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Macronutrient Contents and Yield of Cocoa Resulting from Two Different Rejuvenation Techniques

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Abstract

Cocoa (*Theobroma cacao* L.) is one of Indonesia's leading and important export commodities. The cocoa plant rejuvenation techniques aim to increase cocoa production and quality. Ring budding and side grafting can rejuvenate old and unproductive cocoa plants. Therefore, this research was conducted with objectives: 1) to record macronutrient contents, yield components and yields of three cocoa clones, KKM 22, RCC 70 and RCC 71, rejuvenated with ring budding and side grafting, 2) to determine the best techniques in rejuvenation program for old cocoa stand based on nutrient contents, yield components, and yields indicators of three cocoa clones. This research was carried out from November 2020 to January 2021 at Cocoa Plantation, North Segayung Production Unit, Pagilaran Company. The results showed that ring-budded plants had the highest N nutrients content in leaves, stem organic-C production capacity, pods number per stand per year, dry weight bean per stand per year and dry weight bean per hectare per year, which was significantly better than side grafted plants. The macronutrient of total parts (leaves, stem, and root) trend was leaves > stem > root. The trend of macronutrients in cocoa were organic-C > N > Ca > K > Mg > P. Based on the macronutrient content of cocoa tissue, KKM 22 have a higher content of organic-C, N, P, K, Ca, and Mg compared to RCC 71 and RCC 70. The budding ring technique was the best in the rejuvenation program for old cocoa stands based on nutrient contents, yield components, and yield indicators of three cocoa clones.

Keywords: nutrient content; ring budding; side grafting; vegetative propagation

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INTRODUCTION

In 2018, the world produced 5.3 million tons of cocoa pods (*Theobroma cacao* L.), making it one of the most valuable commodities. After Ivory Coast and Ghana, Indonesia is the world's third-largest cocoa-producing nation. In 2018, cocoa plantations in Indonesia covered an area of 1,6 million hectares and produced 593,8 thousand tons (FAO, 2020). The world's cocoa consumption rose from 2014 to 2018, reaching 3.02 million tons in 2018 (ICCRI, 2020). As a result of a prospective increase in cocoa demand, it was estimated that one million additional tons of cocoa were required in 2020, and at least one million additional tons were required in 2030 (Sena Gomes et al., 2015).

Indonesia has the potential to become a significant global cocoa producer because land availability is substantial. According to Fahmid et al. (2022), Indonesia's cocoa export performance in the world market certainly opens up many opportunities. It is necessary to optimize the potency and competitiveness of its cocoa if Indonesia would make cocoa exports the driving of the national economy. In 2010, the cocoa area in Indonesia reached 1,650,621 ha. With an average annual cocoa productivity of

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0.39 tons ha^{-1}, Indonesia ranks 28th worldwide for cocoa productivity. The production of cocoa in several nations is threatened by aging cocoa plantations and declining productivity (Sena Gomes et al., 2015). According to data from the Indonesian Directorate General of Plantations, 34.67% of cocoa plantations were damaged or comprised of old cocoa trees. Therefore, it is necessary to revitalize old cocoa plantations to increase Indonesia's cocoa yield (Rohmah, 2019). It is common for old cocoa plantations to be revitalized using revitalization techniques the rejuvenation of old cocoa plantations through grafting and budding techniques (Sena Gomes et al., 2015). The technique of side grafting is the most popular among Indonesian cocoa producers (Effendy et al., 2013). According to Putra et al. (2012), the success rate of vegetative propagation for rejuvenating old cocoa plantations using the side grafting technique was 61.20%, 64.23% for shoot grafting, and 55.45% for grafting.

According to Palad (2017), the productivity of five-year-old side-grafting stands is quite adequate during the first five years, but production and productivity decline after that. This condition may be the result of an imbalance between the capacity of the plant shoot to produce biomass and the condition of the plant's root system, which supplies nutrients and water and has aged roots. In light of this, researchers conducted an extensive research program to develop a new technique for revitalizing old cocoa plantations through ring budding to contribute through sustainable cocoa cultivation (Obeng, 2022). Compared to shoot grafting, side grafting, and grafting, this technique produced 91.38% more surviving plants (Putra et al., 2012). In addition, according to Gurrieri et al. (2006) and Saraswati (2018), the budding ring technique has several advantages, including: (1) ring budding can be carried out on old plants by using orthotropic shoots growth from the lower part of the main stem close to the soil surface; (2) orthotropic shoots use to obtain abundant nutrition from the main stem, so it develops and adapts quickly; (3) the growth is fast and vigorous because it still receives nutrients from its main stem; and (4) when new stem start to bear pods, young pips can be harvested.

According to Bekele and Berhan (2021), large amounts of macronutrients, such as organic-C, N, P, K, Ca, and Mg, are necessary for plant growth. Nitrogen is the primary component of amino acids and proteins, which play a crucial role in the growth and development of plants. This element has a greater total quantity and effect on plant growth than others. Phosphorus (in the form of phosphate PO_4^{3-}) is an essential component as a constituent of plant cells, nucleotides (ATP), DNA, and RNA. Calcium is the principal cell wall component of the middle lamella, which regulates membrane integrity. Calcium absorption is influenced by the concentration of the soil solution and the transpiration rate, allowing passive transport of Ca^{2+} ions (El Habbasha and Ibrahim, 2015).

Additionally, antagonist ions such as NO_3^- also affect Ca absorption. Magnesium is the primary photosynthesis-site chlorophyll molecule component. Furthermore, Mg serves as an enzyme activator. Magnesium deficiency can negatively affect plant growth and development, CO_2 fixation will be reduced, and the production of growth-required carbohydrates will drop (Xie et al., 2021).

In the past ten years, the technique of ring budding has been internalized, indicating that most of them are currently at optimum production. In mature plants, root function and transport tissues are anticipated to assimilate and translocate water and nutrients optimally. However, recorded data on mature ring budding stands are limited or nonexistent. Therefore, this research was conducted with two objectives, namely 1) to record data, i.e., macronutrient content, yield components, and yields of cocoa clones KKM 22, RCC 70, and RCC 71 rejuvenated with ring budding and side grafting, and 2) to determine which technique was better to use in rejuvenation program for old cocoa stands based on indicators of nutrient content, yield components, and yields of cocoa clones.

MATERIALS AND METHOD

Study area

This research was conducted from November 2020 to January 2021 at the cocoa plantation of Pagilaran Estate, North Segayung Production Unit, Simbangjati Village, Tulis Sub-district, Batang Regency, Central Java Province. The study site coordinates at latitude 6°56'39.3"S and longitude 109°48'08.3"E.

Experimental design

The research was conducted with a factorial of a nested design. The first factor was the rejuvenation techniques, namely side grafting and ring budding. The second factor was cocoa clones, namely KKM 22, RCC 70, and RCC 71.

Cocoa clones were nested in rejuvenation techniques.

KKM 22 is one of the commercial cocoa clones released by Malaysia. KKM 22 is an acronym for Koko MARDI output number 22, including a Trinitario type clone with an Angoleta fruit shape. This clone has a productivity potential of up to 2,420 kg ha^{-1} year^{-1}, an average fruit value of 24, an average bean weight of 1.09 g, Vascular streak dieback (VSD) resistant and has a fat content of 57% (Lee et al., 1993), and is susceptible to *Phytophthora palmivora* infection (Rubiyo et al., 2020).

RCC 70 (Rispa Cocoa Clone 70) is a type of lindak cocoa (forastero) developed in Indonesia and released by the Ministry of Agriculture through Minister of Agriculture Decree Number 530/Kpts/SR.120/9/2006. Yield potential reaches 2.28 tons ha^{-1} (1,100 trees ha^{-1} population), dry weight bean 1.18 g, bean fat content 57.5%. This clone is somewhat susceptible to fruit rot disease, as well as susceptible to VSD, Heliopolis and Cocoa pod borer (CPB) pests (ICCRI, 2020). Based on the Regulation of the Minister of Agriculture (2013), clone RCC 71 (Ministry of Agriculture through Minister of Agriculture Decree Number 686.a/Kpts-IX/98) is a type of lindak cocoa (forastero) which has a potential yield of 2,639 kg ha^{-1} year^{-1}, calabasilo fruit shape, wet bean color is purple, dry weight bean 1.18 g, fat content 58.1%, moderate resistance to fruit rot disease (*P. palmivora*), resistant to Helopeltis pests.

Procedures

This research used samples of cocoa stands located in Blocks IV and VIIIA. Block IV has a land area of 10.08 ha, cocoa was planted in 1985, with 9,867 plants. The cocoa stands in Block IV represent treatment using ring budding technique which was rejuvenated in 2012. Meanwhile, Block VIIIA has a land area of 6.2 ha, cocoa was planted in 1985 with 3,250 plants. The cocoa stands in Block VIIIA represent treatment using the side grafting technique rejuvenated in 2013. In each block, a square map was then made with a size of 20 m x 20 m contained about 40 cocoa stands, and crop space ± 3 m x 3 m. The location chosen for plots is flat land, situated in the middle of the block with homogeneous stands. The selected stands have an age of about 7 to 8 years after replanting, the plants are in healthy condition without stem or fruit rot disease, have a stem circumference of ± 35 cm and a stand height of ± 3 m. From a total of 40 cocoa stands, three stands were selected for each clone, namely RCC 70, RCC 71 and KKM 22, so in each block number of stands were used as samples.

Ring budding implementation stages

The ring budding technique was implemented by preparing stand to be joined. The orthotropic shoots appearing were selected, with some indicators, namely having vigorous shoot, growing from the bottom of main stem of old cocoa stands, and being located near to soil surface. Then, two shoots were prepared in case one shoot died. After one was confirmed alive, spare shoots might be cut. Procedure for implementing ring budding technique includes: 1) orthotropic shoot was cut as high as 30 cm, 2) stem of the orthotropic shoot was cut in a circle and split open, and skin was peeled off after it had been cut in circles; the incision distance from soil surface was 20 to 30 cm, while from the growing point was 10 to 15 cm, 3) scion to be attached to orthotropic shoots was cut in a circle with two buds and the length was same as the length of orthotropic rod being cut, 4) buds of scion were attached to orthotropic shoot incisions, and then tied, but the buds were ensured unclosed, and 5) after new shoot buds lived, the rope could be removed.

Side grafting stages

The side grafting technique was applied through several stages, including: 1) scions used were from healthy shoots such as being free from disease and pest, fresh green shoots, and from clones having good characteristics; scion consisted of two buds, and then the bottom of scions was cut tapered, 2) main stand bark (stock) of old cocoa stands were cut in a T-shape, 3) after that, shoots were attached to main stem incision (stock) and tied with a rope or plastic, 4) plastic cover was attached to side joint, and 5) after new shoots developed, the plastic cover was removed.

Soil analysis

Soil analysis variables including soil pH, organic-C, and total N were measured using Kjeldahl method, available P and K were measured using Morgan-Wolf extraction method, and total Ca and total Mg were estimated using wet ash extract (HNO_3 and $HClO_4$). Soil sampling was carried out on each plant sample in each block with a depth of 40 cm. After that, the soil samples were ground and mixed until homogeneous. The number of soil samples in each block was two replicates. The type of soil in the location is Latosols (Dewi Hs et al., 2020). Soil analyses were made at Testing Laboratory of Central Java Agricultural Technology Assessment Center.

Tissue analysis

Tissue analysis for macronutrient contents included macronutrient content in root, stem, and leaf tissues. Organic-C, total N, total P, total K, total Ca, and total Mg contents were analyzed using wet ash extract (HNO_3 and $HClO_4$). Tissue analysis was made at the Testing Laboratory of Central Java Agricultural Technology Assessment Center.

Yield components

Yield components and yields included total pods per stand and 100 dry beans. The number of pod per tree was calculated based on the yield obtained in one sample tree. A total of 100 beans were taken at random from the total bean harvested in one tree. Furthermore, the beans per pod were weighed. The pod harvested from each treatment was split and then the beans were dried to reach a moisture content of approximately 7.5% (Directorate General of Plantation, 2014). After being dried, all the beans were weighed for each treatment. The dry bean weight per stand was obtained by the formula: weight of total bean per pod x total pods per stand. The productivity was obtained using the formula: dry weight bean per stand x number of trees per ha.

Data analysis

The comparison between ring budding and side grafting was analyzed using unpaired T-test. Quantitative data from each observed variable were analyzed using ANOVA α 5%. If clone treatments were significantly different, the conclusion was made using Fisher Least Significant Difference (LSD) test. The Correlogram of the relationship between variables used R-studio v 1.0.53 software. The principal component analysis (PCA) used the Minitab v17 software as the analysis tool.

RESULTS AND DISCUSSION

Soil chemical characteristics

The growth and yield of cocoa plantations are influenced by planting materials, soil climate, and agricultural practices. Due to diminished mineral nutrients and organic matter, soil quality is a limiting factor for cocoa growth. According to the T-test, the organic-C elements, total N, available P, and available K, soil chemical properties for ring budding were substantially different from those for side grafting in Table 1. Organic-C and available P were greater on ring budding than side grafting, whereas total N and available P was low. The soil pH of both techniques was 5.36 and 5.46, respectively, so that it was considered acidic. Cocoa-suitable soils with a pH of S2 were classified as quite suitable, and according to Hartati et al. (2018), the potential land suitability for cocoa in Maluku was obtained into class S3. The pH of the soil provides valuable information regarding the availability of nutrients. The soil organic-C content for ring budding was 1.25%, and for side grafting, it was 1.19%, both of which are considered insufficient. Based on the suitability of the land, the organic-C content was classified as class S1 or highly suitable for cocoa. The research site was appropriate for cocoa cultivation based on its pH and organic-C content. As long as nutrient content is sufficient, cocoa can grow in soils with a pH range between 5.0 and 7.5 and is tolerant of acidic soils (ICCO, 2013).

Soil ability to provide sufficient nutrients for plants was affected by four factors: (1) amount of various important elements in soil, (2) form of nutrient combinations, (3) chemical processes so that elements become available to plants, and (4) soil solution and pH (Resh, 2013).

Table 1. Soil characteristics of cocoa stands rejuvenated with ring budding and side grafting

Character	Ring budding	Side grafting	p-value (0.05)	Note	Status of soil[a]	Land suitability class[b]
pH H_2O	5.36±0.01	5.46±0.04	0.16	ns	Acidic	S2
Organic C (%)	1.25±0.00	1.19±0.01	0.05	*	Moderate	S1
Total N (%)	0.17±0.00	0.19±0.00	0.03	*	Low	S2
Available P (ppm)	2.57±0.05	6.87±0.48	0.05	*	Low	S2
Available K (ppm)	413.01±8.32	261.17±17.76	0.02	*	Very high	S1
Total Ca (%)	0.05±0.01	0.09±0.01	0.09	ns	Very high	S1
Total Mg (%)	0.04±0.00	0.05±0.01	0.50	ns	Very high	S1

Note: * = Significantly different; ns = not significantly different based on t-test (α = 0.05); status of soil[a] was based on criteria by Soil Research Institute; Land suitability class[b] was based on Ritung et al. (2011); S1 = very suitable, S2 = moderate, S3 = marginal, N = not suitable

The amounts of soil nutrients fluctuate due to climate change, considering the application of fertilizers (Guyonnet et al., 2018). Soil type at Pagilaran Company is Latosols (Dewi Hs et al., 2020). The main characteristics of Latosols or Inceptisols according to USDA Soil Survey Staff (2022) are: 1) color: yellowish to brownish red shown by whole solum, 2) depth solum: moderate to deep (1.5 to 10 m), 3) horizon: sesquioxide, 4) texture: clay constant through whole solum, 5) structure crumb to weak blocky, 6) consistency: friable to slightly firm, 7) other features: plinthite, sometimes with weak clay coatings, 8) base saturation: 20% to 90%, 9) pH (H_2O): 4.5 to 6.5, and 10) cation exchange capacity: 15 to 25 meq 100 g^{-1}.

Total N contents in the soil on ring budding and side grafting were 0.17% and 0.19%, and thus categorized as low. Based on land suitability, total N content was classified as class S2 for cocoa. The P content of soil on ring budding was 2.57 ppm, which can be categorized as very low so it was included in S3 class land suitability for cocoa, while available P content on side grafting was 6.87 ppm, which was classified as low or in S2 class. Soil K contents on ring budding and side grafting were 413.01 ppm and 261.17 ppm, which can be characterized as very high so they are very suitable for cocoa cultivation (class S1). Total Ca contents on ring budding and side grafting were 0.05% and 0.09%, which are categorized as very high. Total Mg contents of soil on ring budding and side grafting were relatively similar, at 0.04% and 0.05%, which are very high.

Some 91% to 94% of the N in cocoa systems was found in the top soil. Total N content in the upper 30 cm of the soil varied from about 4,800 to 6,700 kg ha^{-1}. The accumulation of P in cocoa ecosystems was small. The P stored in the soil amounted to 30 to 79 kg ha^{-1}. Variability of accumulated K in cocoa systems was extremely large. Stocks of exchangeable K in the top soils of mature cocoa varied from about 100 to 560 kg ha^{-1}, which was between 27% and 61% of total exchangeable K accumulated in the cocoa systems (Hartemink, 2005).

In this study, total N contents of soil were 0.17% and 0.19%, which were categorized as low, and the P contents of soil were 2.57 ppm and so 6.87 ppm, which were very low and low (Table 1). Nitrogen is a highly mobile soil nutrient, which may rapidly transform or leach from the soil. Nitrogen is taken up by plants largely in mineral form, which is only a fraction of the total N content of the soil. Both mineral N and total N contents in the soil at any point in time may have little relation with subsequent N availability to the plant (van Vliet and Giller, 2017). Kumar et al. (2021) reported synergistic interactions between N and P in providing a higher yield under diverse ecosystems. Thus, evidence suggests that N availability modulates P starvation responses (Liang et al., 2015; Medici et al., 2019). Under P-starvation, N supplementation activates P acquisition, while N-starvation represses the P-starvation responses. This indicates that a plant modulates its regulatory system to prioritize N nutrition over P. Three major signaling factors involved in N–P interaction were identified, including SPXs, PHRs, and PHO_2. Otherwise, the soil K, Ca, and Mg contents on ring budding and side grafting were categorized as very high. It is important to highlight that the K^+, Ca^{2+}, Mg^{2+} ions present competitive inhibition in the absorption process, that is, they compete for the same root absorption site, consequently the greater presence of one inhibits the absorption of the other (Bahia et al., 2021).

Tissue nutrient content

Nutrient content differed significantly between tree components. In this research, it was observed that recyclable plant components tend to have higher nutrient content, like leaves, whereas less recyclable components like root and stem, showed a lower value. The macronutrients trend was leaves > stem > root. Mohammed et al. (2020) reported that the cocoa leaf and husk contained on average high elemental nutrient concentrations compared with the root, stem, and branches, according to the results by Romero (2018), were obtained in cacao. As a general trend, the order concentration for macronutrients (N, P, K) in plant components was leaves > bark > roots > wood. Several authors found a similar trend but did not make the distinction between bark and wood, resulting in: leaves > roots > branches > stem. Both stem and branches were composed by a lower proportion of bark and higher of wood. The bark presented significantly higher nutrient content than wood. The bark plays a key role in the storage of water and photosynthates as it contains secondary phloem (Rosell, 2016). This explains its high nutrient content. The nutrient accumulation of the bark is N > Ca > K > Mg > P and the wood K > Ca > N > Mg > P.

Macronutrient content of root tissue

The nutrient contents of cocoa roots rejuvenated with ring budding were not substantially different from side grafting (Table 2). The nutrient contents of roots did not differ significantly between the two rejuvenation techniques. The organic-C content of both methods was 29.87%. The total amounts of N were 0.69% and 0.67%, P were 0.05% and 0.05%, K were 0.55% and 0.60%, Ca were 0.44% and 0.49%, and Mg were 0.42% and 0.43%. In this study, the total P content in the root was lower than other nutrients. Ruseani et al. (2022) observed an antagonistic effect of N treatment on the absorption of P, K, and Mg by cocoa seedling roots and shoots. With the administration of N, roots and shoots absorbed less P and Mg than those of untreated plants. Moreover, the N application reduced root K assimilation. In response to the N treatment, there was an increase in Ca absorption by cocoa seedling roots and shoots. This condition suggests a synergistic effect of N on Ca absorption. These results indicate an interaction between cocoa plants' N, P, K, Ca, and Mg.

Table 3 shows that organic-C content of roots between clones in each method of rejuvenation was not different, but the contents of N, P and K were significantly different. There were differences in root macronutrient contents between clones on ring budding method. By ring budding, KKM 22 had the highest N, P and K contents in the roots, although N and K contents were not significantly different from RCC 71 clone. The RCC 70 clone had the lowest N and K contents, although P content did not differ from the RCC 71 clone. There was a tendency that by side grafting there was no difference in root

Table 2. Macronutrient contents of root tissue on cocoa stands rejuvenated with ring budding and side grafting

Observation variable (%)	Ring budding	Side grafting	P-value (0.05)	Note
Organic-C	29.87±1.73	29.86±1.52	0.98	ns
Total N	0.69±0.07	0.67±0.05	0.65	ns
Total P	0.05±0.02	0.05±0.01	0.70	ns
Total K	0.55±0.11	0.60±0.05	0.37	ns
Total Ca	0.44±0.16	0.49±0.13	0.56	ns
Total Mg	0.42±0.14	0.43±0.04	0.91	ns

Note: * = significantly different; ns = not significantly different based on t-test (α = 0.05)

Table 3. Organic-C, N, P, K, Ca, and Mg total contents of roots of three cocoa clones on ring budding and side grafting rejuvenation

Clone	Macronutrient content of cocoa root tissue			
	Organic-C (%)		Total N (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	30.81^{a}	29.41^{a}	0.75^{a}	0.72^{a}
RCC 70	28.91^{a}	30.62^{a}	0.60^{b}	0.65^{a}
RCC 71	29.89^{a}	29.54^{a}	0.72^{a}	0.65^{a}
	Total P (%)		Total K (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	0.07^{a}	0.06^{a}	0.66^{a}	0.62^{a}
RCC 70	0.04^{b}	0.04^{a}	0.44^{b}	0.63^{a}
RCC 71	0.03^{c}	0.04^{a}	0.54^{ab}	0.53^{b}
	Total Ca (%)		Total Mg (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	0.39^{b}	0.47^{b}	0.30^{b}	0.43^{ab}
RCC 70	0.28^{c}	0.64^{a}	0.37^{b}	0.39^{b}
RCC 71	0.63^{a}	0.35^{c}	0.59^{a}	0.46^{a}

Note: Means in a column followed by a different letter indicating a significant difference based on LSD test at α = 5%

macronutrient content, except that the K content of RCC 71 clone was lower than those of other clones.

The contents of Ca and Mg in the roots between clones in each rejuvenation method were different (Table 3). There were differences of root macronutrient contents in cocoa clones rejuvenated with ring budding. On ring budding, RCC 71 clones had the highest root contents of Ca and Mg. RCC 70 clones had the lowest contents of Ca and Mg. In side grafting, RCC 70 clones had the highest root content of Ca, but the lowest Mg content. On the other hand, the RCC 71 clones had the highest Mg content, although it was not significantly different from KKM 22, but RCC 70 clones had the lowest root content of Ca.

Ruseani et al. (2022) studied shows that cocoa clones in Indonesia vary in their nutrient concentrations in root and shoot. Clone ICS 9 had the lowest root P concentration, which was significantly different from root P concentration in other clones except for TSH 858. MCC 02 had the highest root Mg concentration which significantly differed from that of clones ICS 9 and Sulawesi 2. Root P, K, and Mg uptake significantly differed between scion clones. MCC 02 had the highest P uptake, followed by clone Sulawesi 2. MCC 02 also had the highest root K and Mg uptake, significantly different from those of the other clones.

Macronutrient content of cocoa stem tissue

Table 4 demonstrates that a T-test revealed significant differences between ring blossoming and side grafting techniques for organic-C and total P contents. The level of organic-C in ring budding stem tissue was greater than that of side grafting, whereas the level of total P was lower. The organic-C content of ring-budded stems was greater than that of side-grafted stems. In contrast, the total P content of ring-budded stems was lower than that of side-grafted stems, 0.06% compared to 0.08%. Moreover, there was no significant difference between the root contents of these two elements. Mohammed et al. (2020) reported the following concentrations (mg g^{-1}) of cocoa stem nutrients in cocoa ecosystems of Ghana: total N (3.7 ± 0.6); total P (0.5 ± 0.3); total K (4.0 ± 0.4); total Ca (5.0 ± 1); and total Mg (2.5 ± 0.3). Based on the data on nutrients, it was determined that the chemical properties of the cocoa tree's biomass fractions varied significantly.

Table 5 presents that the contents of N, P, and K in stems differed significantly. Meanwhile, the content of organic-C in stems between clones in each rejuvenation technique was not different. In-ring budding, KKM 22 had the highest N, P, and K contents in stems, although the P content was not significantly different from RCC 71. The RCC 70 had the lowest P content, although N and K contents did not differ from RCC 71. There was a tendency that by the side grafting, there was no difference in stem macronutrient contents.

The Ca and Mg contents of stems differed between clones resulting from each rejuvenation technique. Rejuvenated cocoa stands with ring budding revealed that RCC 71 had the highest Ca and Mg contents, while the Ca content of KKM 22 was not substantially different from RCC 71. KKM 22 contained the least amount of Mg. Side grafting rejuvenation has a similar pattern to ring budding, and RCC 71 had the full Mg content. Similarly, the RCC 70 clone had the lowest Mg content, whereas the Ca content did not differ substantially from KKM 22 and RCC 71. Calsium absorption was similar across all development phases. This constituent is quite mobile in the xylem. In all phases of plant development, Mg absorption was relatively constant. This element was more mobile than Ca in the xylem and phloem.

Macronutrient content of cocoa leaf tissue

The content of macronutrients in cocoa leaves are presented in Table 6. Content of macronutrients in cocoa leaves were similar between ring budding and side grafting, except

Table 4. Macronutrient contents of cocoa stem tissue by ring budding and side grafting rejuvenation

Observation variable (%)	Ring budding	Side grafting	P-value (0.05)	Note
Organic-C	41.06±1.13	38.60±1.69	0.02	*
Total N	1.06±0.09	1.07±0.03	0.78	ns
Total P	0.06±0.01	0.08±0.01	0.04	*
Total K	1.88±0.78	2.26±0.15	0.30	ns
Total Ca	2.67±0.44	2.21±0.30	0.06	ns
Total Mg	0.68±0.12	0.57±0.06	0.08	ns

Note: * = significantly different; ns = not significantly different based on t-test (α = 0.05)

Table 5. Organic-C, N, P, K, Ca, and Mg contents in total stem of three cocoa clones in ring budding and side grafting rejuvenation

Clone	Macronutrient content of cocoa stem tissue			
	Organic-C (%)		Total N (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	40.81[a]	38.89[a]	1.18[a]	1.09[a]
RCC 70	41.49[a]	38.43[a]	1.01[b]	1.08[a]
RCC 71	40.87[a]	38.45[a]	1.00[b]	1.05[a]
	Total P (%)		Total K (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	0.07[a]	0.07[a]	2.89[a]	2.23[a]
RCC 70	0.05[b]	0.08[a]	1.38[b]	2.38[a]
RCC 71	0.06[ab]	0.07[a]	1.37[b]	2.15[a]
	Total Ca (%)		Total Mg (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	2.68[ab]	2.53[a]	0.53[c]	0.57[ab]
RCC 70	2.20[b]	2.16[ab]	0.73[b]	0.51[b]
RCC 71	3.13[a]	1.91[b]	0.77[a]	0.63[a]

Note: Means in a column followed by a different letter indicating a significant difference based on LSD test at α 5%

for total N. Total N of ring budding was higher than that of side grafting, 1.87% and 1.68%, respectively. The organic-C contents of both techniques were 41.62% and 41.45%, respectively. The total P contents were 0.11% and 0.10%; the total K were 1.40% and 1.17%; the total Ca were 1.18% and 1.25%; and the total Mg were 0.55% and 0.59%. The amount of total N, total P, total K, total Ca, and total Mg was within the optimal nutrient for cocoa based on the criteria by Paramo et al. (2016) presented in Table 7. Ipinmoroti and Ogeh (2015), also reported the leaf P contents for old cocoa plants in Nigeria were 0.14% to 0.29%, the K contents were 1.11%, the leaf Ca contents were 0.88% to 2.08%, and the leaf Mg contents were 0.5%.

The results of this study showed that the nutrient accumulation trend of mature leaves was organic-C > N > Ca > K > Mg > P. A similar order of nutrient accumulation had been presented on cocoa by Mohammed et al. (2020). Considering the measured mean concentrations of elemental nutrients for the cocoa tree, the order of nutrient accumulation in cocoa is N > Ca > K > Mg > P > S > Al = Fe > Zn = Mn. Romero (2018), in general terms, reported that the contents of certain nutrients (N, P, K, Cu, Zn) decrease while others (Ca, Mg, B, Fe and Mn) increase in the transition between newly produced leaves (leaves from the current flush) to mature leaves (leaves form the current flush) and senescing leaves. This could give us some insight on the nutrient translocation and nutrient fixation in cocoa leaves.

In this research, the N content of ring budding leaves was higher than that of side grafting, even though the soil N content of ring budding was lower than that of side grafting. This can describe the efficiency of N nutrient absorption

Table 6. Macronutrient contents of cocoa leaf tissue by ring budding and side grafting rejuvenation

Observation variable (%)	Ring budding	Side grafting	P-value (0.05)	Note
Organic-C	41.62±1.67	41.45±2.26	0.88	ns
Total N	1.87±0.11	1.68±0.07	0.01	*
Total P	0.11±0.02	0.10±0.01	0.09	ns
Total K	1.40±0.64	1.17±0.29	0.45	ns
Total Ca	1.18±0.16	1.25±0.09	0.36	ns
Total Mg	0.55±0.09	0.59±0.07	0.33	ns

Note: * = significantly different; ns = not significantly different based on t-test (α = 0.05)

by root of cocoa stands rejuvenated with ring budding, which was then transferred to leaves. In addition, the high N content of cocoa leaves rejuvenated with ring budding indicated that vascular tissue was relatively normal so it did not cause symptoms of incompatibility after 10 years grafted. On the contrary, low translocation of N in cocoa stands rejuvenated with side grafting indicated vascular tissue disorders due to delayed incompatibility between rootstock and scion. This statement was supported by the analysis between nutrients content, biochemical activities, yield, and anatomy variables (unpublished data) (Meilani, 2021). Elemental N is mobile in plant tissues and functions as a constituent of organic compounds, such as amino acids, proteins, coenzymes, nucleic acids, and chlorophyll, which play an important role in plant growth and development (Resh, 2013). A good root system can increase nutrient absorption and nutrient accumulation and this indicates that roots can adapt to soils with low nutrient availability (Baron et al., 2018).

Connection compatibility resulted from perennial plant propagation as well as perennial plant rejuvenation programs that use connection technique occurs if scion and rootstock can unite and develop into one plant. Meanwhile, according to Hartmann et al. (2010), connection incompatibility is a disturbance in transport network that fails. Sometimes, the connection between scion and rootstock is successful in early stages (nursery and immature crops), but symptoms of incompatibility begin to appear (at the mature stage).

Incompatibilities may occur within days or years. Delayed incompatibility can occur years, until 20 years after grafting (Hartmann et al., 2010). Physiological and metabolic mechanisms involved in incompatibility remain unclear (Zarrouk et al., 2006; Melnyk, 2017). This may be due to lack of understanding and adequate technique to analyze metabolic changes that occur during development between fused scion and rootstock. Only a few studies have been conducted on the compatibility of woody plants (Pina and Errea, 2005; Koepke and Dhingra, 2013).

Table 7. Optimal nutrient concentration in cocoa leaves according to Paramo et al. (2016) in Colombia for mature leaves

Nutrient	Range (%)
N	1.61 - 1.83
P	0.12 - 0.19
K	0.90 - 1.27
Ca	1.69 - 2.45
Mg	0.44 - 0.71

The C dan K contents in leaves were significantly different among clones in both ring budding and side grafting. Meanwhile, the contents of total N and P were not different among clones. The C, N, and P contents in leaves of KKM 22 were highest, except for K content. RCC 70 had the lowest organic-C, N, P, and K contents in leaves. Side grafting rejuvenation showed that KKM 22 had the highest organic C, N, P and K contents in leaves. Meanwhile, the P and K contents of RCC 71 were the lowest, although the P content was not different from RCC 70 (Table 8).

Nitrogen content in the leaves varied widely from low (2%) to high (5%) of plant dry weight. Optimum N requirements differed widely between plant species. In general, the plant N content is highest in the early growth stage, then decreased as the plant ages (Yousaf et al., 2021). In general, P content in leaves ranges from 0.2% to 0.5% of dry weight. Meanwhile, P content in young plants ranges from 0.5% to 1%, decreasing with age. The P absorption increases at pod formation, then decreases.

Calcium content in plant leaves ranges from 0.5% to 3% of dry weight depending on plant species. In some species, Ca content is relatively very low (about 0.08%) almost the same as micronutrient. The Mg content in leaves only ranges from 0.3% to 0.5% of dry weight, while S content in leaves ranges from 0.15% to 0.5% of dry weight (Xie et al., 2021). Leaf Ca content between clones in each method of rejuvenation were not different, but Mg content was significantly different. It can be seen that the contents of Ca and Mg in ring budding rejuvenation did not differ between clones.

Macronutrient content of three cocoa clones in each rejuvenation method had a similar trend. Based on macronutrient content of cocoa root tissue, KKM 22 had the highest nutrient contents compared to RCC 71, while RCC 70 had the lowest nutrient contents. The distribution of nutrients in stem and leaf tissue also aligns with nutrient content in the roots. Li et al. (2015) and Cuenca-Cuenca et al. (2019) stated that absorption and translocation of macronutrients were affected by cocoa clones. The absorption of Fe and Mn in Amelonado cocoa clones, which are Amazon forastero cocoa species, was lowest compared to those of EET-400 and ICS 9. Meanwhile, ICS 95 clones, belonging to Trinitario cocoa type, had the largest nutrient uptake, and ETT-400 had average ability to absorb nutrients. In this research, KKM 22 clone was Trinitario type and had highest organic-C, N, P, K, Ca, and Mg contents, while RCC 70 and RCC 71 were Forastero type. De Oliveira et al. (2019) showed that the macronutrient contents of K, Ca, Mg, and S, as well as the micronutrient contents of Fe, and Mn were different between CCN51 and PS1319 clones. Dogbatse et al. (2020) also stated that highest N content were found in crosses with SPD clone, while the highest P and K contents were in PA 150 x Ex 3338 grown on Nzima soil.

Yield component

Table 9 shows that based on T-test, the total pods per stand (per year), dry weight bean (kg per stand per year), and productivity (ton per ha per year) were affected by the rejuvenation method. Meanwhile, the rejuvenation method did not affect 100 bean weight (g), dry weight bean per pod (g), and the bean number per pod. In ring budding, the number of pods was higher than that of side grafting, which was 46 stands year^{-1}, although seed weight and bean number per pod did not differ. The total pods per stand will control cocoa productivity, in the case of the dry weight bean per stand and per hectare. Cocoa stand rejuvenated with ring budding has higher productivity than side grafting, 1.11 kg stand^{-1} year^{-1} (1.12 ton ha^{-1} year^{-1}) and 0.55 kg stand^{-1} year^{-1} (0.55 ton ha^{-1} year^{-1}) respectively.

Table 10 demonstrates that the number of pods per stand of three cocoa clones was relatively similar, especially cocoa stands rejuvenated with in ring budding, ranging from 48 to 55 pods. In cocoa stands rejuvenated with side grafting, total of pods per stand of KKM 22 was relatively high (40 pods), while those of RCC 70 and RCC 71 were lower, 20 and 24 pods, respectively.

Table 8. Organic-C, N, P, K, Ca, and Mg contents of leaves of three cocoa clones in ring budding and side grafting rejuvenation

Clone	Macronutrient contents of cocoa leaf tissue			
	Organic-C (%)		Total N (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	42.38^{a}	40.42^{a}	1.96^{a}	1.75^{a}
RCC 70	39.65^{b}	40.56^{a}	1.72^{b}	1.60^{b}
RCC 71	42.83^{a}	43.37^{a}	1.92^{a}	1.69^{a}
	Total P (%)		Total K (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	0.13^{a}	0.11^{a}	1.69^{b}	1.49^{a}
RCC 70	0.09^{b}	0.09^{b}	0.58^{c}	1.18^{b}
RCC 71	0.11^{b}	0.09^{b}	1.92^{a}	0.84^{c}
	Total Ca (%)		Total Mg (%)	
	Rejuvenation techniques		Rejuvenation techniques	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	1.15^{b}	1.21^{b}	0.50^{b}	0.60^{b}
RCC 70	1.36^{a}	1.36^{a}	0.66^{a}	0.51^{c}
RCC 71	1.02^{b}	1.18^{b}	0.48^{b}	0.66^{a}

Note: Means in a column followed by a different letter indicating a significant difference based on LSD test at α 5%

Table 9. Comparison of cocoa production variables by ring budding and side grafting rejuvenation

Observation variable	Ring budding	Side grafting	P-value (0.05)	Note
Total pods per stand (year^{-1})	46.00±20.61	28.00±9.33	0.05	*
100 bean weight (g)	90.39±15.67	87.75±6.70	0.63	ns
Dry weight bean per pod (g)	25.56±7.18	26.43±2.46	0.75	ns
Bean number per pod	48.69±8.75	35.40±10.64	0.07	ns
Dry-weight bean (kg stand^{-1} year^{-1})	1.11±0.40	0.72±0.24	0.04	*
Productivity (ton ha^{-1} year^{-1})	1.12±0.40	0.72±0.24	0.04	*

Note: * = significantly different; ns = not significantly different based on t-test (α = 0.05)

This is in line with the research of Anita-sari and Susilo (2013), which concluded that the results of analysis of variance of 21 clones combined with the data on the characters of flowering, number of pod, and shoots showed that genotype factors had a significant effect on flowering, number of pod, and shoots in each genotype.

Cocoa stands rejuvenated with side grafting had a similar weighing of 100 beans and weight of each seed in three clones tested. Meanwhile, in cocoa stands rejuvenated with ring budding, the weight of RCC 70 clone 100 beans and dry weight bean per pod were higher when compared to those of KKM 22 and RCC 71. Meanwhile, the number of beans per pod of three clones on ring budding and side grafting was relatively similar, namely 31 to 53 beans. This illustrates that KKM 22 has higher total number of pods but medium-sized bean, while RCC 70 has a lower total number of pods, but big size of bean. RCC 71 has a low number of total pods with a small size of bean.

The total pods per stand and beans weight affected the total productivity of cocoa stands. The productivity of stands rejuvenated with ring budding was higher than side grafting. In ring budding, the bean productivity of three clones tested was relatively similar. In contrast, cocoa stands rejuvenated with side grafting had different trends with ring budding in case of productivity. In side grafting, the bean productivity of KKM 22 was relatively high (1.03 kg stand^{-1} year^{-1} or 1.03 ton ha^{-1} year^{-1}), while those of RCC 70 and RCC 71 were relatively low, ranging from 0.54 to 0.62 kg stand^{-1} year^{-1} or 0.54 to 0.62 ton ha^{-1} year^{-1}. Productivity of cocoa stands rejuvenated with side grafting was quite low compared to potential productivity, especially for RCC 70 and RCC 71.

The nutrient content and productivity of RCC 70 were the lowest, compared to cocoa clones of RCC 71 and KKM 22. On the other hand, KKM 22 had the highest nutrient content and productivity. In addition to plant nutrient content, genetic potential and cultivation management play an important role in cocoa yields (Puentes Paramo et al., 2016). Cuenca-Cuenca et al. (2019) also reported that ICS 95 had the lowest nutrient translocation for N, P, K, Mg, and Cu, while Amelonado had the lowest nutrient translocation for Ca, Fe, and Mn (Li et al., 2015). Meanwhile, CCN-51 could absorb and use nutrients more efficiently, as indicated by optimal yields compared to ETT-575, ETT-575, and ETT-103. The ability of nutrient absorption and distribution from grafted plants is affected by genetics and rootstock ability (Gonçalves et al., 2019). Ruseani et al. (2022) mentioned that the significant clone effects in P, K and Mg uptake suggests a genotypic variability in macronutrient uptake by cocoa seedlings. Rasool et al. (2020) stated that rootstock has an effect on the contents

Table 10. Yield components of three cocoa clones on ring budding and side grafting rejuvenation

Clone	Yield components			
	Total pods per stand (year^{-1})		100 bean weight (g)	
	Rejuvenation technique		Rejuvenation technique	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	48.00[a]	40.00[a]	88.91[b]	88.83[a]
RCC 70	36.00[a]	20.00[b]	106.84[a]	91.21[a]
RCC 71	54.00[a]	24.00[b]	75.43[c]	83.21[a]
	Dry weight bean per pod (g)		Bean number per pod	
	Rejuvenation technique		Rejuvenation technique	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	25.02[b]	25.97[a]	46.22[a]	46.78[a]
RCC 70	33.91[a]	27.97[a]	52.36[a]	28.23[a]
RCC 71	17.75[c]	25.35[a]	47.51[a]	31.19[a]
	Dry-weight bean (kg stand^{-1} year^{-1})		Productivity (ton ha^{-1} year^{-1})	
	Rejuvenation technique		Rejuvenation technique	
	Ring budding	Side grafting	Ring budding	Side grafting
KKM 22	1.17[a]	1.00[a]	1.16[a]	1.03[a]
RCC 70	1.20[a]	0.54[b]	1.23[a]	0.54[b]
RCC 71	0.96[a]	0.61[b]	0.95[a]	0.61[b]

Note: Means in a column followed by a different letter indicating a significant difference based on LSD test at α5%

of element in leaves and scions of apple plants. Rootstock M.9 clone was more effective in absorbing N, Mn, and Fe, while rootstock MM.106 has a high potential to absorb P. Amiri et al. (2014) reported that rootstock is responsible for inducing drought tolerance in scion by regulating transcription of cell wall-related genes, resistance to biotic and abiotic stresses, antioxidant and carbohydrate-soluble systems, ABA signaling pathways, and at same time by down regulating activity of genes involved in light reactions, metabolic processes, and ethylene biosynthesis.

Based on the Figure 1, there is a correlation between the macronutrient levels in the leaves of the cocoa plant and yield components of cocoa. The content of organic-C was positively correlated with total Ca and total Mg contents, but it was insignificant. Meanwhile, the total K content was significantly positively correlated with the N and P contents, with the values of 0.88 and 0.83, respectively. These findings show that the higher contents of N and P is in line with the increasing K content. On the other hand, leaf Mg content was significantly and negatively correlated with N, P, and K contents. According to Dewi Hs et al. (2020), the B content in leaves was significantly and positively correlated with photosynthetic components, but the N, P, and K levels in leaves did not show a significant correlation. Li et al. (2015) also reported that the concentrations of K had significant and negative correlations with shoot concentrations of Ca, Mg, P, B, and Mn. The uptake of P, Ca, Mg, Fe, and Mn decreased and uptake of N, K, Cu and Zn increased with increasing soil K levels.

Leaf macronutrient contents also correlate with yield components of cocoa (Figure 1). The organic-C content was correlated with total pods per stand, dry weight beans per stand, and productivity, with values of 0.49, 0.56, and 0.56, respectively, but this correlation was insignificant. Meanwhile, cocoa productivity was significantly correlated with total pods per stand and total beans per stand with values 0.75 and 0.85. The organic-C and total N contents indicate the type of organic matter present in the soil and, in particular, the degree of humification. Snoeck and Dubos (2018) reported a positive correlation between the organic matter content and the C:N ratio of the same horizon on the one hand, and the cocoa yield on the other. This demonstrates

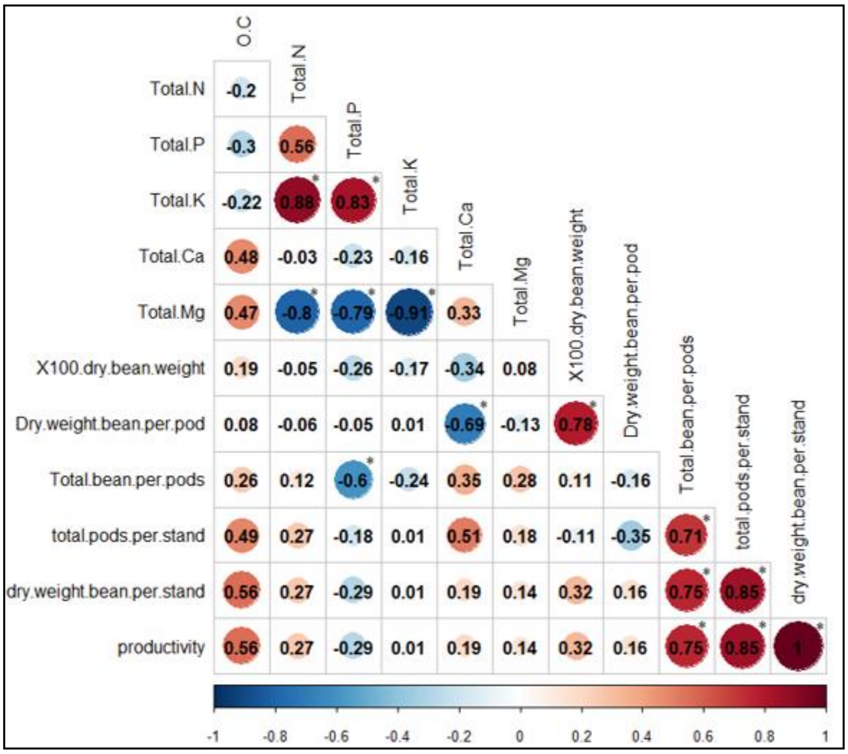

Figure 1. Correlogram of the relationship between macronutrient content of leaf tissue and yield component in two rejuvenation methods and three different clones (Note: red color indicates a positive correlation, while blue color indicates a negative correlation; O.C = organic-C; X100.dry.bean.weight = 100 dry bean weight; sign * = significantly different at 5%)

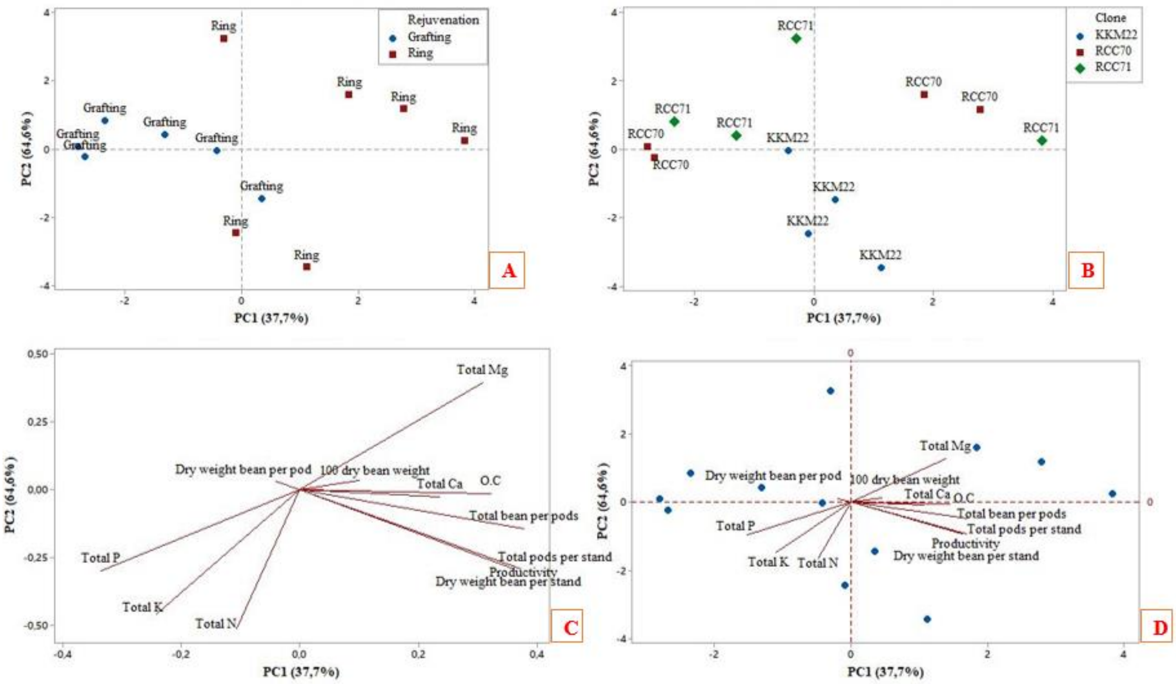

Figure 2. Principal component analysis (PCA) on the crossing combinations of leaf macronutrient content, yield components, and yields of cocoa clones of KKM 22, RCC 70 and RCC 71 rejuvenated with ring budding and side grafting; (A) Score plot of rejuvenation techniques based on leaf macronutrient content, yield components, and yields of cocoa; (B) Score plot of cocoa clones based on leaf macronutrient content, yield components and yields of cocoa; (C) Loading plot of PC1 and PC2; (D) Biplot analysis of leaf macronutrient content, yield components, and yields of cocoa based on rejuvenation techniques and cocoa clones; (Note: O.C = organic-C).

that the C:N ratio of soil can be a useful indicator of soil fertility in cocoa production.

The principal component analyses (PCA) analyses can also give us a good information about the relationship between variables (Mishra et al., 2017). Therefore, in order to study the relationship of the chosen leaf macronutrient content, yield component variables, and the cacao yield, a biplot chart of PC1 and PC2 was used (Figure 2). The results of the PCA biplot show that the total diversity among the leaf macronutrient content, yield components, and yields of cocoa was 64.6%. Leaf macronutrients content i.e., total Mg, total Ca, and organic-C had a close position to the yield component i.e., 100 dry bean weight in quadrant I. Meanwhile, total P, total K, and total N were close to each other in quadrant III. Cocoa yield and yield components i.e., total beans per pod, total pods per stand, dry weight beans per stand, and productivity were close together in the same quadrant IV, while dry weigh beans per pod stand were alone in quadrant II. This shows that the yield components affecting productivity were total beans per pod, pods per stand, and dry-weight beans per stand.

Based on the score plot and biplot analysis (Figure 2), the leaf macronutrient contents such as total N, total Mg, total Ca, and organic-C of ring budding were higher than side grafting, whereas total P and total K were lower. This is in line with the yield and yield components of cocoa in ring budding such as total pods per stand, dry weight beans per stand, and the productivity of ring budding were higher than those in side grafting (Meilani, 2021). Meanwhile, when viewed based on cocoa clones, the KKM 22 had the highest contents of total N, total P, and total K compared to those of RCC 70 and RCC 71. On the other hand, the levels of total Mg, total Ca, and organic-C were lower.

CONCLUSIONS

Cocoa stands rejuvenated with ring budding have higher content of N, stem organic-C, total pods number per stand per year, dry weight bean per stand per year, and dry weight bean per hectare per year in leaves compared to those of side grafting. The macronutrient trend is leaves > stem > root. Macronutrient contents of three cocoa clones in each rejuvenation method have similar trends. The trend of macronutrients in cocoa is organic-C > N > Ca > K > Mg > P. Based on macronutrient content of cocoa tissue, KKM 22 has higher contents of organic-C, N, P, K, Ca, and Mg compared to RCC 71 and RCC 70. Nutrient distribution in stem and leaf tissues is in line with nutrient content in roots. The ring budding technique is effective in rejuvenation program for old cocoa stand based on nutrient contents, yield components, and yields indicators of three cocoa clones.

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