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# The Role of Mycorrhizae on Seedlings and Early Growth of Sugarcane

*Wawan Sulistiono and Taryono*

## Abstract

The role of mycorrhizae in plant growth is well known, such as the ability to increase nutrient uptake, especially phosphate (P), drought tolerance, and resistance to pathogens. It is necessary to understand the application of arbuscular mycorrhizal technology in industrial plant production systems and their impact on agriculture systems. Large-scale nurseries of plantations require proper mycorrhizal application techniques. The relationship of mycorrhizal infection with plant yield (biomass) is known and in the next step, appropriate application time is needed to increase the effectiveness of mycorrhizae in plant growth and yield. Application of mycorrhizal inoculum was more effective in increasing the biomass of sugarcane stem weight to reach 61% with an increase in infection of 41.3%. In addition, the mycorrhizal application increases the root growth of sugarcane seedlings. The root growth promoting ability is important to increase the initial growth of plants after transplanting in dry land under the influence of drought stress, limited nutrients. The application of this technology is expected to increase plant growth, facilitate the maintenance and efficiency of cultivation on an industrial scale.

**Keywords:** mycorrhizae, seedlings, early growth, industrial crops, plantation

## 1. Introduction

Arbuscular mycorrhiza (*Zygomycetes*) is a symbiotic form of mutualism between fungal mycelium and higher plant roots. It is estimated that more than 80% of the higher terrestrial plants have a symbiosis with mycorrhizae [1]. The arbuscular mycorrhizae are known as vesicular-arbuscular mycorrhizae (VAM). VAM infects from outside the root into the root tissue and enter the root cells to form vesicles and arbuscules [2]. Arbuscules is a network of hyphae that penetrates between root cells and plasmalemma. Arbuscules help transport of nutrients to plant cells, especially P elements. In the root cells, the hyphae also form vesicles, small bubbles (granules) in the cytoplasm that contain lipids as a means of asexual reproduction of mycorrhizae. The vesicle plays a role during the reproduction, and when cells are ruptured at damage [2, 3]. Arbuscular mycorrhizae fungi (AMF) have very wide distribution in terrestrial ecosystems in terms of host plants, climate, and soil types [4]. Mycorrhizal infections will change the morphology of plant roots and nutrient absorption. This because their structures ensure the physical expansion of roots and absorption of nutrients from the soil and increase the flow of nutrients to plants [2, 3].

The role of mycorrhizae becomes important in sub-optimal land, dry land and for sustainable agriculture. Utilization of mycorrhizae, especially for plant growth, soil fertility and mitigation of drought stress by heat and climate change. Mycorrhiza becomes a component of future technology for sustainable agriculture [5, 6].

Mycorrhizae in agricultural land, especially sub-optimal land, functions to reduce soil erosion and leaching of nutrients. This condition is caused by the faster nutrient cycling mechanism. Besides, the absorption of nutrients is more due to the higher root surface area, which causes long-term soil fertility or soil productivity [5–7]. The presence of mycorrhizae in sub-optimal dry land of plantation crops is useful for renaturation and afforestation, namely stabilizing degraded land and eroding the soil surface [8]. In areas with high rainfall, plants in symbiosis with mycorrhizae also increase ecosystem repair by reducing the leaching of elements in the soil. Mycorrhizae will suppress the loss of nitrogen (N) and P elements by 40% and 50%, respectively in soil [9].

Mycorrhizal inoculation is important in dealing with drought stress and preparing plants for good growth in the field. Treat mycorrhizal inoculation on plantation seedlings to produce plants that have better root morphology and plant growth. These include root surface area in early coconut growth [10], root length, root diameter, root dry weight, and root dry weight ratio, root surface area, and shoot growth of sugarcane seedlings [11]. Likewise, mycorrhizae play a role in accelerating the growth or emergence of secondary roots in sugarcane seedling [12]. Mycorrhizae also appeared to have a significant role in increasing the growth of forest plant seedlings in the nursery, the increase in leaf chlorophyll content, photosynthesis rate, NPK content in root, stem and leaf compared to plants without mycorrhizal inoculation [13].

Thus mycorrhizal inoculation in plantation crops is needed as an effort to mitigate environmental stresses, both drought and high rainfall. In addition, the impact of mycorrhizal inoculum will increase the nutrients cycle in the soil, prevent excessive leaching of nutrients so that it plays a role in afforestation. In mycorrhizal inoculation, the inoculation time and dose are important. The optimal time of mycorrhizal inoculation is plantation seedlings in the nursery which will increase their colonization by 46% compared to field application [14].

The inoculation of mycorrhizae on seedlings of seedlings is expected to increase the morphological performance and plant physiological performance for early growth and morphological properties, increase the adaptive ability of plants to environmental stress. Based on the above, there are several important benefits for using mycorrhizal inoculation in the nursery of industrial plants.

## **2. Determinants of colonization and colonization patterns of plantation seeds**

Before the inoculation of AMF on plantation seeds, it is necessary to know the determinants of colonization and the pattern of colonization. According to Sieverding [15], the process of colonization or infection progresses through 6 stages, namely: (1) pre-infection, at this stage, the spores are not yet active and AMF hyphae are in the soil; (2) penetration of the fungus to the roots. (3) arbuscules and vesicle formation. Arbuscule is formed after 2–5 days from penetration in the form of a strong band of hyphae growing around the cell plasmalemma. The vesicle at the tip of the hypha consists of lipids and fungal organs; (4) fungal elongation in roots and rhizosphere; (5) Spread of fungi to the soil. Hyphae grow out of

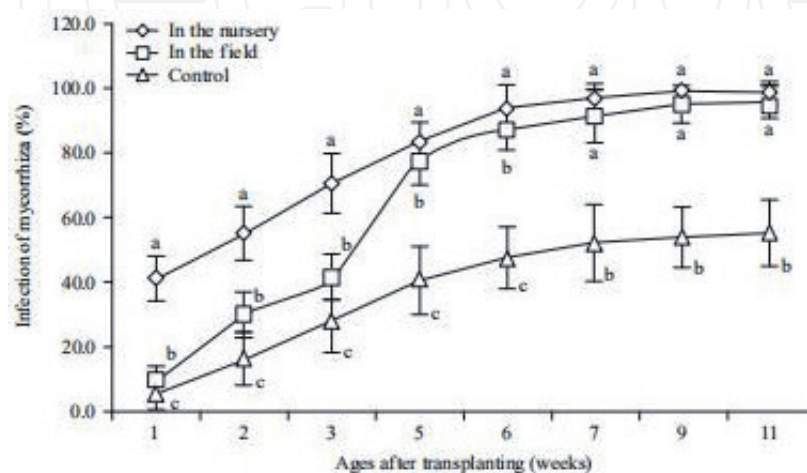
the roots. The hyphae in the rhizosphere form the “external mycelium”; (6) culture of AMF structures into the form of resting spores on the external mycelium.

The elongation of fungi in the roots and rhizosphere consists of 3 stages, namely (a) slow phase, when infection to the target roots begins; (b) an exponential growth phase, maximum at 40 days after infection; (c) slowed growth phase, “plateau phase” balance [15]. Meanwhile, according to the observations of Sulistiono et al. [14] the colonization of sugarcane seedlings will experience a sharp increase at the age of 5–10 days after inoculation, then it will be constant at the age of 10–30 days after inoculation. An interesting point was also conveyed by Sulistiono et al. [14] that the tendency of AMF inoculation of sugarcane seeds in the nursery would result in higher colonization than inoculation carried out in the field when sugarcane at the age of 1–9 weeks after transplanting. However, after 9 weeks of age, the colonization rates of the two differences in inoculation time were similar. This indicates an equilibrium point for colonization or the development of infection at the root (**Figure 1**).

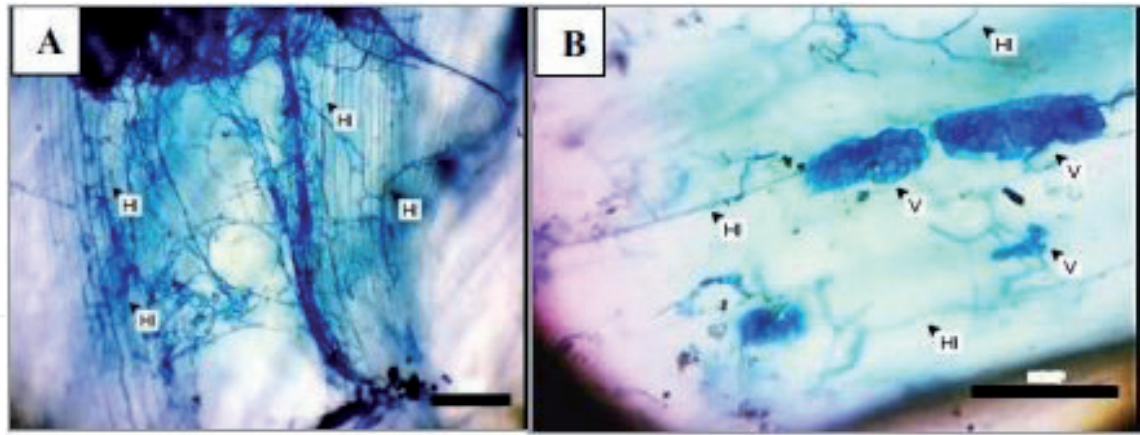
From the results of **Figure 1**, it shows that AMF inoculation in the nursery has several advantages, including:

1. Accelerate colonization when transplanting in the field
2. The AMF inoculated seeds has better growth of roots and shoots of plants
3. It has better adaptability to environmental stresses in the form of soil moisture and low nutrients, and diseases
4. Easy to apply

The higher colonization at the early growth of sugarcane was due to the effect of inoculation time which was applied in the nursery. AMF has infected and further developed which arbuscule and vesicle structures have formed [15] In the nursery, the colonization was optimal at 10–30 days after inoculation [12]. This is characterized by the formation of vesicles and arbuscules. The arbuscules and vesicles forms indicate symbiosis has occurred. This is because arbuscule are used for the transportation of nutrients from AMF to the root cells of host plant, especially P and vesicles are the reproductive organs of AMF and as a food reserve. One of the vesicles or arbuscules on the roots of sugarcane in the nursery as in **Figure 2**.



**Figure 1.**  
The pattern of AMF colonization at different inoculation times.



**Figure 2.** Colonization on sugarcane bud chips at the age of 10 (A) and 20 days after inoculation (B). Hi: Hyphae; V: Vesicles. Scale bar: 10  $\mu$ m. Objective 10 $\times$ .

Therefore, AMF inoculation at seedling time results in an earlier infection growth process. This is indicated by the presence of hyphae structures since the age of 10 days after inoculation and vesicles at the age of 10 days after inoculation [12].

In the next stage, after the AMF structure is formed, it will accelerate the growth of secondary roots in sugarcane seeds, which was significantly different from the control (without inoculation) (**Table 1**).

Secondary roots in sugarcane seedlings are bigger roots and have a role to support the plant's upright and optimal absorption of nutrients. Thus, AMF inoculation in the nursery has the potential to increase plant growth (sugarcane) after transplanting. This is due to an increase in the number of secondary roots that are larger in diameter and also stronger [12].

Seeds/seedlings that have been inoculated with AMF in the nursery will have better growth properties in terms of leaf area, chlorophyll content, photosynthesis rate, and stem biomass in post-transplanting sugarcane seeds. This is because the application of AMF in the nursery increased the colonization by 41.3% at 7 days after transplanting and had the effect of increasing stem biomass from 11 to 61% (depending on sugarcane variety). This condition shows that there is a positive correlation between the rate of colonization and the weight of stem biomass, namely  $r = 0.54$  [14].

AMF inoculation since seedling in forest plants (*Gleditsia sinensis* Lam) was also reported to increase seedling height, stem diameter, dry weight of seed biomass, chlorophyll content, photosynthetic rate, and NPK content in root, stem, and leaf tissue [13]. Likewise, inoculation of AMF in nurseries on tropical plant seeds was also reported to increase plant height, root diameter, and biomass [16] as well as N and P content of seedlings and root dry weight in forest plant seedlings [17].

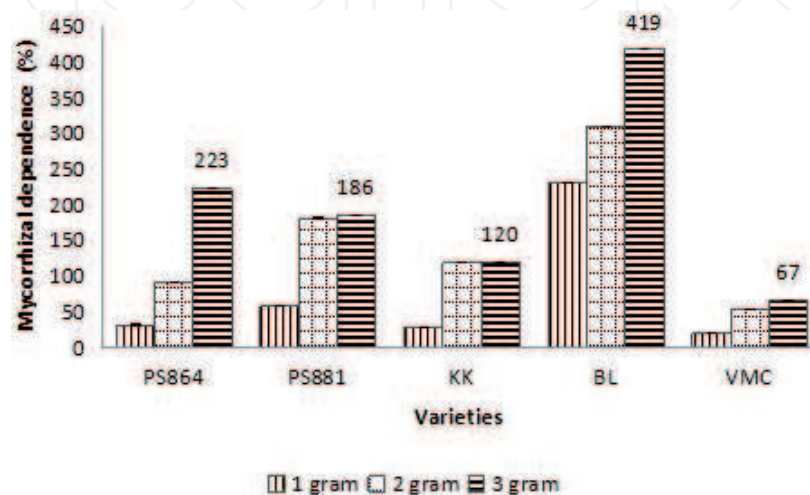
AMF inoculation in perennial/industrial plant nurseries aims to prepare conditions for optimal growth factors, early symbiosis in the rhizosphere. This is because in 7–10 days the AMF structure has been formed, namely hyphae, vesicles, or arbuscule (**Figure 2**) [12]. With this difference in root symbiosis, plants can grow more optimally, uniformly, and faster. In this condition, it will provide an opportunity for healthy seed selection before transplanting in the field.

In the AMF inoculation treatment in the nursery, the things that need to be considered are the inoculum dose and the variety response. For plantation crops such as sugarcane, the optimal inoculation of AMF as much as 2 g of inoculum/seed or 7.8 spores/seedlings. This treatment resulted in significant root growth characteristics, shoot: root ratio and leaf P concentration compared to control [12].

Doses of AMF (g/seeds)	Number of secondary roots
0	1.60 b
1	2.80 ab
2	4.00 a
3	3.95 a

Remarks: Different letters in same column represent significant differences by Duncan's multiple range test at 5% level.

**Table 1.**  
 The effect of AMF doses level on number of secondary roots at the age of 40 days after inoculation and sowing.



**Figure 3.**  
 Mycorrhizal dependence on several varieties of sugarcane.

The application of AMF inoculum is attempted in an optimal amount, in the right dose. The application of a higher dose will cause it to be less economical for a larger scale/volume.

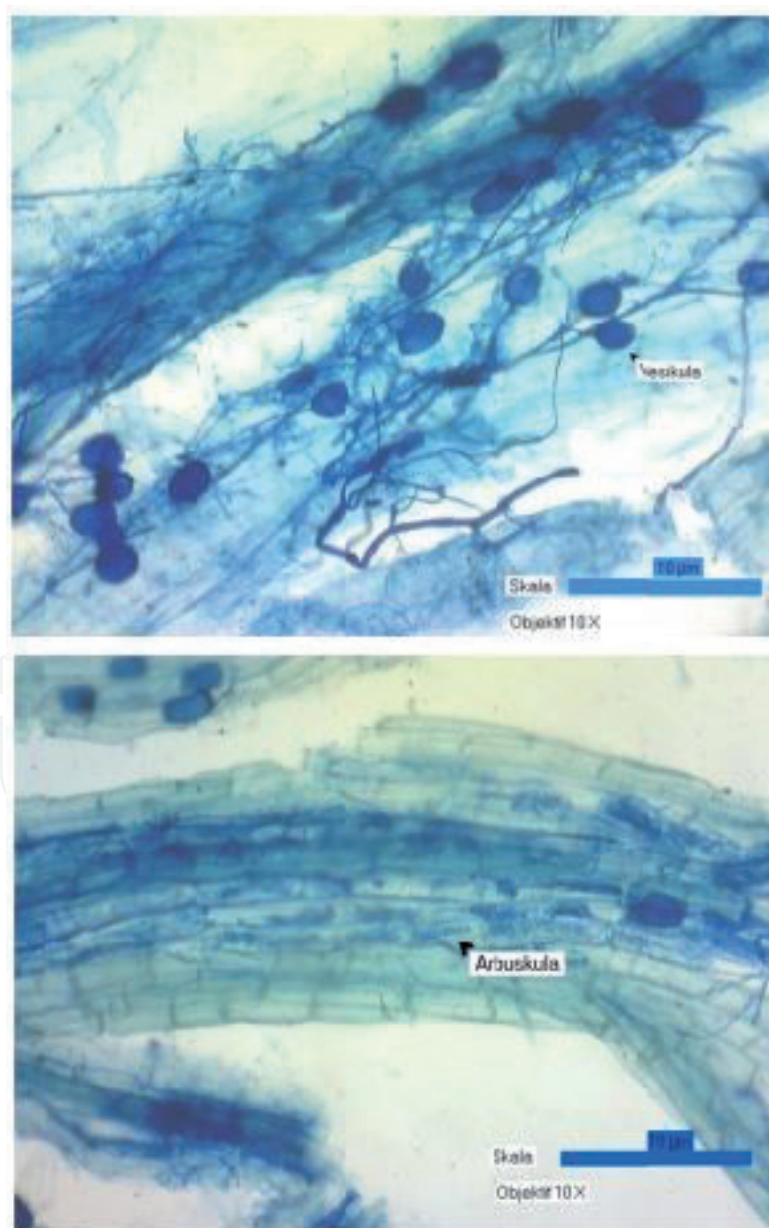
AMF inoculation of industrial plant seedlings in the nursery needs to consider several limiting factors so that the colonization rate is optimal. Environmental factors are prepared since in the nursery. Environmental factors that determine the level of symbiosis with AMF, namely: (1) Light. Konvalinkova and Jansa [18] reported that the decreasing light intensity will decrease mycorrhizal growth (AMF) and decrease P transfer by AMF to host plants. This is because the availability of an energy source in the form of carbon is not sufficient for AMF and plant symbiosis. The light intensity which is only below 65% of a full-beam with 14–84 days shading time decreases the development of AMF in the root transfer of P elements from AMF to the host plant [18]. (2) soil temperature. The optimal soil temperature for AMF symbiosis with host plants is 20° C as indicated by the percentage of arbuscules and vesicles. An increase in temperature of 23–30° C causes a decrease in the arbuscules and vesicles formation [19]. (3) Elemental content of P, The addition of P into the soil showed a decrease in the percentage of mycorrhizal colonization [20]. (4) Host plants. The host plant is in the form of age, species, or variety [20, 21]. Different types of varieties respond to mycorrhizal inoculation as presented in **Figure 3**.

**Figure 3** shows that genetically different varieties (sugarcane) have different mycorrhizal response [22]. These results can be used as the basis for selecting varieties for transplanting in the dry land. It can be concluded that:

1. Mycorrhizal inoculation to increase root and shoot growth
2. Preparation of a nursery that supports the symbiosis of AMF with plants
  - a. Adjustment of nursery shade for colonization activities
  - b. Setting the temperature of the media and nursery room for colonization
  - c. Regulation of nutrient content, especially soil P, it should not excess.

### **3. Transplanting mycorrhizae inoculated seedlings for sustainable agriculture at adverse conditions**

Mycorrhizal inoculation in plantation crops aims to promote good early growth and tolerate environmental stresses. A report shown that AMF inoculated seedlings



**Figure 4.**  
*The structure of arbuscules and vesicles and hyphae formed at the roots of plants infected with mycorrhizae. Field observations.*

were then transplanted had increased leaf nitrate reductase activity (NRA) and root surface area in early coconut growth [10].

The increase in colonization with the formation of arbuscules and vesicles in early plant growth indicated that the symbiosis was optimal (**Figure 4**) [14]. This condition causes the host plant to obtain P elements from AMF transfer, more nutrient uptake by hyphae elongation and plant root structure, thus the plant experiences more optimal growth. Planting mycorrhizal inoculated plant seedlings is to increase nutrition in plants, especially P in cropping area [8], reduce nutrient loss due to leaching [6] so as to support sustainable agriculture [3, 8].

More stable nutrients available in mycorrhizae inoculated plant area resulted in increased soil productivity. Many nutrients are bound by the AMF structural system because there is a glomalin system. Glomalin as a glycoprotein forms chelates with inorganic P. Besides, the hyphae structure is more abundant which can directly absorb more nutrients, especially P [6]. Thus the AMF mycorrhizal inoculation treatment is a mitigation measure against climate change so that plants will continue to grow and survive.

#### **4. Conclusion**

AMF inoculation on seedlings is increased the root and shoot growth as well as increased the colonization. The nursery location is adapted for AMF growth and symbiosis. Plants infected with mycorrhizae had better growth (roots and shoots) ability after transplanting the seedlings. Besides that, it can reduce nutrient loss and maintain soil fertility so that it is an effort to mitigate climate change.

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#### **Conflict of interest**

I declare that I have no conflict of interest as an author on the financial and intellectual processes of the entire manuscript.



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