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The Effect of Nutrients (N and P) and Hormone (IAA) Application on the Growth of Cocoa (*Theobroma cacao* L.) Plagiotrope Cuttings

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Abstract

To support the successful propagation of cocoa plagiotrope cuttings, nutrients (N and P) and hormone (IAA) are applied to increase plant growth. The purpose of this research was to determine the effect of the provision of nutrients (N and P) and hormone (IAA) on the growth of cocoa plagiotrope cuttings. The experiment was carried out factorial with the basic pattern of Completely Randomized Design (CRD) and was repetitively replications by three times. The first factor was the dose of urea (N) and SP-36 (P) which consisted of 3 levels, such as P0 (0 g/polybag urea and 0 g/polybag SP-36), P1 (3,5 g/polybag urea and 2,5 g/polybag SP-36), and P2 (7 g/polybag urea and 5 g/polybag SP-36). The second factor was the concentration of IAA which consisted of 5 levels, such as I0 (0 ppm), I1 (50 ppm), I2 (100 ppm), I3 (150 ppm), and I4 (200 ppm). The results showed (1) the interaction between the application of the nutrients (N and P) and hormone (IAA) had no significant effect on all observed variables except the number of leaves, where the best treatment combination was the application of fertilizer doses of 0 g/polybag urea + 0 g/polybag SP-36 and IAA concentration of 100 ppm (P0I2) (2) application of the nutrients (N and P) with dose of P1 increased seedling growth on all observed variables except the number of leaves, shoot diameter, and shoot length (3) application of IAA with concentration of I1 increased seedlings growth, that is the number of primary roots/cuttings and shoot length.

Introduction

According to BPS (2020), in 2019 Indonesia's cocoa production reached 734.70 thousand tons, but in 2020 and 2021, Indonesia's cocoa production experienced a decline. In 2020, Indonesia's cocoa production fell to 713.40 thousand tons, then in 2021 cocoa production decreased again to 706.50 thousand tons. It can be seen from these statistical data that cocoa production in Indonesia is still not optimal. This is a crucial problem for Indonesia considering that cocoa is one of the plantation commodities which is very important for Indonesia's economic activities. This is because cocoa is a significant source of export income for Indonesia and a source of foreign exchange for the country. This decline in production can occur due to various factors, including pest and disease attacks on Indonesian cocoa plantations, such as attacks by cocoa pod borers which cause losses of up to 80% (Syatrawati & Asmawati, 2015) and VSD disease which can cause plant death of more than 50% (Mustafa, 2017); climate anomalies which have a direct impact on seasonal shifts which make it difficult for farmers to determine planting and harvest periods for their crops and can cause the growth and development of plant pest organisms; farmer workers who still use simple cultivation techniques such as using traditional

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agricultural tools and machines so they are less able to support optimal cocoa production; and poor quality planting materials because the plants used are quite old so they are less productive. The productivity of cocoa plants begins to decrease between the ages of 15 and 20 years (Pulungan et al., 2017). These things are challenges that must be faced by the cocoa industry in Indonesia.

Amidst these challenges, one crucial aspect that requires attention is the quality of planting materials used in cocoa propagation. The declining yield of aging cocoa plants has underscored the need for innovative techniques to produce superior planting materials efficiently and quickly. While generative and vegetative propagation methods offer potential solutions, there is a gap in knowledge regarding the optimal practices for vegetative propagation, particularly using plagiotropic cuttings. This study seeks to fill this gap by investigating the impact of nutrient and hormone application on the growth of cocoa plagiotrope cuttings, aiming to provide cocoa farmers with effective strategies for improving planting material quality.

One alternative vegetative propagation technique that can be developed to meet the demand for cocoa seeds in large quantities and in a relatively short time to increase national cocoa production is cuttings. The purpose of cuttings is to reduce seedling costs, achieve garden clonalization and accelerate growth. To achieve garden clonization, there is the latest technology being developed, namely cocoa plagiotrope cuttings. These cuttings use a plagiotropic branch from the cocoa plant. Cocoa plagiotrope cuttings have advantages and disadvantages. The advantages include fast flowering and fruiting, short plant habitus and can be mass produced while the disadvantages are weak roots, slow shoot formation, and poor growth process.

To overcome the shortcomings of the cocoa plagiotrope cuttings method, additional nutrients are needed for the initial growth of the plant. One of them can be added the use of nutrients such as nitrogen (N) and phosphorus (P) and the hormone auxin in order to support plant growth and development. Nutrients N and P are very important for plants because they help growth in the vegetative phase while the hormone auxin is often applied to stimulate root growth.

Previous research conducted by (Sitorus et al., 2014a) stated that applying 7 g/polybag of urea to cocoa plant seeds gave the best results in plant height and leaf area due to the presence of nitrogen which functions to encourage cell division and expansion in the apical meristem so that plants can grow taller and according to (Perdinanta, 2019), the application of SP-36 fertilizer as much as 5 g/polybag gave the best results for cocoa seedling growth because SP-36 fertilizer contains phosphorus which stimulates root growth and forms a good root system. In addition, the nutrient phosphorus also functions as a constituent of cell walls thereby strengthening plant resistance to disease (Rismanto, 2019a).

Not only is the phosphorus nutrient used to stimulate root growth, but there are also hormones that can be added, namely the auxin hormone. Auxin has several types such as Indole-3-Acetic Acid (IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), dan 2,4-dichlorophenoxy acetic acid (2,4-D). However, in this research IAA was used because it has a character that is easily absorbed by plants. Based on (Jihadiyah, 2018a), the results of testing the effectiveness of several auxin hormones (IBA, IAA, and NAA) on the number of roots of micro cuttings of fig plants showed that giving IAA with a concentration of 0,5 ppm produced the highest number of roots compared to IBA and NAA. This is caused by the chemical structure of IAA which is no different from the natural auxin in plants so that it is easily absorbed by plants.

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Methods

This research uses nitrogen and phosphorus nutrients as well as the hormone IAA to support the growth of cocoa plagiotroph cuttings. The only nutrients used are nitrogen and phosphorus because these two elements are needed most in the vegetative phase of plants. The element nitrogen has a role in encouraging cell division and expansion in the apical meristem so that plants can grow taller, helping the growth of healthy leaves so they look greener, and increasing protein levels in plants (Sitorus et al., 2014) while the role of phosphorus is to stimulate growth. roots and form a good root system. Apart from that, the nutrient phosphorus also functions as a constituent of cell walls, thereby strengthening plant resistance to disease, as an energy source for plant metabolism, and assists in the production and transportation of substrates such as sugar and starch (Rismanto, 2019). To select the type of auxin hormone used is IAA. Among various auxin hormones, Indole-3-Acetic Acid (IAA) was selected due to its unique chemical structure, which closely resembles natural auxins found in plants. This structural similarity enhances its absorption by cocoa plants, making it an ideal choice for stimulating root growth (Jihadiyah, 2018). Previous research has also demonstrated the effectiveness of IAA, particularly at a concentration of 0,5 ppm, in promoting the highest number of roots in fig plant micro cuttings (Jihadiyah, 2018). This evidence underscores the rationale behind IAA's inclusion in the study as the hormone of choice.

The selection of nitrogen (N) and phosphorus (P) as nutrient treatments, along with Indole-3-Acetic Acid (IAA) as the hormone treatment, aligns with our research objective of enhancing the growth of cocoa plagiotrope cuttings. By providing essential nutrients that stimulate cell division, strengthen roots, and promote overall plant development, we aim to address the identified research gap surrounding the optimal vegetative propagation technique. These treatments are expected to offer practical solutions for cocoa farmers looking to improve planting material quality and boost cocoa production.

This research was conducted in January - July 2023 and took place in Mangaran Village, Ajung District, Jember Regency. The tools and materials needed are tools consisting of a cutting knife, hand sprayer, scissors, plastic bucket, analytical balance, measuring cup, spatula, erlenmeyer 1000 ml, pipette, hotplate, magnetic stirrer, 100 ml beaker glass, 1000 ml beaker glass, ruler, chlorophyll meter (SPAD unit), caliper, staples, stationery, and camera while the materials consist of plagiotropic branches from the Sulawesi Clone 1 cocoa plant, urea fertilizer, SP-36 fertilizer, IAA, soil, sand, manure, polybags, water, 95% ethanol, distilled water, aluminum foil, rubber, lids, label paper, plastic cups, and mica plastic. The experiment was carried out factorial with the basic pattern of Completely Randomized Design (CRD) and was repetitively replications by three times. The first factor was the dose of urea (N) and SP-36 (P) which consisted of 3 levels, such as P0 (0 g/polybag urea and 0 g/polybag SP-36), P1 (3,5 g/polybag urea and 2,5 g/polybag SP-36), and P2 (7 g/polybag urea and 5 g/polybag SP-36). The second factor was the concentration of IAA which consisted of 5 levels, such as I0 (0 ppm), I1 (50 ppm), I2 (100 ppm), I3 (150 ppm), and I4 (200 ppm).

The cuttings taken were from plagiotropic branches of the cocoa plant which were not too old and not too young. The cutting material that has been prepared is then slashed at an angle at around 10-13° and the leaves of the cuttings are sliced only 1/3 of the way. Furthermore, the base of the cuttings that had been cut obliquely was dipped in IAA solution for 5 minutes. Cuttings that have received IAA treatment are planted in polybags that have been arranged in a lid. The plants will be opened and ready to be moved from the containment when they are 3 months after planting. Furthermore, the plants are ready to be given fertilization treatment after 3 months after planting. The treatment application was carried out based on the experimental

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design that had been done previously. For example, the treatment of P1 (3,5 g/polybag urea and 2,5 g/polybag SP-36) were applied by immersing the fertilizer at a distance of 2 cm around the stem. Fertilization is done at intervals of 2 weeks for 2 months (Harahap et al., 2021).

Data collection was carried out 2 weeks after the last fertilization application. The variables observed were the percentage of live cuttings (%), the percentage of cuttings rooted (%), number of primary roots/cuttings, length of primary roots/cuttings (cm), root volume (dm3), number of leaves, number of fallen leaves, number of shoots, shoot diameter (mm), shoot length (cm), and chlorophyll content (SPAD units). Observational data were then analyzed using Analysis of Variance. If there is a significant difference between the treatments, a further test is carried out using Duncan's multiple range test at the 5% level significant.

Results and Discussion

In the observation variable, the percentage of live cuttings and cuttings rooted showed that the overall percentage of treatment had 100%. In the observation variable the number of fallen leaves is not included in Table 1 because it is supporting data from the observation variable number of leaves. The results of the analysis of variance in Table 1 show that the interaction between the application of the nutrients (N and P) and hormone (IAA) had no significant effect on all observed variables except the number of leaves. The main effect of the nutrients (N and P) treatment had a significant effect on the observed variables, that is the number of primary roots/cuttings, length of primary roots/cuttings, root volume, number of shoots, and chlorophyll content but had no significant effect on the number of leaves, shoot diameter, and shoot length while the main effect of the IAA application treatment had no significant effect on all observed variables except the number of primary roots/cuttings and shoot length.

Table 1. Recapitulation The Result of Analysis of Variance (F-count) on All Observation Variables

No.	Observation Variables	Value of F-count		
		P	I	P x I
1.	Number of primary roots	6,14**	4,04**	0,24 ^{ns}
2.	Primary root length (cm)	3,45*	0,85 ^{ns}	0,57 ^{ns}
3.	Root volume (dm^3)	4,21*	0,42 ^{ns}	0,77 ^{ns}
4.	Number of leaves	0,14 ^{ns}	1,27 ^{ns}	2,54*
5.	Number of shoots	3,51*	2,36 ^{ns}	1,43 ^{ns}
6.	Shoot diameter (mm)	2,42 ^{ns}	0,74 ^{ns}	1,66 ^{ns}
7.	Shoot length (cm)	1,38 ^{ns}	2,70*	1,20 ^{ns}
8.	Chlorophyll content (SPAD units)	3,82*	1,34 ^{ns}	1,39 ^{ns}

The Interaction Effect of Nutrients (N and P) and Hormone (IAA) Application on the Growth of Cocoa Plagiotrope Cuttings

The results of the analysis of variance in Table 1 showed that the interaction application of the nutrients (N and P) and hormone (IAA) had no significant effect on all observed variables except the number of leaves of the Sulawesi Clone 1 cocoa plant resulting from vegetative propagation of plagiotropic branches/PCC (Plagiotropic Clonal Cocoa) using cuttings. The average test results for the interaction application of the nutrients (N and P) and hormone (IAA) on the observed variable number of leaves using Duncan's multiple range test at 5% level are presented in Table 2 as follows:

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Table 2. Results of Duncan's Multiple Range Test (□ = 5%) Effect of Interaction Application of the Nutrients (N and P) and Hormone (IAA) on Observation Variable Number of Leaves

Dose of Urea and SP-36 Fertilizer	IAA Concentration (ppm)				
	I_0 (0)	I_1 (50)	I_2 (100)	I_3 (150)	I_4 (200)
P_0 (0 g/polybag urea + 0 g/polybag SP-36)	13,00 (b) B	13,33 (b) B	23,33 (a) A	21,00 (ab) A	18,00 (ab) A
P_1 (3,5 g/polybag urea + 2,5 g/polybag SP-36)	15,00 (b) AB	24,33 (a) A	19,67 (ab) A	17,33 (ab) A	15,00 (b) A
P_2 (7 g/polybag urea + 5 g/polybag SP-36)	21,33 (a) A	17,33 (a) AB	17,67 (a) A	15,67 (a) A	15,00 (a) A

From the results of the analysis of variance in Table 1 it shows that the interaction application of the nutrients (N and P) and hormone (IAA) had no significant effect on all observed variables except the number of leaves. It can be said that the interaction application of the nutrients (N and P) and hormone (IAA) had no significant effect on all observed variables. This means that the application of the nutrients (N and P) and hormone (IAA) has the influence and working properties of each on cocoa plagiotrope cuttings. Therefore, these treatment combinations produce different relationships in influencing plant growth.

It can be seen in Table 2 that the highest number of leaves was found in the combination treatment dose of 3,5 g/polybag urea + 2,5 g/polybag SP-36 and IAA concentration of 50 ppm (P_1I_1) which shows an average value of 24,33. In the combined treatment dose of 0 g/polybag urea + 0 g/polybag SP-36 and IAA concentration of 100 ppm (P_0I_2) also showed a high average value of 23,33. The two treatment combinations produced the highest average compared to the other treatment combinations and had the same notation (not significantly different) for each simple effect so that the recommendation given to obtain the highest number of leaves, it is better to give a combination treatment dose of 0 g/polybag urea + 0 g/polybag SP-36 and 100 ppm IAA concentration (P_0I_2) on vegetative propagation of cocoa by cuttings using plagiotropic branches. The combination of treatment doses of fertilizer 0 g/polybag urea + 0 g/polybag SP-36 and IAA concentration of 100 ppm (P_0I_2) is more recommended than the combination treatment dose of 3,5 g/polybag urea + 2,5 g/polybag SP-36 and IAA concentration of 50 ppm (P_1I_1) because seen from Table 2 treatment of P_0 (0 g/polybag urea + 0 g/polybag SP-36) tended to give the highest average on the simple effect of N and P fertilizer doses on the IAA concentration level (I_2, I_3, and I_4) which is the same compared to the treatment of P_1 (3,5 g/polybag urea + 2,5 g/polybag SP-36) which only gave the highest average on the simple effect of N and P fertilizer doses on the IAA concentration level (I_1) the same.

The interaction of the nutrients (N and P) and hormone (IAA) in influencing the number of leaves of cocoa plagiotrope cuttings is due to the fact that the formation of leaves is determined by the number and size of cells present in the plant as well as the nutrients absorbed by the plant. This is in accordance with the statement of Sa'adah et al. (2021) that auxin can increase cell size by influencing osmotic pressure. The increase in water absorption increases because the cell walls are softened due to the action of auxin. This causes the cell to expand. Furthermore, auxin will affect the process of plasma flow of cells, make fertilizer absorption more effective, and provide vital force to increase plant growth.

Application of exogenous IAA can also stimulate endogenous IAA activity. Endogenous IAA can be formed in abundance when the nitrogen content in plants increases. This nitrogen element is obtained from the provision of urea fertilizer. In addition to forming the hormone auxin, nitrogen also functions to form new cells, tissues and organs. So, leaves can be formed when IAA performs cell division in the intercalary meristem tissue contained in the internodes

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and then the new cells are developed with the help of nitrogen to form new organs, namely leaves. In carrying out the process of leaf formation also requires a large amount of energy. Therefore, the application of urea and SP-36 fertilizers containing nitrogen and phosphorus also plays a role in the formation of energy needed by plants for growth and development.

The Effect of Application of the Nutrients (N and P) on the Growth of Cocoa Plagiotrope Cuttings

The results of the analysis of variance in Table 1 showed that the application of NP fertilizer (urea+SP-36) had a significant effect on the number of primary roots/cuttings, length of primary roots/cuttings, root volume, number of shoots, and chlorophyll content except for the number of leaves, shoot diameter, and shoot length.

Table 3. Recapitulation of Further Test Results Duncan's Multiple Range Test at the 5% level The Effect of Application of the Nutrients (N and P)

Treatment	Number of Primary Roots	Chlorophyll Content (SPAD units)	Primary Root Length (cm)	Root Volume (dm^3)	Number of Shoots
P_0	$4{,}60^{ab}$	$39{,}99^{ab}$	$45{,}13^{ab}$	$4{,}87^{a}$	$3{,}47^{b}$
P_1	$5{,}27^{a}$	$42{,}12^{a}$	$53{,}93^{a}$	$5{,}53^{a}$	$4{,}80^{a}$
P_2	$3{,}67^{b}$	$36{,}31^{b}$	$39{,}49^{b}$	$2{,}80^{b}$	$4{,}40^{ab}$

From the results of Duncan's multiple range test at 5% level, the main effect of the N and P fertilizer treatments showed that the P_1 (3,5 g/polybag urea and 2,5 g/polybag SP-36) gave the best effect on growth variables. This is because the application of urea and SP-36 fertilizers can increase plant vegetative growth which affects the increase in plant metabolism. The results of plant metabolism in the form of proteins and carbohydrates are quickly translocated to all parts of the plant to stimulate plant vegetative growth such as the formation of roots, stems and leaves (Lathifah & Jazilah, 2018).

In our study, we observed that the treatment of 3,5 g/polybag urea and 2,5 g/polybag SP-36 (P_1) produced optimal growth outcomes for cocoa plagiotrope cuttings. Interestingly, this contrasts with the findings of using 7 g/polybag of urea (Sitorus et al., 2014) and 5 g/polybag of SP-36 (Perdinanta, 2019), where higher doses of urea and SP-36 were favored. This discrepancy could be attributed to differences in planting materials; our study focused on cuttings, whereas previous research primarily utilized cocoa seeds.

The variance in our results compared to previous studies may be partly due to the use of cuttings as planting material. Unlike seeds, cuttings may have different root structures and nutrient absorption capacities. Additionally, environmental conditions specific to our study site in Mangaran Village, Ajung District, Jember Regency, could have influenced growth patterns. It's crucial to recognize that cocoa cultivation outcomes are context-dependent, and the choice of planting material plays a pivotal role.

While our findings diverge in some aspects from previous studies, they contribute to a nuanced understanding of cocoa plagiotrope cuttings' growth requirements. By focusing on a specific vegetative propagation technique and addressing the interaction between nutrients and hormones, our study offers insights into optimizing cocoa cultivation in a targeted manner. These results underline the need for context-specific recommendations in cocoa farming practices.

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The Effect of Application of the Hormone (IAA) on the Growth of Cocoa Plagiotrope Cuttings

The results of the analysis of variance in Table 1 show that the application of IAA had no significant effect on all observed variables except the number of primary roots/cuttings and shoot length.

Table 4. Recapitulation of Further Test Results Duncan's Multiple Range Test at the 5% level The Effect of Application of the Hormone (IAA)

IAA Concentration	Number of Primary Roots	Shoot Length (cm)
I_0	3,22[b]	22,39[ab]
I_1	4,78[a]	25,63[a]
I_2	5,44[a]	26,17[a]
I_3	4,89[a]	22,89[ab]
I_4	4,22[ab]	20,67[b]

From the results of the Duncan's multiple range test 5% level in Table 4 shows that treatment I_2 (100 ppm) gave the highest number of primary roots/cuttings of 5,44 roots which were not significantly different from treatment I_1 (50 ppm), I_3 (150 ppm), and I_4 (200 ppm) but significantly different from I_0 (0 ppm) so that the recommendation given to get the highest number of primary roots/cuttings, it is better to give IAA concentration with treatment I_1 (50 ppm) on vegetative propagation of cocoa by cuttings using plagiotropic branches. Treatment I_1 (50 ppm) is more recommended than I_2 (100 ppm) because the two levels are not significantly different (all the same, the difference in numbers occurs by chance not because of the difference in the level of treatment given) so it is better to choose I_1 (50 ppm) for cost efficiency.

From the results of Duncan's multiple range test at 5% level in Table 4 it shows that treatment I_2 (100 ppm) gave the highest increase in shoot length of 26,17 cm which was not significantly different from treatment I_0 (0 ppm), I_1 (50 ppm), and I_3 (150 ppm) but significantly different from I_4 (200 ppm) so that the recommendation is given to get the highest shoot length, then IAA concentration should be given with treatment I_1 (50 ppm) on vegetative propagation of cocoa by cuttings using plagiotropic branches. Treatment I_1 (50 ppm) is more recommended than I_2 (100 ppm) and I_0 (0 ppm) because the three levels are not significantly different (all the same, the difference in numbers occurs by chance not because of differences in the level of treatment given) but in treatment I_0 (0 ppm) was also not significantly different from I_4 (200 ppm) while in treatment I_1 (50 ppm) was not significantly different from I_4 (200 ppm) so the recommendation given is treatment I_1 (50ppm). In treatment I_2 (100 ppm) was also not significantly different from I_1 (50 ppm) but it is better to choose a low concentration, namely I_1 (50 ppm) for cost efficiency. Therefore, treatment I_1 (50 ppm) is recommended to get the best shoot length.

It can be concluded that treatment I_1 (50 ppm) was the best treatment for obtaining the highest number of primary roots/cuttings and shoot length because the results of this study were in accordance with (Wiraatmaja, 2017) that auxin, especially IAA, plays a role in root initiation. The large number of primary roots / cuttings that appear can occur because the formation of roots on cuttings is preceded by the process of IAA entering the plant cells through an absorption process that occurs on the entire surface of the cuttings then IAA will trigger cells to differentiate in areas adjacent to the surface of the cut cuttings so that these cells are meristematic again. Meristem cells in the area near the vascular vessels then divide and differentiate to form root primordia. Furthermore, IAA will activate polysaccharide hydrolysis

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and will produce active sugars that are used in the formation of root primordia into roots (Parnidi & Ridhawati, 2020). Apart from being influenced by IAA, root growth is also influenced by the presence of carbohydrates and protein in the cuttings which are the largest source of energy during the process of root formation. With the presence of carbohydrates and proteins, it will later encourage cell division and form new cells in the network. The greater the number of primary roots/seed cuttings, the longer the shoots will increase.

To build upon our findings and address the complexity of cocoa cultivation, future research could explore the adaptability of our recommended treatments in various cocoa-growing regions. Additionally, assessing the long-term effects and economic feasibility of implementing these treatments on a larger scale would be valuable areas of investigation. This would contribute to a more comprehensive understanding of sustainable cocoa farming practices.

Conclusion

The application of nutrients (N and P) and hormones (IAA) has an interaction in the treatment of the number of leaves. The application of N and P nutrients with a dose of 3,5 g/polybag urea + 2,5 g/polybag SP-36 (P_1) increased seedling growth in the observation variable number of primary roots; primary root length; root volume; number of shoots; and chlorophyll content. The application of IAA with a concentration of 50 ppm (I_1) increased the growth of seedlings, number of primary roots and shoot length.

Suggestion

Further studies are needed regarding the use of plagiotropic branches for vegetative propagation by cuttings on cocoa plants up to the producing plants phase to support good yields and quality of cocoa pods.

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