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DEVELOPMENT OF CACAO SEEDLINGS (*Theobroma cacao* L.) AS A FUNCTION OF D'RAZ® BIOSTIMULANT CONCENTRATION

FREITAS, Josivan Lima de[1]; JARDIM, Iselino Nogueira[2]; GUEDES, Mayara Neves Santos [3*]

[1] 0009-0009-0273-9347; Federal University of Pará. Altamira, PA, Brazil. josivanfreitas18@gmail.com
[2] 0000-0001-5159-1654; Federal University of Pará. Altamira, PA, Brazil. jardim@ufpa.br
[3*] 0000-0002-2601-2275; Federal University of Pará. Altamira, PA, Brazil. mayaraguedes@ufpa.br

A B S T R A C T

Recently, the use of bio-stimulants in agriculture has increased due to the need of sustainability in crop production. Additionally, these products are low-cost, easy to handle, environmentally cleaner, and safer for producers regarding health risks. This study aims to test the effectiveness of different doses of the D'Raz® bio-stimulant on the initial growth of cocoa plants (*Theobroma cacao* L.). The study was conducted in a forest nursery at the Federal University of Pará, Altamira. A completely randomized experimental design was used with four doses of D'Raz® bio-stimulant (0; 2; 4; and 8 mL per plant) and 10 replications, totaling 40 plants. Evaluations were carried out 120 days after sowing, including measurements of shoot height (cm), stem diameter (mm), height-to-diameter ratio, number of leaves, dry mass of shoot, root, and total (g), Dickson's quality index, leaf area, chlorophyll **a**, and chlorophyll **b**. The data were subjected to analysis of variance and Pearson correlation, and the means were compared using the Scott-Knott test at 5% significance level. The use of different concentrations provided a statistically significant effect on most variables, except for leaf area and chlorophyll **b**. Overall, the exogenous addition of bio-stimulant to the soil had beneficial effects on seedling development.The study concluded that the D'Raz® bio-stimulant at a concentration of 4 mL per plant was effective in cocoa seedling production.

R E S U M O

Recentemente o uso de bioestimulante na agricultura tem se tornado mais expressivo como resultado da busca pela maior sustentabilidade dos cultivos em geral. Além disso, são produtos considerados de baixo custo, de fácil manuseio, ambientalmente mais limpos e seguros aos produtores quanto aos riscos à saúde. O objetivo deste trabalho foi testar a eficiência de diferentes doses do bioestimulante D'Raz® no crescimento inicial de plantas de cacaueiro (*Theobroma cacao* L.). O trabalho foi realizado em viveiro florestal da Universidade Federal do Pará, Altamira. Utilizou-se o delineamento experimental inteiramente casualizado com quatro doses de bioestimulante D'Raz® (0; 2; 4 e 8 mL.planta^{-1}) e 10 repetições, num total de 40 plantas. Foram realizadas avaliações aos 120 dias após a semeadura, sendo elas: altura da parte aérea (cm), diâmetro do caule (mm), relação entre altura e diâmetro do caule, número de folhas, massa seca da parte aérea, da raiz e total (g), Índice de qualidade de Dickson, área foliar, clorofila **a** e clorofila **b**. Os dados foram submetidos análise de variância e correlação de Pearson e as médias comparadas pelo teste Scott-Knott a 5%. O uso das diferentes concentrações proporcionou efeito estatístico significativo na maioria das variáveis, exceto para a área foliar e clorofila b. De modo geral, a adição exógena de bioestimulante ao solo proporcionou efeito benéficos no desenvolvimento das mudas. Concluiu-se que o bioestimulante D'Raz® na concentração de 4 mL.planta^{-1} apresentou-se como eficiente na produção de mudas de cacau.

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Introduction

The cacao tree (*Theobroma cacao* L.), Malvaceae family, is native to the American continent, with origin in the Amazon (LESSA *et al.*, 2020). It is a perennial plant that blooms in summer and bears fruit in autumn and winter, spreading through seeds. It thrives in warm and humid climates (GARUZZO; PEREIRA, 2018). The cocoa fruit is a premium product, and the seeds are the raw material for chocolate, consumed worldwide, holding significant economic importance. According to the Brazilian Institute of Geography and Statistics (IBGE), the states of Pará and Bahia are the main producers of cocoa, responsible for 52% and 41% of the Brazilian production, respectively (IBGE, 2023). In Pará, the municipalities in the Transamazonian region along the cocoa route stand out, contributing to 63% of the state's total production (JARDIM; RAD, 2023). The increasing demand stimulates the production of seedlings, which producers often prefer to multiply themselves due to cost-saving reasons.

However, the success of cocoa farming ventures relies on the quality of seedlings obtained from forest nurseries. These factors, combined with environmental conditions and silvicultural methods, are crucial for cutting costs and expediting the establishment of orchards (LIMA FILHO *et al.*, 2019). Factors such as seed quality, container type, substrate, fertilization, and management can affect the production of high-quality seedlings. Cocoa is a valuable Brazilian commodity, underscoring the importance of research in seedling production. In this context, research on new products that can enhance quality and expedite seedling growth is crucial (SMIDERLE; SOUZA, 2016).

Biostimulants, natural or synthetic substances applied to seeds, plants, and soil, induce changes in vital and structural processes to increase seed productivity and quality. The application of seaweed-based biostimulants in fruit trees has been the subject of several studies, showing great potential and excellent results in producing quality seedlings. However, its application in cocoa seedling production remains underexplored, requiring studies to demonstrate its efficiency, either positively or negatively, in cultivation.

Therefore, identifying optimal dosages of seaweed extract-based biostimulants applied to each species is important to develop specific biofertilization protocols for each one. In this sense, the following research question was raised: Does the D'Raz® biostimulant influence the growth and development of cocoa seedlings? Consequently, the study hypothesis is that doses of D'Raz® biostimulant influence growth and development, resulting in better quality seedlings. Understanding this topic, seaweed extract-based biofertilizers can be used to benefit cocoa seedling production, thus making it available for ecological restoration and commercial efforts.

Therefore, the objective of this study was to test the effectiveness of different doses of the D'Raz® biostimulant on the initial growth of cocoa plants.

Material and methods

The experiment occurred in a forest nursery belonging to the Federal University of Pará, in the municipality of Altamira, Pará. According to the Köppen classification, Altamira has an equatorial climate type, with an annual average temperature of 26 ºC, annual average precipitation of 1700 mm, and relative air humidity of 80% (ALVARES *et al.*, 2013).

The soil used in the experiment was collected from a depth ranging from 0.0 to 0.20 m of an Ultisol. The collected soil was disaggregated and sieved (2 mm mesh), followed by chemical analysis at Solocria Agricultural Laboratory LTDA, located in Goiânia, GO. The soil showed the following average values: pH ($CaCl_2$) = 5,3; Al^{3+} = 0; H + Al = 2,2 cmolc dm^{-3} Ca^{2+} = 1,2 cmolc dm^{-3}; Mg^{2+} = 0,6 cmolc dm^{-3}; K^{1+} = 1,42 cmolc dm^{-3}; P = 22,7 mg dm^{-3}; C = 4,64 g dm^{-3}; V% = 45,93 e CTC = 4,06 cmolc dm^{-3}; sand = 210 g kg^{-1}; silt = 200 g kg^{-1}; and clay = 590 g kg^{-1}.

The characteristics of the D'Raz® bio-stimulant, according to the manufacturer, are as follows: N = 44.8 g L^{-1}; C = 140.0 g L^{-1}; d = 1.12 kg L^{-1}; pH = 7.5; Salinity index = 12%. The commercial product D'Raz® is a plant bio-stimulant composed of phytohormones, osmoprotective agents, oligopeptides, alginates, mannitol, oligo- and polysaccharides, betaines, polyamines, and vitamins extracted from the seaweed *Ascophyllum nodosum*, with a concentration of 10.7% w/w (12% w/v) of free amino acids, 4.0% w/w (4.5% w/v) of total N, 1.7% w/w (1.9% w/v) of organic N, and 2.3% w/w (2.6% w/v) of ureic N.

The cocoa seeds were treated with the systemic fungicide Cercobin® 875 WG, at the dosage recommended by the manufacturer. The germinated seeds were manually sown in polyethylene plastic bags (18 x 35 cm) with a capacity of two liters each. No sowing fertilizer was applied, regardless of the soil chemical analysis results, to assess the potential efficacy of the commercial bio-stimulant D'Raz®.

The experimental design was completely randomized (CRD) with 4 treatments and 10 repetitions, totaling 40 plots. Different concentrations of the D'Raz® bio-stimulant were used as follows: T1 = 0.0 (control); T2 = 2.0 mL; T3 = 4.0 mL; and T4 = 8.0 mL. Each concentration of the bio-stimulant was diluted in 100 mL of distilled water, forming a solution to be applied. The first application of the D'Raz® bio-stimulant dose to the soil occurred at 30 days after sowing (DAS), with repetitions at 60 and 90 DAS.

At 120 DAS the following parameters were measured: shoot length (H, cm), stem diameter (SD, mm), plant height-to-stem diameter ratio (HSDR), chlorophylls (*a* and *b*), leaf area (LA, cm^2), shoot dry weight (SDW, g), root dry weight (RDW, g), total dry weight (TDW, g), and Dickson Quality Index (DQI).

The shoot length was determined using a graduated ruler in cm, measured from the base of the stem to the terminal bud of the plant. Stem diameter was measured 0.5 cm from the stem base using a Adaskala stainless steel Vernier caliper, a high-precision digital caliper ranging from 0-150mm. Chlorophyll levels were assessed using the portable chlorophyll meter

ClorofiLOG CFL 1030, with the 5th and 6th leaves of each plant selected for measurement. Leaf area (LA) was estimated using the ImageJ software, freely available online (http://rsbweb.nih.gov/ij/), with results expressed in cm². Dry weight of shoot, root, and total mass were determined after drying the material in a forced-air oven at 60°C until a constant weight was achieved, with results expressed in grams. The Dickson Quality Index (DQI) was calculated according to the equation proposed by DICKSON *et al.* (1960):

$$DQI = \frac{TDM}{\frac{SH}{SD} + \frac{SDM}{RDM}} \quad (1)$$

where: DQI: Dickson Quality Index; TDM: Total Dry Mass; SH: Shoot Height; SD: Stem Diameter; SDM: Shoot Dry Mass; and RDM: Root Dry Mass.

Statistical tests were used to validate and compare the data. The Shapiro-Wilk test was employed to assess data normality, while the Bartlett test was used to evaluate data homogeneity. Subsequently, the data underwent analysis of variance, and when a significant difference was observed, the Scott-Knott test ($p < 0.05$) was applied to compare means among treatments. Polynomial regression analysis was conducted for the D'Raz® bio-stimulant doses to determine the maximum technical efficiency dose (MTED). The MTED was calculated using the formula: $X = \frac{-B}{2A}$, where **B** and **A** are values estimated by the polynomial regression equation. Pearson correlation analysis ($p < 0.05$) was performed to determine correlations between the evaluated variables. All analyses were carried out using the SISVAR software (FERREIRA, 2011).

Results and Discussion

The Table 1 presents the mean square (MS) values from the analysis of variance for the morphometric variables and indices evaluated at 120 DAS. For the initial development of *Theobroma cacao* L., the use of the D'Raz® bio-stimulant promoted a significant effect at the 1% probability level ($p<0.01$) for the variables SH, SHDR, Chl *a*, SDM, RDM, TDM, and DQI analyzed, except for SD. It is also possible to observe that the coefficients of variation ranged from 7.28 to 23.80%, considered moderate for a nursery experiment (PIMENTEL-GOMES, 2009).

Table 1.
Summary of the analysis of variance for growth characteristics and seedling quality of *Theobroma cacao* L. as a function of D'Raz® biostimulant doses.

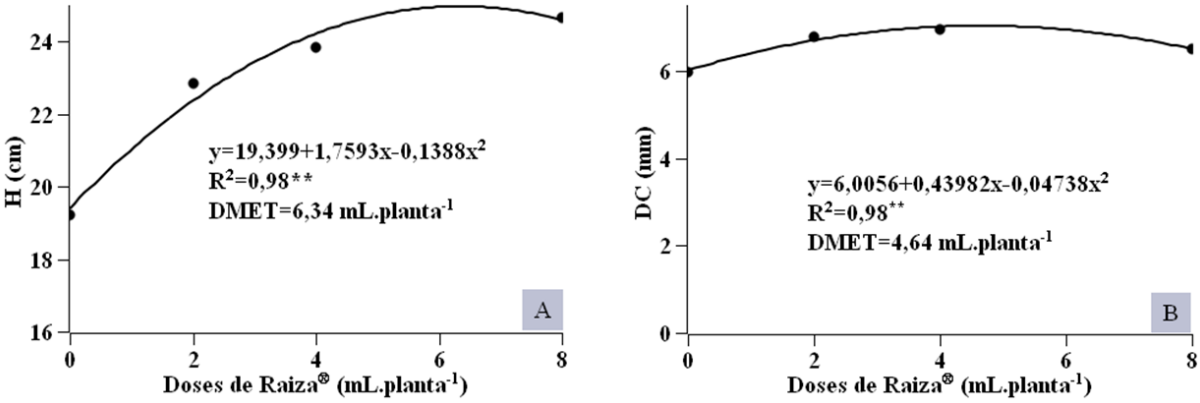
		Mean square (MS)							
SV	DF	SH	SD	SHDR	Chl *a*	SDM	RDM	TDM	DQI
B	3	172.68**	5.42*	1.73**	14.8**	5.52**	1.78**	13.54**	0.50**
Error	116	23.42	2.43	0.22	1.61	0.46	0.13	0.68	0.07
CV(%)		21.37	23.80	13.27	7.28	13.81	12.62	10.57	16.37
OM		22.64	6.55	3.50	17.43	4.89	2.88	7.78	1.62

*SV: Source of Variation; B: D'Raz® biostimulant doses; CV (%): Coefficient of variation in percentage; OM: Overall mean; DF: Degrees of freedom; SH: Seedling height; DC: Stem diameter; SHDR: Stem height and diameter ratio; Chl a: Chlorophyll a; SDM: Shoot dry mass; RDM: Root dry mass; TDM: Total dry mass; DQI: Dickson Quality Index; ns: non-significant and *, **: significant at 5% and 1% probability level, respectively.*

The seedling height variable adjusted to a quadratic model, with a maximum technical efficiency dose (MTED) of 6.34 mL per plant (Figure 1A). The height reached 25.0 cm at the MTED with a 30% percent increase compared to the control. This result is supported by Guimarães *et al.* (2012), who observed papaya seedling growth under the influence of the D'Raz® biostimulant added to the substrate. This phenomenon may be explained by the presence of hormonal precursors and phytohormones contained in the product, which cause cell expansion and growth (ARAÚJO, 2016; SHUKLA *et al.*, 2019).

Figure 1.

Average values of shoot height (A), stem diameter (B) of *Theobroma cacao* L. seedlings.



Note: Primary data, 2022.

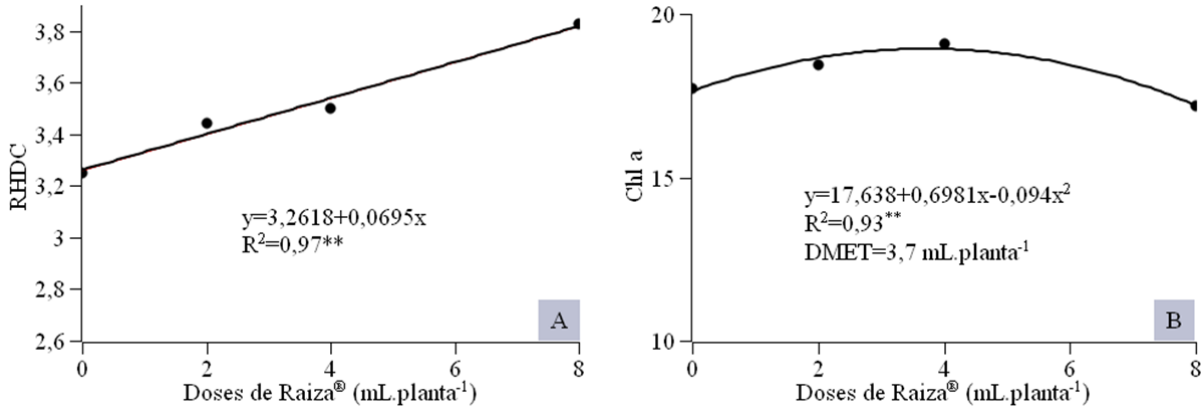
The variable stem diameter (SD) was best fitted to the quadratic order model, where an increase was observed up to the MTED at 4.64 mL per plant (Figure 1B). At MTED, the average value of 7.0 mm was obtained, representing a growth increment of 18% compared to the control. The D'Raz® bio-stimulant has a positive effect on stem diameter, although smaller than the increase observed in seedling height. Koyama et al. (2012), evaluating the effect of *Ascophyllum nodosum* algae extract on tomato plant development, observed an increase in stem diameter over time. According to Mazzoni and Trufem (2004), a larger stem diameter transports a greater volume of nutrients and water to the shoot, aiding in vegetative growth, biomass accumulation, and plant metabolic and photosynthetic processes. In summary, stem height and diameter are easily measurable and non-destructive parameters that, when combined, successfully predict the definitive establishment of seedlings in the field.

The data related to the variable Stem Height to Diameter Ratio (SHDR) fitted best to an increasing linear function with significant differences between treatments (Figure 2A). The SHDR variable showed increments of 0.0695 mL per plant for each unit increase in the bio-stimulant used in the substrate. The SHDR ratio reached a maximum value of 3.83 at the dose of 8 mL per plant of D'Raz® bio-stimulant. According to Cargnelutti Filho *et al.* (2018), this index expresses a growth balance, relating two important morphological parameters into a single index. It is recommended that the lower its value, the greater the ability of the seedlings to survive and establish in the planting area.

Dantas *et al.* (2018) confirm that high-quality seedlings of forest species have SHDR indices lower than 10, and when they meet this index, they show higher survival rates after planting.

Figure 2.

Average values of the stem height to diameter ratio (A) and chlorophyll *a* (B) of *Theobroma cacao* L. seedlings.



Note: Primary data, 2022.

The value of chlorophyll *a* (Chl *a*) differed significantly among the treatments, with a quadratic fit, where the MTED was estimated at 3.7 mL per plant (Figure 2B). The values of chlorophyll *b* (Chl *b*) did not differ among the doses of D'Raz® bio-stimulant used at 120 DAS, with a global mean of 3.88.

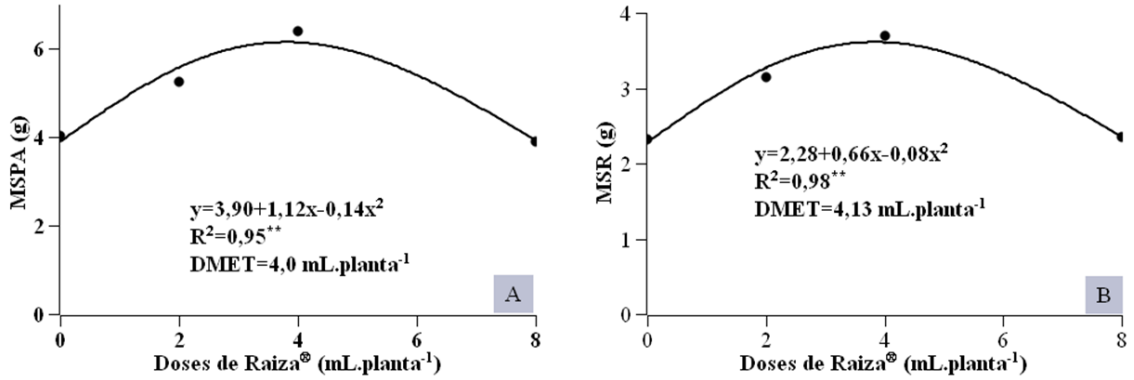
No regression equation could be fitted for leaf area (LA), with an observed average leaf area of 650.0 cm². Despite no significant response for leaf area, the dose of 2 mL per plant of the D'Raz® bio-stimulant stood out among the other treatments. Malta *et al.* (2011) stated that leaf area is one of the most important growth parameters, as it reflects the size of the plant's assimilatory apparatus.

Analysis of variance showed a significant effect of D'Raz® bio-stimulant doses on cocoa seedlings regarding shoot dry mass (SDM) production. In treatments where D'Raz® bio-stimulant was applied, the regression analysis revealed a quadratic response, increasing up to

the maximum technical efficiency dose (MTED) of 4.0 mL per plant of D'Raz® bio-stimulant, decreasing thereafter (Figure 3A). At the MTED, SDM was estimated at 6.14 g, with an increase of 57.4% compared to the absence of D'Raz® bio-stimulant.

Figure 3.

Average values of shoot dry mass (A) and root dry mass (B) of *Theobroma cacao* L. seedlings.



Note: primary data, 2022.

Even though shoot dry mass (SDM) is a destructive method, it should be considered when evaluating seedling biometric characteristics because, according to Gomes and Paiva (2011), shoot dry mass indicates the hardiness of the seedling. Additionally, higher values for SDM indicate more lignified and hardy seedlings, thus showing greater potential for survival in environments with adverse conditions (Gomes; Paiva, 2011).

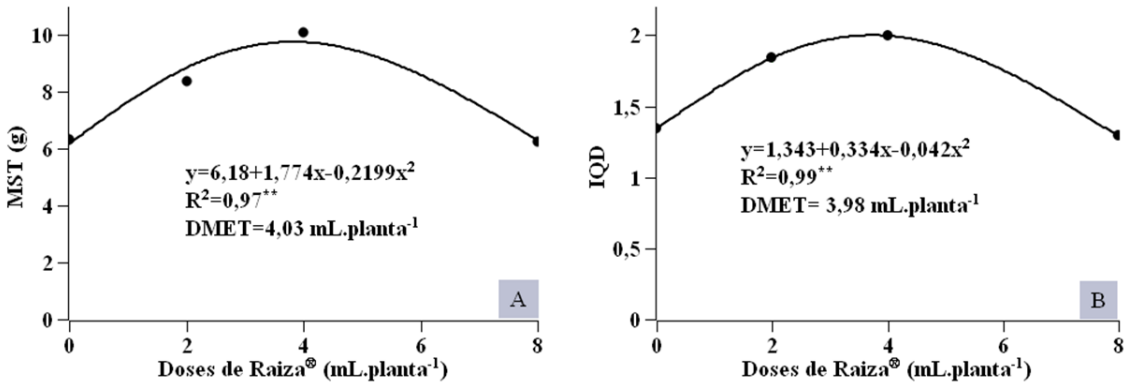
The root dry mass (RDM) showed a significant statistical effect at the tested doses with a quadratic effect (Figure 3B). Increasing doses of D'Raz® bio-stimulant led to an increase in RDM up to the MTED (4.13 mL per plant), resulting in a maximum estimated mass of 3.64 g. Compared to the absence of D'Raz® bio-stimulant, the increase in root dry mass reached 60%. With developed root systems, there's greater stability, as well as increased water and nutrient absorption (FREITAS *et al.*, 2009). The RDM variable holds significant importance in plant development, as greater root formation enhances growth capacity and survival potential in the field (AMORIN *et al.*, 2020).

The total dry mass (TDM) of *Theobroma cacao* seedlings ($p<0.01$) was influenced by the doses of D'Raz® bio-stimulant, with a quadratic equation adjustment (Figure 4A). In this study, the estimated average total dry mass was 9.76 g at the maximum technical efficiency dose. Compared to the absence of extract, the increase in total dry mass of seedlings was 58% at the MTED. This is justified by the D'Raz® bio-stimulant stimulating plant physiological processes such as nutrient absorption and photosynthesis, due to molecules extracted from the algae (GUIMARÃES *et al.*, 2012). The production of total dry mass is a good variable to assess

plant growth, as it is a direct reflection of total net photosynthesis combined with the amount of mineral nutrients absorbed (TAROUCO *et al.*, 2021).

Figure 4.

Mean values of total dry mass (A) and Dickson's Quality Index (B) of *Theobroma cacao* L. seedlings produced with different doses of seaweed extract and evaluated at 120 DAS under nursery conditions, Altamira, PA, 2022.



Note: Primary data, 2022.

Teixeira *et al.* (2009), working with the seaweed *Lithothamnium*, found that the best concentration for producing papaya seedlings was 2 kg.m^{-3} (0.2%). Conversely, Hafle et al. (2009) recommend a concentration of 3 kg.m^{-3} (0.3%) of *Lithothamnium* for the Soil group. Robertson-Andersson *et al.* (2006), in an experiment with a seaweed extract-based product, observed that its application at low concentrations is beneficial for agricultural use. The seaweed extract *Ascophyllum nodosum* has been frequently used by the European community, showing increased vegetative development of plants (ARTHUR *et al.*, 2003).

Freitas *et al.* (2017) stated that the Dickson Quality Index (DQI) is a balanced formula that includes morphological parameter ratios such as TDM, SH/SD, and SDM/RDM. In this study, the regression analysis showed a significant response of the quality index to the D'Raz® bioestimulant applied to *Theobroma cacao* seedlings.

The DQI reached a maximum point (2.0) at the MTED of 3.98 mL per plant of D'Raz® bioestimulant, decreasing beyond this level (Figure 4B). The DQI value at the MTED was considerably higher compared to the absence of extract, resulting in a 49% increase. However, at the other doses of seaweed extract used, the obtained DQI values also classified the seedlings as of good quality.

The DQI can be considered a good parameter to indicate the quality standard of *Theobroma cacao* seedlings developed under different doses of seaweed extract. It confirms that under certain doses of the extract, especially close to 4.0 mL per plant, the seedlings

exhibit better quality for planting, as the value of their constituent parameters is within the limits considered suitable for planting. According to Freitas *et al.* (2017), the higher the DQI value, the better the quality standard of the seedlings.

The Pearson analysis showed the existence of statistically significant correlation between some pairs of investigated variables (Table 2).

The correlation above 0.50 between the dependent variables indicates that observing one variable would allow inferring about the others. In terms of importance, we observed SDM, RDM, IQD, SD, and SH. In this sense, the recommendation of applying D'Raz® bioestimulant to the soil to obtain the highest DQI is justifiable since this index correlates with the evaluated characteristics with a high Pearson correlation. It is also worth noting that SDM was the morphological parameter that correlated well with most other variables, as it showed a significant correlation coefficient higher than 0.5.

The RDM variable of the seedlings showed a high correlation index when related to the DQI, indicating that, in general, seedlings with higher RDM had a higher DQI. For this relationship to be significant, it is important to increase the time that the seedlings remain in the greenhouse so that there is an increase in diameter.

Table 2.

Pearson correlation coefficient between variables in *Theobroma cacao* L. seedlings.

Correlation coefficient								
VAR	SDM	RDM	SD	SH	TDM	SHDR	DQI	LA
SDM	1							
RDM	0.80**	1						
SD	0.56**	0.70**	1					
SH	0.60**	0.52**	0.67**	1				
TDM	0.97**	0.91**	0.64**	0.60**	1			
SHDR	0.34 ns	0.02ns	-0.07 ns	0.50**	0.24 ns	1		
DQI	0.72**	0.93**	0.73**	0.38 ns	0.84**	-0.29 ns	1	
LA	0.37ns	0.24 ns	0.41 ns	0.50**	0.34 ns	0.52**	0.11	1

Note: Primary data, 2022.

Measuring the height and stem diameter of seedlings in the nursery is a method to infer their quality, as these variables showed correlation with other characteristics. Additionally, it is operationally feasible since it is not a destructive method (FREITAS *et al.*, 2017).

Given these aspects, it is evident that the D'Raz® bio-stimulant could be an important biofertilizer for cocoa cultivation. The doses of D'Raz® bio-stimulant used, within the time intervals considered in the study, except for the highest dose applied (8.0 mL per plant),

proved to be effective in the growth of cocoa seedlings in a nursery environment. The extract could represent lower costs for the producer, as well as an alternative to saving on the use of industrial fertilizers during cocoa seedling production. However, further studies should be conducted to assess the influence on the quality of seedlings until field implementation.

Conclusion

More applications of D'Raz® bio-stimulant favor the development of cocoa seedlings.

Concentrations of D'Raz® bio-stimulant in the range of 4 mL per plant can be recommended for the initial development of cocoa in a nursery.

The addition of D'Raz® bio-stimulant based on seaweed provided better cocoa seedlings.

However, further studies should be conducted to assess the influence on the quality of seedlings until field implementation.

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