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REGULAR ARTICLE

IN VITRO PRODUCTION OF *KAEMPFERIA GALANGA* (L.)- AN ENDANGERED MEDICINAL PLANT

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SUMMARY

Kaempferia galanga is an important medicinal plant that is facing threat of extinction owing to indiscriminate and unsustainable harvesting in the wild. Conventional breeding is difficult in this plant, and in vitro multiplication is important to conservation and propagation. Tissue culture techniques are useful for ex situ conservation of rare, endemic or threatened plant species. An efficient protocol has been established for rapid production of plantlets using rhizome tip and lateral bud of the pot cultured plant. The explants were cultured on MS medium with various combinations of 6-benzyladenine (BA), α-naphthalene acetic acid (NAA), indole-3-butric acid (IBA) and indole-3-acetic acid (IAA) at concentrations ranging from 0.1 to 3.0 mg/l. High-frequency organogenesis and multiple shoot regeneration were induced from rhizome explants on MS medium supplemented with 2.0 mg/l of BA and 0.2 mg/l of NAA. Micro-shoots were isolated from the in vitro proliferated cluster of shoots and they produced roots on half strength MS medium supplemented with 1.0 mg/l of IBA. The regenerated plantlets were transferred to plastic pots for hardening in a mixture containing red soil, sand and vermiculate(1:1:1) ratio and established on the soil with 81% of success.

Keywords: Aromatic plants, *Kaempferia galanga*, Invitro production, Rhizome, Multiple shoots, Organogenesis.

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1. Introduction

Kaempferia galanga L. an aromatic rhizomatous herb, belonging to the family Zingiberaceae. This plant is native of South India, but today it is mainly cultivated in Southeast Asia and China. This species is used as the main ingredient of many ayurvedic drugs used for the healing of rheumatism and the aromatic essential oil extracted from the rhizome is a valuable component of perfume production. The extract from rhizome contains n-pentadecane, ethyl p-methoxy cinnamate, ethyl cinnamate, camphene, borneol, cineol, p-methoxy styrene, kaempferol and kaempferide [1, 2]. Generally the rhizome has the medicinal properties of stimulatory, expectorant, carminative and diuretic. In addition, it possesses camphoraceous odour and the decoction prepared from the rhizome is used for dyspepsia, headache and malaria. The

rhizome also contains essential oils, that are used for the preparation of decoction or powder which areused for the healing of indigestion, cold, pectoral pain, abdominal pain, headache and toothache. Its alcoholic maceration has also been applied as liniment for rheumatism [3-5]. It has been reported recently that the rhizomes of *K. galanga* contains the chemicals that are of insecticidal potent [6]. Conventional propagation of *K. galanga* by the splitting of rhizome is not sufficiently rapid to meet the need of propagules for planting, and it takes years to build up commercial quantities. Utilization of the rhizome for drug preparations largely by pharmaceutical industries also makes planting propagules unavailable. About 1500 kg of rhizome is needed to plant 1 ha. India is the leading exporter of medicinal plants and many medicinal plants are harvested from the wild form. Increasing population, urbanization, shrinking forests, over harvesting and related factors have brought several medicinal plants to the very brink of extinction. In this way *K. galanga* is one among 100 red listed medicinal plants to be conserved in southern India [7].

Considering high demand and greater economic and medicinal values of *K. galanga*, it is necessary to develop a suitable protocol for mass production of disease free stocks through tissue culture techniques. Protocol for in vitro propagation of *K. galanga* has been reported earlier [8-10]. The potential of tissue culture for the rapid multiplication of plants and extraction of secondary metabolites from callus and cell cultures has been used for the conservation of *K. galanga* and also for the mass production on a commercial scale [11]. Hence, with the view of above said reasons, the conservation of *K. galanga* is the most important responsibility so that, the in vitro plantlet production of this species was developed from rhizome buds explants by using suitable technique.

2. Materials and methods

Collection of explants and Surface sterilization

Explants were collected from the sprouted rhizomes of pot cultured *K. galanga* plants. They were then brought to the laboratory and processed for aseptic culture. For surface sterilization, the rhizome explants were excised and cleaned thoroughly under continuous flushing of running tap water for 30 min to remove all the mud and then washed with a solution of antiseptic [Dettol 5% (v/v)] for 10 min. The explants were then immersed in 70% ethanol for 2 seconds and then cut into round slices (rhizome tip and lateral bud, 1–2 mm transverse sections) with each of them possessing axillary buds. These pieces were then washed with 5% sodium hypochloride for 10 min followed by rinsed thoroughly in distilled water and finally with surface disinfected with 0.1 % (w/v) aqueous mercuric chloride solution for 5 min. Then they should be rinsed five times with sterile distilled water under laminar air flow chamber.

Culture condition

After surface sterilization, the explants were trimmed to appropriate sizes and inoculated on MS medium [12] for shoot multiplication. To finding out the best results for shooting and rooting, the medium was supplemented with different concentrations of BA either alone or BA with different concentrations of IBA and NAA. The regenerated micro shoots were placed on half-strength MS medium supplemented singly with various concentrations of NAA, IBA or

IAA for rooting. The MS basal medium used for this studies containing standard salts, vitamins, 3% sucrose (w/v) and 0.8% agar (w/v) (Himedia Pvt. Ltd., India). The pH of the medium was adjusted to 5.8 before adding agar and the medium was autoclaved for 20 min at 121°C. Each explant was cultured in a test tube (20 ×150 mm) containing 15ml of sterilized semisolid medium followed by culture bottles (500 ml) containing 50 ml of semisolid medium for in vitro plantlet production.

The cultures were incubated at a temperature of 25±2°C with a photoperiod of 16 hrs per day with photon flux density of about 45µ mol m-2s-1 provided by white fluorescent light. Each experiment was repeated three times and five replications per treatment were taken into account. All the data were analyzed by using ANOVA and the means and the standard errors of data were calculated with SPSS software [13].

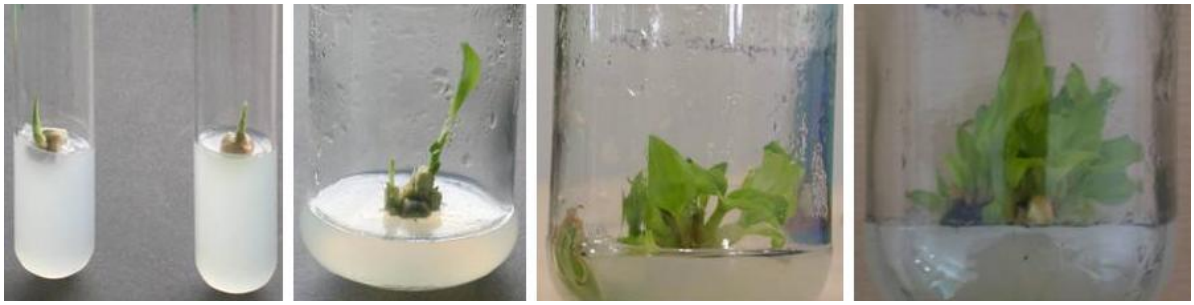
3. Results and Discussion

Proliferation of shoots

A number of experiments were conducted with a view of finding out optimum culture condition for maximum shoot multiplication from the culture of rhizome explants. Multiple shoots were found to develop from the rhizome explants when they were cultured on MS medium supplemented with BA alone and combination with NAA, IBA (Table 1). Generally, in most of the treatments, initiation of multiple shoots was observed within four weeks of culture. The best shoot proliferation from the explants was observed in MS medium with 2.0 mg/l BA and 0.2 mg/l NAA. In this treatment, 85% of the cultured explants produced multiple shoots and the number of shoot per culture was 19.40±0.42 and average length of shoots per culture was 6.10±0.32 (Fig.1).

Similar results were observed on the same medium for shoot multiplication of K. galanga [8, 9, and 14] K. rotunda, Curcuma sp. [15] and *Alpinia calcarata* [16].An alternative approach to increase the number of shoot formation has been reported in ginger where MS medium containing BA with NAA was used to multiply shoots [17] incubated explants showed shoot initiation. Almost similar result was obtained by Haque [18] and Rahman [19] worked with the plants of same nature such as ginger and turmeric.

Fig.1. A-D: Plantlets regeneration from rhizome lateral bud explants of *Kaempferia galanga* L. under *in vitro* condition



A. Shoot buds initiation B. Early stage of development of multiple shoots C. Well development of multiple shoots D. Elongation of multiple shoots

Rooting of the proliferated shoots

Root development was induced in the in vitro proliferated shoots by culturing them on half strength of MS medium with 1.0 mg/l of NAA and 1.0 mg/l of IBA (Table 2). Among different types of auxin used in the present experiment, IBA was found to be the most effective at different concentrations tested for producing roots on bases of micro-shoots. Among different concentrations of IBA, 1.0 mg/l was found to be the best concentration of auxin for proper rooting of *K. galanga* in which 96% shoots rooted within four weeks of culture. The similar results were also reported in *K. galanga* by Shirin [9] and Swapna [20]. The present findings are in agreement with those observed in similar rhizomatous plant species such as ginger [18], *Alpinia calcarata* [16] and *Curcuma longa* [19].

Table 1. Effect of different concentration of BA alone and in combination with NAA, IBA on MS medium for shoot proliferation from rhizome explants.

Growth regulators (mg/l)	% of explants showing proliferation	Number of shoot per culture	Average length of shoot per culture
BA			
0	06	1.20±0.19	2.44±0.36
0.5	57	5.20±0.45	3.24±0.24
1.0	63	6.40±0.35	4.32±0.19
2.0	71	8.20±0.40	5.48±0.26
3.0	50	7.00±0.50	3.70±0.24
BA+NAA			
1.0+ 0.1	73	9.00±0.35	4.62±0.45
+0.2	65	7.60±0.26	4.26±0.21
+0.5	55	5.40±0.30	3.50±0.12
2.0+0.1	80	14.60±0.27	5.78±0.24
+0.2	85	19.40±0.42	6.10±0.32
+0.5	55	7.60±0.15	4.16±0.19
3.0+0.1	47	6.20±0.40	3.92±0.34
+0.2	40	4.20±0.41	3.38±0.24
+0.5	36	3.80±0.31	2.82±0.33
BA+IBA			
1.0+ 0.1	56	4.00±0.30	3.28±0.23
+0.2	45	4.20±0.24	4.00±0.26
+0.5	33	3.40±0.12	2.82±0.42
2.0+0.1	63	5.20 ±0.42	4.90±0.14
+0.2	71	6.40±0.17	5.36±0.17
+0.5	53	4.00±0.35	4.74±0.43
3.0+0.1	42	3.40±0.20	2.14±0.23
+0.2	47	3.80±0.22	2.76±0.21
+0.5	30	2.60±0.35	1.82±0.23

Values are mean±SE(n=5) of two independent experiments.

Table 2. Effect of different concentration of auxins on adventitious root formation from in vitro regenerated shoots.

Conc. of auxin (mg/l)	Percentage of shoots rooted	No. of roots per rooted shoots	Average length of roots
NAA			
0.2	75	5.40±0.16	3.74±0.24
0.5	80	6.00±0.23	4.10±0.24
1.0	90	7.60±0.42	5.22±0.46
2.0	70	4.40±0.52	3.34±0.30
IBA			
0.2	88	6.40±0.35	4.32±0.27
0.5	93	8.20±0.23	5.64±0.23
1.0	96	10.40±0.84	6.30±0.34
2.0	80	5.20±0.28	5.28±0.56
IAA			
0.2	45	3.40±0.29	3.26±0.23
0.5	53	4.80±0.24	3.58±0.42
1.0	67	5.60±0.26	4.10±0.27
2.0	38	3.00±0.31	2.82±0.20

Values are mean±SE(n=5) of two independent experiments.

Establishment of plantlets under ex vitro condition

The in vitro regenerated plantlets were transferred to soil. Before their transplantation, the root systems of the plantlets were made to agar gel free by continuous flushing of running tap water. Then, the regenerated plantlets were transferred to plastic pots for hardening in a mixture containing red soil, sand and vermiculate(1:1:1). It was observed that 81% of the plantlets could be established under ex vitro conditions. The use of sufficiently porous potting mix that allows adequate drainage and aeration has been recommended for fast acclimatization of in vitro regenerated plants [21, 22]. Rest of the transplants could not survive either due to wilting caused of hot and dry atmosphere or microbial overgrowth that caused damping off and necrosis of transplants. These results demonstrate that *K. galanga* can be micropropagated readily with above said methods and medium.

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References

1. Anonymous, 1959.Wealth of India, Raw Materials Volume V. Publ. and Inforn. Direct, CSIR, New Delhi, India, 314 pp.
2. Kirtikar, K.R. and B.D. Basu, 1996. Indian Medicinal Plants. E. Blatter, J. F. Caius and K.S. Mahasker (Eds.), Lalit Mohan Basu, Allahabad, India, IV: 2422-2423.
3. Keys, J.D., 1976. Chinese Herbs (Their Botany, Chemistry, and Pharmaco-dynamics). Charles E. Tuttle Company, Inc., Tokyo.

4. Kirtikar, K.R., and B.D. Basu, 1981. Indian Medicinal Plants, vol. IV B/S Bishen Singh Mahendra Pal Singh, Dehradun, India.
5. Kanjanapothi, D., Panthong, A., Lertprasertsuke, N., Taesotikul, T., Rujjanawate, C. and D. Kaewpinit, 2004. Toxicity of crude rhizome extract of *Kaempferia galanga* L. (Proh Hom), Journal of Ethnopharmacology. 90(2-3): 359-365.
6. Ahn, Young-Joon et al., 2008. Larvicidal activity of *Kaempferia galanga* rhizome phenylpropanoids towards three mosquito species. Pest Management Science. 64(8): 857-862
7. Revikumar, K., and D.K. Ved, 2000. In Red Listed Medicinal Plants of Conservation Concern in Southern India, 1st ed., pp. 212–214.
8. Vincent, K.A., Mathew, K.M., Hariharan, M., 1992. Micropropagation of *Kaempferia galanga* L – a medicinal plant. Plant Cell Tiss. Org. Cult. 28, 229–230.
9. Shirin, F., Kumar, S., Mishra, Y., 2000. In vitro plantlet production system for *Kaempferia galanga*, a rare Indian medicinal herb. Plant Cell Tiss. Org. Cult. 63:193-197.
10. Chithra, M., K.P. Martinb, 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 Horticulturae 104: 113–120.
11. Rajasekharan, P.E., and S. Ganeshan, 2002. J. Med. Aromat. Plant Sci. 24: 132–147.
12. Murashige, T., and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15: 473-479.
13. SPSS., 1999. Statistical Package for Social Sciences (SPSS) v.11.5 for Windows Illinois, USA: SPSS Inc.
14. Rahman, M.M., M. N. Amin, T. Ahamed, S. Ahamad, A. Habib, R. Ahmed, M. B. Ahmed and M. R. Ali, 2005. In vitro Rapid Propagation of Block Thorn (*Kaempferia galanga* L.): A rare medicinal and aromatic plant of Bangladesh. Journal of Biological Sciences. 5(3): 300-304.
15. Yasuda K, T .Tsuda, H. Shimizu and A. Sugaya, 1987. Multiplication of Curcuma species by tissue culture, Plant. Med. 54: 75–78.
16. Amin, M.N., M. A. Islam and M. A. K. Azad, 2001. Micropropagation and conservation of a threatened aromatic medicinal plant, *Alphima calcarata* Rose. 4th Intl. Plant Tissue cult conf. Dhaka. Nov, 1-3: 55.
17. Inden, H., T. Asahira and A. Hirano, 1988. Micropropagation of ginger, Acta Hort. 230:177-184.
18. Haque, M. I., S. Perveen and R. H. Sarker, 1999. In vitro propagation of ginger (*Zingiber officinale* Rose.) Plant Tissue Cult. 9: 44-51.
19. Rahman, M.M., M. N. Amin, H. S. Jahan and R. Ahmed, 2004. In vitro regeneration of plantlets of *Curcuma longa* Linn. A valuable spice plant in Bangladesh. Asian J. Plant Sci., 3: 306-309.
20. Swapna, T.S., M. Binitha and T.S. Manju, 2004. In vitro multiplication in *Kaempferia galanga* Linn. Applied Biochemistry and Biotechnology.118:233-241.
21. Dunstan DI, Turner KE (1984) The Acclimatization of micropropagated plants. In: cell culture and Somatic cell Genetics of plants. Vol. I Laboratory procedures and their applications. Vasil, I.K. (Ed), Academic Press, Orlando.123-129.
22. Kozai T., M. Hayashi, Y. Hirosawa, T. Kodama and I. Watanabe, 1987. Environmental control for acclimatization of in vitro cultured plantlets (1). Development of the acclimatization unit for acceleration the plantlet growth and the test cultivation. J. Agric. Met. Japan. 42: 349-358.