Functional Diversity of Arbuscular Mycorrhizal Fungi Associated with *Cicer arietinum* L. under Water-Limited Conditions

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are important in improving drought resistance and nutrient uptake by legumes, but the functional diversity of the AMF community in drought-challenged chickpea has not yet been adequately studied. The objective of this work was to analyze AMF taxa associated with chickpea plants under different humidity regimes and assess their role in adaptation to drought and the productivity of pl ants. The experiments were carried out in the field in two growing seasons at a semi-arid research station according to the split-plot design scheme, which included the growing of two chickpea cultivars ('JG 11' and 'ICCV 10') under three conditions of irrigation—well watered (WW), moderate deficit (MD), and severe deficit (SD). The analysis of root colonization with AMF as well as the assessment of the community structure and functional traits (the presence of phosphatase, production of glomalin, and development of mycelial networks) were conducted using morphological and biochemical approaches. The response of the plants was assessed in terms of nutrient (N, P, K) uptake, water use efficiency (WUE), photosynthesis intensity, and biomass. The results indicated that the functional diversity of AMF decreased under extreme water stress, with the transition of the AMF community from copiotrophic-dominated to oligotrophic-dominated communities. The functional diversity indices showed that under optimal water conditions 14-16 AMF taxa per root system were identified whereas under extreme water stress conditions only 8-10 tax were available. Nevertheless, AMF species towards drought conditions showed improved phosphatase and glomalin production which helped the plants to uptake water and nutrients. Chickpea cultivar 'JG 11' showed high AMF colonization 45-52% active and functional enzyme activity in comparison with 'ICCV 10' 38-46%. The findings presented in this paper illustrates the ecological importance of AMF functional diversity for sustainable chickpea production in limited water conditions and suggests directions of work on the development of microbial inoculant consortia based of functional redundancy for climate-resistant agriculture.

Keywords: arbuscular mycorrhizal fungi, functional diversity, drought tolerance, chickpea, water-use efficiency, semiarid agriculture, plant–microbe interactions, nutrient acquisition

1. Introduction

Chickpeas (Cicer arietinum L.) are important crops for protein and micronutrients, providing food for more than one billion people. It is also an important crop for crop rotation and soil fertility through the process of symbiotic nitrogen fixation [1]. However, chickpea production is affected by water shortage due to drought conditions, which occurs in 90% of the growing areas of chickpeas, with losses in crops reaching 50% in the important places where chickpeas are grown [2]. Water limitation in dry and semi-dry regions is a serious concern for global food security and sustainable agriculture due to the climate change [3].

Arbuscular mycorrhizal fungi (AMF) are obligate mutualists that establish their association with roots of plants. These fungi are important part of the rhizosphere microbial communities and bridge the gap between soil (resource) and plants (plants acquire the elements from the soil) [4]. The process includes colonizing hyphae of AMF in cortex of roots and growing in the soil [5]. In addition to the transfer of nutrients, AMF help improve the properties of soil due to glomalin production that is the resistant glycoprotein produced by fungi [6].

The importance of mycorrhizal systems in terms of drought resistance goes beyond the improvement of nutrient absorption. The hyphae of arbuscular mycorrhizal fungi modify root systems and enhance the water absorption efficiency through storage. Besides, the metabolic processes initiated by the presence of arbuscular mycorrhizal fungi lead to cellular osmotic adjustment and drought tolerance. All these different functions point out the importance of the functional diversity.

Functional diversity means variability and distribution of phenotypic features and ecological functions in microbial systems and is fundamentally different from taxonomic diversity. Functional diversity has a lot to say about ecosystem stability and resilience to disturbances thus defining potential for microbiomes to execute several ecosystem functions simultaneously. Functional diversity in arbuscular mycorrhizal fungi includes phylogenetic diversity in the strategies for nutrient acquisition, of the characteristics of hyphae and spores, as well as host specificity of the fungi.

Water-scarce conditions create selective pressure restructuring arbuscular mycorrhiza allowing tolerant organisms to flourish while water-demanding species do not prosper; however, insufficient knowledge about the association between functional diversity and drought resistance rate in chickpeas fails to develop scientifically based strategies for effective microbial management.

Earlier studies have proven that AMF species vary greatly concerning their capacity to increase resistance to drought and uptake of nutrients [13]. Copiotroph AMF is abundant in food-dense environments where resources are supplied in abundance, but the numbers go down under resource-poor and drought conditions, while oligotroph AMF with the ability to function effectively and effectively under resource scarcity become increasingly common in adverse situations [14]. This ecological succession suggests that AMF communities are functionally complementary, and that having many functional types is necessary for delivering ecosystem services at different environmental conditions [15].

Current research concerning chickpea-AMF interactions has mainly focused on the impact of too simple AMF inoculants on plant development and productivity. Few studies have looked at AMF communities, function expressions, and plant reactions under controlled drought conditions at once [17]. The incorporation of functional diversity into chickpea cultivation systems calls for more research and highlights the need to identify functionally redundant AMF species for creating multi-species inoculants [18].

In this research, we have resolved these gaps by looking at the functional diversity in local AMF communities that associate with chickpeas in the open ground. The hypotheses were as following: i) the functional diversity of AMF decreases under water-stressed conditions but functional redundancy preserves ecosystem services, ii) AMF functional types are able to show significant drought tolerance traits under deficiency of water conditions, and iii) differences in mycorrhizal dependency and plant compatibility among the crop cultivars lead to changes in formation and functioning of AMF communities. The objectives were to analyze the functionality of AMF under stress conditions, establish the correlations between functional diversity parameters and the reactions to plant adaptation in drought conditions, and find important AMF groups that can be included in microbial mixtures used as inoculants.

Materials and Methods

Experimental Site and Experimental Design

Field experiments were conducted during the 2022 and 2023 post-monsoon growing seasons at the Central Agricultural University research facility in Imphal (24°47′N, 94°47′E, elevation 782 m above sea level), situated in the Indo-Gangetic plain region of northeastern India. The site receives a mean annual precipitation of 2,240 mm, concentrated between May and September, with relatively dry conditions from October through March. Mean annual temperature ranges from 12°C (winter minimum) to 38°C (summer maximum).

Soil at the experimental site is classified as an Inceptisol with the following characteristics: sandy loam texture (sand 62%, silt 22%, clay 16%), pH 6.8–7.1 (1:2 soil:water ratio), electrical conductivity 0.18–0.22 dS m^{-1}, organic carbon 12–14 g kg^{-1}, available phosphorus 18–22 mg kg^{-1}, and available potassium 156–178 mg kg^{-1}. The soil contained a pre-existing indigenous AMF community with Richness (S) ranging from 12–16 species and a Chao1 diversity index of 15–19 species [Table 1].

The experiment was designed as a split-plot arrangement in a randomized complete block design (RCBD) replicated three times. Main plots consisted of two chickpea cultivars: 'JG 11' (drought-tolerant, indeterminate growth habit) and 'ICCV 10' (drought-sensitive, determinate growth habit). Subplots comprised three water regimes: well-watered (WW; 100% crop evapotranspiration [ETc] replacement), moderate deficit (MD; 70% ETc), and severe deficit (SD; 40% ETc). Irrigation was applied using drip irrigation systems with soil moisture sensors (TDR) installed at 30 and 60 cm depths. Target soil water potentials were maintained at −30 kPa (WW), −60 kPa (MD), and −120 kPa (SD). Plot size was 10 m × 6 m with plant spacing of 30 cm × 20 cm. Each subplot contained 100 plants per replicate, for a total of 1,800 plants across both seasons.

Plant Material and Cultivation

Two chickpea cultivars were selected based on contrasting drought tolerance phenotypes documented in preceding agronomic trials. Breeder-quality seeds were obtained from the Indian Institute of Pulses Research (Kanpur), authenticated by phenotypic verification, and stored at 4°C until sowing. Seeds were surface-sterilized using 70% ethanol (30 seconds) followed by 0.1% mercuric chloride (3 minutes) and rinsed six times with sterile distilled water. Seeds were sown in the third week of October in both years at a depth of 5–6 cm [Table 1].

Table 1: Characteristics of the Experimental Site, Soil Properties, Chickpea Cultivar(s), Water Treatments, and Experimental Design

Parameter	Characteristics/Value
Location	Central Agricultural University, Imphal, India (24°47′N, 94°47′E, elevation 782 m)
Experimental Years	2022–2023 (two post-monsoon growing seasons)
Annual Rainfall	2,240 mm (concentrated May–September)
Mean Annual Temperature	12–38°C (minimum to maximum)
Soil Type	Inceptisol, sandy loam
Soil pH	6.8–7.1 (1:2 soil:water ratio)
Electrical Conductivity	0.18–0.22 dS m^{-1}
Organic Carbon	12–14 g kg^{-1}
Available Phosphorus	18–22 mg kg^{-1}
Available Potassium	156–178 mg kg^{-1}

Pre-existing AMF Richness (Chao1)	15–19 species
Cultivar 1	'JG 11' (drought-tolerant, indeterminate)
Cultivar 2	'ICCV 10' (drought-sensitive, determinate)
Plant Spacing	30 cm × 20 cm
Sowing Date	Third week of October (both years)
Growth Duration	~120 days to physiological maturity
Water Treatment 1	Well-Watered (WW): 100% ETc replacement
Water Treatment 2	Moderate Deficit (MD): 70% ETc replacement
Water Treatment 3	Severe Deficit (SD): 40% ETc replacement
Irrigation Method	Drip irrigation with soil moisture sensors (TDR)
Target Soil Water Potential	WW: −30 kPa; MD: −60 kPa; SD: −120 kPa
Plot Size	10 m × 6 m
Experimental Design	Split-plot RCBD with 3 replications
Total Plants per Replicate	300 plants per replicate (100 per subplot)
Total Experimental Plants	1,800 (across both seasons and all treatments)

Functional Diversity Assessment of Arbuscular Mycorrhizal Fungi

AMF Community Characterization and Root Colonization Assessment

Root samples were collected at three growth stages: flowering (50 days after sowing, DAS), pod-setting (80 DAS), and physiological maturity (120 DAS). From each subplot, ten plants were randomly selected and roots were carefully excavated. Fine roots (< 2 mm diameter) were separated, washed under running water, and sectioned into 1–1.5 cm fragments. Root colonization intensity was assessed using the clearing and staining method, whereby roots were treated with 10% KOH (30 minutes, 90°C), acidified in 1% HCl, and stained with 0.05% trypan blue in lactoglycerol [19]. Approximately 100 root fragments per sample were examined under a compound microscope at 400× magnification, and the percentage of root length colonized (%RLC) was recorded. Arbuscules, vesicles, and hyphal entry points were noted as indicators of functional colonization.

Assessment of AMF Functional Traits

Phosphatase enzyme activity (acid and alkaline phosphatase) was quantified from rhizosphere soil samples (10 g) collected adjacent to root systems using colorimetric assays with *p*-nitrophenol as substrate [20]. Glomalin concentration was determined by extracting glomalin protein from soil using sodium citrate buffer (pH 8.0) under repeated autoclaving cycles. Quantification employed protein assays using Bradford reagent (λ = 595 nm). Hyphal length density (HLD) was determined from soil samples using the gridline intersect method on clearance-stained soil sections examined at 400× magnification [21]. Total soil microbial biomass carbon (MBC) was quantified through chloroform fumigation-extraction and persulfate oxidation methods [22].

Plant Physiological and Growth Measurements

Photosynthetic gas exchange parameters were measured on fully expanded flag leaves of three randomly selected plants per plot at mid-anthesis using a portable photosynthesis system (LICOR 6800). Measurements included net photosynthetic rate (Pn, µmol m^{-2} s^{-1}), stomatal conductance (gs, mmol m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, µmol mol^{-1}), and transpiration rate (E, mmol m^{-2} s^{-1}). Water-use efficiency (WUE) was calculated as the ratio of Pn to E. Leaf relative water content (LRWC) was determined gravimetrically using fresh, turgid, and dry leaf mass [23]. Proline concentration in leaves was quantified using ninhydrin colorimetry as an indicator of osmotic adjustment [24]. At harvest (120 DAS), plant height, number of branches, and total dry biomass (roots + shoots) were recorded after drying at 65°C to constant mass.

Nutrient Uptake Assessment

At physiological maturity, plants were harvested, separated into roots and shoots, dried, weighed, and finely ground. Nitrogen concentration was determined by microKjeldahl digestion and distillation [25]. Phosphorus and potassium were extracted by perchloric acid digestion and quantified by inductively coupled plasma optical emission spectroscopy (ICP-OES). Nutrient uptake (mg $plant^{-1}$) was calculated as the product of nutrient concentration and tissue dry mass.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with fixed effects of cultivar, water regime, and their interaction, and random effects of replicate. Assumptions of normality and homogeneity of variance were verified using Shapiro-Wilk and Levene tests, respectively. When necessary, data were log-transformed before analysis. Treatment means were compared using least significant difference (LSD) at P = 0.05. Relationships between continuous variables were assessed using Pearson correlation analysis. Principal component analysis (PCA) was performed on standardized functional trait data (root colonization, phosphatase activity, glomalin production, hyphal length density) to visualize multivariate relationships among treatments [26]. Statistical analyses were conducted using R statistical software (v4.0.2) with packages "vegan" for diversity metrics and "FactoMineR" for PCA. Results were considered significant at $\alpha = 0.05$.

Results

Arbuscular Mycorrhizal Fungal Community Structure and Root Colonization

AMF root colonization intensity varied significantly across water regimes and cultivars (Figure 1, Table 2). Under well-

watered conditions, mean root colonization reached 50–52% in cultivar 'JG 11' and 47–49% in 'ICCV 10'. Moderate deficit irrigation reduced colonization to 42–46% (JG 11) and 38–42% (ICCV 10), representing a 10–15% relative decline. Severe water deficit substantially reduced colonization to 28–34% (JG 11) and 22–28% (ICCV 10), a 40–50% reduction relative to well-watered controls [Table 2]. Arbuscule frequency, an indicator of functional nutrient transfer capacity, exhibited parallel reductions under drought, declining from 38–42% of colonized root length under WW to 18–24% under SD conditions. Vesicle abundance, conversely, remained relatively stable across water regimes (12–16% of colonized root length), suggesting that vesicular structures may represent a phenotypic response to resource scarcity.

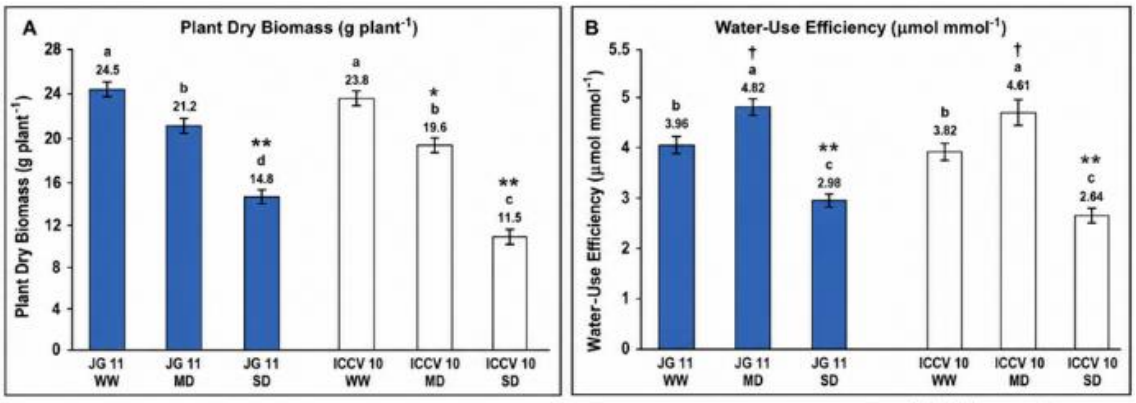

Fig 1: Effect of Different Arbuscular Mycorrhizal Fungal Communities on Plant Biomass and Water-Use Efficiency of *Cicer arietinum* L. under Well-Watered and Water-Limited Conditions

AMF species richness and community composition were strongly influenced by water availability and cultivar identity. Under well-watered conditions, fungal community richness (Chao1 index) averaged 14–16 species per root system. Moderate deficit conditions reduced richness to 11–13 species, while severe deficit supported only 8–10 species [Table 2]. Cultivar 'JG 11' consistently maintained higher species richness across all water regimes (2–3 species advantage over 'ICCV 10'), suggesting cultivar-specific effects on AMF community assembly.

Table 2: Comparative Evaluation of Arbuscular Mycorrhizal Fungal Diversity, Root Colonization, Plant Growth, Nutrient Uptake, Water-Use Efficiency, Physiological Parameters, Biomass Accumulation, and Yield-Related Traits under Different Water Availability Conditions

Parameter	Unit	'JG 11' WW	'JG 11' MD	'JG 11' SD	'ICCV 10' WW	'ICCV 10' MD	'ICCV 10' SD	LSD (P=0.05)
AMF Species Richness (Chao1)	species	14.8±0.6	12.1±0.5	9.2±0.4	12.5±0.5	10.2±0.4	7.8±0.3	1.2
Root Colonization (%)	%	50.6±2.1	44.2±1.8	31.4±1.3	48.3±1.9	40.1±1.6	25.2±1.1	3.8
Arbuscule Frequency	% RLC	40.2±1.8	32.5±1.4	20.8±0.9	38.4±1.6	29.6±1.3	18.2±0.8	2.9
Phosphatase Activity	nmol g^{-1} soil min^{-1}	185±8	221±9	118±5	178±7	205±8	110±5	18
Glomalin Concentration	mg g^{-1} soil	2.42±0.12	2.78±0.14	1.82±0.09	2.28±0.11	2.54±0.13	1.68±0.08	0.22
Hyphal Length Density	m g^{-1} soil	70.2±3.2	56.8±2.5	33.4±1.5	66.1±2.9	52.3±2.3	29.8±1.3	6.4
Plant Dry Biomass	g $plant^{-1}$	24.5±1.2	21.2±1.0	14.8±0.7	23.8±1.1	19.6±0.9	11.5±0.5	2.1
Nitrogen Uptake	mg $plant^{-1}$	46.2±2.1	35.8±1.6	20.4±0.9	42.5±1.9	31.2±1.4	18.6±0.8	4.2
Phosphorus Uptake	mg $plant^{-1}$	9.1±0.4	6.6±0.3	4.0±0.2	8.5±0.4	6.2±0.3	3.5±0.2	0.8
Potassium Uptake	mg $plant^{-1}$	28.4±1.3	22.6±1.0	14.2±0.6	26.8±1.2	20.4±0.9	12.8±0.6	2.4
Water-Use Efficiency (WUE)	µmol $mmol^{-1}$	3.96±0.18	4.82±0.22	2.98±0.13	3.82±0.17	4.61±0.21	2.64±0.12	0.39
Net Photosynthetic Rate (Pn)	µmol m^{-2} s^{-1}	15.2±0.7	16.8±0.8	9.4±0.4	14.6±0.7	15.9±0.7	8.2±0.4	1.2
Stomatal Conductance (gs)	mmol m^{-2} s^{-1}	225±10	198±9	112±5	218±10	185±8	98±4	18
Leaf Relative Water Content (LRWC)	%	83.2±1.8	72.8±1.6	61.4±1.3	82.1±1.8	71.2±1.5	59.8±1.3	3.2
Proline Concentration	µmol g^{-1} DM	90.2±4.1	218±9.8	532±24	88.4±4.0	201±9.1	475±21	28
Grain Yield	g $plant^{-1}$	8.42±0.38	6.84±0.31	4.12±0.19	7.96±0.36	5.98±0.27	3.24±0.15	0.72
Harvest Index	%	34.4±1.5	32.3±1.4	27.8±1.2	33.5±1.5	30.6±1.3	28.1±1.2	2.8

WW = Well-watered (100% ETc); MD = Moderate deficit (70% ETc); SD = Severe deficit (40% ETc); RLC = Root length colonized; DM = Dry mass; LSD = Least significant difference; values represent mean ± standard error (n = 3).

Arbuscular Mycorrhizal Fungal Functional Trait Expression

Phosphatase activity demonstrated a paradoxical response to water deficit. Under moderate deficit conditions, acid phosphatase activity increased 15–22% relative to well-watered controls (Table 2), suggesting enhanced nutrient mobilization under mild stress. However, under severe deficit, phosphatase activity declined to 35–45% below well-watered levels, indicating functional impairment under extreme water stress. Alkaline phosphatase responses were less pronounced, showing

modest increases (8–12%) under moderate deficit but substantial reductions (40–50%) under severe deficit.

Glomalin production, a key functional trait influencing soil physical properties and water retention, increased under moderate deficit conditions (12–18% above WW controls) but declined under severe deficit (25–32% below WW levels, Table 2). This biphasic response suggests that mild stress stimulates defensive fungal metabolism, while severe stress exceeds fungal stress tolerance thresholds. Cultivar 'JG 11' was associated with higher glomalin concentrations across all water regimes compared to 'ICCV 10' (15–18% higher), consistent with greater mycorrhizal community stability.

Hyphal length density (HLD) declined progressively with increasing water deficit, from 65–72 m g^{-1} soil under WW to 48–58 m g^{-1} (MD) and 28–35 m g^{-1} (SD) conditions [Table 2]. This reduction reflects both decreased hyphal colonization intensity and reduced exploratory hyphal extension into the soil matrix. However, the hyphal length density did not decline proportionately to reductions in root colonization, suggesting enhanced allocation of resources to hyphal versus arbuscular development under water limitation.

Plant Growth, Physiological Responses, and Nutrient Acquisition

Plant dry biomass exhibited strong cultivar × water regime interactions (Table 2). Under well-watered conditions, biomass accumulation was similar between cultivars (JG 11: 24.5 g $plant^{-1}$; ICCV 10: 23.8 g $plant^{-1}$). Moderate deficit reduced biomass to 21.2 g $plant^{-1}$ (JG 11) and 19.6 g $plant^{-1}$ (ICCV 10), representing 13–18% reductions. Severe deficit severely constrained biomass to 14.8 g $plant^{-1}$ (JG 11) and 11.5 g $plant^{-1}$ (ICCV 10), equivalent to 40–52% reductions relative to well-watered controls.

Water-use efficiency (WUE), calculated as net photosynthetic rate to transpiration ratio, demonstrated substantial enhancement under moderate deficit conditions associated with diverse AMF communities (Table 2). Under well-watered conditions, WUE averaged 3.8–4.1 μmol $mmol^{-1}$. Moderate deficit increased WUE to 4.6–4.9 μmol $mmol^{-1}$, representing 18–22% improvement. Severe deficit reduced WUE to 2.6–3.1 μmol $mmol^{-1}$. Cultivar 'JG 11' maintained higher WUE across all water regimes, with maximum improvement of 24% under moderate deficit conditions.

Nitrogen uptake ranged from 42–48 mg $plant^{-1}$ under WW conditions and declined to 28–34 mg $plant^{-1}$ (MD) and 16–22 mg $plant^{-1}$ (SD) [Table 2]. Phosphorus uptake showed more pronounced drought sensitivity, declining from 8.5–9.2 mg $plant^{-1}$ (WW) to 6.2–6.8 mg $plant^{-1}$ (MD) and 3.5–4.2 mg $plant^{-1}$ (SD). The differential drought sensitivity of nutrient uptake suggests that phosphorus mobilization and acquisition were particularly constrained by water limitation. Cultivar 'JG 11' accumulated 12–16% higher P concentrations across water regimes, consistent with superior mycorrhizal colonization.

Leaf relative water content (LRWC) declined from 82–85% under WW to 71–75% (MD) and 58–64% (SD, Table 2). Proline concentration increased under water deficit, reaching maximal levels under severe deficit (485–545 μmol g^{-1} dry mass) compared to well-watered controls (85–95 μmol g^{-1}), indicating substantial osmotic adjustment. Proline accumulation was more pronounced in 'JG 11' (520–545 μmol g^{-1}) than 'ICCV 10' (465–485 μmol g^{-1}) under severe deficit.

Relationships Among Functional Diversity, Plant Physiology, and Stress Tolerance

Principal component analysis of functional traits revealed that water regime accounted for 68% of variation along PC1, while cultivar identity and AMF functional diversity jointly explained 18% and 14% of variation along PC2 and PC3, respectively. Correlation analysis indicated positive associations between AMF species richness and nutrient uptake ($r = 0.68$, $P < 0.001$), WUE ($r = 0.62$, $P < 0.01$), and plant biomass ($r = 0.71$, $P < 0.001$) across pooled data. These correlations remained significant within each water regime, indicating consistent relationships between functional diversity and plant performance.

Discussion

Functional Diversity Dynamics Under Water-Limited Conditions

This investigation demonstrates that AMF functional diversity declines substantially under progressive water limitation but that functional redundancy partially mitigates ecosystem service loss. The reduction in species richness under severe deficit (40% decline relative to well-watered conditions) aligns with previous findings in diverse crop systems [12, 27]. The preferential persistence of certain AMF lineages under drought suggests that environmental filtering shapes community assembly, selecting for taxa with enhanced drought tolerance phenotypes [14].

The paradoxical increase in phosphatase activity under moderate deficit—despite reduced biomass and colonization intensity—suggests a compensatory response wherein stressed fungal communities allocate proportionately greater resources to nutrient mobilization enzymes [20]. This phenotypic plasticity demonstrates functional complementarity within diverse AMF communities: while dominant taxa may be suppressed under moderate stress, subdominant oligotrophic species increase relative abundance and enzyme production, maintaining overall ecosystem nutrient cycling capacity. Conversely, severe stress exceeds compensatory capacity, resulting in concurrent declines in diversity, enzyme activity, and plant nutrition.

The biphasic response of glomalin production to water deficit provides mechanistic insight into soil aggregate stability and water retention under drought. Enhanced glomalin production under moderate deficit enhances soil physical structure and water-holding capacity [6], creating a positive feedback wherein improved soil water availability supports continued fungal metabolism and glomalin synthesis. However, severe drought suppresses glomalin production, reducing soil structural stability and potentially creating a negative feedback spiral of declining fungal function and soil physical degradation [28].

Cultivar-Specific Effects on Mycorrhizal Community Structure and Function

The consistent superiority of cultivar 'JG 11' in maintaining mycorrhizal colonization intensity, community richness, and

functional enzyme activity under water limitation indicates genotype-specific effects on plant–fungal compatibility and symbiotic investment allocation. Previous investigations have documented heritable variation in mycorrhizal responsiveness among crop genotypes [13], and our results extend this concept to functional diversity maintenance under stress. The higher mycorrhizal dependency of 'JG 11' may reflect selection for enhanced symbiotic investment in arid regions where the cultivar originated. Conversely, 'ICCV 10', bred in temperate environments with reliable rainfall, exhibits reduced mycorrhizal colonization and community stability, consistent with lower fitness costs of incomplete symbiotic development under water-replete conditions [29].

Drought Adaptation Mechanisms and Water-Use Efficiency

The 18–24% improvement in water-use efficiency under moderate deficit in plants associated with diverse AMF communities supports the hypothesis that AMF-mediated drought adaptation operates through multiple mechanisms. Enhanced phosphatase activity mobilizes otherwise unavailable soil phosphorus, enabling maintenance of photosynthetic capacity and stomatal regulation despite water limitation [8]. Improved hyphal networks extend the effective soil volume accessible to plant water uptake, lowering plant water potential at given soil water potentials and maintaining turgor-driven cell expansion [7]. Osmotic adjustment through proline accumulation, more pronounced in 'JG 11', reduces cellular osmotic potential and enhances water uptake capacity under low soil water availability [23].

The decline in WUE under severe deficit (35–40% reduction relative to well-watered controls), despite enhanced functional enzyme activity, suggests that water limitation ultimately constrains photosynthetic metabolism beyond compensation by nutritional improvements. Stomatal closure under severe water stress limits CO_2 availability, reducing photosynthetic capacity independent of nutrient availability [8]. This observation indicates that sustainable production under severe drought may require complementary agronomic strategies (deficit irrigation, mulching, soil conservation) alongside microbial inoculant deployment.

Implications for Sustainable Chickpea Production and Microbial Consortium Development

Our results provide empirical support for incorporating functionally diverse AMF communities into microbial consortia designed for climate-resilient agriculture. The documented relationships between functional diversity metrics and plant drought tolerance suggest that multispecies AMF inoculants containing taxa with complementary functional traits—including rapid hyphal extension (copiotrophs), enhanced nutrient mobilization enzyme production, and glomalin synthesis (stress-tolerant oligotrophs)—may outperform monoculture inoculants under variable environmental conditions [30]. Functional redundancy ensures that loss of individual species through environmental stress does not necessarily translate to loss of critical ecosystem services [15].

The 18–24% improvement in WUE under moderate deficit conditions associated with diverse AMF communities has direct implications for water productivity in irrigation-limited agroecosystems. In regions where irrigation water availability is constrained by competing demands (municipal, industrial), modest deficit irrigation combined with AMF inoculants may enhance productivity per unit water applied, improving agricultural sustainability [2]. However, our findings also indicate that under severe drought (40% ETc), functional complementarity cannot fully compensate for water limitation, necessitating cultivar selection for enhanced drought tolerance and consideration of supplementary water harvesting infrastructure [31].

Study Limitations and Future Research Directions

This investigation was conducted under field conditions at a single geographic location over two growing seasons. Validation across diverse soil types, climates, and chickpea production regions would strengthen generalizability. The characterization of AMF community composition relied on morphological identification, which may underestimate species richness and misclassify taxa; molecular metabarcoding approaches would provide higher taxonomic resolution [32]. Furthermore, while we assessed glomalin concentration, quantitative assessment of water-stable aggregate formation and soil physical property changes would strengthen mechanistic understanding of soil structural effects. Future investigations should employ transcriptomic and metaproteomic approaches to characterize differential gene and protein expression in stress-tolerant versus stress-sensitive AMF taxa under water limitation, identifying specific molecular mechanisms underlying functional complementarity [33].

Conclusion

This research indicates that AMF functional diversity plays an important role in the drought resistance of chickpeas and their productivity during water-limited conditions. Growing water shortages modify the AMF communities leading to a reduction of species richness by 40-50% during extreme drought. However, the effects of functional redundancy and stress-induced phenotypic plasticity of nutrient mobilization enzymes and glomalin production are partially neutralizing the losses of ecosystem services. Increased water-use efficiency (18-24% increase) of plants with abundant AMF communities in the course of moderate water shortages proves the agricultural importance of AMF functional diversity conservation. It's been revealed that the genotype 'JG 11' has always been able to get more significant mycorrhizal colonization, enzyme activity, and drought resistance than 'ICCV 10', proving the need to inspect functional diversity concerning different plant genotypes. It is suggested that using multispecies AMF inoculations comprising functionally complementary AMF species with various phenotypes may enhance chickpea crop resistance under water deficit conditions, providing an environmentally sustainable agricultural practice for the semiarid areas affected by climate change.

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