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Research article

Mycorrhizal Influence on Irrigation Efficiency: A Study of Maize under Drought Conditions

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Abstract

Drought stress is one of the biggest threats to agriculture in different parts of the world, especially in countries in the Mediterranean climate zone. One of the most natural solutions for agricultural sustainability is to use fungi that can establish symbiotic relationships with agricultural products. In this study, the effectiveness of different arbuscular mycorrhizal fungi that can help reduce drought stress in maize plants was tested. The findings revealed that arbuscular mycorrhiza fungi inoculation significantly improved both irrigation water use efficiency (IWUE) and plant biomass under drought stress compared to non-inoculated controls. Notably, *Rhizophagus intraradices* and *Glomus iranicum* showed the highest enhancements in IWUE and yield. For example, *R. intraradices* achieved an irrigation water use efficiency of 16.2 kg/m³ under low drought stress (70% of field capacity) and a yield of 26.9 t ha^{-1}. Under moderate drought stress (50% of field capacity), this species maintained a high IWUE of 16.1 kg m^{-3} and a yield of 18.4 t ha^{-1}. In severe drought conditions (30% of field capacity), *R. intraradices* still performed well with an IWUE of 13.5 kg m^{-3} and a yield of 10.9 t ha^{-1}. Overall, AMF-treated plants exhibited 30-50% higher WUE compared to controls, with *G. iranicum* and *R. intraradices* being the most effective in enhancing drought tolerance and plant productivity. These results suggest that integrating AMF into maize cultivation can contribute to sustainable agricultural practices, particularly in regions facing water scarcity.

Keywords: arbuscular mycorrhizal fungi; water use efficiency; maize; drought stress; chlorophyll

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are integral components of soil ecosystems, forming symbiotic relationships with the roots of most terrestrial plants. These relationships are vital for enhancing plant growth and stress tolerance, primarily through improved nutrient uptake, disease tolerance, and water relations. In agriculture, AMF are particularly significant as they contribute to sustainable farming practices by reducing the need for

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chemical fertilizers and enhancing plant resilience to environmental stressors such as drought (Kaba et al., 2021).

Water is a critical resource for plant growth, and its efficient use is paramount, especially in regions prone to water scarcity. Water use efficiency (WUE) is a measure of how well a plant uses available water to produce biomass. AMF can significantly influence WUE by enhancing water uptake through increased root surface area and improved soil structure. The hyphal networks of AMF extend beyond the root zone, accessing water from soil pores that roots alone cannot reach. This extended network not only improves water uptake but also facilitates better water retention in the soil, thus enhancing the overall WUE of plants (Duan et al., 2021). In a study conducted by Chen et al. (2025), the effect of arbuscular mycorrhizal fungi (AMF) on microbiome stability in the maize rhizosphere was investigated. The study evaluated the impact of AMF species such as *Rhizophagus intraradices* (syn. *Glomus irregulare*, previously misidentified as *Glomus intraradices*) on sugar transport, water use efficiency, and abiotic stress tolerance in maize plants under water deficit conditions. The genes *SWEET13, CHIT3*, and *RPL23A* exhibited higher expression in AMF-inoculated plants, which contributed to improved plant growth under water stress conditions. In a study by Boomsma and Vyn (2008), the resilience of AMF-inoculated maize plants to water scarcity was assessed. The research determined that increased AMF colonization led to higher soluble sugar and proline content in the plants. Additionally, mycorrhiza-inoculated maize plants exhibited greater biomass and root development under drought conditions. A study conducted by Oliveira et al. (2022) demonstrated that the AMF species *Rhizophagus clarus* enhanced drought tolerance in soybean plants. This mechanism was reported to function by increasing water uptake through the root system while reducing water loss at the leaf level. These findings suggest that AMF applications could similarly confer drought tolerance in maize plants. Several mechanisms are proposed for how AMF improve plant water use. Firstly, the physical extension of the root system by the fungal hyphae increases the soil volume explored for water. Secondly, AMF can alter the root hydraulic properties, enhancing water transport efficiency. Additionally, AMF can influence the expression of aquaporin genes in roots, which are proteins involved in water transport across cell membranes (Liu et al., 2023; Ni et al., 2025). Lastly, AMF associations can lead to improved soil aggregation, which enhances soil water retention and reduces water loss through evaporation (Aminzadeh et al., 2025).

Maize (*Zea mays* L.) is a globally important crop, heavily reliant on adequate water supply for optimal growth and yield. However, water scarcity and drought stress are significant challenges in maize cultivation, affecting both productivity and sustainability. The application of AMF in maize cultivation presents a promising strategy to mitigate the adverse effects of water stress by improving WUE and overall plant health. Previous studies have shown that AMF-inoculated maize plants exhibit improved growth and yield under drought conditions, suggesting a potential role of AMF in sustainable maize farming (Abrar et al., 2024; Li et al., 2025).

In this study, the effects of different AMF species on the water use and WUE of maize plants under varying levels of drought stress was investigated. By examining the interactions between AMF and irrigation levels under controlled conditions, this research seeks to elucidate the potential benefits of AMF applications in enhancing the resilience of maize to water stress. The specific objectives were: to evaluate the impact of different AMF species on the water uptake of maize plants, to determine the effect of AMF on the WUE of maize under different drought stress levels, and to analyze the physiological and morphological changes in maize roots and shoots induced by AMF under water-limited conditions.

Understanding the role of AMF in improving plant water relations is crucial for developing sustainable agricultural practices, particularly in water-scarce regions. This research provides insights into the potential of AMF to enhance crop resilience to drought, thereby contributing to food security and sustainable farming. By identifying effective AMF species and their mechanisms of action, this study offers practical applications for farmers and agricultural practitioners aiming to optimize water use and improve crop productivity under challenging environmental conditions.

2. Materials and Methods

2.1 Site description and experimental design

The study was carried out in 2023 at the Ankara Soil Fertilizer and Water Resources Central Research Institute within a glass greenhouse. Soils were sieved to 4 mm and placed in pots at their natural bulk densities. All soils, except those in the control group, were sterilized in an autoclave to remove existing mycorrhizal fungi prior to pot filling.

In this study, experiment was conducted using different species of arbuscular mycorrhizal fungi (AMF). The *Rhizophagus irregularis* inoculum was initially cultured using maize (*Zea mays* L.) as a host plant through the trap culture technique (Selvakumar et al., 2016) for its use in the project. It was propagated in a sand mixture in a greenhouse for four months in association with *Zea mays* L. This procedure was carried out to facilitate the use of institute products. The experimental groups used in this study were to ensure an equivalent dosage to the calculated propagule amount for *Glomus iranicum var. tenuihypharum*, and the following calculations were performed. Control Group (M0): This group did not receive any mycorrhizal inoculum. *Glomus* spp. (M1, Shubhodaya): The *Glomus* spp. inoculum was commercially sourced from the Shubhodaya product, which contained a mixture of *Glomus intraradices* and *Glomus proliferum* with a density of $1×10^6$ propagules per package. The dosage was calculated to be equivalent to 12.6 propagules, and 1.26 mg of *Glomus* spp. inoculum was applied to each 7 kg soil pot, placed 2 cm below the soil surface along with water in the seedbed. *Rhizophagus irregularis* (M2): Provided by the Soil Fertilizer and Water Resources Central Research Institute, this inoculum had a density of 25 propagules/g. A total of 500 mg of *R. irregularis* inoculum was applied per 7 kg soil pot. *Rhizophagus intraradices* (M3): This inoculum, supplied by the institute and initially cultured with maize (*Zea mays* L.), had a density of 20 propagules/g. The inoculum, propagated in the greenhouse for four months, consisted of spores, mycelium, and fine root segments. A total of 630 mg of *R. intraradices* inoculum was applied per pot. *Glomus fasciculatum* (M4): Commercially obtained from the GROW CARE product, this inoculum had a density of 60 spores/g. A total of 210 mg of *G. fasciculatum* inoculum was applied per 7 kg soil pot. *Glomus iranicum* var. *tenuihypharum* (M5): This inoculum was commercially sourced from the MycoUp® product, which was designed to promote root development and enhance nutrient uptake efficiency in field crops, vegetables, and viticulture. The inoculum consisted of spores (120 propagules/g soil), mycelium, and fine root segments. A total of 105 mg of *G. iranicum* var. *tenuihypharum* inoculum was applied per 7 kg soil pot. All inocula were applied to the seedbed at a depth of 2 cm below the soil surface along with water.

Corn seeds, specifically the Dekalp DKC5685 maize variety, were surface sterilized with 5% sodium hypochlorite solution for 10 min before planting, and then rinsed five times with distilled water. Irrigation was administered to reach field capacity until the maize developed 3-4 leaves. Beyond this growth stage, irrigation was adjusted to maintain soil moisture at 70%, 50%, and 30% of the soil's available water holding capacity. The results of the soil and irrigation water analysis are shown in Tables 1 and 2, respectively.

Table 1. Some characteristics of the soil used in the greenhouse experiment

Characteristics	**Value**
Organic matter, %	1.11
P, kg da-^{1}	39.30
K, kg da-^{1}	143.0
Ca, ppm	7405.0
Mg, ppm	831.0
Zn, ppm	2.08
Mn, ppm	5.24
Fe, ppm	4.76
Texture	Clay
Electrical conductivity, dS m^{-1}	0.62
pH	7.01
Field capacity, %	32.07
Wilting point, %	17.07
Bulk density, g cm^{-3}	1.29

Table 2. The characteristics of the irrigation water used in the greenhouse experiment

pH	**EC dSm^{-1}**	**Cations (meL^{-1})**				**Total**	**Anions (meL^{-1})**				**SAR**	**Boron ppm**	**Class**
		Na^+	K^+	Ca^{++}	Mg^{++}		$CO3^-$	$HCO3^-$	Cl^-	$SO4^=$			
7.53	0.54	3.43	0.13	3.39	1.65	8.6	0	1.71	3.87	3.01	2.16	0	C_2S_1

According to the electrical conductivity (EC) and alkalinity measurements, the water is classified as C2S1. The quality of the irrigation water is considered excellent, characterized by slight alkalinity and minimal salinity hazard. This makes it suitable for irrigating a diverse range of crops with minimal risk of accumulating soil salinity or sodicity, provided that proper drainage systems are in place. Soil moisture levels were monitored using a gravimetric method to determine irrigation timing. At the beginning of the experiment, pots were saturated with water. Once the water had ceased leaking from the pots, their weights were recorded and considered as the field capacity. The study involved five different arbuscular mycorrhizal fungi (AMF) treatments along with a control group, all using maize plants. In the selection of AMF species used in the study, those reported to establish a symbiotic relationship with maize under stress conditions and facilitate water and nutrient uptake were considered. *Rhizophagus irregularis* has been found to enhance phosphorus uptake, thereby improving maize nutrient utilization, and to exhibit aggressive root colonization even in low-phosphorus soils (Basiru et al., 2020). The growth and yield of maize were significantly enhanced by *R. irregularis*. It has been reported that *R. irregularis* improves soil resilience against degradation under both cultivated and uncultivated field conditions while maintaining maize productivity (Basiru et al., 2020). *Glomus iranicum* var. *tenuihypharum* has been shown to enhance drought tolerance, enabling maize plants to better adapt to arid conditions. Additionally, it has been demonstrated to improve plant growth even in high-salinity soils, thereby enhancing maize adaptation capacity. Furthermore, it has been shown to enhance nitrogen, phosphorus, and potassium uptake in maize, leading to improved growth and yield (Martín et al., 2017).

Three levels of drought stress were applied: 30% of field capacity (no drought stress), 50% of field capacity (moderate drought), and 70% of field capacity (severe drought) within the greenhouse environment. Throughout the experiment, pots were periodically weighed to monitor moisture levels, and irrigation water was added to each treatment to maintain the predetermined weight levels (Kiran, 2019). The greenhouse experiment followed a factorial design, comprising 54 pots arranged in randomized plots, with 3 replications of 6 AMF treatments and 3 drought levels. Trial sub and main topics were established as follows.

AMF treatments:

- M0: Control treatment
- M1: *Glomus* spp. (Shubhodaya)
- M2: *Rhizophagus irregularis*
- M3: *Rhizophagus intraradices*
- M4: *Glomus Fasciculatum*
- M5: *Glomus iranicum*

Drought stress levels:

- I70: Treatment where the soil's available water is maintained at 70% (low drought stress)
- I50: Treatment where the soil's available water is maintained at 50% (moderate drought stress)
- I30: Treatment where the soil's available water is maintained at 30% (severe drought stress)

The experiment was terminated when drought symptoms (withering, wilting, drying of lower leaves) were clearly visible on the plants.

2.2. Irrigation

Irrigation was performed at field capacity level for all pots until seed emergence occurred and the plants reached the 3-4 leaf stage. After this phase, subject-specific irrigation treatments were initiated. For this purpose, the pots were monitored daily, and their weights were measured. Irrigation amount for each treatment were calculated according to equation (1) and irrigation water use efficiency (IWUE) was calculated by using equation (2).

$$I = FC_W - (FC_W - WP_w) \times Ic \qquad (1)$$

Where; I: irrigation amount (l), FC_W: pot field capacity weight (g), WP_W: pot wilting point weight (g), Ic: coefficient related to irrigation treatment (30 %, 50%, 70%).

$$IWUE= Y/ I \quad (2)$$

In the equation: IWUE: irrigation water use efficiency (kg m^{-3}), Y: fresh crop weight (kg ha^{-1})

In the study, changes in plant yield at different irrigation levels under different mycorrhizal treatments were calculated with the following equations;

$$YI= Yc-Ya \quad (3)$$

Where, YI: yield increased by irrigation, Yc: crop yield in control treatment, Ya: crop yield in actual treatment.

$$IWP= (Y/I) x100 \quad (4)$$

In the equation, IWP: irrigation water productivity (kg m^{-3}), Y: yield (kg m^{-3}), I: irrigation amount (mm).

$$WP= (YI/I) x100 \quad (5)$$

Where, WP: Water productivity (kg m^{-3})

Mycorrhizal dependency was calculated for the maize by using the following formula (Raya-Hernández et al. 2020):

$$MD\ (\%) = \frac{\text{crop fresh yield (M +)} - \text{ crop fresh (M −)}}{\text{crop fresh (M +)}} x100 \quad (6)$$

Where, MD represents mycorrhizal dependency, for M+ (inoculated) and M− (not inoculated) plants.

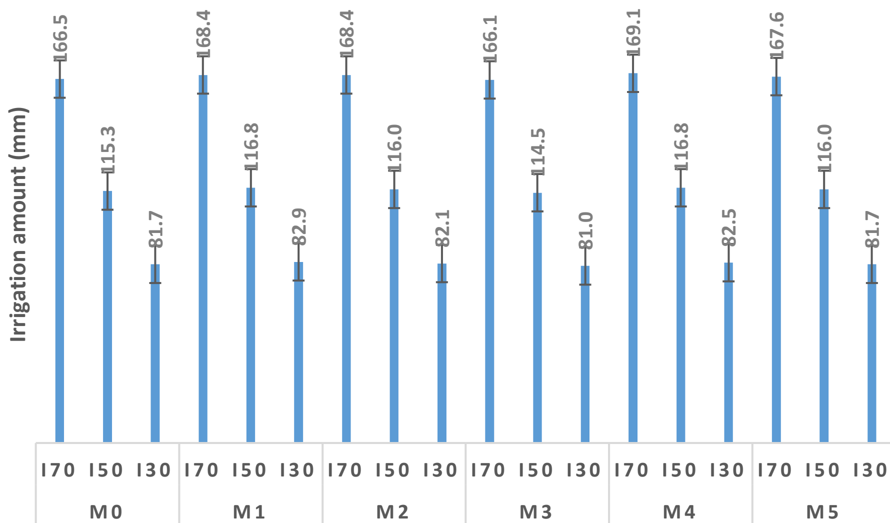
2.3. Statistical analysis

The data obtained from the study were analyzed with JMP v.17 computer software. ANOVA test was applied for variance analysis, while Tukey's multiple comparison test was applied for statistical differences between treatments.

3. Results and Discussion

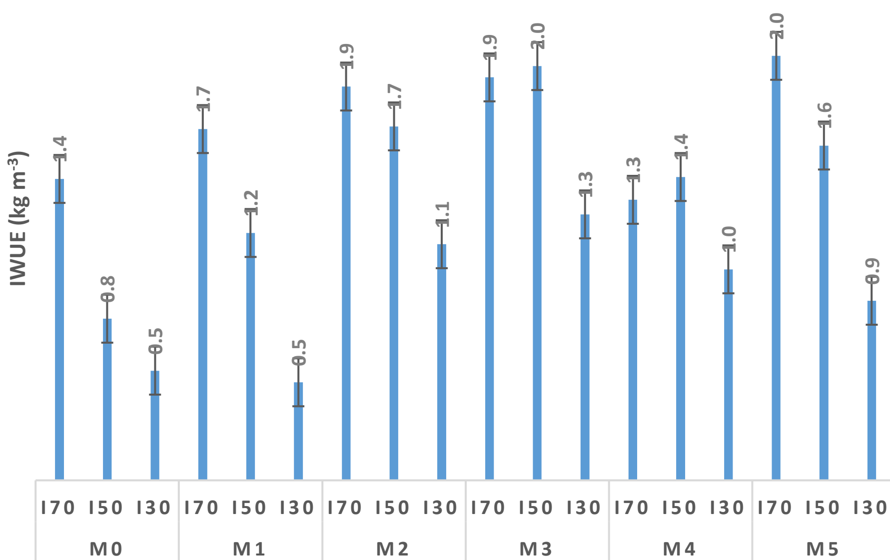
3.1 Irrigation

In the greenhouse study, the amounts of irrigation water given to the subjects and the irrigation water use efficiency calculated based on the plant green mass weight are presented in Figure 1 and Figure 2. Across all mycorrhizal treatments, irrigation at 50% field capacity was roughly 30-31% less than at 70% field capacity, and irrigation at 30% field capacity was about 50-51% less than at 70% field capacity. This indicates that there



I70: irrigation at 70% of field capacity, **I50**: irrigation at 50% of field capacity, **I30**: irrigation at 30% of field capacity, **M0**: Control, **M1**: Glomus spp. (Shubhodaya), **M2**: *Rhizophagus irregularis*, **M3**: *Rhizophagus intraradices*, **M4**: *Glomus Fasciculatum*, **M5**: *Glomus iranicum*

Figure 1. Irrigation amount according to treatments



I70: irrigation at 70% of field capacity, **I50**: irrigation at 50% of field capacity, **I30**: irrigation at 30% of field capacity, **M0**: Control, **M1**: Glomus spp. (Shubhodaya), **M2**: *Rhizophagus irregularis*, **M3**: *Rhizophagus intraradices*, **M4**: *Glomus Fasciculatum*, **M5**: *Glomus iranicum*

Figure 2. Irrigation water use efficiency according to treatments

was a consistent pattern of reduction in irrigation volume as the percentage of field capacity decreased, which was true for all mycorrhizal treatments observed.

In the absence of any mycorrhizal applications, as represented by M0 (the control group), there was a noticeable decrease in IWUE as the amount of irrigation water was reduced. This suggested that plants utilized water less efficiently when provided with less irrigation. The highest IWUE in the M0 group was observed at the S70 irrigation level (1.44 kg m^{-3}), and the lowest at the S30 level (0.52 kg m^{-3}). Mycorrhizal treatments generally exhibited higher IWUE values compared to the control group (M0), indicating that mycorrhizal applications might enhance the water use efficiency of plants. Upon examining individual mycorrhizal treatments, M5 (*Glomus iranicum*) showed the highest IWUE at the S70 irrigation level with a value of 2.03 kg m^{-3}, suggesting that this species of mycorrhiza most effectively improved water use efficiency. Moreover, M5 consistently achieved high IWUE values across different irrigation levels. M3 (*Rhizophagus intraradices*) stood out with the highest IWUE value of 1.98 kg m^{-3} at the S50 irrigation level and also showed increased efficiency compared to the control group at other irrigation levels. The treatment M2 (*Rhizophagus irregularis*) also exhibited prominence, particularly at the S50 and S70 irrigation levels, with high IWUE values. In conclusion, mycorrhizal applications appeared to enhance the water use efficiency of plants, and this enhancement varied depending on the type of mycorrhiza and the level of irrigation. The highest water use efficiency was typically observed at higher irrigation levels, particularly with the application of *G. iranicum* (M5). A general decline in IWUE was noted with the reduction of irrigation levels across all mycorrhizal treatments, but this decline was less pronounced compared to the control group (M0). This suggested that mycorrhizal applications may aid in more efficient water usage by plants even under conditions of water stress. The effect of AMF applications on IWUE depends on the structure of the symbiotic relationship. Significant irrigation water use efficiency can be observed when the symbiotic structure formed between the plant and AMF reaches an optimal level at a certain stress condition. Indeed, in this study, the prominent mycorrhizal species M3 and M5 exhibited significant differences in IWUE compared to the control treatments. In the I30 treatment, M3 and M5 AMF applications resulted in an IWUE increase of 142.5% and 63.7%, respectively, compared to the control. In the I50 treatment, these increases were 156.3% and 107.2%, while in the I70 treatment, IWUE increased by 33.7% and 40.9%, respectively. The highest IWUE values were achieved particularly under moderate drought stress (I50), indicating that the most effective symbiotic relationship was established under this drought condition.

Abrar et al. (2024) found that AMF-inoculated plants exhibited not only increased biomass but also improved water use efficiency under drought conditions, which is consistent with the observed improvements in IWUE. Li et al. (2025) reported that AMF colonization enhanced plant growth and biomass under water-limited conditions, potentially through mechanisms that improved water uptake and conservation. Wu et al. (2024) found that AMF colonization increased plant water use efficiency by enhancing root water uptake capacity. Their study on different plant species under drought stress indicated that AMF treatments could lead to a higher IWUE, aligning with findings where AMF-treated maize showed increased IWUE at reduced irrigation levels. Our study emphasizes the role of AMF in modulating physiological mechanisms that conserve water, such as stomatal conductance and transpiration rates. The data provided in the research, when compared with existing literature, underscores the beneficial role of AMF treatments in enhancing plant growth and water use efficiency under drought stress conditions. This highlights the potential of AMF inoculation as a sustainable agricultural practice to improve crop resilience to water scarcity.

3.2 Water productivity

In the conducted study, water productivity versus maize mass yield was evaluated according to both mycorrhiza and water stress treatments. Accordingly, the water productivity values obtained are given in Table 3. The findings from this study clearly demonstrate the positive effects of arbuscular mycorrhizal fungi (AMF) applications on the irrigation water productivity (IWP) and overall productivity (WP) of maize plants. Specifically, the applications of *R. intraradices* (M3) and *G. iranicum* (M5) yielded remarkable results. The *Rhizophagus intraradices* (M3) application resulted in the highest yield (26.9 t ha^{-1}) and IWP value (16.2 kg m^{-3}) under low water stress conditions (I70). Additionally, a positive WP value (3.72 kg m^{-3}) was recorded under these conditions. These results indicate the potential of *R. intraradices* to optimize water use and plant growth. As water stress increased (I50 and I30), the IWP values for this mycorrhiza species remained relatively high at 16.1 kg m^{-3} and 13.5 kg m^{-3}, respectively, while the WP values decreased to -2.04 kg m^{-3} and -12.09 kg/m^3. This suggests that *R. intraradices* can enhance water efficiency even at lower water levels. Similarly, the *Glomus iranicum* (M5) application also showed high yield (26.1 t ha^{-1}) and IWP value (15.6 kg m^{-3}) under I70 conditions, along with a positive WP value (3.24 kg m^{-3}). This highlights the beneficial effects of *G. iranicum* on water use efficiency and plant performance. Under moderate (I50) and severe (I30) water stress conditions, the IWP values were 13.2 kg m^{-3} and 9.0 kg m^{-3}, respectively, while the WP values were -4.68 kg m^{-3} and -16.31 kg m^{-3}. These findings demonstrate that *G. iranicum* can improve water productivity even under high water stress.

Rhizophagus irregularis and *R. intraradices* are widely used species among arbuscular mycorrhizal fungi (AMF) and these species have been proven to be beneficial in many agricultural systems (Aguégué et al., 2021; Jie at al., 2022; Huang et al., 2024). However, the *Rhizophagus irregularis* and *R. intraradices* strains used in this study were locally isolated and compared with commercially available *G. iranicum* var. *tenuihypharum*, *Glomus* spp. and *G. fasciculatum*. The use of AMF in maize cultivation has been previously investigated in different studies (Kazadi et al., 2022; Fall et al., 2023). However, the originality of this study lies in the fact that it directly compared the performance of a locally isolated *R. intraradices* strain with a commercial AMF strain. Compared to previous studies, the effects of both AMF species on plant growth, water use and nutrient uptake were examined in detail and directly compared in this study. The results of this study are consistent with other studies in the literature that reported the ability of AMF applications to enhance water use efficiency in plants under water stress conditions. For example, Xiao et al. (2023) reported that mycorrhizal fungi improved plant water uptake and enhanced plant growth under water stress conditions. Similarly, Duan et al. (2021) noted that AMF positively affected plant water relations and water use efficiency, increasing plant tolerance to water stress. Kaba et al. (2021) also demonstrated that AMF improved plant nutrient uptake, making plants more resistant to water stress. These findings suggest that AMF applications are an effective method for improving water use efficiency in agriculture. In regions with limited water resources, AMF applications can ensure more efficient water use and maintain plant productivity.

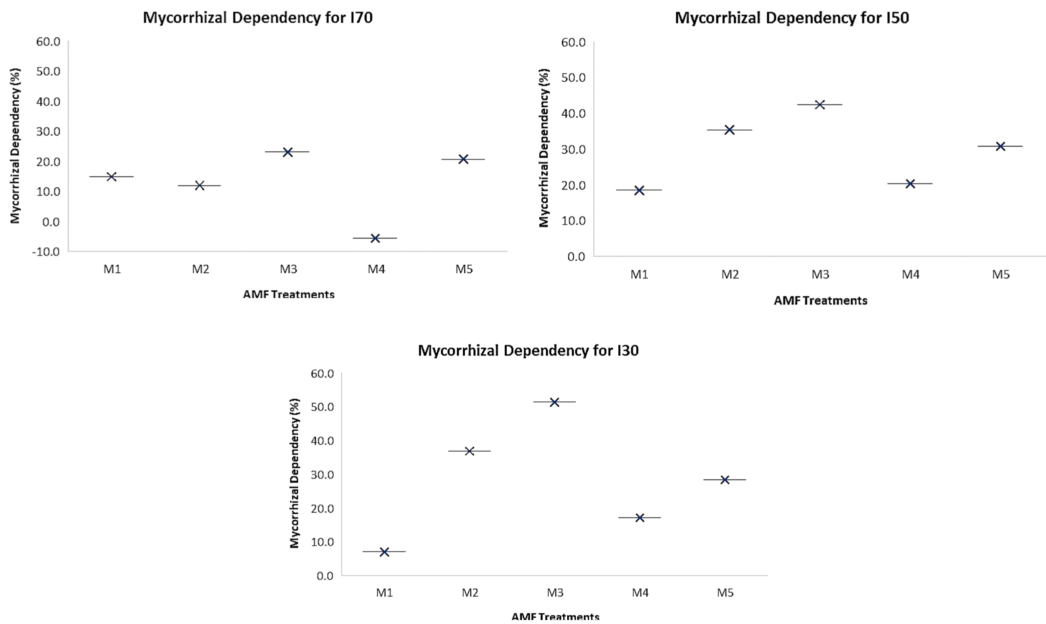
Table 3. Water productivity according to the experimental treatments

1	2	3	4	5	6	7
Treatments		**Y (t ha^{-1})**	**I (mm)**	**YI (t ha^{-1})**	**IWP (kg m^{-3})**	**WP (kg m^{-3})**
Mycorrhiza	**Irrigation**			**(3-3a)**	**(3/4)*100**	**(5/4)*100**
M0	I70 (control)	20.7 b-d	166.5	–	12.4	–
	I50	10.6 h-j	115.3	-10.1	9.2	-8.76
	I30	5.3 k	81.7	-15.4	6.5	-18.80
M1	I70	24.3 a-b	168.4	3.6	14.5	2.15
	I50	13.0 f-ı	116.8	-7.8	11.1	-6.65
	I30	5.7 k	82.9	-15.1	6.8	-18.18
M2	I70	23.5 a-c	168.4	2.8	14.0	1.66
	I50	16.4 d-f	116.0	-4.3	14.2	-3.70
	I30	8.4 ı-k	82.1	-12.4	10.2	-15.05
M3	I70	26.9 a	166.1	6.2	16.2	3.72
	I50	18.4 d-e	114.5	-2.3	16.1	-2.04
	I30	10.9 g-j	81.0	-9.8	13.5	-12.09
M4	I70	19.6 c-e	169.1	-1.1	11.6	-0.67
	I50	13.3 f-h	116.8	-7.4	11.4	-6.32
	I30	6.4 j-k	82.5	-14.3	7.8	-17.35
M5	I70	26.1 a	167.6	5.4	15.6	3.24
	I50	15.3 e-g	116.0	-5.4	13.2	-4.68
	I30	7.4 j-k	81.7	-13.3	9.0	-16.31

I70: irrigation at 70**%** of field capacity, **I50:** irrigation at 50**%** of field capacity, **I30:** irrigation at 30**%** of field capacity, **M0:** Control, **M1:** Glomus spp**.** (Shubhodaya), **M2:** *Rhizophagus irregularis*, **M3:** *Rhizophagus intraradices*, **M4:** *Glomus Fasciculatum*, **M5:** *Glomus iranicum*, **Y:** yield, **YI:** yield increased by irrigation, **IWP:** irrigation water productivity, **WP:** Water productivity, **Letters (a to k):** indicate statistically significant groups

3.3. Mycorrhizal dependency

In the study, the effect of different mycorrhiza applications on plant yield under each water stress condition is given in Figure 3 as mycorrhizal dependency. When analyzing the graphs showing mycorrhizal dependency under different drought stress conditions, it was observed that the M3 application provided the highest mycorrhizal dependency in all conditions. This indicates that M3 was the most efficient AMF in establishing a symbiotic



M1: *Glomus* spp. (Shubhodaya), **M2:** *Rhizophagus irregularis*, **M3:** *Rhizophagus intraradices*, **M4:** *Glomus fasciculatum*, **M5:** *Glomus iranicum*, **I70:** irrigation at 70% of field capacity (no drought stress), **I50:** irrigation at 50% of field capacity (moderate drought stress), and **I30:** irrigation at 30% of field capacity (severe drought stress)

Figure 3. Mycorrhizal dependency according to treatments

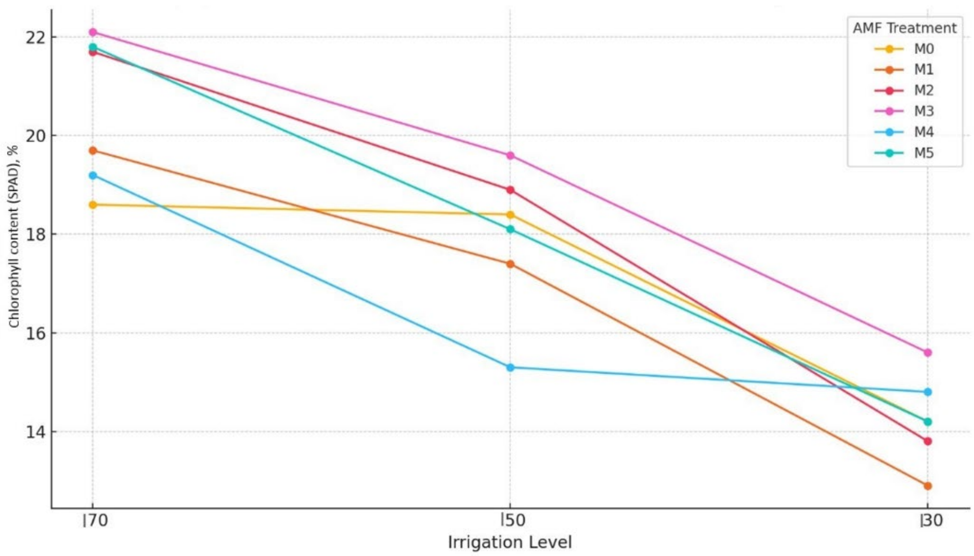
relationship with plants and maintained its effectiveness across different water levels. The M5 application also demonstrated generally high dependency percentages, making it the second most effective AMF option after M3. While M1 and M2 applications exhibited a moderate effect, The M4 application stood out with its negative impact, particularly at the S70 level. This suggests that M4 may not be suitable for plant growth under certain irrigation conditions. In conclusion, M3 and M5 applications appeared to be the best mycorrhizal options for establishing a strong symbiotic relationship with maize across all drought stress levels. Chandrasekaran (2024) reported that under moderate drought stress, AMF increased plant water use efficiency, contributing positively to plant growth. The results of this study support the notion that AMF optimized water use under this stress level, enhancing growth (Duan et al., 2024). In severe drought stress (I30), M3 (51.0%) exhibited the highest dependency, with M2 (36.0%) and M5 (27.6%) also showing high values. Wu et al. (2023) found that AMF can improve plant performance even under severe drought stress.

Arbuscular mycorrhizal fungi (AMF) mitigate this effect by regulating root physiology and enhancing the expression of genes such as *SWEET13, CHIT3*, and *RPL23A*. These genes respectively improve sugar transport, reduce oxidative stress, and support protein synthesis, thereby sustaining plant growth. In AMF-infected plants, root water uptake increases, water loss decreases, and stress management is optimized. Consequently, AMF is considered a crucial symbiotic organism that enhances plant drought tolerance (Chen et al., 2025). The benefits of AMF in mitigating drought stress occur through both direct and indirect mechanisms. Indirect effects include improved mineral nutrition, support for osmoregulation, reduction of oxidative stress levels, and increased root hydraulic conductivity (Quiroga et al., 2019; Madouh and Quoreshi, 2023). Additionally, the hyphal network formed by AMF plays a role in stabilizing soil aggregates, regulating soil porosity, and binding soil particles, thereby improving water retention (Zhang et al., 2022b). This process helps maintain hydraulic continuity around the roots during desiccation and limits the formation of air gaps, reducing water loss. Recent studies have revealed that AMF actively participates in the direct transfer of water to plant roots (Abdalla et al., 2023). AMF hyphae can transport water through air pockets in the soil and extract water from small pores that roots cannot reach, enhancing plant water uptake capacity. This feature is particularly beneficial in arid soil conditions, facilitating access to water and improving plant tolerance to water stress. Moreover, under edaphic stress conditions, AMF helps maintain hydraulic continuity between roots and soil, ensuring sustained water uptake. Specifically, it minimizes the decline in soil matrix potential near the root surface, allowing plants to continue water absorption even under drought conditions (Abdalla & Ahmed, 2021).

In this study, *R. intraradices* and *G. iranicum* were identified as the AMF species that established the most effective symbiotic relationships with maize plants. These species are reported in the literature to be more effective than other AMF species due to their specific mechanisms of action. Study fundings similarly indicate that certain AMF treatments increase plant dependency under severe drought conditions. However, some treatments like M1 (5.3%) and M4 (16.5%) showed lower dependency values, suggesting that the effect of AMF can vary depending on the fungal and plant species involved (Püschel et al., 2021). The mechanisms by which AMF enhance plant performance under drought stress are varied. AMF increase plant water use efficiency by improving root hydraulic conductivity and stomatal conductance (Erice et al., 2024). Additionally, AMF are known to enhance root growth and root surface area, which improves water and nutrient uptake (Xiao et al., 2023). AMF also increase antioxidant enzyme activities and osmolyte accumulation in response to water stress (Li et al., 2019). These findings indicate that mycorrhizal dependency varies significantly with drought stress levels and AMF treatments. Compared to other studies, the results confirm the beneficial effects of AMF on plant water use and growth (Hamedani et al., 2022).

3.4. Chlorophyll content analysis under different AMF treatments and ırrigation levels

Chlorophyll values measured from plants in each mycorrhizal treatment under different drought stresses are presented in Figure 4. Under low drought stress (S70) condition, the highest chlorophyll values were observed with M3 (22.1) and M2 (21.7) AMF treatments. All AMF treatments at this irrigation level showed higher chlorophyll content compared to the control group (M0). Under S50 (moderate drought stress) condition, the highest chlorophyll value was recorded with M3 (19.6) AMF treatment. The M4 treatment showed a lower chlorophyll value (15.3) compared to other treatments, indicating that some AMF treatments are less effective under moderate drought stress. Under severe drought stress condition, the highest chlorophyll value was observed with M3 (15.6) AMF treatment. Chlorophyll content is a critical indicator of a plant's photosynthetic capacity and overall health (Al-Gaadi et al., 2024; Shah et al., 2019; Zhang et al., 2022a). Higher chlorophyll content enables plants to perform more photosynthesis, leading to better growth and yield.



I70: irrigation at 70**%** of field capacity, **I50:** irrigation at 50**%** of field capacity, **I30:** irrigation at 30**%** of field capacity, **M0:** Control, **M1:** *Glomus* spp. (Shubhodaya), **M2:** *Rhizophagus irregularis*, **M3:** *Rhizophagus intraradices*, **M4:** *Glomus fasciculatum*, and **M5:** *Glomus iranicum*

Figure 4. Chlorophyll content under different AMF and irrigation treatments

Numerous studies have confirmed that AMF treatments increase chlorophyll content and promote plant growth (Turhan, 2021; Rasouli et al., 2023). Under drought stress, AMF treatments have been shown to enhance water use efficiency and drought tolerance in plants (Chandrasekaran, 2024). Certain AMF species, such as M2 and M3, appear to be more effective under various stress conditions (Kaba et al., 2021; Wahab et al., 2023). These findings suggest that AMF treatments, especially under low and moderate drought stress, enhance chlorophyll content, thereby supporting plant health and growth. However, under severe drought stress, some AMF treatments may be less effective.

4. Conclusions

The results of this study underscore the significant role of arbuscular mycorrhizal fungi (AMF) in enhancing the water use efficiency and growth performance of maize (*Zea mays* L.) under drought stress conditions. With the application of AMF, particular species like *Rhizophagus intraradices* and *Glomus iranicum* demonstrated a marked improvement in both irrigation water use efficiency (IWUE) and overall plant productivity. These findings are consistent across various levels of drought stress, highlighting the potential of AMF as a sustainable agricultural practice in water-limited environments.

AMF-treated maize plants exhibited higher IWUE compared to the control group, with the most significant improvements observed under higher irrigation levels. This suggests that AMF can optimize water utilization in maize cultivation, making it a valuable strategy for improving crop resilience to water scarcity. The enhancement in plant growth and yield was particularly notable with *R. intraradices* and *G. iranicum*, which achieved the highest productivity and efficiency metrics across different irrigation treatments. The study also revealed higher chlorophyll content in AMF-inoculated plants indicated enhanced photosynthetic capacity and overall plant health, contributing to better growth and yield outcomes under drought stress. Moreover, the dependency of maize on mycorrhizal associations was more pronounced under severe drought conditions, with AMF treatments providing critical support in maintaining plant water relations and mitigating drought stress effects. These findings align with existing literature on the beneficial impacts of AMF on plant water use efficiency and stress tolerance, further validating the efficacy of AMF in sustainable agriculture.

In conclusion, the application of AMF represents a promising approach to enhance maize cultivation in drought-prone areas. By improving water use efficiency and promoting plant growth under water-limited conditions, AMF can significantly contribute to the sustainability and productivity of agricultural systems facing water scarcity challenges.

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6. Authors' Contribution

RG: Lead writer, Review & editing, Methodology. CG: Project administration. TY: Project administration ÇS: Laboratory analysis. KA: Project administration

7. Conflicts of Interest

The authors declare no competing interests.

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