Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2014, 5(3):259-263

ISSN: 0976-8610
CODEN (USA): AASRFC

Micropropagation-an *in vitro* technique for the conservation of *Alpinia galanga*

Nongmaithem M. Singh[1], Lukram A. Chanu[1], Yendrembam P. Devi[1], Wahengbam R.C. Singh[2] and Heigrujam B. Singh[2]

[1]DBT-Institutional Biotech Hub, Pettigrew College, Ukhrul, Manipur
[2]DBT- Institutional Biotech Hub, Deptt. of Biotechnology, S.K. Women's College, Nambol, Manipur

---

ABSTRACT

This study was conducted to develop an efficient protocol for mass propagation of Alpinia galanga L. Explants from rhizome buds were cultured on Murashige and Skoog (MS) medium supplemented with 6-Benzylaminopurine (BAP) alone (0 to 5 mg/l) or a combination of BAP (0 to 5 mg/l) and indole 3-acetic acid (IAA) (0 to 2 mg/l). MS medium supplemented with a combination of 5.0 mg/l BAP and 2.0 mg/l IAA or 3.0 mg/l BAP and 0.5 mg/l IAA produced the highest mean number of shoots per explant as compared to other concentrations. The best shoot length was obtained on the medium containing 1.0 mg/l of BAP and 2.0 mg/l IAA. Thus, combined effects of BAP and IAA improved significantly the shoot growth and proliferation. MS medium supplemented with a combination of 5.0 mg/l BAP and 2 mg/l IAA gave the highest number of roots. However, longest roots per explant were obtained with 1.0 mg/l BAP alone. The proliferated shoots were green and healthy in appearance. Finally, healthy and complete plants with well developed roots were hardened, acclimatized and planted in the field successfully with a survival rate of 80%.

Key words: Mass propagation, *Alpinia galanga* L, Murashige and Skoog (MS) medium, 6-Benzylaminopurine (BAP), Indole 3-acetic acid (IAA)

INTRODUCTION

Micropropagation is the art and science of plant multiplication *in vitro.* The process includes many steps like stock plant care, explant selection and sterilization, media manipulation to obtain proliferation, rooting, acclimation, and growing on of liners. Micropropagation is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods.

The wealth of medicinal plants is an important asset in the flora of a state. As many as 1200 species of medicinal plants are reported by S.C. Sinha (1996) and the local medicinal uses of about 430 speices have been noted. *Alpinia galanga* is also one of the important medicinal plant of Manipur.

Galangal (*Alpinia galanga*), a rhizome closely related to ginger family, is used to flavour Thai food. It pairs well with many ingredients of Thai food that compose of coconut, garlic, chili peppers, kaffir lime leaves, turmeric, fish sauce, tamarind and shallots. In Thailand, galangal is used in curry pastes, Tom-Yam (Thai soup) and many curries. Galangal rhizome has a wide range of application in traditional medicine (Scheffer and Jansen, 1999; Yang and Eilerman, 1999). The traditional medicine from galangal can be used to cure skin diseases, indigestion, colic, dysentery, enlarge spleen, respiratory diseases, cancer of mouth and stomach, and systemic infections and cholera. It can also be used as an expectorant and after childbirth (Scheffer and Jansen, 1999). 1′-Acetoxy-chavicol acetate and 1′-acetoxyeugenol acetate from galangal were reported as anti-tumor substance (Itokawa *et al.*, 1987; Kondo *et al.*, 1993). Essential oil from galangal was reported as a potential anti-carcinogen (Lam and Zheng, 1991; Zheng *et al.*, 1993). The essential oil from both fresh and dried rhizomes of galangal exhibited the antimicrobial activities against

gram-positive bacteria, yeast and some dermatophytes. The most active compound was terpinen-4-ol (Janssen and Scheffer, 1985).

Like ginger, galangal is a 'de-fisher' and so appears frequently in fish and shellfish recipes. It has a unique flavour profile and is described as having more woody, minty and floral aroma than ginger. The potent odourants of galangal are 1,8-cineole, linalool, geranyl acetate, eugenol and chavicol acetate (Mori *et al*., 1995). Compounds causing fishy odour have been extensively reviewed. These compounds are trimethylamine (Cadwallader *et al*., 1995; Prost *et al*., 1998 and Fukami *et al*., 2002), 2,4,7-decatrienal (Meijboom and Stroink, 1972; Karahadian and Lindsay, 1989; Karahadian and Lindsay, 1990), (*Z*)-4-heptenal (Karahadian and Lindsay, 1990; Hartvigsen *et al*., 2000), *trans,cis*-2-4-heptadienal (Hartvigsen *et al*., 2000), dimethyl trisulfide, 4-ethyl-6-hepten-3-one (Fukami *et al*., 2002) and (*E,Z*)-2,6-nonadienal and 1-penten-3-one (Venkateshwarlu *et al*., 2004).

Galangal is a good source of natural antioxidants. The search for natural replacements for synthetic antioxidants has been increased because of the requirement to meet safety standards. The natural antioxidants present in food and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects. Potent sources of antioxidants have been searched in several plant materials.

MATERIALS AND METHODS

Fresh rhizomes of galangal (*Alpinia galanga*) were collected from different districts of Manipur. The explants were washed thoroughly under running tap water to remove adhering soil particles. To control the microbial contamination, a composite approach of washing the explants first with 60% Clorox and addition of 6 to 7 drops of Tween 20 for 30 min was employed. Subsequently, the explants were thoroughly washed once with sterile distilled water. Sterilized explants were then dissected to remove one layer of leaf sheaths under aseptic conditions. Then, the excised explants were immersed again in 20% Clorox with the addition of 6 to 7 drops of Tween 20 for another 15 min and thoroughly washed seven times with sterilized distilled water. Outer leaves were removed aseptically and explants of 1 mm were inoculated on basal Murashige and Skoog (MS) medium supplemented with combination of KIN (3 mg/l), BA (1-3 mg/l), IAA (0.5-1.0 mg/l), IBA (1.0 mg/l), NAA (0.5-2.0 mg/l) and Ads (100 mg/l) for culture establishment. The sucrose amount in the media was 30 gm/lt and agar 0.8% was used as basal. All experiments were conducted in five replicas for each treatment. The pH of the medium was adjusted to 5.7 before adding agar and was autoclaved at 121^0C and 105 kg/cm2 of pressure for 20 minutes. All the cultures were incubated at 25 ± 1^0C under white fluorescent light with 50µ mole m^{-2} s^{-2} light intensity during a photoperiod of 16:8 h light and dark cycles. The *in vitro* derived shoots were cultured on medium [MS +KIN (3 mg/l) + BA (3 mg/l) + NAA (1 mg/l)] for proliferation and multiplication. Further these plantlets were cultured on rooting media containing Benzyladenine (1 mg/l) and Indolebutyric acid (1 mg/l) and the maximum number of root produced was 7.8 ± 0.3.

Plantlets with well developed roots were removed from the medium, then washed thoroughly under running tap water to remove adhering solid MS medium, and transplanted to plastic pots containing sterilized peat moss soil and kept in a 50% shaded net house. The plants were covered by a transparent perforated polyethylene bags in order to maintain a high humidity and to avoid plant dehydration by water loss. The plants were frequently watered to keep high level of humidity. The polyethylene bags were then removed after 7 days and the plantlets were acclimatized for another three weeks.

RESULTS AND DISCUSSION

The explants of *Alpinia galanga* cultured on MS medium supplemented with different concentrations of BAP alone (0.0, 1.0, 3.0 and 5.0 mg/l) or in combination with IAA (0.0, 0.5, 1.0 and 2.0 mg/l) produced both shoots and roots, simultaneously whereas, roots induction was lowest (4.50) for explants cultured on MS medium containing higher concentrations of BAP alone (5 mg/l) and highest (17.10) in medium containing combination of 5 mg/l of BAP and 2 mg/l of IAA (Table 1). Such type of simultaneous production of shoot and roots were reported earlier for *Z. zerumbet* by Stanly and Keng (2007) and on other Zingiberaceae species (Balachandran et al., 1990; Chan and Thong, 2004; Yusuf et al., 2011). This study suggests that the use of combination medium will shorten the time for plant regeneration. When BAP was used alone, the maximum number of shoots (3.6) was obtained from explants on MS medium with 5 mg/l BAP. With an increase in the concentration of BAP, the number and length of shoots per explant increased (Table 1). Therefore, it appears that a higher concentration of BAP would have a positive effect on *in vitro* shoot multiplication of *Alpinia galanga*. The proliferated shoots were green and healthy in appearance. The role of BAP in shoots proliferation has been reported in other Zingiberaceae species (Ikeda and Tambe, 1989; Balachandran et al., 1990; Smith and Hamil, 1996; Rout et al., 2001; Panda et al., 2007; Mohanty et al., 2011; Abdelmageed et al., 2011).The presence of BAP and IAA in the medium markedly improved the number of

proliferating shoots. Besides the number of shoots induced, BAP and IAA accelerated mean shoot length considerably (Table 1). The highest shoot multiplication was found in the medium containing 5.0 mg/l BAP + 2.0 mg/l IAA and also 3.0 mg/l BAP + 0.5 mg/l IAA, which produced nearly 5.65 shoots per explant, whereas, the longest shoots length (9.64) were obtained on the media containing 1.0 mg/l of BAP + 2.0 mg/l of IAA and 5.0 mg/l of BAP + 2.0 mg/l of IAA, respectively (Table 1). It was observed that cytokinin was required in optimal quantity for shoot proliferation in some species of Zingiberaceae, but inclusion of low concentration of auxins along with cytokinin triggered the rate of shoot proliferation (Rout and Das, 1997; Sharma and Singh, 1997). Further, Stanly and Keng (2007) reported that MS medium supplemented with 6 mg/l BAP induced the formation of multiple shoots *of Alpinia galanga*. Earlier, it was reported that the highest numbers of shoots were obtained on MS medium supplemented with 5 to 10 mg/l BAP from *Zingiber officinale* Roscoe (Noguchi and Yamakawa, 1988). Faria and IIIg (1995) noted that the addition of 2.25 mg/l of BAP with 1.0 mg/l of IAA induced a high rate of shoot proliferation of *Zingiber spectabile*. For *Curcuma haritha*, Bejoy et al. (2006) found that the best shoot multiplication and root system were achieved on MS medium supplemented with 1.0 mg/l of BAP and 0.5 mg/l of IAA. This results indicate the importance of IAA hormone in the induction of roots. Moreover, Anish et al. (2008) reported that MS medium supplemented with IAA at 0.5 mg/l in a combination with BAP seems to be optimum for rooting of *Boesenbergia pulcherrima*. In the same trend, Vincent et al. (1992) reported that the highest number of shoots per explant was obtained with cultured axillary buds of *Kaempferia galangal* on MS medium supplemented with 0.50 mg/l of BAP and 3.0 mg/l kinetin after 120 days of incubation. MS medium supplemented with 5 mg/l BAP and 2.0 mg/l IAA produced significantly the highest mean number (17.10) of roots per explant (Table 1) whereas, MS medium supplemented with 1.0 mg/l BAP produced the longest roots (5.40) per explant followed by MS medium without growth regulators (4.95) (Table 2).This results indicate that IAA promoted the growth of roots. Higher concentration of auxin, in the range that normally stimulates elongation of shoots, causes a significant inhibition of root growth (Hopkins and Hüner, 2004). Bejoy et al. (2006) found that the shoot multiplication and root systems were obtained on MS medium supplemented with 1.0 mg/l of BAP and 0.50 mg/l of IAA. This result is comparable to this study, which indicates the importance of IAA hormone in the induction of roots. According to Anish et al. (2008), MS medium supplemented with IAA at 0.50 mg/l in combination with BAP seems to be optimum for rooting of *B. pulcherrima*. In this study, the IAA hormone was found to have a positive effect on root induction, especially when used in combination with BAP. According to Sharma (2006), IAA and IBA are usually used for easier-to-root herbaceous plants and NAA for more recalcitrant woody plants, and they mentioned that the efficacy of different auxins also depends on the explants type and exposure to light. The number of rhizomes per explant showed significant differences among the different treatments (Table 2). The highest number of rhizomes formed was obtained on MS media supplemented with the following combinations: 5.0 mg/l BAP + 0.5 IAA mg/l, 5.0 mg/l BAP + 1.0 mg/l IAA and 5.0 mg/l BAP + 2.0 mg/l IAA, respectively. This result is comparable to those noted on other Zingiberaceae species (Rout et al., 2000; Anisuzzaman et al., 2008). This study revealed that rhizomes induction in *Alpinia galanga* needs further works.

Fig.1. (A) Collecting Site (B) Explant showing shoot initiation from axillary bud

With regards to number of leaves per explant, MS medium containing 5.0 mg/l BAP + 2.0 mg/l produced significantly the highest number of leaves per explants (6.65) (Table 2), which indicate that cytokinin alone or in combination with auxin (BAP and IAA) had a significant response on the number of leaves per explant of this species. This result confirmed earlier findings of Borthakur et al. (1999) for *Alpinia galangal*, Yusuf et al. (2011) for *Boesenbergia rotunda* and Waseem et al. (2011) for *Chrysanthemum morifolium* L. Acclimatization of plantlets can be considered as one of the most important phase in tissue culture techniques.

Table 1. Effect of growth regulators on *in vitro* multiplication of the number of shoots, shoot length and number of roots of *Alpinia galanga* grown on MS medium

Sl. No.	PGRs (mg/l)	No. of shoot	Length of shoot	No. of root
1	MSO	2.00^{e*}	2.80^{g}	4.50^{i}
2	1.0 BAP	2.00^{e}	3.30^{g}	6.20^{h}
3	3.0 BAP	3.30^{d}	5.20^{f}	11.10^{g}
4	5.0 BAP	3.60^{cd}	3.30^{g}	4.30^{i}
5	1.0 BAP + 0.5 IAA	4.00^{cd}	7.57^{e}	13.90^{d}
6	1.0 BAP + 1.0 IAA	4.00^{cd}	7.57^{e}	13.70^{de}
7	1.0 BAP + 2.0 IAA	4.20^{bc}	9.64^{a}	13.80^{de}
8	3.0 BAP + 0.5 IAA	5.70^{a}	9.20^{ab}	12.80^{ef}
9	3.0 BAP + 1.0 IAA	4.80^{ab}	8.29^{cd}	12.40^{fg}
10	3.0 BAP + 2.0 IAA	5.10^{a}	7.75^{de}	15.85^{b}
11	5.0 BAP + 0.5 IAA	5.30^{a}	8.25^{cd}	14.90^{c}
12	5.0 BAP + 1.0 IAA	5.45^{a}	8.82^{bc}	16.10^{b}
13	5.0 BAP + 2.0 IAA	5.65^{a}	9.27^{ab}	17.10^{a}

**Means followed by the same letter(s) within each column are not significantly different at P ≤ 0.05, according to Duncan's multiple range test for each parameter. PGRs, plant growth regulator.*

Table 2. Effect of growth regulators on *in vitro* multiplication of length of roots, number of rhizomes and number of leaves of *Alpinia galanga* grown on MS medium

Sl. No.	PGRs (mg/l)	Length of root	No. of rhizome	No. of leaf
1	MSO	4.95^{ab*}	1.10^{d}	2.10^{g}
2	1.0 BAP	5.40^{a}	1.20^{d}	2.85^{f}
3	3.0 BAP	4.75^{bc}	1.50^{c}	3.30^{ef}
4	5.0 BAP	2.30^{i}	1.40^{c}	3.40^{e}
5	1.0 BAP + 0.5 IAA	3.25^{gh}	1.50^{c}	4.45^{cd}
6	1.0 BAP + 1.0 IAA	3.75^{ef}	2.05^{b}	4.45^{d}
7	1.0 BAP + 2.0 IAA	4.15^{de}	2.10^{b}	4.25^{d}
8	3.0 BAP + 0.5 IAA	3.10^{h}	2.05^{b}	4.45^{cd}
9	3.0 BAP + 1.0 IAA	3.57^{fg}	2.10^{b}	4.85^{bcd}
10	3.0 BAP + 2.0 IAA	3.85^{def}	2.05^{b}	5.10^{bc}
11	5.0 BAP + 0.5 IAA	3.90^{def}	3.12^{a}	5.25^{b}
12	5.0 BAP + 1.0 IAA	4.10^{de}	3.08^{a}	5.50^{b}
13	5.0 BAP + 2.0 IAA	4.25^{cd}	3.07^{a}	6.65^{a}

**Means followed by the same letter(s) within each column are not significantly different at P ≤ 0.05, according to Duncan's multiple range test for each parameter.*

CONCLUSION

In this investigation, the mortality rate of the plantlets with well developed roots that were acclimatized and hardened was low. On average, 80% of *in vitro* transferred plantlets survived in potted soil and did not show any morphological abnormalities. This protocol is a step forward towards improvement of the propagation of *Alpinia galanga*, which have a very important medicinal value.

Acknowledgement
We are grateful to Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India, New Delhi, India for providing official support and laboratory facilities at Institutional Biotech Hub at Pettigrew College, Ukhrul, Manipur and S. Kula Women's College, Nambol, Manipur.

REFERENCES

[1] Abdelmageed AHA, Faridah QZ, Norhana FMA, Julia AA, Kadir MA (**2011**). *J. Med. Plants Res.* 5(13).
[2] Anish NP, Dan M, Bejoy M (**2008**). *Int. J. Bot*. 4(1): 93-98.
[3] Anisuzzaman M, Sharmin SA, Mondal SC, Sultana R, Khalekuzzaman M, Alam I, Alam MF (**2008**). *J. Biol. Sci*. (Faisalabad, Pak.). 8(7): 1216-1220.
[4] Balachandran SM, Bhat SR, Chandel KPS (**1990**). *Plant cell Rep*. 8: 521-524.
[5] Bejoy M, Dan M, Anish NP (**2006**). *Asian J. Plant Sci*. 5: 847-853.
[6] Borthakur M, Hazarika J, Singh RS (**1999**). *Plant Cell Tissue Organ Cult.* 55: 231-233.
[7] Cadwallader, K.R., Qiang Tan, Feng Chen and S. P. Meyers. **1995**. *J. Agric. Food Chem*. 43: 2432-2437.
[8] Chan LK, Thong WH (**2004**). *J. Plant Biotechnol*. 6: 181-188.
[9] Faria RT, Illg RD (**1995**). *Sci. Hortic*. Amsterdam, Neth. 62: 135-137.
[10] Fukami S, Watanabe K, Iwata N, Haraoka J, Lu B, Gerard NP, Gerard C, Fraser P, Westaway D, St. George-Hyslop P, Saido TC (**2002**) *Neurosci Res* 43:39–56.

[11] Hartvigsen J, Leboeuf-Yde C, Lings S, Corder EH **2000**. *Scand J Public Health*, 28(3):230–239.
[12] Hopkins WG, Hüner NP (**2004**). Introduction to Plant Physiology. 3rd Edn., John Wiley and Sons Inc., New York, ISBN: 978-0-471-38915-6, p. 576.
[13] Itokawa, H., H. Morita, T. Sumitomo, N. Totsuka and K. Takeya. **1987**. *Planta Med*. 53: 32-33.
[14] Ikeda IR, Tambe MJ (**1989**). *Hort. Sci*., 24: 142-143.
[15] Janssen, A.M. and J.J.C. Scheffer. **1985**. *Planta Med*. 6: 507-511.
[16] Karahadian, C. and R.C. Lindsay. **1989**. *J. Amer. Oil Chem. Soc*. 66:1302-1308.
[17] Karahadian C, Lindsay RC. **1990**. *J Am Oil Chem Soc* 67:85–91.
[18] Kondo, A., H. Ohigashi, A. Murakami, J. Suratwadee and K. Koshimizu. **1993**. *Biosci. Biotechnol. Biochem*. 57(8): 1344-1345.
[19] Lam, L.K.T.and B.-L. Zheng. **1991**. *J. Agric. Food Chem*. 39: 660-662.
[20] Meijboom, P.W. and Stroink, J.B.A. (**1972**), *J.Am Oil Chem Soc*, 49, 555-558.
[21] Mohanty S, Panda MK., Sahoo S, Nayak S (**2011**). *Biol. Plant*, 55(1): 16-20.
[22] Mori, H., K. Kubota and A. Kobayashi. **1995**. A potent aroma components of rhizomes from *Alipia galanga*. Willd. L. Nippon Shokuhin Kogyo Gakkaishi 42(12): 989-995.
[23] Noguchi Y, Yamakawa O (**1988**). *Jpn. J. Breed*, 38: 437-442.
[24] Panda MK., Mohanty S, Subudhi E, Acharya L, Nayak S (**2007**) *Int. J. Integr. Biol*. 1: 189-195.
[25] Prost, S., Ford, J.M., Taylor, C., Doig, J., and Harrison, D.J.(**1998**). *J. Biol Chem* 273, 33327-32.
[26] Rout GR, Das P (**1997**). *J. Herbs, Spices Medicinal Plants*, 4: 41-51.
[27] Rout GR, Palai SK, Samantaray S, Das P (**2001**). *In vitro Cell. Dev. Biol. Plant*. 37: 814-819.
[28] Rout GR, Samantaray S, Das P (**2000**). *Biotechnol. Adv*. 18: 91-120.
[29] Sinha S.C. **1996**. Medicinal Plants of Manipur, MASS & Sinha, Manipur Cultural Integration Conference, Palace Compound, Imphal, Manipur, 795001
[30] Scheffer, J.J.C. and P.C.M. Jansen. **1999**. *Alpinia galanga* (L.) Wild, pp. 65-68. *In* C.C. de Guzman and J.S. Siemonsma, (eds.). Plant Resources of South-East Asia No 13. Spice. Backhuys Publishers. Leiden, The Netherlands.
[31] Sharma R (**2006**). Biomass and Cell Culturing Techniques. New Delhi: Biotech. Books, p. 287.
[32] Sharma TR, Singh BM (**1997**). *Plant Cell Rep*. 17: 68-72.
[33] Smith MK, Hamil SD (**1996**) *Aust. J. Exp. Agric*. 36: 347-354.
[34] Stanly C, Keng CL (**2007**). *Biotechnology*, 6(4): 555-560.
[35] Venkateswarlu, K., Brandom, K. G., Lawrence, J. L. (**2004**). *J. Biol. Chem*. 279: 6205-6208.
[36] Vincent KA, Mathew KM, Hariharan M (**1992**). *Plant Cell Tissue Organ Cult*. 28: 229-230.
[37] Waseem K, Jilani MS, Khan MS, Kiran M, Khan G (**2011**). *Afr. J. Biotechnol*. 10(8): 1477-1484. Yang, X. and R. G. Eilerman. **1999**. *J. Agric. Food Chem*. 47: 1657-1662.
[38] Yang, X. and R. G. Eilerman. **1999**. *J. Agric. Food Chem*. 47: 1657-1662.
[39] Yusuf NA, Annuar MMS, Khalid N (**2011**). *Afr. J. Biotechnol*. 10(7): 1194-1199.
[40] Zheng, G.-Q., P.M. Kenney and L.K.T. Lam. **1993**. *J. Agric. Food Chem*. 41: 153-156.