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Original article

Indole-3-butyric acid induced adventitious root of *Dendrodium milla nayla* x *Dendrobium striaenopsis* planted on coco-husk and wood charcoal during acclimatization stage

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Abstract

Orchids are one of the most favorite cut flowers, flowering potted plants, and have developed into a highly profitable industry. The development of acclimatization method is a necessity for the high survival rate of plantlets. The aim of this study was to investigate the effect of low indole-3-butyric acid (IBA) concentration on faster root formation of Dendrobium hybrid planted on coco- husk and charcoal in a short time. Plantlets derived from the seed culture of *Dendrodium milla nayla* x *Dendrobium striaenopsis* were harvested and pre-acclimated, planted on charcoal or coco-husk, and regularly sprayed with 0.25, 0.50, 0.75, and 1.0 mg/L IBA twice a week for one month. The results showed that IBA at 0.50 mg/L provided the most appropriate concentration for immediate root induction and growth of Dendrobium hybrid planted on coco-husk. Root number (1.75) and root length (0.28 cm) showed the highest and the most important indicator of adventitious root induction
.

Keywords: IBA, Dendrobium, coco-husk, charcoal

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Introduction

Orchids are among the most popular cut flower and flowering potted plants and have emerged as a good indicator of a healthy, functioning ecosystem, as they interact with other plants, fungi, and animals for germination and specific pollination (An et al., 2021). In fact, the orchid family (Orchidaceae) is one of the largest and most diverse families of flowering plants, with more than 28.000 recognized species comprising 763 genera of 295.383 flowering plants (Christenhusz and Byng, 2016). These species are particularly abundant in the humid tropics worldwide, excluding polar and desert regions (Chase, 2005). The life forms of orchids can be terrestrial, epiphytic, lithophytic, or saprophytic (Zhang et al., 2018). However, as they are the most evolved of all flowering plants, they require optimized conditions to survive in an ecosystem (An et al., 2021).

Commercial production has expanded greatly and has grown into a highly profitable industry (Zhang et al., 2018). It would appear that conventional propagation of orchids from seed fails to meet the demand, thus farmers have been adopting tissue culture to meet the market demands (Santoso et al., 2020). Tissue culture remains the preferred method of selection for orchid propagation and conservation over conventional methods (Restanto et al., 2016). However, the survival rate of plant tissue cultures in the final stage, including Dendrobium hybrids, remains low (da Silva et al., 2015).

Rapidly fluctuating conditions during acclimation to *ex vitro* conditions are very stressful for plants and can cause a high rate of mortality (Poniewozik et al., 2021). The success of any commercial-scale *in vitro* propagation technique relies on the ability to regenerate large numbers of plantlets at low cost (Barry-Etienne et al., 2002) and transfer them to *ex vitro* conditions at high survival rates (Hazarika, 2006). In general, plant tissue cultures exhibit some malformations of the leaf and root system (Hazarika, 2006). Gradual improvement of morphological abilities of orchids during acclimation is a prerequisite for the adaptation process during acclimation (da Silva et al., 2017).

As an epiphytic plant, the exposed roots of Dendrobium orchids absorb moisture from moist air as well as from dew (Atwell et al., 2010). When adapting under artificial conditions, aeration, capillary action, water, and nutrient uptake capacity of the substrate, which is readily available and cost-effective, should be considered (da Silva et al., 2017). The most widely used substrates for acclimation of plant tissue culture are organic materials such as charcoal pieces, pine bark, cycas bark, coco-husk, coconut shells, sawdust, sphagnum moss, fern roots, or mineral materials such as perlite and vermiculite (da Silva et al., 2017), which have different properties in terms of water absorption and retention. Charcoal, which is made from plant residues, usually has higher nutrient content and lower water retention, but is capable of controlling acidity (Hamidi et al., 2021). Coco-husk, on the other hand, can absorb water (da Silva et al., 2017) and store higher levels of nutrients. Developing reliable rooting during acclimation is essential to extend plant longevity and ensure plant survival (Oakes et al., 2020). It has traditionally been known that auxins exert different roles in promoting the root development of different species (Pacurar et al., 2014). Among auxins, Indole-3-butyric acid (IBA) is

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preferable to the others (Wei et al., 2014). Since IBA is the most effective and stable promoter of root formation, it is widely used in clonal propagation for example Camellia sinensis (Wei et al., 2019) and Arabidopsis thaliana (Fattorini et al., 2017). The aim of this research was to investigate the effect of low IBA concentration on faster root formation of Dendrobium hybrids planted on coco-husk and charcoal in a short period of time.

Methods

Experiments were performed from October to December 2021, located in Kebun Anggrek Singosari, Malang. Plants were kept under screen-house condition with 60% filtered sunlight and manually irrigated to manage lower temperature at 27-30°C and higher humidity at 60-80%.

Plant materials and substrate

Plantlets of *Dendrodium milla nayla* x *Dendrobium striaenopsis* seeds culture were collected from Kebun Anggrek Singosari (Fig. 1a), harvested and pre-acclimated in screen-house under controlled conditions. Plants were selected based on similar size, phase, and health from a community pot. Two substrates on which plants material growing that is charcoal (Fig. 1b) and coco-husk (Fig. 1c) were washed and sprayed within minutes with a pesticide solution (0.2% Difenokonasol 250 g and 0.1% Carbosulfan 200,11 g/L) to reduce microbes and insects. The coco-husk and charcoal pieces were then cut into small pieces and placed individually in the pots. The coco-husk were soaked in water for one night and rinsed three times before being placed in the trays

Indole-3-butyric acid and substrate treatment

Plants were planted individually in each hole of the potting trays. Approximately 288 plants were selected and planted to receive four treatments with IBA concentrations of 0.25, 0.50, 0.75, and 1.0 mg/L and two substrates of charcoal and coco-husk. All treatments were assigned as part of a complete block design with three replicates. The IBA treatment at each concentration was regularly sprayed on the samples twice a week for one month. Along with the IBA treatment, a nutrient at a half concentration of Murashige and Skoog and B1 liquinox were also added to the plants. The humidity of the substrate and the environment was regularly controlled at 40-60% by simply watering every two days.

Data analysis

Plantlet survival was observed daily, and after four weeks of incubation, vegetative traits such as new root length (RL), root diameter (RD), number of new roots (RN), shoot basal diameter (SD), number of new leaves (LN), shoot basal color (SC), old leaf color (OLC), and new leaf color (NLC) were observed. Data were assessed using Genstat 64-bit release 19.1 (PC/Windows 8)

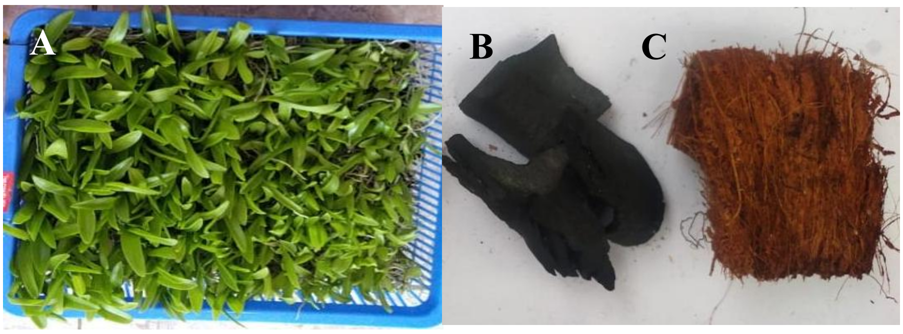

Figure 1. Plantlet of *Dendrodium milla nayla* x *Dendrobium striaenopsis* and its growth medium (a) plantlets, (b) wood charcoal and (c) coco-husk

Results

Application of IBA at different concentrations and growing media affected root induction and growth, albeit it was not statistically significant. The two best treatments, i.e., 0.25 mg/L IBA; charcoal and 0.50 mg/L IBA; coco-husk induced a higher new number of roots (RN) than other treatments. IBA at 0.25 mg/L produced 2 and 3 roots (average 1.75) per plant growing on charcoal. However, IBA at 0.50 mg/L combined with coco-husks induced a maximum of 4 roots per plant earlier and grew longer than IBA at 0.25 mg/L combined with charcoal (Table 1). New root length (RL) reached 0.28 cm and new root diameter (RD) reached 0.10 cm after 2 weeks of spraying IBA at a concentration of 0.50 mg/L in combination with coir husk, corresponding to a 2-fold increase compared with the treatment with 0.25 mg/L IBA and charcoal (0.12 cm new root length and 0.08 cm diameter (SD)). These data suggest that the combination of IBA and growing media plays an important role in immediate root induction and growth.

Further addition of an IBA concentration of 1 mg/L on a similar substrate did not induce significantly more roots in the observed plants. Up to 4 roots per plant were induced on both coco-husk and charcoal, but at lower rates and shorter in terms of new root length.

As reported previously by Wu et al. (2016), the number of roots correlates with the length of the roots. In this study, the number of new roots that grew from the basal part of the plant (Fig. 2 and 3) correlated with root length (0.04) and new leaf color (0.48) (Table 2).

All treatments with IBA and growing media produced an average of 0.75 to 1 leaf per plant of new leaves (LN). The best combination of IBA and coco-husk produced 3 new leaves per plant. After 4 weeks of incubation, the new leaves (LN) produced under ex vitro conditions showed different colors based on the royal color chart. Most of the new leaves color (NLC) showed a strong yellow-green color (SYG) of type A, while a few leaves exhibited type B and C. However, a single sample of 0.50 mg/L IBA and coco-husk showed a moderate olive-green color (Table 3).

Table 1. Root and leaf characters of Dendrodium milla nayla x Dendrobium striaenopsis after 4 weeks of treatment.

Treatment	RL (cm)	RD (cm)	RN	SD (cm)	LN
0.25 mg/L IBA; Charcoal	0.12	0.08	1.75	0.36	1.00
0.50 mg/L IBA; Charcoal	0.13	0.07	1.00	0.36	1.00
0.75 mg/L IBA; Charcoal	-	-	-	0.46	1.00
1.00 mg/L IBA; Charcoal	0.20	0.05	1.00	0.40	1.00
0.25 mg/L IBA; Coco husk	0.25	0.10	1.25	0.43	1.00
0.50 mg/L IBA; Coco husk	0.28	0.10	1.75	0.34	1.00
0.75 mg/L IBA; Coco husk	0.09	0.06	1.25	0.42	1.00
1.00 mg/L IBA; Coco husk	0.22	0.05	1.25	0.42	0.75

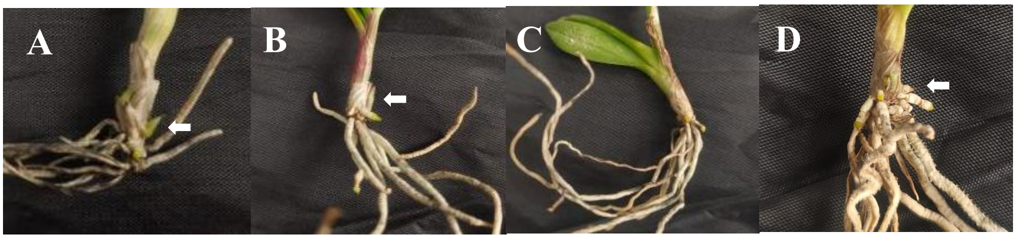

Figure 2. Root formation of Dendrodium milla nayla x Dendrobium striaenopsis on wood charcoal. affected by IBA (a) 0.25, (b) 0.50, (c) 0.75, (d) 1.00 mg/L

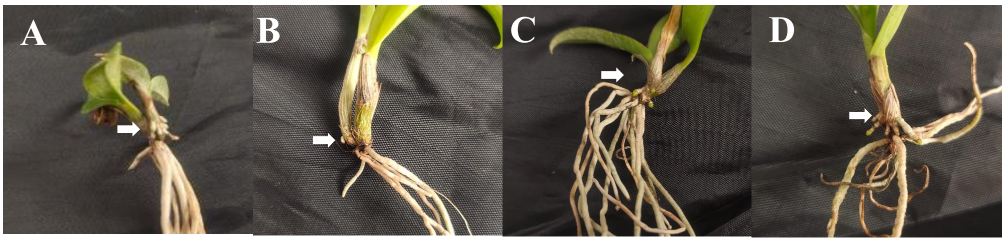

Figure 3. Root formation of Dendrodium milla nayla x Dendrobium striaenopsis on coco-husk affected by IBA (a) 0.25, (b) 0.50, (c) 0.75, (d) 1.00 mg/L.

Discussion

The root system is essential as it anchors a plant to its substrate and plays a role in water and nutrient uptake (Bellini et al., 2014). The tissue culture of plants exhibits malformations of roots and other tissues. The development of a new root system is a prerequisite for plant survival during adaptation and early development *ex vitro*.

Table 2. The correlation (R2) among root and leaf characters of Dendrodium milla nayla x Dendrobium striaenopsis after 4 weeks of treatment.

	RL	LN	SC	OLC	NLC
RN	1.00				
LN	0.04	1.00			
SC	0.09	-	1.00		
OLC	0.12	0.09	0.08	1.00	
NLC	0.48	0.35	0.37	0.34	1.00

Plant hormones play an important role in controlling root development by responding to environmental conditions and sending signals that determine and specify cell fate (Druege et al., 2016). Auxin is an important phytohormone that controls many aspects of plant development and coordinates plant responses to their environment (Brumos et al., 2018). Auxin is essential for regulating root system architecture by controlling primary root elongation and lateral root formation (Alarcón et al., 2019). It is broadly recognized that auxins, especially indole-3-acetic acid (IAA), are effective in stimulating root formation, while high auxin levels at later stages evidently have an inhibitory effect (Ludwig-Müller, 2009). In young seedlings of Arabidopsis, both IBA and IAA were transported only in a basipetal direction. Auxins moved in two distinct polarities and in specific tissues and the kinetics of IBA and IAA transport appear similar, with transport rates of 8 to 10 mm per hour (Rashotte et al., 2003). Apically applied IBA can stimulate elongation of subtending nodes, suggesting IBA is transported basipetally in intact pea plants, but with slower kinetics compared to IAA (Yang and Davies, 1999). Lateral root development approval considerably to root systems architecture and more sensitive to variations in nutrient conditions than primary roots (Tian et al., 2014), therefore more profitable than primary root.

In meristems, auxin has been shown to regulate cell division, elongation, and differentiation, causing downstream organogenesis that shapes shoot and root architecture (Brumos et al., 2018). Local auxin production in roots is required for maintaining functional root meristems (Brumos et al., 2018). However, Exogenous auxin has been reported to inhibit primary root elongation but promote lateral root formation (Alarcón et al., 2019).

Our findings confirmed the effective application of auxin (IBA) on root induction and formation in a short period of time. Roots appeared 2 and 3 weeks after application, followed by new leaves. IBA is widely used in tea propagation to induce rooting (Wei et al., 2014). In different types of explants and species, IAA and its natural precursor IBA (Simon and Petrášek, 2011), are the main root inducers when given exogenously, alone or in combination with other phytohormones (Fattorini et al., 2017) and cytokinin (Wei et al., 2019).

However, the effective application of IBA relies on the growth medium during acclimatization and plant growth. An organic medium capable of retaining more water would effectively retain the IBA solution for absorption by the plants. Our results showed that coco-husk; porous organic structures, promoted root induction and formed more roots per plant than charcoal, indicating its capacity to hold IBA. Charcoal has wide surface, porous structures that retain water but lesser than coco-husk. The selection of medium during acclimation is essential for plant life processes (Hamidi et al., 2021). It is important to understand the characteristics of the substrate in terms of water capacity before administering water and nutrients. Excess water and nutrients would cause higher humidity, which would induce microbes to reside in organic substrates that store more water, such as coco-husk. In further experiments, charcoal and coco-husk could be combined in small pieces. Deb and Imchen (2010) reported that a combination of charcoal pieces (5-7 mm thick), small brick chips, and moss in a 1:1 ratio was suitable for the epiphytic orchids or the terrestrial orchid *Malaxis khasiana* Soland ex Schwartz, a 1:1 ratio of moss to decayed wood/forest litter, along with charcoal fragments and brick chips, was more suitable (Deb and Imchen, 2010).

Finally, we concluded that an IBA concentration of 0.50 mg/L was the most appropriate concentration for immediate root induction and growth of Dendrobium hybrids planted on coir husk. Root number and root length were the most important indicators of adventitious root induction.

Table 3. Different colors of shoot observed from samples of *Dendrodium milla nayla* x *Dendrobium striaenopsis* affected by IBA at 0.25 (a), 0.50 (b), 0.75 (c), 1.00 (d) mg/L on a substrate of coco-husk.

Treatment	SC	OLC	NLC
0.25 mg/L IBA; Charcoal	SYG A, B, C	SYG A, B	SYG A
0.50 mg/L IBA; Charcoal	SYG A, B, C	SYG A, B	SYG A, B, C
0.75 mg/L IBA; Charcoal	SYG B, D	SYG A, C	SYG A
1.00 mg/L IBA; Charcoal	SYG B, C	SYG A, C	SYG A, B
0.25 mg/L IBA; Coco husk	SYG B, C BYG	SYG , SYWG A, C	SYG A, B
0.50 mg/L IBA; Coco husk	SYG A, D	SYWG, SYG A, C	MOG
0.75 mg/L IBA; Coco husk	SYG B,C	SYG A, B	SYG A, B
1.00 mg/L IBA; Coco husk	SYG A, B,C	SYG B	SYG A, B

strong yellow green (SYG) A

Note: strong yellow green (SYG) (picture), strong yellowish green (SYWG), brilliant yellow green (BYG), moderate olive green (MOG)

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