

Article

Effects of Arbuscular Mycorrhizal Fungi on Growth and Nutrient Accumulation of Oat under Drought Conditions

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Abstract: Arbuscular mycorrhizal fungi (AMF) have established themselves as pivotal allies in the realm of plant physiology, renowned for their remarkable contributions to augmenting both growth and resilience against environmental stresses. In this study, we embarked on a comprehensive investigation into the discernible impact of two distinct AMF species on a widely planted oat cultivar, ‘Qingyan No. 1’, when subjected to the austere conditions of a drought. The experimental design included three distinct AMF treatments (inoculation with *Rhizophagus intraradices*, *Funneliformis mosseae*, or not), and the three water treatments were 75% of field capacity (well watered), 50% of field capacity (moderate drought), and 30% of field capacity (severe drought). The obtained results showed that the rate of inoculation under 75% FC for both AMF species was over 74%. Drought stress limited the growth and osmotic regulation of the oat plants. However, AMF inoculation observably increased the above-ground biomass under 75% FC and increased the root biomass under 30% FC. AMF inoculation also increased the root traits under 75% FC and 50% FC. *R. intraradices* inoculation increased the above-ground soluble sugar and soluble protein concentrations, and both AMF species showed decreased malondialdehyde (MDA) concentrations in the roots. Furthermore, the pervasive influence of drought stress exerted a discernible stranglehold on nutrient uptake in the oat plants, profoundly impacting the distribution of nutrients within the shoots and roots. Regardless of the drought stress treatment, the inoculation with both AMF species increased the P concentrations in the roots and the K and Mg concentrations in the roots, and the inoculation with *R. intraradices* increased the Ca concentration in the whole oat plant. Under 75% FC, the N concentration of the whole oat plant was significantly reduced by both AMF species. However, under 50% FC and 30% FC, the N concentrations in the shoots inoculated with both AMF species were close to that of the non-inoculated shoots. In summary, AMF improved the osmotic regulation and nutrient absorption and distribution of oat plants under drought stress and thus promoted the growth and biomass accumulation of oat plants.

Keywords: *Avena sativa*; arbuscular mycorrhizal fungi; drought stress; nutrient uptake



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1. Introduction

In the world cereal production statistics, oats (*Avena sativa* L.) rank around sixth, be-hind wheat, corn, rice, barley, and sorghum [1]. In many parts of the world, oats are grown for use as grains as well as for forage and fodder, as straw for bedding, hay, haylage, silage, and chaff [2]. At the same time, oats are the winter reserve grass for the livestock industry [3,4]. Russia, countries of the former Soviet Union, the US, Canada, Germany, and Poland account for about 75% of the world’s supply of grain oats, seeds, and industrial-grade oats [5]. However, the rates of yield reduction related to drought

disasters for major crops will increase significantly with future climate change [6]. Droughts are one of the factors restricting the growth and production of oats. At the same time, droughts also limit the sustainable development of society and the economy [7,8]. Drought-induced stress orchestrates a series of cascading effects, culminating in the diminishment of soil nutrient availability, a curtailed plant nutrient uptake, and hampered nutrient translocation from the roots to the above-ground portions of plants [9]. The repercussions extend to the cellular realm, where drought-induced cellular dehydration and constrained nutrient assimilation conspire to elevate the presence of reactive oxygen species (ROS). The outcome is a compromised cell membrane permeability, exacerbating plant cell membrane peroxidation and malondialdehyde (MDA) accumulation. Plants have evolved an array of mechanisms to contend with drought-induced damage, encompassing morphological adjustments such as stomatal closure and an augmented wax content [10], physiological adaptations including osmotic regulation and antioxidant responses [11], and molecular responses involving the up-regulation of drought resistance genes [12]. But for some water-sensitive varieties, self-regulation is far from enough to cope with drought damage. Besides variety breeding and transgenic methods, biological interaction contributes to improving plant drought resistance as well.

Arbuscular mycorrhizal fungi (AMF) have garnered attention as formidable facilitators of plant resilience in the face of droughts and other environmental stressors [13]. This biological alliance is considered a pivotal strategy for bolstering plant drought tolerance [14]. AMF enter into a symbiotic relationship with their host plants, exchanging nutrients and water in return for carbon sources such as sugars and lipids [15–19]. Under drought conditions, AMF significantly enhance a plant's N (nitrogen) and P (phosphorus) utilization efficiency [20]. Mild drought stress, as evidenced in inoculation studies with *Rhizophagus irregularis*, notably increases the P and Ca (calcium) contents in plant leaves [21]. AMF interventions have also been shown to ameliorate the water status of crops like wheat, enhancing chlorophyll synthesis under drought conditions, and ultimately leading to increased yields and growth [22,23]. The inoculation with *R. irregularis* has similarly augmented the root length and root volume in *Triticum aestivum* ssp. *spelta* L. [24], while *Glomus mosseae* inoculation has exhibited a promotional effect on the root dry weights and the active and total absorption areas of trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) root systems under drought stress [25]. It is worth noting, however, that while a wealth of research underscores AMF's potential to alter plant root configurations, the colonization of different strains can induce distinct responses within the same species under varying environmental conditions [26,27]. While numerous experiments have corroborated AMF's prowess in enhancing the drought resistance of crops such as tomato [28], wheat [22,29], and rice [30], investigations on the effects of AMF on oats and other forage crops remain comparatively sparse. In the present study, we executed a meticulously designed experiment involving three distinct AMF treatments and three water treatments on cultivated oats. The objective was to assess the impacts of inoculation with *Rhizophagus intraradices* and *Funneliformis mosseae* and no inoculation on drought resistance, as well as the nitrogen, phosphorus, and trace element contents, and any differentiations in the root configurations between the above-ground and underground components of oats.

2. Materials and Methods

2.1. Biological Materials and Experimental Design

The experiment had a complete $1 \times 3 \times 3$ factorial design with one oat cultivar (*Avena sativa* cv. Qinyan No. 1), three arbuscular mycorrhizal fungi treatments (without inoculation and inoculation with *Rhizophagus intraradices* or *Funneliformis mosseae*), and three drought treatments (75% of field capacity, well watered; 50% of field capacity, moderate drought; and 30% of field capacity, severe drought). There were twelve replicate pots in each treatment, totaling 108 pots. Two AM fungi were provided by the Institute of Root Biology, Yangtze University (Jingzhou, China), and were multiplied in our laboratory using maize (*Zea mays* L.) as a host plant. The experiment was conducted in a greenhouse at the Laboratory of

Alpine Grass Resistance Physiological Ecology at Southwest Minzu University (Chengdu, China). The mixture of sand and soil (1:1) was autoclaved at 121 °C for 2 h under pressure (0.11 MPa), and then placed in a storage room for 1 night and oven-dried for 6 h before use. The maximum field capacity (FC) of soil is 20.54%, and the field capacity was calculated as follows:

$$FC = (\text{saturated soil weight} - \text{dry soil weight}) / \text{dry soil weight} \times 100\%. \quad (1)$$

Seeds were disinfected with 1% sodium hypochlorite (Guangzhou Testing Technology Co., Ltd., Guangzhou, China) for 10 min and washed with distilled water, and one seed was sown in each plastic pot containing 3.2 kg of a sterilized mixture of sand and soil. Each pot was supplemented with 115 g sterilized or unsterilized inoculum of *R. intraradices* or *F. mosseae*. All pots were watered regularly to 75% FC (CK, well watered) in the first 2 months, and then two-thirds of non-mycorrhizal (NM) and arbuscular mycorrhizal (*R. intraradices*, *F. mosseae*) pots were exposed to drought stress by reducing the water regime to 50% FC (MD, moderate drought) and 30% FC (SD, severe drought), while the remaining third were kept well watered (75% FC).

2.2. Mycorrhizal Colonization and Plant Growth Parameters

After 15 days of water stress treatment, plants were harvested. Shoots' and roots' fresh materials were separated and dried at 105 °C for 15 min and then at 75 °C for 24 h to record dry weights and conduct nutrient content analyses. Subsamples of fresh roots (0.5 g) were stored in a 4 °C refrigerator to measure mycorrhizal colonization. The rest of the roots and shoots were stored in a –80 °C refrigerator to measure the content of total soluble sugar (TSS), MDA, and soluble protein content.

The fresh, clean roots were soaked in 10% KOH solution at 90 °C for 30 min and then acidified with 1% HCL for 5 min. The cleared roots were stained with 0.05% Trypan blue in lactoglycerol (*v/v*) at 90 °C for 20 min [31]. The rates of AM colonization were examined using the gridline intercept method [32]. The mycorrhizal dependency was calculated according to van der Heijden method [33]. If biomass of $\sum_1^n an > bn$, then mycorrhizal dependency was calculated as follows:

$$\text{mycorrhizal dependency} = (1 - (bn / (\sum_1^n an))) \times 100. \quad (2)$$

If biomass of $\sum_1^n an < bn$, then mycorrhizal dependency was calculated as follows:

$$\text{mycorrhizal dependency} = (-1 + (an / (\sum_1^n bn))) \times 100 \quad (3)$$

where *a* is the plant dry weight of a treatment inoculated with AMF, *n* is the number of treatments where plants were inoculated with AMF, and *b* is the plant dry mass of the non-inoculated treatments.

2.3. Method for Determination of Root Architecture

The root total length, root volume, and root surface were determined using the LA-S plant root analysis system from the company Hangzhou Wseen Testing Technology Co., Ltd. (Hangzhou, China). A total of three replicates were set for the root architecture determination. The supporting roots and all fibrous roots in one root sample were scanned and measured.

2.4. TSS, MDA, and Protein Concentrations

Total soluble sugar, MDA, and soluble protein contents were measured using the assay kits from the company Suzhou Comin Biotechnology Co., Ltd. (Suzhou, China).

2.5. Macronutrient Concentrations in Oats

The concentrations of N, P, potassium (K), magnesium (Mg), and Ca were determined using homogenized dry samples of shoots and roots. The N concentration was determined as described by Kong [34] using 0.1 g of dried shoots and roots, and the P concentration was determined using 1 g of dried shoots and roots that were burned with a Muffle oven and using the vanadomolybdate method [35]. The K, Na, and Ca concentrations were determined as described by Liu and Zhang [36] using an atomic absorption spectrophotometer (Hitachi Z-2000, Tokyo, Japan).

2.6. Statistical Analysis

The effects of mycorrhizal inoculation, drought stress, and their interactions were statistically analyzed with two-way ANOVA using IBM SPSS 26. At least 3 replicates were used for each treatment of all measured parameters. The significance of differences among treatments and interaction between factors was calculated at 5%. Multiple comparisons were performed using Duncan's (HSD) post hoc test $p < 0.05$. Graphpad prism 8.0 was used to make graphs, and the data in the graph were shown as mean value \pm SE.

3. Results

3.1. Mycorrhizal Colonization

The mycorrhizal colonization of oats was significantly affected by the drought treatments (Figure 1). The colonization rates of *R. intraradices* and *F. mosseae* are higher than 74% under well-watered conditions. Compared with CK, the colonization rate of *R. intraradices* under MD and both AMF colonization rates under SD were significantly reduced. However, under MD and SD, the mycorrhizal colonization of all AMF species was not significant (Figure 2).

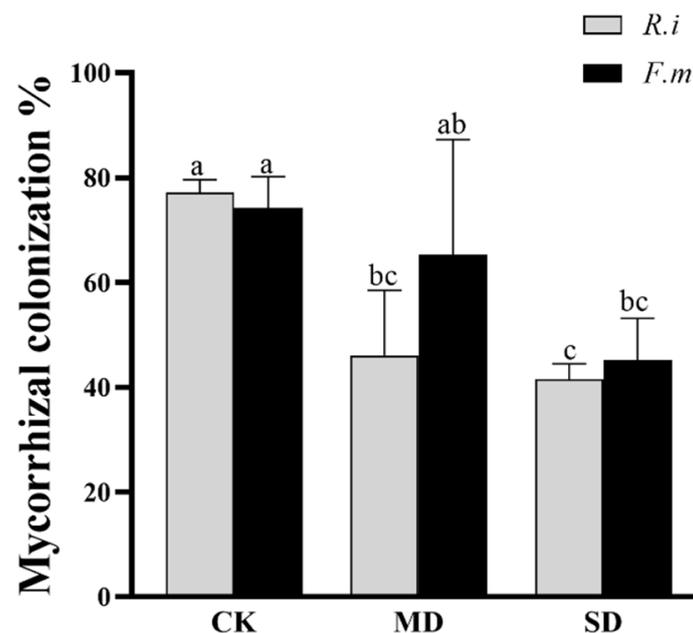


Figure 1. Frequency of mycorrhizal colonization. CK, well watered; MD, moderate drought; SD, severe drought; *R.i.*, inoculated with *Rhizophagus intraradices*; *F.m.*, inoculated with *Funneliformis mosseae*. Values with different letters indicate a significant difference (Duncan's test, $p \leq 0.05$, $n = 3$).



Figure 2. Structures of arbuscular mycorrhizal fungi (AMF) in oat roots inoculated with AMF spores at the end of the experiment: (A) *Funneliformis mosseae* spores (s) and extraradical hyphae (eh); (B) *Rhizophagus intraradices* vesicle (v), arbuscular (a), and intraradical hyphae (ih). Unit: μm .

3.2. Plant Growth of Shoots and Roots

The shoot and root dry weights were significantly influenced by drought treatments and AMF inoculation. However, drought and inoculation had no significant interaction effect on the shoot dry weights and root dry weights (Figure 3). Compared with the non-inoculated treatments, the inoculation with *R. intraradices* and *F. mosseae* increased the shoot dry weights under the CK and MD treatments and increased the root dry weights under the CK and SD treatments (Figure 3A,B), while drought stress decreased the shoot dry weights and root dry weights for both the non-inoculated and inoculated treatments.

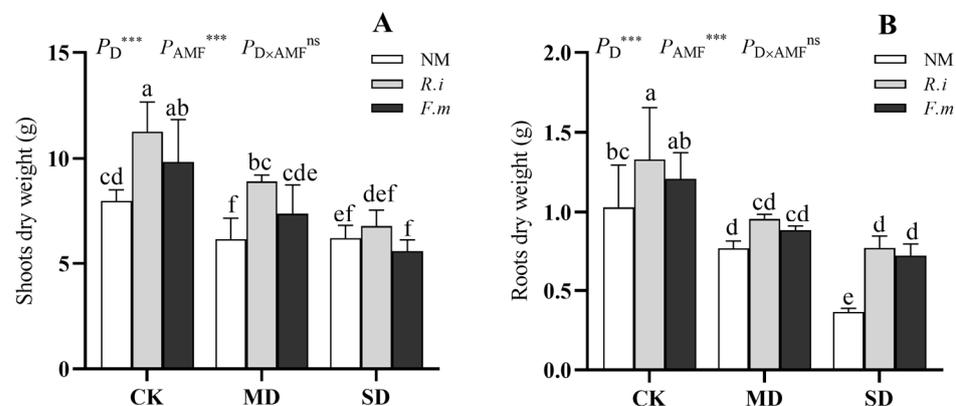


Figure 3. The effects of irrigation regime and inoculation with two AMF species on (A) shoot dry weight and (B) root dry weight of oats. CK, well watered; MD, moderate drought; SD, severe drought; NM, non-mycorrhizal; *R.i*, inoculated with *Rhizophagus intraradices*; *F.m*, inoculated with *Funneliformis mosseae*. Values with different letters indicate a significant difference (Duncan's test, $p \leq 0.05$, $n = 3$). P_{AMF} , probability value for the inoculation with the *G.r* and *G.m* species; P_{AMF} , probability value for the inoculation with the *R.i* and *F.m* species; P_D , probability value for the moisture treatment; $P_{D \times AMF}$, probability value for the AMF \times drought stress. *** $p \leq 0.001$; ns, no significant.

Based on oat shoot and root biomasses, the mycorrhizal dependency was calculated to evaluate the contribution of two AMF inoculations to oat growth. Except for the inoculation with *F. mosseae* under SD in the shoots, the other inoculations under three water regimes had positive contributions to both the shoots and roots (Figure 4). Under the three irrigation regimes, the shoots showed the greatest mycorrhizal dependency on two AMF species in the MD treatment, the inoculation with *R. intraradices* had better mycorrhizal dependency in the shoots under three irrigation regimes, and the inoculation with *F. mosseae* had negative

contributions in the shoots under SD. Under the three irrigation regimes, two AMF species showed positive contributions in the roots. The inoculation with *R. intraradices* had better mycorrhizal dependency in the root under MD and SD.

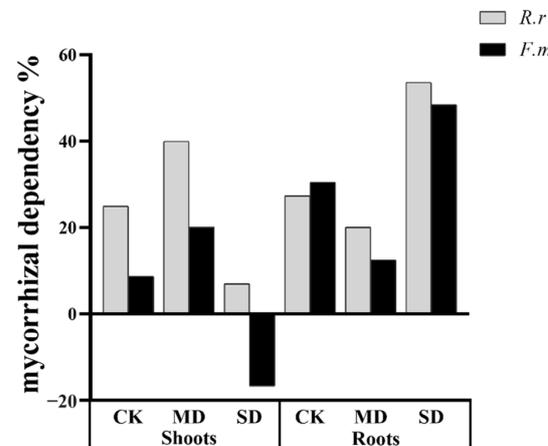


Figure 4. The mycorrhizal dependency of two AMF species in different irrigation regimes based on shoot and root dry weights. CK, well watered; MD, moderate drought; SD, severe drought; *R.i*, inoculated with *Rhizoglyphus intraradices*; *F.m*, inoculated with *Funneliformis mosseae*.

The total root length, root volume, and root surface area of the oats were significantly influenced by the drought and AMF inoculation. The drought and inoculation had significant interaction effects on the total root length and root surface area (Figures 5 and 6). Under inoculated and non-inoculated treatments, the drought significantly decreased the total root length, root volume, and root surface area (Figure 5A–C). The inoculation with *F. mosseae* and *R. intraradices* significantly increased the total root length and root volume under CK and MD (Figure 5A,B). The inoculation with *F. mosseae* and *R. intraradices* significantly increased the root volume under SD (Figure 5B), and the inoculation with *R. intraradices* significantly increased the root surface area under SD (Figure 5C). *F. mosseae* had a more significant effect on the total root length under CK (Figure 5A), and *R. intraradices* had a more significant effect on the root surface area under MD (Figure 5C).

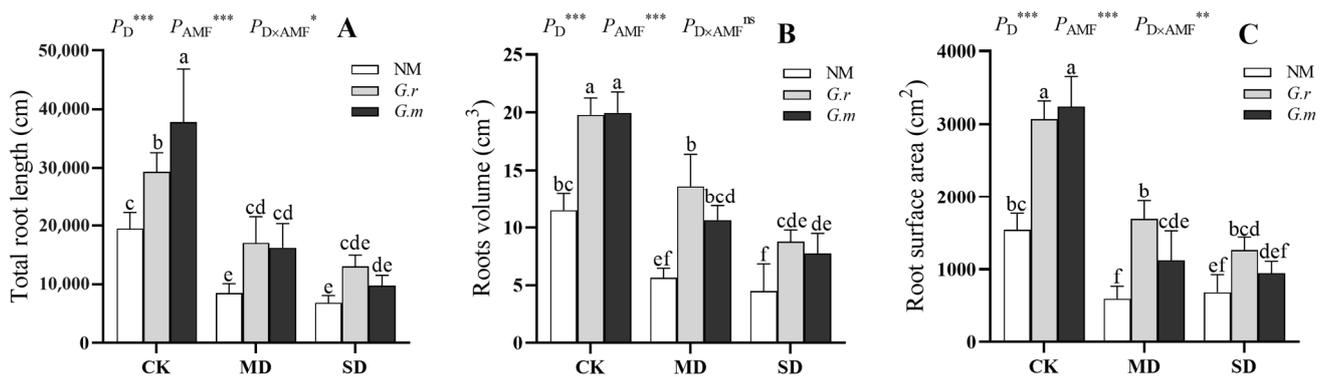


Figure 5. The effects of irrigation regime and inoculation with two AMF species on (A) total root length, (B) root volume, and (C) root surface area of oats. CK, well watered; MD, moderate drought stress; SD, severe drought; NM, non-mycorrhizal, *R.i*, inoculated with *Rhizoglyphus intraradices*; *F.m*, inoculated with *Funneliformis mosseae*. Values with different letters indicate a significant difference (Duncan’s test, $p \leq 0.05$, $n = 3$). P_{AMF} , probability value for the inoculation with the *R.i* and *F.m* species; P_D , probability value for the moisture treatment; $P_{D \times AMF}$, probability value for the AMF \times drought stress. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, no significant.



Figure 6. The pictures of roots under irrigation regime and inoculation with two AMF species. (a–c) non-mycorrhizal; (d–f) i, inoculated with *Rhizoglyphus irregularis*; (g–i) m, inoculated with *Funneliformis mosseae*. B, Qinyan No. 1; CK, well watered; MD, moderate drought; SD, severe drought.

3.3. TSS, MDA, and Protein Contents

The concentrations of soluble sugar, soluble protein, and MDA in the leaves were significantly influenced by the drought treatment and AMF inoculation, except MDA, drought, and inoculation had significant interaction effects on the soluble sugar and soluble protein contents (Table 1). The AMF inoculation had significant effects on the soluble sugar content in the roots, which was the same as in the leaves, and drought and inoculation had significant interaction effects on the soluble sugar and soluble protein contents in the roots. Drought stress significantly increased the accumulation of soluble sugar in oats under non-inoculated and inoculated treatments with *R. irregularis*, and compared with the non-inoculated treatment, the inoculation with *R. irregularis* increased the leaf soluble sugar content by 19%, 38%, and 125% under CK, MD, and SD treatments, and the root soluble sugar content increased by 50% under MD. As a consequence of SD, the protein concentration decreased (by 13.2%) in the non-inoculated leaf. However, it significantly increased in the *R. irregularis* treatments in the leaves, showing increasing rates of 44.4%, and showing increasing rates of 79.2% in the roots under MD. The accumulation of MDA was steeply increased in the non-inoculated leaves as a result of water stress application. However, mycorrhizal colonization significantly reduced the MDA concentration in the leaves induced by water stress (Table 2). The MDA concentration in the leaves significantly decreased by 16% and 18% when inoculated with *R. irregularis* and *F. mosseae* under SD. The inoculation with *F. mosseae* decreased the MDA concentration in the roots by 38% under MD.

Table 1. A two-way ANOVA for the effects of drought, two AMF species inoculation, and their interactions with TSS, MDA, and protein contents of oat.

Part	Treatment	Soluble Sugar	Soluble Protein	MDA
Shoots	Drought	10.63 **	6.46 **	12.13 ***
	AMF	110.97 ***	19.08 ***	8.33 **
	Drought × AMF	16.26 ***	10.45 ***	2.28 ns
Roots	Drought	1.94 ns	2.89 ns	0.54 ns
	AMF	6.34 **	0.13 ns	2.24 ns
	Drought × AMF	11.3 ***	9.09 ***	0.558 ns

ns, not significant; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 2. The effects of irrigation regime and inoculation with two AMF species on TSS, MDA, and protein contents.

Part	Treatment	Soluble Sugar (mg/g)	Soluble Protein (mg/g)	MDA (nmol/g)
Leaves	CK-NM	10.78 ± 0.72 cd	17.69 ± 0.52 cd	8.9 ± 0.36 c
	CK- <i>R.i</i>	12.88 ± 1.59 bc	18.06 ± 1.2 cd	8.67 ± 0.34 c
	CK- <i>F.m</i>	8.43 ± 0.7 de	16.33 ± 0.35 de	8.95 ± 0.37 c

Table 2. Cont.

Part	Treatment	Soluble Sugar (mg/g)	Soluble Protein (mg/g)	MDA (nmol/g)
Leaves	MD-NM	14.58 ± 0.65 b	18.49 ± 0.78 bc	11.26 ± 0.54 ab
	MD- <i>R.i</i>	20.26 ± 0.42 a	20.49 ± 0.68 ab	9.95 ± 0.91 bc
	MD- <i>F.m</i>	6.24 ± 0.28 e	16.67 ± 0.2 cde	8.71 ± 0.51 c
	SD-NM	8.88 ± 0.62 d	15.35 ± 0.76 e	12.22 ± 0.35 a
	SD- <i>R.i</i>	20.06 ± 1.27 a	22.17 ± 0.44 a	10.27 ± 0.51 bc
	SD- <i>F.m</i>	9.27 ± 0.47 d	20.18 ± 0.23 ab	9.97 ± 0.18 bc
Roots	CK-NM	5.95 ± 0.78 ab	8.36 ± 0.91 ab	13.5 ± 0.3 a
	CK- <i>R.i</i>	3.83 ± 0.16 c	4.77 ± 0.85 c	8.27 ± 0.56 ef
	CK- <i>F.m</i>	4.65 ± 0.35 abc	8.89 ± 1.07 ab	11.96 ± 0.84 abc
	MD-NM	4.46 ± 0.21 bc	6.53 ± 0.37 bc	12.66 ± 0.3 ab
	MD- <i>R.i</i>	6.09 ± 0.57 a	11.7 ± 1.94 a	10.84 ± 1.04 bcd
	MD- <i>F.m</i>	1.69 ± 0.1 d	4.17 ± 0.57 c	7.8 ± 0.79 f
	SD-NM	4.34 ± 0.72 c	9.01 ± 1.3 ab	10.17 ± 0.61 cde
	SD- <i>R.i</i>	4.63 ± 0.49 abc	8.28 ± 0.65 ab	9.63 ± 0.46 def
SD- <i>F.m</i>	4.83 ± 0.16 abc	10.38 ± 1.1 a	10.3 ± 0.9 cde	

CK, well watered; MD, moderate drought; SD, severe drought; NM, non-mycorrhizal, *R.i*, inoculated with *Rhizophagus intraradices*; *F.m*, inoculated with *Funneliformis mosseae*. Mean values ± SE with different letters indicate a significant difference (Duncan's test, $p \leq 0.05$, $n = 3$).

3.4. Macronutrient Concentrations in Oats

The concentrations of N, P, K, and Ca in all plant tissues, and the concentration of Mg in the roots were significantly influenced by AMF inoculation. The concentrations of Ca and K in all oat plant tissues, and the concentration of Mg in the roots were significantly influenced by drought treatments. Except for Mg in the shoots, the other macronutrient concentrations were significantly influenced by AMF inoculation. Meanwhile, the K and Mg contents in the roots and the Ca in all oat plant tissues were significantly influenced by drought stress, inoculation, and their interactions (Figure 7). Compared with the non-inoculated treatments, the N concentrations of the shoots in the oats were not significantly changed except under CK. The N concentrations of the roots even decreased via inoculation under CK and MD. Under different water conditions, there was no significant difference in the N concentration of the shoots under two kinds of AMF species inoculation, but *F. mosseae* significantly increased the N content of the roots compared with the inoculation of *R. intraradices* (Figure 7A). Compared with the non-inoculated treatments, only the inoculation with *R. intraradices* significantly increased the concentration of shoot P under CK, while both *R. intraradices* and *F. mosseae* inoculation increased the concentration of root P under different water treatments (Figure 7B). Compared with the non-inoculated treatments, the K contents of the shoots were not significantly changed except for *R. intraradices* inoculation under CK, and there were significant differences in the inoculated and non-inoculated root K concentrations under CK and MD; the *R. intraradices* and *F. mosseae* inoculations increased the concentrations of root K under CK, and *F. mosseae* inoculation increased the concentrations of root K under MD, while SD decreased the concentration of root K for both the non-inoculated and inoculated treatments (Figure 7C). Compared with the non-inoculated treatments, *R. intraradices* inoculation increased the concentrations of Ca in all plant tissues under MD and SD treatments (Figure 7D), and *R. intraradices* inoculation also increased the concentrations of root Mg under three water treatments (Figure 7E).

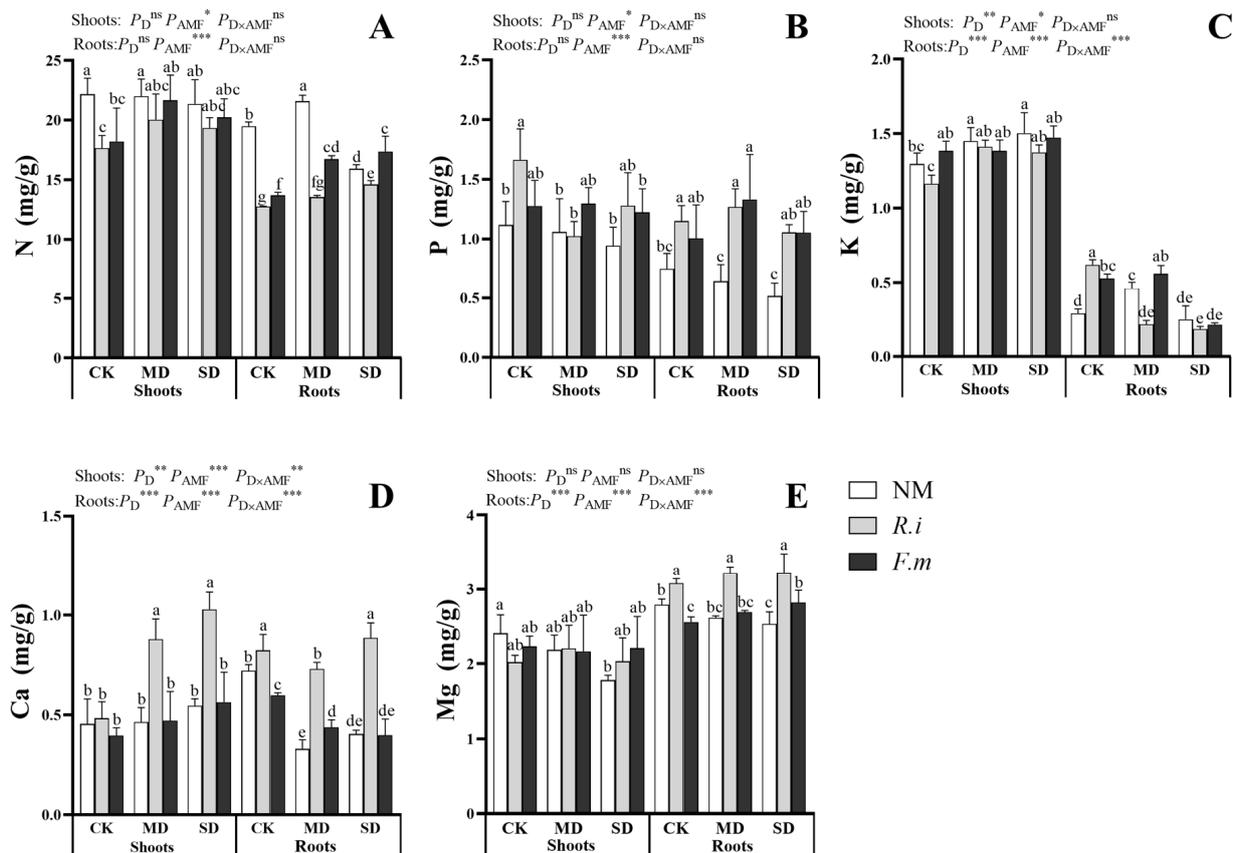


Figure 7. The effects of irrigation regime and inoculation with two AMF species on shoot and root N (A), P (B), K (C), Ca (D), and Mg (E) concentrations of oats. CK, well watered; MD, moderate drought; SD, severe drought; NM, non-mycorrhizal, *R.i.*, inoculated with *Rhizoglyphus intraradices*; *F.m.*, inoculated with *Funneliformis mosseae*. Values with different letters indicate a significant difference (Duncan's test, $p \leq 0.05$, $n = 3$). P_{AMF} , probability value for the inoculation with the *R.i.* and *F.m.* species; P_D , probability value for the moisture treatment; $P_{D \times AMF}$, probability value for the AMF \times drought stress. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant.

4. Discussion

Our experimental results align with those of previous studies, supporting the idea that droughts can decrease the infection rate of AMF [21,30,37]. Despite facing drought conditions, both AMF species had a positive influence on the oat growth, resulting in increased shoot and root biomasses as well as enhanced root architecture. It is worth noting that before the drought treatment, AMF and oat roots demonstrate symbiosis for up to two months, and establish a robust symbiotic relationship. Consequently, under normal water conditions, both AMF treatments exhibited high infection rates exceeding 74%, with *G.r.* achieving a higher rate at 77.21%. Similar observations have been reported in wheat crops under comparable irrigation practices, where *F. mosseae* inoculation achieved an infection rate of 72.5% [37]. However, Zhang [38] observed a lower inoculation rate (below 40%) when oats were inoculated with *Rhizoglyphus intraradices* under 75% FC conditions. Notably, it should be acknowledged that plant mycorrhizal infection rates are influenced by various factors such as the bacterial substrate specificity [39], host genotype variations [40], soil pH levels, and even other microorganisms that are present in the soil ecosystem [41]. The relatively low infection rate observed in Zhang's [38] study may be attributed to the influence exerted by other microorganisms present in non-sterile soil environments. In our study, both AMF species successfully established symbiotic associations with Qingyan No. 1 and positively influenced their biomass accumulation, root growth, nutrient uptake, and stress tolerance capabilities.

Consistent with most mycorrhizal studies, the two AMF species in this research increased the biomasses of different parts of the oat, especially the shoot dry weight under moderate drought conditions and the root weight under severe drought conditions. However, the oats inoculated with *R. intraradices* have a higher mycorrhizal dependency, especially for the shoots, which means *R. intraradices* inoculation has a higher mycorrhizal contribution to oats. The two AMF species also have significant effects on the root growth under well-watered and moderate drought conditions. AMF could up-regulate *PtYUC3* and *PtYU8* genes related to IAA synthesis, thereby increasing the IAA level, root length, and root density in trifoliolate orange root [42].

Despite oats' drought and barren resistance characteristics, water restriction reduces the utilization of N, P, and other nutrients. In this study, AMF inoculation resulted in lower N concentrations in oats compared to those without inoculation. It might be the high biomass that led to N dilution in the shoot tissues [43]. However, under water stress, AMF inoculation may promote N uptake by the roots and maintain the stability of the shoot N concentration. This result may be related to the nitrate reductase activity (NR), a key enzyme in N metabolism, which is greatly affected by droughts. Some research already showed that mycorrhizal plants had higher NR than the uninoculated treatments, particularly under water stress conditions [44,45]. Similar to most mycorrhizal studies, both AMF species enhanced the P concentration in the oat roots; the extracellular hyphae of AMF can reach the soil that cannot be reached by the root system to absorb nutrients. Additionally, the secretion of mycelia can also convert insoluble N and P nutrients into usable forms, which provide more absorbed N and P to the host plants [46].

Previous studies have shown that K plays crucial roles in enzyme activation, membrane transport, osmotic regulation, as well as protein synthesis, including starches, cellulose, and vitamins in plants [47]. Additionally, K^+ also participates in stomatal opening, aiding in plant adaptation to water stress [48]. P, Ca, K, and Mg are mobile ions in the soil that rely on continuous water flow between the soil–root–shoot parts for absorption [49]. Our results demonstrated that both AMF species increased the root K concentration under well-watered conditions and were consistent with the findings of Li [21]. *G. mosseae* increased the K concentration in the roots under moderate drought conditions. *R. irregularis* increased the growth, K content, and K channel gene expression in *Lycium barbarum*, especially under drought conditions [50]. Compared with the well-watered treatment, the K concentration of the shoots under the drought treatment has no significant difference. Therefore, we believe that the two AMF species can maintain higher K concentrations under drought conditions, thereby reducing drought damage.

The inoculation with *R. intraradices* significantly increased the concentration of Ca in oat plants under all water treatments; Ca^{2+} can serve as a mediator for transducing signals released by the AMF to plants, thereby facilitating the establishment of a symbiotic relationship and also transmitting drought signals within mycorrhizal structures under drought conditions [51,52]. Meanwhile, Ca promoted the water retention capacity of leaves and cell membranes to alleviate plant water scarcity under drought stress [53]. In our study, the inoculation with *R. intraradices* had a significant influence on the oat roots under all water treatments. Mg is involved in carbon metabolism by activating the enzyme, RUBISCO, thus promoting mycorrhizal colonization and plant growth [54]. However, Lopes [55] suggested that the higher Mg accumulation in the roots of mycorrhizal plants reduces the energy required for the unnecessary transport of mineral nutrients. Overall, both AMF species had positive effects on ion nutrition accumulation, especially at root level, during drought stress due to the extraneous mycelium extending from the roots absorbing more nutrients and water.

5. Conclusions

In this study, the oats had different responses to drought stress and the colonization of the two AMF species. On the whole, AMF alleviated drought stress to a certain extent, which was manifested in the increase in the biomass, root architecture, soluble sugar,

soluble protein, and the decrease in MDA. In addition, the inoculation of two kinds of AMF increased the accumulation of P, K, Ca, and Mg in different parts of the oat to different degrees, and both AMF species had significant effects on the increase in the root P concentration. Of the two types of AMF, the inoculation with *R. intraradices* was the most effective in the drought tolerance and nutrient absorption of the oats. The results demonstrated that the root architecture and nutrient absorption were enhanced in the mycorrhizal oats, which resulted in enhanced osmotic adjustment, shoot and root growth, and reduced biomass loss during drought stress compared with the non-mycorrhizal oats, leading to an improvement in the drought resistance of oats.

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