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Multiple Shoots from Leaf Explants of *Trichosanthes anguina* L.

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Abstract: Regeneration shoots from leaf explants of callus and In Vitro multiple shoots were obtained on MS Medium within BAP, NAA, L-Glutamic acid and Kinetin, Coconut milk (CM). High frequency plant regeneration from leaf explants of *S. Nigrum* by Ugander *et al* (2010) Callus induction and base explants of *Aloe vera* R. Prasad, Venkateshwarlu M *et al* (2018). MS basal medium supplemented with various Auxons/Cytokinins BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Addition of BAP at 3.0 mg/l and NAA at 2.0 mg/l to the MS basal medium, induced regeneration from the leaf segments. With an increase in the level of BAP 1.0 - 3.0 mg/l the percentage of explants producing shoots also increased. The number of shoots developed on the leaf segments ranged from 1-4 to 2-3 by the addition of BAP at concentration of 1.0 mg/l or NAA at 2.0 mg/l. Among the three concentrations of coconut milk used i.e., 5, 10 and 15% of coconut milk along with 0.5 mg/l BAP proved to be ideal for multiple shoot induction. MS medium fortified with 2.0 mg/l BAP 0.5 mg/l L-2.0mg/l Kn or 3.0 mg/l L-Glutamic acid also induced shoot buds on leaf segments.

Keywords: Multiple shoots, Leaf Explants, NAA, L-Glutamic acid, BAP, *Trichosanthes anguina* L

I. INTRODUCTION

In the present paper, a simple and reproducible procedure was devised to obtain multiple shoots from leaf segments of *Trichosanthes anguina* L on MS medium fortified with plant growth regulators along with coconut milk and amino acids. The main objective of clonal propagation is to establish plants that are uniform and predictable for selected qualities. Growth or *in vitro* propagated plants is often stronger than in those cloned *in vitro* phyto chemical analysis and biological activities in *in vitro* propagation (Venkateshwarlu *et al* (2011)). The plants of Cucurbitaceae suffer from several diseases including the water melon mosaic virus Wayne *et al* (2011) Cucumber green mottle mosaic virus Wayne *et al* (2011) and *Solanum nigrum* also suffers from downy and powdery mildews which seriously limits the crop production. Axillary buds from pump-kin were reported by Ugander *et al* (2019) & Rathore (2010).

II. MATERIALS AND METHODS

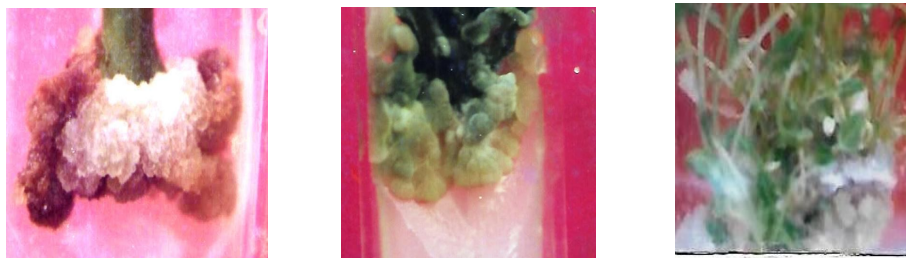
They were cultured on MS medium containing 2.5% sucrose and 0.8% Agar- Agar and different concentrations of BAP, NAA and L-Glutamic acid Leaf segments of *Trichosanthes* were cultured and surface sterilized with 0.1% HgCl for 5-7 minutes and rinsed with sterile distilled water. Cultures were incubated under 16 hrs illumination (250 lux) at 25± 2°C temperature. Each treatment consisted of 10-15 replicates. The data was recorded at the end of eighth week in vitro propagation of *Zizyphus* Sudharsan *et al* (2000) cloning protocol Campstrini (2006). The pH of the medium was adjusted to 5.8 and later was autoclaved at 120 °C for 17 minutes. Rajendraprasad, Venkateshwarlu M (2018) experimental mutagenesis on cicer tissue culture studies stem node explants, multiple shoots in cucumis Venkateshwarlu m (2008) and (2019).

III. RESULTS AND DISCUSSION

The results of the study have shown the initiation of shoot buds and formation of multiple shoots from leaf segments. Addition of NAA failed to produce many shoots, but enlarged the leaf segments. Lower levels of coconut milk (5 & 10%) induced callus formation. Leaf explants were inoculated on MS basal medium fortified with various Auxins cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots Kanna *et al* (2005) *In Vitro* micropropagation *Solanum nigrum* Ram *et al* (2002). The mean number of shoots developed on the leaf segments ranged from 1- 4 to 2 - 3 by the addition of different concentrations of BAP and NAA the level of BAP (3.0 mg/l to 4.0 mg/l) resulted in an increase in the percentage of shoots developed with 10, 15, 20% of coconut milk also triggered the induction of multiple shoots (Plate I). Low concentration of L- Glutamic acid (0.5 - 3.0 mg/l, along with BAP (1.0 mg/l, produced significant mean number of multiple shoots that ranged from 2-3 to 5- Sin the leaf segments. Shoot multiplication was obtained from leaf explants cultured on MS Medium supplemented with 1.0 to 3.0 mg/l BAP. Raising the level of BAP (0.5 to 2.0 mg/l) resulted in an increase in the number of shoots from leaf segments of *Trichosanthes anguina* L suggested that the formation of multiple shoots at the leaf region of the leaf of soybean indicated the existence of totipotency in this region which can be activated with the addition of BAP. (Table-1) (Plate-I)

Table-I Multiple shoots from leaf explants of *Trichosanthes anguina* L

Growth Regulators	Leaf Explants	
	% Frequency of Shoots	Mean no. of Shoots
MS + 0.5 mg/l BAP + NAA	40	Callus
MS + 1.0 mg/l BAP + NAA	35	Callus
MS + 2.0 mg/l BAP + NAA	30	Shoots (1-2)
MS + 3.0 mg/l BAP + NAA	25	Shoots (2-4)
MS + 0.5 mg/l NAA + Kn+CM+L-Glutamic acid	30	Callus+ Small buds
MS + 1.0 mg/l NAA + Kn+CM+L-Glutamic acid	25	Callus + Small buds
MS + 2.0 mg/l NAA + Kn+CM+L-Glutamic acid	20	Shoots (3-5)
MS + 3.0 mg/l NAA + L-Glutamic acid	25	Shoots (3-4)
MS + 4.0 mg/l NAA + L-Glutamic acid	20	Shoots (4-6)

Plate-I Multiple shoots from leaf explants of *Trichosanthes anguina* L.


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