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Arbuscular mycorrhizae alleviate water deficit in *Dipteryx alata* Vogel: seedling quality and resilience

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Abstract

Dipteryx alata Vogel (Fabaceae) is a fruit tree species native to the Cerrado with ecological and economic potential. However, water deficit can be a limiting factor to the initial growth of this species, requiring knowledge on technologies that can alleviate this stressful effect. We hypothesized that inoculation with arbuscular mycorrhizae fungi contributes to stress mitigation during and after water deficit. *D. alata* seedlings were subjected to two water regimes (control: seedlings irrigated daily; and water deficit: irrigation suspension); associated with inoculation with arbuscular mycorrhizal fungi (AMF): AM- = without inoculation; AM+ = inoculation with *Rhizophagus clarum*; and three evaluation periods: T0 - time zero; F0 - zero photosynthesis (seven days of water restriction); REC - recovery (100 days). Water deficit impaired water relations, decreasing the quality of *D. alata* seedlings. AM+ seedlings showed higher relative water content (*RWC*), leaf area ratio, chlorophyll index, and Rubisco carboxylation capacity (A/C_i), which helped in photosynthetic metabolism. Inoculation with *R. clarum* alleviated the impact of stress on water use efficiency, water potential, *RWC*, and A/C_i in REC. Inoculation with AMF is a promising management technique in the production of *D. alata* seedlings for increasing seedling quality and resilience to water deficit.

Keywords: baru; hydric stress; arbuscular mycorrhizal fungi; *Rhizophagus clarum*, greenhouse experiment.

Introduction

Dipteryx alata Vogel (Fabaceae), popularly known as baru, coumaru, and cumbaru, is a fruit tree native to Brazil with wide occurrence in the Cerrado. The economic potential of the species is associated with its wood products, used for manufacturing furniture and coal, among others (Martinotto et al., 2012; Pinho et al., 2019). Its fresh or processed pulp and almonds are rich in starch, fibers, sugars, macro and micronutrients, proteins, oleic and linoleic acids, and phenols, being used in human and animal food, with medicinal and cosmetic properties (Lemos et al., 2012; Boni et al., 2020).

In addition, its seedlings can be indicated for reforestation programs in degraded areas and for landscaping. Scabora et al. (2011) observed that the specie has a lower rate mycorrhizal colonization when compared to other legumes species studied, with values from 30 to 47.5% depending on the growing soil. Pott and Pott (2003) highlighted that *D. alata*, among 116 native tree species, is one of the most promising for use in agroforestry systems.

Generally, *D. alata* plants are found in areas with humid and well-drained soils (Vera and Souza, 2009). However, natural or anthropic climatic fluctuations occur in several tropical and subtropical areas, promoting long drought periods (Matos et al., 2018) that significantly reduce soil water availability, limiting seedling survival and development in the initial phase in these phytoecological regions. Thus, knowledge on ecophysiological responses considering the natural habitat of *D. alata* is essential for decision making regarding its most appropriate management.

Considering the deleterious effects of water deficit on seedling quality (Park Williams et al., 2012; Martin-St.Paul et al., 2017), there is an increasing search for alternatives/technologies that improve seedling vigor and resilience through morpho-

physiological adjustments during and after the stress period. Inoculation with arbuscular mycorrhizal fungi (AMF) has been studied in order to contribute to the production of tree seedlings under water deficit (Birhane et al., 2013; Barros et al., 2018; Wu et al., 2019; Firmino et al., 2020).

The most vascular plants perform symbiosis with mycorrhizal fungi, whereas 72% with AMF, 10% orchid mycorrhizal, 2% ectomycorrhizal, and we highlight that 8% does not perform symbiosis and 7% has inconsistent association (Brundett and Tedersonn, 2018). Symbiose may obtain exudates and photosynthetic products from plants and on the other hand, it helps in the beneficial regulation of their physiological and biochemical processes, such as in water and nutrient absorption, gas exchanges, and enzyme activities, among others (Augé et al., 2015; Ruiz-Lozano et al., 2016; Liu et al., 2020). In addition, under water deficit, AM allow a combination of responsive strategies - escape and tolerance - from plants (Ruiz-Sánchez et al., 2010), which can help the tolerance and physiological recovery of seedlings after the stress period in several tree species (Barros et al., 2018; Wu et al., 2019; Firmino et al., 2020).

D. alata has shown sensitivity to water deficit since it reduces photosynthetic pigment content, transpiration and vegetative growth, in addition to increasing the activity of antioxidant enzymes (Maltarolo et al., 2016; Matos et al., 2018). We aim to evaluate the potential of inoculation of AMF to alleviate the effect of water deficit and contribute to the resilience of *D. alata* seedlings, in order to test the hypothesis that AM could optimize hardening in *D. alata* during and after water deficit by increasing the efficiency of water relations and seedling quality.

Material and Methods

Experimental site and installation

The study was conducted from August 2018 to May 2019, in which fruits of *Dipteryx alata* were randomly collected from matrices in the rural settlement of Dezessete de Abril, located in Casa Verde, district of Nova Andradina, Mato Grosso do Sul state, Brazil, with approximate area of 28.5 thousand hectares where four thousand hectares correspond to legal reserve (LR) area (21° 46' 54.6" S, 53° 13' 23.6" W). The climate of the region is classified as Tropical Monções, type AM (Alvarez et al., 2013), with average temperatures of 20° to 24° C, and with annual averages or precipitation greater than 1,500 mm and, in the driest month, less than 60 mm. The population of *D. alata* observed in the region is abundant, collecting fruits from 20 matrices. The species has been identified and an exsiccatae is deposited at the DDMS herbarium at Federal University of Grande Dourados under no. 5252.

Fruits were pulped and seeds were disinfected for one minute in alcohol (70% v/v) and for five minutes in sodium hypochlorite (2.5% active chlorine). Subsequently, pots with capacity of 7 L were filled with substrate consisting of the mixture of soil and sand (2:1), being sterilized by autoclaving at 120 °C and pressure of 1.05 kg cm⁻² for 60 minutes. The soil used to form the substrate was a Dystroferic Red Latosol with clayey texture (Santos et al., 2018). The analysis of the chemical attributes of the substrate used showed pH (H₂O) = 6.0; P (resin) = 7.5 mg dm⁻³, and organic matter content = 24.1 g dm⁻³. Data in cmol_c dm⁻³ were potassium (K) = 0.18; calcium (Ca) = 5.7; magnesium (Mg) = 1.7; potential acidity (H+Al) = 3.0; sum of exchangeable bases (SB) = 7.6; cation exchange capacity (CEC) = 10.6; and percentage base saturation (V %) = 72.0%.

Three seeds were sown per pot. Thinning was conducted at 18 days after sowing (DAS) in order to standardize one seedling per pot. Daily manual irrigations were conducted, maintaining 75% of water retention capacity in the substrate (75% _C.C.)

(Souza et al., 2000). Pots were maintained in a protected nursery with a transparent 150 μm polyethylene cover and 50% shading from Sombrite® screens, with 865 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Experimental design

The randomized block experimental design was used in a 4 x 3 factorial design consisting of four treatments of the combinations between water regimes and inoculation (CAM-, CAM+, DAM-, and DAM+) x three evaluation periods (T0, F0, and REC) with three replicates, with one pot containing one seedling for each experimental unit.

Arbuscular mycorrhizal fungi (AMF) inoculation

The inoculation treatment with AMF was conducted at 90 DAS, when seedlings of all treatments had average height of 15 cm, being named AM- = without inoculation and AM+ = inoculation with *Rhizophagus clarum* (species selected after pre-tests of frequency (100%) and colonization intensity (11.30% and 34.43% in control seedlings and under water deficit, respectively). Inoculation was conducted by opening two small holes spaced 3 cm from each seedling, in which 15 dm^3 of the inoculum (substrate + spores) hole^{-1} was poured. Inoculants were obtained from the *Brazilian Agricultural Research Corporation - Embrapa - Maize and Sorghum* (Sete Lagoas, Minas Gerais, Brazil).

Water regimes and evaluation periods

When seedlings were approximately five months old and had, on average, 20 cm of shoot length, five leaves, and a leaf area of 143.00 cm^2 , the first evaluation was conducted (T0 - time zero). Seedlings were then divided into two groups according to the

following water regimes: control (C) - seedlings irrigated daily (75% _C.C.); and water deficit (WD) - with irrigation suspension.

Photosynthetic rate (A) was monitored using an infrared gas analyzer (IRGA), ADC, model LCi PRO (Analytical Development Co. Ltd., Hoddesdon, U.K.). When A values close to zero were detected in the first seedlings of at least one WD treatment, which occurred at seven days of water restriction in the DAM+ seedlings (mean of $0.50 \mu\text{mol m}^{-2} \text{s}^{-1}$), the second evaluation period (F0 - zero photosynthesis) was determined. The third evaluation period (REC - recovery) was carried out at the 100 days after the resumption irrigation (75%_C.C.) with C seedlings being also evaluated in all periods. In the evaluation periods, mean values of temperature and relative humidity were determined (Figure 1) using a thermo-hygrometer.

Evaluation parameters

Non-destructive evaluations were conducted between 8 and 11 a.m. under photosynthetic photon flux (PPF) $>700 \text{ mmol m}^{-2} \text{s}^{-1}$, with three readings being conducted in each replicate of treatments on completely expanded leaves that were previously marked, so that all measurements were always conducted on the same leaves. From gas exchanges quantified using the infrared gas analyzer (IRGA), ADC, model LCi PRO (Analytical Development Co. Ltd., Hoddesdon, U.K.), photosynthesis data were only used to calculate the ratios of water use efficiency (WUE) and Rubisco carboxylation capacity (A/C_i), disregarding the isolated form. Chlorophyll index ($SPAD$) was determined by using a portable chlorophyll meter (Konica Minolta, model $SPAD-502$).

Leaf water potential (Ψ_w) was obtained from readings performed immediately after leaf collection (until 8 a.m.), on individual leaves belonging to the third pair of fully expanded leaves (from the apex to the base) using a pressure chamber (Portable Plant

Water Status Console - model 3115). Relative water content (*RWC*) was determined according to Bajji et al. (2001).

Seedling quality (*DQI*) was calculated according to Dickson et al. (1960). We also calculated the root:shoot ratio (*R:S*), with lengths being measured using a graduated ruler, and leaf area ratio (*LAR*), with leaf area being measured in a LI-COR® 3100 area meter. Total dry matter was obtained through drying in a forced air circulation oven at 65° C for 72 hours (Hunt, 1990).

In order to evaluate the impact of AM in the resistance of *D. alata* to water deficit, the relative drought alleviation rate (*RDAR*) (Ouledali et al., 2018) was calculated according to the following equation:

$$RDAR (\%) = \left[\frac{(ID_{AM-} - ID_{AM+})}{(ID_{AM-})} \right] \times 100 \quad [1]$$

Where ID_{AM-} is the impact of water deficit on the physiological characteristic measured in non-mycorrhizal plants (AM-), calculated as the maximum difference found between DAM- and the reference (mean CAM-) for each characteristic measured and evaluation period; and ID_{AM+} is the impact of water deficit on the physiological characteristic measured in mycorrhizal plants (AM+), calculated as the maximum difference found between DAM+ and the reference (mean CAM-) for each characteristic measured and evaluation period. We calculated the *RDAR* for the physiological characteristics *RWC*, *WUE*, and *A/C_i* and for the evaluation periods F0 and REC.

Statistical analyses

The data were subjected to analyses of variance (two-way ANOVA) using the following linear model:

$$Y_{ijk} = \mu + \beta_i + \tau_j + \rho_k + (\tau\rho)_{jk} + \varepsilon_{ijk} \quad [2]$$

Where, Y_{ijk} is the observation of dependent variable of the j^{th} treatment of the k^{th} evaluation period in i^{th} block (replicates), and μ is the overall mean. β_i is the effect of blocks ($i = 1...3$); τ_j is the effect of treatments ($j = 1...4$); ρ_k is the effect of periods ($k = 1...3$); $(\tau\rho)_{jk}$ is the interaction effect of levels j and k of factors τ and ρ , respectively; and ε_{ijk} is the random error associated with Y_{ijk} .

The data satisfied normality and equality of variance assumptions. The ANOVA and the Tukey test ($P \leq 0.05$), to means comparison, were conducted within AgroEstat statistical software (Barbosa and Maldonado, 2015).

Results

General statical results

Interaction between the factors analyzed was significant for Dickson quality index (*DQI*); leaf area ratio (*LAR*), water potential (Ψ_w), relative water content (*RWC*), water use efficiency (*WUE*), and Rubisco carboxylation capacity (A/C_i). Chlorophyll index (*SPAD*) and root:shoot ratio (*R:S*) were only affected by the isolated effect of evaluation period (Table 1).

Quality, growth, and morphology of D. alata seedlings

Differences in the quality of *D. alata* seedlings were only observed in REC, in which the highest *DQI* values were observed for plants subjected to water deficit regardless of inoculation with AMF (AM+). However, in the same period, DAM+ plants showed 30.60% (mean and standard deviation: 1.44 ± 0.04) more quality than DAM- (1.00 ± 0.16) and 23.08% more quality that CAM- seedlings (1.17 ± 0.17) (Figure 2).

In general, *LAR* values decreased throughout evaluations. However, inoculation with *R. clarum* increased the *LAR* of seedlings under DAM+ in F0 by 64.94% ($80.51 \pm 8.44 \text{ dm}^2 \text{ g}^{-1}$) compared to the sum of means ($28.33 \pm 7.28 \text{ dm}^2 \text{ g}^{-1}$) of the other treatments

in the same period. Conversely, these same seedlings presented lower values in REC ($8.98 \pm 1.61 \text{ dm}^2 \text{ g}^{-1}$), being 57.98% lower than the other treatments ($21.18 \pm 1.47 \text{ dm}^2 \text{ g}^{-1}$) (Figure 3).

In T0 (2.61 ± 0.40) for F0 (2.04 ± 0.40), *R:S* ratio means decreased by 28.43% in seedlings under both regimes and inoculation, subsequently increasing by 33.77% in the REC (3.08 ± 0.40) (Figure 4).

Water relations and photosynthetic traits

The *SPAD* index was positively affected especially by inoculation with AMF in *D. alata* seedlings. AM+ and AM- plants differed by 41.00% (33.46 ± 7.86 and 23.73 ± 4.86 , respectively) (Figure 5).

The lowest Ψ_w values were observed for F0 in seedlings under water deficit and for REC in seedlings cultivated in the control treatment and without inoculation, together showing $-1.03 \pm 0.02 \text{ MPa}$ of mean and standard deviation, respectively. The other means of combinations of periods and treatments were similar to each other and 23.00% higher ($-0.88 \pm 0.04 \text{ MPa}$) (Figure 6A).

The *RWC* of AM+ seedlings were negatively affected in T0. However, throughout evaluations, the means of seedlings previously stressed increased until REC, surpassing by 41.0% ($79.28 \pm 4.50\%$) the values of controls in the same period ($46.24 \pm 3.03 \%$), although not differing from DAM- seedlings (Figure 6B).

We observed that *WUE* and *A/C_i* showed a response pattern. In T0, the lowest means were observed in the AM+ seedlings. General decrease was observed in F0 except for seedlings grown under CAM+ when compared to T0, with no difference being observed between treatments. In the REC, the highest values were observed for seedlings inoculated with *R. clarum* (Figures 6C and D). In F0, seedlings under water deficit,

regardless of inoculation or not with AMF, significantly decreased *WUE* values by 62% (1.08 ± 0.15) compared to control plants in the same period (1.75 ± 0.17) (Figure 6C).

Stress alleviation

Regarding the *RDAR* in *D. alata* seedlings, the dotted+hatched area represents the unalleviated part of the impact of drought on the characteristic under analysis and the checkered+hatched area represents the effect of inoculation with *R. clarum* in the alleviation of the stress condition imposed (Figures 7A, B, and C). In general, *RDAR* was higher in REC, and seedlings showed their mycorrhizal dependence in alleviating the water deficit condition, especially through A/C_i and *RWC* (Figures 7A and C).

Discussion

The water deficit causes deleterious effects on the *DQI* and *WUE* of *D. alata* seedlings, reinforcing the sensitivity of this species. However, after resumption of water supply (REC), the species showed resilience, increasing *DQI*, *R:S ratio*, Ψ_w and *WUE* values regardless of treatments, indicating physiological plasticity. Symbiosis with *R. clarum* highlighted this response even more, demonstrating its beneficial effect of promoting tolerance against the stress condition by increasing *DQI*, *LAR*, *SPAD* index, *WUE* and A/C_i . The data obtained in this work provide support for our hypothesis.

Regarding the growth of *D. alata* plants, we expected that the combination of drought and the presence of mycorrhizae would result in an increase of root growth and, consequently, in the *R:S* ratio. However, we did not find a significant effect on this parameter, for none of treatments. Baysal and Emery (2020) also did not observe these differences, regardless of the presence or absence of AMF and water regimes. According to Veresoglou et al., 2012, the absence of net effect on the roots may be a consequence

of multiple regulators and their interactions, such as symbiosis duration, AM fungus and plant species identity, or volume of pot.

Therefore, we suggest that the greatest effect of treatments occurred in the shoot of seedlings. The lowest *LAR* observed in DAM+ seedlings in REC reinforced our idea that the AM *R. clarum* increases the resilience capacity of *D. alata* seedlings. This response may be related to the increased metabolic efficiency in plants promoted by the perception of environmental signal, allowing control of water loss and maintaining some flow of water and nutrients even during drought (Erice et al., 2010; Barros et al., 2018) or possibly accumulating proteins in leaves and increasing their thickness for the action of the AMF on the leaf anatomy and plant morphology.

The discrepant increase in the leaf area:total dry matter ratio (*LAR*) in F0 in DAM+ of *D. alata* seedlings is similar to that observed for plants of *Arundo donax* L. AM+ and according to Romero-Munar et al. (2017), they presented higher total biomass, with higher biomass in shoots and without differences for roots, with the larger leaf area.

Demir (2004) observed the highest *LAR* in *Capsicum annuum* L. cv Cetinel-150 inoculated with AMF due to the increase in the P content of plants. Amanullah et al. (2007) defined *LAR* as a measurement of the photosynthetic machinery per unit of plant biomass. Increased *LAR* positively influences CO₂ assimilation, as well as its efficiency regarding resources such as water. Therefore, *LAR* can be a fundamental characteristic for obtaining ecophysiological information on the species, such as carbon gain and distribution, production, and respiration, helping to determine the photosynthetic and hydraulic response of plants (Weraduwege et al., 2015; Pereira et al., 2019).

Still in this context, the highest *SPAD* index was observed especially in seedlings containing AM under deficit. This increase may be attribute to increased availability and absorption of N, P, and Mg in leaves, which is promoted by AMF as suggested by Zhu et

al. (2014) However, the quantification of these mineral elements in *D. alata* seedlings were not evaluated in our study. Other tree species also had positive responses to AMF, such *Ricinus communis* (Zhang et al., 2018) showing increased pigments under water deficit.

We have seen so far direct, indirect, and probable benefits of the symbiosis with AMF on the quality and tolerance of *D. alata* seedlings under water deficit. However, in fact, was there water deficit? Did AM make seedlings more efficient in their physiological activities as mentioned?

As the lowest Ψ_w values were observed in F0, we can state that water deficit had a negative impact on *D. alata* seedlings, and as this scenario occurred regardless of inoculation or not with *R. clarum*, this sensitivity seems to be related to characteristics inherent to the species. In addition, it is worth mentioning that climatic conditions during the experimental period may have influenced plant responses. In F0, relative humidity decreased by 14% and temperature increased by 3.2 °C compared to T0 (Figure 1). This can be noted in the lower values of some characteristics evaluated even when seedlings were cultivated in the control treatment.

On the other hand, it is noteworthy that even with the stress condition, *RWC* was little changed. Thus, in the REC, seedlings previously subjected to water deficit exhibited higher *RWC* than seedlings in the control treatment, demonstrating the resilience of the species, which can be interesting in the acquisition of responsive memory for subsequent stress episodes. *RDAR_RWC* showed negative effect of AMs in F0 and positive effect in REC, although not showing difference for AM- plants. However, the highest *DQI* occurred only in AM+ plants. According to Moucheshi et al. (2012), the combination of increased biomass accumulation with *RWC* retention is promoted by increased water absorption by mycorrhizal symbiosis.

Even with increase of *DQI* after the resumption of water supply, *WUE* in AM- seedlings did not follow this growth and the water needs of the plants may have not been met, indicating stress occurrence. On the other hand, AM+ seedlings showed increased *WUE*, alleviating the impact of stress (*RDAR_WUE*).

The combination of the results of higher *WUE* and maintenance of *RWC*, according to Zhu et al. (2012), facilitates the movement of water from plants to leaves, which leads to greater balance in the water flow. More specifically, mycorrhizae can improve the hydraulic properties of plants as extra-radial hyphae can reach water reservoirs inaccessible by roots (Augé et al., 2015); by compensatory processes and alternations between the apoplast and the symplast, with the participation of hyphal cells (Bárzana et al., 2012); and also by expressing genes for aquaporin regulation, in order to conserve or increase cellular water, facilitating diffusion across cell membranes (Bárzana et al., 2014; Xu et al., 2018).

In addition, it is known that mycorrhizal roots (AM+) can spend more energy because the fungus acts as a carbon sink (Fracasso et al., 2020) when compared to non-mycorrhized roots. However, this energy expenditure can be compensated with increased photosynthesis and other characteristics related to the shoot for the symbiotic characteristic of the relationship, as observed in increased of A/C_i .

According to Elhindi et al. (2017), these responses combine to improve *WUE* and consequently improve gas exchanges in mycorrhizal plants. This can be evidenced by the increase in A/C_i , especially in the REC for DAM+ plants and shown in *RDAR_A/C_i* as the parameter with the greatest participation of AM to alleviate the impact caused by drought throughout evaluations. In fact, the increase in A/C_i was related to the increased capacity to mobilize water in mycorrhizal roots for maintaining perspiration and the increased

activity of Rubisco and other key enzymes of the Calvin cycle given by AMF (Chen et al., 2017; Quiroga et al., 2019).

Zhu et al. (2017) suggested that the mycorrhizal seedlings of false acacia (Fabaceae: *Robinia pseudoacacia* L.) with higher chlorophyll content showed higher CO₂ assimilation rate, A/C_i , and leaf area, corroborating our *SPAD*, *LAR* and A/C_i results for *D. alata*. Porcel et al. (2015), who studied rice (*Oryza sativa* L. cv. Puntal), found that the increase in A/C_i promoted by the presence of AM triggered biomass accumulation, as also observed in our study by the increase of *DQI*.

According to Ouledali et al. (2018), the *RDAR* represents the percentage of water deficit alleviation in the physiological parameter studied obtained by inoculation with AMF. In this study, alleviations of 6.72% for *WUE*, 23.51% for A/C_i , and 24.45% for *RWC* were observed during the experimental period, with the greatest expression occurring at 100 days after drought conditions, which can be considered moderate to severe in the F0, since the average of the Ψ_w was approximate to the permanent wilting point of -1.50 MPa cited in literature for other species (Slatyer, 1967).

Our study presents the first evidence that the inoculation with AMF, especially with *Rhizophagus clarum*, seems to be a promising management technique in the production of *D. alata* seedlings, which will possibly present increases in quality, resilience, and memory as a response to the adverse drought conditions they may be subjected during development in the field. Acknowledging the limitations of our study, we hope that the same will be complemented with future experiments, addressing issues such as, molecular, signaling and perception.

Considering the fact that *D. alata* in its natural habitat is found in well-drained areas, but subject to environmental adversities, such as water deficit, this information with AMF may contribute to the management of this species in nurseries aiming to increase

tolerance to these conditions for a given period of time, favoring beginning silvicultural practices and biodiversity conservation.

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593

594 **Table and Figure captions**

595 **Table 1.** Results of two-way ANOVA to test the effects of treatment, inoculation with
596 AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*) and water regimes (C:
597 control, D: deficit); evaluation periods (T0: zero time - 150 days after sowing; F0: zero
598 photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0);
599 and interaction: treatment x evaluation periods. *DQI* – Dickson's quality index; *LAR* –
600 Leaf area ratio: total dry mass; Ψ_w – Water potential; *RWC* – relative water content; *WUE*
601 – water use efficiency; *A/C_i* – Rubisco carboxylation capacity; *SPAD* – Chlorophyll
602 index; Root:shoot ratio (*R:S*).

603 **Figure 1.** Relative humidity (RH) and temperature (T) measured during experimental
604 evaluations, zero time (T0); zero photosynthesis (F0); and recovery (REC).

605 **Figure 2.** Dickson's quality index – *DQI* in *Dipteryx alata* Vogel seedlings in relation to
606 water regimes (C: control, D: deficit); evaluation periods (T0: zero time - 150 days after
607 sowing; F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100
608 days after F0); and inoculation with AMF: AM- (without inoculation) and AM+
609 (*Rhizophagus clarum*). Means with same capital letters do not differ for evaluation

periods at each treatment level by Tukey test ($P \leq 0.05$). Same lowercase letters do not differ for treatments at each level of evaluation periods by Tukey test ($P \leq 0.05$).

Figure 3. Leaf area ratio: total dry mass - *LAR* in *Dipteryx alata* Vogel seedlings in relation to water regimes (C: control, D: deficit); evaluation periods (T0: zero time - 150 days after sowing; F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0; and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). Means with same capital letters do not differ for evaluation periods at each treatment level by Tukey test ($P \leq 0.05$). Same lowercase letters do not differ for treatments at each level of evaluation periods by Tukey test ($P \leq 0.05$).

Figure 4. Root: shoot ratio (*R:S*) in *Dipteryx alata* Vogel seedlings in relation to evaluation periods - T0: zero time (150 days after sowing); F0: zero photosynthesis (7 days after irrigation suspension), REC: recovery (100 days after F0). Means with same letter do not differ by Tukey test ($P \leq 0.05$).

Figure 5. Chlorophyll index - *SPAD* in *Dipteryx alata* Vogel seedlings in relation to water regimes (C: control, D: deficit); and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). Means with same letters do not differ by Tukey test ($P \leq 0.05$).

Figure 6. Water potential - Ψ_w (A), Relative water content - *RWC* (B), Water use efficiency - *WUE* (C) and Rubisco carboxylation capacity - A/C_i (D) in *Dipteryx alata* Vogel seedlings in relation to water regimes (C: control, D: deficit); evaluation periods (T0: zero time - 150 days after sowing; F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0); and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). Means with same capital letters do not differ for evaluation periods at each treatment level by Tukey test ($P \leq 0.05$). Same

lowercase letters do not differ for treatments at each level of evaluation periods by Tukey test ($P \leq 0.05$).

Figure 7. Relative drought alleviation rate - RDAR estimated by: relative water content (*RWC*) (A); water use efficiency (*WUE*) (B); and Rubisco carboxylation capacity (*A/C_i*) (C) in *Dipteryx alata* seedlings in relation to water regimes (C: control, D: deficit); evaluation periods (F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0); and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). The dotted+hatched area represents the unalleviated part of the impact of the water deficit on the studied parameter, but the checkered+hatched area represents the alleviation of the stress performed by the inoculation of AMF.

656 **Table and Figures**

Variable	Source of variation					
	Treatment (A)		Evaluation period (B)		Interaction (AxB)	
	F	p-value	F	p-value	F	p-value
<i>DQI</i>	1.13	0.3588	64.38	< 0.0001	5.22	0.0018
<i>LAR</i>	7.62	0.0011	114.78	< 0.0001	23.82	< 0.0001
Ψ_w	7.02	0.0017	15.49	< 0.0001	14.99	< 0.0001
<i>RWC</i>	13.59	<0.0001	13.58	< 0.0001	14.13	< 0.0001
<i>WUE</i>	1.22	0.3275	61.28	< 0.0001	11.03	< 0.0001
<i>A/C_i</i>	4.08	0.0190	21.38	< 0.0001	20.27	<0.0001
<i>SPAD</i>	4.87	0.0096	1.36	0.2785	0.69	0.6563
<i>R:S</i>	0.55	0.6516	5.22	0.0140	1.04	0.4277

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Variable	Source of variation					
	Treatment (A)		Evaluation period (B)		Interaction (AxB)	
	F	p-value	F	p-value	F	p-value
<i>DQI</i>	1.13	0.3588	64.38	<0.0001	5.22	0.0018
<i>LAR</i>	7.62	0.0011	114.78	<0.0001	23.82	<0.0001
Ψ_w	7.02	0.0017	15.49	<0.0001	14.99	<0.0001
<i>RWC</i>	13.59	<0.0001	13.58	<0.0001	14.13	<0.0001
<i>WUE</i>	1.22	0.3275	61.28	<0.0001	11.03	<0.0001
<i>A/C_i</i>	4.08	0.0190	21.38	<0.0001	20.27	<0.0001
<i>SPAD</i>	4.87	0.0096	1.36	0.2785	0.69	0.6563
<i>R:S</i>	0.55	0.6516	5.22	0.0140	1.04	0.4277

Table 1. Results of two-way ANOVA to test the effects of treatment, inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*) and water regimes (C: control, D: deficit); evaluation periods (T0: zero time - 150 days after sowing; F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0); and interaction: treatment x evaluation periods. *DQI* – Dickson's quality index; *LAR* – Leaf area ratio: total dry mass; Ψ_w – Water potential; *RWC* – relative water content; *WUE* – water use efficiency; *A/C_i* – Rubisco carboxylation capacity; *SPAD* – Chlorophyll index; Root:shoot ratio (*R:S*).

Figure 1. Relative humidity (RH) and temperature (T) measured during experimental evaluations, zero time (T0); zero photosynthesis (F0); and recovery (REC).

107x74mm (300 x 300 DPI)

Figure 2. Dickson's quality index – DQI in *Dipteryx alata* Vogel seedlings in relation to water regimes (C: control, D: deficit); evaluation periods (T0: zero time - 150 days after sowing; F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0); and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). Means with same capital letters do not differ for evaluation periods at each treatment level by Tukey test ($P \leq 0.05$). Same lowercase letters do not differ for treatments at each level of evaluation periods by Tukey test ($P \leq 0.05$).

126x89mm (300 x 300 DPI)

Figure 3. Leaf area ratio: total dry mass - LAR in *Dipteryx alata* Vogel seedlings in relation to water regimes (C: control, D: deficit); evaluation periods (T0: zero time - 150 days after sowing; F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0; and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). Means with same capital letters do not differ for evaluation periods at each treatment level by Tukey test ($P \leq 0.05$). Same lowercase letters do not differ for treatments at each level of evaluation periods by Tukey test ($P \leq 0.05$).

128x83mm (300 x 300 DPI)

Figure 4. Root: shoot ratio (R:S) in *Dipteryx alata* Vogel seedlings in relation to evaluation periods - T0: zero time (150 days after sowing); F0: zero photosynthesis (7 days after irrigation suspension), REC: recovery (100 days after F0). Means with same letter do not differ by Tukey test ($P \leq 0.05$).

126x91mm (300 x 300 DPI)

Figure 5. Chlorophyll index - SPAD in *Dipteryx alata* Vogel seedlings in relation to water regimes (C: control, D: deficit); and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). Means with same letters do not differ by Tukey test ($P \leq 0.05$).

128x91mm (300 x 300 DPI)

Figure 6. Water potential - Ψ_w (A), Relative water content - RWC (B), Water use efficiency - WUE (C) and Rubisco carboxylation capacity - A/C_i (D) in *Dipteryx alata* Vogel seedlings in relation to water regimes (C: control, D: deficit); evaluation periods (T0: zero time - 150 days after sowing; F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0); and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). Means with same capital letters do not differ for evaluation periods at each treatment level by Tukey test ($P \leq 0.05$). Same lowercase letters do not differ for treatments at each level of evaluation periods by Tukey test ($P \leq 0.05$).

Figure 7. Relative drought alleviation rate - RDAR estimated by: relative water content (RWC) (A); water use efficiency (WUE) (B); and Rubisco carboxylation capacity (A/C_i) (C) in *Dipteryx alata* seedlings in relation to water regimes (C: control, D: deficit); evaluation periods (F0: zero photosynthesis - 7 days after irrigation suspension, REC: recovery - 100 days after F0); and inoculation with AMF: AM- (without inoculation) and AM+ (*Rhizophagus clarum*). The dotted+hatched area represents the unalleviated part of the impact of the water deficit on the studied parameter, but the checkered+hatched area represents the alleviation of the stress performed by the inoculation of AMF.

166x282mm (300 x 300 DPI)