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IN VITRO PROPAGATION OF COSTUS PICTUS (D. DON.)

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ABSTRACT

Costus pictus a valuable medicinally important 'insulin plant' cultivated in garden as an ornamental belongs to the family Costaceae. During present investigation Micropropagation studies in Costus have been tried using different growth hormones viz. BAP, KIN, IBA, IAA, and NAA. Nodal part of stem was tried and proven suitable for Micropropagation as compare to rhizome, rhizomatic eye, and apical shoot. Suitable combinations of growth regulators recorded for Micropropagation were 2.7 mg/L BAP, 0.2 mg/L IAA from the nodal region. Hence present findings provide the standard *in vitro* protocol for *Costus pictus*.

KEY WORDS: ***Costus pictus,*** **in vitro propagation, nodal explant.**

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INTRODUCTION

The Costus is genus of perennial tropical herbaceous flowering plants belongs to the family Costaceae. It is often characterized and distinguished from relatives such as Zingiber (true ginger) by their spiraling stems. The whole genus is thus often called spiral gingers. It is widely cultivated in south India and also grows wild in many places. It is a recently introduce by America as an herbal cure for diabetes; hence it is commonly known as 'insulin plant.' *Costus pictus* is also referred to as *Costus congestus* and *Costus mexicanus*. *Costus pictus* is a vigorous growing in a shady place. It can be identified by particular characteristic broad, dark green leaves and beautiful solid red stems. It grows with an outstanding spiral effect along with the green inflorescences, with their yellow-with-red-striped flowers. The flowers are produced at the ends of the leafy stalks in summer. Less frequently, they bloom from basally in the spring. *C. pictus* can be growing to about 6 feet in medium sun to part shade; it requires rich, moist soil. In south India it is grown in garden as an ornamental plant especially in Kerela. The major attraction of this plant is its stem with spiral leaves and light airy and tissue paper like flower. Red painted stem enhance the beauty of the glossy leaves and strongly spiralling canes. C. *pictus* is also well known for its medicinal value mainly antiseptic, tonic, aphrodisiac, carminative, stomachic, vermifuge (Beena and Reddy,2010). It is able to prevent the hair turning grey and its root is anodyne, antibacterial properties (Devi VD, Urooj A, 2008). It is widely used as a remedy for diabetes. Powdered leaves of C. *pictus* known to possess therapeutic effect, when supplemented to streptozotocin induced diabetic rats, is found to reduce blood glucose level by 21% after 15 days of supplementation (Jajasri et al, 2008). The Methanolic leaf extract of C. *pictus* is used to lower blood glucose level in alloxan induced diabetic rats (Jothivel et al, 2007). Therefore this important medicinal as well as an ornamental garden plant needs to produce large scale through tissue culture. It is problematic to use of rhizomatous eyes, shoot tips and thin sections rhizome due to higher contamination. Therefore the present research work initiates to produce standard *in vitro* propagation protocol using nodal segments of stem. Use of the nodal segments of stem for Micropropagation overcome problem of the higher microbial contamination.

MATERIALS AND METHODS

Source of Explants

The explants were collected from the Costus plant planted in green house, Botanical garden, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Various explants taken were rhizome, rhizome eye, apical shoot and nodal part of stem. These explants were surface sterilized carefully in running tap water for 10 minute followed by distilled water for 5 minutes. Apart from this for surface sterilization, agents such as 70% ethanol, $Hgcl_2$ (0.3 %) were used. The duration for surface sterilization was for 5 minute by 0.3% mercuric chloride followed by three subsequent rinses with sterilized distilled water. All these explants were dissected into small pieces and inoculated in medium so that maximum part can be exposed to media.

Culture medium and conditions

MS (Murashige and Skoog, 1962) medium along with various combinations of growth regulators was used. The explants used for multiple shoot formation were Rhizome, rhizome eye, apical shoot and nodal part of stem. The growth hormones used were BA, KIN, as cytokinins and for rooting IAA, IBA, and NAA were tried. Apart from this 3% sucrose, 3 gm/L solidified agent Clerigel was added and the pH was adjusted to 5.8 after adding the growth regulators. The media was sterilized in an autoclave under 15 psi and 121° C for 15 minutes. After inoculation cultures were transferred to culture room under a 16 h photoperiod supplied by cool white fluorescent tubes light and the temperature was maintained 25 ±2 ^{0}C. At least five replicates of cultures were raised to minimize the error.

Data record

Readings were taken after 25days taking at least five replicate for shoot multiplication and values of percentage of multiplication and number of shoots multiplied in each culture were tabulated along with the standard error (S.E.).

RESULTS AND DISCUSSION

For *in vitro* multiplication of rhizomatous eyes, shoot tips and thin sections of rhizome were inoculated on MS medium. Heavier contamination was recorded with rhizomatous eye and their thin sections. It was observed that nodal segment of stem were most suitable for in vitro propagation in *Costus pictus*. Callus was recorded from leaf as an explant (Plate B).

Bud initiation using nodal part

After inoculation results were recorded after t second week and observed that no sprouting was recorded on hormone free MS medium, MS along with 1-4 mg/L BAP in combination with 0-1.6 mg/L IAA taking nodal segment of stem as an explant. Further increase in concentration of BAP and IAA there was subsequent increase in sprouting which has been indicated by (+) sign in observation table. Maximum multiple shoots recorded were upto 80 percent with 3.5 mg/L BAP and 1.4 mg/L IAA in MS. There was a subsequent decrease in sprouting by increasing the concentration of hormones.

Table 1

Effect of BAP on sprouting of bud using nodal segment of stem.

Source of explant	Conc. of growth Regulators (mg/L)		Frequency of Sprouting Buds	% of bud initiation
	BAP	IAA		
Leaf	0.0	0.0	Callus	--
	1.0	0.5	-	00
	2.0	1.0	+	10
Nodal segment	3.0	1.2	+	30
	3.5	1.4	+++	80
	4.0	1.6	++	60

Degree of sprouting Low- + , Medium-++-, High - +++.

A B

C D

Fig. A. Leaf From Callus B. Multiplication from nodal segment

C. Multiple shoots D. Plant hardened in pot.

Table 2
Effect of BAP and KIN on multiple shoot formation using sprouted buds

Source of explant	Conc. of growth Regulators (mg/L)				% of shoot formation	No. of shoots per explant (mean± SE)
	BAP	KIN	IBA	IAA		
Nodal segment of Stem	1.0	0.0	0.0	0.0	10	1.12±0.115
	1.5	-	0.2	-	35	2.64±0.256
	2.0	-	0.2	-	80	8.80±0.300
	2.5	-	0.2	-	65	6.64±0.129
	3.0	-	0.2	-	45	4.10±0.113
	-	1.0	-	0.2	35	4.52±0.278
	-	1.5	-	0.2	20	6.12±0.115
	-	2.0	-	0.2	65	3.64±0.256
	-	2.5	-	0.2	50	2.8±0.300
	-	3.0	-	0.2	25	6.64±0.129

After 30 days mean ± SE of 5 replicate

The sprouted buds were subcultured for multiplication on MS media along with various concentrations of plant growth regulators like BAP 1.0 mg/L, 1.5 mg/L, 2.0 mg/L, 2.5 mg/L, 3.0 mg/L as cytokinins along with 0.2 mg/L IAA, 0.2 mg/L IBA, and 0.2 mg/L NAA as auxins shown in the table 2. The highest numbers of shoots recorded were 8.80 with 2.0 mg/L BAP along with 0.2 mg/L IBA. The average numbers of multiplied shoots were 6.64 taking 3.0 mg/L BAP and 0.2 mg/L IAA (Plate A,C). For separation and growth of multiple shoots, these cultures were transferred in MS medium containing 20 mg/l sucrose, 3 mg/l Clerigel as solidifying agent devoid of growth regulators. For getting more number of multiple shoots concentrations KIN was 1.0, 1.5, 2.0, 2.5, 3.0 mg/L along with IBA 0.2 mg/L IAA and 0.2 mg/L. By taking KIN 1.5 mg/L along with 0.2 mg/L IAA, maximum 6.12 shoots were regenerated. From the recorded observations it could be concluded that the multiple shoot formation with cytokinins like BAP was most effective as compare to KIN. For further multiplication separated detopped shoots were transferred to fresh MS medium containing 20 mg/l sucrose, 3 mg/l Clerigel as solidified agent with 0.5 mg/l NAA, 0.5 mg/l IAA. Root initiation was recorded after 25 days. In vitro grown plants were hardened in poly house successfully in root trainer followed by polythene pots (Plate D). The leaves of *Costus pictus* D. Don are used extensively for its anti-hyperglycemic activity by the people in Kerala, India and abroad. The anti- hyperglycemic and insulin secretary activity of an aqueous extract of *Costus pictus* leaf extract was investigated in streptozotocin induced diabetic rats by Geerish et al (2008). Similar kind of work was also recorded by Archana Roy and Amita Pol (1991), Balachandran et al (1990) in the family zingiberaceae by taking nodal segments as an explant. Earlier multiplication studies in *Elletaria cardamom* was studied in our laboratory Pandhure et al (2007) and shown resemblance with present studies. They also tried to get in vitro rhizomes production for large scale propagation in *Costus spacious*.

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