The Journal of Animal & Plant Sciences, 26(5): 2016, Page: 1405-1410
ISSN: 1018-7081

IN VITRO PROPAGATION OF *KAEMPFERIA MARGINATA* CAREY EX ROSCOE, A NATIVE PLANT SPECIES TO THAILAND

P. Saensouk[1*], N. Muangsan[2], S. Saensouk[3]and P. Sirinajun[1]

[1]Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand.
[2]School of Biology, Institute of Science, Suranaree University of Technology, Nakhonratchasima 30000, Thailand.
[3]WalaiRukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham 44150, Thailand.
*Corresponding author; e-mail: pcornukaempferia@yahoo.com

ABSTRACT

Kaempferia marginata Carey ex Roscoe is used in northeastern Thailand as a vegetable. The conventional method for propagation of *K. marginata* Carey ex Roscoe is through the use of rhizomes, but this has low proliferation rates. The tissue culture technique provides an alternative method of propagation for mass production. Callus induction as well as shoot and root formation was studied when culturing microshoots of *K. marginata* Carey ex Roscoe on Murashige and Skoog (MS) medium supplemented with (thidiazuron) TDZ, (6-benzyl adenine) BA plus TDZ and BA plus (6-furfuryl amino purine) Kinetin for 6 weeks. The highest percentage of callus induction was 90% when the microshoot was cultured on MS medium supplemented with 2 mg L^{-1} BA plus 1.5, 3 or 4 mg L^{-1} TDZ. The best result for shoot multiplication was 4.90 shoots/explant achieved on MS medium added with 2 mg L^{-1} BA and 1 mg L^{-1} TDZ. The maximum number of roots were obtained on MS medium without plant growth regulators. Rooted shoots were transplanted into burned rice husk and sand (1:1 w/w) in a greenhouse and their survival was 100%.

Keywords: *In vitro,* Propagation, Microshoot, Callus induction**,** *Kaempferia marginata* Carey ex Roscoe.

INTRODUCTION

Kaempferia L., a genus of the family Zingiberaceae, is widely distributed in tropical Asia. Seventeen species of *Kaempferia* are recorded from Thailand (Larsen and Larsen, 2006), and many species are used as food (*K. galanga* and *K. marginata*), medicinal plants (*K. galanga, K. marginata, K. parviflora* and *K. rotunda*) and ornamental plants (*K. elegans, K. filifolia, K. galanga, K. grandifolia, K. larsenii, K. marginata, K. parviflora, K. pulcha, K. roscoeana* and *K. rotunda*). *Kaempferia marginata* Carey ex Roscoe is commonly known as Prao or Toobmoob (I-San local name), and this species is native to Thailand. The local people in northeastern Thailand use the young leaves of *K. marginata* Carey ex Roscoe for food as a fresh vegetable.

The propagation rate when using the underground rhizome is slow, and the rhizomes from field grown plants are affected by various pathogens such as *Fusarium oxysporum* causing rhizome rot and ginger yellows, *Pseudomonas solanacearum* causing bacterial wilt, *Pythium* spp. and nematodes (*Meloidogyne* spp.) causing soft rot in storage and under field conditions (Sharma and Singh, 1995). Tissue culture has been applied as a potent method for multiplication and conservation of many plant species. Furthermore, this method helps to maintain uniform and consistent production of true-to-type plants within a short period of time (Selvakkumar *et al.*, 2007)

Previous workers have reported the *in vitro* culture through rhizome buds of only three species from the genus *Kaempferia*, i.e. *K. galanga* (Vincent *et al*., 1992a; Greetha *et al*., 1997; Shirin, 2000; Chirangini *et al*., 2005; Chithra *et al*., 2005; Rahman *et al*., 2004, 2005; Swapna *et al*., 2004; Kalpana and Anbazhagan, 2009; Parida *et al*., 2010; Ahmad *et al*., 2011; Kochuthressia *et al*., 2012; Bhattacharya and Sen, 2013; Mohanty *et al*., 2013), *K. rotunda* (Greetha *et al*., 1997; Chirangini *et al*., 2005) and *K. parviflora* (Prathanturarug *et al*., 2007). So far, there has been no report of the *in vitro* propagation of *K. marginata* Carey ex Roscoe. The objective of this study was to investigate the effects of various concentrations of auxins and cytokinins on micropropagation of rhizome bud explants from *K. marginata* Carey ex Roscoe.

MATERIALS AND METHODS

Explant Sources and Sterilization: The rhizome buds of 20 plants of *K. marginata* Carey ex Roscoe were collected from plants growing in Mahasarakham Province, Thailand. The axillary buds (5 mm long) were excised and washed with running tap water for 30 minutes, rinsed with 70% (v/v) ethyl alcohol for 30 seconds, sterilized with 15% sodium hypochlorite containing 2 drops of Tween 20 for 15 seconds and washed three times with sterilized distilled water. The axillary buds then were cut into 2-3 mm sections and cultured on Murashige and Skoog (MS) (1962) medium.

Medium and Culture Conditions: The MS medium supplemented with 3% (w/v) sucrose and 0.7% (w/v) Difcoagar (Hemedie) was used in all experiments. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 1 N HCl prior to autoclaving at 121 ^{o}C for 15 minutes. The cultures were incubated at 25±2 ^{o}C and were exposed to a photoperiod of 16 hours with a light intensity of 2,000 lux using white fluorescent light.

Acclimatization: The *in vitro* regenerated plantlets with well-developed shoots and roots were washed with tap water to remove the agar medium from the roots and transplanted to pots containing three types of potting medium-burned rice husk, sand and burned rice husk:sand (1:1 w/w). The potted plants were maintained under a greenhouse condition at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand and regularly irrigated with tap water.

Statistical Analysis: The experiments were conducted using a completely randomized design (CRD) with 20 plantlets for each treatment. Data were analyzed for significance using ANOVA and the differences contrasted using a Duncan's multiple range test (DMRT). All statistical analyses were performed at the 5% level using the SPSS program (version 11.5).

RESULTS AND DISCUSSION

Microshoots (1 cm long) were cultured on MS medium with various concentrations of TDZ alone, BA plus TDZ, and BA plus Kinetin for callus induction, shoot and root formation. Callus formation occurred on the leaf base surface of the explants after being cultured for 4 weeks. The texture of the callus was soft, light creamy-white and friable (Figure 1B). Calluses did not form on the medium lacking plant growth regulators. The highest percentage of callus induction (90%) was obtained from microshoots cultured on the medium containing 2 mg L^{-1} BA with 1.5, 3 or 4 mg L^{-1} TDZ for 6 weeks (Tables 1-2 and Figures 1-2). In the present study, all the treatments, except the media free of hormones, showed callus formation. When microshoots were cultured on the medium supplemented with various concentrations of TDZ alone, BA plus TDZ, and BA plus Kinetin, the percentage of callus formation ranged from 30 to 90% (Table 2). These results differ from those of Babu *et al.* (1992); Samsudee *et al.* (2000); Prakash *et al.* (2004); and Saensouk (2011) who reported callus formation from plants in the family Zingiberaceae using 2,4-D. The other plant growth regulators used for callus induction in Zingiberaceae are dicamba (Kackar *et al*., 1993), NAA and BA (Salvi *et al*., 2001) BA and 2,4-D (Malamug *et al*., 1991). Young leaves are the most commonly used explants for callus induction in Zingiberaceae. The percentage of explants developing calluses depended on the culture medium. A combination of cytokinins was better for plant regeneration rather than when they were used individually.

When using just TDZ, the maximum number of shoots (2.20±0.20) was obtained from microshoots cultured on MS medium with 0.5 mg L^{-1} TDZ. The best average shoot length was achieved from microshoots culture on MS medium with 1 mg L^{-1} TDZ (3.28±0.43 cm) (Table 1 and Figure 1). Root formation was observed on MS medium without the addition of a plant growth regulator (5.80±0.53) (Table 1).

The effect of BA on clonal propagation of members of the family Zingiberaceae has been reported previously by Tyagi *et al.* (2004) in *Curcuma*; Yusuf *et al.* (2011) in *Boesenbergia*; Mohanty *et al.* (2013) in *Hedychium*; Shirin *et al.* (2000) in *Kaempferia* and Chirangini and Sharma (2005) in *Zingiber*; 2 mg L^{-1} BA is the most common cytokinin hormone used to induced cell division and cell differentiation in the Zingiberaceae. The interactive effect of two cytokinins (BA and TDZ) or (BA and Kinetin) was also determined. Shoots and roots simultaneously formed in the same medium with BA and TDZ within 14 days. The maximum number of shoots (4.90±1.77) was obtained from explants when cultured on MS medium with 2 mg L^{-1} BA plus 1 mg L^{-1} TDZ for 6 weeks (Table 2 and Figure 2). The effect of BA plus Kinetin for an enhanced rate of shoot multiplication has also been reported by Saxena (1990); Bejoy and Hariharan (1993); Vincent *et al.* (1992b); and Jinu and Aravindan (2008). Shoot and roots simultaneously originated in the same medium with TDZ, BA plus TDZ, and BA plus Kinetin.

TDZ act as potent regulator for *in vitro* propagation system and as an effective mean of induction of adventitious shoots in a number of plant species (Huettman and Preece, 1993; Lu, 1993). The presence of BA and TDZ induced a higher number of shoots per explants (Table 2), more than the treatment using BA plus Kinetin (Table 3) or TDZ alone (Table 1). BA plus Kinetin was more effective than TDZ alone. The combination of BA and TDZ was the best. The addition of TDZ combined with BA in MS medium generally gives an effect better than that of TDZ only.

Plantlets with two to three leaves and a well-developed root system were removed and transferred to pots containing (1) burned rice husk, (2) sand and (3) burned rice husk and sand (1:1 w/w) without a hardening process for 6 weeks under a greenhouse condition at the Department of Biology, Faculty of Science, Mahasakham University, Mahasarakham, Thailand. After 2 weeks, plantlets of *K. marginata* Carey ex Roscoe transplanted into the burned rice husk and the mixture of burned rice husk and sand produced new leaves, indicating that they can adapt well to conditions outside the culture environment and be used as planting material. However, plantlets of *K. marginata* Carey ex Roscoe transplanted into sand could not adapt to the *ex vitro* condition. The

leaves started to turn yellow within 2 weeks of transplantation and some plantlets died after 3 to 6 weeks. The highest percentage of surviving plantlets (100%), average number of leaves per shoot (3.39) and average shoot height (9.49 cm) were obtained from plantlets transplanted to burned rice husk:sand (Table 4 and Figure 4). The regenerated plants did not show detectable variation in morphological characteristics compared to the mother plant.

Table 1. Effects of TDZ on callus induction as well as shoot and root formation of *K. marginata* Carey ex Roscoe.

TDZ (mg L^{-1})	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	1.00±0.00^{b}	2.01±0.18^{b}	5.80±0.53^{a}	2.04±0.21^{a}
0.1	60	2.10±0.18^{a}	3.15±0.32^{a}	4.50±0.67^{a}	1.46±0.24^{a}
0.3	70	2.00±0.00^{a}	3.11±0.27^{a}	2.50±0.22^{b}	1.54±0.32^{a}
0.5	70	2.20±0.20^{a}	3.19±0.31^{a}	1.30±0.39^{b}	1.40±0.40^{a}
0.7	80	1.90±0.18^{a}	2.94±0.29^{a}	2.20±0.53^{b}	1.97±0.10^{a}
1.0	70	1.80±0.13^{a}	3.28±0.43^{a}	2.50±0.45^{b}	2.01±0.21^{a}

*Means followed by the same letters within each column are not significantly different at P ≤ 0.05, according to DMRT.

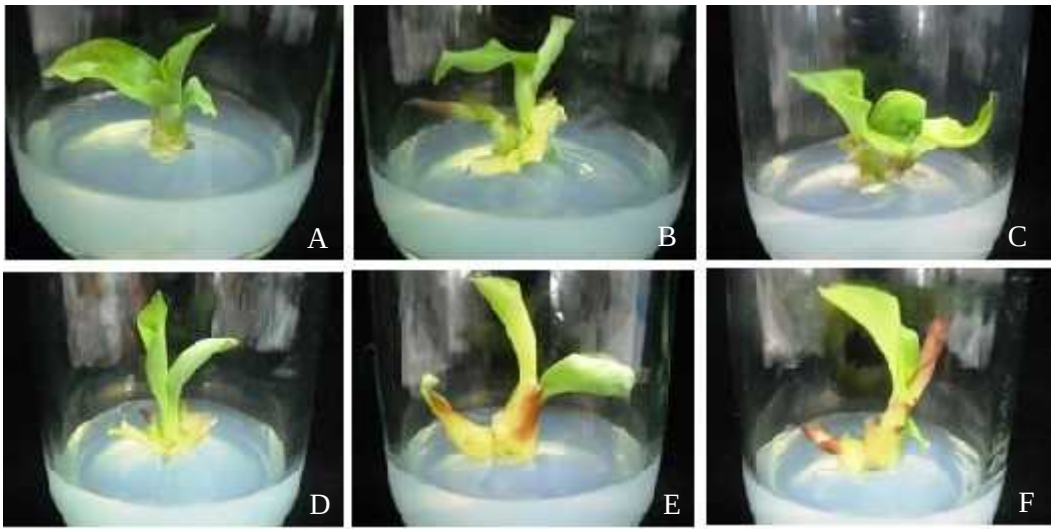

Figure 1. Callus induction as well as shoot and root formation of *K. marginata* Carey ex Roscoe after 6 weeks of culture. (A) hormone free (B) TDZ 0.1 mg L^{-1} (C) TDZ 0.3 mg L^{-1} (D) TDZ 0.5 mg L^{-1} (E) TDZ 0.7 mg L^{-1} (F) TDZ 1 mg L^{-1}.

Table 2. Effects of BA and TDZ on callus induction as well as shoot and root formation of *K. marginata* Carey ex Roscoe.

BA (mg L^{-1})	TDZ (mg L^{-1})	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	1.50±0.26^{b}	1.89±0.11^{ab}	5.40±0.74^{a}	2.21±0.13^{ab}
2	0.1	30	2.50±0.22^{ab}	1.94±0.12^{a}	5.20±0.95^{a}	2.54±0.22^{a}
2	0.5	70	3.50±0.47^{ab}	1.83±0.17^{abc}	4.20±0.97^{a}	1.54±0.30^{b}
2	1.0	80	4.90±1.77^{a}	1.11±0.12^{d}	5.20±1.15^{a}	1.92±0.20^{ab}
2	1.5	90	2.50±0.52^{ab}	1.40±0.17^{cd}	3.70±0.61^{a}	2.13±0.25^{ab}
2	2.0	50	2.40±0.33^{ab}	1.50±0.12^{bcd}	3.60±0.85^{a}	1.92±0.25^{ab}
2	2.5	70	3.30±0.47^{ab}	1.32±0.12^{d}	3.30±0.51^{a}	1.50±0.16^{b}
2	3.0	90	4.50±1.19^{a}	1.48±0.15^{bcd}	5.10±1.06^{a}	2.12±0.19^{ab}
2	3.5	80	3.10±0.52^{ab}	1.84±0.12^{ab}	4.70±0.98^{a}	2.11±0.34^{ab}
2	4.0	90	3.70±0.42^{ab}	1.38±0.12^{d}	4.20±0.48^{a}	2.25±0.29^{ab}

*Means followed by the same letters within each column are not significantly different at P ≤ 0.05, according to DMRT.

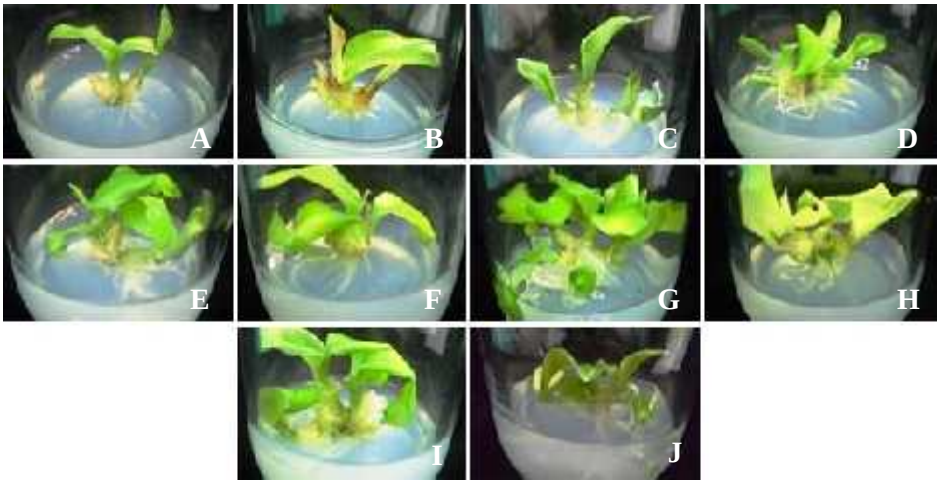

Figure 2. Callus induction as well as shoot and root formation of *K. marginata* Carey ex Roscoe after 6 weeks of culture. (A) hormone free (B) BA 2 mg L^{-1}+TDZ 0.1 mg L^{-1} (C) BA 2 mg L^{-1}+TDZ 0.5 mg L^{-1} (D) BA 2 mg L^{-1}+TDZ 1 mg L^{-1} (E) BA 2 mg L^{-1}+TDZ 1.5 mg L^{-1} (F) BA 2 mg L^{-1}+TDZ 2 mg L^{-1} (G) BA 2 mg L^{-1}+TDZ 2.5 mg L^{-1} (H) BA 2 mg L^{-1}+TDZ 3 mg L^{-1} (I) BA 2 mg L^{-1}+TDZ 3.5 mg L^{-1} (J) BA 2 mg L^{-1}+TDZ 4 mg L^{-1}.

Table 3. Effects of BA and Kinetin on callus induction as well as shoot and root formation of *K. marginata* Carey ex Roscoe.

BA (mg L^{-1})	Kinetin (mg L^{-1})	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	1.30±0.21^{c*}	1.90±0.19^{a}	3.50±0.70^{a}	1.02±0.19^{a}
2	0.1	50	1.80±0.24^{bc}	1.47±0.20^{a}	2.30±0.51^{a}	1.03±0.19^{a}
2	0.5	70	2.20±0.24^{abc}	1.78±0.20^{a}	2.20±0.46^{a}	2.05±0.55^{a}
2	1.0	70	2.90±0.48^{a}	1.46±0.20^{a}	1.70±0.36^{a}	1.13±0.25^{a}
2	1.5	60	2.90±0.34^{a}	1.82±0.22^{a}	2.10±0.50^{a}	1.98±0.27^{a}
2	2.0	70	2.70±0.21^{ab}	1.54±0.12^{a}	2.20±0.75^{a}	1.44±0.34^{a}
2	2.5	80	2.90±0.50^{a}	1.27±0.25^{a}	1.80±0.57^{a}	1.28±0.36^{a}
2	3.0	70	2.70±0.21^{a}	1.42±0.23^{a}	2.00±0.20^{a}	1.52±0.23^{a}
2	3.5	60	2.23±0.27^{abc}	1.34±0.21^{a}	1.98±0.34^{a}	1.49±0.41^{a}
2	4.0	50	2.17±0.26^{abc}	1.27±0.22^{a}	1.86±0.36^{a}	1.32±0.25^{a}

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

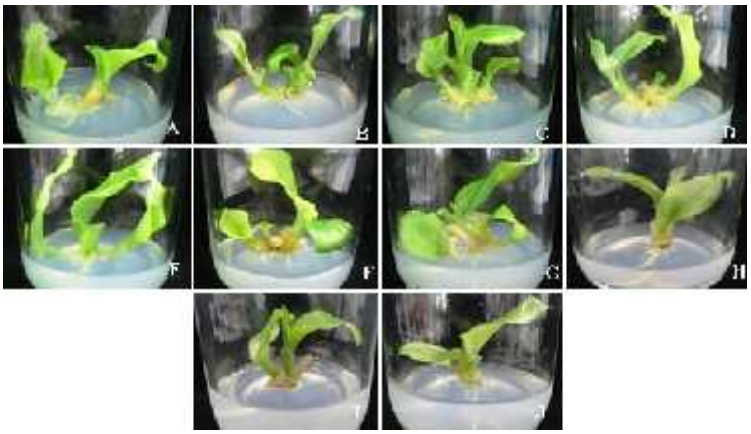

Figure 3. Callus induction as well as shoot and root formation of *K. marginata* Carey ex Roscoe after 6 weeks of culture. (A) hormone free (B) BA 2 mg L^{-1}+Kinetin 0.1 mg L^{-1} (C) BA 2 mg L^{-1}+Kinetin 0.5 mg L^{-1} (D) BA 2 mg L^{-1}+Kinetin 1 mg L^{-1} (E) BA 2 mg L^{-1}+Kinetin 1.5 mg L^{-1} (F) BA 2 mg L^{-1}+Kinetin 2 mg L^{-1} (G) BA 2 mg L^{-1}+Kinetin 2.5 mg L^{-1} (H) BA 2 mg L^{-1}+Kinetin 3 mg L^{-1} (I) BA 2 mg L^{-1}+Kinetin 3.5 mg L^{-1} (J) BA 2 mg L^{-1}+Kinetin 4 mg L^{-1}.

Table 4. Effect of potting media on plantlet performance of *K. marginata* Carey ex Roscoe after 6 weeks of acclimatization.

Potting medium	Percentage of surviving plantlets	Average no. of leaves/shoot mean±SE	Average shoot length mean±SE
Burned rice husk	65	3.11±0.28[a]	8.93±0.81[a]
Sand	20	3.25±0.25[a]	9.25±1.75[a]
Burned rice husk:Sand (1:1 w/w)	100	3.39±0.19[a]	9.49±0.82[a]

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

Figure 4. Acclimatized plantlets 6 weeks after transfer to a pot containing burned rice husk and sand

Conclusion: This is the first report describing tissue culture of *K. marginata* Carey ex Roscoe from rhizome bud explants. We reported that the *in vitro* shoot propagation of *K. marginata* Carey ex Roscoe from rhizome bud explants in MS medium containing 2 mg L^{-1} BA and 0.5 mg L^{-1} TDZ could regenerate multiple shoots (4.9 shoots/explant) after 6 weeks of culture. MS medium without a plant growth regulator was recommended for rooting while the combination of burned rice husk:sand (1:1 w/w) was chosen as the most suitable potting medium for acclimatization.

Acknowledgements: We acknowledge the National Research Council of Thailand for financial support. Warm thanks to the Department of Biology, Faculty of Science, Mahasarakham University for their facilities and support during this study. Also thanks to Dr. Jolyon Dodgson for language editing and suggestions to improve the manuscript.

REFERENCES

Ahmad, D., N. Wicaksana, T. Shimazaki, A. Kikuchi, S. A. Jatoi, and K. N. Watanabe (2011). Environmentally safe *in vitro* regeneration protocol for *Curcuma*, *Kaempferia* and *Zingiber*. Afr J Biotechnol. 10: 8584–8592.

Babu, K. N., K. Samsudeenn, and M. J. Ratnambal (1992). *In vitro* plant regeneration from leaf-derived callus in ginger. Plant Cell Tiss Organ Cult. 29: 71–74.

Bejoy, M., and M. Hariharan (1993). *In vitro* propagation of *Adenanthera pavonina* L.. J Tree Sci. 12: 69–72.

Bhattacharya, M., and A. Sen (2013). *In vitro* regeneration of pathogen free *Kaempferia galanga* L.-a rare medicinal plant. Res Plant Biol. 3: 24–30.

Chirangini P., S. K. Sinha, and G. J. Sharma (2005). *In vitro* propagation and microrhizome induction in *Kaempferia galanga* L. and *K. rotunda* L. Indian J Biotechnol. 4: 404–408.

Chithra, M., K. P. Martin, C. Sunandakumari, and P. V. Madhusoodanan (2005). Protocol for rapid propagation and to overcome delayed rhizome formation in field established *in vitro* derived plantlets of *Kaempferia galanga* L. Scientia Hort. 104: 113–120.

Geetha. S. P., C. Manjula, C. Z. John, D. Minoo, K. N. Babu, and P. N. Ravindran (1997). Micropropagation of *Kaempferia* spp. (*K. galanga* L. and *K. rotunda* L.) J. Spices and Aromatic Crops. 6(2): 129–135.

Huettman, M. J., and J. E. Preece (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss Organ Cult. 33: 105–119.

Jinu, J., and K. M. Aravinthan (2008). Micropropagation of *Alpinia zerumbet* Vaigata through *in vitro* rhizome bud culture. Ind J Bot Res. 4: 197–200.

Kackar, A., S. R. Bhat, K. P. S. Chandel, and S. K. Malik (1993). Plant regeneration via somatic embryogenesis in ginger. Plant Cell Tiss Organ Cult. 32: 289–292.

Kalpana, M., and M. Anbazhagan (2009). *In vitro* production of *Kaempferia galangal* L. an endangered medicinal plant. J. Phytology. 1: 56–61.

Kochuthressia, K. P., S. Britto, and M. O. Jaseentha (2012). *In vitro* multiplication of *Kaempferia galangal* L. an endangered species. J Biotechnol. 3: 27–31.

Larsen, K., and S. Larsen (2006). Gingers of Thailand. Queen Sirikit Botanic Garden. Thailand.

Lu, C. Y. (1993). The use of thidiazuron in tissue culture. In Vitro Cell Dev Biol. 29: 92–96.

Malamug, J. J. F., H. Inden, and T. Asahira (1991). Plant regeneration and propagation from ginger callus. Scientia Hort. 48: 89–97.

Mohanty, P., S. Behera, S. S. Swain, D. P. Barik, and S. K. Naik (2013). Micropropagation of *Hedychium coronarium* J. Koenig through rhizome bud. Physiol Mol Biol Plants. 19(4): 605-610.

Mohanty, S., R. Parida, S. Sahoo, and S. Nayak (2013). *In vitro* conservation of nine medicinally and economically important species of Zingiberaceae from eastern India. Proc Natl Acad Sci India Sect. B Biol Sci. 84: 799–803.

Murashige, T., and F. Skoog (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant. 15: 473–479.

Parida, R., A. Kuanar, S. Mohanty, and S. Nayak (2010). Rapid multiplication and *in vitro* production of leaf biomass in *Kaempferia galanga* through tissue culture. Electron J Biotechnol. 13: 1–8.

Prakash, S., R. Elangomathavan, S. Seshadri, K. Kathiravan, and S. Ignacimuthu (2004). Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. Plant Cell Tiss Organ Cult. 78: 159–165.

Prathanturarug, S., T. Apichartbutra, W. Chuakul, and P. Saralamp (2007). Mass propagation of *Kaempferia parviflora* Wall.ex Baker by *in vitro* regeneration. J Hortic Sci Biotechnol. 82: 179–183.

Rahman, M. M., M. N. Amin, T. Ahamed, M. R. Ali, and A. Habib (2004). Efficient plant regeneration through somatic embryogenesis from leaf base-derived callus of *Kaempferia galanga* L.. Asian J Plant Sci. 3: 675–678.

Rahman, M. M., M. N. Amin, T. Ahamed, and S. Ahmad (2005). Leaf base segments were excised aseptically from *in vitro* grown cultures which were originally established from the rhizome buds of field grown mature plants. J Biol Sci. 5: 300–304.

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

Salvi, N. D., L. George, and S. Eapen (2001). Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. Plant Cell Tiss Organ Cult. 66: 113–119.

Samsudeen, K., K. N. Babu, M. Divakaran, and P.N. Ravindran (2000). Plant regeneration from anther derived callus cultures of ginger (*Zingiber officinale* Rosc.). J Hortic Sci Biotechnol. 75: 447–450.

Saxena, S. (1990). *In vitro* propagation of the Bamboo (*Bambusa tulda* Roxb.) through shoot proliferation. Plant Cell Rep. 9: 431–434.

Selvakkumar, C., A. Balakrishnan, and B. S. Lakshmi (2007). Rapid *in vitro* micropropagation of *Alpinia officinarum* Hance, an important medicinal plant, through rhizome bud explants. Asian J Plant Sci. 6: 1251–1255.

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

Shirin, F., S. Kumar, and Y. Mishra (2000). *In vitro* plantlet production system for *Kaempferia galanga*, a rare indian medicinal herb. Plant Cell Tiss Organ Cult. 63: 193–197.

Swapna, T. S., M. Binitha, and T. S. Manju (2004). *In vitro* multiplication in *Kaempferia galanga* L.. Appl Biochemistry Biotechnology. 118: 233–241.

Tyagi, R. K., A. Yusuf, P. Dua, and A. Agrawal (2004). *In vitro* plant regeneration and genotype conservation of eight wild species of *Curcuma*. Biol Plant. 48: 129–132.

Vincent, K.A., M. Hariharan, and K.M. Mathew (1992a). Embryogenesis and plantlet formation in tissue culture of *Kaempferia galanga* L.: a medicinal plant. Phytomorphology. 42: 253–256.

Vincent, K. A., K. M. Mathew, and M. Hariharan (1992b). Micropropagation of *Kaempferia galanga* L.-a medicinal plant. Plant Cell Tiss Organ Cult. 28: 229–230.

Yusuf, N. A., M. M. Suffian Annuar, and N., Khalid (2011). Rapid micropropagation of *Boesenbergia rotunda* (L.) Mansf. Kulturpfl. (a valuable medicinal plant) from shoot bud explants. Afr J Biotechnol. 10(7): 1194–1199.