

## Full Length Research Paper

# *In Vitro* Regeneration of the Endangered *Kaempferia galanga* L.

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An efficient protocol is out lined for multiple shoot induction of a medicinally valuable *Kaempferia galanga* L. using rhizome segment explants. Murashige and Skoog medium supplemented with BA (2.0 mg/ml) and Kn (1.0 mg/ml) exhibited regeneration rate up to  $10.85 \pm 1.34$  shoot/explants. Spontaneous rooting of shoots occurred in the same concentration of cytokinin. The number of leaves ( $5.21 \pm 0.34$ ) and profuse rooting ( $12.14 \pm 1.67$ ) facilitated 100% of plant recovery on acclimation. *In vitro* derived plants were morphologically identical to the mother plant.

**Keywords:** *Kaempferia galanga*, multiplication, regeneration, rhizome segment.

## INTRODUCTION

*Kaempferia galanga* L. an endangered medicinal plant of Zingiberaceae (Kareem 1997, Shanker *et al.*, 1997), is native to India, and is cultivated mainly in South East Asia and China (Kirtikar and Basu 1996) for its aromatic rhizome. The plant is an economically important medicinal species used in several ayurvedic preparations (Sadiman 1992). The rhizome extract contains n-pentadecane, ethyl p-methoxy cinnamate, ethyl cinnamate, L-  $\Delta^3$ - carene, camphene, borneol, cineol, p-methoxy styrene, kaempferol and kaempferide (Anonymus, 1959). The methanolic extract of rhizome contains ethyl p-methoxy trans-cinnamate, which is highly cytotoxic to HeLa cells (Kosuge *et al.*, 1985). Leaves and flowers contain flavanoids (Ghani 1998). The rhizome and tubers are used for curing bronchitis, asthma, malaria, skin diseases, wounds and splenic disorders (Kirtikar and Basu 1997). Larvicidal and anticancer principles have also been reported from the rhizome extract (Kiuchi *et al.*, 1988).

## Abbreviations

BA; 6 benzyladenine, Kn; Kinetin, MS; Murashige and Skoog.

The plant exhibits dormancy during drought and sprouts only in spring. Conventional propagation of the species is through rhizomes and there is no seed setting under natural conditions. Hence, *in vitro* methods are desirable for conserving this valuable medicinal species. *In vitro* clonal multiplication of *K. galanga* through rhizome buds has been reported (Vincent *et al.*, 1992, Geetha *et al.*, 1997, Lakshmi *et al.*, 2003). A few reports available describe the *K. galanga* micropopagation using rhizome pieces (Shirin *et al.*, 2000, Swapna *et al.*, 2004). In this study, we report an easy propagation protocol of *K. galanga* through rhizome segments within a short duration.

## MATERIALS AND METHOD

Rhizomes of *K. galanga* L. (2 months old) collected from the botanical garden, Carmel College, Mala were used as explants. Each rhizome was sectioned into 1.0 – 1.5 cm long pieces and washed under running tap water for 20 minutes and immersed in detergent (Teepol) for 5 minutes. After 3 sterile distilled water rinses, rhizomes were surface disinfected in 70%(v/v) ethanol for 1 minute and rinsed in sterile distilled water and then soaked in 0.1% (w/v) aqueous Mercuric chloride solution for 5 min and thoroughly rinsed with sterile distilled water for 5 min. 028 Int. Res. J. Biotechnol.

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The rhizome pieces were cultured aseptically on nutrient medium.

### Culture medium

Rhizome explants were cultured on MS medium (Murashige and Skoog, 1962) supplemented with various hormones for shoot initiation and multiplication. The medium also contained 3% sucrose (w/v) and after adjusting the pH to 5.8, it was gelled with 0.7% agar (w/v), (Hi-media, Mumbai) and sterilized by autoclaving at 104 kPa at 121 °C for 20 min. Twenty and thirty ml medium was distributed into 25mm × 150mm test tubes and 100 ml conical flask respectively. For each treatment at least 20 replicate cultures were initiated. Cultures were incubated at 25±2°C under a 16/8h photoperiod under white fluorescent tubes. (Photosynthetic photon flux of 25µmol m<sup>-2</sup>s<sup>-1</sup>)

### Shoot multiplication

MS medium supplemented with BAP (0.5- 5.0 mg/l) was used for shoot initiation. *In vitro* raised shoots produced from rhizome pieces excised and transferred to MS medium with various concentrations and combinations of BAP (0.5- 4.0 mg/l) and Kn (0.1- 2.5) for further multiple shoot proliferation. The multiple shoot obtained after 4 weeks were separated into single shoots (5-7cm height with rootlets) and subcultured on the same medium.

### Acclimatization

The well developed rooted plantlets were removed from the culture tubes and washed in distilled water. The number of leaves and roots per shoots were recorded and dipped in 0.1 (w/v) Bavistin for 5 min. The plantlets were then transferred to plastic cups containing sterilized soil, sand and farmyard manure (1:1:2) nourished with half strength MS basal liquid medium. Each one was covered with polythene wraps and watered on every alternate day to maintain humidity.

### Statistical analysis

Only data which showed some advantageous effect were included in the tables and are presented as mean ± SD of 20 explants per treatment and experiments were repeated thrice. Mean values with the same superscript

were not statistically different (P<0.05%) according to Duncan's Multiple Range Test DMRT (Gomez and Gomez, 1976)

## RESULT AND DISCUSSION

Sprouting of axillary buds followed by shoot initiation was observed 15 days after the cultures with rhizome slices were started. When BA alone was used, the maximum number of shoots (7.14±1.34) was obtained from explants on MS medium with 2mg/l BA with 88.5% shoot regeneration response followed by 6.07±0.61 shoots from 3.0 mg/l BA with 60.0% response. At low BA concentration (0.5 mg/l) fewer shoots were obtained (4.71± 1.11). Maximum shoot length (5.57± 0.97cm) was observed in the presence of 2.0mg/l BA (Table 1)

The interactive effect of two cytokinins, BA (0.5-3.0mg/l) and kinetin (0.1-2.5mg/l) was also determined. Maximum multiple shoots (10.85±1.34) with 88.5% of regeneration response were observed on medium supplemented with BA (2.0mg/l) and kinetin (1.0mg/l). The individual shoot subcultured on same concentrations of cytokinins. Multiple shoot were induced and maximum shoot height obtained was 6.14±1.86 (Table 1, Figure 1)

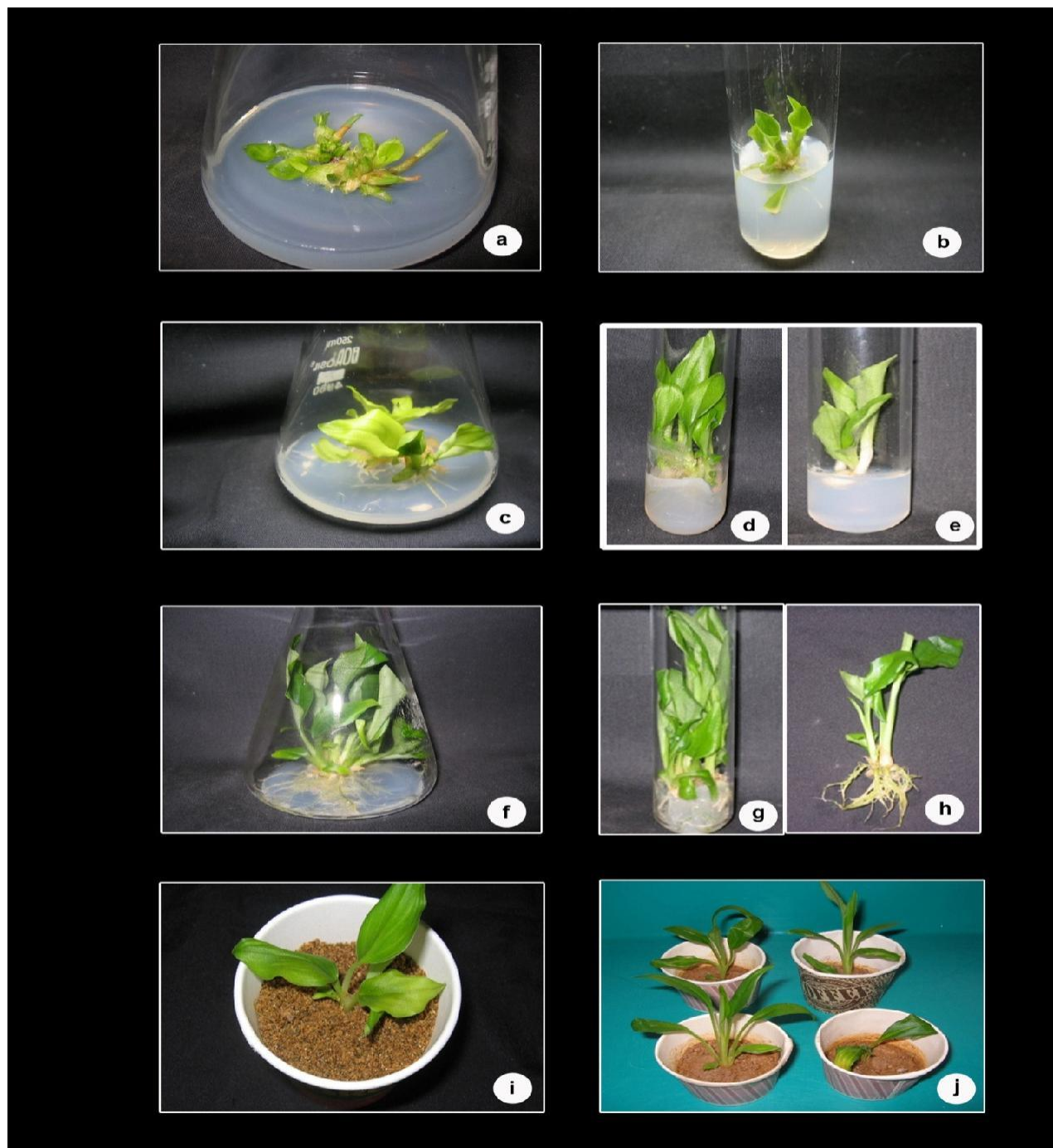
Effect of BA on clonal propagation of members of family Zingiberaceae has been reported earlier by Balachandran *et al.*, (1990) in *Curcuma* spp.; Hosoki and Sagava, (1977) in *Zingiber officinale*; Agreious *et al.*, (1996) in *Alpinia calcarata*. The synergistic effect of two cytokinins (BAP and Kn) for an enhanced rate of shoot multiplication has been reported by Saxena (1990), Bejoy and Hariharan (1993), Vincent *et al.*, (1992), Jinu and Aravindan (2008). No additional step was required for rooting of the shoot. Shoots and roots simultaneously originated in the same medium fortified with BA and KN within 15 days of the second subculture. (Table 2, Figure 1 f & g). Such type of simultaneous production of shoot and roots were reported earlier for a few species of Zingiberaceae by Kurunishetty *et al.*, (1982) in Turmeric, Balachandran *et al.*, (1990) in *Zingiber officinale*, Borthakur *et al.*, (1999) in *Alpinia galanga*. This may be due to the root inducing factors which are intrinsic in the rhizome has been reported in rhizomatous plants (Vincent *et al.*, 1992). A few reports available describe the *K. galanga* micropopagation using rhizome pieces (Shirin *et al.*, 2000, Swapna *et al.*, 2004)

Plantlets with well developed roots and leaves were hardened for 6-8 weeks and then transferred to field. *In vitro* produced plants were easily established in the field with almost 100% survival and this makes an easy propagation of crop within a short duration.

**Table 1.** Effect of different concentrations of plant growth regulators BAP(0.5 mg/l -5.0 mg/l) and KN (0.1 mg/l -2.5mg/l) on multiplication of shoots from rhizome disc explants of *Kaempferia galangal*

Plant Growth Regulators (mg/l)	Percentage of culture responded	Mean no. of shoot /explant	Mean shoot length(cm)	Mean no. of root /explant
<b>BAP</b>				
0.5	51.4 <sup>cd</sup>	4.71±1.11 <sup>de</sup>	2.85±0.89 <sup>ef</sup>	8.04±1.20 <sup>de</sup>
1	57.1 <sup>bc</sup>	6.67±0.44 <sup>ab</sup>	3.28±1.11 <sup>de</sup>	8.85±0.69 <sup>cd</sup>
<b>2</b>	<b>88.5<sup>A</sup></b>	<b>7.14±1.34<sup>A</sup></b>	<b>5.57±0.97<sup>A</sup></b>	<b>14.71±2.13<sup>A</sup></b>
3	60.0 <sup>b</sup>	6.07±0.61 <sup>bc</sup>	4.85±0.69 <sup>b</sup>	12.14±2.03 <sup>b</sup>
4	45.7 <sup>de</sup>	5.14±0.69 <sup>cd</sup>	4.28±0.75 <sup>c</sup>	9.28±1.79 <sup>c</sup>
5	40.0 <sup>ef</sup>	2.28±0.75 <sup>f</sup>	3.64±3.35 <sup>d</sup>	6.85±1.06 <sup>f</sup>
<b>KN</b>				
0.5	65.7 <sup>c</sup>	3.71±0.95 <sup>de</sup>	3.14±0.89 <sup>e</sup>	5.57±1.61 <sup>de</sup>
1	80.0 <sup>b</sup>	4.71±1.60 <sup>c</sup>	4.71±0.75 <sup>bc</sup>	6.14±1.67 <sup>cd</sup>
<b>2</b>	<b>85.7<sup>A</sup></b>	<b>6.42±1.51<sup>A</sup></b>	<b>5.57±1.61<sup>A</sup></b>	<b>9.71±1.38<sup>A</sup></b>
3	71.4 <sup>c</sup>	5.42±0.97 <sup>b</sup>	5.14±1.34 <sup>ab</sup>	6.57±1.61 <sup>bc</sup>
4	68.6 <sup>de</sup>	4.24±0.97 <sup>cd</sup>	4.14±0.69 <sup>d</sup>	6.85±1.06 <sup>b</sup>
5	62.9 <sup>f</sup>	3.28±1.11 <sup>ef</sup>	3.04±0.53 <sup>ef</sup>	4.85±0.69 <sup>ef</sup>
<b>BAP+KN</b>				
2.0+0.1	57.1 <sup>cd</sup>	4.21±0.61 <sup>ef</sup>	3.14±0.69 <sup>ef</sup>	4.71±1.11 <sup>f</sup>
2.0+0.5	65.7 <sup>c</sup>	7.42±0.26 <sup>cd</sup>	4.85±1.67 <sup>cd</sup>	8.28±0.75 <sup>cd</sup>
<b>2.0+1.0</b>	<b>88.5<sup>A</sup></b>	<b>10.85±1.34<sup>A</sup></b>	<b>6.14±1.86<sup>A</sup></b>	<b>12.14±1.67<sup>A</sup></b>
2.0+1.5	80.0 <sup>ab</sup>	9.57±2.37 <sup>ab</sup>	5.85±1.67 <sup>ab</sup>	10.34±1.87 <sup>b</sup>
2.0+2.0	51.4 <sup>de</sup>	7.57±1.39 <sup>c</sup>	5.42±0.97 <sup>bc</sup>	8.57±0.69 <sup>c</sup>
2.0+2.5	40.0 <sup>ef</sup>	4.28±0.75 <sup>e</sup>	3.72±0.74 <sup>e</sup>	7.42±0.97 <sup>de</sup>

Total number of explants taken for observation =35 (each treatment consist of at least 7 explants and the experiments were repeated five time). Mean ± SD each experiment marked with same letter do not differ significantly (p<0.05)



**Figure 1.** Multiple shoot induction from rhizome segment explant (a, b) shoot initiation after 15 days of inoculation in MS + BA (2.0mg/l) and KN 1.0mg/l), (c, d and e) development of shoot, (f, g and h) rhizogenesis after 15 days of first subculture in same concentrations of cytokinins. (i and j) Hardened regenerated plantlets after five weeks from the day of inoculation.

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