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Growth and yield responses of cocoa (*Theobroma cacao L.)* to fungi arbuscular mycorrhizal biofertilizer prototypes with Different Spore Carrier Media and pruning

Nicholas Olga Situmorang, I Nyoman Rai * and I Wayan Wiraatmaja

Faculty of Agriculture, Udayana University, Denpasar (80361), Bali, Indonesia.

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Abstract

Bali is one of the organic cocoa producers in Indonesia, but its production has not met the national demand for cocoa. Bali is one of the cocoa producing centers is located in Buleleng Regency. In an effort to increase the growth and yield of cocoa plants, prototype mycorrhizal biofertilizers with different spore carriers can be used to improve soil fertility and cocoa productivity added with pruning treatment. This study aimed to determine the growth and yield response of cocoa to a prototype of mycorrhizal biofertilizer with different spore-carriers' media and pruning. The study used a randomized block design with 2 factors and 6 replications. The results showed that the prototype of mycorrhizal biofertilizer with volcanic sand carrier media treatment gave the best results compared to others treatments. Pruning new shoots and the end of twigs 2-3 internode can provide the best growth and yield of cocoa. The interaction of mycorrhizal biofertilizer prototypes with different spore carrier media and pruning had a very significant effect only on leaf chlorophyll, leaf reducing sugar, and leaf sucrose content.

Keywords: Cocoa; Mycorrhiza; Pruning; Carrier medium; Volcanic sand; Biofertilizers

1. Introduction

Cocoa (*Theobroma cacao L.)* is one of the plantation commodities that has an important role in economic activities in Indonesia. Indonesia is the third largest cocoa producer and exporter in the world after Ghana and Ivory Coast. Based on data, there was a decrease in the area of cocoa cultivation in Bali reaching 13,927 hectares in 2019 with a yield of 4,951 tons, and 13,866 hectares with a yield of 4,997 in 2020. Despite a decline in cocoa plantations in Bali Province, production and productivity have increased. In line with the policies of the Indonesian and Bali Regional governments, increasing the yield and quality of cocoa has been an Agricultural Development Policy since 2009 Environmental protection, energy efficiency and return to nature issues [1]. The distribution of cocoa plants in the Buleleng region covers several areas, including the Tajun village area, Kubutambahan district in 2014, with a cocoa area of 3 hectares and production of 3.80 quintals. The location in Buleleng has an ideal altitude for growing cocoa at an altitude of 0-500 m above sea level. So far, Buleleng's cocoa plantations have experienced a decline due to old and diseased cocoa trees. It can be seen that the actual productivity of cocoa land in Buleleng produced 4,951 with a productivity of 0.36 tons lower than the national productivity value of 0.49 tons / ha. The government is trying to increase cocoa productivity in Buleleng Regency, especially for rejuvenation and better cocoa production [2].

Strategies to increase the productivity of cocoa plants by carrying out environmentally sound agriculture development. Apart from using organic materials, mycorrhizal biofertilizers can be an effective alternative. In the Regional Regulation of Bali Province Number 8 of 2019 concerning the Organic Farming Systems, that the agricultural system developed in Bali has not been optimal in accordance with the rules regulated in the law. Then the use of organic microbial fertilizer in the soil as a natural fertilizer technology to increase crop yields, especially cocoa. [3] stated in application of mycorrhizal biofertilizers found that the use of Fungi Arbuscular Mycorrhizal (FMA) infection on plant was affected by

* Corresponding author: I Nyoman Rai

availability of nutrients in the soil where the more soil nutrient, the smaller infection FMA in root plant. Meanwhile, the use of mycorrhizal biofertilizer prototypes in the field has a positive effect on cocoa growth and production. Apart from that, cocoa cultivation is almost similar to coffee cultivation. In order for cocoa fruit production to be obtained a lot, cocoa plants must be able to produce a lot of assimilates. As evidence, not all the leaves are crowned plants produce and are able to carry out photosynthesis optimally, leaves Shaded plants actually become assimilated uses (*sinks*). Cocoa pruning is an attempt to speed up photosynthesis to take place optimal, net result photosynthesis maximum, and distribution to organs Which need going on fluent [4].

Plants used as research material are cocoa plant already in production . Prior to the prototyping of biofertilizers, all sample of plants are cleaned of weeds/nuisance plants to prevent nutrient competition or the chance of disease contamination. At the base of the tree is carried out bulking of the soil, as well as pruned branches and twigs. After that, fertilization with organic fertilizers is carried out according to the SOP for good and correct cocoa cultivation / *Good Agricultural Practices (GAP).* The FMA biofertilizer prototype was applied directly to the soil around the base of the stem on during research started, thereby also pruning done moment study started Based on the description above, it is deemed necessary to conduct research to find out how the effectiveness of mycorrhizal biofertilizer prototypes with different carrier media and pruning and the interaction between the two to growth and yield cocoa.

2. Material and methods

2.1. Time and Place of Research

The research was conducted on July 2, 2022 – December 30, 2022 at the cocoa plantation in Tajun Village, Kubutambahan District, Buleleng. FMA spore isolation was carried out at the Agronomy and Horticulture Laboratory as well as the Plant Disease Laboratory, and the Genetic Resources and Molecular Bilogy Laboratory of the Faculty of Agriculture, Udayana University. Meanwhile, the treatment of the application of mycorrhizal biofertilizer prototypes and pruning was carried out at the Cocoa Plantation, Tajun Village, Kubutambahan District, Buleleng.

2.2. Materials and tools

Materials used in this research include soil and cocoa roots from three cocoa production centers in Bali, namely Mendoyo Village, Mendoyo District, Jembrana, Megati Village, West Selemadeg District, Tabanan, and Tajun Village, Kubu Tambahan Buleleng District, 60% glucose, water, *trypanblue*, volcanic sand carrier media, zeolite Powder, sprora carrier media treatment sprinkled 150 spores. The tools used are shovels or hoes, plastics, sprayers, scissors or saws, centrifuse machines, centrifuse tubes, stereo and compound microscopes, ose needles, object glass, petri dishes, glass covers, A set of filtrations (*sieve*) witha hole diameter of 1 mm, 500 µm, 212 µm, 106 µm and 53 µm, a glass glass and a 1000ml beaker glass.

2.3. Research Methodology

This research used Randomized Block Design (RBD) with two factors treatment, There are 6 combinations of treatments and each be repeated as much 6 time so that needed 36 units test. The first factor was prototype of mycorrhizal biofertilizer with different spore carrier media consists of 3 levels i.e., Pt = without prototyping mycorrhizal biofertilizers, Ps = prototype of mycorrhizal biofertilizer with zeolite spore carrier media, and Pv = prototype of mycorrhizal biofertilizer with volcanic sand spore carrier media, while the second factor was pruning consisting of 2 levels i.e., Ct = without pruning, and Cd = new shoots and 2 segment ends of twigs was pruned. Mycorrhizal biofertilizer prototype was done by combining 150 consortiums of spore's mycorrhizal isolate with 500 grams in each level of spore carrier media treatment. Plants that receive pruning according to the level will be pruned at the ends of twigs 2-3 internodes and all of the new shoot. The pruning of cocoa plants was done during the flowering period.

2.4. Variables and data analysis

Variable observed in this study were: Soil fertility level , infection/mycorrhizal colonization rate, leaf P nutrient content, leaf chlorophyll content, leaf relative water content (RWC), total sugar content, reduction sugar, leaf sucrose, number of fruits per tree, number of flowers per tree, and pest attack rate. Data from the observations are tabulated, then analyzed using (*analysis of variance* according to the design experiment. Ifthe treatment interaction has a significant effect, then further analysis will be carried out using the Duncan's 5%, while if the interaction has no significant effect, then to find out the difference in single factor it will be further analyzed using the BNT 5%.

3. Results and discussion

Based on the results of the analysis of variance, it was showed that the interaction between the prototype mycorrhizal biofertilizer with different spore carrier media (P) and pruning (C) had a very significant effect on leaf chlorophyll, reducing sugar, and leaf sucrose content, while the effect on other variables was not significant. In the single factor, the prototype of mycorrhizal biofertilizer with different spore carrier media (P) had a very significant effect on the variables of leaf chlorophyll content, number of flowers per tree, number of fruits per tree, and leaf sucrose content. In the single factor, pruning of cocoa plantations (C) had a very significant effect on the variables leaf relative water content, the number of fruits per tree, and had a significant effect on the leaf P nutrient content variable.

Table 1 Significance of the Effect of Mycorrhizal Biofertilizer Prototypes with Different Spore Carrier Media (P), Pruning (C) and their Interaction (PC) on the Variables Observed

No	Variable	Treatment		
		P	C	PC
1	Nutrient content of P leaves (%)	ns	*	ns
2	Relative water content of leaves (%)	ns	**	ns
3	Chlorophyll content of leaves (SPAD)	**	ns	**
4	Number of flowers per tree (fruit)	**	ns	ns
5	Number of fruits per tree (fruit)	**	**	ns
6	Total sugar content	ns	ns	ns
7	Reduction sugar content	ns	ns	**
8	Leaf sucrose content	**	ns	**

Description: ns: not significant effect (P ≥ 0.05); *: significant effect (P < 0.05); **: very significant effect (P < 0.01)

The treatment of the prototype mycorrhizal biofertilizer (P) showed that the number of fruits per tree formed tended to be higher with mycorrhizal application on volcanic sand media Pv (74 fruits) compared to zeolite powder media Ps (60 fruits), and without Pt prototype application (27 fruits) was the lowest result. The high number of fruits per tree in the Pv treatment was supported by the high number of flowers per Pv tree (418.5 fruits). The growth process of the number of flowers per tree is influenced by the chlorophyll content of the leaves and the relative water content of the leaves. The high value of leaf chlorophyll content (38.73 SPAD) in plants will result in higher photosynthesis so that plants will grow well. It is proven that the correlation value of the number of flowers per tree is supported by leaf chlorophyll from the existence of a real positive correlation between leaf chlorophyll (r = 0.829*) and the number of flowers per tree. The real positive correlation value indicates that the higher the value of leaf chlorophyll, the more the number of flowers per tree. High chlorophyll content has a positive effect on photosynthesis which has implications for increasing crop yields. The high relative water content of the leaves (4.32%) causes the plant to be resistant to drought so that plant metabolism can still take place properly which will affect plant growth. The high content of leaf chlorophyll and relative water content of leaves is due to the provision of mycorrhiza with volcanic sand carrier. This is in line with research conducted [5] Putri (2019) that chlorophyll produced in chloroplasts in leaf photosynthetic tissues is a pigment that has a major role in the photosynthetic process to absorb sunlight so that plants are able to convert light energy into chemical energy stored in photosynthate. The high content of leaf chlorophyll and leaf KAR causes the photosynthesis process in cocoa plants to be more optimal. The content of total leaf sugar and leaf sucrose is the main result of the photosynthesis process, and is a source of energy for plants and materials stored in plant tissues. It is proven that the negative correlation value of leaf P content (r = 0, 388) with total sugar content and leaf sucrose is inversely proportional. This indicates that there is a more effective photosynthate translocation by administering the prototype mycorrhizal biofertilize when using volcanic sand media compared to other media.

In line with the results of research [6] Melani et al. (2022) stated that the prototype of indigenous FMA biofertilizer with volcanic sand as a carrier media provides an increase in soil fertility, and better carrier media for growth and yield on cocoa plantations in Mendoyo District, Jembrana. This is indicated by the highest number of fruits per tree, the highest chlorophyll. Based on research conducted [7] Febriyanti et al. (2020) showed that volcanic sand carrier media caused better growth in salak seedlings than sea sand. The application of volcanic sand as a spore carrier medium is based on the properties of volcanic sand which has a better cation exchange capacity than soil so that it can be used as a medium

for plant growth, besides that it also causes proper aeration for aerobic organisms to live. Volcanic sand with a coarse texture and low nutrients, so it is very good as a growing medium for the production of arbuscular mycorrhizal inoculum (Gunawan, 1993) [8]. The material component of volcanic sand has a low binding capacity to water due to its coarse structure. The silica content in volcanic sand indirectly acts as an adsorbent material, especially for water purification around the roots. Volcanic sand resulted in an increase in the levels of cations (Ca, Mg, K and Na) in the soil by almost 50% from the previous state and had a soil acidity (pH) of 5-6.5. Volcanic sand has fewer micro pores than clay with low moisture but the binding capacity of sand to water is low due to its coarse structure. The results of soil analysis after being treated with the prototype of mycorrhizal biofertilizer with volcanic sand carrier media showed the value of soil moisture content at air dry KU% (15.13%) and field capacity KL% (49.27%) affecting soil texture to sandy clay loam. Low soil water content causes dry land conditions, dry land is very supportive for the development of mycorrhiza, because it has good aeration so that it supports root growth. The surface area of plant roots increases which results in an increase in the amount of nutrients that can be absorbed by plants with the help of mycorrhizal hyphal (Lenny, 2004) [9].

The pruning treatment (Cd) on cocoa plantations in Tajun Village showed that the number of flowers per tree (433.33 pieces) and the number of fruits per tree (69 pieces) formed tended to be higher than without pruning (Pt) with the number of flowers per tree (308.67 pieces) and the number of fruits per tree (37 pieces). This was supported by the other 8 variables that showed that pruning branches and pruning water shoots (Cd) better than those without pruning (Ct). The high content of sugar and chlorophyll in the leaves causes the formation of flowers and the development of fruit will be better. In addition, pruning can reduce competition for nutrients in flower and fruit growth (Efendi, 2015) [10]. Based on the results of the analysis of Cd pruning treatment (twigs and water buds were pruned) caused high results of total leaf sugar content. The high content of total leaf sugar is supported by leaf sucrose, as evidenced by the real positive correlation to the total sugar content variable (r = 0.841*). The real positive correlation value shows that the higher the total sugar content of leaves, and leaf sucrose in this study, the higher the number of fruits per tree and the number of flowers per plant. [11] In line with the research of Rai et al. (2004) that the flowering process of mangosteen is also influenced by the total sugar content of leaves and total sugar of twig skin. At the time of flower induction, total sugar in the shoots increases and a greater increase occurs in the shoots that will induce flowers. Therefore, Cd pruning treatment can increase the accumulation of total sugar, leaf sucrose and chlorophyll. Good cocoa yield is influenced by the success of plants in photosynthesizing which is closely related to the chlorophyll content of the leaves. Reducing sugars are a class of sugars (carbohydrates) that can reduce compounds, for example, sucrose is not a reducing sugar, but when sucrose reacts with acid, sucrose turns into reducing sugars such as glucose and fructose. Through the process of respiration (glycolysis and Kreb's cycle) the reducing sugar (glucose) is broken down and produces energy that will be used for plant growth and development. There is an inverse relationship with a negative correlation value (r = -0.899*), causing high leaf reduction sugar content and decreased leaf sucrose sugar content. Production pruning is also carried out on dead twigs, water buds, overlapping branches, and parts of plants attacked by pests and diseases with the aim of stimulating vegetative growth towards more productive generative growth to produce a higher number of flowers per tree and a higher number of fruits per tree. In line with Nasaruddin's research [12], pruning unproductive plant parts and water buds will minimize the use of photosynthate for vegetative growth so that it can be used for reproductive growth in the form of flower and fruit formation.

The prototype treatment of mycorrhizal biofertilizers (P) and pruning (C) showed a very significant interaction on the sucrose content of the leaves. The best combination of mycorrhizal biofertilizer prototype with volcanic sand media and pruning (PvCd) resulted in the highest leaf sucrose content (21.24%), and the lowest occurred in combination without prototyping with PtCd pruning (15.84%). Mycorrhiza increases the absorption of plant nutrients by infecting the host plant's root system and producing hyphae intensively, infected roots become larger but do not damage the cells in the roots, with the support of pruning branches and water shoots providing PvCd can transfer nutrients to all parts of the plant and optimum photosynthesis. Plants without light can experience a decreased growth and die due to the inability to produce food through photosynthesis. Plants that are trimmed regularly will provide a good microenvironment for the growth of the plant itself. Leaves as photosynthetic organs play a very important role in setting the source to produce photosynthesis. Leaves that are too few will not be effective as photosynthetic organs because there are few photosynthesises, on the contrary, too many leaves and shade each other cause the process of photosynthesis will not be effective, so the reproductive organs lack food and cannot develop properly and may even fall.

Table 2 Effect of Mycorrhizal Biofertilizer Prototypes with Different Spore Carrier Media (P), Pruning (C) and their Interactions (PC) on Observed Variables

No	Variable	Prototype of mycorrhizal biofertilizer with different spore-carrying medium (P)			Pruning (C)	
		Pt	Ps	Pv	Ct	Cd
1	Nutrient content of P leaves (%)	0.88 a	0.93 a	0.92 a	0.96 a	0.85 b
2	Relative water content of leaves (%)	4.39 a	4.50 a	4.72 a	4.75 a	4.32 b
3	Chlorophyll content of leaves (SPAD)	209 c	240 a	228 b	226 a	226 a
4	Number of flowers per tree (fruit)	129 c	565.5 a	418.5 b	308.67 b	433.33 a
5	Number of fruits per tree (fruit)	27 c	60 b	74 a	37 b	69 a
6	Total sugar content (%)	69.14 a	67.54 a	72.07 a	69.21 a	69.96 a
7	Reduced sugar content (%)	17.14 a	14.88 b	14.95 b	16.49 a	14.82 a
8	Leaf sucrose content (%)	52 b	57.19 a	52.59 b	52.72 a	55.14 a

Description: The numbers followed by the same letter on the same treatment and same line showed not significant difference in the Least Significance Different test of 5% level.

The results of isolation and identification of endomycorrhizal fungi from cocoa root three cocoa production centers in Bali, namely the genus Glomus, Acaulospora, and Scutellospora. The Glomus spore type is the most commonly found from three soil sampling sites (Mendoyo District, East Selamadeg District, Kubu Tambahan District). The percentage of root colonization from the three sampling sites is very high at 100% each. Infected roots become bigger and expand but do not damage the root cells even when seen from the outside it looks like there is no change, this is in accordance with research [13] Very high colonization of host plant roots indicates that FMA isolated from cocoa roots can have excellent symbiosis with corn roots. In addition, Leal *et al*. [14] informing that the number of mycorrhizal spores from Acaulospora and Glomus in drought-affected Eucalyptus camaldulensis plants increased by more than 300 times compared to controls.

Table 3 Root Colonization, Identification and Number of Endomycorrhizal Spores in Cocoa Plant

Sample Location	Fungi Mycorrhizae Arbuscular (FMA)				Infection Root	Spore
	S	H	V	A		
East Selemadeg District	*	*	*	*	100%	Glomus
Mendoyo District	*	*	*	*	100%	Glomus, Acaulospores
Kubutambahan District	*	*	*	*	100%	Glomus, Scutellospores

Description: S = Spore, H = Hyphae, * = Infected FMA, V = Vesikular, A = Arbuscular

From the results of the soil analysis carried out, it can be concluded that the application of mycorrhizal biofertilizers can increase the level of soil fertility. Volcanic Sand Media (Pv) is the best medium as a carrier of mycorrhizal spores, this is indicated by a tendency to higher yields compared to other treatments. Mycorrhizal biofertilizers that have been applied increase soil fertility, increase the process of photosynthesis which affects the increase in leaf chlorophyll content, leaf sucrose content, and increasees N, P, K to increase fruit production per tree but also produce off-season fruit in cocoa plants, supported by research [15] with endomycorrhizal biofertilizers at a dose of 75 g / tree increases the amount of fruit and fruit weight per tree and produces fruit in the off-season, Fertilized granulated sugar plants get endomycorrhiza so that infected roots have a greater ability to absorb water and nutrients. Volcanic sand media has good aeration for mycorrhizal microorganisms so as to support root growth. Mycorrhiza infects and expands the surface of plant roots to increase which results in an increase in the amount of nutrients that can be absorbed by plants with the help of mycorrhizal hyphae tissue. In line with the opinion of Mathimaran et al. [16] the presence of an increase in

mycorrhiza caused the colonization of roots by mycorrhiza arbuskula to increase sharply, resulting in an increase in root range of up to 80% compared to the control indicating that the adaptability of FMA derived from cocoa roots to drought was very high.

Table 4 Effect of Mycorrhizal Biofertilizer Prototype with Different Spore Carrier Media (P) on pH, Electrical Conductivity (DHL), Organic C, Total N, Available P, and Available K in Soil

Treatment	pH (1:2,5)	DHL	C-Organic	N-Total	P-available	K-available
	H2O	(mmhos/cm)	(%)	(%)	(ppm)	(ppm)
Ps	6.9	5.43	3.62	0.59	305.24	494.02
	N	Very high	Tall	Tall	Very high	Very high
Pt	6.8	0.52	2.26	0.33	16.81	202.97
	N	Very law	Keep	Keep	Keep	Keep
Pv	7.0	4.35	3.59	0.64	228.27	451.42
	N	Very high	Tall	Tall	Very high	Very high

Table 5 Effect of Mycorrhizal Biofertilizer Prototype with Different Spore Carrier Media (P) on Soil Moisture Content and Soil Texture

Treatment	Moisture content (%)		Texture (%)		
	Dry air (KU%)	Field capacity (KL%)	Sand (%)	Dust (%)	Clay (%)
Ps	16.13	53.18	48.72	27.95	23.33
			Sandy clay		
Pt	15.85	40.29	38.07	28.55	33.38
			clay		
Pv	15.13	49.27	51.61	25.67	22.71
			Sandy clay		

4. Conclusion

The interaction between the treatment of mycorrhizal biological fertilizer prototype (P) and pruning (C) had a very significant effect only on leaf sucrose content (21.24%), leaf reduction sugar content (3.78%), and leaf chlorophyll content (37.18%). The combination of a prototype mycorrhizal biofertilizer with volcanic sand and pruning (PvCd) media can increase the growth and yield of cocoa plants. Treatment of prototype mycorrhizal biofertilizers with different spore carriers had a very significant effect on leaf chlorophyll content, number of flowers per tree, number of fruits per tree, and leaf sucrose. Volcanic sand-carrying media is better used than powdered zeolite, this is indicated by the tendency to have more flowers per tree (74 pieces), the relative water content of leaves (472.32%), P leaf content (0.92%), leaf total sugar content (72.07%), and leaf sucrose content (57.19%), as well as increased soil fertility. The pruning treatment (C) had a very significant effect on the number of fruits per tree (69 pieces) and the relative water content of the leaves (432.02%). And there is a tendency for plants to be pruned (Cd) more number of flowers per tree (433.33 pieces), total sugar content of leaves (69.96%) and sucrose content of leaves (55.14%).

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared that there is no conflict of interest

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