Effects of exogenous auxin on stem cutting growth of tea (Camellia sinensis)

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Abstract. Root growth rate of a stem cutting was affected by the auxin levels absorbed by the stem cutting. Level of endogenous auxin was insufficient to stimulate the root growth, so that exogenous auxin should be applied. As synthetic auxin was highly expensive, natural auxin sources gained more attention recently to be used. This study was aimed to determine the best source of exogenous auxin triggering the stem cutting growth of tea (Camellia sinensis). This research was conducted using a completely randomized design with four replicates of each treatment. Source of exogenous auxin used in this study consisted of both synthetic (IBA and Rootone-F™) and natural (goat urine and shallot tuber extract) types. These exogenous auxins were applied into the tea stem cutting before planting through dipping method. Growth parameters related to root, shoot and leaf development were observed. Data were evaluated statistically using one-way anova. Application of 6.000 ppm IBA enhanced the rooting percentage and leaf area. Increase of root length was found in the stem cutting treated with 10% goat urine. These results suggested that each type of exogenous auxin stimulated the root growth in a different way. Therefore, proportional combination of these auxin sources might provide a greater support on root growth of tea stem cutting.

1. Introduction

Tea plantations have been widely spread in Indonesia to fulfill the demand of tea both domestic and international. However, most of tea plantations in Indonesia showed low productivity due to the lack of regular replanting caused by the limited supply of planting material. The low availability of planting material could be overcome through vegetative propagation. This method provides faster multiplication, higher quantity of seeds and higher similarity between parent and progeny [1, 2].

Cutting is an easy-to-do vegetative propagation method which is able to meet the needs of large amount of plant material. However, the root growth of vegetatively propagated plants showed a very low success rate. Mostly practiced tea cutting only produced less than 50% viable cutting showing proper root growth. Tea cultivation in an official plantation often apply the growth regulator or maintain the level of endogenous hormone to obtain a high quality seedling. For instance, PT Tambi UP Tanjungsari applied Atonik™ to the tea cuttings as it could maintain the endogenous auxin by inhibiting the activity of IAA oxidase [3]. However, according to Gardner et al. [4], the level of endogenous auxin were insufficient to stimulate the root growth optimally, thus the exogenous auxin was highly required.

Plant growth regulator (PGR) is organic compounds, aside from nutrients, that affect the physiological processes of a plant. PGR is naturally found in plants, but mostly in a low level, thus the
external sources are needed to generate the maximum response. During the cutting nursery, the use of PGR in the form of auxin could improve the seedling quality and reduce the number of abnormal seedlings [3]. Based on its source, auxin could be obtained either naturally or synthetically. Synthetic auxin can be either IBA, NAA, or commercial products such as Rootone-F™ and Root-up™ [5, 6]. Zenginbal et al. [7] recommended the application of IBA 6000 ppm in the nursery of tea cutting. Other study reported the success of Rootone-F™ application in improving the percentage of viable Magnolia cuttings [8].

Generally, natural auxin was easily available in nature from organic materials such as coconut water, animal urine or shallot extract [9, 10]. Organic auxin is considered to be more ecological friendly and highly affordable. Widiana and Riza Linda [11] mentioned that the 15 minutes soaking of Anthocephalus cadamba cuttings in 10% goat urine increased its number of roots, plant height, fresh and dry weights. Similar studies also reported that the application of 20% cow urine improved the percentage of viable and rooted cuttings, length and number of roots as well as shoots. This result was found to be higher than the result of cattle urine application [12]. In addition, the application of 70% shallot extract for 10 minutes reported by Alimudin and Ramli [13] exhibited the best result in all root growth parameters of the rose cuttings. Similarly, Siregar et al. [14] revealed the best growth of agarwood seeds due to the application of 1.5-2% shallot extract indicated by the increase of leaf number, leaf area, stem circumference, seedling height and dry weight. Si [15] also reported the effect of 1.0% shallot extract application resulting in 80% viable cuttings. However, scientific report on the application of these natural auxin in the nursery of tea cutting remained less known. Therefore, this study was aimed to determine the best source of auxin stimulating the optimal growth of tea (Camellia sinensis) cuttings.

2. Materials and Methods

2.1. Preparation of Endogenous Auxins
Endogenous auxin used in this study was both synthetic (IBA and Rootone-F™) and natural (shallot extract and goat urine) sources. IBA (indole-3-butyric acid) was prepared by dissolving 0.6 g IBA into 100 ml 95% alcohol to obtain the final concentration of 6,000 ppm. Goat urine used was 10% (v/v) and the preparation followed the recommendation of Widiana and Riza Linda [11]. Shallot extract was prepared using finely ground shallot tuber according to the protocol proposed by Alimudin and Ramli [13]. Mashed shallot was filtered and 70 ml shallot liquid was collected and dissolved with 30 ml water to obtain 70% final concentration. The use of Rootone-F™ was applied directly to the tea cutting in the form of paste without any pre-treatment.

2.2. Preparation of Planting Medium
Planting medium used was the mixture of top soil and sub soil which previously prepared by mixing with fertilizers (KCl, basamid, alum and SP-36) and fungicides (Dithane M-45™). The medium was then composed by filling the 2/3 polybag with the top soil as the lower layer. The remaining space was filled with sub soil as the upper layer. The medium was then watered to provide a sufficient moisture.

2.3. Tea Cutting Preparation and Planting
Cutting used for planting was prepared by cutting the stem on the border of brown and dark green part about 15 cm in length. The center of that dark green part was used as the stem cutting for planting. Each cutting brought a leaf on the upper part (0.5 cm) and below the axilla (4-5 cm) with a slope of 45°. The leaves were cut by 50% to reduce transpiration.

The cuttings were soaked in Atonik™ for 2 minutes then various auxin sources, such as IBA (6,000 ppm), Rootone-F™, goat urine (10% v/v) and shallot extract (70% v/v) were applied. Soaking duration of each auxin sources was performed differently, as followed 5 seconds (IBA), 10 minutes (goat urine) and 15 minutes (shallot extract). Unlike these three auxin sources, the application of Rootone-F™ was carried out by spreading the Rootone-F™ paste onto the cutting wound. The cutting was then grown
for 4 months in the soil medium covered with plastic hood. Several root and shoot growth parameters were observed weekly from 16th to 24th week after planting.

2.4. Statistical Analysis
This study was conducted in a completely randomized design with four treatments of auxin sources repeated in 4 replications. Negative control used was the cutting planted without any exogenous auxin added. Data were statistically analyzed using one-way anova and the significance among treatment was further evaluated using Duncan’s Multiple Range Test with a p<0.05.

3. Results and Discussion

3.1. Effect of Various Auxin Sources on Root Growth of Tea Cuttings
Application of various auxin sources contributed significant increase on the percentage of rooted cutting and root length, but showed no effect on root number and dry weight (Table 1). Among auxin sources tested, IBA exhibited the best result in most of root growth parameters, except in root length. This result indicated the high persistence of IBA within the cutting that mainly contributed to the better stimulation of root growth in the tea cutting. In line with this assumption, Salisbury and Ross [3] mentioned that IBA was the most widely used auxin to stimulate the rooting compared to other types of auxin. Once it was active, IBA would be rapidly converted into IBA-aspartate or conjugated with other amino acids, thus resulting in a more efficient released IBA and provided longer persistence, especially during the root formation stage.

Table 1. Effect of various exogenous auxins on the root growth of tea cutting at the 24th week after. Values were mean of 4 replications.

| Source of Exogenous Auxin | Rooted cutting (%) | Length (cm) | Dry Weight (g) | Number of roots |
|---------------------------|--------------------|-------------|----------------|-----------------|
|                           |                    | Primary     | Secondary      |                 |
| Synthetic                 |                    |             |                |                 |
| IBA                       | 62.50 a            | 13.77 ab    | 0.20 a         | 6.00 a          |
| Rootone-F™                | 43.75 b            | 6.10 bc     | 0.13 a         | 3.42 a          |
| Natural                   |                    |             |                |                 |
| Goat urine                | 43.75 b            | 17.21 a     | 0.19 a         | 5.13 a          |
| Shallot extract           | 31.25 b            | 9.94 bc     | 0.10 a         | 4.13 a          |
| No auxin applied          | 25.00 b            | 2.60 c      | 0.03 a         | 1.75 a          |

Values followed by the same lowercases in the same column were insignificantly different based on DMRT with a p<0.05.

Unlike the effect of IBA, the application of goat urine showed the longest root suggesting the low concentration of auxin absorbed triggered the root extension. Salisbury and Ross [3] stated that the root would be easily elongated under the low concentration of auxin. Kusumo [6] also mentioned that some plant hormones might confer different effect on a cutting, particularly related to the root number and quality. Endogenous auxin derived from shoot and young leaves would provide sufficient amount required to stimulate the root growth as it was transported basipetally.

Root length would play an important role in the water and nutrients uptake. Gardner et al. [4] stated that the efficacy of water and nutrient uptake by plant depended on the depth of root proliferation. The deeper the rooting, the farther the water and nutrients could be accessed by the root. It would also minimize the risk of wilt.
3.2. Effect of Various Auxin Sources on Shoot Growth of Tea Cuttings
The addition of exogenous auxin showed no significant effect on nearly all shoot growth parameters observed, except leaf area (Table 2). These results might indicate the insufficient amount of food storage to trigger the shoot formation. As the planting material used was stem cutting, the food reserved with the cutting might be allocated mostly for the root formation, instead of shoot formation. Shoot acts as an auxin source, particularly the newly growing bud both on the shoot and leaf. However, the auxin produced would be transported downward and accumulated to the bottom of the cutting [1], thus stimulating the root formation. Once the roots were enable to uptake the nutrients, it would be used directly to develop other plant organ, including shoot.

Table 2. Effect of various exogenous auxins on the shoot growth of tea cutting at the 24th week after. Values were mean of 4 replications.

| Source of Exogenous Auxin | Parameters of shoot growth |
|---------------------------|-----------------------------|
|                           | Height (cm) | Number of leaves | Dry Weight (g) | Diameter (mm) | Leaf Area (cm²) |
| Synthetic                 |             |                 |                |               |                |
| IBA                       | 6.82 a      | 3.00 a          | 0.28 a         | 1.96 a        | 35.42 a        |
| Rootone-FTM               | 7.43 a      | 3.04 a          | 0.31 a         | 2.00 a        | 30.29 a        |
| Natural                   |             |                 |                |               |                |
| Goat urine                | 8.05 a      | 3.04 a          | 0.34 a         | 1.88 a        | 28.58 a        |
| Shallot extract           | 5.55 a      | 2.50 a          | 0.18 a         | 1.78a         | 17.00 ab       |
| No auxin applied          | 5.19 a      | 1.50 a          | 0.16 a         | 1.93a         | 7.50 b         |

Values followed by the same lowercases in the same column were insignificantly different based on DMRT with a p<0.05.

Addition of exogenous auxin (except shallot extract) had been successfully broadened the leaf area of tea cutting compared to the control. This result might be associated with the higher number of roots formed (Table 1) due to the application of these auxin sources. The more the roots, the more nutrients and water could be absorbed, thus resulting in better growth of other plant organs, including leaf. Moreover, IBA-treated cutting showed longer roots enabling the broader coverage to uptake water and nutrients from the soil. Sufficient water and nutrients absorbed would be used to produce the photosynthate to support the plant growth, including leaf growth. As the leaf expanded, its ability to capture sunlight would be greater. In line with this interpretation, Gardner et al. [4] reported that some plants tended to invest most of its energy to support the initial growth, such as leaf area expansion leading to the efficient utilization of solar radiation.

4. Conclusion
Of all auxin sources evaluated, the most recommended source used for the nursery of tea cutting was 6,000 ppm IBA and 10% goat urine. Both auxin sources induced the longer root and broader leaf area after 24 weeks of planting, while IBA application improved the percentage of rooted cutting better than other treatments. Based on this study, proportional combination of IBA and goat urine could be developed to stimulate better root growth of tea stem cutting, thus resulting in a higher success rate of its vegetative propagation.

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