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Micropropagation of large cardamom (*Amomum subulatum* Roxb.)

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Abstract

A method for micropropagation of large cardamom (*Amomum subulatum*) is reported. Rhizome buds were activated to grow in Murashige and Skoog basal medium supplemented with 3% sucrose and 0.5 mgl^{1} kinetin. The sprouted buds were multiplied at the rate of 5-10 shoots per culture in Murashige and Skoog medium supplemented with 1.0 mgl^{-1} benzylaminopurine and 0.5 mgl^{-1} indole-3-butyric acid. The shoots rooted in the same medium. The *in vitro* regenerated plantlets could be established in the soil with 90 per cent success.

Key words: *Amomum subulatum*, large cardamom, micropropagation.

Abbreviations

BAP : 6-Benzylaminopurine
IBA : Indole-3-butyric acid
Kin : Kinetin
MS : Murashige and Skoog medium
NAA : ∝-Naphthaleneacetic acid

Large cardamom (*Amomum subulatum* Roxb.), a tall perennial rhizomatous zingiberaceous herb is the most important cash crop of Sikkim and also parts of north eastern states and West Bengal in India. The seeds are used as a flavouring agent in many culinary preparations and also find their application in medicine and industry. This paper reports protocols for micropropagation of large cardamom.

Rhizomes were collected from potted plants, after drenching with 0.3% copper oxychloride at 3 day intervals for obtaining contamination free cultures. These rhizomes were washed throughly in running tap water and treated with 0.3% copper oxychloride and Teepol for 1 h. The rhizomes were then washed and young sprouting buds were excised along with a portion of the rhizome and treated with 0.1% mercuric chloride

solution for 5-7 min. The explants were washed throughly with sterile double distilled water and then inoculated on the culture medium.

Surface sterilised rhizome buds were inoculated on to MS basal medium (Murashige & Skoog 1962) supplemented with 3% sucrose and 0.5% mgl^{-1} Kin and gelled with 0.7% bacteriological grade ager. After establishment of cultures and activation of rhizome buds, they were transferred to fresh media. To select the most suitable medium for multiplication, MS basal medium supplemented with various growth regulators like cytokinins (BA and Kin) and auxins (IBA and NAA) at concentrations of 0.5 and 1.0 mgl^{-1} were tried (Table1) in combinations as well as singly. The pH of the medium was adjusted to 5.8 prior to autoclaving at 15 kg cm^{-2} pressure for 20 min. The cultures were incubated at 22±2°C under a 12 h photoperiod of 2500 lux.

The rhizome buds sprouted within 10-15 days in MS medium supplemented with 0.5 mgl^{-1} Kin. Sufficient number of uniform sized explants could be established to carry out the multiplication experiments. The rhizome buds produced shoots and roots simultaneously in all combinations tried (Table 1). The explants responded to MS medium supplemented with auxins and cytokinins. MS medium supplemented with BAP alone (1.0 mgl^{-1}) or in combination with IBA (0.5 mgl^{-1}) gave the maximum number of shoots. However, MS medium with BAP (1.0 mgl^{-1}) and IBA (0.5 mgl^{-1}) was considered the best as it induced the highest number of shoots as well as roots (Fig.1a). In this combination, 8-12 shoots per culture could be produced with an average of 5 roots per shoot, after 90 days of culture.

Table 1. Effect of growth regulators on *in vitro* responses of shoot bud explants of large cardamom

Growth regulator (mgl^{-1})	No. of shoots/ culture	No. of roots/ shoot
BAP		
0.5	6-8	2
1.0	10-12	2
NAA		
0.5	3-5	3
1.0	3-5	4
BAP+IBA		
1.0+0.5	8-12	5
0.5+1.0	6-10	3
0.5+0.5	4-5	3
BAP+NAA		
1.0+0.5	6-8	3
70.5+1.0	4-5	6
0.5+0.5	2-4	3
IBA+NAA		
1.0+0.5	1-3	8
0.5+0.5	2-3	4

Eighty five per cent of the explants showed good shoot elongation and developed into full-fledged plants. Prolific root system was produced in all the treatments. Thus MS medium supplemented with cytokinins and auxins was favourable for micropropagation of large cardamom. Earlier reports on micropropag- ation of zingiberaceous species like cardamom, ginger and turmeric also indicated the same (Nadgauda *et al.* 1978; 1983; Kumar *et al.* 1985; Ilahi & Jabeen 1987; Balachandran *et al.* 1990; Choi & Kim 1991).

The *in vitro* grown rooted plantlets with 3-5 roots per shoot were transferred to polybags containing a mixture of sand, garden soil and vermiculite in equal proportions and were kept in humid chamber for hardening. The plantlets produced new leaves and roots within

Fig. 1. Micropropagation of large cardamom
a. *In vitro* multiplication b. Tissue cultured plants in pots

20 days in humid chamber. Hardening was easy with 90 per cent establishment due to the well developed root system. The plants were maintained in the nursery for 6 months before transferring to pots maintained in the field (Fig. 1b).

The study indicates that large cardamom is amenable to easy micropropagation from rhizome buds. The protocols standardised for micropropagation through direct regeneration, can be used for large scale production of planting material.

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References

Balachandran S M, Bhat S R & Chandel K P S 1990 *In vitro* clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.). Plant Cell Rep. 8 : 521-524.

Choi S K & Kim D C 1991 The study on the clonal multiplication of

ginger through *in vitro* culture of shoot apex. Research Report of the Rural Development Administration Biotechology 33 (1) : 40-45.

Ilahi I & Jabeen M 1987 Micropropagation of *Zingiber officinale* Rosc. Pak. J. Bot. 19 (1) : 61-65.

Kumar K B, Kumar P P, Balachandran S M & Iyer R D 1985 Development of clonal plantlets from immature panicles of cardamom. J. Plantn. Crops 13 : 31-34.

Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.

Nadgauda R S, Mascarenhas A F, Hender R R & Jaganathan V 1978 Rapid multiplication of turmeric (*Curcuma longa* L.) by tissue culture. Indian J. Exp. Biol. 16 : 120-122.

Nadgauda R S, Mascarenhas A F & Madhusoodanan K J 1983 Clonal multiplication of *Elettaria cardamomum* Maton. by tissue culture. J. Plantn. Crops 11 : 60-64.