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The effect of biological fertilizer application on interest amount and relative water content in rehabilitation effort of old cacao

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Abstract. Purpose This study was to examine the effect of the application of *Trichoderma asperellum* and *Azotobacter chroococcum* on the condition of cacao leaves (RWC and LMA) and their relationship to the interest amount formed, after treatment *Inarching grafting* in the rehabilitation of old cacao plants from side grafting. The method used in this study was Split Plot Design, with 2 factors, namely the use of *T. asperellum* and *A. chroococcum* which were repeated 3 times each, and using *Duncan's New Multiple Range Test* (DNMRT). The best results obtained were the relative water content of the leaves 98.43%; LMA 225.05 mg.mm⁻², which produced an average number of 62 flowers every tree, with bacteria *A.chroococcum* and fungus *T.asperellum* applied twice each using the inarching grafting method which was carried out at the time of the appearance of the flush.

1. Introduction

The development of the cocoa industry in Bantaeng Regency, is supported by the development of new technology and new seeds in the form of a side grafting system that can harvest relatively quickly in 1 year and 4 months, yields can be increased 3-4 times with better quality. However, after the side grafted plants have been producing satisfactorily, then the plants experience a decrease in production and productivity. This is due to the condition of the plant root system that supplies nutrients and water from old roots. Efforts that can be made to improve the root system of plants that have been rehabilitated through side grafting were the implementation of *inarching grafting* by utilizing cacao seeds that have been at least 6 months old planted on both sides of the relatively old cacao plants which were then attached to the plant so that the roots of this young plant were expected to help in the absorption of nutrients and water so that cacao productivity can be increased.

The most practical improvement of soil fertility conditions is the addition of fertilizers to the soil. The addition of inorganic fertilizers that provide mineral ions available to plants only, will damage the physical fertility of the soil where the soil becomes hard and compact. For this reason, the application of organic fertilizers will greatly improve soil conditions. However, organic fertilizers decompose more slowly into mineral ions, especially if the application is only in the form of adding raw organic matter, therefore soil micro-organisms need to be added to accelerate the decomposition process, which will improve soil biological conditions so that soil fertility can be realized.

One of the functional microorganisms which is widely known as soil biological fertilizer is *Trichoderma sp.* These microorganisms are soil-dwelling fungi that can be isolated from the roots of



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field crops, such as cocoa. Species *Trichoderma* as well as decomposing organisms, can also function as biological agents and plant growth stimulators [1][2][3]. As a biofungicide, *Trichoderma* can inhibit the growth of several fungi that cause plant diseases such as, *Fusarium oxysporum*, *Rizoctonia solani*, and *Phytophthora palmivora* which cause fruit rot and stem cancer which are very susceptible to attack on cacao plants that are rehabilitated by side grafting [4][5][6].

Besides *Trichoderma*, the microbial group that has received the most attention and is widely used in plant cultivation systems is *Azotobacter chroococcum*. *Azotobacter* is a species of rhizobacteria that has been recognized as a dinitrogen-fixing biological agent, diazotroph, which converts dinitrogen to ammonium through electron reduction and protonation of dinitrogen gas [7]. Efforts to improve soil biology and at the same time increase plant productivity within oculation *Azotobacter* need to be done because these rhizobacteria act as plant growth enhancing agents through the production of phytohormones (auxins, cytokinins and gibberylins) which are the main substances that can increase and control plant growth [8].

Based on the foregoing, the application of *Trichoderma* and *Azotobacter* to the rhizosphere of sidecocoa plants to be rehabilitated by modifying the *inarching grafting method* needs to be studied and investigated the state of leaf mass and relative water content of leaves in relation to cacao flowering, so that efforts to improve cacao in South Sulawesi are currently can be resolved immediately and further increased production.

2. Research and Methods

The research was carried out in a cacao farm located in Gantarangkeke village, Banyorang sub-district, Bantaeng district, South Sulawesi province, about 300 km from Makassar city, starting from March 2019 to December 2020. Six-month-old cacao seedlings were planted under a cacao tree stand is about 20 years old, with a spacing of 4 x 4 m. The planting holes were made with a size of 60 x 60 x 60 cm as many as three holes and 20 cm apart from the tree old cacao.

This research was conducted using a Split Plot Design, with 2 factors and 3 replications, with the following treatment: As the main plot, *T. asperellum* given were which consisted of 4 levels, namely without *Trichoderma* (T0), giving *Trichoderma* 1 time application (T1), 2 times application (T2) and 3 times application (A3) each as much as 4 gr.L⁻¹per tree. As a sub-plot, the given of *A. chroococcum* consisted of 3 levels, namely without *Azotobacter* (A0), 2 times application (A1), 2 times application (A2), each of 40 ml x 10⁸ CFU.mL⁻¹.tree⁻¹. Thus there were 12 treatment combinations and each treatment combination consisted of 3 trees so that there were 108 experimental trees, and each experimental tree was connected to 3 cacao saplings.

The materials used were cacao plants and cacao seedlings aged 6 months, *Trichoderma asperellum* isolate ART-4, *Azotobacter chroococcum*, manure, cacao husk compost and NPK. Parameters to be observed consisted of: the Amount of interest formed, counting all flowers formed every month, leaf Relative Water Content (RWC) and Leaf Mass Area (LMA). The data of observations of growth parameters were analyzed using analysis of variance at a significant level of $P=0.05$. Statistical analysis was performed by using *Duncan's New Multiple Range Test* (DNMRT), a single-step multiple comparison procedure and statistical test.

Calculating the leaf relative water content (RWC) and leaf mass of area (LMA) at the end of the study, is a measure of the water status of plants as a physiological consequence of soil water content. Leaf samples were weighed as fresh weight (FW), then the leaves were soaked for 12 hours in water, then removed and the water attached to the leaves was impregnated using tissue paper and then weighed as turgid weight (TW). The leaf samples were then oven-dried for 48 hours at 80°C to obtain dry weight (DW) [9] [10]. Relative water content (RWC) of leaves and leaf area per area (LMA) is calculated by the equation:

$$RWC (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100\% \quad LMA (mg \cdot mm^{-2}) = \frac{DW}{FLA}$$

Information:

- 26 RWC = Relative Water Content
- 1 LMA = Leaf Mass Area
- 1 FW = Fresh Weight
- TW = Turgid Weight
- DW = Dry Weight
- FLA = Fresh Leaf Area, measuring leaf area using the millimeter paper method

25 3. Result and Discussion

From the results of SPSS analysis on the number of flowers produced formed from October to January showed that the time factor, application of *A.chroococcum* and *T.asperellum* and their interaction affected the amount of interest formed.

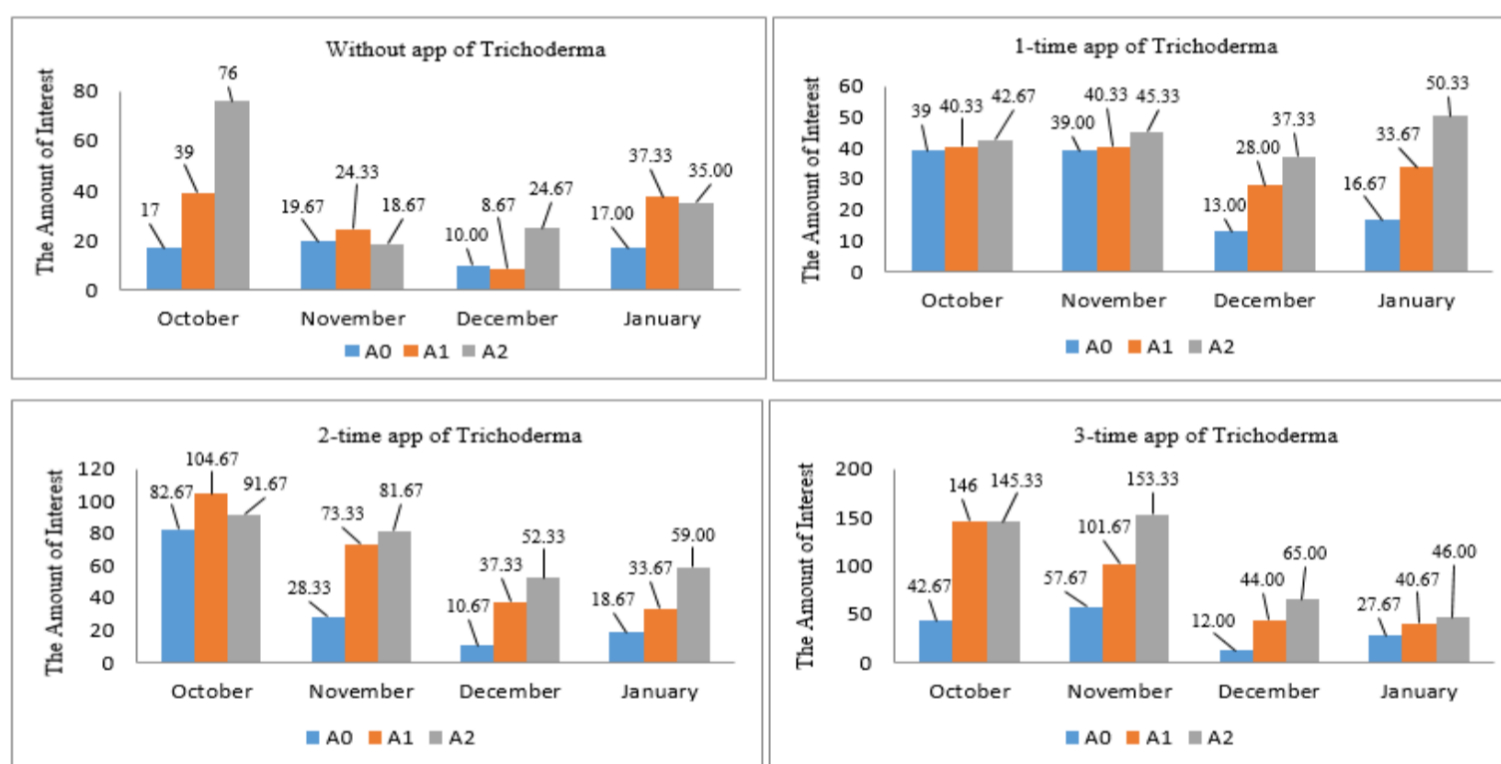


Figure 1. Graph of the average the amount of interest from October to January

24 Based on the results of analysis of variance on monthly observations, the *A.chroococcum* and *T.asperellum* interaction between had a very significant effect ($P < 0.01$) on the amount of interest formed in January, leaf relative water content (RWC) and leaf mass per area (LMA). The average amount of interest formed in January, RWC and LMA are listed in tables 1, 2 and 3.

Table 1. Data on the average amount of interest in January

Azotobacter	Trichoderma				Main Effect A
	Without apl	1x apl	2x apl	3x apl	
without apl	17.00 A aw	18.00 A ax	18.67 A ay	27.67 AB az	20.33 a
1x apl	37.33 BCD bw	33.67 BC bx	34.33 BCD by	40.67 CDE bz	36.33 b
2x apl	35.00 DE bw	50.33 E cx	62.33 F cy	46.00 DE bz	48.42 c
Main Effect T	29.78 x	34.00 x	38.44 y	38.11 y	-

Value with the same letters and the same column were not significantly different from each other within

treatments (one-way Anova DN MRT, $P=0.05$).

The rate of flower formation is in line with the growth of cacao plants and the effect of each single factor frequency of *T.asperellum* and *A.chroococcum* each month of observation (Table 1). From October to November, it was seen that the insertion and application of *T.asperellum* and *A. chroococcum* had no significant effect ($P>0.05$) on flower formation, but from December to January the application of the two single factors applied gave significantly different effects. ($P<0.05$) on the amount of interest formed, where it was seen that the given of *T.asperellum* should be interacted with *A. chroococcum*.

Table 2. Average Leaf Relative Moisture Content (RWC)

Azotobacter	Trichoderma				Main Effect A
	Without apl	1x apl	2x apl	3x apl	
without apl	48.27 A aw	74.17 AB ax	71.40 B ay	85.88 C az	69.93 a
1x apl	64.27 B bw	86.68 C bx	95.62 CD by	96.12 CD bz	85.68 b
2x apl	87.10 C cw	90.06 CD cx	98.43 D by	94.22 CD bz	92.45 b
Main Effect T	66.55 x	83.64 y	88.48 xy	92.07 z	-

Value with the same letters and the same column were not significantly different from each other within treatments (one-way Anova DN MRT, $P=0.05$).

The results of the Duncantest in table 2 show that the frequency treatment of spacing *T.asperellum* and *A.chroococcum* applications gave a higher average relative water content of the leaves, namely 98.35% and significantly different ($P < 0.05$) with treatment without given of *T.asperellum* and *A.chroococcum*, but not significantly different ($P>0.05$) with treatment with three times of *T. asperellum* and one application of *A.chroococcum*. Without the application of *T.asperellum* and *A.chroococcum* lowest gave the average leaf relative moisture content of 48.27%, so that there was an increase in the average leaf relative water content of 51% when applying *T.asperellum* and *A.chroococcum* with a frequency of two times to the cacao plant that is being rehabilitated.

Table 3. Average Leaf Mass of Area (LMA)

Azotobacter	Trichoderma				Main Effect A
	Without apl	1x apl	2x apl	3x apl	
without apl	88.64 A aw	103.29 AB ax	123.25 ABCD ay	137.25 BCD az	113.11 a
1x apl	109.38 ABC bw	108.49 ABC ax	136.67 BCD ay	147.75 CD abz	125.57 b
2x apl	120.01 ABCD bw	114.34 ABC ax	225.05 E by	159.69 D bz	154.77 c
Main Effect T	106.01 x	108.71 x	161.66 y	148.23 y	-

Value with the same letters and the same column were not significantly different from each other within treatments (one-way Anova DN MRT, $P=0.05$).

Based on tables (1, 2 and 3) and figure (1) above, it can be seen that the best treatment and effect on the amount of interest formed in January, RWC and LMA is to combine *T.asperellum* was applied twice and *A.chroococcum* was applied twice, which produced the best amount of interest. The results showed that the inoculation of Azotobacter had a positive correlation with the relative water content of cacao leaves (RWC) and leaf mass area (LMA), namely the more applications of Azotobacter the higher the RWC and LMA either alone or under conditions of application of Trichoderma, as well as their interactions. This is because the increase in plant height and number of leaves is strongly influenced by

the availability of nutrients needed by plants, where *Trichoderma* species are able to stimulate growth by affecting the balance of hormones such as IAA, gibberellic acid and ethylene [11], which creates a favorable environment for nutrient uptake by cacao plants. form optimal plant growth and development through treatment.

The application of the fungus *T.asperellum* into the soil can accelerate the process of decomposition of organic matter, because this fungus can produce three enzymes, namely 1) Cellobiohydrolase (CBH), which actively hydrolyzes cellobiose units into glucose molecules; 2) Endo- β -1,4-glucanase (EBG), which actively breaks down soluble cellulose; and 3) -glucosidase (BGL), which actively hydrolyzes cellobiose units into glucose molecules [12] [13]. This enzyme works synergistically, so that the decomposition process can take place more quickly and intensively, as researched by Zhang [14]. This enzyme hydrolyzes alkali and produces high glucose (99.18%) in corn stover.

The flowering season period for cacao plants is basically closely related to fluctuations in overall plant growth. The amount of interest that formed in the early flowering period in the *A.chroococcum* application *A.chroococcum* due application *A.chroococcum* can meet the needs of water and mineral nutrients, especially N and P. It is seen from the results, that the application *A.chroococcum* increase leaf relative water content (98.43 %), and leaf mass per area (225.05 mg.mm⁻²) compared with without application *A.chroococcum*. Adequate levels of water and nutrients allow plants to be able to form more new leaves and produce assimilate (the result of photosynthesis) and phytohormones to support flowering and fruiting. The nutritional status and assimilate in the plant will affect the flowering and fruiting of cacao plants. Lack of water in the leaf tissue will inhibit the rate of photosynthesis, because the turgidity of the stomata guard cells will decrease, which causes the stomata to close. Closure of stomata on the leaves will reduce the rate of absorption of CO₂ on the same and will ultimately reduce the rate of photosynthesis.

Water status in plants can be determined by measuring the relative water content of leaves as a physiological indicator, which is the interaction between leaf water potential and stomatal conductance, where drought will induce root signals to the canopy to reduce transpiration rate so that stomata close when water supply decreases. High relative water content is a mechanism of plant resistance to drought, and this high relative water content is the result of over-osmotic regulation or reduced elasticity of the cell wall tissue [15]. The results of the study [16] stated that the relative water content was influenced by the harvest season, and irrigation, so that drought stress could significantly reduce the relative water content value. LMA and RWC are highly correlated with leaf processes such as maximum photosynthetic rate [17], and potential growth rate [18] which will affect flowering rate in cacao plants.

4. Conclusion

conclusion from the results of this study is that the rehabilitation of cacao plants that are relatively old can be carried out without the need for felling of cacao trees, by modifying Inarching grafting method with an environmentally friendly agricultural system using bacteria *Azotobacter chroococcum* and the fungus *Trichoderma asperellum*, so that farmers do not need long enough for the cacao plants to remain productive in producing cacao beans. The best treatment yielded an average amount of interest 62.tree⁻¹; leaf relative water content 98.43%; LMA 225.05 mg.mm⁻²; is to apply the bacterium *Azotobacter chroococcum* 2 times and the fungus *Trichoderma asperellum* 2 times by using the *Inarching grafting method* appears which is carried out when the flush.

7 Acknowledgement

The authors would like to thank the Directorate of Research and Community Service, Deputy of Strengthening Research and Development, the Ministry of Technology Research/National Research and Innovation Agency of Indonesia (Grant no.7/E/KPT/2019) for funding this research.

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