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Symbiosis Under Stress:

How Arbuscular Mycorrhizal Fungi and Sorghum Metabolism Shift Under Drought

July 2025

Alina Vulcan

U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Alina Vulcan

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Biological Sciences Division, Earth and Biological Sciences Division

ABSTRACT

As drought becomes more prevalent across the globe, causing billions of dollars in agricultural loss, the need to maintain crop health and productivity grows increasingly important. Out of the most important cereal crops, sorghum shows the greatest drought tolerance, and plant-microbiome interactions at the root region play a crucial role in this. A key microbial player is arbuscular mycorrhizal fungi (AMF), which deliver water and nutrients to plants in exchange for nutrients they cannot produce. Using sorghum as a model plant, we combine mass-spectrometry based proteomics and metabolomics to examine metabolic interactions between sorghum roots and AMF under drought stress. With AMF, sorghum downregulated lipid-related biological processes involving fatty acid biosynthesis and metabolism. Similarly, amino acid biosynthesis pathways were also suppressed; however, metabolite analysis revealed increased amino acid abundance related to the phenylpropanoid pathway. AMF also enhanced the upregulation of drought-protective osmolytes, such as mannitol and sorbitol, suggesting their key role in mediating sorghum's response to drought stress. AMF hyphal biomass also had an increased abundance of key osmoprotectant amino acids, indicating similar mechanisms of drought tolerance between sorghum and AMF. Metabolomic evidence also suggested that carbohydrate exchange between sorghum and AMF shifted under stress, indicating an altered exudation pattern likely driven by drought response.

Our results demonstrate the molecular mechanisms through which AMF modulate sorghum metabolism under drought conditions, highlighting their promising role in improving crop resilience. By identifying the molecular targets that can improve drought tolerance, we can begin engineering drought resistant agricultural biosystems.

I. INTRODUCTION

Drought is one of the most severe environmental stressors that threaten global agricultural productivity and food security. All regions of the United States have faced a negative impact on crop yield to due to drought, with the "breadbasket" of the country, the Midwest, facing the highest reduction over the past decade (Kuwayama et al., 2019). Among staple cereal crops, Sorghum (*Sorghum bicolor*) is one of the most drought and heat tolerant. The U.S. is the lead producer of sorghum, where it is primarily grown as livestock forage, but is also grown as food in semi-arid and arid regions.

To avoid water loss, plants reduce transpiration by closing their stomata, resulting in less photosynthesis and an overall plant decline (Avramova et al., 2016). This is in part because of high accumulation of reactive oxygen species (ROS) that damage the structure and function of plant cells (Avramova et al., 2016). In response, sorghum raises the expression of compounds related to ROS metabolism (Abreha et al., 2022), and increases production of osmolytes, molecules that help maintain osmotic pressure, like proline, polyols, and carbohydrates (Ghosh et al., 2021).Despite its drought tolerance, sorghum still exhibits growth trade-offs under prolonged stress, resulting in reduced harvest yield (Mutava et al., 2011).

The rhizosphere, a narrow zone of soil surrounding the root of plants, plays a critical role in maintaining plant health, as that is where root secretions and associated soil microorganisms communicate. Arbuscular mycorrhizal fungi (AMF) are a widespread species of symbiotic fungi that form associations with plant roots. AMF provide key nutrients, including phosphorous and nitrogen to their host plants (Leake et al., 2004). In return, the AMF receives photosynthetically fixed carbon (Jakobsen & Rosendahl, 1990). AMF mediated growth provides a myriad of benefits, including: improvement of soil structure (Alqarawi et al., 2014; Navarro et al., 2014) , improvement of photosynthetic efficiency (Mathur et al., 2019), and increase of osmolytes and secondary, protective metabolites (Tang et al., 2022; Wahab et al., 2023). Despite this, the functional dynamics of AMF and sorghum under drought conditions remain poorly understood, especially in relation to metabolic exchanges.

In this study, we investigate how AMF (*Rhizophagus irregularis*) and sorghum root-microbe metabolic interactions shift under drought. Through untargeted metabolomics and proteomics in AMF-colonized sorghum roots, exudates, and fungal hyphal biomass under drought and control conditions, we aim to understand the key metabolic pathways involved in AMF-assisted drought resistance.

II. METHODS

A. Data Collection and Processing

Before my involvement, the Sorghum sudangrass (*Sorghum bicolor*) was grown with AMF (*Rhizophagus irregularis*) under drought and control conditions. The Sorghum root

exudates (metabolomics), roots (proteomics) and fungal hyphae (metabolomics, proteomics) were then harvested and analyzed using liquid chromatography-tandem mass spectrometry [LC-MS/MS] for untargeted metabolomics and proteomics. Lists of statistically significant metabolites and proteins were generated for the following comparisons and provided to me: (1) root exudates drought [+AMF] vs control [+AMF], (2) root exudates drought [+AMF] vs drought [-AMF], (3) root exudates [-AMF] drought vs [-AMF] control, (4) hyphal biomass drought vs control.

My work focused on biological interpretation through literature surveys and pathway enrichment analyses as described below.

B. Data Analysis

After the data was processed and classified, I began organizing and analyzing the results of the proteomics and metabolomics. For the proteomics, I used ShinyGO (Ge et al., 2020), a graphical gene-set enrichment tool, to analyze enrichment trends. Significant proteins ($p < 0.05$) upregulated and downregulated under treatment conditions were ran against background genes (all genes collected in the data set) with a false discover rate cut-off of 0.05. These were also mapped to the STRING database, the Search Tool for the Retrieval of Interacting Genes/Proteins, which can create comparison tables for gene ontology (GO) biological processes, GO cellular components, and GO molecular functions.

Significant metabolites ($p < 0.05$) under treatment were analyzed using RStudio (*Posit Team, RStudio: Integrated Development Environment for R*, 2025) and Microsoft Excel and were organized by fold change in the previously mentioned four data sets. I then analyzed the three root exudate pairwise comparisons, looking for similar trends and individual molecules, as well as the hyphal biomass pair-wise sets comparisons.

III. RESULTS & DISCUSSION

A. Root Exudate Metabolomics

1. Increased Exudation of Amino Acids

Metabolomics revealed that AMF significantly enhanced the drought-induced accumulation of two key amino acids in the sorghum root exudates: 3,4-Dihydroxy-L-phenylalanine (L-DOPA) and L-Arginine. Under drought stress without AMF, L-DOPA and L-Arginine showed only modest abundance (L-DOPA FC = 0.90, $p < 0.005$; L-Arginine FC = 1.73, $p < 0.001$), indicating that abundance is partially drought driven. Under drought, AMF-colonized plants show significantly higher levels compared to non-colonized controls (L-DOPA, fold change = 1.13, $p < 0.001$; L-Arginine, fold change = 1.74, $p < 0.001$), indicating that AMF enhances the abundance. Lastly, in AMF plants under drought compared to AMF controls, both were significantly higher (L-DOPA Fold change = 2.23, $p < 0.001$; L-Arginine Fold Change =

2.17, $p < 0.001$). Together these results suggest that AMF colonization amplifies drought-induced biosynthesis or retention of L-DOPA and L-Arginine, beyond the effect of drought alone.

Compared to all other abundant amino acids, L-DOPA and L-Arginine exhibited the strongest AMF-mediated abundance under drought.

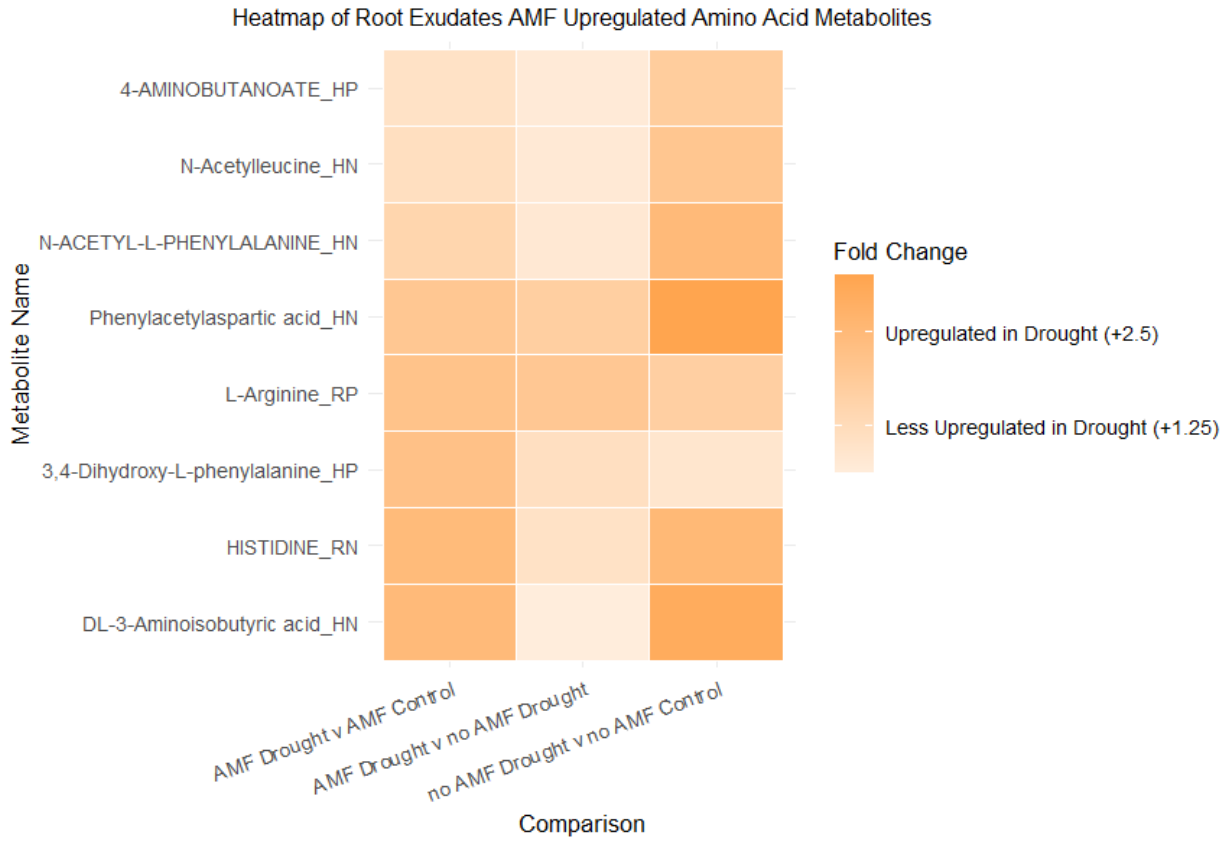

Figure 1. Heatmap comparing abundant amino acid root exudates in (1) root exudates drought [+AMF] vs control [+AMF], (2) root exudates drought [+AMF] vs drought [-AMF], (3) root exudates [-AMF] drought vs [-AMF] control. Each row represents an individual metabolite, with the shade of orange indicating the direction and magnitude of change: darker shades represent more abundance (maximum +3), while lighter shades represent less abundance (minimum +1). Metabolite names are annotated with suffixes indicating experimental analysis method (e.g., _HP, _RN, _RP). All metabolites, except L-Arginine and 3,4-Dihydroxy-L-phenylalanine (L-DOPA), are more abundant without the presence of AMF.

L-DOPA, a non-protein amino acid generated via hydroxylation of tyrosine, is a major part of the phenylpropanoid pathway. The phenylpropanoid pathway metabolites play significant roles in signaling, which is important for plant development and defense (Dixon et al., 2002). In soybeans, L-DOPA application has been shown to significantly impact phenylpropanoid pathways, inhibiting root growth, increasing the concentrations of phenylalanine and tyrosine, and providing precursors for the polymerization of lignin (Soares et al., 2012). Furthermore, L-DOPA reduces root length, shoot length, and fresh weight in weed species while staple crops like wheat and barley remained unaffected, indicating species-specific responses (Topal &

Kocaçalışkan, 2006) There is also evidence that L-DOPA acts as an antioxidant in soybean roots, reducing ROS (Soares et al., 2011). However, L-DOPA is also vital to the biosynthesis of melanin, which is initiated by a cascade of L-DOPA auto-oxidation, causing cell oxidative damage (Soares et al., 2014). Nevertheless, there is no evidence of melanin production in sorghum, and so it seems more likely that L-DOPA plays an antioxidant role. Furthermore, AMF may enhance L-DOPA content in plants. In bean pods of AMF-treated faba beans, there was a substantial increase in L-DOPA levels compared to the controls (Yilmaz, 2025). Together, these findings suggest that AMF driven upregulation of L-DOPA under drought stress may have both an antioxidant and root inhibition effect.

L-Arginine also plays a pivotal role in enhancing plant stress tolerance. When applied exogenously to corn seedlings, it reduces the accumulation of ROS and mitigates the inhibition of photosynthesis (Sun et al., 2023). Similarly, in wheat, barley, and sunflower seedlings L-arginine improves overall plant growth under drought and salt stress (Hussein et al., 2022; Nejadalimoradi et al., 2014; Shalaby et al., 2018). Increased L-arginine production is also linked to AMF mediated drought mitigation in trifoliate orange (Zou et al., 2021). These findings highlight the potential of L-arginine as a key molecule for improving plant drought resilience.

2. Increased Exudation of Carbohydrates

Metabolomics revealed that AMF significantly enhanced the drought-induced accumulation of three key carbohydrates: sorbitol, mannitol, and galactitol (dulcitol)

Under drought stress without AMF, sorbitol levels remain unchanged compared to the control ($p > 0.5$), indicating that drought alone does not drive its abundance. However, drought-stressed plants colonized by AMF show significantly higher levels of sorbitol compared to non-colonized plants ($FC = 1.64$, $p < 0.05$), indicating that AMF enhances sorbitol accumulation under drought conditions. Furthermore, in AMF-colonized plants, sorbitol levels are higher under drought compared to AMF control conditions ($FC = 2.76$, $p < 0.001$), highlighting the combined effect of AMF and drought in driving sorbitol abundance. These findings suggest that AMF colonization induces the production or retention of sorbitol under drought conditions.

Under drought stress without AMF, mannitol and galactitol show some abundance compared to the control (mannitol $FC = 1.05$, $p < 0.05$; galactitol $FC = 1.14$, $p < 0.05$), suggesting a limited response to drought alone. In contrast, drought-stressed plants colonized by AMF exhibit significantly higher levels of both compared to non-colonized plants (mannitol $FC = 2.08$, $p < 0.001$; galactitol $FC = 2.17$, $p < 0.001$), indicating that AMF enhances the abundances under drought. Additionally, AMF-colonized plants under drought increased mannitol and galactitol when compared to AMF control conditions (mannitol $FC = 2.39$, $p < 0.001$; galactitol $FC = 2.69$, $p < 0.001$), demonstrating that AMF and drought drive accumulation. These results suggest that AMF colonization amplifies the drought-induced abundance of mannitol and galactitol.

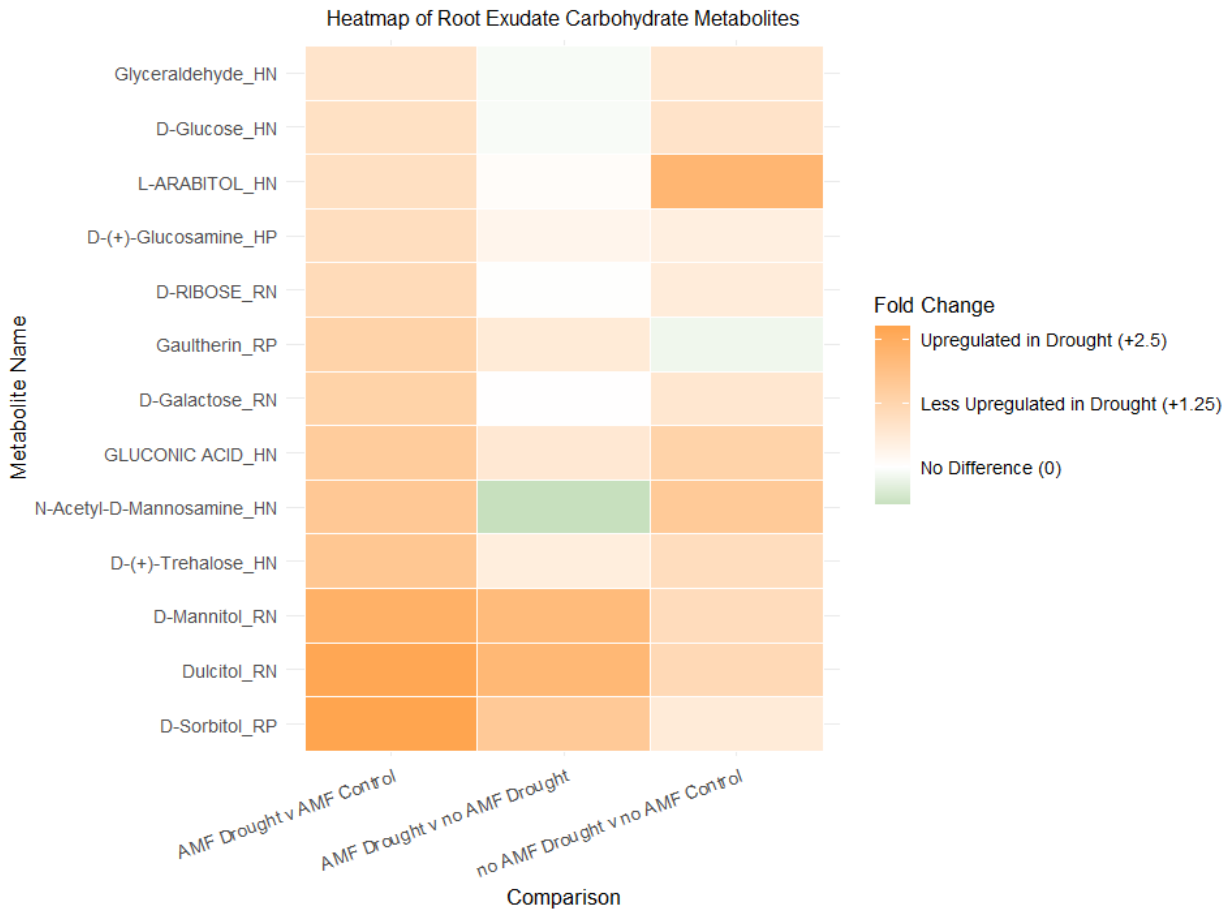

Figure 2. Heatmap comparing abundant carbohydrate root exudates in (1) root exudates drought [+AMF] vs control [+AMF], (2) root exudates drought [+AMF] vs drought [-AMF], (3) root exudates [-AMF] drought vs [-AMF] control. Each row represents an individual metabolite, with colors indicating the direction and magnitude of change: orange shades represent abundance (maximum +3), green shades represent more abundance in the control (minimum -0.8), and white indicates no change (0). Metabolite names are annotated with suffixes indicating experimental analysis method (e.g., _HP, _RN, _RP). Mannitol, dulcitol (galactitol), and sorbitol are more abundant than the other carbohydrates and show AMF-driven increases.

Mannitol and sorbitol, two polyol-type sugar alcohols, were more abundant in comparison to all other carbohydrates under drought stress. Their accumulation is a known stress response across multiple species, particularly under drought-induced osmotic stress, where they function as both osmolytes and antioxidants (Meena et al., 2015). Interestingly, despite their role in receiving photosynthetically fixed carbon, AMF are unable to metabolize mannitol directly, relying instead on glucose and fructose (Pfeffer et al., 1999; Solaimanand & Saito, 1997). As shown in figure 2, glucose levels under drought are not markedly higher in AMF-colonized plants compared to non-colonized ones, suggesting that AMF have limited influence on its exudation despite preferentially utilizing it. These findings point to a host-regulated stress defense mechanism in which AMF colonization may stimulate greater polyol accumulation, enhancing osmotic regulation and ROS detoxification.

In contrast, galactitol, a sugar alcohol derived from galactose, has not been associated with osmoprotection and may instead be an indicator of rhizosphere microbiome shifts under stress conditions. In diseased watermelon systems, galactitol is one of the five key metabolites shown to drive deterministic assembly of rhizosphere microbiota, specifically promoting the enrichment of microbial groups associated with small-molecule sugar and acid metabolism, while simultaneously suppressing autotoxin-degrading taxa (Wen et al., 2022). In the context of sorghum, the drought-induced, AMF enhanced accumulation of galactitol may similarly shift the composition of rhizosphere microbes. However, further research is needed to determine whether galactitol directly influences microbial recruitment or plays a functional role in plant stress signaling and tolerance.

B. Root Proteomics

Pathway analysis revealed that serine protease inhibitors, including members of the potato inhibitor I family, were significantly upregulated in sorghum roots colonized by AMF under drought stress when compared to the control (see figure 3). This accumulation of inhibitors suggests a protective mechanism that may stabilize stress-response proteins by limiting their degradation. This is consistent with patterns observed in drought-tolerant sorghum varieties, which have been shown to upregulate protease inhibitors as part of their adaptive response to abiotic stress (Goche et al., 2020). The retention of functional proteins under drought could help maintain essential metabolic functions and enhance plant survival.

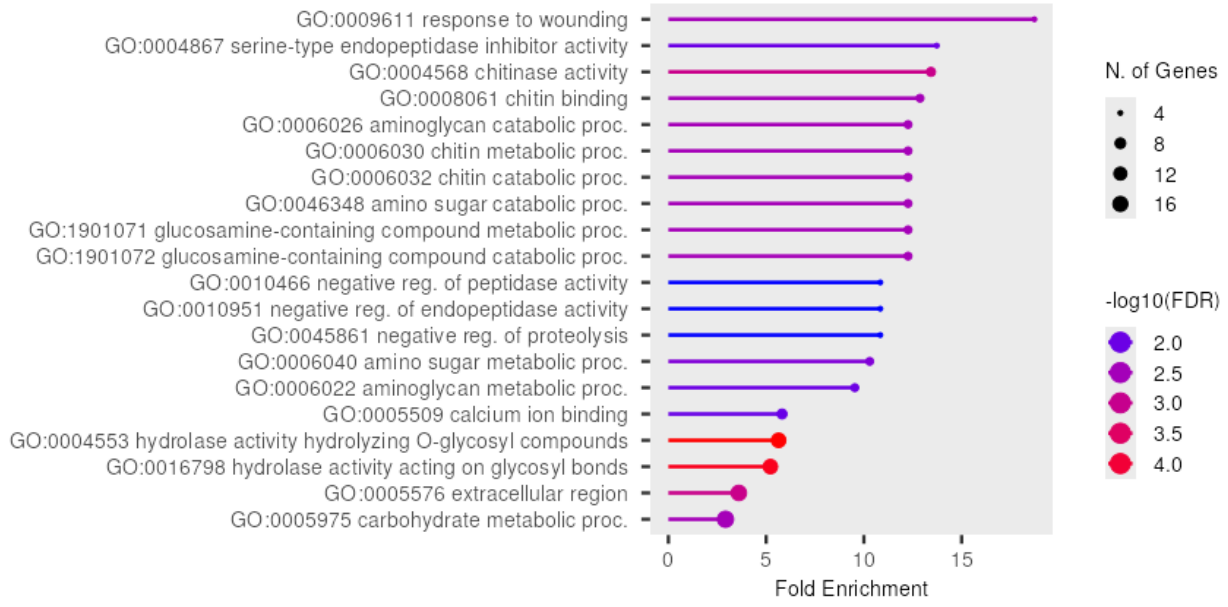

Figure 3. Gene ontology (GO) enrichment analysis of upregulated proteins under drought stress in arbuscular mycorrhizal fungi (AMF)-colonized roots. The plot displays the top enriched GO terms, ranked by fold enrichment. Dot size represents the number of genes associated with each term, while color change indicates statistical significant as measured by -$\log_{10}$(false discover rate). Highly enriched processes include response to wounding, serine-type endopeptidase inhibitor activity, and chitin metabolism, highlighting shifts in cell wall remodeling, stress signaling, and metabolic adaptation.

In contrast, sorghum downregulates lipid-related processes, such as fatty acid biosynthesis and metabolism, and cellular lipid metabolic processes, as see in figure 4. Drought stress is known to cause changes in plant lipid metabolism, overall decreasing the amounts of total lipids while fatty acid composition varied depending on stressors (Sharma et al., 2023). AMF are not able to synthesize fatty acids de novo and instead obtain it from their host plants. The plants may be allocating more fatty acids internally to support the symbiosis, further reducing the amount available for exudation, but the mechanism of transport is currently unknown (Kameoka & Gutjahr, 2022).

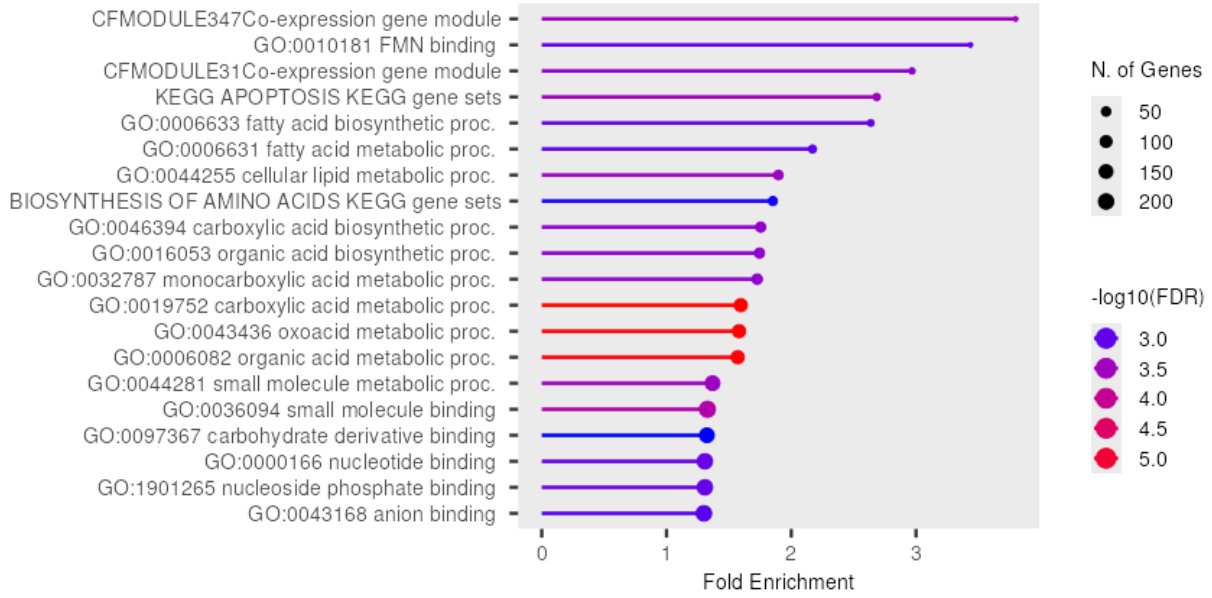

Figure 4 Gene ontology (GO) enrichment analysis of downregulated proteins under drought stress in arbuscular mycorrhizal fungi (AMF)-colonized roots. The plot displays the top enriched GO terms, ranked by fold enrichment. Dot size represents the number of genes associated with each term, while color change indicates statistical significant as measured by -$\log_{10}$(false discover rate). Highly downregulated processes include fatty acid biosynthetic metabolic processes and cellular lipid metabolic processes, highlighting shifts in lipid utilization.

C. Hyphal Biomass: Proteomics & Metabolomics

1. Increased Abundance of Amino Acids

Under drought conditions, amino acid biosynthesis and metabolism was upregulated in AM fungal hyphae, with the top four out of five enriched pathways involving that (figure 5). This upregulation is reflected in metabolomics as well, with certain amino acids increasing in abundance. These abundant amino acids include 3,5-Dihydroxyphenylglycine (DHPG, FC = 3.666, $p < 0.05$), L-arginine (FC = 3.20, $p < 0.05$), tryptophan (FC = 2.17, $p < 0.05$), histidine (FC = 1.87, $P < 0.005$), and proline (FC = 1.519, $p < 0.005$).

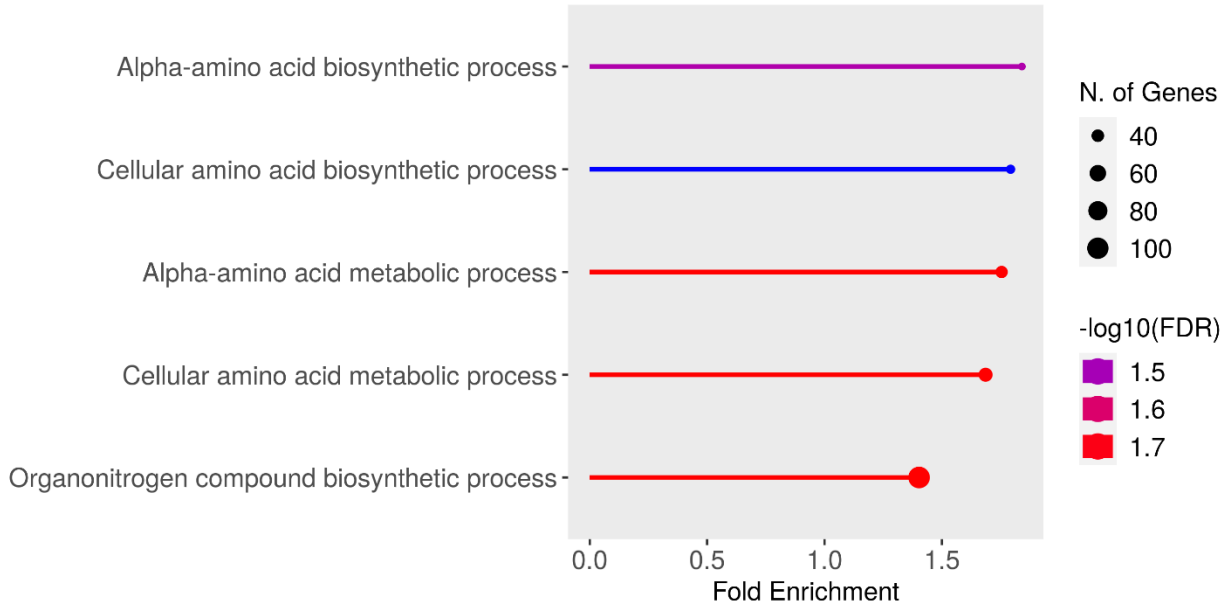

Figure 5. Gene ontology (GO) enrichment analysis of upregulated proteins under drought stress in arbuscular mycorrhizal fungi (AMF) hyphal biomass. The plot displays the top enriched GO terms, ranked by fold enrichment. Dot size represents the number of genes associated with each term, while color change indicates statistical significant as measured by $-\log_{10}$(false discover rate). Highly enriched processes include various biosynthetic and metabolic processes related to amino acids and organonitrogen compounds.

While there is no direct evidence that DHPG is biosynthesized by fungi, bacterial gene sequence analysis identified a predicted DHPG polyketide synthase sharing 20-30% with plant chalcone synthases (Pfeifer et al., 2001). Chalcone synthases are key enzymes in the phenylpropanoid pathway involved in antioxidant production (Dao et al., 2011). Furthermore, phenolic compounds, such as DHPG, are generally increased under drought conditions and are associated with increased tolerance (Mohagheghian et al., 2025). Altogether, these suggest a functional similarity, raising the possibility that DHPG contributes to oxidative stress mitigation. Whether produced by the fungi or resulting from rhizosphere-microbe metabolic interplay, its accumulation points to a possible role in drought adaptation.

L-arginine serves as a major nitrogen storage and transport molecule for AMF. In alfalfa and tea plants, L-arginine increases resulted in better nitrogen uptake by the plants (Wang et al., 2025; Wu et al., 2024). This suggests that the AMF is increasing its supply of nutrients to the plant, even under drought stress. Tryptophan is a precursor for indole-3-acetic acid (IAA), a plant hormone that regulates root growth and architecture. AMF are known to elevate auxin levels in colonized plant roots, promoting lateral root development, which is the preferred site for fungal colonization (Fu et al., 2015). The buildup of tryptophan suggests a possible role in modulating auxin-related pathways, either through direct transfer to the plant or localized auxin synthesis, enhancing fungal development and root system architecture under stress conditions.

Histidine and proline are both known to be upregulated under stress conditions (Whiteside et al., 2012). Histidine plays a role as a signaling molecule (Mongès et al., 2023), so

the increase could reflect upregulated biosynthesis to support stress-related signaling. The fungal proteomics also reflects an upregulation of histidine phosphatases, suggesting enhanced activity of histidine-dependent pathways. Conversely, proline is a known and well-studied osmolyte that has roles in mitigating water stress, balancing turgor pressure, preventing protein aggregation, and reducing oxidative stress (Liang et al., 2013). Its abundance under drought stress suggests that it plays a vital role in maintaining cellular functions for the AMF, much like it does in plants.

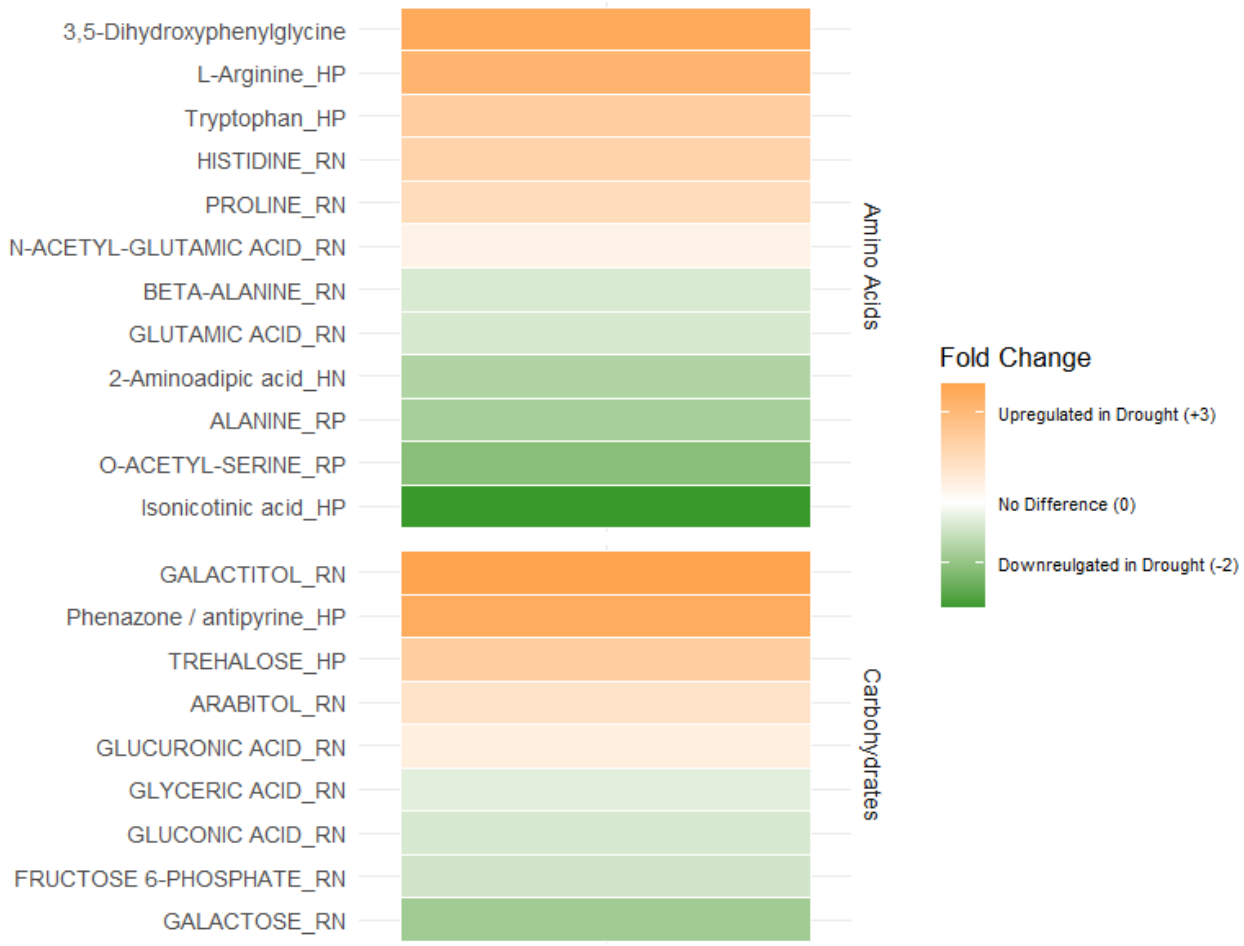

Figure 6. Heatmap showing abundance of amino acid (top) and carbohydrate (bottom) in hyphal biomass. Each row represents an individual metabolite, with colors indicating the direction and magnitude of change: orange shades represent abundance in drought(maximum +3), green shades represent more abundance in control (minimum -2), and white indicates no change (0). Metabolite names are annotated with suffixes indicating experimental analysis method (e.g., _HP, _RN, _RP). Notably, several amino acids such as 3,5-dihydroxyphenylglycine (DHPG) and L-arginine are upregulated, Similarly, drought stress alters levels of specific carbohydrates, with galactitol and trehalose showing high abundance.

2. Changing Abundances of Carbohydrates

Trehalose (FC = 2.12, $p < 0.05$) and galactitol (FC = 3.93, $p < 0.01$) were both abundant under drought conditions in the hyphal biomass. Trehalose, a nonreducing disaccharide consisting of two molecule of glucose, is a common reserve carbohydrate in fungi, but it also

plays an important role as a protectant against abiotic stress (Ocón et al., 2007; Zou et al., 2024). The abundance of trehalose demonstrates that the AMF is still receiving carbon stores from the plant and may similarly reflect a protective role, potentially contributing to osmoprotection, membrane stabilization, and recovery processes during or following water stress.

Despite glucose being the preferred source of carbon for AMF (Solaimanand & Saito, 1997), there is metabolomic evidence that the AMF is also utilizing galactose. Although galactose is not abundant under drought (FC = -1.77, $p < 0.001$), the byproduct, galactitol is increased. As characterized in the filamentous fungi, *Aspergillus nidulans*, fungi are able to utilize and take up galactose (Mojzita et al., 2012), suggesting that the AMF are shifting their preferred carbon source under drought. The sorghum may be altering their carbohydrate exudation patterns in response to stress, releasing more galactose or related sugars into the rhizosphere, which the fungi then preferentially assimilate. This shift could reflect a coordinated metabolic adjustment between the plant and the fungus, where carbon allocation is redirected toward stress-adapted pathways, enhancing the survival and functionality of both partners under drought conditions.

IV. CONCLUSION

Our results indicate that AMF association in sorghum lead to downregulation of lipid-related processes, particularly those involved in fatty acid biosynthesis and metabolism. Amino acid biosynthetic pathways were also suppressed; however, metabolomics revealed an accumulation of amino acids linked to the phenylpropanoid pathway, suggesting their key role under drought. AMF presence further promoted the accumulation of drought-protective carbohydrate osmolytes in sorghum. Additionally, key osmoprotectant amino acids and carbohydrates were more abundant in AMF hyphal biomass, pointing to shared drought adaptation strategies between the host and the symbiont. Metabolomic data also indicated a shift in carbohydrate exchange dynamics between sorghum and AMF under drought conditions, suggesting stress-induced changes in root exudation patterns.

These findings suggest that plant-fungal co-metabolism and AMF colonization enhances specific plant responses to drought.

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