0273-2289/04/118/0233/$25.00

In Vitro Multiplication in *Kaempferia galanga* Linn

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Received June 15, 2003; Revised September 1, 2003; Accepted September 2, 2003

Abstract

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. Leaf and rhizome explants of *Kaempferia* were aseptically cultured on MS medium with various combinations of indole-3-acetic acid (IAA), benzyl amino purine (BAP), napthalene acetic acid (NAA), 2-4-dichlorophenoxy acetic acid (2,4-D) and kinetin at concentrations ranging from 0.5 to 2.5 mg/L. High-frequency organogenesis and multiple shoot regeneration was induced from rhizome explants on MS medium supplemented with 0.5 mg/L of IAA and 2.5 mg/L of BAP. Rooting was induced in MS medium with 0.5 mg/L of IAA and 2 mg/L of BAP.

Index Entries: Medicinal plants; *Kaempferia galanga*; in vitro propagation; multiple shooting; organogenesis.

Introduction

India is the leading exporter of medicinal plants, and a major part of these plants is harvested from the wild. Increasing population, urbanization, shrinking forests, overharvesting, and related factors have brought several medicinal plants to the very brink of extinction. In this context, conservation of threatened medicinal plants is the most important responsibility of biodiversity in rich nations such as India. The potential of the use of tissue culture for the rapid multiplication of plants and extraction of secondary metabolites from callus and cell cultures has been established on a commercial scale *(1)*.

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Kaempferia galanga is best known for its medicinal properties and chemical constituents. Extracts of the plant have shown remarkable resistance to asthma, epilepsy, fever, and spleenic disorders. Some complex chemical substances that are biologically active and highly valuable, such as ethyl-*p*-methoxy cinnamate, ethyl cinnamate, deoxypodophyllotoxin, and transcinnamate, are present in the rhizome of the plant *(2)*. *K. galanga* is a monocotyledonous herbaceous plant with a strongly fragrant tuberous rhizome that belongs to the family Zingiberaceae. The underground part consists of mainly one or more prominent, fairly vertically oriented tuberous rootstocks together with smaller secondary tubers and a cluster of roots most of which are long and narrow. Rhizomes and rootstocks are bitter, thermogenic, carminative, and aromatic and are used as expectorants, digestives, and stimulants. They are good for leprosy, skin diseases, asthma, cough, bronchitis, jaundice, ulcer, fever, and nasal obstructions. *K. galanga* is among 100 red-listed medicinal plants of conservation concern in southern India *(3)*. In view of its economic and ecologic importance, in vitro clonal propagation was developed from rhizome and leaf explants.

Materials and Methods

Healthy leaves and rhizomes harvested from plants grown in the botanical garden at the S. N. College campus were used as explants. The explants were thoroughly washed in soapy water and surface sterilized by immersing in 0.1% (w/v) mercuric chloride solution for 10 min. Murashige and Skoog's *(4)* medium supplemented with 3% sucrose and different plant growth regulators (IAA, NAA, 2,4-D, BAP, and kinetin) at various concentrations (0.5–2.5 mg/L) was used (*see* Tables 1–3). Different combinations of medium were named medium A, B, and C with hormone combinations IAA and BAP, 2,4-D and kinetin, and NAA and BAP, respectively. The pH of the media was adjusted to 5.8 using 0.1*N* NaOH solution, and 0.8% (w/v) agar was added to the media before dispensing (15 mL) to flasks. The media were sterilized by autoclaving at 1.06 kg/cm^2 pressure for 15 min.

Explants were inoculated aseptically onto the media and incubated in a culture room at a 16-h day and 8-h night duration at 22 ± 2°C with a light intensity of 1500 lux from a fluorescent light source for 3–6 wk. Different combinations of medium were placed in 30 culture bottles with one explant each at one time, and experiments were repeated three times. Observations were made at the end of each week, and the mean of three values was taken. Regenerated plants were transferred to basal MS medium with 2.5 mg/L of IAA and 2 mg/L of BAP for root induction. For acclimatization, rooted plants were taken out of the bottles after treating with 0.5% dithane solution. They were subsequently transferred to plastic pots containing autoclaved soil and kept under culture room conditions for 1 wk. The pots were then transferred to the garden and placed under shade for 2 wk before transplanting them to normal garden conditions. The rate of transplantation success was about 65%.

Results and Discussion

Among Zingiberaceae, organogenesis and plant regeneration has been reported in cardamom seedling callus *(5)*, in the callus of ginger shoot bud *(6)* and leaf *(7)*, and in rhizome callus of *Curcuma* sp. and *Kaempferia rotunda (8)*. Multiple shoot formation of *K. galanga* has been reported in young sprouting buds *(9)*. In the present work, multiple shoots were induced from leaf and rhizome explants in an MS medium having a composition of different auxin and cytokinin concentrations. The data revealed that basal MS medium with various combinations of IAA and BAP (medium A) was most suitable for multiple shoot and root induction of *K. galanga*. From the selected explants, rhizome showed good response when compared to leaf explants. In the case of rhizome explants, MS medium with 0.5 mg/L of IAA and 2 mg/L of BAP showed maximum shoot induction (i.e., 79%) (Table 1 and Fig. 1). Medium containing 0.5 mg/L of IAA and 2.5 mg/L of BAP also showed a good response to shoot induction (78.33%). However, the leaves of *K. galanga* showed only 61.6% of shoot induction in MS medium containing 0.5 mg/L of IAA and 2.5 mg/L of BAP, which was the maximum response in leaf explants. Organogenesis was also induced in MS medium with 0.5 mg/L of IAA and 1 mg/L of BAP, and 1 mg/L of IAA and 2.5 mg/L of BAP in both explants. Effective root induction was observed in MS medium with 2.5 mg/L of IAA and 2 mg/L of BAP (78.3%) in rhizome, but induction was only 56.6% in medium with 0.5 mg/L IAA and 1 mg/L of BAP in the case of leaf explants. Anand et al. *(10)* observed similar results in *K. rotunda* rhizome explants. They reported multiple shoot induction in MS medium with 0.5 mg/L of IAA and 2 mg/L of BAP from rhizome. In the present study, the same hormonal combination was found to be suitable in rhizome, but leaf explants showed a better response when IAA concentration was high. MS medium containing 1 mg/L each of IAA and BAP and 2.5 mg/L each of IAA and BAP produced 78 and 70% roots, respectively. Fifty-six percent of inoculated rhizome explants produced callus in the MS medium containing 2.5 mg/L of IAA and 2 mg/L of BAP, but in the case of leaves, only 32.5% produced callus. Rhizome explants were found to give the best response in tissue culture, rather than leaves, in this plant.

Medium containing 2,4-D and kinetin was not effective for organogenesis. Medium with 0.5 mg/L of 2,4-D and 2.5 mg/L of kinetin produced 32.5% shoots in rhizome and 2 mg/L of 2,4-D and 2.5 mg/L of kinetin produced 24.3% shoots in leaves (Table 2 and Fig. 2). Root induction was effective in MS medium with 2.5 mg/L of 2,4-D and 0.5 mg/L of kinetin in rhizome (28.33%), and in leaf it was 20.66% in MS medium with 2 mg/L each of 2,4-D and kinetin. In medium with 2.5 mg/L of 2,4-D and 0.5 mg/L of kinetin, 33 and 20% callusing was found in both rhizome and leaf, respectively.

Geetha et al. *(11)* found that hormonal combinations such as NAA and BAP were successful in inducing multiple shoots in *K. galanga*. They reported multiple shoot induction from young buds in MS medium with

Table 1
Effect of IAA and BAP on Rhizome and Leaf Explants of *K. galanga*

			Rhizome			Leaf		
Medium A	IAA (mg/L)	BAP (mg/L)	Shooting (%)	Rooting (%)	Callusing (%)	Shooting (%)	Rooting (%)	Callusing (%)
1	0.5	0.5	15 ± 5.0	20	0	9.3 ± 1.5	20	0
2	0.5	1	74 ± 3.6	10	0	56.6 ± 2.8	12.5 ± 3.5	0
3	0.5	2	79 ± 1.7	6.6 ± 2.8	0	50	10	0
4	0.5	2.5	78.3 ± 2.8	6 ± 1.7	6.5 ± 2.1	61.6 ± 2.8	5	0
5	1	0.5	37.6 ± 2.5	20	7.6 ± 2.5	30	22.5 ± 2.5	5
6	1	1	16.6 ± 3.5	78 ± 3	6.3 ± 1.5	20	56.6 ± 2.8	2
7	1	2	48.3 ± 2.3	18.3 ± 2.8	5	28.3 ± 2.8	10	5
8	1	2.5	69 ± 3.6	10	5	51.6 ± 2.8	7.5 ± 3.5	5
9	2	0.5	33.3 ± 2.8	27.6 ± 2.5	17.5 ± 3.5	9 ± 1.7	17.5 ± 3.5	10
10	2	1	47 ± 1.7	31 ± 3.6	15	20	22.5 ± 3.5	11.6 ± 2.8
11	2	2	17.5 ± 3.5	36.6 ± 2.8	15	15 ± 7.0	23.3 ± 2.8	12.5 ± 3.5
12	2	2.5	27.6 ± 2.5	22.5 ± 3.5	12.5 ± 3.5	10	17.5 ± 3.5	10
13	2.5	0.5	20	29.3 ± 4.0	25	10	18.3 ± 2.8	12.5 ± 3.5
14	2.5	1	10	22.5 ± 3.5	0	7.5 ± 3.5	10	5
15	2.5	2	10	78.3 ± 2.8	56.6 ± 2.8	12.5 ± 3.5	49 ± 3.6	32.5 ± 3.5
16	2.5	2.5	10	70	10	10	51.6 ± 2.8	0
17	0	1	33.3 ± 2.8	10	0	18.3 ± 2.8	10	0
18	1	0	17.5 ± 3.5	32.5 ± 3.5	10	17.5 ± 3.5	27.5 ± 3.53	10

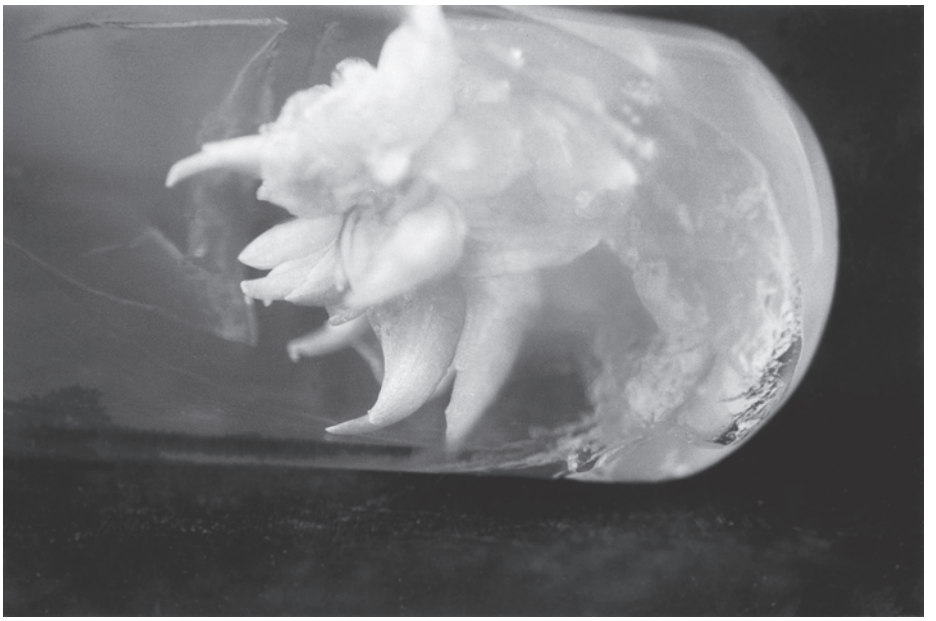

Fig. 1. Multiple organogenesis in MS medium with 0.5 mg/L of IAA and 2.5 mg/L of BAP.

Fig. 2. Multiple shoot formation in MS medium with 0.5 mg/L of IAA and 2 mg/L of BAP.

Table 2
Effect of 2,4-D and Kinetin on Rhizome and Leaf Explants of *K. galanga*

			Rhizome			Leaf		
Medium B	2,4-D (mg/L)	Kinetin (mg/L)	Shooting (%)	Rooting (%)	Callusing (%)	Shooting (%)	Rooting (%)	Callusing (%)
1	0.5	0.5	1.6 ± 1.5	6.6 ± 2.8	2	2.3 ± 0.5	3 ± 1.7	0
2	0.5	1	3 ± 1.7	1.3 ± 0.4	2.6 ± 0.5	2	2	2.3 ± 0.5
3	0.5	2	7.6 ± 2.5	1.3 ± 0.4	2	4.3 ± 1.1	2	3
4	0.5	2.5	32.5 ± 3.5	2	2.6 ± 0.5	11.6 ± 2.8	2.3 ± 0.5	2
5	1	0.5	7.6 ± 2.5	10	9 ± 1.7	5	4.3 ± 1.1	5
6	1	1	5	6.6 ± 2.8	8.3 ± 2.8	5	10	10
7	1	2	12.5 ± 3.5	5	0	6.6 ± 2.8	7 ± 1.7	0
8	1	2.5	17.5 ± 3.5	5	5	12.5 ± 3.5	4.3 ± 1.1	2
9	2	0.5	11.6 ± 2.8	9.3 ± 1.1	9 ± 1.4	22.5 ± 3.5	10	11 ± 1.4
10	2	1	27.6 ± 2.5	10	10	20	5.6 ± 1.1	5
11	2	2	9.3 ± 1.1	18.3 ± 2.8	20	5.6 ± 1.1	20.6 ± 1.5	19.6 ± 0.5
12	2	2.5	10	27.5 ± 3.5	25	24.3 ± 4.0	16.6 ± 5.7	5
13	2.5	0.5	13.6 ± 3.2	28.3 ± 1.5	33.3 ± 2.8	9.3 ± 1.1	10	20
14	2.5	1	10	21.3 ± 1.1	20	5.3 ± 0.5	10	8.6 ± 1.1
15	2.5	2	5	12.3 ± 2.5	10	6 ± 1.7	5	5
16	2.5	2.5	5	6.5 ± 2.1	6 ± 1.4	5	2	0
17	0	1	10	5	0	5	0	0
18	1	0	0	5	10.6 ± 1.1	0	10	10

Table 3
Effect of NAA and BAP on Rhizome and Leaf Explants of *K. galanga*

			Rhizome			Leaf		
Medium C	NAA (mg/L)	BAP (mg/L)	Shooting (%)	Rooting (%)	Callusing (%)	Shooting (%)	Rooting (%)	Callusing (%)
1	0.5	0.5	0	10	0	0	0	0
2	0.5	1	2	0	0	0	0	0
3	0.5	2	22.5 ± 3.5	0	5	12.5 ± 3.5	0	0
4	0.5	2.5	30	0	0	18.3 ± 2.8	0	0
5	1	0.5	16 ± 1.7	10	5	5	7 ± 1.1	0
6	1	1	0	10	16.3 ± 1.5	0	0	7 ± 1.7
7	1	2	10	0	6	1	0	0
8	1	2.5	15	5	5	8.3 ± 2.8	5	0
9	2	0.5	22.5 ± 3.5	7 ± 1.7	9.3 ± 1.1	9 ± 1.7	9 ± 1.7	9.3 ± 1.1
10	2	1	20	10	8.6 ± 1.1	10	5	5
11	2	2	10	10	10	5	10	10
12	2	2.5	10	22.5 ± 3.5	26.6 ± 2.8	0	5	0
13	2.5	0.5	8.3 ± 2.8	20	27.6 ± 2.5	0	0	8.6 ± 1.1
14	2.5	1	10	23.3 ± 2.8	25	5	7 ± 1.1	5
15	2.5	2	8.3 ± 2.8	10	10	0	5	0
16	2.5	2.5	2	2	5	5	2.3 ± 0.57	0
17	0	1	0	5	7	5	0	0

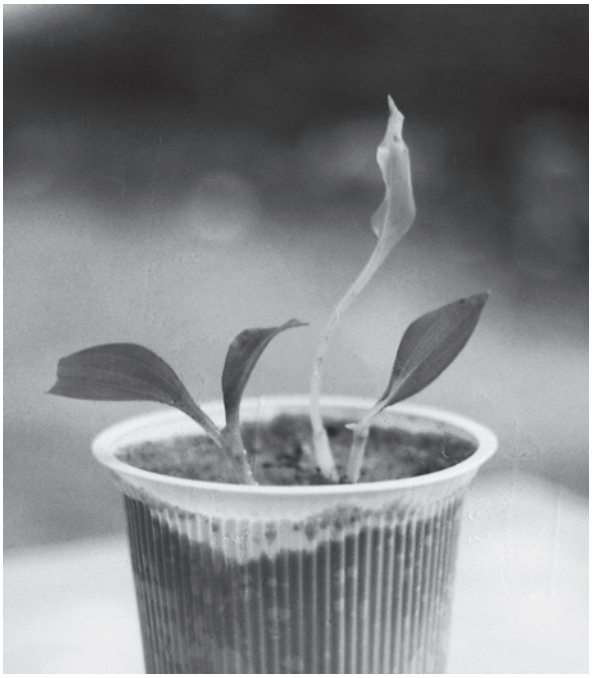

Fig. 3. Regenerated plants growing under natural conditions.

0.5 mg/L of NAA and 1 mg/L of BAP, but this hormonal combination was found to be unsuitable in the present work. MS medium with 0.5 mg/L of NAA and 2.5 mg/L of BAP produced 30 and 18.33% shoots in rhizome and leaf, respectively (Table 3). Twenty-three percent root induction was found with a hormonal combination of 2.5 mg/L of NAA and 1 mg/L of BAP in rhizome, and 10% rooting was found with a combination of 2 mg/L each of NAA and BAP. MS medium with 2.5 mg/L of NAA and 0.5 mg/L of BAP produced 23.33% callusing in rhizome and with 2 mg/L each of NAA and BAP produced 10% callusing in leaf.

The present study confirmed that in *K. galanga*, MS medium with IAA and BAP is best suited for organogenesis, and rhizome explants give a better response than leaf.

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