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IN VITRO REGENERATION AND ACCLIMATIZATION OF *Anthurium scherzerianum* SCHOTT PLANTS

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ABSTRACT

This study investigated the effects of different PGRs on *in vitro* plant regeneration of *Anthurium scherzerianum* Schott and acclimatization in the *ex vitro* stage. Depending on the objective of each experiment, the *in vitro* shoots were induced from callus cultured on a modified Murashige and Skoog (MS) medium containing cytokinins and auxins. Results showed that 90% of leaf explants and 48% of petiole explants produced callus. A half-strength MS medium supplemented with 1.0 mg/l Kinetin (KIN) gave the best result for shoot regeneration and 1.5 mg/l α-naphthalene acetic acid (NAA) is suitable for rooting of *A. scherzerianum.* The culture vessel having a hole on the cap gave the best result on the growth of *A. scherzerianum* plantlets. After 90 days transferred to the greenhouse, *in vitro* plantlets which were cultivated on a mixed substrate of coconut fiber powder: rice husk ash: compost (6:3:1, respectively) had a better growth (shoot length of 8.88 cm, root length of 3.44 cm) than those on another mixed substrate of coconut fiber powder: compost (9:1, respectively).

Keywords: *Anthurium scherzerianum,* callus, root formation, shoot regeneration, ventilation condition.

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INTRODUCTION

The flamingo flower plant (*Anthurium scherzerianum* Schott), named "Vi hoa thon" in Vietnamese, is an ornamental species that is widely appreciated around the world, primarily for its showy and colorful spadix. Reisch (1998) reviewed that, among tropical flowers, its trade value is next to orchids and has increased in recent years in Asian as well as European countries. A successful tissue culture protocol for *Anthurium* would allow for the mass clonal propagation of this plant to serve the floriculture pot-plant and cut-flower markets. Conventional seed propagation of *A. scherzerianum* is quite laborious and often results in poor uniformity of progenies. Due to the long duration of both seed development and juvenile growth phase, progress in the proliferation of improved and uniform cultivars is very slow. The seed propagation is not proper because of cross-pollination, poor germination rate, variation in color, quality and yield of flowers, and low viability (2−3 days). The progenies are heterozygous, and also propagation by seed leads to genetic segregation. For commercial cultivation, large-scale propagation through tissue culture in general, and micropropagation, in particular, have proved to be better techniques (Martin et al., 2003; Jahan et al., 2009). Callus culture is customarily the method used in *Anthurium* micropropagation in the Netherlands and numerous reports have been published utilizing this method. Organogenetic callus and plant regeneration have been successfully achieved by using sexual organs, such as seeds or embryos, and asexual organs, such as leaf lamina, petiole, spadix, spathe, and etiolated shoots (Geier, 1990; Lightboum & Deviprasad, 1990; Liu & Xu, 1992). An embryogenic-like callus of *Anthurium andraeanum*, cultured on a medium containing 2,4-D and BA was described by Kuehnle et al. (1992). Plants were readily obtained from that callus but regeneration from somatic embryos was not demonstrated. Embryo-like structures were observed by Geier (1986), using spadix explants of *A. scherzerianum* , but plants were not proliferated. Recently, somatic embryos and plant regeneration in *A. scherzerianum* were reported using an induction medium containing 2,4-D and high sucrose concentration (Musa et al., 1997). However, few studies have investigated the *in vitro* culture condition which helps to improve the survival rate and growth of these plants when transferred to *ex vitro* conditions. Thus, this study aims to describe callus induction of *A. scherzerianum* from the leaf lamina culture, the shoot regeneration from callus, and especially, *in vitro* plant growth in the acclimatization stage. The results of the study will provide a good application to the large-scale production of this species.

MATERIALS AND METHODS

Callus induction

A red flowering species of *A. scherzerianum* from Tay Nguyen Institute for Scientific Research, Dalat city, Lam Dong province, was chosen as a resource for the experiments. Unfolded leaves of two-month-old plants were used as initial explants for *in vitro* cultures. They were thoroughly rinsed under running tap water for 30 minutes. Next, the materials were soaked in 70% alcohol for 1 minute. For surface disinfection, the explants were dipped in 0.1% $HgCl_2$ (w/v) for 7 minutes, then washed in sterilized water 3−4 times in sterilized water on the clean bench. After sterilization, two types of explants were tested: leaves and petioles. Lamina (approx. 1.0 cm in width - 1.0 cm in length) with veins and petiole explants (approx. 1 cm in length) were used for callus induction.

The explants were cultured on 1/2 MS medium (Murashige & Skoog, 1962) in glass vessels (V = 250 mL) with 30 mL culture solution, supplemented with 6-benzylaminopurine (BAP) (0.5, 1.0, 1.5 and 2.0 mg/L); or 1.5 mg/L BAP in combination with α-naphthalenacetic acid (NAA) (0.3, 0.5, 0.7 and 1.0 mg/L); or 1.5 mg/L BAP and 0.5 mg/L NAA in combination with Kinetin (KIN) (0.3, 0.5, 0.7 and 1.0 mg/L). The medium was added 30 g/L sucrose (Bien Hoa Sugar Factory,

Dong Nai, Vietnam) and 8 g/L agar (Ha Long Food Co., Hai Phong, Vietnam). The pH of the medium was adjusted to 6.0 before autoclaving at 121 ^{o}C, 1 atm in 21 minutes. Twelve treatments were conducted in each of 3 replications. Each treatment comprised five vessels, 2 leaf explants or 1 petiole explant per vessel. Fresh weight and callus induction rate were taken using destructive measurements after 90 days of culture.

$$\%\ \text{callus induction rate, root formation rate} = \frac{\text{Total of explants regeneration callus, formation root}}{\text{Total explants}} \times 100$$

The cultures were incubated at 25 ± 2 ^{o}C, at 10 hours light period by the illumination from white fluorescent lamps (1.2 m long) (Dien Quang Co.).

Effects of cytokinins on the *in vitro* shoot regeneration

The *in vitro* shoots (about 0.5 cm) from callus were cultured on 1/2 MS medium supplemented with KIN (0.5, 0.7, 1.0, 1.5, 2.0 mg/L) and Thidiazuron (TDZ) (0.1, 0.2, 0.3, 0.4, 0.5 mg/L), 30 g/L sucrose (Bien Hoa Sugar Factory, Dong Nai, Vietnam) and 8 g/l agar (Ha Long Food Co., Hai Phong, Vietnam). The pH of the medium was adjusted to 6.0 before autoclaving at 1 atm 121 ^{o}C for 21 minutes. Ten treatments were conducted in each of 3 replications. Each treatment comprised three vessels (V = 250 mL) with 30 mL culture solution, with 5 shoots per vessel. The number of the new shoot (shoots/explant) and shoot length (cm) were taken using destructive measurements after 90 days of culture.

Effects of auxins on the *in vitro* root formation

The *in vitro* shoots (about 1 cm with 1–2 open leaves) were cultured on 1/2 MS medium supplemented with indole-3-butyric acid (IBA) (0.5, 1.0, 1.5, 2.0 mg/L) and NAA (0.5, 1.0, 1.5, 2.0 mg/L), 30 g/L sucrose (Bien Hoa Sugar Factory, Dong Nai, Vietnam) and 8 g/L agar (Ha Long Food Co., Hai Phong, Vietnam). The pH of the medium was adjusted to 6.0 before autoclaving. Eight treatments were conducted in each of 3 replications. Each treatment comprised three vessels (V = 250 mL) with 30 mL culture solution, with 5 shoots per vessel. The effects of culture media on rooting were evaluated by root length (cm) and the number of roots (roots/explant) after 90 days of culture.

Effect of ventilated and non-ventilated vessels on the growth of *A. scherzerianum* plantlets

The *in vitro* shoots (about 1.5 cm with 2−3 open leaves) of *A. scherzerianum* were cultured into a glass vessel (V = 250 mL), which its mouth was sealed by a piece of the thin layer of nylon with having hole covered by a filter paper and fastened by elastic string. Each vessel contained 30 mL 1/2 MS medium without any plant growth regulators, supplemented with 30 g/L sucrose (Bien Hoa Sugar Factory, Dong Nai, Vietnam) and 8 g/L agar (Ha Long Food Co., Hai Phong, Vietnam). The pH of the medium was adjusted to 6.0 before autoclaving. The experiments were repeated three times. Data of three replicates per treatment were used for statistical analysis after 60 days. Each replicate consisted of three vessels with five shoots per vessel. The height of each shoot, the number of roots per explant, and the length of root were determined after 60 days.

Effects of different substrates on the adaptability of plantlets in the greenhouse

A. scherzerianum plantlets at 2−3 cm long stage with 2−3 open leaves, 2−3 roots (average root length of 1 cm) were transplanted onto a mixed substrate of coconut fiber powder: rice husk ash (RHA): compost (6:3:1, respectively), or coconut fiber powder: compost (9:1, respectively). Each treatment comprised three pots, 30 plants per pot. Shoot and root length, survival rate was taken using destructive measurements after 60 days of culture. The greenhouse was equipped with a rain cover and shading net blocking out

50−70% of light. It had a temperature of 20−25 ^{o}C and a humidity of 60−70%.

Statistical analyses

The obtained results were subjected to Analysis of Variance (ANOVA) for one factor per experiment. The significant difference between the means was compared with Duncan's Multiple Range Test (DMRT) at $p \leq 0.01$, $p \leq 0.05$, or T-test for two treatment levels $p \leq 0.05$, performed by the program SPSS 16.0. (Duncan, 1955).

RESULTS AND DISCUSSION

Callus induction

Lamina and petiole explants were applied for the induction of callus. Three months after culture, 90% leaf explants and 48% petiole explants have produced callus (Figs. 1a, 1b). In contrast with our research, petiole explants were not proper for callus formation of *A. andraeanum* (Raad et al., 2012). Lamina explants exhibited more potential for callus formation when they contained midrib (visual observation), which was in agreement with results reported by Kumar et al. (1992) and Bejoy et al. (2008). Several researchers have reported the induction of callus on leaf explants of *Anthuriums*, especially lamina (Martin et al., 2003; Nhut et al., 2006; Bejoy et al., 2008; Jahan et al., 2009). Callus induction and formation were observed after 50 days. In the current study, callus induction was observed on 1/2 MS containing BA (0.5−2 mg/L) in combination with NAA (0.3−1 mg/l) and KIN (0.3–1 mg/L) (Table 1). Raad et al. (2012) obtained callus on MS basal medium containing different concentrations of BA (0.5−3 mg/L) in combination with NAA (0.01−2 mg/L) in leaf segments. Applying plant growth regulators in a culture medium were able to increase the fresh weight of callus. The highest callus fresh weight (0.64 g/explant) was observed on 1/2 MS medium containing 1.5 mg/L BA + 0.5 mg/L NAA + 1 mg/L KIN. The lowest callus fresh weight (0.069 g/explant) was observed on 1/2 MS medium containing 0.5 mg/L BA. There was significant difference between callus fresh weights grown on 1/2 MS media supplemented with BA (0.069–0.08 g/explant), BA + NAA (0.165–0.251 g/explant), BA + NAA + KIN (0.38–0.64 g/explant). The combination of BA + NAA + KIN produced more callus, which is watery friable white translucent, than those between BA and NAA.

Table 1. Effects of PGRs on callus induction

Treatment	BAP (mg/L)	NAA (mg/L)	KIN (mg/L)	Fresh weight (g)	Callus induction rate (%)
B1	0.5			0.069m	31.3l
B2	1.0			0.073l	39k
B3	1.5			0.076k	43i
B4	2.0			0.08i	52h
BN1	1.5	0.3		0.165h	54.3g
BN2	1.5	0.5		0.240g	59.3f
BN3	1.5	0.7		0.249f	61.6e
BN4	1.5	1.0		0.251e	65.7d
BNK1	1.5	0.5	0.3	0.38d	76c
BNK2	1.5	0.5	0.5	0.45c	84.3b
BNK3	1.5	0.5	0.7	0.53b	90.6a
BNK4	1.5	0.5	1	0.64a	91.3a
ANOVA				**	**

Note: **: Significant at $p \leq 0.01$.

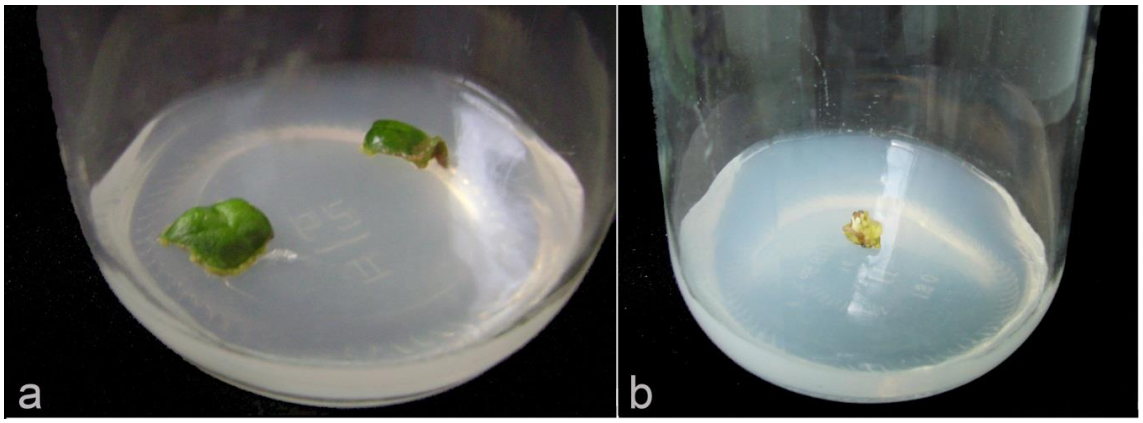

Figure 1. Callus induction and growth after 90 days. a: leaf explants, b: petiole explants

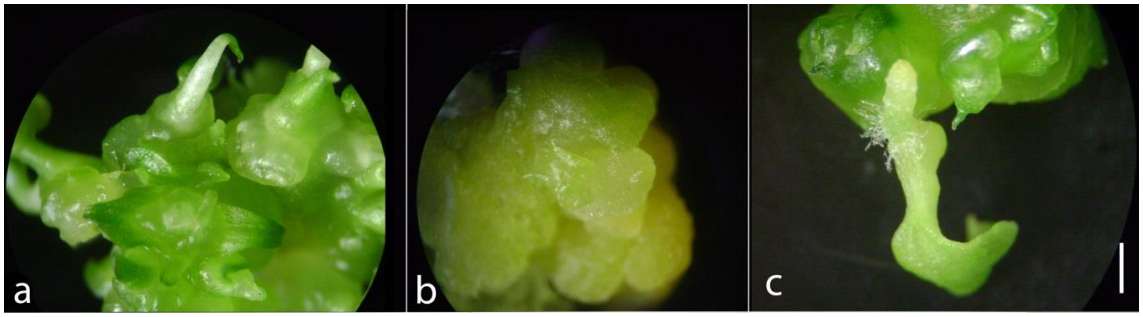

Figure 2. Shoot regeneration. a, b, c shoot regeneration from callus in the medium 1/2 MS + 1.5 mg/L BA + 0.5 mg/L NAA + 1.0 mg/L KIN. Bar = 1 mm

Te-chato et al. (2002, 2006) demonstrated that the kind of genotype effects callus formation in *Anthurium.* Atak and Çelik (2009) showed that while the callus induction rate for the Arizona variety was 80%, this rate was 70% for the Sumi variety. In our study, the highest rate for callus induction was observed in culture medium containing BA 1.5 mg/L + NAA 0.5 mg/L + KIN 0.7 mg/L and BA 1.5 mg/L + NAA 0.5 mg/L + KIN 1.0 mg/L. The lowest rate was found on 1/2 MS + 0.5 mg/L BA (Table 1). Thus, half-strength MS medium supplemented with BA 1.5 mg/L + NAA 0.5 mg/L + KIN 1.0 mg/L is suitable for increasing callus fresh weight and callus induction rate.

Effects of cytokinins on the *in vitro* shoot regeneration

In some reports, KIN or TDZ concentrations have been used alone or in combination with auxin to induce multiple shoots in *Anthurium* (Winarto et al., 2010 a, b; Raad et al., 2012; Cardoso & Habermann, 2014). The presence of KIN and TDZ in 1/2 MS medium improved the shoot proliferation in *A. scherzerianum* after 90 days of culture (Table 2). Statistical analysis of results showed that culture medium had significant effects on shoot number and shoot length ($p \leq 0.01$). In the current study, 1/2 MS containing 1.0 mg/L KIN was found to be the most suitable for shoot regeneration with 9.97 new shoots/explant and 3.98 cm in length. By adding the concentrations from 0.5–0.7 mg/L KIN, the number of new shoots and shoot length were going up (0.6–3.06 shoots/explants; 0.98–2.59 cm, respectively). At the concentrations of 1.5 mg/L and 2.0 mg/L KIN, the number of new shoots increased (15.94 shoots/explant and 25.05 shoots/explant, respectively) but

shoot length decreased (2.56 cm and 1.57 cm, respectively). Our findings were in parallel with Jahan et al. (2009) showing the positive effect of KIN on increasing the number of shoots from the callus of *Anthurium.*

In the medium supplemented with TDZ (0.1–0.5 mg/L), the number of new shoots was greater (0.98–3.47 shoots/explant) and shoot length was lower (0.19–0.66 cm) than that on the medium containing KIN. In contrast with our results, Gu et al. (2012) showed that 83.3% *A. andraeanum* "Alabama" and 77.8% *A. andraeanum* "Sierra" leaf explants produced callus while 24.9 and 24.7 adventitious shoots were produced per callus piece in 1/2 MS plus 1.82 μM TDZ; 0.89 μM BA + 2.32 μM KIN + 0.98 μM IBA. It seems that the main cause of this difference might be the type of cultivars. In summary, 1/2 MS medium supplemented with 1.0 mg/L KIN is suitable for shoot regeneration of *A. scherzerianum.*

Table 2. Effects of cytokinins on the *in vitro* shoot regeneration

Cytokinin	Concentration (mg/L)	New shoot No. (shoots/explant)	Shoot length (cm)
KIN	0.5	0.60k	0.98d
	0.7	3.06e	2.59b
	1.0	9.97c	3.98a
	1.5	15.94b	2.56b
	2.0	25.05a	1.57c
TDZ	0.1	1.0h	0.35fg
	0.2	2.04f	0.66e
	0.3	3.47d	0.50ef
	0.4	1.88g	0.3g
	0.5	0.98h	0.19g
ANOVA		**	**

Note: **: Significant at $p \leq 0.01$.

Effects of auxins on the *in vitro* root formation

Generally, most studies related to the rooting of *Anthuriums* shoots regenerated from callus have been performed when using IBA and NAA (Martin, 2003; Puchooa & Sookun, 2003; Jahan et al., 2009). Investigation of rooting was done by assessment of the number and length of roots. Our study on the effect of culture medium on rooting showed the importance of IBA and NAA. Statistical analysis of results showed that culture medium had significant effects on root number and root length. The largest number of roots per shoot (5.54) was obtained on 1/2 MS medium containing 1.5 mg/L IBA; whereas, root length was the greatest (3.78 cm) on medium added with 2 mg/L IBA (Table 3, Fig. 3a), but shoots seemed short-lived, yellow leaves and short shoots. In this study, IBA was not suitable for rooting from shoot of *A. scherzerianum.* This result also indicated that increasing the concentrations of NAA (0.5–1.5 mg/L) led to a rise in the number of roots and root length (2.43–4.02 roots and 1.11–2.82 cm, respectively). The best medium for increasing root length per explant (2.82 cm) was 1/2 MS medium supplemented with 1.5 mg/L NAA (Table 3, Fig. 3b).

Furthermore, the rooting response (> 90%) was found in this study. Our findings were in parallel with the study of Joseph et al. (2003) when we transferred shoots regenerated from *A. andraeanum* Hort. onto a half-strength MS medium supplemented with 0.54 μM of NAA for rooting. Also, Bejoy et al. (2008) achieved 98% root from shoots in medium supplemented with 0.5 mg/L NAA.

Figure 3. a) Root formation in the medium supplemented with IBA;
b) Root formation in the medium supplemented with NAA

Table 3. Effects of auxins on the *in vitro* root formation

Auxins	Concentration (mg/L)	Root No. (roots/explant)	Root length (cm)	Root formation rate (%)
	0	1.00g^{x}	0.56h	90%
IBA	0.5	2.08f	1.23f	95%
	1.0	2.38e	1.82e	95%
	1.5	5.54a	2.30c	100%
	2.0	3.82c	3.78a	100%
	0	1.00g^{x}	0.56h	90%
NAA	0.5	2.43e	1.11g	95%
	1.0	3.48d	1.87d	95%
	1.5	4.02b	2.82b	100%
	2.0	3.49d	2.31c	100%
ANOVA		*	ns	

Note: ns, *: non-significant, significant at $p \leq 0.05$.

Effect of ventilated or non-ventilated vessels on the growth of *A. scherzerianum* plantlets

The anaerobic conditions *in vitro* caused by root growth in agar can stunt growth and thus decrease the survival of *ex vitro* plantlets in the greenhouse. *A. scherzerianum* plantlets were cultured in vessels covered with a piece of the thin layer of nylon (without a hole) and showed significantly slower growth compared to those grown in vessels with a hole (ϕ = 1 cm) on plastic caps (Table 4, Figs. 4a, 4b). Statistical analysis of

the results showed that cultural conditions had significant effects on the growth of *A. scherzerianum.* After eight weeks of culture, plantlets, cultured in ventilated vessels, attained a greater height (5.01 cm) compared with those in non-ventilated vessels. This result, thus, indicated that higher humidity that was created by the culture vessels with normal caps induced worse growth for *in vitro* of *A. scherzerianum* plantlets. The study of Mills et al. (2004) showed the same result whereby *in vitro* plantlets in non-ventilated vessels produced less elongated shoots and low dry weight biomass compared to those cultured in the intermediate ventilated vessels.

The desiccation of the culture medium was observed in ventilated vessels. This phenomenon was in agreement with other reports that desiccation of the culture medium was observed when vessel ventilation increased (McCown & Sellmer, 1986; Sallanon & Maziere, 1992). Gaseous exchange in culture vessels was found to be higher (Kozai et al., 1986) when the plastic cap was used as vessel closure instead of metal caps. A possible explanation of this phenomenon was that gas exchange occurred through the fitting between the culture vessel and the cap (Fal et al., 2002). Therefore, ventilated caps used in our study increased the gaseous exchange and decreased relative humidity, thus resulting in producing green healthy plantlets with expanded leaves and higher shoot length (Figs. 4a, 4b). In summary, ventilated caps gave a better result in the growing of *A. scherzerianum* plantlets when compared with the non-ventilated cap.

Table 4. Effect of ventilated or non-ventilated vessels on the growth of *Anthurium scherzerianum* plantlets

Ventilation	Shoot length (cm)	Root length (cm)	Root No.
Ventilated cap	5.01*	1.68*	3.31*
Non-ventilated cap	3.25	1.01	1.96

Note: *: Significant at $p \leq 0.05$.

Effects of different substrates on the adaptability of plantlets in the greenhouse

Acclimatization is an equally important stage of the tissue culture process as it guarantees the survival of qualified plantlets. Haploid *Anthurium* plantlets died easily during acclimatization with as much as 90% mortality (Winarto et al., 2009). Thus, the acclimatization step is one of the most important features of the micropropagation protocol. *A. scherzerianum* plantlets at 2−3 cm long stage with 2−3 open leaves and 2−3 roots (average root length of 1 cm) were transplanted onto two kinds of substrate, including: (1) coconut fiber powder mixed with compost at the ratio of 9:1, or (2) coconut fiber powder mixed with rice husk ash (RHA) and compost at the ratio of 6:3:1. The results after 90 days of cultivation revealed that the survival rate of *in vitro* plantlets on the two substrates was significantly different, 65% in the first substrate and 80% in the second. These rates were lower than those in the study of Raad et al. (2012), which showed that 96% of plantlets survived when the plantlets were transferred to greenhouse conditions, the plantlets were transferred to the plastic pots filled with a mixture of peat: perlite: sand (1:1:1) and placed into the greenhouse at 27± 1 ^{o}C, the light density of 4,000 lux and 70% RH. In our work, the plantlets were grown on the mixed substrate of coconut fiber powder, RHA, and compost and grew faster, with 8.88 cm in shoot length and 3.44 cm in root length, than another substrate. The average root length of *in vitro* plantlets cultivated on coconut fiber powder, RHA, and compost significantly increased by 2.44 cm more than that on another substrate (1.14 cm) (Table 5, Fig. 4c). Using a combination of coconut fiber powder, RHA and compost as the substrate makes the root strongly developed.

Table 5. Effects of substrates on the plantlets in the greenhouse

Substrate	Shoot length (cm)	Root length (cm)	Increased root length (cm)	Survival rate (%)
Coconut fiber powder: compost (9:1)	6.16	2.12	1.14	65
Coconut fiber powder: RHA: compost (6:3:1)	8.88^{ns}	3.44^{ns}	2.44*	80*

Note: ns, *: non-significant, significant at $p \leq 0.05$.

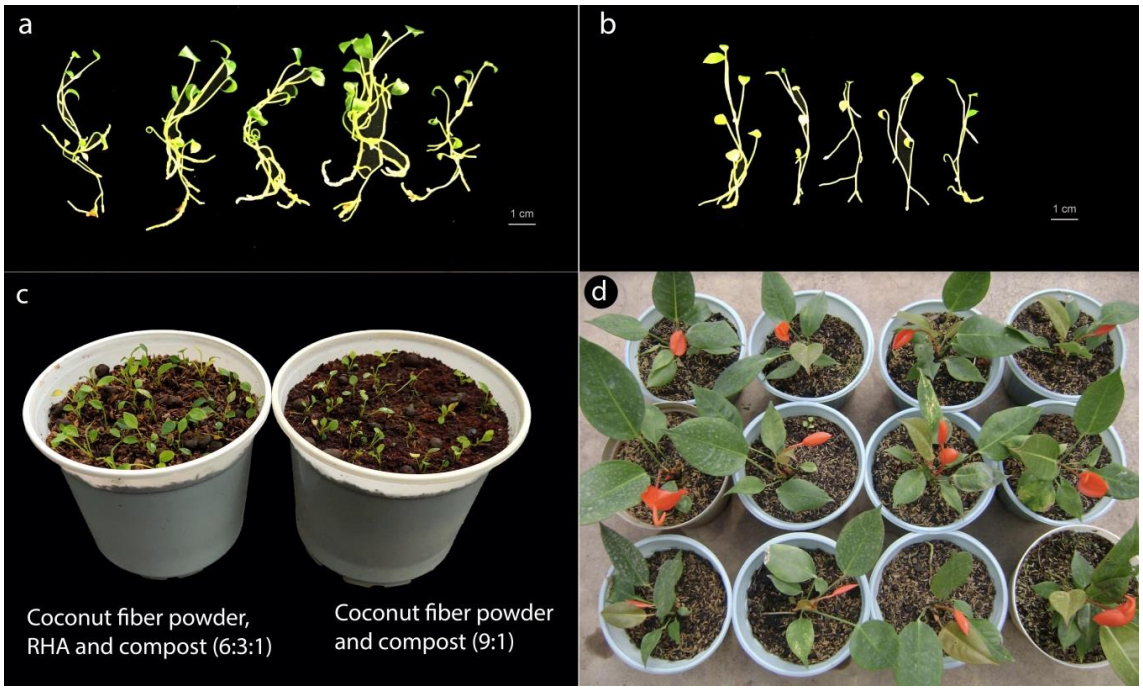

Figure 4. In vitro growth of *Anthurium scherzerianum* in (a) ventilated vessels; (b) non-ventilated vessels. Growth of *Anthurium scherzerianum* in the greenhouse on two kinds of mixed substrates on (c) day 90; (d) 12 months

CONCLUSION

Half-strength MS medium supplemented with BA 1.5 mg/L + NAA 0.5 mg/L + KIN 1.0 mg/L was suitable for increasing callus fresh weight and producing watery friable callus. The medium containing 1.0 mg/L KIN gave the best result on shoot regeneration; whereas, the medium added with 1.5 mg/L NAA was suitable for the rooting stage. Ventilated vessels were better for the growth of *A. scherzerianum* plantlets. Being transferred to the greenhouse, *Anthurium* plantlets on the substrate of coconut fiber powder: RHA: compost at the ratio 6:3:1, respectively, grew better than those on coconut fiber power: compost at 9:1.

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REFERENCES

Atak C., Çelik O., 2009. Micropropagation of *Anthurium andraeanum* from leaf explant. *Pak. J. Bot.*, 41(3): 1155−1161

Bejoy M., Sumitha V. R., Anish N. P., 2008. Foliar regeneration in *Anthurium andraeanum* Hort cv. Agnihothri.

Biotech., 7(1): 134−138. https://doi.org/10.3923/biotech.2008.134.138

Duncan D. B., 1955. Multiple range and F tests. *Biometrics*, 11: 1−42. https://doi.org/10.2307/3001478

Cardoso J. C., Habermann G., 2014. Adventitious shoot induction from leaf segments in *Anthurium andreanum* is affected by age of explant, leaf orientation and plant growth regulator. Hortic. Environ. *Biotechnol.*, 55(1): 56–62. https://doi.org/10.1007/s13580-014-0022-9

Fal M., Majada J., Sánchez Tamés R., 2002. Physical environment in non-ventilated culture vessels affects *in vitro* growth and morphogenesis of several cultivars of *Dianthus Caryophyllus* L. *In vitro Cellular and Developmental Biology-Plant*, 38(6): 589−594.

Gu A., Liu W. F., Ma C., Cui J., Henny R. J., Chen J. J., 2012. Regeneration of *Anthurium andraeanum* from leaf explants and evaluation of microcutting rooting and growth under different light qualities. *HortScience*, 47: 88−92. https://doi.org/10.21273/HORTSCI.47.1.88

Geier T., 1986. Factors affecting plant regeneration from leaf segments of *Anthurium scherzerianum* Schott (Araceae) cultured *in vitro*. *Plant Cell Tissue Organ Cult.*, 6: 115−125.

Geier T., 1990. Anthurium. In: Amirato P. V., Evans D. A., Sharp W. R., Bajaj Y. P. S. (Eds.), Handbook of Plant Cell Culture, Ornamental Species, Vol. 5. McGraw-Hill, New York, pp. 228−252.

Kozai T., Fujiwara K., Watanabe I., 1986. Fundamental studies on environments in plant tissue cultue vessels. (2) Effects of stoppers and vessels on gas exchange rates between inside and outside of vessels closed with stoppers. *J. Agric. Met*., 42(2): 119−127 (in Japanese with English abstract).

Kuehnle A. R., Chen F. C., Sugii N., 1992. Somatic embryogenesis and plant regeneration in *Anthurium andraeanum* L. hybrids. *Plant Cell Rep.*, 11: 438−442. https://doi.org/10.1007/BF00232686

Kumar S. S., Deth S. K., Seeni S., 1992. Development of floricultural resources in Kerala-rapid micropropagation of Anthuriums. In: Proceedings of the Forth Kerala Science Congress, Thrissur Kerala, pp 347−350.

Jahan M. T., Islam M. R., Khan R., Mamun A. N. K., Ahmed G., Hakim H., 2009. *In vitro* clonal propagation of Anthurium (*Anthurium andraeanum* Lind) using callus culture. *Plant Tiss Cult Biotech*, 19(1): 61−69. https://doi.org/10.3329/ptcb.v19i1.4961

Joseph D., Martin K. P., Madassery J., Philip V. J., 2003. *In vitro* propagation of three commercial cut flower cultivars of *Anthurium andraeanum* Hort. *Indian J. Exp. Biol*., 41(2): 154−159.

Lightbourn G. J., Deviprasad P. V., 1990. *In vitro* techniques for rapid multiplication of four varieties of *Anthurium andraeanum* in Jamaica. Proc Interam Soc Trop Hortic., 34: 3−5.

Liu C. M., Xu Z. H., 1992. An efficient procedure for micropropagation of *Anthurium scherzerianum* Schott (flamingo flower). *Chin. J. Bot*., 4: 49−55 (in Chinese with English abstract).

Martin K. P., Joseph D., Madassery J., Philip V. J., 2003. Direct shoot regeneration from lamina explants of two commercial cut flower cultivars of *Anthurium andraeanum* Hort. *In Vitro Cellular and Developmental Biology-Plant*, 39(5): 500–504. doi:10.1079/IVP2003460.

McCown B. H., Sellmer J. C., 1986. General media and vessels suitable for woody plant culture, Ed, Vol 1 General Principles and Biotechnology. Martinus Nijhoff Publishers, The Netherlands.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bio-assays

with tobacco tissue cultures. *Physiol. Plant.*, 15: 473−497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Musa H., Abdul Ghani A. K., Pierre D., 1997. Somatic embryogenesis and plant regeneration in *Anthurium scherzerianum*. *Plant Cell, Tissue and Organ Culture*, 48: 189−193.

Mills D., Yanqing Z., Benzioni A., 2004. Improvement of jojoba shoot multiplication *in vitro* by ventilation. *In Vitro Cellular and Developmental Biology-Plant*, 40(4): 396−402. https://doi.org/10.1079/IVP2004537.

Nhut D.T., Duy N., Vy A. N. H., Khue C.D., Khiem D. V., Vinh D. N., 2006. Impact of *Anthurium* spp. genotype on callus induction derived from leaf explants, shoot and root regeneration capacity from callus. *J. Appl. Hort*. 8(2): 135−137. https://doi.org/10.37855/jah.2006.v08i02.31

Raad M. K., Zanjani S. B., Shoor M., Hamidoghli Y., Sayyad A. R., Harabian Masouleh A., Kaviani B., 2012. Callus induction and organogenesis capacity from lamina and petiole explants of *Anthurium andraeanum* Linden (Casino and Antadra). *Aust. J. Crop. Sci*., 6(5): 928−937.

Puchooa D., Sookun D., 2003. Induced mutation and *in vitro* cultured of *Anthurium andraeanum*. *AMAS, Food and Agricultural Research Council, Reduit, Mauritius*: 17−27. https://doi.org/10.1.1.611.7415

Reisch L., 1998. Effect of media on production of *Anthurium*. Hawaii Agr. Exp. Sta. Prog. Notes No. 94.

Sallanon H., Maziere Y., 1992. Influence of growth room and vessel humidity on the *in vitro* development of rose plants. *Plant Cell, Tissue Organ Cult.*, 30(2): 121−125.

Te-Chato S., Naksombut S., Boonsiri J., 2002. Effect of variety and explants on callus formation and micropropagation of *Anthurium*. *Songklanakarin J Sci Technol*, 24: 569−578.

Te-Chato S., Susanon T., Sontikun Y., 2006. 20 medium influencing embryogenesis and organogenesis in *Anthurium* spp. cultivar, explants type and culture. *Songklanakarin J Sci Technol*, 28 (4): 717−722.

Winarto B., Hayati N. A., Purwito A., Marwoto B., 2009. Effect of sucrose and glucose on successful induction and regeneration of callus derived from anther culture of *Anthurium*. *J Biol Res*, 14(2): 165−172 (in Indonesian with English abstract).

Winarto B., Hayati N. A., Purwito A., Marwoto B., 2010a. Improvement of selected induction culture media on callus induction in anther culture of *Anthurium* and a histological study on its callus formation. *J. Nat. Indones*, 12(2): 93−10 (in Indonesian with English abstract).

Winarto, B., Hayati, N. A., Purwito, A., Marwoto, B., 2010b. Application of 2,4-D and TDZ in formation and regeneration of callus derived from anther culture of *Anthurium*. *J. Hortik.*, 20(1): 1−9 (in Indonesian with English abstract).