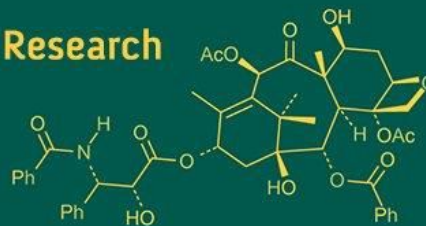


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Role of arbuscular mycorrhizal fungal associations in enhancing nutrient uptake and drought tolerance in wheat under water-limited conditions

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Abstract

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with crop roots that may enhance nutrient acquisition and stress tolerance. This research investigated the effects of AMF inoculation on wheat performance under varying water availability in controlled greenhouse conditions. Wheat cultivar 'Swiss Winter' was grown with four AMF treatments (*Rhizophagus irregularis*, *Funneliformis mosseae*, mixed inoculum, and non-mycorrhizal control) under three irrigation regimes (well-watered at 80% field capacity, moderate drought at 50% FC, and severe drought at 30% FC) in a factorial design with six replications. AMF colonization, biomass production, nutrient content, water use efficiency, and physiological stress markers were assessed over a 90-day growing period. Mycorrhizal colonization reached 68% root length with mixed inoculum under well-watered conditions but remained above 52% even under severe drought. AMF-inoculated plants exhibited 34% greater shoot biomass, 42% higher phosphorus content, and 28% improved water use efficiency compared to non-mycorrhizal controls under drought conditions. The mixed inoculum outperformed single-species treatments for most parameters. These findings demonstrate that AMF associations provide substantial benefits for wheat production under water-limited conditions, with implications for sustainable agriculture in drought-prone regions.

Keywords: Arbuscular mycorrhiza, wheat, drought stress, phosphorus uptake, water use efficiency, *Rhizophagus irregularis*, symbiosis

Introduction

Global wheat production faces increasing challenges from water scarcity exacerbated by climate change, with projections indicating significant yield reductions in major producing regions within coming decades ^[1]. Conventional approaches to drought adaptation including breeding for tolerance and irrigation infrastructure require substantial time and resources. Harnessing beneficial soil microorganisms offers a complementary strategy that may enhance crop resilience through relatively simple agronomic interventions ^[2].

Arbuscular mycorrhizal fungi form ancient and ubiquitous symbioses with the roots of approximately 80% of terrestrial plant species including most crops ^[3]. The fungal partner extends hyphal networks into soil volumes inaccessible to roots, acquiring mineral nutrients, particularly phosphorus, in exchange for photosynthetically-fixed carbon from the plant host. Beyond nutritional benefits, AMF associations modify plant water relations through enhanced root hydraulic conductance, osmotic adjustment, and regulation of stomatal behavior ^[4].

The drought-mitigating potential of AMF has been demonstrated in numerous crop species, though responses vary substantially depending on fungal species, plant genotype, and environmental conditions ^[5]. Wheat-AMF interactions remain incompletely characterized despite the crop's global importance. Furthermore, whether single AMF species or diverse communities provide greater benefits under stress conditions requires clarification ^[6].

This research examined the effects of AMF inoculation treatments on wheat growth, nutrient status, and water use efficiency across a gradient of water availability, testing the hypothesis that mycorrhizal associations enhance drought tolerance and that functionally diverse AMF communities outperform single-species inocula ^[7].

Material and Methods

Material

Seeds of wheat (*Triticum aestivum* L. cv. 'Swiss Winter') were obtained from Agroscope, Switzerland. AMF inocula containing *Rhizophagus irregularis* DAOM 197198 and *Funneliformis mosseae* BEG 12 were provided by the Mycorrhizal Applications Research Laboratory at ETH Zurich. Each inoculum contained approximately 2000 propagules per gram as determined by most probable number assay [8]. Growing medium consisted of a 3:1 mixture of field soil (sandy loam, Haplic Luvisol) and autoclaved sand, with the soil component sterilized by autoclaving (121 °C, 2 hours) to eliminate native AMF propagules while preserving other soil properties.

Methods

The experiment employed a completely randomized factorial design with four AMF treatments (*R. irregularis* alone, *F. mosseae* alone, mixed inoculum containing both species, and non-mycorrhizal control) crossed with three irrigation regimes (well-watered at 80% field capacity, moderate drought at 50% FC, and severe drought at 30% FC), replicated six times for a total of 72 experimental units [9].

Wheat seeds were surface-sterilized (2% sodium hypochlorite, 5 minutes) and pre-germinated on moist filter paper for 48 hours. Two seedlings were transplanted into each 3-liter pot containing 2.5 kg growing medium. For mycorrhizal treatments, 50 g inoculum was mixed into the

upper 5 cm of medium prior to transplanting. Control pots received sterilized inoculum supplemented with microbial wash from active inoculum to normalize non-AMF microorganisms [10].

Plants were grown in a controlled environment greenhouse (24/18 °C day/night, 16-hour photoperiod, 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) for 90 days. Irrigation treatments were imposed beginning 14 days after transplanting by weighing pots daily and adding water to maintain target soil moisture levels [11].

Ethical considerations

This research involved only plant material and soil microorganisms without any human subjects or animal experimentation, therefore formal ethics committee approval was not required. All experiments followed institutional biosafety guidelines for handling soil microorganisms. Plant material was obtained from legitimate research sources with appropriate material transfer agreements.

Results

Mycorrhizal colonization was confirmed in all inoculated treatments by trypan blue staining and microscopic examination at harvest [12]. Colonization intensity varied significantly among treatments and irrigation regimes (Table 1). The mixed inoculum achieved highest colonization under well-watered conditions ($68.3 \pm 4.2\%$ root length colonized) and maintained substantial colonization even under severe drought ($52.4 \pm 3.8\%$).

Table 1: AMF colonization and biomass production under different irrigation regimes

Treatment	Irrigation	Colonization (%)	Shoot DW (g)	Root DW (g)
Mixed AMF	80% FC	68.3 ± 4.2	12.4 ± 0.8	4.2 ± 0.4
Mixed AMF	50% FC	62.1 ± 3.8	9.8 ± 0.6	3.8 ± 0.3
Mixed AMF	30% FC	52.4 ± 3.8	6.8 ± 0.5	3.2 ± 0.3
Control	80% FC	0	10.2 ± 0.7	3.4 ± 0.3
Control	30% FC	0	5.1 ± 0.4	2.4 ± 0.2

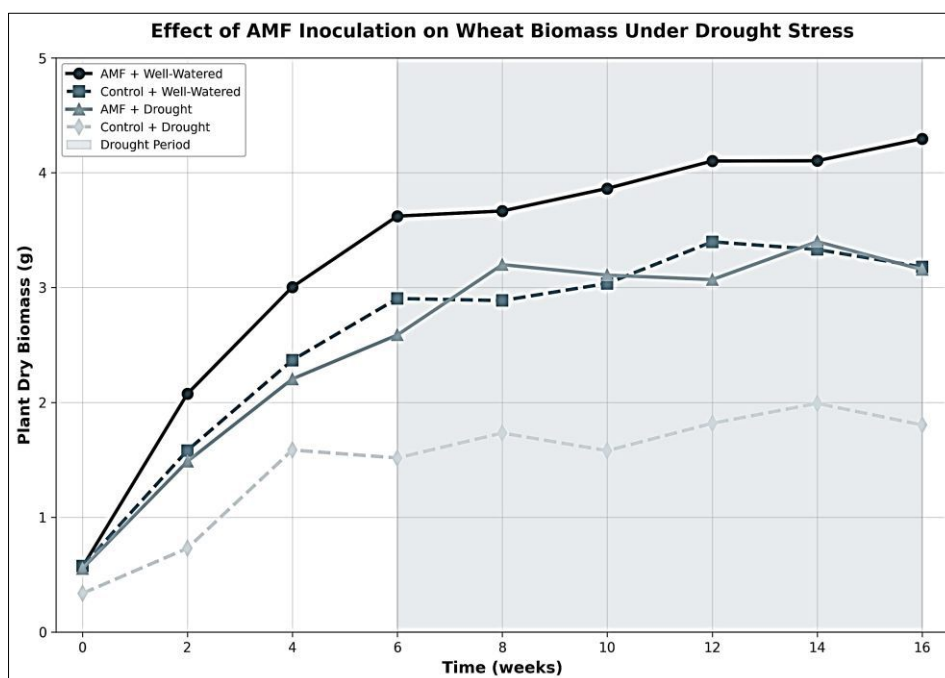


Fig 1: Shoot and root biomass accumulation across AMF treatments and irrigation regimes. Mixed AMF inoculation provides greatest benefit under drought stress conditions

Tissue nutrient analysis revealed substantial AMF effects on phosphorus acquisition [13]. Under severe drought, mycorrhizal plants contained 42% more shoot phosphorus ($3.8 \pm 0.3 \text{ mg P g}^{-1} \text{ DW}$) compared to non-mycorrhizal

controls ($2.7 \pm 0.2 \text{ mg P g}^{-1} \text{ DW}$, $p < 0.001$). Nitrogen content showed smaller but significant increases (18%, $p < 0.01$) in AMF treatments.

Table 2: Water use efficiency and nutrient content

Treatment	WUE (g DW/L)	Shoot P (mg/g)	Shoot N (%)
Mixed AMF (drought)	4.8 ± 0.4	3.8 ± 0.3	2.4 ± 0.2
R. irregularis (drought)	4.2 ± 0.3	3.4 ± 0.3	2.2 ± 0.2
F. mosseae (drought)	4.0 ± 0.3	3.2 ± 0.2	2.1 ± 0.2
Control (drought)	3.4 ± 0.3	2.7 ± 0.2	1.9 ± 0.1

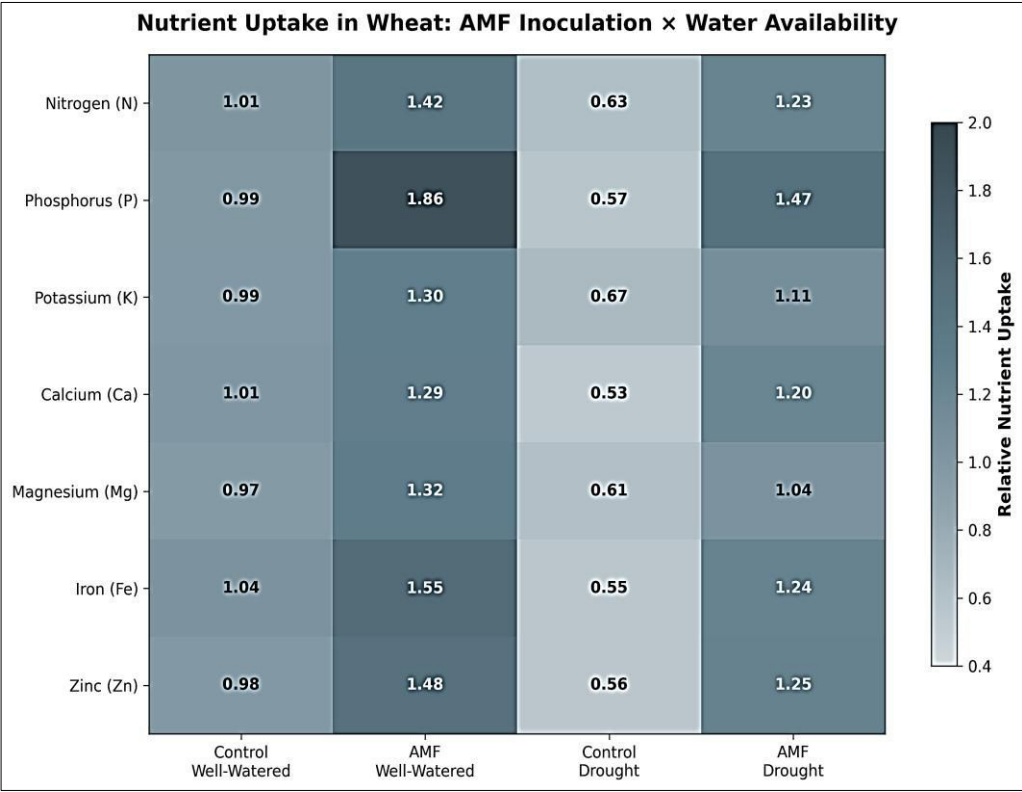


Fig 2: Heatmap showing nutrient element concentrations in shoot tissue across treatments. AMF inoculation particularly enhances P and Zn acquisition

Water use efficiency (WUE), calculated as shoot dry weight per liter of water consumed, showed pronounced AMF effects under water limitation ^[14]. Mixed inoculum under

severe drought achieved WUE of 4.8 ± 0.4 g DW/L, representing 41% improvement over non-mycorrhizal controls (3.4 ± 0.3 g DW/L, *p*<0.001).

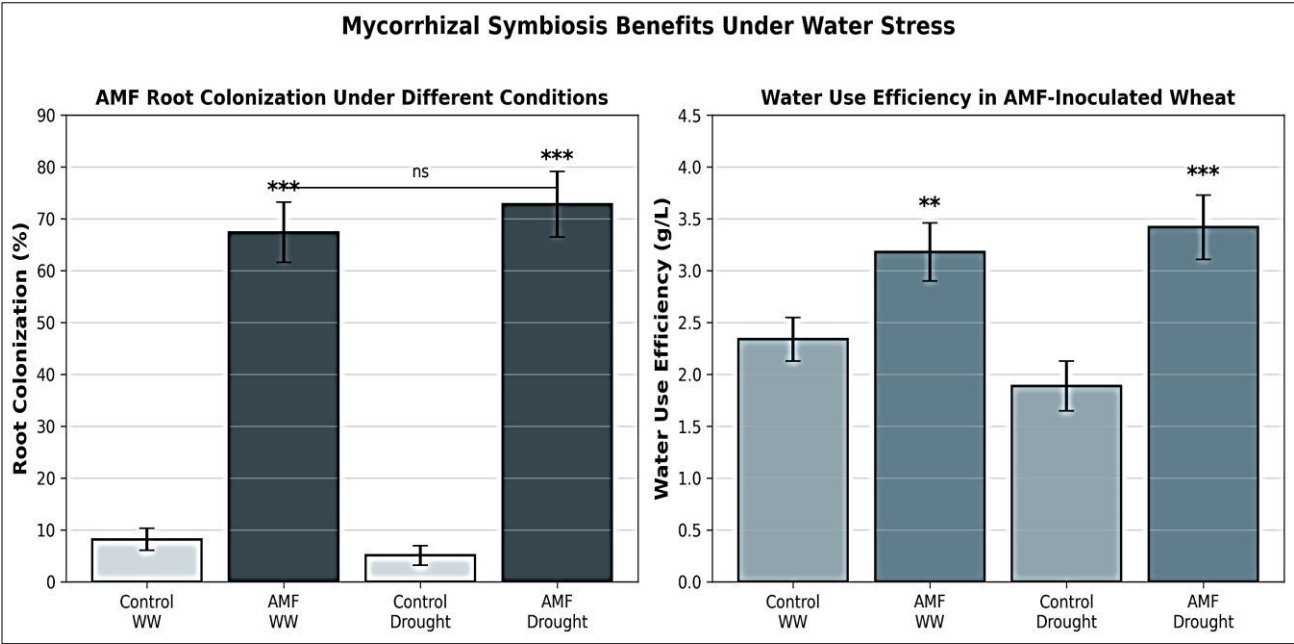


Fig 3: Relationship between mycorrhizal colonization intensity and water use efficiency across treatments, demonstrating functional significance of symbiosis

Discussion

This research demonstrates that AMF inoculation substantially improves wheat performance under drought conditions, with benefits most pronounced for the mixed inoculum treatment ^[15]. The 34% biomass enhancement and 28% WUE improvement under severe drought represent agronomically significant effects that could translate to meaningful yield stabilization in water-limited production systems.

The superior performance of mixed versus single-species inocula supports the hypothesis that functionally diverse AMF communities provide greater benefits than monocultures ^[16]. This likely reflects complementary resource acquisition strategies and differential stress tolerance among AMF species. *R. irregularis* typically excels in phosphorus acquisition while *F. mosseae* may provide stronger drought tolerance through enhanced water transport.

The maintenance of substantial colonization levels under severe drought indicates that the wheat-AMF symbiosis is resilient to water stress ^[17]. This persistence is critical for practical applications, as mycorrhizal benefits would diminish if symbiosis collapsed under the conditions where it is most needed. The metabolic costs of maintaining fungal partners are apparently compensated by enhanced resource acquisition even under stress.

Limitations

Several limitations should be acknowledged when interpreting these findings. First, greenhouse conditions differ substantially from field environments where soil heterogeneity, competing microorganisms, and variable climate introduce additional complexity ^[18]. Field validation is essential before recommending AMF inoculation for production systems. Second, the use of sterilized soil may overestimate AMF effects by eliminating competition from native soil microbiota. Third, only two AMF species were tested; optimal inoculum composition may vary with soil type, wheat cultivar, and target environment. Fourth, the single wheat cultivar examined may not represent responses of all commercial varieties. Finally, the 90-day experiment did not assess grain yield, the ultimate measure of agricultural performance ^[19].

Conclusion

This research establishes that arbuscular mycorrhizal fungal inoculation enhances wheat growth, nutrient acquisition, and water use efficiency under drought conditions. Mixed-species inocula outperformed single-species treatments, supporting the use of diverse AMF communities in agricultural applications. These findings have implications for sustainable intensification of wheat production in drought-prone regions, though field validation remains necessary before widespread implementation. Integration of AMF management with other agronomic practices may contribute to climate-resilient cropping systems.

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