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Arbuscular Mycorrhizal Fungi Improve Growth, Photosynthetic Activity, and Chlorophyll Fluorescence of *Vitis vinifera* L. cv. Ecolly under Drought Stress

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Abstract: Drought stress has become a limiting factor for viticulture with climate change. The influence of arbuscular mycorrhizal fungi (AMF) on grapevine *Vitis vinifera* L. cv. Ecolly's leaf water content, chlorophyll concentration, photosynthesis activity, and chlorophyll fluorescence under drought stress was studied in the greenhouse. The experiment was designed as a randomized complete block with four treatments: AMF colonization, well-watered; non-AMF colonization, well-watered; AMF colonization with drought stress; and non-AMF colonization with drought stress. The grapevines inoculated with mycorrhiza had a higher water content in the leaves and higher chlorophyll concentration under drought stress than those without mycorrhiza inoculation. AMF colonization increased the dry biomass of shoots and roots, photosynthetic rate, stomatal conductance, and transpiration rate and decreased intercellular CO_2 concentration. Mycorrhizal grapevines had higher non-photochemistry efficiency, higher photochemistry efficiency, and higher actual quantum yield than non-mycorrhizal grapevines. The results show that AMF alleviated the negative effects of drought stress on grapevines. The alleviation improved leaf water status, chlorophyll concentration, and photosynthetic capacity. Altogether, the results of our study indicate that AMF inoculation has the potential to protect grapevines under drought stress.

Keywords: arbuscular mycorrhizal fungi; grapevine; drought stress; photosynthesis; chlorophyll fluorescence

Citation: Ye, Q.; Wang, H.; Li, H. Arbuscular Mycorrhizal Fungi Improve Growth, Photosynthetic Activity, and Chlorophyll Fluorescence of *Vitis vinifera* L. cv. Ecolly under Drought Stress. *Agronomy* **2022**, *12*, 1563. https://doi.org/10.3390/agronomy12071563

Received: 26 May 2022
Accepted: 27 June 2022
Published: 29 June 2022

1. Introduction

Nearly half of the land area in China is arid or semi-arid. These arid and semi-arid areas are mainly in the northwest regions of China. Moreover, most wine grapes are cultivated in the northwest regions of China, which are generally areas plagued by droughts [1]. Although moderate water stress during maturation usually positively influences grape quality, continued water stress could have a strong negative effect on photosynthesis and the yield of grapevines [2]. With climate change, water availability is expected to decrease in many viticultural regions [3]. Moreover, more extreme weather brings water stress, which leads to great losses in viticulture. Drought has been a limiting factor for agriculture development, including for vineyard management.

Arbuscular mycorrhizal fungi (AMF) are members of the phylum Glomeromycota and abundantly present in the soil of most ecosystems [4]. They form mutualistic symbioses with roots of more than 80% of terrestrial plants [5]. Abundant research has identified that AMF could enhance the ability of plants such as apple [6], citrus [7,8], maize [9], white clove [10], *Zenia insignis* [11], *Knautia arvensis* [12], wheat [13], carob [14], rice [15], rose [16], and blueberry to cope with water stress [17]. The mechanisms behind this action include the enhancement of apples' drought tolerance by improving their gas exchange capacity, increasing chlorophyll fluorescence parameters, creating a greater osmotic adjustment capacity, increasing the scavenging of reactive oxygen species (ROS), and using MAPK signals for interactions between AMF and their apple plant hosts [6]. Citrus tolerance to

drought is improved based on improved water uptake via extended extraradical hyphae, improving nutrient uptake, better root system architecture, polyamine regulation, higher osmotic adjustments, developed enzymatic and non-enzymatic antioxidant defense systems, and improving glomalin-induced soil structural development [7,8].Drought tolerance is improved in maize by improving chlorophyll content, improving mineral uptake and assimilation, increasing growth and photosynthesis, increasing the content of compatible solutes, up-regulating the antioxidant system, and eliminating ROS, which leads to the protection of major metabolic pathways [9]. Enhanced drought tolerance in white clove is led by greater nutrient absorption and the accumulation of protective substances (soluble protein, proline, and flavonoids) [10]. A positive effect on plant biomass, osmolytes, and antioxidant enzyme activity under drought was noted in *Zenia insignis* [11], while alleviating the effect of AMF on *Knautia arvensis* drought stress was supported by lower proline accumulation [12]. Higher water-use efficiency in mycorrhizal wheat was also noted [13], and a few pieces of research have reported that AMF symbiosis increased grapevine tolerance to water stress and increased water-use efficiency [2,18].

Photosynthesis is one of the most important basic physiological activities of plants and is highly sensitive to environmental changes [19]. Some research has comprehensively investigated the influence of arbuscular mycorrhizae on the photosynthesis of plants under abiotic stress, such as its effects on maize under salt stress [20], on black locust under salt stress [21], and on peppermint being irrigated with saline water [22]. The potential of arbuscular mycorrhizal (AM) fungi to enhance citrus growth and photosynthesis has been documented well [23]. However, few studies have systematically investigated the effect of AM colonization on photosynthesis in grapevines. Nikolaos Nikolaou et al. [24] explored the physiological process of mycorrhizal and non-mycorrhizal 'Cabernet Sauvignon' grafted on different rootstocks under drought stress. Only stomatal conductance, net photosynthesis, and CO_2 assimilation rates were compared in terms of photosynthesis. The effect of mycorrhizal inoculation on photosynthesis differed by rootstock. Marko Karoglan et al. [25] assessed the influence of the application of mycorrhizal fungal inoculum on 'Cabernet Sauvignon' leaf gas exchange and concluded that inoculated grapevines expressed improved leaf gas-exchange parameters in general.

Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologists [26]. Many fluorescence applications detecting and analyzing stress effects on plants are nondestructive and sensitive [27]. Chlorophyll fluorescence shows the state of photosystem II and reflects the extent to which PS II uses the energy absorbed by chlorophyll. A combination of chlorophyll fluorescence and gas-exchange measurements could obtain a full picture of the response of plants to a changing environment [26]. Moreover, the sensitivity of chlorophyll fluorescence to even minor alterations in plant metabolism makes this technique suitable for identifying plant–stress factor interactions.

This study aimed to explore the influence of an established AMF association on chlorophyll, photosynthesis, and chlorophyll fluorescence in *Vitis vinifera* L. cv. Ecolly under water stress.

2. Materials and Methods

2.1. Experimental Design and Materials

Two-year own-rooted *Vitis vinifera* L. cv. Ecolly grapevines were each potted in 5 kg Lou soil (Eum-Orthic Anthrosol in FAO soil taxonomy). The soil was collected from the top layer (0–20 cm) of land in Yangling, Shannxi Province, China. The soil (pH 7.6) containing 3.6 mg/kg available nitrogen, 4.2 mg/kg available phosphorus, and 132.3 mg/kg available potassium was measured according to the method of Shidan Bao [28]. The soil was autoclaved at 121 °C for 2 h. The *Vitis vinifera* L. cv. Ecolly with good resistance to disease were selected by the College of Enology at Northwest A & F University [29]. The grapevines were grown under controlled conditions for the greenhouse experiment from January 2021

to September 2021. The grapevines were potted at the end of January, then inoculated in March. The greenhouse was set at a temperature of 20–25 °C and 12–14 h light.

One commercial AMF (MycoApply® Ultrafine Endo, Compostwerks Company, Mount Kisco, NY, USA), which had a good performance in previous experiments [30], was selected to inoculate the vines. The commercial AMF contained four endomycorrhizal fungi species: *Rhizophagus irregularis*, *Funneliformis mosseae*, *G. aggregatum*, and *Claroideoglomus etunicatum*. AMF was inoculated according to specification of the product on 25 March 2021. A hole was dug at the same position in the pot, and 5 g inoculum was put in the hole of each pot. Then, the hole was covered with excavated soil and the pot was watered. The grapevines were colonized with AMF under regular water management for 5 months. The water stress treatment was conducted after 5 months.

Four treatments were conducted in a randomized complete block design: AMF colonization, well-watered (AW); non-AMF colonization, well-watered (NW); AMF colonization with drought stress (AD); and non-AMF colonization with drought stress (ND). Each treatment had 15 pots for replication. Well-watered treatments were watered regularly, and water stress treatments did not receive watering. Well-watered potted soil maintained around 70% maximum field water-holding capacity, determined by weighing the pots. Measurements were started 7 days after watering was stopped in the pretest. Pot soils were at around 40% maximum field water-holding capacity 7 days after watering was stopped.

2.2. AMF Colonization Measurement and Biomass

The trypan blue method of Koske and Gemma [31] was used with some modifications. Roots were cut into 2 cm fragments and incubated for 20 min at 90 °C in 10% KOH, bleached for 30 min with alkaline H_2O_2 solution, acidified in 1% HCL at 90° for 10 min, and stained for 25 min at 90° in 0.05% trypan blue in acidic glycerol solution. Stained root samples were stored in an acidic glycerol solution for 72 h before being mounted in the same solution on a microscope slide. Stained roots of 2 cm were cut into 1 cm segments. A total of 60 stained root segments per plant were quantified under a compound microscope (Olympus-Japan) using the intersection method [32]. Four plants' roots were sampled for each treatment for quantifying colonization.

Shoots, roots, and roots with stakes were weighed after being oven-dried at 70 °C for 72 h until reaching a constant mass.

2.3. Relative Water Content (RWC)

Six pots were randomly selected for each treatment to determine the leaf relative water content (RWC). The leaf RWC was determined as described by Barrs and Weatherley [33,34]. The discs were detached from fresh leaves to calculate fresh weight (FW). Afterward, the same discs were allowed to achieve turgidity by keeping them in Petri dishes filled with distilled water to record the turgid weight (TW). After oven-drying, the dry weight (DW) of discs was recorded. RWC was calculated with the given formula: RWC (%) = (FW − DW)/(TW − DW) × 100. Another alternative parameter that expresses the water content on the basis of the water content at full turgor is water saturation deficit (WSD): WSD (%) = (TW − FW)/(TW − DW) × 100 or WSD = 100 − RWC. Leaf water content (LWC) was expressed by the fresh weight: LWC (%) = (FW − DW)/FW × 100.

2.4. Chlorophyll Concentration

Six pots were randomly selected in each treatment to determine the chlorophyll concentration. The chlorophyll concentration (chlorophylls a and b) was quantified according to Lichtenthaler and Wellburn [35]. Leaf tissue was homogenized in 80% acetone (v/v), and the absorbance was recorded at 480 nm, 663 nm, and 645 nm.

2.5. Photosynthesis Measurements

A portable GFS-3000 gas-exchange and fluorescence system (Heinz Walz GmbH, Effeltrich, Germany) was used for photosynthetic investigations. Three leaves from each grapevine and three pots for each treatment were measured. Mature and healthy leaves from the 8th–12th nodes were used for analysis. Measurements of the photosynthetic activity of leaves were carried out from 9 a.m. to 12 p.m. on a sunny day (12 September 2021).

Measurements of maximal photochemical efficiency (Fv/Fm), actual photosystem efficiency II (ΦPSII), and non-photochemical quenching (NPQ) were conducted using GFS-3000 system after dark adaptation of leaves for 30 min.

2.6. Chlorophyll Fluorescence Imaging

Chlorophyll fluorescence imaging was performed using a FluorCam FC 800-C/1010 (Photon Systems Instruments, Brno, Czech Republic). The shutter time and sensitivity of the charge-coupled device camera (SN_FC800) and light intensities were optimized for the experiment object in the pretest. Basic fluorescence F_0 was induced by two panels of super-bright orange-light-emitting diodes (λmax = 620 nm, 345 LED per panel; approx. 3 μmol m^{-2} s^{-1}). Maximum fluorescence (Fm) was triggered by short-term (1 s) saturation light pulses (max. 2500 μmol photons m^{-2} s^{-1}) generated by an electronic shutter-equipped halogen lamp (250 W). The grapevines were dark-adapted for 40 min before measurement. Three leaves from each grapevine and three pots for each treatment were sampled for imaging. The leaves were removed from the plants after being dark-adapted and then immediately put under the FluorCam FC 800-C/1010 camera.

2.7. Data Analysis

Statistical analyses were performed with the open-source R statistical computing environment (R Development Core team 2010). Two-way analysis of variance (ANOVA) and means were compared by Tukey's test at the 5% level. Graphs were made with GraphPad prism 9 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. AMF Colonization and Plant Growth

None of the grapevine plants in the non-inoculated treatments was colonized. Plants in the inoculated treatments had 89.2% AM fungal colonization and 76.8% arbuscules colonization (Table 1 and Supplementary Figure S1).

Table 1. Arbuscular mycorrhizal fungi colonization: percentage of mycorrhizal and non-mycorrhizal grapevine 'Ecolly' roots. A: AMF colonization; N: non-AMF colonization. Data are means ± SE (n = 4). Different letters indicate significant differences between treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$).

Treatment	Mycorrhizal Colonization %		Arbuscules Colonization %	
A	89.2 ± 2.9	a	76.8 ± 4.7	a
N	0	b	0	b

The mycorrhizal grapevines were more vigorous than the non-mycorrhizal grapevines during the growing period. The dry biomass of the shoots, roots, and roots with stakes was significantly higher in mycorrhizal than non-mycorrhizal plants regardless of water stress treatments (Figure 1). There were no significant differences between the well-watered treatment and the water stress treatment in terms of the dry biomass of shoots, roots, and roots with stakes.

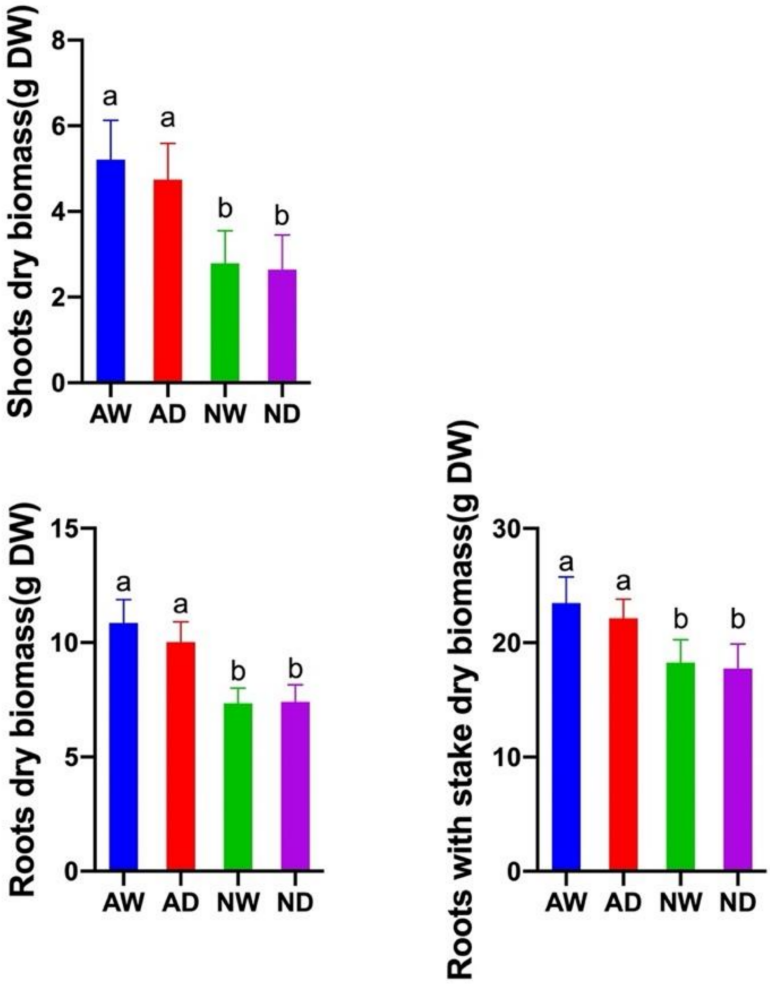

Figure 1. Shoot dry biomass, root dry biomass, and root-with-stake dry biomass of mycorrhizal and non-mycorrhizal grapevines under drought stress and well-watered conditions. AW: AMF colonization, well-watered; AD: AMF colonization, drought stress; NW: non-AMF colonization, well-watered; ND: non-AMF colonization, drought stress. Data are means ± SE (n = 6). Different letters indicate significant differences between treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$).

3.2. Leaf Water Content

The relative water content in the leaves was significantly higher in the mycorrhizal than the non-mycorrhizal plants under water stress (Figure 2). The relative water content in the leaves of the mycorrhizal and non-mycorrhizal plants showed no significant difference. Water stress decreased the relative water content in the leaves. The relative water content in the leaves was significantly lower in the plants with water stress treatment than in the well-watered plants, regardless of whether plants were inoculated with AM fungi. However, plants with AM fungi inoculation showed smaller relative water content decreases in leaves under water stress compared with plants without AM fungi inoculation.

The leaf water content (FW basis) had similar results to those for relative water content among the treatments. The leaf water content (FW basis) was significantly higher in the mycorrhizal than the non-mycorrhizal plants under water stress. Plants with AM fungi inoculation showed smaller leaf water content (FW basis) decreases under water stress compared with plants without AM fungi inoculation.

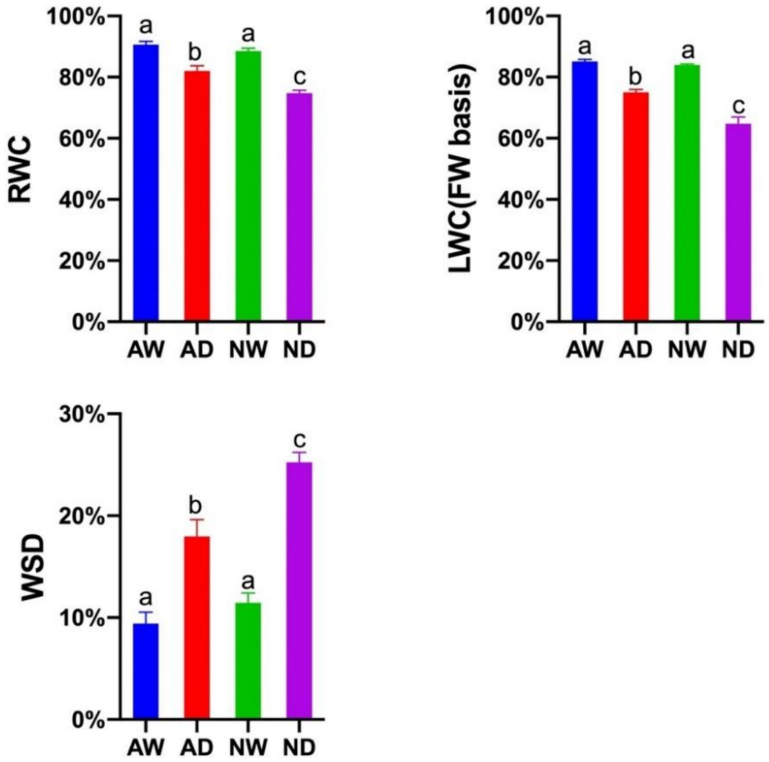

Figure 2. Relative water content, leaf water content, and water saturation deficit of mycorrhizal and non-mycorrhizal grapevines under drought stress and well-watered conditions. RWC: relative water content; LWC: leaf water content; WSD: water saturation deficit. AW: AMF colonization, well-watered; AD: AMF colonization, drought stress; NW: non-AMF colonization, well-watered; ND: non-AMF colonization, drought stress. Data are means ± SE (n = 6). Different letters indicate significant differences between treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$).

Water saturation deficit in the leaves was significantly lower in the mycorrhizal than the non-mycorrhizal plants under water stress. Plants with AM fungi inoculation showed smaller water saturation deficits under water stress compared with plants without AM fungi inoculation.

3.3. Chlorophyll Content

Inoculation of AMF improved the contents of chlorophylls a and b and total chlorophyll compared to non-inoculation. AW exhibited significantly higher chlorophyll a content and total chlorophyll content than control NW; chlorophyll b content of AW and NW exhibited no significant difference (Table 2). AD exhibited significantly higher chlorophyll a and b contents and total chlorophyll contents than ND.

Drought-exposed plants exhibited significantly lower contents of chlorophylls a and b and total chlorophyll than plants with no water stress. AD exhibited reduced content of chlorophyll a compared to AW. ND exhibited reduced contents of chlorophylls a and b and total chlorophyll compared to NW. However, AMF application mitigated the decline in pigments. Drought-exposed plants with AMF inoculation had a significantly higher content of chlorophylls a and b and total chlorophyll than drought-exposed plants without inoculation.

Table 2. Leaf chlorophyll concentrations of mycorrhizal and non-mycorrhizal grapevine 'Ecolly' seedlings under drought stress and well-watered conditions. AW: AMF colonization, well-watered; AD: AMF colonization, drought stress; NW: non-AMF colonization, well-watered; ND: non-AMF colonization, drought stress. Data are means ± SE (n = 6). Different letters indicate significant differences between treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$).

Treatment	Chlorophyll a (mg/g FW)		Chlorophyll b (mg/g FW)		Total Chlorophyll (mg/g FW)	
AD	0.670 ± 0.08	b	0.344 ± 0.05	a	1.014 ± 0.13	a
AW	0.899 ± 0.20	a	0.433 ± 0.08	a	1.332 ± 0.27	a
ND	0.330 ± 0.04	c	0.220 ± 0.04	b	0.550 ± 0.07	b
NW	0.689 ± 0.08	b	0.365 ± 0.03	a	1.054 ± 0.08	a

3.4. Photosynthesis Activity

AM symbiosis significantly enhanced the net photosynthetic rate (A), stomatal conductance (GH_2O), and transpiration rate (E) and reduced the intercellular CO_2 concentration(ci) of grapevine leaves under water stress (Figure 3). AM symbiosis also enhanced the net photosynthetic rate (A) and reduced the intercellular CO_2 concentration (ci) of grapevine leaves in the well-watered condition. Water stress dramatically decreased photosynthesis activity, while AM fungi inoculation ameliorated the decrease caused by drought stress. The vapor pressure deficit (VPD) had no significant impact in either treatment.

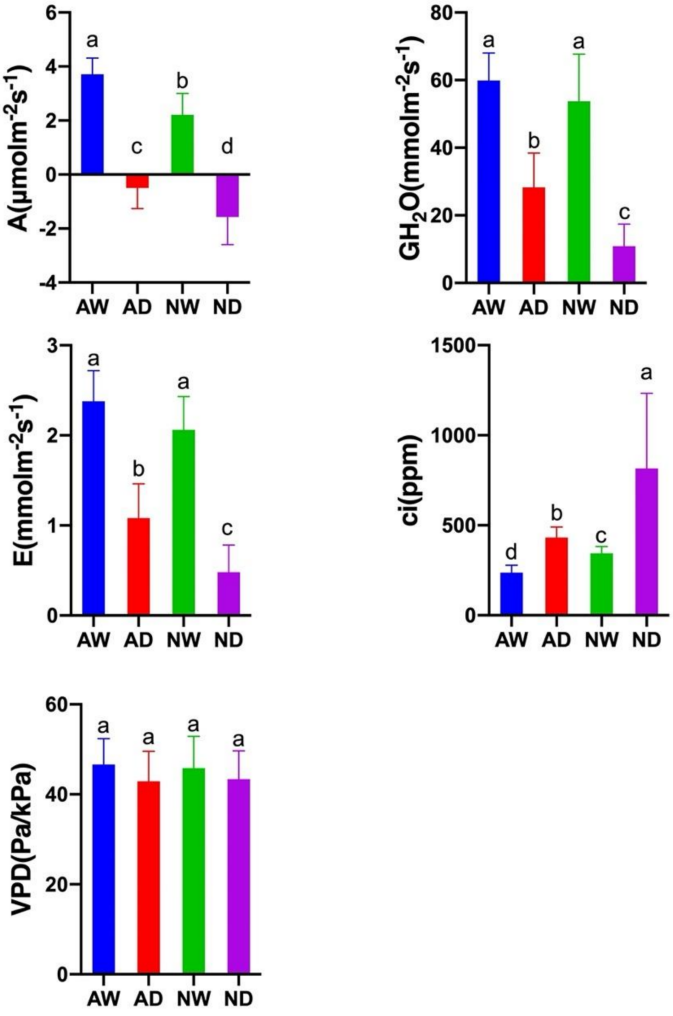

Figure 3. Photosynthesis parameters of mycorrhizal and non-mycorrhizal grapevines under drought stress and well-watered conditions. A: Assimilation rate/ photosynthetic rate; GH_2O: water vapor

conductance/stomatal conductance; E: transpiration rate; ci: intercellular CO_2 mole fraction; VPD: vapor pressure deficit. AW: AMF colonization, well-watered; AD: AMF colonization, drought stress; NW: non-AMF colonization, well-watered; ND: non-AMF colonization, drought stress. Data are means ± SE (n = 9). Different letters indicate significant differences between treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$).

3.5. Chlorophyll Fluorescence

Chlorophyll fluorescence was measured both by the GFS-3000 system and FluorCam FC 800-C/1010. FluorCam FC 800-C/1010 was more sensitive than the GFS-3000 system. Water stress was detected by FluorCam FC 800-C/1010 7 days after stopping watering (around 40% maximum field water-holding capacity), while the GFS-3000 system could detect the water stress when the stress treatment was prolonged (around 35% maximum field water-holding capacity). Both systems determined that AM fungi inoculation increased photochemical efficiency (Fv/Fm) under water stress (Figures 4 and 5). AM fungi inoculation increased the parameters of the potential photosynthetic activity of PSII Fv/F_0, the effective quantum yield of PSII ϕPSII, and non-photochemical quenching NPQ compared with the non-mycorrhizal plants under water stress. There were no significant differences in Fv/Fm, Fv/F_0, ϕPSII, and NPQ between the mycorrhizal and the non-mycorrhizal plants under well-watered conditions, while ϕPSII and NPQ exhibited a trend that increased in the mycorrhizal plants compared to the non-mycorrhizal plants.

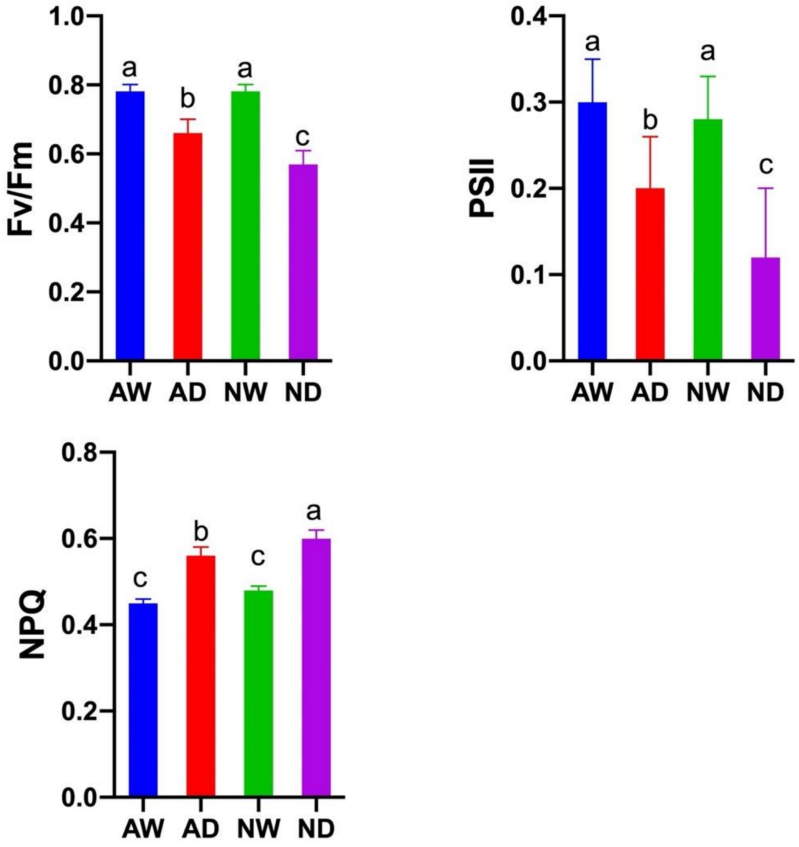

Figure 4. Chlorophyll fluorescence of mycorrhizal and non-mycorrhizal grapevines under drought stress and well-watered conditions measured by GFS-3000 fluorescence system. Fv/Fm: maximal photochemical efficiency; ΦPSII: actual photosystem efficiency II; NPQ: non-photochemical quenching. AW: AMF colonization, well-watered; AD: AMF colonization, drought stress; NW: non-AMF colonization, well-watered; ND: non-AMF colonization, drought stress. Data are means ± SE (n = 9). Different letters indicate significant differences between treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$).

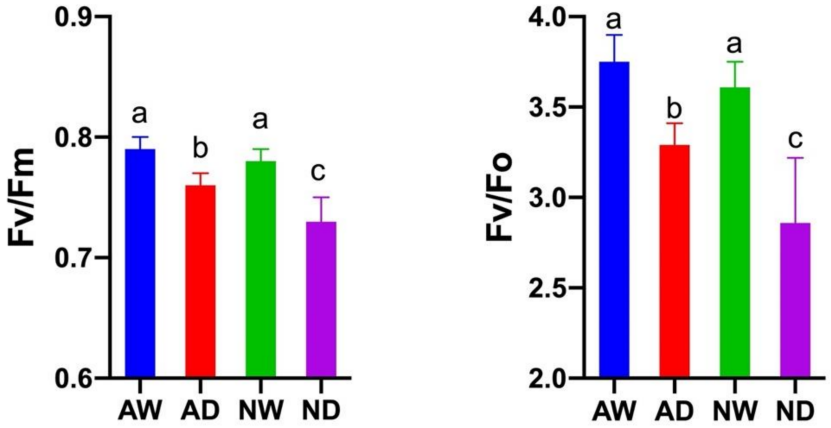

Figure 5. Chlorophyll fluorescence of mycorrhizal and non-mycorrhizal grapevines under drought stress and well-watered conditions measured by FluorCam FC 800-C/1010 system. Fv/Fm: maximal photochemical efficiency; Fv/F_0: potential photosynthetic activity of PSII AW: AMF colonization, well-watered; AD: AMF colonization, drought stress; NW: non-AMF colonization, well-watered; ND: non-AMF colonization, drought stress. Data are means ± SE ($n = 9$). Different letters indicate significant differences between treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$).

Photos of chlorophyll fluorescence imaging directly reflect the stress that leaves suffered through color-coding of the images (Supplementary Figure S2). The imaging results indicated that the AM fungi inoculation increased the parameters of the maximal photochemical efficiency of PSII Fv/Fm compared with the non-mycorrhizal plants.

4. Discussion

Globally, during the growing season, most vineyards are located in arid and semi-arid areas or dry climate areas. Due to these conditions, water deficits easily and frequently occur. With climate change, water deficits will likely happen more and become a limiting factor for viticulture [36]. AMF are increasingly used as natural biofertilizers and a sustainable alternative to improve plant health and growth [37]. The potential of AMF to enhance plant tolerance to abiotic stress conditions, including water stress, has been widely known and represents a promising sustainable solution to increase crop production without compromising the environment.

The AMF used in this study was selected based on a previous study [30]. This kind of commercial AMF had the highest colonization rate and significantly improved the growth of grapevines. This study observed the same good colonization rate. This showed that all inoculated grapevines roots were effectively colonized, and the cultivar 'Ecolly' was compatible with this inoculum in the experimental conditions. Natural soil contains various microorganisms, including mycorrhizas. The soil was sterilized to minimize the effect of the microorganisms from nature. The water stress treatment conducted in this study withheld watering until the plants started to become wilted, which was a short time compared with treatments that withhold irrigation for several weeks. The short-time water stress treatment did not influence the AMF colonization, which was also observed by Naheeda Begum et al. [38], Armada et al. [39], and Kong et al. [40].

Due to the relatively short drought-stress treatment time, drought stress also did not affect the dry biomass of shoots and roots. The results showed that the mycorrhizal grapevines had higher shoot and root dry biomasses than the non-mycorrhizal grapevines. This is in agreement with a previous study [30] and many others, such as studies on grapevine rootstock SO_4 [41], *Knautia arvensis* [12], and maize [42]. Some studies have also found that AMF inoculation did not significantly increase plants' dry biomass. A study on wheat found no significant effect of AMF inoculation on ear or grain weight. However, AMF inoculation influenced the number of ears and grains [43]. A study on olive cuttings that

used a mixed community inocula of AMF found that some inocula significantly increased plants' dry biomass, and some did not; however, they all improved plant growth [44].

AMF inoculation increased the relative water content in leaves. The enhancement of water status by AM symbiosis was related to the improved water uptake due to AM fungal hypha, better root architecture, and enhanced root activity due to the colonization of AM fungi [7]. The improvement in water status by AM symbiosis could indirectly enhance the capacity of gas exchange and the efficiency of photochemistry and non-photochemistry of PSII. Foliar water potential is another more specific parameter that indicates water stress and should be included in further studies.

Drought stress decreased chlorophyll concentrations, especially in plants without AMF inoculation. Some leaves of the grapevines appeared wilted 7 days after watering was stopped. Water stress influences the synthesis of chlorophyll pigments and induces pigment degradation by upregulating chlorophyllase activity and downregulating the enzymes involved in the biosynthesis of chlorophyll [45]. The influence of water stress on the metabolism of chlorophyll pigments affects chlorophyll fluorescence and net photosynthesis [38,46].

The mycorrhizal grapevines had significantly higher chlorophyll a content in leaves than the non-mycorrhizal grapevines, not only under drought stress but also under well-watered conditions. The net photosynthetic rate also showed the same results. Other gas-exchange parameters showed similar differences between the mycorrhizal grapevines and the non-mycorrhizal grapevines. This suggests that this difference is not just induced by drought stress but also accumulated by AMF inoculation. Many studies have found that AMF inoculation can improve photosynthesis capacity and chlorophyll content, such as studies on black locust [47], rice [48], and citrus tangerine seedlings [49]. Some studies have also indicated that AMF inoculation did not increase chlorophyll content. A study on four Iranian grape varieties recorded that AMF inoculation maintained total chlorophyll [50]. Related to photosynthesis, studies on grapevines are not as numerous as those on other plants, while E. Nicolás et al. [51] found that AMF inoculation improved the photosynthetic performance and plant water status in Crimson seedless grapevines. AMF inoculation improving photosynthesis was also found in a study on 'Paulsen 1103' (*Vitis berlandieri* × *rupestris*), and it alleviated the toxic effects of heavy metals [52]. A study demonstrated that inoculated 'Cabernet Sauvignon' grapevines expressed improved leaf gas-exchange parameters in general [25]. In a study by Valentine A. J. [53], although AM plants had lower stomatal conductances and substomatal CO_2 concentrations, similar photosynthetic rates were found between AM and non-AM plants under drought stress.

Mycorrhizal grapevines had higher stomatal conductance, a higher transpiration rate, a higher net photosynthetic rate, and lower intercellular CO_2 concentrations than those of non-mycorrhizal grapevines under water stress. This suggests that AMF colonization could elevate photosynthesis by improving the gas-exchange capacity of grapevines under water stress. The AMF is considered a metabolic sink for photosynthate mobilization to the plant roots, hence providing signals for greater photosynthesis activity [54].

Fv/Fm has been known as a reliable indicator of stress due to its particular sensitivity to a variety of environmental-stress-inducing factors [55]. The response of Fv/Fm to water stress is more sensitive than the photosynthesis parameters measured in this study. However, related studies reported that the net assimilation rate and stomatal conductance decreased earlier than the chlorophyll fluorescence in stressed plants [56,57]. There were several reasons that could lead to this contrary result. First, the chlorophyll fluorescence was measured by a closed system in a room with little disturbance; however, the gas-exchange parameter was measured outdoors with more disturbances. Second, the sensitivities of the machines are different. The FluorCam FC 800-C/1010 may be more sensitive than the GFS-3000 system in this experiment. Third, the variability between plants could be great or small. The variance in the data from the chlorophyll fluorescence was smaller than that in the gas-exchange parameters. Chlorophyll fluorescence imaging could directly reflect via images the stress that plants suffer. Our results showed that the efficiency of

PSII photochemistry (indicated by Fv/Fm) in the leaves of the mycorrhizal grapevines was significantly higher than that in the non-mycorrhizal grapevines. The mycorrhizal plants had a higher potential photosynthetic activity of PSII (Fv/F_0) than that of the non-mycorrhizal plants. Additionally, we can discern that the mycorrhizal plants had a higher effective quantum yield of PSII (ϕPSII) and non-photochemical quenching (NPQ).

Drought decreased photosynthesis and PSII activity significantly; however, AMF inoculation alleviated the decline efficiently, which is consistent with studies of peanuts and tomatoes [58] and watermelon seedlings [59]. Mo et al. [59] demonstrated that inoculation of AMF improves photosynthesis and PSII functioning under drought by improving water uptake, stabilizing chloroplasts and membrane structure, enhancing Rubisco content, and reducing the accumulation of free radicals. The present results suggest that AMF inoculation can improve photosystem functioning and mitigate the inhibition of photosynthesis caused by drought stress in AMF-inoculated plants.

The positive effect of AMF on grapevines in this study was obtained under the conditions of a controlled greenhouse. Many factors affect the success of inoculation and AMF persistence in soil, such as the AMF species' compatibility with the environment, competition with other soil organisms, and the timing of inoculation [37]. Thus, more research needs to be completed under different conditions in the field.

5. Conclusions

This study intensively analyzed the effect of AMF inoculation on the photosynthesis activity of grapevines under greenhouse conditions. AMF symbiosis increased water content in the leaves and chlorophyll concentration under drought stress. AMF colonization increased the photosynthetic rate, stomatal conductance, and transpiration rate and decreased the intercellular CO_2 concentration. The mycorrhizal grapevines had higher non-photochemistry efficiency, photochemistry efficiency, and actual quantum yield compared with the non-mycorrhizal grapevines. These results show that AMF inoculation could mitigate the negative effects caused by drought stress on photosynthesis. Further studies are needed to investigate the effect of AMF inoculation on vines under different conditions in the field.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071563/s1, Figure S1: Photos of mycorrhizal and non-mycorrhizal grapevine 'Ecolly' roots observed with optical microscope; Figure S2: Photos of Chlorophyll fluorescence imaging taken by FluorCam FC 800-C/1010 system.

Author Contributions: Conceptualization, formal analysis, investigation, methodology, software, and writing—original draft preparation, Q.Y.; writing—review and editing, supervision, project administration, and funding acquisition, H.W. and H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the research and application of key technologies for the sustainable development of the wine industry program, grant number LYNJ202110.

Data Availability Statement: Not Applicable.

Acknowledgments: We thank the large-scale instrument sharing platform of the College of Life Science at Northwest A & F University for providing the FluorCam FC 800-C/1010 system.

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

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