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Research Article

Growth of cocoa seedlings (*Theobroma cacao* L.) in application of cow manure compost and density of *Bacillus subtilis* bacteria

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ABSTRACT

Cocoa rejuvenation is important to maintain high productivity. The rejuvenation mainly uses seedlings; thus providing high-quality seedlings is important. This research aimed to determine the effect of various doses of cow manure compost and the density of Bacillus subtilis bacteria on the growth of cocoa seedlings. The research was implemented at the Teaching Farm, Faculty of Agriculture, Hasanuddin University, Makassar City, from 2021 to 2022. The experiment was arranged in a split-plot design with three replications. The main plot was the dose of cow manure (without compost, 1.25 kg per polybag, and 2.5 kg per polybag). The subplot was the density of Bacillus subtilis (without bacteria, 10^4 CFU.mL^{-1}, 10^8 CFU.mL^{-1}, and 10^{12} CFU.mL^{-1}). The results showed an interaction between the dose of cow manure 2.5 kg per polybag and the bacterial density 10^{12} CFU.mL^{-1} on chlorophyll a (347.91 mol.m^{-2}), chlorophyll b (158.33 mol.m^{-2}), total chlorophyll (444.57 mol.m^{-2}), and leaf area of cocoa seedlings (813.96 cm^2). A compost dose of 2.5 kg per polybag showed the best results for plant height (8.98 cm).

Keywords: bacteria density; bivariate correlation; chlorophyll content; manure dose; organic fertilizer

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an annual crop with high economic value in the world, including in Indonesia. Cocoa plays an important role in the Indonesian economy as a foreign exchange earner, a source of farmer income, and a source of encouragement for domestic agribusiness and agro-industry. The cocoa plantations are spread throughout Indonesia, with its center in Sulawesi. Cocoa production in the Central Sulawesi region is 18%, Southeast Sulawesi is 16%, South Sulawesi is 15%, West Sulawesi is 11% and other regions are 40% (FAO, 2021). In 2016 cocoa production in Indonesia was 658,399 tons, in 2017 production was 590,684 tons, in 2018 it was 767,280 tons. In 2019, cocoa production in Indonesia decreased to 734,796 tons, and in 2020, cocoa production in Indonesia decreased again to 713,378 tons (DGPI, 2020).

The decline in cocoa production and productivity in Indonesia is mainly due to old trees causing unproductive plants (Sulistyowati, 2014; Fahmid et al., 2018; Daymond et al., 2020). One of the efforts that can be made to improve production is through rehabilitation and rejuvenation by providing quality seedlings (Safaruddin et al., 2023; Djuideu et al., 2021; Somarriba et al., 2021). Cocoa nurseries play an important role in producing good-quality seedlings (Santosa et al., 2023). Many efforts have been made to obtain quality seeds, one of which is improving the planting media with a mixture of organic matter and water management (Anthonio et al., 2018; Kafrawi et al., 2018; Mintah et al., 2022; Abri & Amirudin, 2023; Innaya et al., 2023; Santosa et al., 2023).

Various types of mixed planting media can be used, such as livestock waste, one of which is cow manure. Cow manure has the potential to be used as compost because it contains 0.4-1.0% nitrogen, 0.2-0.5% phosphorus, 0.1-1.5% potassium, and other microelements (Gupta et al., 2016; Maulana et al., 2023; Ren et al., 2023). Cow manure compost as a planting medium can be applied independently or together with biological agents (microbes) beneficial to plants. According to Cardoso et al. (2013), increasing soil organic matter content will stimulate soil microbial activity. To support this, cow manure compost is combined with *Bacillus subtilis* bacteria, which are classified as saprophytic bacteria, with the purpose of using organic matter to obtain food. These bacteria can dissolve minerals in the form of complex compounds into ions so that they can be absorbed by plants (Ma et al., 2017; Bai et al., 2019; Han et al., 2019; Lozano-Andrade et al., 2023; Sun et al., 2023).

The combination treatment of 2 kg of soil growing media added with 1 kg of compost significantly affected stem diameter, root length, fresh weight, dry weight, and total leaf area of cocoa plants (Kafrawi et al., 2018). Application of *Bacillus subtilis* bacteria with a density of 10^{11} CFU.mL^{-1} increased the growth of cocoa seedlings through the increase in height, stem diameter, number, and leaf area of 4-month-old cocoa seedlings (Puspita et al., 2018).

Based on the above description, research was conducted by applying cow manure that can provide nutrients for plants and microorganisms in the soil and *Bacillus subtilis* bacteria that can affect the growth of cocoa plant seedlings. Therefore, this study aimed to determine the effect of cow manure compost dosage and *Bacillus subtilis* bacteria density on the growth of cocoa seedlings.

MATERIALS AND METHODS

The research was held at a screen house of the Teaching Farm Faculty of Agriculture Hasanuddin University, Subdistrict Tamalanrea, Makassar City, South Sulawesi Province. The study was conducted from early November 2021 to February 2022.

Research methods

This experiment used a split-plot design with three replications. The main plot, namely the dose of cow manure compost (K), consisted of three levels: K0 (without compost), K1 (1.25 kg per polybag), and K2 (2.5 kg per polybag). The subplot, namely the density of *Bacillus subtilis* bacteria (B), consisted of four levels: B0 (without *Bacillus subtilis* bacteria), B1 (10^4 CFU.mL^{-1} per polybag), B2 (10^8 CFU.mL^{-1} per polybag) and B3 (10^{12} CFU.mL^{-1} per polybag). Therefore, 12 treatment combinations were obtained so there were 36 experimental units. Each experimental unit consisted of 2 sample units, resulting in 72 5-month-old shoot-grafted cocoa seedlings of clones (the scion used was done MCC 02 and the rootstock was Sulawesi 2).

Procedures and data analysis

Compost was applied two weeks before planting by mixing top soil and cow manure compost according to the treatment. Polybags were filled with 5 kg top soil (K0), 3.75 kg top soil + 1.25 kg cow manure compost (K1), and 2.5 kg top soil + 2.5 kg cow manure compost (K2). The application of *Bacillus subtilis* bacterial inoculant was carried out three times, namely one week after transplanting (WAT), 5 WAT, and 9 WAT. *Bacillus subtilis* bacteria density was applied by showering surrounding plant roots according to the predetermined treatment level. Each application of *Bacillus subtilis* bacteria was given as much as 35 mL, so the total *Bacillus subtilis* was 10^5 mL per polybag.

The variables observed in this study were plant height, stem diameter, number of flush leaves, leaf area and chlorophyll a, chlorophyll b, and total chlorophyll content in the leaves calculated using CCM-200 plus using the formula: Leaf chlorophyll content (y) = a + b (CCI)c, where a, b, and c are constants and CCI is the leaf chlorophyll index data read on CCM 200+ (Table 1). Chlorophyll content observations were made at week 12 after planting. Sampling on the 3rd leaf from the shoot. Data were analyzed using bivariate correlation analysis.

Table 1. Chlorophyll content formula and constanta.

Parameters	Constanta		
	a	b	c
Chl a	-421.35	375.02	0.1863
Chl b	38.23	4.03	0.88
Chl tot	-283.20	269.96	0.277

Source: Goncalves et al., 2008.

RESULTS AND DISCUSSION

Bivariate correlation analysis in Figure 1 showed that the density of *Bacillus subtilis* bacteria and the administration of 0 kg of cow manure compost had a moderate correlation linearly by following the equation y = 0.2383x + 5.02 with a coefficient of determination R^2 = 0.5745 and a correlation coefficient r = 0.75*. The density of *Bacillus subtilis* bacteria and the application of 1.25 kg of cow manure compost had a very significant positive correlation linearly by following the equation y = 0.1233x + 7.6933 with a coefficient of determination R^2 = 0.5094 and a correlation coefficient r=0.71**. Bivariate correlation analysis showed that the density of *Bacillus subtilis* bacteria and the application of 2.5 kg of compost had a significant linear positive correlation by following the equation y = 0.135x + 8.2567 with a coefficient of determination R^2 = 0.4116 and a correlation coefficient r = 0.64*.

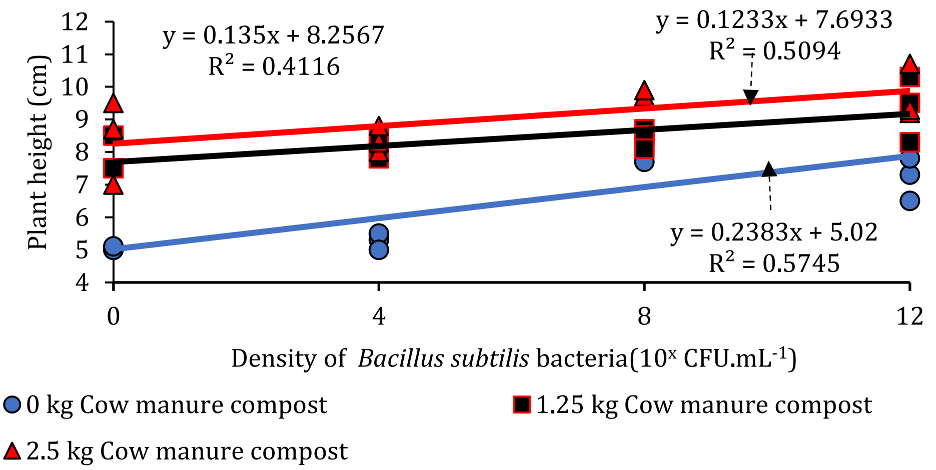

Figure 1. Bivariate correlation of plant height with several doses of cow manure compost and the density of *Bacillus subtilis* bacteria.

The bivariate correlation analysis in Figure 2 showed that the given density of *Bacillus subtilis* bacteria and 0 kg of compost had a very significant positive correlation linearly by following the equation y = 9.1755x + 205.14 with a coefficient of determination R^2 = 0.8681 and a correlation coefficient r = 0.93**. The density of the bacteria *Bacillus subtilis* and 1.25 kg of compost correlates very significantly quadratic by following the equation y = $-1.2221x^2$ + 14.505x + 281.8 with a coefficient of determination R^2 = 0.7009 and a correlation coefficient r =0.83**. This equation is differentiated into 2.444x = 14.505 to obtain *Bacillus subtilis* bacteria with a density of 10^6 $CFU.mL^{-1}$, producing a maximum chlorophyll of 324.81 $\mu mol.m^{-2}$. The bivariate analysis showed that the density of *Bacillus subtilis* bacteria and 2.5 kg of compost had a very significant positive correlation linearly by following the equation y = 6.7554x + 259.9 with a coefficient of determination R^2 = 0.8915 and a correlation coefficient r = 0.94**.

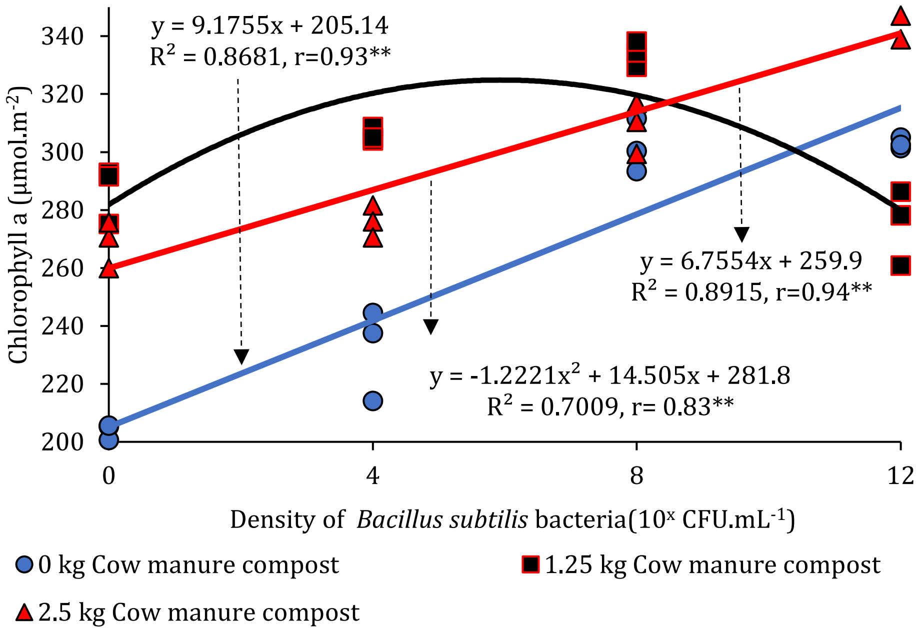

Figure 2. Bivariate correlation of chlorophyll to applying several doses of cow manure compost and the density of *Bacillus subtilis* bacteria.

The bivariate correlation analysis in Figure 3 indicated the density of *Bacillus subtilis* bacteria and 0 kg of compost had a very significant positive correlation linearly by following the equation y = 4.242x + 82.973 with a coefficient of determination R^2 = 0.8603 and a correlation coefficient r = 0.92**. The density of the bacteria *Bacillus subtilis* and 1.25 kg of compost correlated very significantly quadratic by following the equation y = -0.7161x^2 + 8.5972x + 116.36 with a coefficient of determination R^2 = 0.6905 and a correlation coefficient r=0.83**. This equation was differentiated into 1.4322x = 8.5972 so that *Bacillus subtilis* bacteria with a density of 10^6 CFU.mL^{-1} were obtained, producing a maximum chlorophyll b of 142.16 µmol.m^{-2}. Then, the bivariate correlation analysis shows that the density of *Bacillus subtilis* bacteria and 2.5 kg of compost had a very significant positive correlation linearly by following the equation y = 4.0738x + 104.16 with a coefficient of determination R^2 = 0.8713 and a correlation coefficient r =0.93**.

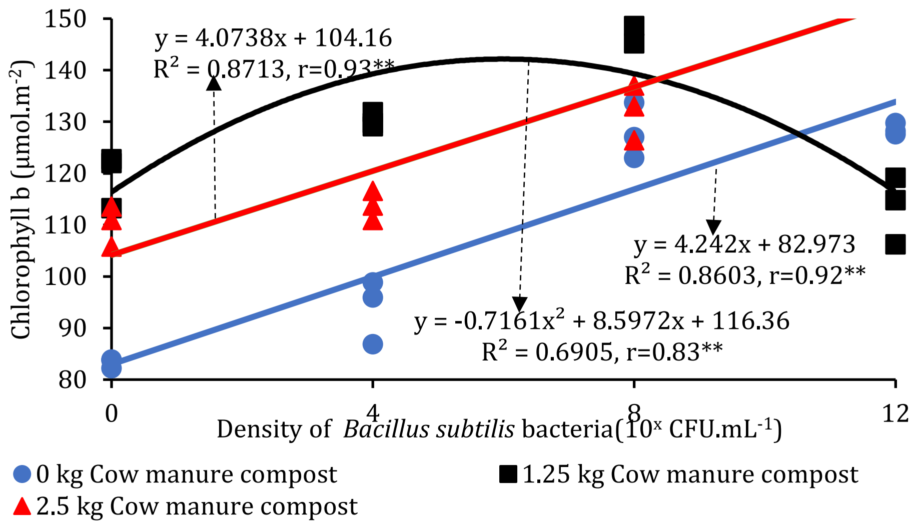

Figure 3. Bivariate correlation of chlorophyll b on the application of several doses of cow manure compost and the density of inoculation of *Bacillus subtilis* bacteria.

The bivariate correlation analysis in Figure 4 showed that the given density of *Bacillus subtilis* bacteria and 0 kg has a very significant positive correlation linearly by following the equation y = 13.091x + 295.45 with a coefficient of determination R^2 = 0.8674 and a correlation coefficient r = 0.93**. the density of the bacteria *Bacillus subtilis* and 1.25 kg of compost correlates very significantly quadratic by following the equation y = -1.8002x2 + 21.4x + 404.08 with a coefficient of determination R^2 = 0.6999 and a correlation coefficient r = 0.83**. This equation is differentiated into 3.6004x = 21.4 so that *Bacillus subtilis* bacteria with a density of 106 CFU.mL^{-1} produce a maximum total chlorophyll of 467.68 µmol.m^{-2}. Further, the bivariate correlation analysis shows that the density of *Bacillus subtilis* bacteria and 2.5 kg of compost had a very significant positive correlation linearly by following the equation y = 5.6437x + 383.61 with a coefficient of determination R^2 = 0.7984 and a correlation coefficient r =0.89**.

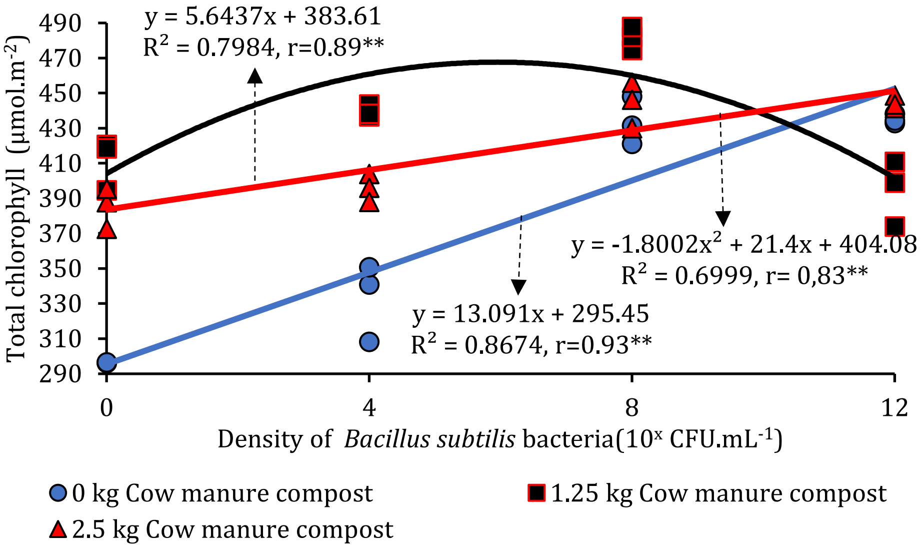

Figure 4. Bivariate correlation of total chlorophyll to the application of several doses of cow manure compost and the density of inoculation of *Bacillus subtilis* bacteria.

The bivariate correlation analysis in Figure 5 showed that the density of *Bacillus subtilis* bacteria and 0 kg of compost has a very significant positive correlation linearly by following the equation y = 7.9033x + 302.71 with a coefficient of determination R^2 = 0.6466 and a correlation coefficient r = 0.80**. The bivariate correlation analysis in Figure 5 shows that the density of *Bacillus subtilis* bacteria and 1.25 kg of compost had a very significant positive correlation linearly by following the equation y = 27.146x + 409.82 with a coefficient of determination R^2 = 0.8908 and a correlation coefficient r = 0.94**.

Figure 5 showed that the density of *Bacillus subtilis* bacteria and a dose of 2.5 kg of compost correlated not quadratic significantly by following the equation y = 1.2478x^2 - 5.0322x + 708.34 with a coefficient of determination R^2 = 0.1548 and a correlation coefficient r=0.39^{ns}. This equation is differentiated into 2.4956x = 5.0322 so that the bacteria *Bacillus subtilis*, with a density of 10^2 CFU.mL-1, is obtained, resulting in a maximum leaf area of 703.26 cm^2.

The application of compost and *Bacillus subtilis* bacteria to cocoa seedlings showed that there was a very significant interaction with chlorophyll a (Figure 2), chlorophyll b (Figure 3), total chlorophyll (Figure 4), and significant interaction with leaf area (Figure 5) in cocoa seedlings. The compost is rich in essential nutrients like nitrogen (N), phosphorus (P), and potassium (K), vital for plant growth, and contains organic matter that supports the activity of the *Bacillus subtilis* bacteria, making nutrition more available for plants. This corroborates the research by Kafrawi et al. (2018) the presence of *Bacillus subtilis* bacteria is related to a large amount of organic matter, which directly affects the amount and activity of its life, which significantly supports the growth of aerobic *Bacillus subtilis* bacteria. According to Lozano-Andrade et al. (2023) and Ibrahim et al. (2022), compost adds to the organic matter content in the soil that plants need and can provide food for microorganisms. Organic matter contained in compost can bind soil particles. This soil particle bond can facilitate root penetration and improve soil air exchange (aeration), supporting plant growth.

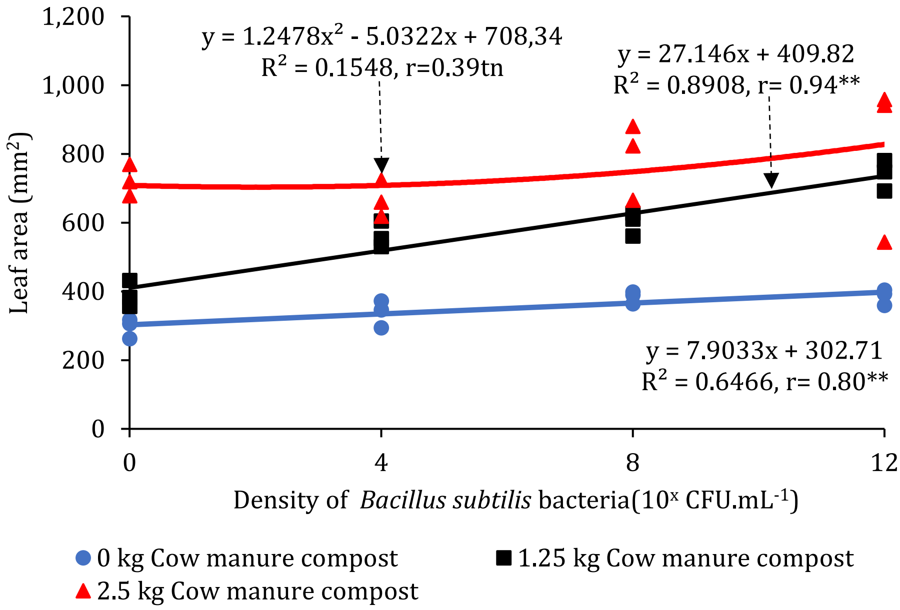

Figure 5. Bivariate correlation of leaf area with the application of several doses of cow manure compost and the density of inoculation of *Bacillus subtilis* bacteria.

Bivariate correlation analysis in Figure 6 showed that total chlorophyll to plant height is highly correlated quadratic by following the equation $y = -0.0002x^2 + 0.1494x - 25.051$ with a coefficient of determination $R^2 = 0.6752$ and a correlation coefficient r = 0.82**.

Figure 6 showed that total chlorophyll to number of leaves had a non-significant positive correlation linearly by following the equation y = 0.0034x + 0.7236 with a coefficient of determination $R^2 = 0.2238$ and a correlation coefficient r = 0.47^{ns}. Bivariate correlation analysis in Figure 6 showed that total chlorophyll to stem diameter had a highly significant positive correlation linearly by following the equation y = 0.0008x + 0.601 with a coefficient of determination $R^2 = 0.6086$ and a correlation coefficient r = 0.78**. Figure 6 shows that total chlorophyll to leaf area was not significantly correlated quadratic by following the equation $y = -0.016x^2 + 14.03x - 2461.4$ with a coefficient of determination $R^2 = 0.2755$ and a correlation coefficient r = 0.52^{ns}.

Total chlorophyll in bivariate correlation analysis (Figure 6) had a highly significant correlation with plant height gain and no significant correlation with leaf area quadratic. Total chlorophyll had a highly significant positive correlation with stem diameter increase and no significant positive correlation with the number of leaves parameter. Bivariate correlation analysis showed that the higher the total chlorophyll, the greater the effect on height gain, increase in stem diameter, number of flush leaves, and leaf area of cocoa seedlings. The resulting photosynthate will be stored in plant tissues, namely roots and stems, to affect the height, stem diameter, number of flush leaves, and leaf area of cocoa seedlings. *Bacillus subtilis* bacteria plays a role in producing growth hormones that can stimulate the growth of cocoa plant seeds, which functions for more optimal absorption of water and nutrients so that the process of photosynthesis will increase (Albayani et al., 2022).

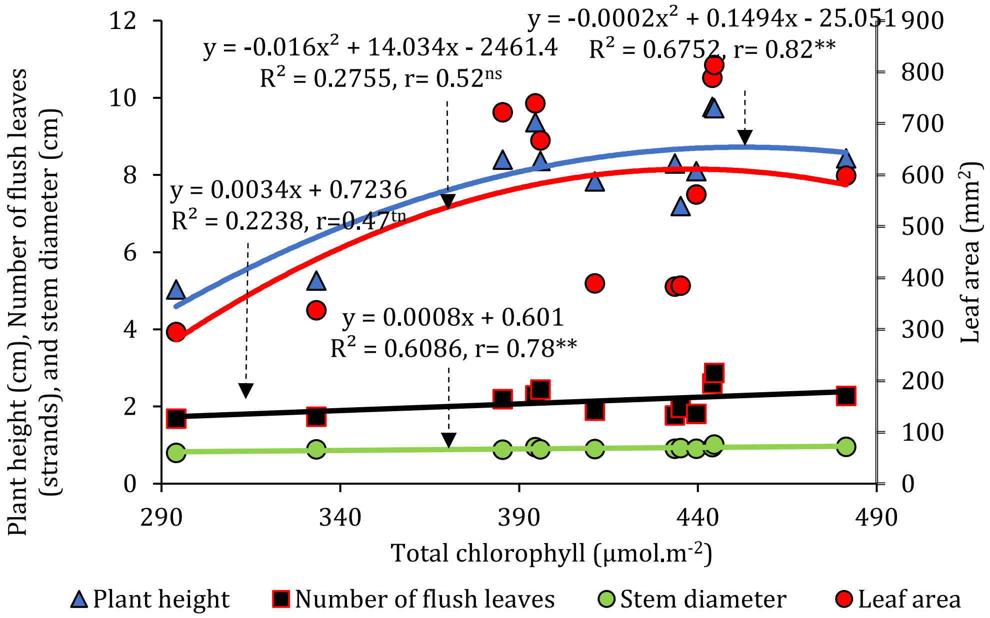

Figure 6. Bivariate correlation between total chlorophyll and plant height, stem diameter, number, and area of cocoa leaves.

Bacillus subtilis bacteria influence on increasing the growth of cocoa seedlings because of the ability of the bacteria as a phosphate solubilizer. This is in accordance with the opinion of Tian et al. (2021) that phosphate-solubilizing microbes produce phosphatase enzymes that produce organic acids, which can mineralize organic phosphates in the soil. The phosphatase enzyme is affected by the presence of acid and base reactions in the soil. It will affect the transformation of phosphate synthesized by phosphate-solubilizing bacteria in the soil. This enzyme also plays a role in the process of hydrolyzing organic phosphate into inorganic phosphate, which is available to plants. Phosphorus in plants plays an important role as an energy-carrying compound for various metabolic processes, both as a constituent of ATP derivatives and as a constituent of NADP (Nasaruddin & Yunus, 2012).

CONCLUSIONS

There was an interaction between the dose of cow manure and the density of *Bacillus subtilis* bacteria on cocoa seedlings in the parameters of chlorophyll a, chlorophyll b, total chlorophyll, and leaf area. The compost at a dose of 2.5 kg per polybag gave the best results on the parameters of plant height and the number of flush leaves. *Bacillus subtilis* at a density of 10^8 CFU.mL^{-1} gave the best results in increasing plant height. It is probable that compost application at seedlings might support the activity of *Bacillus subtilis* resulting in higher nutrient availability.

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