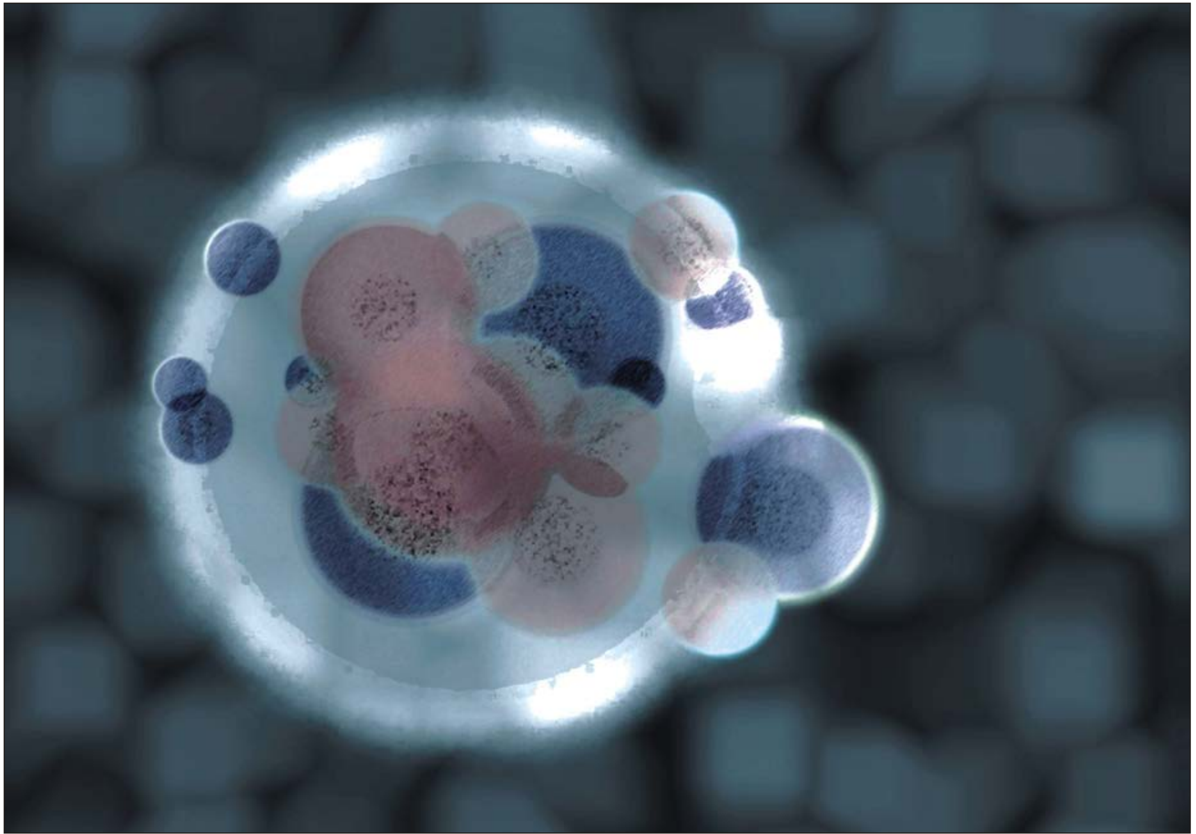

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In vitro Rapid Propagation of Black Thorn (*Kaempferia galanga* L.): A Rare Medicinal and Aromatic Plant of Bangladesh

M.M. Rahman, M.N. Amin, T. Ahamed, S. Ahmad,
[1]A. Habib, R. Ahmed, M.B. Ahmed and [1]M.R. Ali
Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh
[1]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

Abstract: An efficient protocol has been established for rapid production of plantlets using rhizome tip and lateral bud explants of the field grown plant. The explants were cultured on MS medium with auxins (NAA, IBA and IAA) and cytokinins (BA and Kn). Cent percent of the explants produced two or three shoot buds in each culture when they were cultured on MS medium containing 1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA within three weeks of culture. The number of shoots per culture increased gradually when the primary cultures were subcultured in two weeks intervals. Highest number of 20.50±1.80 shoots proliferated in each culture when the explants of initially sprouted shoots were subcultured at three times on the same medium. Microshoots were isolated from the *in vitro* proliferated cluster of shoots produced roots in 100% cases on modified (MMS_2) medium supplemented with 0.2 mg L^{-1} of IBA. Maximum number of 12.4±1.23 roots per microshoot were recorded on the medium containing 0.2 mg L^{-1} IBA. The regenerated plantlets were acclimatized and established on the soil with eighty five percent success.

Key words: *In vitro,* propagation, *Kaempferia galanga*

INTRODUCTION

Kaempferia galanga L. is a perennial rhizomatous plant, commonly known as Black Thorn (Eng.) and Ekangi or Chandramul (Beng.), belonging to the family Zingiberaceae. The plant is a native to India, but currently it is cultivated mainly in South East Asia and China[1]. In Bangladesh, the plant is specially found in Sal forest of Dhaka and grown in limited scale at different parts of the country, Maymensingh[2]. *Kaempferia galanga* is an important medicinal plant. The rhizomes of the plant are widely used in East Asia for a wide range of medicinal applications[3]. Since the rhizomes of this plant contain volatile oil and other important compounds of enormous medicinal values, they are very demanding to the traditional health care practitioner[4]. The rhizomes have immense importance in traditional health care system as a carminative, diuretic and stomachic as well as in the diseases of coughs, pectoral affections and stoppage of nasal blocks[2]. They are also used in perfumery and cosmetics. The plants grow semi-wild in a limited area and is considered as one of the threatened plants of Bangladesh.

Kaempferia galanga is propagated conventionally by vegetative means using perennial rhizomes. Low productivity, disease susceptibility and higher cost of seed rhizome production are major constraints faced by the growers. In the recent decades micro-propagation techniques are being profitably used to overcome such constraints in various vegetatively propagated crops as well as ornamental and horticultural plants[5]. Considering high demand and greeter economic and medicinal values of *Kaempferia galanga*, it is necessary to develop a suitable protocol for mass production of disease free stocks through tissue culture technique. There are many reports on *in vitro* propagation of some rhizomatous plants like ginger[6-8], cardamom[9], *Alphinia calcarata*[10] and *Curcuma longa*[11-13]. However, there are a few reports on tissue culture of *K. galanga*[14,15].

In the present study an effort was made to establish a protocol for the *in vitro* propagation of *Kaempferia galanga* from rhizome tip and lateral bud explants. This study is obviously a first step in the advancement of *Kaempferia galanga* tissue culture in Bangladesh.

MATERIALS AND METHODS

Rhizome explants were collected from the sprouted rhizomes as well as from field grown plants of

Corresponding Author: Md. Mahabubur Rahman, Department of Botany, University of Rajshahi, Bangladesh
E-mail: spmahabub@yahoo.com

Kaempferia galanga. They were brought to the laboratory and processed for aseptic culture. For surface sterilization the rhizome explants were excised, cleaned thoroughly under continuous flushing of running tap water for 20 min and then washed with a solution of antiseptic [Savlon 5% (v/v)] for 10 min. The explants (rhizome tip and lateral bud) were then washed repeatedly with distilled water and finally surface terilized with $HgCl_2$ (0.1%) for 12 min in a laminar flow cabinet and washed three times with autoclaved distilled water to remove any trace of $HgCl_2$.

After surface sterilization, rhizome explants were trimmed to appropriate sizes and cultured on the MS medium for shoot multiplication. The basal medium used for all the experiments were Murashige and Skoog mineral formulation (MS) containing standard salts and vitamins, 30 g L^{-1} sucrose and 7 g L^{-1} agar. The media were variously supplemented with 6-benzyl adenine (BA) either individually or in different combinations with auxins, α-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA). Regenerated microshoots were placed on MMS_2 medium (half strength of both major and minor salts) supplemented with various concentrations of auxins (NAA, IBA or IAA) singly for rooting. The media pH was adjusted to 5.7±0.1 before adding agar and the media were autoclaved at 1.1 kg cm^{-2} for 20 min at 121°C. Cultures were incubated at 25±1°C with a photoperiod of 16 h with photon flux density of about 70 μ mol m^{-2} s^{-1} provided by cool white fluorescent light.

RESULTS AND DISCUSSION

Proliferation of shoots: A number of experiments were conducted with a view to finding out optimum culture condition for maximum shoot multiplication from the rhizome cultured explants. Multiple shoots were found to develop from rhizome explants when they were cultured on MS medium supplemented with BA alone or in combination with NAA or IBA (Table 1). However, in most of treatments initiation of multiple shoots was observed within four weeks of culture. The best shoot proliferation from the explants was observed in MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA. In this treatment, 100% of the cultured explants produced multiple shoots where number of shoot per culture was 20.5±1.80 and average length of shoots per culture was 6.31±0.24 cm (Fig. 1A-D). Similar result was observed on the same medium for shoot multiplication of *Alpinia calcarata*[10]. 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[16].

Table 1: Effects of different concentrations of BA alone and in combination with NAA on shoot proliferation from rhizome explants. There were 20 explants in each treatment and data (Mean±SE) were recorded after 6 weeks of culture

Growth regulators (mg L^{-1})	% of explant showing proliferation	No. of shoot per culture	Average length of shoot per culture
BA			
0.2	47	4.70±0.45	3.85±0.24
0.5	60	5.45±0.35	4.00±0.20
1.0	67	8.50±0.40	5.57±0.10
2.0	40	4.00±0.50	3.50±0.15
3.0	33	2.90±0.24	3.00±0.20
BA+NAA			
0.5+0.1	80	14.30±0.27	4.50±0.45
+0.2	53	7.86±0.26	4.00±0.21
+0.5	47	3.45±0.31	3.65±0.12
1.0+0.1	100	20.50±1.80	6.31±0.24
+0.2	87	16.40±0.31	5.33±0.19
+0.5	73	10.60±0.10	4.18±0.32
+1.0	53	6.05±0.17	3.50±0.14
2.0+0.1	73	11.20±0.35	4.22±0.19
+0.2	65	6.55±0.14	3.70±0.34
+0.5	55	5.50±0.30	3.35±0.24
+1.0	40	4.20±0.41	2.91±0.33
BA+IBA			
0.5+0.1	53	3.44±0.20	3.14±0.45
+0.2	40	3.23±0.24	2.80±0.23
+0.5	33	1.50±0.12	2.36±0.21
1.0+0.1	47	3.25±0.17	3.50±0.26
+0.2	73	5.24±0.63	5.00±0.24
+0.5	53	4.20±0.32	4.05±0.43
+1.0	33	3.00±0.20	2.11±0.12
2.0+0.1	33	2.66±0.17	1.82±0.17
+0.2	47	3.42±0.22	2.74±0.26
+0.5	40	2.84±0.42	2.15±0.14
+1.0	20	2.16±0.35	1.50±0.23

Table 2: Effects of different concentrations of auxins on adventitious root formation from *in vitro* regenerated shoots. There were 20 shoots in each treatment and data (Mean±SE) were recorded after 6 weeks of culture

Conc. of auxin (mg L^{-1})	% of shoots rooted	No. of roots per rooted shoots	Average length of roots (cm)
NAA			
0.1	90	7.42±0.16	4.00±0.24
0.2	100	9.66±1.23	6.50±0.24
0.5	95	6.00±0.42	4.80±0.46
1.0	80	4.44±0.52	3.85±0.30
IBA			
0.1	100	8.60±0.35	5.10±0.27
0.2	100	12.40±1.23	7.20±0.23
0.5	80	6.57±0.35	4.80±0.34
1.0	70	4.80±0.28	4.00±0.86
IAA			
0.1	50	3.30±0.29	3.20±0.23
0.2	55	4.63±0.24	3.42±0.42
0.5	70	5.90±0.26	4.80±0.27
1.0	40	3.10±0.31	2.95±0.20

The effects of different concentrations of BA (0.2-3.0 mg L^{-1}) on shoot initiation and multiplication from the cultured rhizome explants on MS medium were also studied in the present experiment. The best response of shoot proliferation was observed in 1.0 mg L^{-1} BA supplemented on MS medium and almost 67% of the

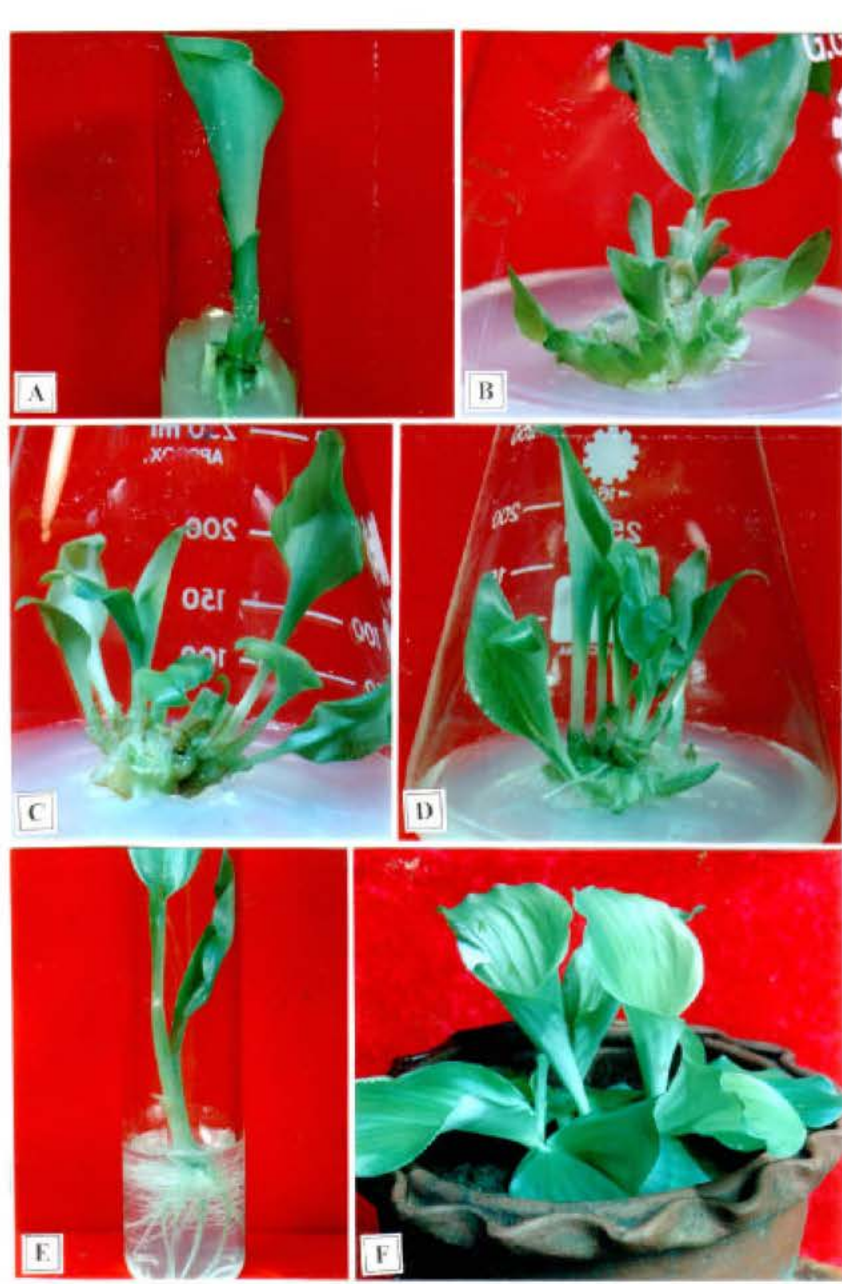

Fig. 1A-F: Plantlets regeneration from rhizome bud explants of *Kaempferia galanga* L. under *in vitro* condition

- A): Shoot buds initiation from rhizome bud explant on MS containing 1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA after two weeks in culture.
- B): Development of multiple shoot buds on same medium after six weeks in culture, respectively.
- C-D): Development and elongation of multiple shoots on same medium after eight and ten weeks in culture, respectively.
- E): Roots development in *Kaempferia galanga* on MMS_2 medium 0.2 mg L^{-1} IBA after four weeks in culture
- F): Establishment of *in vitro* grown plantlets under *ex vitro* condition after six weeks of culture.

incubated explants showed shoot initiation. Almost similar result was obtained by Balachandran *et al.*[11], Haque *et al.*[8], Rahman *et al.* [13], Keshavachandaran and Khader[17] worked, while working on the plants of same nature such as, ginger and turmeric.

Rooting of the proliferated shoots: Root development was induced in the *in vitro* proliferated shoots by culturing them on MMS_2 medium with 0.1-1.0 mg L^{-1} either of NAA, IBA or IAA (Table 2). Among the three types of auxins used in the present experiment, IBA was found to be the most effective at different concentrations tested for producing roots on the bases of microshoots. Among different concentrations of IBA, 0.2 mg L^{-1} IBA was found to be the best concentration of auxin for proper rooting of *K. galanga* in which 100% shoots rooted within six weeks of culture (Fig. 1E). The findings are in agreement with those observed in similar rhizomatous plant species such as ginger[8], *Alpinia calcarata*[10], *Curcuma longa*[13].

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 flashing of running tap water. Then the plantlets were transferred gradually to the different potting mix for hardening. About 100% of the plantlets established under *ex vitro* condition when they were initially transferred on coco-peat as potting mix. On the other hand, 90% survival rate of the plantlets was observed when they were transferred on ice cream pots containing garden soil, compost and sand (2:2:1). It was also observed 85% of the plantlets could be established under *ex vitro* conditions when they were transferred on earthen pots containing soil and organic manure (Fig. 1F). The reason behind variation in survival rate of the plantlets on different potting mix is that coco-peat is a soil-free material with very porous but good water holding capacity and do not allow microbial growth easily, while a mix of garden soil, compost and sand has a moderate water holding capacity and allows microbial growth in the interface of plantlet and potting mix. The third category potting mix, fresh soil plus organic manure has least water retaining capacity and allows rapid microbial growth over the surface. The use of sufficiently porous potting mix that allows adequate drainage and aeration has been recommended for fast acclimatization of *in vitro* regenerated plants[18,19]. Nevertheless, excellent success has been obtained with soil-free potting mix like vermiculite, perlite, peat plugs or small foam blocks[20,21]. Rest of the transplants could not survive either due to wilting during hot, dry atmosphere or microbial over growth that caused damping off and necrosis. After the plantlets are acclimatized, the supplemental bottom heat is no longer necessary[18]. Damping off and necrosis of the transplants were also observed during acclimatization in *ex vitro* condition of Thankuni[22].

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