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In Vitro Callus Induction in Medicinal Plant Aerva lanata (L.) Juss.ex Schult.

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ABSTRACT

Aerva lanata (L.) Juss.ex Schult.is an important medicinal plant belongs to family Amaranthaceae. The plant is extensively used in traditional medicines and highly demandable in Ayurvedic medicine. In the present work protocol has been established for callus induction by using different explants leaves, nodes,etc. These explants were tried by using various concentrations and combinations of growth regulators .Stock callus developed from leaf and nodal segments on MS media by using BAP, KIN and IAA,NAAetc. Developed callus was healthy,soft, green, pinkish, cream in color. After every four weeks so formed callus was sub-cultured on MS media.

KEYWORDS : *In vitro, Aerva lanata*, callus, explant, subculture etc.

Introduction

Aerva lanata is an important medicinal plant belongs to family Amaranthaceae .It is commonly known as Kapurmadhuri and Pandharivasu in Maharashtra.It is mainly indigenous to South Africa, Saudi Arabia, South Asia, Srilanka and India[1].It is aperinnialher baceous plant which grows erect sometimes in prostrate habit. Leaves are simple and stalkless, entire, ovate to oblong in shape, apexobtuse, base cuneate, pubescent or wooly tomantose. Leaves arranged alternately on stem, stem branched, flowers yellowish white in colour, small and sessile. Inflo were sence axillary spike, seeds are tiny, bean shaped, smooth and black in color.Medicinal plants are the huge resource of valuable drugs which has demands in traditional healers, Ayurveda, siddha, unani medicines and in pharmaceutical industries also . It is a medicinal plant which is extensively used in Ayurvedic medicine.The plant has various important uses such as hepatoprotective[2], antidibetic[3], antimicrobial[4] and antiasthamatic[5],etc. In traditional medicines it is used for cough,sore throat, wound healing, indigestion. It is mainly used for treating reanal diseases. It is also used for curing urinary calculi *A. lanata* comprises of alkaloids,tannins,flavonoids,phenol,proteins and carbohydrates [5].The isolated alkaloids fromaerva lanata are canthin-6-one, *β*-carboline,Hentriacontane, β-sitosterol,*α-amyrin and Betuline*[6]. The plant also consist of campesterol, chrysin, narcissi, stigmasterol, stigmasterol acetate, daucosterol, erosterol, kaemferol-3-galactoside, kaemferol-3-rahmno-galacto- side & starch. It also consist of free sugars like fructose,galactose, rhamnose& sucrose [7][8].

Plant Tissue culture is one of the valuable techniques for the sustainable conservationof medicinal plants which are overexploited by the peoples for their medicinal use. It is one of theimportant tools which has tremendous potential to produce high quality plant based drugs utilized in pharmaceutical industries. It is again an important aspect that through in vitro regeneration technique clonal propagation of the plant is possible due to which genetic stability also remain conserved. So, the tissue culture is powerful bioweapon for enhancing the secondary metabolite production from medicinal plants. In the present work callus induction was achieved by using different growth hormones with base MS media.

Materials and Methods

All the experimental material (Explants) of *A.lanata was* collected from botanical garden Dr. Babasaheb Ambedkar Marathwada University Aurangabad. Leaves and nodal segment were collected from previously grown plants and used as source of explants. These explants were surface sterilized with 0.3% (w/v) $HgCl_2$ (RFCL Ltd, India) for four - six min. followed by washing with sterile distilled water 4-5 times. Leaves and Nodal segments were cut (0.5 cm) and cultured on MS-media [9] contain 0.3% (w/v) clerigar (Himedia Pvt. Ltd.,India) as a solidifying agent supplemented with plant growth regulators viz. NAA ,IAA as an auxins& BAP, KIN as a cytokinines at different combinations were tried.

Culture condition

The present research work was carried out under controlled conditions. MS medium was fortified with 3% sucrose and 0.3% Clerigar. After the addition of phytohormones the pH was adjusted to 5.8 after that MS medium was sterilized in an autoclave under 15 psi pressures and 121° C temperature for 20 minutes. Sterilized medium was transferred into laminar air flow for inoculation. After inoculation culture vessels were transferred into culture room contains 25± 2°C temperatures for 3- 4 weeks with 16 hours of photoperiod and 70% relative humidity. Data was recorded after every week and analyze by five replicated with mean ± SE.

Result and discussion

During the present research investigation both such as Nodal and leaf explants were potentially capable to regenerate the callus. These explant required as an efficiency of MS medium with optimization fortified different phytohormones. For the formation of callus MS medium enriched with different concentration and combination of growth hormoneswere tried on leaf and stem segment as an explants. The callus could be differentiated by structure and color of the callus.Suitable callus was induced on MS medium supplemented with 0.5mg BAP with 0.2 mg/L IAA and 1.0 mg BAP with 0.2 mg/L IAA. All the explantswere shown good response for the callus induction. Nodal explants inoculated on MS media with BAP and KIN gives pink color callus(A) ,green color callus(B), sometimes slightly whitish colored callus(C). But the explants treated with NAA shows only formation of cream colored callus(D). Nature of the callus also gets changed with the color of the callus .pink or green colored callus is soft in nature whereas cream colored callus is friable in nature. Callus induction was started within 10-12 days of inoculation.Within 4-6 weeks maximum callus was obtained. After 4 weeks fresh callus was collected and transferred to petriplates. Fresh weight of callus was taken and further callus was shade dried. After complete drying of callus i.e. till callus shown constant weight. Dry weight of callus was calculated (Table). BAP 1.5 mg/L along with 0.2 IAA mg/L and KIN 3.0 mg/L in combination with 0.5 IAA mg/L shows maximum callus weight. Such type of work was reported by Dalilah Abu Baker(2014) in *Celosia argentea* (var.) cristata[10].Leaf as an explant treated with NAA and IAA also shown noticeable callus weight.Similar type of study was reported by Rajanna L. et al.,(2011) in *A. lanata* by using MS medium + 2.5 mg/l 2, 4-D + 1.5 mg/l and L_2 medium from shoots and leaf segment explant[11].

Varutharaju et, al., 2014 reported direct organogenesis from leaf explant in *A. lanata* with fortified 3% of sucrose MS basal medium alone 0.25-2.0 mg/l TDZ+NAA and IBA[12]. The regeneration of shoots (86%) recorded. But in the present study direct shoot organogenesis was achieved from nodal segment explant by using BAP 1.0-3.0 mg/l + IAA 0.2-1.0 and KIN 0.6-3.0 mg/l + 0.2-1.0 IAA mg/l, both combination and optimized concentration induce callus alone with proliferation of shoots. After the 21 days nodal and leaf derived callus was sub-cultured on above standardized MS medium, maximum regeneration percentage were obtained on MS medium+BAP 1.5mg/L + IAA 0.2 mg/L & minimum regeneration percentage were obtained on MS media along with BAP 1 mg/L & NAA 2.5 mg/L.

Table 1.Effect of different concentrations and combinations of growth regulators on MS for the induction of callus from the plant Aerva *lanata*.

Growth regulators(mg/l)				Explant Used	Fresh weight of callus	Dry weight of callus	Texture of callus	Colour of Callus
BAP	Kin	NAA	IAA					
0.5			0.2	Node	9±0.57	1.3±0.30	Soft	Pink
1.0			0.2	Node	7.66±1.20	1.23±0.39	Soft	Pink
1.5			0.2	Node	11±0.57	2.2±0.14	Soft	Slightly whitish
2.0			0.2	Node	8.6±0.66	1.8±0.1	Soft	Light green
2.5			0.2	Node	10.6±0.33	2.13±0.06	Soft	Green
	0.6		0.5	Node	10±1	1.96±0.26	Soft	Light green
	1.2		0.5	Node	8.3±1.45	1.7±0.5	Soft	Light green
	1.8		0.5	Node	9.3±0.88	1.8±2.0	Soft	Slight pink
	2.4		0.5	Node	8.6±0.33	1.63±0.06	Soft	Light green
	3.0		0.5	Node	7±1	1.03±0.33	Soft	Slightly pink
1.0		0.5		Leaf	8.66±0.66	1.66±0.66	Friable	Cream
1.0		1.0		Leaf	8±1	1.33±0.33	Friable	Cream
1.0		1.5		Leaf	8±1.15	1.4±0.35	Friable	Cream
1.0		2.0		Leaf	7±0.57	1.06±0.23	Friable	Cream
1.0		2.5		Leaf	6.6±1.20	0.96±0.37	Friable	Cream

Abbreviations used in the Table: **BAP:**Benzyl Amino Purine**KIN**:Kinetin**IAA:**Indole-3-Acetic Acid **NAA:**Naphthalene Acetic Acid.

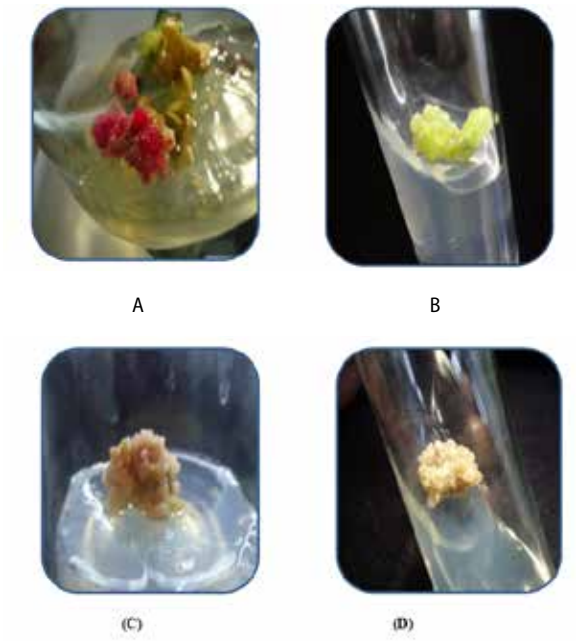

Figure 1: Different colored callus induced in *A.lanata* on MS medium

Conclusion

Present research work was concluded that *in vitro* propagation of *A. lanata required* optimum concentration of plant phytohormones as well as MS medium was the best medium induction of various types callus.BAP and KIN both are effective for callus induction in combination with IAA and NAA. Most suitable combination and concentration of BAP and IAA was 1.5 mg/l and 0.2 mg/l for KIN and IAA it was 1.8mg/l and 0.5 mg/lwas recorded respectively with maximum fresh and dry callus weight.

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