

## Interactive Effects of Arbuscular Mycorrhizal Fungi and Different Phosphorus Levels on Faba Bean (*Vicia faba* L.) under Drought Stress Conditions

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### ABSTRACT

*This greenhouse study, conducted in Darab County in 2023, aimed to investigate the effects of drought stress, different phosphorus levels, and mycorrhizal inoculation on morphological and physiological indices and nutrient uptake in plants. The experiment was laid out as a factorial design with three drought stress levels (normal irrigation, moderate stress, and severe stress), three phosphorus levels (0, 10, and 20 mg/kg soil), and three arbuscular mycorrhizal fungal levels (0, 75, and 150 g). The results showed that drought stress significantly impacted plant growth and development. At the maximum stress level, leaf number, stem height, leaf area, and chlorophyll index decreased by 51.8%, 36.3%, 40.5%, and 40.4%, respectively, compared to normal conditions. Furthermore, shoot and root dry weights significantly declined, indicating a reduction in water and organic matter content in plant tissues. Additionally, drought stress led to decreased phosphorus uptake and translocation in the plant, resulting in a significant reduction in phosphorus concentration. Increasing phosphorus levels significantly improved plant morphological and physiological indices. Leaf number, stem height, leaf area, and chlorophyll index increased by 37.4%, 15.02%, 19.05%, and 26.5%, respectively. The most substantial change was observed in leaf number, highlighting phosphorus's crucial role in producing new leaves and developing the plant's vegetative system. The application of mycorrhizal fungi also enhanced plant morphological indices and chlorophyll content. Leaf number, stem height, leaf area, and chlorophyll index increased by 12.3%, 4.97%, 5.7%, and 9.5%, respectively. The most prominent effect of mycorrhiza was on leaf number, demonstrating its vital role in increasing phosphorus uptake and improving vegetative growth. Under water stress conditions, plants inoculated with arbuscular mycorrhizal fungi exhibited improved growth characteristics and enhanced nutrient uptake, especially for phosphorus. Mycorrhizal dependency was maximal under moderate stress conditions and decreased with increasing stress intensity and under normal irrigation. Increased phosphorus levels also led to increased mycorrhizal dependency. Overall, the findings of this research indicate that drought stress significantly negatively impacts plant growth and development, but increasing phosphorus levels and applying mycorrhizal fungi can largely mitigate these adverse effects.*

**Keywords:** Drought stress, Phosphorus, Mycorrhiza, Morphological indices, Nutrient content

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### 1. INTRODUCTION

According to statistics from Iran's Ministry of Agriculture Jihad, the cultivated area for faba bean (broad bean) in Iran during the 2022-2023 cropping year was 30,000 hectares, with an average yield of 5 tons per hectare (green weight) and an annual production of 150,000 tons. Faba bean (*Vicia faba* L.), as one of the most important legumes for human and animal nutrition, holds significant importance due to its high nutritional value and its role in crop rotation and improving soil structure. However, the production of this crop in many parts of the world, especially in arid and semi-arid regions, is increasingly affected by drought stress. As a major limiting factor, drought stress can lead to a significant reduction in faba bean growth, yield, and seed quality.

On the other hand, the use of beneficial soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF), has gained attention as a sustainable biological strategy to increase plant resistance to environmental stresses. Mycorrhizal fungi, by establishing a symbiotic relationship with plant roots, can enhance the uptake of water and nutrients, especially phosphorus, thereby increasing the plant's tolerance to drought stress (Yang

*et al.*, 2023). Numerous studies have demonstrated the positive effects of mycorrhiza on various plants under drought stress. However, a precise and comprehensive investigation of these effects on faba bean, particularly under field conditions and considering different stress levels, still requires further research.

Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, are the most prevalent symbiotic agents in soil, making them of significant ecological and economic importance (Ajema Gebisa, 2024). These fungi establish obligate symbiotic relationships with plant roots, providing a portion of the necessary nutrients through their hyphae. Conversely, these fungi depend on the host plant's roots and cells for their growth and completion of their life cycle (Wahab *et al.*, 2023). Research indicates that these fungi are capable of forming symbiotic relationships with most vascular plants. This highlights the potential to effectively utilize this symbiosis for increased agricultural production by selecting and applying the optimal combination of host plant and symbiotic fungus.

Plants with mycorrhizal symbiosis exhibit improved growth due to enhanced absorption of nutrients and water from the soil, leading to higher yields and increased resistance to both biotic (pathogens that attack plant roots) and abiotic stresses (drought, cold, and salinity). Therefore, their use as a novel and important strategy to combat drought stress can lead to significant water conservation. Numerous studies have demonstrated that mycorrhizal symbiosis increases host plant resistance to drought conditions. However, the underlying mechanisms are still debated, although their effects are primarily attributed to nutritional changes within the host plant.

These fungi aid in phosphorus uptake by forming extensive hyphal networks in the soil. The mechanisms of phosphorus absorption by arbuscular mycorrhizal fungi involve extraradical hyphal networks that extend into the soil, increasing the volume of soil explored for phosphorus uptake (Jin *et al.*, 2024). This process helps plants absorb more phosphorus from the soil. Additionally, arbuscular mycorrhizal fungi convert organic phosphorus into absorbable inorganic forms by secreting enzymes and root exudates (Zhu *et al.*, 2025).

Faba bean, like many legumes, primarily forms a symbiotic relationship with arbuscular mycorrhiza (Ardakani *et al.*, 2014). In contrast, ectomycorrhiza is characterized by forming a hyphal network around roots and between cortical cells and is more commonly observed in forest trees (Martin *et al.*, 2017). The importance of arbuscular mycorrhiza in sustainable agriculture stems from its role in increasing plant access to nutrients, particularly phosphorus, and improving plant resistance to environmental stresses (Rai, 2006). Multiple studies have shown that the use of mycorrhiza can significantly increase the vegetative growth of legumes (Beltayef *et al.*, 2023). This increased growth is observable in various parameters such as shoot and leaf dry weight, plant height, and leaf number. This positive effect is attributed to increased nutrient and water absorption by mycorrhiza, leading to enhanced photosynthesis and biomass production (Beltayef *et al.*, 2023). For example, a study by Ardakani *et al.* (2014) demonstrated that mycorrhizal inoculation significantly increased the shoot and root dry weight of faba bean. This increase in dry weight indicates enhanced vegetative growth, resulting from better access to nutrients, especially phosphorus. Furthermore, some studies have indicated that mycorrhiza can influence the production of plant hormones like auxins and cytokinins, which can play a role in plant vegetative growth (Rai, 2006).

The use of mycorrhiza can have a significant impact on the growth, nutrition, yield, and resistance to environmental stresses in faba bean. Mycorrhiza can contribute to increased faba bean yield and seed quality by enhancing nutrient absorption, particularly phosphorus, increasing water uptake, boosting resistance to environmental stresses, and improving interactions with other soil microorganisms. Selecting the appropriate mycorrhizal species and considering environmental conditions are crucial for achieving the best results. Further studies are needed to investigate the effects of different mycorrhizal species and various environmental conditions on faba bean, as well as to examine the complex interactions of mycorrhiza with other soil microorganisms. The use of mycorrhiza as a sustainable agricultural practice can help increase faba bean production and improve soil fertility.

This study was designed and implemented to investigate the interactive effects of drought stress and different phosphorus levels, along with inoculation with arbuscular mycorrhizal fungi, on the morphological, physiological, and nutrient uptake characteristics of faba bean. The impact of different levels of these factors on growth indices (stem height, leaf number, leaf area, shoot and root dry weight), chlorophyll index, nutrient uptake, and mycorrhizal dependency in faba bean were measured and analyzed.

This research was designed and conducted with the hypothesis that drought stress at different levels would lead to a significant reduction in the examined indices, and that the growth and inoculation of faba bean with arbuscular mycorrhizal fungi would mitigate the negative effects of this stress. Furthermore, attention was given to the increased mycorrhizal dependency due to drought stress and increased phosphorus. By providing comprehensive information on the effects of drought stress and the role of mycorrhiza in improving faba bean's tolerance, this research aims to offer suitable strategies for the sustainable management of this crop's production in drought-prone areas.

## 2- MATERIALS AND METHODS

This greenhouse experiment was conducted at the Bakhtajerd Darab Research Station, located 250 km southeast of Shiraz, Iran, at an elevation of 1110 meters above sea level, 57°17' East longitude, and 28°47' North latitude. A 3x3x3 factorial experiment was implemented in a completely randomized design with four replicates. The factors investigated in the experiment included inoculation with arbuscular mycorrhizal fungi of the species *Glomus mosseae* at three levels: 1) control (no inoculation), 2) inoculation with 75 g of mycorrhizal fungal inoculum, and 3) inoculation with 150 g of mycorrhizal fungal inoculum. Drought stress was applied at three levels: 1) no stress (normal irrigation), 2) moderate stress, and 3) severe stress. These stress levels were defined as percentages of plant available water based on permissible moisture depletion coefficients of 0.40, 0.55, and 0.70, respectively. Phosphorus levels were also tested at three concentrations: 0, 10, and 20 mg/kg soil, sourced from triple superphosphate fertilizer.

For the experiment, plastic pots with a 20 cm top diameter and 25 cm height were uniformly filled with 8 kg of a growing medium consisting of field soil and sand in a 2:1 ratio. Based on soil test results and the nutritional requirements of the faba bean, any nutrient deficiencies were corrected by adding sufficient amounts of the necessary elements to the soil. To ensure proper drainage, holes were made at the bottom of the pots, and a 2 cm layer of gravel (4-6 mm diameter) was placed at the base of each pot. Four-leaf stage faba bean plants of the 'Shoushtari' cultivar, which were visibly uniform, were then transplanted into the main pots. Simultaneously, inoculation with the designated mycorrhizal fungal inoculum was performed according to the treatment plan. Two weeks after transplanting, drought stress treatments were initiated. This involved daily weighing of the pots and calculating the appropriate amount of water for each treatment based on the Management Allowed Depletion (MAD) percentages (40% for no stress, 55% for moderate stress, and 70% for severe stress). Water was added to bring the soil moisture content up to 85% of field capacity (F.C.). The experiment continued for 90 days (until before faba bean flowering).

At the end of the experimental period, vegetative growth characteristics such as height, stem diameter, leaf number, and fresh and dry weights of shoots and roots were measured separately. Dry weights were determined by placing samples in an oven at 65°C until a constant weight was achieved. Leaf chlorophyll content was measured using a SPAD meter (model CL-01). Mycorrhizal dependency was calculated by subtracting the dry weight of non-mycorrhizal control seedlings from the dry weight of mycorrhizal seedlings, divided by the dry weight of non-mycorrhizal control seedlings, and multiplied by 100.

For leaf phosphorus measurement, a dry ashing extraction method was performed, and leaf phosphorus concentration was determined spectrophotometrically using a UV-2100 model instrument, based on the yellow colorimetric method (Subramanian and Charest, 1997). Finally, statistical analysis was performed using MSTATC software, and mean comparisons were conducted using Duncan's multiple range test at a 5% probability level.

## 3- RESULTS

### 3-1- Faba Bean Leaf Number

The analysis of variance revealed a significant effect of the studied factors on **leaf number**. Both two-way and three-way interactions among the factors also significantly influenced leaf number.

The results showed that the average leaf number per plant decreased with increasing **drought stress**. This reduction amounted to 32% and 51.8% under moderate and severe stress, respectively, compared to the control (no stress).

**Mycorrhizal inoculation** generally increased the average leaf number in faba bean plants. Using 75 g and 150 g of mycorrhizal inoculum led to an average increase in leaf number of 6.6% and 12.4%, respectively, compared to the uninoculated control.

Adding **phosphorus** to the soil also increased faba bean leaves. This increase was 19.9% and 34.7% at 10 mg and 20 mg phosphorus levels, respectively, compared to the control without phosphorus fertilizer.

An increase in phosphorus at each stress level consistently led to an increase in plant leaf number. For the 20 mg phosphorus level, the increase in leaf number was 44.7%, 45.8%, and 54.7% under normal conditions, moderate stress, and maximum stress, respectively. As the results indicate, increasing phosphorus levels positively impacted leaf number under stressed conditions. The highest average leaf number in this study (13.4 leaves per plant) was observed from the interaction of 20 mg phosphorus under minimum stress conditions.

The interaction between mycorrhizal use and drought stress showed that the highest leaf number, with an average of 12 leaves per plant, was associated with the 150 g mycorrhiza treatment under minimum stress conditions. Although leaf number per plant decreased with increasing stress, increased mycorrhizal levels compensated for this reduction. Specifically, under maximum stress conditions, using 75 g and 150 g of mycorrhiza increased leaf number by 8.2% and 15.8%, respectively.

The highest leaf number resulted from the three-way interaction of 150 g mycorrhiza, 20 mg phosphorus, and normal irrigation conditions. At every stress level, the addition of both mycorrhiza and phosphorus increased leaf number. However, none of the mycorrhiza or phosphorus treatments were able to increase leaf number to the extent of normal irrigation conditions or completely prevent the damage caused by stress (Figure 1).

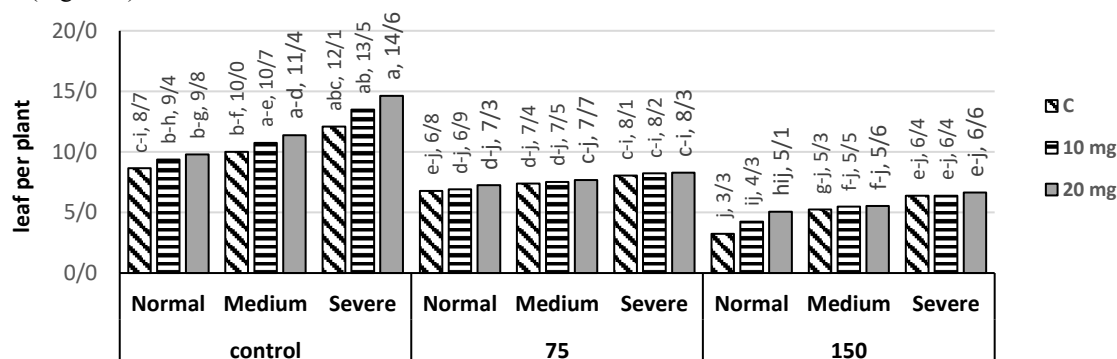


Figure 1- Interaction of drought stress, phosphorus, and mycorrhiza on the mean number of leaves per faba bean plant.

Means with at least one letter in common do not have significant differences (Duncan 5%).

### 3-2- Plant Height

Plant height, based on the analysis of variance results, was significantly affected by the studied factors. According to these results, all two- and three-way interactions of these factors on leaf number were significant. The obtained results showed that the mean plant height decreased with increasing drought stress. This reduction amounted to 20.7% and 36.3% under moderate and severe stress, respectively, compared to the control.

Mycorrhizal application increased the mean plant height in faba bean plants. Specifically, applying 75 and 150 grams of mycorrhizal inoculum led to an increase in mean plant height by 1.3% and 5%, respectively, compared to the non-mycorrhizal control. Adding phosphorus to the soil also increased the mean plant height in faba bean. This increase was 8.6% and 15% at 10 and 20 mg phosphorus levels, respectively, compared to the control without phosphorus fertilizer. An increase in phosphorus at each stress level consistently led to an increase in the mean plant height. For the 20 mg phosphorus level, this increase was 24.11%, 7.88%, and 15.44% under normal, moderate, and maximum stress conditions, respectively, compared to the phosphorus-free control. Based on these results, increasing phosphorus levels positively impacted plant height under stress conditions. The highest mean plant height in this study, averaging 21.91 cm, was achieved from the interaction of 20 mg phosphorus under minimal stress conditions.

The interaction between mycorrhizal application and drought stress revealed that the highest mean plant height, averaging 21.11 cm, corresponded to the 150 g mycorrhiza treatment under non-stress conditions. No significant difference was observed between treatments under normal and moderate stress conditions in the presence of the specified mycorrhizal levels. The highest mean plant height in the current experiment resulted from the three-way interaction of 150 g mycorrhiza, 20 mg phosphorus, and normal irrigation conditions (22.3 cm). The results of the three-way interaction analysis of the studied factors indicated that under normal irrigation conditions, other factors did not significantly affect faba bean plant height (Figure 3).

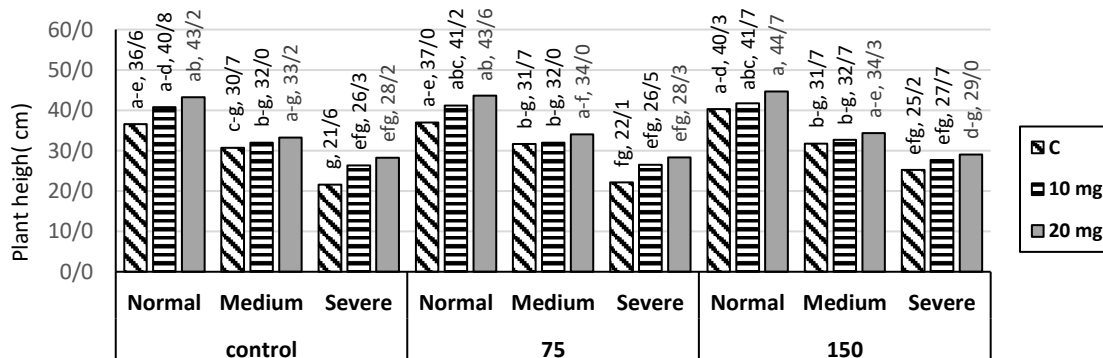


Figure 2- Interaction of drought stress, phosphorus, and mycorrhiza on the plant height of faba bean plant. Means with at least one letter in common do not have significant differences (Duncan 5%).

### 3-3- Faba Bean Leaf Area

Based on the variance analysis results, all simple, two-way, and three-way interaction effects of the investigated factors significantly influenced faba bean leaf area. The results indicated that the mean faba bean leaf area significantly decreased with increasing drought stress. Leaf area reduction under moderate and severe stress was 23.47% and 40.52%, respectively, compared to the unstressed control. Mycorrhizal application, at the predicted rates in the current experiment, increased leaf area. At 75 and 150 grams of mycorrhizae, leaf area increased by 2.5% and 5.7%, respectively, compared to plants without mycorrhizal application. Furthermore, adding phosphorus at 20 and 30 mg per kg of soil resulted in a 7.7% and 19.1% increase in faba bean leaf area.

Mean faba bean leaf area increased with mycorrhizal application at each phosphorus level. This increase, particularly at 150 grams of mycorrhizae, was 4.4%, 8.4%, and 7.9% compared to the unstressed control with 0, 10, and 20 mg of phosphorus, respectively. These findings suggest a positive effect of phosphorus on the ability of mycorrhizae to enhance faba bean leaf area.

The three-way interaction of the investigated factors on faba bean leaf area revealed that the highest leaf area was observed under the triple interaction of normal stress, 20 mg phosphorus, and 150 g mycorrhizae. At each stress level, the maximum leaf area was obtained from the two-way interaction of 20 mg phosphorus and 150 g mycorrhizae, which showed no significant difference from the top-performing treatment and fell into the same statistical group (Figure 3).

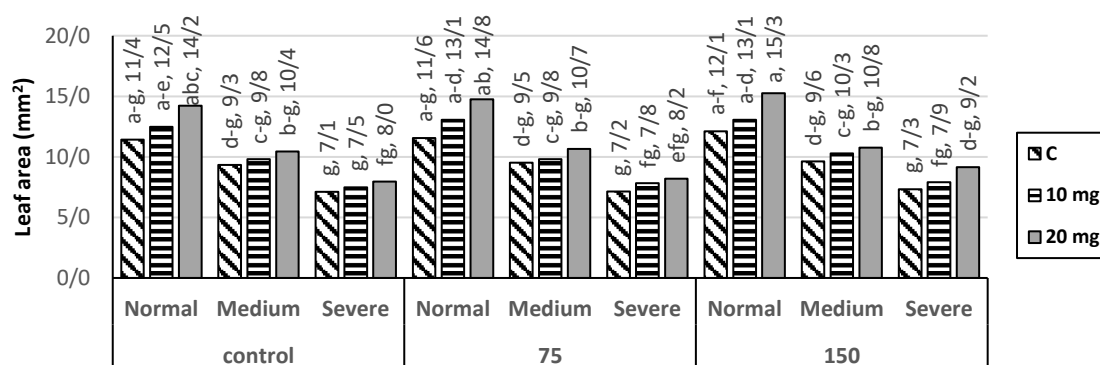


Figure 3- Interaction of drought stress, phosphorus, and mycorrhiza on the Leaf area of faba bean plant. Means with at least one letter in common do not have significant differences (Duncan 5%).

### 3-4- Faba Bean Leaf Chlorophyll Index

The results from the analysis of variance revealed a significant effect of all investigated factors, as well as their interactions, on the faba bean leaf chlorophyll index. Drought stress at moderate and severe levels reduced the chlorophyll index by 21.41% and 40.42%, respectively. Mycorrhizal application significantly increased the leaf chlorophyll index; this increase was 11.24% and 26.55% for 75 and 150 grams of mycorrhizae, respectively, compared to the control. Increasing phosphorus to 10 and 20 mg per kg of soil led to a significant increase in the faba bean leaf area index by 3.26% and 9.5%, respectively, compared to the phosphorus-free control.

Figure 4 illustrates the three-way interaction of the investigated factors on the leaf chlorophyll index. Based on these results, the highest leaf chlorophyll index was obtained from the interaction of normal irrigation, 150 grams of mycorrhizae, and 20 grams of phosphorus (25.13 units). The findings also indicated that under normal irrigation conditions, there was no significant difference in the chlorophyll index among the interactions of other phosphorus and mycorrhizae levels. The lowest chlorophyll index was observed under severe stress conditions, and the interaction of mycorrhizae and phosphorus levels could not significantly influence it (fig 4).

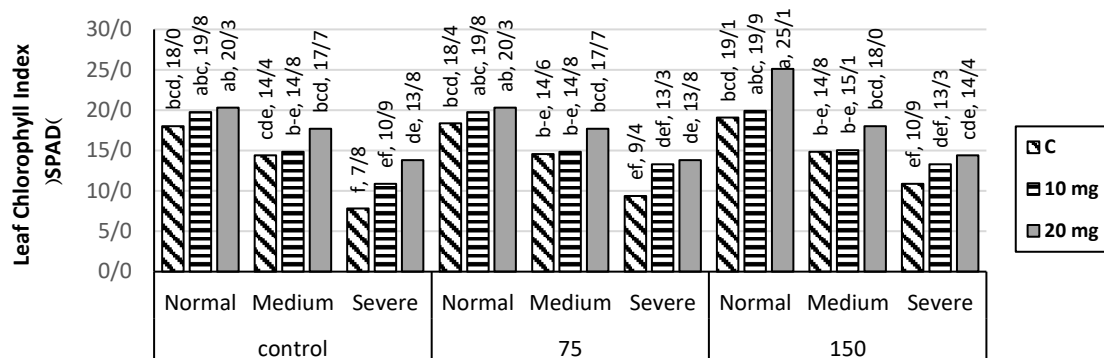


Figure 4 Interaction of drought stress, phosphorus, and mycorrhiza on the Leaf Chlorophyll Index (SPAD) of faba bean. Means with at least one letter in common do not have significant differences (Duncan 5%).

### 3-5- Faba Bean Shoot and Root Dry Weight

The analysis of variance revealed that shoot dry weight was significantly impacted by stress levels. The highest shoot dry weight, averaging 5.69 grams, was observed under normal irrigation. Severe stress led to a significant 55.6% reduction in this parameter compared to normal irrigation. Furthermore, shoot dry weight was significantly influenced by phosphorus levels. The highest shoot dry weight, also averaging 5.69 grams, was achieved with 20 mg/kg of phosphorus. Conversely, the absence of phosphorus resulted in a significant 42.27% decrease in shoot dry weight compared to the 20 mg phosphorus treatment. Applying 10 mg of phosphorus increased shoot dry weight by 16.3% relative to the phosphorus-free control.

The use of 75 and 150 grams of mycorrhizae increased shoot dry weight by 4.5% and 13.2%, respectively, compared to the non-mycorrhizal treatment. The simple effect of drought **stress** on both fresh and dry root weight also showed a significant negative impact. Moderate and severe stress reduced shoot dry weight by 31.8% and 47.5%, respectively, compared to the normal irrigation control.

Based on the analysis of variance, fresh and dry root weight were significantly affected by phosphorus levels. The highest root dry weight, averaging 20.47 grams, was obtained with 20 mg/kg of phosphorus. The absence of phosphorus resulted in a significant 27.9% reduction in root dry weight compared to the 20 mg phosphorus treatment. Applying 10 mg of phosphorus increased root dry weight by 10.2% compared to the phosphorus-free control. Similarly, the use of 75 and 150 grams of mycorrhizae increased shoot dry weight by 8.9% and 4.8%, respectively, compared to the non-mycorrhizal treatment.

The analysis of variance indicated that the three-way interaction of the examined factors significantly influenced both shoot and root dry weight. An investigation into shoot dry weight revealed that the highest shoot dry weight, averaging 18.56 grams, was attributed to the interaction of 150 grams of mycorrhizae and 20 grams of phosphorus under normal irrigation conditions. The highest root dry weight, averaging 6.13 grams, was obtained from this same treatment, which independently grouped into the highest statistical category (Table 1).

Table 1 - Interactive Effect of Drought Stress, Phosphorus, and Mycorrhiza on Root and Shoot Dry Weight

| Mycorrhiza (gr) | Water stress | P (mg/kg)   | Shoot Dry weight(gr) | Root dry weight(gr) |
|-----------------|--------------|-------------|----------------------|---------------------|
| 0               | Normal       | Control (0) | 8.17 g               | 4.61 def            |

|     |        |             |         |          |
|-----|--------|-------------|---------|----------|
| 75  | Medium | 10          | 10.63 e | 4.86 cd  |
|     |        | 20          | 13.14 c | 5.63 b   |
|     |        | Control (0) | 6.64 j  | 3.07 hij |
|     |        | 10          | 6.85 ij | 3.21 hi  |
|     |        | 20          | 7.06 i  | 3.85 g   |
|     |        | Control (0) | 4.96 n  | 2.42 m   |
|     | Severe | 10          | 5.74 kl | 2.66 klm |
|     |        | 20          | 5.95 kl | 2.92 ijk |
|     | Normal | Control (0) | 8.23 g  | 4.7 de   |
|     |        | 10          | 10.74 e | 5.08 c   |
|     |        | 20          | 15.62 b | 6.03 a   |
|     |        | Control (0) | 6.67 ij | 3.12 hij |
| 150 | Medium | 10          | 6.94 ij | 3.33 h   |
|     |        | 20          | 7.07 i  | 4.35 f   |
|     |        | Control (0) | 5.29 mn | 2.46 m   |
|     | Severe | 10          | 5.77 kl | 2.82 jkl |
|     |        | 20          | 5.98 kl | 2.96 ijk |
|     | Normal | Control (0) | 9.02 f  | 4.73 de  |
|     |        | 10          | 11.74 d | 5.5 b    |
|     |        | 20          | 18.56 a | 6.13 a   |
|     | Medium | Control (0) | 6.7 ij  | 3.14 hi  |
|     |        | 10          | 7.04 ij | 3.62 g   |
|     |        | 20          | 7.73 h  | 4.52 ef  |
|     | Severe | Control (0) | 5.59 lm | 2.6 lm   |
|     |        | 10          | 5.84 kl | 2.91 ijk |
|     |        | 20          | 6.06 k  | 3.05 hij |

Means with at least one common letter in each column do not show statistically significant differences (Duncan 5%)

### 3-6- Phosphorus Concentration in Shoots

The average phosphorus concentration in shoots, influenced by the factors under investigation, was 1.6% under the interaction of normal irrigation, 150g mycorrhiza, and 20mg phosphorus.

Under maximum stress conditions, increasing phosphorus and mycorrhiza levels at each individual level could not significantly increase the phosphorus concentration in shoots. The most significant changes in aerial organ phosphorus concentration due to increased mycorrhiza and phosphorus levels occurred under minimum stress conditions (Figure 5).

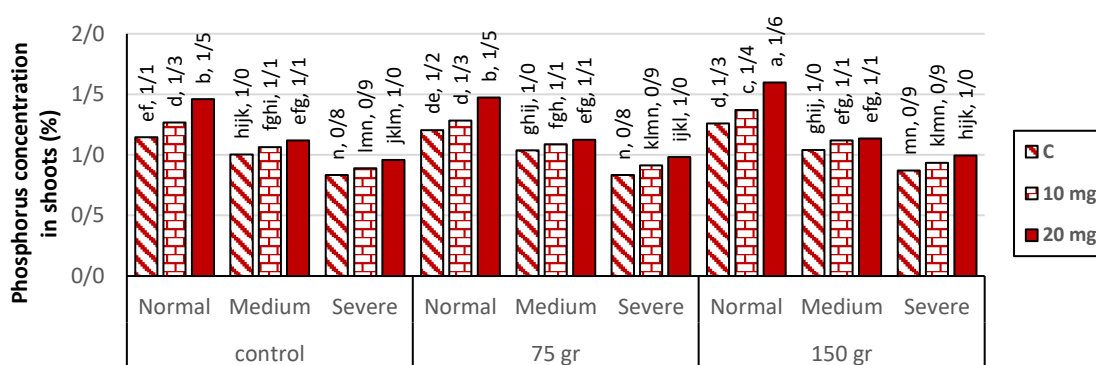


Figure5- Interaction of drought stress, phosphorus, and mycorrhiza on the Phosphorus concentration in shoots of faba bean plant.

Means with at least one letter in common do not have significant differences (Duncan 5%).

### 3-7- Mycorrhizal Dependency

The analysis of variance results showed a significant effect of all investigated factors and their two-way and three-way interactions on the mycorrhizal dependency of faba bean plants.

Under moderate stress conditions, the highest mycorrhizal dependency (10.31%) was observed in faba bean plants. Mycorrhizal dependency under severe stress and normal irrigation conditions showed no significant difference, decreasing to 3.32% and 3.23% respectively, placing them in the next statistical group.

Increasing phosphorus significantly increased mycorrhizal dependency compared to the control. This increase was 2.4 and 2.3 times greater than the phosphorus-free control at 10 mg and 20 mg levels, respectively. Mycorrhizal dependency at 75g and 150g levels was measured at 5.6% and 11.13%, respectively, with significant differences placing them in independent statistical groups.

The highest mycorrhizal dependency (17.81%) was obtained from the interaction of 20 mg phosphorus under moderate stress conditions. The results indicated the lowest dependency in the phosphorus-free control under normal and moderate irrigation conditions. The decrease in mycorrhizal dependency under stress conditions with increasing phosphorus levels aligns with the results reported by Subramanian et al. (2008).

Increasing mycorrhiza levels increased mycorrhizal dependency. The 150g level, compared to 75g mycorrhiza, showed increases of 2.4, 2.6, and 1.4 times for 0g, 10g, and 20g phosphorus concentrations, respectively. The highest mycorrhizal dependency was obtained from the interaction of 10 mg phosphorus and 150g mycorrhiza, with an average of 15.01%.

At each stress level, increasing mycorrhiza increased mycorrhizal dependency. The results showed that the growth in mycorrhizal dependency was higher under **maximum stress** conditions compared to other stress levels. The increase in mycorrhizal dependency at the 150g mycorrhiza level compared to the 75g level of the same factor was 1.82, 1.79, and 2.4 times for normal irrigation, moderate stress, and severe stress, respectively.

The effect of the three-way interaction of the investigated factors on mycorrhizal dependency indicated the highest dependency in the interaction of severe stress levels, 20 mg phosphorus, and 150g mycorrhiza. In this study, the lowest mycorrhizal dependency was obtained from using 75g mycorrhiza and 20 mg phosphorus under severe stress conditions (Figure 6).

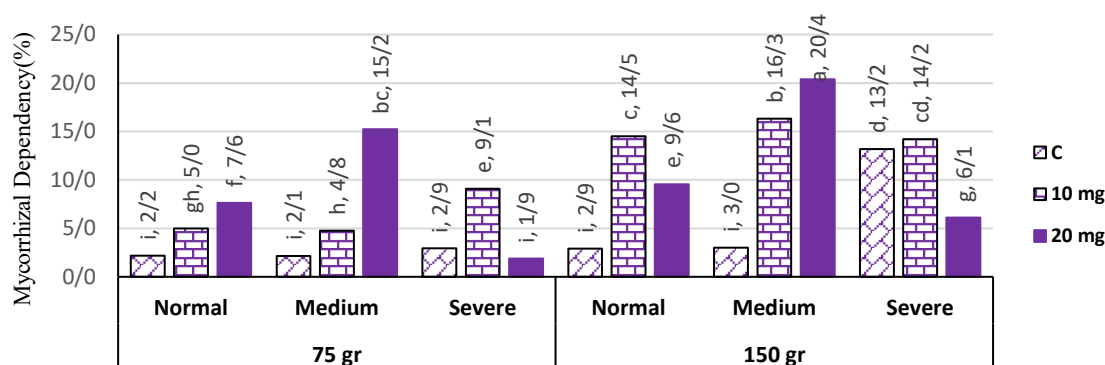


Figure6- Interaction of drought stress, phosphorus, and mycorrhiza on mycorrhizal dependency of faba bean Means with at least one letter in common do not have significant differences (Duncan 5%).

#### 4- DISCUSSION AND CONCLUSION

Under maximum stress conditions, leaf count, stem height, internode count, leaf area, and chlorophyll index decreased by 51.8%, 36.3%, 40.5%, and 40.4% respectively, compared to normal conditions. Based on these results, leaf count was the most sensitive indicator to stress in the plant. Since leaves, as the primary photosynthetic organs, play a vital role in plant survival, under stress conditions, the plant attempted to prevent water and nutrient loss by reducing the number of leaves. Our findings suggest that leaves are more sensitive to stress than other plant organs, and they experienced damage earlier.

Drought stress in faba bean plants led to a reduction in leaf number through leaf senescence, decreased photosynthetic efficiency, and changes in leaf morphology. When faced with water scarcity, the plant senesced older leaves and reabsorbed their nutrients to preserve resources for younger leaves and reproduction. This process was accelerated by chlorophyll degradation and an increase in reactive oxygen species, leading to leaf loss (Munné-Bosch & Alegre, 2004).

Despite the important role of phosphorus in increasing the height of faba bean plants under drought stress, our experimental results showed that the phosphorus levels used could not fully compensate for the height reduction caused by drought stress. Previous studies have indicated that phosphorus application can mitigate the negative effects of water deficit and improve plant growth and development. For instance, studies by Mataa et al. (2019) demonstrated a significant improvement in common bean plant height under drought with phosphorus application. Similarly, bean genotypes receiving sufficient phosphorus showed faster growth and reduced maturity periods under drought stress (Namugwanya et al., 2018), which helped maintain their height.

Based on our experimental results, increasing drought stress led to a reduction in faba bean plant height, and mycorrhizal inoculation could not significantly compensate for this decrease, especially under severe stress where the effect of mycorrhiza on plant height was not significant compared to the control. This contrasts with other studies suggesting that mycorrhiza can help increase the height of bean plants even under drought stress due to enhanced nutrient and water uptake and improved physiological responses (Zaman et al., 2024). Additionally, plants inoculated with arbuscular mycorrhiza exhibited better leaf water relations and vascular conductivity, which are crucial for maintaining plant height during drought stress (Al-Karaki & Williams, 2021).

Drought stress significantly reduced leaf chlorophyll content. This reduction was greater in mesophyll cells than in bundle sheath cells in corn and was primarily due to a decrease in chlorophyll a/b protein (Arabshahi & Mobasser, 2014). However, increasing phosphorus levels managed to mitigate the negative effects of drought stress, leading to an increase in leaf count, stem height, leaf area, and chlorophyll index. The most significant effect of increased phosphorus in this study was observed on leaf count, indicating the importance of this element in producing new leaves and developing the plant's vegetative system, thereby increasing the photosynthetic surface area. Phosphorus plays a vital role in cell division and the growth of new tissues.

Phosphorus significantly increased leaf count by promoting vegetative growth and leaf surface development. Phosphorus is crucial for various metabolic processes, including photosynthesis and energy transfer, which directly affect leaf expansion and overall plant health. According to Lynch et al. (1991), adequate phosphorus supply is essential for leaf growth, as it influences the number of leaves and nodes, consequently increasing branching and leaf appearance.

Phosphorus deficiency significantly reduced leaf area in plants through multiple mechanisms. It decreased the rate of leaf expansion and final leaf size, particularly in lower leaves (Colomb et al., 2000). The reduction in leaf area was primarily due to a lower rate of cell production and a decrease in final cell length. Phosphorus deficiency slowed down cell division and elongation processes, leading to smaller leaves (Kavanová et al., 2006).

The use of mycorrhizal fungi increased morphological indices and the chlorophyll index of the plant. The increases in measured indices at the highest mycorrhiza level compared to the control without it were 12.3% for leaf count, 4.97% for stem height, 5.7% for leaf area, and 9.5% for chlorophyll index, respectively. As the results show, the most affected index by mycorrhiza was leaf count. Mycorrhizal fungi led to the production of more leaves by increasing phosphorus uptake, which plays a crucial role in cell division and the growth of new tissues. They also enhanced overall plant vegetative growth and consequently produced more leaves by improving water and nutrient absorption.

The increased leaf count in mycorrhiza-inoculated plants was due to improved growth and nutrient uptake. These fungi, by establishing a symbiosis with the roots, enhanced nutrient uptake (Selvaraj & Thangavel, 2022), water relations (Lü et al., 2018), and photosynthetic efficiency (Ballhorn & Millar, 2013), directly contributing to leaf growth.

Although mycorrhizal inoculation in this experiment did not lead to a significant difference in plant height compared to the control, mycorrhizal inoculation generally enhances plant growth and nutrient uptake in legumes, especially faba beans (*Vicia faba* L.). Other studies have shown that mycorrhizal treatment can significantly increase faba bean plant height (Aldeen and Sultan, 2019), an increase attributed to improved uptake of nutrients such as phosphorus, nitrogen, and potassium (Dubova et al., 2015).

Increasing drought stress in this experiment led to a 47.5% reduction in aerial dry weight and a 51.6% reduction in root dry weight of faba bean plants, indicating a decrease in water and organic matter in tissues and reduced root growth. Drought stress reduced dry weight and overall plant growth by disrupting photosynthesis, nutrient uptake and transport, and increasing respiration and the accumulation of harmful compounds (Siddiqui et al., 2015; Abid et al., 2017). However, some genotypes showed drought tolerance mechanisms (Abid et al., 2017).

On the other hand, arbuscular mycorrhizal fungi, by forming a beneficial symbiosis with faba bean roots, increase biomass and nutrient uptake, especially under nutrient-deficient conditions (Almethyeb et al., 2013). These fungi improve the uptake of phosphorus, iron, and zinc, increase biomass and root density, and enhance nitrogen fixation (Ingraffia et al., 2019). Mycorrhizal inoculation increases plant height, leaf area index, and

grain yield in faba beans, and by improving photosynthesis and antioxidant systems, it enhances drought tolerance and soil remediation (Gholamhoseini et al., 2022). The positive effects of mycorrhiza are more pronounced in nutrient-poor soils (Almethyeb et al., 2013), demonstrating its potential in sustainable agriculture and increasing crop productivity.

Drought stress reduced phosphorus uptake and transport in the plant. The reduction in phosphorus was 20% compared to the non-stressed control. The decreased percentage of this element indicates a disruption in the plant's physiological and metabolic processes under stress conditions. Drought stress reduced soil moisture, consequently limiting the movement and absorption of nutrients by the plant's roots. This stress led to reduced root growth and, subsequently, a decreased surface area for nutrient absorption.

Arbuscular mycorrhizal fungi increased phosphorus uptake in the plant and drought resistance. Under water stress conditions, plants inoculated with arbuscular mycorrhizal fungi showed improved growth characteristics and increased nutrient uptake. Under moderate stress conditions, the maximum mycorrhizal dependency was observed in faba bean plants (10.31%). Mycorrhizal dependency under severe stress and normal irrigation decreased by 32.4% and 23.3%, respectively, compared to this treatment. Increased phosphorus increased mycorrhizal dependency compared to the control. This increase was 2.4 and 2.3 times greater than the phosphorus-free control at 10 mg and 20 mg levels, respectively. Mycorrhizal dependency was measured at 5.6% and 11.13% at 75 and 150 mycorrhiza levels, respectively.

The interaction of drought stress and mycorrhiza significantly affected the aerial dry weight of beans, with arbuscular mycorrhiza increasing aerial biomass by enhancing plant growth and resilience under drought, and improving nutrient uptake and physiological responses (Recchia et al., 2018). Mycorrhiza-inoculated plants under drought stress showed a 54% increase in shoot dry weight, and specific mycorrhizal compounds significantly increased biomass and nutrient uptake. Mycorrhiza provides physiological and nutritional benefits by improving chlorophyll, stomatal conductance, and glucose secretion, and enhances plant adaptation to water stress by regulating drought-related genes. However, severe drought stress can overcome the benefits of mycorrhiza (Chandrasekaran, 2022).

Arbuscular mycorrhiza plays a crucial role in increasing nutrient uptake and drought resistance. Inoculated plants under water stress show improved growth and greater uptake of N, P, and K (Wu and Zou, 2009). Mycorrhiza-inoculated plants under drought stress show a 49% increase in growth, increased biomass, improved root morphology, and increased nutrient uptake, especially an 86% increase in phosphorus uptake (Chandrasekaran, 2022). This symbiosis improves drought tolerance through increased nutrient uptake, especially phosphorus, and root modifications, contributing to sustainable agriculture in water-scarce regions.

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