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# THE EFFECT OF INDIGENOUS AMF APPLICATIONS ON THE MORPHO-PHYSIOLOGICAL CHARACTERISTICS OF TWO VARIETIES OF SHALLOTS ON DROUGHT STRESS CONDITIONS

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***Abstract***. *One of the limiting conditions for shallot plants to grow optimally is dry land conditions. Indigenous AMF application is one way to overcome this condition. This study aimed to determine the effect of indigenous AMF application on the morpho-physiological characters of two shallot varieties which are sensitive and tolerant to drought stress conditions. The study was carried out for 6 months on a wirehouse and laboratory scale. The Experiment used a completely randomized design with two factors. The first factor was varieties of shallots, i.e. Brebes (Sensitive) and Kuning (Tolerant) varieties of shallots. The second factor was the application of indigenous AMF which consisted of 5 levels i.e. Glomus sp1, Glomus sp2, Glomus sp3, a mixed those three isolates and control treatment (without application of AMF). The morpho-physiological observation parameters included header dry weight, root weight, and leaf proline content. From the observations, it can be concluded that under stressed conditions, the leaf proline content of the sensitive variety accumulated higher in the header than the tolerant variety, because the tolerant variety was better able to produce higher root and header weights when adapting than the sensitive. AMF inoculation did not show significant differences with the treatment without AMF inoculation on leaf proline. However, there was a tendency that inoculation of a mix of AMF isolates (Glomus sp1+Glomus sp2+ Glomus sp3) decrease the proline content in the leaves, both in sensitive and tolerant varieties so that plants are more resistant to drought stress.*
***Keywords:*** drought stress; Indigenous AMF; morpho-physiological characteristics; shallots,

## 1. Introduction

One of the horticultural commodities belonging to spiced vegetables is shallot (*Allium ascalonicum* L.). This vegetable is needed as a cooking spice, to add flavor to almost all dishes around the world. Other uses are as traditional and modern medicine because: Contains antiseptic and allicin compounds (Shahrajabian *et al.*, 2020).

Las *et al*. (2014) reported that dry land in Indonesia has not been cultivated intensively. Dry land is a stretch of land that is never flooded or flooded most of the time of the year (BPS, 2017). This means that the use of water on dry land is limited and rainfall is the main source of water. Drought stress is a problem that needs attention in shallot cultivation, especially in lowland dry land. Water loss due to high temperatures and large infiltration of sandy soil, resulting in reduced water availability for plants.



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Another problem is when dry land in the lowlands with wet climatic conditions causes the formation of soil types that react acid (Pusat Penelitian dan Pengembangan Tanah, 2012). Agricultural businesses on acid dry land will face difficulties in increasing and maintaining their productivity. The low productivity of acid dry land, especially in lowland areas is caused by rapid weathering of soil organic matter, erosion and nutrient leaching due to rainfall and high temperatures, resulting in poor soil organic matter and nutrients. According to Prashad & Chakraborty (2019), in acid dry land, the problem of low availability of phosphorus (P), high Al content and low pH values are the main obstacles in increasing yields. According to Kim *et al.* (2018), this condition causes the distribution of plant roots to be relatively shallow so that plants are less resistant to drought stress.

The morphological and physiological characteristics of roots play a major role in determining growth and production (Ghosh *et al.*, 2014). Shallots are herbaceous plants that have short roots, but like a lot of water during the vegetative period. During the growth and development of tubers, a lot of water is needed. Drought conditions that start from the vegetative phase) negative effect on reproductive growth.

Cultivation using a "leb" system for water supply and drainage costs money and water sources are always available. Crop lands that rely on rainfall as a water source and low nutrient availability, especially P element, will disrupt the function and growth of plant roots. Drought stress is an unavoidable follow-up problem, especially during the dry season. One of the efforts to cultivate shallots on dry land is the use of Arbuscular Mycorrhizal Fungi (AMF).

Mycorrhizae is a form of mutualistic symbiotic relationship between fungi and plant roots. These infections include uptake of nutrients, especially P and better plant adaptation to drought stress. On the other hand, fungi can also meet the necessities of life (carbohydrates and other growing needs) from the host plant (LibreTexts, 2022).

The existence of AMF is very beneficial for plants that grow on acid dry land with shallow roots. In a wire house scale experiment by Susila *et al.* (2018) it was reported that AMF was effective in increasing the growth and yield of shallots with drought treatment. AMF can increase the ability of plants to grow and survive in conditions of limited water and nutrients. The external hyphae of AMF can able to absorb water and nutrients in the soil pores when plant roots are no longer able to absorb water and nutrients. The wide spread of hyphae in the soil causes the amount of water and nutrients taken to increase (Khaliq *et al.*, 2022).

## 2. Methods

The study was carried out for 6 months on a wire house and laboratory scale. Experiment used a completely randomized design with two factors and three replications. The first factor was the sensitive (cv. Brebes) and tolerant (cv. Kuning) varieties of shallots. The second factor was the application of indigenous AMF which consisted of 5 levels, i.e. *Glomus* sp1, *Glomus* sp2, *Glomus* sp3, a mixed those three isolates and control treatment (without applications of AMF*)*. Source of isolates from the results of research by Susila *et al.* (2017). Data analyzed used the Statistical Tool for Agricultural Research (STAR) program. The results of the variance were significantly different, followed by the Duncan New Multiple Range Test (DNMRT) at the 5% level. The morpho-physiological observation parameters included header dry weight, root weight, and leaf proline content. Proline levels were analyzed based on the Bates *et al*. (1973) method. Observations were also made on root colonization with AMF.

## 3. Results and Discussion

### 3.1. Root Weight (g) and Shoot Dry Weight (g)

The symbiosis of shallots inoculated with AMF was able to increase root weight and shoot dry weight header in both sensitive and tolerant varieties. Inoculation of various types of AMF, either applied single or in a mixture, is provided.

Table 1. Root weight and header dry weight in two shallot varieties by inoculation of various types of AMF under drought stress conditions.

| AMF Inoculation | Root weight | | | Header dry weight | | |
|---|---|---|---|---|---|---|
| | Varieties | | Means | Varieties | | Means |
| | Sensitive (cv.Brebes) | Tolerant (cv.Kuning) | | Sensitive (cv.Brebes) | Tolerant cv. Kuning) | |
| | ….g… | | | …..g…. | | |
| Without AMF | 0.84 | 1.15 | 0.99 c | 0.35 | 0.37 | 0.36 c |
| *Glomus sp1* | 1.23 | 1.26 | 1.25 bc | 0.55 | 0.73 | 0.64 b |
| *Glomus sp2* | 1.30 | 1.46 | 1.38 b | 0.58 | 0.89 | 0.74 b |
| *Glomus sp3* | 1.08 | 1.53 | 1.31 bc | 0.80 | 0.61 | 0.71 b |
| Mixed isolate | 2.03 | 2.49 | 2.26 a | 1.06 | 1.17 | 1.12 a |
| Means | 1.296 B | 1.579 A | | 0.67 | 0.76 | |

*The numbers in the column followed by the same lowercase letter, and the numbers in the row followed by the same uppercase letter were not significantly different according to the DNMRT test at a significance level of 5%.*

Significantly different effect with the treatment without AMF inoculation. The mixed isolate treatment (*Glomus sp1*+*Glomus sp2*+*Glomus sp3*) gave the highest root weight and shoot dry weight, followed by single isolate treatment; *Glomus sp2*, *Glomus sp1* dan *Glomus sp3*, gave statistically equal root weights. The contribution of nutrients and water provided by AMF to plants, caused plant growth to increase, including root growth.

The absorption of nutrients from mycorrhizal plants was greater than that of non-mycorrhizal plants because mycorrhizae can reduce the absorption range of nutrients that must be diffused into plant roots. Mycorrhizal plant roots had a wider absorption surface due to the presence of mycorrhizal external hyphae. The association with mycorrhizae can ameliorate the adverse effects of the unfavorable plant-growing environment.

The results of Samanhudi *et al*. (2017) showed that mycorrhizal application had a significant effect on plant dry mass parameters, as well as root length trends and that P nutrient uptake was higher in soybeans inoculated with mycorrhizae compared to treatment without mycorrhizae. This increase in root weight is indicated the potential for AMF colonization to protect plants from drought stress. Al-Hmoud & Al-Momany (2015); Huey *et al*. (2020) stated that mycorrhizal plants are more adaptive to acid soils through increased nutrient uptake and through the formation of protective root membranes.

Roots are organs that play an important role in the absorption of nutrients, water and other minerals. Root growth according to Khalil *et al*. (2020) also responds to water shortages. Lack of water caused shallow root growth to be inhibited because the cells cannot maintain the turgor required for elongation. Deeper roots surrounded by still moist soil that continues to grow. The Increase in root weighted along with the application of AMF inoculation. Treatment without AMF inoculation gave the lowest root weight, proving that there was no assistance from AMF hyphae on water uptake, so the plants were in a state of water shortage. One of the factors that affected root weight/length was water availability. Reinforced by the opinion of Zulkarnain (2018), root growth is the result of the vegetative phase through cell elongation, stimulation of certain hormones and the presence of sufficient water.

Almost all plant growth variables under normal conditions decreased due to the drought stress. Root growth was the most responsive variable to drought stress. Gupta *et al*. (2020) state that one of the responses of plants experiencing drought stress is plants alter their physiology, modify root growth and architecture. One of the abilities of tolerant varieties was to increase root weight to avoid drought stress. According to Swasono (2012b), there was a sharp decrease in sensitive varieties from normal conditions to drought stress conditions by 46.15% compared to tolerant varieties which were only 8.33%. In the tolerant varieties, the root system will reproduce by maximizing exposure to groundwater. Drought conditions increased root retention to prevent water loss due to the absorption by dry soil. Such adaptations can indeed help plants to survive, but in extreme droughts, such adaptations are not sufficient to protect plants (Salisbury & Ross, 1995). In this condition, the role of AMF with the help of its hyphae was highly expected to expand the area of water absorption so that the availability of water was sufficient for plants.

To the increase in growth tended to be determined by the shallot variety. The use of sensitive varieties in drought stress conditions gave significant different effects with tolerant varieties.

Tolerant varieties gave higher scores than sensitive varieties. However, the increase in root weight in sensitive varieties with mixed isolate inoculation treatment (*Glomus sp1+Glomus sp2+Glomus sp3*) gave a higher percentage of 58.62% compared to tolerant varieties, which was 53.82%. This result suggested that AMF inoculation treatment on sensitive varieties was more effective in increasing root weight in shallots under drought stress conditions.

Inoculation of AMF mixed isolate can increase shoot dry weight under drought stress conditions compared to treatment without AMF inoculation (Table 1). The lowest shoot dry weight was indicated by the treatment without AMF inoculation. The highest increase in shoot dry weight was shown by the inoculation treatment of mixed AMF isolates (*Glomus sp1+Glomus sp2+Glomus sp3*) which was 67.68%. There was a significant difference with the application of single isolates with an increase in dry weight (43.76–51.35%), both on sensitive and tolerant varieties. This condition was related to the ability of mycorrhizal plants to absorb nutrients.

Several studies have shown the contribution of AMF to plant growth through the ability to take up nutrients, especially immobilized nutrients (P, Cu and Zn) (Sarkar *et al.*, 2015). In addition to the ability to absorb nutrients, AMF also helped the translocation of these nutrients (Sané *et al.*, 2022). Nutrients that were absorbed through the roots will be carried to the plant parts, especially the leaves for photosynthesis. Assimilation would increase plant biomass and affected the partition assimilate between shoots and roots, which in turn will have an impact on increasing canopy dry weight.

The header dry weight of sensitive and the tolerant varieties of shallots showed no statistically significant difference. In line with the observation of root weight (Table 1), although not statistically significant, there was a tendency for the tolerant variety to show the higher shoot dry weight than sensitive varieties. Pressure on roots due to drought stress resulted in decreased plant growth which was indicated by lower dry weight in the sensitive variety than in the tolerant variety. However, inoculation AMF isolates that was application as single or mix improvement an increase in shoot dry weight, both in sensitive and tolerant varieties. The highest increase in header dry weight was indicated by the inoculation treatment of mixed isolates (*Glomus sp1+ Glomus sp2+Glomus sp3*), namely 66.98% in the sensitive variety and 68.38% in the tolerant variety.

### 3.2. Leaf proline content (μmol/g.bb)

There was no interaction between variety and indigenous AMF on leaf proline content. The experimental results showed that the variety factor showed significant differences in the proline content of the leaves. Under stressed conditions, the leaf proline in sensitive variety accumulated higher than the tolerant variety. This was due to the condition of drought, the tolerant variety was able to adapt by producing higher root weights than the sensitive variety. Therefore, there was only a slight increase in leaf proline in the tolerant variety. Indigenous AMF application, either singly or mixed, did not show a significant difference in leaf proline content compared to no inoculation AMF. However, there was a tendency that the application of mixed isolates (*Glomus sp1+Glomus sp2+Glomus sp3*) to show the lowest accumulated leaf proline content, both in the sensitive and the tolerant variety.

Table 2. Leaf proline content in sensitive and tolerant shallots varieties by inoculation of various types of AMF under drought stress conditions

| AMF Inoculation | Varieties | | Influence AMF inoculation |
|---|---|---|---|
| | Sensitive (cv. Brebes) | Tolerant (cv. Kuning) | |
| | ……… µmol/g.bb | ……… | |
| Without AMF | 16,84 | 7,79 | 12,32 |
| *Glomus sp1* | 13,80 | 11,36 | 12,58 |
| *Glomus sp2* | 12,85 | 12,41 | 12,63 |
| *Glomus sp3* | 12,59 | 10,77 | 11,68 |
| Mixed isolate | 12,35 | 8,61 | 10,48 |
| Influence varieties | 13,69 A | 10,19 B | |

*The numbers in the row followed by the same capital letter were not significantly different according to the DNMRT Test at a significance level of 5%.*

Swasono (2012a) stated that drought stress on coastal sandy soils (at 60% available water content) caused an increase in crown proline content in shallot plants, both in sensitive and tolerant varieties. The increase proline in the sensitive variety was 428.54%, while in the tolerant variety was 225.16%. This situation illustrated that leaf proline was an indicator compound that marks plants experiencing drought stress and a significant increase in the sensitive varieties.

AMF inoculation treatments, either singly or mixed, did not show a significant difference in leaf proline content compared to treatments without AMF inoculation (Table 2). However, there was a tendency that the inoculation treatment of mixed isolates (*Glomus sp1+Glomus sp2+Glomus sp3*) to show the lowest accumulated leaf proline content, both in sensitive and tolerant varieties. This showed the effectiveness of the AMF mix isolate symbiosis with plants, so it was not in a state of stress. Likewise, the treatment of single isolate inoculation did not maximally help plant growth, so the accumulation of proline was almost the same value as the treatment without AMF inoculation on sensitive varieties.

It is to observe that under stressed conditions, AMF application in tolerant varieties increased in leaf proline content. The increase in proline content did not occur in sensitive varieties. This condition indicates that the leaf proline content was not affected by drought stress. The same result was shown by Swasono (2012a), the application of AMF to tolerant varieties resulted in an increase in shoot proline in drought stress (60%), but not in sensitive varieties. It was suspected that only specific proteins (not total proteins) are active in influencing plant tolerance to drought stress in coastal sandy soils.

The adaptability of each plant is different to stress conditions, including drought stress. Sensitive varieties characterized the increase in proline content in the leaves when there was stress. In the unstressed condition, there was no increase in the proline content. This can be seen in the AMF inoculation treatment under drought stress conditions, there was no increase in the proline content due to the plants being under drought stress conditions with the help of AMF. There were differences in the adaptability of the Kuning variety. It showed adaptability with the ability to multiply roots under drought stress conditions. The Condition of shallot plants aged 45 DAP under drought stress conditions on sensitive varieties and tolerant varieties is seen in Figur 1.

As confirmed by Battaglia and Covarrubias (2013), it is revealed that plants try to adapt to drought stress through increased synthesis and activation of certain proteins. According to Battaglia *et al.* (2008), these specific proteins are called LEA proteins (the late-embryogenesis-abundant proteins) which will be active as protectors when plants experience abiotic stress, including drought stress. It was further disclosed that the LEA protein was composed of 11 amino acids arranged in an amphiphilic-helical form, and was present in the leaves in an amount ranging from 0.5-2.5% of the total protein. LEA protein functions in a protection system against superoxide compounds that are often formed in drought-stressed plant conditions. This is in line with the opinion of Das and Roychoudhury (2014) which states that superoxide formed during stress will greatly damage lipids and proteins.

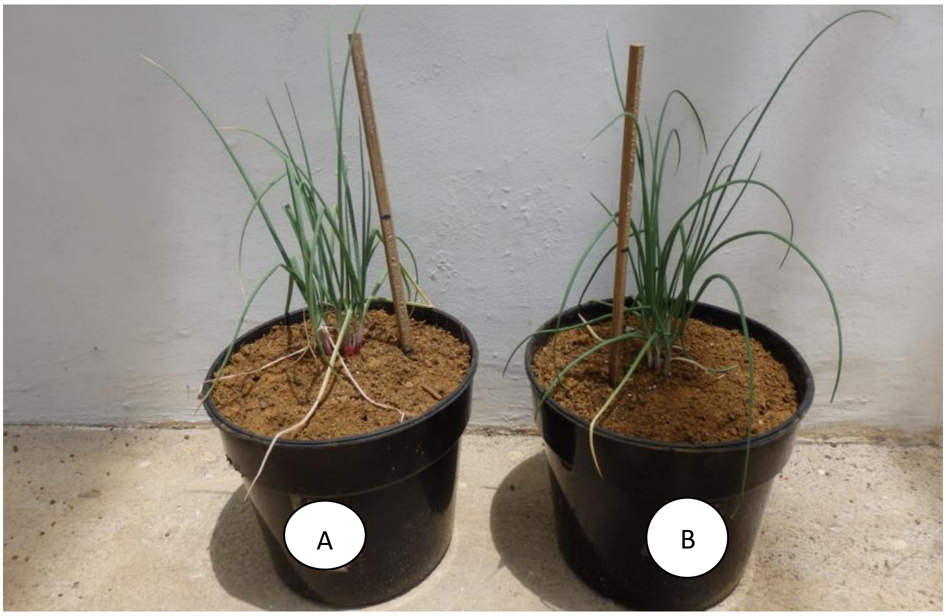


Figure 1. Shallot plants aged 45 DAP under drought stress conditions. Note; (A) sensitive varieties and (B) tolerant varieties

### 3.3. Mycorrhizal Activity on Shallot Roots

There were significant differences in AMF activity in plant roots as seen from the parameters of the infection intensity of sensitive and tolerant varieties (Table 3). Sensitive varieties showed higher infection intensity than tolerant varieties under drought-stress conditions. Swasono (2012a) reported that shallot varieties (sensitive and tolerant) had different responses to drought stress in coastal sandy soils. The something was reported from this experiment that there were differences in the response of shallots (sensitive and tolerant varieties) to acid soils under drought-stress conditions. In the following, Swasono (2012a) said that under drought-stressed conditions, sensitive varieties are more responsive to AMF than tolerant varieties. The application of mixed AMF isolates increases the adaptability of shallot plants to drought stress which is related to increased water and nutrient absorption, especially phosphorus and nitrogen (Rouphael *et al.*, 2015). This can be seen from the activity of AMF (infection intensity) which is relatively high in the roots of shallot plants (Table 3). For more details, AMF colonization on plant roots with high and low intensity can be seen in Figure 2.

Table 3. Colonization of shallot roots by various types of indigenous AMF aged 35 HST under drought stress conditions.

| AMF Inoculation | Infection Percentage | | | | Infection Intensity | | | |
|---|---|---|---|---|---|---|---|---|
| | Varieties | | Influence AMF Inoculation | | Varieties | | Influence AMF inoculaion | |
| | Sensitive | Tolerant | | | Sensitive | Tolerant | | |
| | ….%…. | | | | ……%….. | | | |
| Without AMF | 1.67 | 3.33 | 2.50 | c | 3.33 | 1.67 | 2.50 | d |
| *Glomus sp1* | 93.33 | 86.67 | 90.00 | b | 50.33 | 45.67 | 48.00 | c |
| *Glomus sp2* | 93.33 | 96.67 | 95.00 | ab | 5.67 | 37.67 | 44.17 | c |
| *Glomus sp3* | 96.67 | 100.00 | 98.33 | a | 64.67 | 63.00 | 63.83 | b |
| Mixed isolates | 96.67 | 96.67 | 96.67 | ab | 90.00 | 77.00 | 83.50 | a |
| Influence of varieties | 76.33 | 76.67 | | | 51.80 A | 45.00 B | | |

*Numbers in columns followed by the same lowercase letters, and numbers in rows followed by the same uppercase letters are not significantly different according to the DNMRT test at a significant level of 5%.*

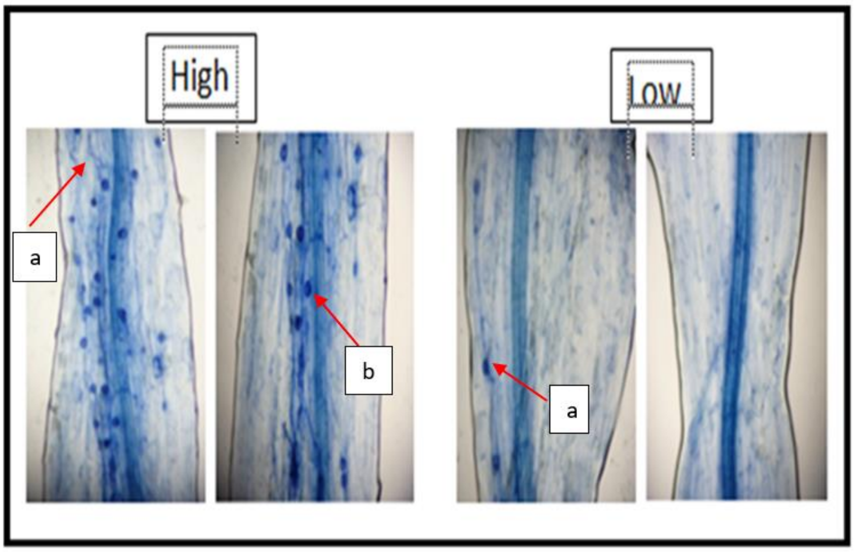


Figure 2. AMF colonization in shallot root with high and low intensity; a) hyfa Internal, b) vesicle, (magnification 100 x)(Source ; Susila *et al*., 2018)

## 4. Conclusions

It can be concluded that under the stressed conditions, the leaf proline content of the sensitive variety accumulated higher in the header than the tolerant variety, because the tolerant variety were able to produce higher root and header weights when adapting than the sensitive. AMF inoculation did not show significant differences with the treatment without AMF inoculation on leaf proline. However, there was a tendency that the inoculation of a mix of AMF isolates (*Glomus sp1+Glomus sp2+ Glomus sp3*) to decrease the proline content in the leaves, both in sensitive and tolerant varieties, so that plants are more resistant to drought stress.

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