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Micropropagation of *Kaempferia* spp. (*K. galanga* L. and *K. rotunda* L.)

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Abstract

Protocols were standardised for micropropagation of *Kaempferia galanga* and *K. rotunda*. Young sprouting buds of both the species could be established in Murashige and Skoog basal medium supplemented with 0.5 mgl^{-1} kinetin and 1.5% sucrose solidified with 0.7% agar. The buds produced multiple shoots and well developed roots in Murashige and Skoog medium supplemented with 0.5mgl^{-1} α-naphthaleneacetic acid and 1.0 mgl^{-1} 6-benzylaminopurine. A multiplication ratio of 1:10 and 1:6 with an average of 7 and 5 roots per shoot was obtained in *K. galanga* and *K. rotunda,* respectively. The micropropagated plants were successfully planted out in pots with over 90 per cent survival. The morphological characters and yield of these two species for three crop seasons were also studied.

Key words: *Kaempferia galanga, Kaempferia rotunda,* micropropagation.

Abbreviations

BAP : 6-Benzylaminopurine

Kin : Kinetin

MS : Murashige and Skoog (1962)

NAA : α-Naphthaleneacetic acid

Introduction

Kaempferia (Zingiberaceae) is a genus of rhizomatous herbs distributed in the tropics and sub tropics of Asia and Africa. About 10 species occur in India of which *K. galanga* and *K. rotunda* are important (Gamble 1956; CSIR 1959). *K. galanga* is found throughout the plains of India and is cultivated for its aromatic rhizomes. *K. rotunda* is cultivated for its use in traditional Ayurvedic medicine. Insecticidal principles have also been isolated from both the species.

In Kerala, cultivation of *K. galanga* is restricted to some localised tracts and the productivity of the crop is low ranging from 2 to 5 tonnes of fresh rhizomes per hectare. There is acute shortage of planting material and the absence of seed set limits the scope for generative breeding (Kurien *et al.* 1993). Though micropropagation protocols were reported in *K. galanga* (Vincent *et al.* 1992) there are no reports of the same in *K. rotunda*. This paper reports micropropagation of *K. galanga* and *K. rotunda* for large scale propagation as well as evaluation of performance of tissue cultured plants, in potted conditions, for three crop seasons.

Materials and methods

Explant source and surface sterilization

The rhizomes were collected from field grown plants, washed thoroughly in running tap water to remove soil and other dust particles. The cleaned rhizome pieces were treated with a dilute detergent (Teepol) and 0.3% copper oxychloride for 1 h. After thorough washing, young sprouting buds were excised along with a small portion of the rhizome. Surface sterilization was carried out using 0.5% sodium hypochlorite solution followed by 0.1% mercuric chloride for 10 min and 8 min, respectively. The material was rinsed with sterile double distilled water after every treatment to remove all traces of surface sterilant.

Culture media and culture conditions

Surface disinfected explants of 1-2 cm size were inoculated on basal MS medium (Murashige & Skoog 1962) supplemented with 0.5 mgl^{-1} Kin and 1.5% sucrose solidified with 0.7% agar for culture initiation. After initiation, the explants were transferred to multiplication medium. To identify a suitable medium for multiplication and rooting, MS medium supplemented with NAA (0.5 mgl^{-1} and 1.0 mgl^{-1}), IBA (0.5 mgl^{-1} and 1.0 mgl^{-1}), BAP (0.5 mgl^{-1} and 1.0 mgl^{-1}) and Kin (0.5 $mgl^{-1}$and 1.0 mgl^{-1}) were tried singly and in combination (Table 1). The pH of the medium was adjusted to 5.8 and was autoclaved at 15 kg/cm^2 pressure and 120°C for 20 min. All the cultures were incubated at 25°C under 12 h photoperiod of 2500 lux. Observations on initial response of the explant and average number of shoots per explant, roots per shoot and shoot length at the time of transplating were recorded to assess the *in vitro* responses.

Planting out and field establishment

The *in vitro* grown plants were transferred to polybags containing a mixture of sand, garden soil and vermiculite in equal proportions and were kept in humid chamber for 20 days for hardening. The plants were taken out and maintained in the nursery for the first season and then transferred to pots (12 inch diameter) and maintained in the field. Morphological characters like plant length, number of tillers, leaf length and breadth, nature and size of rhizomes and tubers were recorded in the plants grown under potted conditions for three crop seasons and in the third season the characters were compared with conventionally propagated plants (control) to assess the performance of micropropagated plants *ex vitro*.

Results and discussion

Culture initiation

Only 60-70 per cent of the cultures could be established; culture loss was mainly due to bacterial contamination since the explants were taken from rhizomes. A

Table 1. Effect of growth regulators on the *in vitro* responses of *Kaempferia galanga* and *K. rotunda**

Growth regulator	*K . galanga*			*K. rotunda*		
(mgl^{-1})**	Cultures responded (%)	No. of shoots	No. of roots/shoot	Cultures responded (%)	No.of shoots	No. of roots/ shoot
Kin						
0.5	75	1	12	60	1	7
1.0	80	5	4	80	3	5
BAP						
0.5	80	2	6	70	1	8
1.0	90	3	6	75	3	4
NAA						
0.5	90	4	5	90	3	4
1.0	100	6	8	90	4	4
IBA						
0.5	80	3	2	50	1	3
1.0	80	1	3	50	1	4
Kin+BAP						
0.5+0.5	90	5	6	70	5	5
0.5+1.0	90	3	5	85	3	6
1.0+0.5	85	3	4	70	3	4
Kin+NAA						
0.5+0.5	70	4	4	65	4	6
0.5+1.0	75	5	6	60	4	6
1.0+0.5	75	4	4	60	3	4
Kin+IBA						
0.5+0.5	65	3	2	60	1	3
0.5+1.0	70	1	6	50	2	3
1.0+0.5	60	2	4	70	2	4
BAP+IBA						
0.5+0.5	75	3	4	60	4	3
0.5+1.0	60	1	3	65	1	4
1.0+0.5	70	5	4	50	3	4
BAP+NAA						
0.5+0.5	90	8	7	80	4	3
0.5+1.0	100	5	7	90	4	5
1.0+0.5	100	8	10	90	6	8
NAA+IBA						
0.5+0.5	70	4	3	60	1	3
0.5+1.0	75	3	4	60	1	4
1.0+0.5	75	5	4	60	2	3

* Values are averages of 20 cultures
** In MS basal medium

similar difficulty in establishing contamination free cultures from rhizomes was reported in ginger and turmeric (Balachandran *et al.* 1990). Rhizome buds of both the species sprouted within 10 days in MS medium containing 0.5 mgl^{-1} Kin and 1.5% sucrose solidified with 0.7% agar and required number of uniform size explants could be established to carry out the multiplication trials. Shoot growth as well as root initiation were observed in the initiation medium within 2 weeks. The cultures were transferred to multiplication medium with different combinations of growth regulators at this stage (Table 1).

Multiplication

The results obtained from the experiments using 26 combinations of media are given in Table 1. Differences were observed in the percentage of cultures that responded and the rate of multiplication and rooting, between two species. When cytokinins (BAP and Kin) were used separately, Kin at 1 mgl^{-1} responded more favourably in *K. galanga* with regard to multiple shoot production. The medium containing either BAP or Kin at 1 mgl^{-1} was equally good for *K. rotunda*. When both the cytokinins were used in combination, the medium supplemented with 0.5 mgl^{-1} each of Kin and BAP yielded an average of 5 harvestable shoots with good rooting (1:6 and 1:5) in both the species. When cytokinins were used in combination there was slight increase in the production of multiple shoot formation and rooting. Nadgauda *et al.* (1978) reported good growth and plantlet formation in turmeric in media containing combinations of Kin and BAP.

The effect of auxins (NAA and IBA) alone and in combination were studied. IBA alone did not give a favourable response. The root system produced was generally poor as they were very slender and not vigorous. Up to 6 and 4 harvestable shoots with very good root system was produced in *K. galanga* and *K rotunda* respectively, in the medium with NAA at 1.0 mgl^{-1}. When IBA at 0.5 mgl^{-1} and NAA at 1 mgl^{1} were used together, 4-5 shoots and good rooting was observed in *K. galanga*, whereas in all other auxin combinations the root system produced was short and stout without secondary roots. Among the cytokinin-auxin combinations used, NAA at 0.5 mgl^{-1} and BAP at 1 mgl^{1} were optimum for the two species. In this medium, *K. galanga* produced 8-10 harvestable shoots (Fig. 1a) and *K. rotunda* produced 6-7 shoots (Fig. 1b). Good rooting of 1:10 and 1:6 was achieved in *K. galanga* and *K. rotunda* respectively, in the same medium (Table 1). In all the combinations of growth regulators used, except those containing IBA alone, the regenerated plants produced good root system. Multiple shoot formation and prolific rooting was achieved in a single medium, so that an additional step was not required for rooting of shoots. Though only slight species specific differences were obtained in the rate of multiplication, the explants of *K. galanga* responded faster and showed comparatively higher rate of multiplication.

Within 8 weeks, all the shoots that developed grew vigorously and regular sub-culturing was essential to promote further growth and development. For continuous multiplication, the plantlets were transferred to fresh medium and within 8 weeks, 18 and 6 shoots were produced through axillary bud proliferation in *K. galanga* and *K. rotunda*, respectively. Shoot multiplication occurred by axillary bud proliferation in

Table 2. Morphological characters and yield of micropropagated plants of *Kaempferia galanga* and *K. rotunda* in the field*

Species	Season/Age	Plant length (cm)	Number of tillers	Leaf length (cm)	Leaf breadth (cm)	Rooting and rhizome/tuber formation	No.of tubers	Weight of tuber and rhizome (g)
K. galanga	First season**							
	At the time of transfer	12.0	1	3.0	1.5	*In vitro* formed roots	Nil	Nil
	2 months	12.0	1	5.0	1.5	New roots	Nil	Nil
	4 months	12.0	1	5.0	3.0	New roots, tubers	5	3
	At maturity	14.0	4	8.0	4.3	Very small rhizomes, root tubers	12	15
	Second season	14.0	12	12.5	9.0	Root tubers, bigger rhizomes	22	75
	Third season	15.0	15	13.5	14.0	Root tubers, normal sized rhizomes	32	350
Control ***		15.0	14	14.0	14.5	Root tubers, rhizomes	30	348
K. rotunda	First season **							
	At the time of transfer	13.0	1	7.0	2.0	*In vitro* formed roots	Nil	Nil
	2 months	13.0	1	7.0	2.0	New roots	Nil	Nil
	4 months	15.3	1	9.4	3.5	Root tubers	8	12
	At maturity	30.0	3	20.0	8.5	Root tubers, very small rhizomes	45	102
	Second season	42.0	7	22.0	10.0	Root tubers, bigger rhizomes	135	400
	Third season	58.0	9	30.0	12.0	Root tubers, normal sized rhizomes	155	450
Control ***		60.0	11	35.0	13.0	Root tubers, rhizomes	163	446

* Average of 20 plants
** In the first season the plants were grown in polybags
*** Conventionally propagated plants

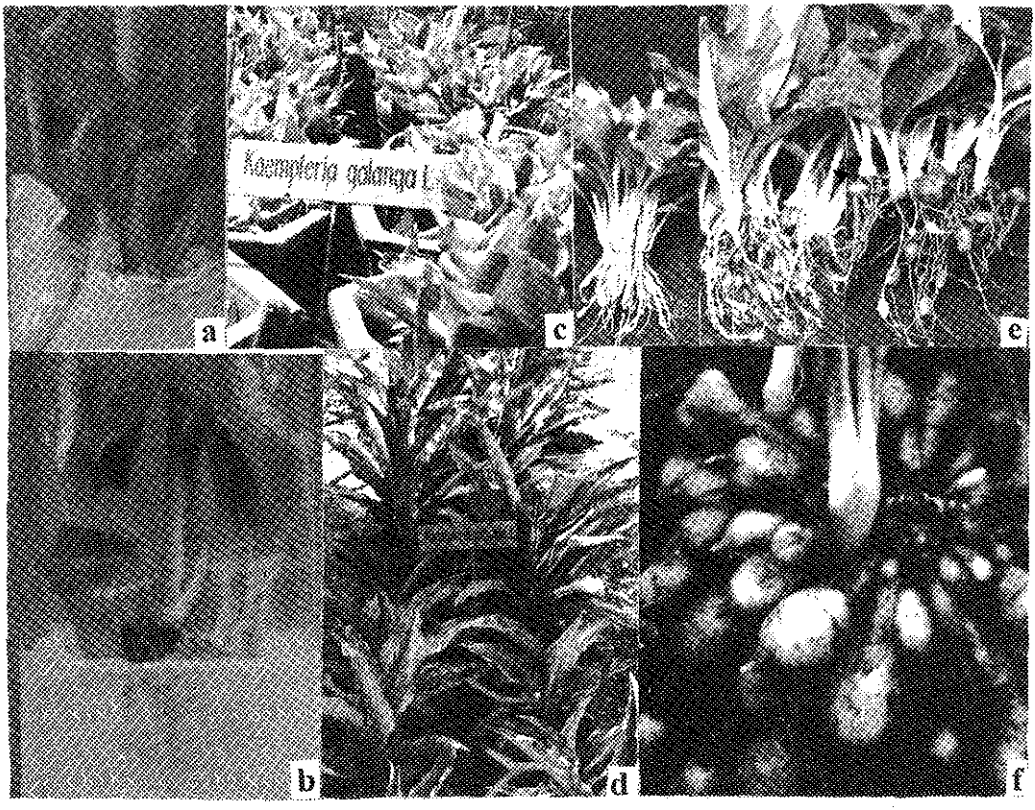

Fig. 1. Micropropagation of *Kaempferia* spp.
a. Multiple shoots in *K. galanga* b. Multiple shoots in *K. rotunda* c. *K. galanga* plants in pots d. *K. rotunda* tissue cultured plants in pots e. Rhizome production in *K. galanga* during 1st, 2nd and 3rd seasons f. Rhizome production in *K. rotunda* during 3rd season.

both the species which has been reported to maintain genetic stability in tissue cultures than adventitious bud formation (Balachandran *et al.* 1990)

Planting out and field establishment

Plantlets with 3-5 leaves and with 6-10 roots per shoot could be harvested and transferred to soil within 12-14 weeks. The presence of well developed root system made hardening and establishment in the soil easy with 90-95 per cent survival (Fig. 1 c & d). The plantlets produced new leaves and roots within 20-30 days. *K. rotunda* plantlets lacked the pink colouration of the plant in *in vitro*, but after planting out and establishment the plants gradually developed the colouration.

During the first season of planting, *K. galanga* and *K. rotunda* produced 4 and 3 tillers respectively, in polybags. Plant length, leaf length and leaf breadth slightly increased at maturity in the first season in both the species. In *K. galanga*, the leaves were narrow compared to conventionally propagated plants up to two seasons. In the second season, an average of 12 and 7 tillers were produced in *K. galanga* and *K. rotunda*, respectively. The number of tillers increased up to 15 and 9 in the

third season (Table 2). During the first season only minute rhizomes and root tubers were produced. The production of rhizomes increased over years and only after the third season rhizome production was similar to that of control. An average of 350 g and 450 g rhizomes and tubers per pot were harvested from tissue cultured plants of *K. galanga* and *K rotunda* respectively, after the third season (Fig. 1 e & f). Hence, tissue cultured plants cannot be used directly for commercial planting but can be used after three seasons in the nursery for the production of sufficient amount of rhizomes to be used as planting material. However, this method can be used for large scale production of planting material within a short period of time.

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