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Adventitious shoots derived from leaf explants in *in vitro* mass propagation of indonesian selected *anthurium* clones

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Abstract

A new *in vitro* mass propagation protocol for Indonesian *Anthurium* was successfully established using different selected clones and culture media from initiation to acclimatization stages. The Kar-005 clone cultured on IM-2 (WT medium containing 0.5 mg/L TDZ, 0.1 mg/l BA and 0.2 mg/l NAA) was produced green fast growth compact callus. Friable yellow callus derived from leaf explants of M-11 clone with moderate growth type was the fastest growth responsive. The callus was successfully initiated in 1.2 months after culture of shoot regeneration time with 25.3 shoots per explant, 0.79 cm shoot height, and 3.6 number of leaves per shoot on the WT medium supplemented with 0.5 mg/l TDZ, 1.0 mg/l BAP and 0.02 mg/l NAA. The shoots of M-11 clone gave the best root response on WT medium hormone-free with 1.5 g/l AC with 3.2 roots per shoot and 0.86 cm root length. The plantlet acclimatization was carried out on burned-rice husk with 87.3% plantlet survivability. The results of the study imply that the protocol can be applied to propagate other clones and varieties by paying attention to the growth type of callus.

Key words: *Anthurium initation*, regeneration, callus, in vitro, medium

Introduction

Anthurium andraeanum Linden. ex Andre is one of the most popular Araceae family members, which has high economic values both in global and local markets (Thokchom and Maitra, 2017; Saptari et al, 2017). The plants have beautiful and attractive flowers, varied color of spates and spadices, more extended longevity and generally used as cut flower, potted and garden plants (Bejoy et al., 2008; Winarto et al., 2011). In the global market, the *Anthurium* cut flowers are ranked in the second position among tropical cut flowers (Atak and Celik, 2012), while in Indonesia, the cut flower occupies the eighth position after *Dracaena* cut flower (BPSDJH, 2019). Though the flower has high economic values, the development of the flower in Indonesia is constrained by the availability of qualified planting materials continually. Therefore, preparing high quality and quantity planting materials is importantly addressed.

Conventionally, *Anthurium* is propagated asexually and sexually. Both methods have limitations in providing good quality and sufficient quantity (Martin et al., 2003; Vargas et al., 2004; Atak and Celik, 2012; Saptari et al., 2017). Applying and developing an *in vitro* mass propagation method are primarily addressed to prepare high-qualified planting materials continuously for commercial flower production

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Recently, several *in vitro* propagation works on *Anthurium* were successfully established. Successfully Adventitious shoots were derived from callus regenerated from leaf explants of *Anthurium andreanum* 'Alabama' and 'Sierra' on MS medium (1962) containing 0.2 mg/l BA, 0.5 mg/l Kinetin and 0.2 mg/l IBA. The shoots were rooted on MS medium supplemented with 0.2 mg/l IBA (Gu et al., 2012). In different studies, shoots of *A. antioquennse* were produced on half-strength MS (half-salt content) medium with 1 mg/l BAP. The shoots were then easily rooted in a growth regulator free-medium (Murillo-Gómez et al., 2014). Regenerative callus derived from mature endosperm explants up to 100% was determined on VW medium (Vacin and Went, 1949) fortified by 0.75 mg/l TDZ and 0.15 mg/l BAP then periodical subcultured on half-strength MS medium supplemented with 0.12 mg/l 2,4-D, 0.75 mg/l TDZ and 0.5 mg/l BAP. High regeneration of shoots up to 13.5 shoots per callus was proved in NWT medium (Winarto et al., 2011)supplemented with 0.25 mg/l 2,4-D, 1.5 mg/l TDZ, 0.75 mg/l BAP and 0.02 mg/l NAA. The shoots were easily rooted on NWT medium containing 0.06 mg/l 2,4-D, 0.37 mg/l TDZ, 0.5 mg/l BAP and transferred *ex vitro* condition in a mixture of burned-rice husk, raw rice husk and organic manure with highly survival plantlets (Winarto et al., 2018). In these studies, it was revealed that each *in vitro* propagation protocol in each *Anthurium* cultivar needs different routes and specific medium. In this study, we reported a reliable new route of an *in vitro Anthurium* propagation protocol initiation to acclimatization by using young leaves via adventitious shoot formation. All steps of the new protocol were described clearly in this paper.

Materials and Methods

Callus initiation

In the study, leaf explants prepared from five different clones of Kar-005, M-11, S-2005-6, S-2005-7, and S-2005-26 were cultured on two different initiation media (IM) *viz*. (1) MS medium (Murashige & Skoog, 1962) containing 1.0 mg/l KIN and 1.0 mg/l BA (IM-1), (2) Winarto and Teixeira (Winarto et al., 2011) (WT) medium supplemented with 0.5 mg/l TDZ, 0.1 mg/l BA and 0.2 mg/l NAA (IM-2). All media were added by 3% sucrose and 0.3% gelrite. The pH media were adjusted at 5.8. Explants were incubated in dark conditions at 24 ±1°C until callus regenerated for ± 4 weeks. After four weeks, cultures were transferred to light conditions with 12 h photoperiod under the cool fluorescent lamp with 13 µmol/m2/s for ± 1.5 months. The split-plot experiment was arranged in a completely randomized design (CRD) with three replications. Each treatment consisted of 4 bottles. Each bottle contained three explants cultured. The total number of explants used in the step was 180 explants, with 36 explants per clone.
Variables observed in this experiment were: 1) explant growth potential (%), calculated by counting total number of swelling explant and keeping green divided by total number of explants cultured time by 100%; 2) callus initiation period (week after culture-WAC), counted started from initial culture till callus initiated; 3). Percentage of explant regeneration (%), calculated by counting a total number of regenerated-explants divided by a total number of explants cultured time by 100%; 4) diameter of callus (cm), 5) type of callus, 6) color of callus and 7) callus growth type. Periodical observations were carried out to know any alteration of explant during incubation. All data were recorded ± 2.5 months after culture initiation.

Shoot regeneration

In shoot regeneration, experiment was carried out by subculturing callus derived from the five clones in the previous experiment on two different regeneration media (RM) of (1) WT medium supplemented with 0.5 mg/l TDZ and 0.01 mg/l NAA (RM-1) and (2) WT medium augmented with 0.5 mgL-1 TDZ, 1.0 mgL-1 BA and 0.02 mgL-1 NAA (RM-2). The split-plot experiment was arranged in a CRD with two factors (media and clones), and three replications with the media were used as the main plot and callus derived from five clones as a subplot. Each treatment consisted of 3 bottles, and each bottle contained 3 callus clumps. The variables observed were: 1) shoot regeneration time (a month after subculture-MAS), the shoot regeneration time was observed started from initial culture till initial shoots regenerated; 2) a number of shoots per explant; 4) height of shoots (cm) and (5) number of leaves per explant. The periodical observation was conducted to observe the response of explant on shoot regeneration while data in all variables were observed, measured, and noted 2.0 - 5.0 months after culture.

Shoot rooting and plantlet acclimatization

Rooting of shoots was carried out by culturing shoots (± 1.5 cm in height with 1-3 leaves) derived from 5 clones studied on WT medium hormone-free with 1.5 g/l activated charcoal (Fig.3F). The cultured-shoots were then incubated under light incubation as described previously for ± 2 months. The experiment was arranged in CRD with three replications. Each treatment consisted of 10 bottles, and each bottle contained 5 shoots cultured. Total shoots cultured per clone were 150 shoots. At the end of the experiment, a number of roots per shoot and root length were recorded after ± 2 months after culture.
Plantlet acclimatization was carried out after well, and vigor plantlet performances were successfully prepared. Plantlets in almost similar size and performance pulled out from the culture vessel using blunt tweezers. The plantlets were then washed under running tap water gently to remove attaching agars on root surfaces (Fig. 3G), immersed them in 1 % pesticide solution of bactericide and fungicide for 3-5 minutes (Fig. 3H) and then placed on dry paper for a while to reduce water attaching plantlets. Furthermore, the plantlets were planted in burned rice husk media (Fig 3IJ) and covered with transparent plastic for about 6 weeks to protect plantlets from higher transpiration (Fig 3K).
The experiment was arranged in CRD with three replications. Each treatment consisted of a plastic box, and each plastic box contained 50 plantlets cultured. Total numbers of plantlets acclimatized were 150 plantlets in each clone. Variables observed in the study were the percentage of survivability (%), and several survival plantlets recorded 1.5 months after acclimatization.

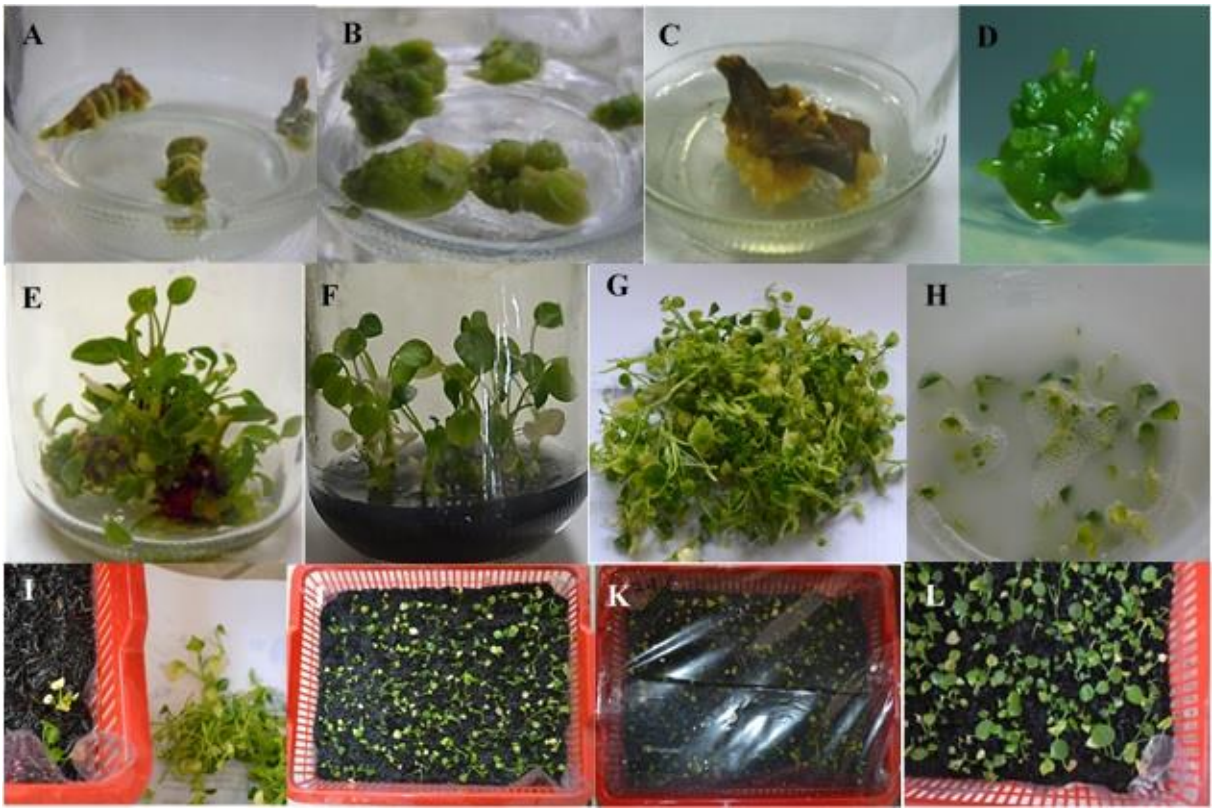

Figure 3. Adventitious shoots derive from leaf explants in *in vitro* propagation of *Anthurium* clones. (A) High potential growth of plantlets ± 8 days after culture of Kar-005 clone on WT medium containing 0.5 mg/l TDZ, 0.1 mg/l BA and 0.2 mg/l NAA. (B) Fast growth performance of Kar-005 clones one month after light incubation on the same medium. (C) Friable callus derived from leaf explant of M-11 clone 2 months after culture in a similar medium. (D) Initial shoots derived from leaf explant of M-11 clone on WT medium supplemented with 0.5 mg/l TDZ, 1.0 mg/l BA, and. 0.02 mg/l NAA 1.0 MAS. (E) Regenerated shoots on friable callus of M-11 on the same regeneration medium 2 MAS. (f) Rooted-shoots on WT medium hormone-free with 1.5 g/l AC. (G) Plantlets ready for pesticide solution. (h) Immersing plantlets in a 1% bactericide and fungicide solution for 3 minutes. (I) Planting plantlets in burned rice husk prepared on a plastic tray and watered sufficiently. (J) Plantlets culture on burned rice husk for acclimatization purpose in initial culture. (K) Plantlets in plastic trays covered by transparent plastic for 6 weeks. (L) Plantlets growth ± 2 months after acclimatization.

Data Analysis

Data collected from the study were analyzed using analysis of variance (ANOVA) with SAS Release Window 9.12 program. If there were significant effects among the treatments, the mean values of the treatments were further tested using Tukey (HSD) at $p=0.05$ (Mattjik and Sumertajaya, 2006).

Results and Discussion

Callus Initiation

Following the periodic observation, initiation of callus was clearly observed from 6.7-14.7 weeks after culture (Fig. 3A). The initiated callus grew continually and increased in size and volume. After ± 2 months after culture, the percentage of potential growth of explant was varied from 58.0-91.3%, 2.7-18.0% explant regenerated callus, and 0.2 – 0.8 cm in diameter size. Each clone was also produced callus in different types and colors (Table 2).

In the first experiment, it was clearly revealed that two initiation media and five clones indicated a significant effect on callus formation statistically. IM-2 (WT medium containing 0.5 mg/l TDZ, 0.1 mg/l BA and 0.2 mg/l NAA) was suitable medium for callus initiation. On average, the medium had 74.9% potential growth of explant, 7.3 weeks of callus initiation time, 12.9% explants regenerated callus and 0.59 cm in callus size (Fig. 1A; Table 1). Kar-005 clone was the most responsive clone in callus initiation with 87% potential growth of explant, 9 weeks callus initiation period, 11.7% explant regenerated callus and 0.60 cm in callus size (Fig. 1B; Table 1). Though the Kar-005 callus grew faster than others, the callus was clustered in compact callus that was difficult to induce in regenerating shoots. The friable callus in yellow color with moderate growth was noted on M-11 clone callus, and the lowest callus growth was performed by callus derived from S-2005-6 clone.

The best combination treatment in callus initiation was determined on leaf explants of Kar-005 clone cultured on IM-2 (Fig 3B; Table 1). The combination treatment stimulated potential growth of explants up to 91.3% with 6.7 WAC of callus initiation time, 18% explants regenerated callus, and 0.80 cm in callus diameter. Though the combination treatment was the best combination, compact callus let to less and/or no shoots successfully regenerated in the callus. The second best treatment was yellow friable callus derived from leaf explants of M-11 clone cultured on IM-2 medium (Fig. 3C; Table 1). While other combinations indicated lower results than the previous combination.

Table 1. Interaction effect of initiation media and five genotypes on the callus initiation

Clones/ Media	Potential growth of explant (%)		Callus initiation period (week)		Percentage of callus regeneration (%)		Callus diameter (cm)	
	IM-1	**IM-2**	**IM-1**	**IM-2**	**IM-1**	**IM-2**	**IM-1**	**IM-2**
Kar-005	82.70 a	91.33 a	11.30 b	6.70 a	5.30 a	18.00 a	0.40 a	0.80 a
M-11	70.30 b	68.00 b	0.00 c	7.30 a	0.00 b	12.70 ab	0.00 c	0.60 ab
S-2005-6	58.00 d	72.00 b	14.70 a	8.30 a	2.70 ab	9.30 b	0.20 b	0.40 b
S-2005-7	65.30 bc	70.30 b	14.30 a	7.30 a	4.70 a	11.30 b	0.30 ab	0.50 b
S-2005-26	60.70 cd	72.70 b	0.00 c	7.00 a	0.00 b	13.30 ab	0.00 c	0.60 ab

Means followed by the same letter in the same column are not significantly different based on the Tukey test, *p=0.05*. IM-1, MS medium augmented with 1.0 mg/l Kin and 1.0 mg/l BA; IM-2, WT medium fortified by 0.5 mg/l TDZ, 0.1 mg/l BA and 0.2 mg/l NAA.

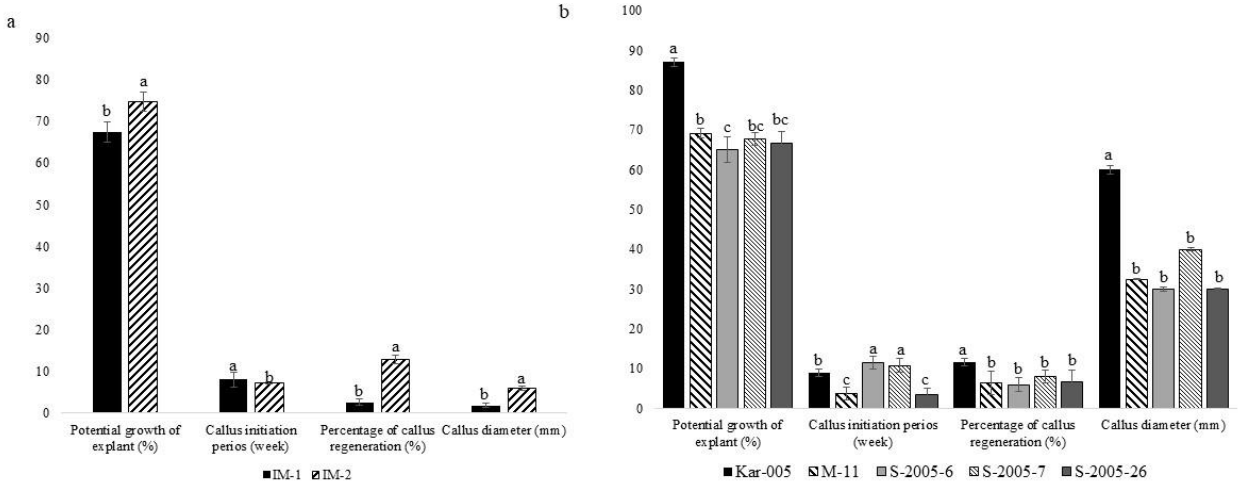

Figure 1. Effect of media (a) and responses of *Anthurium* clones (b) on callus initiation. A. Histograms followed by the same letter in the same cluster are not significantly different based on Tukey test at *p=0.05*. IM-1, MS medium containing 1.0 mg/l kin and 1.0 mg/l BA; IM-2, WT medium supplemented with 0.5 mg/l TDZ, 0.1 mg/l BA and0.2 mg/l NAA.

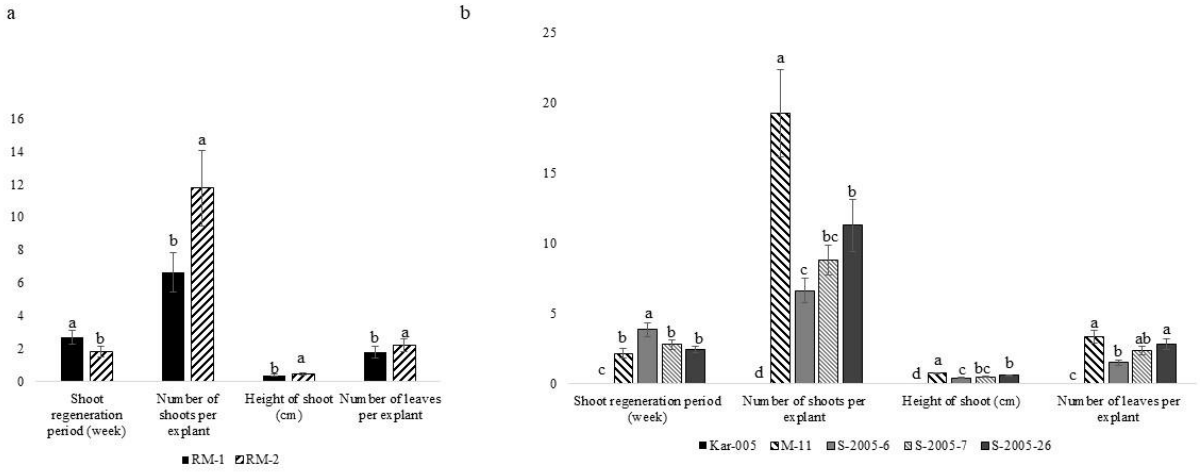

Figure 2. Effect of media (a) and leaf callus of five anthurium clones (b) on shoot regeneration. Histograms followed by the same letter in the same cluster are not significantly different based on the Tukey test at *p=0.05*. RM-1: WT medium containing 0.5 mg/l TDZ and 0.01 mg/l NAA; RM-2: WT medium supplemented with 0.5 mg/l TDZ, 1.0 mg/l BA and 0.02 mg/l NAA.

From the study, a new route on *in vitro* propagation protocol for *Anthurium* clone was successfully established with unique and interesting evidence in each stage from the initiation to acclimatization. Initiation of callus up to 63.4% with varied results, as a crucial stage *in vitro* culture of the plant, was established by culturing fruit explant on MS medium containing 4.0 mg/l 2,4-D; but only one callus grew and was successfully regenerated to produce plantlet on MS medium

fortified by 0.2 mg/l BA; other callus become brown and necrotic (Alves dos Santos et al., 2005). Creamy and compact callus induction up to 94.5% and 74.7% was recorded on petioles and leaf lamina of *A. andreanum* 'Esmeralda' cultured on MS salts, 100 mg/l inositol, 0.4 mg/l thiamine HCL, 30 g/l sucrose, 0.2 mg/l 2,4-D and 1.0 mg/l BAP and 0.6 g/l agar incubated in the dark condition. The callus was successfully regenerated to produce shoot after six subculture on the same medium with a varied number of shoots from 30-39 shoot per explant and 1.5-2.5 cm in height (Prakasha et al., 2017). Compact and green callus was observed in MS medium containing 2.0 mgL-1 NAA (77.33%). The callus was stimulated to produce shoots up to 5.8 shoots per explant for 27.8 days with 98.9% percentage of regeneration on MS medium containing 3.0 mg/l BAP and 0.5 mg/l NAA (Thokchom and Maitra, 2017). Half-strength modified MS (lowering ammonium nitrate to 250 mg/l and for the first time - 0.1% EDTA Ferric Sodium) medium supplemented with 1.0 mg/l 2, 4-D and 1.0 mg/l BAP successfully induced high percentage of callus formation up to 82.6% after 30 days in dark conditions. The callus was regenerated to produce shoots up to 29.1 shoots per explant on A2W after 6 weeks in 16/8 h light and dark cycle under a photoperiod of 50 µmol/m2/s (Bhavana et al., 2018). These results gave evidence that regenerated callus successfully induced shoots in different culture media.

In the study, friable and regeneratable callus were successfully induced on leaf explants of 4 clones with varied growth types. According to Moon and Stomp (Moon and Stomp, 1997), slow, moderate, or fast growth types of callus were affected by the origin of cell types, and higher meristematic cells let to regenerating faster growth types of callus than the organized cells. Regeneration of shoots was significantly noted due to increasing BAP concentration from 0.1 to 1.0 mg/l and reducing NAA from 0.2 to 0.02 mg/l. Atak and Celik (Atak & Celik, 2009) reduced 2,4-D concentration from 0.6 to 0.1 mg/l and maintained BA in the same concentration at 1.0 mg/l BA, reduction of BAP from 2.5 to 1.0 mg/l and omission of 2,4-D (Jahan et al., 2009), reduction of BA from 3.0 to 1.0 mg/l; NAA from 0.5 to 0.1 mg/l (Raad et al., 2012). In the alteration BAP and NAA concentration, Friable callus of M-11 clone induced on WT medium supplemented with 0.5 mg/l TDZ, 0.1 mg/l BA, and 0.2 mg/l NAA with 68% callus formation in 7.3 WAC, 12.7% regeneratable callus and 0.6 cm3 callus size. The callus was then regenerated to produce shoots in 1.2 MAS up to 25.3 shoots per explant with 0.79 cm height of shoots and 3.6 leaves per shoot on WT medium containing 0.5 mg/l TDZ, 1.0 mg/l BA and 0.02 mg/l NAA. Callus with 81.3% rate was induced in half-strength MS basal salt with 0.6 mg/l 2,4-D, 1 mg/l BA. The callus was regenerated and produced 26.9 shoots per explant on modified half-strength MS salts with NH_4NO_3 lowered to 250 mg/l, 0.1 mg/l 2, 4-D, and 1.0 mg/l BA (Atak & Celik, 2009). High frequency of calli was obtained from leaf and spadix segments of *A. andraeanum* L. cultured on N6 medium containing 2.5 mg/l BAP and 0.2 mg/l 2,4-D in dark condition. The callus was regenerated on MS medium fortified by 1.0 mg/l BAP and produced 18 shoots per explant (Jahan et al., 2009). High callus production was established on young explants of lamina and petiole incubated in dark condition on MS medium supplemented with 3.0 mg/l BA and 0.5 mg/l NAA after 65 days of culture. The 22.8 shoots per cm2 of callus were observed on medium augmented with 0.01 mg/l NAA + 1.0 mg/l BA after 8 weeks in a 16/8 h light and dark cycle under a photoperiod of 50 µmol/m2/ (Raad et al., 2012).

Shoot Regeneration

In the initiation step, there was determined different callus regenerated from five different clones. Leaf explants from Kar-005 regenerated green compact callus with faster growth than another callus (Table 2). Most of the leaf explants dominantly produced friable callus with varied colors and different growth types from slow to moderate. Compact callus frequently had low regeneration capacity, while friable callus is easily stimulated to produce shoots. The regeneration capacity of this callus was proven in the regeneration stage. Similar results in local accessions of *Anthurium* were also reported by Winarto and Mattjik (Winarto and Mattjik, 2009). This phenomenon was presumably occurred due to compact callus needs periodical subculture before can be regenerated to produce shoots. The compact callus derived from petioles and leaf lamina of *A. andreanum* 'Esmeralda' produced shoots after six subculture in dark condition (Prakasha et al., 2017); or need different concentration and combination of hormone as reported on *A. andreanum* 'Jewel' using MS medium supplemented with 2 mg/l NAA for callus initiation and 3.0 mg/l BAP and 0.5 mg/l NAA for shoot regeneration (Thokchom & Maitra, 2017).

Table 2. Callus type, color, and growth type successfully regenerated on initiation stage

Clone	Callus type	Color of callus	Callus growth type
Kar.005	Compact	Green	Fast
M-11	Friable	Yellow	Moderate
S-2005.6	Friable	Yellowish green	Slow
S-2005-7	Friable	Green	Slow
S-2005-26	Friable	Green	Moderate

The shoots were ini tiated at 1-2 months after the callus subculture (Fig 3D). The number of shoots produced per explant was varied from 1-28 shoots with a range of shoot regeneration period from 1.0-4.5 months. The number of leaves and size of petioles increased gradually, and at the end of the experiment, shoots with 0.1 - 1.5 cm in height and 1-5 leaves were noted. Regeneration of callus to produce shoots was significantly affected by media and the response of different clones. RM-2 medium (WT medium containing 0.5 mg/l TDZ, 1.0 mg/l BA and 0.02 mg/l NAA) was the most appropriate medium to induce shoot regeneration. The medium initiated shoot formation in 1.8 MAS with 11.8 shoots per explant, 0.49 cm shoot height, and 2.2 leaves per explant (Fig 2A). Clone of M-11 was the most responsive clone on shoot regeneration with 2.0 MAS shoot regeneration time, 19.3 shoots per explant, 0.75 cm height of shoots, and 3.3

leaves per explant (Fig. 2B) while other clones induced lower results in all variables observed.

Regeneration media and different clones gave significant interaction effects in all variables observed. Leaf explants of M-11 clone cultured on RM-2 medium (WT medium supplemented with 0.5 mg/l TDZ, 1.0 mg/l BA, and 0.02 mg/l NAA) were the most appropriate combination treatment for shoot regeneration. The combination treatment stimulated shoot formation in 1.2 MAS and produced a number of shoots per explant up to 25.3 shoots with 0.79 cm shoot height and 3.6 leaves per shoot (Table 3; Fig. 3C). The second best combination treatment was recorded at S-2005-26 clone. The lowest results were indicated by S-2005-6 clone, while no-shoot regeneration was noted on callus derived from compact callus of Kar-005 clone. From the shoot regeneration study, it was revealed that friable callus had a high regeneration capacity to produce shoots, while compact callus had less and/or no regeneration capacity on shoot regeneration.

Table 3. Effect initiation media and genotypes interaction on the shoots regeneration

Clones/ Media	Shoot regeneration time (MAS)		Number of shoots per explant		Height of shoot (cm)		Number of leaves per shoot	
	RM-1	**RM-2**	**RM-1**	**RM-2**	**RM-1**	**RM-2**	**RM-1**	**RM-2**
Kar.005	0.00 c	0.00 c	0.00 c	0.00 d	0.00 c	0.00 d	0.00 c	0.00 c
M-11	2.80 b	1.20 b	13.30 a	25.30 a	0.70 a	0.79 a	3.10 a	3.60 a
S-2005.6	4.50 a	3.20 a	5.30 b	8.00 c	0.36 b	0.45 c	1.20 bc	1.80 b
S-2005-7	3.30 ab	2.30 ab	6.70 b	10.90 bc	0.45 b	0.56 bc	2.20 ab	2.50 ab
S-2005-26	2.80 b	2.10 ab	8.00 b	14.60 b	0.53 ab	0.65 ab	2.50 ab	3.10 ab

Means followed by the same letter in the same column are not significantly different based on the Tukey test, p=0.05. Media: RM-1-WT medium containing 0.5 mg/l TDZ and 0.01 mg/l NAA, RM-2, WT medium supplemented with 0.5 mg/l TDZ, 1.0 mg/l BA and. 0.02 mg/l NAA.

Shoot rooting and plantlet acclimatization

Shoot rooting in the study was efficiently carried out by culturing them in WT medium hormone-free with 1.5 g/l AC under light incubation (Fig 3F). Primary roots were recorded from 5-8 days after culture at the basal part of cut stem and node. The initial roots grew continually and increased in size and length. At the end of the experiment number of roots per shoot was varied from 1-4 roots with 0.4 – 1.0 cm in root length. Higher root capacity was described on M-11 clone with 3.2 roots per shoot and 0.86 cm length of roots (Table 4; Fig 3F-G). While low response on root formation was noted on S-2005-6.

Table. 4. Shoot rooting and acclimatization of of shoots on five *Anthurium* clones

Clone	Number of root	Length of root (cm)	Survivability (%)	Number survival plantlets
M-11	3.20 a	0.86 a	87.30 a	43.60 a
S-2005.6	1.70 c	0.52 b	81.00 a	40.50 a
S-2005-7	2.10 bc	0.64 ab	81.60 a	40.80 a
S-2005-26	2.70 ab	0.70 ab	83.80 a	41.90 a

Means followed by the same letter in the same column are not significantly different based on the Tukey test, p=0.05.

The acclimatization of well-developed plantlets was also successfully established in the study. All clones had survivability of plantlets more than 81% and several survival plantlets of 40.5-43.6 plantlets. Though there was no significant difference in all clones statistically, M-11 clone indicated the highest results with 87.3% survivability and 43.6 survival plantlets. The acclimatized plantlets grew continually indicated by increasing number of leaves and size of them (Table 4; Fig. 3L).

The rooting and acclimatization play an essential role and in several cases to be the most critical stages in establishing an *in vitro* mass propagation protocol of plant (Petrova et al., 2011; Muniz et al., 2013; Kadam et al., 2017; Winarto and Yufdy, 2017). However, the stages were quickly prepared and carried out for *Anthurium* (Saptari et al., 2017;Thokchom and Maitra, 2017; Winarto et al., 2018). In the study, root formation was easily prepared by culturing shoots on WT medium hormone-free, while plantlet acclimatization was carried out in burned-rice husk with 87.3% survivability and 43.6 survival plantlets at 6 weeks after culture of M-11 clone. *A. andreanum* 'Nitta' shoots were matured in MS medium with a half-strength of macro minerals added with 1 mg/l paclobutrazol and plantlets were acclimatized in a mixture of soil, dung manure and cocopeat (1:1:1, v/v/v) with 89.3% survival rate for 8 weeks (Saptari et al., 2017). Half strength MS medium with 1.0 mg/l NAA was a maximum rooting medium for A. andreanum 'Jewel' shoots with a 96.2% survival rate of the plantlets cultivated on vermiculite and cocopeat (1:1, v/v) (Thokchom and Maitra, 2017). While Winarto et al., (Winarto et al., 2018) established rooted-shoots on New Winarto and Teixeira da Silva (NWT) medium (Winarto et al., 2011) containing 0.06 mg/l 2,4-D, 0.37 mg/l TDZ and 0.5 mg/l BAP and transferred plantlets to *ex vitro* condition in a mixture of burned rice husk, raw rice husk and organic manure (2:2:1, v/v/v) with 74% survivability of them. These results clear informed that the preparation of plantlets and their acclimatization were not a critical problem in *in vitro* culture of *Anthurium.*

Conclusions

The present study exhibited that a new route of *in vitro* propagation protocol for *Anthurium* clones was successfully established using young leaves and WT medium as an explant source and basic medium. Friable yellow callus derived from leaf explants of M-11 clone was the best callus type stimulated on WT medium supplemented with 0.5 mg/l TDZ, 0.1 mg/l BA, and 0.2 mg/l NAA with moderate growth type and easily regenerated to produce shoots. The M-11 clone callus produced shoots in 1.2 MAS of shoot regeneration time with 25.3 shoots per explant, 0.79 cm shoot height, and 3.6 number of leaves per shoot on WT medium containing 0.5 mg/l TDZ, 1.0 mg/l BA and 0.02 mg/l NAA. The shoots were easily rooted on WT medium hormone-free with 1.5 g/l AC with 3.2 roots per shoot and 0.86 cm root length. The plantlets were then acclimatized on burned-rice husk medium with 87.3% plantlet survivability and 150 plantlets acclimatized per clone.

Author contributions

BW and FR designed the experiments, and DP, FR, carried out the lab work; FR took the images and prepared statistical analysis. FR, DP and BW wrote the manuscript. BW is also authorship in preparing the manuscript till final review and revisions. All authors read and approved the final text.

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Conflict of interest

All authors have no financial or commercial conflicts of interest for this particular study.4

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