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In vitro Multiplication of *Kaempferia Galanga* L.- An Important Medicinal Plant

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ABSTRACT

An efficient *in vitro* propagation protocol was standardized *in K. galanga*, wherein shoot cultures were raised from rhizome with axillary bud explants in Murashige and Skoog (MS) medium supplemented with different hormonal regimes. The best medium identified for shoot initiation as well as multiplication was MS supplemented with 2 mg/l BAP and 1 mg/l Kin. The high rates of rooting in Half MS media supplemented with 1 mg l^{-1} IBA and successful transfer to polybags make this production system useful for ex-situ conservation and large scale multiplication of kacholam.

Key Words: *Kaempferia*, Kacholam, *in- vitro*, micropropagation, medicinal plant.

INTRODUCTION

Kaempferia galanga L. is an herbaceous medicinal species with pale-green, fragrant rhizomes. It is regarded as a cash plant as its rhizomes are used for the essential oil extraction as well as for direct uses in the preparation of ayurvedic drugs, perfumery and cosmetics, spices. The plant is wild in nature and exhibits poor natural regeneration by rhizomes thus reaching the threshold of being recognized as an endangered species. Large scale deforestation and over exploitation further reduces the plant population. *K. galanga* is rarely cultivated at some places for medicinal purposes. Though it is currently recognized as an ideal medicinal plant for intercropping in the coconut gardens of Kerala but conventional propagation of *Kaempferia* by the splitting of rhizome is not sufficiently rapid to meet the need of planting materials, and it takes years to build up for the commercial quantities. In the recent years micropropagation techniques are being profitably used to overcome the present demand of medicinal plants. Considering the present demand (both for economic and medicinal values) and propagation problem of the plant, development of suitable protocols for rapid multiplication of existing elite cultivars has become crucial for meeting the market demand and to replenish highly impoverished populations. *In vitro* regeneration of kacholam grown under Indian conditions is limited and hence an attempt was taken up to standardize the *in vitro* propagation techniques for rapid multiplication of elite kacholam genotypes.

MATERIALS AND METHODS

Plant material

Plant materials of *Kaempferia galanga* were collected from the Jawaharlal Nehru Tropical Botanic & Research Institute (JNTBGRI), Palode, Thiruvananthapuram; National Bureau of Plant Genetic Resources (NBPGR), Thrissur, Kerala Agricultural University (KAU); and Farmer's fields. The Director, Jawaharlal Nehru Tropical Botanic & Research Institute, authenticated the samples. Morphological characterization was done based on standard descriptors for kacholam.

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Table 1. Effect of different concentrations of BAP and Kin on the initiation and shoot proliferation of Kacholam.

(Medium –MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 7.50 g l^{-1})

Hormonal concentrations (mg/l)	No. of explants cultured	Mean number of explants regenerated	Percentage of explants showing proliferation	Days to shoot initiation	Number of shoots per explant		Average length of shoot per culture
					Initiation	Proliferation	
BAP							
0.5	20	13	65	35-37	1	1.20± 0.19	1.7
1.0	20	14	70	32-35	1	3.40± 0.12	2.3
1.5	20	15	75	30-32	1	4.00± 0.35	2.5
2.0	20	18	90	28-30	3	5.20± 0.45	2.7
2.5	20	16	80	30-31	1	3.80± 0.31	2.0
3.0	20	14	70	33-35	1	2.60± 0.35	1.8
BAP + Kin							
2.0 + 1.0	20	19	95	28-30	3	6.40± 0.17	3.4
2.0 + 2.0	20	14	70	30-35	1	3.80± 0.22	2.6

Explant source and Surface sterilization of explants

Healthy rhizome sprouts with active buds were collected from the rhizome of plant material germplasm maintained at Onattukara Regional Agricultural Research Station, Kayamkulam. Rhizome buds of about 1-2 cm length were cleaned and washed in running tap water and then soaked with laboratory detergent (Tween -20) for 5 minutes followed by 3-4 washes with water. They were surface sterilized with Bavistin 0.3% for 10 minutes and then washed with sterile distilled water. Further sterilization procedures were carried out in laminar air flow chamber by using 0.1% $HgCl_2$ for 15 minutes. Finally, the explants were rinsed three times with sterile distilled water to remove traces of mercuric chloride and placed on sterile blotting paper prior to the inoculation.

Culture media and conditions

The basal medium used for all the experiments were Murashige and Skoog (1962) mineral formulation containing standard salts and vitamins, 30 g/l sucrose and 7.5 g/l agar fortified with various concentrations/combinations of growth hormones. Specific quantities of the stock solutions were pipetted out into a 1000 ml beaker. Sucrose and inositol were added fresh. After making up the volume to 1000 ml using distilled water, the pH of the medium was adjusted to 5.7 using 0.1 N NaOH/ HCl. The medium was autoclaved at 15 Ibs/sq. inch for 20 min at 121ºC. Cultures were incubated at a temperature of 20±2°C and 16 h photoperiod. The autoclaved media were kept for 1-2 days before inoculations to screen for inherent contamination.

***In vitro* propagation and rooting**

Sterilized explants were cut into 2-3 mm sized pieces using sterile scalpel under laminar air flow chamber and inoculated into autoclaved basal MS media with different growth regulators singly or in combinations to standardize bud initiation. For shoot induction and multiplication, the media was supplemented with 0.5 - 3.0 mg/l BAP and 1.0 - 2.0 mg/l Kin either individually or in combination. Basal MS medium devoid of growth regulators were kept as Control. For root induction, *in vitro* raised shoots measuring about 4-5 cm length were excised into single shoots, and cultured on half-strength MS media supplemented with either NAA or IBA

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

Concenration of auxin (mg/l)	Percentage of shoots rooted	Number of roots per rooted shoots	Average length of Roots
NAA			
0.2	75	2.17± 0.02	3.18± 0.14
0.5	80	2.25± 0.07	3.59± 0.05
1.0	90	3.54± 0.06	3.84± 0.07
2.0	70	3.10± 0.04	3.61± 0.04
IBA			
0.2	88	2.63± 0.07	3.22± 0.01
0.5	93	3.38± 0.11	4.23± 0.08
1.0	98	4.71± 0.12	6.21± 0.02
2.0	80	3.88± 0.09	5.90± 0.05

(Medium –Half MS + inositol 100 mg l-1 + sucrose 30.00 g l-1 + agar 7.50 g l-1)

in concentration of 0.2 - 2.0 mg/l. Each treatment had twenty culture tubes and the experiment was repeated thrice. The cultures were maintained by regular subcultures at 4 weeks intervals on fresh medium with the same compositions. Subcultures were carried out when the leaves had elongated and filled up the culture tubes. At each subsequent subculture, the clump of shoots was taken out and the leaves cut to a length of 2-3 cm to facilitate transfer. The individual shoots were separated from each other by gently pulling them apart.

Hardening and field transfer

In vitro grown plants having well developed shoots and roots were washed gently under running water to remove all traces of agar from roots and planted in small polycups containing soil, vermicompost and sand mixture in 1:1:1 proportion. The plantlets were kept in shade net house under 50% sunlight for acclimatization to the outside environmental conditions for 30 days with regular watering and transferred to normal field condition for growth until maturity. The percentage of survival was noted.

Statistical analysis

Observations were recorded after a month and analysed statistically (Panse and Sukhatme, 1985). Responses of the cultures were observed and recorded over a period of four weeks. The shoot length and number of shoots and roots formed were observed.

RESULTS AND DISCUSSION

Culture establishment

Establishment of aseptic cultures was found to be very difficult in target species of kacholam, because the explants were taken from underground rhizomes. Nearly 56% of the cultures were found to be contaminated and culture establishment was only 44% in Kacholam by adopting the three level surface sterilization techniques. Multilevel decontamination procedures were found beneficial to improve disinfection of explants of underground origin (Anish *et al*., 2008). Difficulty in eliminating infection from Zingiberaceous explants of underground origin were reported from *C. longa* (Naz *et al*., 2009). For other studies that evaluated asepsis in Zingiberaceae buds,

stronger substances were required, such as ethanol immersion (70%) for 2 minutes and two drops of mercury chloride solution at 0.1 (%) in turmeric (Islam *et al.*,, 2004), and mercury chloride solution at 0.1 (%) in wild turmeric (Nayak, 2000). Clorox at 60% was used for 30 min on bitter ginger, then all bud external tissues were removed and the explants were again subjected to asepsis with 20% of Clorox (Faridah *et al*., 2011).

Culture initiation and multiplication

The response of kacholam rhizome sprouting bud explants cultured on different shoot initiation media over a period of four weeks is presented in Table 1. The explant responded after 15 days of culture by breaking the outer thick sheath followed by emergence of shoot primordium. The explants produced 1-3 shoots by four weeks. All the shoots developed were healthy with well developed leaves.

Sprouted rhizome buds inoculated to MS agar medium with different phytohormone combinations of BAP (0.5 - 3.0 mg/l) and Kin (1.0 - 2.0 mg/l) showed initiation in all hormone combinations in media. The best medium identified for initiation of shoot buds was MS supplemented with 2 mg/l BA and 1 mg/l Kin. In this treatment, 90% of the cultured explants showed initiation. Culture medium devoid of growth regulators (Control) failed to stimulate the bud break response in the explants even when the cultures were maintained beyond the normal observation period of four weeks. In general, herbaceous plants are highly responsive to BAP treatments and most cultured herbaceous species produced robust, well formed shoots suitable for further multiplication (Debergh and Zimmerman, 1991). Pareeda et al (2010) reported that highest rate of shoot multiplication (11.5 ± 0.6) shoot/explant as well as leaf biomass production (7.4 ± 0.3) gram/explant in *K. galanga* was observed on Murashige and Skoog medium supplemented with Benzyladenine (1 mg/l) and IAA (0.5 mg/l). Best response (96%) of rhizome explants for shoot proliferation in *K. galanga* was obtained in a concentration of 1.0mg/l BA +0.1mg/l IAA supplemented MS medium (Bindu, 2015)

From the first subculture onwards multiple shoot formation was evident in all the cultures. The best medium identified for shoot multiplication was also MS supplemented with 2 mg/l BA and 1 mg/l Kin and 95% multiple shooting was obtained in this treatment (Plate 1). The entire procedure could be completed without callus formation, an advantage, since callus may cause genetic variability. The high rates of rooting and successful transfer to polybags make this production system useful for ex-situ conservation and large scale multiplication of kacholam.

Rooting of the proliferated shoots

Root development was induced in the *in vitro* proliferated shoots by culturing them on half strength MS medium with 0.2 - 2.0 mg/l either of NAA or IBA (Table 2) along with the control (Half MS). Among these two types of auxins used in the present experiment, IBA was found to be the most effective at different concentrations tested for producing roots in micro shoots. Among the different concentrations of IBA, 1 mg/l IBA was found to be the best concentration of auxin for proper rooting of kacholam in which 98 % shoots rooted within three - four weeks after placing on the rooting medium. IBA is a notable auxin that can stimulate rhizogenesis of diverse plant species (Song *et al*, 2020). The optimal response was observed on Half MS + IBA (1 mg l^{-1}) in terms of earlier root initiation, better rooting percentage (98%), average number of roots (4.71) with mean root length of 6.21 cm. Senarath *et al.* (2017) identified that MS medium supplement with 1.0mg/L IAA and 0.2mg/L Indol-3-butric acid (IBA) as the best medium for root induction in *K. galanga*. Delayed rooting response, even after 4 weeks in root induction medium with lower concentration of IBA was observed. Shoot exposed to higher concentrations of IBA (2.00 mg l^{-1}) even though produced thick, short roots, the shoot became necrotic, lost its leaves and

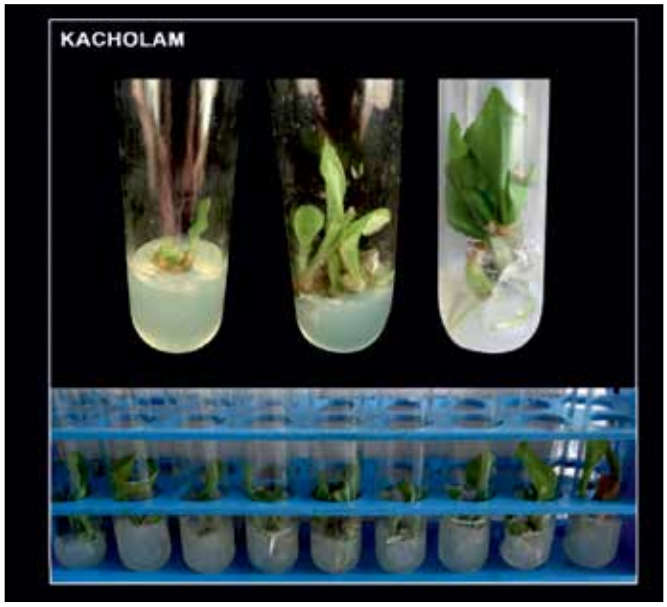

Plate 1. Standardization of micropropagation Shoot multiplication in *K. galanga*

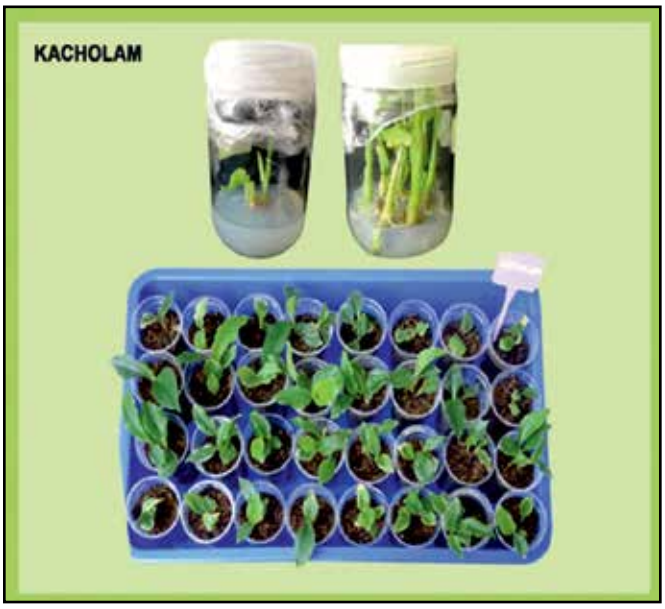

Plate 2. Hardening of *K. galanga* plants in polycups

the shoot tips died gradually. With regard to number of roots in control (MS medium without any plant growth regulator), no root formation was observed. The findings were in agreement with those observed in similar rhizomatous plant species such as ginger and turmeric.

Hardening and Field transfer

The *in vitro* propagated plantlets with well developed root and shoot system were successfully transferred to small polycups containing soil, vermicompost and sand mixture in the 1:1:1 proportion (Plate 2). They were maintained in shade net for 1 month and they were transferred to normal field condition. About 96% of plants survived and grew to maturity in field condition.

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