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INDUCTION OF CALLOGENESIS AND SHOOTING IN *ASTERACANTHA LONGIFOLIA*(L.) NEES –A MEDICINAL HERB

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ABSTRACT: This study is concerned to develop a rapid system for regenerating shoots and callus from mature shoot tip explants of *Asteracantha longifolia* (L.) Nees, a medicinally important annual herb (family acanthaceae). Effective shoot and callus regeneration was observed by using several concentrations of cytokinins and auxins with MS medium. Shoot tips responded better for callusing almost in all combinations such as BA + IAA (1.0+1.0, 1.5+1.0, 1.5+1.5, 2.0+1.5 mg/lit) and BA + NAA (1.0+1.0, 1.5+1.0, 1.5+1.5, 2.0+1.5 mg/lit). Shooting was observed only in shoot tip explants when media was supplemented with growth regulators in different combinations at different concentrations such as BA (0.5, 1.0, 1.5, 2.0 mg/lit) and BA+NAA (1.0+0.5, 1.5+0.5, 1.0+1.0, 1.5+1.0). However no shooting was observed in MS media by using nodal explants containing different concentrations of BA +NAA + IAA. This approach of plant tissue culture may proved to be a helpful method to develop a rapid system of regeneration for production of medicinally important products. Also the production of aseptic plants which may decline the over exploitation of plants growing under natural condition.

INTRODUCTION: *Asteracantha longifolia* Nees is a, medicinal herb belongs to the family acanthaceae. The plant in India is distributed throughout tropical and sub tropical regions and other parts of the world including Burma, Malaya, Nepal and Phillippines, Srilanka and in many other parts of the world. It is usually found in ponds, freshwater swamps and stagnant streams and alongside river beds. *Asteracantha longifolia* (L.) Nees, finds mention in Ayurvedic treatise like 'Sushruta Samhita' and 'Charak Samhita' as Rasayan or rejuvenator.

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Different parts of plant including leaves, inflorescence, seeds, roots and ashes are diuretic in nature and have been extensively used in the preparation of herbal medicine for various ailments including jaundice, diopesy, rheumatism, hepatic obstructions and dissolutions of gallstones, kidney stones, liver disfunction and diseases of urino-genital tract [1, 2].

Asteracantha longifolia contains adiversity of biologically active compounds such as alkaloids [3], waxy substances, gum [4], minerals as Ca, Mg, K, Fe, Cu, Zn Mn, Co and Cr [5] and phytosterols [6], essential oil, a straight chain ketone [7], flavonoids, terpenoids, manganese salts, potassium chloride and sulphate and fixed oils [8]. Ethanol extract of whole plant of *Asteracantha longifolia* was examined for its anti-inflammatory and analgesic properties [9]. The plant is known to possess

antitumor [10, 11], hypoglycaemic [12], Antibacterial [13], Free radical scavenging and lipid peroxidation activities [14], hepatoprotective [15,16] and anti-nociceptive properties [17]. The methanolic extract of leaves contain phenolic and flavonoid shows promising antioxidant activity [18]. Aqueous extract of leaves of *A. longifolia* shows potent antioxidant aactivity in various *in vitro* model [8]. Speman a polyherbal formulation containig *Asteracantha longifolia* improving number and morphology of sperms [20]. Ethanolic extract of *A.Longefolia* treatment clearly affect sexual behaviour of the animals and improved attractability towards females and considerable increase in the sperm count as well as fructose levels of seminal vesicles [21]. *Asteracantha longifolia* leaf extracts may be prove to be effective in the treatment of diabetes mellitus owing to its ability to increase insulin secretion and enhance the antioxidant activity.

MATERIALS AND METHODS:

The plants were collected from Amravati region and planted in the Departmental garden of of Botany, Sant Gadge Baba Amravati University Amravati.

Excision and surface sterilization of explants: All aseptic operations were performed in laminar airflow cabinet in order to avoid contamination. Explants such as node, internode, shoot tip and leaf were selected from healthy and disease free plants. The explants were subjected to wash thoroughly with running tap water and then with sterile distilled water for 10 minutes. The explants then subjected to washing with soap solution for 2-3 minutes and followed by washing with sterile distilled water. Explants were surface sterilized with 70% alcohol for nearly 30 second , followed washing sterile distilled water, then immersed in 0.1% mercuric chloride ($HgCl_2$) for 2-3 minutes followed by rinsing with sterile double distilled water for 3-4 times in laminar air flow cabinet. Then explants were soaked by placing them on sterile tissue paper and cut the edges of the explants with sterile scalpel.

Media preparation and inoculation:

Murashige and Skoog's (1962) basal medium was prepared in sterile double distilled water and pH was adjusted to 5.8. After completion of sterilization, the explants inoculated on MS medium with different concentrations and combinations of auxin and cytokinin.

Culture conditions:

All the standard physical conditions were provided to culture *in- vitro.* The photoperiod was adjusted as 16 hours light and 8 hours dark, as per the requirement. The culture was kept at 27 $^{\pm}$ 2^0C temperature and 70% humidity.

RESULTS AND DISCUSSION:

Present investigation was carried out in the well equipped plant tissue culture laboratory. MS media supplemented with different combination of growth regulators including auxin and cytokinin i.e. IAA, NAA, and BAP respectively. Nodal explants, shoot tip and leaf were used, among these only shoot tip explants responded better.

Maximum callogenic response was seen by using shoot tip explants in different combinations of growth regulators. It was observed that the callus obtained from the shoot tip explants took nearly 20-25 days after inoculation. BA 1.5 mg/ l + NAA 1.5 mg/ l and BA 1.5 mg/ l + IAA 1.5 mg/ l showed better response. Callus obtained from this combination was healthy, yellowish white and fragile, as mentioned in **Table 1** and shown in **photo plate 1**, **Fig. A, B**. Other combination of BA + IAA and BA + NAA such as (1.0+1.0, 1.5+1.0, 2.0+1.5 mg/lit) also responded better, as mentioned in **Table 1**.

Shoot tip explants responded better also for shoot initiation and shooting in MS medium with different concentrations and combination of growth regulators as BA(0.5,1.0, 1.5, 2.0 mg/lit) and BA+NAA (1.0+0.5, 1.5+0.5, 1.0+1.0, 1.5+1.0) **Table 2**, **Fig. C, D**. No shooting was observed by using other explants in such concentrations**.**

CONCLUSION: From overall observation using different combinations of growth regulators at different concentrations. It was concluded that shoot tip responded for callusing almost in all combinations and the callus obtained was yellowish white/Yellowish green/Greenish and fragile. Also shooting was observed only in shoot tip explants when media was supplemented with growth

regulators in different combinations at different concentrations. Callus obtained through this study may prove a good tool for *in vitro* production of secondary metabolites.

TABLE 1: RESPONSE OF SHOOT TIP FOR CALLUSING TO DIFFERENT CONCENTRATIONS OF GROWTH REGULATORS SUCH AS BA + NAA, BA + IAA.

Explant used	Growth regulators in different combination and concentrations mg/l			Callus formation response %	Result	Colour and texture of callus
	BA	NAA	IAA			
Shoot tip	1.0	0.5	-	-	-	-
	1.0	1.0	-	74.73	++	yellowish green/ Fragile
	1.5	1.0	-	68.80	++	greenish/ Fragile
	1.5	1.5	-	75.92	++	yellowish white/ Fragile
	2.0	1.5	-	67.85	++	greenish/ Fragile
	1.0	-	0.5	-	++	-
	1.0	-	1.0	71.75	++	Yellowish green/ Fragile
	1.5	-	1.0	57.50	++	greenish/ Fragile
	1.5	-	1.5	73.21	++	Yellowish white/ Fragile
	2.0	-	1.5	63.50	+	Yellowish white / Fragile

+ = Small callus
++ = Large callus

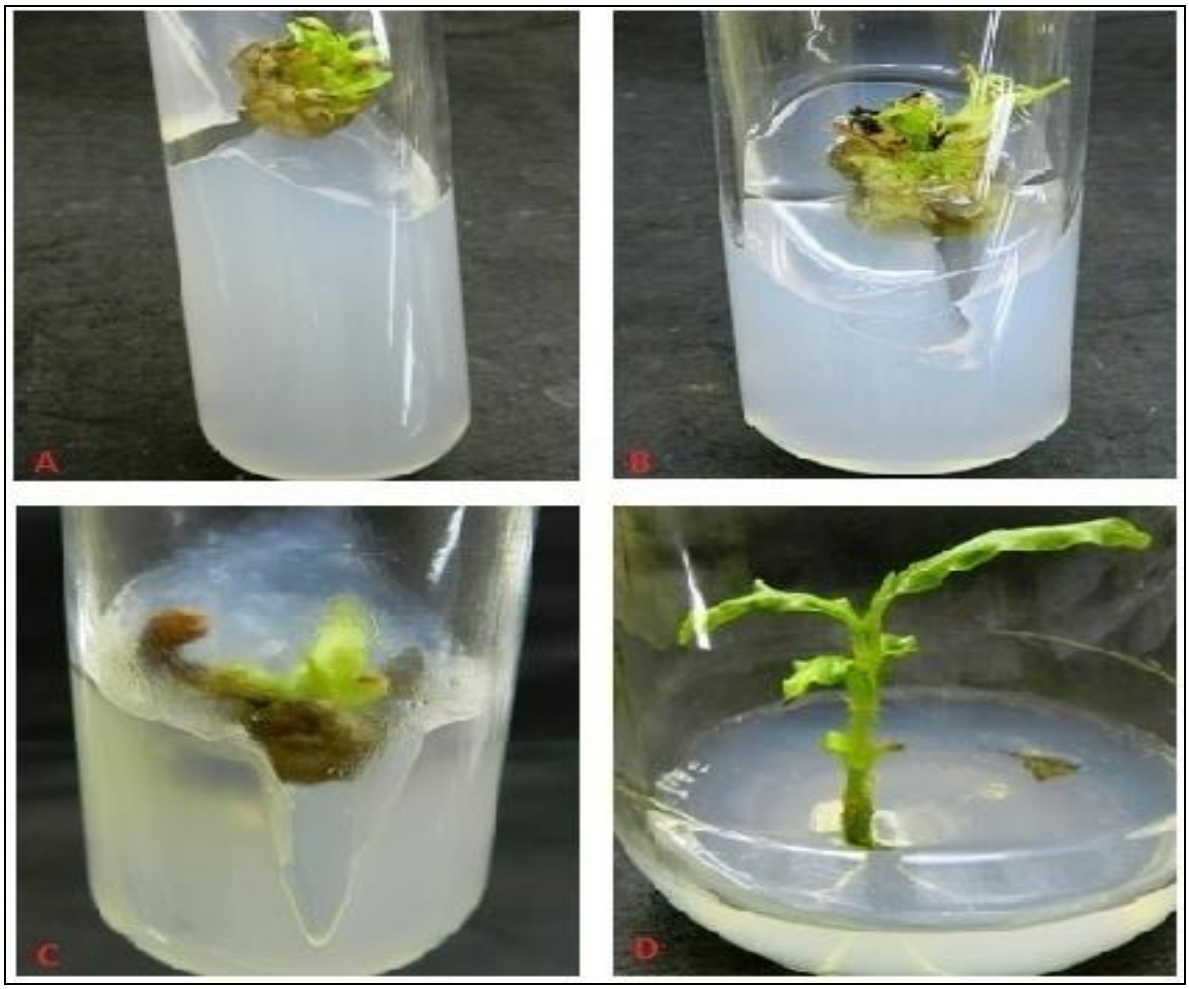

FIG. 1: CALLUS FROM SHOOT- TIP

A = Callus from shoot-tip explants at BAA 1.5 mg/ l + NAA 1.5 mg/ l.
B = Callus from shoot-tip explants at BAA 1.5 mg/ l + IAA 1.5 mg/ l.
C= Initiation of shooting from shoot tip explants at BA 1.5 mg/l.
D=Shooting from shoot tip explants at BA 1.5 mg/l + 1.0mg/l.

TABLE 2: RESPONSE OF SHOOT TIP EXPLANTS FOR SHOOTING TO DIFFERENT CONCENTRATIONS OF GROWTH REGULATORS SUCH AS BA, BA + NAA.

Explants used	Growth regulators in different combinations and concentrations (mg/lit)		Frequency of regeneration	Result
	BA	NAA		
Shoot tip	0.5	-	-	-
	1.0	-	59.72	
	1.5	-	63.42	
	2.0	-	57.50	
	1.0	0.5	67.85	Shooting
	1.5	0.5	75.92	
	1.0	1.0	79.50	
	1.5	1.0	84.16	

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