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Improved Waterlogging Tolerance in Roots of Cucumber Plants after Inoculation with Arbuscular Mycorrhizal Fungi

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Abstract: Mycorrhizal symbiosis enhances host plant resistance to various unfavorable environmental stresses, but whether and how it also enhances waterlogging tolerance in cucumber plants is not known. The objective of this study was to analyze the effect of *Paraglomus occultum* inoculation on biomass production, osmolyte levels, and the expression of 12 heat shock protein 70 (*Hsp70*) genes and 14 plasma membrane intrinsic protein (*PIP*) genes in the roots of cucumber plants under a short-term waterlogging stress (WS) (5 days) condition. Although the short-term WS treatment significantly inhibited the arbuscular mycorrhizal fungal colonization of roots, the inoculation with arbuscular mycorrhizal fungi (AMFs) significantly increased leaf, stem, and root biomass under WS. AMF inoculation also significantly increased root glucose, sucrose, betaine, and proline contents, along with decreased fructose levels, compared with the uninoculated control. More *CsHsp70* and *CsPIP* genes were up-regulated in AMF-inoculated plants than in AMF-uninoculated plants in response to WS. AMF inoculation showed no significant effect on the expression of any of the examined *CsHsp70* genes under no-waterlogging stress, but it did raise the expression of 11 of 12 *CsHsp70* genes under WS. AMF colonization also down-regulated or had no effect on *CsPIP* expression under no-waterlogging stress, whereas it up-regulated the expression of 12 of the 14 *CsPIP* genes under WS. It is concluded that AMF inoculation enhances waterlogging tolerance in cucumber plants by increasing osmolyte levels and stress-responsive gene (*CsPIP* and *CsHsp70*) expression.

Keywords: flooding; mycorrhizal symbiosis; stress-responsive gene; vegetable

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1. Introduction

Cucumber (*Cucumis sativus* L.) is an important cash crop grown worldwide. Due to its aerobic roots and shallow distribution, cucumber is susceptible to waterlogging stress (WS) [1]. In China, seasonal rainfall, poor drainage and irrigation systems, and rising groundwater levels in the Yangtze River region frequently result in the WS of cucumber in the open field, which seriously affects cucumber production [2]. Therefore, enhancing cucumber waterlogging tolerance is critical for improving cucumber production and ensuring food supply.

Soil microorganisms are known to assume a crucial role in regulating ecosystem function and promoting sustainable agricultural development, and to have a profound impact on ecosystem evolution by regulating nutrient cycling pathways. The plant rhizosphere inhabits arbuscular mycorrhizal fungi (AMFs) to establish reciprocal symbiosis with their roots, where the host plant supplies the fungi with organic carbon and the fungi return nutrients to the host [3]. AMFs are present in most natural habitats and have a wide range of favorable effects on the host, including improved plant nutrient acquisition and enhanced

stress tolerance [4]. Several studies have also confirmed that AMFs improve waterlogging tolerance in host plants. Yang et al. [5] reported that a 15-day waterlogging treatment significantly increased AMF abundance in the roots of *Populus deltoides* from 4 OTUs before the treatment to 17 OTUs. However, long-term waterlogging treatments (e.g., 90 days) significantly inhibited the mycorrhizal colonization rate of the roots of lonkida (*Nauclea orientalis* L.) [6]. The response of AMF to waterlogging varies depending on host plant species and soil types [7].

Although AMFs affect the AMF diversity and root infection rate of host plants, AMF inoculation still regulates the physiological activity of waterlogged hosts. Matsumura et al. [8] found that, under waterlogging conditions, trifoliate orange seedlings inoculated with *Gigaspora margarit* could survive by obtaining additional oxygen from the aerenchyma of the intercropped bahiagrass roots through the mycorrhizal extraradical hyphal network. In peach plants, inoculation with AMFs significantly improved their flooding tolerance, which was closely related to enhanced chlorophyll component concentrations and antioxidant defense systems [9,10]. However, the AMF inoculation of *Citrus junos* did not enhance root peroxidase activity under waterlogging [11]. These results show the variability in the physiological response of mycorrhizae to waterlogged hosts.

AMFs have been applied to cucumber plants and confirmed in the field to promote cucumber survival, fruit yield, and nutrient acquisition (e.g., P and Zn) [12]. Moreover, mycorrhizal cucumber plants presented better adaptation under both NaCl stress and heat stress, as evidenced by better growth and higher plant gas exchange [13,14]. However, how mycorrhizal cucumber responds to waterlogging treatment has not been reported. Since AMFs have been demonstrated to improve host waterlogging tolerance to some extent [8–11], we hypothesized that AMFs could enhance cucumber waterlogging tolerance, which is closely related to changes in osmolytes and stress-responsive gene expression. To test this hypothesis, this study inoculated cucumber with AMFs and examined the changes in cucumber growth, five osmolytes, and the expression of stress-responsive genes (heat shock protein 70, *Hsp70*; plasma membrane intrinsic proteins, *PIPs*) under WS conditions.

2. Materials and Methods

2.1. Mycorrhizal Fungal Preparations

In this study, we selected the *Paraglomus occultum* strain (BGC BJ04B), which was isolated from the rhizosphere of a peach tree in Pinggu, Beijing, China. Following morphological identification (spores are solitary or sparsely attached to the mycelium tips in the soil, colorless to transparent-white, pear-shaped, and debris is often attached to the outside of the spores. In Melzer's reagents, the spores are orange in color, with 1–2 layers of spore walls, straight or small funnel-shaped hyphal commissure, and 8–9 μm wide at the commissure) [15], these spores were subjected to single-spore cultures using sorghum as host plants. After preservation at the Institute of Root Biology, Yangtze University, this fungal strain was trapped by white clover in pots, and the root segments and growth substrates were collected as mycorrhizal fungal inoculum containing 25 spores/g.

2.2. Plant Arrangement and Design

Seeds of the cucumber variety "Pamandi" were germinated at 28 °C in Petri dishes with wet gauze. After two days, these seeds were sown in 16-hole trays pre-filled with sterilized substrates. After one month, the cucumber seedlings were transplanted into plastic pots (20.5 cm × 9.8 cm × 11.5 cm). Approximately 1.8 kg of growth substrates (soil/sand = 3:1, by volume) was autoclaved for 2 h and then filled into plastic pots. Two seedlings were planted in each pot. Then, 120 g of *P. occultum* inoculum/pot was applied near the potted cucumber roots at the time of cucumber seedling transplantation. The treatment not inoculated with *P. occultum* received the same amount of autoclaved inoculum, and an additional 3 mL of the fungal inoculum filtrate through a 25 μm size nylon mesh was also applied to the rhizosphere, with the aim of maintaining a consistent microbial community, except for *P. occultum*.

The treated seedlings were acclimated indoors for 1 w before being placed in the chamber described by Tian et al. [14]. Seventy-five days after *P. occultum* inoculation, half of the plants were selected for waterlogging treatment, and the other half were not. To address waterlogging, the tested pots were placed in a larger plastic container (116 cm × 40 cm × 23 cm) filled with tap water at a level 2 cm above the topsoil of the pots. The intensity of the waterlogging was maintained for 5 days by replenishing the lost water daily. The plant culture was performed from June to August 2023.

Here, this study consisted of a total of four treatments, including inoculation with *P. occultum* under no-waterlogging stress (NS+AMF), no inoculation with *P. occultum* under no-waterlogging stress (NS-AMF), inoculation with *P. occultum* under waterlogging stress (WS+AMF), and no inoculation with *P. occultum* under waterlogging stress (WS-AMF). Each treatment repeated four times for a total of 32 plants with two plants per pot.

2.3. Determination of Plant Biomass and Root Mycorrhizal Infestation Rate

At the time of harvesting, the plants were removed from the pots, avoiding damage to the roots as much as possible. After removing the soil attached to the roots, the four plants from each treatment were separated into the roots, stems, and leaves and their fresh biomass was immediately weighed. One plant from each pot was frozen in liquid nitrogen for subsequent molecular determination; the other plant was used for the analysis of physiological variables. The roots obtained were immediately cut into 1–1.5 cm lengths, and approx. 12 root segments per plant were stained for root arbuscular mycorrhizae using the trypan blue procedure, as outlined by Phillips and Hayman [16], followed by microscopic examination. Mycorrhizal infestation rate was defined as the ratio of the length of *P. occultum*-infected root segments to the total root segment length.

2.4. Determination of Five Osmolytes in Roots

Contents (mg/g DW) of sucrose, fructose, and glucose in roots were measured using the colorimetric method described by Wu et al. [17]. Root proline content (μg/g FW) was determined using the acidic ninhydrin colorimetric method described by Bates et al. [18]. Root betaine content (μg/g FW) was assayed by the colorimetric method described by Liu et al. [19]. The osmolyte measurements were repeated four times for each variable, using four plants per treatment.

2.5. Relative Expression of CsPIPs and CsHsp70 Genes in Roots

Total RNA was extracted from the roots of treated cucumber plants using the Plant RNA Rapid Extraction Kit (Aidlab, Beijing, China). The extracted RNA was tested for integrity by agarose gel electrophoresis, and its purity was assessed by spectrophotometry. The extracted RNA was reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit with a gDNA Eraser (TaKaRa, Dalian, China). Primer Premier 5.0 software (http://www.premierbiosoft.com, accessed on 2 February 2024) was used to design specific primers (Supplementary Material Table S1) for selected *CsHsp70* and *CsPIP* homologs for subsequent qRT-PCR analysis. In the qRT-PCR system, the reaction composition had been described by Tian et al. [14], accompanied by a run on a Bio-Rad CFX system and UBI-ep as an internal reference. The $2^{-\Delta\Delta Ct}$ method [20] was used to compare the relative expression levels of target genes in different samples. In these gene expression assay, there were three biological replicates (plants) per treatment.

2.6. Data Analysis

The analysis of variance (ANOVA) of the experimental data was carried out under SAS software (v8.1), and significant differences between treatments were compared with Duncan's multiple range test at $P < 0.05$ levels. SigmaPlot 13.0 was utilized to generate the figures.

3. Results

3.1. Response of Root AMF Infection and Biomass Production to Waterlogging Stress

Root AMF infestation was only seen in the roots of AMF-inoculated plants (Figure 1a), with the root infection rate ranging from 26.07% to 44.72% (Figure 1b). The root infestation rate was significantly inhibited by the waterlogging treatment.

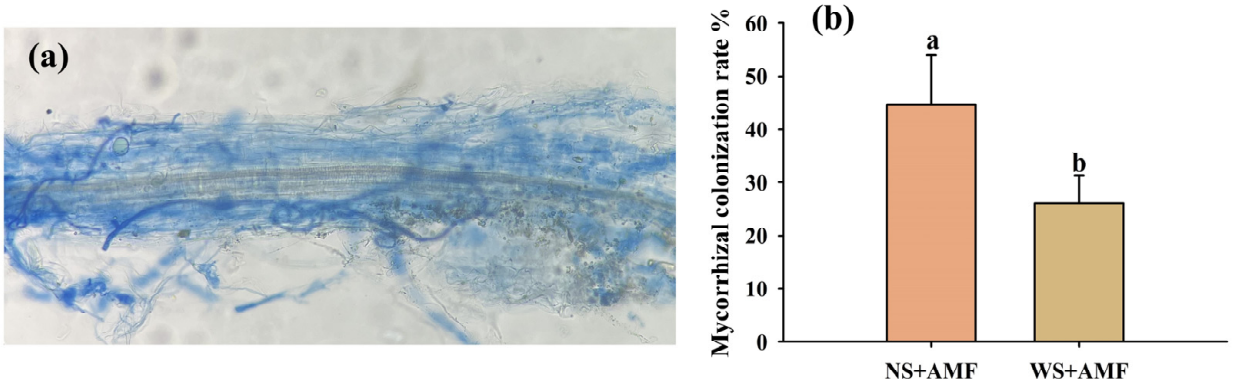

Figure 1. Root infection of *Paraglomus occultum* in cucumber under waterlogging tolerance (**a**) and changes in root mycorrhizal infestation rate (**b**). Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($P < 0.05$) differences. Abbreviations: +AMF, inoculation with *P. occultum*; NS, no-waterlogging stress; WS, waterlogging stress.

WS also significantly inhibited biomass production on the leaves, stems, and roots, whereas inoculation with AMF significantly increased the leaf, stem, and root biomass, as evidenced by values of 294.7%, 140.2%, and 222.4% in the absence of WS (NS+AMF) and 159.7%, 97.2%, and 238.1% in the presence of WS (WS+AMF), respectively, compared to the no-inoculation treatment (Figure 2a–c).

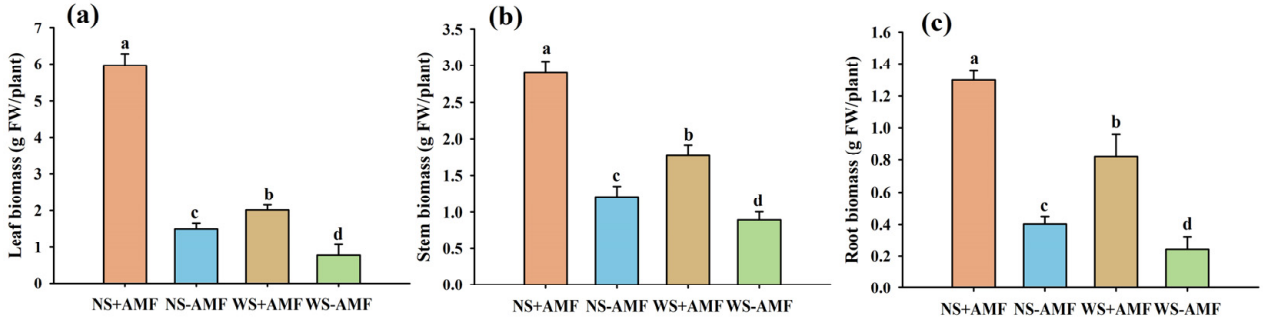

Figure 2. Changes in leaf (**a**), stem (**b**), and root (**c**) biomass of cucumber plants exposed to waterlogging stress and non-waterlogging stress after AMF inoculation. Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($P < 0.05$) differences. Abbreviations: −AMF, no inoculation with *P. occultum*; +AMF, inoculation with *P. occultum*; NS, no-waterlogging stress; WS, waterlogging stress.

3.2. Response of Osmolytes in Roots to Waterlogging Stress

The glucose, fructose, and sucrose contents in the roots were significantly reduced by WS by 52.5%, 12.5%, and 20.0% in uninoculated plants and by 30.6%, 52.5%, and 21.9% in AMF-inoculated plants, respectively (Figure 3a–c). In contrast, AMF inoculation presented a significantly increased effect in glucose and sucrose contents in roots, as demonstrated under NS by 40.6% and 38.8%, and under WS by 105.7% and 35.5%, respectively, compared to the no-inoculation treatment. However, the root fructose contents were suppressed by AMF treatment by 48.0% and 71.8% under NS and WS, respectively. The WS treatment did not affect the root proline contents in AMF-inoculated and uninoculated plants and

betaine contents in AMF-inoculated plants, but it reduced root betaine contents in uninoculated plants by 24.8% compared with the NS treatment (Figure 3d,e). The effect of AMF inoculation on root betaine contents was dependent on soil moisture: it was significantly reduced by 16.9% under NS and increased by 15.1% under WS. AMF treatment significantly increased the root proline contents by 11.2% under NS and 17.9% under WS, respectively.

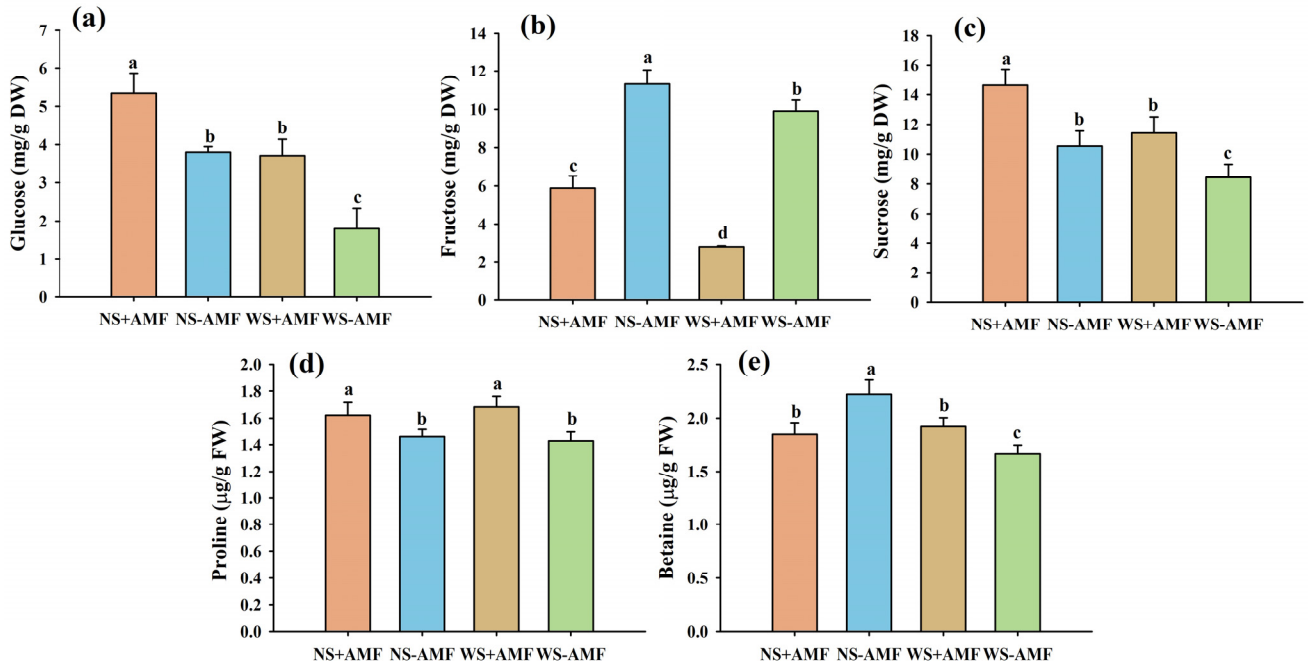

Figure 3. Changes in root glucose (**a**), fructose (**b**), sucrose (**c**), proline (**d**), and betaine (**e**) contents of cucumber plants exposed to waterlogging stress and no-waterlogging stress after AMF inoculation. Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($P < 0.05$) differences. Abbreviations: −AMF, no inoculation with *P. occultum*; +AMF, inoculation with *P. occultum*; NS, no-waterlogging stress; WS, waterlogging stress.

3.3. Response of CsHsp70s Gene Expression Levels in Roots to Waterlogging Stress

In uninoculated plants, the WS treatment did not significantly affect the expression of *CsHsp70-2*, *CsHsp70-3*, *CsHsp70-4*, *CsHsp70-9*, *CsHsp70-11*, or *CsHsp70-12*, while *CsHsp70-2*, *CsHsp70-5*, *CsHsp70-6*, *CsHsp70-7*, *CsHsp70-8*, and *CsHsp70-10* expression was significantly up-regulated by 212.27-, 3.24-, 9.50-, 8.67-, 2.26-, and 20.89-fold, respectively, compared with the NS treatment (Figure 4). In inoculated plants, the WS treatment significantly up-regulated the expression of *CsHsp70-2*, *CsHsp70-3*, *CsHsp70-4*, *CsHsp70-5*, *CsHsp70-6*, *CsHsp70-7*, *CsHsp70-8*, *CsHsp70-9*, *CsHsp70-10*, *CsHsp70-11*, and *CsHsp70-12* by 1227.00-, 148.23-, 36.27-, 541.5-, 2582-, 1234-, 3.23-, 466-, 91.13-, 824.25-, and 1616-fold, respectively, compared with the NS treatment. In addition, compared with uninoculated treatment, AMF inoculation had no significant effect on the expression of any of the tested *CsHsp70* genes under NS treatment; under WS, AMF inoculation caused the increased expression of *CsHsp70-2*, *CsHsp70-3*, *CsHsp70-4*, *CsHsp70-5*, *CsHsp70-6*, *CsHsp70-7*, *CsHsp70-8*, *CsHsp70-9*, *CsHsp70-10*, *CsHsp70-11*, and *CsHsp70-12* by 12.21-, 12.73-, 5.29-, 3.34-, 2.66-, 1.36-, 1.67-, 466.00-, 1.63-, 274.75-, and 3.85-fold, respectively, accompanied by a 0.91-fold decrease in *CsHSP70-1* expression.

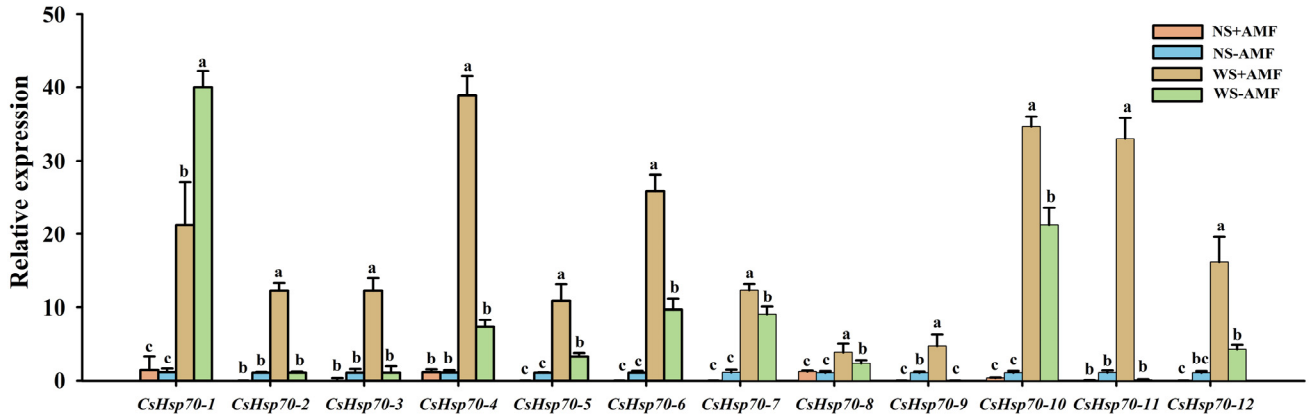

Figure 4. Changes in 12 *Hsp70* gene expression in roots of cucumber plants exposed to waterlogging stress and no-waterlogging stress after AMF inoculation. Data (means ± SD, n = 3) followed by different letters above the bars indicate significant ($P < 0.05$) differences. Abbreviations: −AMF, no inoculation with *P. occultum*; +AMF, inoculation with *P. occultum*; NS, no-waterlogging stress; *Hsp70*, heat shock protein 70; WS, waterlogging stress.

3.4. Response of CsPIP Gene Expression Levels in Roots to Waterlogging Stress

In the roots of plants not inoculated with AMF, the expression of *CsPIP1;1*, *CsPIP1;3*, *CsPIP1;4*, *CsPIP2;1*, *CsPIP2;3*, *CsPIP2;4*, *CsPIP2;5*, *CsPIP2;7*, and *CsPIP2;8* distinctly decreased under WS versus NS by 0.75-, 0.99-, 0.76-, 0.88-, 0.98-, 0.83-, 0.96-, 0.98-, and 0.98-fold, respectively, whereas the expression of the other *CsPIP* genes was not affected (Figure 5). In the roots of AMF-inoculated plants, *CsPIP1;1*, *CsPIP1;2*, *CsPIP1;3*, *CsPIP1;4*, *CsPIP1;5*, *CsPIP1;6*, *CsPIP2;1*, *CsPIP2;3*, *CsPIP2;4*, *CsPIP2;5*, *CsPIP2;6*, *CsPIP2;7*, and *CsPIP2;8* expression were up-regulated by 32.47-, 9.78-, 80.03-, 71.08-, 24.62-, 23.48-, 62.29-, 39.35-, 31.15-, 626.58-, 17.01-, 40.30-, and 1325.89-fold under WS versus NS, respectively, whereas there was no significant effect on *CsPIP2;2* expression.

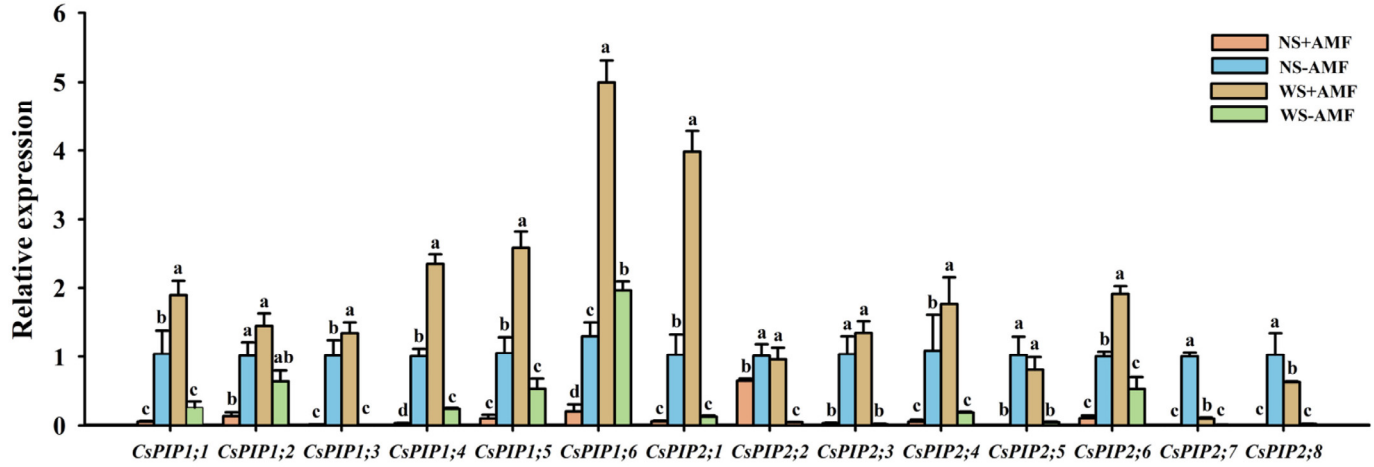

Figure 5. Changes in 14 *PIP* gene expression in roots of cucumber plants exposed to waterlogging stress and no-waterlogging stress after AMF inoculation. Data (means ± SD, n = 3) followed by different letters above the bars indicate significant ($P < 0.05$) differences. Abbreviations: -AMF, no inoculation with *P. occultum*; +AMF, inoculation with *P. occultum*; NS, no-waterlogging stress; *PIP*, plasma membrane intrinsic protein; WS, waterlogging stress.

In addition, AMF inoculation down-regulated the expression levels of *CsPIP1;1*, *CsPIP1;2*, *CsPIP1;3*, *CsPIP1;4*, *CsPIP1;5*, *CsPIP2;1*, *CsPIP2;3*, *CsPIP2;4*, *CsPIP2;5*, *CsPIP2;7*, and *CsPIP2;8* under NS conditions 0.95-, 0.87-, 0.98-, 0.97-, 0.90-, 0.94-, 0.97-, 0.95-, 1.00-, 1.00-, and 1.00-fold, respectively, whereas the expression levels of *CsPIP1;5*,*CsPIP1;6*,

CsPIP2;2, and *CsPIP2;6* were not significantly affected (Figure 5). Under WS conditions, AMF inoculation up-regulated the expression of *CsPIP1;1*, *CsPIP1;3*, *CsPIP1;4*, *CsPIP1;5*, *CsPIP1;6*, *CsPIP2;1*, *CsPIP2;3*, *CsPIP2;4*, *CsPIP2;5*, *CsPIP2;6*, *CsPIP2;7*, and *CsPIP2;8* 6.29-, 201.49-, 8.76-, 3.85-, 1.55-, 30.25-, 72.76-, 8.51-, 16.67-, 2.61-,7.78-, and 27.71-fold, respectively, while there was no significant effect on *CsPIP1;2* and *CsPIP2;2* expression.

4. Discussion

The results of this study showed that the root AMF infection rate was significantly inhibited by a short-term WS treatment on cucumber plants compared with the NS treatment, which is consistent with the results of Cheng et al. [21] on trifoliate orange inoculated with *Funneliformis mosseae* after a 10-day WS treatment. This inhibition is attributed to the fact that waterlogging creates a hypoxic environment that decreases the respiratory energy required by AMF as an aerobic organism [22], which, in turn, reduces its root colonization.

In addition, the WS treatment also significantly inhibited cucumber biomass production, which may originate from the WS-triggered reduction in root respiration and accumulation of toxic substances [23]. As a result, the cucumber variety is a waterlogging-sensitive plant. Inoculation with AMF, however, significantly improved cucumber biomass production under the short-term WS treatment, demonstrating that AMF can stimulate host growth under waterlogging. However, in another experiment, *Rhizophagus irregularis* inoculation did not increase tomato biomass production under a 6-day waterlogging period [24]. This shows that the growth on waterlogged hosts is dependent on both AMF species and host species. Interestingly, AMF in combination with other non-microbial biostimulants (e.g., plant protein hydrolysates) resulted in a synergistic effect on improving nutrient acquisition and plant growth in vegetable crops (e.g., eggplant) [25]. Therefore, the benefits of combining AMF with other biostimulants in cucumber waterlogging tolerance can be investigated in the future.

In the present study, root glucose, fructose, and sucrose contents were significantly suppressed by WS versus NS, in both AMF-inoculated and uninoculated plants, owing to the fact that WS limits photosynthesis in the plants, leading to a decrease in photosynthetic products [26]. AMF inoculation, however, significantly increased root sucrose and glucose contents and decreased fructose content under both NS and WS. It is well known that the growth of AMF depends on organic compounds, such as lipids and glucose, supplied by the host plant [27]. Thus, the presence of a source of sucrose cleavage in mycorrhizal roots allows for a greater supply of sucrose from host leaves to roots [28], which is one of the reasons why the root sucrose content was elevated in mycorrhizal plants. In addition, AMF also affects the cleavage of sucrose into glucose and fructose, with mycorrhizal fungi preferring glucose [17,29]. As a consequence, more glucose in mycorrhizal roots can provide respiratory energy for the function of mycorrhizae.

Two other organic osmolytes, betaine and proline, were also involved in response to WS under mycorrhizalization. AMF treatment significantly increased the contents of both under WS. This is similar to previous results from testing proline on salt-stressed chickpeas inoculated with *Funneliformis mosseae* and analyzing betaine on salt-stressed pistachios inoculated with *Rhizophagus irregularis* [30,31]. Thus, mycorrhizal cucumber plants maintained higher contents of glucose, sucrose, proline, and betaine under WS than non-mycorrhizal controls, which allows mycorrhizal plants to modulate osmoregulation by promoting these solutes to better cope with WS. However, the response of stressed plants to AMF is dependent on their tissues, with leaves and roots sometimes producing different effects [11]. Therefore, future studies need to consider leaves and roots together to analyze their response patterns to AMFs under waterlogging.

Abiotic stresses usually cause protein dysfunction, and molecular chaperones such as heat-shock proteins are responsible for helping to maintain protein structure and function [32]. *Hsp70* aids cell organelles to validate protein quality by stabilizing the proteins, thus regulating protein degradation, accelerating protein maturation, etc., which makes it involved in plant abiotic stress resistance [33]. In the present study, we found that more

genes (11 in inoculated plants versus 6 in uninoculated plants) were up-regulated in inoculated plants under WS versus NS, suggesting that the inoculated plants were able to be more active in resistance to WS by up-regulating more *CsHsp70* gene expression. Furthermore, under NS, AMF inoculation had no effect on *CsHsp70* gene expression, but this effect was reversed under WS, as evidenced by the up-regulated expression of 11 *CsHsp70* genes, with the exception of *CsHsp70-1*, which was down-regulated. This implies that the mycorrhiza-regulated *CsHsp70* expression pattern is affected by soil moisture. On the other hand, it also suggests that the inoculated plants are more active to resist WS by up-regulating the expression of *CsHsp70* genes. Liu et al. [34] reported that exogenous spermidine was able to activate the expression of *Hsp70* genes and protein abundance in maize. And the presence of mycorrhizae can promote polyamine synthesis in host plants, especially under soil flooding [35]. This implies that AMF inoculation promotes the formation of polyamines to activate *Hsp70* gene expression, thereby protecting protein stability and reducing their degradation, but this remains to be further confirmed in mycorrhizal cucumber plants.

In plant aquaporins, the PIP1 group usually exhibits inactive, low-efficiency water channels, whereas the PIP2 group represents high-efficiency water channels [36]. The response pattern of aquaporins to stress depends on the type of stress, intensity of stress, members of aquaporins, plant tissues, mycorrhizal symbionts, etc. [37]. In this study, WS decreased the expression of three *CsPIP1* genes and six *CsPIP2* genes in uninoculated plants, but it up-regulated the expression of 13 of 14 *CsPIPs* in inoculated plants, which further revealed that inoculated plants had higher WS tolerance by initiating *CsPIPs*. Cheng et al. [21] also reported that four of six *CsPtPIPs* were up-regulated by WS treatment in mycorrhizal trifoliate orange seedlings. On the other hand, cucumber plants inoculated with AMFs showed down-regulation or no effect on the expression of *CsPIPs*, which indicates that mycorrhizal plants reduce water loss by down-regulating *CsPIPs* under normal environmental conditions and preventing water loss [36,38]. The *CsPIP* expression patterns by AMFs under NS was reversed under WS, as evidenced by the up-regulated expression of 12 of the 14 *CsPIP* genes. In *Populus* × *canadensis* plants, AMF inoculation also up-regulated the expression of six *PIP* genes in leaves under drought stress [39]. Tian et al. [14] also reported that 12 of 14 *CsPIP* genes in cucumber were up-regulated under heat stress. These results imply that mycorrhizal plants resist WS by activating the expression of large amounts of aquaporin genes. Meanwhile, AMF itself has aquaporins, which synergize with host aquaporins to regulate host water status [40,41], which remains to be further verified.

5. Conclusions

This study confirmed the hypothesis mentioned earlier, namely, that AMF-inoculated cucumber plants were more resistant to WS than uninoculated plants, which was closely related to increased osmolyte content and stress-responsive gene (e.g., *CsPIPs* and *CsHsp70*) expression. Therefore, the mycorrhization of cucumber, especially at the seedling stage, will promote cucumber growth and stress resistance. However, future studies are needed around whether aquaporin genes in both the host and AMFs are synergistic or competitive under WS. A *CsHsp70* target gene needs to be selected to clarify its biological function as well as its role in arbuscule-containing root cortex cells.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10050478/s1, Table S1: The primer sequence of genes used in qRT-PCR.

Author Contributions: Conceptualization, Y.-N.Z.; Data curation, N.X. and D.W.; Investigation, N.X., X.T., D.W., Z.L.; Methodology, N.X., D.W., Z.L.; Resources, Y.-N.Z.; Supervision, Y.-N.Z.; Writing—original draft, N.X. and Z.L.; Writing—review and editing, A.H., E.F.A. and Q.-S.W. All authors have read and agreed to the published version of the manuscript.

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