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Effects of auxin and cytokinin levels on the success of air layering in tea plant clones of GMB 7 and GMB 9 using husk charcoal, cocopeat and moss media

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ABSTRACT

The research aimed to propagate tea plants by air layering in order to obtain new plants with shorter immature plants period which is 1.5 years, by utilizing wasted branches from routine clean pruning activities in tea plantations. The research was conducted from August 2018 to January 2019 at tea plantation of PT. Pagilaran, Batang, Central Java. Experiment using single factor treatment was arranged in a completely randomized design with three replications. The treatment was layering media, consisting of husk charcoal, moss, and cocopeat, which were applied on GMB 7 and GMB 9 clones. The results showed significant effects of the layering media on the levels of auxin and cytokinin in GMB 7. The highest levels refers to the husk charcoal and moss media. Analysis of sucrose, glucose and total sugar as well as the physiological analysis of the air layering roots showed no significant difference in the fresh weight, dry weight, volume, surface area, diameter, and length of the roots. Husk charcoal resulted in the highest success rate of the air layering in GMB 7, which was 100%. Meanwhile, GMB 9 showed significant difference only in the auxin levels. There was no significant effect of layering media on the analysis of sucrose, glucose and total sugar as well as on the physiological analysis of the air layering roots. Both husk charcoal and moss media resulted in the highest success rate of the air layering in GMB 9, which was 58.33%.

INTRODUCTION

Tea plant (*Camellia sinensis* (L.) O. Kuntze) is one of the main plantation commodities. It plays important roles not only in economic aspects, but also in sociological, ecological, and geographical aspects, including being the source of foreign exchange, farmer income, employment, agroindustry encouragement, regional development, and environmental preservation. However, the tea agroindustry in Indonesia has experienced a decline due to various problems that have not been able to be coped with, such as the low crop productivity due to the lack of the superior seeds use in most of the tea plantation, limited mastery of product processing technology, and the farmers inability to follow the recommended technologies based on Good Agriculture Practices (GAP) and Good Manufacture Process (GMP) of product quality standards as required by ISO (Ministry of Agriculture, 2014). The circle of national tea problems starts from unwell-maintained plantation causing low productivity as well as high production costs but producing low-quality tea with a low selling price. Field identification showed that the condition of tea industry in Indonesia comprised 60% old or damaged tea plants, low productivity (only 50% of the potential production), old and unqualified processing machines based on GMP/ISO/SNI standards, the increasing production costs of > 10% per year, and the selling price that only

increased by ± 4% per year (Arifin, 2014).

The air layering techniques is an innovation opportunity to propagate tea plant by utilizing parts of the wasted branches resulted from routine clean pruning activities in the tea plantation. The specialty of air layering propagation is that it can shorten the immature plant period to ±1.5 years. The tea propagation using air layering techniques is not commonly used because it is unable to produce many new plants from a parent tree (Adisewojo, 1964). However, the air layered seeds are already in the form of mature plants that can more quickly be used to embroider or replace old/damaged/dead plants.

Air layering is carried out by peeling the skin and scraping the cambium of the branch then wrapping it with the media. Exfoliation of the branch aims to cut off the flow of food from the tip of the stem to the incised branch. The root will be formed due to the flow of food substances (carbohydrates) and auxins (growth hormones that encourage the release of roots), which then flow down through the bark (phloem). These nutrients will be retained in the upper part of the incised section to enable the accumulation of carbohydrates and hormones. Heaps of carbohydrates and hormones in the incision will form a callus that will eventually turn into plant roots. When the roots have filled the media, the air layering is considered successful (Prastowo and Roshetko, 2006).

In the description of the clones, it is known that pruned shoots of GMB 7 have quick vegetative growth characteristic with average production of 5.8 tons.ha^{-1} per year. Meanwhile, GMB 9 has after-pruning moderate growths with average production of 4.7 tons.ha^{-1} per year. Air layering media is an important factor. Good media provides sufficient water for the formation and development process of the roots. When media has good aeration and can provide sufficient moisture, the roots can develop well (Adinugraha et al., 2007). It is expected that this study is able to provide information about the most appropriate media to support successful air layering.

MATERIALS AND METHODS

This research was carried out in PT. Pagilaran, Batang, Central Java within 5 months from August 2018 to January 2019. Analysis of auxin and cytokinin hormones was carried out in the Agrochemical Residue Material Laboratory in Bogor. The observation of root physiology was carried out in the Plant Physiology Laboratory, while the analysis of sucrose, glucose and total sugar was carried out in the Horticulture Laboratory, Faculty of Agriculture, Universitas Gadjah Mada.

Materials used in this study consisted of branch of the tea plant and the media for the air layering such as cocopeat, husk charcoal and sphagnum/moss. The instruments used were layering tools, cooler box, ice gel, digital scale, oven, and other laboratory instruments.

Air layering of the selected branches was carried out in the area to be pruned. Branches were incised as long as ±10 cm, cleaned from their cambium, and left for 2–3 days. Then, the branches were given with air-layering media in plastic wrap tightly bound on both ends so as not to shift and then covered with black plastic. Water was given regularly to media when dry. After five months, the branches were cut.

The study used a field research method arranged in a Completely Randomized Design (CRD) with layering media (husk charcoal, cocopeat and moss) as treatments applied to two clones (GMB 7 and GMB 9). For each treatment, two parent plants were selected and replicated three times, in which two branches were air layered within each plant.

The data obtained were subjected to analysis for variance (ANOVA) at α= 5%. When the result indicated a difference between the planting media treatments, then the analysis was continued to Duncan's Multiple Range Test (DMRT) at α= 5%. Data transformation was performed on data with too high CV value.

RESULTS AND DISCUSSIONS

The GMB 7 Clone

The layering media applied to GMB 7 clone did not give significant effect on the levels of sucrose, glucose, and total sugar (Table 1). The highest sucrose level was observed in the husk charcoal media. Meanwhile, the highest level of glucose and total sugar was found in cocopeat. Carbohydrates can also be used for endogenous metabolic processes and biosynthesis of hormones such as auxin, cytokinin, and gibberellins. Auxin, cytokinin and gibberellins can interact in the growth process (Widiastoety and Nurmalinda, 2010). Sucrose will be hydrolyzed into

Table 1. Levels of sucrose, glucose and total sugar in GMB 7 bark as affected by three media types

Media	Sucrose (%)	Glucose (%)	Total sugar (%)
Husk charcoal	3.67 a	4.02 a	2.43 a
Cocopeat	3.49 a	5.31 a	2.53 a
Moss	3.50 a	4.83 a	2.20 a
Coefficient of variation (%)	11.43	18.89	11.61

Remarks: Means followed by the same letters in the same column were not significantly different based on DMRT at α= 5%.

Table 2. Levels of auxin and cytokinin in air layering root of GMB 7 as affected by three media types

Media	Average		Cytokinin/Auxin
	Auxin (M)	Cytokinin (M)	
Husk charcoal	2.22×10^{-8} a	1.26×10^{-10} a	0.006
Moss	1.90×10^{-8} a	1.37×10^{-10} a	0.007
Cocopeat	1.31×10^{-8} b	5.78×10^{-11} b	0.004

Remarks: Means followed by the same letters in the same column were not significantly different based on DMRT at α= 5%.

glucose and fructose. Glucose will be broken down through cellular respiration, which will produce carbon and energy. This energy will be used by cells for callus formation (Shofiyani and Purnawanto, 2017).

There was significant difference in the level of auxin and cytokinin hormones resulted in the three media with the highest results was obtained in the husk charcoal and moss media. Therefore, it can be assumed that the moss and husk charcoal media were equally suitable for producing auxin and cytokinin hormones.

Based on Table 2, it can be seen that the level of auxin in the three media were higher than that of cytokinin. Such ratio would stimulate the formation of roots. If the level of auxin hormone was higher than the level of cytokinin, the two hormones would jointly support the root growth. The combination of cytokinin and auxin can induce callus formation (Miyashita et al., 2009). Auxin does not function properly when it does not interact with other growth regulators such as cytokinin and other nutrients. Cytokinin/Auxin ratios in GMB 7 clone indicate the support in the formation of layering roots because when the auxin level > cytokinin level, the roots are formed. Meanwhile, when the auxin level < cytokinin level, the crown is formed. In all three media, the auxin level was higher than cytokinin level. The lowest cytokinin/auxin value was in cocopeat media. Based on the cytokinin/auxin ratio, cocopeat showed the highest ability for root formation. However, the ratio of cytokinin/auxin between the three media was not significantly different, which means the ability to form roots is almost the same.

The Yin-Yang Chinese concept might illustrate how the relationship between auxin and cytokinin work more accurately, that although opposites, the strength of auxin and cytokinin are complementary. As in yin-yang, auxin and cytokinin work together dynamically, with roles that are opposite but able to support the development process of plant cells (Schaller et al., 2015). The research by Samudin (2009) showed that the use of media with different combinations of auxin-cytokinin resulted in different responses to the growth of dragon fruit. Allegedly, there had been a balance between cytokinins and auxins, enabling cell division that can stimulate the formation of shoots, thorns, and roots of the dragon fruit.

Cytokinin is generally used for shoot formation. Conversely, auxin isused for the formation of roots or callus. However, both are often required depending on the ratio of cytokinin to auxins. The existence of one of specific growth regulators can increase the activity of other growth regulators. The suitable types and concentrations of growth regulators for each plant

are not the same because it depends on the genotype and physiological conditions of the plant tissue (Lestari, 2011). A more efficient balance of concentrations of auxin and cytokinin cannot be certainly determined because the same source of PGR in different plants can have different effects. According to Hartmann et al. (1997), different plant will also respond differently to various concentrations of auxin and cytokinin hormones.

Analysis of air-layering root growth did not show any significant difference between the three media, as shown in Figure 1. The husk charcoal media gave the highest result in the fresh weight, dry weight, length and surface area of the roots, while the cocopeat media gave the highest value of root diameter and volume. The formation of roots in air layering occurs due to a build-up of food substances derived from the leaves at the top of the incision that cannot move towards the bottom of the incision. Thus, at the top, the bark will bulge due to a build-up of auxin and carbohydrates. With the planting media, these substances will stimulate the formation of roots (Rochiman and Harjadi, 1974).

Hasriani et al. (2012), state that cocopeat growing media has water content and storage capacity of 119% and 695.4%, respectively. The disadvantage of cocopeat media is that it has a micro pore that is able to inhibit the movement of water resulting in higher availability of water. At certain times, these conditions cause obstacles of gas exchange in the media because the media begins to saturate with water. This is because the macro pore space is filled with water instead of air, which prevents the roots to breathe and consequently inhibits the plant growth (Istomo and Valentino, 2012). This result relates to this research that was conducted in the rainy season with high rainy day, thereby causing the cocopeat media to be saturated with water. The husk charcoal media holds less water, and so does moss media. Moss media did not give highest results in root physiology, but air layering using moss media showed better success than cocopeat media. The best result of air layering of husk charcoal, moss, and cocopeat was 100%, 83.33%, and 50%, respectively.

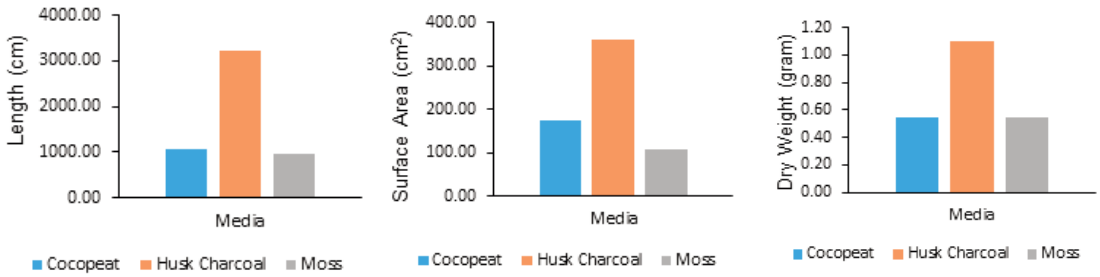

Figure 1. The morphology of air layering roots of GMB 7 as affected by the three media types

The GMB 9 Clone

Carbohydrates can be used for metabolism and biosynthesis process of endogenous hormones such as auxin, cytokinin, and gibberellins, which can interact in the growth process (Widiastoety and Nurmalinda, 2010). Data from the observations of sucrose, glucose and total sugar level were presented in Table 3. Meanwhile, the analysis of auxin and cytokinin level showed that significant difference was only observed in auxin level (Table 4).

The husk charcoal and moss media showed the highest auxin level compared to the cocopeat media. Thus, it can be assumed that both husk charcoal and moss media were good for GMB 9 clone to produce the auxin hormone.

According to Table 4, the auxin level in GMB 9 clone was higher than cytokinin level, either in cocopeat, husk charcoal, or moss media. Such ratio would lead to the formation of roots. However, in contrast to GMB 7, the cytokinin level in GMB 9 was not significantly affected by the three media. Together with auxin, cytokinin stimulate cell division and affect the path of differentiation. Antagonistic interaction between auxin and cytokinin is also one of the ways in which plants regulate the degree of root growth,

Table 3. Levels of sucrose, glucose and total sugar of GMB 9 bark as affected by three media types

Media	Sucrose (%)	Glucose (%)	Total sugar (%)
Husk charcoal	3.84 a	4.39 a	1.00 b
Cocopeat	3.15 a	3.63 ab	1.23 b
Moss	3.33 a	4.47 a	1.20 b
Coefficient of variation (%)	17.69	19.53	10.50

Remark: Means followed by the same letters in the same column were not significantly different based on DMRT at 5%.

Table 4. Levels of auxin and cytokinin in air layering root of GMB 9 as affected by three media types

Media	Average		Cytokinin/Auxin
	Auxin (M)	Cytokinin (M)	
Husk charcoal	1.81×10^{-8} a	4.71×10^{-8} a	0.003
Moss	1.75×10^{-8} a	7.45×10^{-8} a	0.004
Cocopeat	1.31×10^{-8} b	1.22×10^{-7} a	0.009

Remark: Means followed by the same letters in the same column were not significantly different based on DMRT at 5%.

for example, a large number of roots will produce cytokinin in large quantities (Widyastuti and Donowati, 2007).

Comparison of auxin and cytokinin levels in GMB 9 showed that the auxin level was higher than cytokinin level in cocopeat, husk charcoal, and moss media. According to the calculation, the lowest cytokinin/auxin ratio was observed in the husk charcoal and moss media, while the highest cytokinin/auxin ratio was in the husk charcoal media. The low ratio of cytokines/auxins will support root formation (Table 4).

Woodward et al. (2005) explain that auxin plays a role in triggering the formation of lateral roots of undifferentiated callus. The explant response to auxin decreases even in the media exposed to light.

Auxin and cytokinin used in the media are synergistic. Auxin plays a role in regulating cell growth and elongation, while cytokinin plays a role in cell division. This is easily understood because cellular auxin plays a role in cell elongation, while cytokinin triggers cell division, morphogenesis, and growth regulation that are very important in callus formation. Results of the study by Angelina et al. (2017) in the Bangun-Bangun plant showed that leaf explants cultured on MS medium + 3 $mg.L^{-1}$ NAA + 0.1 $mg.L^{-1}$ kinetin could induce roots optimally as indicated by the percentage of root formation, root fresh weight, and number of roots. It is suspected that the antagonistic interaction between auxin and cytokinin is one of the ways of plants to regulate the growth degree and development of the root.

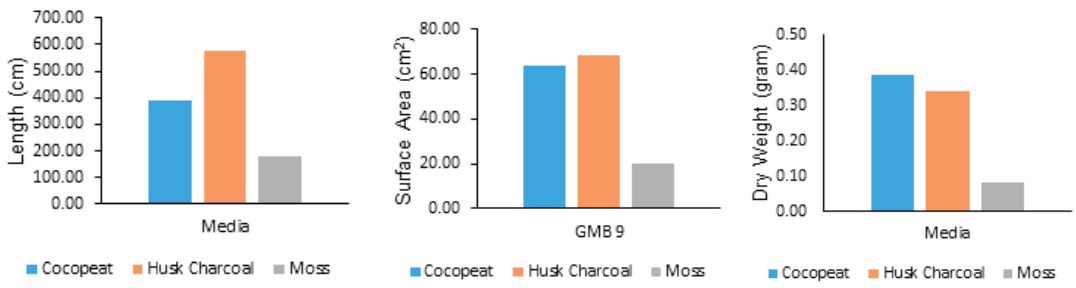

Figure 2. The morphology of air layering roots of GMB 9 as affected by the three media types

Data of the growth of air-layering roots were presented in Figure 2. Analysis of the growth of air-layering root in GMB 9 did not show any significant difference between the three media. However, the graph above shows that cocopeat media showed the highest results on the parameters of wet weight, dry weight, volume and diameter of the air-layering roots. Meanwhile, the husk charcoal showed the highest results in the length and surface area of the air-layering roots.

However, based on the calculation of the success of air-layering, husk charcoal and moss media showed the higher success rate than cocopeat, which was 58.33% and 50%, respectively. Root growth in the three media showed uniformity although there were still many undifferentiated callus forming roots, which were still in the form of bulges on the stem. The undifferentiated callus was predominantly in cocopeat media.

CONCLUSIONS

The type of media significantly affected the auxin and cytokinin level in GMB 7 clone, while in GMB 9 clone, only the auxin was significantly affected by the type of media. The best air-layering media with 100% success rate for GMB 7 clone was the husk charcoal. Meanwhile, for GMB 9 clone, the best air-layering media were husk charcoal and moss with the same success rate of 58.33%.

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