Conference Paper

Growth of Andalas (*Morus macroura* Miq.) seedlings from shoot cutting inoculated with Arbuscular Mycorrhiza Fungi

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Abstract

Andalas is the mascot flora of West Sumatera because its closely related to Minangkabau's Culture. Andalas stems are used as a material for "Rumah Gadang's pole" which is a traditional house of Minangkabau. Exploitation and its nature as a dioecious plant cause the population to be increasingly threatened. Shoot cutting is an alternative to multiply this plant and Arbuscular Mycorrhiza Fungi is commonly used to increase roots growth in cuttings. The research about the growth of Andalas (*Morus macroura* Miq.) seedlings from shoot cutting inoculated with Arbuscular Mycorrhiza Fungi was conducted from March to August 2016 at green house and Plant Physiology and Tissue Culture Laboratory, Biology Department, Mathematics and Natural Science Faculty, Andalas university, Padang. The aim of this research was to found the compatible types of AMF to increase the growth of Andalas seedlings from shoot cutting. The research used Completely Randomized Design (CRD) with 4 treatments and 6 replications. The treatments were without inoculant (control), *Glomus* sp., *Gigaspora* sp. and *Acaulospora* sp. The result showed that *Acaulospora* sp. significantly increased the height of plant, the length of root and very high criteria of mycorrhiza dependency.

Keywords: Arbuscular Mycorrhiza Fungi, *Morus macroura* Miq., Shoot cutting

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1. Introduction

Shoot cutting is one of the method in vegetative reproduction. Vegetative reproduction is which multicellular structures become detached from the parent plant and develop into new individuals that are genetically identical to its parent plant [1]. Vegetative reproduction is more effective and efficient because it can produce the amount (mass) of seed in a short time. Vegetative reproduction with shoot cuttings method is one of good ways in plants nursery, especially Andalas (*M. macroura* Miq.). Andalas is a dioceous plant in which the male and female reproductive structures are found in different individuals [2]. According to Amperawati and Sapulete [3], the reproductive system of Andalas often do not coincide between the availability of pollen and the stigma that affecting the process of pollination. These obstacles cause the generative multiplication to be very difficult to do. The alternative that is expected for the multiplication of Andalas plants is vegetatively through shoot cuttings. However, roots formed in cuttings are short and have a few roots. The short roots cause low absorption of nutrients, less effective and efficient and the plant become vulnerable to environmental influences especially in harsh environment [4]. These obstacles caused plants experienced the stress which lead into mortality of seedlings after planting [5].

Improving the quality of growing plants is one way to guarantee success in overcoming the limiting factors in the environment [6]. Improving seedling quality can be pursued by providing seedlings with AMF [7]. AMF is a mutualistic symbiosis between fungi and the roots of high-level plants. AMF on shoot cuttings plays a role in initiating adventitious root formation. It has an ability to change the morphological architecture of plant roots because it synthesizes auxins released around the base of cuttings that affect changes that involved in adventitious root formation through hormone-mediated [8].

Various types of AMF that was given to pepper cuttings seedling infecting and increasing the growth of seedlings. *Glomus manihotis* was the best type that produces the highest percentage and intensity of infection (32.72% and 1.28%) and significantly increases the stem length, number of leaves, leaf area, number of roots and dry biomass of pepper cuttings, and significantly shortens the nursery [9]. On the other research, the inoculation of *G. etunicatum* on Muna Teak cuttings (*Tectona grandis* Linn.) was able to change the system pattern of the adventitious roots of Muna Teak shoots cuttings as well as IBA [10]. Based on the description above, a research was conducted to determine the effect of AMF on the growth of Andalas seedlings from shoot cuttings which aimed to determine the type of AMF that compatible to increase the growth of Andalas shoot cuttings.

2. Materials and Methods

The study was conducted from March to August 2016 at the Greenhouse and the Research Laboratory of Plant Physiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang. This study used an experimental method that was compiled in a Completely Randomized Design (CRD) with 4 treatments (Without mycorrhizae / control, *Glomus* sp., *Gigaspora* sp. and *Acaulospora* sp.) with 6 replications.

2.1. Preparation of media for planting

Sand mixed with compost in a ratio of 2: 1 and put in a polybag with the same volume for cuttings seedling. For seedlings with inoculant treatment used topsoil as palnting media.

2.2. Propagation of Andalas cuttings

Cuttings are taken by slanting 45^0 with sharp and clean blades so the slice surface is wider and more roots are formed. The cuttings were soaked in IBA for $\pm$ 15 minutes, then transferred to the planting media.

2.3. Preparation of AMF inoculants

FMA inoculants are obtained from Seameo Biotrop. To determine the weight of the inoculant to be applied, the spore density calculation must be done in advance. The calculation of spore density based on Mansur (2014) [11].

2.4. Inoculation

AMF is inoculated by spreading AMF inoculants to the area around the roots according to the treatment given and covered with the planting media.

2.5. Observation parameters

2.5.1. Percentage of AMF infection

The calculation of the percentage of AMF infections based on Sieverding (1991) [12] technique. The percentage of infections is calculated using the equation (Equation 1) and the percentage of AMF infections was catogorized based on its values (Table 1).

$$\text{Infection percentage} = \frac{\text{Number of infected roots}}{\text{Number of observed roots}} \times 100 \quad (1)$$

Table 1: Criteria for the Effectiveness of the Degree of Root Colonization.

The percentage of infections	Criteria
76 – 100 %	Very High
51 – 75 %	High
27 – 50 %	Medium
6 – 26 %	Low
0 – 5 %	Very Low
(Sieverding, 1991).	

2.5.2. The increase of plants height, number of leaves, length and number of roots

The heigh of plant was measured from the base of the plant to the point where it grows. As for the increase in the number of leaves, the calculated leaves are the leaves that have perfectly opened. Root length is measured from the base to the tip of roots.

2.5.3. Dry biomass

Calculation of biomass is carried out at the end of the observation. The weight sample of wet plants consisting of roots, stems and leaves is cut into pieces and put in an oven at 80 °C until the weight is constant.

2.5.4. Mycorrhiza dependency

The calculation of the mycorrhiza dependency based on Habte and Manjunath (1991) [13]. Mycorrhiza dependency is calculated using the equation (Equation 2) and mycorrhiza dependency was catogorized based on its values (Table 2).

$$\text{MD} = \frac{\text{BM of Inoculated plant} - \text{BM of Noninoculated plant}}{\text{BM of Noninoculated plant}} \times 100\% \quad (2)$$

Descriptions:

MD = *Myccorrhiza Dependency*

BM = Dry Biomass

TABLE 2: The criteria of *Myccorrhiza Dependency*.

Values of *Myccorrhiza Dependency*	Criteria
> 70 %	Very high
50 – 75 %	High
25 – 50 %	Medium
< 25 %	Low
0 %	Nil
(Habte and Manjunath, 1991).	

2.6. Analysis

Data analysis was carried out on the average increase in plant height, average number of leaves, average number and length of roots and biomass by using variance analysis, the significantly different result was analysis with Duncan's New Multiple Range Test (DNMRT) at $p < 0,05$ level test. As for the percentage of AMF colonization and plant dependence on mycorrhiza, it was carried out by descriptive test.

3. Results and Discussions

3.1. Percentage of FMA colonization and plants mycorrhiza dependency

TABLE 3: The percentage of AMF colonization at Andalas (*M. macroura* Miq.) seedling from shoot cuttings And mycorrhiza dependency.

Treatments	Root Colonization (%)	Criteria	*Myccorrhiza Dependency* (%)	Criteria
Glomus sp.	43,3	Medium	17,59	Low
Gigaspora sp.	60,0	High	54,62	High
Acaulospora sp.	56,6	High	91,67	Very high

The percentage of colonization of seedling roots of Andalas from shoot cuttings shows medium-high criteria. The criteria for high mycorrhizal colonization were found in *Gigaspora* sp. and *Acaulospora* sp. (Table 3). The high level of infection shows high adaptability of *Gigaspora* sp. and *Acaulospora* sp. against Andalas plants. Suitability

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of host plants with mycorrhizae will cause spores to infect roots, germinate, and then spread more widely to other plant root tissues [14]. Infection in the root of Andalas seedlings from shoot cuttings is possible due to the presence of plant root exudates that stimulate the growth of mycorrhizal inoculants. Root exudates contain carbohydrates, amino acids and other substances needed by mycorrhizae [15]. In this associations, AMF derives photosynthate from the host plant and host plant gains the nutrients through AMF [16]. The different percentage of infections and plants responses to AMF highly dependent on the compatabilty between AMF and host plan species [17].

Mycorrhizal infection is known by the formation of structures such as hyphae, mycelia, arbuscus, vesicles or spores [18]. Hyphae is a structure to absorb nutrients, while mycelia is a collection of hyphae. The arbuscule is the structure of hyphae for nutrient exchange between symbiotic plants and mycorrhizae. The vesicle is an oval-shaped structure, containing fatty fluid, as a food storage organ or develops into klamidospora, which is a reproductive organ and structure of to maintain the life of fungi [19]. From the results of observations found several mycorrhizal structures in the roots of Andalas seedlings from shoot cuttings, hyphae and vesicles (Fig.1).

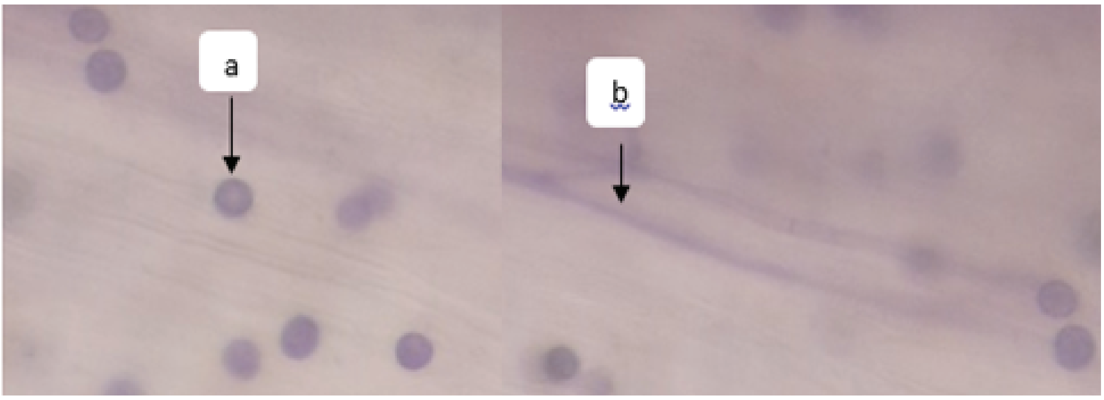

Figure 1: Seedling roots of Andalas from shoot cuttings colonized by mycorrhizae (a) hyphae (b) Vesicles (4×10 Magnification).

The dependence of seedlings from the shoot cuttings of Andalas plants on mycorrhizae ranged from 17.59 to 91.67% which was categorized as low-very high criteria, which is *Acaulospora* sp. showed very high criteria for mycorrhizal dependency (Table 3). This shows that the type of *Acaulospora* sp. have a symbiotic ability and better compatibility with Andalas seedling from shoot cuttings. The differences in compatibility levels are caused by exudates of glucose and organic acids found in the roots and types of AMF [20]. High compatibility causes *Acaulospora* sp. more able to increase the growth of shoot cuttings Andalas. The high dependency of shoot cuttings on mycorrhizae was also caused by the root system formed on shoot cuttings. The roots formed in the cuttings are fewer and have short roots which cause the absorption of nutrients and water are less effective and efficient [4]. Plants that has high dependence on mycorrhizae will showed a well growth performance and when there is no association with mycorrhizae the plant will show imperfect growth. The high mycorrhizal infection in plant roots does not necessarily indicate high dependence on mycorrhizae [21].

3.2. The average increase of height plant, number of leaves, root length and number of roots

The results of analysis of variance on the average increase of height plants, the increase in the number of leaves, root length and number of roots in each treatment (Table 4).

Table 4: The average increase of height, leaves number, length and number of roots from Andalas shoot cuttings seedlings inoculated with Arbuscular Mycorrhizal Fungi.

Treatments	height (cm)	leaves number (sheet)	roots length (cm)	roots number	Shoots Biomass (g)	Roots Biomass (g)
Control	12,15 b	5,50 a	17,80 b	12,00 a	0,94 a	0,14 a
Glomus sp.	10,69 b	5,67 a	21,02 ab	13,00 a	1,09 a	0,18 a
Gigaspora sp.	11,45 b	6,83 a	18,73 b	15,00 a	1,45 a	0,22 a
Acaulospora sp.	19,50 a	6,83 a	28,85 a	15,00 a	1,80 a	0,27 a

The treatment followed by the same letter in the same column is not significantly different at $p < 0,05$ level by Duncan's Multiple Range Test

The results of the analysis of variance showed that the inoculant treatment of *Acaulospora* sp. gives a significantly different effect on plant height and root length. *Glomus* sp. And *Gigaspora* sp. does not show a significantly different effect than control (Table 4). This shows that *Acaulospora* sp. has better compatibility with Andalas seedling from shoot cuttings compared to other inoculants found in the treatment. It is more efficient in increasing the height of seedlings from Andalas shoot cuttings. Symbiotic relationship between plants and mycorrhizae is strongly influenced by the level of compatability type of mycorrhizal fungi with host plants [9]. The compatability of mycorrhizae in association with plants is influenced by several things such as the environment, mycorrhizal species and types of plants [22].

Compatible mycorrhizae will be more effective in optimizing the growh of plants. Compatible isolates can support symbiosis to work effectively [23]. The symbiosis between mycorrhizae and plants can maintain the balance of the physiological processes of plant [24]. *Acaulospora* sp. as one type of mycorrhizae will be able to increase the absorption of macro nutrients and micro nutrients. Increased nutrient uptake is a result of the formation of thick sheaths of hyphae and increased absorption [25]. Mycorrhiza also plays a role in stimulating the formation of growing hormones, such as cytokines, gibberellins and auxins, this hormones able to increase plant height and root length. Cytokinin and auxin hormones play a role in cell division and elongation, while giberelin is more dominant in stimulating the extension of the inter node, causing an increase in plant height [26].

The association between *Acaulospora* sp. with the roots of Andalas shoot cuttings caused the formation of hyphae that was able to increase the ability of plant roots to get nutrients in the soil, especially P elements. The principle of mycorrhizae is to infect the root system of the host plant, producing hyphae intensively so that the plants containing mycorrhizae will be able to increase capacity in nutrient absorption [27]. The supply of nutrients increasing the activity of cell protoplasm and it supports cell to growth [28].

In the results it is known that although *Acaulospora* sp. showed a significantly different effect on plants height, but there was no statistically significant difference in the number of leaves. This is because the seedlings have a fairly wide gap between the nodes, so the high increase in the treated seedling is not always followed by the increase in the number of leaves. Nonoptimal mycorrhizae in increasing the number of leaves is also possible because of the insufficient nutrients needed by plants for leaf formation, such as N element. Although mycorrhizae are able to increase the absorption of macro and micro nutrients, the nutrients that are mainly increased is P elements [25].

At the number of roots also obtained an effect that was not significantly different statistically. This is possible because there is insufficient (little) auxin produced by *Acaulospora* sp. to trigger an increase in the number of roots. On patchouli stem cuttings (*Pogostemon cablin*), that IBA (auxin) at a low concentration of 25 ppm had no significant effect on the number of roots but significantly affected the root length. This is because at low concentrations, IBA will encourage root lengthening [29]. Another factor that is also considered as a factor that caused no significant difference in the number of roots is the presence of rizocalin hormone. The activity of rhizocalin causes no effect of IBA hormone (auxin) on the number of roots Jabon cuttings. Rizokalin is a hormone that is naturally produced by plants and functions in stimulating root lengthening [30].

In the dry biomass also found an effect that is not significantly different, both on the dry biomass of the upper part of the plant and the roots. This is presumably because the observation time is relatively short so the role of mycorrhizae in helping nutrient absorption to increase the dry biomass of the plant has not been seen. The dry biomass of plants reflects the status of nutrients and the amount of nutrients absorbed by plants and the rate of photosynthesis. Apart from P elements, the dry biomass of plants is also strongly influenced by other nutrients, especially elements of N and K. Nitrogen serves to increase vegetative growth [31]. Uptake K will increase the physiological work of plants such as in photosynthesis and respiration processes. It can increase the accumulation of carbohydrates in the process of cell division [32]. Growing and forming of vegetative organs of plants greatly affect dry biomass [33].

4. Conclusions

Acaulospora sp. is the most compatible type of AMF for the growth of Andalas shoot cuttings and able to increase the height and root length 1.6 times better than controls and show very high dependency criteria.

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