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IN VITRO PROPAGATION OF *STAHLIANTHUS CAMPANULATUS* KUNTZE, A RARE PLANT SPECIES FROM THAILAND

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ABSTRACT

A study was conducted to determine the effect of plant growth regulators on the initiation and growth of *Stahlianthus campanulatus* Kuntze, a rare plant species from Thailand. Rhizome buds derived from shoots were cut and cultured on modified MS medium supplemented with various concentrations of auxin, cytokinin and their combinations. The cultures were kept under a temperature of 25±2 °C with fluorescent light for a 16 hours photoperiod/day for 12 weeks. Multiple shoots cultured on MS medium supplemented with 4 mgL^{-1} BAP, produced the highest number of shoots per explant (5 shoots/explant). The highest lengths of shoots, 20.20, 20.06 and 20.12 cm, were obtained on the MS medium supplemented with 4 mgL^{-1} kinetin, 2 and 4 mgL^{-1} BAP, respectively. The best rooting was observed in MS medium supplemented with 1, 2 and 4 mgL^{-1} kinetin, which produced 32.40-37.50 roots per shoot. MS medium supplemented with a combination of 4 mgL^{-1} kinetin and 0.5 mgL^{-1} IAA gave an average number of 3.0 shoots per explant with a length of 21.28 cm. Acclimatization was successful when transplanted in soil and coconut fiber with 91.6% survivability potential.

Key words: *Stahlianthus campanulatus*, Propagation, Zingiberaceae, Rare Plant, Plant Tissue Culture

INTRODUCTION

Stahlianthus campanulatus Kuntze, belonging to the family Zingiberaceae, is known as Wan Pet Noi in Thailand. The genus *Stahlianthus* is a small genus in the tribe Hedychieae and has a distribution range in China, India, Laos, Myanmar, Sikkim, Thailand, Vietnam and Cambodia Currently, there are seven accepted names (Wu and Larsen, 2000). Five species were recorded in Thailand i.e. *S. campanulatus, S. involucratus*, *S. thorelii*, *S. pediculatus* and *S. macrochlamys*. The other two species were reported from adjacent places, *S. andersonii* in Myanmar and *S. philipianus* in Indo-china (Maknoi, 2011). It is a perennial medicinal herb and rare species (Saensouk, 2011). The rhizome is commonly used as ananti-inflammatory and antinociceptive medicine (Pingsusaen, 2015). Moreover, owing to its beautiful leaf and flower it is used as an ornamental plant. *S. campanulatus* Kuntze is normally propagated by its rhizome with a low proliferation rate and this family is easily infected by soil borne pathogens, such as bacterial wilt (*Pseudomonas solanacearum*), nematodes (*Meloidogyne* sp.) and soft rot (*Pythium aphanidermatum*), which cause heavy losses in yield due to the rhizome being small with fewer buds per plant (Taha *et al*., 2013). The tissue culture technique allows rapid clonal propagation and conservation as this technique can be used for multiple shoot induction without the restriction of the season. Several investigators reported micropropagation of Zingiberaceae either from buds (Abdelmageed *et al*., 2011; Mohamed *et al*., 2011; Sharma and Singh, 1995), shoot tips (Anjumanara *et al*., 2003), meristems (Bhagyalakshmi and Singh, 1988), or leaves (Prakash *et al*., 2004; Saensouk, 2011). So far no research or micropropagation protocols has been reported for this species. The objective of this study was to develop a rapid *in vitro* propagation protocol for *S. campanulatus*, using the tissue culture technique.

MATERIALS AND METHODS

Plant Materials: Rhizomes of *S. campanulatus* were collected from Phu Hin Lat Cho Fa, Nong Bua Lam Phu Province, Thailand and used in the experiment (Figure 1). Rhizome buds were rinsed with running tap water for 60 minutes, then surface sterilized by using 10% Clorox with three drops of tween-20 for 10 minutes and 5 minutes respectively and finally rinsed three times in sterilized distilled water. Then, rhizome buds were initially cultured on basal MS (Murashige and Skoog, 1962). After one month of bud culture, excised rhizome buds (1-1.5 cm in diameter) were used as explants for the experiments.

Culture Media and Conditions: The explants were transferred to MS medium supplemented with 3% (W/V) sucrose, 8 g/l agar and different concentrations of plant growth regulators. The plant growth regulators were BAP (0, 1.0, 2.0, 4.0 and 8.0 mgL^{-1}), kinetin (0, 1.0, 2.0, 4.0

and 8.0 mgL^{-1}), NAA (0, 0.5, 1.0, 2.0 and 4.0 mgL^{-1}) and IAA (0, 0.5, 1.0, 2.0 and 4.0 mgL^{-1}) and their combinations. The pH of the medium was adjusted to 5.7. The cultures were kept under a temperature of 25±2 °C with florescent light for 16 hours photoperiod/day for 12 weeks.

***Ex Vitro* Transplantation:** Plantlets, 20-22 cm in height, with well developed roots were removed from the culture medium. The roots were washed gently under running tap water and transferred into plastic pots containing autoclaved soil, sand, burned rice husk or coconut fiber for four weeks before being transplanted into soil under field conditions.

Statistical Analysis: The experimental design used was completely randomized design (CRD). The data on mean number of shoots, roots, leaves and length of shoots and roots after 12 weeks were analyzed statistically by Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$, using the Statistical Package for Social Sciences (SPSS) Version 11.5.

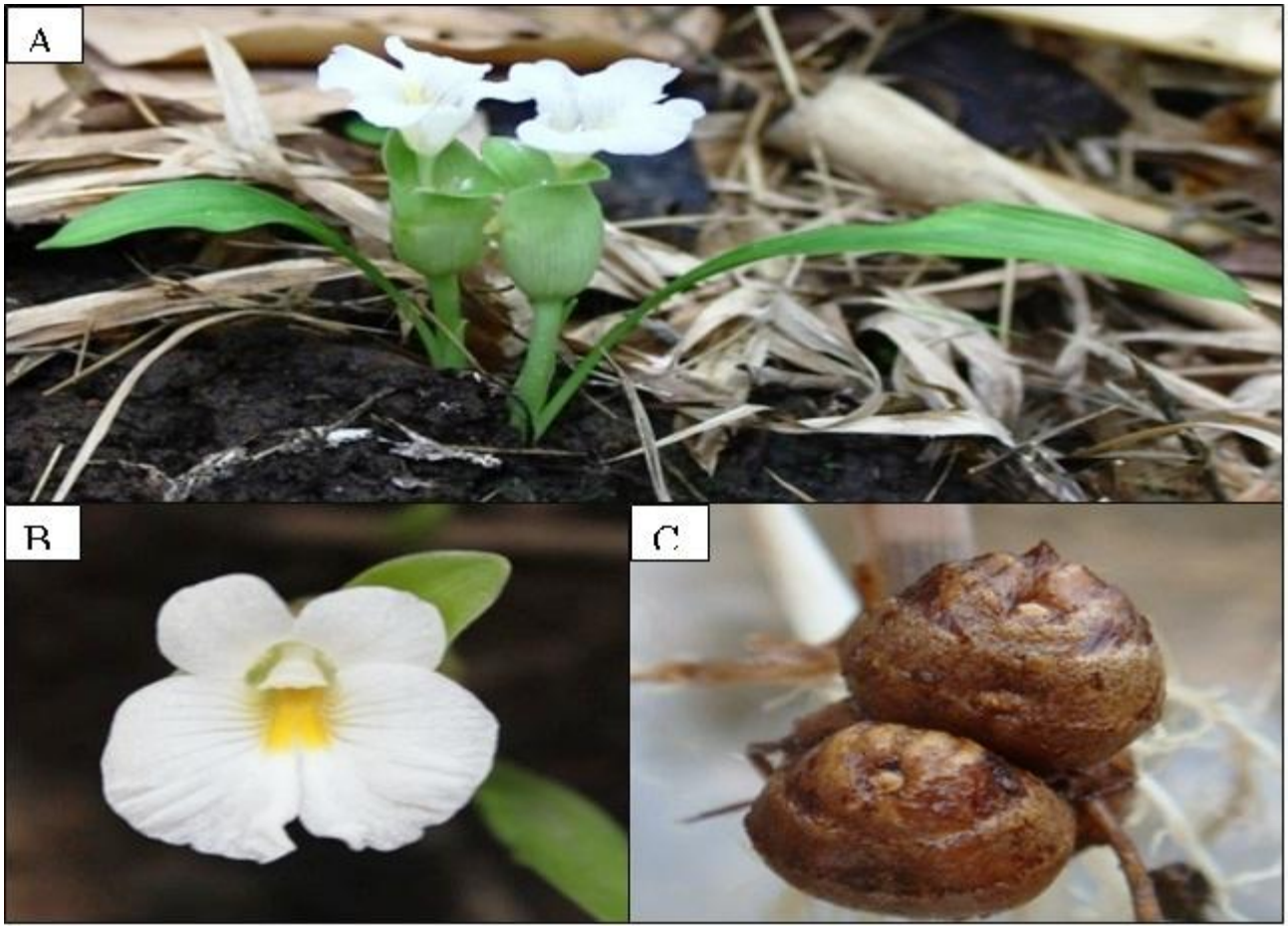

Figure 1. *S. campanulatus* Kuntze (A) Flowering of *S. campanulatus* Kuntze in the natural habitats (B) A closed view of *S. campanulatus* Kuntze flower (C) Rhizome buds as a source of explants.

RESULTS AND DISCUSSION

Multiple shoots were cultured from the rhizome bud explants of *S. campanulatus* on MS medium supplemented with various concentrations (0, 1.0, 2.0, 4.0 and 8.0 mgL^{-1}) of BAP and kinetin for 12 weeks. The highest number of shoots was obtained on the MS medium supplemented with 4 mgL^{-1} BAP (average number of shoots 5.0) and best shoot length was observed on medium containing 4 mgL^{-1} kinetin, 2 and 4 mgL^{-1} BAP (average shoot length 20.20, 20.06 and 20.12 cm respectively). The best rooting was observed in MS medium supplemented with 1, 2 and 4 mgL^{-1} kinetin and the highest number of average roots per shoots were 32.40, 36.75 and 37.50 roots per shoot respectively.

However, the average number of leaves per shoot was not significantly different in all treatments, except the MS medium without plant growth regulators. These results imply that BAP and kinetin as plant growth regulators play an important role in shoot multiplication

(Table 1 and Figure 2). In general, herbaceous plants are highly responsive to BAP treatment and most cultured herbaceous species produces robust, well-formed shoots suitable for further shoot proliferation (Debergh and Zimmerman, 1999). This is in agreement with the study done by Gomathy *et al.* (2014) that, reported high BAP (2.0 mgL^{-1}) produced multiple shoots from *Curcuma longa*. Furthermore, our results are is agreement with Goyal *et al.* (2010) who found that MS medium supplemented with 2 mgL^{-1} BAP was the best medium for shoot multiplication in *C. longa*. Nayak (2000) reported that shoot production by *Curcuma aromatic* was stimulated by increasing the concentration of BA to 5 mgL^{-1}. While, Balachandran *et al.* (1990) found that a concentration of 3.0 mgL^{-1} BAP was the optimum for *in vitro* multiplication of turmeric and ginger species. On the other hand, Bejoy *et al.* (2012) reported that *Curcuma vamana* Sabu and Mangaly cultivated on MS medium supplemented with 0.5 mgL^{-1} TDZ gave the highest shoot induction (9.6 per explant).

The effects of NAA and IAA on root induction were investigated and found that the MS medium supplemented with 2.0 and 4.0 mgL^{-1} NAA and 4.0 mgL^{-1} IAA produced the highest number of roots (15.16 and 16.40) per explants, but the greatest length of roots was 5.28 cm observed with the concentration of 1.0 mgL^{-1} NAA. The addition of NAA and IAA did not affect shoot formation. In this study, the IAA and NAA hormones were found to have positive effects on root induction but less than kinetin alone (Table 2 and Figure 3). According to Loc *et al.* (2005), MS medium supplemented with 2 mgL^{-1} NAA gave 18.5 roots per explants for root induction in *Curcuma zedoaria*.

MS medium supplemented with different concentrations of BAP, kinetin in combination with NAA and IAA resulted in the initiation of shoots and roots from rhizome bud explants. The highest number of shoots was induced in MS medium supplemented with 4 mgL^{-1} kinetin and 0.5 mgL^{-1} IAA, with an average number of 3.0 shoots per explant and length of 21.28 cm. The best rooting was observed in MS medium supplemented with 4.0 mgL^{-1} BAP and 1.0 mgL^{-1} NAA, which produced the highest number of roots (31.50) per explant and greatest length of root (5.62 cm). Under this condition, the development of the shoots and number of leaves were as in a normal plant (Table 3 and Figure 4). This result is confirmed in this study, and this indicated the importance of IAA and NAA hormones in the induction of roots. It was observed that cytokinin was required in the optimal quantity for shoot proliferation in some species of Zingiberaceae, but the inclusion of a low concentration of auxins along with cytokinin triggered the best rate of shoot proliferation (Rout and Das, 1997; Sharma and Singh, 1997; Raihana *et al.*, 2011). Similar results were also observed in rhizome bud explants. These results follow the observations made by Faridah *et al.* (2011), who found that MS medium supplemented with a combination of 5.0 mgL^{-1} BAP and 2.0 mgL^{-1} IAA or 3.0 mgL^{-1} BAP and 0.5 mgL^{-1} IAA were the best for shoot multiplication of *Zingiber zerumbet*. Moreover, Bharalee *et al.* (2005) reported that shoot multiplication of *Curcuma caesia* was induced in MS medium supplemented with 4 mgL^{-1} BAP and 0.5 mgL^{-1} NAA. Raihana *et al.* (2011) reported that *Curcuma manga* cultivated on MS medium supplemented with a combination of 3 mgL^{-1} BAP and 1 mgL^{-1} NAA gave the highest number of shoots.

The plantlets with well developed shoot and roots after acclimatization were successfully transplanted in soil and coconut fiber with 91.6% survivability potential (Table 4 and Figure 5). The survival of *in vitro* regenerated plants to *ex vitro* conditions depends on the conditions during transfer of *in vitro* plantlets and soil substrates, plant survival may be increased by the addition of moisture absorbing material to soil (Seran *et al*., 2005).

Table 1. Effects of cytokinins (BAP and kinetin) on shoot culture from rhizome buds of *Stahlianthus campanulatus* Kuntze after 12 weeks.

Plant growth regulator	Concentration (mgL^{-1})	No. of shoots/explant mean±S.E.	Length of shoots/explant (cm) mean±S.E.	No. of roots/shoot mean ±S.E.	Length of roots/shoot (cm) mean±S.E.	No. of leaves/shoot mean±S.E.
Control	0	1.20±0.16 ^{d}	15.40±0.50 ^{bc}	6.00±1.14 ^{cd}	3.25±0.47 ^{d}	3.40±0.50 ^{a}
BAP	1	2.20±0.20 ^{cd}	14.60±0.50 ^{c}	10.80±1.06^{bc}	3.31±0.43 ^{d}	3.60±0.40 ^{a}
	2	3.75±0.31 ^{b}	20.06±0.64 ^{a}	14.37±1.48 ^{b}	3.70±0.42 ^{cd}	3.75±0.25 ^{a}
	4	5.00±0.26 ^{a}	20.12±0.69 ^{a}	15.87±0.93 ^{b}	3.32±0.23 ^{d}	3.87±0.22 ^{a}
	8	1.75±0.31 ^{cd}	6.75±0.52 ^{d}	3.12±0.39 ^{d}	1.62±0.20 ^{e}	2.75±0.36 ^{a}
Kinetin	1	2.60±0.67 ^{c}	14.40±2.11 ^{c}	32.40±0.65 ^{a}	5.00±0.54 ^{c}	3.80±0.37 ^{a}
	2	3.83±0.30 ^{b}	18.22±1.16 ^{ab}	36.75±1.96 ^{a}	6.67±0.61 ^{b}	3.83±0.47 ^{a}
	4	4.20±0.58 ^{ab}	20.20±1.59 ^{a}	37.50±1.96 ^{a}	12.00±0.70 ^{a}	3.33±0.76 ^{a}
	8	1.30±0.21 ^{d}	14.28±0.74 ^{c}	7.65±0.32 ^{cd}	7.33±0.96 ^{b}	3.33±0.49 ^{a}

Means followed by the same latter are not significantly different at $p< 0.05$.

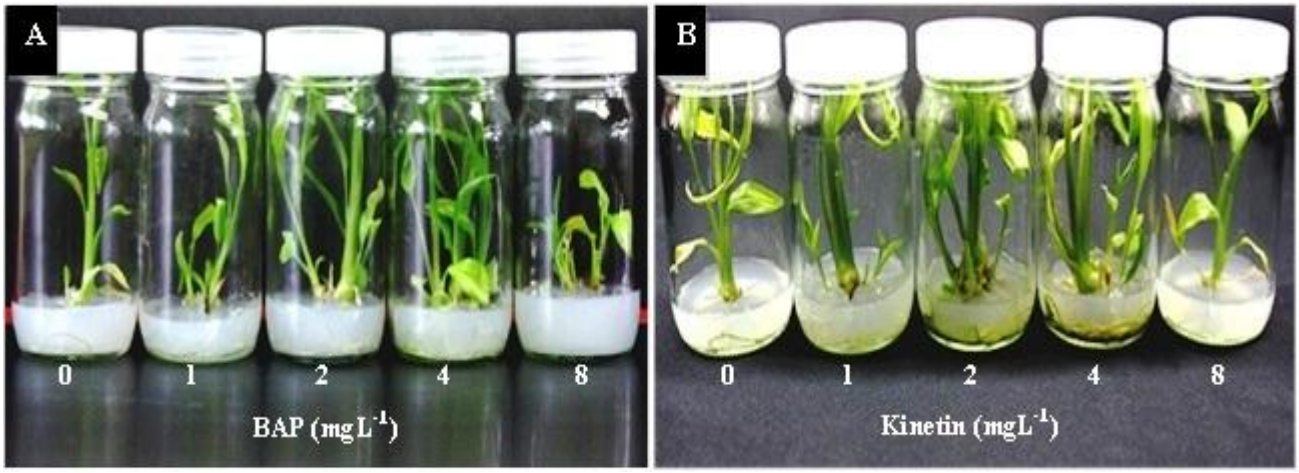

Figure 2. Multiple shoot formation of *Stahlianthus campanulatus* Kuntze after 12 weeks of cultivation on MS medium supplemented with (A) BAP and (B) kinetin.

Table 2. Effects of auxin (NAA and IAA) on shoot culture from rhizome buds of *Stahlianthus campanulatus* Kuntze after 12 weeks.

Plant growth regulator	Concentration (mgL^{-1})	No. of shoots/explant mean±S.E.	Length of shoots/explant (cm) mean±S.E.	No. of roots/shoot mean±S.E.	Length of roots/shoot (cm) mean±S.E.	No. of leaves/shoot mean±S.E.
Control	0	1.00±0.00 ^{b}	16.06±0.37 ^{c}	7.80±0.81 ^{c}	4.60±0.71 ^{ab}	4.50±0.34 ^{bc}
NAA	0.5	1.01±0.16 ^{b}	16.83±0.66 ^{bc}	10.00±1.54 ^{abc}	4.58±0.32 ^{ab}	6.08±0.45 ^{a}
	1	1.57±0.29 ^{b}	18.71±1.25 ^{abc}	12.42±1.77 ^{bc}	5.28±0.30 ^{a}	5.85±0.38 ^{a}
	2	1.80±0.58 ^{ab}	18.81±0.66 ^{abc}	15.16±1.54 ^{a}	3.85±0.33 ^{bc}	5.16±0.47 ^{abc}
	4	2.20±0.58 ^{ab}	18.56±1.40 ^{abc}	16.40±3.72 ^{a}	2.43±0.36 ^{d}	4.28±0.29 ^{c}
IAA	0.5	1.00±0.00 ^{b}	15.40±0.79 ^{c}	5.20±0.48 ^{c}	3.04±0.53 ^{cd}	6.00±0.40 ^{a}
	1	1.16±0.16 ^{b}	18.32±2.34 ^{abc}	8.80±1.15 ^{bc}	3.57±0.35 ^{bcd}	5.60±0.24 ^{ab}
	2	1.20±0.20 ^{b}	21.48±0.90 ^{a}	10.00±1.14 ^{abc}	4.45±0.27 ^{ab}	5.80±0.48 ^{a}
	4	1.14±0.14 ^{b}	20.31±1.68 ^{ab}	16.33±3.07 ^{a}	4.74±0.50 ^{ab}	6.40±0.40 ^{a}

Means followed by the same latter are not significantly different at $p< 0.05$.

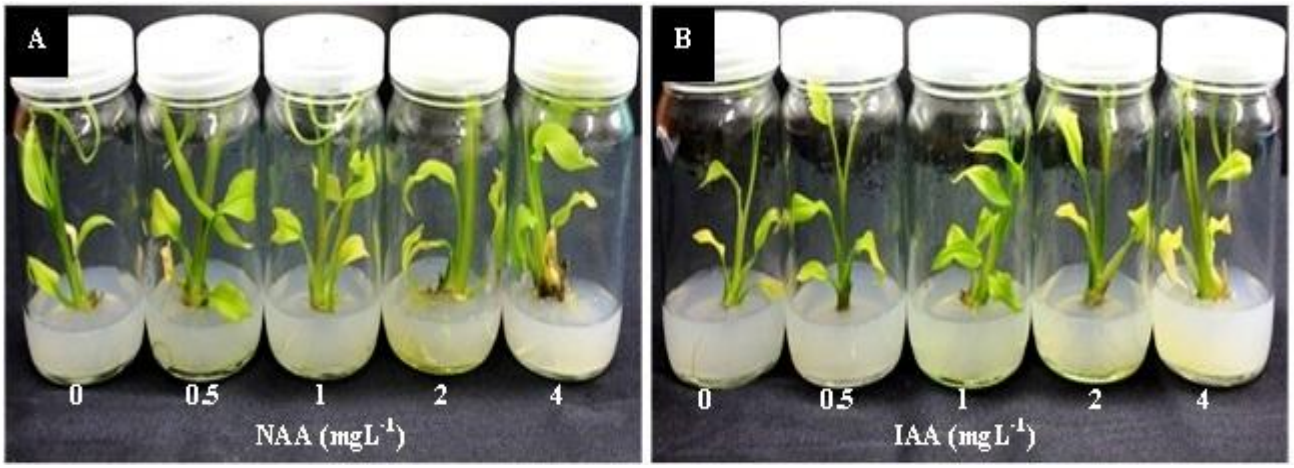

Figure 3. Multiple shoot formation of *Stahlianthus campanulatus* Kuntze after 12 weeks of cultivation on MS medium supplemented with (A) NAA and (B) IAA.

Table 3. Effect of combinations of different levels of cytokinins (BAP and kinetin) and auxin (NAA and IAA) on shoot culture from rhizome buds of *Stahlianthus campanulatus* Kuntze after 12 weeks.

Plant growth regulator	Concentration (mgL^{-1})	No. of shoots/explant mean±S.E.	Length of shoots/explant (cm) mean±S.E.	No. of roots/shoot mean±S.E.	Length of roots/shoot (cm) mean±S.E.	No. of leaves/ shoots mean±S.E.
Control	0	1.00±0.00 ^{f}	15.60±0.40 ^{f}	3.40±0.16 ^{f}	4.40±0.34 ^{c}	5.30±0.15 ^{a}
BAP+NAA	2 + 0.5	1.50±0.15 ^{def}	19.40±0.95 ^{cde}	13.20±0.86 ^{de}	4.50±0.28 ^{bc}	5.87±0.24 ^{a}
	2 + 1	1.37±0.17 ^{def}	20.78±0.90 ^{bcd}	24.85±3.29 ^{b}	5.43±0.36 ^{ab}	5.57±0.33 ^{a}
	4 + 0.5	2.28±0.12 ^{bc}	18.85±0.61 ^{de}	22.14±3.21 ^{bc}	4.53±0.18 ^{bc}	5.48±0.34 ^{a}
	4 + 1	2.75±0.21 ^{ab}	20.57±0.84 ^{bcd}	31.50±3.00 ^{a}	5.62±0.43 ^{a}	5.36±0.26 ^{a}
BAP+IAA	2 + 0.5	1.20±0.13 ^{ef}	17.70±0.50 ^{ef}	11.80±1.23 ^{e}	4.26±0.16 ^{c}	5.40±0.34 ^{a}
	2 + 1	1.16±0.11 ^{ef}	17.86±0.48 ^{ef}	11.40±1.02 ^{e}	4.30±0.13 ^{c}	5.85±0.35 ^{a}
	4 + 0.5	1.40±0.16 ^{def}	15.80±0.99 ^{f}	15.00±2.09 ^{de}	4.43±0.14 ^{c}	5.28±0.35 ^{a}
	4 + 1	1.57±0.13 ^{def}	15.85±0.40 ^{f}	15.20±1.82 ^{de}	4.16±0.18 ^{c}	5.00±0.30 ^{a}
Kinetin+NAA	2 + 0.5	1.16±0.11 ^{ef}	22.41±0.53 ^{ab}	15.50±2.05 ^{cde}	4.19±0.34 ^{c}	5.83±0.21 ^{a}
	2 + 1	1.40±0.16 ^{def}	17.52±1.38 ^{ef}	10.40±1.68 ^{e}	4.44±0.23 ^{c}	5.50±0.23 ^{a}
	4 + 0.5	1.12±0.08 ^{ef}	21.62±0.94 ^{abc}	12.57±0.84 ^{de}	5.43±0.29 ^{ab}	5.93±0.37 ^{a}
	4 + 1	1.40±0.26 ^{def}	19.81±1.02 ^{cde}	10.60±1.60 ^{e}	4.98±0.21 ^{bc}	5.20±0.13 ^{a}
Kinetin+IAA	2 + 0.5	1.80±0.38 ^{cde}	23.96±0.34 ^{a}	19.20±0.77 ^{bcd}	4.28±0.17 ^{c}	5.80±0.25 ^{a}
	2 + 1	2.83±0.32 ^{ab}	21.25±0.76 ^{bcd}	24.50±2.72 ^{b}	5.79±0.24 ^{a}	5.66±0.35 ^{a}
	4 + 0.5	3.00±0.33 ^{a}	21.28±1.18 ^{bcd}	19.00±1.95 ^{bcd}	4.24±0.32 ^{c}	5.76±0.26 ^{a}
	4 + 1	2.00±0.46 ^{cd}	19.07±0.49 ^{cde}	15.57±1.01 ^{cde}	4.99±0.35 ^{bc}	5.35±0.28 ^{a}

Means followed by the same latter are not significantly different at $p< 0.05$.

Table 4. Effect of various potting mixtures on hardening of *in vitro* rooted plantlets of *Stahlianthus campanulatus* Kuntze after 4 weeks.

Potting mixture	No. of plantlets transferred	Survival rate (%)	New leaf formation (%)	Shoot length (cm) (mean±S.E.)
Burned rice husk	36	16.6	0	12.50±0.54 ^{c}
Soil	36	91.6	66.6	21.50±1.43 ^{b}
Coconut fiber	36	91.6	88.8	45.60±0.89 ^{a}
Sand	36	58.3	60.0	18.70±1.12 ^{bc}

Means followed by the same latter are not significantly different at $p< 0.05$.

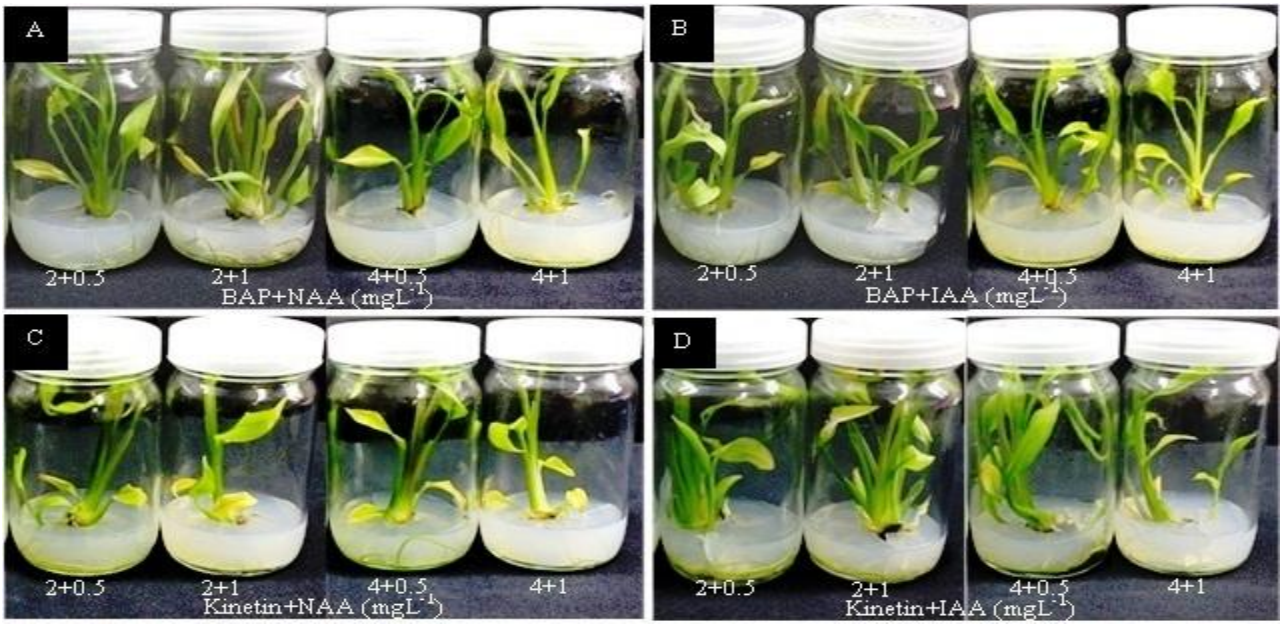

Figure 4. Multiple shoot formation of *Stahlianthus campanulatus* Kuntze after 12 weeks of cultivation on MS medium supplemented with (A) BAP+NAA, (B) BAP+IAA, (C) kinetin+NAA and (D) kinetin+IAA.

Figure 5. Effects of different potting mixtures (A) burned rice husk, (B) soil, (C) coconut fiber and (D) sand 4 weeks after transplantation.

Conclusion: The tissue culture protocol has been developed for rapid and large scale micropropagation and conservation of *S. campanulatus* Kuntze. The shoot multiplication mainly depends on type and strength of BAP incorporated in MS medium. Easy rooting of the microshoots and the plantlets were easily acclimatized to the external environment.

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REFERENCES

Abdelmageed, A.H.A., Q.Z. Faridah, F.M.A. Norhana, A.A. Julia, and A.K. Midhzar (2011). Micropropagation of *Etlingera elatior* (Zingiberaceae) by using axillary bud explants. J Med Plants Res. 5: 4465-4469.

Anjumanara, K., N. Shamima, and M. Tojammal Hossain (2003). Large scale multiplication of Ginger (*Zingiber officinale* Rosc.) from shoot-tip culture. J Biol Sci. 3: 59-64.

Balachandran, S.M., S.R. Bhat, and K.P.S. Chandel (1990). *In vitro* clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.). Plant Cell Rep. 8: 521-524.

Bejoy, M., M. Dan, N.P. Anish, Anjana R.G. Nair, B.J. Radhika, and K. Manesh (2012). Micropropagation of an Indian Ginger (*Curcuma vamana* Sabu and Mangaly): A wild relative of Turmeric. Biotechnology. 11: 333-338.

Bhagyalakshmi, B., and N.S. Singh (1988). Meristem culture and propagation of a variety of ginger (*Zingiber officinale* Rosc.) with a high yield of oleoresin. J Hortic Sci Biotechnol. 63:321-327.

Bharalee, R., A. Das, and M.C. Kalita (2005). *In vitro* clonal propagation of *Curcuma caesia* Roxb. and *Curcuma zedoaria* Rosc. from rhizome bud explants. J Plant Biochem Biot. 14: 61-63.

Debergh, P.C., and R.H. Zimmerman (1999). Micropropagation Technology and Application. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Faridah, Q.Z., A.H.A. Abdelmageed, A.A. Julia, and H.R. Nor (2011). Efficient *in vitro* regeneration of *Zingiber zerumbet* Smith (a valuable medicinal plant) plantlets from rhizome bud explants. Afr J Biotechnol. 10: 9303-9308.

Gomathy, V., M. Anbazhagan, and K. Arumugam (2014). Effect of BAP on *in vitro* regeneration of *Curcuma longa* (Turmeric). Int J Plant Sci. 4: 34-37.

Goyal, A.K., K. Ganguly, T. Mishra, and A. Sen (2010). *In vitro* multiplication of *Curcuma longa* Linn.– an important medicinal zingiber. NBU J Plant Sci. 4: 21-24.

Loc, N.H., D.T. Duc, T.H. Kwon, and M.S. Yang (2005). Micropropagation of zedoary (*Curcuma zedoaria* Roscoe) – a valuable medicinal plant. Plant Cell Tissue Org Cult. 81: 119-122.

Maknoi, C. (2011). The genus *Stahlianthus* Kuntze (Zingiberaceae) in Thailand. 15th Flora of Thailand Meeting, Chiang Mai.

Mohamed, S.A., S.T. Hussein, I.A. Usama, M.E. Hattem, and I.G. El-Sayed (2011). *In vitro* propagation of ginger (*Zingiber officinale* Rosco). J Gen Eng Biot. 9: 165-172.

Murashige, T., and F. Skoog (1962). A revised medium for rapid growth and bioassay tobacco tissue culture. J Plant Physiol. 15: 473-497.

Nayak, S. (2000). *In vitro* multiplication and microrhizome induction in *Curcuma aromatic* Salisb. Plant Growth Regul. 32: 41-47.

Pingsusaen, P. , P. Kunanusorn, P. Seshadri, K. Kathiravan, and S. Ignacimuthu (2004). Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. Plant Cell Tissue Org Cult. 78: 159-165.

Raihana, R., Q.Z. Faridah, A.A. Julia, A.H.A. Abdelmageed, and A.K. Mihdzar (2011). *In vitro* culture of *Curcuma manga* from rhizome bud. J Med Plants Res. 5: 6418-6422.

Rout, G.R., and P. Das (1997). *In vitro* organogenesis in ginger (*Zingiber officinale* Rosc.). J Herbs, Spices Medicinal Plants. 4: 41-51.

Saensouk, P. (2011). Callus induction and plant regeneration from leaf explants of *Cornukaempferia aurantiflora* Mood & Larsen. Pak J Bot. 43: 2415-2418.

Saensouk, S. (2011). Endemic and rare plants of Ginger Family in Thailand, KKU Res J. 16:306-330.

Seran, T.H., K. Hirimburegama and M.T.K. Gunasekare (2005). Encapsulation of embryonic axes of *Camellia sinensis* (L.) O. Kuntze (tea) and subsequent *in vitro* germination. J Hortic Sci Biotechnol. 80: 154-158.

Sharma, T.R., and B.M. Singh (1995). *In vitro* microrhizome production in *Zingiber officinale* Rosc. Plant Cell Rep. 15: 274-277.

Sharma, T.R., and B.M. Singh (1997). High frequency *in vitro* multiplication of disease-free *Zingiber officinale* Rosc. Plant Cell Rep. 17: 68-72.

Taha, H.S., M.S. Abbas, U.I. Aly and I. Gaber (2013). New aspects for callus production, regeneration and molecular characterization of Ginger (*Zingiber officinale* Rosc.). Med Aromat Plants. 6:2-8.

Wu, T.L., and K. Larsen (2000). Zingiberaceae. In: Raven, P.H. & T.L. Wu (Ed.). Flora of China. Science Press, Missouri Botanical Garden, St. Louis. 14: 322-378.