

# ***In vitro* Propagation of Insulin Plant (*Costus pictus*): a Medicinal boon with an Antidiabetic Activity**

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**ABSTRACT:** The present investigation was undertaken with objective to study the rapid multiplication of Insulin plant within a short span time to produce large number of sprouting buds and disease free plantlets, irrespective of the season through direct organogenesis.

Rapid propagation was done by using nodal segments as explant which were initiated on Murashige and Skoog (1962) medium supplemented with five different concentrations of BAP (0.0-2.5mg/l) in combination with IAA (0.0, 0.5, 1.0, 1.2, 1.4mg/l). After the seven weeks of initiation maximum number of sprouting bud were obtained in medium supplemented with 1.5mg/l BAP + 1.0mg/l IAA. (average number of buds  $3 \pm 0.40$  and survival percentage was 75%). Shootlets were regenerated from nodal explants of *Costus pictus* through axillary shoot proliferation. Best shoot multiplication was observed on medium containing 3.0mg/l BAP + 0.2mg/l IBA average number of shoots  $4 \pm 0.40$  and shoot length about  $5.5 \pm 1.08$  in cm within 35 days. The most efficient rooting response obtained on MS supplemented with 1.5mg/l IBA + 1.0mg/l IAA + 0.5gm/l Activated charcoal with highest number of roots  $5 \pm 1.08$  and average root length  $4.5 \pm 1.10$  cm. The well rooted Insulin plantlets transferred to green house condition for primary hardening with promising survival rate.

**KEYWORDS:** *Costus pictus*, rapid propagation, nodal segment, IBA, BAP

## **I. 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. It is widely cultivated in south India and also grows wild in many places. It is a recently introduced by America as an herbal cure for diabetes; hence it is commonly known as 'Insulin plant.' *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. *C. pictus* is also well known for its medicinal value mainly antiseptic, tonic, aphrodisiac, carminative, stomachic, and vermifuge [1]. 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 [2]. The Methanolic leaf extract of *C. pictus* used to lower blood glucose level in alloxan induced diabetic rats [3].

**Traditional propagation:** *Costus pictus* propagated by different methods such as vegetative methods using tubers, rhizomes, rhizomatic eye and division of clumps stem cutting. But stem cutting sprout in about 120 days required. Propagation by seeds, but the percentage of seed germination is very low [4].

**Need of micropropagation of Insulin plant:** *Costus pictus* natural strands are fast disappeared and threatened with extinction due to its indiscriminate collection and over exploitation, natural resources for commercial purposes and to meet the requirement of the pharmaceutical industry. Commercial exploitation for production and conventional propagation is hampered due to its poor seed viability, low rate of seed germination (about 62% only) and poor rooting

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ability of vegetative cutting. Stem cutting required 120 days for sprouting. The lack of seed setting spoiled the breeding of this plant. Micropropagation method would be beneficial in accelerating large scale multiplication, improvement and conservation of the plants. The objective of current study to develop a large scale production and aseptic growth of *Costus pictus*. Tissue culture of such important *Costus* species is recommended for faster growth and large scale production in minimum time period.

**Insulin plant for diabetes:** The patients who are suffering from acute diabetes are advised to eat these leaves by thoroughly chewing, twice a day that is in the morning and in the evening to control the blood sugar level. After swallowing these leaves little amount of water should be consumed. The leaves of insulin plant contain mostly of Carsolic Acid which is beneficial in controlling diabetic. The carsolic acid present in the green leaves induces the insulin production and thus controls hyperglycemia in the blood.

## II. MATERIAL AND METHODS

**Source of explant and surface sterilization:** The nodal segments as explant of size 1-2 cm were collected from mature, healthy Insulin plant grown in Plant Paradise Nursery, Parandwadi, Tal- Maval, Dist- Pune and brought to the tissue culture laboratory of Department of Plant Biotechnology, College of Agril. Biotech. Loni. These explants were thoroughly washed under running tap water for 20 min. then they were treated with 0.5% Bavistin + 0.5% Redomil for 15 min. followed by treatment with 1% Dettol for 10 min (Twice). In next step nodal segments were washed with detergent Tween 80 (10 drops) for 15 min. Then surface sterilization was done with 0.3% Mercuric chloride for 5 min. followed by treatment with 70% Ethanol for 30 sec. in LAF. Finally three subsequent rinses with sterile distilled water and these sterilized explants were cultured on MS medium with different concentrations of growth regulators.

**Initiation stage and culture conditions:** For establishment of nodal segments in initiation stage were transferred to five different MS basal medium supplemented with BAP (0.0-2.5 mg/l) in combination with IAA (0.0, 0.5, 1.0, 1.2, 1.4 mg/l). Apart from this 3% sucrose, 5 gm/l Agar (Himedia, India) gelling agent were added and pH of medium was adjusted upto 5.7 with 1N HCl/NaOH. The media was sterilized in an autoclave under 15 psi and 121° C for 15 minutes. All the experiments were done using analytical grade chemicals (Himedia, Qualigens, Merkard and Sigma). The explants were implanted vertically on culture medium in sterile culture bottles (Borosil, Mumbai, India). The inoculated culture bottles were incubated in incubation room having sterile controlled environmental conditions such as temperature 25±2°C, relative humidity (RH) 70%, 16 hours photoperiod/day with 2000-3000 lux light intensity under cool white fluorescent tubes (Philips, India). At least five replicates of cultures were raised to minimize the error. Data were recorded after seven weeks of initiation in terms of average number of buds per explants, survival percentage and days required.

**Shoot multiplication:** Shoot multiplication was carried out using the 2-3 cm long and most vigorous bud from the initiation stage. The buds which had grown up to suitable sizes were excised and were cut in to 1.5-2 cm size and cultured on shoot multiplication media and incubated in the culture room. Shoot multiplication media (5 lit. as given in table 2) were divided in to 5 parts. Each part contains 1 liter MS medium. First part was free from growth regulators and remaining four parts contains different concentrations of BAP (1.0-4.0 mg/l) in combination with IBA (0.2 mg/l). Data such as average number of shoots per explants average length of shoots and number of days required were recorded after six weeks of culture.

**Rooting and acclimatization:** In rooting regenerated multiple shoots (4-5 cm long) achieved from nodal segment explants were excised and individually transferred to MS medium fortified with various concentrations of IBA (0.5-2.0 mg/l) and IAA (0.0-1.5 mg/l) with activated charcoal 0.5 gm/l. The root induction was observed in five weeks. For acclimatization rooted micro shoots were washed in sterile distilled water. The rooted plantlets then transferred to plastic pots containing mixture of soil, vermiculate and sand (2:1:1) and healthy roots appeared after two weeks.

**Statistical analysis:** Various observations were recorded in terms of number of shoots, shoot length, root length and number of roots per explant and percentage survival. Means and standard deviation were used throughout the each study. All the studied were made with 8 replicates.

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### III. RESULTS AND DISCUSSION

Data were obtained after 7 weeks of initiation of culture showed that sprouted buds of *Costus pictus* could be established at all tested media including the control medium (free from growth regulators). The best result was obtained on MS medium supplemented with 1.5mg/l BAP + 1.0mg/l IAA. (Table 1). The sprouting of bud number or shoot length (average shoot length 2.5 cm) was observed on medium supplemented with 1.5mg/l BAP + 1.0mg/l IAA showed in (Table 1).

**Table 1 Observation of various combinations of hormone BAP and IAA on sprouting of bud by using nodal segment as explants of *Costus pictus* after 7 weeks.**

Sr. no.	Explant Source	Media code	MS + concentration of growth hormones mg/l						No. of bud Sprouting	% Of bud initiation	Days Required		
			B	A	P	I	A	A					
1	Nodal Segm	I 1	0	.	0	0	.	0	1	2	0	5	0
2		I 2	1	.	0	0	.	5	2	4	0	4	7
3		I 3	1	.	5	1	.	0	3	7	5	3	9
4		I 4	2	.	0	1	.	2	2	6	0	4	5
5		I 5	2	.	5	1	.	4	1	4	0	4	3

The buds sprouting in the initiation were subjected to the multiplication on the different multiplication media. According to Firoozabady and Gutterson, 2003 addition of BAP in MS-medium was essential for the regeneration of plantlets. In present study the average no. of shoots formed on all concentrations of BAP and IBA was ranging from 2 to  $4 \pm 0.40$  shoots per explants. Best growth was observed on the 3mg/l BAP + 0.2 IBA mg/l concentrations where the average no. of shoots generated per plant was  $4 \pm 0.40$ . The results showed that the ratio of no. of shoot formed per explants was increased with the increasing concentration of the BAP and IBA. The BAP promotes cell division and shoot formation. Exceptionally the media with 1.0 mg/l BAP was showed slightly low numbers of shoots per explants.

**Table 2 Observation of various combinations of hormone BAP and IBA on multiple shoot formation by using sprouted bud of nodal segment of *Costus pictus* after 6 weeks.**

Sr. no.	Media code	MS + concentration of growth hormones mg/l			Avg. no. of shoots per explants (mean ± SD)	Average shoot length (cm) (mean ± SD)	Days Required		
		BAP	I	B A					
1	I M 1	1.0	0	.	0	$2 \pm 0.40$	$3.0 \pm 0.45$	4	2
2	I M 2	2	.	0	0	$3 \pm 0.40$	$3.5 \pm 0.91$	4	0
3	I M 3	3	.	0	0	$4 \pm 0.40$	$5.5 \pm 1.08$	3	5
4	I M 4	4	.	0	0	$3 \pm 0.70$	$4.0 \pm 0.74$	3	8

\*IM: Insulin Multiplication

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Grown shoots were inoculated in the media containing various IBA and IAA concentrations with activated charcoal for root induction. High concentration of IBA was showed better root induction than low concentration of IBA in the medium supplemented with IAA. MS-medium supplemented with 1.5 mg/l IBA along with 1.0mg/l IAA +0.5gm/l AC per litre of media produced an average no of roots is 5 roots with an average length  $4.5 \pm 1.10$  cm. IAA and IBA are known as root inducing growth regulators and have been used either alone or in combination for root initiation in many cultures (Gupta et al., 1981; Be and Debergh, 2006; Dansoet *al.*, 2008).The plants were hardened for 10-15 days before being transferred to the green house. Later acclimatized plants were shifted to field conditions.

**Table 3 Observation of various combinations of auxins IBA and IAA with AC on root induction from isolated multiple shoots of *Costuspictus* after 5 weeks.**

Sr. no.	Media code	MS + concentration of growth hormones mg/l + Activated charcoal gm/l					Avg. no. of root per explants(mean±SD)	Average root length (cm)(mean±SD)	Days Required						
		I	B	A	I	A					A	A	C		
1	I R 1	0	.	5	0	.	0	0	.	5	2 ± 0.40	1.5 ± 0.33	3	5	
2	I R 2	1	.	0	0	.	5	0	.	5	3 ± 0.40	2.5 ± 0.40	3	1	
<b>3</b>	<b>I R 3</b>	<b>1</b>	<b>.</b>	<b>5</b>	<b>1</b>	<b>.</b>	<b>0</b>	<b>0</b>	<b>.</b>	<b>5</b>	<b>5 ± 1.08</b>	<b>4.5 ± 1.10</b>	<b>2</b>	<b>7</b>	
4	I R 4	1	2	.	0	1	.	5	0	.	5	4 ± 0.40	3.0 ± 0.38	3	2

\* IR: Insulin Rooting

\*AC: Activated Charcoal



(fig.1)

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(fig.3)



(fig.2)

**Fig A: 1-3 In vitro stages: Initiation and multiplication. (fig.1) Sprouting of bud by using nodal segment as explant from various combinations of hormone BAP and IAA of *Costuspictusa* after 7 weeks. (fig.2-3) Multiple shoot formation on MS medium supplemented with various combinations of hormone BAP and IBA from sprouted bud of nodal segment of *Costuspictusa* after 6 weeks.**



(fig.4)



(fig.5)



(fig.6)

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(fig.7)

(fig.8)

**Fig B: 4-8. Rooting and primary hardening.(fig.4-7) Root induction from isolated multiple shoots with varying concentration of auxin: IBA, IAA and activated charcoal.(fig.8) Primary hardening under controlled greenhouse conditions.**

### IV.CONCLUSION

As this herb, is an important medicinal plant, it is becoming a vulnerable species due to its low seed germination percentage and poor seed viability. The methods of vegetative propagation are not efficient to save this rare species. The productions of large numbers of Insulin plants were possible through *in vitro* propagation technique.

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