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THE EFFECT OF STEM SEGMENT CUTTINGS OF ROBUSTA COFFEE (*Coffea canephora*) ON GROWTH OF ROOT AND LEAF SPROUT

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ABSTRACT

Robusta coffee plant propagation is recommended to be performed by vegetative methods, one of which is cuttings. Among plant organs that can be used for cuttings is the stem. The research objective was to determine which stem segment from cuttings was able to provide the best root and leaf growth. The layout study used a completely randomized block design (RBD) with an experimental treatment consisting of five stem segments, namely (1) the first stem segment from the upper shoot (A); (2) the second stem segment from the upper shoot (B); (3) the third stem segment from the upper shoot (C); (4) the fourth stem segment from the upper shoot (D); and (5) the fifth stem segment from the upper shoot (E); each treatment had five replications. Data analysis was performed statistically with analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) test with a significance level of $P < 0.05$. Robusta coffee cuttings stems from the second stem segment from the upper shoot were able to provide the optimal root growth (root length) and shoot growth (leaf sprout height and leaf sprout growth rate). Leaf sprout number derived from the cuttings was not affected by the stem segment.

Contribution/Originality: This study provides important literature for selecting the best stem segment for the propagation of Robusta coffee stem cuttings. Robusta coffee cuttings from the second stem segment from the upper shoot were able to provide optimal root length, leaf sprout height and leaf sprout growth rate.

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1. INTRODUCTION

One type of coffee widely cultivated is Robusta (*Coffea canephora*). World coffee production in the year 2018/2019 is estimated at 169 million bags, which is 5.4% higher than in 2017/2018. Production of Robusta increased by 11% in 2018/2019 to 70.67 million bags (International Coffee Organization, 2019), so Robusta coffee has an important role in improving the economy of coffee farmers. Robusta coffee is cultivated at low to moderate altitudes in tropical Africa, America, and Asia (Cubry, de Bellis, Pot, Musoli, & Leroy, 2013). The main problem with Robusta coffee cultivation is the availability of superior seeds for plant propagation, because propagation by seed will produce new plants that have different genetic properties from the parent plant. The nature of Robusta coffee is self-incompatible, so it tends to be cross-pollinated (Cubry et al., 2013; Hetzel, 2015; Moraes et al., 2018). The results of cross-pollination tend to produce seeds with different genetic characteristics from the parent plant. Therefore, Robusta coffee plant propagation is recommended to be conducted by vegetative methods (Angelo et al., 2018) and is mostly done by vegetative methods such as shoot grafting or cutting. Cuttings are a method of plant propagation that uses the vegetative part of the plant, and the new plants produced have the same properties as the parent plant. The vegetative parts of the plant used for cuttings are generally stems, leaves, and roots.

Cutting is among the extensively practiced means of vegetative propagation in plants. This has many advantages, such as ensuring maximum new crop homogeneity, besides other desirable traits: it is economical, requiring only limited space, and is rapid and simple (Dadashpour, Reza, Behrooz, & Yousef, 2011; Partelli et al., 2014). Stem cuttings in Robusta coffee plants are aimed at producing new plants as a source of superior shoots that will later be used for propagation of shoot grafting methods. New plants from stem cuttings have the same genetic characteristics as the parent plant and, in a short time, can produce large numbers and uniform characteristics in new plants. Stem cuttings will grow successfully if the cuttings form a callus, and the callus will develop into adventitious roots. Adventitious rooting growth plays an important role in the cuttings method of propagation. Adventitious root formation is a complex biological process affected by the age of the mother tree, stem juvenility, plant regulator concentration, seasonality, and environmental conditions during rooting growth (De Oliveira et al., 2015; Stuepp, Juliany, Henrique, Katia, & Ribas, 2017; Zem, Weiser, Zuffellato-Ribas, & Radomski, 2015). Thus, more juvenile branches in good nutritional condition tend to be ideal for rooting (Pijut, Woeste, & Michler, 2011; Wendling, Trueman, & Xavier, 2014).

Stem cuttings are classified based on juvenility or maturity as either softwood, semi-hardwood, or hardwood growth (Hartmann, Kester, Davies, & Geneve, 2011). Cuttings from the upper stem are softwood, and semi-hardwoods can make better roots than those from basal stems, which are hardwood. Also, the basal stem has a thicker diameter in comparison with the upper stem (Soundy, Kwena, Elsa, & Mudau, 2008). Kraiem, Aidi, Zairi, and Ezzili (2010) stated that the establishment and growth rate of stem cuttings depend on branch age, stem segment position, and stem diameter. Stem juvenility or maturity depends on branch age and stem segment position on the branch. Stem juvenility or maturity relates to endogenous hormones and nutrition in stem tissue. Upper stem cuttings have higher rates of auxin synthesis, less tissue differentiation, and are more sensitive to dehydration. Basal stem cuttings, despite lower levels of endogenous auxin, have a greater capacity to provide the necessary reserves of carbohydrate and nitrogen for the formation and growth of roots and shoots (Izadi, Shahsavar, & Mirsoleimani, 2016; Pigatto, Erik, Jéssica, Aurea, & Cícero, 2018). Those stem origins have a different C:N ratio and likelihood of growing cuttings (Pratama et al., 2018). Mauro, Nilton, Alexandre, Júnior, and Antonio (2002), reporting on plum cuttings (*Prunus salicina*), found that semi-hardwood cuttings provided the best results for rooting over the whole rooting process. Softwood, semi-hardwood, and hardwood cuttings have variable carbohydrate and nitrogen ratios along their branches, causing rooting differences in cuttings originating from different stem segments. This was supported by Araya, Du Toit, Mudau, and Soundy (2007), who showed that successful vegetative propagation of bush tea could be achieved by propagating upper stem cuttings. This is because the upper stem is softwood while semi-hardwood has low levels of lignin and high levels of auxin hormones.

In coffee plants, orthotropic branches can be used to prepare cuttings (Angelo et al., 2018) and plant types naturally have good root formation in stem cuttings; nevertheless, some types readily grow roots from the upper, middle, or basal stem (Erdiansyah, Rokhani, & Waluyo, 2016). Teruel Rezende et al. (2010) observed in *Coffea arabica* L. that the longest roots from semi-hardwood cuttings yielded the highest average, differing significantly from other stem cuttings. For total dry matter, semi-hardwood and hardwood cuttings did not present significant differences but they were superior to softwood cuttings. Research conducted by Sumirat, Yuliasmara, and Priyono (2013) on Robusta coffee cuttings showed that root growth characteristics can be grouped into three rooting categories: easy, moderate, and difficult. On the other hand, shoot growth in Robusta coffee cuttings showed easy growth from leaf sprouts. Therefore, in Robusta coffee cuttings, root growth is the critical criterion for success. Adventitious rooting of cutting is controlled by the genetics and physiological age of the stem or branch. The rooting ability of cuttings from perennial plants declines with increase in the age of the stem or branch. For Robusta coffee, there are currently no research results available on the optimal stem segment as a source of stem cuttings to provide the best root and shoot growth for plant propagation. Based on the above, the aim of the present study was to determine which stem segment would provide optimal growth in the roots and shoots of Robusta coffee cuttings.

2. MATERIALS AND METHODS

2.1. Time and Site Research

The research was conducted at laboratory plant production of the Agrotechnology Department, Faculty of Agriculture and Business, Universitas Kristen Satya Wacana (580 m above sea level) in Salatiga, Indonesia, from February to May 2018. The research location with climate classification proposed by Koppen is Am (the tropical, warmest, and short dry season), with an average annual temperature of about 23.10°C and precipitation about 2668 mm per year.

2.2. Treatment Research

The stem material for experimental treatment was taken from orthotropic branches aged 5–6 months, both softwood and semi-hardwood. The Robusta coffee clone used was BP358. The research used a Randomized Completely Block Design (RCBD) with the experimental treatment consisting of five stem segments: (1) first stem segment from the upper shoot (A); (2) second stem segment from the upper shoot (B); (3) third stem segment from the upper shoot (C); (4) fourth stem segment from the upper shoot (D); and (5) fifth stem segment from the upper shoot (E) (see Figure 1), and each treatment had five replications.

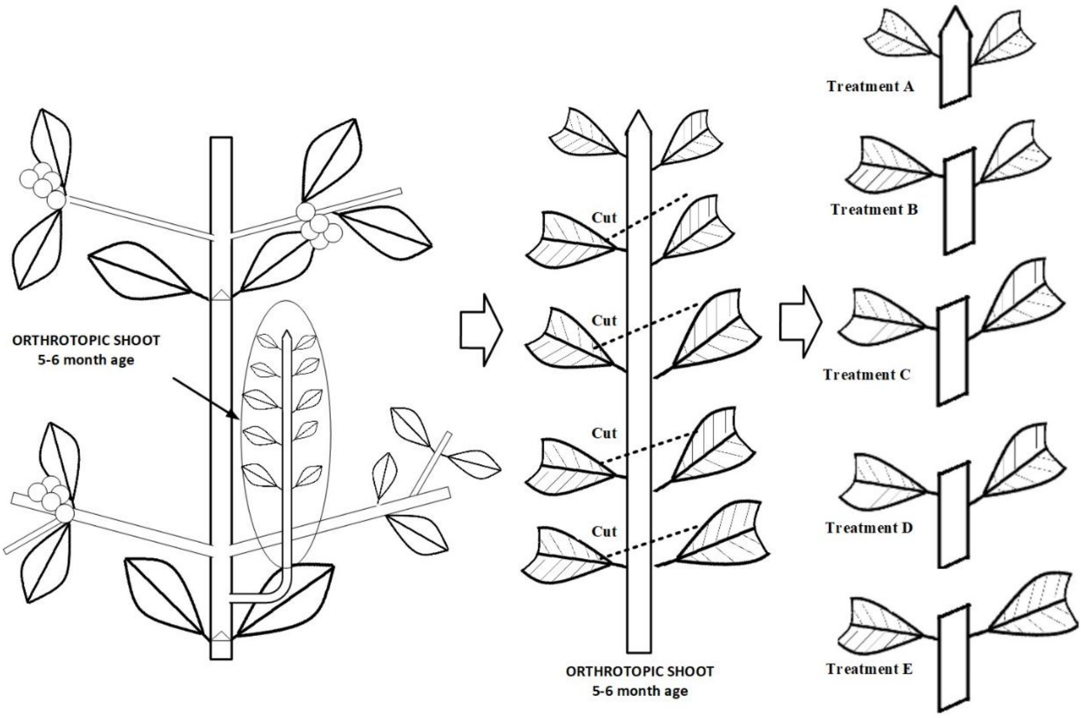

Figure 1. Stem segment treatment from orthotropic branches.
Source: Drawing adapted from Cannell (1985).

Stem cuttings were about 7–10 cm long and accompanied by a pair of leaves cut in two. Cutting was done at an angle of about 45°, then cuttings were planted in growing medium. Vertical planting of stem cuttings was done in sterile growing medium with a particle size of 5-mesh and consisting of a mixture of topsoil, sand, and compost in a ratio of 1:1:1.

The cuttings for each treatment were planted in pots (polybags) and the pots placed in a shading house. During the growth process, the microclimatic conditions in the shading house were adjusted to humidity 90–96% by providing misted water; air temperature was 23–26°C, provided by by a fan; and the level of sun intensity entering the shading house was around 40%, by providing black shading 1.0 m away from the roof of the shading house.

2.3. Data Collection

Evaluation of root and leaf sprout growth was made every day till the end of the experiment, 90 days after planting. The following variables were evaluated: (1) total organic C (C-ot) in cuttings was measured early in the experiment, with $K_2Cr_2O_7$ oxidation under acidic conditions, by spectrophotometry (Walkley and Black method). (2) Total N (N-t) in cuttings was measured early in the experiment, by wet ashing with H_2SO_4 and measurement by distillation (Kjeldahl method). (3) Root length was measured with a ruler (in cm) every day, from the root base to the root apex. (4) Leaf sprout numbers on each cutting were counted every day. (5) Leaf sprout height was measured with a ruler (in cm) every day – from base to apex. (6) Leaf sprout growth rate (cm per week) was calculated based on the absolute growth rate (AGR) formula of plants, as follows: $V = \frac{dS}{dT}$

V = leaf sprout growth rate (cm per week).
dS = leaf sprout height at the last week of measurement – leaf sprout height at the first week of measurement (cm).
dT = observation week length – 1 (weeks).

2.4. Data Analysis

The data were analyzed by analysis of variance (ANOVA) with the F-test at a significance level of at least $P < 0.05$, and the mean value of data comparison was done by Tukey's HSD test with a significance level of at least $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. C-ot, N-t, and C:N ratio

Plant tissue consists of two main groups of organic compounds: (1) a group of carbohydrate compounds including starch, sugar, lignins, tannins, fats, oils, waxes, and other compounds; and (2) the amino acid/protein group. The concentration of organic compounds from the first group can be estimated from the C-ot concentration, and that of the second group from N-t (Gan, Liang, Liu, Wang, & McDonald, 2011), so C and N are the most important plant elements (Zhang, Wang, Wang, & Wang, 2014). C and N, as the major elements, contribute roughly half of dry biomass. C-ot content varies markedly among soft woody and hardwood stems, whereas C content is significantly higher in hard woody stems than in herbaceous-softwood stems. This is consistent with their different degrees of lignification (Ma et al., 2018). Table 1 shows that treatment E has the highest C-ot content and the lowest N-t content, so that it has the highest C:N ratio. This demonstrates that the woodier the stem, the higher the lignin content.

Table 1. C-ot, N-t, and C:N ratio of various stem segment cuttings.

Stem segment of cutting	C-ot (%)	N-t (%)	C:N ratio
First stem segment from the upper shoot (A)	2.13	0.43	4.98
Second stem segment from the upper shoot (B)	2.15	0.41	5.84
Third stem segment from the upper shoot (C)	2.16	0.36	6.20
Fourth stem segment from the upper shoot (D)	2.66	0.34	7.98
Fifth stem segment from the upper shoot (E)	2.84	0.21	13.83

The formation of adventitious roots in plant cuttings is a complex physiological process involving plant growth substances, water, and nutritional status in the cutting tissue (Hartmann et al., 2011). The key nutrients are C and N, where C and N content in the cuttings will affect the growth of roots and shoots. The C:N ratio affects root formation in cuttings, where carbohydrate/protein imbalance will inhibit cell growth and development but a high C:N ratio will help and encourage root formation (Kastono, Sawitri, & Siswandono, 2005; Siregar, 2014; Tombesi, Palliotti, Poni, & Farinelli, 2015).

The position on the branch of stem cutting affects roots because this is associated with growth substances and tissue juvenility. Cuttings from the upper stem have a high rate of auxin synthesis and less differentiated tissue, but are more sensitive to dehydration. Cuttings from the basal stem, although having low levels of endogenous auxin, have a greater capacity to provide the carbohydrate reserves necessary for the formation and growth of roots and shoots (Cuncha, Chaves, Batista, & Hidalgo, 2015). Cuttings from the upper, middle, and lower stem have the highest, medium and lowest root length, respectively; this is related to the C and N content of cutting tissue and growth substances (Pratama, Yunus, Purwanto, & Widyastuti, 2018).

3.2. Root Length

Early growth of cuttings is indicated by callus appearance at the wound. When a callus is formed, auxins accumulate at the callus (Ditengou et al., 2008) and this will differentiate cells to form roots. Therefore, root growth on cuttings is an indicator of cutting growth. Not all callus tissue will develop to root tissue because this is influenced by the auxin/cytokinin balance, the fertility and humidity of the planting medium, and the C:N ratio (Gonin, Bergougnoux, Nguyen, Gantet, & Champion, 2019; Li, Xue, Xu, Feng, & An, 2009; Pijut et al., 2011; Tajbakhsh, Korkan, & Ghiyasi, 2009). Root length in Robusta coffee cuttings is shown in Table 2, from which it will be noted that treatment of the second stem segment from the upper shoot (B) resulted in a root length significantly higher than that with treatments D and E, but was not significantly different from treatments A and C. This shows that cuttings using the basal stem – which is hardwood – can form roots longer than that from the upper stem – which is softwood or semi-hardwood. This finding is the same as research conducted by Benbya, Alaoui, Gaboun, Delporte, and Cherkaoui (2018), which showed that semi-hardwood cuttings yielded the longest roots, followed by softwood.

Cuttings from the basal stem are hardwood and have a high C and low N content, i.e., a high C:N ratio (see Table 1). Stem cuttings with a high C:N ratio have reduced ability to form roots. Carbon and nitrogen in cutting tissue play key roles in root growth, with those from basal stem having low N and high C (Zerche, Haensch, Druege, & Hajirezaei, 2016). Because N is a precursor of amino acids in the production of protein and auxins, stem cuttings with a low N content have a reduced ability to form roots. Adventitious root growth is an intensive metabolic process determined by the presence of auxins and other plant growth substances, enzymes, carbohydrates, and proteins (Legue, Rigal, & Bhalerao, 2014).

Table 2. Effects of stem segment cuttings on Robusta coffee (*C. canephora*) root length, leaf sprout height, and leaf sprout number.

Stem segment of cutting	Root length (cm)	Leaf sprout height (cm)	Leaf sprout number
First stem segment from the upper shoot (A)	6.51^{bc}	2.49^{ab}	2.60^{a}
Second stem segment from the upper shoot (B)	8.40^{c}	4.98^{b}	4.20^{a}
Third stem segment from the upper shoot (C)	7.23^{bc}	3.59^{ab}	3.93^{a}
Fourth stem segment from the upper shoot (D)	4.77^{ab}	3.39^{ab}	3.40^{a}
Fifth stem segment from the upper shoot (E)	2.67^{a}	2.03^{a}	3.20^{a}

Note: Mean values within a column followed by the same letters are not significantly different by Tukey's HSD with a significance level of at least $P < 0.05$.

3.3. Leaf Sprout Height

The carbohydrate content in cuttings decreases during root growth because this provides energy during the root formation process and promotes root growth (Druege, Zerche, & Kadner, 2004). After the roots are formed, the leaf sprout will appear and the subsequent photosynthetic process in the leaves will trigger an increase in carbohydrates in the cuttings, so that the energy needs for root and leaf growth will be supplied by photosynthesis from the leaves that have grown from the cuttings. Thus, photosynthesis by leaves on cuttings can play an important role in providing carbohydrates for the formation and growth of adventitious roots.

Table 2 shows that treatment of cuttings from the second stem segment from the upper shoot (treatment B) yielded the highest leaf sprout height (significantly) when compared to treatment E, but was not significantly different from treatments A, C, and D. Leaf sprout growth from stem cuttings is determined by water, nutrients, root growth, auxins, cytokinins, carbohydrates, and proteins (Kurepa, Shull, & Smalle, 2019). Optimal leaf sprout growth in Robusta coffee cuttings was seen when they were grown from semi-hardwood stems (treatment B, Table 2), because semi-hardwood stems have good root growth, auxin:cytokinin ratio, and C:N ratio.

3.4. Leaf Sprout Number

Leaf size and number are determined by the plant's metabolic processes and C uptake (photosynthesis) (Dombroskie, Tracey, & Aarssen, 2016; Kleiman & Aarssen, 2007). From Table 2 it will be seen that leaf sprout numbers in all treatments were not significantly different. Leaf growth on cuttings is strictly controlled by external and internal factors. External factors are environmental factors, including temperature and sunlight (Gonzalez et al., 2010; Repkova, Brestic, & Olsovska, 2009), while internal factors are plant factors including genetics, auxins, cytokinins, number of bud nodes, and C and N content in stem tissue (Caplan, Stemeroff, Dixon, & Zheng, 2018; Kurepa et al., 2019; Zheng, 2009).

Due to these external and internal factors, leaf sprout number from stem cuttings will be determined by the type of clone, type of branch, and age of branch. In this study, Robusta coffee cuttings were orthotropic branches of the same age – about 5–6 months, and the cutting growth environment was also the same, so that leaf sprout number was not significantly different between treatments.

3.5. Leaf Sprout Growth Rate

Leaf growth determines the subsequent growth of cuttings, with the leaves being the organ where photosynthesis occurs to supply energy for growth (Kotting, Kossmann, Zeeman, & Lloyd, 2010). Leaf growth rates on cuttings depend on root growth to supply water and nutrients, and the rate of C uptake (photosynthesis) by the leaves. If leaf growth is slow so that C fixation is low, this reduces photosynthesis so that photo-assimilation is preferably translocated to the shoots rather than the roots and, consequently, root growth decreases.

Table 3 shows that treatment of the second stem segment from the upper shoot (B) yielded a significantly higher growth rate than treatment E, but was not significantly different compared to treatments A, C, and D. This occurred is because treatment B resulted in good root growth (Table 2) so that the rate of shoot growth was also enhanced.

Table 3. Effects of stem segment on leaf sprout growth rate (cm per week) in cuttings of *C. canephora*.

Stem segment of cutting	Leaf sprout growth rate (cm per week)
First stem segment from the upper shoot (A)	1.97[ab]
Second stem segment from the upper shoot (B)	3.38[b]
Third stem segment from the upper shoot (C)	2.74[ab]
Fourth stem segment from the upper shoot (D)	2.52[ab]
Fifth stem segment from the upper shoot (E)	1.28[a]

Note: Mean values within a column followed by the same letters are not significantly different by Turkey's HSD with a significance level of at least $P < 0.05$.

Based on Table 3, it can be seen that Robusta coffee cuttings from the basal stem (hardwood) have a low leaf sprout growth rate because they have poor root growth. Leaf sprout growth rate is influenced by root growth, leaf number, and nutrients in stem tissue – especially C and N content.

4. CONCLUSION

Robusta coffee cuttings from the second stem segment from the upper shoot yielded optimal root growth (root length) and shoot growth (leaf sprout height and leaf sprout growth rate). The leaf sprout number of cuttings was not affected by the stem segment utilized but was influenced by plant genetics and bud node number on the cutting.

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