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Successful shoot tip grafting of cacao (*Theobroma cacao* L.) due to the application of plant growth regulators on various concentrations

Abstract. Cacao plays an important role in the Indonesia economy. Therefore, its production and area expansion have to increase to achieve maximum cacao productivity by the quality and superior cacao clones. Shoot tip grafting is one of the propagation methods to obtain superior cacao plants by applying Plant Growth Regulators (PGRs). Research on the effect of several PGRs with various concentrations on cacao (*Theobroma cacao* L.) shoot tip grafting was conducted at the Integrated Innovation Farmer Group, Belubus, Sungai Talang Village, Guguak District, Lima Puluh Kota Regency, West Sumatra from February until June 2022. This study aimed to determine the interaction between PGR type and concentration on the grafting success and growth of cacao shoot tip grafted seedling. The research was designed by a Factorial Completely Randomized Design with three replications. The first factor was the type of PGR, i.e., synthetic PGR (BAP), young corn seed extract, shallot extract, and young coconut water. The second factor was the concentration of PGR, i.e., 0%, 25%, 50%, 75% and 100%. The results showed an interaction between PGRs with some concentrations on the number of shoots, shoot length, and leaf length on the grafted cacao seedling. The natural PGR from young coconut water at a concentration of 75% gave the best effect on the number of shoots (3.33 shoots) and shoot length (24.50 cm) of cacao seedling.

Keywords: Cacao · Cytokinin · Plant growth regulators · Shoot tip grafting · Young coconut water

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Introduction

Cacao is an important plantation commodity for the national economy, due to it is the main income for most farmers in Indonesia and potentially developed into the agro-industrial sector. One of the provinces in Indonesia as a center for cocoa production is West Sumatra, however, the cocoa production in this area is still low (productivity of cocoa only reached 200 kg/ha/year). Meanwhile, the average of national cocoa productivity reaches 802 kg/ha/year (Sumilia et al., 2019). It shows that cocoa productivity in West Sumatra has not yet reached the standard of average national cocoa productivity.

The efforts to increase cacao production to achieve maximum productivity should concern to the cacao clones used. The shoot tip grafting method is one way to get superior and quality cacao plants, because there is a combination of different rootstocks and scions with their respective advantages. According to Rahardjo (2011), the advantages of this grafting technique included faster yields and production of plants that are genetically similar with the parent so that cacao plants with uniform productivity and quality will be obtained.

The success of the cacao shoot tip grafting technique is due to the cell response of the scion and rootstock which are interlocked. The treatment that is usually used for the scion of shoot tip grafting is the budding immersion in solutions such as growth regulators. The application of PGRs depends on several factors, such as the duration of soaking in solution and the accuracy of the amount given. Using fewer amounts will cause the effect of PGR to disappear, whereas in high amounts it will inhibit the growth and development of a plant (Gusmawan & Wardiyati, 2019).

Several types of natural PGRs contain the hormones of cytokinin, auxin, and gibberellin, which can support the success and growth of shoot tip grafting of cacao. Types of plants that contain bioactive compounds that can be used as extraction materials as growth regulators include compound extracts from young corn seeds, shallots and young coconut water. Pagalla et al. (2015) detected that corn seed extract contained PGRs, namely zeatin (cytokinin) 53.94 ppm, auxin 1.67 ppm and gibberellin 41.23 ppm. In addition, Trifaldi (2021) found that young corn extract at a concentration of 75% has the best effect on shoot length and leaves number of side grafting of cacao.

According to Kurniati et al. (2019), shallots is a natural PGR that contains the hormone auxin (156.01 ppm in the form of IAA), cytokinin (zeatin: 122.34 ppm and kinetin: 140.11 ppm) and gibberellin (230.67 ppm). The research of Darojat et al. (2014) revealed that soaking cacao seeds in shallot extract at a concentration of 10% was able to significantly increase the percentage of germination, growth speed, and the length of hypocotyl and root.

Furthermore, Djamhuri (2011) revealed that young coconut water contains the hormone gibberellins (0.460 ppm GA_3; 0.255 ppm GA_5; 0.053 ppm GA_7), cytokinin (0.441 ppm kinetin; 0.247 ppm zeatin) and auxin (0.237 ppm IAA). Application of young coconut water at a concentration of 75% has an effect on the number of leaves and stem diameter of cocoa seedlings (Yassi et al., 2016). Moreover, Putri et al. (2021) found that application of coconut water at a concentration of 75 - 100% increase the growth of cacao seedlings especially on plant height, stem diameter, leaf area, and dry weight.

It is presumption that there is an interaction effect between the natural PGRs types and their concentrations, therefore the objective of this study was to determine the interaction effect between those two factors on the success and growth of shoot-tip grafted cacao seedling. The finding of present study will provide valuable information regarding the potential use of natural PGRs which are more affordable and environmental-friendly than the synthetic ones for boosting cacao seedling production.

Materials and Methods

This research was carried out on February until June 2022 at the Integrated Innovation Farmer Group, Belubus, Sungai Talang Village, Guguak District, Lima Puluh Kota Regency, West Sumatra. The materials used in this study were MCC 02 cacao seedlings as rootstock, BL 50 cacao clone as scion, extract natural PGRs (young corn seed extract, shallot extract, and young coconut water), synthetic PGR (Benzyl Amino Purine/BAP), distilled water, label paper and plastic bags. The tools used in this study were grafting knives, blenders, filters for natural

PGR, containers, measuring cups, shading net 75%, tape measure, rulers, camera, and stationery.

This study was arranged in a Factorial CRD consisting of two factors which were repeated three times. The first factor is the type of PGRs, namely:

T0 : synthetic PGR (BAP)
T1 : young corn seed extract
T2 : shallot extract
T3 : young coconut water

The second factor is the concentration of each PGR given, consisted of 5 levels, namely:

C0 : 0% (without PGR)
C1 : 25% (25 ml PGR + 75 ml water)
C2 : 50% (50 ml PGR + 50 ml water)
C3 : 75% (75 ml PGR + 25 ml water)
C4 : 100% (100 ml PGR)

Observed data were analyzed by analysis of variance at the 5% level of significance. If the results are significantly different (F count > F table), continue with Duncan's New Multiple Range Test (DNMRT) at the 5% level.

The research was begun by the preparation of location and MCC 02 cacao seedlings, which were 4 months old as rootstock (seedlings healthy, free from pests and diseases, fresh green leaf color, minimum number of 6 leaves, and stem diameter of ± 1 cm). Then, in preparation for the scion in the afternoon, it was a BL 50 clone derived from a tree that was 10 years old. The production branch used as the 2.5-month-old scion was taken in the middle part of the branch where all the leaf stalks were cut, the color was brownish green and the scion length was 10 cm with 3 buds.

Natural PGR is prepared from young corn seed extract, shallot extract, and young coconut water. It was pureed with a blender without adding water then filtered to separate the liquid extract from the pulp. The concentration of each PRG stock solution was 100%, and then it was made according to each treatment. PGR application was carried out before grafting by immersion of the cacao shoots/scion for 12 hours in a PGR solution according to each treatment.

The V-grafting technique carried out the shoot tip grafting. The rootstock seedlings (4 months old) were cut at a height of 25 cm, and the rootstock was split from top to bottom along 3 cm. Meanwhile, the scion used had been treated with PGR, and then the scion was sliced to form V with an even incision area. Furthermore, the grafts are inserted into the rootstock gap that has been prepared and tied with grafting plastic. Watering once a day. In addition, the shoots that grown on the rootstock are removed to avoid competition for scion growth. Finally, the bond graft was opened about three months after grafting.

Observed variables were the age of shoot emergence, number of shoots, shoot length, number of leaves, leaf length and width were measured on a same leaf which is the longest leaf at the beginning of the observation, percentage of success graft and percentage of survival graft.

Results and Discussion

The Age of Shoot Emergence. The results of analysis of variance showed that there was no interaction effect between the application of natural PGR at various concentrations on the age cacao shoots appeared. However, it was affected only by the application of PGR and was not affected by its concentration given (Table 1).

Table 1. The independent effect of several PGRs with various concentrations on the age of shoot emergence (days) of cacao shoot tip grafting

PGR Types	Concentration of PGR (%)					Mean
	0	25	50	75	100	
T0	17.16	19.16	18.33	22.50	17.00	18.83 a
T1	18.33	17.00	16.50	18.16	21.33	18.27 a
T2	13.00	14.16	11.16	15.83	16.50	14.13 b
T3	18.22	15.83	19.00	18.50	18.50	18.03 a
Coefficient of Variance = 8.44 %						

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's new multiple range test at the level of 5 %.

Table 1 showed that the natural PGRs from shallot extract (T2) generates the fastest cacao shoots emergence (14,13 days after grafting). The application of synthetic PGR (T0) and natural PGR from extracts of young corn seeds (T1) and young coconut water (T3) had the same effect on the age of shoot emergence of cacao shoot tip grafting.

According to Nur et al. (2023), the average emergence of buds on shoot tip grafting of cacao plants is around 30 days. This indicates that the growth regulators in the present study able to accelerate the emergence of shoots, and natural growth regulator from shallot extract was better

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than others, because the content of cytokinin hormone in shallot extract is more than on young corn seed extract and young coconut water. It is supported by Kurniati et al. (2019) revealed that shallot is a natural growth regulator that contains a cytokinin of 262.45 ppm. Meanwhile, the cytokinin content in young corn seed extract is 53.94 ppm (Pagalla et al., 2015) and 0.688 ppm in young coconut water (Djamhuri, 2011).

Growth regulators are organic compounds that affect physiological processes, such as growth, cell differentiation and plant development (Asra et al., 2020). Applying natural PGR on cacao shoot tip grafting gave a better effect on the age of shoot emergence compared to synthetic growth regulators (BAP). Besides that, utilization of natural growth regulators can support earth conservation because it being environmentally friendly. It was propped by Pinto et al. (2012) that the application of natural growth regulators does not leave residues that disturb the environment and human health.

Number of Shoots. Based on the observation of the number of shoots on 12 weeks after shoot tip grafting, it indicated that there was an interaction between the PGR types and their concentrations (Table 2). It shows that the average number of shoots on 12 weeks after grafting ranges from 2.00 – 3.33 shoots. Application of natural growth regulators from young coconut water (T3) with a concentration of 75% showed the best results for the number of grafted shoots (3.33 shoots).

Table 2. The interaction effect of several PGRs with various concentrations on the number of shoots at 12 weeks after shoot tip grafting

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	2.00 a B	2.83 a A	2.16 a AB	2.33 b AB	2.00 b B
T1	2.16 a AB	2.00 b B	2.00 a B	2.83 ab A	2.33 ab AB
T2	2.00 a B	2.33 ab AB	2.33 a AB	2.50 b AB	3.00 a A
T3	2.00 a C	2.00 c C	2.50 a BC	3.33 a A	2.83 a AB
Coefficient of Variance = 7.82 %					

Note: Mean followed by the same lowercase alphabet in the same column and followed by the same uppercase alphabet in the same row is not significantly different based on Duncan's new multiple range test at the level of 5 %.

Application of young coconut water PGR at a concentration of 75% was significantly different with other concentrations (0%, 25% and 50%). However, it had the same effect with the concentration of 100% on the number of shoots. At a concentration of 75%, utilization of young coconut water PGR was also significantly different from the synthetic PGR (T0) and PGR from shallot extract (T2), but had the same effect with young corn seed extract (T1).

Application of young coconut water as growth regulators at a concentration of 75% can increase the number of shoots because of the presence of growth hormones i.e., auxins and cytokinins that stimulate shoot growth if given in the precise amount and concentration. It contains cytokinin hormone 5.8 mg/L that stimulate shoot growth and encourage the activities of living cells or tissues, the auxin hormone 0.07 mg/L and a few gibberellins as well as other compounds that can stimulate germination and growth (Setiawan et al., 2013).

Asra et al. (2020) revealed that the increase of the number of shoots is related to the function of cytokinins in the process of cell division, formation of plant bud and organ. In addition, auxin plays a role in mobilizing cells to form new shoots, because auxin can induce rapid cell enlargement due to the activation of ATP-ase which pumps protons in the cell membrane that cause activation of cell expansion (increase the number and size of cells).

A concentration of 75% is the right amount that needed by cacao shoot grafting in the formation of shoots. This is in accordance with Pamungkas et al. (2020) stated that growth regulators are only effective in certain amounts, while the mistake amount will result in stunted plant growth.

Shoot Length. Analysis of variance results on the shoot length on 12 weeks after grafting showed that there was an interaction between the application of some types growth regulators with various concentrations on cacao shoot tip grafting (Table 3). The high shoot growth that occurs is due to the right interaction between those.

Table 3 shows that the average shoot length ranges from 12.16 – 24.50 cm and 75% young coconut water PGR gave the best results for shoot length (24.50 cm). Utilization of growth regulators (PGR) of young coconut water at a concentration of 75% had a highly significant different effect with synthetic ZPT/BAP (T0),

young corn seed extract (T1) and shallot extract (T2). Likewise, its application also had a significant different effect with others concentrations (0%, 25%, 50% and 100%) on the shoot length.

Table 3. The interaction effect of several PGRs with various concentrations on the shoot length (cm) at 12 weeks after shoot tip grafting

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	14.83 a AB	19.00 a A	15.00 ab AB	17.00 b AB	13.16 ab B
T1	17.08 a A	17.83 a A	18.33 a A	16.00 b AB	12.16 b B
T2	13.00 a B	14.50 a AB	12.66 b B	18.33 b A	13.66 ab B
T3	14.16 a B	16.16 a B	16.50 ab B	24.50 a A	17.41 a B
Coefficient of Variance = 6.97 %					

Note: Mean followed by the same lowercase alphabet in the same column and followed by the same uppercase alphabet in the same row is not significantly different based on Duncan's new multiple range test at the level of 5 %.

This is in line with Yassi et al. (2016) that giving young coconut water at a concentration of 75% has the best effect on the growth of cacao seedlings. According to Manurung et al. (2017), young coconut water can encourage shoot growth because it contains the cytokinin hormone of 5.8 mg/L higher than the auxin content which only 0.07 mg/L. It absorbed by plant tissues to activate food reserve energy which can encourage cell division and tissue differentiation, which forms buds and plays on shoot elongation.

On the other hand, at higher concentrations auxin can inhibit cell elongation. The shoot growth occurs is due to the proper interaction between the added exogenous hormones. The balance of auxin and cytokinin concentrations added resulted in good shoot growth (Manurung et al., 2017). The auxin content on young corn and shallot extract are relatively high that causing the shoots growth slower than shoots given young coconut water.

Number of Leaves. Table 4 shows that the average number of leaves on shoot grafting ranged 6.50 – 16.66 leaves and the application some growth regulators with various concentrations did not affect the number of leaves on cacao shoot tip grafting, automatically there is no interaction between them.

The number of leaves were not significant in this study suspected because the erratic weather when the plants generate leaves. Most of the leaves fall at transition from rainy season to the dry season. The monthly rainfall when the research was carried out (February – June 2022) was ranges from 205-260 mm which is the medium category (Center for Water Resources Management Limapuluh Kota Regency, 2022). Besides that, intercepted sunlight is around 25% can stimulate the activity of hormones that encourages the cell division which has a direct effect on leaf growth. On the other hand, Rosniawaty et al. (2020) revealed that coconut water at a concentration of 50% was able to increase the growth of cacao seedlings of the ICCRI 08 H cultivar and showed the best effect on the variable number of leaves.

Table 4. The independent effect of several PGRs with various concentrations on the number of leaves on 12 weeks after shoot tip grafting.

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	11.33	9.50	10.16	6.50	10.16
T1	10.50	13.16	13.16	9.33	7.83
T2	13.50	16.66	7.66	7.33	13.16
T3	12.83	13.00	11.00	10.50	12.00
Coefficient of Variance = 17.49 %					

Cacao is classified as C3 plants which able to photosynthesize at low temperatures. Excessive photosynthesis will be cause leaf fall, whereas high temperatures over a long period will cause the shoots death. In addition, the increase in leaves is influenced by genetic and environment where the leaf blade will develop according to a certain pattern according to its habitus and will stop at the maximum leaf size limit (Gusmawan & Wardiyati, 2019). This is in accordance with the opinion of Wulandari et al. (2013) that the number of leaves is influenced by genotype and environment, on relatively constant environmental conditions enabling leaf primordial to grow with a constant growth rate.

Leaf Length. The linkage between the scion and rootstock will affect the growth of the grafted leaves, where the leaves as a producer of energy and food reserves (photosynthates) for plant growth and development. Based on the analysis of variance, it indicated that there was an interaction between the types of growth regulators with various concentrations on the leaf length of cacao shoot tip grafting as present in Table below.

Table 5 shows that the effect of some PGR on the average of cacao leaf length aged 12

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weeks after grafting ranged from 11.08 – 21.33 cm. Application of young corn seed extract (T1) at a concentration of 75% was shown the longest leaf length (21.33 cm), although the effect was the same with synthetic PGR (T0) and shallot extract (T2). Otherwise, it was significantly different effect with concentrations of 0% and 100%, but had the same effect with concentrations of 25% and 50% on the leaf length of grafted cacao shoots.

Table 5. The interaction effect of several PGRs with various concentrations on the leaf length (cm) at 12 weeks after shoot tip grafting

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	16.25 ab AB	18.50 a AB	15.50 b B	18.00 ab AB	20.50 a A
T1	15.33 bc BC	17.58 ab AB	17.75 a AB	21.33 a A	11.08 c C
T2	17.75 a A	17.41 ab A	15.66 b A	16.08 ab A	16.91 b A
T3	14.75 c A	15.16 b A	16.83 ab A	14.58 b A	16.83 b A
Coefficient of Variance = 10.45 %					

Note: Mean followed by the same lowercase alphabet in the same column and followed by the same uppercase alphabet in the same row is not significantly different based on Duncan's new multiple range test at the level of 5 %.

The results of this study are in line with Trifaldi (2021) that the highest value of leaf length (26,94 cm) on the side-grafted cacao was obtained from the application PGR at a concentration of 75%. This is because the leaf growth is strongly influenced by the genetic nature of the plant. The scions for grafting in the present study was BL 50 clone, where it has the characteristic shape of leaves that are long and pointed.

Leaf Width. Application of some growth regulators at various concentrations did not affect the leaf width on cacao shoot tip grafting at 12 weeks after grafting (Table 6). The average leaf width ranges 4.75 – 7.25 cm that were relatively the same each other's. This is in accordance with the opinion of Trifaldi (2021) that the average leaf width is relatively the same due to the same genetic characteristics, as the scions used in this research was only one type, namely BL 50 clone.

The effect of growth regulators, such as cytokinin has not been able to support the growth of leaf width, suspected that it used for the growth of other grafted parts, such as shoot length and number of leaves. Provision of growth regulators will be effective if used at certain growth phases and under the supportive environmental conditions. Moreover, according to Sumilia et al. (2019), the light requirement that can be sufficient for the assimilation process of the cacao plant is around 75% of the total full sunlight. Sunlight will affect photosynthetic activity and the speed of plant growth in leaves. The higher the photosynthetic activity, the growth of leaves will also increase and bigger. Sunlight also stimulates the activity of endogenous hormones that encourages the process of cell division, enlargement and forms new cells that affect the leaf width.

Table 6. The average cacao leaf width (cm) on 12 weeks after shoot tip grafting

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	5.75	7.25	6.33	6.45	7.16
T1	6.58	6.41	6.91	6.75	4.75
T2	7.08	7.00	5.66	6.91	6.66
T3	6.00	6.25	5.83	6.50	6.91
CV = 17.10 %					

Percentage of Success Graft. Based on analysis of variance noted that application of some PGR with various concentrations had no effect on the percentage of success cacao shoot tip grafting. Observation data on the average percentage of success graft show in Table 7.

Table 7. The average percentage of success (%) of cacao shoot tip grafting

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	100	66.67	100	83.33	83.33
T1	83.33	100	83.33	66.67	83.33
T2	100	100	83.33	83.33	83.33
T3	100	100	66.67	100	83.33
CV = 15.56 %					

Table 7 shows that the success rate of cacao shoot tip grafting ranged from 66.67 – 100%. It was quite high if compared to previous research conducted by Limbongan & Djufry (2013) who reported that the success percentage of cacao side grafting was around 69.90 – 75.40%, and even decreased by 2.00 – 41.80% due to high rainfall after grafting. Many things can affect the success rate of grafting, including the skill of the person doing the grafting, the time of grafting, the weather conditions, and the compatibility between the rootstock and scion.

This was confirmed by Trifaldi (2021) who stated that the compatibility between scion and

rootstock involves a relationship between them, even though the varieties are different, but if they are still in the same genus, so compatible. Nurhalim & Nuha (2022) also revealed that scion and rootstock relationships affect nutrient distribution patterns, the ability of nutrients to move across the graft link and regulation of hormone transport. The use of growth regulators which contain the hormone cytokinin (zeatin) also provides the same nutrition for the linkage process of the scion with the rootstock.

Percentage of Survival Graft. Table 8 shows that the application of growth regulators with various concentrations did not significantly affect the percentage of survival cacao grafting. The average percentage of survival grafting ranged from 33.33 – 83.33 %. The low percentage of survival grafting is thought due to grafting was carried out in the rainy season, then during shoot growth there was a transition from the rainy season to the dry season (monthly rainfall ranges from 205-260 mm), causing abnormal grafting growth and lots of fallen leaves.

Table 8. The average percentage of survival (%) of cacao shoot tip grafting

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	66.67	66.67	83.33	66.67	50.00
T1	83.33	83.33	83.33	66.67	33.33
T2	50.00	66.67	50.00	83.33	50.00
T3	50.00	66.67	50.00	83.33	66.67
CV = 25.64 %					

According to Trifaldi (2021) the high risk of death for scion stems is due to the penetration of rainwater at the connection (enclosed part) causes decay (death) of cells or plant tissue there. In addition, covering the buds can also increase moisture so that the risk of fungal attack on the joints also increases. Fungus attack on shoots is characterized by symptoms of wilting and drying.

The average percentage of survival was highest at a concentration of 75%, while the lowest was at a concentration of 0% and 100%. This is presumably because growth regulators at certain concentrations can increase the percentage of survival grafting. As stated by Nurlaini & Imam (2015) that utilization of PGR at the right concentration can increase the growth percentage, while at the wrong concentration cause stunted or abnormal plant growth.

Conclusion

1. There was an interaction effect between the types of PGR with their concentrations on varibles of the number of shoots, shoot length and leaf length of cacao shoot tip grafting.
2. Natural growth regulators from young coconut water at a concentration of 75% had the best effect on the number of shoots and shoot length of cacao shoot tip grafting.

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