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Mycorrhizal Symbiosis and Water Deficit: Morphophysiological and Gene Expression Responses in Caatinga Passion Fruit

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Abstract: The advancement of global warming and climate change requires strategic actions in understanding and seeking interactions between plant species and microorganisms that are more tolerant to water deficit. This research assessed the morpho-agronomic, physiological, and gene expression responses of two *Passiflora cincinnata* accessions (tolerant and sensitive) to water deficit, focusing on their relationship with mycorrhization. A randomized design with two accessions, two field capacities, and four AMF inoculation treatments was used to compare drought and control conditions. Differential gene expression was analyzed under drought stress, and the effect of mycorrhization on stress tolerance was evaluated. The results showed that inoculation with native arbuscular mycorrhizal fungi (AMF) communities, especially those from water-deficit conditions (AMF25), resulted in greater increases in height, number of leaves, stem diameter, number of tendrils, leaf area, and fresh biomass of root and shoot, with increases ranging from 50% to 300% compared to the control (non-inoculated) and monospecific inoculation (*Entrophospora etunicata*). Higher photosynthetic rate and water use efficiency were observed in the tolerant accession. Mycorrhizal inoculation increased the total chlorophyll content in both accessions, especially when inoculated with native AMF communities. Overall, *P. cincinnata* showed higher mycorrhizal responsiveness when inoculated with native AMF communities compared to monospecific inoculation with *E. etunicata*. The tolerant accession showed overexpression of the genes *PcbZIP*, *PcSIP*, and *PcSTK*, which are associated with signal transduction, water deficit tolerance, osmoregulation, and water transport. In contrast, the water deficit-sensitive accession showed repression of the *PcSIP* and *PcSTK* genes, indicating their potential use for distinguishing tolerant and sensitive accessions of the species. The tolerance of *P. cincinnata* to water deficit is directly related to physiological responses, increased photosynthetic rate, efficient water use, and regulation of gene expression.

Keywords: caatinga passion fruit; water deficit; mycorrhizal responsiveness; qPCR

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1. Introduction

Global warming has been gradually intensifying, mainly due to the steady rise in global temperature and decreased precipitation, resulting in the expansion of arid regions [1]. Additionally, some projections indicate an increase in the frequency and intensity of droughts, mainly related to the rise in potential evapotranspiration of surface waters [2].

Therefore, there is an urgent need to find alternatives for maintaining food production, including the search for plants tolerant to water deficit and their cultivation in arid and semi-arid conditions [3,4].

To sustain plant production in water-scarce areas, the exploration and identification of plant species resilient to water deficits are crucial for ensuring plant survival and productivity. This exploration entails assessing morpho-agronomic descriptors [5] alongside physiological and molecular evaluations [6], with these tools being essential in genotype selection for the development of cultivars or lines tolerant to water deficit [7,8].

The low availability of water affects plants in various ways, leading to diverse responses. Morphologically, this can manifest as a decrease in aboveground biomass and alterations in developmental patterns. Additionally, there may be changes in leaf substance production, resulting in an augmented leaf cuticle and modifications in stomatal development, including variations in size, density, and aperture [9]. Recently, Yu et al. [10] reported that water deficit can cause leaf curling and root thinning, resulting in thinner and more dispersed roots. Physiologically, there is a notable decrease in stomatal conductance, transpiration, internal CO_2 concentration, and relative water content [11]. Biochemically, there may be an increase in reactive oxygen species (ROS) and production of antioxidant enzymes, as well as a reduction in photosynthetic efficiency due to chloroplast deformations [10,12].

At the molecular level, the differential expression of various genes related to responses to water deficit and tolerance mechanisms is observed. Among these, aquaporins and late embryogenesis abundant (LEA) genes, induced by water deficit, facilitate water transport and osmoregulation and confer tolerance [13,14]. Some regulatory genes and transcription factors, such as bZIP, MYB, WRKY, NAC, and AP2/ERF, are differentially expressed under water-deficit conditions due to their involvement in the expression of genes related to the biosynthesis of abscisic acid (ABA) and brassinosteroids [15–17]. Additionally, genes associated with photosynthesis and ABA biosynthesis, such as 9-cis-epoxycarotenoid dioxygenase (NCED), exhibit differential expression during periods of reduced water availability, contributing to ABA catalysis from carotenoids in the initial step of the ABA biosynthesis pathway [9,18–21].

On the other hand, changes in plant morphology and physiology in response to water deficit may be influenced by another component of the soil, namely, arbuscular mycorrhizal fungi (AMF). These fungi can significantly alleviate the adverse impacts of water scarcity on plants by forming mutualistic symbiotic relationships with the majority of plant species, constituting over 72% of mycorrhizal symbioses formed with plants [22]. This mutualistic symbiosis facilitates bidirectional exchange, wherein AMF enhance water and nutrient availability, primarily absorbing phosphorus and nitrogen, while plants reciprocate by providing carbon derived from sugars or lipids [23]. The main mechanisms of water deficit attenuation promoted by AMF reported in the literature are the increase in biomass production, nutrient concentration, photosynthetic rate, efficient water use by plants [24], and increased stomatal conductance under drought conditions [25]. An increase in stomatal conductance, transpiration, and a reduction in internal CO_2 concentration in mycorrhizal plants under water deficit have also been reported, thus increasing their tolerance to these conditions [26].

There are numerous reports that demonstrate the relationships between water deficit and morpho-agronomic, physiological, biochemical, and molecular responses in various economically important species. However, there are few studies that highlight the relationship between water deficit and species of the genus *Passiflora*. In this case, the biometric, physiological, and anatomical responses to water deficit depend on the *Passiflora* species, with some exhibiting quicker responses (e.g., *P. edulis*), while others respond more slowly (e.g., *P. cincinnata*) [27]. Additionally, Lozano-Montaña et al. [28] further define the evasive

character of *P. edulis* by avoiding water loss through stomatal closure, growth modulation, accumulation of proline and sugars, and promotion of root growth.

Recently, Qi et al. [29] reported that *P. edulis* plants subjected to water deficit exhibit reductions in fresh weight and chlorophyll concentration, alongside increased activity of peroxidase, superoxide dismutase, and catalase enzymes, as well as elevated levels of total proteins, proline, and malondialdehyde (MDA) to counteract the adverse effects of water deficit. Lozano-Montaña et al. [28] and Song et al. [30] demonstrated that the genes *DREB2A*, *RD21A*, *GOLS1*, *AFL1*, and *TIP3-2*, all involved in drought tolerance, are overexpressed in *P. edulis* under both severe and moderate water deficit conditions. This upregulation leads to the regulation of other genes directly involved in the biosynthesis of ABA, ethylene, and methyl jasmonate [31], as well as the production of osmoprotectors [32] and water transport and osmoregulation [14]. On the other hand, there are no studies involving genes related to water deficit tolerance in *P. cincinnata* plants, despite their economic importance in the Brazilian semi-arid region, where they are valued for their drought tolerance and high market demand, contributing to local agriculture and the production of fruits and by-products such as juices and jams.

Therefore, the objective of this study was to evaluate the differences in morpho-agronomic, physiological, and gene expression responses in two contrasting accessions of *P. cincinnata* under water deficit and to understand if such responses could be related to mycorrhization. Our hypotheses are as follows: (i) *P. cincinnata* accessions with contrasting characteristics of water deficit tolerance respond differentially to water scarcity, meaning that the tolerant accession is less affected or exhibits better responses under water deficit; (ii) native AMF communities, originating from the rhizosphere of *P. cincinnata* accessions, promote greater development and responsiveness of passion fruit plants compared to non-mycorrhized plants and those inoculated with monospecific inoculum; and (iii) the most water deficit-tolerant *P. cincinnata* accession will show overexpression of genes related to water deficit tolerance, water transport, and osmoregulatory control when grown under water stress.

2. Results

2.1. Morpho-Agronomic and Physiological Descriptors of P. cincinnata with Different Inoculation Treatments Under Water Stress

2.1.1. Morpho-Agronomic Descriptors

The analysis of morpho-agronomic descriptors of the two accessions of *P. cincinnata* showed an interaction between the identity of the accession and the inoculation treatments concerning height, number of tendrils, and stem diameter (Table 1). For the number of leaves, fresh aboveground weight, and leaf area, there was an influence of all three factors evaluated independently (Table 1). Total chlorophyll showed a significant triple interaction among accession identity, inoculation treatments, and field capacity (Table 1). For each *P. cincinnata* accession, a significant double interaction was observed between field capacity and inoculation treatments. Pairwise comparisons between inoculation treatments are detailed in Table S1.

Inoculation with native AMF communities promoted greater increases in passion fruit plant height, stem diameter, and number of tendrils compared to the mono-specific inoculation (EE) and non-inoculation treatments (Control) (Figure 1A–C). Also, native AMF communities from water deficit conditions (AMF25) promoted greater development of the sensitive accession (A48) compared to the tolerant one (A01) regarding those descriptors. However, for the tolerant accession (A01), the origin of the native AMF community did not result in differences in those descriptors (Figure 1A–C). Height, stem diameter, number of

leaves, leaf area, and fresh aboveground weight showed greater increments when at field capacity above 75%.

Table 1. Three-way ANOVA and comparison of means of morphoagronomic descriptors of two accessions (A) of *P. cincinnata* under different inoculation treatments (I) with AMF in contrasting water availability conditions (FC), with the values presented in the table corresponding to the F-test results.

Morphoagronomic	ANOVA						
	A	**I**	**FC**	**A × I**	**A × FC**	**I × FC**	**A × I × FC**
Height (cm)	5.671 *	67.57 ***	17.10 ***	5.05 **	1.58 ^{ns}	1.10 ^{ns}	0.89 ^{ns}
Number of leaves	13.91 ***	77.15 ***	14.81 ***	2.67 ^{ns}	0.15 ^{ns}	1.67 ^{ns}	0.70 ^{ns}
Number of tendrils	21.30 ***	2.86 ^{ns}	0.37 ^{ns}	5.32 *	2.36 ^{ns}	0.59 ^{ns}	0.59 ^{ns}
Stem diameter (mm)	0.056 ^{ns}	63.94 ***	15.23 ***	3.28 *	0.03 ^{ns}	0.56 ^{ns}	2.57 ^{ns}
Total Chlorophyll (a + b)	5.17 *	11.68 ***	6.03 *	4.15 **	0.11 ^{ns}	10.33 ***	2.88 *
Fresh weight (aboveground part) (g)	5.79 *	90.43 ***	63.30 **	1.65 ^{ns}	0.07 ^{ns}	1.35 ^{ns}	1.60 ^{ns}
Fresh weight (root) (g)	2.69 ^{ns}	39.17 ***	5.08 ^{ns}	4.37 ^{ns}	8.34 ^{ns}	3.49 ^{ns}	2.53 ^{ns}
Leaf area (cm^2)	14.43 ***	101.07 ***	83.35 ***	1.14 ^{ns}	0.51 ^{ns}	0.36 ^{ns}	1.12 ^{ns}

^{ns}, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

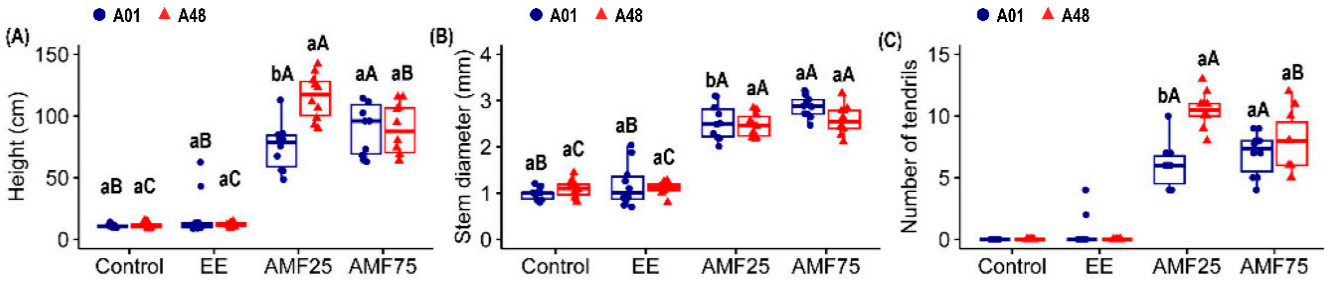


Figure 1. Growth of passion fruit plants cultivated under two irrigation regimes (up to 25% of field capacity and 75–100% of field capacity) in greenhouse. (**A**) Height, (**B**) stem diameter, and (**C**) number of tendrils in tolerant (A01—blue color) and sensitive (A48—red color) passion fruit accessions, either uninoculated (Control) or inoculated with *E. etunicata* (EE), native AMF communities from soil under water deficit (AMF25) or without water deficit (AMF75). In each boxplot, data points represent n = 10 samples from the two-way ANOVA interaction between accession and inoculation. Different letters denote significant differences between treatments, as determined by Tukey's test ($p < 0.05$). Lowercase letters compare accessions within each treatment, and uppercase letters compare inoculation treatments within each accession.

Accessions inoculated with native AMF communities showed a higher number of leaves, leaf area, and fresh aboveground and root weight compared to non-mycorrhized plants or those inoculated with *E. etunicata* (EE) (Figure 2A–D). The sensitive accession (A48) presented a higher leaf count than the tolerant accession (A01) (Figure 2E). This trend was similar for leaf area (Figure 2F) and shoot fresh weight (Figure 2G).

For the tolerant accession (A01), inoculation with native AMF communities equally increased total chlorophyll under conditions of low water availability (i.e., field capacity < 25%) but differed from both the control and the mono-specific inoculation treatments with *E. etunicata* (EE) (Table S1; Figure 3). It was also observed that under field capacity < 25%, mono-specific inoculation with *E. etunicata* in the tolerant accession (A01) resulted in a lower concentration of total chlorophyll compared to the control. In contrast, inoculation with *E. etunicata* in the sensitive accession (A48) resulted in a total chlorophyll concentration similar to that found in plants mycorrhized by native AMF communities but differed from the control treatment (Table S1; Figure 3). Overall, increased water availability led to an

increase in total chlorophyll concentration in *P. cincinnata* plants, except in the treatments with the native AMF community AMF25 in the tolerant accession (A01) and the *E. etunicata* in the sensitive accession (A48) (Table S1; Figure 3).

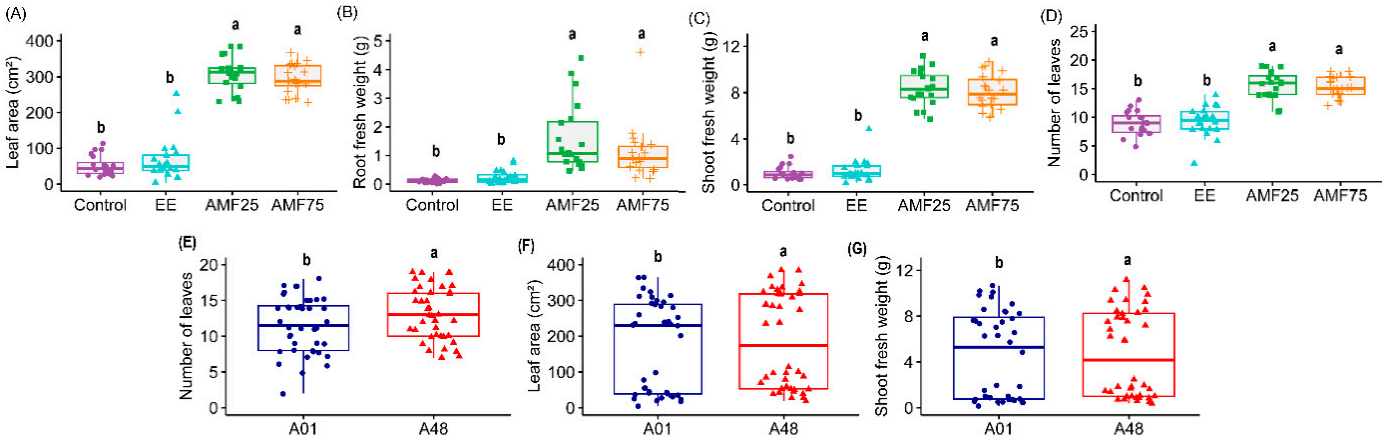


Figure 2. Comparison of morpho-agronomic variables in passion fruit accessions using a one-way ANOVA. (**A**–**D**) Growth of plants uninoculated (Control) or inoculated with *E. etunicata* (EE) or with native AMF communities from soil under water deficit (AMF25) or without water deficit (AMF75). (**E**–**G**) Differences between tolerant (A01) and sensitive (A48) passion fruit accessions. In each boxplot, data points represent n = 20 samples for inoculation and n = 40 for accession, based on one-way ANOVA. Different letters denote significant differences between treatments, as determined by Tukey's test ($p < 0.05$).

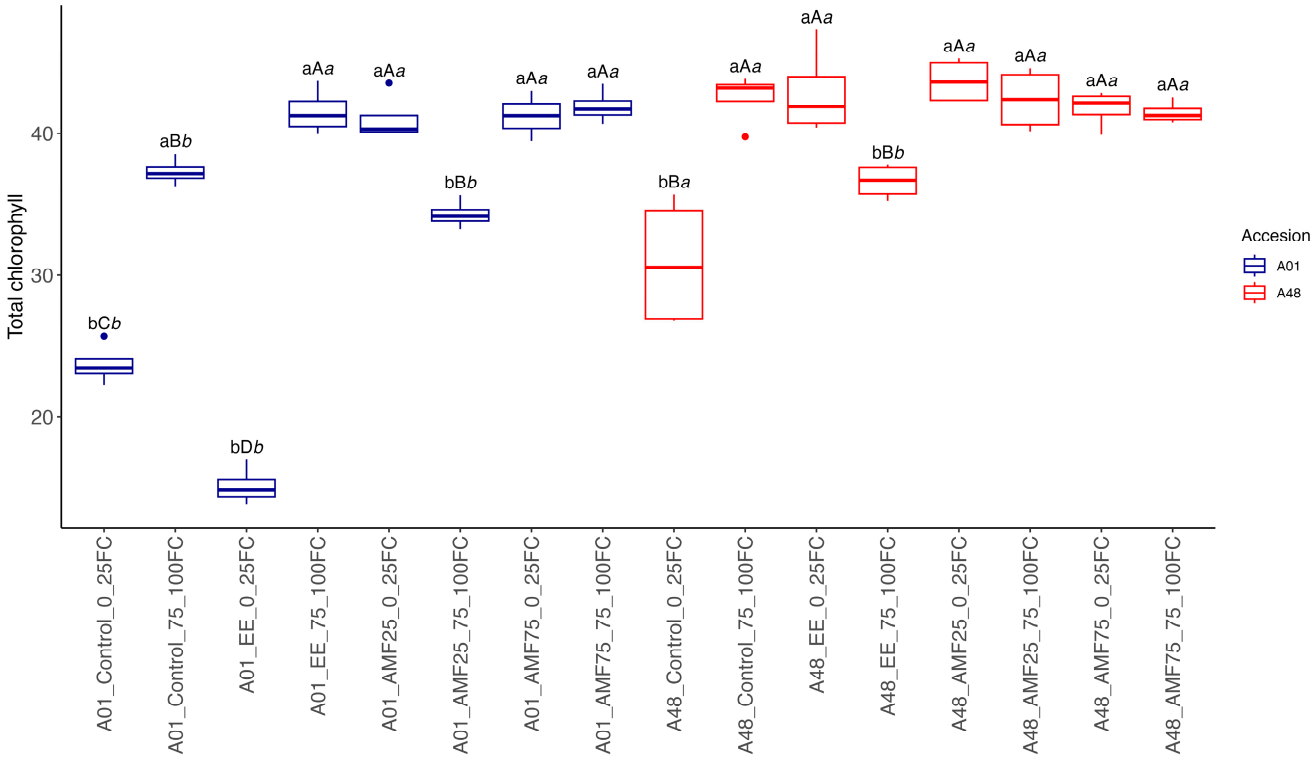


Figure 3. Graph showing total chlorophyll content in two passion fruit accessions: tolerant (A01) and sensitive (A48). Plants were inoculated with three different types of arbuscular mycorrhizal fungi (AMF): *E. etunicata* (EE), AMF communities from soil subjected to water deficit (AMF25), and AMF communities from soil without water deficit (AMF75). Irrigation conditions were applied to maintain

either 25% field capacity (low) or 75–100% field capacity (high). Each boxplot represents data from $n = 5$ samples and illustrates the three-way interaction between accession, field capacity, and inoculation, as determined by ANOVA analysis. Different letters denote significant differences between treatments, as determined by Tukey's test ($p < 0.05$). Lowercase letters compare field capacity within each access individually; uppercase letters compare the interaction between field capacity and inoculation within each access; italicized lowercase letters compare the interaction between field capacity and inoculation between contrasting accesses.

2.1.2. Physiological Descriptors

Among the physiological descriptors evaluated, photosynthesis showed significant differences for all three factors evaluated independently (Table 2), with the tolerant accession (A01) exhibiting a higher photosynthetic rate compared to the sensitive accession (A48) (Figure 4B). Accessions inoculated with native AMF communities from non-water-deficit soil (AMF75) showed higher photosynthetic rates compared to both the control and the *E. etunicata* (EE) inoculated treatments, with no statistical difference from the treatment inoculated with native AMF communities from soil with water deficit (AMF25) (Figure 4A). There was a higher photosynthetic rate at field capacity above 75% compared to irrigation below 25% of field capacity for both accessions.

Table 2. Three-way ANOVA and comparison of means of physiological descriptors of two accessions (A) of *P. cincinnata* under different inoculation treatments (I) with AMF in contrasting water availability conditions (FC), with the values presented in the table corresponding to the F-test results.

Physiological	**ANOVA**						
	A	**I**	**FC**	**A × I**	**A × FC**	**I × FC**	**A × I × FC**
Photosynthetic rate	8.29 **	5.95 **	5.87 *	0.97 ^{ns}	1.94 ^{ns}	0.55 ^{ns}	0.53 ^{ns}
Stomatal conductance	0.69 ^{ns}	0.46 ^{ns}	5.73 *	1.05 ^{ns}	0.001 ^{ns}	1.85 ^{ns}	1.59 ^{ns}
Transpiration	0.02 ^{ns}	0.14 ^{ns}	4.29 *	1.02 ^{ns}	0.43 ^{ns}	0.16 ^{ns}	1.04 ^{ns}
Leaf temperature	0.45 ^{ns}	0.50 ^{ns}	0.001 ^{ns}	0.23 ^{ns}	0.49 ^{ns}	0.93 ^{ns}	1.29 ^{ns}
WUE	4.57 *	7.28 ***	0.48 ^{ns}	0.35 ^{ns}	0.97 ^{ns}	0.66 ^{ns}	0.96 ^{ns}
iWUE	4.05 ***	9.15 ***	9.37 **	0.33 ^{ns}	1.60 ^{ns}	0.45 ^{ns}	0.42 ^{ns}

^{ns}, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Stomatal conductance and transpiration were influenced only by the field capacity factor, showing higher rates when the field capacity was above 75%. Leaf temperature did not show significant differences among the evaluated factors (Table 2).

Water use efficiency (Photosynthesis/Transpiration) (WUE) and intrinsic water use efficiency (Photosynthesis/Stomatal conductance) (iWUE) showed differences both between accessions and among the inoculation treatments used (Table 2). The tolerant accession (A01) exhibited higher WUE and iWUE values compared to the sensitive accession (A48) (Figure 4D,F), indicating that inoculation with native AMF communities favored both water use efficiency ratios (WUE and iWUE) compared to the control treatment and inoculation with *E. etunicata* (EE) (Figure 4C,E).

2.1.3. PCA Analysis of Morpho-Agronomic and Physiological Descriptors

The PCA analysis of morpho-agronomic and physiological descriptors shows that 63.9% of the variation is explained by the two axes, and there is a clear separation between two main groups formed by the contribution of the variables leaf area, fresh shoot weight, height, stem diameter, number of tendrils, and number of leaves. The first group involves the accessions inoculated with native AMF communities from both water deficit (AMF25) and non-water deficit (AMF75) conditions, showing a stronger association with the growth descriptors. The second group is more closely linked to accessions that were either not

inoculated (Control) or inoculated with *E. etunicata* (EE), exhibiting negative correlations with most of the assessed descriptors (Figure 5).

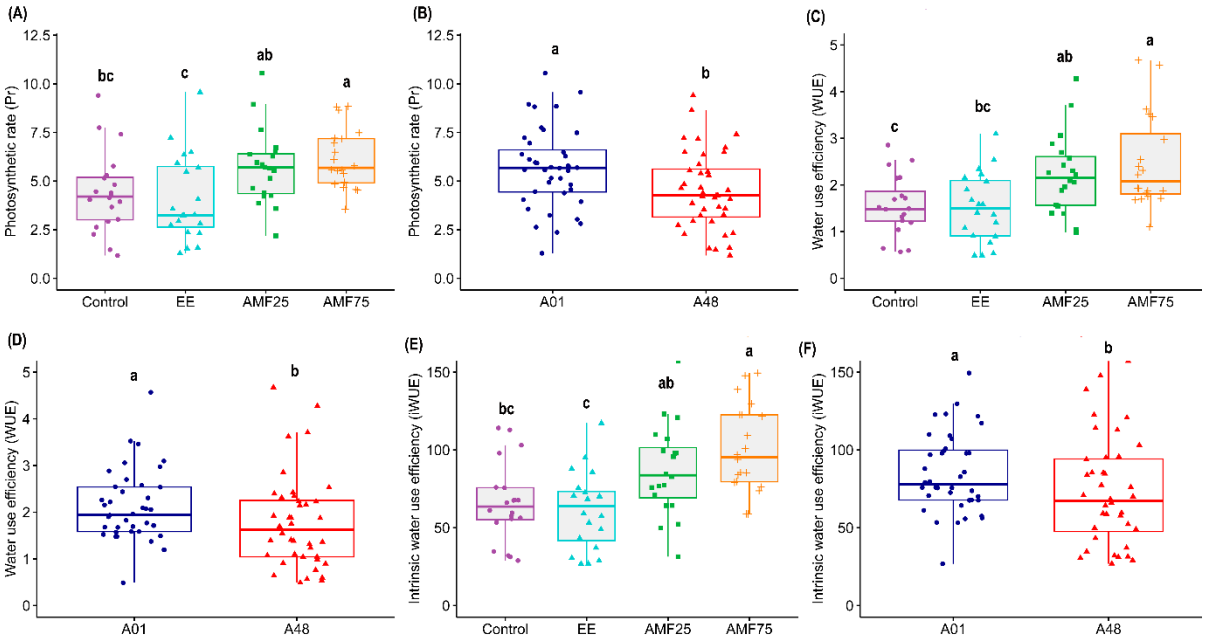


Figure 4. Physiological status of passion fruit plants inoculated or non-inoculated with arbuscular mycorrhizal fungi (AMF), cultivated under two irrigation conditions: 25% field capacity (low) and 75–100% field capacity (high). (**A**,**C**,**E**) Water use efficiency, intrinsic water use efficiency, and photosynthetic rate in plants not inoculated (Control) or inoculated with *E. etunicata* (EE) or AMF communities from soil under water deficit (AMF25) or without water deficit (AMF75). (**B**,**D**,**F**) The same physiological parameters in tolerant (A01) and sensitive (A48) passion fruit accessions. In each boxplot, data points represent $n = 20$ samples for inoculation and $n = 40$ samples for accession, based on one-way ANOVA. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

2.1.4. Relative Mycorrhizal Responsiveness

The analysis of relative mycorrhizal responsiveness based on height, leaf area, fresh aboveground weight, and root biomass showed that *P. cincinnata* accessions benefited from AMF inoculation (Table 3). Overall, the tolerant accession (A01) showed higher mycorrhizal responsiveness compared to the sensitive accession (A48), with average values of 784.3 and 455%, respectively.

Table 3. Relative mycorrhizal responsiveness (%) of *P. cincinnata* to inoculation with monospecific AMF and with a pool of native community from cultivation with (<25%) and without (>75%) water deficit.

Accession	Inoculation	Height	Leaf Area	Shoot Fresh Biomass	Root Fresh Biomass
01	EE	12.71	81.57	50.94	192.3
	C25	586.60	671.38	1102.76	2119.9
	C75	790.46	680.8	1131.8	1990.9
48	EE	10.09	−13.38	−12.35	−38
	C25	987.54	479.57	769.92	1095.3
	C75	719.5	429.07	679.6	352.36

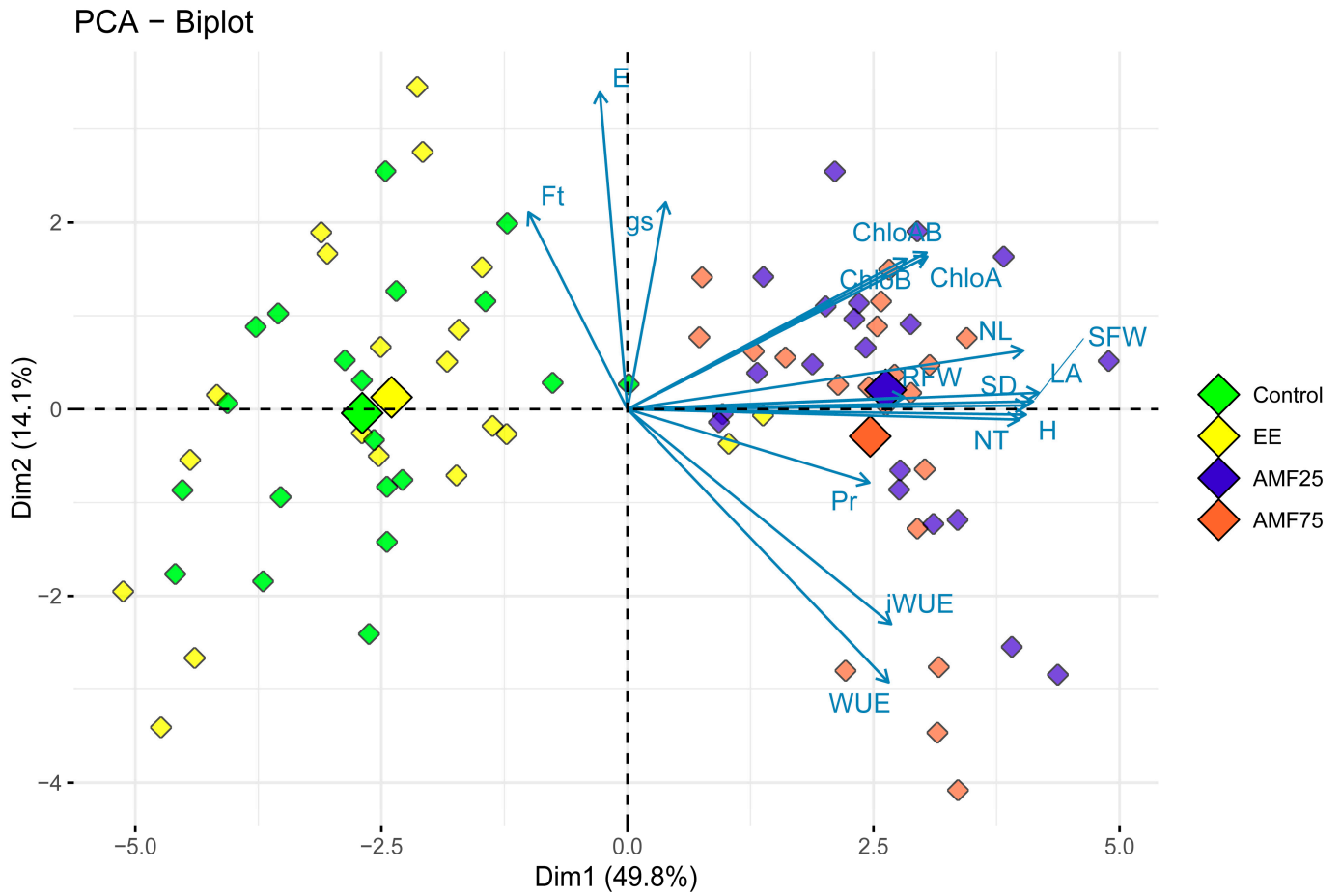


Figure 5. PCA of morpho-agronomic and physiological descriptors across different inoculation treatments: Control (non-inoculated), *E. etunicata* inoculation (EE), AMF communities from soil under water deficit conditions (AMF25), and AMF communities from soil under irrigated conditions (AMF75). Photosynthesis rate (Pr), Stomatal conductance (gs), Transpiration rate (E), Leaf temperature (Lt), Height (H), Number of leaves (NL), Number of tendrils (NT), Stem diameter (SD), Chlorophyll a (ChloA), Chlorophyll b (ChloB), Total Chlorophyll (ChloAB), Shoot Fresh Weight (SFW), Root Fresh Weight (RFW), Leaf area (LA), Water-use efficiency (WUE), and Intrinsic water-use efficiency (iWUE).

This benefit in the development of *P. cincinnata* accessions may also vary depending on the inoculum used, as accessions inoculated with native AMF communities, derived from conditions with (AMF25) and without (AMF75) water deficit, showed high mycorrhizal responsiveness, ranging from 352.36 to 2119.9% (Table 3).

On the other hand, inoculation with *E. etunicata* (EE) resulted in negative values of mycorrhizal responsiveness, ranging from −38 to 10.09% for the sensitive accession (A48), whereas the tolerant accession (A01) showed mycorrhizal responsiveness values ranging from 12.71 to 193.3% (Table 3).

2.2. Gene Expression, Colonization, and Mycorrhizal Abundance of P. cincinnata Inoculated with AMF Under Water Deficit Condition

2.2.1. Differential Gene Expression in *P. cincinnata* Accessions Inoculated with Native AMF Communities from Water Deficit Conditions (AMF25)

Gene expression analysis revealed contrasting patterns between *P. cincinnata* accessions for the genes *PcbZIP*, *PcSIP*, and *PcSTK*. Comparison between water deficit (<25% field capacity) and control (>75% field capacity) conditions showed that in the tolerant accession (A01), the genes *PcbZIP*, *PcSIP*, and *PcSTK* were up-regulated, respectively, 2.43, 2.28, and 2.25 times under water deficit conditions (Table 4 and Table S2). On the other hand, in

the sensitive accession (A48), the genes *PcSIP* and *PcSTK* were down-regulated 0.53 and 0.72 times, respectively (Tables 4 and S3). The genes *PcCAT*, *PcLEA*, and *PcSOD* showed constitutive expression in both accessions under water deficit (Tables 4, S2 and S3).

Table 4. Relative gene expression of *PcbZIP*, *PcCAT*, *PcLEA*, *PcSIP*, *PcSOD*, and *PcSTK* in accessions A01 (tolerant) and A48 (sensitive) of *P. cincinnata*. A comparison was made between treatments subjected to <25% field capacity (treatment) and >75% field capacity (control) within each accession. Data were analyzed using qPCR, with 3 biological replicates (n = 3) and 3 technical replicates (n = 3) per treatment.

Genes	Accession	Relative Expression	Std. Error	95% Confidence Interval	p	Result
*Pc*bZIP	A01	2.433	1.613–3.598	1.261–5.521	0.001 ***	Up-regulated
*Pc*CAT	A01	1.292	0.304–3.329	0.239–4.837	0.469 ^{ns}	Constitutive
*Pc*LEA	A01	1.064	0.659–1.922	0.360–2.355	0.742 ^{ns}	Constitutive
*Pc*SIP	A01	2.278	0.762–6.489	0.276–7.974	0.04 *	Up-regulated
*Pc*SOD	A01	0.97	0.657–1.283	0.525–1.664	0.769 ^{ns}	Constitutive
*Pc*STK	A01	2.252	1.634–3.225	1.091–4.098	0.001 ***	Up-regulated
*Pc*bZIP	A48	1.672	0.289–6.867	0.184–49.351	0.371 ^{ns}	Constitutive
*Pc*CAT	A48	0.502	0.160–1.357	0.082–1.699	0.052 ^{ns}	Constitutive
*Pc*LEA	A48	0.699	0.360–1.427	0.311–1.860	0.078 ^{ns}	Constitutive
*Pc*SIP	A48	0.531	0.276–1.260	0.170–1.368	0.014 *	Down-regulated
*Pc*SOD	A48	1.004	0.677–1.513	0.434–1.972	0.985 ^{ns}	Constitutive
*Pc*STK	A48	0.724	0.594–0.856	0.548–1.029	0.001 ***	Down-regulated

^{ns}, *, *** Nonsignificant or significant at $p \leq 0.05$, and 0.001, respectively.

2.2.2. Mycorrhizal Colonization in *P. cincinnata* Accessions Inoculated with AMF Communities from Water Deficit Conditions (AMF25)

The intensity of mycorrhizal colonization (M%) did not show significant differences in field capacity (F: 0.585; p: 0.466) and interaction between the factors (F: 2.02; p: 0.19). Similarly, mycorrhizal frequency (F%) did not show differences in field capacities (F: 0.13; p: 0.721) and interaction between the factors (F: 0.42; p: 0.413). However, there was a significant difference between passion fruit accessions for the intensity of mycorrhizal colonization M (F: 19.177; p: 0.002) and for mycorrhizal frequency (F) (F: 5.46; p: 0.047), with the tolerant accession (A01) displaying higher frequency and intensity of mycorrhizal colonization compared to the sensitive one (A48) (Figure 6A,B).

There was an interaction between the factors for both the intensity of arbuscules per root fragment (a%) (F = 25.50; p = 0.0009) (Figure 6C) and the intensity of arbuscules in the root system (A%) (F = 30.348; p = 0.0005) (Figure 6D). There was a contrasting response in arbuscule formation between the passion fruit accessions, as the tolerant accession (A01) showed an increase in arbuscules under water deficit conditions (up to 25% FC), while the sensitive accession (A48) only showed an increase under non-water deficit conditions (>75% FC) (Figure 6C,D). It is worth noting that although the native inoculated AMF community (AMF25) originated from conditions under water deficit, the increment provided in arbuscular formation occurred only in the tolerant accession (A01).

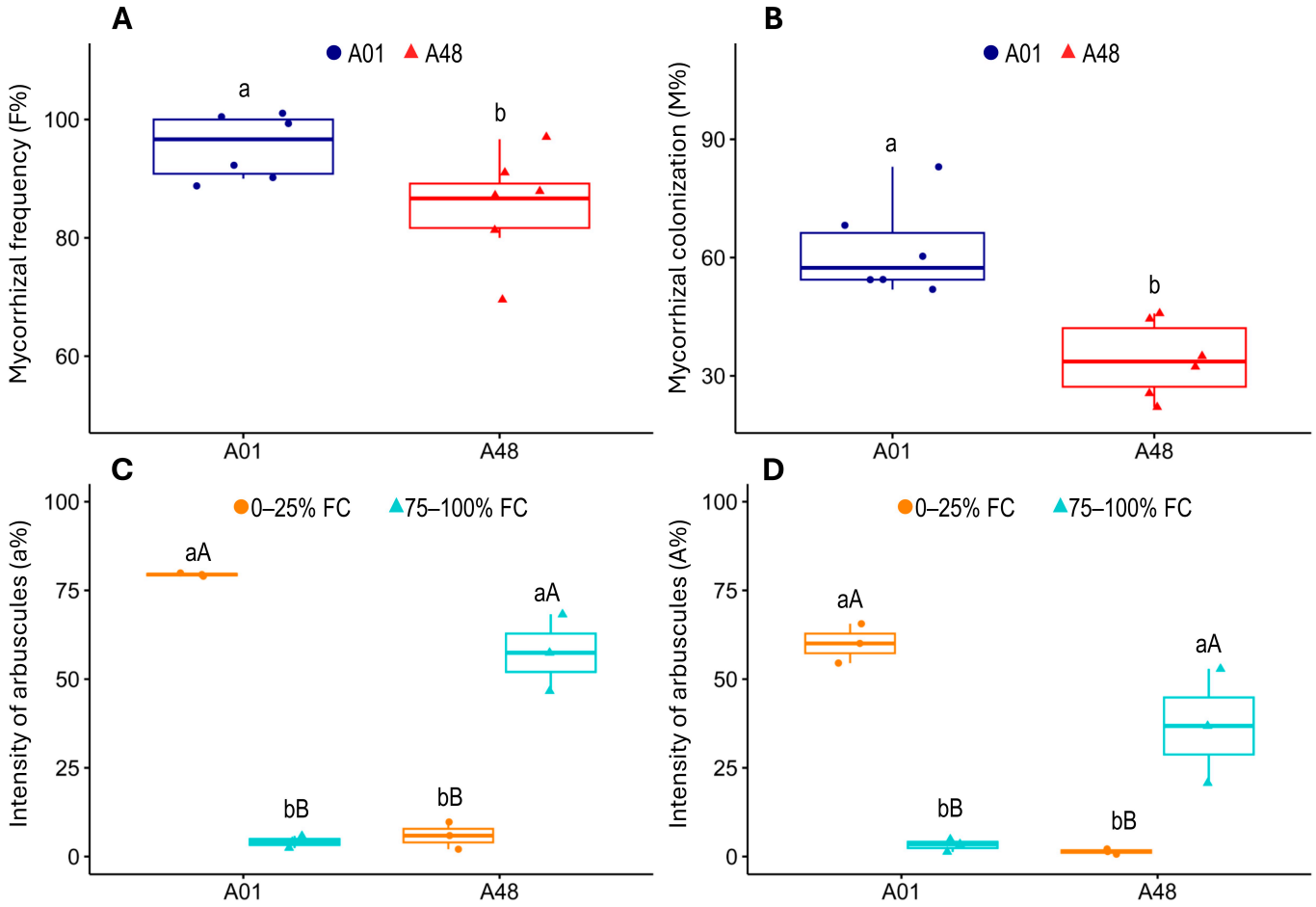
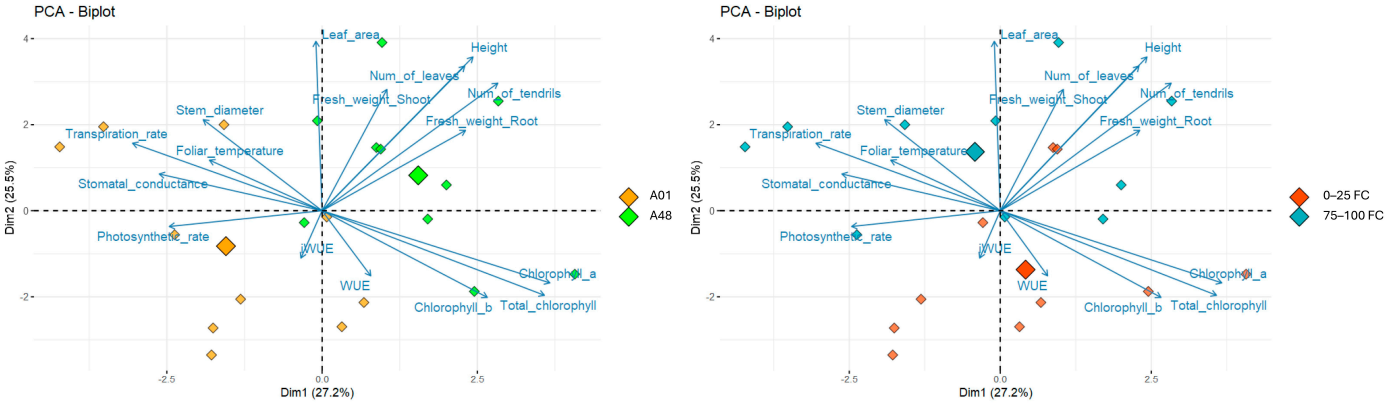


Figure 6. Mycorrhizal colonization of *P. cincinnata* accessions inoculated with AMF communities from the C25 water deficit condition under contrasting water availability conditions. Mycorrhizal frequency (**A**), mycorrhizal colonization intensity (**B**), intensity of arbuscules per root fragment (**C**), and intensity of arbuscules in the root system (**D**) are expressed as percentages. Lowercase letters indicate significant differences within accessions, while uppercase letters denote differences between accessions. In Figures (**C**,**D**), orange represents <25% field capacity, and ciano represents > 75% field capacity.

2.2.3. PCA in *P. cincinnata* Accessions Inoculated with AMF Communities from Water Deficit Conditions (AMF25)

The PCA analysis of morpho-agronomic and physiological descriptors using only the native AMF communities from water-deficit conditions (AMF25) showed that 52.7% of the variation is explained by two axes. It clearly separates the accessions, with the sensitive accession (A48) associated with most morphological descriptors, while the tolerant accession (A01) is more related to physiological descriptors (Figure 7A). Between the two water availability conditions, it was observed that the treatment with field capacity > 75% showed a positive correlation with most evaluated descriptors, except for total chlorophyll (a + b), which was more related to field capacity < 25% (Figure 7B).



(**A**) (**B**)

Figure 7. PCA of morpho-agronomic and physiological descriptors among different *P. cincinnata* accessions: A01 (tolerant) and A48 (sensitive) to water deficit (**A**), and under two irrigation conditions (0–25% field capacity and 75–100% field capacity) (**B**), using samples from the treatment with native AMF communities from soil under water deficit (AMF25). This analysis includes treatments evaluated in the differential gene expression study.

2.2.4. Glomerospore Abundance and Native AMF Communities in *P. cincinnata* Accessions Inoculated with AMF Communities from Water Deficit Conditions (AMF25)

The number of glomerospores did not show significant differences between irrigated conditions (FC > 75%) and water deficit conditions (FC < 25%) for the two passion fruit accessions (sensitive [A48] = F: 6.11; $p > 0.05$; tolerant [A01] = F: 3.26; $p > 0.05$). Similarly, there was no difference in AMF richness, Shannon diversity, Pielou's evenness, and Simpson dominance between the rhizospheres of the tolerant (A01) and sensitive (A48) accessions. PERMANOVA analysis showed that the AMF communities were not influenced by either the accessions (R^2: 0.07; F: 0.81; $p > 0.05$) or the field capacities (R^2: 0.11; F: 1.19; $p > 0.05$) used in this comparison.

3. Discussion

3.1. Morpho-Agronomic and Physiological Descriptors of P. cincinnata Inoculated with AMF or Not, Under Contrasting Water Availability Conditions

The hypothesis that *P. cincinnata* accessions with contrasting drought tolerance characteristics respond differently to water deficit—specifically that the tolerant accession is less affected or shows better responses under water deficit—was partially confirmed in this study. We found that the sensitive accession achieved greater increases in the number of leaves, leaf area, and fresh shoot biomass, while the tolerant accession exhibited higher values for photosynthesis, WUE, and iWUE. Several reports have documented different responses between tolerant and sensitive genotypes under water stress. For example, studies on *Coffea arabica* [33] and *Musa acuminata* [34] highlighted distinct morpho-agronomic responses associated with these contrasting genotypes. However, our study did not reveal significant morpho-agronomic differences among *P. cincinnata* accessions. It is important to note that there is genetic variability among the accessions of *P. cincinnata*, as confirmed by [35]. The lack of significant differences in morpho-agronomic variables has also been observed by [36] in their comparative analysis of bean (*Phaseolus vulgaris* L.) genotypes that are tolerant and sensitive to water stress. Nevertheless, tolerant genotypes of *P. vulgaris* demonstrated better physiological efficiency compared to sensitive ones, which aligns with the results obtained in *P. cincinnata*.

Geographic differentiation, natural selection, and the species' history of regional adaptation are known to drive genetic diversity, as demonstrated by [37] in *Camelina sativa*

and [38] in *Sorghum bicolor*, using SNP markers. Similarly, Qahtan et al. [39] employed ISSR markers to assess genetic diversity in *Vicia faba* while underscoring the importance of integrating additional descriptors, such as morpho-agronomic traits. Furthermore, Dantas et al. [40] investigated *P. cincinnata* accessions through select morpho-agronomic descriptors and identified distinct responses under water deficit conditions.

Responses to water deficit vary in plants, especially in arid environments, where they may exhibit escape (evasive) strategies, tolerance, or both, depending on the species [41]. In *P. edulis*, water deficit resulted in decreased height, leaf area, fresh shoot biomass, total chlorophyll, and stomatal conductance, suggesting an evasive strategy by this species in response to water stress [28,29]. Our results showed a similar pattern for *P. cincinnata*, particularly regarding height, leaf area, stem diameter, number of leaves, fresh shoot weight, photosynthetic rate, stomatal conductance, and transpiration, with reductions in these descriptors regardless of accession identity. Additionally, Valladares et al. [42], in a review on ecophysiology and scales of drought responses, reported that leaf drop and reduced leaf quantity in *P. edulis* are responses to water deficit, characteristics also observed in *P. cincinnata* as they reduce the number of leaves under low water availability conditions.

In general, physiological descriptors are negatively impacted by water deficit. However, tolerant genotypes may exhibit a smaller reduction in stomatal conductance, photosynthetic rate, and transpiration. They also tend to maintain a balanced water relationship in the plant, including lower water consumption, as observed in *Cicer arietinum* [43].

In the present study, the tolerant *P. cincinnata* accession exhibited a higher photosynthetic rate and more efficient water use under water deficit compared to the sensitive accession, suggesting that these factors may be associated with the accession's tolerance. Additionally, the water consumption relationship, quantified by water use efficiency and intrinsic water use efficiency, indicated a greater adaptation to water deficit in the tolerant accession compared to the sensitive one, as seen by [44] in *Oryza sativa*, and higher photosynthesis ability by [45] in *Vitis* cultivars. Furthermore, according to Conti et al. [46], better functioning of the photosynthetic apparatus, based on Fv/Fm ratios (variable fluorescence by maximum fluorescence) and performance indices, would explain the tolerance of *Solanum lycopersicum* genotypes to water deficit.

In our study, both *P. cincinnata* accessions behaved similarly in terms of stomatal conductance and transpiration rate, being more influenced by water availability. A similar pattern was observed in *Eucalyptus globulus*, where the genotypes showed similar behavior concerning physiological descriptors (predawn leaf water potential, conductance, and maximum quantum yield of PSII), being more influenced by water availability, especially when the water deficit was more severe, with irrigation deprivation reaching −1.5 to −1.8 MPa [47].

The second hypothesis that native AMF communities, originating from the rhizosphere of *P. cincinnata* accessions, promote greater development and responsiveness of passion fruit plants compared to non-mycorrhized plants and those inoculated with monospecific inoculum, respectively, was confirmed in the present study.

The treatments with inoculation of native AMF communities reinforce the importance of this association for *P. cincinnata*, as most morpho-agronomic and physiological descriptors were influenced by the association. The discrepancy in these descriptors between treatments with and without AMF resulted in high relative mycorrhizal responsiveness, especially with the use of native AMF communities originating from cultivation with *P. cincinnata*, a fact that was not observed with the use of *E. etunicata*, even though it is an isolate from the rhizosphere of *P. edulis.*

The efficiency of native AMF in promoting increased growth and flower quality under water deficit was demonstrated in *Rosa damascena*, and such improvements are related

to the increase in water relations and photosynthetic status, leading to the tolerance of the species to water deficit [48]. Other plant species also benefited from inoculation with native AMF under water deficit conditions, as reported by [49], who found higher growth of quinoa (*Chenopodium quinoa* Willd.) with the use of native AMF communities isolated from the rhizosphere of *Phoenix dactylifera*, suggesting that the activation of photosynthetic mechanisms and antioxidant enzyme activity in leaves and roots would be related to the benefits provided by AMF. In *Ceratonia siliqua*, native AMF communities, with or without the addition of biological residues, were also efficient in promoting growth and improvement in biochemical and physiological descriptors, leading to increased plant tolerance to water deficit [50]. In our study, the native AMF communities (AMF75 and AMF25) were able to promote greater benefit in many morpho-agronomic and physiological descriptors, forming a distinct grouping from the treatments not inoculated or inoculated only with *E. etunicata*, demonstrating that the choice of AMF isolates or communities can be decisive to bring the expected benefits of the inoculum application of these fungi.

Greater responsiveness of *P. cincinnata* to inoculation with AMF, especially with the use of AMF species native to the Caatinga, may be due to intrinsic factors of the species being naturally responsive to AMF, even under optimal P conditions [51]. Furthermore, a greater benefit of AMF communities from water deficit conditions was observed in the sensitive accession, compared to the similarity of responses of the tolerant accession to inoculation with both communities used.

The importance of using native AMF groups as an inoculant was confirmed by [52] when comparing commercial and native AMF inoculants in the cultivation of *Hordeum vulgare* spp. *nudum.* These authors demonstrated that inoculation with native AMF mitigated the negative effects of water deficit, enhancing growth and biochemical and physiological responses. They attributed these improvements to the intensity of extraradicular colonization and better soil exploration, suggesting the use of native AMF species as a sustainable technology for promoting plant growth in arid and semi-arid regions. These results highlighted the importance of prospecting native AMF as a strategy to enhance plant tolerance to water deficit, as suggested by [49] for quinoa cultivation. Considering that water limitation is one of the main factors reducing field production, the increase in biomass and the biochemical and physiological mechanisms provided by AMF, even under water deficit, could be crucial for a drought resilience strategy.

Another aspect that must be considered is the responsiveness of plants to mycorrhization. Clearly, there is a high responsiveness of *P. cincinnata* to mycorrhization, especially with the use of a diverse native AMF community. In this study, we demonstrated that the tolerant accession (A01) showed higher responsiveness compared to the sensitive accession (A48), particularly when compared to the monospecific inoculation with *E. etunicata*. Among the limited studies on responsiveness in *Passiflora*, the most studied species has been *P. edulis*. Soares and Martins [53] demonstrated that *P. edulis* f. *flavicarpa* showed a mycorrhizal dependency of over 400%, with control plants displaying signs of deficiency and low biomass increment. Cavalcante et al. [54] found higher responsiveness of *P. edulis* f. *flavicarpa* in soils with low phosphorus content (<11 mg/dm^3), while [55] reported an 80% mycorrhizal dependency of *P. edulis* f. *edulis* under cultivation with a maximum phosphorus concentration of 0.02 mg L^{-1}. Other studies have found that different *Passiflora* species also show mycorrhizal responsiveness, such as *P. alata* cultivated in soils with low phosphorus content [56] and *P. setacea* with dry biomass production in mycorrhizal plants superior to that found with phosphorus fertilization, suggesting a reduction in the use of this element [57]. However, it is known that the responsiveness of plants to mycorrhization is influenced by the soil moisture history in successive droughts and water availability [58] or by the genotype [59,60].

Studies with different genotypes of *Phaseolus vulgaris* [61], *Capsicum annuum* [62], *Allium cepa* [63], and *Sorghum bicolor* [64] demonstrate that the responses of genotypes differ in mycorrhizal responsiveness, a fact also observed among *P. cincinnata* accessions. In addition to plant genotype, responsiveness to AMF is also influenced by other biotic factors [65], such as the choice of isolates or composition of the AMF inoculum.

The mycorrhizal responsiveness of *Zea mays* genotypes has been reported by [59,60], who demonstrated that maize responsiveness to AMF should be considered in breeding programs, especially in the search for varieties more resilient in nutrient-deficient soils, and that this responsiveness is genotype-dependent. However, studies on mycorrhizal responsiveness with accessions of a plant species are still scarce, although they provide important information in the characterization of material kept in Active Germplasm Banks, especially regarding the generation of technology to produce mycorrhized seedlings.

3.2. Differential Gene Expression in P. cincinnata Inoculated with AMF Communities Originated from Water Deficit Conditions

The hypothesis that the drought-tolerant *P. cincinnata* accession would exhibit overexpression of genes related to drought tolerance was confirmed, as the influence of water availability on the upregulation of *PcbZIP*, *PcSIP,* and *PcSTK* genes was observed exclusively in the tolerant accession. The importance of prospecting genes related to drought tolerance in *P. cincinnata* is essential for the cultivation and production in areas increasingly impacted by water scarcity.

Some families of transcription factors have been studied under water deficit conditions, such as bZIP, one of the targets of our study. Soleimani et al. [66] demonstrated the induction of bZIP genes in leaves and roots of drought-tolerant soybean (*Glycine max*) genotypes subjected to irrigation suppression. Tu et al. [67] showed that the *VlbZIP36* gene from *Vitis* spp. plays a role in drought tolerance by improving water status by limiting water loss and mitigating cellular damage in transgenic *Arabidopsis thaliana* lines. The authors concluded that *Vl*bZIP36 enhanced drought tolerance through the transcriptional regulation of stress- and ABA-related genes. Other authors also reported the overexpression of bZIP genes in different species and cultivation conditions, such as *Solanum tuberosum* under drought stress, mainly relating to the regulation of ABA-dependent stress-signaling pathways [68] in *Panax ginseng,* playing an important role in the species' response to water deficit [69]; in *Chrysanthemum grandiflora,* under saline stress and water deficit due to irrigation suppression, being associated with tolerance to both abiotic factors [70], and in *Dendrobium catenatum,* in response to drought stress [71]. Thus, it can be inferred that the exclusive overexpression of *PcbZIP* in the tolerant accession of *P. cincinnata* indicates its involvement in the species' mechanisms of tolerance to water deficit.

In addition to the bZIP transcription factors, genes encoding oxidoreductase proteins and those related to carbohydrate metabolism and osmotic adjustment, such as LEA proteins and aquaporins (AQPs), were also evaluated in our study. We observed that the expression of the AQP *PcSIP* was contrasting between the two *P. cincinnata* accessions; while its expression was reduced in the sensitive accession, induction was observed in the tolerant accession, suggesting that the overexpression of *PcSIP* may increase drought tolerance in *P. cincinnata*. Some studies report the involvement of AQPs in the adaptation of plant species to water stress, such as that of [72], who reported significant positive correlations between AQP transcript levels and seed water content and proposed that AQPs likely play an important role in mediating water accumulation and outer seed coating in *P. granatum.* He et al. [73] also associated the upregulation of genes *PIP* and *TIP* to alleviate a reduction in stomatic and mesophyll conductance in *Oryza sativa*.

In *P. edulis*, Song et al. [30] evaluated the *NIP*, *PIP*, *SIP,* and *TIP* genes from the AQP family under different abiotic stresses, such as drought (50 and 10% FC), salinity (300 mM

NaCl on different days), freezing (0 °C for 24 and 48 h), and temperature (45 °C for 2, 4 and 24 h), and most AQPs were induced by water deficit, including *SIP*, suggesting that these AQPs can respond to various abiotic stresses, including drought, corroborating the results obtained in our study with *P. cincinnata*.

Different expression patterns have been reported for *LEA* genes in plants. The overexpression of these genes in tolerant genotypes of *Gossypium* subjected to water deficit through irrigation suppression was reported by [74], suggesting that such genotypes had a greater capacity to modulate *LEA* expression under drought conditions. In *Oryza sativa*, differences in *LEA* gene expression related to genotype identity, or the type of *LEA* gene analyzed, with some genes being induced while others maintained constitutive expression under drought stress tolerance, were observed by [75]. Similar results were found by [76], who analyzed the transcriptional profile of *LEA* genes in *S. lycopersicum* under drought, salinity, high temperatures, and treatments with phytohormones (ABA, MeJa, *rac*-GR24, and GABA) in various tissues (seeds, roots, meristem, leaves, flowers, and fruits), showing that most *LEA* genes responded to abiotic stress and water deficit. The antioxidant capacity and resilience to drought, cold, and heat in transgenic tobacco plants were reported by [77], who investigated the gene LEA in abiotic stress response in *Panax ginseng*.

In *P. cincinnata*, *PcLEA*, *PcSOD*, and *PcCAT* showed constitutive expressions in both passion fruit accessions, suggesting that the plants might not be experiencing severe oxidative damage due to ROS accumulation that would require the activity of these enzymes under the stress conditions evaluated in this study.

The *PcSTK* gene showed induction in the tolerant accession and repression in the sensitive accession of *P. cincinnata*, similar to what was observed for *PcSIP*. Our results are consistent with previous studies that reported the involvement of serine/threonine kinase proteins in signaling pathways related to water-stress tolerance in various plant species, as demonstrated by [78], who observed high induction of *STK* (14.4-fold) in leaf tissues of *Withania somnifera* under water deficit. Muhammad et al. [79] presented findings on overexpression in *Solanum lycopersicum* L. under different abiotic stresses (cadmium response, dehydration, salinity, and heat) and hormonal treatments (ABA, MeJa, and SA), which conferred an increase in plant tolerance to cadmium and drought, reflected by increased germination rate and improvements in seedling growth. Furthermore, this gene plays an important role in regulating water use efficiency and growth of *Xanthoceras sorbifolium* under water stress and is associated with drought resistance [80]. Enhanced tolerance to salt and oxidative stress through improved ROS scavenging ability and increased sensitivity to ABA is also reported due to the overexpression of *STK* genes in *Oryza sativa* [81].

Our studies highlight the practical significance of using specific AMF communities, especially those from dry environments, to enhance drought tolerance in plants like *P. cincinnata*. By treating plants with AMF from arid regions, we can help mitigate the adverse effects of water scarcity exacerbated by climate change. Our findings also show that native AMF communities promote better plant development than single AMF species, such as *E. etunicata*. This research provides valuable insights into how manipulating the plant microbiome can improve plant resilience, offering a potential strategy to secure food production in increasingly dry and variable conditions.

4. Materials and Methods

4.1. Accessions of P. cincinnata

Two accessions of *P. cincinnata* from the Active Germplasm Bank (BAG) of Embrapa Semiárido were used, chosen based on their tolerance or sensitivity to water deficit: accession 01 (CBAC0701—Tremedal-BA), classified as tolerant, and accession 48 (CPIF2648—Patos do Piauí-PI), classified as sensitive to water deficit [40].

4.2. Inoculation with AMF

The *P. cincinnata* accessions received four inoculation treatments: negative control without AMF (Control); monospecific inoculation with *Entrophospora etunicata* (EE Univasf 72) isolated from the rhizosphere of *P. edulis*; and treatments with native AMF communities from two water deficit conditions: field capacity below 25% (AMF25) and above 75% (AMF75). The soil inoculum of the native AMF communities consisted of approximately 750 glomerosporos, represented by *Glomus* spp., *Gigaspora decipiens* I.R. Hall and L.K. Abbott, *Gigaspora gigantea* (T.H. Nicolson and Gerd.) Gerd. and Trappe, *Gigaspora margarita* W.N. Becker and I.R. Hall, *Cetraspora gilmorei* (Trappe and Gerd.) Oehl. F.A. Souza and Sieverd, *Acaulospora* spp., and *Ambispora appendicula* (Spain. Sieverd. and N.C. Schenck) C. Walker [40]. The *E. etunicata* inoculum was provided by the Inoculum Bank of the Microbiology Laboratory at the Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, Brazil (9°19′29″ S e 40°32′57″ O), using soil inoculum with approximately 750 glomerospores per repetition.

4.3. Microbial Filtrate

To equalize the soil microbiota, a microbial filtrate derived from the soil inocula, excluding AMF, was applied to all treatments. This filtrate was prepared by mixing the soil inocula used in the experiment to ensure homogenization of the microbiota across all pots, thereby eliminating the effect of this variable. The soil inoculum and distilled water were mixed at a 1:10 (w/v) ratio, vigorously agitated, and then filtered using two 80 G paper filters, which retained particles between 4 and 12 µm, following the methodology described by [82]. One liter of microbial filtrate was prepared, and 10 mL was applied per pot immediately after transplanting the *P. cincinnata* seedlings.

4.4. Microcosm Experiment Imposing Water Deficit on P. cincinnata Accessions

The soil for the experiment was collected from a native Caatinga area in Petrolina, Pernambuco, Brazil (9°19′10″ S e 40°33′39″ O), at an average altitude of 376 m, with a climate classified as BSh according to Köppen. The experiment was conducted under similar conditions in a greenhouse located at the Agricultural Sciences Campus of the Universidade Federal do Vale do São Francisco (9°19′32″ S e 40°33′33″ O).

Seedlings from two different accessions of *P. cincinnata* were cultivated at Embrapa Semiárido. The soil used in the experiment, eutrophic red–yellow argisol, was mixed with sand at a 2:1 (v/v) ratio and autoclaved at 121 °C for 1 h in two cycles. After 30 days of seed germination, the seedlings were transplanted into two-liter pots, with one plant per pot.

Irrigation was maintained at field capacity between 70 and 80% for 30 days for acclimatization. After this period, two contrasting field capacity treatments were applied: 0–25% and 75–100%. Field capacity levels were maintained and monitored using data from the TDR 100—Time Domain Reflectometry equipment. During this experiment, two applications of the [83] solution, as modified by [84], were required in all treatments 15 days after transplanting the seedlings.

4.5. Morpho-Agronomic Descriptors

Measurements of plant height were taken using a tape measure; stem diameter was measured using a digital caliper, and leaf and tendril counts were recorded. Total chlorophyll (a + b) was measured in vivo using a digital chlorophyll meter (chlorofiLOG). After 25 days of water deficit conditions, biological samples were collected for destructive analysis, including leaf area, fresh aboveground biomass, and root biomass. Leaf area was determined by photographing freshly harvested leaves with a fixed-distance scale and analyzing them using the ImageJ software version 1.53k.

4.6. Physiological Descriptors

The physiological descriptors measured were the net photosynthesis rate (Pr), stomatal conductance (gs), transpiration rate (E), and leaf temperature. The gas exchange of *P. cincinnata* leaves was evaluated after 25 days under water deficit using the IRGA equipment—Li-6400, Licor®, Lincoln, NE, USA. The evaluations were carried out between 7 and 9 in the morning only at the end of this experiment. In addition to these descriptors, the efficient use of water was quantified by the ratio (Pr/E)—WUE and the intrinsic water use efficiency by the ratio (Pr/gs)—iWUE [85,86].

4.7. Mycorrhizal Responsiveness

The mycorrhizal responsiveness (MR) analysis was conducted following the methodology used by [87,88]. The formula employed by these authors was: [descriptor with AMF—descriptor without AMF (Control)/descriptor without AMF (Control)] × 100, yielding results in percentage.

4.8. Experimental Design

4.8.1. Morpho-Agronomic and Physiological Characteristics and Mycorrhizal Responsiveness of *P. cincinnata*

To assess the mycorrhizal responsiveness and evaluate the morpho-agronomic and physiological characteristics of *P. cincinnata*, a completely randomized design was employed with a factorial arrangement consisting of three factors: (i) two *P. cincinnata* accessions, (ii) two field capacities, and (iii) four AMF inoculation treatments. Each treatment combination was replicated five times, resulting in a total of 80 experimental units (Figure 8).

4.8.2. Analysis of Differential Gene Expression in *P. cincinnata*

For the analysis of differential gene expression, the AMF25 inoculation treatment was selected. Gene expression was compared under two conditions: drought stress (0–25% field capacity, FC) and control (irrigated, 75–100% FC). Each condition was tested with three biological replicates, leading to a total of six experimental units per *P. cincinnata* accession (Figure 8).

4.9. Differential Gene Expression Analysis and Its Relationship with Mycorrhizal Colonization and AMF Community

The comparisons used in the analysis of relative expression were delimited to treatments with different field capacities (0 to 25% of field capacity representing the treatment group, and 75 to 100% of field capacity representing the control group) and inoculated with the AMF community from the water deficit condition (AMF25) for each *P. cincinnata* accession. We opted for the AMF25 inoculation for two main reasons: (a) it resulted in superior plant development (height, number of tendrils, stem diameter, fresh root weight) compared to the AMF75 inoculation; (b) this condition is common in the Brazilian semi-arid region, meaning AMF communities naturally exist in conditions of low water availability.

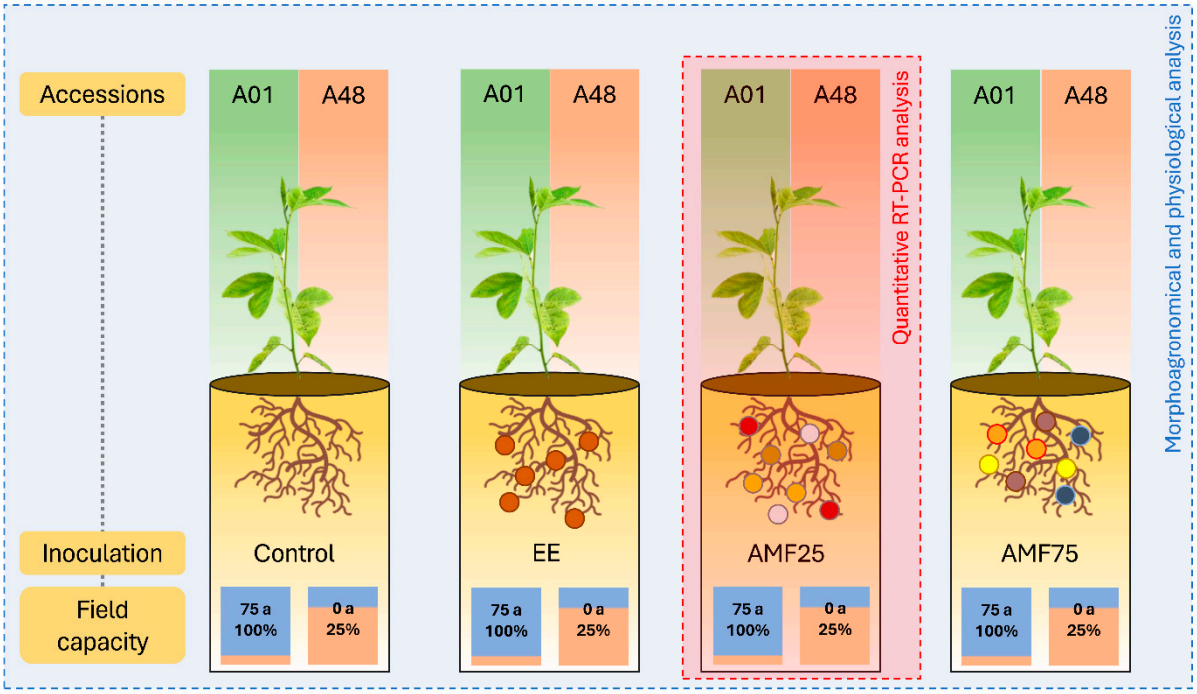


Figure 8. Experimental design diagram featuring two *P. cincinnata* accessions (A01 and A48), two irrigation conditions (<25% and >75% field capacity), and four AMF inoculation treatments: Control (no inoculation), EE (inoculation with *Entrophospora etunicata*), AMF25 (native AMF communities from drought-stressed plants), and AMF75 (native AMF communities from irrigated plants). The box outlined with dashed blue lines represents the morphophysiological analyses, while the box outlined with dashed red lines indicates the treatments used for gene expression analysis.

4.10. Molecular Analyses and Validation by Quantitative Real-Time PCR (qPCR)

Leaf tissues from the *P. cincinnata* accessions were collected 25 days after the treatments were imposed, immediately frozen in liquid nitrogen, and stored in an ultra-freezer at −80 °C. Three biological replicates were selected for each treatment and *P. cincinnata* accessions.

Total RNA was extracted using approximately 200 mg of leaf tissue, following the technical manual recommendations of the ReliaPrep™ RNA Tissue Miniprep System kit (Promega, Madison, WI, USA). RNA integrity was verified using a 1% agarose gel stained with ethidium bromide run at 70 V, 120 A, for 90 min.

The concentration and quality of the RNA samples were analyzed using a Nanodrop™ One C UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) (Table S4). cDNA synthesis was performed using the GoScript™ Reverse Transcription Mix, Oligo(dT) kit (Promega, Madison, WI, USA), according to the manufacturer's protocol, with 1000 ng/µL of RNA used for a final reaction mix volume of 20 µL.

Primers were designed using the Primer3 Plus program [89], with the following modifications to the default descriptors: GC content of 50%, fragment size between 70 and 150 base pairs (bp), melting temperature between 57 °C and 60 °C, and exon–exon junctions. Specificity tests were conducted in Primer-BLAST using *P. edulis* sequences deposited in GenBank (NCBI). Additionally, primer dimer formation was analyzed using PerlPrimer (http://perlprimer.sourceforge.net accessed on 28 June 2024). Six pairs of primers related to genes associated with water deficit tolerance were designed (Table 5): *PcSIP*, *PcLEA*, *PcbZIP*, *PcCAT*, *PcSOD*, *PcSTK*. The reference genes *PcEF1α* and *PcNDID* were selected for normalization of relative expression data based on the literature findings [90] (Table 5). All primers were synthesized and purified by desalting by Exxtend Biotecnologia Ltda. (Paulínia-SP, Brazil).

qPCR validations followed the MIQE (The Minimum Information for Publication of Quantitative RealTime PCR Experiments) guidelines [91]. Reactions were performed on the QuantStudio™ 5 system (Thermo Fisher Scientific, Waltham, MA, USA), using biological and technical triplicates, with detection by SYBR Green. The reactions followed these conditions: initial denaturation at 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min, with a final volume of 10 µL, 5 µL of GoTaq qPCR *Master mix* (Promega, Madison, WI, USA), 1 µL of cDNA, 0.6 µL of primers (5 µM), and 3.4 µL of ultrapure water. Melting curves were analyzed from 65 to 95 °C for 20 min after the 40 cycles to confirm primer specificity. Negative controls (NTC) were used in all reactions. Amplification efficiency (E = 10^{-1}/slope), correlation coefficient (R), interception (y), and slope values were calculated using the standard curve method with serial dilutions of an equimolar pool containing aliquots of all samples (Figures S1–S3).

4.11. Communities of Arbuscular Mycorrhizal Fungi and Mycorrhizal Colonization

The AMF community characterization and the assessment of mycorrhizal colonization intensity in the microcosm experiment were exclusively conducted on the samples utilized for the analysis of differential gene expression. This was performed to ascertain whether the differential gene expression could be linked to the different AMF taxa present in the rhizosphere of *P. cincinnata* and mycorrhizal colonization.

Glomerospores were extracted from the soil using the wet sieving and decanting technique [92], followed by centrifugation in water and sucrose [93], and then quantified. After counting, the glomerospores were placed on slides with PVLG (polyvinyl alcohol-lactoglycerol) and PVLG + Melzer's reagent (1:1 v/v) for better observation of color, size, spore quantity, and germination structures to aid in identification. Identification Keys [94–98], the International AMF Collection (INVAM), and recent publications were utilized for taxonomic identification. This study adopted the classification proposed by [99,100] with some updates on families and genera. AMF communities were assessed for species richness, Shannon diversity, Pielou's evenness, and Simpson's dominance.

For mycorrhizal colonization analysis, 0.5 g of fresh roots from each replicate of *P. cincinnata* accessions were isolated. The roots were washed to remove soil fragments, cleared in KOH 10% for 24 h at room temperature, washed again, and acidified in 1% HCl, followed by staining in Trypan blue solution in lactoglycerol (0.05%), following the method proposed by [101] with modifications.

The quantification of AMF colonization was performed following the methodology proposed by [102]. Thirty root fragments, each measuring 1 cm in length, were separated from each sample. Each fragment was then assessed for total mycorrhizal colonization, rated on a scale from 0 (absent), 1 (<1%), 2 (<10%), 3 (<50%), 4 (>50%), and 5 (>90%), and for arbuscular colonization, graded on a scale from A0 (none), A1 (few), A2 (frequent), and A3 (very abundant). Mycorrhizal frequency (F), mycorrhizal intensity (M), arbuscular intensity per fragment (a), and the root system (A) were presented as percentages (%).

4.12. Statistical Analyses

The data obtained in this study were tested for normality and homogeneity of variances, and when significant, they were subjected to three-way ANOVA and compared by Tukey's test ($p < 0.05$) using the gsheet [103] and ExpDes.pt [104] packages. Principal component analysis (PCA) was conducted to ascertain the correlation between morpho-agronomic and physiological descriptors, with the principal components derived using the vegan [105], permute [106], and lattice [107] packages.

For the analyses presented alongside the gene expression results, the morpho-agronomic and physiological data of *P. cincinnata*, glomerospores abundance, ecological

indices, mycorrhizal colonization, and the PCA corresponding to the samples used in the gene expression analysis were filtered and reassessed.

Ecological indices, including species richness, Shannon diversity (which is the sum of the proportional abundance of each species weighted by its natural logarithm), Pielou's evenness (which is the ratio of observed diversity to the maximum possible diversity based on the number of species), and Simpson's dominance (which is the sum of the squared proportions of the relative abundance of species), were analyzed using the vegan package [105]. Beta diversity was assessed using species abundance data transformed by the Hellinger method to reduce the influence of rare species and was ordinated based on Bray–Curtis dissimilarity. PERMANOVA was conducted to determine the influence of water deficit treatments and *P. cincinnata* accessions on the composition of AMF communities using the vegan package [105], with the *adonis2* and *pairwiseAdonis* functions. The mycorrhizal colonization analysis was conducted using the [102] with the Ramf [108], BiocManager [109], devtools [110], and ggplot2 [111] packages. All analyses were performed using the R Statistical Interface v4.2.3 and RStudio v2023.03.0. The genes of interest were validated via qPCR and analyzed using the REST software (Relative Expression Software Tool, v.2.0.13) [112]. Such analysis is based on paired comparisons (of target transcript and reference genes under stress conditions and controls) using randomization and bootstrapping—Pair-wise Fixed Reallocation Randomization Test [112]. Hypothesis testing ($p < 0.05$) was used to determine if differences in expression of target transcripts under control and treated conditions were significant.

Table 5. Primers used in the gene expression analysis of passionfruit accessions (*P. cincinnata*) (tolerant-A01 and sensitive-A48) under contrasting water availability conditions (field capacity <25% or >75%).

Gene (Access Number)	Description	Function	Primers	Amplicon (bp)	Reference
RG—*Pc*NDID (AB304270.1)	NADP-Dependent Isocitrate Dehydrogenase (IDH)	Responses to abiotic stress and associated with drought tolerance [1]	F: GTCGTCACTCTCTCTTTACG R: TCATTTCATCACCGTCCATC	155	[90]
RG—*Pc*EF1α1 (DQ447160.1)	Translation Elongation Factor 1α-1	Exhibits stable expression in drought and oxidative stress experiments [2]	F: GTTAAGGATTTGAAGCGTGG R: ATGTGTGATGTGTGGCAGT	172	[90]
*Pc*SIP (JAEPBF010000225.1)	Small and basic intrinsic protein	Mobilizes water and responds to drought stress [3]	F: CGTGTCTCTCTTGTCGATGG R: TCACTTGCAGAATTGCCTTG	83	This study
*Pc*LEA (JAEPBF010000087.1)	Late Embryogenesis Abundant	Involved in signaling pathways for abiotic stress responses [4]	F: GCAACAGGAGGGTCAAAATC R: ACCGTTGTCTTTGTGTCGTG	118	This study
*Pc*bZIP (JAEPBF010000054.1)	Basic leucine zipper	Enhances expression of genes related to abiotic stress tolerance [5]	F: CAAAACGTGTGAGGAGGATG R: CAGATGGGCTTGCTTTCTTC	74	This study
*Pc*CAT (JAEPBF010000191.1)	Catalase	Induced by ABA and linked to drought stress tolerance [6]	F: GAACAACACGCTCAGGGATG R: GCCCTATTCTGCTCGAGGAC	81	This study
*Pc*SOD (JAEPBF010000343.1)	Superoxide dismutases	Responses to drought stress [7]	F: CAAAACCCATGGTGCTCCTG R: GCAGTGCCATCATCACCAAC	81	This study
*Pc*STK (JAEPBF010000187.1)	Serine/threonine protein kinase	Regulates drought and osmotic-stress tolerance [8]	F: AGTCGGCTCTATTGGCCTTC R: ACCGGGAAGGCTACAACAAG	90	This study

bp = base pairs; [1] [113]; [2] [114]; [3] [115]; [4] [116]; [5] [117]; [6] [118]; [7] [119]; [8] [120].

5. Conclusions

Although the sensitive accession showed greater increases in morpho-agronomic variables in response to inoculation with AMF communities under water deficit conditions, its sensitivity is reinforced by the superiority of the tolerant accession in exhibiting a higher photosynthetic rate, greater water use efficiency, and the recruitment of genes *PcbZIP*, *PcSIP,* and *PcSTK*, which play a relevant role in the molecular response to water deficit. Additionally, greater mycorrhizal colonization and arbuscular abundance found in the tolerant accession strongly contribute to maintaining the exchange capacity between the

symbionts under water deficit conditions since the arbuscule is the main site for nutrient and water exchange.

The discrepancy in responses observed between the control treatment and those inoculated with AMF communities strongly suggests the hypothesis that *P. cincinnata* is highly responsive to mycorrhization, especially when native AMF communities are used instead of a monospecific inoculum application.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/stresses5010018/s1, Table S1: Multiple comparisons among different inoculation treatments within the *Passiflora cincinnata* accession and field capacity descriptors; Table S2: Cycle of quantification (Cq) obtained from RT-qPCR reactions using cDNA from *Passiflora cincinnata* accession A01 tolerant to drought stresss; Table S3: Cycle of quantification (Cq) obtained from RT-qPCR reactions using cDNA from *Passiflora cincinnata* accession A48 sensitive to drought stress; Table S4: RNA quantification (concentration and purity) of biological replicates of *Passiflora cincinnata* under contrasting water availability conditions used in the differential gene expression analyses; Figure S1: Amplification curves of *PcZIP*, *Pc*SIP, *Pc*LEA, *Pc*CAT, *Pc*SOD, *Pc*STK genes, the reference genes EF1α, and NDID genes in cDNA samples of leaf tissues of Caatinga passionfruit (*Passiflora cincinnata*) under abiotic stresses; Figure S2: Melting curves of *PcZIP*, *Pc*SIP, *Pc*LEA, *Pc*CAT, *Pc*SOD, *Pc*STK genes, the reference genes EF1α, and NDID genes in cDNA samples of leaf tissues of Caatinga passionfruit (*Passiflora cincinnata*) under abiotic stresses; Figure S3: Efficiency curves of of of *PcZIP*, *Pc*SIP, *Pc*LEA, *Pc*CAT, *Pc*SOD, *Pc*STK genes, the reference genes EF1α, and NDID genes in cDNA samples of leaf tissues of Caatinga passionfruit (*Passiflora cincinnata*) under abiotic stresses.

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