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Micro Propagation Studies in Insulin Plant Costus Pictus D. Don

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Abstract: Costus pictus ispopularly known as 'insulin plant'. It is one of the medicinally important plants cultivated in garden as an ornamental which is belong to family Costaceae. It is also used as a munching dietary supplement for the treatment of diabetes in Southern India. The oral feeding of aqueous leaf extract of this plant in diabetic patients for subsequent 28 days (2gm/kg body weight) exhibited a significant reduction in fasting blood glucose level and a remarkable increase in serum insulin level. Looking towards the importance Micropropagational studies in C. pictus have been carried out using various growth regulators viz. BAP, KIN, and IAA Various explants viz. shoot tip, leaf, nodal segment, eye of rhizome were tried among which nodal part of stem was proven suitable for Micropropagation. Rate of Micropropagation was higher using 3.0 mg/L BAP and 0.5 mg/L IAA. Present piece of work provides the suitable in vitro protocol for regeneration of C. pictus.

Key words: Costus pictus, Insulin plant, Micropropagation.

I. INTRODUCTION

The genus *Costus* is 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.'

Costuspictus is also well known for its medicinal value mainly antiseptic, tonic, aphrodisiac, carminative, stomachicand vermifuge (Beena and Reddy, 2010). It is able to prevent the hair turning grey and its root is anodyne, antibacterial properties. 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 (Jayasri 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). *C. pictus* is natural strands are fast disappeared and threatened with extinction due to its indiscriminate collection, over exploitation, natural resources for commercial purposes and to meet the requirements of the pharmaceutical industry. Conventional propagation is hampered due to its poor seed viability, low rate of germination and poor rooting ability of vegetative cuttings. Therefore alternative propagation methods would be beneficial in accelerating large scale multiplication, improvement and conservation of the plant. The present study aims to developthe rapid *in vitro* methods of propagation in *C. pictus*.

II. MATERIALS AND METHODS

Preparation of Explant:

The explants were collected from elite plants growing in green house, botanical garden, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Young leaves and nodal part of stem were taken as explants. Explants were surface sterilized in running tap water for 10 minute followed by sterilized distilled water for 5 minutes. Apart from this sterilizing agents such as 70% ethanol, $Hgcl_2$ (0.3 %) were tried. The duration for surface sterilization was 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 MS medium so that maximum part could be inserted in media.

Culture conditions:

MS medium (Murashige and Skoog, 1962) fortified with various combinations of growth regulators weretried. The growth hormones added in MS were BAPand KIN as cytokinins and for rooting IAA was tried. Apart from this 3% sucrose and0.3 % Clerigel as solidifying agent were added in media.The pH was adjusted to 5.8 before autoclave. Media was sterilized in an autoclave under 15 lb. pressures and at 121°C temperature for 15 minutes. The media was transferred to laminar air flow. After inoculation cultures were transferred to culture room under a 16 h photoperiod supplied by cool white fluorescent tubes (Philips) light and the temperature was maintained 25 $\pm 2^0$C. At least five replicates were maintained to minimize the error.Observations wererecorded after 15 days and statistical analysiswas also done.

III. RESULTS AND DISCUSSION

The results were obtained in present research investigation have been divided into two parts such as callus induction and shoot multiplication in *C. pictus*.

A. Induction of callus:

During present piece of work, explants viz. leaf and nodal segments were inoculatedon MS medium.After 21 days callus was noticed at the cut end of the explants. The callus was white in color along withluster. It was turned green after 40 days. Maximum amount of callus formation was recorded on MS with 0.5mg/L IAA and 2.5mg/L BAP. Induction of callus was also noticed by taking IAA and KIN on MS but the rate of callus induction was less as compare to IAA and BAP.

Frequencyof callus induction was maximumonhalfstrength MS adding1.0 mg/L of 2-4-D in combination with 0.5 mg/L Kin first time reported by (Wani et al., 2014). During the present piece of work, highest frequency of callus formation was noticed on MS media. This might be due to inclusion of BAP 2.5 mg/L + IAA 0.5 mg/L by taking leaf disk as an explant.Similar kind of growth pattern of callus was recorded by Biradar et al., 2013.

Table.1. Induction of callus using different growth hormones on MS

Source of Explant	Conc. of growth regulators (mg/L)			% of Callus/Shoot Formation
	IAA	BAP	KIN	
Leaf	0.5	2.0	-	40
	0.5	2.5	-	**70**
	0.5	3.0	-	50
	0.5	-	2.0	00
	0.5	-	2.5	20
	0.5	-	3.0	**30**
Nodal Segment	0.5	2.0	-	40
	0.5	2.5	-	50
	0.5	3.0	-	**80**
	0.5	-	2.0	20
	0.5	-	2.5	20
	0.5	-	3.0	**30**

Plate.1. Shoot initiation using leaves andnodal shoot as explants:

A-Callus

B-Initiation of Shoots

C-Multiple shoots

D-Rhizogenesis *in vitro*

B. Formation of multiple shoots and rhizogenesis:

Secondly nodal segments were tried for multiplication and it was observed that theyare more suitable for *in vitro*multiplication in *C. pictus.*80 % of the explants were capable of multiplication using nodal segment as an explants on MS along with 2.0 mg/L BAP and 0.5 mg/L IAA. Further increase in concentration of BAP and IAA,there was subsequent increase in number of shoots. Higher rate of multiplication was in 3.0 mg/L BAP and 0.5 mg/L IAA rather than combination of IAA and KIN.Similar kind of results was recorded by Kshetrimayum using NAA for rhizogenesis.Bakrudeenand Arun,(2009) reported multiple shoot from rhizomatic eye explants in *C. pictus* on MS medium incorporated fortified with BAP 2.5 mg/L and Kin 1.0 mg/L. Philip et al.,(2009) also shown*in vitro* multiplication in *C. pictus* on MS medium supplemented with 0.05 mg/L.

IV. CONCLUSION

Now days there is a growing interest in herbal remedies for the treatment of several disorders like diabetes mellitus as oral hypoglycemic agents cause side effects. Many herbal preparations are used in ayurvedic medicine to manage diabetes mellitus. New oral hypoglycemic compounds from medicinal plants may provide a useful source for development of pharmaceutical entities or as a dietary adjunct to existing therapies. Drugs obtained from plant origin are considered to be safer. From the present findings it could be concluded that, for rapid propagation and conservation of *C. pictus* tissue culture is the unique method. MS medium along with 3.5mg/L of BAP and 0.5mg/L of IAA facilitate the highest shoot proliferation. Nodal segment explants were most suitable for multiplication of *C. pictus* as compared to other excised parts.The method is viable and cost effective as well.

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