Enhancing Wheat Drought Tolerance Through Mycorrhizal Symbiosis: A Sustainable Approach to Mitigating Water Stress

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***Abstract*—The output of wheat (*Triticum aestivum* L.) is seriously threatened by drought stress, a problem that is made worse by climate change. In specifically, *Glomus fasciculatus* and *Glomus mosseae* are two vesicular-arbuscular mycorrhizal (VAM) fungi that have shown promise as biological agents for enhancing drought tolerance in wheat. The purpose of this study was to assess how VAM fungus might improve wheat's resistance to drought in different soil moisture levels. *G. fasciculatus* and *G. mosseae*-inoculated wheat plants were evaluated in both drought-stressed and well-watered environments using a randomized full block design. In comparison to non-mycorrhizal controls, mycorrhizal plants showed better water retention and osmotic adjustment under drought conditions. The results showed that mycorrhizal inoculation significantly improved root colonization, biomass accumulation, stomatal conductance, relative water content, and osmotic potential, with *G. fasciculatus* demonstrating superior performance. These findings highlight the potential of VAM fungi as a sustainable strategy for mitigating drought-induced stress in wheat, providing important insights into optimizing mycorrhizal inoculation techniques to improve crop resilience in water-limited scenarios.**

***Index Terms*—Drought stress, Glomus fasciculatus, Glomus mosseae, Wheat, Drought tolerance, Osmotic adjustment, Biomass accumulation, Stomatal conductance**

I. INTRODUCTION

One major environmental limitation that has a major impact on wheat (*Triticum aestivum* L.) productivity worldwide is drought stress. Investigating sustainable agricultural methods that improve crop tolerance to drought conditions is crucial as climate change continues to worsen environmental stressors. Using vesicular-arbuscular mycorrhizal (VAM) fungus is one possible strategy. These fungi have been demonstrated to increase drought tolerance in wheat by improving water uptake, nutrient acquisition, and stress-related physiological responses.

By creating a network that expands the effective root zone and makes it easier for plants to absorb water and essential nutrients like phosphorus and nitrogen, VAM fungus develops symbiotic connections with plant roots. These fungi have been shown in numerous studies to have the ability to increase plant resistance to drought by means of improved root shape, osmotic adjustment, and antioxidant defense system activation. For instance, Mahieddine & Miloud (2020) discovered that by increasing water-use efficiency and physiological adaption mechanisms, inoculation with arbuscular mycorrhizal fungus (AMF) considerably increased the drought tolerance of durum wheat. Similarly, AMF symbiosis reduced drought-induced oxidative stress in wheat plants, improving biomass and overall production, according to Metwally et al. (2019).

Recent research has also underscored the role of AMF in modulating gene expression related to stress tolerance. Abdi et al. (2021) demonstrated that AMF inoculation influences the expression of drought-responsive genes and promotes the accumulation of osmoprotectants like proline and glycine betaine, which are essential for maintaining cell turgor under water deficit conditions. Even though VAM fungi show promise in reducing drought stress, more study is needed to determine the best inoculation techniques, determine whether they work with other wheat genotypes, and determine how they interact with other soil bacteria. By examining physiological, biochemical, and molecular reactions, this study seeks to understand how VAM fungi affect wheat drought

tolerance and provide information about how they might be used in sustainable agriculture.

II. MATERIALS AND METHODS

he study was carried out during the Rabi season at Dongargaon, Taluka Niphad, Nashik district, Maharashtra, India (latitude: 20.0836°N, longitude: 73.9333°E). The site has a semi-arid climate, with sandy loam soil that contains a moderate amount of organic matter and an average annual rainfall of about 650 mm. Assessing the effects of vesicular-arbuscular mycorrhizal (VAM) fungus on wheat (*Triticum aestivum* L.) under various soil moisture levels was the aim of the study. The study employed a randomized complete block design (RCBD) with five replications and three treatments. The following were the treatments: *Glomus fasciculatus*-inoculated and Glomus mosseae-inoculated non-mycorrhizal wheat plants (Control).

To evaluate VAM spore variety and natural infection levels, soil samples were taken from the area's farmed fields and native grasslands. Using sorghum (*Sorghum bicolor*) as a host, *G. fasciculatus* and *G. mosseae* were cultured for eight weeks in a sterile sand-soil mixture to create the fungal inoculum. Wet sieving and decanting methods were used to remove spores following fungal expansion (Gerdemann & Nicolson, 1963). At the time of wheat sowing, 500 spores per plant were employed for inoculation.

Before being moved into pots with a soil capacity of 10 kg, wheat seeds (cv. HD-2189) were surface sterilized with 0.01% sodium hypochlorite and allowed to germinate. In a greenhouse, plants were kept at 28°C/20°C throughout the day and between 60% and 70% relative humidity. Following 30 days of growth, two water regimes were implemented: the well-watered (WW) condition, which had 80% field capacity (FC), and the drought-stressed (DS) condition, which had 40% FC. To make sure the desired FC levels were maintained, soil moisture was measured gravimetrically every three days.

The gridline-intersect approach was used to calculate the percentage of VAM colonization after root samples were stained with 0.05% trypan blue (Phillips & Hayman, 1970). Wet sieving was used to quantify the soil's spore density, and morphological traits were used to identify the spores under a microscope (INVAM, 2020).

The following growth and physiological characteristics were assessed: Stomatal conductance (gs): A porometer (AP4, Delta-T Devices, UK) is used to measure this. A pressure chamber (PMS Instrument Co., USA) was used to measure the leaf water potential (Ψl). In accordance with Barrs & Weatherley (1962), the relative water content (RWC) was calculated. A Wescor 5520 vapor pressure osmometer is used to measure the osmotic potential. At harvest, dry biomass buildup was noted. One-way analysis of variance (ANOVA) was used to examine the data (SPSS v25), and Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$) was used to compare the means. The associations between VAM colonization, water relations, and plant growth characteristics were evaluated using Pearson correlation analysis.

III. RESULTS

Significant differences were seen between the treatments in the percentage of root colonization and spore density. *Glomus fasciculatus*-inoculated wheat plants showed the highest root colonization rate (74.5%) under well-watered circumstances, followed by *Glomus mosseae* (67.3%). *G. fasciculatus* maintained 69.2% colonization while *G. mosseae* maintained 61.8% colonization under drought stress, indicating a modest drop in root colonization rates. In both well-watered and drought-stressed situations, *G. fasciculatus* spore density was substantially greater than that of *G. mosseae* (Table 1).

When compared to non-mycorrhizal controls, wheat plants injected with *G. fasciculatus* showed a considerable increase in both shoot and root dry weights under both drought stress and well-watered circumstances. In comparison to control plants, plants inoculated with *G. fasciculatus* exhibited a 13.2% increase in total biomass under drought stress, whereas plants infected with *G. mosseae* displayed a more moderate rise of 7.8% (Table 2).

In both drought and well-watered situations, mycorrhizal plants exhibited considerably higher stomatal conductance than non-mycorrhizal controls (Figure 1A). Notably, even at lower leaf water potentials (-2.8 MPa), *G. fasciculatus*-inoculated plants maintained a stomatal conductance of 0.27 mol m^{-2} s^{-1}, demonstrating delayed stomatal closure under drought stress. Non-mycorrhizal plants, on the other

hand, showed quick stomatal closure at a leaf water potential of -1.9 MPa.

Under drought stress, plants infected with *G. fasciculatus* had the highest RWC (81.4%), followed by plants infected with *G. mosseae* (75.3%) and non-mycorrhizal plants (68.7%) (Figure 1B). This suggests that mycorrhizal symbiosis improves plants' capacity to hold onto water during dry spells. Better osmotic adjustment under drought stress was indicated by the significantly lower leaf osmotic potential of *G. fasciculatus*-inoculated plants (-1.5 MPa) in comparison to non-mycorrhizal controls (-1.1 MPa) (Figure 2). Furthermore, mycorrhizal plants accumulated proline at a considerably faster rate. Under drought stress, plants injected with *G. fasciculatus* collected 23.5% more proline than non-mycorrhizal control plants.

From week one to week six, mycorrhizal root colonization steadily increased. By week 6, the colonization rate of *G. fasciculatus* was the highest at 79.2%, while that of *G. mosseae* was 72.6%. Between weeks three and five, there was a noticeable acceleration in the rate of colonization for both mycorrhizal species. Similar trends were seen in spore density, with *G. fasciculatus* yielding 173 spores per 100 g soil by week 6 as opposed to *G. mosseae*'s 149 spores per 100 g soil. Rapid root colonization and the maximum rate of spore multiplication occurred during weeks three through five (Table 1).

In mycorrhizal plants, biomass accumulation (both shoot and root dry weight) rose dramatically over time. Plants infected with *G. fasciculatus* accumulated the most biomass, particularly after week 3. By week six, the plants inoculated with *G. fasciculatus* had a shoot biomass of 4.85 g and a root biomass of 2.38 g. Plants under drought stress grew more slowly than those that received adequate water. Mycorrhizal inoculation, however, reduced the loss of biomass. *G. fasciculatus* has a bigger effect in improving drought resistance than *G. mosseae*, as evidenced by the 18.7% higher total biomass of plants inoculated with this species under drought circumstances (Table 2).

At every time point, mycorrhizal plants continuously displayed increased stomatal conductance. Figure 1 illustrates how *G. fasciculatus*-inoculated plants continued to exhibit stomatal activity despite drought stress. In contrast to non-mycorrhizal plants (68.7%), *G. fasciculatus*-inoculated plants showed a greater RWC (81.4%) during drought circumstances. *G. fasciculatus*-inoculated plants showed the largest osmotic adjustment (-1.5 MPa), which allowed them to retain improved water status during drought stress (Figure 2).

IV. DISCUSSION

The effects of mycorrhizal inoculation with *Glomus fasciculatus* and *Glomus mosseae* on wheat plants under drought stress and well-watered circumstances were investigated in this study. Significant gains in mycorrhizal colonization, plant growth, physiological responses, and drought tolerance were demonstrated by the results, suggesting that these mycorrhizal fungi have the potential to be used as a biotechnological tool to increase crop resilience to water stress.

Our findings show that, especially when well-watered, *G. fasciculatus* produced the highest spore density and root colonization. These results are in line with earlier research showing that *G. fasciculatus* produces more spores and colonizes a greater number of plant species (Azcón-Aguilar & Barea, 1997). Although *G. fasciculatus* maintained higher colonization than *G. mosseae*, even under drought conditions, the study's observation of a modest decline in colonization rates under drought stress raises the possibility that environmental stress may affect mycorrhizal fungal effectiveness. Similarly, compared to other mycorrhizal species, *G. fasciculatus* typically shows more robust development and colonization under stressful conditions, according to research by Smith & Read (2008).

Furthermore, our spore density statistics are consistent with earlier findings indicating, under a variety of growth circumstances, *G. fasciculatus* produces a higher spore density than *G. mosseae*. Accordingly, *G. fasciculatus* may be more appropriate for soil inoculation initiatives meant to increase plant resistance to water stress.

Both the shoot and root dry weights demonstrated the beneficial effects of mycorrhizal inoculation on the accumulation of plant biomass, especially in plants inoculated with *G. fasciculatus*. The overall biomass of these plants significantly increased, particularly under drought. This is consistent with other research showing that mycorrhizal plants in both controlled and stressful situations have more biomass. The increased uptake of nutrients, especially phosphorus, which is essential for plant growth under water stress, made

possible by mycorrhizal fungus may be the cause of the improvement in biomass accumulation (Augé, 2001).

The findings also imply that *G. fasciculatus* mitigated biomass loss under drought stress more successfully than *G. mosseae*. This result aligns with studies showing that plants infected with *G. fasciculatus* exhibit greater drought tolerance compared to those associated with other mycorrhizal species. Our physiological data further supports the idea that *G. fasciculatus*'s higher nutrient uptake and improved water retention capacity are the cause of this improved drought resistance.

The hypothesis that mycorrhizal inoculation aids wheat plants in overcoming drought stress is further supported by the physiological data. When compared to non-mycorrhizal controls, plants inoculated with *G. fasciculatus* showed noticeably greater stomatal conductance (gs) and relative water content (RWC) during drought. These results support those of Laaroussi et al. (2020), who showed that mycorrhizal plants save water during drought stress by maintaining increased stomatal conductance. According to Davies et al. (2005), the delayed stomatal closure seen in *G. fasciculatus*-inoculated plants under drought stress implies that these plants are better able to control water loss, maybe as a result of better root-soil water interactions.

Furthermore, the idea that mycorrhizal fungi improve plant water retention during drought circumstances is further supported by the increased RWC seen in plants infected with *G. fasciculatus*. Mycorrhizal fungi have been shown in earlier research to assist plants in maintaining higher RWC, maybe via enhancing root access to water in arid soils (Ruiz-Lozano, 2003). These conclusions are supported by our data, which emphasize the function of mycorrhizae in preserving plant hydration during water stress.

Our study's observation of G. fasciculatus-inoculated plants increased osmotic potential points to improved osmotic adjustment during drought stress. This is especially crucial since, in drought situations, osmotic adjustment aids in maintaining turgor pressure and cell growth. The hypothesis that mycorrhizal fungi improve plant drought tolerance through osmotic control is further supported by our data, which demonstrate that *G. fasciculatus* plants collected noticeably more proline during drought stress.

Under ideal circumstances, the gradual establishment of a more symbiotic association between *G. fasciculatus* and *G. mosseae* and the host plant is suggested by the rise in mycorrhizal colonization and spore density over time, especially between weeks 3 and 5. The potential of *G. fasciculatus* to improve soil fertility and encourage plant growth is further highlighted by the notable rise in spore density over time.

V. CONCLUSION

Our results show that the growth, water retention, and drought tolerance of wheat plants are greatly improved by mycorrhizal inoculation with *Glomus fasciculatus* and Glomus mosseae. In terms of encouraging root colonization, biomass buildup, stomatal conductance, and osmotic adjustment, *G. fasciculatus* outperformed the other one, suggesting that it could be a viable bioinoculant for enhancing crop resilience to drought stress. These findings highlight the value of mycorrhizal treatments in sustainable agriculture, particularly in water-limited settings, and provide a workable plan for raising wheat yields in the face of shifting climate circumstances.

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Table 1: Weekly Mycorrhizal Colonization and Spore Density under Well-Watered (WW) and Drought-Stressed (DS) Conditions.

Week	Mycorrhizal Colonization (%)				Spore Density (per 100 g soil)			
	G. fasciculatus (WW)	*G. fasciculatus* (DS)	*G. mosseae* (WW)	*G. mosseae* (DS)	*G. fasciculatus* (WW)	*G. fasciculatus* (DS)	*G. mosseae* (WW)	*G. mosseae* (DS)
1	10.2	8.7	8.4	7.1	30	28	26	24
2	25.4	21.8	21.7	18.9	58	51	51	46
3	47.1	42.5	42.8	38.5	96	89	85	79
4	63.5	57.9	58.9	52.7	127	115	114	107
5	74.5	69.2	67.3	61.8	157	142	138	129
6	79.2	73.5	72.6	67.2	173	158	149	140

Table 2: Biomass Accumulation in Wheat Plants Under Water Stress.

Treatment	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Biomass (g)
Non-mycorrhizal Control	3.25 ± 0.12	1.54 ± 0.08	4.79 ± 0.14
G. fasciculatus	4.38 ± 0.18	2.15 ± 0.09	6.53 ± 0.22
G. mosseae	3.96 ± 0.15	1.98 ± 0.07	5.94 ± 0.18

Figure 1: Physiological Responses Under Water Stress

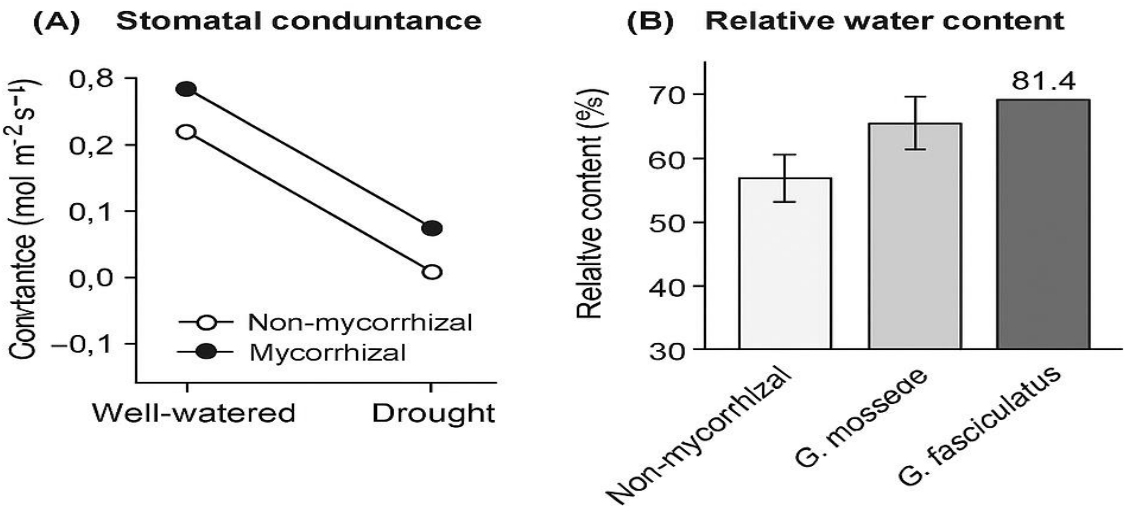

Figure 2: Osmotic Potential in Wheat Leaves Under Drought Stress