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Different Concentrations and Combinations of Cytokinins from Leaf Explants of *Trichosanthes Cucumerina* (L) A Medicinal Plant

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Abstract*-- MS medium supplemented with 1.0 mg/l BAP + 2.0 mg/l NAA and 3.0 mg/l L-Glutamicacid was found to be optimum to induce shoots. The present study established reliable and reproducible protocol for rapid multiple shoot induction from leaf explants of *Trichosanthes cucumerina* using different concentrations and combinations of Cytokinins. Plantlet regeneration through Cotyledon and shoottip explants and streptomycin resistant plantlet using in vitro mutagenesis was also developed in S. Surattence (Muukherjee *et al* 2011, Swamy *et.al* 2005, Nasar *et.al* 1997) plant regeneration callus induction. *In vitro* regeneration in the *Trichosanthes cucumerina* (L) study of multiple shoots MS Containing media with different and combinations of growth regulators. The effect of plant growth regulators on rooting was investigated 4-6 weeks after culture on MS medium (Subashri *et.al* 2014: Devendra *et.al* 2011: Verma 1996: Sunderraj *et.al* 1989). Due to over exploitation for high medicinal values and destruction of the habit this Sunderraj *et.al* 1989, Nasir *et.al* 1997) meristem shoottip cultures hence there is need for *ex situ* conservation through tissue culture methods. The leaf explants were inoculated on MS medium supplemented with various cytokinins i.e., BAP and NAA. Addition of BAP at 3.0 mgl/L concentration or NAA at 3.0 mgl/L to the MS basal medium, induced regeneration from the leaf explants. Micro propagation involves multiplication of genetically identical individual by asexual reproduction within a short span of time with tremendous potential for the production of high quality plant based medicines (Murch *et al.*, 2000). Multiple shoot induction was achieved in one of the important medicinal plant of *Trichosanthes Cucumerina* (L) The effect of BAP and NAA + Kn combination on *In vitro* shoot regeneration and elongation was analysed in leaf explants. The percentage of response was increased up to 2.0mgl/L to 3.0 mgl/L Kn and later gradually decreased at high concentrations.

***Keywords*-- leaf, explants, NAA BAP, *Trichosanthes Cucumerina*,**

I. INTRODUCTION

The development of protoplast systems has increased the plants for use in both Biochemical and genetic research. Ugandhar *et.al* (2011) has been also reported that high amount of Cytokinin and lower amount of Auxins is the best combination for Somatic embryogenesis which is in accordance of our study.

Leaf explants of *Trichosanthes cucumerina* (L) on MS medium fortified with plant growth regulators along with coconut milk and Amino acids. The plants of Cucurbitaceae suffer from several diseases including the water melon mosoic virus (Greber, 1978), Cucumber green mottle mosoic virus (Nijsden, 1984) and *Trichosanthes Cucumerina* (L) also suffers from downey and powdery mildews which seriously limits the crop production. Auxiliary buds from pumpkin were reported by Jelaska (1972). *In Vitro* Regeneration of plants via Somatic embryo genesis has much potential for plant propagation and gene transfer In Soybean somatic embryo have been obtained from cultured immature cotyledons Soybean from embryogenic callus line derived from the shoot tip explants (Parvathi, Venkateshwarlu M-2018) *In Vitro* regeneration Via somatic embryo genesis has drawn more attention than other methods because it can produce a large number of plants in a relatively short time (walker *et.al* 2001) The number of protoplasts showed increase during shorter treatment time and reaches a peak at 4-5 hours of after this many reports of soyabean somatic embryogeneis were published (Pathak. *et.al* 2014)

II. MATERIALS AND METHODS

MS Medium was supplemented with various plant growth regulators and 3.0% Sucrose. The PH of the media was adjusted to 5.8, solidified with 0.8% Difco-bacto Agar and Autocloved at 103.4 KPa or 121°C for 15-20min.

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A single explants was placed in each culture tube and incubated (at25± 1°C with a 16h photoperiod under fluorescent light (40-50m^2 s^{-1}). Explants with In Vitro multiple shoots proliferated on TD2-containing media were transferred to MS Medium containing different concentrations (BAP2.0-3.0mgl+) Multiple shoot intonation from shoot tip explants was observed within 20-25days after inoculation. The presence of Cytokinin unducing shoot organogenesis well developed shoot lets from our *Trichosanthes cucumerina* experimental data. The capacity of shoot bud differentiation and shoot proliferation from leaf explants of Coconut milk (CM) depended on hormonal variation. The percentage of response was increased gradually from 2.0 to 3.0 mgl/L BAP, the shoots raised *in vitro* 2-3 cm long were cultured on MS medium supplemented with various concentrations multiple shoot formation from shoot apics was obtained on MS medium supplemented with 1.0mgl/L NAA (Deca *et.al* 1999 and Anju *et.al* 2005) shoottip explants showed a positive response on MS medium containing BAP (1.0 mgl/L-2.0mgl/L) combination with NAA (1.0mgl/L to 2.0 mgl/l) That clearly implies the additive effect of IAA (1.0mgl/l to 2.0 mgl/L) combination of auxin and Cytokinin favored shoot bud differentiation in many plants. BAP was superior to Kn in inducing high frequency shoot regeneration in many number of plants. (Srilatha, venkateshwarlu m 2019) planlet regeneration from Cytoledon culture Soybean All media were adjusted to PH 5-8 before addition of 0.8% agar-agar and auto claved at 121°C and 103K Pg for 20Min Cultures 25x 150nm cultures tubes. The effect of different five types of growth regulators on direct plantlef regeneration of tamato from leaf explants (T Ugandar & M Venkateshwarlu-2018)

The Result from this study has shown that BAP induced the activation of Totipotency at the stem node explants, which resulted in the formation of multiple shoots. The stem node segments of 2.0 – 3.0 cm long were cultured and surface sterilized with 0.1% $HgCl_2$ for 5-7 minutes and rinsed with sterile distilled water. They were cultured on MS medium containing 2.5% sucrose and 0.8% Agar-Agar and different concentrations of BAP, NAA and L-Glutamic acid. The pH of the medium was adjusted to 5.8 and later was autoclaved at 120^0C for 17 minutes. Cultures were incubated under 16 hrs, illumination (250 lux) at 25 ± 2^0C temperature. Raising the level of BAP (0.5 to 2.0 mg/l) resulted in the increase in the number of shoots from hypocotyls and cotyledon explants of Niger (Nikam and Shitole, 1993).

Cotyledonary explants (3-4 weeks old) of different sizes (0.5-1.0mm) were cultured with the abaxial surface in contact with induction Ms Medium consisting (1.0-2.0 mgl/L BAP) and (1.0 to 3.0 mgl/L) NAA for maturation and plant regeneration.

III. Results And Discussion

The Regenerated plants will be useful for constant supply of uniform raw materials for commercial secondary metabolic extraction according to their observation. BAP, Kn were superior for multiple shoot formation has been reported as it was observed in the present investigations. The hormonal supplement was selected because it was optimum for callus formation leaf explants of *Trichosanthes cucumerina* developed (60%) with increase in concentration of NAA in MS Medium. Then the plantlets were transferred to polypots containing pre chamber set at 28°C and 60-70% relative humidity. After 2-4 weeks they were transplanted to polybags containing mixture of soil+sand+manure in 1:1:1 ratio and kept under shade house for a period of 6 days to 4 weeks. Multiple shoot buds were initiated on the callus cultured in MS Medium supplemented with different combinations (BAP, NAA & Kn).The regenerated elongated shoots were transferred to Indole Byutyric acid (IBA) (2.0mgl/L-4.0mgl/L) for root induction. Sub cutting of callus into fresh medium containing the same concentrations of growth regulates resulted in the emergence of callus. The leaf explants were inoculated on MS medium fortified with various cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Raising the level of BAP (3 mg/l to 4 mg/l) resulted in an increase in the percentage of shoots developed from Stem node cuttings. There was no significant increase in the number of shoots on NAA at low and high concentration. Low concentration of L-glutamic acid (0.5 – 1.0 mg/l, along with BAP (2.0 mg/l, has produced significant mean number of multiple shoots that ranged from 2-3 to 5-6 in both the explants.

The mean number of shoots developed on the explants ranged from 2-4 to 3-6 by the addition of different concentrations of BAP and NAA. The number of shoots developed on the explants ranged from 1-4 to 2-3 by the addition of BAP at a concentration of 2.0 mg/l or NAA at 2.5 mg/l. (Plate – I, Fig 1, 2 & 3 Table – I). MS medium fortified with 1.0 mg/l BAP or 2.0 mg/l L-Glutamic acid also induced shoot buds on Stem node explants. Addition of NAA failed to produce many shoots but enlarged the stem node segments.

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Lower levels of coconut milk (6, 12%) induced callus formation. The results from study have shown the initiation of shoot buds and formation of multiple shoots from different explants i.e. leaf explants of *Trichosanthes Cucumerina* (L).

Among all explants used Stem node segments were the best for multiple shoot induction. With an increase in the level of BAP 2.0 – 3.0 mg/l the percentage of explants producing shoots also increased.

Table 1:
Shoot induction from leaf explants of *Trichosanthes Cucumerina* (L).

Growth Regulators	leaf explants	
	% frequency of Shoots	Mean No. of Shoots
MS + 0.5 mg/l BAP + 1.0 L-Glutamic acid +IBA	50	Green Callus
MS + 1.0 mg/l BAP + 2.0 L-Glutamic acid+IBA	45	Green Callus
MS + 2.0 mg/l BAP + 3.0 L-Glutamic acid+IBA	40	Callus + shoots (1-3)
MS + 3.0 mg/l BAP + 4.0 L-Glutamic acid+IBA	30	Callus + shoots (4-6)
MS + 1.5 mg/l NAA + 0.5mg/l	25	Green Callus
MS + 1.0 mg/l NAA + 2.0mg/l	20	Green Callus
MS + 2.5 mg/l NAA + 2.5mg/l	22	Callus + shoots (3-6)
MS + 3.0 Mg/l NAA + 3.0mg/l	15	Callus + shoots (2-4)
MS + 3.5 mg/l NAA + 3.0mg/l	10	Callus + shoots (1-3)

CM = Coconut milk water

Plate I. shoot Induction from leaf explants of Trichosanthes Cucumerina (L).

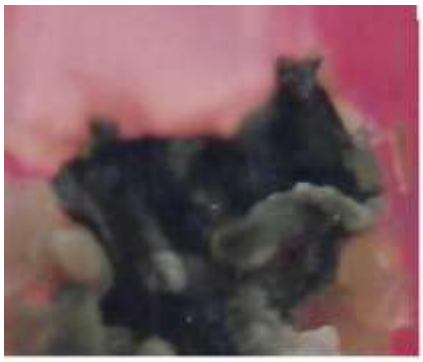

Fig:1 Leaf explant

Fig:2 callus

Fig:3 plantlets

IV. CONCLUSION

The purpose of this work was to study the effect of different concentrations of growth regulators on direct plantlet regeneration of *Trichosanthes cucumerina* from leaf explants explants. Rooted plantlets were successfully hardened under culture conditions and established in the field conditions.The callus showed maximum number of shoot bus (2-4). The method of repeated transfer of explant is considered to be useful for large scale production of plants, as it avoids isolation and culture of new explants. This is considered as one of the methods to increase the response in explants has suggested that repeated transfer of explants on multiplication media containing cytokinins succeeds in activating the plant materials.

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