standardization due to different scale magnitudes. The level of importance of each variable was determined by eigenvector values (McGarigal et al., 2000), with substantial correlation values determined for each attribute in relation to principal components (PC). The level of importance of each PC was determined by the Broken-stick method, where eigenvalues exceeding the expected values were kept for interpretation. Analyses were performed using the software Fitopac 2.1.2.85 (Shepherd, 2010).

265 **3. Results**

266 *3.1. Mycorrhizal colonization*

267 No mycorrhizal colonization was observed in non-inoculated treatments (-AMF). In
268 inoculated treatments (+AMF), the highest percentage of colonization occurred in AMF and
269 Salt*AMF*P_i, followed by AMF*P_i and Salt*AMF plants (Table 1).

270 *3.2. Leaf relative water content (RWC) and soil electrical conductivity (EC)*

271 Under maximum stress day, there was a 4.4% decrease in RWC in AMF*P_i, Salt*P_i and
272 Salt*AMF treatments and 10% in Salt*AMF*P_i compared to Control plants. Despite the
273 reductions observed in these treatments, RWC was above 85% (Table 1). The EC was seven
274 times higher in Salt*P_i, Salt*AMF and Salt*AMF*P_i and 21 times higher in the Salt treatment
275 compared to Control plants (Table 1).

276 *3.3. Dry biomass*

277 Shoot biomass increased in non-saline treatments compared to the Control plants, with the
278 highest values observed for the AMF treatment (Table 1). Under saline conditions, only
279 Salt*AMF plants increased shoot biomass compared to Control treatment (Table 1).

280 *3.4. Gas exchange and environmental variables*

281 Gas exchange were higher throughout the experiment under non-saline and saline
282 conditions in the treatments with isolated AMF (AMF and Salt*AMF). Under maximum
283 stress day, the g_s had increases in non-saline treatments compared to Control plants, with
284 higher values for AMF and AMF* P_i plants. Under saline conditions, g_s decreased in all
285 treatments compared to the Control treatment, except for Salt*AMF plants, with higher
286 decreases observed for Salt plants, 86% (Fig. 1a). During the same period, *A* increased by 27
287 and 49% in AMF*Pi and AMF treatments, respectively, compared to Control plants. Under

288 saline conditions, all treatments decreased *A*. However, Salt*AMF plants decreased by 49%,
289 while Salt plants decreased by 80% compared to the Control treatment (Fig. 1b). The *E* was
290 higher in non-saline treatments compared to Control plants, with higher increases in the
291 AMF*Pi treatment, by 115%. Saline treatments decreased *E* in comparison to Control plants,
292 except Salt*AMF plants, which had higher decreases in Salt plants, by 69%, a behavior
293 similar to g_s (Fig. 1c).

294 The PPFD varied between 1,000 and 1,400 μmol $m^{-2}s^{-1}$ and the VPD varied between 1.63
295 and 3.9 kPa throughout the experiment (Fig. 1d,e). The SM differed from that of the second
296 day of the experiment. It was always higher in saline stress treatments, where the maximum
297 stress values were 28% higher in saline treatments compared to non-saline treatments (Fig.
298 1f).

299 *3.5. Chlorophyll a fluorescence*

300 Under maximum stress day, the F_v/F_m decreased by 11% only in Salt*P_i and Salt*AMF*P_i
301 treatments, compared to Control plants. The F_v'/F_m', $F_{q'}/F_m'$ and ETR decreased in all
302 treatments in comparison to the Control treatment. In the F_v'/F_m', the highest decreases were
303 observed for all saline treatments, by 49%, while $F_{q'}/F_m'$ decreased by 15% in AMF*P_i plants
304 and by 26% in the other treatments. ETR had the highest decreases in Salt*AMF*P_i. The qP
305 decreased by 31% only in AMF plants compared to the Control treatment, while NPQ
306 increased in all treatments, except for AMF*P_i and Salt*AMF*P_i plants (Table 1).

307 *3.6. Organic solutes and photosynthetic pigments*

308 Starch decreased in all treatments compared to Control plants, with the highest reduction
309 occurring in the Salt*P_i treatment (Fig. 2a). . The SS increased by 24% only in Salt plants, ,
310 and decreased in other treatments, except for the P_i treatment, compared to Control plants
311 (Fig. 2b). Sucrose increased by 4% in AMF*Pi and Salt*AMF*Pi plants, and decreased by

312 10% in other treatments, except for Salt plants, compared to the Control treatment (Fig. 2c).
313 Fructose increased by 61% in AMF*Pi plants and by 106% in Salt*AMF and Salt*AMF*Pi
314 plants compared to the Control treatment (Fig. 2d).

315 The FAA increased only P_i plants, by 38%, compared to the Control treatment (Fig. 3a),
316 while the TSP was higher only in Salt plants (Fig. 3b). Proline increased in all saline
317 treatments and AMF plants, with the highest concentration observed for Salt*AMF*Pi plants
318 compared to the Control treatment (Fig. 3c).

319 The Chl *a* decreased by 28% in Salt*AMF*Pi and increased in other treatments, except for
320 Salt*AMF plants. There were higher increases in P_i and AMF treatments, by 86%, compared
321 to Control plants (Fig. 4a). The Chl *b* increased in all treatments, except for Salt*AMF and
322 Salt*AMF*P_i, with higher values observed for P_i, AMF and Salt treatments, which was 92%
323 higher compared to Control plants (Fig. 4b). The Car increased in P_i, Salt and Salt*P_i
324 treatments compared to Control plants (Fig. 4c).

325 *3.7. Nutrients*

326 Shoot N was higher in AMF*P_i, Salt*P_i and Salt*AMF*P_i treatments, with highest
327 increases in the AMF*P_i treatment, by 66%, compared to Control plants. The P in shoots and
328 roots was higher in treatments that received the leaf P_i supply compared with other treatments.
329 In shoots, the highest increases were observed for AMF*P_i plants, by 563%, compared to the
330 Control treatment. In roots, the higher increases occurred in P_i and Salt*P_i plants. Shoot K^+
331 was higher only in Salt* P_i plants, by 27%, compared to the Control treatment. Shoot Na^+
332 decreased in AMF and AMF*P_i treatments compared to Control plants, and increased in
333 saline treatments, except for Salt*AMF plants. In roots, the Na^+ was higher only in Salt
334 plants, by 30%, compared to the Control treatment, while AMF, AMF*P_i and Salt*AMF
335 decreased. Shoot Cl^- increased in all saline treatments and the highest values occurred for

Salt*P_i plants, by 269%, and the lowest increases occurred for Salt*AMF treatment, by 100%, compared to the Control treatment, while AMF and AMF*P_i plants, showed decreases. In roots, as in shoot, the Cl^- was higher in all saline treatments, in addition to AMF and AMF*P_i plants compared to the Control treatment. There were higher increases in Salt plants, while P_i plants showed reductions. The shoot Na^+/K^+ ratio was 13% higher in saline treatments and 38% lower in AMF and AMF*P_i treatments compared to Control plants. In roots, the Na^+/K^+ ratio was higher only in Salt plants and lower in non-saline treatments compared to Control plants (Table 2).

3.8. Principal component analysis (PCA)

At ordination, the variables accounted for 49.85% of the total data variation. The most relevant attributes in distinguishing group had eigenvectors values >0.21. Treatments were separated according to salinity into PC1 and according to AMF and P_i into PC2. For PC1, the most relevant attributes and their correlation coefficients were *A* (0.98), shoot Na^+ (-0.94), shoot Cl^- (-0.90), g_s (0.90), *E* (0.88), proline (-0.76), F_v/F_m (0.76), F_v'/F_m' (0.76), starch (0.72), shoot dry biomass (0.67), root Na^+ (-0.66) and root Cl^- (-0.66). For PC2, the most relevant attributes were Chl *b* (0.86), fructose (-0.82), Chl *a* (0.81), SS (0.78), Car (0.77), RWC (0.75), shoot N (-0.63) (Fig. 5). The Salt treatment showed greater decreases in gas exchange, with accumulation of Na^+ and Cl^- in roots and higher concentrations of photosynthetic pigments and SS, forming a more distanced and different group at ordination. The Salt*P_i plants had greater increases in shoot Cl^- and higher decreases in starch, while Salt*AMF*P_i had greater decreases in Chl *a*, RWC and SS and increases in fructose and proline, forming two different groups at ordination. Salt*AMF plants had no decreases in g_s and *E*, smaller decreases of *A*, and greater increases in shoot dry biomass compared to other salinity treatments. There was

less accumulation of Cl^- in shoots, without an accumulation of Na^+ in shoots. There was a decrease of this ion in roots, forming a distinct grouping at ordination closer to Control plants (Fig. 5).

Among non-saline treatments, AMF plants showed less reduction of SS, with a higher *A* and shoot dry biomass. AMF* P_i had a higher *E*, fructose and shoot N, and a lower shoot Cl^- and SS, differentiating at ordination. P_i plants had a higher Car content in relation to other non-saline treatments, with a lower increase of g_s and shoot dry biomass, forming a distinct group at ordination (Fig. 5).

4. Discussion

In our study, *P. pyramidalis* plants were favored in growth both under non-saline and saline conditions in the presence of P_i, AMF or both simultaneously. Such higher increases were observed for plants with only AMF under both conditions. Salt*AMF plants had a higher selectivity regarding the absorption of Na^+ and Cl^- ions in relation to other saline treatments, reducing their harmful effects and promoting a greater tolerance.

4.1. Plant-AMF relation

Plants inoculated with AMF under non-saline conditions had increases in gas exchange. Under saline conditions, they had lower decreases in gas exchange. The positive effects on gas exchange by the association with AMF are related to the promotion of water flow inside plants due to the increased the hydraulic conductivity of roots (Ruiz-Lozano et al., 2012) and the activity of aquaporins (Bárzana et al., 2014). In addition, the greater assimilations in mycorrhizal plants are also related to a strong drain of C, which is characteristic of AMF (Kaschuk et al., 2009). The increased assimilation of AMF also correlates with the increase in the concentration of chlorophylls (Sheng et al., 2008) and with the increase in NPQ, which is

383 involved in the dissipation of excess energy to prevent damage to the photosynthetic
384 machinery (Baker, 2008).

385 Such higher photosynthetic rates in inoculated plants caused a higher growth under non-
386 saline and saline conditions, with increases in shoot biomass (Table 2). Querejeta et al. (2007)
387 also stressed the relation between photosynthesis and higher gains in growth in woody plants
388 (Oak savanna) associated with AMFs. Similar results obtained with *P. pyramidalis* under
389 well-watered condition were observed by Frosi et al. (2016) and under drought conditions by
390 Frosi et al. (unpublished data).

391 The leaf primary metabolism of AMF and Salt*AMF plants decreasedstarch, sucrose and
392 SS. It was associated with an increase in biomass, indicating that these metabolites were
393 related to growth. However, in Salt*AMF plants, the decrease in sucrose was accompanied by
394 an increase in fructose, a monosaccharide constituent of sucrose involved in responses
395 signaling abiotic stress (Liu et al., 2013). These plants also accumulated proline, as well
396 plants from other saline treatments, corroborating studies which reported that the presence of
397 AMF promotes an increase in the levels of this amino acid (Ruiz-Lozano et al., 2012). Proline
398 is usually accumulated in plants subjected to salinity. This amino acid can perform functions
399 such as osmotic protection, energy reserve and nitrogen source, stabilization of sub-cellular
400 structures, energy drain and sequestration of reactive oxygen species (Evelin et al., 2009;
401 Szabados and Savoure, 2010).

402 Another key point in the growth of Salt*AMF plants was the lowest concentrations of the
403 ions Na^+ and Cl^- both in shoots and roots compared to other saline treatments. Studies show
404 that plants associated to AMF have lower levels of Na^+ (Zuccarini and Okurowska, 2008),
405 and that colonization decreases the translocation of this ion to shoots (Talaat and Shawky,
406 2011). Wu et al. (2010) also found a decreased absorption of Na^+ in citrus plants colonized by
407 AMF, alleviating the negative effects of salinity. This selectivity regarding the absorption of

Na^+ promoted the lowest Na^+/K^+ ratios in Salt*AMF plant roots compared with Salt plants. These decreased ratios are beneficial because Na^+ competes with K^+ binding sites, but does not perform the same function (Giri et al., 2007). AMF may also promote a lower absorption of Cl^- by roots (Zuccarini and Okurowska, 2008), a situation that wasobserved in this study. An x-ray analysis of the dispersive energy revealed low amounts of Na^+ and Cl^- in plants colonized by AMF by the legume *Trigonella foenum-graecum*, confirming that these fungi induce a buffering effect on the absorption of these ions (Evelin et al., 2012). Studies with a mycorrhizal legume species under salinity also found a lower accumulation of Na^+ in shoots, suggesting that AMF are able to protect leaves by regulating the absorption of toxic soil ions (Shokri and Maadi, 2009). Such lower accumulation of Na^+ and Cl^- in mycorrhizal plants may also be related to the regulation of gene expression in roots and leaves involved in the homeostasis of these ions due to volatile compounds produced by AMF (Ansari et al., 2013; Contreras-Cornejo et al., 2009).

4.2. Foliar P_i supply

P. pyramidalis plants with only the supply of P_i in leaves were favored regarding photosynthesis throughout the experiment, which was reflected in a higher shoot biomass under non-saline conditions compared to Control plants. This is a different behavior from that observed in a previous study on this same species under well-watered conditions, where no gains were observed for shoot dry biomass (Frosi et al., unpublished). Several studies have shown that plants with a P_i supply increase growth (Oliveira et al., 2014; Santos et al., 2004, 2006; Singh et al., 2013), and that such increase in growth may be related to the increased production of ATP, NADPH and the regeneration of phosphate sugars by the increased activity of enzymes related to carboxylation (Rao and Terry, 1995).

Salt*P_i plants had a greater accumulation of Cl^- in shoots compared to other salinity treatments. It was associated with higher concentrations of K^+. Cl^- has been considered an inert anion tolerated in a wide range of salt concentrations, and it acts as an ion counterbalancing K^+, responsible for regulating cell turgor and depolarization of membranes (Barbier-Brygoo et al., 2000). Its accumulation may have favored a greater g_s compared to Salt plants. Studies indicate that the higher P supply under salinity conditions promotes a reduction in the negative effects of Na^+ and Cl^- in plants because it can promote the integrity of the tonoplast (Rinaldelli and Mancuso, 1996), which would facilitate the compartmentalization of these ions in the vacuole, preventing their actions under metabolic processes (Cantrell and Lindemann, 2001). Moreover, Salt*P_i had a lower Na^+/K^+ ratio in roots compared to Salt, promoting improvements in cell operation, as Na^+ competes with the active K^+ sites, causing damage to the metabolism (Evelin et al., 2009).

Another factor associated with photosynthetic improvement is the high concentration of chlorophyll, a situation observed for P_i and Salt*P_i. High levels of chlorophyll have been attributed to nutritional improvements in P and N (Ruiz-Sánchez et al., 2010), a situation observed for plants with isolated P_i under both conditions. The higher chlorophyll concentrations may have offset the decrease in chlorophyll fluorescence parameters associated with an increased NPQ because salt stress is able to destroy PSII reaction centers and hinder the electron transport in the photosynthetic apparatus (Evelin et al., 2009).

In both treatments with leaf P_i, starch and sucrose decreased, in addition to SS in Salt*P_i, combined with an increase in shoot dry biomass under non-saline conditions and no decreases under salinity conditions, suggesting an investment in growth. The application of P_i may act by regulating the synthesis or degradation of starch under stress conditions through β-amylases (Liu et al., 2015). This lower concentration of sugars may be related to an increase in the enzymatic activity of invertase and sucrose phosphate synthase (Liu et al., 2015).

4.3. Relations of plants with AMFP_i*

Plants with a combination of AMF and leaf P_i increased gas exchange under non-saline conditions and decreased it under saline conditions. AMF*P_i plants had a photosynthesis higher than P_i, which may have been caused by the lower decreases in photochemical attributes without increases in NPQ. Compared to AMF, AMF*P_i plants had a lower photosynthesis. This may be related to a lower concentration of chlorophylls in relation to AMF. The stability of NPQ was also observed for Salt*AMF*P_i plants, providing lower photosynthesis decreases caused by a reduction in chlorophyll fluorescence attributes. Usually, increases in NPQ act as an energy dissipation mechanism to prevent damage to the photosynthetic apparatus (Baker, 2008), while other saline treatments had decreased photochemical attributes and an increased non-photochemical dissipation.

The higher photosynthetic rates in AMF*P_i plants compared to Control and P_i treatments and the higher concentrations of P and N in shoots (Table 2) were reflected in gains in shoot dry biomass (Table 2). On the other hand, plants under saline conditions had no increases in biomass compared to Salt. This gain in shoot dry biomass under non-saline conditions in AMF*P_i may be related to lower concentrations of SS and the mobilization of reserves (starch) compared to other non-saline treatments (Table 1), indicating that these metabolites were related to growth. Under saline conditions, Salt*AMF*P_i plants had greater decreases in photosynthesis, combined with a chlorophyll decrease, compared to isolated AMF plants, and an increased energy storage as proline. This treatment did not increase in shoot biomass when compared to the Control treatment. However, when compared to the Salt treatment, there were no decreases.

Under saline conditions, Salt*AMF*P_i had a high concentration of shoot Cl^-, similar to the treatment Salt*P_i. As discussed above, Cl^- is an anion involved in membrane depolarization and cell turgor (Barbier-Brygoo et al., 2000), achieving a greater stomatal conductance, as

observed in this treatment in relation to Salt. Moreover, a higher P content in these plants may have promoted a greater stability in tonoplasts, favoring the compartmentalization of the ions and avoiding damage to metabolic processes (Cantrell and Lindemann, 2001; Rinaldelli and Mancuso, 1996). As with other saline treatments with AMF or P_i, these plants had a higher Na^+/K^+ ratio only in roots, which would represent an advance in tolerance to salinity by favoring various metabolic processes, since Na^+ competes with K^+ sites, damaging the enzymes and the activation of protein synthesis (Evelin et al., 2009).

Plants without AMF or P_i under salinity had greater decreases regarding gas exchange, photochemistry, mobilization of reserves and SS accumulation, which may be involved in responses signaling stress (Keunen et al., 2013). Furthermore, the plants showed a concentration of shoot Na^+ similar to Salt*P_i and Salt*AMF*P_i plants, with less accumulation of Cl^- in relation to these treatments in the region, even with higher concentrations of these two ions in roots, especially Cl^-. The lower transfer of Cl^- from roots to shoots may be due to a lower carrying capability of this ion through anion channels (Munns and Tester, 2008), indicating that this species already has some degree of tolerance to salinity, without decreasing shoot dry biomass compared to the Control treatment.

In summary, plants inoculated with AMF in non-saline and saline situations had higher increases in photosynthetic rates and shoot biomass, being higher than the other treatments, and presenting a greater selectivity regarding the absorption of Na^+ and Cl^- ions in saline situations. Plants with a supply of isolated leaf P_i were also benefited in both situations, with increases and higher concentrations of chlorophylls, Car and shoot biomass under non-saline conditions. There was a greater accumulation of Cl^- and K^+ in shoots under salinity conditions. On the other hand, plants with the combination of AMF and P_i were benefited under both conditions, with increases in photosynthesis, photochemical apparatus and biochemistry. There was an increase in shoot biomass under non-saline conditions.

Therefore, our hypothesis 1 (AMF or supply of leaf P_i promotes a greater growth and a higher tolerance of plants under salinity) was partially corroborated, with an improvement in gas exchange under saline conditions and increases in shoot biomass only in AMF plants and no reductions in growth in P_i plants, with AMF plants showed a less accumulation of Na^+ and Cl^-. The hypothesis 2 (the combination of AMF and supply of leaf P_i promoting a greater growth and a greater tolerance under saline conditions compared with plants with isolated AMF or P_i) was not confirmed. Despite increases in gas exchange, photochemistry and biochemical metabolism in these plants, Salt*AMF*P_i plants did not showed gains in biomass and higher photosynthetic rates compared to Salt*AMF*. The absence of a synergistic effect between AMF and leaf P_i could be due to AMF efficiency in supplying plants with nutrients, especially P_i, which showed a low content in the soil at the end of the experiment.

5. Conclusion

P. pyramidalis plants were benefited in presence of leaf P_i supply or AMF*P_i regarding gas exchange under non-saline and saline conditions, and increased in shoot biomass under non-saline conditions. However, the isolated AMF promoted a higher photosynthesis and increase in shoot biomass under non-saline and saline conditions, with a greater tolerance under salt stress when compared with plants without supply of P_i, AMF or both. That is, both leaf P_i and AMF could be tools to increase the tolerance of this species when exposed to semiarid conditions aiming a successful regeneration of degraded areas in seasonally dry tropical forests. However, the highest increase in biomass and the tolerance to salinity of this species occurred in the isolated presence of AMF.

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Figure legend

Fig. 1. a - Stomatal conductance (g_s), b - net photosynthetic rate (A), c - transpiration rate (E), d - photosynthetic photon flux density (PPFD), e - vapor pressure deficit (VPD) and f - soil moisture (SM) in young *Poincianella pyramidalis* plants at two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply levels (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions. Asterisks compare daily treatments and the differences according to Student Newman Keuls test ($P < 0.05$) (n = 4 ± SE).

Fig. 2. a - starch, b - soluble sugars (SS), c – sucrose and d – fructose in young *Poincianella pyramidalis* plants at two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply levels (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions. Different letters indicated differences according to Student Newman Keuls test ($P < 0.05$) (n = 4 ± SE).

Fig. 3. a – amino acids (FAA), b – total soluble proteins (TSP) and c - proline in young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply levels (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions. Different letters indicated differences according to Student Newman Keuls test ($P < 0.05$) (n = 4 ± SE).

Fig. 4. a – chlorophyll *a* (Chl *a*), b – chlorophyll *b* (Chl *b*) and c – carotenoids (Car) in young *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply levels (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions. Different letters indicate differences according to Student Newman Keuls test ($P < 0.05$) (n = 4 ± SE).

Fig. 5. Principal component analysis (PCA) based on the whole dataset of the study on young *Poincianella pyramidalis* at two mycorrhizal levels (+AMF and –AMF), two leaf P_i supply levels (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) in maximum stress day under greenhouse conditions (n = 4).

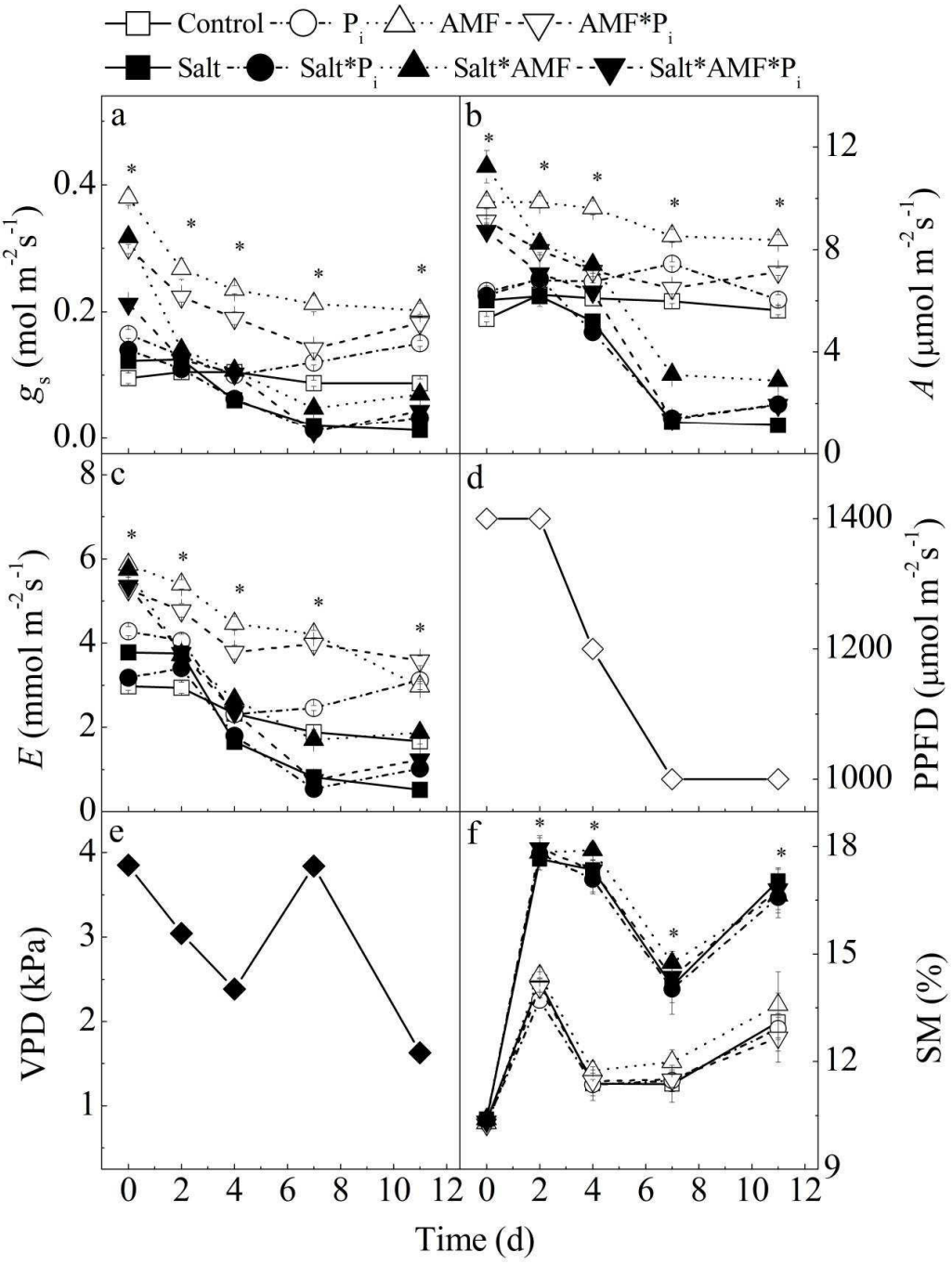
Control
P$_i$
AMF
AMF*P$_i$
Salt
Salt*P$_i$
Salt*AMF
Salt*AMF*P$_i$
a
b
c
d
e
f
g_s (mol m^{-2} s^{-1})
A (μmol m^{-2} s^{-1})
E (mmol m^{-2} s^{-1})
PPFD (μmol m^{-2} s^{-1})
VPD (kPa)
SM (%)
Time (d)

Fig. 1

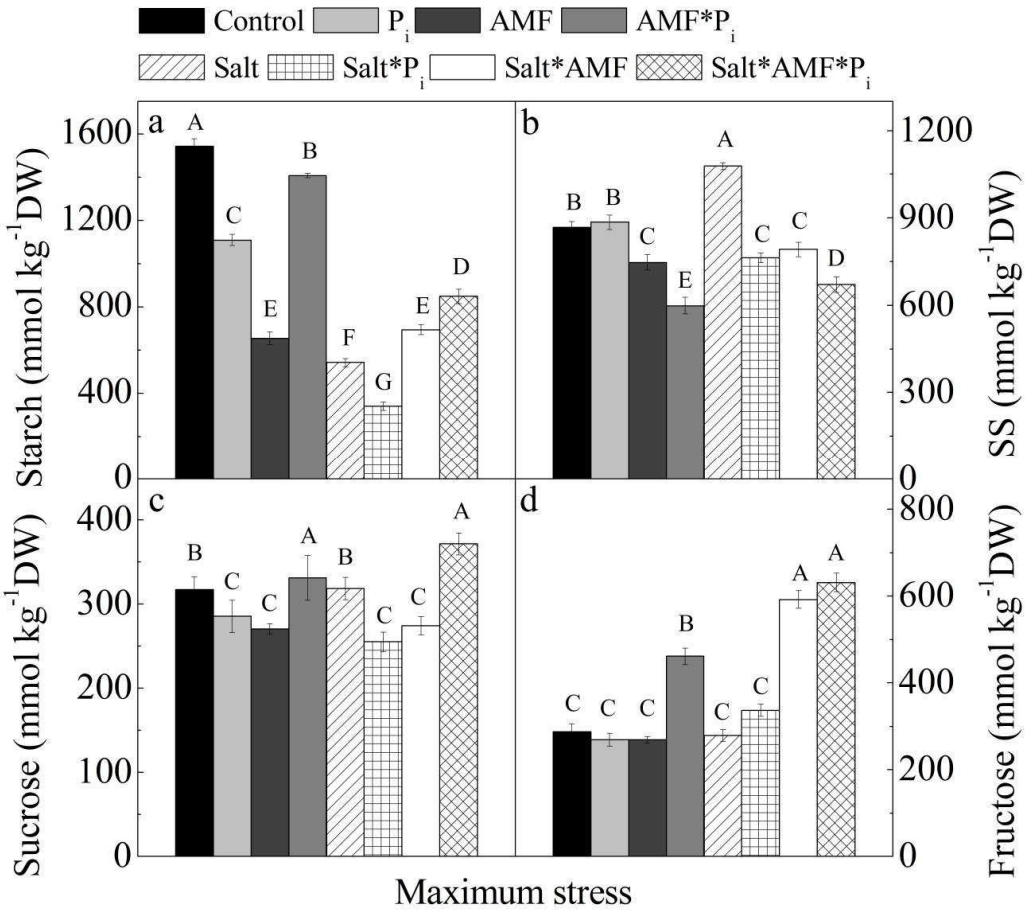
Control
P i
AMF
AMF*P i
Salt
Salt*P i
Salt*AMF
Salt*AMF*P i
a
b
c
d
Starch (mmol kg-1DW)
SS (mmol kg-1DW)
Sucrose (mmol kg-1DW)
Fructose (mmol kg-1DW)
Maximum stress

Fig. 2

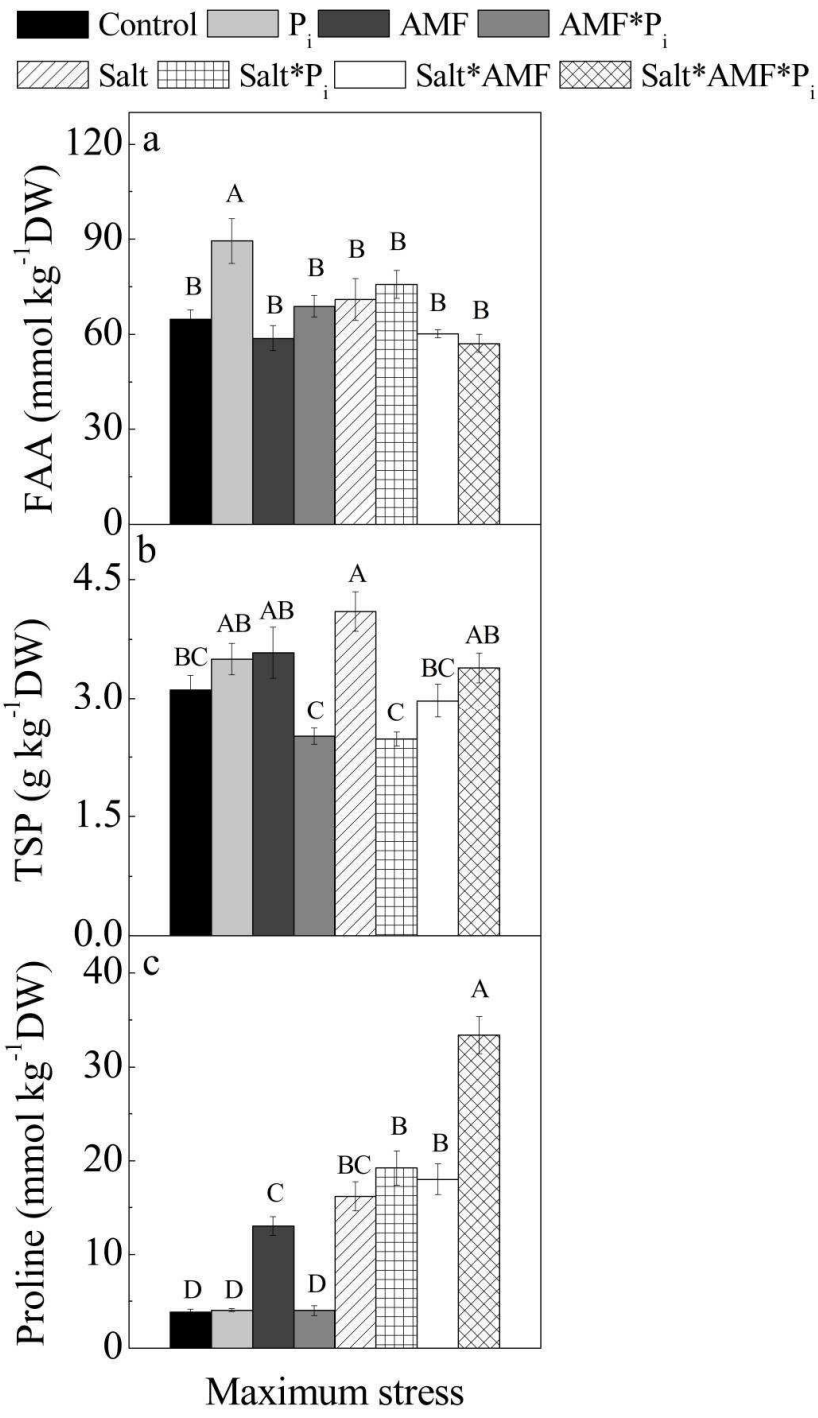
Control
P i
AMF
AMF*P i
Salt
Salt*P i
Salt*AMF
Salt*AMF*P i
a
FAA (mmol kg-1DW)
120
90
60
30
0
B
A
B
B
B
B
B
B
b
TSP (g kg-1DW)
4.5
3.0
1.5
0.0
BC
AB
AB
C
A
C
BC
AB
c
Proline (mmol kg-1DW)
40
30
20
10
0
D
D
C
D
BC
B
B
A
Maximum stress

Fig. 3

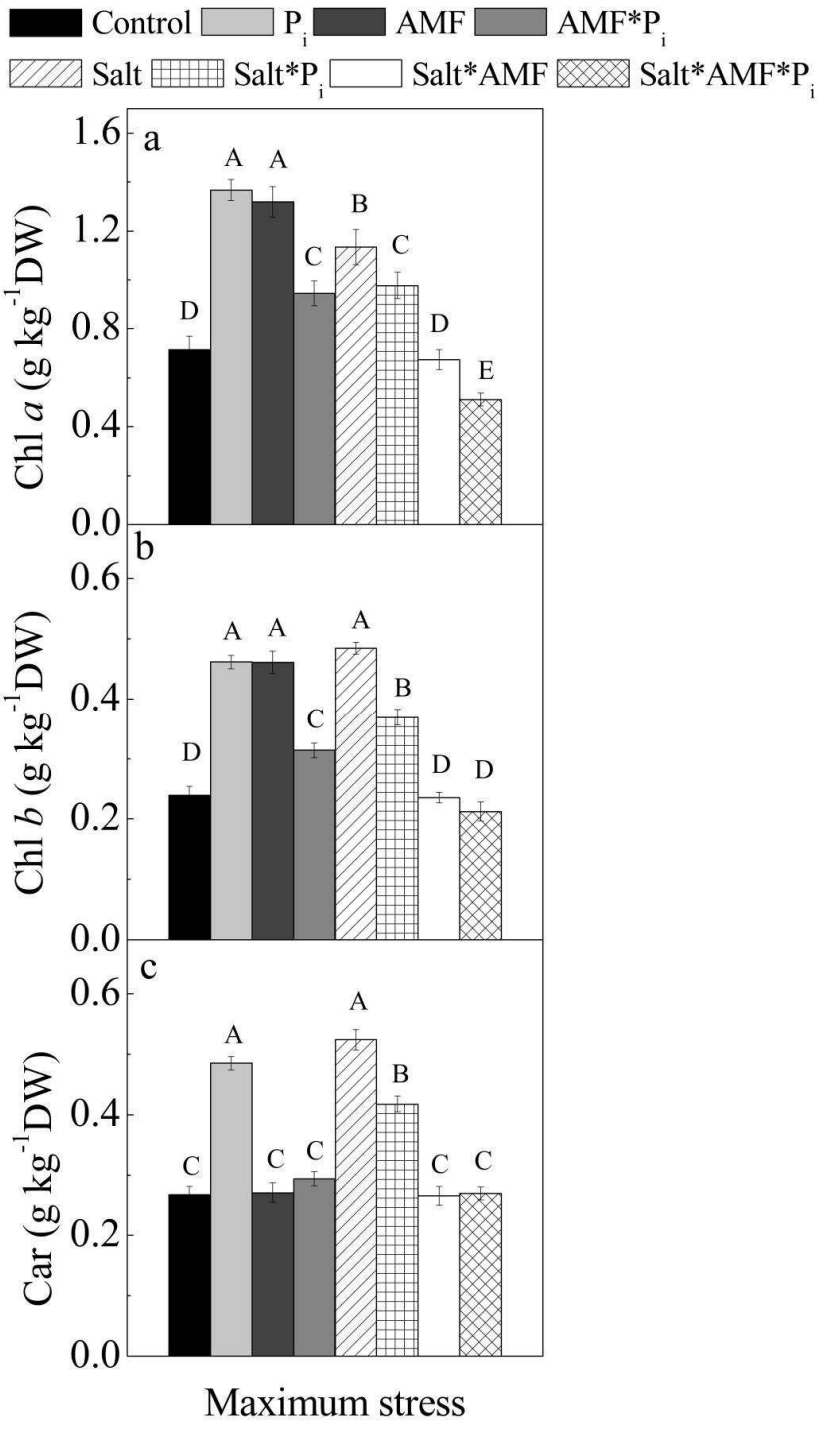
Control
P i
AMF
AMF*P i
Salt
Salt*P i
Salt*AMF
Salt*AMF*P i
a
b
c
Chl a (g kg-1DW)
Chl b (g kg-1DW)
Car (g kg-1DW)
Maximum stress

Fig. 4

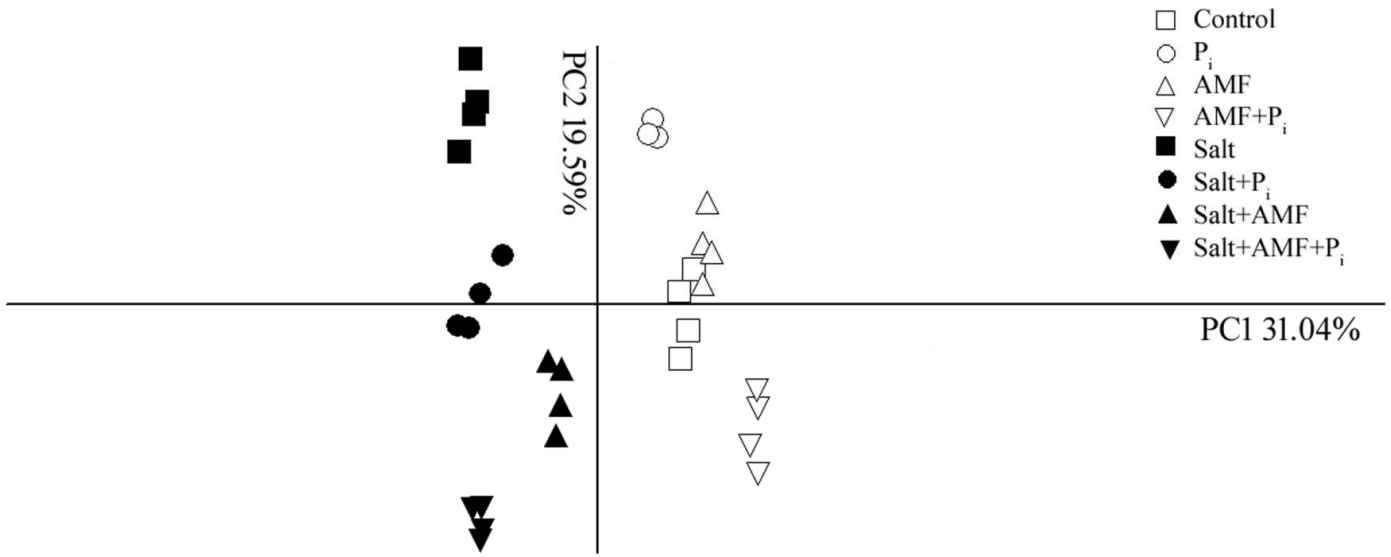
Control
P_i
AMF
AMF+P_i
Salt
Salt+P_i
Salt+AMF
Salt+AMF+P_i
PC2 19.59%
PC1 31.04%

Fig. 5

Table 1

Attributes measurements (RWC - relative water content; EC – electrical conductivity; F_v'/F_m' - maximum efficiency of PSII; F_q'/F_m'- operating efficiency of PSII; ETR - electron transport rate; qP - photochemical quenching; NPQ - non-photochemical quenching) in maximum stress day (11th day of experiment) in young *P. pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two foliar P_i supply (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions.

Attributes	**Treatments**							
	Control	**P_i**	**AMF**	**AMF*P_i**	**Salt**	**Salt*P_i**	**Salt*AMF**	**Salt*AMF*P_i**
Colonization (%)	0.0 ± 0.00^{D}	0.0 ± 0.00^{D}	44.2 ± 1.46^{A}	25.0 ± 0.32^{B}	0.0 ± 0.00^{D}	0.0 ± 0.00^{D}	22.4 ± 0.87^{C}	44.0 ± 1.34^{A}
RWC (%)	94.8 ± 0.64^{A}	95.7 ± 0.66^{A}	93.0 ± 2.37^{AB}	90.2 ± 3.52^{B}	92.8 ± 1.57^{AB}	89.6 ± 0.31^{B}	90.6 ± 0.80^{B}	85.1 ± 0.53^{C}
EC (mS cm^{-1})	0.31 ± 0.01^{C}	0.31 ± 0.01^{C}	0.30 ± 0.01^{C}	0.33 ± 0.04^{C}	6.53 ± 0.12^{A}	2.19 ± 0.07^{B}	2.21 ± 0.03^{B}	2.46 ± 0.20^{B}
Shoot biomass (g)	1.03 ± 0.01^{D}	1.60 ± 0.02^{C}	2.73 ± 0.17^{A}	2.06 ± 0.01^{B}	1.05 ± 0.01^{D}	1.03 ± 0.01^{D}	1.73 ± 0.02^{C}	0.96 ± 0.01^{D}
F_v/F_m	0.75 ± 0.00^{A}	0.74 ± 0.01^{A}	0.77 ± 0.01^{A}	0.77 ± 0.01^{A}	0.73 ± 0.01^{A}	0.67 ± 0.02^{B}	0.74 ± 0.01^{A}	0.65 ± 0.01^{B}
F_v'/F_m'	0.79 ± 0.00^{A}	0.52 ± 0.00^{C}	0.46 ± 0.00^{D}	0.68 ± 0.03^{B}	0.39 ± 0.01^{E}	0.40 ± 0.02^{E}	0.35 ± 0.02^{E}	0.34 ± 0.01^{E}
F_q'/F_m'	0.27 ± 0.01^{A}	0.18 ± 0.01^{C}	0.20 ± 0.00^{C}	0.23 ± 0.01^{B}	0.19 ± 0.01^{C}	0.17 ± 0.01^{C}	0.17 ± 0.01^{C}	0.16 ± 0.01^{C}
ETR	118.9 ± 2.23^{A}	75.9 ± 2.38^{C}	53.0 ± 2.68^{E}	93.2 ± 3.30^{B}	72.9 ± 2.32^{CD}	54.3 ± 1.13^{E}	65.1 ± 5.06^{D}	37.3 ± 2.53^{F}
qP	0.48 ± 0.01^{A}	0.45 ± 0.01^{A}	0.33 ± 0.03^{B}	0.46 ± 0.01^{A}	0.46 ± 0.02^{A}	0.44 ± 0.02^{A}	0.46 ± 0.01^{A}	0.44 ± 0.01^{A}
NPQ	1.03 ± 0.05^{E}	1.71 ± 0.06^{D}	2.14 ± 0.10^{C}	1.27 ± 0.10^{E}	2.89 ± 0.10^{A}	2.47 ± 0.07^{B}	2.91 ± 0.08^{A}	1.17 ± 0.01^{E}

Different letters indicate statistically significant differences by Student Newman Keuls test at a 5% significance level between treatments (lines) (n = 4 ± SE).

Table 2

Shoot dry biomass and nutrients in shoot and root (N - nitrogen; P - phosphorus; K^+ - potassium; Na^+ - sodium; Cl^- - chloride; Na^+/K^+ ratio) in *Poincianella pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two foliar P_i supply (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions in maximum stress day (11th day of experiment).

Treatments	Nutrients (g kg^{-1} DM)											
	N		P		K^+		Na^+		Cl^-		Na^+/K^+	Na^+/K^+
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	50.9±2.6^{C}	38.5±3.5^{A}	6.0±0.4^{D}	6.4±0.4^{C}	8.5±0.3^{B}	8.3±0.4^{AB}	6.5±0.1^{B}	6.6±0.1^{B}	2.6±0.1^{E}	2.2±0.0^{F}	0.8±0.0^{B}	0.8±0.0^{B}
P_i	45.8±1.2^{C}	45.4±4.0^{A}	16.5±0.4^{C}	12.8±0.9^{A}	10.4±0.9^{AB}	9.7±0.8^{A}	6.8±0.5^{B}	6.0±0.2^{B}	2.9±0.1^{E}	1.8±0.0^{G}	0.7±0.1^{B}	0.6±0.1^{C}
AMF	53.6±3.1^{C}	45.2±4.1^{A}	7.8±0.7^{D}	6.6±0.4^{C}	9.2±0.5^{AB}	8.0±0.4^{AB}	4.2±0.5^{C}	3.5±0.3^{D}	2.1±0.1^{F}	2.7±0.1^{E}	0.5±0.1^{C}	0.4±0.1^{D}
AMF*P_i	84.5±2.2^{A}	44.8±1.3^{A}	39.8±1.3^{A}	8.3±0.3^{B}	9.9±0.2^{AB}	8.8±0.1^{AB}	3.4±0.3^{C}	4.0±0.1^{D}	1.5±0.1^{G}	2.7±0.0^{E}	0.3±0.0^{C}	0.5±0.0^{D}
Salt	48.2±3.6^{C}	44.0±2.2^{A}	6.2±0.2^{D}	7.6±0.3^{C}	9.5±0.5^{AB}	8.7±0.2^{AB}	9.8±0.2^{A}	8.6±0.4^{A}	5.7±0.1^{C}	13.4±0.1^{A}	1.0±0.1^{A}	1.0±0.0^{A}
Salt*P_i	65.2±2.9^{B}	45.6±1.5^{A}	23.2±0.5^{B}	15.1±0.6^{A}	11.6±0.8^{A}	8.6±0.5^{AB}	10.3±0.5^{A}	6.7±0.5^{B}	9.6±0.2^{A}	4.4±0.0^{C}	0.9±0.0^{A}	0.8±0.0^{B}
Salt*AMF	52.5±3.9^{C}	40.8±1.6^{A}	5.5±0.3^{D}	7.3±0.1^{C}	8.8±0.8^{B}	7.3±0.2^{B}	7.8±0.7^{B}	4.9±0.1^{C}	5.2±0.1^{D}	3.6±0.0^{D}	0.9±0.2^{A}	0.7±0.0^{BC}
Salt*AMF*P_i	62.8±0.8^{B}	44.8±1.0^{A}	17.0±0.0^{C}	8.4±0.2^{B}	9.6±0.2^{AB}	8.5±0.2^{AB}	10.2±0.4^{A}	6.2±0.0^{B}	6.5±0.1^{B}	5.3±0.0^{B}	1.1±0.0^{A}	0.7±0.0^{BC}

Different letters indicate statistically significant differences by Student Newman Keuls test at a 5% significance level between treatments (columns) (n = 4 ± SE).

Supplementary 1 Biochemical characteristics (P- phosphorus; Ca – calcium; Mg – magnesium; K – potassium) of soil used in experiment. The soil was collected in last day of experiment (11th day).

Treatments	**P (mg dm^{-3})**	**Ca ($cmol_c$ dm^{-3})**	**Mg ($cmol_c$ dm^{-3})**	**K ($cmol_c$ dm^{-3})**	**pH (H_2O)**
Original soil	3	0.65	0.6	0.07	5.7
Control	29	1.15	0.85	0.07	5.9
AMF	3	0.6	0.65	0.05	6.4
Salt	11	1.3	0.85	0.08	6.6
Salt*AMF	5	0.7	0.6	0.06	6.1

Supplementary 2

Statistical summary of effects in variables (RWC - relative water content; g_s - stomatal conductance; *A* - net photosynthetic rate; *E* - transpiration rate) of young *P. pyramidalis* plants in two mycorrhizal levels (+AMF and –AMF), two foliar P_i supply (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions. The values represents the 0, 2, 4 and 7 days of experiment.

Attributes	Effect						
	Salt	**AMF**	**P_i**	**Salt vs AMF**	**Salt vs P_i**	**AMF vs P_i**	**Salt vs AMF vs P_i**
Day 0							
RWC	0.320	4.190	1.630	0.030	0.020	**26.940*****§	0.000
g_s	**34.377*****§	**744.729*****§	**14.544*****§	**37.111*****§	**9.736****§	**115.509*****§	1.082
A	3.300	**362.590*****§	3.210	0.040	**11.090****§	**29.570*****§	0.350
E	1.052	**63.660*****§	1.084	1.289	0.625	1.289	0.010
Day 2							
g_s	**92.480*****§	**164.230*****§	3.380	**94.230*****§	1.720	**8.490****§	**9.040****§
A	**13.941****§	**109.306*****§	**7.000***	**13.830****§	1.470	**40.628*****§	0.784
E	**37.423*****§	**72.850*****§	0.267	**48.045*****§	3.615	**10.642****§	**29.601*****§
Day 4							
g_s	**351.099*****§	**375.099*****§	**10.934****§	**69.645*****§	**8.750****§	**8.950****§	4.190
A	**64.488*****§	**108.928*****§	**19.543*****§	0.050	0.243	**25.360*****§	**10.971****§
E	**154.323*****§	**212.473*****§	**5.760***§	**32.905*****§	2.333	**9.722****§	0.410
Day 7							
g_s	**1168.558*****§	**95.405*****§	**45.574*****§	**24.234*****§	**18.577*****§	**67.737*****§	3.354
A	**4644.250*****§	**167.800*****§	**78.020*****§	**61.780*****§	**56.960*****§	**290.270*****§	**30.84*****§
E	**1931.264*****§	**432.005*****§	**87.242*****§	4.211	**172.601*****§	**42.637*****§	0.536

Boldface denotes significant results. *P < 0.05; **P < 0.01; ***P < 0.001. §Denotes *p*-values significant at α=0.05 when corrected for 4 comparisons in day 0 and 3 comparisons in other days according to the Benjamini-Hochberg procedure

Supplementary 3

Statistical summary of effects in variables (RWC - relative water content; g_s - stomatal conductance; EC – electrical conductivity; *A* - net photosynthetic rate; *E* - transpiration rate; F_v/F_m - maximum quantum efficiency of PSII photochemistry; F_v'/F_m' - maximum efficiency of PSII; F_q'/F_m'- operating efficiency of PSII; ETR - electron transport rate; qP - photochemical quenching; NPQ - non-photochemical quenching; SS - soluble sugars; FAA- free amino acids; TSP- total soluble proteins; Chl *a* - chlorophyll *a*; Chl *b* - chlorophyll *b*; Car - carotenoids; N- nitrogen; P - phosphorus; K^+ - potassium; Na^+ – sodium; Cl^- – chloride; Na^+/K^+ - sodium/potassium rate) of young *P. pyramidalis* plants in two mycorrhizal (+AMF and –AMF), two foliar P_i supply (+P_i and –P_i) and two salinity levels (+NaCl and –NaCl) under greenhouse conditions. The values represents the maximum stress day (11th day).

Attributes	Effect						
	Salt	AMF	P_i	Salt vs AMF	Salt vs P_i	AMF vs P_i	Salt vs AMF vs P_i
RWC	**39.120*****§	**31.760*****§	**14.760*****§	0.590	**6.620****§	**4.670***	0.900
EC	**2696.870*****§	**986.828*****§	**986.641*****§	**999.501*****§	**1016.144*****§	**1113.117*****§	**1085.562*****§
g_s	**683.684*****§	**153.846*****§	**7.229***§	0.094	3.366	**61.480*****§	0.078
A	**1978.692*****§	**166.615*****§	**4.583***	**23.214*****§	2.555	**62.766*****§	0.108
E	**1568.831*****§	**515.327*****§	**122.861*****§	**61.292*****§	**33.903*****§	**250.063*****§	**44.773*****§
F_v/F_m	**66.130*****§	0.880	**28.540*****§	**5.300***§	**17.360*****§	0.900	1.540
F_v'/F_m'	**481.238*****§	**35.937*****§	0.441	2.136	1.270	**112.007*****§	**128.847*****§
F_q'/F_m'	**46.950*****§	**4.730***§	**14.742*****§	0.266	2.367	**27.694*****§	**20.032*****§
ETR	**184.163*****§	**80.066*****§	**35.674*****§	**8.363****§	**28.154*****§	**81.050*****§	**126.849*****§
qP	3.482	**8.667****§	2.052	**11.393****§	**12.525****§	**12.935****§	**14.193*****§
NPQ	**36.450*****§	**24.670*****§	**4.470***	**231.650*****§	**864.160*****§	**72.000*****§	**8.470****§
Starch	**1002.182*****§	0.894	**14.123*****§	**298.183*****§	**25.601*****§	**454.457*****§	**132.140*****§
SS	**9.790****§	**144.540*****§	**75.174*****§	0.211	**21.745*****§	0.174	**30.092*****§
Sucrose	0.112	2.626	2.009	2.806	0.012	**33.283*****§	2.395
Fructose	**137.092*****§	**274.028*****§	**32.912*****§	**84.299*****§	2.652	**16.493*****§	**23.570*****§
FAA	1.979	**19.825*****§	**8.392****§	0.058	**6.935***§	3.179	0.300

Proline	**278.662*****§	**46.085*****§	**6.719***§	3.454	**54.149*****§	0.720	**33.862*****§
TSP	0.156	1.610	**10.616****§	0.262	0.839	1.024	**36.157*****§
Chl *a*	**50.139*****§	**25.278*****§	0.078	**55.094*****§	**16.531*****§	**48.769*****§	**47.687*****§
Chl *b*	**21.411*****§	**78.292*****§	2.828	**162.677*****§	**32.550*****§	**54.114*****§	**151.139*****§
Car	**16.798*****§	**233.788*****§	**12.537****§	**31.397*****§	**77.641*****§	**4.731***	**61.340*****§
Biomass (shoot)	**531.258*****§	**536.275*****§	**36.781*****§	**124.635*****§	**117.522*****§	**328.109*****§	**4.346***
N (shoot)	0.589	**31.041*****§	**46.811*****§	**25.865*****§	0.037	**14.278*****§	**30.416*****§
N (root)	0.027	0.079	2.589	1.837	0.016	0.453	1.695
P (shoot)	**74.440*****§	**82.500*****§	**2057.360*****§	**273.620*****§	**29.220*****§	**41.060*****§	**159.110*****§
P (root)	**10.287****§	**75.754*****§	**166.788*****§	**4.525***	0.201	**74.421*****§	1.936
K^+ (shoot)	0.772	2.109	**11.269****§	3.063	0.049	2.379	0.023
K^+ (root)	2.061	**5.315***§	**8.552****§	0.045	0.678	0.453	2.311
Na^+ (shoot)	**215.383*****§	**44.914*****§	3.635	**9.240****§	**8.048****§	0.559	**6.595***§
Na^+ (root)	**74.630*****§	**165.720*****§	0.300	**4.480***	0.160	**33.060*****§	**6.020***§
Cl^- (shoot)	**3072.220*****§	**289.340*****§	**234.920*****§	**28.090*****§	**276.590*****§	**109.220*****§	**27.820*****§
Cl^- (root)	**223557.129*****§	**4388.883*****§	**14828.295*****§	**52733.203*****§	**3928.318*****§	**49349.535*****§	**27673.465*****§
Na^+/K^+(shoot)	**52.300*****§	**10.691****§	2.006	**13.345****§	1.312	0.194	1.353
Na^+/K^+(root)	**73.691*****§	**88.351*****§	**11.834****§	2.545	0.000	**21.218*****§	1.194
Root colonization	**8.66****§	**24178.540*****§	0.040	**8.660****§	**583.460*****§	0.040	**583.460*****§

Boldface denotes significant results. *P < 0.05; **P < 0.01; ***P < 0.001. §Denotes *p*-values significant at α=0.05 when corrected for 5 comparisons in day 0 and 4 comparisons in other days according to the Benjamini-Hochberg procedure when corrected for 35 comparisons.

7. CONCLUSÃO

Diante da realização dos três estudos propostos, foi possível concluir que duas espécies nativas, lenhosas e representativas da Caatinga, *P. pyramidalis* e *C. quercifolius* realizam associação com fungos micorrízicos arbusculares (FMAs) e que são beneficiadas quando micorrizadas. Além disso, o nível ideal de P no solo, fator crucial que influencia diretamente na efetividade da micorrização, foi indicado para cada espécie no primeiro estudo, sendo 33 mg dm^{-3} para *P. pyramidalis* e 15 mg dm^{-3} para *C. quercifolius*, níveis definidos devido aos maiores ganhos no crescimento e taxas fotossintéticas. Devido aos ganhos mais representativos do crescimento em *P. pyramidalis*, essa espécie foi escolhida para ser submetida à seca e salinidade, em um fatorial de presença e ausência de FMA, suprimento de P_i foliar, e de forma inovadora, com a presença simultânea do FMA e dosagem de P_i foliar. Foi observado que a presença do P_i foliar e FMA+P_i promoveram ganhos nas taxas fotossintéticas das plantas sob condição hidratada em ambos os experimentos e uma maior tolerância aos dois estresses estudados. Houve melhorias na parte fotoquímica e bioquímica sob estresse severo, e uma recuperação mais rápida do metabolismo primário foliar após reidratação no experimento de seca, sem reduções na biomassa aérea comparado às plantas controle tanto no experimento de deficiência hídrica como no experimento de salinidade. Entretanto, a presença isolada do FMA promoveu maiores ganhos no crescimento e taxas fotossintéticas sob condição hidratada, e maior tolerância à seca e salinidade. Quando as plantas foram expostas a cada estresse, tiveram menores reduções nas taxas fotossintéticas comparadas aos demais tratamentos sob seca ou salinidade e recuperação mais acentuada do metabolismo primário foliar após a reidratação no experimento de seca. Sob salinidade, as plantas com FMA isolado tiveram ganhos na biomassa da parte aérea comparado às plantas Controle, com uma maior seletividade na absorção dos íons Na^+ e Cl^-, que em níveis elevados são tóxicos para o vegetal. Esses resultados não corroboram a nossa hipótese inicial, onde, em um primeiro momento, foi esperado que plantas com FMA+P_i apresentassem maiores ganhos em crescimento sob condição hidratada e uma maior tolerância à seca e salinidade comparado os demais tratamentos, com uma recuperação mais rápida do metabolismo primário foliar após o déficit hídrico. Portanto, tanto o P_i foliar quanto FMA podem ser ferramentas utilizadas para aumentar a tolerância de *P. pyramidalis* quando exposta a estresses abióticos comuns no seminárido, objetivando o sucesso da regeneração de áreas degradadas em Florestas Tropicais Sazonalmente Secas, como a Caatinga. Contudo, a presença isolada dos

FMAs foi mais eficiente, promovendo ganhos no crescimento e tolerância à seca e salinidade nessa espécie.

ANEXO A – Normas da revista Applied Soil Ecology

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APPLIED SOIL ECOLOGY

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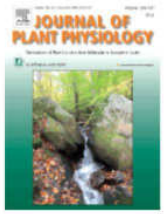

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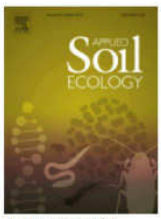

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