# High Frequency Regeneration and Hardening of Insulin Plant *Costus pictus D.Don*

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***Abstract:*** *Costus pictus* is one of the important medicinal plants and cultivated as ornamental plant belonging to family Costaceae. It is popularly known as 'insulin plant' due to its potential of reducing sugar level in oral consumption of aqueous leaf extraction to 2 mg/kg body weight significantly reduced in fasting blood glucose level. Considering importance of plant multiple shoot induction and hardening of plant has carried out using various growth viz. BAP, KIN, and IAA with different explants leaf, nodal segment for multiple shoot induction. It was notice that 2.5 mg/L of BAP in combination of 0.5 mg/L of IAA was most suitable for multiple shoot induction from nodal explants whereas in case of Kinetin it was 3.0 mg/L of KIN in combination of 0.5 mg/L of IAA exhibit maximum number of shoots. The well rooted *in vitro* grown cultures were transferred for hardening and nearly 60.00% of the plantlet was found successful during the present work in *C. pictus*.

***Key words*** **-** *Costus pictus*, Insulin plant, High Frequency Regeneration.

## I. INTRODUCTION

The genus Costus is of perennial tropical herbaceous flowering plants belongs to the family Costaceae. 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.' C. *pictus* is also well known for its medicinal value mainly antiseptic, tonic, aphrodisiac, carminative, stomachic and 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). Its natural strands are fast disappeared due to its indiscriminate collection, over exploitation, natural resources for commercial purposes and to meet the requirements whereas 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 develop the high frequency regeneration in *C. pictus*.

## II. MATERIALS AND METHODS

**Preparation of explants and Culture conditions:** Young leaves and nodal part of stem were taken as explants from potted plants. These explants were surface sterilized in running tap water for 10 minute followed by sterilized distilled water for 5 minutes. Apart from this sterilizing agent 0.3% mercuric chloride was used for 5 minute followed by three subsequent rinses with sterilized distilled water. All these explants were dissected into small pieces and inoculated in MS medium (Murashige and Skoog, 1962) along with various combinations of growth regulators BAP & KIN in combination with IAA. Apart from this media was fortified with 3% sucrose and 0.3 % Clerigel as solidifying agent. The pH was adjusted to 5.8 before autoclave.

The media was sterilized in an autoclave under 15 Lb pressure 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 0C. At least five replicates of cultures were raised to minimize the error.

**Organogenesis and Hardening**: The nodal segments, leaf explants were collected from plants growing in green house for direct organogenesis and rapid multiplication. Callus induced was cut in small pieces and were inoculated on MS basal medium containing cytokinins (BAP and KIN) with auxins as IAA aseptically. After four to five weeks, observations were recorded in regard to number of shoots per explant and percentage of multiple shoot formation. The micropropagated *in vitro* shoots of about

4-5 cm height were carefully isolated from the clumps or callus and transferred to the MS media devoid of plant growth regulator or the one supplemented with IAA.

The *in vitro* grown plantlets after rooting were removed from the culture bottles/test tubes and were carefully washed in running tap water to remove the media. They were treated with antibiotic agent to avoid attack of pathogens. The plantlets were transferred to plastic root trainer/bags/pots containing coco-peat mixture to help hardening by maintaining high humidity 90 ± 5% and 26 ± $4^0$C temperature. The plants were placed in the carry bags containing 1:1 proportion of soil and FYM. After 11 weeks, the seedlings were transferred to field.

## III. RESULTS AND DISCUSSION

**Multiple Shoot induction using leaves & nodal shoot as explants:** Various explants viz. Leaf and nodal segments were tried for inoculation in MS medium. Callus was noticed at the cut end of explants after 21 days. The callus was white in color along with luster. Maximum amount of Callus Formation was recorded in MS with 0.5mg/L IAA and 1.5mg/L BAP. Callus induction was also noticed taking IAA & KIN at 2.5mg/L KIN along with 0.5mg/L IAA but the rate of callus induction was less as compare to BAP in combination with IAA on both explants.

### Response BAP and Kin on Multiple shoot induction from Leaf and Nodal segment explants

| Concentration of plant growth regulators (PGRs) (mg/l) | | Leaf | | Nodal segment | |
|---|---|---|---|---|---|
| | | No. of shoots/ explants | Shoot induction (%) | No. of shoots/ explants | Shoot induction (%) |
| IAA 0.5 | BAP | | | | |
| | 0.5 | 1.06±0.266 | 46.66 | 1.06±0.266 | 60.00 |
| | 1.0 | 1.20±0.261 | 46.66 | 1.26±0.283 | 60.00 |
| | 1.5 | 1.33±0.251 | 53.33 | 1.33±0.251 | 66.66 |
| | 2.0 | 1.33±0.251 | 53.33 | 1.73±0.181 | 66.66 |
| | 2.5 | 2.86±0.445 | 73.33 | 3.86±0.336 | 93.33 |
| | 3.0 | 1.80±0.380 | 66.66 | 2.86±0.434 | 80.00 |
| | 3.5 | 1.46±0.388 | 53.33 | 1.33±0.251 | 66.66 |
| | KIN | | | | |
| | 1.0 | 0.73±0.283 | 33.33 | 1.20±0.311 | 53.33 |
| | 2.0 | 1.00±0.333 | 50.00 | 1.40±0.272 | 60.00 |
| | 3.0 | 1.06±0.248 | 60.00 | 2.53±0.236 | 93.33 |
| | 4.0 | 1.60±0.289 | 73.33 | 2.40±0.213 | 93.33 |
| | 5.0 | 1.53±0.306 | 66.66 | 1.86±0.273 | 80.00 |

Values represent the mean ± SE and percentage response on three separate experiments, each based on minimum of five replicates.

A-Callus Induction

B- Shoot Induction

C- Multiple Shoot Induction

D- Rhizogenesis

E- Primary Hardening

F- Secondary Hardening

Callus derived from both explants as well leaf and nodal segments were tested for multiple shoot induction on MS medium incorporation of various concentrations of BAP and KIN along with IAA. It was observed that nodal segment explants was most suitable for multiple shoots induction in *C. pictus* 93.33 % of the explants showed multiplication. Highest frequency of shoot induction was recorded on 2.5 mg/L BAP in combination with 0.5 mg/L IAA with 3.86±0.336 number of shoots with 93.33% of shoot induction using nodal segment explants whereas in case of leaf explants it was recorded 2.86±0.445 number of shoots with 73.33% of shoot induction at same concentration of growth regulators. Multiple shoots induction was also achieved by using KIN in combination with IAA. Maximum numbers of shoots were recorded 2.53±0.236 with 93.33 % shoot induction on 3.0 mg/L of KIN in combination of 0.5 mg/L of IAA, followed by 4.0mg/L KIN in combination of 0.5mg/L of IAA with 2.40±0.213 number of shoot with 93.33% shoot induction using nodal segment explants. Leaf explants shown maximum 73.33% of shoot induction with 2.86±0.445 number of shoot using 2.5 mg/L of BAP in combination of 0.5 mg/L of IAA whereas 73.33 % shoot induction with 1.60±0.289 number of shoot using 4mg/L of KIN in combination of 0.5mg/L of IAA.

Rhizogenesis were obtained by transferring cultures on MS medium supplemented with IAA and NAA, Similar kind of results was obtained by Kshetrimayum punyarani using NAA as rooting hormone. These well cultures were tried for hardening, 80% of primary and 60% of secondary hardening were successfully achieved in *C. pictus* plant. Present study revealed that for multiple shoots induction nodal segment were most suitable explants on MS medium supplemented with 2.5mg/L of BAP in combination of 0.5 mg/L of IAA, similar result were obtained by Bakrudeen Ali Ahmed et al 2009 using BAP and KIN.

## IV. REFERENCES


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