A Combination of IBA and NAA Resulted in Better Rooting and Shoot Sprouting than Single Auxin on Malay Apple [Syzygium malaccense (L.) Merr. & Perry] Stem Cuttings

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ABSTRACT

This research aimed to study effects of IBA, NAA and their combination on rooting and shoot sprouting in Malay apple cuttings. Cuttings from superior genotype were collected and treated with (in ppm w/w): 0, 2000 IBA, 4000 IBA, 2000 NAA, 4000 NAA, 1000 IBA+1000 NAA, 2000 IBA+2000 NAA. To record the timing and percentage of rooting, cuttings were treated with (in ppm w/w) 1000 IBA+1000 NAA or without auxin as control. The results revealed that application of auxin was significantly enhanced root formation as shown by the significant increases in rooting percentage and number of roots. NAA at 2000 or 4000 ppm was the most effective auxin to promote root formation (100 %, 17.8–25.5 roots per cuttings), followed by NAA+IBA (100 %, 16.8– 9.8 roots per cuttings) and the least effective was IBA alone (79-100 %, 3.2–7.1 roots per cutting). The best treatment for rooting and shoot sprouting were (in ppm) 1000 IBA+1000 NAA, since it produced higher root length, better root morphology and higher shoot sprouting. It was also found that a combination of IBA+NAA each at 1000 ppm not only enhanced root percentage, but also shortened the time for root formation.

INTRODUCTION

Malay apple [*Syzygium malaccense* (L.) Merr. &. Perry], (synonym: *Eugenia malaccensis* L.) is a tree species, a member of Myrtaceae (myrtle) family native to South East Asia (Indo-Malayan region) which has relatively large-ovoid berry 5-8 cm in diameter, dark red skin and thick white juicy fleshed nutritious fruits. It has several common names, including *jambu bol* or *jambu Jamaica* (Indonesia), *otaheite cashew* (Jamaica). The tree has broadly ovoid canopy, pyramidal or cylindrical, and has many small horizontal to ascending branches, grows up to 20 m tall and potentially yields 21-85 kg fresh fruit per tree. Its fruits are sour to slightly sweet, juicy, delicate but crisp and has a short shelf life. The dark-red skinned mature fruits of Malay apple have moderate to high contents in vitamines (A, C, niacin), minerals (phosphorus, calcium and iron), and dietary fiber as well as other organic compounds that act as antioxidant. In some local wisdoms, it was believed that Malay apple has some health benefits such as reducing inflammation, curing mouth infection, coughs, trush and as a purgative (Whistler & Elevitch, 2006). In other societies, it is believed that Malay apple is beneficial to improve the health of the skin, boost blood circulation, build stronger bones, prevent the development or worsening of diabetes, improve vision health, prevent the development of cataract, strengthen and improve hair quality. Currently, Malay apple fruits becomes one of exotic tropical fruits in some provinces of Indonesia. The price of mature fruits of Malay apple is economically reasonable to be used as a significant cash crop, ideal for either homegardens or casual intercrop plantings to support and enhance food security program.

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Propagation of Malay apple generally can be done through seeds, semi-hardwood cuttings, and air layering. Propagation through seeds is less desireable, since a large Malay apple fruit often contains only one large subglobose or two (a pair) of subglobose to hemispherical seeds, and the fruits of some tree are even completely seedless. Besides, the Malay apple seeds have short viability period. Therefore, right after being taken from the fruits, the seeds should be planted right away. Malay apple has a long juvenile phase of the tree and most progeny plants obtained from seeds generally have off-type characters (Whistler & Elevitch, 2006).

The more successful plant propagation by air-layering compared to cutting might due to the fact that the air-layered branches are still in connection with the mother plant, so that their supply of water, as well as assimilates and natural rooting hormones is more sufficient for rooting. Furthermore, the dark condition surrounding the rooting zone covered with rooting medium might also stimulate the activity of rooting hormones (Hartmann, Kester, Davies Jr., & Geneve, 2010). However, this method is slower, more labor-intensive and potentially could damage the architec of the mother plants.

Progation by cuttings is probably the easiest, most efficient and cheapest method to produce true-to-type and uniform plant materials. However, cuttings from different species have different capacity to form roots. Woody plant stem cuttings are often difficult-to-root, and this difficulty is still one of the major obstacles for economical propagation. In our preliminary study, it was found that without application of root promoting substance, Malay apple cuttings were difficult to root. Several factors are widely doccumented to affect adventitious root formation in stem cuttings, i., e., genotypes (Rahdari, Khosroabadi, Delfani, & Hoseini, 2014), physiological age (Santoso & Parwata, 2014), ontogenetic age of the trees (Rasmussen, Hosseini, Hajirezaei, Druege, & Geelen, 2015), preconditioning of cuttings with blanching and girdling in combination with auxin (Thakur, Sharma, & Singh, 2014), seasons, substrate or media for cuttings (Benti, 2014), and the use of exogenous root promoting substances, especially auxins (Seyedi, Esmaeili, & Mostafavi, 2013). Among these factors, application of synthetic auxins as root promoting substances such as indole-acetic acid (IAA), indole-butyric acid (IBA), or naphthaleneacetic acid (NAA) The basal portions of the stem cuttings appeared to be an effective method to induce root formation. Mixtures of two types of auxins are sometimes more effective than either component alone (Hartmann, Kester, Davies Jr., & Geneve, 2010). However, succesful results were often species or even clone-dependent (Abu-Zahra, Al-Shadaideh, Abubaker, & Qrunfleh, 2013).

Ryadin, Ranamukaarachchi, Soni, & Shrestha (2014) found that cuttings of five local cultivars of Malay apple in a mix media of top soil and sand with application of 1000 ppm NAA resulted in the best performance in producing shoots and roots, compared to 1000 ppm IAA with other media mixtures. However, the percent of rooted cuttings, number of roots per cuttings, and the percentage of the cuttings forming shoots which were needed to evaluate the success of propagation by cuttings were not recorded. Furthermore, there was no control treatment in the experiment, so it could not be concluded if the rooting was due to the application of auxins. In some difficult-to-propagate species, shoots might grow earlier, but roots did not form in months. The success of propagation by cuttings is indicated by high percentage of rooted cuttings and shoot growth. Application of auxins to stem cuttings at high concentration may inhibit shoot sprouting and its development, sometimes to the point at which there is no shoot growth at all, eventhough the root formation has been adequate (Hartmann, Kester, Davies Jr., & Geneve, 2010). Therefore, the right choice of auxin type and its optimum concentration for a given species need to be investigated. Since the price of NAA is much cheaper than IBA, application a combination of IBA + NAA or NAA alone as a root-promoting substances can be more economical, provided that the root formation and shoot growth are comparable. The objectives of this study were to develop an efficient clonal propagation method of Malay apple by observing the effects of various concentrations of IBA, NAA or NAA+IBA in the form of talcum powder on rooting and shoot sprouting in cuttings, and to study effects of a combination of IBA and NAA, each at 1000 ppm on the percentage of rooting and the timing of root formation in Malay apple cuttings.

MATERIALS AND METHODS

This research was conducted in the experiment station of Lampung State Polytechnic, Lampung, Indonesia from May to August 2016. Semi-hardwood cuttings without leaves of approximately 20-22 cm long were taken from healthy branches of elite mature Malay apple trees (6-8 years old) in homegardens in Bandar Lampung, Indonesia during the month of May 2016. Two consecutive experiments were conducted to study (1) effects of various concentrations of IBA, NAA and their combinations on rooting, and (2) effects of IBA + NAA each at 1000 ppm w/w on rooting and the timing of root emmergence of cuttings of Malay apple. In both experiments, the basal 1 cm parts of the cuttings were treated with auxins in the form of a paste from auxin talcum powder and water (1 g of talc with 1 mL of water). The control treatment was cuttings without growth regulator. Both experiments were done using completely randomized design with three replicates. The treatments assigned in the first experiment were (in ppm w/w): control (without auxin), 2000 IBA, 2000 NAA, 1000 IBA+1000 NAA, 4000 IBA, 4000 NAA and 2000 IBA+2000 NAA. Each experimental unit consisted of ten semi-harwood cuttings of Malay apple (30 cuttings for each treatment in three replicates). After the application of exogenous auxins (except for the control treatment), the ten cuttings for each replication were then planted vertically in polythene bags (20 cm diameter x 25 cm height) containing a mixture of substrate consisted of rice husk charcoal: compost: sand (1:1:1 v/v). The best treatment obtained from the first experiment, namely (in ppm w/w) 1000 IBA+1000 NAA was further investigated in the second experiment to study its effects on rooting percentage and the timing of root formation in Malay apple cuttings. In this experiment, each experimental unit consisted of 5 hardwood cuttings of Malay apple, treated with (in ppm w/w) 1000 IBA+1000 NAA or without auxin as the control treatment. Since the observation is conducted every week destructively, starting from the end of the first week to the end of the fifth week, there would be overall 150 cuttings for two treatments in three replications.

All cuttings were maintained under shaded benches, approximately 60 % full sun shine and watered regularly every day. Relative humidity in the bench was kept high (≥ 80 %). After 12 weeks (for experiment 1), the percentage of rooted cuttings and cuttings with shoots were recorded, followed by observing the number of roots per cutting, length of three primary longest roots in each cuttings, number of leaves per shoot and length of shoots of the responding cuttings. In the second experiment, observations were conducted from the end of the first week until the fifth week with one week intervals to record the percentage of rooted cuttings. The second experiment was terminated at the end of the fifth week, since the auxin-treated cuttings has rooted constantly more than 90 % at the end of the fourth to the end of the fifth weeks after planting. Analysis of variance was carried out to analyse the data and the least significant diference was used to separate means at 0.05 significant level.

RESULTS AND DISCUSSION

Analysis of variance of the data showed that different types and concentrations of auxins applied to Malay apple semi-hardwood cuttings resulted in significant differences at $P < 0.01$ for all variables measured for rooting and shoot sprouting, namely percent of rooted cuttings, number of roots per cuttings, root length, percentage of cuttings forming shoots, number of shoots per cutting, shoot length and number of leaves (Table 1). Furthermore, a mean separation test by LSD for those variables showed that the degree of responses of cuttings to form roots and shoots as well as shoot growth were different depending upon the types and concentrations of auxin applied.

Percent of Rooting and Root Number

Results of the first experiment revealed that Malay apple semi-hardwood cuttings could be categorized as a difficult-to-root species, since after 8 weeks, the control treatment or without auxin application produced only 25 % of the cuttings rooted, with the average of only 1 (one) primary root per cutting, while application of auxins (IBA, NAA or their combinations) were significantly enhanced root formation as shown by the significant increases in both rooting percentage (79.2 % - 100 %) and average number of roots (3.2 – 25.5 roots per cutting), regardless of the concentrations applied (Table 1, Fig. 1).

Table 1. Effects of IBA, NAA or IBA+NAA concentrations on the percentage of rooted cuttings, percentage of shoot formation, number of shoots per cuttings, length of shoots and number of leaves of malay cuttings observed at 8 weeks after planting

Auxin Types and Concentrations (ppm w/w)	Percentage of Rooted Cuttings (%)	Percentage of Cuttings forming Shoots (%)	Number of Shoots per cuttings	Length of shoots (cm)	Number of leaves per shoot
Control	25 c	29.2 b	1.0 cd	1.7 bc	3.5 ab
IBA 2000	79.2 b	41.7 ab	4.8 a	3.3 a	4.3 a
IBA 4000	100 a	25.0 b	3.2 ab	3.3 a	3.7 ab
NAA 2000	100 a	8.3 c	1.0 cd	0.8 cd	1.0 cd
NAA 4000	100 a	8.3 c	1.3 c	0.7 cd	2.0 bc
IBA 1000+NAA 1000	100 a	50.0 a	4.0 a	2.5 ab	3.3 ab
IBA 2000+NAA 2000	100 a	-	-	-	-
**Significant at P = 0.01	**	**	**	**	**

Remarks: Means in each column followed by different letters are significantly different based on LSD at P < 0.05)

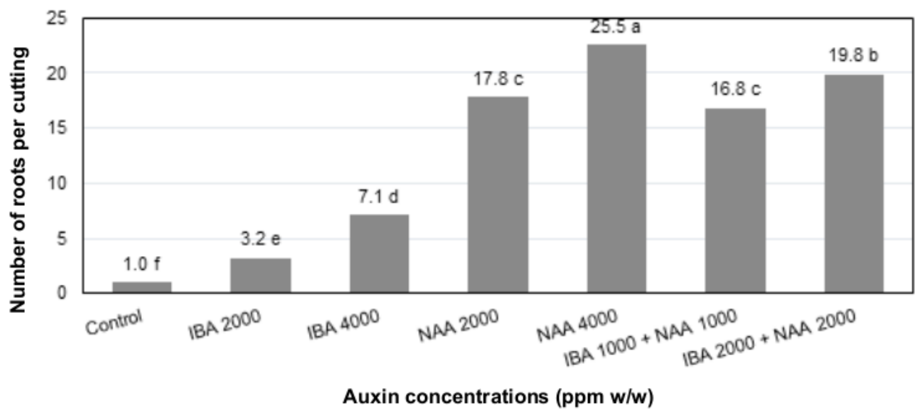

Fig. 1. Effects of IBA, NAA or IBA+NAA concentrations (ppm w/w) on the number of malay apple roots per cutting observed at 8 weeks after planting (Anova was significant at P < 0.01). Means in each column followed by different letters are significantly different based on LSD at P<0.05).

The results also showed that NAA was the most effective auxin treatment to promote root initiation in Malay apple semi-hardwood cuttings, followed by IBA+NAA and IBA alone being the least effective ones. The treatment with 2000 ppm of IBA resulted in 79 % root formation and 4000 ppm of IBA led to 100 % root formation. Mixture of IBA and NAA, regardless of the total concentrations (2000 or 4000 ppm), all resulted in 100 % root formation. In addition, the number of roots per cutting stimulated by 2000 or 4000 ppm NAA (17.8 – 25.5 roots per cuttings) were much higher than those stimulated by 2000 or 4000 ppm IBA alone (3.2 – 7.1 roots per cuttings), i.e., more than five-folds or more than three-folds, respectively (Fig. 1). In addition, the data also showed that increasing concentration of either IBA, NAA or IBA + NAA from 2000 to 4000 ppm consistantly resulted in significant increases of primary root numbers per cutting.

Root Length and Morphology

In general, the more the root number formed per cutting, the less was the average root length. Without the application of auxin, the average root number was only 1 root per cutting. However, the root length was the highest (17.3 cm). With the increase of root numbers as affected by application of IBA at 2000 and 4000 ppm, the root lengths decreased accordingly from 14.6 to 12.1 cm. In addition, the root length in NAA treated cuttings was even less than the IBA-treated cuttings (8.0 – 8.8 cm), since NAA was much more effective to induce root formation than IBA. However, application of 1000 ppm IBA+1000 ppm NAA in a combination, resulted in longer roots (10.5 cm) than those affected by NAA alone (Fig. 2). Fig. 3 shows the root performances of Malay apple cuttings under the control and all different types and concentrations of auxin treatments at 8 weeks after planting. Furthermore, the adventitious root morphologies among IBA-treated, or lower concentration of IBA+NAA-treated cuttings were different from those induced by NAA alone or higher concentration of IBA + NAA. Adventitious roots formed in former tend to have better morphologies with more branched and more fibrous secondary and tertiary roots (Fig. 4-a,b,c), compared to the unbranched, bigger in diameter of individual roots and more compact root ball in NAA alone or higher concentration of IBA + NAA-treated cuttings (Fig. 4-d,e,f).

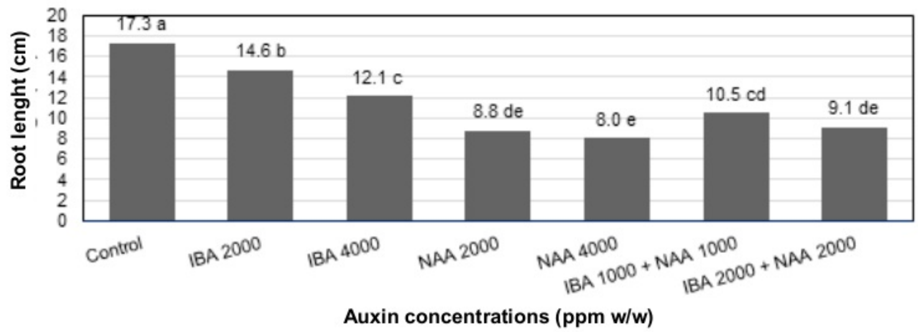

Fig. 2. Effects of IBA, NAA or IBA+NAA (in ppm w/w) on primary root length of malay apple cuttings observed at 8 weeks after planting (Anova was significant at $P < 0.01$). Means in each column followed by different letters are significantly different based on LSD at $P < 0.05$).

Fig. 3. Root performance of malay apple cuttings as affected by applied auxins (in ppm w/w): Control (without auxin), IBA 2000, IBA 4000, NAA 2000, NAA 4000, IBA 1000 + NAA 1000, IBA 2000 + NAA 2000

Fig. 4. Differences in root morphologies among roots induced by (a) 1000 ppm IBA, (b) 2000 ppm IBA, or (c) 1000 ppm IBA+1000 ppm NAA and roots induced by (d) 2000 ppm NAA, (e) 4000 ppm NAA, or (f) 2000 ppm IBA+2000 ppm NAA in malay apple cuttings. Roots induced by 2000, 4000 ppm IBA and 1000 ppm IBA+1000 ppm NAA have better morphologies with more branches or secondary roots, compared to roots induced by 2000 ppm NAA, 4000 ppm NAA, or 2000 ppm IBA+2000 ppm NAA, which have less or no secondary roots (arrows)

Shoot Sprouting and Growth

At 8 weeks after planting, a combination application of IBA + NAA each at 1000 ppm and IBA alone at 2000 ppm to Malay apple cuttings resulted the highest shoot sprouting percentage (50 % and 41.7 %, respectively), followed by the control treatment (29.2 %) and and 4000 ppm IBA (25 %), while NAA applied alone at 2000 or 4000 ppm produced only 8.3 % shoot sprouting, and IBA + NAA at higher level (a total of 4000 ppm) did not form shoot at all. The shoot growth as represented by number of shoots per cuttings and length of shoots in IBA alone or IBA+NAA (in a total concentration of 2000 ppm) treated cuttings were significantly higher or similar to those of control, while those values in NAA-treated cuttings was similar to those of control treatment (Table 1). Furthermore, cuttings of control and those applied with IBA alone or a combination of IBA and NAA each at 1000 ppm exhibited similar number of leaves per shoot, while those of NAA-treated cuttings was similar or lower than the control treatment.

Effects IBA + NAA on the Timing and Pecentage of Root Formation

Results of the second experiment revealed that when applied in a combination, IBA+NAA each at 1000 ppm not only enhanced rooting percentage, but also shortened the timing of root formation. Fig. 5 shows that cuttings without auxin rooted at very low frequencies after 4 weeks, i.e. only 13 % and these figure became 27 % after 5 weeks. On the other hand, application of 1000 ppm IBA+1000 ppm NAA resulted in 60 % of cuttings forming roots after 3 weeks and became constant at 93 % after 4 and 5 weeks. These data indicated that a combination of IBA and NAA each at 1000 ppm not only promoted root initiation, but also shortened the time needed for root formation and development in Malay apple semi-hardwood cuttings.

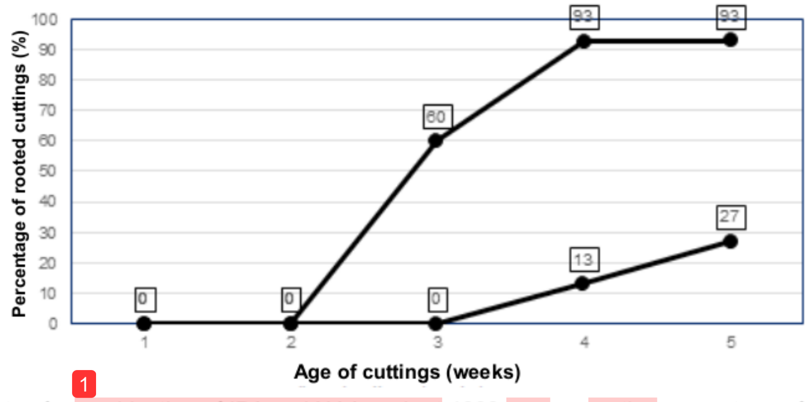

Fig. 5. Effects of a combination of IBA and NAA each at 1000 ppm on rooting percentage of malay apple semi-hardwood cuttings during five weeks after planting

Plant propagation by cuttings has several advantages including maintenance the specific characteristics of the plant from which cutting was collected, giving the true-to-type plant materials, it is simple and easy to conduct, it does not need a large space and it is a cheaper propagation method compared to layering, grafting or budding. The success of plant propagation by cuttings is shown by the root and shoot formation and growth which is well documented to be affected by several factors, including genotypes, physiological and ontogenetic age of cuttings, endogenous hormone contents, type of woods, carbohydrate contents, preconditioning treatment of cuttings and external factors such as micro-environment of cuttings and the use of root promoting substances (Dirr & Heuser, 2006; Hartmann, Kester, Davies Jr., & Geneve, 2010). One of the most important and widely reported factors is the use of auxin such as IBA, NAA or a mixture of the two auxins as root promoting substance (Abu-Zahra, Al-Shadaideh, Abubaker, & Qrunfleh, 2013; Rahdari, Khosroabadi, Delfani, & Hoseini, 2014; Costa, Loss, Pereira, & Almeida, 2015). Vegetative propagation of five cultivars of local Malay apple by cuttings as affected by 1000 ppm NAA or 1000 ppm IAA with various media mixture was reported by Ryadin, Ranamukaarachchi, Soni, & Shrestha (2014), however, the rooting percentage and average number of roots were not recorded and since there was no control treatment for the auxins, it could not be concluded that the rooting of Malay apple cuttings was influenced by application of auxins.

In this experiment, IBA, NAA and combinations of IBA+NAA each of which at two total concentrations: 2000 and 4000 ppm w/w, were tested for their effectiveness in rooting of semi-hardwood Malay apple cuttings. Results of this experiment showed that all auxin types treatments (IBA, NAA or IBA + NAA), at total concentrations of 2000 or 4000 ppm significantly stimulated adventitious root formation as shown by the marked increases in rooting percentage from 25 % with only 1.0 root per cutting of control (without auxin) to 79 % - 100 % with 3.2 to 25.5 roots per cutting in the auxins treated cuttings. It was also shown that regardless of the types of auxins applied, increasing concentration from 2000 to 4000 ppm caused significant increases in the number of roots per cuttings, all with 100% rooting percentage. These findings agree with previous statement that for more than 70 years, IBA, NAA or their combination were and are still effectively used as root promoting substance for cuttings from various species commercially, and have been the back bone of cutting propagation success (Dirr & Heuser, 2006). It has been repeatedly confirmed that the formation and divisions of the first root initial cells need the existence of either endogenous or applied auxins. The mechanisms by which IBA stimulate root formation in stem cuttings, as reviewed by Hartmann, Kester, Davies Jr., & Geneve (2010) were through it conversion to IAA, increasing internal free-IBA, enhance tissue sensitivity to IAA, enhance the endogenous IAA synthesis or the action of IAA synergistically, while the stimulatory effect of NAA to induce root formation was probably associated with inhibition of IAA-oxidase (IAAO) activity, thus preventing IAA degradation and increase its activity.

Application of IBA Alone

In this experiment, application of IBA alone at 2000 or 4000 ppm w/w significantly promoted root formation in Malay apple cuttings compared to the control treatment. However, IBA at 4000 ppm was much more effective than at 2000 ppm, since there was an increase of rooting percentage from 79 % to 100 % and an increase of root number from 3.2 to 7.1 roots per cutting. This finding was consistent with previous ones, that IBA at 4000 ppm caused maximum root formation in cuttings of *Dodoneae viscosa* L (Saffari, M. & Saffari, V., 2012), *Bougainvillea glabra* (Seyedi, Esmaeili, & Mostafavi, 2013), *Olea europaea* L. (Thakur, Sharma, & Singh, 2014) and *Schleichera oleosa* Lour. (Jannat, Hossain, & Kamruzzaman, 2016). Accumulating evidence showed that because of the genotype effects, the concentrations of IBA that caused the maximum rooting in cuttings from different species varies. Thakur, Sharma, & Singh (2014) found that application of 4000 ppm IBA was the best treatment for rooting of olive (*Olea europaea* L.) cuttings, while Costa, Loss, Pereira, & Almeida (2015) reported that 2000 ppm IBA resulted in the best root formation in *Bougainvillea spectabilis* Wild. cuttings. Yusnita, Pungkastiani, & Hapsoro (2011) also found that *ex vitro* rooting of two cultivars *Sansevieria* shoots was succesfully performed using 2000 ppm IBA. It was stated by Dirr & Heuser (2006) that nowdays IBA is the most widely used commercial root promoting substance, followed by IAA and NAA. Application of exogenous IBA on cuttings caused the endogenous IAA reaches peak (Krajnc, Turinek, & Ivančič, 2013). In a biochemical study to elucidate IBA mode of action in various plants and the use of *Arabidopsis*-IBA mutants, it was found that IBA acts mainly through its conversion to IAA. However, some scientific findings indicated that IBA exert a hormonal action as an auxin (Pop, Pamfil, & Bellini, 2011).

Effectiveness of NAA on Rootings of Malay Apple

The data from this experiment explained that the order of effectiveness from the highest to the lowest of the auxin types to induce root formation in Malay apple cuttings as indicated by the number of roots per cutting, at concentration 2000 ppm were NAA alone or IBA+NAA as the more effective, followed by IBA alone as the less effective, while at 4000 ppm, NAA was the most effective, followed by IBA+NAA, then followed by IBA as the least effective. Although IBA has long been used to promote rooting in cuttings of various plant species, NAA was found to be more effective than IBA in some plants which respond unsatisfactorily to IBA. In this experiment, Malay apple semi-hardwood cuttings were more responsive to NAA in root formation than to IBA. Similar results were obtained in water apple (*Syzygium javanica* L.) air-layer, that 1000 ppm NAA was found to be more effective than IBA to promote root formation (Paul & Aditi, 2009). Appplication of various concentrations of NAA to cuttings of diffrerent ornamental plants resulted in the best root fomation of different plants at different levels, i.e., 1000 ppm for syngonium, 3000 ppm for rosemary and *Hedera helix*, and 4000 ppm for gardenia (Abu-Zahra, Al-Shadaideh, Abubaker, & Qrunfleh, 2013). In a physiological study using *Hemarthria compressa* stem cuttings, Yan, Li, Zhang, Yang, Wan, Ma, Zhu, Peng, & Huang (2014) reported that the stimulatory effect of NAA to induce root formation was associated with a remarkable increase of polyfenol oxidase (POD) and inhibition of IAA-oxidase (IAAO) activities. IAAO is an enzyme which catalyzes oxidation of IAA, or breaks down endogenous auxin (Hartmann, Kester, Davies Jr., & Geneve, 2010). The activity of POD is reported to be involved in an important step of root formation, during the metabolism of auxin and the process of lignification (Rout, 2006). PPO involved in metabolism of mono-and polyphenols during lignification process in plant cells (Khorsheduzzaman, Alam, Rahman, Mian, & Mian, 2010).

Effects of IBA+NAA in A Combination

As mentioned earlier, the effect of a combinaton of IBA and NAA on rooting of Malay apple cuttings was comparably effective to NAA alone at 2000 ppm, in which both resulted in 100 % rooting percentage and the similar average of root numbers per cutting (16.8-17.8). However, application of 1000 ppm IBA + 1000 ppm NAA in a combination, resulted in a greater root length (10.5 cm), and the adventitious root morphologies were more branched and more fibrous, compared to the unbranched, bigger in diameter of NAA-treated cuttings. The superiority of 1000 ppm IBA+1000 ppm NAA treatment over NAA alone at 2000 or 4000 ppm, or the combination of 2000 ppm IBA+2000 ppm NAA was indicated by the highest percentage of shoot sprouting and its development which was shown by the number of shoots per cuttings, length of shoot and number of leaves per shoots.

The data showed that although NAA at 2000 or 4000 ppm were the most effective to stimulate root formation, they significantly reduced the frequency of the cutting forming shoots (8.3 %). The shoots were even inhibited to the level of zero when the cuttings were given with IBA+NAA each at 2000 ppm. Therefore, it was concluded that among other auxin treatments applied in this experiment, the combination of IBA and NAA at a total concentration of 2000 ppm was the best treatment to promote adventitious root formation in Malay apple semi-hardwood cuttings. This finding was in line with previous results reported by Rahdari, Khosroabadi, Delfani, & Hoseini (2014) that application of IBA + NAA in a combination was better than IBA or NAA alone for rooting of *Cordyline terminalis* and Mohana, Majd, Jafari, Kiabi, & Paivandi (2014) for *Azalea alexander* L.

In this experiment, the fact that a combination of 1000 ppm IBA+1000 ppm NAA stimulated better root formation in Malay apple cuttings was a valuable finding, as in regard to cost consideration, the use of a combination of IBA+NAA would be cheaper than the use of IBA alone. This because the price of IBA is about seven to fourteen-fold more expensive than that of NAA for the same amount. For an illustration, the price of 5 g and 25 g of IBA in the TCI (Tokyo Chemical Industry Co., LTD.) catalogue (Product no. I0026) in December 2017 were US$ 29.00 and US$ 87.00, respectively, while those for 25 g and 500 g of NAA (product number N0005) were US$ 20.00 and US$ 121.00, respectively (www.tcichemicals.com).

Effects IBA + NAA on the Timing and Pecentage of Root and Shoot Formation

Results of the second experiment showed that when compared to control, application of 1000 ppm IBA+1000 ppm NAA not only significantly enhanced rooting percentage, but also shorten the timing needed for root formation. This indicated that Malay apple semi hardwood cuttings lack of endogenous auxin needed during the auxin-active stage of the root initiation stage. Hartmann, Kester, Davies Jr., & Geneve (2010) explained that root formation in cuttings consists of two stages, namely root initiation stage, during which dedifferentiation, followed by initiation of root and development of root primordia occur, and elongation of root primordia stage. The root initiation stage could be devided into an auxin-active stage, which lasted about 4 days, during which the existence of auxin was required continuously for roots to form, and an auxin-inactive state, during which the absence of auxin did not significantly influence root formation. During the stage when root primordia elongated, the root tips grow outward through the cortex, and finally emerging from the epidermis of the stem. Apparently the exogenous application of IBA and NAA to the cuttings has ensured the availability of auxin during the auxin-active stage so that the formation of root initials occured readily, which in turned shortened the overall root formation process compared to the presumeably auxin-lacked of untreated Malay apple semi-hardwood cuttings.

In softwood cuttings, the existance of shoot meristems and young leaves could still supply endogenous auxin continuously. Therefore, the need for exogenous auxin for root initiation was often not essential. However, in most of the cuttings lack of growing tip such as leafless semi-hardwood cuttings of Malay apple used in this experiment, the continuous supply of auxin for the auxin-active stage might become the limiting factor. Therefore, application of exogenous auxin (s) such as a combination of 1000 ppm IBA + 1000 ppm NAA would not only shorten the time needed for formation of adventitious roots, but also enhance rooting percentage and markedly increase root number per cutting, compared to the control treatment (Hartmann, Kester, Davies Jr., & Geneve, 2010).

CONCLUSION AND SUGGESTION

Application of NAA at 2000 or 4000 ppm resulted the highest number of roots, followed by 1000 ppm NAA+1000 ppm IBA, both with 100 % rooting. However, the best treatment for Malay apple cuttings was 1000 ppm IBA+1000 ppm NAA, since it produced greater root length, better root morphology, highest shoot sprouting and shortening the time for root formation.

Further research is needed to investigate effectiveness of auxin application methods in relation to physiological changes during root initiation.

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