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Research Article

Transcriptome analysis of wheat (*Triticum aestivum*) reveals-regulatory mechanisms of adaptation to water deficit stress induced by arbuscular mycorrhizal fungi

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Abstract

RNA sequencing (RNA-Seq) is a powerful tool for exploring transcriptional responses to environmental cues and elucidating gene regulatory networks. This study investigates how arbuscular mycorrhizal fungi (AMF) alleviate water deficit (WD) stress in *Triticum aestivum* seedlings through transcriptomic analysis. A comparative transcriptome analysis was performed on wheat roots under two irrigation regimes, well-watered (WW) and WD, and/or AMF inoculation, to identify differentially expressed genes (DEGs). AMF inoculation modulated the expression of genes involved in osmotic adjustment and protective metabolite biosynthesis. While genes such as *P5CS*, *ARG*, *OAT*, and *TaPROT2* were downregulated in AMF-treated plants under WD, *asparagine synthase (ASNS)* was notably upregulated. Furthermore, AMF symbiosis enhanced the expression of genes related to polyamine and GABA metabolism under WD stress. A significant upregulation of antioxidant-related genes, particularly *GSTU1*, indicated an AMF-induced strengthening of the antioxidant defense system. Additionally, AMF treatment upregulated multiple nutrient transporter genes, including *PHT*, *AMT*, *NPF*, *NRT*, *HAK/AKT*, aquaporins, sugar transporters, and ABC transporters, thereby contributing to improved nutritional status. AMF also influenced carbohydrate metabolism to promote cell wall (CW) biosynthesis and remodelling, highlighting its role in structural adaptation to drought. These findings offer key molecular insights into the mechanisms by which AMF symbiosis modulates gene expression to improve wheat drought tolerance under varying irrigation conditions.

Keywords: arbuscular mycorrhizal fungi (AMF); cell wall; osmotic regulation; RNA-seq; transporters; *Triticum aestivum*; water deficit

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Introduction

Arbuscular mycorrhizal fungi (AMF) establish one of the most widespread and beneficial symbiotic relationships between plant roots and soil microorganisms. In this mutualistic interaction, AMF enhance plant growth and metabolism by improving the uptake of water and nutrients, particularly nitrogen and phosphorus (Diagne *et al*., 2020; Dowarah *et al*., 2022). Beyond nutritional benefits, AMF substantially enhance plant tolerance to various abiotic stresses, including drought, salinity, extreme temperatures, and heavy metal toxicity (Diagne *et al*., 2020). These protective effects are attributed to improved hydromineral nutrition, stomatal regulation, modulation of gene expression, accumulation of osmolytes, reinforcement of antioxidant systems, and hormonal balance (Diagne *et al*., 2020). Consequently, AMF help plants mitigate the adverse effects of drought (Zou *et al*., 2021), salinity (Boorboori and Lackóová, 2025) and heavy metal toxicity (Riaz *et al*., 2021).

Molecular studies indicate that AMF symbiosis confers stress tolerance through multiple mechanisms, including transcriptional reprogramming (Chen *et al*., 2021; Ran *et al*., 2021; Qin *et al*., 2023; Zhang *et al*., 2025); enhancement of osmotic adjustment (Zong *et al*., 2023); hormonal regulation (Khalloufi *et al*., 2024); improved nutrient status (Zhang *et al*., 2025); and bolstering of defense responses (Romero-Muñoz *et al*., 2022). Elucidating these molecular mechanisms is crucial for improving crop resilience and advancing sustainable agriculture in the face of environmental stress.

Climate change and increasing water scarcity present critical threats to global food security by negatively impacting crop productivity (Bapela *et al*., 2022; Yuan *et al*., 2024). Drought events are becoming more frequent and severe, making it essential to understand and mitigate their impact on major crops such as wheat (Acevedo *et al*., 2018; Qasim *et al*., 2022; Yanagi, 2024). Wheat (*Triticum aestivum* L.), the second most important staple crop worldwide after rice, is particularly vulnerable to water deficit (WD) stress (Nyaupane *et al*., 2024). WD severely impairs photosynthesis, carbohydrate metabolism, and growth in wheat, while triggering the accumulation of osmoprotectants as a defense response (Ahmad *et al*., 2018; Oguz *et al*., 2022). However, these physiological adjustments are often insufficient to prevent significant yield losses.

Breeding drought-tolerant wheat varieties remains challenging due to the complex genetic and environmental interactions governing this trait (Varshney *et al*., 2021; Ma *et al*., 2024). As a result, sustainable strategies such as the application of beneficial soil microorganisms like AMF are increasingly being explored as complementary or alternative approaches to conventional breeding.

In this context, RNA sequencing (RNA-Seq) has emerged as a powerful tool for dissecting transcriptional responses to environmental cues and uncovering gene regulatory networks involved in stress adaptation. Whole-transcriptome analyses have been extensively used to identify genes and pathways related to abiotic stress responses and plant–microbe interactions (Bahadur *et al*., 2019). Previous transcriptomic studies have shown that AMF colonization influences root architecture (Chen *et al*., 2021), secondary metabolism (Ran *et al*., 2021), nutrient transporter gene expression (Qin *et al*., 2023; Zhang *et al*., 2025), and osmotic stress responses (Puccio *et al*., 2023). RNA-Seq has been particularly instrumental in identifying stress-responsive genes under conditions such as drought (Chaichi *et al*., 2019; Rasool *et al*., 2022) and AMF colonization (Hu *et al*., 2018; Puccio *et al*., 2023). Breeding drought-tolerant wheat varieties remains challenging due to the complex genetic and environmental interactions governing this trait (Varshney *et al*., 2021; Ma *et al*., 2024). As a result, sustainable strategies such as the application of beneficial soil microorganisms like AMF are increasingly being explored as complementary or alternative approaches to conventional breeding. Despite these advances, transcriptomic investigations addressing the combined effects of AMF symbiosis and WD stress in wheat remain limited. Knowledge gaps persist regarding how AMF modulate drought-responsive molecular pathways in this crop. Since both conserved and species-specific mechanisms operate under stress, further molecular research is needed to support the development of drought-resilient wheat varieties.

In this study, we performed RNA-Seq analysis on the roots of *T. aestivum* cv. 'Chamran', a widely cultivated Iranian wheat variety, inoculated with *Funneliformis mosseae* under well-watered and water-deficit conditions. This research aimed to identify key molecular pathways by which AMF confer drought tolerance in wheat. While prior studies have established the general benefits of AMF, comprehensive transcriptomic insights into their influence on osmotic regulation, antioxidant defense, nutrient metabolism, hormonal signaling, and cell wall (CW) modification under drought stress remain scarce.

Here, we sought to identify genes responsive to both mycorrhizal symbiosis and irrigation regimes, particularly those involved in osmotic regulation, polyamine and GABA metabolism, antioxidant systems, nutrient uptake, and primary and secondary metabolism. Moreover, we explored genes associated with CW biosynthesis and remodeling, to determine whether AMF influence structural and metabolic adaptation under drought. Our findings contribute to a better understanding of the molecular mechanisms underlying AMF-induced drought tolerance and provide a foundation for future studies aiming to enhance crop performance under water-limited conditions.

Material and Methods

Plant materials and experimental conditions

Seeds of *Triticum aestivum* L. cv. 'Chamran', a widely cultivated Iranian wheat variety, were obtained from the Seed and Plant Improvement Institute (SPII), Karaj, Iran. The seeds were surface-sterilized by immersion in 70% ethanol for 2 minutes, followed by 1% sodium hypochlorite for 10 minutes, and then rinsed three times with sterile distilled water. Sterilized seeds were soaked in distilled water overnight in the dark at room temperature (RT), and germinated for three days. The germinated seeds were sown in pots filled with a sterilized mixture of field soil (pH 8.0; texture: 35.5% sand, 25.4% clay, and 38.7% silt), sand, and perlite in a 2:1:1 (v/v/v) ratio. Four experimental treatment groups were established: (i) well-watered control (Ctrl), (ii) water-deficit (WD), (iii) AMF-inoculated seedlings under well-watered conditions (AMF), and (iv) AMF-inoculated seedlings under WD conditions (AMF+WD).

The arbuscular mycorrhizal (AM) fungus *F. mosseae* (isolate Gmb17), provided by the Department of Plant Protection, Faculty of Agriculture, Rafsanjan University, Iran, was used as inoculum. A total of 100 g of AM inoculum was applied to each pot assigned to AMF treatments at the time of sowing.

Irrigation was carried out at two levels: well-watered (WW) condition (twice weekly, to maintain field capacity), and water-deficit (WD) condition (twice weekly, at 50% of field capacity). Plants were maintained under these irrigation regimes for seven weeks. At harvest, root samples from selected plants were immediately frozen in liquid nitrogen and stored at −80 °C for subsequent RNA extraction, cDNA library construction, and transcriptome sequencing.

Physiological assessments

This study employed a series of standard spectrophotometric assays to quantify key biochemical constituents and antioxidant enzyme activities in root samples, including proline content, insoluble and soluble sugars, soluble protein, phosphate, malondialdehyde (MDA), and the activities of peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD). Proline content was measured using the method of Bates *et al*. (1973), which involves tissue homogenization in sulfosalicylic acid, reaction with acid ninhydrin, and absorbance measurement at 520 nm. Soluble protein content was determined according to the Bradford method (Bradford, 1976), in which homogenized tissue extracts were reacted with Bradford reagent, and absorbance was recorded at 595 nm using bovine serum albumin (BSA) as the standard. Soluble sugars were quantified using the anthrone method described by Yemm and Willis (1954), following ethanol extraction and absorbance measurement at 620 nm, with glucose as the standard. Insoluble sugars (starch) were

estimated by hydrolyzing the ethanol-insoluble residue and applying the anthrone reagent, with absorbance recorded at 620 nm (McCready *et al*., 1950). Phosphate content was assessed using the molybdenum blue method following nitric–perchloric acid digestion, with absorbance of the resulting complex measured at 882 nm (Murphy and Riley, 1962). Lipid peroxidation was evaluated by determining malondialdehyde (MDA) levels via the thiobarbituric acid (TBA) reaction, with absorbance read at 532 nm (Heath and Packer, 1968).

The activities of antioxidant enzymes were determined as follows: peroxidase (POD) activity was measured based on guaiacol oxidation at 470 nm (Chance and Maehly, 1955); catalase (CAT) activity by monitoring the decomposition of hydrogen peroxide at 240 nm (Aebi, 1984); superoxide dismutase (SOD) activity by evaluating the inhibition of nitroblue tetrazolium (NBT) photoreduction at 560 nm (Beauchamp and Fridovich, 1971); and ascorbate peroxidase (APX) activity by monitoring the decrease in absorbance due to ascorbate oxidation at 290 nm (Nakano and Asada, 1981). All experiments were conducted in triplicate. Statistical analysis was performed using multiple t-tests ($P < 0.05$) with GraphPad Prism 9. Results are presented as mean ± standard error (SE), and different lowercase letters indicate statistically significant differences among treatments.

RNA extraction and cDNA library construction

Whole root tissues were harvested for RNA-Seq analysis. Roots from ten plants per biological replicate were pooled for RNA extraction. Total RNA was extracted from each pooled root sample using the RNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. RNA concentration and integrity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). Only RNA samples with a RNA Integrity Number (RIN) ≥ 7 were selected for downstream analysis. cDNA libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit v2 (Illumina, San Diego, USA), according to the manufacturer's protocol. Final libraries were sequenced on the Illumina NextSeq 500 platform (Illumina, San Diego, USA) to generate 360 bp paired-end reads.

Pre-processing data

The quality of raw FASTQ files was assessed using FastQC (*http://www.bioinformatics.babraham.ac.uk/projects/fastqc/*). Adapter sequences and low-quality reads were removed using the BBMap software package (*https://sourceforge.net/projects/bbmap/*), resulting in clean libraries for downstream analysis. High-quality 360-bp paired-end reads were generated from a total of eight cDNA libraries. On average, 41.6% (Ctrl), 55.62% (WD), 50.55% (AMF+WD), and 50.27% (AMF) of the clean reads were successfully mapped to the wheat reference genome (*Triticum aestivum*, *https://plants.ensembl.org/Triticum_aestivum/Info/Index*) using HISAT2 (*https://ccb.jhu.edu/software/hisat2/*) (Kim *et al*., 2015).

After stringent quality filtering, high-quality clean reads were used for transcriptome assembly and expression analysis. Gene expression levels were quantified as FPKM (Fragments Per Kilobase of transcript per Million mapped reads) using Cufflinks v2.2.1 (*http://cole-trapnell-lab.github.io/cufflinks/*). Differentially expressed genes (DEGs) were identified using the DESeq2 R package. Genes with an absolute value of $|\log_2$ fold change$| \geq 2$ and an adjusted p-value (q-value) < 0.05 were considered significantly differentially expressed (Supplementary Table S1).

The raw RNA-Seq data have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA867352**.**

Functional annotations, GO enrichment, and pathway analysis

The functional annotation of differentially expressed genes (DEGs) was performed through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. GO

enrichment analysis was carried out using the PANTHER 18.0 platform (*http://www.pantherdb.org/*) and g:Profiler web tool (*https://biit.cs.ut.ee/gprofiler/gost*). Additional GO and KEGG pathway analyses were performed using ShinyGO v0.80 (*http://www.bioinformatics.sdstate.edu/go/*) to identify significantly enriched GO terms and KEGG pathways among the DEGs. GO and KEGG terms with an adjusted p-value < 0.05 were considered statistically significant.

qRT-PCR Validation

To validate the RNA-Seq results, five DEGs from the WD vs. AMF+WD comparison, related to CW metabolism and transporter activity, were selected for quantitative real-time PCR (qRT-PCR) analysis (Table S2). Reverse transcription was performed using total RNA and the GoScript™ Reverse Transcription System (Promega, Madison, WI, USA), following the manufacturer's protocol. qRT-PCR was conducted using the StepOne™ Real-Time PCR System (Applied Biosystems™, Waltham, MA, USA) and Power SYBR™ Green PCR Master Mix (Applied Biosystems, USA). Each 10 µL reaction contained: 1 µL diluted cDNA template, 0.5 µL each of forward and reverse primers (10 µM), 5 µL 2× SYBR Green Master Mix, and 3 µL sterile ddH_2O. The thermal cycling conditions included an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 92 °C for 45 seconds, annealing at the gene-specific annealing temperature for 10 seconds, and extension at 72 °C for 45 seconds. Gene-specific primers were designed using Primer3 software and are listed in Supplementary Table S2.

The relative expression of target genes was normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference gene using the $2^{-\Delta\Delta Ct}$ method. Each qRT-PCR reaction was performed in two technical replicates per sample.

Statistical Analysis and Data Visualization

All data were statistically analyzed using the Hochberg false discovery rate (FDR) correction, with a significance threshold set at $p < 0.05$ after adjustment. Statistical analyses and graph generation were performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Heatmaps of gene expression patterns were generated using the heatmap.2 function from the gplots R package (v2.12.1) in R software (version i386 3.2.0).

Results

Biochemical parameters

Water deficit (WD) significantly altered several key biochemical parameters in wheat roots (Figure 1a-e). Proline content (Figure 1a), a well-known osmoprotectant, increased significantly in WD-treated roots compared to control plants, indicating an active osmotic adjustment mechanism. Interestingly, $AMF^{+}WD$ plants exhibited significantly lower proline accumulation than non-mycorrhizal WD-treated plants, suggesting that AMF inoculation mitigated the severity of osmotic stress. Similarly, soluble sugar content (Figure 1c), another important osmolyte, was higher in WD-treated plants compared to controls. Nevertheless, $AMF^{+}WD$ plants accumulated less soluble sugar than non-mycorrhizal WD-treated roots, further demonstrating the ability of AMF to enhance water status and reduce the need for excessive osmotic adjustment. Insoluble sugar content (Figure 1b) also increased under WD conditions. Insoluble sugar content (Figure 1b) also increased significantly under WD conditions compared to the control. While $AMF^{+}WD$ plants exhibited similar levels of insoluble sugars as WD-treated plants, AMF treatment alone also led to an increase in insoluble sugar content compared to the control, suggesting a potential role of AMF in carbon allocation or storage.

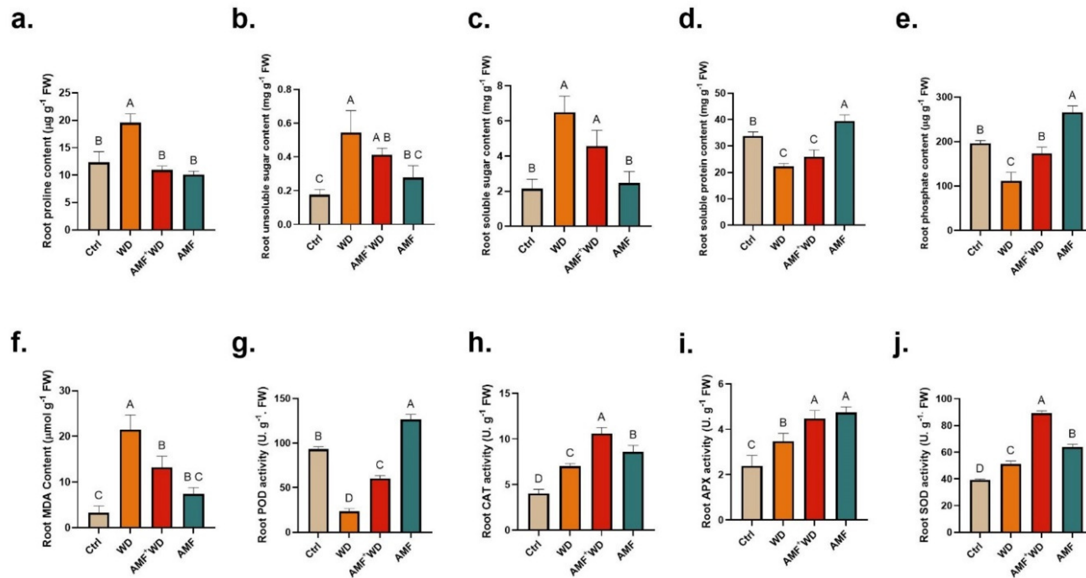

Figure 1. Physiological and biochemical characteristics of wheat roots under two irrigation regimes, and/or AMF inoculation. Wheat plants were subjected to four treatments: Ctrl, WD, AMF⁺WD, and AMF. (a) Root proline content (µg g^{-1} FW); (b) Root insoluble sugar content (mg g^{-1} FW); (c) Root soluble sugar content (mg g^{-1} FW); (d) Root soluble protein content (mg g^{-1} FW); (e) Root phosphate content (mg g^{-1}FW); (f) Root malondialdehyde (MDA) content (µmol g^{-1} FW); (g) Root peroxidase (POD) activity (U g^{-1} FW); (h) Root catalase (CAT) activity (U g^{-1} FW); (i) Root ascorbate peroxidase (APX) activity (U g^{-1} FW); (j) Root superoxide dismutase (SOD) activity (U g^{-1} FW)

Data are presented as mean ± standard deviation (SD). Different lowercase letters above bars indicate statistically significant differences among treatments ($P < 0.05$), based on one-way ANOVA followed by Tukey's post-hoc test

Soluble protein content (Figure 1d), on the other hand, decreased markedly in WD-treated roots compared to the control. In contrast, AMF^+WD plants maintained significantly higher levels of soluble protein than non-mycorrhizal WD-treated plants, approaching (or even surpassing) those observed in the control group. A particularly notable finding was the significant increase in root phosphate content (Figure 1e) in both AMF and AMF^+WD treatments compared to the control. While phosphate levels decreased under WD conditions, AMF^+WD plants exhibited a dramatic increase, significantly surpassing even the control group.

Oxidative stress indicators and antioxidant enzyme activities

Water deficit (WD) induced significant oxidative stress in wheat roots, as indicated by elevated malondialdehyde (MDA) levels (Figure 1f). MDA, a byproduct of lipid peroxidation, was markedly higher in WD-treated roots compared to the control. Notably, AMF^+WD plants exhibited significantly lower MDA levels than non-mycorrhizal WD-treated plants, suggesting that AMF inoculation effectively mitigated oxidative damage to cell membranes.

In response to the oxidative stress, wheat roots activated their antioxidant defense system, as reflected by increased activities of key antioxidant enzymes (Figure 1g-j). The activities of peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) were all significantly elevated under WD compared to control conditions, indicating an intrinsic response to reactive oxygen species (ROS) accumulation. Importantly, AMF^+WD plants exhibited consistently higher activities of all four enzymes compared to non-mycorrhizal WD-treated plants, suggesting that AMF inoculation further enhanced the antioxidant defense system. This elevated enzymatic activity implies that AMF priming promotes more efficient ROS scavenging and offers improved protection against oxidative damage under drought conditions.

Even under non-stressed conditions, AMF inoculation led to modest increases in soluble protein, phosphate content, and antioxidant enzyme activities, indicating an overall enhancement of plant physiological performance and resilience.

RNA-Seq Results

Differentially expressed genes were identified through pairwise comparisons, including "AMF vs. AMF$^+$WD", "WD vs. AMF$^+$WD", "Ctrl vs. AMF", and "Ctrl vs. WD" (Table 1). The DEGs were visualized with Venn diagrams and Volcano plots (Figure 2,3).

Table 1. Number of up-regulated and down-regulated DEGs across four comparisons

Comparisons	Total DEG	Up-regulated DEGs	Down-regulated DEGs
Ctrl vs. WD	7,637	2,474	5,163
Ctrl vs. AMF	3,125	1,731	1,394
WD vs. AMF$^+$WD	4,513	3,465	1,048
AMF vs. AMF$^+$WD	3,087	1,444	1,643

Under well-watered condition, 3,125 DEGs were identified in the "Ctrl vs. AMF" comparison. Under non-inoculated condition, the "Ctrl vs. WD" comparison revealed a total of 7,637 DEGs, indicating that WD stress induced a significantly higher number of DEGs compared to AMF inoculation. In AMF vs. AMF$^+$WD comparison, 3,087 DEGs were identified. In the WD vs. AMF$^+$WD comparison, 4,513 DEGs were identified comprising 3,465 up-regulated DEGs and 1,048 down-regulated genes. These AMF-induced DEGs under WD stress may play a crucial role in enhancing plant tolerance to WD stress. The highest number of DEGs was observed in the "Ctrl vs. WD". Notably, in the Ctrl vs. WD comparison, the number of down-regulated DEGs (5,163) was more than twice the number of up-regulated DEGs (2,474). Conversely, in AMF-inoculated plants exposed to WD stress (WD vs. AMF$^+$WD), the number of up-regulated DEGs number (3,465) was more than three times that of down-regulated DEGs number (1,048), suggesting that WD stress primarily down-regulated gene expression, while under WD stress, AMF inoculation markedly enhances gene up-regulation (Table 1). Venn diagram (Figure 3a, Table S4) identified a total of 11,380 DEGs across the four comparisons. In the comparison assessing the effects of WD with and without AMF inoculation ("Ctrl vs. WD" and "AMF vs. AMF$^+$WD"), 8,973 DEGs were influenced by WD stress, with 1,751 DEGs being shared between the two comparisons. The number of total DEGs in each comparison was generally higher than the shared DEGs, which may indicate distinct adaptive mechanisms induced by AMF. In the comparison assessing the impact of AMF inoculation under well-watered and without WD stress conditions ("Ctrl vs. AMF" and "WD vs. AMF$^+$WD"), *F. mosseae* inoculation altered the expression of 5,909 DEGs. Among these, 1,729 DEGs were shared between the two comparisons, with 1,467 DEGs displaying consistent expression patterns regardless of WD stress, suggesting their involvement in symbiosis establishment (Figure 3b, Table S4). Additionally, 262 DEGs exhibited opposite regulatory trends under WD stress and well-watered condition, indicating their potential role in both symbiotic relationships and WD stress responses. Furthermore, 2,784 DEGs were uniquely induced by AMF under WD stress. In total, 3046 DEGs (2,784 exclusive DEGs and 262 DEGs with opposite trends) were implicated in adaptive response of AMF-inoculated wheat to WD stress (Figure 3b, Table S4).

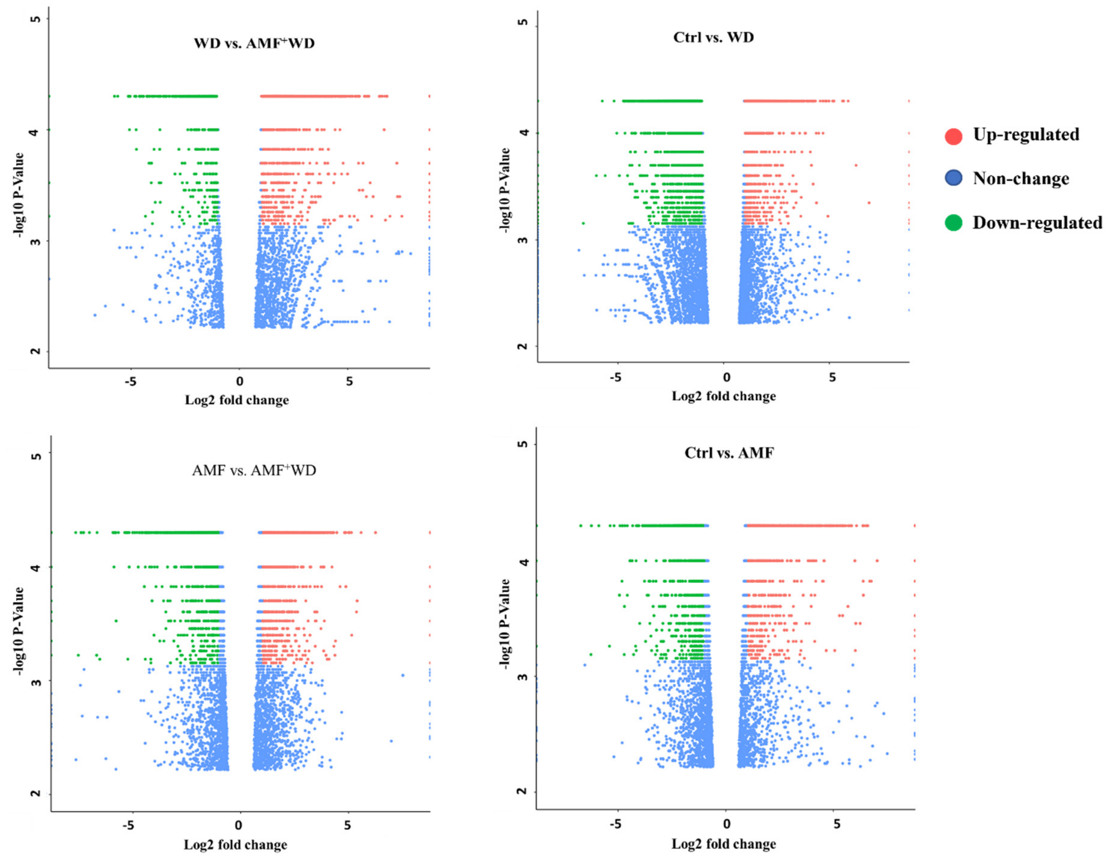

Figure 2. Volcano plots illustrate the up- and downregulated DEGs in the comparisons of Ctrl vs. WD, WD vs. AMF$^+$WD, AMF vs. AMF$^+$WD, and Ctrl vs. AMF
Each dot represents a gene: red dots indicate upregulated genes, green dots indicate downregulated genes, and blue dots represent genes with no significant differential expression

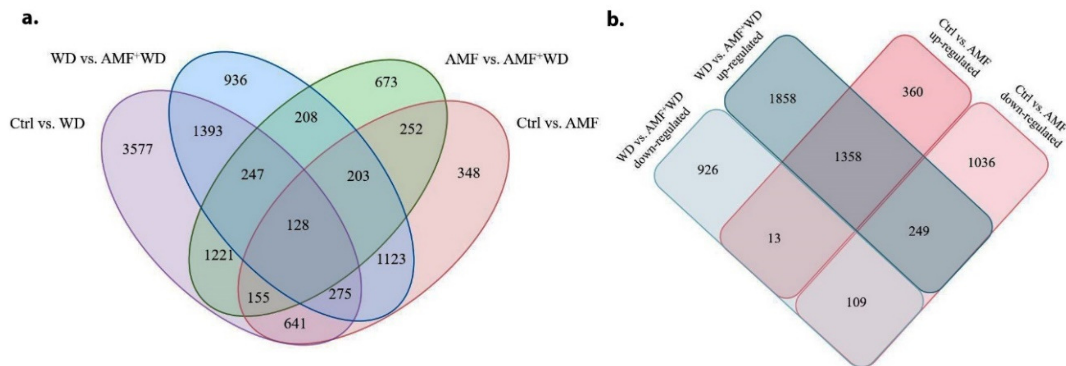

Figure 3. Venn diagram analysis of DEGs. (a) DEGs identified in four comparisons. (b) DEGs between two pairwise comparisons (WD vs. AMF$^+$WD) and (Ctrl vs. AMF)
Green-ellipsoid: (AMF vs. AMF$^+$WD); Purple-ellipsoid: (Ctrl vs. WD); Pink-ellipsoid: (Ctrl vs. AMF); Blue-ellipsoid: (WD vs. AMF$^+$WD)

Functional enrichment analysis of DEGs induced by AMF under WD stress

To elucidate the protective mechanisms of AMF in mitigating WD stress, 2,784 exclusive DEGs were subjected to Gene ontology (GO) and Kyto Encyclopedia of Genes and Genomes (KEGG) enrichment

analyses (Table S5). GO analysis showed revealed significant enrichment in biological processes, molecular functions and cellular components terms associated with functional categories: response to stimulus (e. g., “antioxidant activity”, “response to abiotic stimulus”, and “response to biotic stimulus”), transport (e. g., “transporter activity”, “transmembrane transporter activity”), and several metabolic and catabolic processes (e. g., “cell wall organization or biogenesis”, “positive regulation of metabolic process”, and “hormone metabolic process”) were significantly enriched. GO enrichment analysis suggested that the up-regulated genes were associated with metabolic process (such as “alpha-amino acid metabolic process”, “phenylpropanoid metabolic process”, “secondary metabolite process”, “cellulose metabolic process”), redox (such as “oxidoreductase activity, acting on peroxide as acceptor”), antioxidation (such as “hydrogen peroxide metabolic process”, “peroxidase activity”, “response to oxidative stress”), CW activities (such as “cell wall organization or biogenesis”, “cell wall macromolecule metabolic process”), and transport (such as, “transporter activity”, “water transmembrane transporter activity”) (Figure 4a; Table S6). On the other hand, categories of “metabolic process” (e. g., “cellular amino acid metabolic process”, “glutamine family amino acid metabolic process”, and “arginine metabolic process”, “cellular carbohydrate metabolic process”), response to stimulus (e. g., “response to osmotic stress”, “response to oxidative stress,” “antioxidant activity”), transport (e.g., “ion transport”, “lipid transport”, “carbohydrate transport”) were significantly overrepresented among the down-regulated genes (Figure 4a; TableS7). KEGG pathway analysis highlighted significant enrichment in the phenylpropanoid biosynthesis, biosynthesis of secondary metabolites and metabolic pathways (Figure 4b; Table S8).

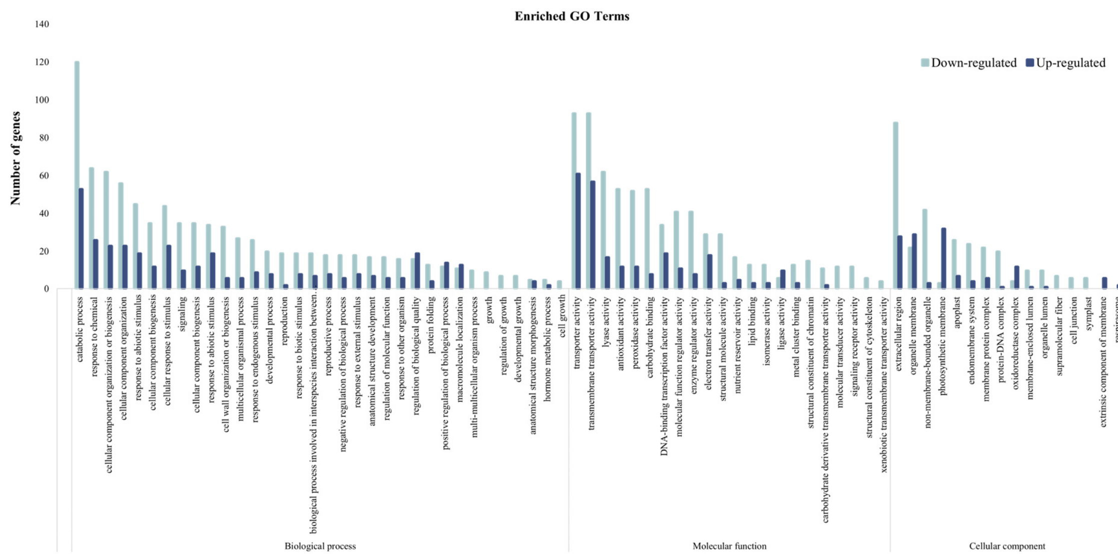

Figure 4(a). Functional enrichment analysis of DEGs. - GO enrichment of exclusive DEGs in biological processes, molecular functions and cellular components aspects in “WD vs. AMF^{+}WD”

The complete listing of enriched GO terms can be found in Supplementary Tables S5-S7; Significantly enriched GO and KEGG terms were selected based on FDR < 0.05

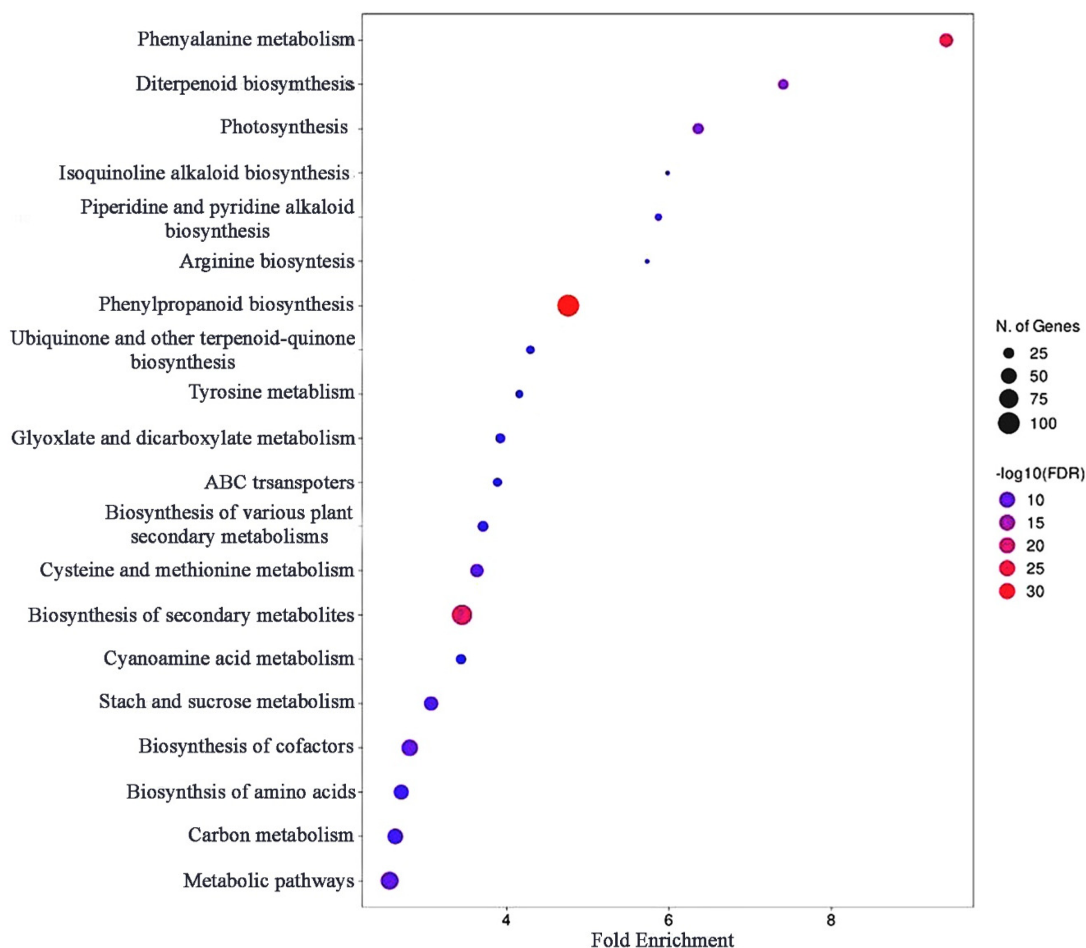

Figure 4(b). Functional enrichment analysis of DEGs - Top KEEG pathways enrichment for exclusive DEGs in "WD vs. AMF⁺WD"
The complete listing of enriched pathways can be found in Supplementary Table S8; Significantly enriched GO and KEGG terms were selected based on FDR < 0.05

Additionally, functional enrichment analysis was performed for DEGs in the "Ctrl vs. WD" and the "AMF vs. AMF⁺WD" comparisons (Figure 4c; Table S9) to further delineate differences between AMF-inoculated and non-inoculated plants under WD stress. GO terms related to response to stimulus (e.g., "response to water", "response to abiotic stimulus", "response to oxidative stress"), metabolic process (e.g., "carbohydrate biosynthetic process", "phenylpropanoid metabolic process" , "secondary metabolic process"), antioxidant (e.g., "response to oxidative stress", "reactive oxygen species metabolic process", "antioxidant activity"), transport (e.g., "ion transport", "anion transport", "organic acid transport"), CW activities (such as "cell wall organization or biogenesis", "cell wall macromolecule metabolic process"), were significantly enriched in both the comparisons. However, AMF-inoculated plants exhibited a greater number of enriched GO terms and DEGs per term under WD stress. Moreover, certain GO terms such as those related to metabolic processes (e.g., asparagine metabolic process, terpene metabolic process, spermine metabolic process), transport (e.g., carbohydrate transport and amino acid transport) and enzyme inhibitor activity (e.g., "peptidase activity", "endopeptidase inhibitor activity" "serine-type endopeptidase inhibitor activity") were uniquely enriched in the "AMF vs. AMF⁺WD" comparison, while GO terms such as metabolic processes (e.g., "cellulose metabolic process", "hemicellulose metabolic process", "putrescine metabolic process"), ion transport (e.g., "metal ion transport", "potassium ion transmembrane transport") in "Ctrl vs. WD" comparison.

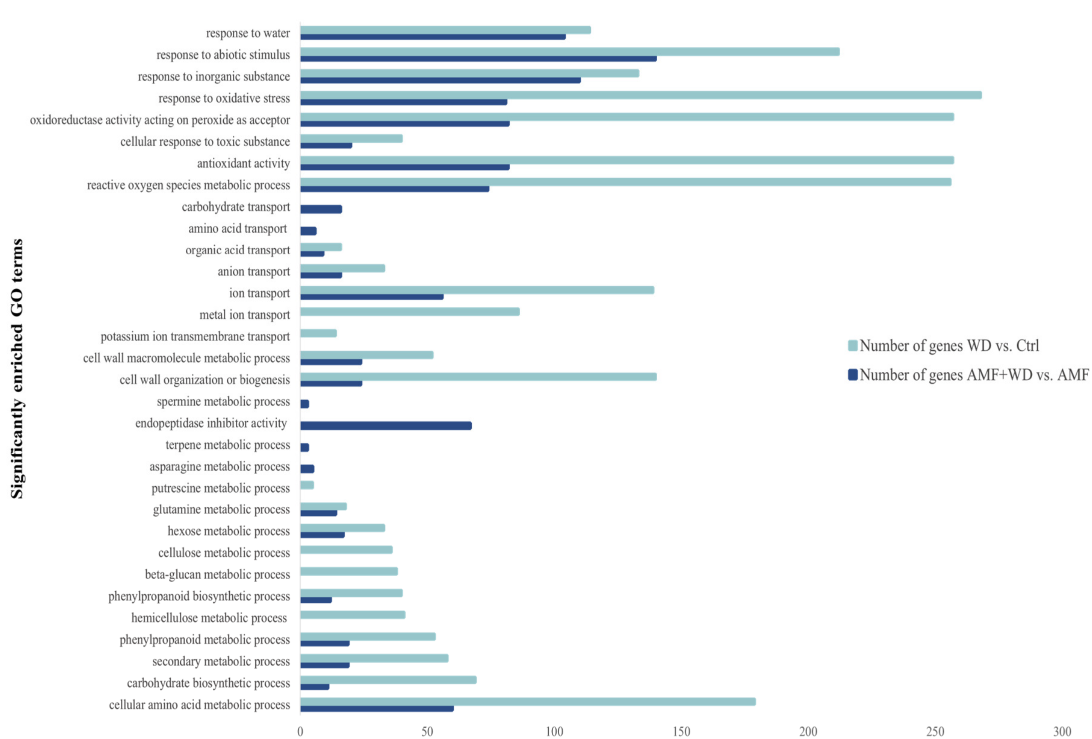

Figure 4(c.) Functional enrichment analysis of DEGs - Significantly enriched GO terms distribution for DEGs in “AMF vs. AMF⁺WD” and “Ctrl vs. WD”
The complete listing of enriched GO terms can be found in Supplementary Table S9; Significantly enriched GO and KEGG terms were selected based on FDR < 0.05

Expression patterns of DEGs involved in osmotic regulation, ROS scavenging, transporter proteins, and CW biosynthesis regulated by AMF under WD stress (WD vs. AMF⁺WD)

To better understand how *F. mosseae* mitigates WD stress-induced damage, we analyzed expression patterns of DEGs related to osmotic regulation, antioxidant activity, ion transport, and CW biosynthesis, were analyzed, especially in “WD vs. AMF⁺WD” comparison.

Our analysis revealed that, AMF inoculation significantly affected the expression involved in osmotic adjustment, particularly those related to sugars, amino acids, and trehalose metabolism. Specifically, genes associated with arginine and proline metabolism, including one *delta-1-pyrroline-5-carboxylate synthetase* (*TaP5CS1*), three *arginase* (*ARG*), two *ornithine aminotransferase* (*OAT*), three *Glutamate dehydrogenase* (*TaGDH2*), four *Glutamine synthetase* (*TaGS1*), three *acetylornithine transaminase* (*AcOAT*), three *aspartate aminotransferase* (*TaASP1*), four *tyrosine aminotransferase* (*TaTAT*), and three *4-hydroxyphenylpyruvate dioxygenase* (*TaHPD*) were significantly down-regulated. Additionally *probable proline transporter 2* (*TaPROT2*) was suppressed. Conversely, four genes encoding *asparagine synthetase1* (*TaASNS1*), a gene encoding *glutamate decarboxylase* (*TaGAD1*), one gene encoding *Glutamine synthetase* (*TaGS1*), one gene encoding *agmatine deiminase* (*aguA*) and three *GABA transporter 2* (*GAT2*) genes were up-regulated (Table S10). Notably, most of these DEGs displayed opposite expression trends in Ctrl vs. WD treatment.

AMF inoculation also significantly upregulated gene involved in sugar metabolism, including six *sucrose-1-fructosyltransferase* (*1-SST*), and three *fructan-6-fructosyltransferase* (*6-SFT)*, promoting the biosynthesis of fructan, under WD stress. However. These genes were downregulated in the non-inoculated plants in response to WD stress. Similarly, seven genes associated with trehalose metabolism, significantly affected by AMF

inoculation under *WD* stress. Specifically, three *trehalose-6-P phosphatases* (*TaTPPE*) and one *trehalase* (*TaTRE1*) were upregulated, whereas three *α-α-trehalose-phosphate* (*TaTPS*) genes were downregulated under *WD* stress. These genes displayed inverse expression patterns in non-mycorrhizal wheat roots exposed to WD stress, except for *TaTRE1*, which remain unchanged. Under non-inoculation condition WD stress led to the down-regulation of eight *TPP* genes and up-regulation six *TPS* (Table S10). Furthermore, AMF inoculation upregulated seven *sugar transport protein* (*TaSTP1*) genes along with two SWEET transporters (*TaSWEET15* and *TaSWEET1b*), while down-regulating two *vacuolar invertases* (*TaVAC-INV*), one *CW invertases* (*TaCWINV2*), multiple *SWEET* transporters (one *TaSWEET2a,* three *TaSWEET12*, and one *TaSWEET17*) (Table S10). AMF inoculation also triggered the expression of three *sucrose synthase 1* (*SuSy 1*), three *hexokinase* (*TaHXK3)*, two *fructokinase* (*FK*), and two *UDP-glucose pyrophosphorylase* (*UGP*) and repressed the expression of 1 three *sucrose synthase 5* (*SuSy 5*).

AMF-induced expression of genes involved in the antioxidant defense systems in T. aestivum roots under WD stress

AMF inoculation enhanced the expression of ROS scavenging-related genes under WD stress. Seventy-eight *peroxidases* (*POD)*, thirty-two *glutathione S-transferases* (*GST*), three *glutaredoxins* (*GRX*), two *Catalase* (*CAT*) genes, one *thioredoxin* (*TRX*) three *adenosylhomocysteinases* (*AHCY*), and two *superoxide dismutase* (*SOD*) genes were up-regulated by AMF inoculation under WD stress. Conversely, fifteen *POD*, nine *GST* and seven *TRX* were down-regulated by AMF inoculation under WD stress. Notably, the expression of three *probable glutathione S-transferase* (*GSTU1*) (*TraesCS5A02G374800*, *TraesCS5B02G376800*, *TraesCS5D02G384400*) were strongly induced (6.9-, 5.9- and 4.0-fold, respectively) by AMF inoculation under the WD stress (Table S11). Among these, *TraesCS5B02G376800* and *TraesCS5D02G384400* were exclusively expressed in AMF-inoculated roots, suggesting they are AMF-specific responsive genes. In the non-inoculated plants, most DEGs encoding *POD* (238 of 252 transcripts), *GST* (50 of 55 transcripts), *TRX* (3 of 14 transcripts), and three *AHCY* genes were down-regulated under WD stress.

AMF-induced expression of transporter genes under WD stress

AMF appears to enhance water and nutrient homeostasis during WD stress, aiding plants in coping with WD stress. In this study, AMF inoculation up-regulated numerous genes encoding various transporters, including ABC transporters, aquaporins, phosphate, ammonium, potassium, sugar and nitrate transporters under WD stress (Table S12).

AMF inoculation significantly up-regulated the expression of seven *phosphate transporter (PHT1)* (*TaPHT1.8-D1*, *TaPHT1.11-A1*, *TaPHT1.11-B1*, *TaPHT1.11-D1* (known as *TRIae;Pht1; 12*, *TRIae;Pht1;11*, *TRIae;Pht1; 10*, respectively)) genes, and *TaPT13*, *TaPT14* and *TaPT15*) eight *low-affinity NRT1/PTR family (NPF) transporters* (*TaNPF1.1*, *TaNPF2.4*, *TaNPF4.10*, *TaNPF5.7*, *TaNPF5.10*, *TaNPF6.4*) gens, six *ammonium transporter* (*TaAMT2;1*, *TaAMT2;3, TaAMT2;6*) genes, five *potassium transporter* (*TaHAK5, TaHAK1*) genes and only one *potassium channel* (*AKT3*). However, eight *PHT1* (*TaPHT1.2-A1*, *TaPHT1.2-B1*, *TaPHT1.2-D1*, *TaPHT1.9-D1*, *TaPHT1.10-D2*, *TaPHT1.10-D1*, *TaPT7, TaPT8*; ten *high-affinity nitrate transporters* (*TaNRT2*) genes: six *TaNRT2.1,* one *TaNRT2.3* and three *TaNRT3.2*, three *TaAMT1.2*; three *TaHAK5,* and four *potassium channel* (*AKT*)**,** three *TaSKOR* and a *TaKAT1* genes under AMF$^+$WD stress.

Following that, the expression of genes coding for *TaHAK1b-2DL* (*TraesCS2D02G287300*), and four *low-affinity NRT1* (*TraesCS1D02G147400*, *TraesCS7B02G101800*, *TraesCS4D02G361500*, *TraesCS5A02G537100*), and four *TaAMT2* (*TraesCS3B02G414300*, *TraesCS3A02G381700*,

TraesCS3A02G381600, *TraesCS3D02G374800*) were induced by AMF, especially under WD stress, indicating a possible function of these genes specifically in AMF-mediated under WD stress.

F. mosseae inoculation also up-regulated the expression of forty-nine *ABC* transporter genes and down-regulated ten ABC transporter genes in wheat roots under WD stress. The majority of these genes are in the *ABCG* subfamily, with twenty-four being upregulated and three being downregulated. *F. mosseae* inoculation also induced the expression of twenty-four members in ABCG family and repressed the expression of three *ABCG* genes under WD stress.

Moreover, the upregulation of twenty aquaporin-encoding genes in response to WD stress induced by *F. mosseae* was also observed. In particular, nine nodulin 26-like intrinsic proteins (NIP) genes (*TaNIP1-5*, *TaNIP1-10*, *TaNIP2-2*, *TaNIP3-3*), ten tonoplast intrinsic protein (*TIP*) genes *(TaTIP1-2, TaTIP1-4, TaTIP2-5, TaTIP2-7, TaAQP5*), and one plasma membrane intrinsic protein *PIP* gene (*TaPIP2-13*) were upregulated in mycorrhizal *T. aestivum* under WD stress. Moreover, the AMF inoculation led to the up-regulation the expression of two DEGs encoding *calcium-transporting ATPases* (*ACAs*) and two *plasma membrane* H^+*-ATPases ATPase* (*PMA*) genes under WD stress (Table S12).

AMF-induced the expression of genes related to the CW metabolism under WD Stress

Expression of genes relevant to the CW metabolism were changed by the AMF inoculation in the WD conditions. Taking CW biogenesis and modification into account, most DEGs were enriched in the process of carbohydrate metabolism. In addition to carbohydrate metabolism-related genes, several other genes required for CW synthesis and modification were also identified to be responsive to the AMF inoculation under the WD stress.

Analysis showed that inoculation with *F. mosseae* changes the expression of genes involved in cellulose synthesis and deposition. Under WD stress, *F. mosseae* inoculation up-regulated the expression of ten *cellulose synthase* (*CesA*), one *cellulose synthase-like* (*Csl*), and four *COBRA-like* (*COBL*) family genes. Moreover, the expression of eleven *fasciclin-like arabinogalactan proteins* (*FLAs*) DEGs coding for *FLAs 1*, *11*, and *16* was upregulated by AMF under WD stress. We also found that AMF inoculation induced the expression of four *trichome birefringence-like* (*TBL*) genes involved in the acetylation of xylan and secondary CW deposition and repressed the expression of only one *TBL* encoding gene. Likewise, the expression of three *UDP-arabinopyranose mutase 3* (*RGP3*), a *mixed-linkage glucan* (*MLG*) and a *mannan synthesis-related 1 like* (*MSR*), involved in pectin and hemicellulose biosynthesis were up-regulated by AMF inoculation under WD stress (Table S13).

Regarding the CW loosening and degradation, the expression of twenty-one *beta-glucosidase* (*BGLU*), five *endoglucanase* (*Eg*), two *beta-D-xylosidase* (*XYL*), six *beta-galactosidase* (*BGLA*), three *alpha-mannosidase (MNS)*, five *xyloglucan endotransglucosylase/hydrolase* (*XTH8*), five *pectin acetylesterases* (*PAE*), two *pectate lyase* (*PL*) and fourteen *UDP-glycosyltransferase* (*UGT*) encoding genes were up-regulated by *F. mosseae* inoculation under WD stress. In addition, under WD stress, *F. mosseae* inoculation down-regulated the expression of four *beta-glucosidase* (*BGLU*), three *endoglucanase*, two *xyloglucan endotransglucosylase/hydrolase* (*XTH1*), a *pectate lyase* (*PL*), a *pectinesterase inhibitor* and four *UDP-glycosyltransferase* (*UGT*) encoding genes. We also found that nine *beta-hexosaminidase* (*HEXO2*), forty *chitinase* (*Cht*) and *endochitinase* encoding genes were up-regulated and only one *chitinase* was down-regulated by AMF inoculation under WD stress (Table S13). Among the genes involved in CW loosening, we found eleven genes encoding *expansin-like EG45-like protein,* five *expansin* encoding-genes, and two *leucine-rich repeat extensin-like protein* were up-regulated by AMF under WD stress and only *expansin-like EG45-like protein* (*TaEXLA1*) and *TaEXPA8P* were down-regulated (Table S13).

Moreover, most of the DEGs involved in phenylpropanoid pathway were also identified to be induced by *F. mosseae* inoculation under WD stress. Under WD stress, *F. mosseae* inoculation triggered the expression of twenty-eight *phenylalanine ammonia lyase 1* (*PAL*), thirteen *4-coumarate-CoA ligases* (*4CL*), a *Caffeoyl-CoA O-methyltransferase* (*CCoAMT*), eleven *caffeic acid 3-O methyltransferases* (*COMT*), eight *Cinnamyl alcohol dehydrogenases* (*CAD*), and four *cinnamoyl-CoA reductase 1* (*CCR1*), and repressed the expression of two *4-coumarate-CoA ligases* (*4CL*).

Among the genes mediating CW lignification and suberization, we found that *F. mosseae* induced the expression of nineteen *dirigent protein* encoding genes (*DIR*), sixteen *CASP-like proteins* encoding genes, two *Laccase* (*LAC*), eight *omega-hydroxypalmitate O-feruloyl transferase (HHT1*), and seventy-eight *peroxidases* (*POD*), as well as eighty-nine *germin-like proteins* (*GLP*), under WD stress and downregulated the expression of one *DIR,* three *CASP-like proteins,* two *LAC* and fifteen *POD* encoding genes. Likewise, under WD stress, twenty-one *GDSL esterase/lipase* (*GDSL*), five genes encoding *glycerol-3-phosphate acyl transferase RAM2/GPAT* were found to be up-regulated and only one *GDSL* gene was down-regulated by AMF inoculation for suberin and cutin metabolism-related genes. Moreover, a large number of *cytochromeP450* family (*CYP*) genes (60 out of 78) were found to be highly up-regulated by AMF inoculation under WD stress, including *CYP71A1*, *CYP71*, *CYP71B11, CYP71E1, CYP72A123, CYP72A15, CYP76C, CYP86B1, CYP87A3, CYP90B1, CYP704C1, CYP709H1, CYP711A1, CYP714C, CYP722, CYP81Q32.*

Moreover, our expression analyses also showed a significant change in the expression of sixteen *strigolactone synthesis-related* genes after AMF inoculation under WD stress. Among these DEGs, eight were up-regulated and two *strigolactone esterase* encoding genes which were repressed (Table S14). Considering protease activity, the expression of seventy *cysteine proteases*, four *metalloproteases,* thirty-one *aspartic proteases,* eighteen *Subtilisin-like* and forty-one *carboxypeptidase* encoding genes triggered by AMF inoculation under WD stress and repressed the expression of thirteen *cysteine proteases*, two *metalloproteases,* an *aspartic protease,* two *Subtilisin-like* and six *carboxypeptidase* encoding genes. In addition, inoculation with AMF induced the expression of various DEGs encoding *protease inhibitors* (62 out of 68) by AMF in WD conditions (Table S14).

Interestingly we found three genes *NIP* class of aquaporins encoding genes, including *TaNIP1-10* (TraesCS7B02G272700, TraesCS7D02G367800, TraesCS7A02G355000), three *inorganic phosphate transporter*s (*TRIae;Pht1;11*), one *potassium transporter 5* (*TaHAK5;* TraesCS3A02G446700), two *plasma membrane ATPase* (*TraesCS4B02G376400, TraesCS5A02G543300*), eight *ATP-binding cassette* (*ABCG*) transporters (Three DEGs encoding *ABCG38* (TraesCS3D02G284900, TraesCS3A02G285100, TraesCS3B02G319000), and five DEGs encoding *ABCG28* (ABC transporter G family member STR/STR2) (TraesCS5D02G211000, TraesCS5A02G205100, TraesCS5B02G203200, TraesCS3A02G253700, TraesCS3B02G285600), and three genes encoding for *GSTU1* (TraesCS5A02G374800, TssraesCS5B02G376800 and TraesCS5D02G384400), showed higher expression than the other genes with more than a 10-fold change (Table S15). Moreover, a large change was observed for a *probable proline transporter 2* (*TaPROT2*) (TRAESCS4A02G179500) whose expression was ten-fold (log2FC > 10) more inhibited after AMF inoculation under WD stress (Table S15).

Validation of RNA-seq data by qRT-PCR

To confirm the accuracy of data obtained by RNA-seq analysis, experimental validation by qRT-PCR was performed on five selected DEGs under WD stress, with comparable $\log_2$ fold change (Figure 5a-e). These DEGs were selected based on their putative functions involved in transport and synthesis of CW.

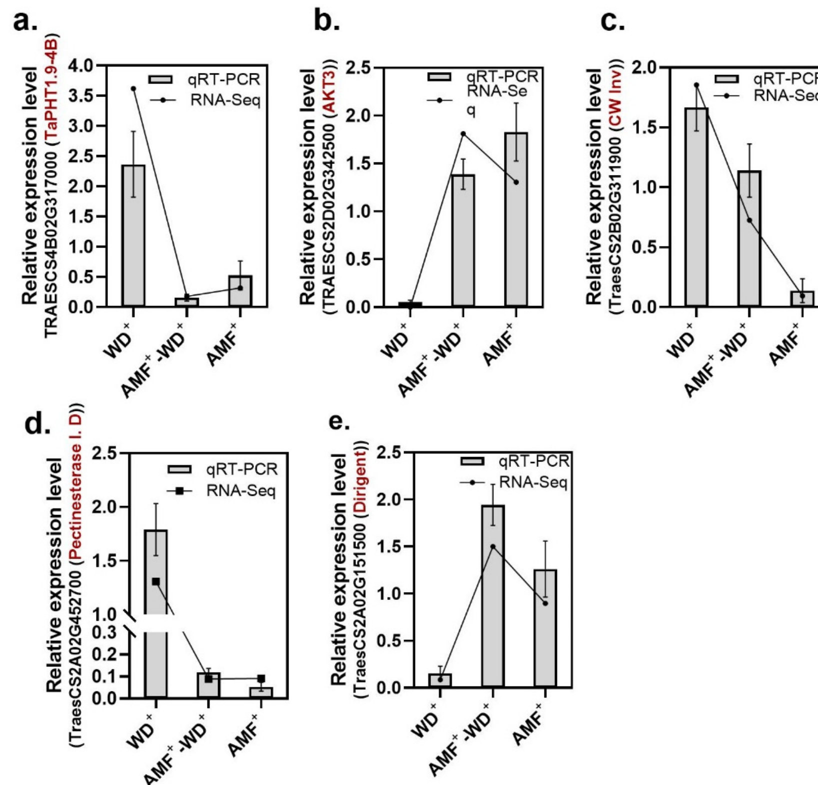

Figure 5. The verification of RNA-Seq sequencing data by the qRT-PCR assay. (a) *TaPHT1;9-D1*, (b) *AKT3*, (c) *CWINV1-RELATED,* (d) *Pectinestrase I. D*, (e) *Dirigent*

Discussion

This study provides robust evidence that *F. mosseae* enhances drought tolerance in wheat by triggering wide-ranging physiological and molecular responses. AMF inoculation improved water status and nutrient acquisition, and enhanced antioxidant enzyme activities, collectively contributing to drought resilience. The observed increase in insoluble sugars and phosphate in AMF-treated plants under WD suggests efficient carbon storage and mycorrhiza-mediated phosphorus uptake. Notably, AMF inoculation reduced the accumulation of proline and soluble sugars under drought stress, indicating improved water status and a decreased reliance on osmotic adjustment. These findings are in line with previous studies (Tang *et al*., 2022; Abdelaal *et al*., 2024), underscoring the potential of AMF as a sustainable approach to improve crop performance under water-limited conditions. These physiological traits, in conjunction with a large number of differentially expressed genes (DEGs), highlight the extensive impact of AMF on wheat stress responses.

RNA-Seq analysis revealed 11,380 DEGs across treatments, with significant reprogramming under both WD and AMF inoculation. While WD stress alone led to a pronounced downregulation of core metabolic genes, likely reflecting a conserved energy-saving response under stress, as reported by Chaichi *et al*. (2019), and Cao *et al*. (2022), AMF colonization effectively mitigated these effects by sustaining or even enhancing the expression of stress-responsive and resource acquisition pathways. This ability to preserve active metabolic and physiological functions under drought highlights the critical role of AMF in minimizing growth trade-offs. The observed transcriptional reprogramming encompassed networks related to osmotic regulation, water and nutrient transport, antioxidant defense, and CW remodeling, reflecting a coordinated AMF-mediated drought adaptation mechanism.

Transcriptomic analysis revealed that *F. mosseae* significantly modulated the expression of a wide array of transporter genes under WD stress, including *PHT*, *NPF*, *NRT*, *AMT*, *HAK/KUP/KT*, *PMH⁺-ATPase*, *Ca²⁺-ATPase*, and *aquaporins* (Figure 6). These changes reflect substantial reprogramming of the plant's nutritional strategies, particularly in phosphorus, nitrogen, potassium, and water uptake. AMF symbiosis upregulated mycorrhiza-associated phosphate transporters (*TaPHT1.8-D1*, *TaPHT1.11-A1/B1/D1*, *TaPT13–15*), while downregulating direct-uptake transporters (*TaPHT1.2*, *PHT1.9*, *PHT1.10*), indicating a functional shift toward fungal-mediated Pi uptake, consistent with previous studies (Smith and Read, 2011; Teng *et al*., 2017; Zhang *et al*., 2022). Similarly, AMF inoculation induced several low-affinity NPF-type nitrate transporters (e.g., *TaNPF1.1*, *TaNPF2.4*, *TaNPF4.10*), while repressing high-affinity *NRT2* transporters. Notably, *TaNPF4.10* showed exclusive expression in mycorrhizal roots, suggesting a mycorrhiza-specific nitrate uptake function. For ammonium uptake, *TaAMT2;3* and *TaAMT2;6* were strongly upregulated in AMF-inoculated roots; these transporters are known to facilitate nitrogen transfer from the fungus to the host plant (Loqué and von Wirén, 2004; Guether *et al*., 2009; Handa *et al*., 2015; Duan *et al*., 2016; Porras-Murillo *et al*., 2023). In contrast, the root-intrinsic *TaAMT1;2* was suppressed by AMF, further highlighting the role of mycorrhizal symbiosis in enabling fungal-derived NH_4^+ acquisition under drought stress (Loqué and von Wirén, 2004; Duan *et al*., 2016).

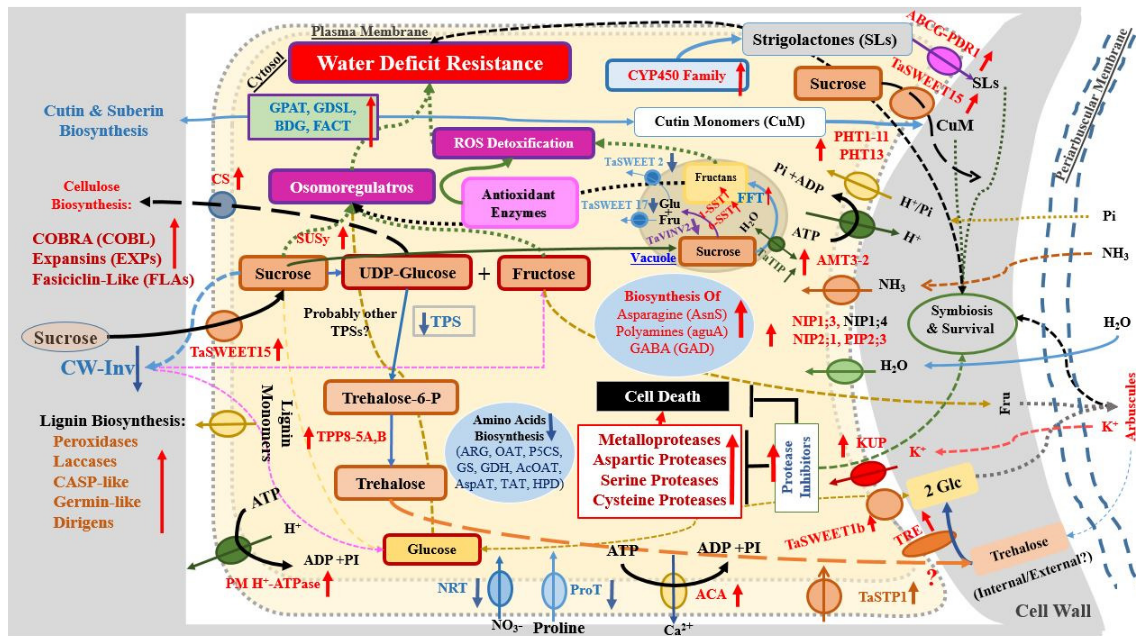

Figure 6. Proposed schematic model illustrating the molecular and physiological mechanisms by which F. *mosseae* enhances drought tolerance in wheat roots under WD stress

This integrative model summarizes transcriptomic responses in mycorrhizal wheat roots under water-deficit (WD) conditions. AMF symbiosis modulates multiple stress-responsive pathways, including enhanced nutrient transport (Pi, K^+, NH_3, H_2O), reinforced CW biosynthesis and remodeling (cellulose, lignin, suberin), regulation of antioxidant enzymes and osmoprotectants (trehalose, proline, polyamines, asparagine), and balanced proteolysis via coordinated induction of proteases and their inhibitors. Altered sugar metabolism and transport (*SWEETs*, *SuSy*, *CW-Inv*, *STPs*) support carbon redistribution toward symbiosis maintenance and stress resilience. Collectively, these changes contribute to improved cellular homeostasis, structural fortification, and metabolic flexibility under drought stress

Potassium transport also underwent clear reprogramming. While genes like *TaHAK5* were upregulated in non-inoculated WD-stressed roots, their expression was reduced in AMF-colonized roots. Instead, AMF specifically induced *TaHAK1*, *TaHAK5*, and inward-rectifying *TaAKT3*, while repressing outward-rectifying

TaSKOR and efflux-related *TaKAT1*. This indicates a shift from direct K^+ uptake to symbiotic pathways, promoting root K^+ retention and osmotic regulation (Wang *et al*., 2021). Additionally, increased expression of *PM H⁺-ATPases* and *Ca²⁺-ATPases* in mycorrhizal roots under WD suggests greater energy investment to support active ion uptake and transport at the symbiotic interface. These proton pumps facilitate electrochemical gradients necessary for nutrient acquisition and ionic homeostasis (Wang *et al*., 2014; Cheng *et al*., 2021). Overall, these transcriptional shifts reveal a coordinated AMF-driven strategy to optimize nutrient uptake and maintain cellular stability under drought conditions.

Drought stress commonly suppresses aquaporins (AQP) expression, disrupting water transport. However, *F. mosseae* inoculation reversed this pattern by upregulating multiple AQP subfamilies (PIPs, TIPs, NIPs), notably *TaTIP* isoforms, *TaPIP2*-13, and *TaNIP1-5/3-3*, This AQP induction suggests improved water and nutrient transport, contributing to AMF-mediated drought resilience (Maurel *et al*., 2015; Asadollahi *et al*., 2023).

Osmoprotectant accumulation, such as proline and soluble sugars, is a typical drought response. In non-mycorrhizal roots, drought induced strong upregulation of proline biosynthetic genes (e.g., *TaP5CS1*, *OAT*, *PROT2*), consistent with elevated proline levels (Yang *et al*., 2021). By contrast, AMF inoculation suppressed these genes under WD, suggesting improved hydration and nutrient balance reduced osmotic stress corroborated by biochemical assays and P5CS activity data. Transcriptome analysis revealed AMF-triggered reprogramming of sugar metabolism in wheat roots under drought, optimizing carbon partitioning to support symbiosis and stress adaptation. Trehalose cycling was particularly active; *TPP* and *TRE* were upregulated, enabling dynamic turnover of trehalose, while repression of class II *TPS* genes (e.g., *TaTPS6*, *TaTPS11*) may relieve T6P-mediated inhibition of SnRK1, facilitating energy-saving responses (Lunn *et al*., 2014; Kosar *et al*., 2019; Paul *et al*., 2020; Avidan *et al*., 2024). Sucrose metabolism was also modulated: downregulation of *TaCWINV2*, *TaVAC-INV*, and *TaSuSy5* alongside upregulation of *STPs* and *TaSuSy1* suggests a shift toward efficient sugar transport and minimal sucrose cleavage. This strategy ensures sugar availability for both host and AMF, while reducing metabolic costs under drought. At the symbiotic interface, CW invertases (cwINVs) hydrolyze sucrose into hexoses, which are subsequently imported via sugar transport proteins (STPs), a process essential for both AMF sustenance and host stress tolerance (Verbančič *et al*., 2018). Downstream, enzymes such as hexokinase, fructokinase, and UDP-glucose pyrophosphorylase (UGP) further metabolize these sugars into precursors required for structural reinforcement, CW biosynthesis, and osmoprotection, highlighting a tightly coordinated sugar economy under AMF symbiosis. AMF symbiosis modulated sugar transporter expression, with upregulation of *TaSWEET1b* and *TaSWEET15*, and repression of vacuolar-localized *TaSWEET2a* and *TaSWEET17*, indicating cytoplasmic sucrose retention and targeted allocation towards fungal arbuscules and host metabolism. These shifts likely promote both symbiotic efficiency and drought adaptation, consistent with previous reports of SWEET1-like induction in arbuscule-containing cells (Handa *et al*., 2015; An *et al*., 2019). In addition to sugars, AMF influenced nitrogen-rich osmolytes. Drought-stressed AMF-inoculated roots showed elevated expression of *ASNS* (asparagine synthetase), supporting nitrogen storage, osmoprotection, and energy balance. Asparagine accumulation has been linked to enhanced drought resilience in crops like sweet potato (Akin and Kaya, 2024; Yin *et al*., 2024).

AMF colonization also upregulated antioxidant enzymes and genes involved in GABA and polyamine metabolism (e.g., *GAD*, *aguA*), contributing to osmotic adjustment, redox regulation, and stomatal control. These responses reflect a coordinated modulation of carbon - nitrogen metabolism and stress preparedness. Exogenous GABA has been shown to reinforce antioxidant defenses and enhance drought tolerance in wheat (Zhao *et al*., 2023), while AMF-mediated GABA accumulation and *Put* degradation in maize similarly improved drought resilience (Hu and Chen, 2020). The combined induction of enzymatic and non-enzymatic defenses underscores the role of AMF in mitigating oxidative damage across diverse systems (Afshari *et al*., 2022; Chandrasekaran, 2022). Osmotic adjustment and oxidative stress management are essential for drought

tolerance, and AMF symbiosis markedly modulates these pathways in wheat roots. Drought-induced oxidative stress typically leads to ROS accumulation, which compromises cellular integrity unless scavenged by antioxidant systems (Miller *et al*., 2010). In our study, WD stress downregulated genes encoding key antioxidants, peroxidases, GSTs, TRXs, and CAT, suggesting metabolic suppression or post-transcriptional regulation. However, *F. mosseae* inoculation reversed this trend, significantly upregulating *POD*, *GRX*, *TRX*, *SOD*, *CAT*, and particularly *TaGSTU1* under drought. This transcriptional activation is consistent with reports in other AMF-host systems (e.g., trifoliate orange, apple), where symbiosis enhances ROS detoxification and drought resilience (Huang *et al*., 2020; He *et al*., 2020; Zhang *et al*., 2020).

F. mosseae significantly modulated CW biosynthesis and remodeling in drought-stressed wheat roots. AMF symbiosis induced genes associated with both wall stiffening (e.g., *CesA*, *Csl*, *COBRA-like*) and loosening (e.g., *XTH*, *expansin*, *PAE*, *BGLA*, *UGT*, *chitinase*), thereby facilitating structural reinforcement while sustaining root elongation under reduced turgor pressure. This dynamic balance between rigidity and flexibility reflects adaptive strategies previously reported in maize and wheat (Tenhaken, 2015; Gall *et al*., 2015; Li *et al*., 2019; Han *et al*., 2023).

AMF also activated the phenylpropanoid pathway, with strong upregulation of *PAL*, *4CL*, *COMT*, *CAD*, and *CCR*, facilitating lignin and flavonoid biosynthesis for antioxidant defense and CW fortification (Begum *et al*., 2021; Tang *et al*., 2022). Genes related to lignification (*DIR*, *LAC*, *POD*) and suberization (*GPAT*, *GDSL*, *HHT1*, *CYP86B1* and multiple *CYP450s*) were induced, promoting deposition of suberin lamellae and ferulate linkages (Kashyap *et al*., 2022). These modifications establish hydrophobic barriers that limit water loss and enhance pathogen resistance. Collectively, these transcriptomic responses suggest that *F. mosseae* symbiosis drives a coordinated architectural and biochemical reinforcement of wheat roots, optimizing growth, water retention, and stress resilience under drought.

Regulated proteolysis plays a key role in stress adaptation by degrading damaged proteins and recycling amino acids. In our study, AMF significantly upregulated various protease genes in wheat roots, including cysteine proteases (linked to PCD and storage mobilization), serine/aspartate proteases (involved in signaling and metabolism), and metalloproteases (associated with CW remodeling and peptide signaling) (Fanourakis *et al*., 2020; Sharma and Gayen, 2021). This coordinated induction may contribute to both elimination of misfolded proteins and remodeling of the extracellular matrix under drought. Interestingly, we also observed a strong activation of protease inhibitor genes (notably cystatins and serpins) in mycorrhizal roots, suggesting a tightly regulated balance to prevent excessive proteolysis, which could otherwise lead to cell loosening or uncontrolled cell death (Habib and Fazili, 2007; Moloi and Ngara, 2023). This finely tuned protease/inhibitor equilibrium appears crucial for preserving cellular integrity during water deficit while supporting mycorrhizal colonization by avoiding detrimental remodeling of root tissues.

Moreover, *F. mosseae* notably induced the expression of strigolactone biosynthesis genes under WD, many of which were specific to the mycorrhizal condition (Figure 6). Since strigolactones act as key regulators of root architecture, stomatal conductance, and AMF recruitment, their mycorrhiza-specific induction reflects a positive feedback loop: drought-stressed roots increase strigolactone levels to promote AMF colonization, which in turn further boosts strigolactone signaling (Ruiz-Lozano *et al*., 2016; Daszkowska-Golec *et al*., 2023).

Altogether, these findings indicate that AMF symbiosis reprograms both proteolytic activity and hormonal signaling, notably strigolactone pathways, to optimize stress adaptation and symbiotic development, thereby enhancing host drought resilience at both the structural and regulatory levels.

Conclusion

This study provides strong evidence that colonization by *F. mosseae* significantly improves drought resilience in wheat (*Triticum aestivum*) by reshaping physiological, biochemical, and transcriptomic responses. RNA-Seq analysis revealed that arbuscular mycorrhizal symbiosis regulates a wide array of drought-responsive genes associated with nutrient transport, osmolyte accumulation, antioxidant defense, sugar metabolism, and CW dynamics.

Notably, AMF enhanced nutrient uptake through the activation of mycorrhiza-specific transporters and improved water status by modulating aquaporin expression. It also contributed to osmotic adjustment by promoting alternative sugar allocation strategies. The symbiosis further strengthened CW structure via the upregulation of phenylpropanoid pathway genes and the stimulation of lignin and suberin biosynthesis. Simultaneously, the induction of both proteases and their inhibitors suggest a finely tuned balance of stress-responsive protein turnover. Importantly, AMF also modulated key hormonal signaling pathways, particularly strigolactone biosynthesis, which not only facilitates symbiosis establishment but also enhances plant adaptation to water deficit. Altogether, these findings underscore the crucial role of AMF in coordinating a multifaceted molecular and metabolic network that enhances wheat tolerance to drought. They also highlight the promise of AMF-based approaches as sustainable strategies to improve crop resilience under climate-related water limitations.

Authors' Contributions

Conceptualization, Data curation and Project administration: AI Alireza; Formal analysis: ME; Methodology: ED; Software: RA; Supervision: AI; Visualization: RA; Roles/Writing - original draft: IM; Writing - review & editing: AI.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdelaal K, Alaskar A, Hafez Y (2024). Effect of arbuscular mycorrhizal fungi on physiological, bio-chemical and yield characters of wheat plants (*Triticum aestivum* L.) under drought stress conditions. BMC Plant Biology 24(1):1119. *https://doi.org/10.1186/s12870-024-05824-9*

Acevedo M, Zurn JD, Molero G, Singh P, He X, Aoun M … McCandless L (2018). The role of wheat in global food security. In Nagothu US (Ed). Agricultural Development and Sustainable Intensification. Routledge, London pp 81-110.

Aebi H (1984). [13] Catalase in vitro. In: Packer L (Ed). Methods in Enzymology. Academic Press 105:121-126. *https://doi.org/10.1016/S0076-6879(84)05016-3*

Afshari M, Rahimmalek M, Sabzalian MR, Szumny A, Matkowski A, Jezierska Domaradzka A (2022). Mycorrhizal colonization modulates the essential oil profile and enzymatic and non-enzymatic antioxidants to mitigate the adverse effects of water deficit in *Salvia* subg. *Perovskia*. Biology 11(12):1757. *https://doi.org/10.3390/biology11121757*

Ahmad Z, Waraich EA, Akhtar S, Anjum S, Ahmad T, Mahboob W … Rizwan M (2018). Physiological responses of wheat to drought stress and its mitigation approaches. Acta Physiologiae Plantarum 40:80. *https://doi.org/10.1007/s11738-018-2651-6*

Akin S, Kaya C (2023). Asparagine and nitric oxide jointly enhance antioxidant capacity and nitrogen metabolism to improve drought resistance in cotton: Evidence from long term field trials. Food and Energy Security 13(2):e502. *https://doi.org/10.1002/fes3.502*

An J, Zeng T, Ji C, de Graaf S, Zheng Z, Xiao TT … Pan Z (2019). A *Medicago truncatula* SWEET transporter implicated in arbuscule maintenance during arbuscular mycorrhizal symbiosis. New Phytologist 224(1):396-408. *https://doi.org/10.1111/nph.15975*

Asadollahi M, Iranbakhsh A, Ahmadvand R, Ebadi M, Mehregan I (2023). Synergetic effect of water deficit and arbuscular mycorrhizal symbiosis on the expression of aquaporins in wheat (*Triticum* aestivum L.) roots: insights from NGS RNA-sequencing. Physiology and Molecular Biology of Plants 29(2):195-208. *https://doi.org/10.1007/s12298-023-01285-w*

Avidan O, Martins MCM, Feil R, Lohse M, Giorgi FM, Schlereth A … Stitt M (2024). Direct and indirect responses of the Arabidopsis transcriptome to an induced increase in trehalose 6-phosphate. Plant Physiology 196(1):409-431. *https://doi.org/10.1093/plphys/kiae196*

Bahadur A, Batool A, Nasir F, Jiang S, Mingsen Q, Zhang Q … Feng H (2019). Mechanistic insights into arbuscular mycorrhizal fungi mediated drought stress tolerance in plants. International Journal of Molecular Sciences 20(17):4199. *https://doi.org/10.3390/ijms20174199*

Bapela T, Shimelis H, Tsilo TJ, Mathew I (2022). Genetic improvement of wheat for drought tolerance: Progress, challenges and opportunities. Plants 11(10):1331. *https://doi.org/10.3390/plants11101331*

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. Plant and Soil 39(1):205-207. *https://doi.org/10.1007/BF00018060*

Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44(1):276-287. *https://doi.org/10.1016/0003-2697(71)90370-8*

Begum N, Akhtar K, Ahanger MA, Iqbal M, Wang P, Mustafa NS … Zhang L (2021). Arbuscular mycorrhizal fungi improve growth, essential oil, secondary metabolism, and yield of tobacco (*Nicotiana tabacum* L.) under drought stress conditions. Environmental Science and Pollution Research 28(33):45276-45295. *https://doi.org/10.1007/s11356-021-13755-3*

Bernardo L, Carletti P, Badeck FW, Rizza F, Morcia C, Ghizzoni R … Lucini L (2019). Metabolomic responses triggered by arbuscular mycorrhiza enhance tolerance to water stress in wheat cultivars. Plant Physiology and Biochemistry 137:203-212. *https://doi.org/10.1016/j.plaphy.2019.02.007*

Boorboori MR, Lackóová L (2025). Arbuscular mycorrhizal fungi and salinity stress mitigation in plants. Frontiers in Plant Science 15:1504970. *https://doi.org/10.3389/fpls.2024.1504970*

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2):248-254. *https://doi.org/10.1016/0003-2697(76)90527-3*

Cao X, Hu Y, Song J, Feng H, Wang J, Chen L … Qiao Z (2022). Transcriptome sequencing and metabolome analysis reveals the molecular mechanism of drought stress in millet. International Journal of Molecular Sciences 23(18):10792. *https://doi.org/10.3390/ijms231810792*

Chaichi M, Sanjarian F, Razavi K, Gonzalez Hernandez JL (2019). analysis of transcriptional responses in root tissue of bread wheat landrace (*Triticum aestivum* L.) reveals drought avoidance mechanisms under water scarcity. PLoS One 14(3):e0212671. *https://doi.org/10.1371/journal.pone.0212671*

Chance B, Maehly A (1955). Assay of catalases and peroxidases. Methods in Enzymology 2:764-775. *https://doi.org/10.1016/S0076-6879(55)02300-8*

Chandrasekaran M (2022). Arbuscular mycorrhizal fungi mediated alleviation of drought stress via non-enzymatic antioxidants: a meta-analysis. Plants 11(19):2448. *https://doi.org/10.3390/plants11192448*

Chen W, Ye T, Sun Q, Niu T, Zhang J (2021). Arbuscular mycorrhizal fungus alters root system architecture in *Camellia sinensis* L. as revealed by RNA-Seq analysis. Frontiers in Plant Science 12:777357. *https://doi.org/10.3389/fpls.2021.777357*

Cheng S, Zou YN, Kuča K, Hashem AF, Abd Allah EF … Wu QS (2021). Elucidating the mechanisms underlying enhanced drought tolerance in plants mediated by arbuscular mycorrhizal fungi. Frontiers in Microbiology 12:809473. *https://doi.org/10.3389/fmicb.2021.809473*

Daszkowska Golec A, Mehta D, Uhrig RG, Brąszewska A, Novak O, Fontana IM … Marzec M (2023). Multi-omics insights into the positive role of strigolactone perception in barley drought response. BMC Plant Biology 23(1):445. *https://doi.org/10.1186/s12870-023-04450-1*

Diagne N, Ngom M, Djighaly PI, Fall D, Hocher V … Svistoonoff S (2020). Roles of arbuscular mycorrhizal fungi on plant growth and performance: importance in biotic and abiotic stressed regulation. Diversity 12(10):370. *https://doi.org/10.3390/d12100370*

Dowarah B, Gill SS, Agarwala N (2022). Arbuscular mycorrhizal fungi in conferring tolerance to biotic stresses in plants. Journal of Plant Growth Regulation 41(4):1429-1444. *https://doi.org/10.1007/s00344-021-10392-5*

Duan J, Tian H, Gao Y (2016). expression of nitrogen transporter genes in roots of winter wheat (*Triticum aestivum* L.) in response to soil drought with contrasting nitrogen supplies. Crop and Pasture Science 67(2):128-136. *https://doi.org/10.1071/CP15152*

Fanourakis D, Nikoloudakis N, Pappi P, Markakis E, Doupis G, Charova SN … Tsaniklidis G (2020). The role of proteases in determining stomatal development and tuning pore aperture: a review. Plants 9(3):340. *https://doi.org/10.3390/plants9030340*

Gall HL, Philippe F, Domon JM, Gillet F, Pelloux J, Rayon C (2015). Cell wall metabolism in response to abiotic stress. Plants 4(1):112-166. *https://doi.org/10.3390/plants4010112*

Gao C, Courty PE, Varoquaux N, Cole B, Montoya L, Xu L … Taylor JW (2023). Successional adaptive strategies revealed by correlating arbuscular mycorrhizal fungal abundance with host plant gene expression. Molecular Ecology 32(10):2674-2687. *https://doi.org/10.1111/mec.16343*

Guether M, Neuhäuser B, Balestrini R, Dynowski M, Ludewig U … Bonfante P (2009). A mycorrhizal-specific ammonium transporter from Lotus japonicus acquires nitrogen released by arbuscular mycorrhizal fungi. Plant Physiology 150(1):73-83. *https://doi.org/10.1104/pp.109.136390*

Habib H, Fazili KM (2007). Plant protease inhibitors: A defense strategy in plants. Biotechnology and Molecular Biology Review 2(3):68-85.

Han J, Liu Y, Shen Y, Li W (2023). A surprising diversity of xyloglucan endotransglucosylase/hydrolase in wheat: new in sight to the roles in drought tolerance. International Journal of Molecular Sciences 24(12):9886. *https://doi.org/10.3390/ijms24129886*

Handa Y, Nishide H, Takeda N, Suzuki Y, Kawaguchi M, Saito K (2015). RNA-seq transcriptional profiling of an arbuscular mycorrhiza provides insights into regulated and coordinated gene expression in Lotus japonicus and Rhizophagus irregularis. Plant and Cell Physiology 56(8):1490-1511. *https://doi.org/10.1093/pcp/pcv071*

He JD, Zou YN, Wu QS, Kuča K (2020). Mycorrhizas enhance drought tolerance of trifoliate orange by enhancing activities and gene expression of antioxidant enzymes. Scientia Horticulturae 262:108745. *https://doi.org/10.1016/j.scienta.2019.108745*

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125(1):189-198. *https://doi.org/10.1016/0003-9861(68)90654-1*

Hu L, Xie Y, Fan S, Wang Z, Wang F, Zhang B … Kong L (2018). Comparative analysis of root transcriptome profiles between drought tolerant and susceptible wheat genotypes in response to water stress. Plant Science 272:276-293. *https://doi.org/10.1016/j.plantsci.2018.03.036*

Hu Y, Chen B (2020). Arbuscular mycorrhiza induced putrescine degradation into γ aminobutyric acid, malic acid accumulation, and improvement of nitrogen assimilation in roots of water stressed maize plants. Mycorrhiza 30(2-3):329-339. *https://doi.org/10.1007/s00572-020-00952-0*

Huang D, Ma M, Wang Q, Zhang M, Jing G, Li C … Ma F (2020). Arbuscular mycorrhizal fungi enhanced drought resistance in apple by regulating genes in the MAPK pathway. Plant Physiology and Biochemistry 149:245-255. *https://doi.org/10.1016/j.plaphy.2020.02.020*

Kashyap A, Jiménez Jiménez ÁL, Zhang W, Capellades M, Srinivasan S, Laromaine A … Coll NS (2022). Induced ligno-suberin vascular coating and tyramine-derived hydroxycinnamic acid amides restrict *Ralstonia solanacearum* colonization in resistant tomato. New Phytologist 234(4):1411-1429. *https://doi.org/10.1111/nph.17982*

Khalloufi M, Martínez-Andújar C, Karray-Bouraouib N, Pérez-Alfocea F … Albacete A (2024). The crosstalk interaction of ethylene, gibberellins, and arbuscular mycorrhiza improves growth in salinized tomato plants by modulating the hormonal balance. Journal of Plant Physiology 303:154336. *https://doi.org/10.1016/j.jplph.2024.154336*

Kim D, Langmead B, Salzberg SL (2015). HISAT: A fast spliced aligner with low memory requirements. Nature Methods 12(4):357-360. *https://doi.org/10.1038/nmeth.3317*

Kosar F, Akram NA, Sadiq M, Al-Qurainy F, Ashraf M (2019). Trehalose: A key organic osmolyte effectively involved in plant abiotic stress tolerance. Journal of Plant Growth Regulation 38(2):606-18. *https://doi.org/10.1007/s00344-018-9876-x*

Li P, Liu Y, Tan W, Chen J, Zhu M, Lv Y … Cai H (2019). Brittle Culm 1 encodes a COBRA-like protein involved in secondary cell wall cellulose biosynthesis in sorghum. Plant and Cell Physiology 60(4):788-801. *https://doi.org/10.1093/pcp/pcy246*

Loqué D, von Wirén N (2004). Regulatory levels for the transport of ammonium in plant roots. Journal of Experimental Botany 55(401):1293-1305. *https://doi.org/10.1093/jxb/erh147*

Lunn JE, Delorge I, Figueroa CM, Van Dijck P, Stitt M (2014). Trehalose metabolism in plants. The Plant Journal 79(4):544-567. *https://doi.org/10.1111/tpj.12509*

Ma Y, Li Y (2024). The research progress on genomic selection and breeding for drought resistance in wheat: from genetic analysis to smart breeding applications. Geographical Research Bulletin 3:625-646. *https://doi.org/10.50908/grb.3.0_625*

Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L (2015). Aquaporins in plants. Physiological Reviews 95(4):1321-58. *https://doi.org/10.1152/physrev.00008.2015*

McCready RM, Guggolz J, Silviera V, Owens HS (1950). Determination of starch and amylose in vegetables. Analytical Chemistry 22(9):1156-8. *https://doi.org/10.1021/ac60045a016*

Miller GA, Suzuki N, Ciftci-Yilmaz SU, Mittler RO (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell & Environment 33(4):453-67. *https://doi.org/10.1111/j.1365-3040.2009.02041.x*

Moloi SJ, Ngara R (2023). The roles of plant proteases and protease inhibitors in drought response: a review. Frontiers in Plant Science 14:1165845. *https://doi.org/10.3389/fpls.2023.1165845*

Murphy JAM, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36. *https://doi.org/10.1016/S0003-2670(00)88444-5*

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22(5):867-880. *https://doi.org/10.1093/oxfordjournals.pcp.a076140*

Nyaupane S, Poudel MR, Panthi B, Dhakal A, Paudel H, Bhandari R (2024). Drought stress effect, tolerance, and management in wheat - a review. Cogent Food and Agriculture 10(1):2296094. *https://doi.org/10.1080/23311932.2023.2296094*

Oguz MC, Aycan M, Oguz E, Poyraz I, Yildiz M (2022). Drought stress tolerance in plants: Interplay of molecular, biochemical and physiological responses in important development stages. Physiologia 2(4):180-197. *https://doi.org/10.3390/physiologia2040015*

Paul MJ, Watson A, Griffiths CA (2020). Linking fundamental science to crop improvement through understanding source and sink traits and their integration for yield enhancement. Journal of Experimental Botany 71(7):2270-2280. *https://doi.org/10.1093/jxb/erz480*

Porras-Murillo R, Zhao Y, Hu J, Ijato T, Retamal JP, Ludewig U, NeuhÃ¤user B (2023). The wheat AMT2 (AMmonium Transporter) family, possible functions in ammonium uptake and pathogenic/symbiotic interactions. Journal of Plant Nutrition and Soil Science 186(2):164-168. *https://doi.org/10.1002/jpln.202200362*

Puccio G, Ingraffia R, Mercati F, Amato G, Giambalvo D, Martinelli F, Frenda AS (2023). Transcriptome changes induced by Arbuscular mycorrhizal symbiosis in leaves of durum wheat (*Triticum durum* Desf.) promote higher salt tolerance. Scientific Reports 13(1):116. *https://doi.org/10.1038/s41598-022-26903-7*

Qasim M, Ahmed W, Safdar U, Dilshad R, Sajid HB, Noor H, Haq MIU (2022). Effect of drought stress on fertile tillers of wheat genotypes (*Triticum aestivum* L.). International Journal of Agricultural and Bioscience 11(3):172-180. *https://doi.org/10.47278/journal.ijab/2022.024*

Qin Y, Cai Q, Ling Y, Chen X, Xu J, Huang G, Liang S, Yuan X, Yang XM, Lu D, Wang X (2023). Arbuscular mycorrhizal fungi improve selenium uptake by modulating root transcriptome of rice (*Oryza sativa* L.). Frontiers in Plant Science 14:1242463. *https://doi.org/10.3389/fpls.2023.1242463*

Ran Z, Chen X, Li R, Duan W, Zhang Y, Fang L, Zhou J (2023). Transcriptomics and metabolomics reveal the changes induced by arbuscular mycorrhizal fungi in *Panax quinquefolius* L. Journal of the Science of Food and Agriculture 103(10):4919-4933. *https://doi.org/10.1002/jsfa.12563*

Rasool F, Khan MR, Schneider M, Uzair M, Aqeel M, Ajmal W, Naz AA (2022). Transcriptome unveiled the gene expression patterns of root architecture in drought-tolerant and sensitive wheat genotypes. Plant Physiology and Biochemistry 178:20-30. *https://doi.org/10.1016/j.plaphy.2022.02.025*

Riaz M, Kamran M, Fang Y, Wang Q, Cao H, Yang G … Wang X (2021). Arbuscular mycorrhizal fungi induced mitigation of heavy metal phytotoxicity in metal contaminated soils: a critical review. Journal of Hazardous Materials 402:123919. *https://doi.org/10.1016/j.jhazmat.2020.123919*

Romero Muñoz M, Gálvez A, Martínez Melgarejo PA, Piñero MC, del Amor FM, Albacete A … López Marín J (2022). The interaction between hydromulching and arbuscular mycorrhiza improves escarole growth and productivity by regulating nutrient uptake and hormonal balance. Plants 11(20):2795. *https://doi.org/10.3390/plants11202795*

Ruiz Lozano JM, Aroca R, Zamarreño ÁM, Molina S, Andreo Jiménez B, Porcel R … López Ráez JA (2016). Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant, Cell & Environment 39(2):441-452. *https://doi.org/10.1111/pce.12631*

Santander C, Aroca R, Ruiz Lozano JM, Olave J, Cartes P, Borie F … Cornejo P (2017). Arbuscular mycorrhiza effects on plant performance under osmotic stress. Mycorrhiza 27(7):639-657. *https://doi.org/10.1007/s00572-017-0784-x*

Sharma P, Gayen D (2021). Plant protease as regulator and signaling molecule for enhancing environmental stress tolerance. Plant Cell Reports 40(11):2081-2095. *https://doi.org/10.1007/s00299-021-02739-9*

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology 156(3):1050-1057. *https://doi.org/10.1104/pp.111.174581*

Tang H, Hassan MU, Feng L, Nawaz M, Shah AN, Qari SH … Miao J (2022). The critical role of arbuscular mycorrhizal fungi to improve drought tolerance and nitrogen use efficiency in crops. Frontiers in Plant Science 13:919166. *https://doi.org/10.3389/fpls.2022.919166*

Teng W, Zhao YY, Zhao XQ, He X, Ma WY, Deng Y … Tong YP (2017). Genome wide identification, characterization, and expression analysis of PHT1 phosphate transporters in wheat. Frontiers in Plant Science 8:543. *https://doi.org/10.3389/fpls.2017.00543*

Tenhaken R (2015). Cell wall remodeling under abiotic stress. Frontiers in Plant Science 5:771. *https://doi.org/10.3389/fpls.2014.00771*

Varshney RK, Barmukh R, Roorkiwal M, Qi Y, Kholova J, Tuberosa R … Siddique KH (2021). Breeding custom designed crops for improved drought adaptation. Advanced Genetics 2(3):e202100017. *https://doi.org/10.1002/ggn2.202100017*

Verbančič J, Lunn JE, Stitt M, Persson S (2018). Carbon supply and the regulation of cell wall synthesis. Molecular Plant 11(1):75-94. *https://doi.org/10.1016/j.molp.2017.10.004*

Wang E, Yu N, Bano SA, Liu C, Miller AJ, Cousins D … Schultze M (2014). A H^+-ATPase that energizes nutrient uptake during mycorrhizal symbioses in rice and *Medicago truncatula*. The Plant Cell 26(4):1818-1830. *https://doi.org/10.1105/tpc.113.120527*

Wang M, Wang Z, Guo M, Qu L … Biere A (2023). Effects of arbuscular mycorrhizal fungi on plant growth and herbivore infestation depend on availability of soil water and nutrients. Frontiers in Plant Science 14:1101932. *https://doi.org/10.3389/fpls.2023.1101932*

Wang Y, Chen YF, Wu WH (2021). Potassium and phosphorus transport and signaling in plants. Journal of Integrative Plant Biology 63(1):34-52. *https://doi.org/10.1111/jipb.13053*

Yanagi M (2024). Climate change impacts on wheat production: Reviewing challenges and adaptation strategies. Advances in Resources Research 4(1):89-107. *https://doi.org/10.50908/arr.4.1_89*

Yang X, Lu M, Wang Y, Wang Y, Liu Z, Chen S (2021). Response mechanism of plants to drought stress. Horticulturae 7(3):50. *https://doi.org/10.3390/horticulturae7030050*

Yemm EW, Willis AJ (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57(3):508-514. *https://doi.org/10.1042/bj0570508*

Yin Y, Qiao S, Kang Z, Luo F, Bian Q, Cao G, Yang Y (2024). Transcriptome and metabolome analyses reflect the molecular mechanism of drought tolerance in sweet potato. Plants 13(3):351. *https://doi.org/10.3390/plants13030351*

Yuan X, Li S, Chen J, Yu H, Yang T, Wang C … Ao X (2024). Impacts of global climate change on agricultural production: a comprehensive review. Agronomy 14(7):1360. *https://doi.org/10.3390/agronomy14071360*

Zhang F, Zou YN, Wu QS, Kuča K (2020). Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. Environmental and Experimental Botany 171:103926. *https://doi.org/10.1016/j.envexpbot.2019.103926*

Zhang Q, Yang W, Wang M, Chen J, Zhang Z, Wei Y … Gong M (2025). Transcriptome analysis reveals the molecular mechanisms for mycorrhiza enhanced drought tolerance in maize by regulating the Ca2+ signaling pathway. Journal of Fungi 11(5):375. *https://doi.org/10.3390/jof11050375*

Zhang X, Li C, Lu W, Wang X, Ma B, Fu K … Li C (2022). Comparative analysis of combined phosphorus and drought stress responses in two winter wheat. PeerJ 10:e13887. *https://doi.org/10.7717/peerj.13887*

Zhao Q, Ma Y, Huang X, Song L, Li N, Qiao M … Cheng Y (2023). GABA application enhances drought stress tolerance in wheat seedlings (*Triticum aestivum L*.). Plants 12(13):2495. *https://doi.org/10.3390/plants12132495*

Zong J, Zhang Z, Huang P … Yang Y (2023). Arbuscular mycorrhizal fungi alleviate salt stress in *Xanthoceras sorbifolium* through improved osmotic tolerance, antioxidant activity, and photosynthesis. Frontiers in Microbiology 14:1138771. *https://doi.org/10.3389/fmicb.2023.1138771*

Zou YN, Wu QS, Kuča K (2021). Unravelling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress. Plant Biology 23:50-57. *https://doi.org/10.1111/plb.13161*

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