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In vitro propagation of Monocot (*Costus pictus* D. Don) – An antidiabetic medicinal plant

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The nature of the explant, medium type, plant growth regulators, complex extracts (casein hydrolysate, coconut milk, malt extract and yeast extract) and antioxidants (activated charcoal, ascorbic acid, citric acid and polyvinylpyrrolidone) markedly influenced *in vitro* propagation of *Costus pictus*. A maximum number of shoots percentage termed (50.8) from rhizome explants and multiple shoots (4.7) observed from Murashige and Skoog (MS) medium containing 6-benzyladenine (2.5 mg/1) and KN (1.0 mg/1). High frequency of rooting was obtained in rhizome explant derived shoots (50%) on half strength MS medium supplemented with IAA (1.5 mg/l) propagated plantlets were successfully acclimatized in the greenhouse. All the plantlets established in the field exhibited morphological characters similar to those of the mother plant.

Key words: *Costus pictus*, *Costaceae*, plant growth regulators, rhizome explants

Introduction

Costus pictus (D.Don) belongs to the family *Costaceae* and it is called as Insulin plant in English, Keu – Hindi, Kottam – Tamil, Kemuka - Sanskrit. It is a vulnerable species, slow growing, perennial herb of tropical and subtropical regions. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine (Joshi, 2000). It also used asthma, eye complaints and snake bite and 18 chemical analyzed and identified from leaves of *Costus pictus* (George *et al.*, 2007).

Costus pictus natural strands are fast disappeared and threatened with extinction due to its indiscriminate collection and over exploitation and natural resources for commercial purposes and to meet the requirements of the pharmaceutical industry. Commercial exploitation for production and

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conventional propagation is hampered due to its poor seed viability, low rate of germination and poor rooting ability of vegetative cuttings. Alternative propagation methods would be beneficial in accelerating large scale multiplication, improvement and conservation of the plant. Limited tissue culture work has been done on *Costus* species. However, the multiplication rate achieved by this investigation was very low. The objective of the current study was to develop a system for the mass propagation and aseptic growth of *Costus pictus*. Shoot cultures are well established in a wide range of plant species and can be used for the clonal propagation. In the present study, shoots were regenerated from excised rhizome segments on cytokinins supplemented medium. The literature reveals that there are different regeneration systems for mass propagation of many *Costus* plantlets for different purposes. But this is the first report on *in vitro* culture of *Costus pictus*.

Materials and methods

Plant material

Healthy and young rhizome explants (3-6 months old) of *Costus pictus* (Fig. 1a) were selected and maintained in the Medicinal Plant Botanical Garden, Department of Biotechnology, Bharath College of Science and Management, Thanjavur.

Surface sterilization

Costus pictus rhizome explants were trimmed and washed under running tap water for 5 min followed by a rinse solution of liquid detergent 2 drops Teepol for 1 min, followed by rinse with sterile distilled water. The washed explants were surface sterilized with 70% (V/V) ethanol for 30 sec followed by 5-6 times rinse with double sterile distilled water and sterilized with 3% of sodium hypochlorite (V/V) for 2 min followed by 5-6 rinse with double distilled water and also sterilized with 0.1% of mercuric chloride (W/V) for 2 min followed by 5-6 rinse double sterile distilled water. The final rhizome was (0.5 - 1.0 cm long) and inoculated into MS medium for multiple shoot induction.

Costus pictus rhizome node explants used MS medium (Murashige and Skoog, 1962) fortified with various concentrations of cytokinins (BA and KN: 0.5-2.5 mg/l) either individually or in combination with auxins (IAA and IBA: 0.5-2.5 mg/l) were investigated to optimize salt and hormonal requirements for bud sprouting and multiple shoot induction. The pH of the media was adjusted to 5.7 with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid before autoclaving at 1.06 Kg cm^{-2} and 121°C for15 min. All culture media contained

3% sucrose (w/v) and solidified with 0.8% agar (Bacteriological grade, Hi media, India). Explants were placed vertically in to the culture tubes (150×25 mm), the culture tubes containing 20 ml of culture medium and plugged tightly with non absorbent cotton.

Rooting and transfer of plantlets to soil

The regenerated multiple shoots (4–5 cm long) achieved from rhizome explants were excised and individually transferred to MS medium fortified with various concentrations [0.5-2.5 mg/l (w/v)] of auxins (IAA and IBA) and onto varying strengths of MS medium (full, 3/4, 1/2, ¼ and 1/8) for root induction. The root induction was observed in 50 days, the rooted micro shoots were removed from the culture medium and the roots were washed in sterile distilled water to remove all traces of agar. The micro shoots were placed on filter paper supports in test tubes. The plantlets were then transferred to plastic pots containing garden soil mixed with vermiculite and sand (1:1:1) under control growth chamber conditions (26 ± 2 °C, 16-h photoperiod, 80–85% relative humidity and 50 μ mol m^{-2} s^{-1} light intensity). The potted plants were irrigated with MS basal salts solution (1/8 strength) devoid of sucrose and myo-inositol every 4 days for 3 weeks. After 30 days, the plants were kept under shade for 4 weeks and then the developed plant lets were transfer to field.

Culture conditions and statistical analysis

Cultures were maintained in a culture room at 25±2°C in darkness for one week (for both multiple shoots and rooting cultures) and then under 16-h photoperiod provided by cool white fluorescent tubes (60 μ mol m^{-2} s^{-1}) with 55–60% relative humidity. Explants were subcultured on every 4 week. Data were scored after 30 and 50 days for multiple shoot induction and rooting respectively. Only data which showed some advantageous effect were included in the tables and presented in mean ± SE of 20 explants per treatment and repeated three times. Mean values with the same superscript were not significantly different (p=0.05%) according to Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1976).

Results and discussion

Influence of medium

Costus pictus rhizome multiple shoot proliferation failed in MS medium fortified without growth regulators. The MS medium was found to be the best

basal medium for shoot sprouting (50%), number (2.9±0.16) and length (4.8±0.17cm) with little callus formation. The shoot buds sprouted on MS medium with auxins showed only limited development even if they were maintained for longer period in culture (data not shown). Axillary bud sprouting was initiated at all concentrations of BA and KN (alone and in combinations). The shoots developed longer rhizome on KN fortified medium than on BA supplemented medium (Table 1). In the present study, combined BA (2.5 mg/l) and KN (1.0 mg/l) in the culture medium promoted the shoot sprouting frequency and multiple shoot induction (Table 2, Fig. 1 b, c and d). KN at high concentration (1.0 mg/l), resulted in suppression of shoot sprouting and domination of callus growth. The shoot number increased when auxins (IAA and IBA at 0.5 mg/l) combined with optimized BA and KN, where as auxin combined with either BA or KN alone resulted in low propagation rate (Table 3). Though both IAA and IBA increased the shoot number, IAA showed better overall growth response. High concentrations of IAA (above 2.5 mg/l), and IBA induced callus, occasionally root formation occurred at excised ends and prevented multiple shoot induction. The shoots formed exhibited phenolic exudation and leaf drop, which inhibited the conversions of multiple buds into shoots. The degree of growth and differentiation varied considerably with the medium constitution (Shekhawat *et al*., 1993; Das *et al*., 1996). The need of MS salts for shoot sprouting and proliferation shows the high salt requirement for the growth of *Costus pictus*. Axillary bud sprouting was initiated at all concentrations of BA and KN (alone and in combinations). MS medium containing BA was more effective than KN for inducing proliferation of axillary buds (Reddy *et al*., 1998). Superiority of BA and KN in combination has been found for micropropagation of other woody perennials (Das *et al*., 1996; Komalavalli and Rao, 1997). Reddy *et al.,* (1998) reported that KN did not improve significantly the shoot length and the number of proliferating shoots. High concentrations of IAA (above 2.5 mg/l), and IBA induced callus, occasionally root formation occurred at excised ends and prevented multiple shoot induction. Similar response was also observed in the propagation of *Asclepias* (Chi and John, 1985), *Hemidesmus* (Patnaik and Debata, 1996) and *Costus pictus* (Arun *et al.*, 2007) species.

Excised rhizome derived *in vitro* shoots (3–4 cm long) were rooted only upon transfer to full strength MS medium containing auxins, whereas low frequency of rooting was noted in hormone free 1/2 MS medium. MS medium supplemented with auxins at different concentrations showed varied effect on rooting. Of the three auxins tested, IAA (1.5 mg/l) was most effective for root induction (Table 4, Fig. 1 e and f) and survival in the field with minimum callus formation. Rooting was not observed from the cut ends of the micro shoots

within 10 days. However, a single root emerged after 20 days in the presence of IAA that continued its linear growth with further production of branches when isolated micro shoots were maintained on media containing IAA (1.5 mg/l). Extensive callusing at the base without root formation and thin, delicate roots with intervention of callus were noticed when the medium was supplemented with IBA respectively (data not shown). No significant difference was observed regarding auxin type and concentration requirement between C. *species* and *C. pictus*. The shoots were cultured on various strengths of MS basal medium fortified with 1.5 mg/l IAA to improve the overall growth of roots and to reduce the time duration of root induction. There was a considerable improvement in rooting as about 50% shoots could be induced to root on 1/2 MS medium within 50 days with a fairly good length and number of roots per shoot. Thus improvement in overall quality of roots was observed at 1/2 MS strength medium. In contrast, a drastic inhibitory effect on both root formation and elongation was noted at 1/4 MS strength medium. About 100 rooted plantlets (5-6 cm height) with 6-10 fully expanded leaves and well developed roots were successfully transferred to soil (Figure g and h). Normal growth of the potted plants was shown visible 10–15 days after transfer to field conditions. The transplantation success was about 80% - 85%. All the potted plants placed outdoors under full sun survived (Fig. 1 i and j). The regenerated plants of both the species did not show detectable variation in morphological or growth characteristics studied (Fig. 1 k) when compared with the donor plants (Fig. l). The highest root induction was observed on half-strength MS basal medium supplemented with auxins. The root lengths were varied in all MS basal strength with IAA or IBA concentrations. Similar results were observed in *Madhuca longifolia* (Rout and Das, 1993), *Gymnema sylvestre* (Komalavali and Rao, 2000) and *Eclipta alba* (Baskaran and Jeyabalan, 2005).

In conclusion, the outlined procedure offers a potential system for improvement, conservation and mass propagation of *C. pictus* from pre-existing meristems of explants. MS medium containing 2.5 mg/l BA + 1.0 mg/l KN is the best for shoot proliferation. The use of rhizome for micropropagation is beneficial than other explants. MS basal medium supplemented with 1.5 mg/ l IAA is the best for root induction.

Table 1. Effect of multiple shoot induction on MS medium supplemented with cytokinin on *Costus pictus.*

Plant growth regulators (mg/l)	Percentage of multiple shoot (%)	Multiple shoot number Mean ± S.E	Multiple shoot length (cm) Mean ± S.E
BA - 0.5	32.5^{de}	5.8 ± 0.59^{de}	2.1 ± 0.29^{e}
1.0	34.6^{d}	6.1 ± 0.28^{d}	2.4 ± 0.30^{d}
1.5	39.2^{c}	6.9 ± 0.36^{c}	2.8 ± 0.41^{b}
2.0	48.9^{a}	8.4 ± 0.34^{a}	3.4 ± 0.22^{a}
2.5	44.6^{b}	7.5 ± 0.52^{b}	2.7 ± 0.23^{bc}
KN – 0.5	45.3^{e}	4.2 ± 0.21^{dc}	3.6 ± 0.36^{b}
1.0	56.0^{a}	5.5 ± 0.33^{a}	4.8 ± 0.34^{a}
1.5	51.6^{b}	5.1 ± 0.29^{b}	3.3 ± 0.12^{bc}
2.0	48.4^{bc}	4.7 ± 0.38^{c}	2.1 ± 0.32^{de}
2.5	47.9^{d}	4.3 ± 0.40^{d}	2.6 ± 0.18^{d}

Values are mean of 30 replicates per treatment and repeated thrice. Values with the same superscript are not significantly different at 5% probability level according to DMRT.

Table 2. Effect of multiple shoot and shoot length induction on supplemented with cytokinins combinations on *Costus pictus.*

Plant growth regulators (mg/l)	Percentage of multiple shoot (%)	Multiple shoot number Mean ± S.E	Multiple shoot length (cm) Mean ± S.E
BA + KN			
2.0 + 0.5	18.0^{e}	2.5 ± 0.14^{e}	2.0 ± 0.18^{e}
1.0	20.5^{cd}	3.7 ± 0.18^{cd}	2.9 ± 0.22^{b}
1.5	22.4^{c}	4.2 ± 0.21^{ab}	3.6 ± 0.26^{b}
2.0	35.7^{a}	4.4 ± 0.19^{a}	4.1 ± 0.21^{a}
2.5	32.9^{ab}	3.8 ± 0.20^{c}	2.8 ± 0.10^{cd}
BA + KN			
2.5 + 0.5	28.5^{e}	1.6 ± 0.18^{de}	1.0 ± 0.12^{de}
1.0	34.6^{bc}	1.9 ± 0.24^{d}	1.3 ± 0.28^{d}
1.5	35.8^{b}	2.4 ± 0.20^{c}	1.5 ± 0.20^{bc}
2.0	39.7^{a}	3.6 ± 0.12^{a}	2.6 ± 0.22^{a}
2.5	33.4^{d}	3.4 ± 0.19^{ab}	1.8 ± 0.12^{b}
KN + BA			
2.5 + 0.5	43.4^{de}	2.9 ± 0.19^{de}	3.5 ± 0.12^{e}
1.0	48.8^{c}	3.5 ± 0.28^{b}	4.0 ± 0.14^{c}
1.5	50.8^{a}	4.7 ± 0.24^{a}	4.5 ± 0.16^{b}
2.0	49.7^{ab}	3.2 ± 0.14^{bc}	3.9 ± 0.18^{cd}
2.5	43.7^{d}	2.9 ± 0.16^{d}	4.8 ± 0.17^{a}

Values are mean of 30 replicates per treatment and repeated thrice. Values with the samesuperscript are not significantly different at 5% probability level according to DMRT

Table 3. Effect of multiple shoot and shoot length induction on MS medium supplemented with IAA combination with cytokinins on *Costus pictus.*

Plant growth regulators (mg/l)	Percentage of multiple shoot (%)	Multiple shoot number Mean ± S.E	Multiple shoot length (cm) Mean ± S.E
IAA + BA			
0.5 + 0.5	28.5^{ab}	2.5 ± 0.18^{de}	2.6 ± 0.10^{e}
1.0	30.0^{a}	3.5 ± 0.23^{a}	3.9 ± 0.15^{a}
1.5	26.5^{c}	3.1 ± 0.14^{b}	3.6 ± 0.20^{b}
2.0	22.5^{d}	2.8 ± 0.25^{c}	3.3 ± 0.18^{c}
2.5	19.0^{e}	2.6 ± 0.15^{d}	3.2 ± 0.19^{cd}
IAA + KN			
0.5 + 0.5	14.0^{e}	1.0 ± 0.21^{e}	1.0 ± 0.10^{e}
1.0	18.5^{cd}	1.3 ± 0.14^{cd}	1.6 ± 0.18^{cd}
1.5	20.6^{c}	1.6 ± 0.26^{c}	1.9 ± 0.26^{c}
2.0	23.6^{a}	2.1 ± 0.14^{a}	2.5 ± 0.15^{a}
2.5	21.5^{ab}	1.8 ± 0.15^{b}	2.2 ± 0.13^{b}

Values are mean of 30 replicates per treatment and repeated thrice. Values with the same superscript are not significantly different at 5% probability level according to DMRT.

Table 4. Effect of root induction on MS medium supplemented with IAA and IBA in *Costus pictus.*

Plant growth regulators (mg/l)	Percentage of rooting (%)	Number of roots	Root length (cm)	Root weight (mg)
MS + IAA				
0.5	42.5^{d}	6.1^{d}	4.1 ± 0.16^{c}	8.2 ± 0.24^{b}
1.0	46.2^{b}	7.2^{bc}	5.1 ± 0.17^{ab}	8.0 ± 0.14^{bc}
1.5	50.8^{a}	8.1^{a}	6.1 ± 0.18^{a}	9.1 ± 0.14^{a}
2.0	44.6^{bc}	7.5^{b}	4.1 ± 0.14^{ed}	6.1 ± 0.23^{e}
2.5	37.8^{e}	6.0^{de}	3.1 ± 0.14^{e}	7.4 ± 0.16^{d}
MS + IBA				
0.5	17.2^{ab}	2.4^{cd}	1.1 ± 0.13^{d}	1.2 ± 0.15^{de}
1.0	18.4^{a}	4.7^{ab}	3.1 ± 0.10^{a}	2.8 ± 0.24^{a}
1.5	16.6^{c}	5.2^{a}	2.5 ± 0.20^{b}	2.2 ± 0.16^{b}
2.0	15.8^{cd}	2.8^{c}	2.2 ± 0.16^{bc}	2.1 ± 0.14^{bc}
2.5	13.4^{e}	2.3^{e}	1.1 ± 0.15^{de}	1.4 ± 0.15^{d}

Values are mean of 30 replicates per treatment and repeated thrice. Values with the same superscript are not significantly different at 5% probability level according to DMRT.

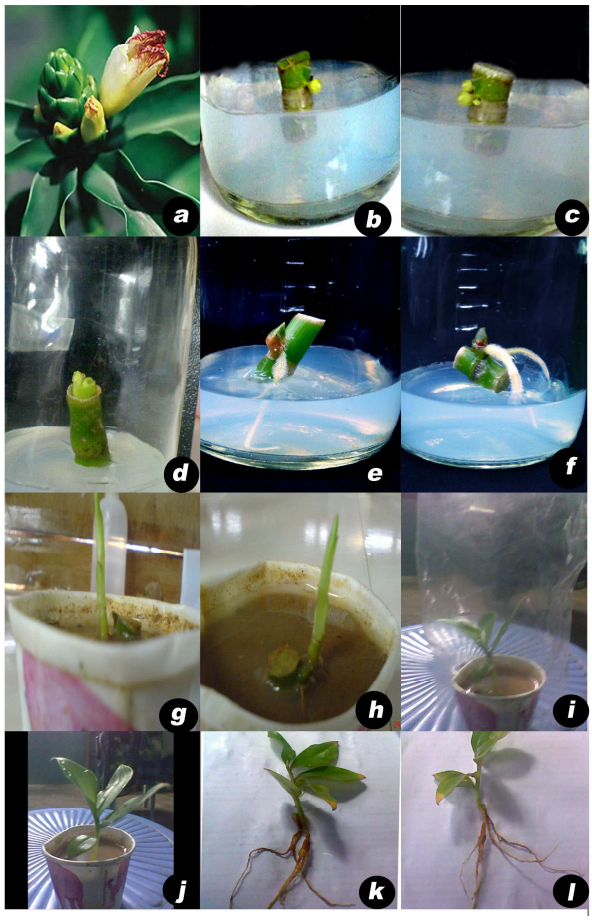

Fig. 1. *In vitro* studies on *Costus pictus* (D. Don)
a. habit; b. multiple shoot initiation; c. multiple shoot maturation; d. shoot elongation; e. root initiation; f. root maturation; g. and h. maturation plant maintained; i. and j. hardening; k. and l. before field transfer plant.

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