Application of Trichoderma on single bud Sugarcane (Saccharum officinarum L) seedlings originated from different stems

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Abstract. The aim of this study was to determine the growth of sugarcane seeds using Trichoderma application and different parts of the stems using the single bud method. The research was conducted at the Experimental Garden at the Faculty of Agriculture, Hasanuddin University, Tamalanrea, Makassar. The research took place from July to November 2018. This research was conducted in the form of a two-factor factorial experiment which was arranged based on a randomized block design. The first factor is Trichoderma with 3 levels of doses, namely without Trichoderma, 15 g / polybag and 30 g / polybag, while the second factor is the stem which consists of the upper stem, middle stem and lower stem which consists of 9 treatment combinations, respectively. -Each treatment was repeated 3 times (3 polybags / treatment) so that the total poly bag used was 81. The results showed that treatment without Trichoderma gave the best results on plant height, namely 13.91 cm at 2 WAP. While the treatment of the middle stem gave the best results on the number of tillers, namely 10.71.

1. Introduction
Sugarcane (Saccharum officinarum L.) is a strategic commodity for Indonesia because it has high economic value. Indonesia experienced the heyday of the sugar industry in the 1930s with 179 factories operating (PG), a productivity of around 14.80%, and a yield of 11% −13.80%. Peak production reached up to 3 million tonnes and sugar exports amounted to 2.40 million tonnes. This success is supported by the ease of obtaining fertile land, cheap labor, priority for irrigation, and discipline in the application of technology [1]. Sugar cane as a raw material for the sugar industry is one of the plantation commodities that have a strategic role in the economy in Indonesia. With an area of approximately 420.15 thousand hectares in 2017, the sugar cane industry is a source of income for thousands of sugarcane farmers and workers in the sugar industry [2].

The development of sugar production for large estates (PB) and smallholder plantations (PR) from 2013 to 2017 tends to decline. Sugar production from PB and PR has decreased due to a decrease in acreage. In 2017, sugar production decreased to 2.19 million tons or decreased by 172.06 thousand tons (7.28 percent) compared to 2016. The shortage of domestic sugar supply requires Indonesia to import sugar from various countries. In 2017, 13 countries were recorded as suppliers of Indonesian sugar [2].

According to the Center for Agricultural Data and Information Systems [3], in 2017-2021 the projection of sugar imports in the form of raw sugar in Indonesia is estimated to increase by 1.6% per year. In 2017, Indonesia’s sugar imports amounted to 2.80 million tons, then the projected sugar imports
will continue to increase until 2021. In 2018 Indonesia's sugar imports amounted to 2.84 million tons, until in 2021 sugar imports will be 2.99 million tons.

The increase in sugar consumption in Indonesia from year to year provides ample opportunities to increase the production capacity of sugar factories. Apart from that, the amount of domestic sugar production currently feels unable to meet the demand for sugar in Indonesia due to several factors, including the lack of sugarcane planting area, low productivity, and low sugar yield. Problems that often appear in the low sugar production include, among others, from the perspective of sugarcane cultivation, namely the preparation of seeds, the quality of the seeds and the varieties used. The preparation of seeds that are often used is mule seeds. Mules are very influential on nursery time because they take between 6 and 8 months for one planting period. Seed quality affects production, because seed quality is one of the determining factors for the success of sugarcane cultivation. In addition, the varieties used are of course a major factor in the success of sugarcane cultivation. Selection of varieties must be in accordance with the planting area, because varieties are only superior for one location (echolocation). From these problems, a short, high quality seed preparation technology is needed as well as the selection of the right varieties according to the planting location.

Vegetatively, sugarcane is propagated using stem cuttings or known as mule seeds. The need for planting material uses mule seeds with 2-3 buds, which is around 6-8 tons / ha. The large amount of planting material is a major problem in transportation, handling and storage of sugarcane seeds. In addition, the lack of land availability causes the need for land for nurseries to become increasingly difficult. Quality seedlings are characterized by good adaptation to new environments, can grow well if planted in the field, are healthy, and uniformly [4].

The single bud nursery technique is one of the nursery methods used as a method of developing superior seedlings. The superior seeds produced are propagated using a single bud technique. This method can be said to be a new nursery method, especially in Indonesia. The single bud nursery technique is a nursery with one bud that does not require a long time, which is about three months. The seedlings can be planted in the field. In addition, this single bud nursery technique will produce uniform growth, increase the number of tillers and save space and costs because it can be planted using small polybags [5].

In addition, the level of soil fertility is one of the problems that becomes an obstacle to increasing production. Less optimal cultivated land conditions can reduce sugarcane production. One way to increase soil fertility is to enrich the planting medium using microbes. Trichoderma is a microorganism that has the ability to accelerate the process of decomposing organic matter and is a symbiotic fungus that is harmless, even mutually beneficial between soil-borne fungi and plant roots [6].

Trichoderma fungi are abundant in nature and agricultural soils, and generally colonize with the roots of many plant species. Trichoderma is also one of the microbes that can overcome the pokahbung disease caused by the pathogenic fungus *Fusarium moniliformae*. In certain circumstances the *F. moniliformae* fungus attacks the growing point and causes rot which is accompanied by an unpleasant odor and a further attack can cause the death of the plant. In accordance with the results of research conducted by Birtha et al. [7], that the administration of Trichoderma antagonistic fungi in in vitro tests with various concentrations of spore density was able to suppress the growth of pathogenic fungi *F. moniliformae* equally well. While in the in vivo test *Trichoderma sp.* is able to protect pokahbung attacks on sugar cane.

Besides its ability as biological control, Trichoderma has a positive influence on plant roots, plant growth, and crop production. This property indicates that Trichoderma also acts as a Plant Growth Enhancer [8]. The ability of Trichoderma to increase plant growth has been proven by Windham et al., [9] that the application of Trichoderma on sterile soil can increase the germination rate of tomatoes and tobacco. Meanwhile, Haryuni's research [10] by giving 25 g and 50 g of Trichoderma shows that Trichoderma fungus is able to infect the roots and stems of 3 months old seeds, in the plant tissue there is an increase in protein and proline levels and a decrease in glucose levels thereby increasing resistance and health in growth the beginning of sugarcane seeds before planting in the field.
The use of good shoots for planting also determines the success of cultivation. Sugarcane plant growth conditions are needed for the uniform growth of shoots. The buds that are located on the young and not colored internodes will germinate faster than the older ones. The buds of the upper, middle and rootstock stems have different available nutrients and sucrose, which have an effect on the vegetative growth of sugarcane. As the results of research by Adinugraha et al. [11] show that the seedling treatment has a significant effect on the vegetative growth of sugarcane. The buds on the scion stem had better growth than the buds on the rootstock. The use of the correct position of the buds is a very decisive first step for the success of sugarcane cultivation so that it can encourage an increase in sugar productivity.

Based on the description above, this study was conducted to determine the effect of Trichoderma treatment on the growth of sugarcane seeds by using different parts of the stem using the single bud method.

2. Methodology
This research was conducted at the Experimental Garden, Faculty of Agriculture, Hasanuddin Tamalanrea University, Makassar. The research took place from July to November 2018. The materials used in this study were Kidang Kencana, Trichoderma, compost, soil, water, atonics, fungicides and insecticides of sugarcane seeds. The tools used in this study were hoes, rulers, hoes, saws, buckets, scales, calipers, care nameplate, polybags size 30 x 40 cm and stationery.

2.1. Research design
This research was set based on a factorial design using a randomized block design (RBD) with two factors, namely: The first factor is the provision of Trichoderma (T) which consists of (t0): Control (without Trichoderma), (t1): 15 g / polybag, and (t2): 30 g / polybag. Second factor: stem (B), namely (b1): scion, (b2): middle stem, and (b3): rootstock. Each treatment was repeated 3 times (3 polybags / treatment). So the total poly bag used is 81.

2.2. Preparation of planting materials
The planting material used is sugarcane stalks which are cut into three parts, namely the top, middle and bottom stems using a saw. Cut length + 5 cm. At the time of cutting, the shoots should not be damaged because they are the growing points of the sugarcane. The planting material taken is 6 - 7 months old because at that age the shoots can grow optimally.

2.3. Nurseries
Before sowing, the sugar cane planting material is soaked in warm water (Hot Water Treatment) using warm water (50 ºC) for 15 minutes. The purpose of immersion in warm water is to free the planting material (shoots) that will be used from pathogens (fungi, bacteria, or viruses). After chilling, the shoots that have been treated (HWT) are then carried out again by soaking in a nordox fungicide solution to avoid fungal and insect attack that causes the seeds to not grow properly. Furthermore, atonic growth stimulants are soaked for 15 minutes to accelerate germination.

After being treated with HWT and immersed in a solution, the planting material is dried and then sown in the prepared beds. Shoots are sown together and straight over the surface of the bed with a depth of ± 2 cm. The planting time for planting material is 15 days and then transferred to the prepared nursery media.

2.4. Preparation of planting media and planting
The planting medium consists of a mixture of soil and compost with a ratio of 3: 1. After mixing, the media is put into a poly bag then arranged in the research location according to the research design and labeled according to the treatment. Seedlings that are 15 days old are characterized by the appearance of 5-10 cm shoots or 1-2 leaflets starting to open and then transferred to polybags measuring 30 x 40 cm containing mixed soil and compost in a ratio of 3: 1.
2.5. Trichoderma application
Application of Trichoderma was conducted according to the treatment, namely 15 g / polybag and 30 g / polybag in the planting hole which was carried out at the time of planting, by sprinkling Trichoderma in each planting hole. So that when planting seeds, the position of Trichoderma will be right at the root of the plant.

2.6. Plant maintenance
Maintenance includes watering, weeding and controlling pests and diseases. Watering is done 2 times a day, namely in the morning and evening or depending on the conditions of the planting medium on the experimental poly bag. Weeding is done by removing weeds that grow around the plant.

2.7. Parameter observation and data analysis
The parameters observed were plant height, stem diameter, number of leaves, leaf area, number of tillers, number of internodes, root length and root volume. Data was analysed using analysis of variance for a two factors factorial design with randomized block design (RBD). A further test was conducted if the treatment show a significant effect using the Least Significane Defference (LSD) test at a confidence level of 5%.

3. Results and discussion
3.1. Effect of Trichoderma and origin of bud segment on the growth of seedlings
Growth parameters of the sugarcane seedlings are shown in table 1.

| Bud section | Trichoderma dosage | Average |  |
|-------------|--------------------|---------|---|
|             | 0 g polybag⁻¹ | 15 g polybag⁻¹ | 30 g polybag⁻¹ | |
| Top         | Plant height (cm) | 27.78  | 26.11  | 23.33  | |
| Middle      | 24.06  | 25.39  | 25.89  | |
| Basal       | 27.00  | 25.94  | 26.17  | |
| Top         | Number of leaves (leaves) | 8.33  | 7.89  | 8.67  | |
| Middle      | 8.22  | 8.44  | 8.22  | |
| Basal       | 7.89  | 7.89  | 8.00  | |
| Top         | Stem diameter (mm) | 17.47  | 16.17  | 16.47  | |
| Middle      | 16.89  | 17.08  | 17.02  | |
| Basal       | 16.11  | 17.36  | 16.63  | |
| Top         | Number of tillers (tillers) | 8.74  | 7.00  | 11.00  | 8.91 ab |
| Middle      | 11.22  | 10.67  | 10.25  | 10.71 a |
| Basal       | 7.33  | 8.11  | 9.44  | 8.30 b |
| LSD _α=0.05_ | 2.24  | | |

Numbers followed by the same letter at the same age show the results are not significantly different based on the LSD test 0.05.
Treatment of Trichoderma application and use of different sources of buds position did not have any significant effect on the growth of seedlings shown by parameter plant height, number of leaves, stem diameter and number of segment at 12 weeks after planting (WAP). Despite this, the origin of the buds had a significant effect on number of tillers produced by the seedlings.

3.2. *Effect of Trichoderma and origin of bud on the root growth of sugarcane seedlings.*

This recent study show that root growth of sugarcane seedlings were not affected significantly by the application of Trichoderma and the origin of the buds. No difference found in the root length and root volume between sugarcane seedlings grown from the apical, middle and basal buds. Similarly, no effect of the Trichoderma on these two parameters of root growth (figure 2).

*Figure 1.* Effect of application of Trichoderma and the origin of bud on the root length (A) and root volume (B) of sugarcane seedlings.
Trichoderma treatment did not have a significant effect on plant growth either shown by above ground (plant height, number of leaves, stem diameter, and number of segments parameters) or by root growth parameters. The absence of effect of this fungus might be due to external factors so that the Trichoderma fungus could not release certain substances that could increase plant growth. This is in accordance with Rao's opinion in Yudha et al. [12] which stated that several factors such as soil type, humidity, pH, temperature, and plant age and condition affect the rhizosphere. One of the most influential factors is the availability of water in the planting medium. This is supported by the opinion of Agrios in Yudha et al. [12] that generally Trichoderma lives in slightly humid areas, while in dry soil conditions the Trichoderma population will decline after some time. In addition, Baihaqi et al. [13] also stated that the insufficient growing space for the growth of Trichoderma, food sources, and relatively fluctuating humidity can affect the efficiency of Trichoderma application that in turn will also affect cropping.

One of the factors that causes the use of Trichoderma to not have an optimal effect is the improper application method. In this study, Trichoderma was applied by placing it in the hole of the plant when planting in the field. This is supported by the opinion of Simarmata et al. [14] which stated that in order to increase the effectiveness of using Trichoderma, it is recommended that the use of Trichoderma fungi in tomato plants be applied while the plants are still in the nursery so that when the plants are transferred to the field the Trichoderma fungus has infected the plant roots.

The number of tillers was affected significantly by the treatment of the middle stem shoots (b2) had an average number of more tillers compared to the treatment of the upper stem (b1) and the lower stem (b3). This is because the middle stem has a balanced ratio of amino acids and carbohydrates so that it can grow well. In accordance with the opinion of Hardjanti [15], that the ability of buds to grow and develop is influenced by carbohydrate content and hormonal balance. Buds that contain high carbohydrates will facilitate the germination and sprouting process so that the buds can grow well.

The use of the upper stem (b1) produced the largest average number of leaves, stem diameter and root volume, namely 8.67 cm3, 17.47 cm3 and 162.22 cm3, respectively. The middle stem (b2) produced the highest average number of internodes, namely 7.00, while the lower stem (b3) produced the highest average root length of 55.11 cm. The upper and middle stems are the parts that contain the most growth hormones, one of which is auxin. The hormone auxin plays a role in accelerating cell elongation, so that the auxin hormone can help the process of growth and development of stem diameter.

4. Conclusion

Based on the results of the research that has been done, it can be concluded that the buds from the middle segments resulted in the best results for the number of tillers of the sugarcane seedlings, namely 10.71.

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