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

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Abstract. *VOne 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 aims 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 wire house and laboratory scale. Experiment using a completely randomized design with two factors.The first factor is the Brebes (Sensitive) and Kuning (Tolerant) varieties of shallots. The second factor is the application of indigenous AMF which consists of 5 levels, namely 3 levels are given singly; Glomus sp1, Glomus sp2, Glomus sp3, one level is a mixed isolate and as a control treatment without aplications 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 varieties accumulated higher in the header than the tolerant varieties, because the tolerant varieties were 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 mixed isolate inoculation (Glomus sp1+Glomus sp2+ Glomus sp3) reduced the proline content in the leaves, both in sensitive and tolerant varieties. This shows the effectiveness of the symbiosis of various types of AMF with plants so that plants not under stress.*

Keywords: *Indigenous AMF, morfo-physiological characteristics, shallots, drought stress*

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 alicin compounds (Kumar, Debjit, Hiranjib, Biswajit and Pankaj, 2010).

Agricultural Research and Development Agency (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 onion cultivation, especially in lowland dry land, due to water loss due to high temperatures and large infiltration of sandy soil, resulting in reduced water availability for plants. (Swasono, 2006).

Another problem is when dry land is in the lowlands with wet climatic conditions causes the formation of soil types that react acid (Center for Soil and Agroclimate Research and Development, 2014) 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 (Hidayat, Hikmatullah and Santoso, 2000) According to Hilman (2005), 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. This condition causes the distribution of plant roots to be relatively shallow so that plants are less resistant to drought stress.

Shallots are herbaceous plants that have short roots, but like a lot of water during the vegetative period (Wibowo (2003); Director General of Horticultural Production Development (2003). 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 or fungi can also meet the necessities of life (carbohydrates and other growing needs) from the host plant (Anas, 1997).

The existence of AMF is very beneficial for plants that grow on acid dry land with shallow roots. AMF can increase the ability of plants to grow and survive in conditions of limited water and nutrients. The external hyphae of AMF are 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 (Finlay, 2004).

This study aimed to determine the effect of indigenous AMF inoculation on the morpho-physiological characters of two shallot varieties which are sensitive and tolerant to drought stress conditions.

2. Methods

The study was carried out for 6 months on a wire house and laboratory scale. Experiment using a completely randomized design with two factors.The first factor is the Sensitive (Brebes) and Tolerant (Kuning) varieties of shallots. The second factor is the application of indigenous AMF which consists of 5 levels, namely 3 levels are given singly; *Glomus sp1, Glomus sp2, Glomus sp3,* one level is a mixed isolate and as a control treatment without aplications of AMF. Data were analyzed using 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.

3. Results and Discussion

Root Weight (g) and Top Dry Weight (g)

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

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	tolerant		sensitive	tolerant	
		….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 weightsThe contribution of nutrients and water provided by AMF to plants, causes plant growth to increase, including root growth.

The absorption of nutrients from mycorrhizal plants is 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 have 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 Hanum's research (2007) showed that root dry weight and P nutrient content were higher in mycorrhizal soybeans compared to treatment without mycorrhizae. This increase in root weight indicates the potential for AMF colonization to protect plants from drought stress. Howeler, Sieverding and Saif (1987), 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 Campbell, Reece and Mitcell (2003), also responds to water shortages. Lack of water causes 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. Increase in root weight 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. Lee (2012) stated that one of the factors that affect root weight/length is water availability. Reinforced by the opinion of

Zulkarnain (2010), 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 drought stress. Root growth is the most responsive variable to drought stress. Tardieu (1996) states that one of the responses of plants experiencing drought stress is marked by inhibition of root growth. One of the ability of tolerant varieties is 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 tolerant varieties, the root system will reproduce by maximizing exposure to groundwater (Campbell *et al*., 2003). Drought conditions increase root retention to prevent water loss due to 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 and Ross, 1995). In this condition, the role of AMF with the help of its hyphae is highly expected to expand the area of water absorption so that the availability of water is sufficient for plants.

The increase in growth tends to be determined by the onion variety. The use of sensitive varieties in drought stress conditions gave significantly 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 means that AMF inoculation treatment on sensitive varieties was more effective in increasing root weight in shallots under drought stress conditions.

Inoculation of AMF isolates singly or mixed can increase canopy dry weight under drought stress conditions compared to treatment without AMF inoculation (Table 1). The lowest canopy dry weight was indicated by the treatment without AMF inoculation. The highest increase in canopy dry weight was shown by the inoculation treatment of mixed AMF isolates (*Glomus sp1+Glomus sp2+Glomus sp3*) which was 67.68%, significantly different from the application of single isolates with an increase in dry weight (43.76–51.35%), both on sensitive and tolerant varieties. This condition is 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, Asaet, Wang and Rasyid. 2015). In addition to the ability to absorb nutrients, AMF also helps the translocation of these nutrients (Al-Karaky, 2000). Nutrients that are absorbed through the roots will be carried to the plant parts, especially the leaves for photosynthesis. Assimilate will increase plant biomass and affect the partition of assimilate between shoots and roots (Baon, 2000), which in turn will have an impact on increasing canopy dry weight.

The header dry weight of sensitive and tolerant varieties of shallots showed no statistically significant difference. In line with the observation of root weight (Table 1), there was a tendency for tolerant varieties to show higher crown dry weight than sensitive varieties, although not statistically significant. Pressure on roots due to drought stress resulted in decreased plant growth which was indicated by lower dry weight in sensitive varieties than in tolerant varieties. However, single or mixed inoculation of AMF isolates gave an increase in canopy 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 sensitive varieties and 68.38% in tolerant varieties.

Leaf proline content (μmol/g bb)

There was no interaction between varietal treatment and indigenous AMF on leaf proline content. The experimental results showed that the varietal factor showed significant differences in the proline content of the leaves. Under stressed conditions, the proline in the leek sensitive varieties accumulated higher than the tolerant varieties. This is due to the condition drought, tolerant varieties were able to adapt by producing higher root weights than sensitive varieties. Therefore, there was only a slight increase in leaf proline in the tolerant varieties. 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*) showed the lowest accumulated leaf proline content, both in sensitive and tolerant varieties.

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

AMF Inoculation	Varieties		Influence AMF inoculation
	Sensitive	Tolerant	
	……… 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 in proline in the sensitive variety was 428.54%, while in the tolerant variety, the increase in proline was 225.16%. This situation illustrates that leaf proline is 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 without AMF inoculation (Table 31). However, there was a tendency that the inoculation treatment of mixed isolates (*Glomus sp1+Glomus sp2+Glomus sp3*) showed the lowest accumulated leaf proline content, both in sensitive and tolerant varieties. This shows the effectiveness of the mixed isolate symbiosis with plants, so that the plants are not in a state of stress. Likewise, the treatment of single isolate inoculation did not maximally help plant growth, so that the accumulation of proline was almost the same value as the treatment without AMF inoculation on sensitive varieties.

It is interesting 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 is not affected by drought stress. The same result was shown by Swasono's study (2012a), the application of AMF to tolerant varieties resulted in an increase in crown proline in drought stress (60%), but not in sensitive varieties. It is 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 are differences in the adaptability of the Yellow variety. The Yellow variety showed adaptability with the ability to multiply roots under drought stress conditions.

As confirmed by Pattanagul and Madore (1999) which revealed that plants try to adapt to drought stress through increased synthesis and activation of certain proteins. According to Holmberg and Bullow (1998) 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 Cornic and Briantais (1991) which states that superoxide formed during stress will greatly damage lipids and proteins.

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

4. Conclusions

From the observations, it can be concluded that under stressed conditions, the leaf proline content of the sensitive varieties accumulated higher in the header than the tolerant varieties, because the tolerant varieties were 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 mixed isolate inoculation (*Glomus sp1+Glomus sp2+ Glomus sp3*) reduced the proline content in the leaves, both in sensitive and tolerant varieties. This shows the effectiveness of the symbiosis of various types of AMF with plants so that plants are not under stress.

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