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Growth and yield response of organic cocoa (*Theobroma cacao* L.) to mycorrhizal biofertilizer prototype and pruning

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Abstract

Cocoa is one of the industrial plants that have a high market demand. Bali as a cocoa center requires efforts to improve the quality and quantity of cocoa production. Efforts to increase organic cocoa production can be gave by applying mycorrhizal biofertilizers and pruning. This study aimed to find prototypes of mycorrhizal biofertilizers with different carrier media and the best pruning on organic cocoa growth and yield. This study used a randomized block design with 2 factors and 6 replications. The first factor was the prototype of mycorrhizal biofertilizer consists of 3 levels, i.e., without mycorrhizal biofertilizer prototype/control, mycorrhizal biofertilizer prototype with zeolite spore carrier media, and mycorrhizal biofertilizer prototype with volcanic sand spore media. The second factor was pruning which consists of 2 levels, i.e., without pruning and pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pests and diseases and unproductive twigs. The results showed that mycorrhizal biofertilizer prototype with zeolite spore carrier media gave the highest weight of sun-dried seed per plant and number of seed per plant, while pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs gave higher cocoa yield in the form of weight of sun-dried seed per plant than that of without pruning. The interaction between the mycorrhizal biofertilizer prototype and pruning had a significant effect on leaf chlorophyll variables.

Keywords: Biofertilizer prototype; Cocoa; Carrier media; Mycorrhiza; Pruning

1. Introduction

Cocoa (*Theobroma cacao* L.) is one of the industrial crops whose market needs are quite high so it has great potential to be developed into a superior commodity in the plantation sector [1]. Bali is a contributor to national cocoa production with the highest production in Jembrana Regency; Tabanan Regency; and Buleleng Regency [2]. Cocoa production in Bali has increased but the productivity and quality of production are still low so efforts need to be made to increase.

Efforts to increase the quality and quantity of cocoa production can be made by paying attention to all aspects involved in cocoa farming such as fertilization and pruning. The limited availability of fertilizer at high prices has resulted in efforts to increase production not running optimally. Central and local government policies to improve the quality and production of cocoa since 2009 as well as environmentally sound agricultural development with organic farming systems following Bali Province Regional Regulation No. 8 of 2019 mandates the use of biofertilizers as an alternative effort to increase production and maintain the productivity of cropland [3]. The principle of biofertilizer is the empowerment of indigenous microbes or local microbes found in plant roots. Biofertilizers play a role in the provision and absorption of nutrients; decompose organic matter and help provide good aeration conditions in plant roots to increase plant growth and yield. Mycorrhiza is one example of a biofertilizer that can associate with plants naturally.

The association between plants and mycorrhiza will increase the absorption of phosphate and other macronutrients such as nitrogen; potassium; and magnesium [4]. The application of biofertilizer is carried out by making a biofertilizer

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prototype that combines mycorrhizal spore inoculum and spore carrier media. The function of spore carrier media is as an agent and a place for mycorrhizal spore inoculum to live before being applied to plants so that it has a shelf life for a certain period [5]. Spore carrier media that can be used such as zeolite powder and volcanic sand. Zeolite powder has a high porosity derived from the crystal structure of zeolite so it can hold up to 60% air. Air molecules can be easily absorbed and evaporated from inside the zeolite pores without damaging the structure and its high cation exchange capacity between 200 - 300 meq/100 g. Volcanic sand is dominated by the sand fraction with a high silica content and base saturation so that it can improve soil structure; and reduce the toxic effects of compounds and soil organic acids [6].

Plant productivity can be maintained and increased through sustainable plant maintenance such as by pruning plants. Pruning cocoa plants aims to optimize LAI (Leaf Area Index) by cutting consumptive; unproductive twigs and water shoots so that nutrients will be channeled to the reproductive phase which is characterized by the formation of flowers and fruits [7]. Overlapping plant leaves are also classified as unproductive and tend to be consumptive because they do not get light in the photosynthesis process [8]. Pruning plays a role in reducing plant moisture; providing a suitable microclimate; and suppressing and making it easier to control pests and diseases in cocoa plants. Pruning is done in the form of shape pruning; maintenance pruning; and production pruning. Shape pruning is done to form a balanced plant framework; maintenance pruning is done to maintain the framework and remove unnecessary branches and production pruning is done to spur flower and fruit growth [9]. Based on the description above; this study aims to determine the effect of the interaction of mycorrhizal biofertilizer prototypes and pruning and to obtain mycorrhizal biofertilizer prototypes with the best carrier media and the best pruning in increasing cocoa growth and yield.

2. Material and methods

2.1. Location and experimental design

Soil and root samples were taken from cocoa plantations in Selemadeg Timur District Tabanan, Mendoyo District Jembrana, and Kubutambahan District Buleleng. Isolation, identification, and prototyping of mycorrhizal biofertilizers were carried out at the Agronomy and Horticulture Laboratory, Faculty of Agriculture, Udayana University, Genetic Resources and Molecular Biology Laboratory, Udayana University, and Plant Disease Laboratory, Faculty of Agriculture, Udayana University. The application of the prototype of mycorrhizal biofertilizer and pruning was carried out in the Cocoa Plantation of Gadungan Village, Selemadeg Timur District, Tabanan Regency. This research was conducted from July to December 2022.

This study used a 2-factor Randomized Group Design with 6 replicates. The first factor is the prototype of mycorrhizal biofertilizer (P) consists of 3 levels, i.e., without mycorrhizal biofertilizer prototype/control (Pt), mycorrhizal biofertilizer prototype with zeolite powder spore carrier media (Ps), and mycorrhizal biofertilizer prototype with volcanic sand spore media (Pv), while the second factor is pruning (C) consist of 2 levels, i.e., without pruning(Ct), and pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs (Cp).

2.2. Endomycorrhizal isolation

Spores were isolated using a set of Pacioni (1992) wet filters, where 100 g of soil sample was dissolved in 1000 - 1200 ml of water and stirred well, then filtered with a sieve of 1 mm, 500 µm, 212 µm, 106 µm, and 53 µm, from large to small holes 2 - 3 times and repeated 10 times on the same soil sample. The soil remaining in the 500 µm, 212 µm, 106 µm, and 53 µm sieves was transferred to a centrifuge tube and 25 - 40 ml of distilled water was added, after which it was centrifuged using the Brunndret et al. centrifugation technique for 5 minutes at 2000 rpm. The results of the centrifuge were discarded supernate and then added 60% glucose and centrifuged again for 1 minute at 2000 rpm. The supernatant containing glucose was rinsed with water on a 53 µm filter and the results of the rinse were placed in a petri dish and then observed for the number and type of spores.

2.3. Manufacture and application of mycorrhizal biofertilizer prototypes

The manufacture of a mycorrhizal biofertilizer prototype is carried out by combining the type of mycorrhizal isolate/genus with the type of carrier media (carrier) with the composition of each 500 grams of carrier media according to the level of spore carrier media treatment sown 150 consortium spores from various and genus. The carrier media used are zeolite and volcanic sand.

The application of the prototype mycorrhizal biofertilizer was carried out when the study began. The treated plants were cocoa plants that were already in production. Before treatment, all sample plants were cleaned of weeds and the soil was loosened around the crown. After that, fertilization with organic fertilizer was carried out on all trees and continued with sowing mycorrhizal biofertilizer according to the treatment on the soil around the base of the cocoa trunk.

2.4. Pruning cocoa plants

Plant pruning is done according to the treatment level. Plants that receive pruning according to the level will be pruned at the ends of twigs (2-3 segments), dead twigs, twigs attacked by pests and diseases and unproductive twigs. The pruning of cocoa plants is done during the flowering period.

2.5. Observed variable

2.5.1. Leaf chlorophyll content

Leaf chlorophyll content was observed twice during the study, namely in the fifth and tenth weeks after treatment with the Chlorophyll Meter SPAD-502 tool. Measurements were made by taking a sample of the upper, middle, and lower leaves of each 2 leaves, then each leaf was measured as many as 10 points. By pressing the average button on the tool, the average value of chlorophyll content is obtained.

2.5.2. Weight of sun-dried seeds per plant

Weight of sun-dried seeds per plant was calculated by weighing all seeds at each harvest on each tree according to the treatment and then accumulated until the last harvest.

2.5.3. Number of seed per plant

Observations of the number of seeds per plant were made by counting the number of seeds in each fruit and then summing them according to the number of fruits in the same treatment.

2.5.4. Weight per seed

Weight per seed was calculated by dividing the weight of all seeds by the number of seeds in the same treatment.

2.5.5. Leaf P nutrient content

Analysis of leaf P nutrient content was carried out using samples of terminal shoot leaves (the most terminal leaves of the shoots/twigs) and analyzed once during the fruit enlargement phase using the Olsen and Bray method. Samples that have been taken are then dried in an oven and mashed if the weight is constant.

2.5.6. Relative water content of leaves

Measuring the relative water content of leaves requires 6 leaves from each tree with the provisions of 2 sheets from the upper twigs, 2 sheets from the middle twigs, and 2 sheets from the lower twigs. The leaves were picked and then put into aluminum foil to make it watertight and then put into a thermos filled with ice. From the six leaves, 24 pieces of leaves with a size of 1 x 1 cm were taken and then weighed fresh weight (FW). After weighing, it was put into a Petri dish filled with water and irradiated with 40-watt fluorescent light at room temperature for 5 hours. After that, it was removed and cleaned from the remaining sir attached and weighed the turgid weight (TW). After that, the leaf pieces were baked for 24 hours at 700 C until they reached a constant dry weight (DW). The value is calculated by the formula:

$$\text{Leaves RWC (\%)} = \frac{Fresh\ Weight\ (FW) - Dry\ Weight\ (DW)}{Turgid\ Weight\ (TW) - Dry\ Weight\ (DW)} \times 100\ \%$$

2.5.7. Total sugar, reducing sugar and leaf sucrose content

Analysis of total sugar, reducing sugar and leaf sucrose content aims to determine the adequacy of photosynthate to induce flowering. Total sugar analysis was carried out by the Anthrone method, reducing the sugar by the Nelson - Somogyi method, while leaf sucrose was calculated from total sugar minus reducing sugar multiplied by 0.95. The leaves to be analyzed were harvested in the morning and immediately put into a thermos filled with ice. After arriving at the laboratory, the samples were oven dried and pulverized, and then stored in the freezer.

2.6. Statistical analysis

Data were analysed using analysis of variance (Anova). If the interaction between the prototype mycorrhizal biofertilizer and pruning had a significant effect, it was continued with the Duncan's Multiple Range Test at the 5% level, but if the interaction had no significant effect, then the single factor influence was tested with the Low Significant Difference) Test at the 5% level.

3. Results and discussion

3.1. Endomycorrhizal isolation and identification results

The results of isolation and identification of endomycorrhizal at three locations of cocoa plantation found that the glomus type was found in all three-soil sampling location (Selemadeg Timur District, Mendoyo District and Kubutambahan Dictrict), while the Scutellospora type was only found in soil samples from Kubutambahan District and Acaulospora type was onlu found in Mendoyo District soil sample (Table 1). This is in accordance with the results of research by Rai et al. [10] that endomycorrhizal spores in several places in the province of Bali are dominated by the genus Glomus, especially at Pupuan, Payangan, Selat, and Bebandem. Glomus genus spores are also found in coal mining areas which show that Glomus has a better adaptation and resistance mechanism compared to other spore genera [11].

Table 1 Results of isolation and identification of endomycorrhizas in soil and root samples from three cocoa plantation location

Location Sample	Fungi Mikoriza Arbuskula (FMA)				Root Infection	Genus of Mycorrhizal
	S	H	V	A		
Selemadeg Timur District	*	*	*	*	100%	Glomus
Mendoyo District	*	*	*	*	100%	Glomus, Acaulospora
Kubutambahan District	*	*	*	*	100%	Glomus, Scutellospora

Description: S = Spore, H = Hifa, * = Infected FMA; V = Vesikula, A = Arbuskula

3.2. Significance of treatment on Observation variable

The results of the analysis of variance showed that the interaction between mycorrhizal biofertilizer prototype treatment (P) and pruning (C) had a very significant effect on leaf chlorophyll content. Mycorrhizal biofertilizer prototype (P) had a very significant effect on leaf chlorophyll content, weight of sun-dried seeds per plant, number of seeds per plant, and relative water content of leaves and had a significant effect on reducing sugar content. Pruning treatment had a very significant effect on leaf chlorophyll content and the number of seeds per plant (Table 2).

Table 2 Significance of the Effect of Mycorrhizal Biofertilizer Prototype (P) and Pruning (C) on Observation Variables

No	Variables	Treatment		Interaction
		P	C	
1	Leaf Chlorophyll Content (SPAD)	**	**	**
2	Weight of sun-dried seeds per plant (g)	**	ns	ns
3	Number of seeds per plant (Unit)	**	**	ns
4	Weight per seed (g)	ns	ns	ns
5	Leaf P nutrient content (%)	ns	ns	ns
6	Relative water content of leaves (%)	**	ns	ns
7	Total sugar content (%)	ns	ns	ns
8	Reducing sugar content (%)	*	ns	ns
9	Leaf sucrose content (%)	ns	ns	ns

Description: ns: not significantly effect (P<0,05); *: significant effect (P>0,05); **: very significant effect (P>0,01).

3.3. Treatment interaction on leaf chlorophyll content

Leaf chlorophyll content showed the highest results in the combination of mycorrhizal biofertilizer prototype with Ps zeolite spore carrier media with pruning Cp (PsCp) with a value of 88.26 SPAD, the lowest at PtCt with a value of 55.70 SPAD and significantly different from all combination treatments, both Pt, Ps and Pv leaf chlorophyll content is higher in Cp compared to Ct (Table 3).

Table 3 Interaction of Mycorrhizal Biofertilizer (P) and Pruning (C) on Leaf Chlorophyll Content

Treatment	Leaf Chlorophyll Content (SPAD)	
	Ct	**Cp**
Pt	55.70 f	60.42 e
Ps	85.82 b	88.26 a
Pv	62.20 d	72.19 c

Description: Numbers followed by different letters in the same treatment and column indicate significant differences in Duncan's multiple range test (DMRT) at the 5% level.

3.4. Effect of mycorrhizal biofertilizer prototype and pruning on observed variables

Mycorrhizal biofertilizer prototype (P) gave the highest weight of sun-dried seed per plant on zeolite carrier media (Ps) (265.42 g) than volcanic sand media (Pv) and without prototype application (Pt) i.e., 177.08 g and 53.33 g, respectively. The high weight of sun-dried seeds per plant in the Ps treatment is supported by the value of the number of seeds per plant (329,50 unit) and weight per seed (0.80 g). The high cocoa yield in the treatment was supported by the high value of leaf chlorophyll content (87,04 SPAD) and Relative Water Content (RWC) of leaves value (84.59%) (Table 4). Chlorophyll can absorb sunlight which plays an important role in the photosynthesis process, especially as a source of energy so that plants can convert photosynthetic substrates into photosynthate or sugar [12]. High chlorophyll content has a positive influence on photosynthesis results which have implications for increasing crop yields [13]. This is also supported by a very significant positive correlation coefficient value between leaf chlorophyll content and weight of sun-dried seed per plant (r = 0.882**) and a very significant positive with weight per seed (r = 0.757**).

The high relative water content of leaves value indicates that the application of mycorrhiza with zeolite carrier media can overcome water stress in cocoa plants. The symbiosis between plants and mycorrhizae will meet the water and nutrient needs of the host plant even under water stress conditions [14]. Sufficient RWC will ensure the stability of plant metabolism including enzyme formation and photosynthesis so it will have implications for increasing plant growth and yield [15,16]. Leaf P nutrient content also showed a significant effect on cocoa yield. Phosphorus is one of the nutrients whose role is very important to form energized compounds such as ATP [17]. ATP plays an important role in plant metabolisms such as photosynthesis and photosynthate translocation in plants [18]. This is in line with the results of the study where the highest leaf P content was obtained in Ps (0,253%) and inversely proportional to the value of total sugar, reducing sugar, and leaf sucrose content which were lowest in Ps. If the plant is in the reproductive phase, the photosynthate produced will be channeled to the fruit as a generative organ [19]. This indicates that there is a translocation of photosynthate to the fruit that is more effective with the application of mycorrhizal biofertilizer prototypes using zeolite spore carrier media compared to other media.

Pruning treatment (C) showed higher weight of sun-dried seeds per plant with application pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs (Cp) (191.11 g) than that of on without pruning (Ct) (139.44 g). This is also supported by the value of the other 4 variables which show that the Cp treatment is better than Ct namely number of seeds per plant, Leaf chlorophyll content, RWC, and leaf P nutrient content (Table 4). Weight of sun-dried seeds per plant is influenced by yield components such as number of seeds per plant. A good cocoa yield component is influenced by the success of plants in photosynthesizing which is closely related to the chlorophyll content of the leaves. The Cp treatment shows a higher leaf chlorophyll content of 7.8% compared to Ct and has a very significant correlation value with dry bean weight per tree (r = 0.882**). A more even and efficient distribution of cocoa leaves will affect the distribution of light in the canopy, the effectiveness of light energy absorption, water availability in the leaves, photosynthesis, and photosynthate use efficiency [20]. Pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs provide ideal plant canopy conditions so that photosynthesis will be optimum and minimize unproductive leaves that have the potential to become consumptive leaves (sinks) [21]. The use of photosynthate by unproductive leaves will affect the formation of flowers and fruits as sink storage. Pruning will minimize the use of photosynthate by unproductive parts of the plant in the form of shaded crowns, leaves covered by other leaves, and water shoots so that the translocation of assimilates for vegetative growth can be used for reproductive growth in the form of flower and fruit formation [9].

Table 4 Effect of Mycorrhizal Biofertilizer Prototype (P) and Pruning (C) on observed variables

No	Parameter	Mycorrhizal Biofertilizer Prototype			LSD 5 %	Pruning		LSD 5%
		Pt	Ps	Pv		Ct	Cp	
1	Leaf chlorophyll content	58.06 c	87.04 a	67.20 b	1.72	67.91 b	73.62 a	1.72
2	Weight of sun-dried seeds per plant (g)	53.33 b	256.42 a	177.08 a	91.2	139.44 a	191.11 a	ns
3	Number of seeds per plant (unit)	77.00 b	329.50 a	241.83 a	88.56	173.11 a	259.11 a	88.56
4	Weight per seed (g)	0.69 a	0.80 a	0.72 a	ns	0.76 a	0.71 a	ns
5	Leaf P nutrient content (%)	0.250 a	0.253 a	0.230 a	ns	0.240 a	0.248 a	ns
6	Relative water content of leaves (%)	80.66 b	84.59 a	84.44 a	0.03	83.81 a	82.65 a	ns
7	Total sugar content (%)	25.06 a	23.93 a	24.52 a	ns	25.29 a	23.72 a	ns
8	Reducing sugar content (%)	2.86 a	2.22 b	2.66 ab	0.42	2.57 a	2.59 a	ns
9	Leaf sucrose content (%)	22.19 a	21.71 a	21.85 a	ns	22.71 a	21.13 a	ns

Description: Numbers followed by the same letter in the same row and treatment show no significant difference in the 5% level of low significant difference test (LSD).

The treatment of mycorrhizal biofertilizer prototype (P) and pruning (C) showed the best interaction in the mycorrhizal biofertilizer prototype with zeolite spore carrier media and pruning (PsCp) on leaf chlorophyll content. The leaf chlorophyll content is a very important supporting factor in determining yield components. High chlorophyll content will affect the effectiveness of plants in carrying out photosynthesis to produce photosynthates and store them as seeds. Prototypes with zeolite spore media give plants the ability to absorb nutrients through a symbiosis between mycorrhiza and plant roots. Pruning gives plants effectiveness in photosynthesis and photosynthate translocation so that plant yields will increase. This is supported by the value of dry seed weight per tree and the higher number of seeds per tree in Cp due to more optimum photosynthate translocation through the provision of pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs. Shaded the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs are sinks that have the potential to reduce the amount of photosynthate stored in the form of fruit and seeds by plants or sink storage so that it needs to be eliminated by providing pruning.

4. Conclusion

Mycorrhizal biofertilizer prototype with zeolite spore carrier media gave the highest weight of sun-dried seeds per plant was 256.42 g, and the highest number of seeds per plant (329.50 units). In addition, the treatment level also gave the highest value for the variable weight per seed (0.80 g), relative water content of leaves (84.59%), leaf P content (0,253%), and leaf chlorophyll content (87.04 SPAD). Prototype mycorrhizal biofertilizer with volcanic sand carrier media gave the highest value only for the variable total sugar content. Pruning on the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs gave the higher weight of sun-dried seeds per plant, number of seeds per plant, leaf P nutrient content, leaf chlorophyll content, and reducing sugar content, than those of on without pruning. The interaction between mycorrhizal biofertilizer prototype with zeolite powder spore carrier media and pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs had a very significant effect on leaf chlorophyll content and had no significant effect on other variables. Base on this result, it can be recommended to use mycorrhizal biofertilizers with powdered zeolite media and pruning the ends of twigs (2-3 segments), dead twigs, twigs attacked by pest and diseases and unproductive twigs to achieve higher growth and yield of organic cocoa.

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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