forests

MDPI

Article

The Synergistic Effects of AMF Inoculation and Boron Deficiency on the Growth and Physiology of *Camellia oleifera* Seedlings

Junying Liu [1], Mengxue Zhang [1], Jie Fan [1], Wenna Ding [1], Longsheng Chen [2], Jie Luo [1], Yongzhong Liu [1] and Li Mei [1,*]

[1] College of Horticulture and Forestry Sciences, Hubei Engineering Technology Research Center for Forestry Information, Huazhong Agricultural University, Wuhan 430070, China; liujunying@webmail.hzau.edu.cn (J.L.)
[2] Research Institute of Oil Tea Camellia, Hunan Academy of Forestry, Changsha 410004, China
* Correspondence: meili@mail.hzau.edu.cn; Tel.: +86-13871496862

Abstract: Arbuscular mycorrhizal fungi (AMF) symbiosis has been shown to improve the ability to obtain nutrients and resist adverse environmental conditions. However, there are few studies on the functions of AMF in the absorption and accumulation of boron (B). Moreover, it is still unclear whether the root colonization rates of AMF are limited by B deficiency. In this study, *Camellia oleifera* seedlings were planted in normal and boron-deficient substrates, and the seedlings were inoculated with *Funneliformis mosseae* or left uninoculated. The growth and physiological indices of *C. oleifera* seedlings were determined. The results of this experiment indicate that AMF inoculation increased the plant biomass, B content, B accumulation, and antioxidant enzyme activity in both normal and boron-deficient *C. oleifera* seedlings. Furthermore, boron deficiency resulted in a decrease in the AMF root colonization efficiency and the inhibition of *C. oleifera* seedlings' growth and physiological activity. These findings suggest that AMF inoculation could improve the resistance to B-deficiency stress. Additionally, the colonization efficiency of AMF was adversely affected by B deficiency; thus, AMF play a cooperative role with B in the growth and physiological functions of plants. The results provide a theoretical basis for taking measures to solve B-deficiency stress in *C. oleifera* and other plants' cultivation.

Keywords: *Camellia oleifera*; boron deficiency; arbuscular mycorrhizal fungi (AMF); root morphology; physiological parameters

Citation: Liu, J.; Zhang, M.; Fan, J.; Ding, W.; Chen, L.; Luo, J.; Liu, Y.; Mei, L. The Synergistic Effects of AMF Inoculation and Boron Deficiency on the Growth and Physiology of *Camellia oleifera* Seedlings. *Forests* **2023**, *14*, 1126. https://doi.org/10.3390/f14061126

Academic Editor: Stefan Arndt

Received: 27 March 2023
Revised: 23 May 2023
Accepted: 23 May 2023
Published: 30 May 2023

1. Introduction

Boron (B), as one of the essential trace elements, plays an important role in sugar transportation and hormone and phenolic metabolism for the growth and development of higher plants [1,2]. B deficiency induces the accumulation of reactive oxygen species and accelerates peroxidation in the root membrane system. B deficiency also affects the levels of phenylalanine and tyrosine in the roots, promotes the biosynthesis of salicylic acid, caffeic acid, and ferulic acid, and increases lignin contents in plant roots, which can lead to structural and morphological changes [3]. The typical symptoms of B deficiency in crops include bud development without flowering in *Gossypium hirsutum* and flowering without seed production in *Brassica napus* [4,5]. Boron application, on the other hand, has been shown to increase the grain size, reduce ear sterility, and increase the yield of *Oryza sativa* [6], as well as reduce crop sensitivity to abiotic stresses, such as drought, salinity, or heavy-metal toxicity [7]. However, the excessive application of B can lead to B toxicity due to the narrow suitable range of B demand in plants. Therefore, alleviating the adverse effects of B deficiency by improving the root absorption and utilization efficiency of B in plants is very crucial.

Arbuscular mycorrhizal fungi (AMF) are symbiotic with approximately 80% of terrestrial plants, increasing the efficiency of soil water and nutrient absorption while mitigating

the negative effects of stress on plants [8,9]. AMF colonization can improve the plant's uptake and effective utilization of mineral nutrients, such as nitrogen and phosphorus. A few studies have demonstrated that AMF can alleviate excess B toxicity symptoms in plants by increasing biomass and decreasing the tissue B concentration [10,11]. AMF inoculation could also be an effective method to promote the phytoremediation of B-contaminated soils under salt and drought stresses [10]. However, the roles of AMF in B uptake and utilization under B-deficient conditions are poorly understood [12].

Previous research has confirmed that AMF can obtain carbohydrates from host plants [8,13], but it is still unclear whether the symbiosis between AMF and host plants is affected by the levels of mineral nutrients such as B. Recent studies have shown that B deficiency can negatively affect plant growth and the AMF colonization rate. For example, B deficiency reduced root growth and the AMF colonization rate of Norway spruce (*Picea abies*) [4,14,15]. These findings suggest that B may be necessary for effective symbiosis between AMF and host plants [16]. Moreover, some fungi can transport B in their hyphae, indicating that B may play an important role in the relationship between fungi and plants [11]. However, the specific effects of B-deficiency stress on plant AMF colonization in B-deficient environments and the physiological mechanism of the interaction between B deficiency and AMF remain unclear.

The plantations of *Camellia oleifera* cover an expansive area of over 5 million hectares in southern China. These plantations serve crucial functions in resolving the conflict between the demand for and supply of edible oil, boosting the income of farmers, and preserving the ecological balance of the region. The *C. oleifera* planting area is located in the area with red and yellow soil in southern China. The soil's B content in this region, affected by heavy rainfall and leaching, was generally below the normal range of 0.5–1 mg kg^{-1} because of the heavy rainfall leaching influence [17]. B deficiency results in *C. oleifera* plantations exhibiting symptoms such as flowering without bearing fruit, buds without blossoming, and cracks in fruit shells. These symptoms significantly impact the quality and productivity of plantations and serve as the primary inhibitors of the industry's growth.

It has been proven that *C. oleifera* roots can live in symbiosis with AMF [18]. To what extent AMF infection alleviates the symptoms of boron deficiency in *C. oleifera* and whether the AMF infection efficiency is limited by boron deficiency, however, are not clear yet. Clarifying the effects of AMF on B absorption and accumulation, as well as the effects of B deficiency on the AMF colonization efficiency, will provide a basis for understanding the interaction between B and AMF and its physiological mechanism. Additionally, the insights from this study will underpin the development of effective cultivation strategies and address the issue of B-deficiency stress in the *C. oleifera* industry.

2. Materials and Methods

2.1. Experimental Materials and Treatments

C. oleifera 'Chang Lin 40′ (CL40) seedlings were planted in the greenhouse with a plastic board roof, with no additional temperature and humidity control measures other than natural ventilation with open windows. The roots of 2-year-old seedlings were sterilized with 10% hydrogen peroxide (H_2O_2) for 15 s and then repeatedly washed with distilled water before transplanting them to bigger pots (5 L). The culture substrate was a mixture of pearlite and sand (pearlite–sand = 3:1, *v*/*v*), sterilized by high-pressure steam (121 °C, 0.11 Mpa, 2 h). *C. oleifera* seedlings were inoculated with or without *Funneliformis mosseae* on 1 July 2021. The inoculant was composed of fungal spores, mycelia, an inoculated root segment and other propagules, and a mixed matrix, which was purchased from the Institute of Root Research, Yangtze University. The active spore count in the inoculant was 70–80 per gram. To ensure full contact between the AMF agent and the root system, 25 g of the dried fungal agent was spread evenly onto the rhizosphere per pot of inoculant-treated *C. oleifera* seedlings. The same amount of sterilized inactive AMF agent was added in the same way in the non-AMF-inoculated treatments.

Inoculated and uninoculated *C. oleifera* seedlings were irrigated daily with 40 mL of Hoagland nutrient solution with normal B (2.86 mg L^{-1}) or deficient B (0 mg L^{-1}) per plant once a day. Four treatments were applied: AMF inoculation and normal B (+AM+B), AMF inoculation and B deficiency (+AM−B), no AMF inoculation and normal B (−AM+B), and no AMF inoculation and B deficiency (−AM−B). There were 30 replicate seedlings for each treatment.

2.2. Sampling and Plant Growth Parameters

Three replicate seedlings of *C. oleifera* were randomly selected for the measurement of plant height, ground diameter, and other growth indices 60 days after treatment. All plant samples were separated into roots, stems, and leaves and then killed at 105 °C for 30 min and dried at 75 °C until reaching constant weight. Subsequently, the biomass and root-to-shoot (R/S) ratio were calculated. The dried samples were ground into powder for the determination of B content. Three more replicate plants were chosen at random and separated into roots, stems, and leaves. The fresh roots were scanned using an Epson scanner (12000 XL), and the root length, surface area, and volume were examined using the WinRHIZO analysis software (Pro 32-bit 2019a). Some fresh roots were isolated from three plants to determine root vitality and the mycorrhizal infection rate, while root activity was evaluated using 2,3,5-triphenyl-tetrazolium chloride staining [19]. The fresh samples were put in buffer solution and then stored at −80°C in the refrigerator for the determination of physiological indicators.

The AMF root colonization rate was determined using the method outlined by Vierheilig et al. [20]. Specifically, 0.2 g of a fresh, fine root was cut into 1 cm sections, fixed in FAA until transparent, and then stained. Fifteen stained root segments were arranged neatly on glass slides, covered with lactic acid and a slide, and gently squeezed. Pictures were taken using a light microscope to visualize invasion points, mycelium, arbuscules, vesicles, and spores. The colonization rate of each root was determined using a grading score of 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, and the number of root segments at each level was recorded to calculate the AMF root colonization rate for each treatment.

$$AMF\ colonization\ rate\ (\%) = \sum(0 \times N1 + 10 \times N2 + \ldots\ \ldots + 100 \times N11)/N \times 100\% \quad (1)$$

2.3. Boron Analysis and Physiological Parameter Evaluation

Three plants were mixed into one sample, and three duplicate samples were used for boron and physiological parameter determination. The boron contents were determined using the curcumin colorimetric method as described by Bingham [21]. Each dry sample weighed 0.533 g and was dry-ashed in a muffle furnace at 550 °C for 5 h. It was then dissolved in a 0.1 mol L^{-1} HCL solution. The supernatant was absorbed with a curcumin-oxalic acid solution, dried using a water bath, dissolved with alcohol, and filtered with dry filter paper into a light contrast groove. Finally, the boron content and accumulation were calculated.

The malondialdehyde (MDA) content was determined with the thiobarbituric acid reaction [22]. Into a graduated test tube, 2 mL of crude enzyme solution and 3 mL of a 5% trichloroacetic acid solution of 0.5% sulfobarbituric acid were added, and the mixture was heated in a boiling water bath for 10 min. After quickly cooling, it was centrifuged at 4500 r min^{-1} for 10 min. The supernatant was then measured for absorbance using a spectrophotometer at 600 nm and 532 nm (UV-4802), with distilled water as a 100% blank transmittance.

The soluble sugar content was determined using the method outlined by Zhang et al. [23]. Fresh and clean plant samples weighing 0.1 g were extracted two times in boiling water for 30 min each time with 5 mL of distilled water. After filtration into a 25 mL volumetric flask, 0.5 mL of the sample extract was combined with 1.5 mL of distilled water in a 10 mL graduated tube, and the absorbance was measured with a spectrophotometer (UV-4802) at a 630 nm wavelength to determine the soluble sugar content.

The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were determined using the method described by Zhou and Leul [24]. Specifically, 0.5 g of the plant sample was placed in a pre-cooled mortar for enzyme solution extraction. Then, 1 mL of phosphate buffer was added to an ice bath, and the volume was increased to 5 mL, after which it was centrifuged at 4000 r.p.m. for 10 min.

SOD activity was determined by adding 0.05 mol L^{-1} phosphate buffer, 130 mmol L^{-1} methionine solution, 750 µmol L^{-1} tetrazole solution, 100 µmol L^{-1} EDTA-Na_2 solution, 20 µmol L^{-1} riboflavin solution, and crude enzyme solution to a test tube containing phosphate buffer. The reaction mixture was then exposed to 4000 lux daylight for 20 min. The absorbance of the reaction mixture was measured at 560 nm using a spectrophotometer (UV-4802).

POD activity was determined by adding 3 mL of 25 mmol L^{-1} guaiacol solution ($C_6H_4(OH)(OCH_3)$) to a centrifuge tube, followed by the addition of 0.2 mL of 250 mmol L^{-1} hydrogen peroxide solution. The reaction mixture was mixed thoroughly to make a homogeneous solution. Then, 0.1 mL of crude enzyme solution was added to the reaction mixture as a substrate and introduced evenly into the spectrophotometer (UV-4802). The absorbance of the reaction mixture was measured at 470 nm every 1 min for 10 min.

CAT activity was determined by adding 3 mL of 20 mmol L^{-1} hydrogen peroxide solution to a 10 mL centrifuge tube. Then, 50 µL of crude enzyme solution was quickly mixed with the reaction mixture and transferred to a quartz cuvette. The absorbance of the reaction mixture was continuously measured at 240 nm using a spectrophotometer (UV-4802) for 3 min, and the initial and final values were recorded.

2.4. Statistical Analysis

All data were analyzed using IBM SPSS Statistics 26 software. Before statistical analysis, data were tested for normality using appropriate tests. If normality assumption was not met, data were transformed before being subjected to significance analysis. Significance was analyzed using the Duncan method with a significance level of $p < 0.05$. The effect of B-deficiency stress treatment on morphological and physiological parameters was determined using two-way ANOVA.

3. Results

3.1. The Effect of Boron Deficiency and AMF Inoculation on the Growth Parameters

Among the four treatments, +AM+B-treated seedlings showed the highest height elongation, ground diameter thickening, shoot and root biomass, total biomass, and R/S ratio. They were followed by those obtained with +AM−B and −AM+B treatments, while the lowest values were observed in −AM−B-treated seedlings. The biomass and R/S ratio showed significant differences ($p < 0.05$) between the +AM+B and −AM−B treatments. Statistical analysis of the data presented in Table 1 indicated that AMF colonization significantly benefits the growth parameters under B-deficiency stress treatment.

Table 1. Effects of boron deficiency and AMF inoculation on growth parameters (n = 3).

Treatments	+AM+B	+AM−B	−AM+B	−AM−B
Height elongation (cm)	3.45 ± 0.21 ^{a}	2.81 ± 0.29 ^{a}	1.35 ± 0.08 ^{b}	1.26 ± 0.18 ^{b}
Ground diameter thickening (mm)	1.28 ± 0.14 ^{a}	1.04 ± 0.18 ^{a}	1.05 ± 0.11 ^{a}	0.42 ± 0.04 ^{b}
Root biomass (g)	5.15 ± 0.51 ^{a}	4.85 ± 0.40 ^{a}	4.57 ± 1.00 ^{a}	3.40 ± 0.50 ^{b}
Shoot biomass (g)	10.03 ± 0.36 ^{a}	9.47 ± 0.16 ^{a}	9.64 ± 0.16 ^{a}	7.29 ± 0.46 ^{b}
Total biomass (g)	16.02 ± 0.81 ^{a}	13.47 ± 0.74 ^{ab}	15.56 ± 0.82 ^{a}	11.29 ± 0.97 ^{b}
R/S ratio	0.69 ± 0.01 ^{a}	0.69 ± 0.01 ^{a}	0.67 ± 0.03 ^{a}	0.43 ± 0.05 ^{b}

Note: +AM+B: AMF inoculation and normal B; +AM−B: AMF inoculation and B deficiency; −AM+B: no AMF inoculation and normal B; −AM−B: no AMF inoculation and B deficiency. The same applies to tables below. Values are expressed as means ± SE. Significant differences between treatments were determined using Tukey's honestly significant difference test. Different lowercase letters indicate significant differences between treatments, $p < 0.05$.

3.2. Boron-Deficiency Effect on AMF Root Colonization Rate, Activity, and Morphology

The AMF colonization rate of +AM+B-treated seedlings was 46.1%, significantly higher than that of seedlings in the +AM−B treatment (35.6%). In contrast, no AMF colonization was observed in the roots of AMF non-inoculated *C. oleifera* seedlings. This result indicates that B deficiency could inhibit the development of the AMF symbionts (Figure 1). The root sections of AMF-inoculated plants showed the presence of mycelia, follicles, arbuscular, and spores of AMF. The inoculated roots were closely attached to the surface by hyphae and kept growing along the root surface and branches, forming multiple invasion points. The hyphae grew in the form of a binary fork, forming a dendritic structure. Root cortical cells produced multiple round or oval vesicles filled with cellular chambers (Figure 1B–D).

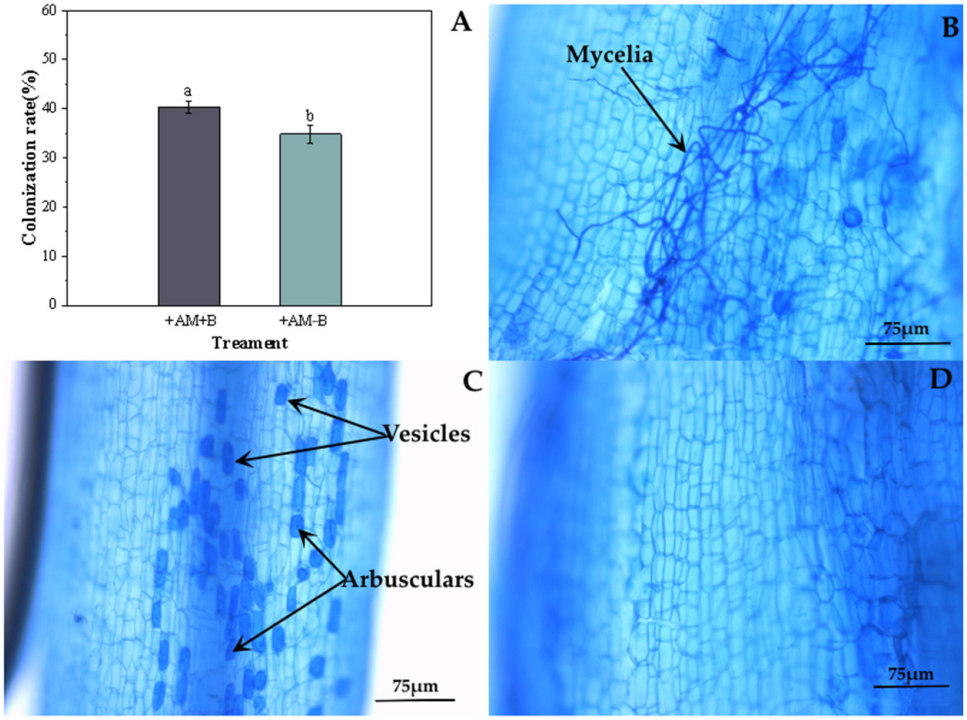

Figure 1. AMF root colonization rate (**A**), light micrographs of AMF-inoculated root (**B**,**C**), and AMF non-inoculated root (**D**) of *C. oleifera* seedlings. +AM+B: AMF inoculation and normal B; +AM−B: AMF inoculation and B deficiency. Values (**A**) are expressed as means ± SE. Significant differences between means were determined using significant difference T-test. Different lowercase letters indicate significant differences between treatments, $p < 0.05$.

The +AM+B-treated seedlings had the maximum root activity after treatment (Figure 2). AMF inoculation improved the root activity in both B-deficient and normal-B-supplied *C. oleifera* seedlings ($p < 0.05$).

The +AM+B-treated *C. oleifera* seedlings had the maximum total root length, root surface area, and root volume (Figure 2). The total root length, root surface area, and root volume of −AM+B-treated seedlings decreased by 5.9%, 33.6%, and 23%, respectively, and those in the +AM−B treatment decreased by 7.2%, 34.9%, and 36.2%, respectively, compared with +AM+B. Under B-deficient conditions, the total root length, root surface area, and root volume of AMF-inoculated *C. oleifera* seedlings were significantly higher than those of uninoculated seedlings 60 days after treatment ($p < 0.05$).

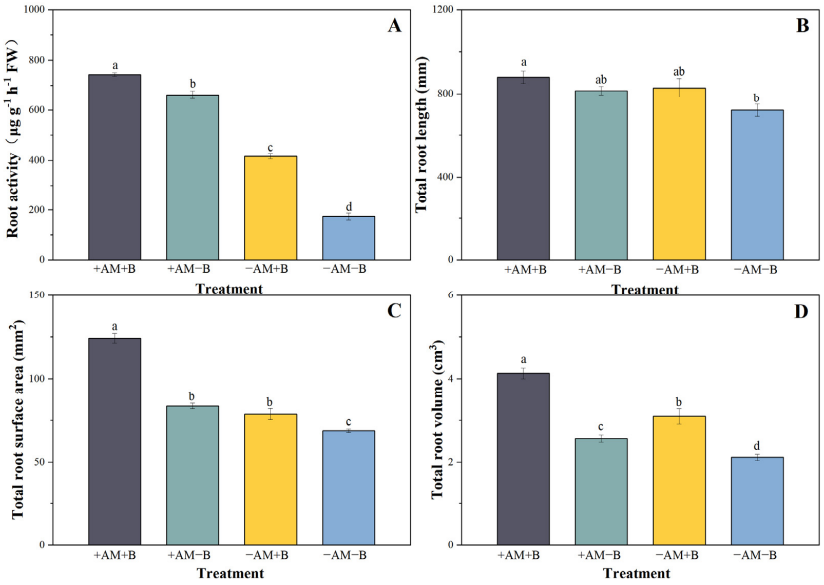

Figure 2. Effects of boron deficiency and AMF inoculation on root activity and root morphology of *C. oleifera* seedlings (n = 3). Root activity (**A**), total root length (**B**), total root surface area (**C**) and total root volume (**D**) of *C. oleifera* seedlings. +AM+B: AMF inoculation and normal B; +AM−B: AMF inoculation and B deficiency; −AM+B: no AMF inoculation and normal B; −AM−B: no AMF inoculation and B deficiency. Values are expressed as means ± SE. Significant differences between means were determined using Tukey's honestly significant difference test. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

3.3. Effect of Boron Deficiency and AMF Inoculation on Boron Content and Accumulation

AMF inoculation improved the B content in stems and leaves in both B-deficiency- and normal-B-supply-treated *C. oleifera* seedlings ($p < 0.05$) (Figure 3B,C). Under normal B treatment conditions, the B content in the roots, stems, and leaves of uninoculated seedlings decreased by 18.7%, 12.5%, and 12.1%, respectively, compared with AMF-inoculated treatments (Figure 3A–C). B accumulation in normal-B-treated plants was higher than that in B-deficiency-treated *C. oleifera* seedlings ($p < 0.05$). AMF inoculation significantly increased the plant's B accumulation in normal-B treatments (Figure 3D).

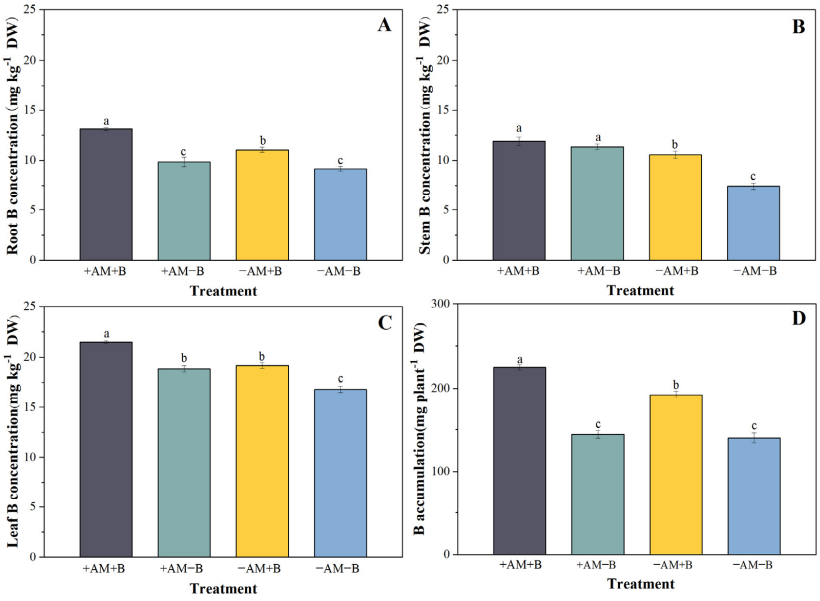

Figure 3. Effects of boron deficiency and AMF inoculation on boron concentration and accumulation in *C. oleifera* seedlings (n = 3). Root B concentration (**A**), stem B concentration (**B**), leaf B concentration

(**C**) and B accumulation (**D**) of *C. oleifera* seedlings. +AM+B: AMF inoculation and normal B; +AM−B: AMF inoculation and B deficiency; −AM+B: no AMF inoculation and normal B; −AM−B: no AMF inoculation and B deficiency. Values are expressed as means ± SE. Significant differences between means were determined using Tukey's honestly significant difference test. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

3.4. Effect of Boron Deficiency and AMF Inoculation on Antioxidant Enzyme Activity

AMF inoculation significantly increased soluble sugar contents in roots and leaves under different boron treatments ($p < 0.05$) of *C. oleifera* seedlings (Figure 4A,B). The MDA contents in roots and leaves significantly differed among the four treatments, and the MDA contents in leaves were significantly higher than that in the roots of *C. oleifera* seedlings ($p < 0.05$). B deficiency increased the MDA content, while AMF inoculation significantly decreased MDA contents in both the leaves and roots of *C. oleifera* seedlings (Figure 4C,D).

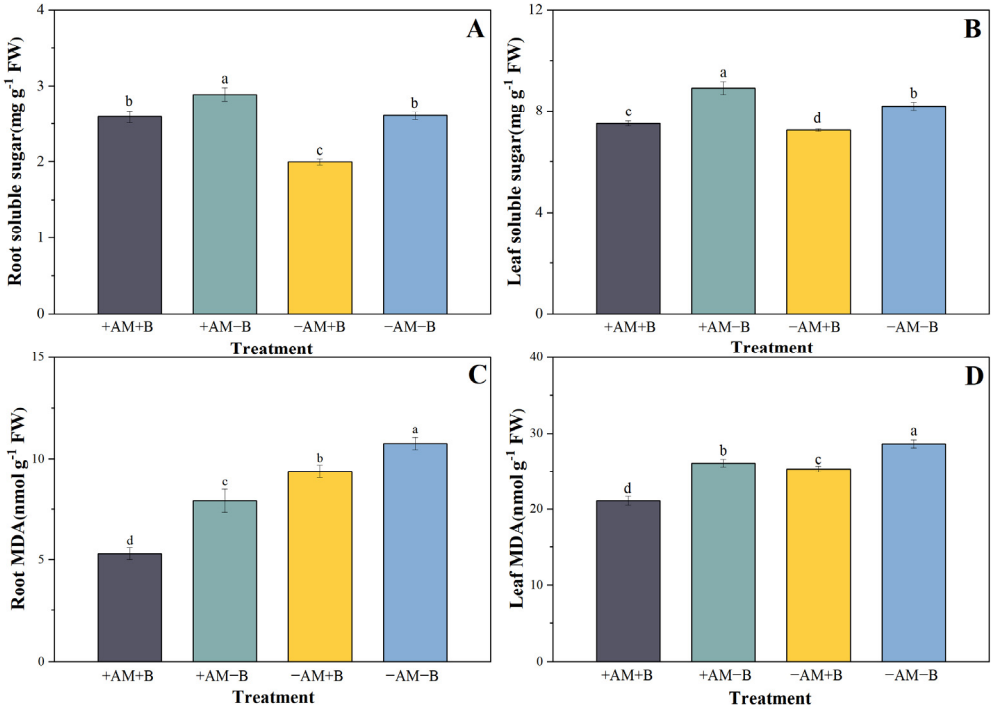

Figure 4. Effects of boron deficiency and AMF inoculation on soluble sugar and MDA contents of *C. oleifera* seedlings (n = 3). Root soluble sugar (**A**), leaf soluble sugar (**B**), Root MDA (**C**) and leaf MDA (**D**) of *C. oleifera* seedlings. +AM+B: AMF inoculation and normal B; +AM−B: AMF inoculation and B deficiency; −AM+B: no AMF inoculation and normal B; −AM−B: no AMF inoculation and B deficiency. Values are expressed as means ± SE. Significant differences between means were determined using Tukey's honestly significant difference test. Different lowercase letters indicate significant differences between treatments, $p < 0.05$.

The SOD activities in roots and leaves significantly differed among the four treatments of *C. oleifera* seedlings ($p < 0.05$). The +AM−B-treated plants had the highest SOD activity in roots and leaves (Figure 5A). The B-deficiency treatment significantly improved the SOD, POD, and CAT activities in the roots and leaves in both AMF-inoculated and non-inoculated *C. oleifera* seedlings. AMF inoculation enhanced SOD, POD, and CAT activities in roots and leaves in B-deficiency-treated *C. oleifera* seedlings ($p < 0.05$).

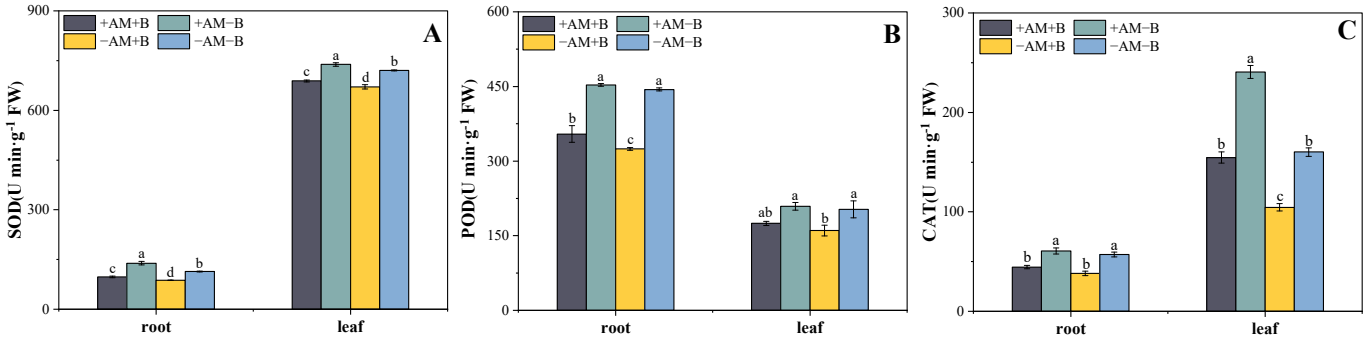

Figure 5. Effects of boron deficiency and AMF inoculation on the SOD, POD, and CAT activities of *C. oleifera* seedlings (n = 3). Root and leaf SOD (**A**), root and leaf POD (**B**), root and leaf CAT (**C**) of *C. oleifera* seedlings. +AM+B: AMF inoculation and normal B; +AM−B: AMF inoculation and B deficiency; −AM+B: no AMF inoculation and normal B; −AM−B: no AMF inoculation and B deficiency. Values are expressed as means ± SE. Significant differences between means were determined using Tukey's honestly significant difference test. Different lowercase letters indicate significant differences between treatments of roots or leaves ($p < 0.05$).

3.5. Principal Component Analysis

A principal component analysis of 14 indicators was performed for dimension reduction. There are two components with eigenvalues greater than 1, and PCA scores for the four treatments are shown in Figure 6. The first principal component eigenvalue is 8.610, and the larger contributing indexes are total root length and soluble sugar; the second principal component eigenvalue is 5.614, and the larger contributors are SOD and CAT. The main variables with high loads on axis 1 are the R/S ratio, root length, root surface area, root volume, root vitality, and malondialdehyde. The main variables with high loads on axis 2 are mainly soluble sugar, soluble protein, SOD, POD, CAT, and boron accumulation. There is a positive correlation between root activity and the mycorrhizal infection rate and R/S ratio. The four treatments are distributed in different quadrants, indicating significant differences between treatments.

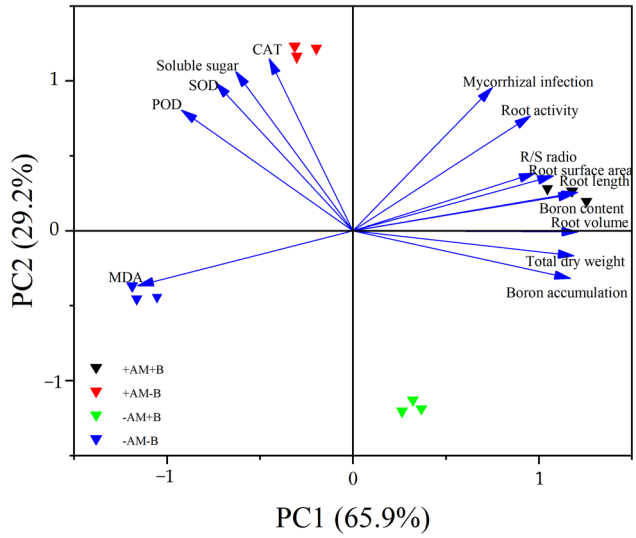

Figure 6. Principal component analysis of variables. The arrow lines of the variables are plotted as the correlation coefficients between them and the first two principal components. CAT, SOD, POD, and MDA are catalase, superoxide dismutase, peroxidase, and malondialdehyde, respectively, in *C. oleifera* plants.

According to the principal component analysis, a comprehensive evaluation model of *C. oleifera* quality under different treatments was established (F = 0.66F1 + 0.29F2), from

which the comprehensive scores of four treatments (Table 2) were calculated, and the scores were ranked from high to low: +AM+B, +AM−B, −AM+B, and −AM−B.

Table 2. Scores and sorting of the principal components of all detected indexes in 4 treatments.

Treatment	Principal Component Score		Scores	Sort
	F1	F2		
+AM+B	3.58	0.54	2.52	1
+AM−B	−0.96	2.50	0.09	2
−AM+B	0.99	−2.30	−0.01	3
−AM−B	−3.61	−0.74	−2.59	4

Note: +AM+B: AMF inoculation and normal B; +AM−B: AMF inoculation and B deficiency; −AM+B: no AMF inoculation and normal B; −AM−B: no AMF inoculation and B deficiency.

The relationships between 14 indexes and 4 treatments were analyzed by principal component analysis. The AMF colonization rate, total biomass, root activity, soluble sugar, root length, root surface area, and root volume made significant contributions to PC1, while there was a large negative correlation between POD and PC1 (Figure 6).

4. Discussion

4.1. Effect of Boron Deficiency on AMF Colonization

The colonization of AMF depends on the host plant species, the classification of AMF, and the soil environment. Several studies have reported that soil environmental factors affect the growth of the AMF symbiont, such as low phosphorus levels, which inhibit plant AMF root colonization [25–27]. This result showed that B deficiency significantly reduced the AMF colonization rate, which expanded our knowledge by showing that the soil B level is also an important factor affecting AMF colonization.

Boron deficiency significantly decreases the plant's total root length, which will limit the contact chances between the root system and AMF in the soil to a certain extent. Many researchers have reported that B deficiency damages the root cell structure [28], which may hinder the invasion and decrease the colonization rate of AMF. In addition, B has been proven to be transported in the hyphae of most fungi, and B also plays a role in maintaining the cell wall structure of hyphae [11]. Under the condition of B deficiency, the stability of the AMF fungal vesicle envelope was impaired, resulting in the production of nitrogenase, and nitrogen fixation was almost non-existent [29]. Therefore, B deficiency inhibits hyphal growth, which is also the main reason that AMF reduce their ability to infect plants [16]. These results further confirm that B is necessary for the establishment of effective symbiosis between AMF and plants.

4.2. Effect of AMF on Plant Growth, Boron Absorption, and Utilization

B deficiency has been proven to inhibit the growth and elongation of plant roots [30]. These research results also showed that the total root length, total root surface area, total root volume, and root biomass of AMF-inoculated *C. oleifera* seedlings were significantly higher than those of non-inoculated seedlings, which is consistent with other research results showing that B deficiency limited the growth and development of plant roots [28,30]. AMF inoculation significantly increased the total root length, surface area, volume, and biomass of *C. oleifera* seedlings under B-deficient conditions, which is consistent with published studies [31,32]. The symbiotic relationship between AMF and plant roots effectively increased the root length, root surface area, root volume, and biomass, which facilitates plant adaptation to B deficiency and other stresses. AMF inoculation increased the B accumulation in both normal-B-supply and B-deficient conditions. The colonization of AMF promotes B absorption and accumulation and effectively alleviates growth and other physiological symptoms in B-deficient environments.

4.3. Effect of AMF and Boron Deficiency on Plant Physiological Characteristics

Plants under stress conditions will produce a series of physiological responses to reduce the damage to themselves. Soluble sugars, as important carbohydrates in plants, increase plant resistance to environmental stress through their osmoregulatory function [33]. Our results showed that AMF inoculation significantly increased the contents of soluble sugars and enhanced the cell stability of *C. oleifera* seedlings under different boron-supply treatments, the same result as in *C. sinensis* [34]. Moreover, the soluble sugar content was significantly higher in boron-deficient than normal-boron-treated *C. oleifera* seedlings. It has been speculated that the accumulation of soluble sugars may result in an increase in osmotic regulators and carbohydrate transportation to improve the adaptation to B deficiency and other stresses [35].

MDA is the final decomposition product of membrane lipid peroxidation, which suppresses the activity of cell-protective enzymes and antioxidant contents, resulting in membrane lipid peroxidation and severe plant cell death [36]. The root and leaf MDA contents of *C. oleifera* seedlings inoculated with AMF were significantly lower than those of non-AMF-inoculated seedlings, indicating that AMF could protect the plant membrane system and thus relieve the stress symptoms of B deficiency in *C. oleifera* seedlings.

The plant exoenzyme protection system consists of SOD, POD, and CAT [22]. CAT is a redox enzyme that converts H_2O_2 into H_2O and O_2, thus preventing H_2O_2 accumulation in the cell, and SOD acts as a key defensive enzyme against free radicals in catalyzing superoxide dismutation to H_2O_2 and O_2 [37], thus functioning to eliminate excess superoxide anions and sustain normal physiological metabolism under stress [38]. POD has a wide variety of physiological roles, including lignification, the crosslinking of polymers in cell walls, the formation of suberin, and resistance to stress [39]. The SOD, POD, and CAT activities were higher in the roots and leaves of AMF-inoculated seedlings than in those without AMF, indicating that AMF could improve antioxidant enzyme activity and protect plants from adversity [40,41]. The SOD, POD, and CAT activities in roots and leaves were significantly higher in B-deficiency-treated seedlings than in normal-B-treated *Brassica* seedlings, which is consistent with the findings of Pandey and Archana [42]. Another study also confirmed that AMF can effectively improve the activities of SOD, POD, and CAT in the leaves of tobacco (*Nicotiana tabacum* L.) and further improve the activities of plant antioxidant enzymes to alleviate toxic effects and enhance plant stress resistance [43].

5. Conclusions

The results demonstrated that AMF inoculation improved both seedling growth and B absorption and utilization efficiency, in addition to enhancing the physiological functions of *C. oleifera* seedlings under both normal and B-deficiency stress conditions. B deficiency had a direct and negative impact on growth and physiological parameters and also reduced the AMF root colonization rate of *C. oleifera* seedlings. These conclusions provide an important theoretical basis for understanding the effect of the interaction between B and AMF on plant growth, providing ideas for solving the problem of B deficiency in *C. oleifera* by AMF inoculation.

Author Contributions: Conceptualization, L.M. and J.L. (Jie Luo); methodology, J.L. (Junying Liu); software, J.F. and Y.L.; validation, J.L. (Junying Liu), M.Z. and W.D.; formal analysis, J.F.; investigation, J.L. (Junying Liu); resources, L.C.; data curation, J.L. (Junying Liu); writing—original draft preparation, J.L. (Junying Liu).; writing—review and editing, L.M.; visualization, J.L. (Junying Liu); supervision, L.M.; project administration, L.M.; funding acquisition, L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grants from the National Key Research Program (2017YFC0505503) and the earmarked fund for CARS26.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yan, L.; Li, S.; Muhammad, R.; Jiang, C.C. Proline metabolism and biosynthesis behave differently in response to boron-deficiency and toxicity in *Brassica napus*. *Plant Physiol. Biochem.* **2021**, *167*, 529–540. [CrossRef] [PubMed]
2. Mehboob, N.; Yasirasir, T.A.; Ul-Allah, S.; Nawaz, A.; Ahmad, N.; Hussain, M. Interactive effect of boron application methods and boron-tolerant bacteria (*Bacillus* sp. MN54) improves nodulation, grain yield, profitability and biofortification of kabuli chickpea grown under irrigated and rainfed conditions. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 5068–5085. [CrossRef]
3. Wu, X.W.; Riaz, M.; Yan, L.; Jiang, C.C. Distribution and mobility of foliar-applied boron (^{10}B) in citrange rootstock under different boron conditions. *Plant Growth Regul.* **2020**, *39*, 575–582. [CrossRef]
4. Dell, B.; Huang, L.B. Physiological response of plants to low boron. *Plant Soil* **1997**, *193*, 103–120. [CrossRef]
5. Subedi, K.D.; Gregory, P.J.; Gooding, M.J. Boron accumulation and partitioning in wheat cultivars with contrasting tolerance to boron deficiency. *Plant Soil* **1999**, *214*, 141–152. [CrossRef]
6. Atique-ur-Rehman; Farooq, M.; Nawaz, A.; Rehman, A.; Iqbal, S. Soil application of boron improves the tillering, leaf elongation, panicle fertility, yield and its grain enrichment in fine-grain aromatic rice. *J. Plant Nutr.* **2015**, *38*, 338–354. [CrossRef]
7. García-Sánchez, F.; Simón-Grao, S.; Martínez-Nicolás, J.J.; Alfosea-Simón, M.; Liu, C.G.; Chatzissavvidis, C.; Pérez-Pérez, J.G.; Cámara-Zapata, J.M. Multiple stresses occurring with boron toxicity and deficiency in plants. *J. Hazard. Mater.* **2020**, *397*, 122713. [CrossRef]
8. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: London, UK, 2008.
9. Lehto, T.; Zwiazek, J.J. Ectomycorrhizas and water relations of trees, a review. *Mycorrhiza* **2011**, *21*, 71–90. [CrossRef]
10. Liu, C.G.; Dai, Z.; Cui, M.Y.; Lu, W.K.; Sun, H.W. Arbuscular mycorrhizal fungi alleviate boron toxicity in *Puccinellia tenuiflora* under the combined stresses of salt and drought. *Environ. Pollut.* **2018**, *240*, 557–565. [CrossRef]
11. Simón-Grao, S.; Nieves, M.; Martínez-Nicolás, J.J.; Alfosea-Simón, M.; Cámara-Zapata, J.M.; Fernández-Zapata, J.C.; García-Sánchez, F. Arbuscular mycorrhizal symbiosis improves tolerance of Carrizo citrange to excess boron supply by reducing leaf B concentration and toxicity in the leaves and roots. *Ecotox. Environ. Saf.* **2019**, *173*, 322–330. [CrossRef]
12. Lavola, A.; Aphalo, P.J.; Lehto, T. Boron and other elements in sporophores of ectomycorrhizal and saprotrophic fungi. *Mycorrhiza* **2001**, *21*, 155–165. [CrossRef]
13. Nehls, U. Mastering ectomycorrhizal symbiosis: The impact of carbohydrates. *J. Exp. Bot.* **2008**, *59*, 1097–1108. [CrossRef]
14. Möttönen, M.; Lehto, T.; Aphalo, P.J. Growth dynamics and mycorrhizas of Norway spruce (*Picea abies)* seedings in relation to boron supply. *Trees* **2001**, *15*, 319–326. [CrossRef]
15. Räisänen, M.; Repo, T.; Lehto, T. Cold acclimation was partially impaired in boron deficient Norway spruce seedings. *Plant Soil* **2007**, *292*, 271–282. [CrossRef]
16. Bolaños, L.; Redondo-Nieto, M.; Bonilla, I.; Wall, L.G. Boron requirement in the *Discaria trinervis* (Rhamnaceae) and Frankia symbiotic relationship. Its essentiality for Frankia BCU110501 growth and nitrogen fixation. *Physiol. Plant.* **2002**, *115*, 563–570. [CrossRef]
17. Zhang, Z.Q.; Mao, P.; Yu, D.S.; Xu, L. Typical soil pH change characteristics in red soil area in the past 25 years—Taking Yujiang County of Jiangxi Province as an example. *Act. Pedol. Sin.* **2018**, *55*, 1545–1553. (In Chinese)
18. Lin, Y.L.; Li, Z.Y.; Zhang, L.P.; Wu, F.; Yang, Y.; Tan, M.X.; Hu, D.N. Effects of organic phosphorus and AM fungi on growth, root morphology and photosynthetic characteristics of *Camellia oleifera*. *Non-Wood For. Res.* **2021**, *39*, 121–128+210. (In Chinese)
19. Wang, J.E.; Li, D.W.; Zhang, Y.L.; Zhao, Q.; He, Y.M.; Gong, Z.H. Defence responses of pepper (*Capsicum annuum* L.) infected with incompatible and compatible strains of *Phytophthora capsici*. *Eur. J. Plant Pathol.* **2013**, *136*, 625–638. [CrossRef]
20. Vierheilig, H.; Coughlan, A.P.; Wyss, U.; Piche, Y. Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. *Appl. Environ. Microbiol.* **1998**, *64*, 5004–5007. [CrossRef] [PubMed]
21. Bingham, F.T. Boron. In *Methods of Soil Analysis Part 2: Chemical and Microbiological Properties*; American Society of Agronomy: Madison, WI, USA, 1982; pp. 431–446.
22. Carmak, I.; Horst, J.H. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant.* **1991**, *83*, 463–468. [CrossRef]
23. Zhang, P.; Zhang, H.; Liu, J.N.; Huan, X.J.; Wang, Q.C.; Li, L.; Liu, Y.J.; Qin, P. Effects of drought and re-watering treatment on physiological and biochemical indexes of different drought-resistant wheat varieties/lines at seedling stage. *Acta Agric. Boreali-Occident. Sin.* **2020**, *29*, 1795–1802. (In Chinese)
24. Zhou, W.; Leul, M. Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. *Plant Growth Regul.* **1999**, *27*, 99–104. [CrossRef]
25. Tarafdar, J.C.; Marschner, H. Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biol. Biochem.* **1994**, *26*, 387–395. [CrossRef]
26. Tanaka, Y.; Yano, K. Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. *Plant Cell Environ.* **2005**, *28*, 1247–1254. [CrossRef]
27. Kazadi, T.A.; Lwalaba, J.L.W.; Ansey, B.K.; Muzulukwau, J.M.; Katabe, G.M.; Karul, M.I.; Baert, G.; Haesaert, G.; Mundende, R.P.M. Effect of phosphorus and arbuscular mycorrhizal fungi (AMF) inoculation on growth and productivity of maize (*Zea mays* L.) in a tropical ferralsol. *Gesunde Pflanz.* **2022**, *74*, 159–165. [CrossRef]

28. Camacho-Cristóbal, J.J.; Martín-Rejano, E.M.; Herrera-Rodríguez, M.B.; Navarro-Gochicoa, M.T.; Rexach, J.; González-Fontes, A. Boron deficiency inhibits root cell elongation via an ethylene/auxin/ros-dependent pathway in Arabidopsis seedlings. *J. Exp. Bot.* **2015**, *66*, 3831–3840. [CrossRef] [PubMed]
29. Zarea, M.J.; Karimi, N.; Goltapeh, E.M.; Ghalavand, A. Effect of cropping systems and arbuscular mycorrhizal fungi on soil microbial activity and root nodule nitrogenase. *J. Saudi Soc. Agric. Sci.* **2011**, *10*, 109–120. [CrossRef]
30. Chen, L.S.; Han, S.; Qi, Y.P.; Yang, L.T. Boron stresses and tolerance in citrus. *Afr. J. Biotechnol.* **2012**, *11*, 5961–5969.
31. Schellenbaum, L.; Berta, G.; Ravolanirina, F.; Tisserant, B.; Gianinazzi, S.; Fitter, A.H. Influence of endomycorrhizal infection on root morphology in a micropropagated woody plant species (*Vitis vinifera* L.). *Ann. Bot.* **1991**, *68*, 135–141. [CrossRef]
32. Borkowska, B. Growth and photosynthetic activity of micropropagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress. *Acta Physiol. Plant.* **2002**, *24*, 365–370. [CrossRef]
33. Tisarum, R.; Theerawitaya, C.; Samphumphuang, T.; Singh, H.P.; Chaum, S. Foliar application of glycinebetaine regulates soluble sugars and modulates physiological adaptations in sweet potato (*Ipomoea batatas*) under water deficit. *Protoplasma* **2020**, *257*, 197–211. [CrossRef] [PubMed]
34. Singh, S.; Pandey, A.; Kumar, B.; Palni, L.M.S. Enhancement in growth and quality parameters of tea [*Camellia sinensis* (L.) O. Kuntze] through inoculation with arbuscular mycorrhizal fungi in an acid soil. *Biol. Fertil. Soils* **2010**, *46*, 427–433. [CrossRef]
35. Huang, Z.; Zou, Z.; He, C.; He, Z.; Zhang, Z.; Li, J. Physiological and photosynthetic responses of melon (*Cucumis melo* L.) seedlings to three *Glomus* species under water deficit. *Plant Soil* **2011**, *339*, 391–399. [CrossRef]
36. Prasad, T.K. Role of catalase in inducing chilling tolerance in pre-emergent maize seedlings. *Plant Physiol.* **1997**, *114*, 1369–1376. [CrossRef] [PubMed]
37. Sreenivasulu, N.; Grimm, B.; Wobus, U.; Weschke, W. Differential response of antioxidant compounds to salinity stress in salt tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). *Physiol. Plant.* **2000**, *109*, 435–442. [CrossRef]
38. Singh, K.; Yadav, S.K.; Mishra, R.K. Differential effect of salinity on thermotolerance of SOD isoforms in seven varieties of *Vigna mungo* (L.) Hepper. *Plant Physiol. Rep.* **2019**, *24*, 279–288. [CrossRef]
39. Mazza, G.; Charles, C.; Ouchet, M.; Ricard, J.; Raynaud, J. Isolation, purification and physico-chemical properties of turnip peroxidases. *Biochim. Biophys. Acta* **1968**, *167*, 89–98. [CrossRef]
40. Akensous, F.Z.; Anli, M.; Boutasknit, A.; Ben-Laouane, R.; Ait-Rahou, Y.; Ahmed, H.B.; Nasri, N.; Hafidi, M.; Meddich, A. Boosting date palm (*Phoenix dactylifera* L.) growth under drought stress: Effects of innovative biostimulants. *Gesunde Pflanz.* **2022**, *74*, 961–982. [CrossRef]
41. Wang, J.P.; Yuan, J.H.; Ren, Q.; Zhang, B.; Zhang, J.C.; Huang, R.Z.; Wang, G.G. Arbuscular mycorrhizal fungi enhanced salt tolerance of *Gleditsia sinensis* by modulating antioxidant activity, ion balance and P/N ratio. *Plant Growth Regul.* **2022**, *97*, 33–49. [CrossRef]
42. Pandey, N.; Archana. Antioxidant responses and water status in *Brassica* seedlings subjected to boron stress. *Acta Physiol. Plant.* **2013**, *35*, 697–706. [CrossRef]
43. Begum, N.; Akhtar, K.; Ahanger, M.A.; Iqbal, M.; Wang, P.P.; Mustafa, N.S.; Zhang, L.X. Arbuscular mycorrhizal fungi improve growth, essential oil, secondary metabolism, and yield of tobacco (*Nicotiana tabacum* L.) under drought stress conditions. *Environ. Sci. Pollut. Res.* **2021**, *28*, 45276–45295. [CrossRef] [PubMed]