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In vitro Propagation of *Globba schomburgkii* Hook. f. via Bulbil Explants

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Abstract

An efficient and rapid protocol for the micropropagation of *Globba schomburgkii* Hook. f. via bulbil explants was investigated. The long divided and undivided bubils of *G. schomburgkii* Hook. f. were cultured on MS medium (Murashige and Skoog) that had either 3 mg/l benzyladenine (BA) or 0.5 mg/l naphthaleneacetic acid (NAA) added for 8 weeks. The results indicated that the long divided bulbils of *G. schomburgkii* Hook. f. showed a greater amount of plant regeneration than the undivided bulbils. Callus induction, as well as shoot and root formation, were observed when culturing microshoots of 1 cm in length on media (MS) that had Thidiazuron (TDZ) or NAA plus BA added at a range of concentrations for 8 weeks. The highest percentage of callus induction was 40 % when culturing the microshoots on MS medium supplemented with NAA and BA. The best result for shoot formation was achieved when culturing the microshoots on MS medium with TDZ added. The highest number of roots was obtained when culturing the microshoots on MS medium with NAA and BA added. The *in vitro*-derived plantlets of *G. schomburgkii* Hook. f. were transplanted to pots containing different types of potting mixture in a greenhouse. The survival rates were 80 % when *G. schomburgkii* Hook. f. was transplanted to sand.

Keywords: *Globba schomburgkii* Hook. f., propagation, callus induction, bulbil, acclimatization

Introduction

Globba schomburgkii Hook. f. is an attractive ornamental plant of the family Zingiberaceae and is found in Thailand. The center of diversity is found in continental monsoon Asia, where Thailand and neighboring countries are particularly rich in species. The plant is used as an ornamental plant. In Saraburi Province, Thailand, the flowers of *Globba* are bound together with candles during the Buddhist Lent. The genus *Globba* comprises 89 species worldwide, while there are 42 species of perennial rhizomatous herbs in Thailand that have uses as dyes, spices, or medicine. Another use of the species in Thailand is as cut flowers or ornamental plants, due to the inflorescences having yellowish bracts [1].

Under the conventional method, *Globba* species are propagated vegetatively by the underground rhizome, but this is disease susceptible and has a low propagation rate. The plants in the genus *Globba* are propagated using the underground rhizome, fruit, and bulbil. Considering the present demand (economic value) and propagation problem of the plant, it is necessary to develop a suitable protocol for mass propagation. The tissue culture technique is a suitable method of mass propagation. There are a few reports available that describe the *Globba* species' micropropagation, namely, *Globba brachyanthera* K. Schum. [2], *G.* sp. [3], *Globba magnifica*, *G. winitii* C.H. Wright, *G. schomburkii* cultivar "Burmese Dancing Girl" [4] and *G. marantina* L. [5].

In Zingiberaceae, different plant organs have been used as explants for tissue culture: rhizome [6], anther [7], leaves [8], leaf sheath [9], inflorescence bud [10], seeds [11], embryo [4], and bulbil [2]. Only

some species of the genus *Globba* have bulbils, such as *G. marantina* L., *G. flagellaris* Larsen, and *G. schomburgkii* Hook. f. This paper reports the *in vitro* propagation of *G. schomburgkii* Hook. f. through bulbil explants. This study's objective was to develop, for the first time, and then present a fast *in vitro* system for the micropropagation of *G. schomburgkii* Hook. f., an attractive ornamental plant from Thailand, using the tissue culture technique.

Materials and methods

Explant sources and sterilization

Bulbils of *G. schomburgkii* Hook. f. (5 mm long) were collected from plants growing in Mahasarakham Province, Thailand (**Figure 1**). Running tap water was used to clean the bulbils for 30 min, before rinsing them using 70 % (v/v) ethyl alcohol for 30 s, and sterilizing for 15 s with 15 % sodium hypochlorite containing Tween 20 (2 drops), after which sterilized distilled water was used for 3 washes before culturing for 8 weeks on media (MS [12]) that was amended with either 3 mg/l BA or 0.5 mg/l NAA.

Medium and culture condition

All experiments had 3 % (w/v) sucrose added to the 0.7 % (w/v) agar in the MS medium. Either 1 N NaOH or 1 N HCl was used to ensure that the medium's pH was 5.8 before autoclaving for 15 min at 121 °C. During the incubation of the cultures they were exposed to a 15 µmol/m^2/s light intensity (white fluorescent light) for a 16 h photoperiod at a temperature of 25±2 °C. Observations on the percentage of callus induction, average number of shoots per explant, average number of roots per shoot, and average shoot and root length were recorded after 8 weeks of incubation.

Acclimatization

The *in vitro* regenerated plantlets (7 cm long), which had both roots and shoots that were well developed, had the agar removed from the roots by washing with tap water and were transplanted to pots containing soil, sand, burned rice husk: soil:sand (1:1), soil:burned rice husk (1:1) and sand:burned rice husk (1:1), sand:burned rice husk (1:1), and soil:sand:burned rice husk (1:1:1). The potted plants were maintained under greenhouse conditions at Mahasarakham University, Thailand, in the Faculty of Science (Department of Biology), and were regularly irrigated with tap water.

Statistical analysis

The experiments were conducted using a completely randomized design (CRD) with 20 plantlets for each treatment. Each experiment was repeated 3 times. ANOVA tests were used to determine the results' significance, and the DMRT (Duncan's multiple range test) was used to observe the differences. All tests were considered significant at the 5 % level, and were conducted using the SPSS program (version 11.5).

Results and discussion

The long divided and undivided bulbils of *G. schomburgkii* Hook. f. were used to evaluate the effects of explant type. After 8 weeks of culturing on the media (MS) that had either 3 mg/l BA or 0.5 mg/l NAA added, the number of shoots (1.00 shoot/explant) and the number of roots (1.10 roots/explant) were found on the long divided bulbil (**Table 1** and **Figure 2**). However, the undivided bulbils that were cultured on the media (MS) supplemented with 3 mg/l BA or 0.5 mg/l NAA could not grow. This result indicated that the long divided bulbils of *G. schomburgkii* Hook. f., which was cultured with a 3 mg/l BA and 0.5 mg/l NAA supplementation of the media, resulted in the amount of shoot, root, and callus formation being significantly greater than when compared to that of the undivided bulbils. Kho *et al.* [2] presented work related to the propagation *in vitro* of *G. brachyanthera* K. Schum. by adventitious bulbils. Jala *et al.* [4] inoculated immature embryos of *G. winitii* C.H. Wright by trimming different parts of their seed coat. These results are similar to the observations of Pimmuen *et al.* [5] who cultured divided and undivided bulbils of *G. marantina* L. on MS medium and found that divided bulbils showed better growth

than undivided bulbils. The results showed that the divided bulbils method could break dormancy because, when the bulbil was divided, it let water, oxygen, nutrients, and plant growth regulators pass though the bulbil to reach the micropyle directly. Chong *et al.* [13] also found that dividing the shoot explants of *Curcuma zedoria* Roscoe longitudinally into halves could enhance the formation of multiple shoots.

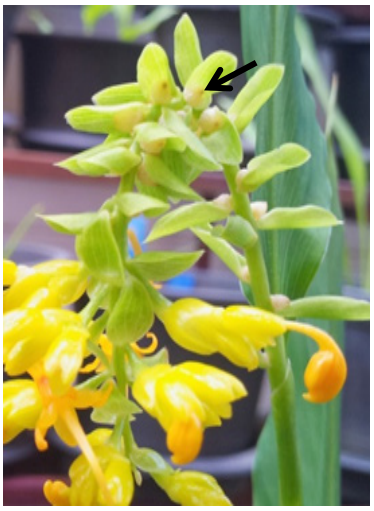

Figure 1 Bulbils of *G. schomburgkii* Hook. f. (arrow).

Table 1 Effect of explant incision on callus induction and shoot and root formation of *G. schomburgkii* Hook. f.

Explant type	Percentage of callus induction	Average no. of shoots/explant mean±SE	Average shoot length (cm) mean±SE	Average no. of roots/explant mean±SE	Average root length (cm) mean±SE
Divided bulbil	50	1.0±0.44[a]	0.54±0.22[a]	1.10±0.72[a]	0.70±0.22[a]
Undivided bulbil	0	0±0.00[b]	0±0.00[b]	0±0.00[b]	0±0.00[b]

*Means followed by the same letters within each column are not significantly different at $p \leq 0.05$, according to DMRT.

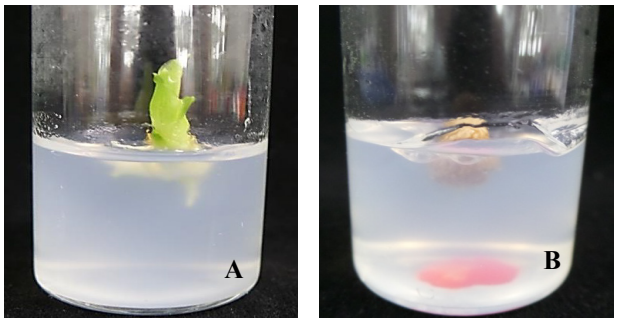

Figure 2 New shoot of *G. schomburgkii* Hook. f. germinated from bulbil after 8 weeks of culture. (A) Divided bulbil, (B) undivided bulbil.

Callus formation occurred at the shoot base of the microshoot when the MS medium had TDZ (0.1, 0.5 or 1 mg/l) added. The callus was soft in texture, friable in structure, and yellowish white. When there was no growth regulator added to the medium, there were no calluses formed. The highest percentage of callus formation (30 %) came from the microshoots being cultured on a media for 8 weeks that had 0.1 mg/l TDZ added (**Table 2** and **Figure 3**). When considering the multiplication of the shoots, TDZ (0.1 - 2 mg/l) produced 6.30 - 9.10 shoots/explant. Srirat *et al.* [14] reported that the suitable conditions for shoot propagation of *Curcuma longa* L. were obtained when sucrose (60 g/l) and TDZ (5 mg/l) were part of the media. Lo-apirukkul *et al.* [15] found that, when the terminal bud explants were cultured, they could obtain the greatest rate for the shoot multiplication (11.82 shoots/explant) for *Curcuma comosa* Roxb. on MS media that was semi-solid with the addition of TDZ (18.16 µM). Hamirah *et al.* [16] also reported that TDZ at 0.5 mg/l was found to induce the highest shoot multiplication of *Zingiber montanum* Koenig. with a mean of 8.1 shoots/explant. These studies are similar to that of Zhang *et al.* [17], in which it was found that, when TDZ was added to the growing medium of *Curcuma soloensis* Valeton., it resulted in over 3 times the number of shoots as BA (18.7 or 5.0 shoots/explant from media, with 2.5 µM TDZ or 40 µM BA, respectively). Higher concentrations of TDZ had no inhibitory effects on shoot multiplication but decreased the callus formation percentage. At a low concentration of, or without, TDZ in the culture media, numerous lengths of shoots and roots, as well as the number of roots, were produced. These results agree with those of Pimmuen *et al.* [5] that TDZ is suitable for shoot multiplication of *G. marantina* L.

Table 2 Effects of TDZ on callus induction and shoot and root formation of *G. schomburgkii* Hook. f.

TDZ (mg/l)	Percentage of callus induction	Average no. of shoots/explant mean±SE	Average shoot length (cm) mean±SE	Average no. of roots/explant mean±SE	Average root length (cm) mean±SE
0	0	1.50±0.16^{b}	5.89±0.43^{b}	6.40±0.66^{b}	2.36±0.22^{a}
0.1	30	6.30±1.36^{a}	2.34±0.18^{a}	8.60±2.07^{a}	1.45±0.08^{b}
0.25	0	8.30±1.01^{a}	1.70±0.09^{a}	6.30±1.28^{b}	1.07±0.07^{c}
0.5	20	7.00±0.80^{a}	2.03±0.12^{a}	3.90±0.79^{b}	0.71±0.06^{d}
1	20	9.10±1.12^{a}	1.94±0.06^{a}	2.29±0.56^{b}	0.65±0.05^{d}
2	0	9.10±1.37^{a}	2.02±0.05^{a}	2.90±0.76^{b}	0.63±0.09^{d}

*Means followed by the same letters within each column are not significantly different at $p \leq 0.05$, according to DMRT.

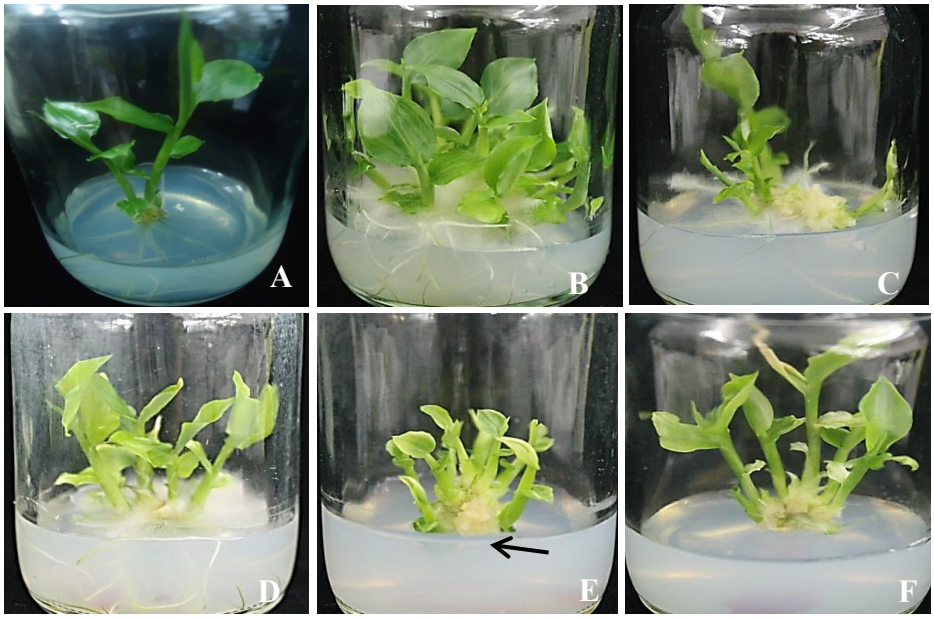

Figure 3 Multiple shoot formation and callus induction of *G. schomburgkii* Hook. f. after 8 weeks of culture on MS medium with various concentrations of TDZ added. (A) 0 mg/l, (B) 0.1 mg/l, (C) 0.25 mg/l, (D) 0.5 mg/l, (E) 1 mg/l, (F) 2 mg/l (callus indicated with arrow).

Callus formation occurred at the shoot base of the microshoot when cultured on the media (MS) with either 4 mg/l BA or 0.5 mg/l NAA with 3 mg/l BA, as well as 1 mg/l NAA with 0.5 mg/l BA added. The callus was soft in texture, friable in structure, and yellowish white. The highest percentage of callus formation (40 %) was obtained from microshoots that came from the media that had 0.5 mg/l NAA plus 3 mg/l BA added, which was more effective than the addition of TDZ at 0.1 mg/l that resulted in a callus induction of 30 %. In this study, the microshoots were cultured on MS medium supplemented with NAA and BA; the shoots were taller than the microshoots that were cultured on the medium added with TDZ. However, the average number of shoots was lower than the microshoots when cultured on media added with TDZ. These results are in agreement with those of Kho *et al.* [2], who reported *in vitro* propagation of *Globba brachyanthera* K. Schum. on MS medium supplemented with NAA and BA. The best response for the number of shoots per explant (5.4 shoots/explant) was obtained on MS medium supplemented with 3 mg/l BA and 0.5 mg/l NAA for 8 weeks. There is only one report from Jala *et al.* [4], who studied embryos of *G. schomburgkii* Hook. f. cultured on MS medium supplemented with 5 mg/l BA for 8 weeks, producing new shoots at 5.33 shoots/explant. Rahman *et al.* [18] also reported that 0.1 mg/l NAA and 1.0 mg/l BA showed the highest number of shoots per culture from *Kaempferia galanga* L. rhizome tip and lateral bud explants of the field grown plant. Kambaska *et al.* [19] found that NAA and BA induced shoot formation in rhizome sprouting of *Zingiber officinale* Rosc. Zhang *et al.* [20] reported that NAA and BA could be used for callus induction of *Curcuma kwangsiensis* Lindl. for root formation. The microshoots of *G. schomburgkii* Hook. f. cultured on NAA and BA, produced 13.60 - 24.30 roots/explant; however, the microshoots cultured on TDZ produced 2.29 - 18.60 roots/explant (**Tables 2** and **3** and **Figures 3** and **4**).

Table 3 Effects of NAA and BA on callus induction and shoot and root formation of *G. schomburgkii* Hook. f.

NAA (mg/l)	BA (mg/l)	Percentage of callus induction	Average no. of shoots/explant mean±SE	Average shoot length (cm) mean±SE	Average no. of roots/explant mean±SE	Average root length (cm) mean±SE
0	0	0	2.40±0.63^{ab}	6.32±0.35^{a}	7.90±1.11^{h}	2.94±0.12^{a}
0	0.5	0	2.20±0.44^{ab}	4.06±0.44^{bcd}	11.80±1.43^{fgh}	1.78±0.09^{bcd}
0	1	0	2.20±0.64^{ab}	3.80±0.57^{fg}	11.50±1.99^{fgh}	1.26±0.17^{f}
0	2	0	3.00±0.57^{ab}	4.01±0.29^{efg}	14.80±2.36^{defg}	1.43±0.08^{def}
0	3	0	3.80±0.80^{a}	3.72±0.38^{fg}	16.00±2.38^{bcdef}	1.40±0.04^{def}
0	4	10	3.30±0.95^{ab}	3.31±0.49^{g}	15.40±3.24^{defg}	1.34±0.16^{ef}
0.5	0	0	2.40±0.42^{ab}	4.65±0.67^{cdefg}	13.40±2.31^{defg}	1.54±0.19^{cdef}
0.5	0.5	0	1.90±0.40^{b}	5.43±0.38^{abcd}	13.60±1.10^{degh}	2.02±0.12^{b}
0.5	1	0	2.60±0.37^{ab}	5.24±0.13^{abcde}	14.20±1.30^{defg}	1.68±0.11^{bcde}
0.5	2	0	2.20±0.32^{ab}	5.72±0.37^{abc}	14.10±1.34^{defg}	1.69±0.08^{bcde}
0.5	3	40	2.40±0.65^{ab}	5.40±0.46^{abcd}	13.90±0.80^{defg}	2.05±0.12^{b}
0.5	4	0	2.70±0.33^{ab}	5.06±0.47^{abcdef}	15.50±2.36^{defg}	1.85±0.09^{bc}
1	0	0	2.10±0.40^{ab}	5.25±0.48^{abcde}	21.40±1.71^{ab}	1.68±0.09^{bcde}
1	0.5	20	2.80±0.51^{ab}	4.84±0.37^{bcdef}	18.80±1.72^{abcd}	1.71±0.07^{bcd}
1	1	0	2.90±0.37^{ab}	5.63±0.30^{abc}	19.80±1.52^{abcd}	1.73±0.12^{bcd}
1	2	0	2.60±0.45^{ab}	6.14±0.25^{ab}	16.20±1.59^{bcdef}	1.84±0.07^{bc}
1	3	0	3.50±0.47^{ab}	5.41±0.42^{abcd}	24.30±1.69^{a}	1.68±0.07^{bcde}
1	4	0	2.90±0.37^{ab}	6.01±0.33^{abc}	20.80±2.15^{abc}	1.94±0.08^{b}

*Means followed by the same letters within each column are not significantly different at $p \leq 0.05$, according to DMRT.

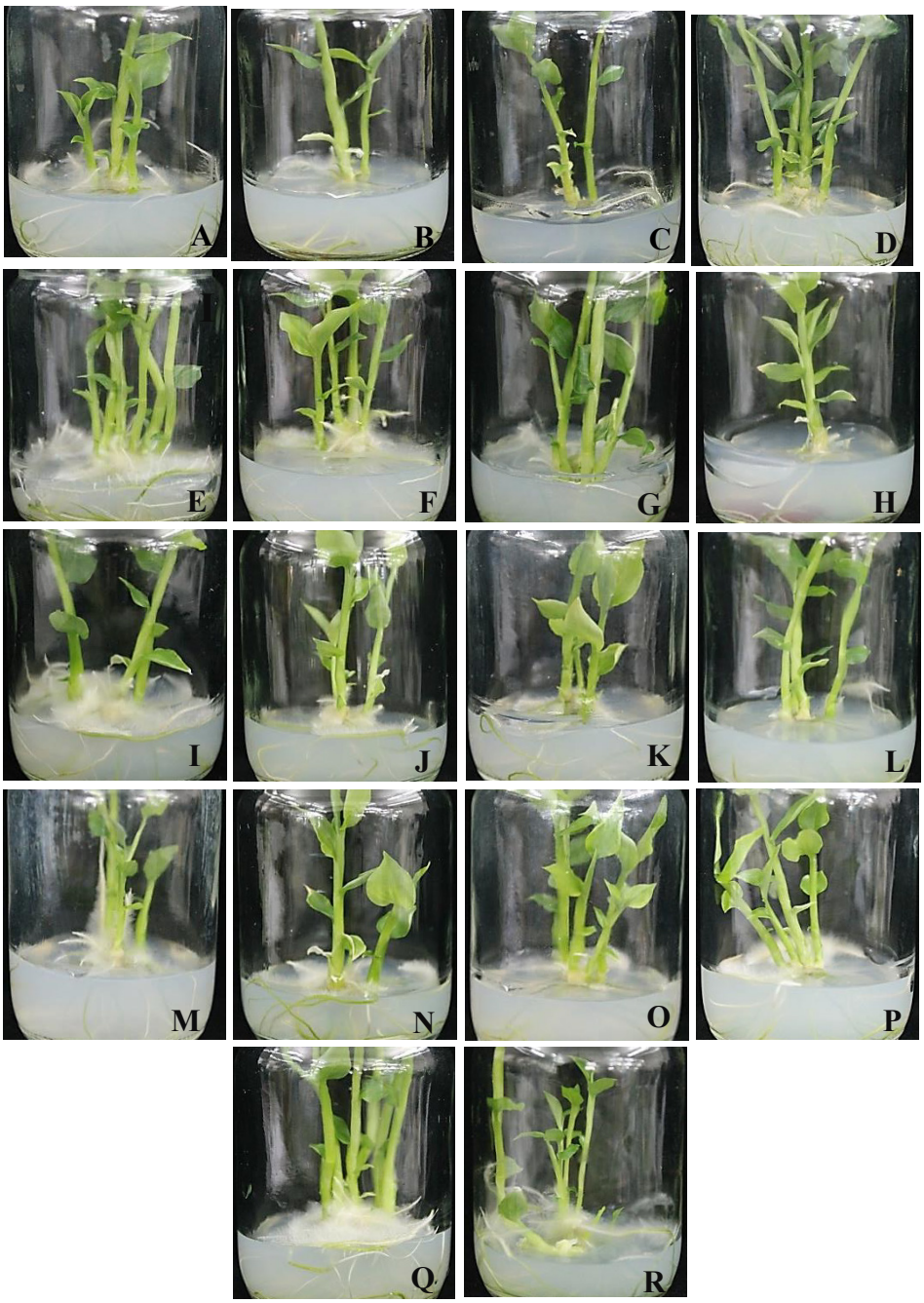

Figure 4 Callus induction and shoot and root formation of *G. schomburgkii* Hook. f. after 8 weeks of culture (A) free hormone (B) BA 0.5 mg/l (C) BA 1 mg/l (D) BA 2 mg/l (E) BA 3 mg/l (F) BA 4 mg/l (G) NAA 0.5 mg/l +BA 0 mg/l (H) NAA 0.5 mg/l +BA 1 mg/l (I) NAA 0.5 mg/l +BA 2 mg/l (J) NAA 0.5 mg/l +BA 3 mg/l (K) NAA 0.5 mg/l + BA 4 mg/l (L) NAA 1 mg/l + BA 0 mg/l (M) NAA 1 mg/l + BA 0.5 mg/l (N) NAA 1 mg/l + BA 0.5mg/l (O) NAA 1 mg/l + BA 1 mg/l (P) NAA 1 mg/l + BA 2 mg/l (Q) NAA 1 mg/l + BA 3 mg/l (R) NAA 1 mg/l + BA 4 mg/l.

Plantlets (10 cm height) with 5 to 6 leaves and well-developed root systems were removed and transferred to pots containing soil, sand, burned rice husk, soil:sand (1:1), soil:burned rice husk (1:1) and sand:burned rice husk (1:1), sand:burned rice husk (1:1) and soil:sand:burned rice husk (1:1:1) without a hardening process for 8 weeks under greenhouse conditions at the Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand. The highest percentage of surviving plantlets (80 %), average number of leaves per shoot (21.62), and average shoot length (15.00 cm) were obtained from plantlets of *G. schomburgkii* Hook. f. transplanted to sand (**Table 4** and **Figure 5**). These results are different from other authors, who successfully grew *in vitro* regenerated plants of the ginger family in potting mixtures containing soils. Our results are in agreement with Pimmuen *et al.* [5], who found that combinations of sand:soil (1:1) and sand:burned rice husk (1:1) were the best acclimatization medium for *Globba marantina* L., with a survival percentage of 100 %. When comparing them with the mother plant, there were no observable differences in the growth characteristics or morphology of the regenerated plants.

Table 4 Effect of potting media on plantlet performance of *G. schomburgkii* Hook. f. after 8 weeks of acclimatization.

Potting medium	Percentage of surviving plantlets	Average no. of leaves/shoot mean±SE	Average shoot length (cm) mean±SE
Soil	73	14.98±0.85^{d}	10.00±0.40^{b}
Sand	80	21.62±1.39^{a}	15.00±0.75^{a}
Burned rice husk	60	17.70±0.49^{c}	11.00±0.44^{b}
Soil:Sand	66	21.96±1.08^{a}	11.40±0.47^{b}
Soil:Burned rice husk	73	19.56±0.44^{ab}	9.90±0.39^{b}
Sand:Burned rice husk	73	19.95±0.84^{ab}	10.09±0.39^{b}
Soil:Sand:Burned rice husk	60	12.00±0.00^{d}	9.00±0.00^{b}

*Means followed by the same letters within each column are not significantly different at $p \leq 0.05$, according to DMRT.

Figure 5 Acclimatized plantlets 8 weeks after transfer to a pot containing soil (A), sand (B), and burned rice husk (C).

Conclusions

The long divided bulbils of *G. schomburgkii* Hook. f. showed a better percentage of plant regeneration than the undivided bulbils. Callus formation was found on the MS medium with TDZ, BA alone, or NAA plus BA added. Shoot multiplication of *G. schomburgkii* Hook. f. can be obtained using 0.1 - 2 mg/l TDZ. Root formation was significantly better when NAA and BA were added to the media (MS). *In vitro* regenerated plantlets of *G. schomburgkii* Hook. f. that were planted in sand had a survival rate of 80 %. This study presents the first report of callus induction, shoot and root formation, and transplantation of *G. schomburgkii* Hook. f., which is an attractive ornamental plant from Thailand. This will provide a foundation for propagating plants on a large scale and for genetic manipulation.

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