Application of Arbuscular Mycorrhizal Fungus (AMF) improves the growth of single-bud sugarcane (*Saccharum officinarum* L.) seedlings from different bud location

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Abstract. This study aimed to study the effect of different doses of arbuscular mycorrhizal fungi (AMF) on the growth of sugarcane seedlings (*Saccharum officinarum* L.) planted through a single-bud method with different buds location. The study was conducted at the Faculty of Agriculture's Experimental Garden, Universitas Hasanuddin, Makassar, South Sulawesi. The study was carried out from July to November 2018 and set using a Randomized Block Design. Four doses of AMF were used i.e. 2, 4, 6, and 8 g/polybag, respectively and three sugar cane seedling buds location namely apical, median and basal end were used. The results show that the use of single buds from the upper buds showed better growth of shoots at the beginning of growth up to 4 weeks after planting (WAP), which was shown by shoot height parameters. In addition, the location of the upper bud also produced highest number of leaves and number of bud segments. The application of AMF with a dose of 8 g/polybag on the single-bud seedling of upper shoot showed the biggest stem diameter parameter values.

1. Introduction
Sugar cane (*Saccharum officinarum* L.) is an important crop of high economic value in various countries, especially in developing countries with a tropical climate such as Indonesia because of its high sugar content in the stem. Sugar cane as a raw material for the sugar industry where sugar is one of the basic needs for most people and a relatively cheap source of calories [1,2]. With an area of around 420.15 thousand hectares in 2017, the sugar industry made from sugar cane is one source of income for thousands of sugar cane farmers and workers in the sugar industry [3]. The need for sugar in Indonesia has not been fulfilled because the efficiency of sugar cane plantations in Indonesia is relatively low. The low efficiency of sugar cane plantations is due to the neglect of the basic rules of sugarcane cultivation by farmers, in terms of seed use, maintenance, harvesting that does not pay attention to proper techniques and age of harvest and management of transportation (felling, loading, transporting) [4].

Efforts to increase sugarcane production are by using superior seeds and implementing good cultivation techniques. Single bud nursery technique does not require a long time, which is about three months and seedlings can be planted in the field. In addition, nurseries with this single bud technique
will produce uniform growth, a greater number of tillers and can save space and costs because they can be planted using small sized polybags. This single bud technique is a nursery technique that can be used to produce large quantities of mule seedlings [5]. Establishment of seedlings using a conventional system (mule) is often constrained by the low production of seedlings from breeders, besides the health and purity of seedlings is not guaranteed. The research results of Adinugraha, Nugroho and Wicaksono [6], that studied the effect of bud chip seed origin on the vegetative phase of three varieties of sugarcane (*Saccharum officinarum* L.), showed that the treatment of seed origin significantly influences the vegetative growth of sugarcane. Buds on the upper stem have better growth than buds on the lower stem. Origin of seedlings from the upper trunk is the origin of the right seeds in sugarcane planting for varieties PSJT 941, VMC 76-16 and Bululawang. Research conducted by Anindita et al., [7] on varieties of Bululawang and ps862 suggested that sugarcane seeds with eye number 7, 8, 9 and 10 were located in the upper stem. While bud numbers 11, 12 and 13 were located on the middle stem and bud number 14, 15 and 16 were located at the basal segment of the sugarcane stem. The stems at the top segments is assumed to have good growth, this is because at the top it has younger shoots, more auxin content while the number of shoots at the lower stem shows lower growth compared to the number of buds at the top.

One way to increase plant growth and development in the field is by symbiosis of Arbuscular Mycorrhizal Fungi (AMF). Arbuscular mycorrhizae are mutualistic symbiotic fungi with plants[8]. The fungus's external mycelium has the role of expanding the root absorption area so that nutrient absorption, especially P, becomes greater [9–12]. The presence of mycorrhizae is important for ecosystem resilience, plant stability and maintenance of biological diversity. The role of mycorrhizae in maintaining biodiversity and ecosystems is now beginning to be recognized, especially because of the influence of mycorrhizae to maintain plant diversity and increase productivity [9]. The use of mycorrhizae provides several advantages, among others: agroecosystem mycorrhiza will help the absorption of nutrients and water through the expansion of roots, and increase the efficiency of the use of phosphate fertilizer [13].

Mycorrhiza can also produce growth regulating hormones such as cytokinins and gibberellins and can improve phosphate metabolism[11]. Increased nutrient absorption and growth due to mycorrhizal application there is an increase in crop yield [14]. Arbuscular mycorrhizal fungi are found in many plants in the tropics and subtropics, and do not have specific symbionts [15]. The use of arbuscular mycorrhizal fungi with a dose of 5 g, can increase plant height by 48.41%, and leaf width 22.2% compared to without the use of mycorrhizae. Sugarcane colonized with AMF has twice the root wet weight compared to that which is not colonized by the fungi. The higher wet weight of the roots of sugarcane seedlings with micoriza shows that more plants store water by 48%, compared to without AMF [16].

Previous studies [15,17,18] showed that mycorrhizal inoculation in cultivated plants has a positive influence on plant growth and production. Sastrahidayat [13] also suggested various types of agricultural plants such as chili, asparagus, corn, grapes, oranges, mangoes, and papayas gave positive responses to the application of mycorrhizae for their growth. Study on the use of this fungi on sugarcane seedlings (*Saccharum officinarum* L.) showed a positive response on number of leaves, number of stem segments, length of stem segments, leaf area, root volume, root fresh weight, crown fresh weight, total fresh weight, root dry weight, crown dry weight, total dry weight, and crown/root ratio [19].

2. Methodology

2.1. Methods

The research was conducted at the Experimental Farm, Faculty of Agriculture, Universitas Hasanuddin, Makassar, South Sulawesi. The research site is located at an altitude of 22 m above sea level (asl) with a temperature of 25 °C- 32 °C and humidity reaching 98%. The trial was conducted from July to November 2018 in the form of an experiment using a randomized group design. The first factor was the application of Arbuscular Mycorrhizal Fungi (M) with various doses consisting of: 2, 4,
6 and 8 g/polybag. The second factor was the treatment of the location of sugarcane buds (B) which consisted of: the upper buds (b1), middle buds (b2), and lower buds (b3). Each treatment consisted of three units so that the total polybag used was 108.

2.2. Preparation of seedling materials

Planting materials were taken from the nursery of Takalar Sugar Factory, PT. Perkebunan Nusantara XIV, Takalar regency, South Sulawesi. The sugarcane plants taken for bud sources were 6-7 months old to ensure optimal growth of the buds. Stem segment containing buds was cut using a saw or can also use mechanical equipment, the length of cut ± 2.5 cm at the time of cutting the bud should not be damaged because it is the point of growth of sugar cane. The seed media used were soil and compost, in seed beds of 2 x 1 meter size. Prior to sowing, buds were soaked in warm water for Hot Water Treatment (HWT) with a temperature of 50 ºC for 15 minutes then left for cooling down. Following the HWT, the buds then soaked again using composition of the Nordox 56 wp fungicide solution. Subsequently, the single bud seeds were drained and then sorted and sown on prepared media.

The single bud seedlings are planted on the seedling media with the buds facing upward with a depth of ± 2 cm and should not be buried by the soil. Daily watering was carried out routinely for 15 days then selected for the emergence of buds 5-10 cm or leaf buds begin to open 1-2 strands. Selected the single bud seedlings were transferred to a polybag containing nursery media that has been mixed previously. The nursery media used were soil, and compost in a ratio of 2: 1 mixed evenly using a 25 x 30 cm polybag.

2.3. Preparation and application of AMF

The AMF used in this study was inoculant mixture from corn and sorghum plants. Species: Acaulaspora tuberculata, family: Acaulasporaceae contains Zeolite media. Application of the mycorrhizae fungi was carried out according to the treatment of 2 g, 4 g, 6 g and 8 g per plant in each planting hole prior to planting. The AMF was placed in the rhizospher of the sugarcane seedlings.

2.4. Data analysis

Data obtained from observations were analyzed using Analysis of variance (ANOVA) for two-factor factorial design. A significant effect of the treatments was tested further using Least Significant Difference (LSD) test (α = 0.05).

3. Results

3.1. Effect of AMF and bud location on plant height and number of segments of the sugarcane seedlings.

Application of AMF did not affect the plant height of sugarcane seedlings parameter. The plant height parameter was only affected by the bud location (p<0.01) in the earlier growth phase up to 4 weeks after planting (WAP), after that, the plant height were not significantly different (Table 1). Seedlings planted from bud located at the apical end of the sugarcane stem showed higher plant at the first month compared to seedlings planted from buds at the median and basal end. However, started from six WAP, seedlings from these location showed no difference in plant height to the seedlings grown from apical buds using single bud method. Faster growing at earlier stage also implied to the number of segments formed on the stem of the seedlings. Higher segment numbers were found on seedlings from the apical bud location treatment (Table 1) at the end of the trial.

Table 1. Average of plant height (cm) and the number of segments of single bud sugarcane seedlings from different bud location

| Bud Location | Weeks After Planting (WAP) | Number of segments |
|--------------|---------------------------|--------------------|
|              | 2  | 4  | 6  | 8  | 10 | 12 | 12 WAP |
| Apical       | 19.5a | 25.8a | 30.4 | 31.7 | 39.4 | 50.8 | 10.00 a |
| Median       | 18.5a | 25.6a | 29.9 | 31.3 | 38.2 | 47.9 | 9.08 b  |
3.2. **Effect of AMF on leaf growth and development of the sugarcane seedlings.**

Application of AMF and bud location had no significant effect on leaf growth and development of sugarcane seedlings. The average number of leaves of the seedlings applied with different doses of AMF show no difference (Table 2) while for leaf area parameter, widest leaf area was resulted from the application of the fungi as much as 8 g per plant.

**Table 2.** Average number of leaves (leaves) and leaf area (cm$^2$) of single bud sugarcane seedlings from different bud location on different dose of Arbuscular Mycorrhizal Fungi (AMF)

| Arbuscular Mycorrhizal Fungi (AMF) dose | Number of leaves (leaves) | Leaf area (cm$^2$) |
|----------------------------------------|---------------------------|-------------------|
| m1 (2 g/polybag)                       | 10.67                     | 32.52             |
| m2 (4 g/polybag)                       | 10.44                     | 28.62             |
| m3 (6 g/polybag)                       | 10.30                     | 31.44             |
| m4 (8 g/polybag)                       | 10.06                     | **36.00**         |

3.3. **Effect of AMF on stem growth and development of the sugarcane seedlings.**

Similarly with the leaf growth parameter, the growth and development of the stem of sugarcane seedlings did not affected by the application of different dose of the fungi. No significant differences found between parameters of stem diameter, number of segments on the stem, and length of each segment as results of the application of different dose of AMF (table 3). Better stem growth and development showed by the higher dose of the AM fungi indicated by the value of the parameters.

**Table 3.** Average stem diameter (mm), number and length of segment (cm) of single bud sugarcane seedlings from different bud location on different dose of Arbuscular Mycorrhizal Fungi (AMF).

| Arbuscular Mycorrhizal Fungi (AMF) dose | Stem diameter (mm) | Number of stem segments | Length of stem segment (cm) |
|----------------------------------------|-------------------|-------------------------|---------------------------|
| m1 (2 g/polybag)                       | 20.1              | 9.33                    | 5.29                      |
| m2 (4 g/polybag)                       | 19.9              | 8.93                    | 5.51                      |
| m3 (6 g/polybag)                       | 20.3              | 8.78                    | **5.61**                  |
| m4 (8 g/polybag)                       | **20.4**          | **10.02**               | 5.10                      |

3.4. **Effect of AMF on root growth and development of the sugarcane seedlings.**

In the recent trial, growth and development of sugarcane seedlings root were not affected by the treatment applied. No difference found between the root volume and length of the seedlings in different doses of mycorrhiza fungi (Table 4). Higher dose of AMF application did not necessarily improve the growth of the sugarcane seedlings. Biggest root volume was shown by application of 4 g per polybag AMF and the longest root was found at the highest AMF dose (8 g per polybag) which was only slightly differed with the seedlings applied with AMF of 2 g per polybag.

**Table 4.** Average root volume (ml) and root length (cm) of single bud sugarcane seedlings from different bud location on different dose of Arbuscular Mycorrhizal Fungi (AMF).

| Arbuscular Mycorrhizal Fungi (AMF) dose | Root volume (ml) | Root length (cm) |
|----------------------------------------|-----------------|------------------|

4
3.5. **Effect of AMF on tillers number of the sugarcane seedlings.**

Analysis of variance show that number of tillers of the sugarcane seedlings from single bud method was significantly affected by application of AMF (p ≤ 0.05) and no significant effect of bud location treatment on this parameter. Number of tillers of the seedlings varied between the dose of AM fungi with highest tillers number shown by the application of 4 g per polybag AMF (Table 5).

**Table 5.** Average of number of tillers of single bud sugarcane seedlings 12 weeks after planting (WAP) from different bud location on different dose of Arbuscular Mycorrhizal Fungi (AMF).

| Arbuscular Mycorrhizal Fungi (AMF) dose | Bud location | Mean   |
|----------------------------------------|--------------|--------|
| m1 (2 g/polybag)                       | b1 (Apical)  | 3.56(1.88) |
|                                        | b2 (Median)  | 6.11(2.42) |
|                                        | b3 (Basal)   | 2.78(1.66) |
|                                        |              | 4.15(1.90)c |
| m2 (4 g/polybag)                       | b1 (Apical)  | 4.22(2.04) |
|                                        | b2 (Median)  | 6.00(2.44) |
|                                        | b3 (Basal)   | 5.89(2.42) |
|                                        |              | 5.37(2.30)a |
| m3 (6 g/polybag)                       | b1 (Apical)  | 4.89(2.21) |
|                                        | b2 (Median)  | 4.33(1.97) |
|                                        | b3 (Basal)   | 5.89(2.41) |
|                                        |              | 5.04(2.20)b |
| m4 (8 g/polybag)                       | b1 (Apical)  | 3.44(1.85) |
|                                        | b2 (Median)  | 2.56(1.60) |
|                                        | b3 (Basal)   | 3.28(1.80) |
|                                        |              | 3.09(1.75)d |

Numbers followed by different letters (a, b, c, d) are significantly different at LSD$_{0.05}$ = 0.07. Number in parentheses are transformed values using $\sqrt{x}$.

4. **Discussion**

The results showed that the location of sugarcane buds significantly affected plant height at ages 2 and 4 WAP and on the number of segments. The results of the analysis of the growth of sugarcane plant, it is known that the highest average height parameters of plant ages 2 and 4 MST (Table 1) is the treatment of the location of the upper buds (Figure 1) where with an average of 19.51 cm respectively and 25.83 cm which is significantly different from the treatment of the location of the lower buds. This is in accordance with the opinion of Anindita [7], which states that the buds located on the young and un-colored segments will germinate faster than the older ones. However, the higher the water content is still high and the lower the longer the germination, this is because in the lower part of the sugar cane there is a high sugar so that it will cause the duration of germination. Then it was strengthened by research of Putri et al., [20] in which the position of the shoots from the upper stem was higher in growth hormone content compared to the position of the buds from the middle and lower stem. Bud cuttings chips top is the part that contains the most growth hormones one of which is auxin. Auxin hormone plays a role in accelerating cell elongation, so auxin hormone can help the process of growth and development of stem diameter. The lower bud chips cuttings have less auxin hormone, so that the growth of stem height on the lower bud chips cuttings is lower than the bud chips cuttings derived from the upper stem.
Figure 1. Growth of sugarcane seedlings 2 weeks after planting based on the location of buds on the seedling media. Apical bud (A), Median bud (B) and Basal bud (C).

Location of the upper buds (b1) produces the highest number of segments (10.00) and significantly different from the location of the middle buds (m2) and the location of the eyes the bottom bud (m3). It is presumed that the location of buds originating from the upper stem is part of plants whose cells are still actively dividing and producing hormones, so the position of the upper buds that are planted grows more quickly [21]. The stem at the top has good growth, this is because at the top it has younger shoots, more auxin content. Auxin on the sugarcane stem serves to stimulate cell lengthening and enlargement. The bud number of the lower stem shows lower growth than that of the bud number. This is in addition to the auxin content in the lower stem which is less than the upper stem, the number of buds on the lower stem has a higher sucrose content. The high content of sucrose will inhibit the buds to germinate, this is because sucrose must first be overhauled into a simple sugar that is glucose. Glucose serves as a food reserve in the germination process. On the young stem that contains simple carbohydrates such as glucose and fructose which is very instrumental in the germination process. Glucose is converted in the process of respiration into energy (ATP) and amino acid compounds that function to form new cells so that the roots in sugarcane seeds grow [22].

Arbuscular mycorrhizal fungi can increase the availability of macro nutrients, especially N, P, and K so that the availability of nutrients is better. The increase in the number of tillers is directly proportional to the increase in sugarcane production, this is because if more tillers increase the population in one plant will increase, so that the resulting fresh weight will certainly increase. N is the most dominant element among the elements needed by sugarcane, which functions among others to encourage the formation of tillers which will eventually increase the number of stems and stem weight per ha [23]. Although a large number of tillers with small diameters the end result will produce a high weight, therefore productivity will also be high [24].

In the recent study, in is shown that application of AMF improve leaf area of the sugarcane seedlings. The leaf is a plant organ that has the function to carry out photosynthesis. The increasing number of leaves is inseparable from the activity of cell elongation which stimulates the formation of leaves as photosynthetic organs. The leaves are generally seen as the main photosynthetic organ. Observation of leaf variables is needed, namely as an indicator of growth and supporting data to explain the growth process that occurs, for example in the formation of biomass [25]. The number of leaves and high levels of chlorophyll can increase yield, because the photosynthesis process is going well. Sugarcane productivity is mainly determined by the process of photosynthesis, bearing in mind that the accumulation of carbon (sugar) skeleton is found in the stem and its size is proportional to the photosynthetic activity during the plant cycle [26].

According to Syamsiyah et al., [27], high N and P nutrient uptake is found in plants that are given mycorrhiza, because mycorrhizae will encourage the development of hyphae in plant roots which in turn will help nutrient absorption. Roots infected with arbuscular mycorrhizal fungi will have greater home range due to external hyphae that develop outside the roots, so that plant nutrient uptake increases. The availability of nutrients is one of the environmental factors that determine the rate of
plant growth, so it takes more essential nutrients available. The availability of nutrients in sufficient quantities and balanced for plant growth, can cause the process of division, enlargement and elongation of cells will take place quickly which results in several plant organs growing quickly [28].

5. Conclusions
The use of buds from the apical and median section of seedlings material in the single bud propagation of sugarcane gives better earlier growth of the seedlings indicated by the plant height parameters 2 and 4 WAP and the number of stem segments 12 WAP. Application of Arbuscular mycorrhizal fungi improves the growth and development of sugarcane seedling from single bud propagation method.

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