

Micropropagation of *Costus speciosus* (Koen.) Sm. Using Nodal Segment Culture

Kshetrimayum PUNYARANI, G. Jitendra SHARMA

Manipur University, Department of Life Sciences, Imphal-795003, India; punyaniksb@gmail.com; gjs1951@yahoo.com

Abstract

Nodal segments of *Costus speciosus* (Koen.) Sm. containing single axillary buds were cultured on Murashige and Skoog medium (MS medium) supplemented with plant growth regulators for inducing plantlets. For breaking of axillary bud dormancy, nodal segments were cultured on 40-70g^l⁻¹ sucrose or 1-13 μM adenine sulphate (AdS) supplemented MS basal medium containing 5 μM 6-benzylaminopurine (BAP) and 1 μM α-naphthalene acetic acid (NAA). The nodal segments cultured on 1-13 μM AdS, 5 μM BAP, 1 μM NAA and 50g^l⁻¹ sucrose showed simultaneous production of shoots and roots while those cultured on 5 μM BAP, 1 μM NAA and 40-70g^l⁻¹ sucrose produced shoots only. The most effective media for breaking axillary bud dormancy was 5 μM BAP, 1 μM NAA, 50 g^l⁻¹ sucrose and 10 μM AdS supplemented medium. The propagules from 40-70g^l⁻¹ sucrose produced roots in shoot multiplication medium, i.e., 10 μM AdS, 1 μM NAA, 50g^l⁻¹ sucrose and 3-11 μM BAP supplemented medium. The best response for shoot multiplication was on 10 μM AdS, 1 μM NAA, 50g^l⁻¹ sucrose and 7 μM BAP. The well-rooted shoots were hardened and transferred to the soil where they showed 95% survival rate. Results show that axillary bud can be used for micropropagation of *Costus speciosus*.

Keywords: *costus speciosus*, micropropagation, adenine sulphate, axillary bud

Introduction

About 150 species of *Costus speciosus* (Koen.) Sm. (family: *Costaceae*) have been reported from the tropical regions of the world (Deb, 1983). The decoction of roots of *Costus speciosus* are prescribed in urinary complaints and are stimulant, tonic, and antihelminthic. Rhizome extract, paste and juice are applied to cure white leprosy, juice poured in earache, eaten with sugar to expel intestinal worms, cooked and eaten as laxative (Prakash and Mehrotra, 1996). The root extract acts as an astringent, aphrodisiac, depurative, purgative and is useful in catarrhal fever, cough, skin diseases and snake bites (Khanna *et al.*, 1977; Rathore and Khanna, 1978; Rastogi and Mehrotra, 1991). The rhizomes can be used as an alternative source of diosgenin (Chopra *et al.*, 1956; Dasgupta and Pandey, 1970). The main advantage of *Costus speciosus* over the two species of *Dioscorea* is that *C. speciosus* grows abundantly in the plains, whereas both *D. prazeri* and *D. deltoidea* grow only at high altitudes of the Himalayas (Dasgupta and Pandey, 1970). Eremanthin and costunolide isolated from *Costus speciosus* possessed hypoglycemic and hypolipidemic activities (Eliza *et al.*, 2009 a and b). However, the existence of this species has been threatened due to deforestation, jhum cultivation, habitat disturbance for conversion of wetland ecosystem into agricultural land and uncontrolled plucking/uprooting of these plants. Shoot multiplication in *Costus speciosus* have been achieved using shoot tips (Chaturvedi *et al.*, 1984) and rhizome thin sec-

tions (Malabadi *et al.*, 2005). Micropropagation through rhizomatous eyes/ buds/ shoot tips or rhizome thin sections has disadvantages: the uprooted rhizomes usually fail to survive after rhizomatous eyes/ shoot tips are decapitated, and establishment of *in vitro* culture is usually difficult due to higher contamination. The present investigation, therefore, is an attempt to develop mass propagation protocol using nodal segments of stem. Micropropagation using nodal segments overcomes many disadvantages of rhizomatous eye/bud/shoots tip culture. In nodal segment culture, contamination is less, mature stem which have produced seeds are used and one mature stem usually contain 15-20 axillary buds in comparison to 4-5 rhizomatous eyes in one rhizome.

Materials and methods

Stems of *C. speciosus* (Koen.) Sm. were collected from the Experimental Field of Manipur University. The leaf sheaths covering the stem were removed and then the stems were washed under running tap water for 15 mins. The stems were then cut into segments, each segment containing one axillary bud. The nodal segments were surface decontaminated in 0.1% HgCl₂ (10 mins.) before rinsing with sterilized distilled water. Nodal segments were cultured in 250 ml conical flasks (Borosil), each containing 15 ml of MS medium supplemented with various plant growth regulators (PGRs) and sucrose concentration. The pH of the media was adjusted to 5.8 before being auto-

claved at 121°C for 20 min and 15 lbs sq inch⁻¹ pressure. All cultures were incubated at 25 ± 2°C under 16 h (day/night) photoperiod with light supplied by white fluorescent tubes (3000 lux). Subculturing was carried out after every 4 weeks by trimming-off leaves and roots. For breaking the dormancy of the bud, the nodal segments were cultured on MS medium supplemented with 5 µM BAP, 1 µM NAA, 50 g⁻¹ sucrose and 1-13 µM AdS or 5 µM BAP, 1 µM NAA and 30-70 g⁻¹ sucrose. The cultures were maintained for induction of bud-break. The freshly initiated plantlets/ shoots were subcultured on MS medium (Murashige and Skoog, 1962) supplemented with 10 µM AdS, 1 µM NAA, 50 g⁻¹ sucrose and 3-11 µM BAP for shoot multiplication.

Each treatment had 10 replicates and data were recorded after 4 weeks. For bud break induction, it was repeated three times while for shoot multiplication experiment, it was repeated two times. All the data were scored after four weeks of culture. Data were analyzed for significance using ANOVA and the differences contrasted using Tukey's comparison tests at 5% probability test. All statistical analysis was performed using the SPSS statistical software package.

Results and discussion

In natural habitats, the axillary buds of *Costus speciosus* are dormant. The present investigation has shown that cutting the stem into segments and culturing them on suitable medium supplemented with suitable PGRs can break the dormancy of the bud. Literature surveys have revealed many possible reasons for this. Auxin makes the shoot apex a sink for cytokinin from the roots and decapitation increases the accumulation of cytokinin in axillary

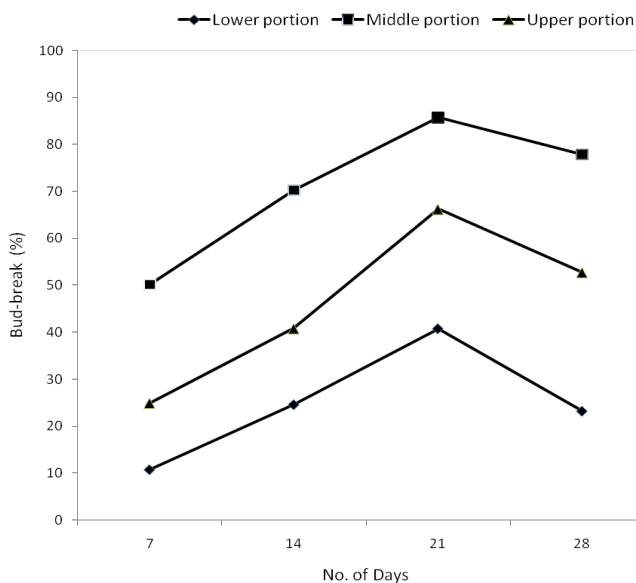


Fig. 1: Effect of the position of the node in the stem on bud-break

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Tab. 1. Effect of MS medium supplemented with different concentration of sucrose on bud-break in the single node segment culture of *Costus speciosus*

Sucrose(g ⁻¹)	Response	Bud-break (%)
30	bud enlarged	51.12
40	bud differentiated to shoots	57.36
50	bud differentiated to shoots	69.05
60	bud differentiated to shoots	61.48
70	bud differentiated to shoots but the leaves turned colorless	53.31

bud (Pilate *et al.*, 1989). Langridge *et al.* (1989) demonstrated with transgenic plant which contained the genes for bacterial luciferase (LUX A and LUX B) under the control of an auxin responsive promoter that auxin content of the axillary bud increases after shoot apex were decapitated. Hence, it was possible to break bud dormancy in cultured nodal segments due to separation from shoot apex. The position of the nodal buds on the stem did affect bud-break (Fig. 1). Nodal segments taken from the upper and middle portion of the stem showed higher bud-break compared to the nodal segments taken from lower portion of the stem. In *Flytrigia repens* (quackgrass), the abscisic acid (ABA) level which is usually high in dormant lateral buds declined to 20% of control level within 24 hrs after the rhizomes were decapitated (Pearce *et al.*, 1995). Hence, the higher bud-break frequency in nodal segments from middle portion of stem may be due to lower level of ABA, in comparison to lower portion of stem; also they are more mature than the upper portion of the stem.

The explants cultured on MS basal medium did not show initiation of bud-break. Success was achieved when the nodal segments were cultured on MS medium supplemented with 1 µM NAA, 5 µM BAP and 30-70 g⁻¹ sucrose. After 3 weeks, the highest percentage of shoot induction was observed in the presence of 50 g⁻¹ sucrose (Tab. 1, Fig. 2 a). However, increased percentage of differentiated buds along with roots was observed in MS medium supplemented with 1 µM NAA, 5 µM BAP, 50 g⁻¹ sucrose and 1-13 µM AdS (Tab. 2, Fig. 2 b). The highest percentage was found in 1 µM NAA, 5 µM BAP, 50 g⁻¹ sucrose and 10 µM AdS supplemented medium (Tab. 2, Fig. 2 b). The length of the plantlets increased as the concentration of AdS increased. However, 13 µM AdS was inhibitory, as decreased in shoot length was observed after achieving maximum length in 1 µM NAA, 5 µM BAP, 50 g⁻¹ sucrose and 10 µM AdS (Tab. 2, Fig. 2b). The average number of roots decreased as the concentration of AdS increased (Tab. 2). The maximum number of roots was on 1 µM NAA, 5 µM BAP, 50 g⁻¹ sucrose and 1 µM AdS supplemented media, but the axillary bud failed to differentiate to shoots. Therefore, 50 g⁻¹ sucrose and 10 µM AdS were considered as optimal for bud-break in *C. speciosus*.

Tab. 2. Effect of MS medium supplemented with different concentration of adenine sulphate (AdS) and 50 gl⁻¹ sucrose on bud-break frequency of single node segment of *Costus speciosus*

Treatment with AdS (μM)	Bud-break (%)	Response	Length of the plantlets (cm) (mean ± SE)	No. of roots (mean ± SE)
1	71.50	bud failed to differentiate but rooting was observed	-	8.70 ^{ab} ± 0.26
4	78.53	bud differentiated to plantlets	2.12 ^a ± 0.16	7.70 ^b ± 0.34
7	83.13	bud differentiated to plantlets	2.62 ^{ab} ± 0.14	6.70 ^b ± 0.37
10	85.73	bud differentiated to plantlets	3.94 ^c ± 0.11	4.40 ^a ± 0.37
13	82.53	bud differentiated to plantlets	3.24 ^b ± 0.06	3.40 ^a ± 0.48

Means followed by same letters are not significantly different at p < 0.05, according to Tukey's comparison test

The possible growth regulatory effect caused by adenine was first noted by Bonner and Huagen-Smith (1939). It could induce bud formation in both tobacco stem segments and elm and tobacco callus in vitro (Bonner *et al.*, 1939; Skoog and Tsui, 1948; Jacquiot, 1951; Miller and Skoog, 1953). In *Nicotina tabacum*, medium containing Kn and AdS showed marked increase in the activities of two enzymes of the oxidative pentose phosphate pathway, compared to their activities in non-shoot forming medium (Scott *et al.*, 1964). Hence, it may be inferred that presence of BAP, NAA and AdS in the medium increases enzymes of the oxidative pentose phosphate pathway which provide activation energy for breaking bud dormancy through sucrose metabolism. After 4 weeks, propagules from 5 μM BAP, 1 μM NAA and 30-70 gl⁻¹ sucrose and from 1μM NAA, 5 μM BAP, 1-13 μM AdS and 50 gl⁻¹ sucrose supplemented MS medium were cultured on MS medium supplemented with 10 μM AdS, 1 μM NAA, 50 gl⁻¹ sucrose and 3-11 μM BAP for shoots multiplication. The average number of multiple shoot increased as the concentration of BAP increased from 3-7 μM. However, further increase was inhibitory (Tab. 3 and 4, Fig. 2 c and d). Maximum average number of shoots, in both cases, was achieved in 7 μM BAP (Tab. 3 and 4). BAP has almost the same effect on shoot multiplication of the propagules

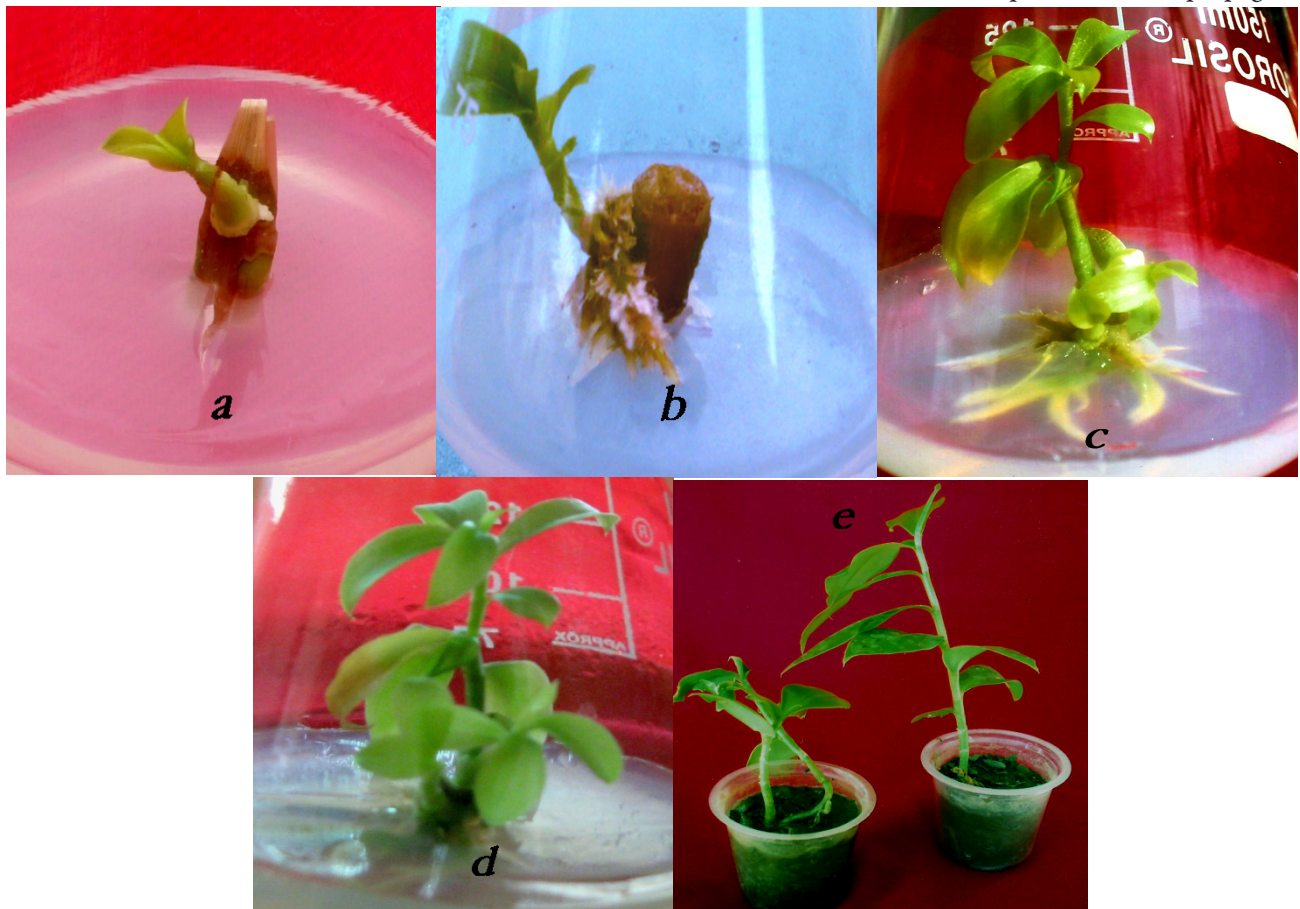


Fig. 2. Micropropagation of *Costus speciosus*; (a) Axillary bud-break in MS + 50gl⁻¹ sucrose; (b) Axillary bud-break with roots in MS + 10μM AdS; (c) Proliferation of propagules from 1-13 μM AdS in MS + 3 μM BAP; (d) Proliferation of propagules from 1-13 μM AdS in MS + 7 μM BAP; (e) Transplanted plantlets established on soil

Tab. 3. Effect of different concentration of BAP on shoot multiplication and rooting of propagules of *Costus speciosus* from 40-60 g^l⁻¹ sucrose enriched medium in MS medium

Treatment with BAP (μM)	No. of shoots (mean ± SE)	No. of roots (mean ± SE)	No. of leaves (mean ± SE)
3	1.20 ^a ± 0.25	3.60 ^b ± 0.27	2.17 ^a ± 0.23
5	1.80 ^a ± 0.26	3.10 ^b ± 0.23	2.96 ^a ± 0.20
7	3.30 ^b ± 0.25	2.20 ^a ± 0.25	3.23 ^a ± 0.24
9	2.20 ^b ± 0.30	1.90 ^a ± 0.23	3.25 ^a ± 0.22
11	1.60 ^a ± 0.34	1.50 ^a ± 0.34	3.16 ^a ± 0.24

Mean followed by the same letters are not significantly different at p < 0.05, according to Tukey's comparison test

derived from 40-60 g^l⁻¹ sucrose and 1-13 μM AdS supplemented medium. Although, there was no significant difference between the average shoot numbers of two types of propagules, the plantlets produced from propagules of AdS supplemented medium were healthier and average number of leaves was also higher (Tab. 3 and 4). This is in agreement with the findings on *Brassica campestris* in which adding adenine sulphate to medium containing Kn and IBA did not increase shoot multiplication, but shoot weight increased, leaves were dark green and the cultures were healthy (Paek *et al.*, 1987).

During bud-break and shoot multiplication experiment, rooting was observed only on medium supplemented with AdS in conjunction with BAP and NAA. The propagules from 40-60g^l⁻¹ sucrose supplemented medium were successfully rooted when transferred on to medium supplemented with 10 μM AdS, 1 μM NAA and 3-11 μM BAP. The maximum average number of roots was found in 3 μM BAP treatment (Tab. 3). In the case of propagules from 1-13 μM AdS supplemented medium, further increase in average number of roots was observed, when transferred on to a medium supplemented with 10 μM AdS, 1 μM NAA, 3-11 μM BAP (Tab. 4). Start and Cumming (1976) have reported positive effect of AdS on rooting in *Saintpaulia ionantha* in the same trend as shown in the current results. The plant survival touches about 95%

Tab. 4. Effect of different concentration of BAP on shoot multiplication and rooting of propagules of *Costus speciosus* from 1-13 μM AdS enriched medium in MS medium supplemented with 50g^l⁻¹ sucrose

Treatment with BAP (μM)	No. of shoots (Mean ± SE)	No. of roots (Mean ± SE)	Rooting rate (Mean ± SE)	No. of leaves (Mean ± SE)
3	0.90 ^a ±0.28	12.80 ^{ab} ± 0.33	3.20 ^{ab} ± 0.09	9.80 ^b ± 0.33
5	2.00 ^b ±0.21	11.50 ^{ab} ± 0.50	2.87 ^{ab} ± 0.13	8.76 ^a ± 0.38
7	3.60 ^{ab} ± 0.27	9.82 ^b ± 0.48	2.45 ^b ± 0.12	8.58 ^a ± 0.34
9	2.70 ^b ± 0.34	8.88 ^b ± 0.54	2.22 ^b ± 0.14	8.06 ^a ± 0.35
11	1.40 ^a ± 0.31	7.30 ^a ± 0.37	1.82 ^a ± 0.10	7.39 ^a ± 0.36

Mean followed by the same letters are not significantly different at p < 0.05, according to Tukey's comparison test

when acclimatization is carried out by transferring the plantlets to potting mixture (Fig. 2 e).

Conclusions

Nodal segment culture provides new technique for micropropagation of *Costus speciosus*. It also overcomes many disadvantages of rhizomatous eye/bud or shoots tip culture, while one mature stem usually contain 15-20 axillary buds in comparison to 4-5 rhizomatous eye in one rhizome. Further induction of multiple shoot from plantlets resulted in regeneration of a large number of plantlets from a single stem. The plant survival touches about 95% when acclimatization is carried out by transferring the plantlets to potting mixture.

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