



***In Vitro* Propagation Studies In Insulin Plant (*Costus pictus*): An Antidiabetic Plant.**

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Abstract: The present study entitled “*In vitro* propagation of Insulin plant (*Costus pictus*)” was conducted in the tissue culture laboratory for rapid multiplication of Insulin plant to produce large number of sprouting buds and disease free plantlets. *In vitro* propagation was done by using nodal segments as explants which were initiated on Murashige and Skoog (1962) medium supplemented with different concentrations of BAP in combination with IAA. After the seven weeks of initiation maximum number of sprouting bud was obtained in medium supplemented with 1.5mg/l BAP + 1.0mg/l IAA with survival percentage 75%. Buds or shoot lets were regenerated from nodal explants through shoot proliferation. The best response of rooting obtained on MS supplemented with 1.5mg/l IBA + 1.0mg/l IAA + 0.5gm/l AC. The well rooted plantlets transferred to green house condition for primary hardening.

Index Terms - *in vitro* propagation, Nodal segment, BAP, IBA, and IAA.

I. INTRODUCTION

The *Costus* is genus belongs to the family Costaceae. The whole genus is 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.’ Insulin plant is originated in Mexico. In India it is grown in gardens as ornamental plant especially in Kerala in every home. “Insulin plant” is indigenous to Southeast Asia, particularly on the Greater Sunda Islands situated in Indonesia.

C. pictus is also well known for its medicinal value mainly antiseptic, tonic, aphrodisiac, carminative, stomachic, and vermifuge[1]. The Methanolic leaf extract of *C. pictus* is used to lower blood glucose level in alloxan induced diabetic rats [3]. It is a perennial, upright, spreading plant reaching about two feet tall, with the tallest stems falling over and lying on the ground. Leaves are simple, alternate, entire, oblong, evergreen, 4-8 inches in length with parallel venation. Conservation need due to low multiplication rate, poor seed viability, low seed germination percentage 62% and scanty delayed rooting of vegetative cutting there is need for alternative propagation methods of these plants. Different plant biotechnological approaches such as micropropagation, germplasm preservation and various tissue culture techniques which results in large scale production of uniform planting material can be used for commercial cultivation and preservation of this species. In India most population is affected by the disease called Diabetes, it is nothing but the increase of sugar in the blood because if the insufficient secretion hormone

called Insulin. Here is a natural way to get cure from diabetes by using a miraculous plant called Insulin Plant. An insulin plant is a nature's gift for the people who are suffering from Diabetes. Both ayurvedic and allopathic practitioners recommend chewing the insulin leaves twice a day to bring the blood sugar level totally under control. If it is difficult to get the fresh insulin leaves, for the convenience of the patients the dried leaves powder is also available commercially. The Methanolic leaf extract of *C. pictus* is used to lower blood glucose level in alloxan induced diabetic rats [3].

II. RESEARCH METHODOLOGY

2.1 Collection of Explants: Healthy Insulin Plants were selected from the Nursery. The nodal part obtained after careful excision of leaves of size about 1-2 cm height which were healthy and free from disease and suitable for sterilization and initiation.

2.2 Sterilization of glassware's: The glassware's such as culture bottles, measuring cylinders and the other equipment like forceps, cutting paper and blade holder were washed in running tap water using detergent followed by rinsing with double distilled water and then wrapped in aluminum foil and subsequently autoclaved at 121° C at 15 lbs. pressure for 20 minutes.

2.3 Preparation of stock solutions: Separate stock solutions of macronutrients, micronutrients, potassium iodide, iron, glycine and various vitamins were prepared by dissolving each chemical separately in small quantity of double distilled water and making up the required volume with double distilled water.

2.4 Media preparation: According to the available literature on in vitro propagation of Insulin Murashige and Skoog's (1962) medium is the most commonly used growing medium for Insulin Plant.

2.5 Surface sterilization: Healthy nodal explants brought to the tissue culture laboratory. 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.

2.6 Initiation stage: The inoculation of nodal segments in initiation stage were transferred to different MS basal medium supplemented with BAP (0.0-2.5mg/l) in combination with IAA (0.0, 0.5, 1.0, 1.2, 1.4mg/l). Apart from this 3% sucrose, 5gm/l Agar 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.

2.7 Culture incubation condition: 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. Data were recorded after seven weeks of initiation in terms of average number of buds per explants, survival percentage and days required.

2.8Shoot multiplication: Shoot multiplication was carried out using the 2-3cm 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 (table 2) were divided into different parts. First part was free from growth regulators and remaining 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.

2.9Rooting and hardening: In rooting regenerated multiple shoots (4-5cm long) achieved from nodal segment explants were excised and individually transferred to MS medium fortified with various concentrations of IBA (0.5-3.0mg/l) and IAA (0.0-2.5mg/l) with activated charcoal 0.5gm/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.

2.10Statistical analysis: Various observations were recorded in terms of number of shoots, shoot length, root length and number of roots per explants and percentage survival. Means and standard deviation were used throughout the each study.

III. RESULTS AND DISCUSSION

3.1Establishment of Shoots: Shoot initiation and establishment from nodal segment explants are cultured on MS basal and MS supplemented with different concentration of growth hormones. Data were obtained after 7 weeks of initiation of culture showed that sprouted buds of *Costus pictus* could be established at all tested media. The best result was obtained on MS medium supplemented with 1.5mg/l BAP + 1.0mg/l IAA. (Table.3.1)

3.2Shoot Multiplication: The buds sprouting in the initiation were subjected to the multiplication on the different multiplication media and the growth was observed after six weeks of sub culturing. The media with all concentrations of BAP and IBA showed multiple shoot generation and proliferation. 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 ± 0.40 .

3.3Rooting: The regenerated multiple shoots or buds achieved from nodal part of explants were excised and individually transferred to MS medium combination with various auxins IBA and IAA. The shoots were successfully rooted after the six weeks. The induced roots were hairy and short. The best result of rooting observed in MS media containing 1.5mg/l IBA+1.0mg/l IAA.

3.4Discussion: During the *in vitro* propagation of Insulin plant, stem consisting of nodal segment of size 1-2 cm were inoculated on the MS-media supplemented with different concentrations of BAP along with IAA for bud initiation where all the media showed shoots or bud induction. Generally, cytokinins are known to stimulate cell division and auxillary bud proliferation (Kyte and Kleyn, 1996). The plants were subjected to multiplication in MS-media with different concentration of BAP and IBA. According to Firoozabady and

Gutterson, 2003 addition of BAP in MS-medium was essential for the regeneration of plantlets. As recorded in the initiation the buds were subjected to the controlled MS-medium to attain shoot elongation. Grown shoots were inoculated in the media containing various IBA and IAA concentrations with AC for root induction. High concentration of IBA was showed better root induction than low concentration of IBA in the medium supplemented with IAA. 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; Danso *et al.*, 2008).

Tables and Figures:

Table.3.1: Observation of various combinations of 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
			BAP	IAA			
1	Nodal Segment	I1	0.0	0.0	1	20	50
2		I2	1.0	0.5	2	40	47
3		I3	1.2	0.5	2	50	45
4		I4	1.5	1.0	3	75	39
5		I5	1.7	1.0	2	60	43
6		I6	2.0	1.2	2	60	45
7		I7	2.2	1.2	2	60	47
8		I8	2.5	1.4	1	40	43
9		I9	2.7	1.4	1	30	45

Table.3.2: Observation of various combinations of BAP and IBA on multiple shoot formation by using sprouted bud after 6 weeks.

Sr. no.	Media code	MS + concentration of growth hormones mg/l		Avg. no. of shoots per explants	Average shoot length (cm)	Days Required
		BAP	IBA			
1	IM1	1.0	0.0	2 ± 0.40	3.0 ± 0.45	42
2	IM2	1.5	0.2	2± 0.40	3.0 ± 0.68	41
3	IM3	2.0	0.2	3 ± 0.40	3.5 ± 0.91	40
4	IM4	2.5	0.2	3± 0.70	4.0± 0.35	38

5	IM5	3.0	0.2	4 ± 0.40	5.5 ± 1.08	35
6	IM6	3.5	0.2	4 ± 0.14	4.0 ± 0.48	39
7	IM7	4.0	0.2	3 ± 0.70	4.0 ± 0.74	38

Table.3.3: Observation of combinations of auxins IBA and IAA with AC on root induction from isolated multiple shoots of *Costus pictus* after 6 weeks.

Sr. no.	Media code	MS + concentration of growth hormones mg/l + Activated charcoal gm/l			Avg. no. of root per explants	Average root length (cm)	Days Required
		IBA	IAA	AC			
1	IR1	0.5	0.0	0.5	2 ± 0.40	1.5 ± 0.33	35
2	IR2	1.0	0.5	0.5	3 ± 0.40	2.5 ± 0.40	31
3	IR3	1.5	1.0	0.5	5 ± 1.08	4.5 ± 1.10	27
4	IR4	2.0	1.5	0.5	4 ± 0.40	3.0 ± 0.38	32
5	IR5	2.5	2.0	0.5	3 ± 0.08	2.6 ± 0.16	30
6	IR6	3.0	2.5	0.5	3 ± 0.14	2.2 ± 0.58	30

Figures:



A



B



C



D



E



F



G

Fig: A) Insulin mother Plant, **Fig: B)** Nodal segment of Stem used as explants. **Fig: C)** Sprouting of bud by using nodal segment as explants from combinations of BAP and IAA of *Costus pictus* after 7 weeks. **Fig: D)** Multiple shoot formation on MS medium supplemented with combinations of BAP and IBA from sprouted bud of nodal segment of *Costus pictus* after 6 weeks. **Fig: E and F)** Root induction from isolated multiple shoots with varying concentration of auxin: IBA, IAA and activated charcoal. **Fig: G):** Primary hardening under controlled greenhouse conditions.

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