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In Vitro Micropropagation Studies Cotyledonary Explants of Trichosanthes Anguina (L)

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***Abstract*— A method for Invitro Micropropagation Through Cotyledonary Explants of Trichosanthes anguina was exercised from 10-15 days of young stage explants [1, 2]. Developed a protocol for cotyledonary explants cultures, callus induction, and regeneration through cotyledon callus tissue [3, 4]. Multiple shoot formation was promoted by BAP 1.0 mg/l to 3.0 to 5.0 mg/l and higher combination with NAA 3.0 to 4.0 mg/l Kn [5, 6]. Invitro produced small shoots were green callus induced to cotyledonary callus cultures green compact small shoots buds were induced in 16-20 days cultures respectively on Ms. Medium.**

Keywords- Micropropagation, Cotyledon Explants, Invitro, BAP, NAA & Kn.

I. INTRODUCTION

The plants are grown in the garden as a dual-purpose vegetable and ornamental for white flowers, fragrant a night, and decorative fruits. It is cultivated particularly in tropical Asia, but also in many other areas of the tropics for its edible fruit, and leaves plus its local medicinal applications. some botanists recognize it as a distinct as Trichosanthes anguina L. whilst others see it as a form of trichosquites Cucurbitaceae the treatment we have used common name is Snake ground belonging to the family cucurbit cease snake ground is an annual, climbing plant producing stem up to 5 meters long. The plant does not tolerate frost it prefers a mean annual rainfall in the range2.00,2500mm, it grows best in areas where annual daytime temperatures are within the range of 22-35OC. The fruit can be used in curries or eaten as vegetables like green beans.

Medicinal Uses: The seed is said to be cooling, and the fruits are considered to be anthelmintic, emetic, and purgative peptides in the plant are used as an abortifacient in china. According to Ayurveda, the plant pacifies vitiated pitta, constipation, skin diseases, burning sensation, diabetes, anorexia, flatulence constipatic fever, worm infestation, and general weakness it is a popular vegetable in South India.

II. MATERIALS AND METHODS

Trichosanthes anguina cotyledonary explants were collected from the Department of botany, kakatiya university campus. Explants were initially washed under young seeding plants (one week) in running tap water and with Teepol solution (5%) for 3-10 min. Followed by 3-4 times washing with distilled water. Finally, the explants were immersed in 0.1 $Hgcl_2$ mercuric chlorides for 2-3min. The surface sterilization was followed by 2-4 rinses in sterile distilled water MS basal medium (1962) containing 3%surcose and 8% Agar-Agar. All cultures were incubated in a culture room at 25oc to2ocwith relative humidity of 50-60- percent and 16 h photoperiod data photon flux density of 15-20 min from whole cool fluorescent tubes. The PH of the medium was adjuestedto5.8 using 0.1 NHCL or 0.1 N. NaOH Sodium hydroxide solutions before Auto cloving.

III. RESULTS AND DISCUSSIONS

In the present study among the different types of sterilization methods used an appropriate surface sterilization agent for producing [15, 16, 17]. Aseptic cotyledonary explants [9, 10, 11, 12] MS medium has been designated for Trichosanthes anguina cultures [7, 8, 19]. Cotyledonary explants regeneration [13, 14] Supplementing different cytokines BAP, Kn 0.5 to5.2 mg/l at various concentrations [18] Either used alone or in combination with Anxious (NAA) 3.0 mg/l – 5.0 mg/l, Table-I, Plate-I, II Figure-1-3. The production of multiple shoots from cotyledon callus explants through In vitro propagation was green called cultures to overcome the affecting regeneration of multiple shoots (3-4). In the present study, it was found that BAP, NAA, and KN were more effective for multiple shoots from cotyledon explants [12, 18]. The primary target of a micropropagation system was the best acclimatization and field established of regenerated plants (4–6-week cultures) Figure-1 explants; Figure-2 callus induction and Figure-3 Multiple Shoots.

The results showed variable shoot forming capacity depending on the combination of growth regulators used in the culture medium. The efficiency of the plant growth regulators was assessed by counting the number of shoots (3-4) per cotyledon explants cultures, as well as showed that 2 .0 mg/l NAA and 3.0 mg/l BAP were found for multiple shoots producing callus induction. A high level of NAA 4 .0 mg/l and BAP 5.0mg/l was found best for multiple shoots. The present study demon started the successful multiple shoot regeneration from cotyledon explants in-vitro cultures. PLATE-I Figure-1 Young Plant, Figure-2 Fruit, Figure-3 Crop Production.

TABLE I. INVITRO MACROPROPAGATION STUDIES COTYLEDONARY EXPLANTS OF TRICHOSANTHES ANGUINA (L)

S.No	Regulators / Mg/l	Cotyledonary explants	
		% of the plantlet production	Callus and regeneration
1	NAA+0.5+BAP+1.5 ,KN+ **Mg/l**	35	Callus
2	NAA+2.0+BAP+3.0	30	Green Callus

	,KN+ **Mg/l**		
3	NAA+3.0+BAP+4.0 ,KN+ **Mg/l**	25	Green Callus+Shoots (2-4)
4	IBA+1.0+BAP+2.0 KN,+ **Mg/l**	20	Callus with shoots (1-2)
5	IBA+2.0+BAP+3.0, KN+ **Mg/l**	15	Callus with the shoot (3-4)
6	IBA+3.0+BPA+4.0, KN+ **Mg/l**	30	Small shoot bud (3-5)
7	IBA+4.0+BPA+5.0, KN+ **Mg/l**	25	Shoot buds (4-6)



Figure-1 Figure-1 Figure-1

Figure 1. PLATE-I Invitro Macropropagation Studies Cotyledonary explants of Trichosanthes anguina (L)



Figure-1 Figure-2 Figure-3

Figure 2. PLATE-II Invitro Macropropagation Studies Cotyledonary explants of Trichosanthes anguina (L)

IV. CONCLUSION

In vitro plant, lets were gradually acclimatized with an increase in temperature from 25-28oC and a decrease in relative humidity40-60 percent for a period of 15–30-day cultures. These plants were irrigated with ¼ strength MS salt and exposed gradually to the external environment. Rooted plants were removed from the culture medium and the roots were washed under running tap water to remove Agar. After two weeks. They were transplanted to polybags containing a mixture of 1:1:1 ratio of Soil+Sand+Manure and kept shade house for three weeks.

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