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The use of sucrose and indole-3-butyric acid for increasing quantity of root and acclimatization of ant plant (*Myrmecodia pendans*)

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Abstract

Ant plant (*Myrmecodia pendans*) is an epiphytic with cavity-bulging trunks inhabited by a colony of ants. This plant is widely used as raw material for natural medicines, which effect in its massive exploitation. In vitro culture becomes one of the ant plant preservation efforts. The research aimed to determine the effect of sucrose and indole-3-butyric acid in improving the quantity of roots and on the success of ant plant plantlets acclimatization. This research was done in 2 stages, namely increasing the quantity of roots and acclimatization of ant plant plantlets. Each step was performed using single factor experiment arranged in a randomized complete block design. The treatments tested were the addition of sucrose (30 and 40 g L⁻¹) and indole-3-butyric acid (0, 0.5 and 1 mg L⁻¹), whereas the acclimatization stage was made the same in all treatments. Each treatment was repeated 5 times. Parameters observed in step one were the percentage of live plantlets, the accretion of plantlets height, the accretion of root number, the accretion of shoot number, the percentage of contaminated plantlets and the percentage of browning plantlets. Parameters observed in acclimatization stage were the percentage of live plantlets, the accretion of root number and the percentage of dead plantlets. The result showed that the addition of sucrose and indole-3-butyric acid gave insignificant effect on increasing the quantity of ant plant plantlets roots. The best treatment for acclimatization of ant plant plantlets was the addition of sucrose 40 g L⁻¹ + indole-3-butyric acid 0.5 mg L⁻¹ with the accretion of root number i.e., 7.2 roots for each plantlet.

Keywords: *Myrmecodia pendans*, sucrose, indole-3-butyric acid, root quantity, acclimatization

INTRODUCTION

Ant plant (*Myrmecodia pendans*) is one of the epiphytes that is used as raw material for medicine because it contains flavonoids, tannins and polyphenols (Subroto and Saputro, 2006). The ant plant has high economic value as selling price reached around \$ 80 kg⁻¹ (Detik Forum, 2015). Propagation of ant plant has some problems, such as ants which eat the seeds, untrue to type seedlings and a limited number of seeds produced. Utilization of ant plants in the long term without the accompaniment of preservation can lead to scarcity and the extinction of ant plants.

In vitro propagation is one alternative to conserve ant plants. In vitro culture provides large quantities of plants in a relatively short time, free of pathogens with the same characteristics as their mother plant (Gunawan, 1992). Sukarjan et al. (2012) stated that the best explants for ant plant in vitro culture was leaves grown on VW medium. Supriyadi (2014) had multiplied ant plant derived from seed explant cultured on medium supplemented with Thidiazuron 1 mg L⁻¹ and NAA 0.1 mg L⁻¹. On the other hand, Thidiazuron 3 mg L⁻¹ + NAA 0.5 mg L⁻¹ could induce multiplication of ant plant as shown by the number of shoot buds (15.33 shoot buds) (Rineksane et al., 2015).

Plantlets must have strong roots for adapting in uncontrolled environment. In vitro shoots should be rooted before being acclimatized in non-aseptic condition. Acclimatization is the crucial phase for in vitro plantlets. The success of in vitro culture is determined by this



phase. Therefore efforts to increase the quantity of plantlet roots will be done by supplementing sucrose and IBA on culture medium. Sucrose will act as source of energy while IBA as source of auxin which is needed for root growth.

The objectives of this study were (1) to determine the effect of sucrose and IBA in increasing the quantity of roots of in vitro ant plant plantlets; (2) to determine the best treatment of sucrose and IBA on the success of acclimatization of ant plants.

MATERIALS AND METHODS

The materials used in this experiment were ant plant plantlets, MS medium, activated charcoal, sucrose, indole-3-butyric acid, ferns and moss. The plantlets were used as materials because the plantlets only have small and few numbers of roots. The treatments were proposed to induce more roots on those plantlets, hence the plantlets have vigorous roots for acclimatization. The ant plant plantlets were inoculated on the liquid MS medium + 100 mg L⁻¹ IBA for one week, before their inoculation on the rooting medium.

This research consisted of two stages, increasing quantity of root and acclimatization. Increasing quantity of root and acclimatization was arranged in a randomized completely block design (RCBD). The treatments were the addition of sucrose (30 and 40 g L⁻¹) and IBA (0, 0.5 and 1 mg L⁻¹) on MS medium + active charcoal 2 g L⁻¹. Each treatment was replicated 5 times. Increasing quantity of root was carried out by transferring the ant plant plantlets to a rooting treatment medium. After incubation in rooting medium for 8 weeks, the plantlets were washed thoroughly before their transfer to an acclimatization medium. The acclimatization stage used the same medium for all plantlets, i.e., ferns and moss.

The parameters observed in increasing quantity of root were the percentage of live plantlets, the percentage of contaminated plantlets, the percentage of browning plantlets, the accretion of plantlets height, the accretion of leaves number, the accretion of shoots number and the accretion of roots number. The parameters observed in acclimatization stage were the percentage of life plantlets, the accretion of plantlets height, the accretion of leaves number, the accretion of roots number and the percentage of dead plantlets. Data were collected every week and analyzed using SAS program. The difference among treatment mean values were analyzed using DMRT at level $\alpha=5\%$.

RESULTS AND DISCUSSION

Increasing quantity of root

The data percentage of live explants, percentage of contaminated explants and percentage of browning explants of ant plant plantlets after 8 weeks of culture are presented in Table 1.

Table 1. The effect of sucrose and IBA on the percentage of life explant, percentage of contaminated explants and percentage of browning explants of ant plant plantlets after 8 weeks of culture.

Treatment	Percentage of live explants (%)	Percentage of contaminated explants (%)	Percentage of browning explants (%)
Sucrose 30 g L ⁻¹ + IBA 0 mg L ⁻¹	80	20	0
Sucrose 30 g L ⁻¹ + IBA 0.5 mg L ⁻¹	80	20	0
Sucrose 30 g L ⁻¹ + IBA 1 mg L ⁻¹	100	0	0
Sucrose 40 g L ⁻¹ + IBA 0 mg L ⁻¹	80	20	0
Sucrose 40 g L ⁻¹ + IBA 0.5 mg L ⁻¹	100	0	0
Sucrose 40 g L ⁻¹ + IBA 1 mg L ⁻¹	100	0	0

Based on Table 1, all treatments showed high percentage of live explants, namely about 80-100%. This result was caused by the sterile plantlets used in this experiment which were derived from the previous in vitro culture. It means that the plantlets were not contaminated by microorganisms as bacteria or fungi. The sterile plantlets were very important in in vitro culture as the plantlets must absorb nutrient from the medium for their

growth. The result also showed that browning plantlets were not found in all treatments. It can be explained because the plantlets were not wounded before being planted into treatment medium. So that there was no phenolic compound released from the plantlets. The phenolic compound is known as browning starter while it is released by plant tissue and oxidized by oxygen in the air. Moreover, the addition of active charcoal 2 g L^{-1} in all treatments medium in this experiment were able to absorb the phenol either on medium or coming out of the plant tissue (Fridborg et al., 1978).

1. The accretion of plantlet height.

Growth is an irreversible increase in the size of plant. One of the plant growth parameters is plant height. The initial plant height of explants used here was 4.9 cm. The plant became higher which could be caused by cell division and an increase in cell size. The analysis of variance showed that the use of sucrose and IBA did not significantly influence the accretion of ant plant plantlet height after 8 weeks of culture as presented in Figure 1. The addition of IBA as source of auxin that influences the cell elongation was not detected in this experiment. Likewise the addition of sucrose till 40 g L^{-1} as source of energy in the medium did not influence the plantlets growth until 8 weeks of culture.

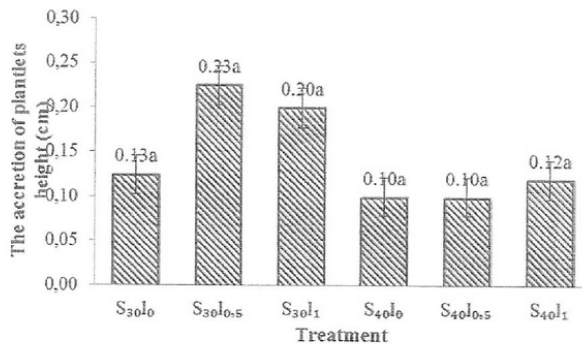


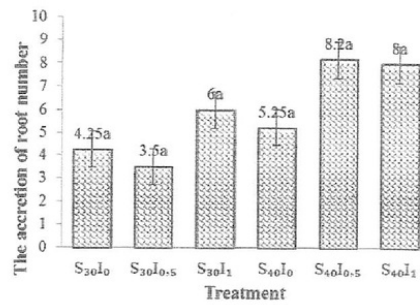
Figure 1. The effect of sucrose and IBA on the accretion on plantlet height ant plant after 8 weeks of culture (S: Sucrose, I: IBA).

The treatment sucrose 30 g L^{-1} + IBA 0.5 mg L^{-1} showed a relatively higher accretion of plantlet height ($0.23 \text{ cm plantlet}^{-1}$) as compared to the other treatment. On the other hand, the use of sucrose 40 g L^{-1} + IBA 0 mg L^{-1} and sucrose 40 g L^{-1} + IBA 0.5 mg L^{-1} showed relatively lower accretion of plantlet height ($0.1 \text{ cm plantlet}^{-1}$) as compared to the other treatment (Figure 1). The accretion of plantlet height was caused by the plant elongation which was triggered by sucrose in the medium. The concentration of sucrose 30 g L^{-1} showed the highest growth of plantlet height of orchid (Batubara et al., 2013) and ginger (Kaisar, 2014). However, the increase of sucrose as 40 g L^{-1} suppressed the accretion of ant plant plantlet height as shown in this experiment.

2. The accretion of root number.

The accretion of root number is the main parameter in this research as effect of the auxin IBA added into the treatment medium. IBA is known as an auxin that produces roots. The analysis of variance showed that the use of sucrose and IBA did not significantly influence the accretion of ant plant root number after 8 weeks of culture as presented in Figure 2.





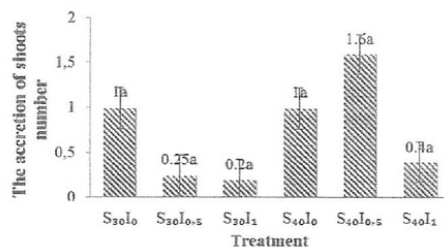
1 Figure 2. The effect of sucrose and IBA on the accretion of root number ant plant after 8 weeks of culture (S: Sucrose, I: IBA).

Based on Figure 2, it could be seen that the treatment sucrose 40 g L⁻¹ + IBA 0.5 mg L⁻¹ produced a relatively higher accretion of root number (8.2 roots plantlet⁻¹) as compared to the other treatment. On the other hand, the use of sucrose 30 g L⁻¹ + IBA 0.5 mg L⁻¹ produced a relatively lower accretion of root number (3.5 roots plantlet⁻¹) as compared to the other treatment. The use of sucrose 40 g L⁻¹ produced a relatively higher accretion of root number than those that used sucrose 30 g L⁻¹. Sucrose is used as source of energy and building block during adventitious root formation in apple (Calamar and de Klerk, 2002), so that the higher concentration of sucrose increased the energy which is used to form roots.

This research also showed that the use of higher concentration of sucrose and IBA tend to increase the accretion of root number of ant plant plantlets. Gunawan (1992) stated that the use of IBA can induce the formation of adventitious roots.

3. The accretion of shoot number.

The regeneration of plants through micropropagation involves five stages as stated by Razdan (2005). One of those stages is the multiplication of shoots. Plant growth regulators (PGRs) which are added into plant culture medium have been known to interact with endogenous PGRs which are responsible for shoot and root formations. Auxins like IAA, IBA, 2,4-D and NAA (Centeno et al., 2003) are among the PGRs frequently used in plant tissue culture. The analysis of variance showed that the use of sucrose and IBA did not significantly influence the accretion of ant plant shoot number after 8 weeks of culture as presented in Figure 3.



1 Figure 3. The effect of sucrose and IBA on the accretion of shoots number ant plant after 8 weeks of culture (S: Sucrose, I: IBA).

Based on Figure 3, the treatment sucrose 40 g L⁻¹ + IBA 0.5 mg L⁻¹ produced a relatively higher accretion of shoot number (1.6 shoots plantlet⁻¹) as compared to the other treatment. On the other hand, the use of sucrose 30 g L⁻¹ + IBA 1 mg L⁻¹ produced a relatively lower accretion of shoot number (0.2 shoots plantlet⁻¹) as compared to the other treatment. The use of sucrose 40 g L⁻¹ produced a higher shoot number than sucrose 30 g L⁻¹, because the root number produced in medium supplemented with sucrose 40 g L⁻¹ was more than those with sucrose 30 g L⁻¹. The more roots produced, the more nutrient could be absorbed by the plantlets to form the adventitious shoots.

Acclimatization

Acclimatization is defined as the process of adaptation of an organism to an environmental change (Dunstan and Turner, 1984). The bottles of plantlets were transferred to a non-sterile incubation room for three days. After three days, plantlets were washed thoroughly before their transfer to a potting mix which contains ferns and moss. The data percentage of live plantlets and dead plantlets is shown in Table 2.

Table 2. The effect of sucrose and IBA on the percentage of life plantlets and the percentage of die plantlets ant plant after 3 weeks of acclimatization.

Treatment	Percentage of live plantlets (%)	Percentage of dead plantlets (%)
Sucrose 30 g L ⁻¹ + IBA 0 mg L ⁻¹	80	0
Sucrose 30 g L ⁻¹ + IBA 0.5 mg L ⁻¹	60	20
Sucrose 30 g L ⁻¹ + IBA 1 mg L ⁻¹	60	40
Sucrose 40 g L ⁻¹ + IBA 0 mg L ⁻¹	80	0
Sucrose 40 g L ⁻¹ + IBA 0.5 mg L ⁻¹	100	0
Sucrose 40 g L ⁻¹ + IBA 1 mg L ⁻¹	80	20

Data in Table 2 showed that all treatments had percentage of life plantlets more than 50% which performed the success of ant plant acclimatization. This phenomenon was caused by supplementing with sucrose and IBA in the previous culture medium which increased quantity of roots.

1. The accretion of root number:

The accretion of root number was used to determine the effect of all treatments in the previous increasing quantity of root stage on the root number produced at acclimatization stage. The analysis of variance showed that the use of sucrose and IBA significantly influenced the accretion of root number after 3 weeks of acclimatization as presented in Figure 4. This result showed that the use of sucrose and IBA in the rooting medium could induce the different response of plantlets on the root formation.

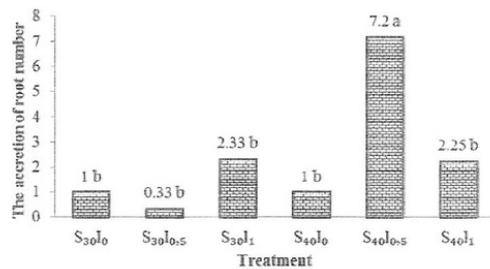


Figure 4. The use of sucrose and IBA on the accretion of root number ant plant after 3 weeks of acclimatization (S: Sucrose, I: IBA).

The data in Figure 4 show that the treatment sucrose 40 g L⁻¹ + IBA 0.5 mg L⁻¹ performed the highest accretion of root number as 7.2 roots plantlet⁻¹. It can be explained that the addition of IBA in increasing quantity of root stage had become the reserve of auxin which then stimulated roots formation during acclimatization stage. Wattimena (1992) stated that IBA had characteristic as slow translocation, high persistence but lower activity, so that promoted the roots formation. This reason was also supported by the use of higher concentration of sucrose 40 g L⁻¹ which provides more energy to the plantlets in producing more roots.

Based on the parameters observed in acclimatization stages, the use of sucrose and auxin IBA significantly increased the root number of ant plant plantlets. The higher root number of plantlets caused a wider zone of roots for nutrient absorption. This phenomenon increased the photosynthate produced which influenced the growth and organ formation of ant plant plantlets.

CONCLUSIONS

It can be concluded that the use of sucrose and IBA had not increased the quantity of roots number of ant plant plantlets in vitro. However, the use of sucrose 40 g L⁻¹ + IBA 0.5 mg L⁻¹ was the best treatment for ant plant plantlets acclimatization as shown by the accretion of ant plant root number as 7.2 roots for each plantlet.

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