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Physiological activities of cocoa trees induced by soil and foliar applications of boron fertilizer

Endang Sri Dewi HS[1]*, Prapto Yudono[2], Eka Tarwaca Susilaputra[2], Benito Heru Purwanto[3]

[1] Departement of Agrotechnology, Faculty of Agriculture, Universitas Sintuwu Maroso, Central Sulawesi, Indonesia
[2] Departement of Agrotechnology, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3] Department of Soil Science, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia

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***** *Corresponding Author*
Email address:
sridewihs83@gmail.com

ABSTRACT

This study investigates the impact of boron fertilizer on physiological activities of cocoa trees, specifically focusing on boron content, nitrogen content, nitrate reductase activity, chlorophyll content, and photosynthesis rate in cocoa plant leaves. This research was arranged in a randomized complete block design with two treatment factors, which were the type of boron fertilizer application (soil and foliar fertilizer), and the dose of boron fertilizer (1.5, 3, 4.5, and 6 g plant^{-1} with 0 g plant^{-1} as a control). Data were then analyzed for variance differences (ANOVA) with α = 5%, followed by the Tukey test, and contrast orthogonal for comparing treated and control plants. The results showed that the dose of boron fertilizer and the type of fertilizer application have a significant effect on the physiological activity of the cocoa plant. The dose of boron with soil application affects physiological activity in a linear pattern where each additional dose of boron will increase the activity of nitrate reductase, chlorophyll content, and photosynthetic rate. The dose of boron with foliar application affects physiological activity in a quadratic pattern, where the dose of boron in the range of 3 g plant^{-1} is the optimum dose that gives maximum results on nitrate reductase activity, chlorophyll content, and photosynthetic rate in the cocoa leaves. Therefore, it is considered that the application of boron fertilizer at a dose of 3 g plants^{-1} with the foliar application is more efficient in increasing physiological activity compared to the dose of boron with soil application.

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1. INTRODUCTION

Cocoa is one of the most significant commercial plantation crops in Indonesia. Indonesia is the third-largest country as an exporter of cocoa beans after the Ivory Coast and Ghana until 2019 (ICCO, 2020). Cocoa productivity in Indonesia is only around 0.7 tons ha^{-1} year^{-1}, lower than its potential productivity, which can reach more than 2 tons ha^{-1} year^{-1} (Rubiyo & Siswanto, 2012). Several factors cause this problem to occur. One of the factors is the lack of nutritional intake from the soil during the cocoa growth and development phase (Kongor et al., 2019; Mulia et al., 2019; Risnah et al., 2013). This results in a decrease in the physiological activity of cocoa plants and their ability to produce optimally. The cocoa plant bears fruit throughout the year, but the highest harvests occur between July and December. The plant is highly responsive to nutrients due to its intensive annual crops that fruit throughout the year (Wahyudi et al., 2015). Moreover, the nutrients in the soil can be supplied in the form of fertilizer. Soil nutrients can be supplied in the form of fertilizer. The addition of nutrients aims to support the sustainability of crop cultivation (Koko, 2014).

The application of nutrients to cocoa plants is still limited to macroelements, whereas microelements are crucial and should be particularly considered. Boron is one of the microelements that have an important role in crop growth and development (Marschner, 2011). The availability of boron is essential for crops because it plays a significant role in cell development, protein metabolism, amino acids, nitrates, fats, carbohydrates, auxin, and phenols, membrane function, flower formation, fruit fertilization, and fruit development (Koko, 2014; Li et al., 2017; Marschner, 2011;

Xiao et al., 2022). Boron deficiency inhibits water transportation from the roots to the top of the plant by inhibiting and damaging the phloem tissue system (Liu et al., 2022; Marschner, 2011).

The deficiency of boron has been reported to damage and cause stunted crop growth in various plants, including necrosis on citrus (Huang et al., 2014); stomata tissue damage in Soybean and Lychee plants (Will et al., 2012); reduction of the chlorophyll content and soluble protein in mustard (Meriño-Gergichevich et al., 2017; Mukhopadhyay & Mondal, 2015; Wimmer & Eichert, 2013); a decrease in the mesophyll content of kiwi leaves (Seth & Aery, 2014); and chlorosis on watermelon leaves (Moustafa-Farag et al., 2016). Furthermore, boron deficiency can affect pollen viability, seed filling, and the amount of fruit that is formed at the stage of fruit development. Additionally, boron may inhibit the transport of carbohydrates, the synthesis of hormones, and the enzyme dehydrogenase (Ali et al., 2019; Marschner, 2011; Seilsepour et al., 2013). Moreover, nitrate reductase is one of the enzymes that convert nitrates to nitrites (Meriño-Gergichevich et al., 2017; Mukhopadhyay & Mondal, 2015). Reducing nitrate to nitrite is the first step in forming amino acids, which will be used as constituents of proteins and other plant parts (Marschner, 2011). Chlorophyll is the pigment that gives green colour to plant. This pigment converts solar energy into chemical energy in the process of photosynthesis. Chlorophyll in higher plants is divided into two namely chlorophyll a ($C_{55}H_{72}O_5N_4Mg$) and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$). Chlorophyll a and chlorophyll b are very strong at absorbing light at a wavelength of 600-700 (red light) nm and slightly absorbing sunlight at wavelengths of 500-600 nm (green light) (Dewi HS et al., 2020a; Li & Chen, 2015). Chlorophyll b absorbs more photons than chlorophyll a, photons absorbed by chlorophyll b will be transferred to chlorophyll A. Chlorophyll is most commonly found in photosystem II (Dewi HS et al., 2020b). In Indonesia, there has been limited research on the role of boron in the physiological activity of cocoa plants. The study aims to observe the effect on the chlorophyll content and nitrate reductase activity in cocoa on the application of boron fertilizer through the leaves and the soil.

2. MATERIAL AND METHODS

2.1. Materials

The cocoa plants selected as samples were healthy and had the same degree of uniformity. The clone used was the RCC 71 clone. The soil at the research location is low (0.043 ppm (Dewi HS et al., 2020b). The research was conducted from June 2018 to February 2019. The research site is located in the PT Pagilaran North Segayung Production Unit cocoa plantation in Simbangjati Village, Tulis, Batang, Central Java, Indonesia.

2.2. Methods

This field research was arranged in a randomized complete block design with two treatment factors. The first factor was the type of boron fertilizer application, i.e., soil fertilizer and foliar fertilizer. Boron was applied in solid form as soil fertilizer, and boron was applied to the leaves in liquid form for foliar application. The second factor was doses of boron fertilizer (1.5 g plant^{-1}, 3 g plant^{-1}, 4.5 g plant^{-1}, 6 g plant^{-1}, and 0 g plant^{-1} as a control). There were 10 combinations of treatments; each combination had three replications with five plants, for a total of 150 samples.

2.3. Nitrogen and Boron content in the leaves

The Kjeldahl method was used for the estimation of the total nitrogen (N) (Dewi HS et al., 2020b), and Morgan-Wolf extraction was employed for the determination of boron (Dewi HS et al., 2020b).

2.4. Chlorophyll content

The chlorophyll contents of leaves was measured by using Harbon spectrophotometer. 0,1 gram fresh cacao leaves was taken and ground with help of pestle and mortar, solution by 50 mL acetone 80%. The extract was separated from its residue by filtrate paper on Erlenmeyer glass. The extract was utilized for chlorophyll estimation. The UV-spectrophotometer read absorbance at 645 and 663 nm (Dewi HS et al., 2020b). Chlorophyll content was counted by Formula 1.

$$Chlorophyll\ a = 12.21 \times A663 - 2.81 \times A646\, x\, 50;$$
$$Chlorophyll\ b = 20{,}13 \times A646 - A663 \times 50;$$
$$Chlorophyll\ total = Chlorophyll\ a + Chlorophyll\ b \text{ .... [1]}$$

2.5. Nitrate reductase activity

Nitrate reductase activity was measured using the spectrophotometric method. Briefly, 0.2 g of cacao leaves were cut into small pieces with a size of 0.5 cm and put into a dark bottle containing 5 mL of 0.1 M phosphate buffer (pH 7.0) for 24 hours. Later, the leaves were placed in a buffer solution containing 0.1 mL of 5 M sodium nitrate ($NaNO_3$) as a substrate and incubated for 2 hours. After the incubation, the filtrate was collected into a tube containing 0.2 mL of 1% sulphanilamide in, 3 N hydrochloric acid and 0.2 mL of 0.02% N-Naphtylethylene, and incubated until pink coloration appeared, indicating the change of nitrate to nitrite by the nitrate reductase enzyme. The color change was recorded at a wavelength of 540 nm using a spectrophotometer (Dewi HS et al., 2020b).

2.6. Photosynthesis rate

Photosynthesis rate was measured by using Licor 6400.

Table 1. Average total nitrogen content of leaves (%) by the type of application and doses of boron fertilizer treatments.

Type of boron fertilizer application	Total nitrogen content of leaves				
	Doses of boron fertilizer (g plant^{-1})				
	0	1.5	3	4.5	6
Soil application	1.80^{e}	2.45^{cd}	2.76^{bc}	3.26^{ab}	3.29^{a}
Foliar application	1.81^{e}	2.64^{c}	3.13^{ab}	2.46^{cd}	2.21^{e}

Note: the average number followed by the same letter shows no significant difference in the HSD test at the 95% significance level.

Table 2. Relations between the nitrogen content of leaves and the type of application and doses of boron fertilizer treatments

Observed variables	Equation	Optimum dose of boron	Maximum nitrogen in leaves
Soil application			
N leaves	$y = 0.2537x + 1.9549$, $R^2 = 0.94$	-	-
Foliar application			
N leaves	$y = -0.1057x^2 + 0.6755x + 1.8532$, $R^2 = 0.86$	3.19	2.93

Note: the boron fertilizer with soil application has a linear equating formula and the boron fertilizer with the foliar application has quadratic equation formula.

Table 3. The average total boron content of leaves (%) on type of application and doses of boron fertilizer treatments

Type of application boron fertilizer	Total boron Content of Leave (%)				
	Doses of boron fertilizer (g plant^{-1})				
	0	1.5	3	4.5	6
Soil application	0.010^{d}	0.018^{c}	0.017^{c}	0.022^{abc}	0.033^{ab}
Foliar application	0.011^{d}	0.017^{c}	0.022^{b}	0.035^{abc}	0.042^{a}

Note: the average number followed by the same letter shows no significant difference in the HSD test at the 95% significance level.

3. RESULTS

3.1. Nitrogen and boron content of the leaves (%)

The results demonstrated that the total N content of the leaves varied depending on the dose and type of boron administration. The soil application with doses of 6 and 4.5 g boron plant^{-1}, and the foliar application with a dose of 3 g boron plant^{-1} resulted in the highest total N content in the leaves. The lowest total N leaf content was observed in the control treatment for both types of applications (Table 1). There is a different relationship pattern for the two types of fertilizer application given boron fertilizer with soil applied gives a quadratic increase in total N, which means that each additional dose of boron in the foliar application increases the number of N leaves by the R-value, which is 0.93%. Moreover, boron fertilizer with foliar application results in a quadratic growth pattern, with a leaf N content of 2.93% after applying 3.19 g of boron fertilizer (Table 2).

Table 2 shows a significant relationship between the total boron content of leaves and the total N content of leaves in boron fertilizer with soil application (R-value = 0.86), in contrast with boron fertilizer with the foliar application, which was not significantly correlated with N uptake (R-value = 0.04). This difference occurs due to the foliar application causing boron uptake on leaves to increase dramatically (Table 3).

3.2. Nitrate reductase activity

The results showed an interaction between the dose and the type of boron application on the nitrate reductase activity of cocoa plant leaves. The highest nitrate reductase activity was shown in foliar application with doses of boron 3, 1.5, and 4 g plant^{-1} and the treatment of soil application with a dose of boron 6 g plant^{-1}. (Table 4). There is a different relationship pattern for the two types of fertilizer application. Soil fertilizer application provides a linear relationship with nitrate reductase activity, i.e., each additional dose of boron in the application increases leaf nitrate reductase activity by an R-value of 0.03 μmoll NO_2 g^{-1} h^{-1}. Meanwhile, boron fertilization with foliar applications gave a quadratic pattern of nitrate reductase activity. This suggests that there is a minimum dose of boron fertilizer (2.87 g plant^{-1}) that produces maximum nitrate reductase activity (1.80 moll NO_2 g^{-1} h^{-1}) (Table 5).

The results showed an interaction between the dose and the type of application of boron fertilizer on the chlorophyll a, chlorophyll b, and total chlorophyll content of cocoa plant leaves. The highest levels of chlorophyll a were shown in foliar applications with doses of boron 3 and 4.5 g plant^{-1} as well as in the treatment of soil applications with doses of boron 4.5 and 6 g plant^{-1}. The highest chlorophyll b was shown in the soil application with a dose of boron between 3 and 4.5 g. The highest total chlorophyll was shown in the foliar application with doses of boron 1.5, 3, and 4.5 g and in the treatment of soil application with doses of boron 4.5 and 6 g plant^{-1} (Table 6). There are different relationship patterns for the two types of fertilization applications. The soil application of boron provided a linear relationship between increasing chlorophyll a, chlorophyll b, and total chlorophyll content on the cocoa leaves, suggesting that each additional dose of boron in the application increases the chlorophyll content of leaves by the R-value (0.78 mg g^{-1}, 0.94 mg g^{-1}, 0.87 mg g^{-1}, respectively). Moreover, foliar application of boron increased the concentration of chlorophyll a, chlorophyll b, and total chlorophyll in a quadratic pattern. The dose of boron fertilizer, i.e., 3.16, 2.74, and 2.95 g plant^{-1}, produced maximum levels of chlorophyll a, chlorophyll b, and total chlorophyll content in the cocoa leaves of 0.60 mg g^{-1}, 0.52 mg g^{-1}, 1.12 mg g^{-1}, respectively (Table 7).

3.3. Photosynthesis rate

The results indicate an interaction between the dose and the type of boron application on the photosynthesis rate of cocoa plant leaves. The highest photosynthesis rate was shown in foliar application with doses of boron 3, 1.5, and 4.5 g plant^{-1} and the treatment of soil application with doses of boron 4.5 and 6 g plant^{-1} (Table 8). There is a different relationship pattern for the two types of fertilizer applications. Soil fertilizer application provides a linear relationship with the photosynthesis rate. Furthermore, each additional dose of boron in the application increases the leaf photosynthesis rate by an R-value of 0.94 μmoll CO_2 m^{2-1-1} s^{-1}. Moreover, boron fertilization with foliar application gave a quadratic pattern of photosynthesis rate, indicating that there is a minimum dose of boron fertilizer (3.04 g plant^{-1}) that produces a maximum photosynthesis rate (166.97 μmoll CO_2.m^{2-1-1} s^{-1}) (Table 9).

Table 4. Average of nitrate reductase activity on type of application and doses of boron fertilizer treatments

Type of application boron fertilizer	Nitrate Reductase Activity (μmoll NO_2 g^{-1} h^{-1})				
	Doses of boron fertilizer (g $plant^{-1}$)				
	0	1.5	3	4.5	6
Soil application	1.4847^{e}	1.4964^{de}	1.6170^{bcde}	1.6561^{bcd}	1.6960^{abc}
Foliar application	$1.558d^{e}$	1.7341^{ab}	1.8427^{a}	1.6989^{abc}	1.5199^{de}

Note: the average number followed by the same letter shows no significant difference in the HSD test at the 95% significance level.

Table 5. Relationship between the type of application and doses of boron fertilizer treatments with nitrate reductase activity (μmoll NO_2 g^{-1} h^{-1}) in the cocoa leaves.

Observed variables	Equation	Optimum doses of boron	Maximum nitrate reductase activity
Soil fertilizer			
Nitrate Reductase Activity	$y = 0.0068x + 1.5696$, $R^2 = 0.03$	-	-
Foliar fertilizer			
Nitrate Reductase Activity	$y = -0.0601x^2 + 0.3607x + 1.6011$, $R^2 = 0.93$	3.00	2.14

Note: the boron fertilizer with soil application has a linear equating formula and the boron fertilizer with the foliar application has quadratic equation formula.

Table 6. Average chlorophyll content on type of application and doses of boron fertilizer treatments

Type of application boron fertilizer	Chlorophyll content (mg g^{-1})				
	Doses of boron fertilizer (g $plant^{-1}$)				
	0	1.5	3	4.5	6
Chlorophyll a					
Soil application	0.3089^{d}	0.3877^{bcd}	0.4889^{abc}	0.5478^{a}	0.4976^{abc}
Foliar application	0.3310^{d}	0.5077^{ab}	0.6020^{a}	0.5789^{a}	0.3632^{cd}
Chlorophyll b					
Soil application	0.3147^{c}	0.3473^{c}	0.3544^{c}	0.4115^{b}	0.4180^{b}
Foliar application	0.2983^{c}	0.4979^{b}	0.5843^{a}	0.3329^{c}	0.2800^{c}
Total chlorophyll					
Soil application	0.6234^{d}	0.7347^{bcd}	0.8430^{bcd}	0.9591^{ab}	0.9154^{abc}
Foliar application	0.6292^{cd}	1.0053^{ab}	1.1860^{a}	0.9116^{abc}	0.6431^{cd}

Note: The average number followed by the same letter shows no significant difference in the HSD test at the 95% significance level.

Table 7. Relationship between the type of application and doses of boron fertilizer treatments on the content of chlorophyll a, b, and total (mg g^{-1} dw^{-1}) in the cacao leaves

Chlorophyll type	Equation	Optimum dose of boron	Maximum chlorophyll content
Soil fertilizer			
Chlorophyll a	$y = 0.0358x + 0.3387$, $R^2 = 0.78$	-	-
Chlorophyll b	$y = 0.0181x + 0.315$, $R^2 = 0.94$	-	-
Total	$y = 0.0539x + 0.6535$, $R^2 = 0.87$	-	-
Foliar fertilizer			
Chlorophyll a	$y = -0.0286x^2 + 0.1809x + 0.3206$, $R^2 = 0.98$	3.16	0.60
Chlorophyll b	$y = -0.0268x^2 + 0.1471x + 0.3186$, $R^2 = 0.75$	2.74	0.52
Total	$y = -0.0554x^2 + 0.3279x + 0.6391$, $R^2 = 0.95$	2.95	1.12

Note: the boron fertilizer with soil application has a linear equating formula and the boron fertilizer with the foliar application has quadratic equation formula.

Table 8. Average photosynthesis rate (µmoll CO_2 m^{2-1-1} s^{-1}) on type of application and doses of boron fertilizer treatments

Type of application boron fertilizer	Photosynthesis Rate (µmoll CO_2 m^{2-1-1} s^{-1})				
	Doses of boron fertilizer (g $plant^{-1}$)				
	0	1.5	3	4.5	6
Soil application	106.13^{f}	131.40^{cde}	140.20^{bcd}	160.60^{ab}	163.53^{ab}
Foliar application	112.07^{ef}	156.53^{ab}	170.87^{a}	146.53^{abc}	120.53^{def}

Note: the average number followed by the same letter shows no significant difference in the HSD test at the 95% significance level.

Table 9. Relationship between type of application and doses of boron fertilizer treatments on photosynthesis rate (µmoll CO_2 m^{2-1-1} s^{-1}) on the cocoa leaves

Observed variables	Equation	Optimum dose of boron	Maximum photosynthesis rate
Soil fertilizer			
Photosynthesis rate	$y = 9.6x + 111.57$, $R^2 = 0.94$	-	-
Foliar fertilizer			
Photosynthesis rate	$y = -5.7016x^2 + 34.672x + 114.26$, $R^2 = 0.95$	3.04	166.97

4. DISCUSSION

The different types of applications of boron at the same dose produce different physiological responses in cocoa plants. The dose of boron fertilizer with soil application had a linear effect, as evidenced by an increase in the physiological activity of the cocoa plant with each additional dose of boron fertilizer. Furthermore, the dose of boron fertilizer with foliar application gives a growth response with a quadratic pattern. The growth pattern illustrates that the plant achieved maximum yield at the optimum dose, and growth inhibition was experienced when the dose exceeded the optimum dose. These different physiological responses result from the soil application of boron, which results in a long series of reactions before reaching the leaves, where foliar applications result in processes such as chlorophyll formation, nitrate reductase activity, and photosynthesis. Furthermore, different doses of boron fertilizer applied to the soil had the possibility of being lost through the processes of volatilization, leaching, and surface evaporation from the soil. This is consistent with several studies that reported that boron fertilizer applied to the soil can also be reduced due to leaching (Dhassi et al., 2019) It was further reported that the absorption of boron fertilizer applied to the soil is strongly influenced by the availability of exchangeable ions, soil pH, and the amount of boron contained in the soil solution, soil organic matters content, humidity, and water conditions in the field (Abat et al., 2015; Liu et al., 2022).

The results suggest that the total boron content and total nitrogen in the soil application are positively correlated (Table 10), whereas no correlation was found in the foliar application (Table 11). This is because adding boron to the soil stimulates the growth of plant roots, which in turn increases water absorption and facilitates the transport of other nutrients, such as nitrogen. Several studies reported that boron added to the soil increases the number of roots and plant nutrient uptake (Dewi HS et al., 2020b; Yang et al., 2013). Moreover, the foliar application of boron does not positively correlate with the total nitrogen content.

Table 10. Relationship between each physiological parameter on soil application of boron fertilizer

	Bc	Nc	Chl a	Chl b	Total Chl	NRA	Pn
Bc	1	0.86^{*}	0.69^{*}	0.90^{*}	0.78^{*}	0.03^{ns}	0.89^{*}
Nc		1	0.95^{*}	0.96^{*}	0.98^{*}	0.98^{*}	0.43^{ns}
Chl a			1	0.87^{*}	0.98^{*}	0.59^{*}	0.93^{*}
Chl b				1	0.94^{*}	0.32^{ns}	0.97^{*}
Total Chl					1	0.52^{ns}	0.97^{*}
NRA						1	0.40^{ns}
Pn							1

Note: the boron fertilizer with soil application has a linear equating formula and the boron fertilizer with the foliar application has quadratic equation formula. Bc = boron content of leaves, Nc = N content of leaves, Clh a = chlorophyll a content, Clh b = chlorophyll b content, Clh total = total chlorophyll content, NRA = nitrate reductase activity, Pn = photosynthesis rate.

Table 11. Relationship between each physiological parameter and foliar application of boron fertilizer.

	Bc	Nc	Chl a	Chl b	Total Chl	ANR	Pn
Bc	1	0.04^{ns}	0.07^{ns}	0.38^{ns}	0.17^{ns}	0.09^{ns}	0.08^{ns}
Nc		1	0.87^{*}	0.89^{*}	0.95^{*}	0.83^{*}	0.96^{*}
Chl a			1	0.71^{*}	0.91^{*}	0.97^{*}	0.93^{*}
Chl b				1	0.93^{*}	0.74^{*}	0.90^{*}
Total Chl					1	0.92^{*}	0.92^{*}
ANR						1	0.90^{*}
Pn							1

Note: the boron fertilizer with soil application has a linear equating formula and the boron fertilizer with the foliar application has quadratic equation formula.

However, it increases the boron content in the leaves but does not increase nitrogen uptake in the leaves. Boron given to the leaves is directly used as an enzyme cofactor in various physiological activities, so only a small amount is translocated to the roots. thus it has no be correlated with N uptake. Several studies have reported that Foliar fertilizer is more quickly absorbed by plants (Guan et al., 2014; Niu et al., 2021), and is more efficient (Gao et al., 2018; Kentelky & Szekely-Varga, 2021). can affect physiological activity faster because it increases the mineral content in leaves more quickly (Davarpanah et al., 2020; Kentelky & Szekely-Varga, 2021).

The type of application of boron fertilizer through the soil and leaves resulted in increased activity of nitrate reductase. Furthermore, boron is strongly suspected of stimulating the activity of the enzyme nitrate reductase in converting nitrate into nitrite compounds and amino acids. The activity of nitrate reductase provides nitrite and amino acids for use in the synthesis of chlorophyll (Dewi HS et al., 2020b). Nitrite is used as a constituent of chlorophyll while amino acids are used to form enzymes for synthesis chlorophyll. In addition to this role, several studies have reported that boron plays a role in maintaining the structure of chlorophyll (Lu et al., 2014), but in these studies the mechanism of boron in maintaining the structure of chlorophyll has not been explained. This logical and temporary explanation can be accepted is that in plants boron acts as a constituent of cell walls (Marschner, 2011). Thus boron keeps the chlorophyll cell wall from being damaged easily.

The correlation results demonstrate that each increase in N levels in the leaves of the cocoa plant increased the levels of chlorophyll a, chlorophyll b, and total chlorophyll of 0.95 mg g^{-1}, 0.96 mg g^{-1} and 0.98 mg g^{-1}, respectively, on soil application of boron (Table 10), and 0.87 mg g^{-1}, 0.89 mg g^{-1}, and 0.95 mg g^{-1} for the foliar application of boron (Table 11). The increased N content induced the synthesis of chlorophyll content, this is because N is the main constituent of chlorophyll. Several studies report that sufficient N in the leaves increased the chlorophyll content synthesis (Han et al., 2016; Wen et al., 2019). Low N levels in plants will inhibit the formation of chlorophyll in the leaves. Soil and foliar application of Boron fertilizer regulates chlorophyll formation by increasing N uptake in cocoa leaves. In addition, soil and foliar application of boron fertilizer affects the activity of the nitrate reductase enzyme. Nitrate reductase enzyme is an enzyme that converts nitrate into nitrite and amino acids. Nitrite and amino acids will be converted into the new enzymes that will be used in the chlorophyll synthesis process. Therefore, increased activity of nitrate reductase will increase the number of enzymes for synthesizing chlorophyll content in the leaves cocoa plants. Previous study reported that boron plays a role in maintaining the structure of chlorophyll (Lu et al., 2014), through strengthening the cell wall (Marschner, 2011).

5. CONCLUSIONS

The dose of boron fertilizer and the type of fertilizer application used significantly affect the cocoa plant's physiological activity. The dose of boron with soil application affects physiological activity in a linear pattern where each additional dose of boron increased the activity of nitrate reductase, the content of chlorophyll a, chlorophyll b, and total chlorophyll and photosynthetic rate of 0.03 μmoll NO_2^- g^{-1} h^{-1}, 0.78 mg g^{-1}, 0.94 mg g^{-1}, 0.87 mg g^{-1}, and 0.94 μmoll CO_2 m^{2-1-1} $s^{61,}$ respectively. The dose of boron with foliar application affects physiological activity in a quadratic pattern, where the dose of boron in the range of 3 g $plant^{-1}$ is the optimum dose that gives maximum results on nitrate reductase activity (2.14 μmoll NO_2^- g^{-1} h^{-1}), the content of chlorophyll a (0.60 mg g^{-1}), chlorophyll b (0.52 mg g^{-1}), and total chlorophyll (1.12 mg g^{-1}), and photosynthetic rate (166.97 94 μmoll CO_2 m^{2-1-1} s^{-1}) cocoa leaves. Therefore, it is considered that the application of boron fertilizer at a dose of 3 g $plants^{-1}$ with the foliar application is more efficient in increasing physiological activity than the dose of boron with soil application.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests that may appear and influence the work reported in this paper.

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