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Anatomical responses of roots and yield of cocoa (*Theobroma cacao* **L.) to K fertilization doses**

Muhammad Syaiful Shodiq^{1*}, Eka Tarwaca Susila Putra², and Siti Nurul Rofiqo Irwan²

¹Department of Agronomy, Faculty of Agriculture, Universitas Lambung Mangkurat Jln. Jend. A. Yani KM 36, Kota Banjarbaru, Kalimantan Selatan 70714, Indonesia ²Departement of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada Jl. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281, Indonesia *Corresponding author: syaifulshodiq@ulm.ac.id

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Article Info Abstract

Cocoa is one of important commodities for the economy of Indonesia. However, national exports of the commodity has declined due to decreasing of domestic cocoa production. For enhancing cocoa production, potassium fertilization is required. Potassium (K) is an essential macronutrient in the physiology, anatomy, and yields processes. Anatomically, K is needed in the elongation of meristem tissue as well as for increasing the yields of fruit and seed. The objectives of this research included the study of the effect of K fertilizer dose and determination of optimum K doses on root anatomy development as well as the yield of cocoa pods. This research was conducted on the cocoa plantation of PT. Pagilaran. Applied doses of K fertilization consisted of 0, 150, 300, 450, and 600 g KCl/plant in Randomized Complete Block Design (RCBD) with 5 replications. Observed variables consisted of root anatomy and yield of cocoa pods. The results indicated that the dose of K fertilization significantly influenced both stele and xylem diameters of cocoa roots but insignificantly effected the thickness of cork tissue, phloem diameter, and the thickness of cambium tissue. Moreover, the dose also showed significant effect on the number of beans per pod, the weight of 100 beans, as well as the fresh and dry weight of beans per plant. It was also revealed that the dose showed no significant effect on length, diameter, and weight of the cocoa pods. It implied that K fertilization dose had effect on root anatomical properties and yield of cacao.

INTRODUCTION

Located in the equatorial region with a tropical climate and promoted as ideal land for cacao plantations, Indonesia is one of the largest cocoa producers in the world. In 2019, the Food and Agriculture Organization (FAO) released the ranks of the world's largest cocoa producers. Ivory Coast was in the first place with 2,235,043 tons (39.8%) of cocoa production, Ghana in second place (811,700 tons or 14.45%), Indonesia in the third place (774,195 tons or13.78%) and consecutively followed with Nigeria (348,448 tons or 6.2%), Ecuador (283,680

tons or 5.05%), Cameroon (280,000 tons or 4.98%), Brazil (259,451 tons or 4.6%), Peru (141,775 tons or 2.52%), Colombia (102,154 tons or 1.81%), and the Dominican Republic (76,113 tons or 1.35%). As the third-largest cocoa producer in the world after Ivory Coast and Ghana, the production volume in Indonesia reached 774.000 tons. The center of cocoa production of Indonesia is located on the island of Sulawesi, specifically the provinces of Sulawesi Tengah, Sulawesi Barat, Sulawesi Selatan, and Sulawesi Tenggara.

Since 1930, cocoa (*Theobroma cacao* L.) has been one of the plantation commodities with strategic role in the economy of Indonesia. It also indicates its

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crucial role as source of employment and income for the farmers. According to Mastuti and Alfiansyah (2016), cocoa is a commodity with significant role in the national economy. It encourages regional development and agro-industry development. However, the exports volume has been decreasing due to the declining domestic cocoa production and export restrictions. Data by the Ministry of Agriculture (2017) indicated that the average national productivity of cocoa plantations was 982 kg/ha with total production of 688,345 tons in 2017. Thus, cocoa productivity in Indonesia was still considered as quite low compared to its potential, which was >2 tons of dry beans/ha.

Some of the problems causing such low productivity included: (a) the use of random seeds instead of widely used clonal seeds, (b) lack of knowledge and skills of cultivation techniques and product processing, (c) most of the plantations consisting of smallholder estate with traditional management, and (d) widely used old cocoa plants (over 25 years old), exceedingly far above the most productive age of 13−19 years (Wahyudi and Raharjo, 2008). The problem of low productivity can be coped with fertilization as an effort to provide nutrients for soils having insufficiency for cultivated plants. Maximum results from fertilization are achievable when done correctly. This includes the correct fertilization dose, type, timing, and methods (Indonesia Coffee and Cocoa Research Center, 2004).

It is commonly known that K plays important role in stomata opening and transport, increasing the water potential as well as the ability to absorb water from the soil, in addition to uptaking water by the roots and regulating the water movement from root cells to xylem tissues (Jasmi, 2016). Moreover, K essentially affects the elongation rate of cocoa stems, especially in actively dividing tissues on the tips of the plants (meristem tissue).

Another important role of K includes improving the quantity and quality of cocoa production which is done by using K fertilizer to influence the arrangement and translocation of carbohydrates in the plant body, to accelerate nitrogen metabolism and to prevent flowers and fruit from fallen (Wibowo, 2015).

Based on source-sink physiology approach, the contribution of K to the formation of sinks (fruits and seeds) is inseparable from the ability of roots to absorb K and to translocate it to leaves (source). In addition, K plays important role in stimulating the root growth of cocoa plants. Optimum root growth will support the supply of nutrients into plant tissue that it will also support the plant production. Jasmi (2016) states that K plays a role in the uptake of water by the plant roots. Increasing application of K fertilizer will enhance the water potential of the leaves, thus enhancing the ability of plants to extract water through the roots. The extraction of water and nutrients as well as the transport by roots depend on the size, arrangement, and changes in shape of cells and tissues in roots. In order to determine the effect of K on the anatomical characteristics of the cocoa root and its relation to production, anatomical studies are required.

MATERIALS AND METHODS

This research was conducted at the cocoa plantation of PT. Pagilaran at the North Segayung Production Unit, Tulis Subdistrict, Batang Regency, the Province of Jawa Tengah, in September 2020 – May 2021. Randomized Complete Block Design (RCBD) was applied with a single factor and 5 replications consisting of 3 samples in each replication. The RCC71 clones of \pm 20 years of age were selected as research objects because of their superior clones and high production. The single factor tested was 5 levels of K fertilization dose: 0 g KCl/plant, 150 g KCl/plant, 300 g KCl/plant, 450 g KCl/plant, and 600 g KCl/plant. The type of K fertilizer used was muriate of potash /potassium chloride (KCl) fertilizer with 60% of K content. The dose was based on the standard published by the Indonesian Coffee and Cocoa Research Institute (ICCRI), which has become the reference standard for fertilization in cocoa. The K Fertilization treatment was carried out early in the experiment, in September 2020. The fertilization method included the placement under the soil at 10 cm depth. Plant maintenance activities included pruning, fertilizing, and controlling plant pest organisms.

The materials used in this study consisted of analytical weight at 0.05 g accuracy, digital caliper, cutter, razor or scalpel, ruler, stationery, microscope, OptiLab, digital microscope, coolbox, transparent plastic, labels, plastic preparat, and styrofoam. In addition, the RCC71 clones aged ±20 years in the North Segayung Production Unit, urea fertilizer, SP-36 fertilizer, KCl fertilizer, herbicides, and pesticides were also used.

The observed variables comprised of two aspects: the anatomical character of roots and the yield of cocoa pods. Observations of root anatomical characters

were done to thickness of cork tissue, diameters of stele, xylem, and phloem, as well as the cambium thickness. Meanwhile, cocoa fruit yields were observed by their pod length, pod diameter, pod weight, number of beans per pod, the weight of 100 beans, the fresh weight of beans, and the dry weight of beans per plant.

The root anatomy sampling to all of the plants was carried on 14 weeks after K fertilization treatment by using soil drill. This was done by making 50 cm depth of soil drilling at 12 points on each plant to have representation of the cocoa roots growth. The next step was preparing anatomical preparats which was destructively done by making vertical and transverse incisions using the paraffin embedding method according to Maiti et al., (2012). The anatomical preparats were then counted and observed by using the Optilab Viewer at 10x and 40x magnifications.

The cocoa pods observation was done by harvesting the fruit once in 42 weeks after fertilization treatment. The ripe cocoa fruit was harvested manually. Sign of ripe cocoa is when red pods change color from red to orange. Observations on harvested fruit included pod length, diameter and weight, as well as the number of beans per pod, the weight of 100 beans, the fresh weight of beans, and the weight of dry beans per plant. The data of root anatomical properties and the cocoa yield was displayed in graph and analysis of variance (ANOVA) at a 95% confidence level. If the ANOVA results showed significant difference between treatments, the data was then tested using a polynomial (regression) test at 95% confidence level in order to determine optimal K dose for the development of the anatomical properties of cocoa roots and yields. Then, SAS program version 6.12 was applied for data analysis.

RESULTS AND DISCUSSION

Root anatomical properties

As the main vegetative organs, roots supply water, minerals, and other essential materials for the growth and development of the plant. Root growth is regulated by a combination of cell enlargement and production. Cell enlargement is generally more sensitive to water and nutrient stress than cell production or division. The inhibition of cell elongation or enlargement activity impacts differences in plant growth (Miyamoto et al., 2005). Sufficient nutrients for cocoa plants are

expected to contribute to optimal activity of cell elongation and division. Moreover, optimal elongation and division of cells and tissues ensure the plants to perform maximum metabolic activities (respiration, transpiration, photosynthesis) and stimulate good plant growth which contributes to optimal fruit or bean yield in cocoa.

Cork tissue or periderm is a protective tissue formed secondary, replacing the epidermis of stems and roots thickened due to secondary growth. Cork tissue is visible in *Dicotyledonae* and *Gymnosperm*. Cocoa (*Theobroma cacao* L.) is classified as a *Dicotyledonae* (two-piece-seed). Its structure consists of phellogen (cork cambium) which will form phellem (cork) to the outside and phelloderm to the inside. Depending on the plant species, phellogen is produced by the epidermis, parenchyma under the epidermis, collenchyma, pericycle, or phloem parenchyma. In transverse incisions, phellogen cells are rectangular or polygonal and meristematic.

Table 1 shows the effect of K fertilization dose on the cork tissue thickness in cocoa roots. The dose showed no significant effect on the cork tissue thickness. Increasing the K fertilization dose from 0 g KCl/plant to 600 g KCl/plant did not significantly affect the cork tissue thickness either because it is located in outer layer of roots that formed itself continuously to protect the epidermis layer.

Application of K to plants can stimulate the activity of secondary meristem cambium contributing to root development. This causes the epidermis layer which is the only layer that is easily broken and eventually peeled off. The protective role carried out by the epidermis is then replaced by cork tissue that is formed from the cork cambium located on the outer side of the root cortex. Cork tissue is found on the edge of plant organs and composed of cork parenchyma cells. It also contains suberin and cutin which are stronger than the epidermis. It is also a tissue consisting of cork cells that functions as protection for the underlying tissue to prevent too much water loose (Bot and Benites, 2005).

The stele consists of a pericycle, vascular bundle, and pith. The vascular bundles are arranged in a circle, and each bundle consists of xylem, cambium, and phloem. Dicot roots are different from monocot roots because they have an epidermis, cortex, and stele. Figure 2 shows that the K fertilization dose significantly (p-value \leq 0.05) affects the stele diameter of the cacao root. Increasing the K fertilization dose from

Figure 1. Cross section (10 x 10) of cacao roots in various K fertilization dose

Remarks: The means followed by the same letters in one column indicate no significant difference based on analysis of variance (ANOVA) at 95% confidence levell.

0 g KCl/plant to 600 g KCl/plant significantly affects the stele diameter. The K fertilization dose on cocoa affected the stele diameter of the root by 82.27% $(R² = 0.8227)$ in a positive linear curve with the equation $y = 0.1569x + 420.43$. Higher K fertilization dose applied to cocoa results in a larger stele diameter. The stele diameter continued to increase until the K fertilization dose reached 600 g KCl/plant. Distribution of K at a dose of 150 g KCl/plant was sufficient to increase the stele diameter larger than the control (0 g KCl/plant). In this study, the stele diameter continued to increase in direct proportion to the increasing K fertilization dose up to 600 g KCl/plant. Such increase of stele diameter helps to improve the plants ability to absorb water and nutrients carried to the leaves for their metabolism.

After absorbed into the root hairs and epidermis, K⁺ is transported to the root stele tissue and then translocated from the roots to the shoots via the xylem vessels, which function as a long-distance K⁺ translocation highway. The K⁺ contributes to the expression of the AtSKOR transporter gene in the root stele. It is suspected that the increase of stele diameter is related to the expression of the Shaker K⁺ AtSKOR transporter involved in this process. AtSKOR is expressed in stele tissue and responsible for releasing K⁺ into the xylem sap (Gaymard et al., 1998 in Wang et al., 2021).

Figure 2. Regression result of the effect of K fertilization dose (g KCl/plant) on stele diameter (μm)

Figure 3. Cross section (10 x 10) of stele affected by various K fertilization dose

Sinaga (2007) revealed that the stele diameter is related to the ability of plants to absorb water and nutrients transported to the leaves and are needed by plants for their metabolism. The stele consists of ground and vascular tissue, including xylem and phloem. The stele in the cacao root is separated from the nearest part of the root cortex. Moreover, the stele has a peripheral area with plenty of vascular tissue, especially phloem, the primary key in transporting assimilation throughout the plant body (Corley and Tinker, 2010). Such increase of stele diameter helps to improve the plant ability to absorb water and nutrients transported to the leaves for metabolism, considering that there are xylem vessels in the stele. In addition, it is also linearly related to the xylem diameter, meaning that an increasing stele diameter will be followed by increasing size of the xylem.

Figure 4 shows that the K fertilization dose (p-value ≤ 0.05) significantly affected the xylem diameter of cocoa roots. The application of K fertilization dose from 0 g KCl/plant to 600 g KCl/plant significantly affected the xylem diameter of cocoa roots by 50.61% (R^2 = 0.5061) quadratic. Higher application of K fertilization dose up to 476.25 g KCl/plant increased the xylem diameter of the cocoa roots. However, when the K fertilization dose >476.25 g KCl/plant was applied, the xylem diameter of the roots decreased, but not lower than the control treatment of 0 g KCl/plant. Based on the regression equation obtained $y = -4E-05x^2 + 0.0381x + 34,277$, the optimum K fertilization dose was reached at 476.25 g KCl/plant, resulting in the highest root xylem diameter of 43.35 μm.

Figure 4. Regression result of the effect of K fertilization dose (g KCl/plant) on xylem diameter (μm)

Figure 5. Cross section (10 x 10) of xylem affected by various K fertilization dose

K also affects the size of xylem diameter in the roots. Figure 4 shows increasing diameter of xylem of the roots at K fertilization dose of 150 g KCl/plant, which was higher than the control treatment (0 g KCl/plant). The size of xylem diameter continued to increase until the K fertilization dose reached 476.25 g KCl/plant. Theoretically, K can neutralize reactions in cells, especially from organic acids, and promote the growth of meristem tissue and cell membranes. Furthermore, elongation and enlargement of root xylem vessels indicate root elongation providing more vital ability to exploit water resources (Campbell et al., 2003).

Moreover, K plays significant role in transporting water and plant nutrients through the xylem. When its availability decreases, the transport of nitrate,

phosphate, calcium, magnesium, and amino acids will also decrease (Schwartzkopf, 1972). In phloem tissue, K also regulates transport in xylem tissue. Adequacy of K is essential to maintain efficient transport system (Thomas and Thomas, 2009).

Table 2 shows the effect of the K fertilization dose on the phloem diameter of the cocoa roots. The further test results in the above-mentioned table showed that K fertilization did not significantly affect the phloem diameter of the cocoa roots. Increasing the dose of K fertilizer from 0 g KCl/plant to 600 g KCl/plant did not significantly affect the phloem diameter of cocoa roots. The increase in K fertilization dose from 0–600 g KCl/plant did not result in changing size of the phloem diameter.

Remarks: The means followed by the same letters in one column indicate no significant difference based on analysis of variance (ANOVA) at 95% confidence level.

After leaving the xylem, K is transported to different sinks through the phloem, such as by developing wood tissues, leaves and roots, and raw fruits. The K fertilization did not significantly affect the phloem diameter of cocoa roots. It is suspected that calcium nutrients influence the elongation and differentiation of phloem. Fromm (2010) suggested that in the formation of phloem, calcium (Ca) is more important than potassium (K). K accumulates more and becomes an enzyme activator in the xylem, while in the phloem, Ca ions in the form of calcium oxalate ($CaC₂O₄$) accumulate more than K ions.

The K fertilization dose did not significantly affect the thickness of the cambium in cacao roots (Table 3). Increasing the dose from 0 g KCl/plant to 600 g KCl/plant did not have significant affect to the cambium thickness in the cocoa roots.

The cambium is a layer of meristematic tissue in plants whose cells actively divide and play a role in plant secondary growth. Cambium is found in the stems and roots of *Dicotyledonae* and *Gymnosperms*, including cocoa. Based on the fixed tissue that is formed, there are two groups of cambiums: the cork cambium (phellogen) and the vascular cambium. The cork cambium is part of the cortex. Its outwards activity produces cork tissue (phellem or cork). It Its functions include regulating water entrance and exits, preventing pest attacks, and several other mechanical functions. Meanwhile, the towards inside activity include the cork cambium in some plant species that produces a layer of cork skin called the phelloderm. The cambium observed in the anatomical character of cocoa roots was vascular (intravascular) cambium which is the cambium contained in the transport

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bundle (between the phloem and xylem). Its function in cell division activity is secondary growth of plants in the formation of secondary xylem and secondary phloem. This activity generates secondary growth to enable enlargement of plant stems (in *Dicotyledonae* and *Gymnosperms*).

The vascular cambium thickness in cocoa roots was not significantly affected by the K fertilization dose. Increasing the dose did not significantly affect the thickness of the cocoa root cambium. The high differentiation activity of the vascular cambium outward and inward is the suspected cause. The activity of the vascular cambium towards the outside will form secondary phloem, while the activity of the vascular cambium towards the inside will form secondary xylem. K regulates the water movement from root cells to xylem tissue. The K⁺ elements are initially accumulated in the cytoplasm and vacuoles of root parenchyma cells moving into the xylem vessels through plasmodesmata (Jasmi, 2016). Sufficient K available in plants stimulates water movement from root cells to the xylem. Increasing K will lead to water accumulated in the primary xylem to also increases and stimulates the formation of secondary xylem originating from differentiated vascular cambium towards the inside. There was no significant effect of K fertilization dose on cambium thickness.

Cocoa yield

The effect of K fertilization dose as shown in Table 4 and Table 5 indicates no significant effect on cocoa pod length and diameter. Cocoa pods showed the same length and diameter even after the dose was increased to 600 g KCl/plant.

Cocoa pods are ripe and ready for harvest after 5–6 months of age (Prawoto and Winarsih, 2010). Many factors are considered in testing the quality of harvested fruit. Fruit size is one of the main indicators in determining fruit quality that includes fruit length, width, and diameter. Cocoa pods reach 12–22 cm long and 6–10 cm diameter with green, yellow, red, or purple colors depending on their variety (Backer and Bakhuizen van den Brink, 1963). The results showed that the length and diameter of harvested RCC71 cocoa pods ranged from 12–17 cm and 7–10 cm, respectively. There was no significant effect of K fertilization dose on pod length and diameter. Applying minerals such as proper K nutrient to the plants can play an important role in stimulating plant growth and development as well as fruit size and quality.

Table 6 shows that the K fertilization dose showed no significant effect on cocoa pod weight. Increasing

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K fertilization dose from 0 g KCl/plant to 600 g KCl/plant did not significantly affect the cocoa pod weight. Cocoa pods showed the same weight even after the K fertilization dose was increased to 600 g KCl/plant. Mulato et al., (2009) stated that the weight of cocoa pods is strongly influenced by environmental conditions during fruit development and agronomic actions on plants such as fertilization and selection of plant varieties. Appropriate K fertilization can play important role in stimulating the plant growth and development, as well as the size and quality of fruit yields. Deficiency of K can lead to reducing photosynthesis rate of plant growth and the weight of harvested fruit (Suwanti et al., 2017).

The results showed that K fertilization dose (p-value ≤ 0.05) significantly affected the number of beans per pod. Increasing the dose from 0 g KCl/plant to 600 g KCl/plant significantly affected the number of

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Table 6. The effect of K fertilization dose (g KCl

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beans per pod. In Figure 6, based on the regression equation, it was obtained that $y = 0.0174x + 33.033$, known that K fertilization dose on cocoa plants affects the number of beans per pod by 60.34% (R^2 = 0.6034) in the positive linear curve. Higher K fertilization dose applied to cacao plants resulted in higher number of beans per pod. The number of beans per pod continued to increase up to K fertilization dose of 600 g KCl/plant.

Cacao beans contain water, fat, ash, nitrogen, carbohydrates, and tannins. Beans are spherical with a size of 2.5 x 1.5 cm and arranged in five rows around the axis of the pod. The number of beans per pod is about 20–60, with 40–59% of bean fat content (Mulato, 2005). Higher K fertilization dose contributes to higher number of beans per pod. Salih et al., (2016) stated that K plays an important role in increasing the size and grain filling. It increased significantly at 90 kg/ha KCl application. On the other hand, N and P fertilization showed no significant effect. The K also plays a role in seed formation in oil crops, such as sunflower (Helianthus annuus). It stimulates the sunflower big head and increases the number of seeds (Salih et al., 2016).

The application of the K fertilization dose showed significant effect on the weight of 100 beans. The increase in K contributes to the increase in weight of 100 cocoa beans. Uddin et., al (2013) suggested that the weight of 100 seeds and rice grain yield increased significantly due to the application of K. It was reported that when other nutrients were adequate, K played an important role in increasing the yield of NERICA 1 rice. On the other hand, although other nutrients were available and sufficient, the increase in yield

Figure 6. Regression result of the effect of K fertilization dose (g KCl/plant) on number of beans per pod

Figure 7. Regression result of the effect of K fertilization dose (g KCl/plant) on the weight of 100 beans (g)

was not significant if without K nutrient.

The application of K fertilization dose from 0 g KCl/plant to 600 g KCl/plant indicated significant effect (p-value \leq 0.05) on the fresh weight of the beans. Figure 8 showed that K fertilization on cocoa plants affects the fresh weight of beans by 54.16% $(R² = 0.5416)$ in a quadratic curve. Higher K fertilization dose applied up to 464.5 g KCl/plant resulted in higher fresh weight of the beans. However, when the dose >464.5 g KCl/plant was applied, it decreased although not lower than the control treatment 0 g KCl/plant. The optimum fresh weight of beans was at the dose of 464.5 g KCl/plant which resulted in the highest fresh weight of beans of 229.6 g.

The application of K fertilization dose from 0 g KCl/plant to 600 g KCl/plant indicated a significant effect (p-value \leq 0.05) on the dry weight of beans. Figure 9 showed that K fertilization on cocoa plants affects the dry weight of beans by 71.22% (R^2 = 0.7122) in a quadratic curve. Higher K fertilization dose applied up to 364.25 g KCl/plant resulted in higher dry weight of beans. On the other hand, when the dose was applied >364.25 g KCl/plant, the dry weight of beans decreased although not lower than the control treatment of 0 g KCl/plant. The optimum dry weight of beans was at a K fertilization dose of 364.25 g KCl/plant. The optimum dose was 364.25 g KCl/plant that resulted in the highest dry weight of beans of 133.26 g.

After absorbed by root hairs, nutrients move in the xylem passively (mass flow) along with water following the transpiration flow. Immediately after entering the xylem vessels, nutrients will follow the water flow to the shoots (sinks) through the mass flow. The inner wall of the xylem has a negative charge which is able to absorb cations to enable absorption area or Cation Exchange Capacity. The K is in the form of K + cations so that the inner wall of the xylem which has a negative charge that easily absorbs K nutrients. In addition, the thickening of the root xylem vessels also indicates root elongation so that its ability becomes stronger in exploiting water resources (Campbell et al., 2003). The water availability in sufficient quantities can ensure cocoa plants to carry out optimal photosynthesis that contributes to the formation of optimal biomass, especially in this study, namely beans.

The interaction between the yield of beans and the dose of K fertilization was quadratic. Yield of beans increased in linear with the increasing dose of K

fertilization up to optimum point before decreased afterward. Increasing yield of beans certainly represented the source-sink relationship. The strategies included the more physiological processes related to the role of sinks in influencing and utilizing assimilation from sources and biosynthesis of the yield of beans. The results of photosynthesis from the main source (leaves) were partitioned into various organs or sink tissues. To obtain high productivity, the largest partition was targeted in the main sink, which was the beans. Relatively high strength of sink could be indicated by the high growth rate or bean filling. Longer bean development period to support of high supply of assimilate from sources will increase bean production (Mastur, 2015).

CONCLUSIONS

Increasing K level through KCl fertilization significantly affects the anatomical character of the roots. Application of various doses of K fertilization significantly affects anatomical characteristics of cacao roots, including both xylem and stele diameters. Increasing K level through the dose of KCl indicated significant effect on the cocoa yield component. Applying various doses of K fertilization significantly affected the number of beans per pod, the weight of 100 beans, as well as the fresh and dry weight of the beans. The K fertilization at 364.25 g KCl/plant was the optimum dose for improving root anatomical characters and increasing cocoa yield components.

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