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Original article

Effects of Indole-3-Butyric Acid (IBA) and rooting media on rooting and survival of air layered wax apple (*Syzygium samarangense*) CV Jambu Madu

Efeitos do Ácido Indol-3-Butírico (AIB) e meio de enraizamento no enraizamento e sobrevivência de maçã de cera em camada de ar (*Syzygium samarangense*) CV Jambu Madu

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Abstract

The wax apple or *jambu madu*, is a non-climacteric tropical fruit from Myrtaceae family and widely cultivated in South East Asia. The limited availability of good quality seedlings of wax apple is the main problem to development of flourish it's market share in the current fruit industry. Therefore, in order to produce good quality planting materials, a study aimed at optimizing propagation and adventitious rooting technique and survivability of wax apple air layer was conducted. In this study, four different levels of Indole-3-Butyric Acid (IBA) concentration (0, 1000, 1500 and 2000 mg L^{-1}) and three rooting media (sphagnum moss, vermicompost and garden soil) were applied after removal of bark (phloem) on the shoot to determine the effect on rooting and survivability of the wax apple air layer under field conditions. The results showed that the wax apple shoots treated with 2000 mg L^{-1} IBA produced the significantly higher number of roots, increased length of root, diameter of branch, length of branch, number of leaf and leaf area of air layers. In addition, the highest chlorophyll content and stomatal aperture were recorded in 2000 mg L^{-1} IBA treatment compared to other treatments including control. Vermicompost medium was better than garden soil and sphagnum moss in respect of rooting and survivability of air layers. The results showed that the combination of 2000 mg L^{-1} IBA and vermicompost as rooting media give the best combination to root initiation, root number, root length and survival rate (100%) of wax apple air layers. From this study, it can be concluded that 2000 mg L^{-1} IBA and vermicompost treatment enhance the root initiation, early establishment and survivability of wax apple air layered under field conditions.

Keywords: vegetative propagation, fruit, auxin, rooting media, survivability, wax apple.

Resumo

A maçã de cera, ou jambu madu, é uma fruta tropical não climatérica da família Myrtaceae e amplamente cultivada no Sudeste Asiático. A disponibilidade limitada de mudas de macieira de boa qualidade é o principal problema para o desenvolvimento de sua participação de mercado na fruticultura atual. Portanto, com o objetivo de produzir materiais de plantio de boa qualidade, foi realizado um estudo visando otimizar a técnica de propagação e enraizamento adventício e a sobrevivência da camada aérea da cera de macieira. Neste estudo, quatro diferentes níveis de concentração de ácido indol-3-butírico (AIB) (0, 1000, 1500 e 2000 mg L-1) e três meios de enraizamento (musgo esfagno, vermicomposto e solo de jardim) foram aplicados após a remoção da casca (floema) na parte aérea para determinar o efeito no enraizamento e capacidade de sobrevivência da camada de ar da macieira em condições de campo. Os resultados mostraram que os brotos de macieira tratados com 2000 mg L-1 de AIB produziram significativamente maior número de raízes, maior comprimento de raiz, diâmetro de galho, comprimento de galho, número de folhas e área foliar das camadas aéreas. Além disso, o maior teor de clorofila e abertura estomática foram registrados no tratamento 2000 mg L-1 IBA em comparação com outros tratamentos, incluindo o controle.

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O meio de vermicomposto foi melhor do que o solo de jardim e o musgo esfagno em relação ao enraizamento e capacidade de sobrevivência das camadas aéreas. Os resultados mostraram que a combinação de 2000 mg L-1 de AIB e vermicomposto como meio de enraizamento proporciona a melhor combinação para iniciação radicular, número de raízes, comprimento radicular e taxa de sobrevivência (100%) das camadas aéreas de macieira. A partir deste estudo, pode-se concluir que 2.000 mg L-1 de AIB e tratamento com vermicomposto melhoram a iniciação radicular, o estabelecimento precoce e a capacidade de sobrevivência de macieiras em camadas de ar em condições de campo.

Palavras-chave: propagação vegetativa, fruto, auxina, meio de enraizamento, sobrevivência, maçã de cera.

1. Introduction

The wax apple (*Syzygium samarangense*) is a non-climacteric tropical fruit which belongs to the family of Myrtaceae (Zen-hong et al., 2006). Rose apple, Java apple and Water apple are vernacular names of wax apple (Pan and Shu, 2007). In Malaysia, *Syzygium aqueum* (water apple), *Syzygium malaccense* (Malay apple) and *Syzygium samarangense* (wax apple (jambu air madu red, masam manis pink and giant green)) are the three species of wax apple which bear edible fruits. The species wax apple (*Syzygium samarangense*) has become frugally important in Thailand, Taiwan, Malaysia, Indonesia and other Southeast Asian countries (Hwang and Shieh, 2001). The fruits of wax apple are a rich source of polyphenolic antioxidant and have potential benefits for human health (Moneruzzaman et al., 2015). The fruit pulp of the wax apple is a potential source of phenols, flavonoids and antioxidant compounds (Khandaker and Boyce, 2016). The wax apple fruit is a popular fruit in tropical and subtropical areas where it can draw a price up to 3 USD per kilogram and has the potential to carry great benefits to farmers and the national economy (Khandaker et al., 2012). The wax apple can be propagated sexually and asexually by using seed, grafting, budding and air layering. The inner white flesh of wax apple fruits is spongy and light, containing one or two small brown rounded seeds. The viability of wax apple seeds is very low and erratic. It is very hard to produce seedlings from the seed. The sexual method is time-consuming, late bearing and recalcitrant as the seeds naturally lose their viability within a short period of time and multiplication through seed creates wide genetic variability in fruit shape, size, and peel colour. In order to obtain uniform planting and to maintain genetic purity, the asexual method of propagation such as air layering is necessary. One problem in developing the wax apple fruit industry is the limited availability of good quality seedlings.

Air layering is a technique of asexual propagation (vegetative) indicated by the initiation of adventitious roots on an insitu tree shoot or branch. Air layering is applied to mature branches which are capable of flowering and fruiting within the tree canopy, usually horizontal and slightly upright from the ground (Tchoundjeu et al., 2010). The rooted branch is cut from the tree after rooting and transplanted in a polybag containing growing media. Like other techniques of asexual propagation, the main benefits of air layering are the cloning with desired characters of the selected mother trees and shortening the cycle of fruit production (Hartmann et al., 1997). For getting true types trees with precocity, early fruiting, high yielding quality fruits, air layering is a more convenient, cheaper and common method for propagating wax apple trees as well as other fruit trees. Air layering is more successful than stem cutting due to continuous attachment of the air layered branch to the mother trees during rooting, which allows a continuous supply of water, minerals, hormones and photosynthates through the intact xylem and phloem (Alam et al., 2004). It is anticipated that promoting rooting and survival rate of air layered *S. samarangense* can be increased easily if optimum propagation methods have been developed. Thus, to support rapid and mass multiplication of planting materials, propagation of wax apple trees by air layering using different concentrations of IBA and rooting media can be a viable option.

Shekhawat and Manokari (2016) stated that the exogenous application of Indole-3-Butyric Acid (IBA) stimulates the translocation speed and movement of photosynthates to the shoot and promotes root growth and development. Air layering is a popular technique for multiplication of many trees and this vegetative propagation method can perform better when rooting hormone and proper media are used to enhance root formation (Leonardi et al., 2001). Singh (2002) reported that IBA is a widely used rooting hormone which promotes rooting in stem cuttings or air layering, enhances early establishment, bud formation, vegetative growth and increases survival of seedlings. Alam et al. (2004) reported that the rooting media and materials of wrapping affect the root emergence time, number, length and thickness of air layer roots. The rooting performance of air layers depends on the medium types used in the rooting ball (Ofori-Gyamfi, 1998). The physical support, oxygen and water available from the media used in air layering affects rooting and survivability (Kester et al., 1990; Larsen and Guse, 1997). It has been reported that physiology of mother trees, nutritional condition of the tree and soil, wrapping materials, ringing or girdling, etiolation, exogenous rooting hormone, time of air layering and rooting medium used can affect the rooting behavior of air layers (Naithani et al., 2018). They also reported that rooting success in air layering largely depends on the use of rooting hormones, concentration of hormones and growing medium used which may differ from plant to plant. This current study investigated the impacts of different concentrations of IBA and rooting media on the root initiation, early establishment and survivability of air layered wax apple. It is proposed that IBA hormone and rooting media can increase the rooting, growth and survivability of wax apple layer.

2. Materials and Methods

2.1. Experimental design

The experiment was performed at a mini-orchard located in front of the Faculty of Informatics and Computing at Universiti Sultan Zainal Abidin, Besut campus, 22200 Besut, Terengganu, Malaysia. Randomised Complete Block Design (RCBD) was used to arrange the treatments with three (3) replications. This involved four levels of Indole-3-butyric acid hormone concentration (0 mg L^{-1} IBA, 1000 mg L^{-1} IBA, 1500 mg L^{-1} IBA and 2000 mg L^{-1} IBA) and three types of rooting media (sphagnum moss, vermicompost and garden soil). The experiment consisted of twelve treatments of combination between the concentration of IBA and the rooting media of sphagnum moss (wet) + IBA 0 mg L^{-1}, vermicompost + IBA 0 mg L^{-1}, garden soil + IBA 0 mg L^{-1}, sphagnum moss (wet) + IBA 1000 mg L^{-1}, vermicompost + IBA 1000 mg L^{-1}, garden soil + IBA 1000 mg L^{-1}, sphagnum moss (wet) + IBA 1500 mg L^{-1}, vermicompost + IBA 1500 mg L^{-1}, garden soil + IBA 1500 mg L^{-1}, sphagnum moss (wet) + IBA 2000 mg L^{-1}, vermicompost + IBA 2000 mg L^{-1}, and garden soil + IBA 2000 mg L^{-1}. There were three replicates for each treatment combination and a total number of 36 shoots were selected for the treatment application.

2.2. Preparation of Indole-3-Butyric Acid (IBA) solution

As IBA hormone is available in powder form, 1 mg of IBA hormone was dissolved in fifty (50) ml of 95% absolute ethyl alcohol. After that, distilled water of the required amount was added into the conical flax until it reached 1 litre of volume. 1 mg L^{-1} of powdered IBA equal to 1 mg L^{-1} IBA solution. For preparation of 2000 mg L^{-1} IBA solution, IBA powder 2 g was dissolved in 50 ml of 95% alcohol, then the final volume of IBA stock solution was filled up to 1 liter by adding 950 ml of distilled water. The same steps were repeated for preparation of 1500 mg L^{-1} and 1000 mg L^{-1} IBA solution.

2.3. Preparation of rooting media and treatment application on the twig

One (1) kg of sphagnum moss was soaked into water for half an hour until the wet sphagnum moss was ready to be use. Vermicompost was mixed properly to make a friable medium for rooting and seedling establishment. Garden soil was also mixed thoroughly to develop a friable medium for air layering. For this study, nine wax apple trees were selected from the mini orchard which were healthy, vigorous and young stage. After the selection of trees, one-year old twigs around 30 cm in length and pencil thickness were selected randomly for air layering. All the selected twigs had an average diameter 0.8 to 1 cm and were tagged properly before air layering. The leaves were detached from the ringing point of the selected twigs, then a ring of bark (phloem) was removed around 2.7 to 3 cm long by giving two round cuts at both ends of a ring form with a sharp blade. The ring was made around 55 to 65 cm away from the twig tip of the wax apple trees. The cambium layer from the ringed portion of the twig was removed by rubbing it gently with cutting blades. After removal of the cambium layer, the ringed portion of the twigs was treated only one time with IBA hormone using rubbing and swabbing methods and then the rooting media around one (1) kg was placed on the ringed portion and wrapped according to the treatment. The ringed portion of the treated branches were covered and wrapped with white transparent plastic (15 × 15 cm) to see the new roots and tied tightly with plastic string. Treated and untreated branches produced roots around one month after ringing at the abaxial sides of the branches.

2.4. Detachment of air layers from the mother trees

Forty-five (45) days after treatment application, all the air layered produced roots and the twigs were ready for detachment. The rooted air layers were detached from the mother trees when the roots were brown in colour and well developed. The twigs were detached from the trees by making a cut with a sharp saw just below the lower end of the ringed surface. The plastic wrap was removed gently and the air layers were transplanted in well-prepared polybags containing mixture of top soil and cow dung (4:1).

2.5. Measurement of morphological and physiological parameters of air layer

Root number was counted manually at 45 days of layering by taking 3 sample air layers per treatment after detachment of air layers from the mother tree. The length of root of air layers was measured using a measuring tap in cm from the root base up to the root tip. The observations of root number were recorded after detachment of air layer from the mother trees. The number of leaves per air layer was counted manually for each treatment at 45 days after air layering. The length of air layered shoots was measured with a measuring tap in cm for all the treatments. Diameter of air layered shoots was measured by using a vernier caliper.

2.6. Leaf area, chlorophyll content and stomatal conductance

The leaf area of air layer was calculated and recorded by measuring the leaf length and width (average width value of 3 spots in the leaf) for every leaf. The leaf area was calculated by using the following formula: $\text{Leaf area} = \text{length} \times \text{width}\left(\text{cm2}\right)$. The chlorophyll content of air layered leaves was measured using a Konica Minolta Chlorophyll meter (Model: SPAD-502 plus). Five mature leaves from the air layering branch were selected and cleaned randomly for data recording. The chlorophyll content was measured around 11 am and data shown as SPAD value index. A portable leaf porometer (Model SC-1) was used for measurement of stomatal conductance of leaves of air layers. Leaf chamber of porometer was attached to leaf and kept at an ambient temperature for 12 to 15 mins to maintain the sunlight adaptation. Five leaves were selected per treatment and stomatal aperture was from 3 different spots on a single leaf.

2.7. Survival of air layered wax apple

All the cut air layers were transplanted into polybags containing garden soil as a growing medium. The survival rate of transplanted air layers was calculated using the following equation at 60 days after transplanting.

$$\text{Survival rate of air layers}(\%) = \frac{\text{Total number of survived air layers}}{\text{Total transplanted air layers}} \times 100 \quad (1)$$

2.8. Statistical analysis

In this study all the treatments were arranged using a Randomized Complete Block Design (RCBD) with three (3) replicates. Statistical analysis was performed using SPSS 20 software (SPPS Inc). All data were analysed according to two-way ANOVA. Fisher's protected Least Significant Difference (LSD) test was used to separate the mean when F test indicated significance at p ≤ 0.05.

3. Results

The results showed that the application of 2000 mg L^{-1} IBA significantly increased the rooting and survival rate of wax apple air layered compared to 1500 mg L^{-1}, 1000 mg L^{-1} and 0 mg L^{-1} IBA (control), respectively. The use of three rooting media which was vermicompost, sphagnum moss and garden soil produced a significant effect on rooting and survival of air layers. Vermicompost proved to be superior over the sphagnum moss and garden soil media in respect of root characteristics and survivability.

3.1. Number of roots

A two-way analysis of variance was conducted to determine the effects of rooting media and IBA hormone on the root initiation of wax apple air layers. Rooting media and hormone produced significant effects on root production, whereas the interaction effect was non-significant (Table 1). The main effect for IBA concentration yielded an F ratio of F (3, 24) = 75.06, $p < .05$, indicating that the effect for IBA treatment produced a significant effect on the root production of air layered branch. The result indicates that all the concentrations of IBA significantly increased the number of roots of air layer branch over the control (Table 1). The highest number of roots was recorded in the 2000 mg L^{-1} IBA treatment (4.87-fold higher over the control), while, the lowest number of roots was found in the 0 mg L^{-1} IBA (control) air layered branch (Table 1 & Figure 1). It was also found that root initiation of air layered increased with IBA concentration.

The main effect for rooting media yielded an F ratio of F= 36.49, $p < 0.05$, which indicates that the effect of rooting media on root production was also significantly different. Rooting media vermicompost produced the highest number of roots, whereas the lowest number of roots was found in garden soil (Table 1). The interaction effect was not significant, F = 2.09, $p > 0.05$ indicating that the concentration of IBA and rooting media showed a non-significant effect on the root production of air layered branches. The highest number of roots counted (31.33) was with treatment combination M_1C_3 (Sphagnum moss with treatment of 2000 mg L^{-1} IBA), whereas, the minimum number of roots recorded (0.67) was with treatment combination M_3C_0 (Garden soil with treatment of 0 mg L^{-1} IBA) (Table 1).

3.2. Root length

The results show that root length of wax apple air layered was significantly affected by rooting hormone and rooting media although their interactions effect were insignificant (Table 2). The main effect for IBA concentration yielded an F ratio of F (3, 24) = 62.90, $p < 0.05$, indicating that the effect for IBA concentration has a significant difference between 0 mg L^{-1} (M = 2.40), 1000 mg L^{-1} (M = 6.03), 1500 mg L^{-1} (M = 10.07) and 2000 mg L^{-1} (M = 13.11).

The results indicate that the root length of air layer was increased with the concentration of IBA solution. Among different concentrations of IBA, the maximum length of roots (13.11 cm) was recorded in the case of 2000 mg L^{-1} IBA treatment when compared to other concentrations of IBA as well as from control. The main effect for media type yielded an F ratio of F (2, 24) = 4.40, $p < 0.05$, indicating that the effect for media type on root length was significant. The maximum root length of air layers (8.99 cm) was recorded in vermicompost media followed by sphagnum moss media with a root length of 7.6 cm. The lowest root length was recorded in garden soil with a value of 6.86 cm (Table 2). The interaction effect was not significant, F (6, 24) = 0.63, $p > 0.05$ indicating the interaction between IBA and rooting media was found not significant in length of roots. The treatment combination M_2C_3 (Vermicompost with 2000 mg L^{-1} IBA) gave the maximum length of roots (14.11cm) which was found to be significantly higher

Table 1. The influence of different concentrations of IBA, different rooting media and their interaction on number of roots counted.

Media	IBA Concentrations (Number of roots)				
	C_0	C_1	C_2	C_3	Average
M_1	5.00 ± 5.56^{fg}	13.00 ± 2.64^{de}	16.00 ± 1.73^{cd}	24.33 ± 3.05^{b}	14.58 ± 7.83
M_2	9.00 ± 1.73^{ef}	11.67 ± 2.88^{de}	26.33 ± 3.78^{ab}	31.33 ± 3.51^{a}	19.58 ± 10.22
M_3	0.67 ± 1.15^{g}	3.00 ± 3.60^{g}	12.33 ± 3.21^{de}	19.00 ± 1.73^{c}	8.75 ± 8.00
Average	4.89 ± 4.67	9.22 ± 5.40	18.22 ± 6.81	24.89 ± 5.90	14.31 ± 9.61

Means (± S. D) followed by different letters of the alphabet indicates statistically significant at $p < 0.05$. S. D. = Standard deviation. [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3= 2000 mg L^{-1}IBA]

Figure 1. Effects of different rooting media and various concentrations of IBA on the rooting of wax apple air layers. M_1C_0: Sphagnum moss (wet) + IBA 0 mg L^{-1}, M_2C_0: Vermicompost + IBA 0 mg L^{-1}, M_3C_0: Garden soil + IBA 0 mg L^{-1}, M_1C_1: Sphagnum moss (wet) + IBA 1000 mg L^{-1}, M_2C_1: Vermicompost + IBA 1000 mg L^{-1}, M_3C_1: Garden soil + IBA 1000 mg L^{-1}, M_1C_2: Sphagnum moss (wet) + IBA 1500 mg L^{-1}, M_2C_2: Vermicompost + IBA 1500 mg L^{-1}, M_3C_2: Garden soil + IBA 1500 mg L^{-1}, M_1C_3: Sphagnum moss (wet) + IBA 2000 mg L^{-1}, M_2C_3: Vermicompost + IBA 2000 mg L^{-1} and M_3C_3: Garden soil + IBA 2000 mg L^{-1}.

Table 2. The effect of different concentrations of IBA, different rooting media and their interaction on length of wax apple roots.

Media	IBA Concentrations (Length of air layer roots)				
	C_0	C_1	C_2	C_3	Average
M_1	3.11 ± 3.16^{f}	5.66 ± 2.96^{ef}	9.66 ± 3.28^{cd}	13.00 ± 1.67^{ab}	7.86 ± 4.63
M_2	4.11 ±0.69^{ef}	7.00 ± 0.88^{de}	10.77 ± 0.77^{bc}	14.11 ± 0.84^{a}	8.99 ± 4.00
M_3	0.00^{g}	5.44 ± 0.83^{ef}	9.77 ± 1.34^{cd}	12.22 ± 0.19^{ac}	6.86 ± 4.89
Average	2.40 ± 2.46	6.03 ± 1.76	10.07 ± 1.89	13.11 ± 1.24	7.90 ± 4.48

*Means (± S. D) within the same column followed by the same letter, do not differ significantly according to LSD test at *ά=0.05*. . [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3= 2000 mg L^{-1}IBA]

than the rest of the treatment combinations. Minimum length of roots (0 cm) was recorded under the treatment combination M_3C_0.

3.3. Branch length

The main effect for IBA concentration yielded an F ratio of $F (3, 24) = 2.02$, $p > 0.05$, indicating that the effect for IBA concentration was not significant between the control and different treatments. The maximum length of branch (38.05 cm) was recorded in the case of 2000 mg L^{-1} IBA treatment, whereas, the minimum length of branch (35.33 cm) was observed in the control treatment (0 mg L^{-1} IBA). The main effect for media type yielded an F ratio of $F (2, 24) = 1.79$, $p > 0.05$, indicating that the effect for media type was not significant between sphagnum moss (M = 37.40 cm), vermicompost (M = 35.90 cm) and garden soil (M = 35.40 cm). The results show that the length of branch was higher in Sphagnum moss (M_1) than in Vermicompost (M_2) and garden soil (M_3) respectively (Table 3). The interaction effect was not significant, $F (6, 24) = 0.75$, $p > 0.05$ indicating that IBA concentrations and rooting media did not affect the length of branch. The branch length was 1.2 times greater in the M_1C_3 treatment compared to the M_2C_1 treatment, although their differences were statistically non-significant (Table 3).

3.4. Branch diameter

The main effect for IBA concentration yielded an F ratio of F (3, 24) = 2.55, $p > 0.05$, indicating that the effect for IBA concentration was not significant between the treatments and control. The maximum diameter of branch (15.88 mm) was recorded in C_3 (2000 mg L^{-1} IBA), while, the minimum diameter of branch (15.27 mm) was recorded with treatment C_1 (1000 mg L^{-1} IBA) (Table 4). The main effect for rooting media yielded an F ratio of F (2, 24) = 2.05, $p > 0.05$, indicating that the effect for media type on branch diameter of air layers was not significant between treatments. The largest branch diameter was recorded in garden soil (M_3), while the smallest branch diameter was recorded in Sphagnum moss (M_1) rooting media. In this study, rooting media produced a significant effect on the rooting of air layered wax apple. The interaction effect of IBA and rooting media for branch diameter has a significant difference, F (6, 24) = 2.74, $p < 0.05$ indicating the IBA concentration has a greater effect in vermicompost than in sphagnum moss and garden soil, respectively. The branch diameter was 10% greater in treatment combination M_2C_3 compared to M_2C_1 (Table 4).

3.5. Leaf number

The main effect for IBA concentration yielded an F ratio of F (3, 24) = 6.077, $p < 0.05$, indicating that the effect for IBA concentration has a significant difference between treatments and control (Table 5). The highest number of leaves (39.78) was recorded with treatment C_3 (2000 mg L-1 IBA), whereas, the minimum number of leaves (37.00) was recorded with the control treatment (C_0). It was also found that leaf number increased with hormone concentration. The main effect of media type yielded an F ratio of F (2, 24) = 5.36, $p > 0.05$, indicating that the effect for media type was not significant between the media used in this study. The garden soil (M_3) treatment produced 3.4% more leaves compared to the rooting media vermicompost (M_2). The interaction effect was not significant, F (6, 24) =0.23, $p > 0.05$ as the effect of one variable has less effect on the number of leaves (Table 5). The maximum number of leaves (40.67) was obtained from the treatment combination M_3C_3 (Garden Soil with 2000 mg L^{-1} IBA). This hormone and rooting media combination proved significantly superior than the other treatment combinations with regards to leaf number.

Table 3. The influence of different treatments of IBA, different rooting media and their interaction on branch length.

Media	IBA Concentrations (Branch length)				
	C_0	C_1	C_2	C_3	Average
M_1	36.36 ± 1.73^{ab}	36.56 ± 3.10^{ab}	37.10 ± 3.29^{ab}	39.60 ± 3.81^{a}	37.40 ± 2.95
M_2	36.00 ± 3.12^{ab}	33.06 ± 2.28^{b}	36.20 ± 1.20^{ab}	38.33 ± 2.72^{a}	35.90 ± 2.85
M_3	33.63 ± 2.00^{b}	36.43 ± 2.3^{7ab}	35.33 ± 3.46^{ab}	36.23 ± 1.84^{ab}	35.40 ± 2.42
Average	35.33 ± 2.41	35.35 ± 2.84	36.21 ± 2.57	38.05 ± 2.91	36.23 ± 2.81

Means (± S. D) followed by different letters of the alphabet indicates statistically significant at $p < 0.05$. S. D. = Standard deviation. [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3 = 2000 mg L^{-1}IBA]

Table 4. Effects of various concentrations of IBA, different rooting media and their interaction on branch diameter.

Media	IBA Concentrations (Branch diameter)				
	C_0	C_1	C_2	C_3	Average
M_1	15.61 ± 0.08^{bc}	15.46 ± 1.43^{bc}	15.26 ± 0.02^{bc}	15.59 ± 0.22^{bc}	15.48 ± 0.63
M_2	15.09 ± 0.01^{c}	14.94 ± 0.05^{c}	15.33 ± 0.04^{bc}	16.60 ± 0.34^{a}	15.49 ± 0.70
M_3	16.05 ± 0.18^{ab}	15.42 ± 0.02^{bc}	15.49 ± 0.12^{bc}	15.45 ± 0.89^{bc}	15.60 ± 0.47
Average	15.58 ± 0.42	15.27 ± 0.76	15.36 ± 0.12	15.88 ± 0.73	15.52 ± 0.59

Means (± S. D) followed by different letters of the alphabet indicates statistically significant at $p < 0.05$. S. D. = Standard deviation. [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3 = 2000 mg L^{-1}IBA]

Table 5. Effects of various concentrations of IBA, different rooting media and their interaction on number of leaves.

Media	IBA Concentrations (Number of leaves)				
	C_0	C_1	C_2	C_3	Average
M_1	37.33±2.08b^{cd}	36.67 ± 0.57^{cd}	38.00 ± 1.00^{ad}	40.00 ± 0.00^{ab}	38.00 ± 1.65
M_2	36.00 ± 3.60^{d}	36.67 ± 2.30^{cd}	38.33 ± 0.57^{ad}	38.67 ± 1.52^{ad}	37.42 ± 2.27
M_3	37.67 ±1.52^{bcd}	37.67± 0.57^{bcd}	39.00 ± 1.00^{ac}	40.67 ± 1.15^{a}	38.75 ± 1.60
Average	37.00 ± 2.34	37.00 ± 1.32	38.44 ± 0.88	39.78 ± 1.30	38.06 ± 1.89

Means (± S. D) followed by different letters of the alphabet indicates statistically significant at $p < 0.05$. S. D. = Standard deviation. [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3 = 2000 mg L^{-1}IBA]

3.6. Leaf area

The main effect for IBA concentration yielded an F ratio of F (3, 24) =170.56, $p < 0.05$, indicating that the effect for IBA concentration has a significant difference between the control and IBA treatments. All the treatments of IBA gave a higher leaf area over the control. The maximum leaf area (4.56 cm^2) was recorded at treatment C_3 (2000 mg L^{-1} IBA), while, the control treatment produced the leaf with the smallest area (4.26 cm^2). The main effect for media type yielded an F ratio of F (2, 24) = 32.53, $p < 0.05$, indicating that the type of media has a significant effect on the leaf area of air layers. The maximum leaf area (4.44 cm^2) was observed in vermicompost treatment (M_2), whereas, the minimum leaf area (4.35cm^2) was recorded in sphagnum moss (M_1) (Table 6). The interaction effect has a significant difference, F (6, 24) = 2.84, $p < 0.05$ indicating the IBA concentration and rooting media show significant effects to leaf area. The maximum leaf area (4.64 cm^2) was recorded with treatment combination M_2C_3 and the minimum leaf area (4.23 cm^2) was recorded with treatment combination M_1C_0 (Table 6).

3.7. Chlorophyll content

The main effect for IBA concentration yielded an F ratio of F (3, 24) = 13.43, $p < 0.05$, indicating that the effect for IBA concentration has a significant difference between the treatments and control (Table 7). The chlorophyll content was highest (39.51 SPAD) in treatment C_2 (1500 mg L^{-1} IBA), while the minimum amount of chlorophyll content (30.47 SPAD) was found with treatment C_1 (1000 mg L^{-1} IBA).

The main effect for media type yielded an F ratio of F (2, 24) = 5.77, $p < 0.05$, indicating that the effect for media type has a significant effect on leaf chlorophyll content. The maximum chlorophyll content (38.12) was observed in garden soil (M_3) followed by vermicompost treatment (M_2), whereas, the minimum chlorophyll content (33.68) was recorded with treatment using Sphagnum moss (M_1). The interaction effect was significant, F (6, 24) = 5.70, $p < 0.05$ indicating the IBA concentration and rooting media show significant effect to the leaf chlorophyll content. The highest chlorophyll content (43.68) was recorded with treatment combination M_3C_3 and the lowest chlorophyll content (29.68) was recorded with treatment combination M_1C_0 (Table 7). Sphagnum moss acts as a soil conditioner and contributes significantly to provide a reservoir of soil water available to plants for plant physiological function.

3.8. Stomatal conductance

The main effect for IBA concentration yielded an F ratio of F (3, 24) = 1.481, $p > 0.05$, indicating that the effect for IBA concentration was not significant. Leaf stomatal conductance was the highest in C_3 treatment with a value of 150.14 mmol m^{-2} s^{-1}, while, the least stomatal conductance (124.19 mmol m^{-2} s^{-1}) was recorded in the control treatment (C_0). The main effect for media type yielded an F ratio of F (2, 24) = 2.288, $p > 0.05$, indicating that the effect for media type on stomatal conductance was not significant. The maximum stomatal conductance recorded (149.61 mmol m^{-2} s^{-1}) was with treatment of vermicompost (M_2) and the minimum stomatal conductance recorded (125.57) mmol m^{-2} s^{-1} was with sphagnum moss treatment (M_1) (Table 8). The interaction effect was also not significant, F (6, 24) = 0.525, $p > 0.05$ indicating the IBA concentration and rooting media did not affect the leaf stomatal conductance significantly.

Table 6. Effects of IBA concentration, rooting media and their interaction on leaf area (cm^2) of wax apple seedlings.

Media	IBA Concentrations (Leaf area of air layer seedlings)				
	C_0	C_1	C_2	C_3	Average
M_1	4.23 ± 0.01^{f}	4.31 ± 0.01^{e}	4.38 ± 0.01^{d}	4.48 ± 0.01^{c}	4.35 ± 0.09
M_2	4.28 ± 0.01^{e}	4.40 ± 0.04^{d}	4.45 ± 0.04^{c}	4.64 ± 0.01^{a}	4.44 ± 0.13
M_3	4.28 ± 0.01^{e}	4.37 ± 0.06^{d}	4.38 ± 0.01^{d}	4.57 ± 0.02^{b}	4.40 ± 0.11
Average	4.26 ± 0.02	4.36 ± 0.05	4.40 ± 0.04	4.56 ± 0.07	4.40 ± 0.12

Means (± S. D) followed by different letters of the alphabet indicates statistically significant at $p < 0.05$. S. D. = Standard deviation. [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3 = 2000 mg L^{-1}IBA]

Table 7. The effect of different concentration of IBA and rooting media and their interaction on the leaf chlorophyll content (SPAD value) of wax apple air layers.

Media	IBA Concentrations (Leaf chlorophyll content of air layer seedlings)				
	C_0	C_1	C_2	C_3	Average
M_1	29.68 ± 1.51^{cf}	33.50 ± 1.90^{e}	38.81 ± 1.12^{abc}	32.74 ± 4.03^{df}	33.68 ± 4.00
M_2	41.52 ± 2.02^{ab}	30.27 ± 1.35^{f}	36.66 ± 2.54^{bd}	40.73 ± 6.96^{ab}	37.29 ± 5.72
M_3	38.11 ± 2.89^{ad}	27.64 ± 2.28^{f}	43.06 ± 2.65^{a}	43.68 ± 5.81^{a}	38.12 ± 7.41
Average	36.43 ± 5.61	30.47 ± 3.02	39.51 ± 3.41	39.05 ± 6.97	36.37 ± 6.03

Means (± S. D) followed by different letters of the alphabet indicates statistically significant at $p < 0.05$. S. D. = Standard deviation. [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3 = 2000 mg L^{-1}IBA]

Table 8. The influence of IBA concentrations, different rooting media and their interaction on the leaf stomatal conductance.

Media	IBA Concentrations (Leaf stomatal conductance of air layer seedlings)				
	C_0	C_1	C_2	C_3	Average
M_1	119.69± 47.8^{b}	135.70 ±34.8^{ab}	124.7 ±19.50^{ab}	126.2 ±19.12^{ab}	126.5 ±28.45
M_2	130.9 ± 34.0^{ab}	153.25 ±34.2^{ab}	158.3 ±19.66^{ab}	155.9 ±25.98^{ab}	149.6 ±27.30
M_3	121.91± 18.8^{b}	133.34 ±10.3^{ab}	138.1 ± 9.51^{ab}	168.3 ± 16.68^{a}	140.4 ±21.71
Average	124.19 ± 31.2	135.70 ± 34.87	140.3 ± 20.72	150.1 ± 26.09	138.8 ±27.00

Means (± S. D) followed by different letters of the alphabet indicates statistically significant at $p < 0.05$. S. D. = Standard deviation. [M_1 = sphagnum moss, M_2 = vermicompost, M_3 = garden soil, C_0 = 0 mg L^{-1}, C_1 = 1000 mg L^{-1}, C_2 = 1500 mg L^{-1}, & C_3 = 2000 mg L^{-1}IBA]

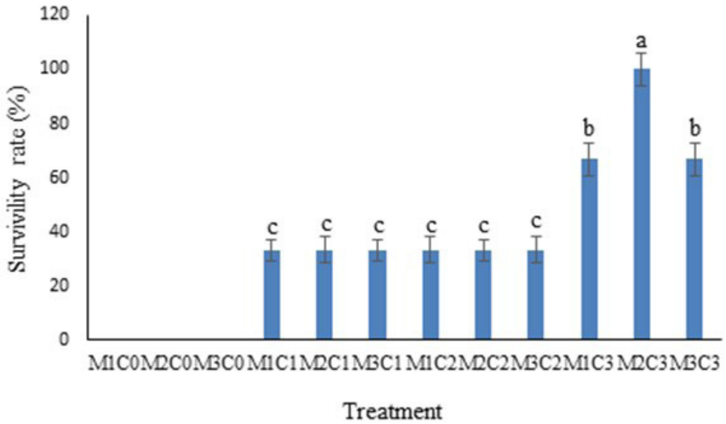

Figure 2. Effects of IBA hormones and different rooting media on the survivability of rate of wax apple air layered. M_1C_0: Sphagnum moss (wet) + IBA 0 mg L^{-1}, M_2C_0: Vermicompost + IBA 0 mg L^{-1}, M_3C_0: Garden soil + IBA 0 mg L^{-1}, M_1C_1: Sphagnum moss (wet) + IBA 1000 mg L^{-1}, M_2C_1: Vermicompost + IBA 1000 mg L^{-1}, M_3C_1: Garden soil + IBA 1000 mg L^{-1}, M_1C_2: Sphagnum moss (wet) + IBA 1500 mg L^{-1}, M_2C_2: Vermicompost + IBA 1500 mg L^{-1}, M_3C_2: Garden soil + IBA 1500 mg L^{-1}, M_1C_3: Sphagnum moss (wet) + IBA 2000 mg L^{-1}, M_2C_3: Vermicompost + IBA 2000 mg L^{-1} and M_3C_3: Garden soil + IBA 2000 mg L^{-1}. Bars represent means (n = 7) ± standard error (S.E.). a, b, and c indicate significant differences within each growth period (p < 0.05).

3.9. Survival of air layers

The survival rate of wax apple air layers was calculated in this study (Equation 1). The results show that the survivability of wax apple air layers is significantly affected by rooting hormone and rooting media (Figures 2 and 3). The highest survival rate of air layers (100%) were found in treatment C_3 (IBA 2000 mg L-1) followed by C_2 and C_1 treatments, while, all the air layers died in the control treatment (C_0). The result of the experiment show that the combination treatment with 2000 mg L^{-1} IBA and vermicompost as the rooting media for air layering have 100% survival percentage. This proved that all three replicates under the treatment managed to survived when the air layers were planted in the polybags (Figure 3). All the control replicates without hormone (0 mg L^{-1} IBA) could not survive when planted in the polybags (Not shown in figure 3).

4. Discussion

In this study, the effects of Indole-3-Butyric Acid (IBA) and rooting media were investigated on the rooting and survival of wax apple air layers under field conditions. Our results show that 2000 mg L^{-1} IBA treatment produced the highest number of roots of wax apple air layers. It was also indicated that an optimum level of IBA, increased the breakdown of amyloplast of the cell wall and stimulated the activity of cambium, thus increasing the mobilisation of photosynthates to the root formation sites of air layers, hence promoting root formation. The exogenous applied IBA stimulated the formation of callus, boosted interfascicular cambium dedifferentiation and produced many cells which differentiated in root primordia and root cells as per findings of Singh et al. (2014). They also reported that optimum levels of exogenous IBA application increase the breakdown of starch and the decline in levels of amyloplast which promote rooting in stem cuttings of duranta golden. An optimum concentration of IBA produced the highest root number in *Vitis vinifera* compared to the control treatment (Shahzad et al., 2019). This is due to the exogenous application of IBA may increase the speed of translocation and movement of carbohydrates from the leaf to the cutting area of the stem or air layering and promote root growth and development of seedlings (Shekhawat and Manokari, 2016).

Different types of rooting media also produced significant effects on the rooting of air layers. The highest

Figure 3. Survival rate of air layers (%) of wax apple as affected by different concentrations of IBA and growing media after detaching from mother plants. M_1C_1: Sphagnum moss (wet) + IBA 1000 mg L^{-1}, M_2C_1: Vermicompost + IBA 1000 mg L^{-1}, M_3C_1: Garden soil + IBA 1000 mg L^{-1}, M_1C_2: Sphagnum moss (wet) + IBA 1500 mg L^{-1}, M_2C_2: Vermicompost + IBA 1500 mg L^{-1}, M_3C_2: Garden soil + IBA 1500 mg L^{-1}, M_1C_3: Sphagnum moss (wet) + IBA 2000 mg L^{-1}, M_2C_3: Vermicompost + IBA 2000 mg L^{-1} and M_3C_3: Garden soil + IBA 2000 mg L^{-1}.

number of roots was recorded in vermicompost media as compared to garden soil and Sphagnum moss used in this study. The porosity and aeration characters of the growing media may affect the root development and growth of air-layered branch (Amri et al. 2009). Rymbai et al. (2012) also found that coco peat and sphagnum moss increased the root initiation and the number of primary and secondary roots within 45 days after transplanting (DAT) of guava air layer. The results show that IBA treatment increased the root length of treated air layers while the minimum length of roots was observed in the control treatment (0 mg L^{-1} IBA). The IBA protein breaks the hydrogen bonds between the cellulose micro fibrils and thus promotes cell wall loosening and elongates the root cells (Kumar et al., 2015; Ouyang et al., 2015). The rate of cambium cell differentiation, hydrolytic activity and callus formation are increased at an optimal concentration of IBA hormone, which increases root length and develops a root system (Gilani et al., 2019). The hormone activity and benefit of exogenous plant growth regulators depends on the right concentration and application technique (Nasir et al., 2020). Exogenous IBA hormone increases the root number and length of roots of the air layers thus enhancing the vegetative growth of seedlings (Singh et al., 2014). This improved root system may enhance water and nutrient uptake, thus increasing the survivability of the air layers under potted condition.

The treatment combination 2000 mg L^{-1} IBA and vermicompost increased significantly the root length of wax apple air layers. Atiyeh et al. (2000) stated that vermicompost contains a considerable amount of potential nutrients and minerals which can improve plant growth and development. It has been reported that the addition of vermicompost to rooting media decreases bulk and particle density but increases water holding capacity (Sinha et al., 2010). Thus this technique is supportive to create a favourable condition for growth and development of stem cuttings or air layering. In addition, the balance between IBA and other constituents in plant cells or tissues regulates organ formation and are the source for root initiations and root development (Westwood, 1973). The balance of hormone and other essential elements may be accomplished by a combination of genetics as well as chemical and environmental factors. Our results showed that IBA treatment increased branch length and diameter of air layers but the effect was not statistically different. Gond et al. (2017) also stated that the IBA significantly increased the length of branches over the control. They also stated that shoot length of air layers was affected insignificantly by rooting media and sphagnum moss and which was comparatively better than the vermicompost or farm yard manure. In this study, the interaction effect of rooting hormone and media on length of air layers was also insignificant, however, Gond et al. (2017) reported that IBA and rooting media significantly affected the length of air layered branches of cape gooseberry.

IBA (auxin), a root inducing hormone, had no direct effect on shoot bud development, branch length and diameter of wax apple air layers. It was stated earlier that the optimum concentration of IBA increased the number and length of roots in wax apple air layers. Thus, a better root system increases the surface area of nutrient and water absorption from the rooting media and creates an impact on branch length and diameter of air layers (Gilani et al., 2019). Akwatulira et al. (2011) also stated that exogenous application of IBA at the growing points of cuttings enhances the mineral nutrients transport and promotes growth of the cuttings. Chalapathi et al. (2001) reported that exogenous IBA treatment increased root initiation, roots number, length and thickness of roots, as well as fresh and dry biomass of stem cuttings of stevia plants. Exogenous application of plant growth regulators produces effects on vegetative growth of plant parts or organ (Pessarakli, 2002). Ofori et al. (1996) reported that the aeration and water holding capacity of the rooting media affects the root formation and a balance between hormone and media must be maintained to ensure maximum rooting which can lead to healthy seedlings. May be the application of exogenous IBA enhanced the endogenous levels of NO and H_2O_2 in the air layers. This elevated level of H_2O_2 activated protein related to cell division, cell elongation, plant signalling and development (Barba-Espin et al., 2010; Ismail et al., 2015). The optimum combination of IBA and vermicompost increases the number of roots of air layers of cape gooseberry, and the improved root system creates positive effects on seedling length and shoot diameter (Gond et al., 2017).

Our results showed that IBA application produced a significant effect on the leaf number of air layers. It has been reported that application of auxin hormone increased the leaf number of air layers (Gond et al., 2017). Vyas et al. (2017) also found that an optimum concentration of IBA increased the number of leaves and stimulated shoot growth of air layering. The exogenous IBA hormone with cytokinin increased the cell differentiations and produced more leaf primordia in the air layer branch. IBA application with rooting medium also increased the seedlings height, number of shoots, leaves per rooted layer and physiological characteristics of plants (Mousa et al., 2019; Khandaker et al., 2017). Gilani et al. (2019) also reported that adding IBA at 150 mgL^{-1} into the rooting media increased the root and shoot characteristics, maximum survival rate and number of new leaves in guava air layers. IBA probably enhanced the translocation of carbohydrates, plant growth regulators and nutrients to the root formation zone for rooting and establishment of the air layers (Yeboah et al., 2014).

In this study, the rooting media and interaction of rooting media and hormones produced a significant effect on leaf area of rooted wax apple air layers. With root inducing hormone applied with rooting medium produced more roots in cuttings, thus absorbing more nutrients and water, and increased the leaf area stem cuttings (Stancato et al., 2003; Mahmod et al., 2020). The aeration capacity of the rooting media is accountable for improving metabolic activities, enhancing vegetative growth and survivability of air layers (Yeboah et al., 2009). The highest shoot number and shoot length recorded in milled pine bark media might also be due to speedy translocation of water and mineral elements from the below ground parts to the stem of the cuttings. Thus, leading to rapid growth and development which contributed to higher leaf area of leaves (Akwatulira et al., 2011). Mahmod et al. (2020) also reported that plant growth regulators application improved callus multiplication and differentiation in the stem culture of ubi gadong. Our study indicated that rooting hormone, rooting medium and their interaction produced significant effects on leaf chlorophyll content of wax apple air layers. Ludwig-Muller (2000) also explained that the IBA treatment increases chlorophyll accumulation in leaves of air layers. Vermicompost is a source of mineral nutrients and the N, P, K, Fe, Mg and Cu content of vermicompost enhance chlorophyll formation, which is required for light harvesting and conversion of light energy to chemical energy via photo assimilation (Tanaka et al., 1998). Vermicompost improves the soil physical properties, serves as buffer against drought stress, maintains pigments content, increases early establishment and survivability of plants (Johnson and Leah, 1990). In our study, it was found that IBA added with rooting media did not affect the leaf stomatal conductance of wax apple air layers. Borah and Baruah (2016) also reported that exogenous application of IBA on the rooting media did not produce positive effects on the stomatal conductance of air layers.

The results show that IBA application with the rooting medium increases the survivability of air layers of wax apple. The exogenous IBA hydrolyse the cuticle layer of the epidermis makes a tight seal at the basal end of stem cuttings or air layers soon after callusing thus preventing it from drying and decay, thus increasing the survival rate of air layers. The K^+ ions in IBA plays a significant role in root initiation and development by dissolving the root epidermal or protodermal layer. Sharma et al. (2009) also reported that IBA at 5000 mg L^{-1} treatment increases the survivability rate of pomegranate cuttings. Piotrowska-Niczyporuk and Bajguz (2014) reported that exogenous auxin added to the growth medium increased the chlorophyll, carotenoid, protein, and simple sugar content which plays a substantial role in the growth of development of *C. vulgaris*. Moreover, IBA or IAA treatment stimulates enzymatic and non-enzymatic antioxidant systems in plants, therefore repressed lipid peroxidation and H_2O_2 accumulation, and increases the survivability rate of air layered seedlings. Bhojvaid and Seema (2003), also reported that IBA with garden soil and farm yard manure stimulated root formation and development within a short time and increased the survivability of rooted air layers.

This study provided evidence that the type of rooting media is one of the most important factors that affects the rooting and survival of air layers. Our results showed that the survivability rate in vermicompost media was higher than the garden soil and sphagnum moss media. Vermicompost is nutritive growing media rich in nitrogen, phosphorus, potassium, calcium, magnesium, zinc and manganese (Kale and Bano, 1986). Amylase, lipase, cellulase and chitinase enzymes of the vermicompost break down the organic matter of the soil into humas which is readily available for the plant. On top of that, vermicompost is a source of auxins, cytokinin and gibberellins which regulate the plant growth and development (Tomati et al., 1998). The aeration, water holding capacity and nutrient availability of the rooting media affects the root development and survivability of stem cuttings or air layers (Alikhani et al., 2011). Akwatulira et al. (2011) also reported that rooting media affect the survivability of stem cuttings of Ugandan green heart, a species of evergreen tree, and the highest survivability was recorded in pine bark media compared to mixtures of sand and top soil. The success of rooting and survivability of wax apple air layers using vermicompost could be attributed to the positive influence of aeration, water holding capacity and nutrient availability. This allows more translocation of water and nutrient elements to the upper part of the air layers leading to form shoot bud and rapid bud break. All the nutrient elements, enzymes and growth regulators present in vermicompost may stimulate root initiation, development and bud break and thus enhance the survivability of wax apple air layers.

5. Conclusions

The main objective of the study was find the best way of enhancing the root initiation, growth and survivability of wax apple air layers by using Indole-3-Butyric Acid (IBA) and suitable rooting media. The exogenous application of IBA with rooting media, particularly IBA at 2000 mgL^{-1} increased the rooting, chlorophyll content, vegetative growth and survival rate of wax apple air layers. Among the rooting media, vermicompost was the utmost effective treatment in promoting the rooting, leaf and shoot growth, chlorophyll accumulation, and survival of air layers. The highest survival rate (100%) of wax apple air layers was recorded in the treatment combination of 2000 mg L^{-1} IBA and vermicompost medium. As a result, it is concluded that the combination of 2000 mgL^{-1} IBA with vermicompost medium is the best option for air layering in wax apple fruit trees to produce quality planting materials. The identification of internal plant growth regulators responsible for root initiation, development and early establishment of air layers merit further study.

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