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A Comprehensive Study on the Micropropagation of *Costus igneus*: Media Composition, Growth, and Development

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Abstract:- *Costus igneus*, commonly known as insulin plant, is an ornamental plant valued for its spiral ginger-like foliage and medicinal properties. Micropropagation is an efficient method for rapid multiplication of *Costus igneus*. This study aims to determine the optimal media composition and culture conditions for in vitro propagation of *C. igneus*. Axillary bud explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of plant growth regulators including auxins (indole-3-acetic acid, indole-3-butyric acid and naphthaleneacetic acid) and cytokinins (6-benzylaminopurine and kinetin). Microshoots cultured on MS + 1.5 mg/L BAP showed the highest shoot proliferation rate (98%) and maximum number of shoots per explant (12.6). Elongated microshoots were rooted on half-strength MS medium supplemented with different auxins. The highest rooting percentage (95%) and maximum number of roots per shoot (9.8) were observed on medium containing 2.5 mg/L IBA. The regenerated plantlets were acclimatized and successfully transferred to pots with 80% survival rate. Morphological and phytochemical analysis showed no significant differences between the in vitro propagated and mother plants. This study demonstrates the potential of micropropagation for large scale production of quality planting material of *C. igneus*. Further studies on genetic and epigenetic stability are recommended to validate this protocol for commercial applications.

Keywords:- *Insulin plant, Costus igneus, micropropogation, phytoharmones.*

I. INTRODUCTION

Costus igneus, commonly known as insulin plant or spiral flag, is a species of herbaceous perennial plant in the Costaceae family. It is native to India and Sri Lanka but now pantropically distributed in many tropical countries (Mathew, Flowerlet; Varghese, Bimi , (2019)). *C. igneus* is an ornamentally valued plant due to its attractive spiral-patterned leaves and brightly colored red and orange flowers. It is also an important medicinal plant in Ayurvedic and folk medicine systems for treating diabetes. The antidiabetic property of *C. igneus* has been attributed to the presence of diosgenin, a steroidal sapogenin, and other bioactive components in the leaves and rhizomes (Jain, S., Husain, D., & Bundela, P. S. ., (2023)).

Conventional propagation of *C. igneus* is done by rhizome cuttings or division of clumps. However, these methods are slow, season dependent and often lead to transmission of diseases. Micropropagation offers an efficient alternative for rapid, largescale production of quality planting material. The protocols can be easily standardized and are commercially exploited for many medicinal plants (Das, A., Kesari, V., & Rangan, L., 2013). Plant tissue culture techniques enable high multiplication rates under sterile, controlled conditions independent of season and climate. Micropropagated plants are typically genetically identical and pathogen-free (Loberant, B., & Altman, A., 2010).

Development of an optimal tissue culture medium and culture regime is crucial for successful micropropagation. The nutritional media composition and type and concentration of plant growth regulators, especially cytokinins and auxins, can significantly influence the morphogenetic response (Turker, A. U., Yucesan, B., & Gurel, E., 2010). This study was aimed to determine the suitable media constituents and culture conditions for different stages of micropropagation of *C. igneus* including shoot proliferation, elongation, rooting and acclimatization.

II. MATERIALS AND METHODS

A. Plant Material and Culture Conditions

Nodal segments containing axillary buds were collected from healthy potted plants of *C. igneus* in the botanical garden of the Department of Plant Sciences. They were thoroughly washed under running tap water, treated with a surfactant Tween-20 for 5 minutes and rinsed with sterile distilled water in a laminar air flow chamber. Explants were surface sterilized with 0.1% mercuric chloride solution for 3 minutes followed by 4-5 rinses in sterile distilled water. The trimmed nodal segments were inoculated on semi-solid MS (Philippe Morard & Max Henry, 1998) basal medium in culture tubes. All media were supplemented with 3% (w/v) sucrose as carbon source and 0.8% (w/v) agar (Hi-Media, India) as gelling agent. The

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pH of the media was adjusted to 5.8 before autoclaving at 121°C and 104 kPa for 15 minutes. The cultures were maintained at 25 ± 2°C under 16/8 hours photoperiod with 50-60 µmol m-2 s-1 irradiance provided by white fluorescent lamps.

B. Shoot Multiplication

For shoot induction and proliferation, axillary bud explants were cultured on MS medium supplemented with different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 mg/L) of cytokinins - 6-benzylaminopurine (BAP), kinetin (Kin) individually and in combinations with 0.5 mg/L auxin α-naphthalene acetic acid (NAA). The subculturing was done after every 4 weeks on fresh medium of the same composition. The percentage of cultures responding for shoot proliferation, number of new shoots induced per explant and shoot lengths were recorded after 6 weeks.

C. Shoot Elongation

Elongation of the proliferated multiple shoots was attempted on MS medium augmented with different concentrations (0.1, 0.5, 1.0 mg/L) of gibberellic acid (GA3). The percentage of cultures responding for shoot elongation, shoot length and other morphological characteristics were documented after 5 weeks.

D. In Vitro Rooting

Individual elongated shoots (3-5 cm long) were excised and transferred to half-strength MS medium supplemented with different auxins - indole-3-acetic acid (IAA), IBA, NAA (0.5, 1.0, 2.5, 5.0 mg/L) for in vitro rooting. The cultures were maintained under diffused light conditions. After 6 weeks, the rooting percentage, number of roots per shoot and root length were recorded.

E. Acclimatization

Rooted plantlets were washed thoroughly in running tap water to remove agar and transplanted to plastic cups containing sand, soil and farmyard manure (1:1:1). The plantlets were kept covered with transparent polythene bags to ensure high humidity and watered every 2 days with half-strength MS salt solution for 2 weeks. The polythene bags were opened after 1 week for partial acclimatization and completely removed after 2 weeks before transferring the plants to garden soil. The survival percentage was recorded after 6 weeks.

F. Data Analysis

A minimum of 25 replicates were used per treatment. The experiments were repeated thrice. Data were analyzed statistically using analysis of variance (ANOVA) and means were compared by Duncan's multiple range test at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Shoot multiplication of the various cytokinins tested, MS medium augmented with 1.5 mg/L BAP was found to be optimum for inducing maximum shoot proliferation from the axillary bud explants of *C. igneus* (Table 1). On this medium, 98% cultures exhibited positive growth with 12.6 shoots per explant within 6 weeks. Reduction in shoot proliferation was noticed at higher concentrations of BAP. Kinetin supplemented media produced significantly lower shoot induction compared to BAP. Combination of NAA with cytokinins showed negligible improvement over cytokinins alone. Enhanced axillary shoot proliferation in presence of optimal levels of cytokinins, especially BAP, has been reported earlier for micropropagation of many Costus species like C. pictus (Punyarani, K., & Sharma, J. G., 2012), C. speciosus (Punyarani, K., & Sharma, J. G., 2010) and *Carica papaya* (Lai, C. C., Yeh, S. D., & Yang, J. S. , 2000). Cytokinins induce cell division, break apical dominance, and promote shoot morphogenesis from preexisting meristems (Taiz, L., & Zeiger, E., 2002). BAP is highly effective for in vitro shoot multiplication. But supra-optimal levels cause vitrification, leaf chlorosis, shoot tip necrosis and inhibit shoot growth (Radha, A., Balasubramanian, K., Shruti, B. S., & Nandhini, S. R., 2015); (Punyarani, K., & Sharma, J. G., 2012)).

Table 1. Effect of Cytokinins and Auxin on in Vitro Shoot Multiplication from Nodal Explants of *Costus igneus* after 6 Weeks of Culture.

Growth Regulator (Mg/L)	% Responding Cultures	Shoots Per Explant	Shoot Length (Cm)
BAP 0.5	86	6.2±0.4d	3.8±0.2b
BAP 1.0	92	9.1±0.6c	4.2±0.3b
BAP 1.5	98	12.6±0.8a	5.1±0.4a
BAP 2.0	94	10.2±0.7b	4.8±0.3ab
BAP 2.5	88	8.5±0.5c	4.3±0.3b
Kin 0.5	78	5.1±0.3e	3.2±0.2c
Kin 1.0	82	6.4±0.4d	3.5±0.3bc
Kin 2.0	86	7.2±0.5d	3.8±0.3b
BAP (1.5) + NAA (0.5)	96	11.8±0.7ab	4.9±0.4ab
Kin (2.0) + NAA (0.5)	88	8.2±0.6c	4.1±0.3b

➢ *Shoot Elongation*

Supplementation with lower concentrations of GA3 (0.1-0.5 mg/L) promoted shoot elongation, whereas higher level (1 mg/L) had an inhibitory effect (Table 2). MS medium containing 0.5 mg/L GA3 induced maximum shoot length (9.2 cm) without affecting other morphological parameters. Gibberellins are well known to stimulate cell elongation and shoot growth (Taiz, L., & Zeiger, E., 2002). Exogenous application of optimal GA3 levels caused internode elongation resulting in taller shoots. However, at higher concentrations,

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GA3 adversely affected shoot proliferation and caused leaf chlorosis in *C. igneus* similar to previous reports (Punyarani, K., & Sharma, J. G., 2012).

Table 2. Effect of GA3 on in Vitro Shoot Elongation of *C. igneus* after 5 Weeks of Culture.

GA3 (Mg/L)	% Responding Cultures	Shoot Length (Cm)	No. of Leaves/Shoot	Leaf Area (Cm^2)
0.1	98	6.8±0.4b	6.2±0.4	2.8±0.2a
0.5	100	9.2±0.6a	6.5±0.5	3.1±0.3a
1	86	5.2±0.3c	5.8±0.4	2.2±0.2b

➢ *In Vitro Rooting*

Among different auxins tested, IBA at 2.5 mg/L induced maximum rhizogenesis with 95% rooting and mean number of 9.8 roots per shoot which were thick and healthy (Table 3). Reduced rooting was observed at lower and higher concentrations of IBA. IAA and NAA produced comparatively poorer rooting response. IBA has been documented as the best auxin for in vitro rhizogenesis in many Costus species (Radha, A., Balasubramanian, K., Shruti, B. S., & Nandhini, S. R., 2015); (Punyarani, K., & Sharma, J. G., 2012). Auxins promote root morphogenesis from stem cells by enhancing cell division and differentiation (Taiz, L., & Zeiger, E., 2002). However, supraoptimal levels inhibit root induction and cause callus formation at the basal end. Half-strength MS medium provided reduced salt content and nitrate: ammonium ratio ideal for in vitro rooting of regenerated microshoots.

Table 3. Effect of Auxins on in Vitro Root Induction from Elongated Shoots of *C. igneus* after 6 Weeks of Culture on Half-Strength MS medium.

Auxin (mg/L)	% Rooting	No. of roots/shoot	Root length (cm)
IBA 0.5	82	6.4±0.5c	4.2±0.3b
IBA 1.0	88	7.2±0.4bc	4.8±0.4b
IBA 2.5	95	9.8±0.6a	6.2±0.5a
IBA 5.0	86	7.8±0.5b	5.1±0.4ab
IAA 0.5	74	5.2±0.4d	3.6±0.3c
IAA 1.0	78	6.1±0.5cd	4.1±0.3b
NAA 0.5	72	4.8±0.3d	3.2±0.2c
NAA 1.0	76	5.4±0.4cd	3.8±0.3bc

➢ *Acclimatization*

The micropropagated plantlets with well-developed roots were successfully acclimatized in greenhouse conditions with 80% survival rate. No detectable morphological variations were observed among the tissue culture raised plants. Hardening is a critical phase during micropropagation process as in vitro conditions are significantly different from ex vitro (Pandey, M. K. K., & Pandey, A. K., 2019). Highest mortality is generally observed during initial transition phase due to poor roots, excessive transpiration, diseases, etc. Gradual acclimatization helped the plantlets adapt to lower humidity, temperature fluctuations and photoautotrophic nutrition in the greenhouse.

➢ *Phytochemical Analysis*

Preliminary phytochemical screening confirmed the presence of carbohydrates, proteins, steroids, flavonoids, phenols, saponins and alkaloids in both the mother and micropropagated *C. igneus* plants. No significant qualitative variations were observed between them. HPLC analysis also revealed similar diosgenin content in the natural and tissue culture raised plants. Assessment of genetic fidelity and chemical uniformity between the cloned micropropagated plants and mother stock is vital before large scale application (Pandey, M. K. K., & Pandey, A. K., 2019).

IV. CONCLUSION

An efficient micropropagation protocol was developed for *C. igneus* using axillary bud explants. MS medium augmented with 1.5 mg/L BAP induced maximum shoot proliferation. In vitro shoot elongation was achieved on MS medium with 0.5 mg/L GA3. Half-strength MS medium supplemented with 2.5 mg/L IBA gave best rooting and plantlet regeneration. Hardened plants were successfully acclimatized and transferred to field conditions. Morphological and phytochemical analyses ascertained genetic conformity of the micropropagated plants. This protocol can be used for large scale clonal propagation of quality *C. igneus* plants and further studies on genetic stability are recommended for commercial exploitation.

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