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Morpho-physiological traits of soybean plants in symbiosis with *Gigaspora* sp. and submitted to water restriction

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In agricultural production, periods in which there is a lack of water can affect the productivity of soybean crops. One alternative is the use of arbuscular mycorrhizal fungi (AMF), which maximize water absorption, biochemical regulation, leaf elasticity and transpiration, and water use regulation. The present study aimed to analyze the morphological and physiological traits of soybean plants associated with *Gigaspora margarita* and *Gigaspora gigantea* submitted to water restriction in nonsterilized soil. The soybean plants received 31 g of the AMF *Gigaspora margarita* or 46 g of *Gigaspora gigantea* separately at sowing and were cultivated in a greenhouse under natural light conditions with controlled relative humidity and temperature. Water restriction was imposed when the plants reached the V3 stage and were divided into three levels: irrigated (80%), moderate (60%), and severe (40%) field capacity (FC). The experimental design was completely randomized in a 3 × 3 factorial design (three inoculation treatments × three water restriction levels). Physiological and morphological parameters, photosynthetic pigments, electrolyte leakage, root colonization of soybean plants, and percentage of fungal spores were evaluated. The inoculation of *Gigaspora gigantea* promoted the adaptation of physiological (photosynthesis rate, transpiration, stomatal conductance, Ci/Ca ratio, and carboxylation) and morphological traits (plant height and stem diameter), with greater colonization of soybean roots under conditions of water restriction, and maximized the tolerance of plants to drought, mitigating the negative effects of these conditions regardless of the level of water restriction. Mycorrhizal inoculation promoted better functioning of the photosynthetic apparatus and growth of soybean plants.

Keywords Drought, Mycorrhizal symbiosis, *Glycine max*, Abiotic stress., *Gigaspora gigantea*

Climatic conditions directly affect agricultural production and drought, one of the main abiotic factors, can limit plant growth and development and negatively affect grain production[1,2]. This unpredictability of the climate is a risk factor that accounts for a considerable part of the failure of some crops because climatic stresses reduce crop yield and seed quality, restricting production sites and sowing dates of the second crop[2]. The influence of climate change on crop production is manifested in several ways, including changes in seasonal temperature, water availability, and radiation incidence, which can have direct effects on crop growth and biomass production through their influence on crop physiological processes[3–5].

Brazil, which is the largest producer and exporter of grain, has significant importance in global soybean production[6]. The maintenance of productivity, as well as the seed quality and grain yield of this crop, depends on weather conditions[7–9], and water restriction can reduce the yield of this grain by approximately 40%[10–12].

Drought affects a number of processes and can cause changes in plant metabolism, limiting the growth of the root system, leaf expansion[13], and stomatal activity, inducing plant closure due to changes in the turgor pressure of guard cells, which consequently reduces CO_2 assimilation, inhibits photosynthesis and restricts grain growth and production in the affected crops[14–17]. These processes range from the perception of stress by the plant to signal transduction, the regulation of gene expression, and possible changes metabolism[18].

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The roots are the first parts of plants to face drought, making them sensitive and receptive to these deficit conditions and establishing a link associated with plant adaptation[19]. The rhizosphere, the region where soil and roots come into contact, can be colonized by various microorganisms, such as arbuscular mycorrhizal fungi (AMF). These AMF have become an alternative for maximizing plant tolerance to water restriction because they can form symbiotic associations with the majority of plant species. This symbiosis allows the AMF to extend the plant's root system, thereby increasing the uptake of water and nutrients that are crucial plant survival and productivity during periods of drought stress[20–23]. In addition, the hyphae of AMF can bridge the gap between the soil and roots that occurs when the soil and roots shrink away from each other under dry conditions[24].

The success of this symbiosis between AMF and plants is scientifically recognized because of the significant benefits it provides. The AMF association leads to an increase in root volume and leaf turgor, as well as a reduction in osmotic potential and oxidative damage. Additionally, AMF symbiosis induces changes in phytohormone levels, which in turn influence stomatal conductance and ultimately improve plant nutrition. These multifaceted effects are decisive factors that enable plants to overcome various biotic and abiotic stresses and adversities[25–30]. In addition to increasing the tolerance of plants to water restriction, the symbiotic association of plants with AMF is a tool that favors plant development and physiology[31–33].

The present study aimed to analyze the morphological and physiological traits of soybean plants associated with *Gigaspora margarita* and *Gigaspora gigantea* submitted to water restriction in nonsterilized soil.

Results

Mycorrhizal colonization and spores density

Regarding mycorrhizal colonization ($F_{2.27} = 4.303$, $p = 0.030$), in soybean roots, higher values occurred at the severe water level (15.33%) (Fig. 1a), and there was a greater number of spores ($F_{2.27} = 31.264$, $p < 0.0001$). This was observed after inoculation with *G. gigantea* (100.67 100 g^{-1} soil) and *G. margarita* (99.56 100 g^{-1} soil) (Fig. 1b).

The microscopy images revealed a greater predominance of arbuscules after inoculation with *G. gigantea* (Fig. 2c) and a lower predominance in the control treatment (nonsterilized soil) (Fig. 2a).

Photosynthetic traits

In terms of physiological characteristics, the photosynthetic rate (*A*) ($F_{2.27} = 17.333$, $p < 0.0001$) (Fig. 3a), transpiration rate (*E*) ($F_{2.27} = 18.719$, $p < 0.0001$) (Fig. 3b), stomatal conductance ($F_{2.27} = 17.969$, $p < 0.0001$) (Fig. 3c), Ci/Ca ratio ($F_{2.27} = 5.565$, $p = 0.013$) (Fig. 3d) and carboxylation rate ($F_{2.27} = 14.432$, $p = 0.0002$) (Fig. 3e) were influenced by the inoculation factor, where plants in symbiosis with the AMF *Gigaspora gigantea* obtained higher means (17.64 μmol CO_2 m^{-2} s^{-1}, 11.27 mol H_2O m^{-2} s^{-1}, 0.94 mol H_2O m^{-2} s^{-1}, 0.8808 and 0.0523, respectively).

In contrast, when the soybean plants were subjected to severe water restriction, the mean effective quantum efficiencies of photosystem II (YII) ($F_{2.27} = 5.153$, $p = 0.017$) (Fig. 4a) and chlorophyll *b* ($F_{2.27} = 7.946$, $p = 0.003$) (Fig. 4b) were highest when the soybean plants were exposed to severe restriction (0.15 and 8.35 μg cm^{-2}, respectively).

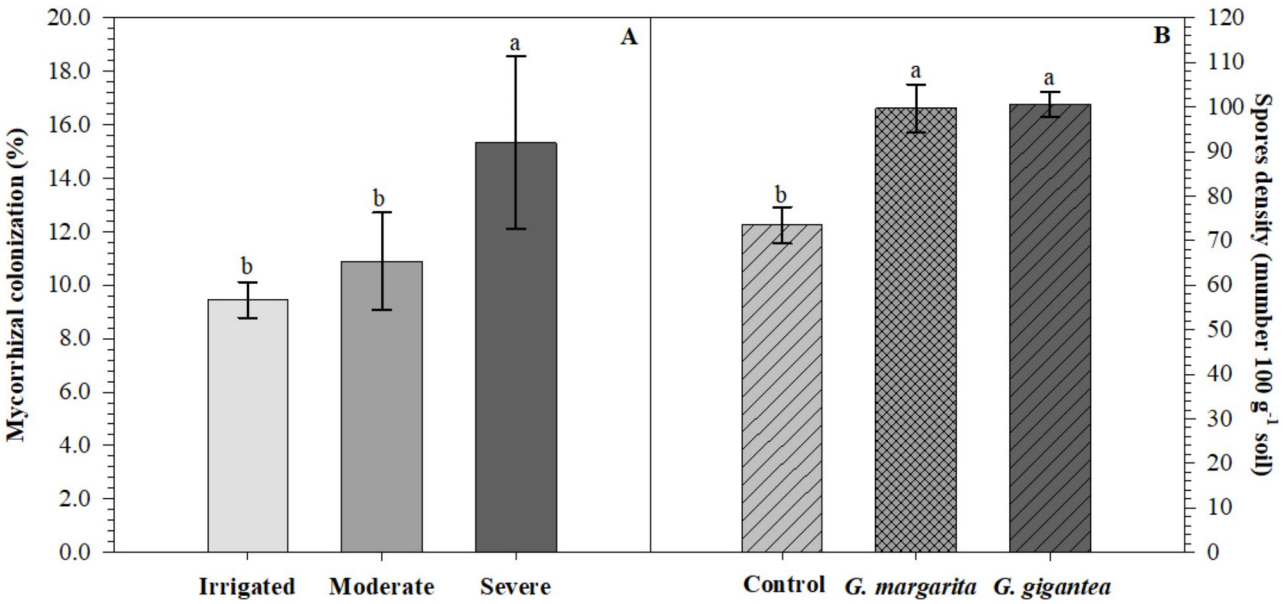

Fig. 1. Mycorrhizal colonization (**A**) and number of AMF spores (**B**) of soybean plants subjected to different levels of hydric restriction after reirrigation. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same letter do not differ ($p > 0.05$) according to Tukey's test.

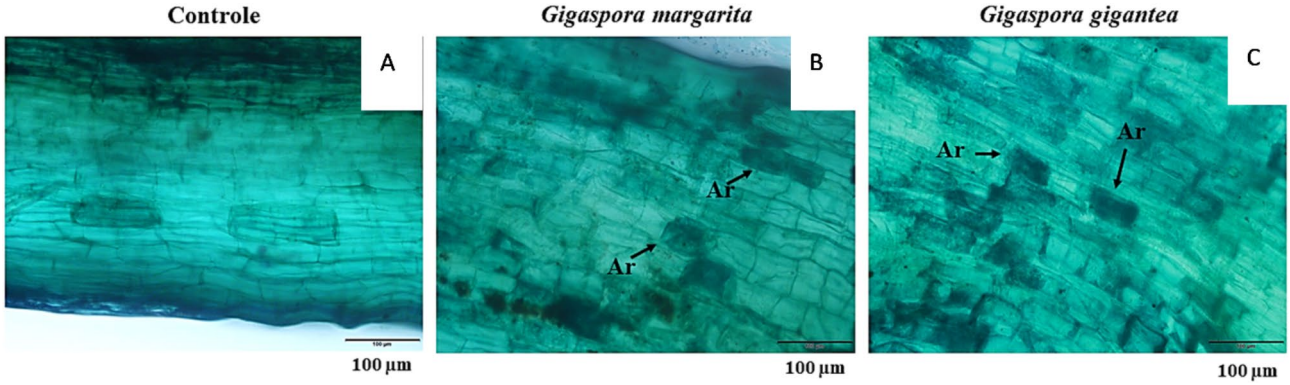

Fig. 2. Root cortex of soybean plants indicating AMF colonization in the control (**A**), *Gigaspora margarita* (**B**), and *Gigaspora gigantea* (**C**) treatments. Ar = arbuscules.

The highest carboxylation rate ($F_{2.27} = 4.574$, $p = 0.025$) of the soybean plants occurred under irrigated conditions (0.0494) and moderate water restriction (0.0486) (Fig. 4c), and the highest carboxylation rate of the carotenoids occurred under irrigated conditions ($F_{2.27} = 3.692$, $p = 0.045$). In plants under moderate restriction (5.51 µg cm^{-2}) (Fig. 4d).

Photosynthetic pigments

Chlorophyll *a* ($F_{4.27} = 3.431$, $p = 0.030$) and the pheophytinization index ($F_{4.27} = 2.999$, $p = 0.046$) exhibited interactions between the water restriction and inoculation treatments. The plants that were not inoculated under severe conditions presented a relatively high chlorophyll *a* concentration (22.96 µg cm^{-2}) (Fig. 5a), whereas the highest pheophytinization index occurred in the plants inoculated with *G. gigantea* in the same water treatment (1.29) (Fig. 5b).

Morphological traits

The greatest increase in soybean plant height (27.31 cm) ($F_{2.27} = 9327$, $p = 0.002$) (Fig. 6a) and stem diameter (5.39 mm) ($F_{2.27} = 5766$, $p = 0.012$) (Fig. 6b) occurred with the inoculation of *G. gigantea*.

Concerning water restriction levels, greater dry weights of roots ($F_{2.27} = 6.688$, $p = 0.007$) (6.94 g) (Fig. 7a) and total plants ($F_{2.27} = 12.031$, $p = 0.0004$) (14.53 g) (Fig. 7b) occurred at the severe level, whereas greater heights ($F_{2.27} = 13.179$, $p = 0.002$) were obtained at the moderate level of water restriction (27.54 cm) (Fig. 7c).

Electrolyte leakage

The greatest amount of electrolyte leakage ($F_{2.27} = 12.999$, $p = 0.0003$) was detected in plants inoculated with *G. gigantea* (68.52%) (Fig. 8).

Water relations

The water potential (Ψw) of soybean plants under water deficit ($F_{2.27} = 6.639$, $p = 0.007$) differed among the inoculation treatments. The presence of the AMF *G. margarita* resulted in greater Ψw values (− 0.253) (Fig. 9), whereas under reirrigation ($F_{2.27} = 2.145$, $p = 0.146$), the values under AMF inoculation did not differ from each other.

Principal components analysis

The first two components explained 44.1% (PC1 29.5% and PC2 14.6%) of the variance, demonstrating the variations in the parameters of the soybean plants and allowing us to observe the relationships between the variables, the inoculation treatments (Fig. 10a) and the water restriction levels (Fig. 10b). Most variables correlated significantly ($p < 0.05$) with the first axis of the PCA. In contrast, variables such as Clb, Cla, Carot, IF, Roots, and Total dry were negatively correlated with this axis in the inoculation treatments (Fig. 10a) and water restriction levels (Fig. 10b).

The other dimensions explained 55.9% of the variance as follows: PC3, 11.92%; PC4, 9.93%; PC5, 7.71%; PC6, 6.27%; PC7, 5.62%; PC8, 4.56%; PC9, 2.37%; PC10, 2.02%; PC11, 1.87%; PC12, 1.28%; PC13, 0.85%; PC14, 0.64%; PC15, 0.40%; PC16, 0.30%; PC17, 0.099%; and PC18, 0.017.

Contributions of variables

Regarding the individual contributions of the variables in PC1, the following trend was observed: A > Carbox > gsw > E > Electro > Spores > Ci/Ca > Clb > PhiPS2 > Cla > Height > Diam > PH. Before > Coloniz > Carot > Total Dry > Roots > IF (Fig. 11a). PC2 exhibited the following trend: total dry weight > roots > Carot > Cla > IF > Coloniz > Clb > Electro > Diam > PhiPS2 > Carbox > A > Spores > E > PH. Before > Height > Ci/Ca > gsw (Fig. 11b).

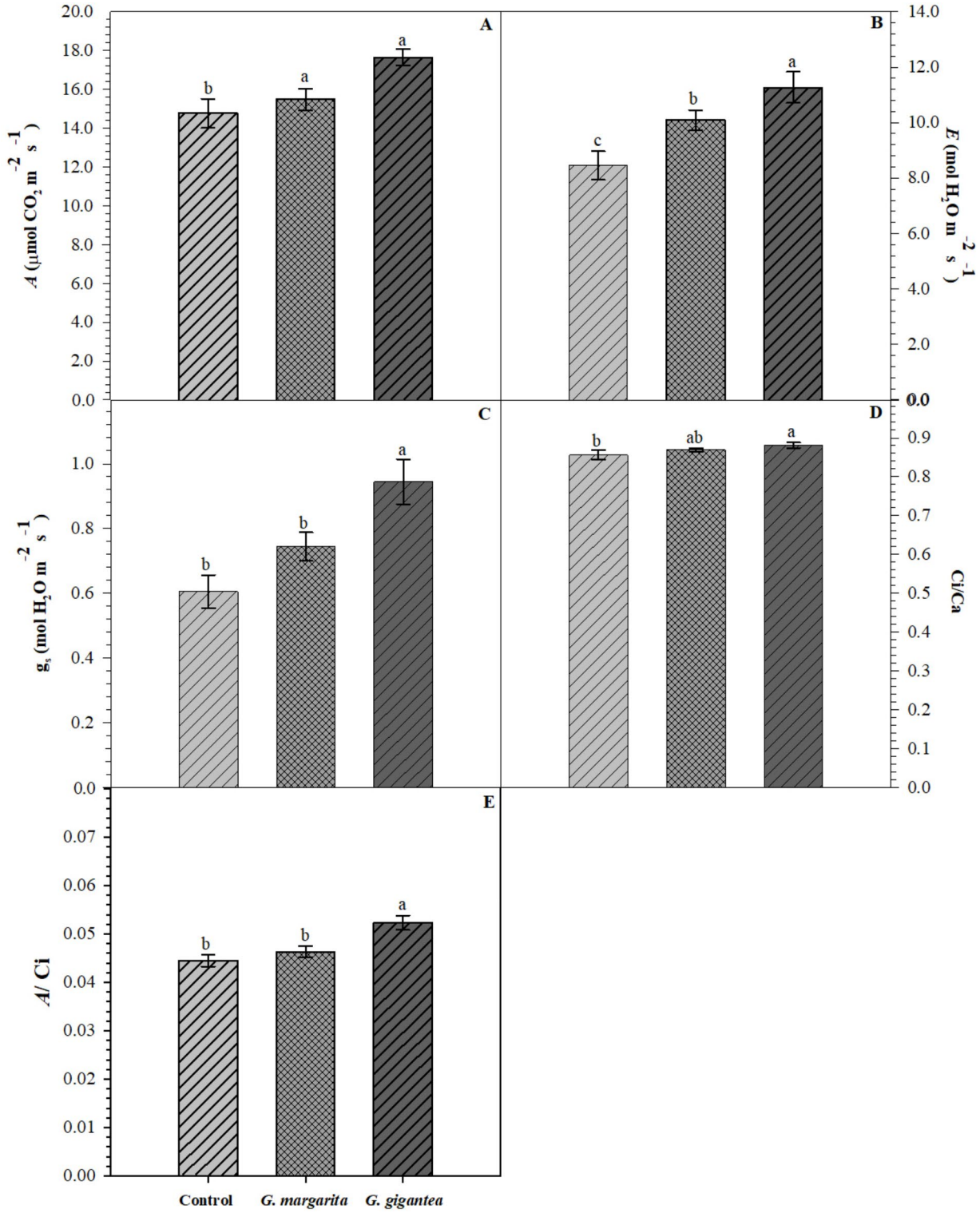

Fig. 3. Photosynthetic rate (**A**), transpiration (**B**), stomatal conductance (**C**), ratio between internal and external CO_2 concentrations (**D**), and carboxylation rate (**E**) of soybean plants inoculated with AMF after reirrigation. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same letter do not differ ($p > 0.05$) according to Tukey's test.

Effects of inoculation treatments and water restriction levels

Moreover, for the individual contributions of the inoculation treatments in PC1, the following trend was observed: *Gigaspora gigantea* > Control > *Gigaspora margarita* (Fig. 12a). In contrast, PC2 exhibited the following trend: *Gigaspora margarita* > control > *Gigaspora gigantea* (Fig. 12b).

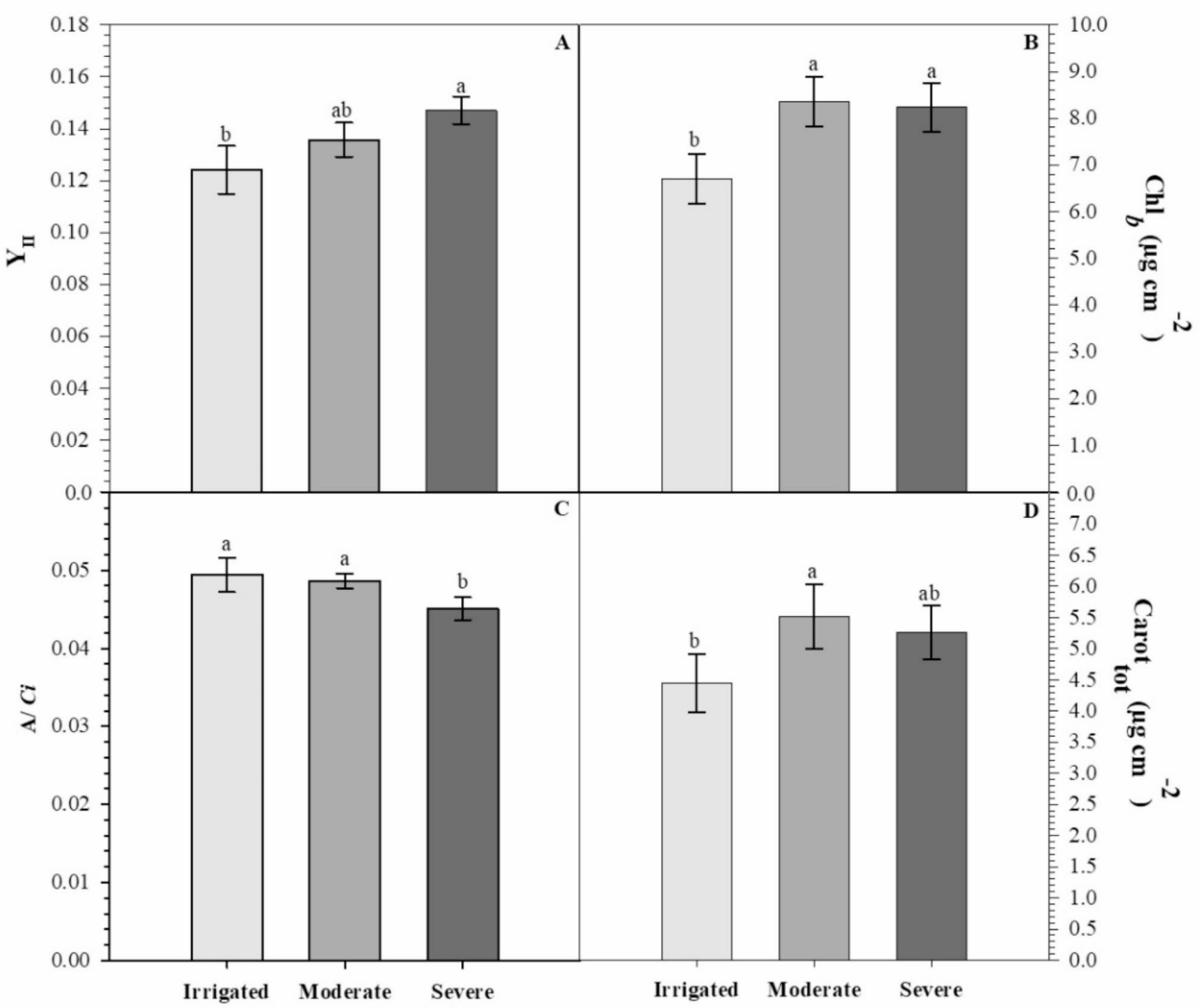

Fig. 4. The effective quantum yield of PSII (YII) (**A**), chlorophyll *b* (**B**), carboxylation rate (**C**), and carotenoids (**D**) and soybean plants subjected to different levels of hydric restriction after reirrigation. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same letter do not differ ($p > 0.05$) among themselves according to Tukey's test.

For the individual contributions of water restriction levels in PC1, the following trend was observed: severe > irrigated > moderate (Fig. 13a). The trend observed in PC2 was as follows: moderate > irrigated > severe (Fig. 13b).

Discussion

The connection between AMF and plants helps in the adaptation of plant physiological and morphological traits (biochemical and osmotic regulation, changes in plant water relationships, leaf elasticity, transpiration regulation, plant height, and dry weight of roots and plants) in environments with water deficit. This adaptation favours increased growth, plant productivity and plant tolerance to drought[34–38].

This connection initially develops through attractive chemotaxis through mutual recognition of chemical signals between fungi and plant roots, with the latter being the first plant organ to be affected by water shortages. A chain of events is triggered, with the roots releasing molecules that stimulate hyphal expansion and the fungi releasing signals that induce symbiosis and, in association with the plant, adapt to mechanisms such as increased sporulation in water-restricted environments[39], as observed in the present work (Fig. 1b) and corroborated by[23,40–42], demonstrating that the plant seeks help from AMF to alleviate the adverse conditions of the environment in which they are[35]. In addition, higher colonization rates are observed in the roots of plants, as observed in the present work (Fig. 1a) and by[43].

AMF penetrate the cortical cells of the roots, and in sequence, structures such as arbuscules (Fig. 2), vesicles and hyphae are formed[44]. With this, the degree of specificity, compatibility and symbiosis between the fungal species and the genetic characteristics of the host, the degree of colonization and the production of propagules

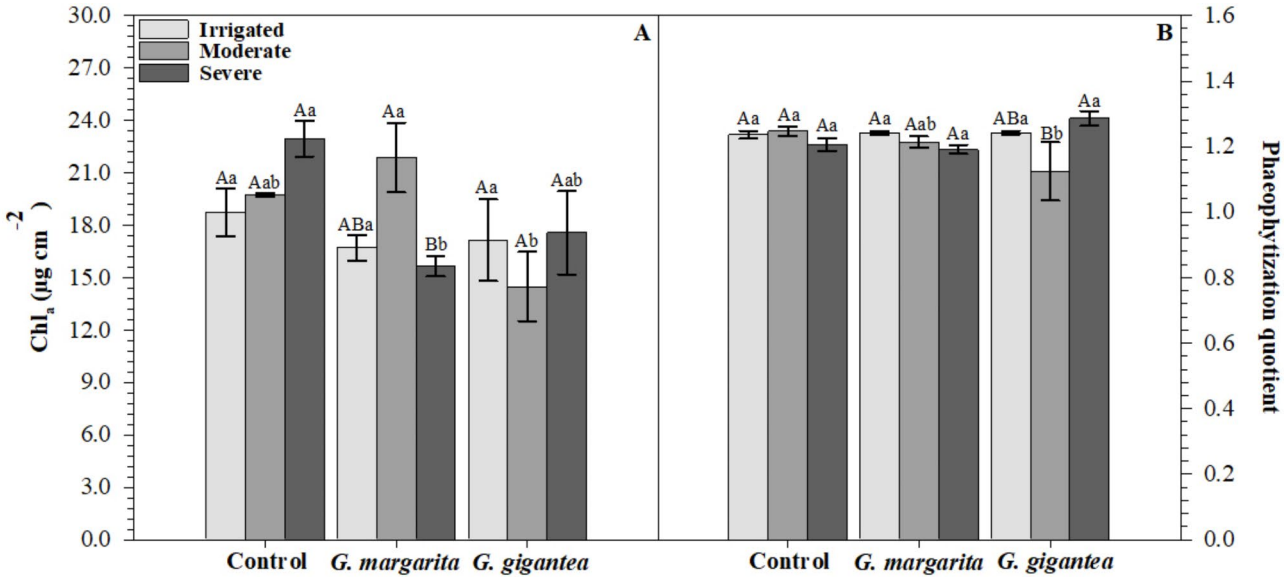

Fig. 5. Chlorophyll *a* (**A**) and phaeophytization quotient (**B**) of soybean plants inoculated with AMF after reirrigation. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same capital letters among hydric restriction treatments and lowercase letters between inoculation treatments do not differ ($p > 0.05$) according to Tukey's test.

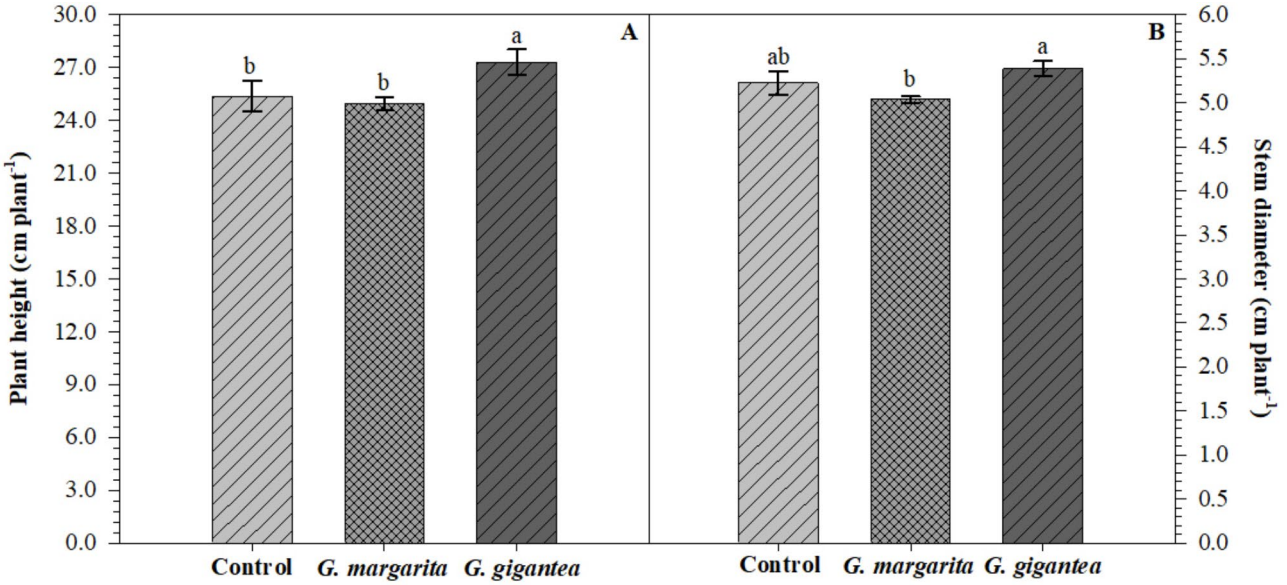

Fig. 6. Plant height (**A**) and stem diameter (**B**) of soybean plants inoculated with AMF after reirrigation. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same letter do not differ ($p > 0.05$) according to Tukey's test.

(Fig. 1) are observed[25,28,45–47]. However, the success of mycorrhizal inoculation depends on fungus–plant–soil relationships, and AMF species act differently according to the host plants and soil conditions[48,49]. The inoculation of the fungal species compatible with plants is crucial for the initiation of the infection process and the colonization of roots under drought conditions[50–52].

Each host plant species has a different dependence[53,54]. Therefore, it is recommended to compare different AMF species, to determine which species effectively promote plant development[55], since an efficient mycorrhizal colonization favors plants under stress[56], as observed in the present work, where the inoculation of *Gigaspora gigantea* favored the physiological and morphological traits of soybean plants (Figs. 3 and 6), under the conditions of this work, and had a relatively high percentage of individual contributions among the inoculation treatments (Fig. 12a).

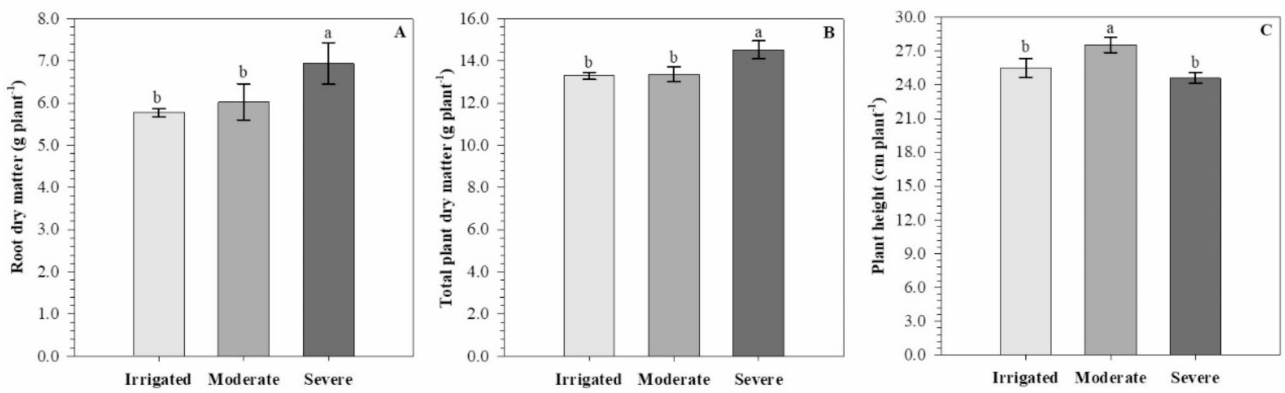

Fig. 7. Root dry matter (**A**), total plant dry matter (**B**), and plant height (**C**) of soybean plants subjected to different levels of water restriction after reirrigation. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same letter do not differ ($p > 0.05$) according to Tukey's test.

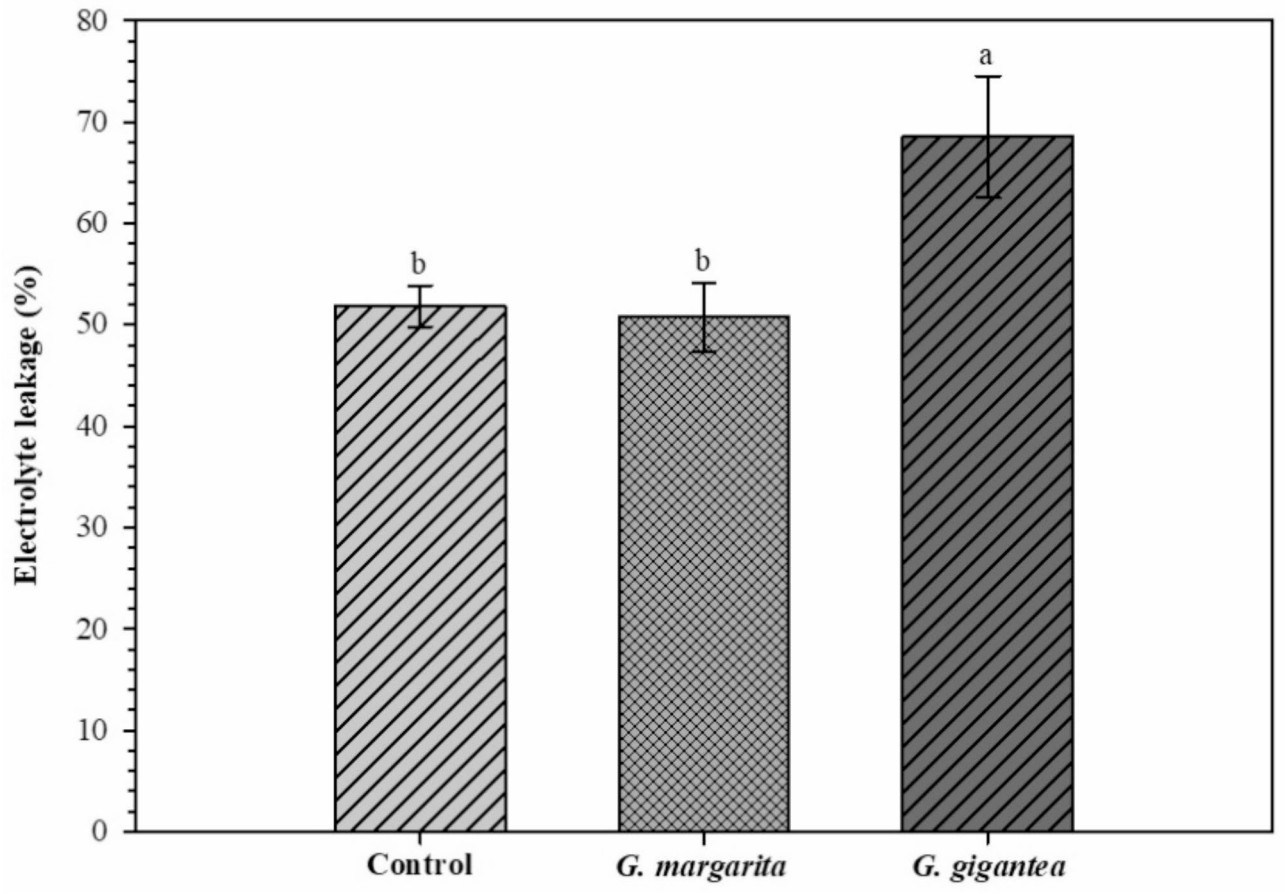

Fig. 8. Electrolyte leakage of soybean plants inoculated with AMF after reirrigation. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same letter do not differ ($p > 0.05$) according to Tukey's test.

In the present study, after reirrigation, soybean plants inoculated with *G. gigantea* had higher photosynthetic rate, transpiration rate, stomatal conductance, Ci/Ca ratio and carboxylation rate (Fig. 3), indicating that inoculation with this species helped in the adaptation of the physiological traits of plants subjected to water restriction. These results can be explained by the fact AMF have a well-developed mycelial network, which improves the shape and distribution of roots in the soil, promotes the expansion of the absorption area and helps in the development of host plants, making them more metabolically active[57], favoring photosynthesis (Fig. 1)

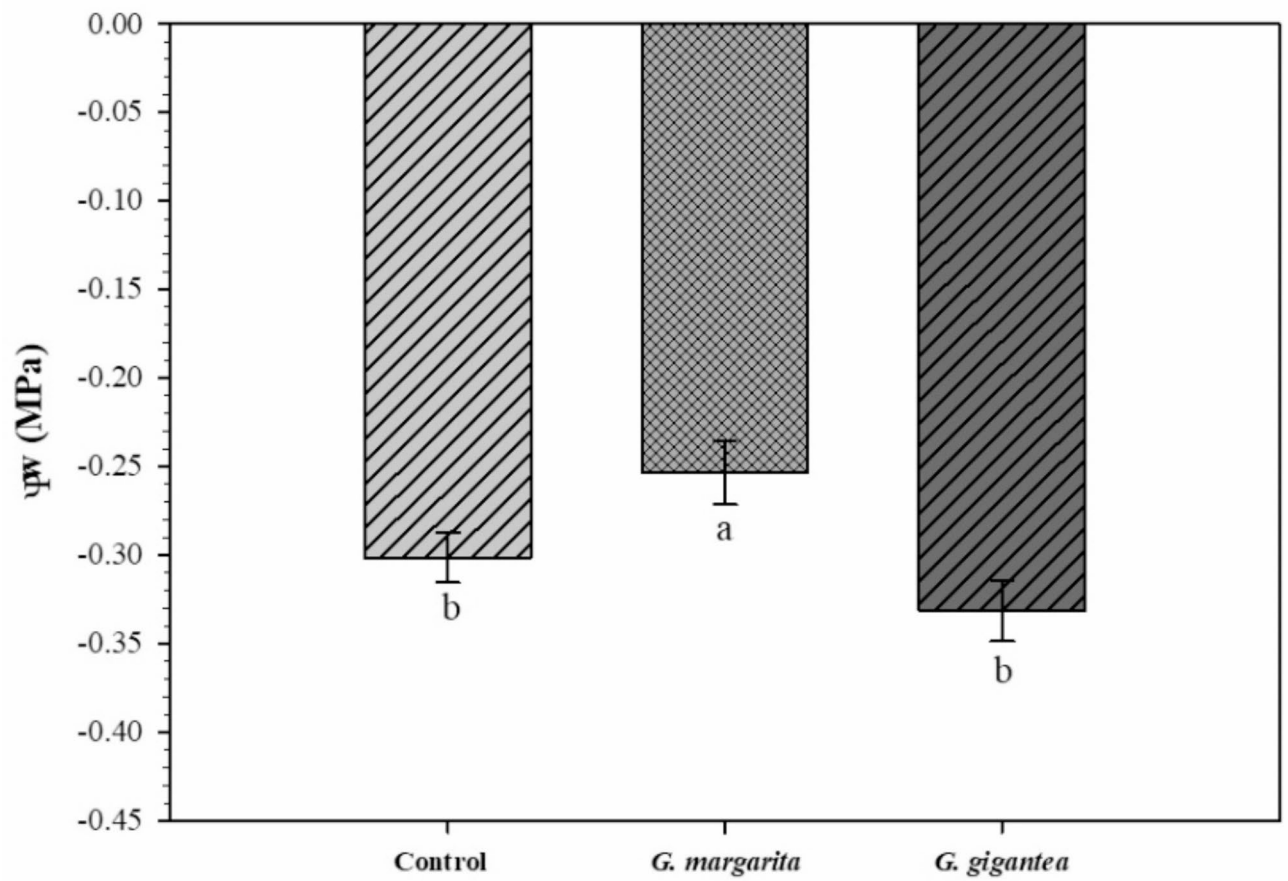

Fig. 9. Water potential of soybean plants inoculated with AMF under water deficit conditions. The bars represent the mean ± standard error of the mean (SEM) ($n = 4$). Means followed by the same letter do not differ ($p > 0.05$) according to Tukey's test.

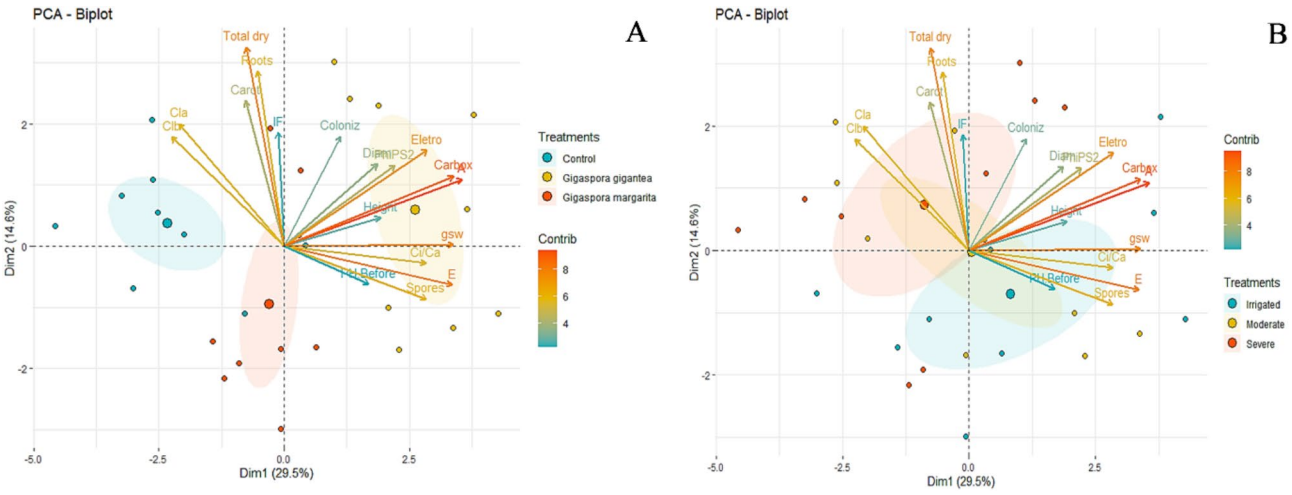

Fig. 10. PCA biplot of the first and second dimensions of soybean plants (*Glycine max*) inoculated with AMF (**A**) and subjected to different levels of water restriction (**B**) after reirrigation.

under drought conditions due to water absorption, which can be proven with the water potential of soybean plants in deficit in the present work (Fig. 9), in addition to stabilizing the chloroplast (Fig. 3) and the structure of the cell membrane, as observed with the inoculation of *G. margarita* which reduces electrolyte leakage (Fig. 8), and promotes an increase in rubisco activity through carboxylation (Fig. 3).

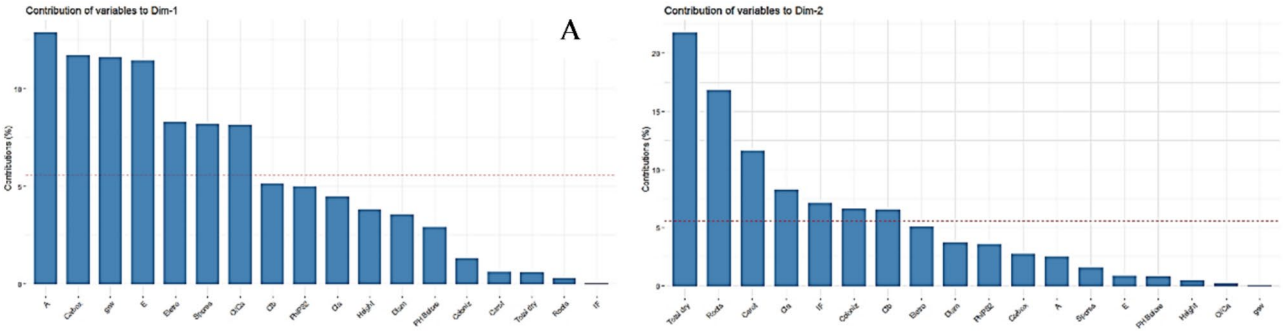

Fig. 11. Contributions of variables in soybean plants (*Glycine max*) inoculated with AMF and subjected to different levels of water restriction after reirrigation. First dimension (**A**) and second dimension (**B**). *A* = Photosynthetic rate, Carbox = carboxylation rate, gsw = stomatal conductance, *E* = transpiration, Electro = electrolyte leakage, Spores = spore density, Ci/Ca = ratio between internal and external CO_2 concentrations, Cl *b* = chlorophyll *b,* PhiPS2 = effective photochemical efficiency of photosystem II, Cl *a* = chlorophyll a, height = plant height, Diam = stem diameter, PH. Before = Water potential, Coloniz. = mycorrhizal colonization, Carot = carotenoid, Total dry = total dry weight, Roots = Root dry matter and IF = phaeophytinization index.

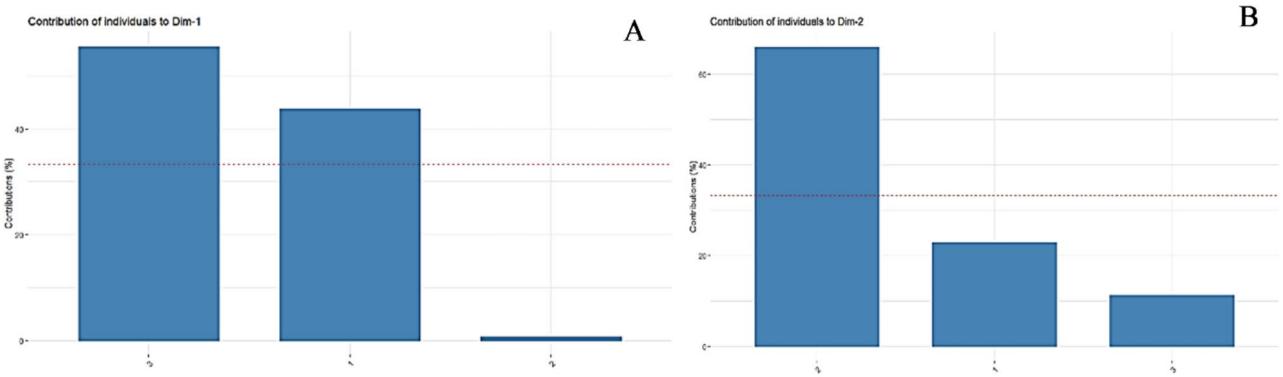

Fig. 12. Graphical illustration of the individual effects of inoculation treatments on soybean plants. (1) Control; (2) *Gigaspora margarita*; (3) *Gigaspora gigantea*. First dimension (**A**) and second dimension (**B**).

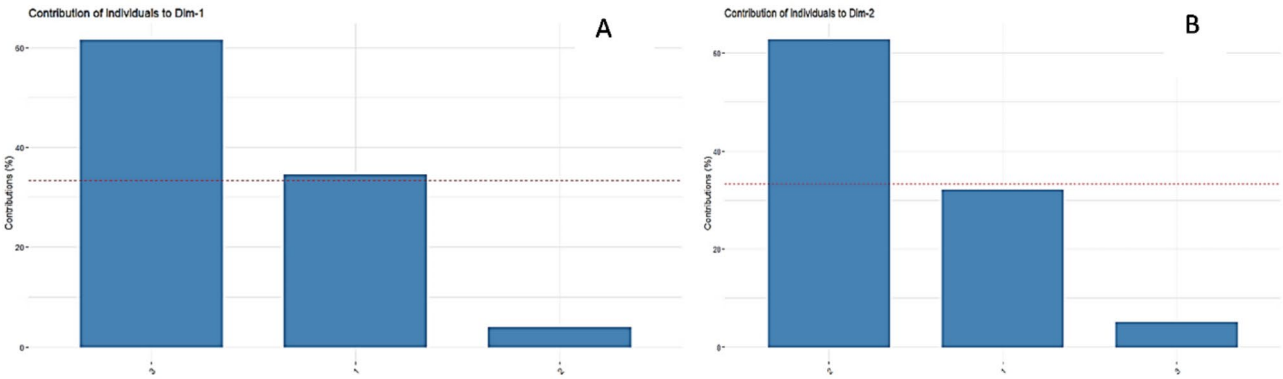

Fig. 13. Graphical illustration of the individual contributions of water restriction levels in soybean plants. (1) Irrigated; (2) Moderate; (3) Severe. First dimension (**A**) and second dimension (**B**).

As a response mechanism to water deficit, plants maximize their root system, to seek better conditions for water absorption, but limit the growth of aerial parts[58], as observed in soybean plants (Fig. 7a). A reduction in the leaf area of a plant under water stress aims to reduce transpiration and protect the plant from potential oxidative damage caused by a smaller surface area of light, but these changes almost always indicate lower

biomass production[59]. In addition to producing higher concentrations of carotenoids and chlorophyll *b*, as observed in the soybean plants of the present study (Fig. 4b and 4d, respectively), under stress conditions, these compounds are photoprotective pigments and, therefore, prevent the photooxidation of chlorophyll *a* during photosynthesis[60,61], a result confirmed in the present study (Fig. 5b). The effects of water deficit on plant pigments (chlorophylls and carotenoids) can cause disturbances in osmotic balance, such as water loss, reduced turgor and growth, leading to the degradation of chlorophyll *a*, which directly affects chlorophyll fluorescence and net photosynthesis[62–64].

However, the inoculation of AMF in plants subjected to drought environments results in increased concentrations of photosynthetic pigments. In the present study, the inoculation of *G. margarita* in soybean plants at a moderate water level favored a higher concentration of chlorophyll *a* (Fig. 5a), and the presence of *G. gigantea* at the same water level reduced the pheophytinization index (Fig. 5b), thus oxidizing the pigment, the results of which are corroborated by[65,66]. This occurs because plants under water deficit lose carbohydrates to AMF, but do not suffer damage from this loss, as it increases their capacity to produce photosynthesis and, consequently, light receptors for the assimilation and dissipation of light energy[60].

In fungus-plant symbiosis, plants exhibit greater growth[38], as observed in the morphological traits of the soybean plants in the present study, with greater shoot height and stem diameter (Fig. 6). The lack of water reduces the water potential, which results in a decrease in cell turgor and stomatal conductance, limiting photosynthesis and water use efficiency, reducing leaf expansion and stem elongation, and consequently, disfavoring growth, as observed in soybean plants with lower shoot height at severe water levels (Fig. 7c), and crop productivity[67,68]. Because of these negative effects, the importance of mycorrhizal inoculation and the selection of AMF species that favor the mitigation of the negative effects of water deficit on plants is justified. In the inoculant market, 63% of AMF-based products increase plant resilience to climate stresses, but these products have a predominance of some AMF species, making it necessary to include new species, and observe the degree of specificity and fungus-plant compatibility[69–71].

For plants, the presence of AMF alters their metabolism and protein synthesis, which maximizes plant growth and productivity, which is related to biochemical regulation, changes in the water relationship in plants and protection against abiotic stresses, such as water deficit[34,72]. Physiological traits can be used as positive indicators of the symbiosis between plants and AMF in situations of water scarcity. In the present work, in order of contribution, the photosynthetic rate (A), carboxylation (A/Ci), stomatal conductance (gsw) and transpiration (E) (Fig. 11a) were good evaluation indicators. In terms of morphology traits, the total dry weight of the plants and roots had the greatest individual contribution to the results (Fig. 11b). In addition, they are indicators for the selection of AMF species according to their compatibility with the plant and adaptations to drought conditions.

The inoculation of *G. gigantea* in soybean plants subjected to water restriction optimizes physiological traits, resulting in better functioning of the photosynthetic apparatus of the plants, maximizing the tolerance of this crop to water scarcity conditions and mitigating the negative effects of these conditions. These results provide information for the development of field research in regions subject to the impact of climate change with longer periods of drought using AMF species according to their compatibility with the plant and soil conditions.

Conclusion

The inoculation of *Gigaspora gigantea* promoted the adaptation of the physiological and morphological traits of soybean plants under conditions of water restriction.

The inoculation of *Gigaspora gigantea* maximized the tolerance of soybean plants to drought, mitigating the negative effects of this condition regardless of the level of water restriction.

Mycorrhizal inoculation promoted better functioning of the photosynthetic apparatus and growth of soybean plants.

Materials and methods

Plant growth conditions and inoculation with AMF

A soil mixture (Red Latosol—typical soil of the Brazilian Cerrado savanna) collected in an area of the IF Goiano—Campus Rio Verde, with average sand (2:1–soil: sand) was used. A sample of the mixture was removed for chemical analysis (Table 1S), and before sowing, liming was performed for 20 days with li. €mestone (Filler dolomitic limestone 100% PRNT) to increase the base saturation to 60%, which is suitable for soybean. The substrate was fertilized on the basis of chemical analysis (Supplementary Table 1S) and recommendations for Cerrado soils[73].

After 20 days, soybean seeds (cv. BMX Flecha 6266, precocious, with an indeterminate growth habit) treated with an inoculant based on *Bradyrhizobium japonicum* to provide nitrogen (N), were germinated in 3 dm^3 pots, and the plants were subsequently grown in a greenhouse (under natural conditions of light, a relative humidity of 65–85%, and an average temperature of 28 °C) of the IF Goiano—Campus Rio Verde.

The noncommercial AMF inoculants used are part of the collection of the Laboratory of Soil Microbiology UNESP, Ilha Solteira, which was donated to the IF Goiano – Campus Rio Verde. The multiplication method was performed according to[23]. Soybean seeds were inoculated in the sowing furrow and received 31 g of *Gigaspora margarita* (3.3 spores g^{-1}) or 46 g of *Gigaspora gigantea* (2.2 spores g^{-1}) separately. The control consisted of no inoculation.

Induction of water restriction levels in soybean plants

The plants were maintained at 80% field capacity (FC) until the V3 stage (40 days after sowing). Then, they were submitted to water restriction levels: control (80% FC), moderate (60% FC), and severe (40% FC). When the

plants reached their respective FC and showed symptoms of water deficit, they were reirrigated for 48 h until they reached 80% of the FC, after which the evaluations were performed.

The water content was controlled once a day using an irrigation sensor, model 10 HS (METER Group, Inc., USA) after the field capacity (FC) was measured via the gravimetric method.

Physiological measurements

The analyses of the parameters linked to photosynthesis were performed using an infrared gas concentration measurement system (IRGA, LI-COR-Li6800). Parameters such as the net photosynthetic rate (*A,* μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*g* s, mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (*Ci*, μmol CO_2 mol^{-1}) and transpiration (*E,* mmol m^{-2} s^{-1}) were determined for all the treatments. An irradiance of 1000 μmol m^{-2} s^{-1} was used throughout the experiment. All the measurements were performed between 8:00 and 11:00 am.

The water potential (Ψw) was measured in the morning using a Scholander pump under deficit conditions and after reirrigation. The determination consisted of collecting fully expanded leaves, which were placed in the pressure pump chamber, where pressure was then applied until exudation occurred through a cut made in the leaf petiole, for reading the applied pressure.[74].

Determination of chlorophyll concentration

The concentrations of carotenoids and chlorophyll *a* and *b* and the pheophytinization index were determined spectrophotometrically at 480 nm, 649.1 nm, 665.1 nm, 435 nm, and 415 nm. Pigment extraction was performed from leaf discs (~ 5 cm^2) immersed in 5 mL of dimethyl sulfoxide (DMSO) with calcium carbonate ($CaCO_3$) (50 g/L) at 65 °C in a water bath. The discs remained in the solution for 24 h. The values were transformed into chlorophyll *a*, *b,* and total contents in the leaves, expressed in area units (μg cm^{-2}). Total chlorophyll was obtained by summing the Chl *a* and Chl *b* values.

Electrolyte extravasation rate

The methodology for determining the electrolyte extravasation rate (ETL) was described previously[75,76]. Fifteen leaf discs were collected for each replicate, placed in amber glass flasks with 30 mL of deionized water, and immersed for 24 h in the dark at room temperature. After this period, the free conductivity (CL, μS/cm) was measured with a portable digital conductivity meter, model CD-850. The flasks were subsequently placed in an oven for 1 h at 100 °C for subsequent measurement of the total conductivity (TC, μS/cm). The sensor was washed between each reading with deionized water. The following formula was used to express the result as a percentage: TLE (%) = CL/TC * 100.

Morphological characteristics

The average length of the aerial parts was obtained with the aid of a ruler. The stems, leaves, and roots were subsequently separately dried in an oven at 65 °C with forced air circulation until a constant mass was reached to obtain the dry mass of each plant separately.

Spore density

The spore density was evaluated using the wet sieving technique[77]. Then, 100 g of the soil was washed and sifted 6 times, placed in a Falcon tube with water, and centrifuged at 3000 rpm for 3 min. Then, water was added, and a 50% sucrose solution was added and centrifuged for another 2 min. Subsequently, the liquid with the spores was poured into sieves with meshes of 710, 425, and 53 mm/μm to wash the samples. Then, the liquid containing the spores was poured into the sieve to wash the sample, which was subsequently stored in a container until analysis in the laboratory. The number of spores was determined on an acrylic plate with concentric rings under a SteREO Discovery. A V8 stereomicroscope (Zeiss, Göttingen, Germany) was used.

Mycorrhizal colonization in soybean roots

Fractions of the roots of the plants in each treatment group were separated and preserved in a 50% alcohol solution. To estimate root colonization by AMF, the roots were depigmented according to a modified method of[78].

The roots (~ 0.4 g) were weighed, immersed in KOH (2%), and placed in an oven at 90 °C for 60 min. After removal from the oven, the roots were washed with distilled water and transferred to a solution of HCl (1%) for 5 min. Then, the HCl was removed, and the dye trypan blue (0.05%) in lactoglycerol was added[79]. Microscopic slides were prepared with root fragments to visualize the structures and percentage of root colonization evaluated under a light microscope at 200 × magnification, according to[80].

Statistical analysis

The experimental design was completely randomized in a 3 × 3 factorial design (three inoculation treatments × three water restriction levels), with 8 replicates of each treatment, each consisting of a pot containing 3 plants. The homogeneity of variance was confirmed by Bartlett's test. The numerical data were statistically evaluated by analysis of variance, and the means were tested by the Tukey test (5%) using the SISVAR software[81].

Principal component analysis (PCA) was performed on the dataset using inoculation treatments and hydric restriction levels. These analyses used the *FactoMineR*[82] and *extrafact*[83] packages in R software[84]. First, the data were scaled using the *scale* function, and the analyses were performed using the *PCA* function. Eigenvalues were evaluated to determine the number of dimensions to be evaluated.

Data availability

All data generated or analyzed during this study are included in this published article. The raw datasets are available from the corresponding author on reasonable request.

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References

1. EEA, E. Global and European temperature (CSI 012/CLIM 001). *Cph. WWW Doc.* (2011).
2. de Barros França-Neto, J. *et al.* Tecnologia da produção de semente de soja de alta qualidade. (2016).
3. Bhattacharya, A. Global Climate Change and Its Impact on Agriculture. in 1–50 (2019). https://doi.org/10.1016/B978-0-12-816209-5.00001-5.
4. Singh, A. K. et al. Impact of climate change on productivity of tropical rice-wheat-jute system under long term fertilizer management in alluvial soils. *Int J Curr Microbiol Appl Sci* **7**, 1623–1632 (2018).
5. Hatfield, J. L. et al. Climate impacts on agriculture: Implications for crop production. *Agron. J.* **103**, 351–370 (2011).
6. Conab. Acompanhamento da safra brasileira de grãos, Terceiro levantamento, 8, 1–86. (2020).
7. Sprent, J. I. & Platzmann, J. *Nodulation in Legumes* (Royal Botanic Gardens Kew, 2001).
8. Stacey, G., Vodkin, L., Parrott, W. A. & Shoemaker, R. C. National Science Foundation-sponsored workshop report. Draft plan for soybean genomics. (2004).
9. St-Marseille, A.-F.G., Bourgeois, G., Brodeur, J. & Mimee, B. Simulating the impacts of climate change on soybean cyst nematode and the distribution of soybean. *Agric. For. Meteorol.* **264**, 178–187 (2019).
10. Clement, M., Lambert, A., Herouart, D. & Boncompagni, E. Identification of new up-regulated genes under drought stress in soybean nodules. *Gene* **426**, 15–22 (2008).
11. Abid, M. et al. Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Sci. Rep.* **8**, 4615 (2018).
12. Ltaief, S. & Krouma, A. Functional dissection of the physiological traits promoting durum wheat (*Triticum durum* Desf.) tolerance to drought stress. *Plants* **12**, 1420 (2023).
13. Ku, Y. S. *et al.* A comprehensive survey of international soybean research—genetics, physiology, agronomy and nitrogen relationships. *Vol. InTech* 13 (2013).
14. Peak, D., West, J. D., Messinger, S. M. & Mott, K. A. Evidence for complex, collective dynamics and emergent, distributed computation in plants. *Proc. Natl. Acad. Sci.* **101**, 918–922 (2004).
15. Behnam, B. et al. Characterization of the promoter region of an Arabidopsis gene for 9-cis-epoxycarotenoid dioxygenase involved in dehydration-inducible transcription. *DNA Res.* **20**, 315–324 (2013).
16. Eldakak, M., Milad, S. I., Nawar, A. I. & Rohila, J. S. Proteomics: a biotechnology tool for crop improvement. *Front. Plant Sci.* **4**, 35 (2013).
17. Ghosh, A., Agrawal, M. & Agrawal, S. B. Effect of water deficit stress on an Indian wheat cultivar (*Triticum aestivum* L. HD 2967) under ambient and elevated level of ozone. *Sci. Total Environ.* **714**, 136837 (2020).
18. Deeba, F. et al. Physiological and proteomic responses of cotton (*Gossypium herbaceum* L.) to drought stress. *Plant Physiol. Biochem.* **53**, 6–18 (2012).
19. Xiong, L., Wang, R.-G., Mao, G. & Koczan, J. M. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiol.* **142**, 1065–1074 (2006).
20. Querejeta, J., Egerton-Warburton, L. M. & Allen, M. F. Topographic position modulates the mycorrhizal response of oak trees to interannual rainfall variability. *Ecology* **90**, 649–662 (2009).
21. Smith, S. E. & Read, D. J. *Mycorrhizal Symbiosis* (Academic press, 2010).
22. Smith, S. E., Facelli, E., Pope, S. & Smith, F. A. Plant performance in stressful environments: Interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil* **326**, 3–20 (2010).
23. Santana, L. R. et al. Arbuscular mycorrhizal fungi associated with maize plants during hydric deficit. *Sci. Rep.* **13**, 1519 (2023).
24. Dar, Z. M., Masood, A., Asif, M. & Malik, M. A. Review on arbuscular mycorrhizal fungi: An approach to overcome drought adversities in plants. *Int J Curr Microbiol Appl Sci* **7**, 1040–1049 (2018).
25. Cavalcante, U. M. T., Goto, B. T. & Maia, L. C. Aspectos da simbiose micorrízica arbuscular. *An. Acad. Pernambucana Ciênc. Agronômica* **5**, 180–208 (2008).
26. Piccoli, P. et al. An endophytic bacterium isolated from roots of the halophyte Prosopis strombulifera produces ABA, IAA, gibberellins A 1 and A 3 and jasmonic acid in chemically-defined culture medium. *Plant Growth Regul.* **64**, 207–210 (2011).
27. Bárzana, G. et al. Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Ann. Bot.* **109**, 1009–1017 (2012).
28. Folli-Pereira, M. D. S., Meira-Haddad, L. S., Bazzolli, D. M. S. & Kasuya, M. C. M. Micorriza arbuscular e a tolerância das plantas ao estresse. *Rev. Bras. Ciênc. Solo* **36**, 1663–1679 (2012).
29. Liu, T. et al. Impact of arbuscular mycorrhizal fungi on the growth, water status, and photosynthesis of hybrid poplar under drought stress and recovery. *Photosynthetica* **53**, 250–258 (2015).
30. Volpe, V. et al. The association with two different arbuscular mycorrhizal fungi differently affects water stress tolerance in tomato. *Front. Plant Sci.* **9**, 1480 (2018).
31. Augé, R. M. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**, 3–42 (2001).
32. Ruiz-Lozano, J. M., Porcel, R., Azcón, C. & Aroca, R. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *J. Exp. Bot.* **63**, 4033–4044 (2012).
33. Chen, M., Arato, M., Borghi, L., Nouri, E. & Reinhardt, D. Beneficial services of arbuscular mycorrhizal fungi–from ecology to application. *Front. Plant Sci.* **9**, 1270 (2018).
34. Miransari, M. Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. *Plant Biol.* **12**, 563–569 (2010).
35. Colodete, C. M., Dobbss, L. B. & Ramos, A. C. Aplicação das micorrizas arbusculares na recuperação de áreas impactadas. *Nat. Line* **12**, 31–37 (2014).
36. Bahadur, A. et al. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. *Int. J. Mol. Sci.* **20**, 4199 (2019).
37. Cheng, S. et al. Elucidating the mechanisms underlying enhanced drought tolerance in plants mediated by arbuscular mycorrhizal fungi. *Front. Microbiol.* **12**, 809473 (2021).
38. Van Der Heijden, M. G. A., Martin, F. M., Selosse, M. & Sanders, I. R. Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytol.* **205**, 1406–1423 (2015).
39. Barros, J. M. T. de M. Fungos micorrízicos em mudas de Jatropha curcas L. submetidas a estresse hídrico. *Mycorrhizal fungi in Jatropha curcas L. seedlings submitted to water stress* (2018).
40. Mo, Y. et al. Regulation of plant growth, photosynthesis, antioxidation and osmosis by an arbuscular mycorrhizal fungus in watermelon seedlings under well-watered and drought conditions. *Front. Plant Sci.* **7**, 644 (2016).

41. Coscolin, R. B. S., Gomes, E. R., Magalhães, V. M. S. & Broetto, F. Associação de fungos micorrízicos no cultivo do amendoim sob deficiência hídrica. *Rev. AgroFIB* **1**, (2019).
42. Oliveira, T. C. et al. The arbuscular mycorrhizal fungus Rhizophagus clarus improves physiological tolerance to drought stress in soybean plants. *Sci. Rep.* **12**, 1–15 (2022).
43. Hu, W., Zhang, H., Chen, H. & Tang, M. Arbuscular mycorrhizas influence *Lycium barbarum* tolerance of water stress in a hot environment. *Mycorrhiza* **27**, 451–463 (2017).
44. Smith, S. E. & Smith, F. A. Roles of arbuscular mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystem scales. *Annu. Rev. Plant Biol.* **62**, 227–250 (2011).
45. Canton, G. C. Efeito do manganês sobre a ecofisiologia e bioquímica de ectomicorrizas. (brasil, 2012).
46. Kiriachek, S. G., de Azevedo, L. C. B., Peres, L. E. P. & Lambais, M. R. Regulação do desenvolvimento de micorrizas arbusculares. *Rev. Bras. Ciênc. Solo* **33**, 1–16 (2009).
47. Guigard, L., Jobert, L., Busset, N., Moulin, L. & Czernic, P. Symbiotic compatibility between rice cultivars and arbuscular mycorrhizal fungi genotypes affects rice growth and mycorrhiza-induced resistance. *Front. Plant Sci.* **14**, 1278990 (2023).
48. Mang'erere Nyamwange, M. et al. Soil management practices affect arbuscular mycorrhizal fungi propagules, root colonization and growth of rainfed maize. *AIMS Agric. Food* **3**, 120–134 (2018).
49. Serpe, M. D., Thompson, A. & Petzinger, E. Effects of a companion plant on the formation of Mycorrhizal propagules in artemisia tridentata seedlings. *Rangel. Ecol. Manag.* **73**, 138–146 (2020).
50. Nunes, J. da S., Souza, P. de, Marodin, G. A. B. & Fachinello, J. C. Development increase of 'Okinawa'peach rootstocks by indigenous arbuscular mycorrhizal fungi. (2011).
51. Berruti, A., Lumini, E., Balestrini, R. & Bianciotto, V. Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2015.01559 (2016).
52. Ronga, D. et al. Interaction of tomato genotypes and arbuscular mycorrhizal fungi under reduced irrigation. *Horticulturae* **5**, 79 (2019).
53. Hoeksema, J. D. et al. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecol. Lett.* **13**, 394–407 (2010).
54. Jiang, F. et al. Mycorrhizal type influences plant density dependence and species richness across 15 temperate forests. *Ecology* **102**, e03259 (2021).
55. Balota, E. L., Machineski, O. & Stenzel, N. M. C. Resposta da acerola à inoculação de fungos micorrízicos arbusculares em solo com diferentes níveis de fósforo. *Bragantia* **70**, 166–175 (2011).
56. Boyer, L. R., Brain, P., Xu, X.-M. & Jeffries, P. Inoculation of drought-stressed strawberry with a mixed inoculum of two arbuscular mycorrhizal fungi: Effects on population dynamics of fungal species in roots and consequential plant tolerance to water deficiency. *Mycorrhiza* **25**, 215–227 (2015).
57. Zou, Y.-N., Srivastava, A. K., Ni, Q.-D. & Wu, Q.-S. Disruption of mycorrhizal extraradical mycelium and changes in leaf water status and soil aggregate stability in rootbox-grown trifoliate orange. *Front. Microbiol.* **6**, 203 (2015).
58. Jin, K. et al. Wheat root growth responses to horizontal stratification of fertiliser in a water-limited environment. *Plant Soil* **386**, 77–88 (2015).
59. Chen, X., Min, D., Yasir, T. A. & Hu, Y.-G. Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). *Field Crops Res.* **137**, 195–201 (2012).
60. Kaschuk, G., Kuyper, T. W., Leffelaar, P. A., Hungria, M. & Giller, K. E. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses?. *Soil Biol. Biochem.* **41**, 1233–1244 (2009).
61. Mathur, S., Sharma, M. P. & Jajoo, A. Improved photosynthetic efficacy of maize (*Zea mays*) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. *J. Photochem. Photobiol. B* **180**, 149–154 (2018).
62. Marques, R. P., Freire, C. S., do Nascimento, H. H. C. & Nogueira, R. J. M. C. Relações Hídricas e Produção de Pigmentos Fostossinteticos em Mudas de Eugenia Uniflora l. Sob Condições de Salinidade (Water Relations and Production of Pigments in Seedlings Photosynthetic Eugenia Uniflora l. Under Salinity Conditions). *Rev. Bras. Geogr. Física* **4**, 497–509 (2011).
63. Dalal, V. K. & Tripathy, B. C. Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis. *Plant Cell Environ.* **35**, 1685–1703 (2012).
64. Egbe, E. A., Forkwa, E. Y. & Enow, E. A. Evaluation of seedlings of three woody species under four soil moisture capacities. *Br. J. Appl. Sci. Technol.* **4**, 3455–3472 (2014).
65. Baslam, M. & Goicoechea, N. Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. *Mycorrhiza* **22**, 347–359 (2012).
66. Yooyongwech, S., Samphumphuang, T., Tisarum, R., Theerawitaya, C. & Cha-Um, S. Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline. *Sci. Hortic.* **198**, 107–117 (2015).
67. Seleiman, M. F. et al. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* **10**, 259 (2021).
68. Hemati, A., Moghiseh, E., Amirifar, A., Mofidi-Chelan, M. & Asgari Lajayer, B. Physiological effects of drought stress in plants. In *Plant Stress Mitigators* (eds Vaishnav, A. et al.) 113–124 (Springer, 2022). https://doi.org/10.1007/978-981-16-7759-5_6.
69. Begum, N. et al. Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. *Plants* **8**, 579 (2019).
70. Basiru, S., Mwanza, H. P. & Hijri, M. Analysis of arbuscular mycorrhizal fungal inoculant benchmarks. *Microorganisms* **9**, 81 (2020).
71. Wahab, A. et al. Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. *Plants* **12**, 3102 (2023).
72. Gill, S. S. et al. Piriformospora indica: Potential and significance in plant stress tolerance. *Front. Microbiol.* **7**, 332 (2016).
73. Sousa, D. de & Lobato, E. Correção do solo e adubação da cultura da soja. *Planaltina Embrapa-CPAC* (1996).
74. Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A. & Hammel, H. T. Sap pressure in vascular plants: Negative hydrostatic pressure can be measured in plants. *Science* **148**, 339–346 (1965).
75. Vasquez-Tello, A., Zuily-Fodil, Y., Thi, A. P. & da Silva, J. V. Electrolyte and Pi leakages and soluble sugar content as physiological tests for screening resistance to water stress in Phaseolus and Vigna species. *J. Exp. Bot.* **41**, 827–832 (1990).
76. Pimentel, C., Sarr, B., Diouf, O., Abboud, A. D. S. & Roy-Macauley, H. Tolerância protoplasmática foliar à seca, em dois genótipos de caupi cultivados em campo. *Rev. Universidade Rural Sér. Ciênc. Vida* **22**, 7–14 (2002).
77. Gerdemann, J. W. & Nicolson, T. H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46**, 235–244 (1963).
78. Koske, R. E. & Gemma, J. N. A modified procedure for staining roots to detect VA mycorrhizas. *Mycol. Res.* **92**, 486 (1989).
79. Phillips, J. M. & Hayman, D. S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55**, 158–161 (1970).
80. McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. & Swan, J. A. A new method which gives an objective measure of colonization of roots by vesicular—Arbuscular mycorrhizal fungi. *New Phytol.* **115**, 495–501 (1990).
81. Ferreira, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs: Sisvar. *Braz. J. Biom.* **37**, 529–535 (2019).
82. Lê, S., Josse, J. & Husson, F. FactoMineR: An R package for multivariate analysis. *J. Stat. Softw.* **25**, 1–18 (2008).

83. Kassambara, A. & Mundt, F. Factoextra: Extract and visualize the results of multivariate data analyses. *R Package Version* **1**, 337–354 (2020).
84. Team, R. C. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *Httpwww R-Proj. Org* (2016).

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Author contributions
E. L S and J.S.R.C. designed and supervised the research. Material preparation and data collection were performed by G.G.T., J.S.R.C., L.R.S. and L.N.S. Analyses were performed by G.G.T., J.S.R.C., L.R.S. A.A.S. cooperated with the physiological analyses of the plants. M. B. T. cooperated with the field capacity analyses. The first draft of the manuscript was written by G. G. T., with contributions from J. S. R. C., L.R.S. and E.L.S. J. S. R. C and E. L. S. substantially revised and edited the manuscript. All the authors have read and approved the final version of the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Plant-guideline statement
Experimental research and field studies on cultivated plants, including the collection of plant material, comply with the required institutional, national and international guidelines and legislation. The seeds used in the experiment were donated by the first author, and the cultivars are described in the methodology section. The greenhouse used was from the institution where the study was carried out, as described in the methodology section.

Additional information
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