IN VTIRO CALLUS PROLIFERATION STUDIES OF SPILANTHES ACMELLA L. (MURR.) IN DIFFERENT PLANT GROWTH REGULATORS.

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Abstract : Ayuerveda is historical gift given by India to the world. Where, it is huge compilation and work of medicinal practices and herbal drugs. Akarkarbha is also named as Akalkara which is Spilanthes acmella (Murr.) also mentioned in some texts of ayurvedic book. Its propagation by seed has so many consequences hence by using in vitro prorogation techniques this plant can be conserved. Micropropogation studies on various growth hormones reported callus formation for mass multiplication and in vitro pharmaceutical drug preparation. Experiment reported that among different explants leaf explant takes lower days (9.90 ± 0.40))for callus formation with 80% callus response in MS medium fortified with BAP (2mg/l) than other combination of Auxins. The callus growth reported is massive healthy with 2300 mg fresh weight. Higher callus fresh weight (2600 mg) observed in BAP (3mg/l) in leaf explants. The callus is firm and friable texture with greenish and yellow with slight white in color. After callus formation this callus is supplemented with Auxin and Cytokinin for root and shoots formation, then after transfer it for hardening when good rooted plants are formed. Hardening response is seen higher 80% (Survival rate) in 2:1 proportion of cocopeat and soil in-vitro potting media.

Index Terms - Spilanthes acmella, Callus, BAP, Kinetin. etc.

I. INTRODUCTION

Among 35 biodiversity hotspots of the world India has four hotspots Himalaya, Indo-Burma, Sundaland and Western Ghats with rich flora and fauna, some of them not found elsewhere. This important herbs and plants are going to be in endangered and threatened category if not conserved properly. One reason of its exploitation is vast harvesting of herbs for pharmaceutical drug preparation. Among these medicinal plants Akalkarara which widely called as antitoothache plant also found in endangered category. Biotechnological tool such as tissue culture is helpful to avoid such exploitation with in-vitro production of plant and callus which are helpful for preparation of pharmaceutical drugs in large quantity.

In ayuerveda some texts like Charaksahmita, Shusuritsahmita etc. akkalkara plant is mentioned as akkarkarbha in Sanskrit text [18]. These plant recorded so many drug formulations and remedies on various diseases. One important remedy is on sexual disorders hence now medicinally over exploited for pharmaceutical drugs and medicines like in Viagra [15]. Botanically *Spilanthes* plant belongs to the Asteraceae family [12] with herbaceous in nature and grown up to 2- 3 feet tall. *Spilanthes acmella* plant has recorded more than 300 species [2] [8] but in India there are nine species recorded among which *Spilanthes acmella* is most acute threatened species which is similar to *Acmella oleracea* [16] [17]. It has golden flower buds with pink top [09] and numbness or acrid in taste when chewed [4] and [05] [19].

Medicinally it is used in various herbal tooth paste and powder but it also widely used in other diseases like mouth ulcers, wounds and boils [11], stomatitis, stammering, gingivitis, and throat complaints [09] [13], snake bite [1] and [14] etc.

This plant has low germination and availability of seed in large quantity and its storage difficulty in humid condition is hammering its propagation. Hence for conservation point of view, pharmaceutical and propagation point of view this plant is cultivated in in-vitro condition.

II. Materials and Methods

Healthy seeds are procured from Dhanvantari udyan, Rahuri and germinated in well potted condition. These seedlings are raised in various pots with using suitable media and their nodes, leaf and meristem are excised and used as explants for further tissue culture process. Healthy diseases free explants are washed under running tap water for 15-20 minutes to remove its dirt and debris. Then these explants are again three times washed with distilled water each for 5 minutes and then surface sterilized with using 1 % Bavistin solution and 0.3 % Streptomycin solution each for 10 minutes. The excess quality of chemicals is diluted by washing all explants in distilled water for 10 minutes each two times. MS media with different proportion (1-5 mg/l) of BAP and Kinetin are inoculated and sterilized with hot bath, autoclaved method at 15 lb pressure and 1210C for 15 minutes. These bottles are UV sterilized under laminar air flow and all operations are carried out under this chamber. Explants are surface sterilized with 0.1 % $HgCl_2$ for 4 minutes and then after three times distilled water washing is carried out of 10 minutes each to remove excess part of chemicals. All explants are trimmed at the end in cross section to expose their ends with using ethano sterilized and heated scissor and forceps. These explants are carefully inoculated in tissue culture bottle containing MS media and cytokinin All bottles kept under 16/8 of light /dark condition environment with 25±2 0C temperature and 80-90 % relative humidity.

The callus observations are recorded after initiation of callus process. This callus is again supplied with different root and shoot formation media for further organogenesis and the rooted plants are shifted in various potting media for hardening response.

III. Statistical analysis.

Callus proliferation observations are recorded at periodic intervals and data are analyzed with one way ANOVA and Duncan Multiple Range Test (DMRT). Statistical Package for Social Science (SPSS, version 11.5) software at 5% level ($p<0.05$) are used for statistical analysis All observations are initially calculated with their mean ± SE. Each treatment is repeated three times with 20 bottles each.

IV. Result:

In vitro callus formation studies are studied on *Spilanthes acmella Murr.*, with using different types and combination of BAP and Kinetin (1-5 mg.l) on three explants i.e. leaf, nodal segment and meristem. Results (Table and Graph No.1 & 2) reported that among three types o explants leaf are takes lower days for callus formation with higher callus fresh weight followed by nodal segments. Among all explants and plant growth regulators, leaf explants in MS media fortified with BAP (2mg/l) takes lower day (9.90 ± 0.40) for callus formation along with 80 % callus formation response (Plate No 1). Callus obtained in these media gives 2300 mg of fresh weight and massive growth with firm and friable texture and greenish creamy callus color. Higher callus fresh weight (2600 mg) obtained in BAP (3 mg/l) in leaf explants where callus is green, yellowish and white.

In nodal segment lower days (11.37 ± 0.39) are reported in Kinetin (2mg/l) supplemented media with 60% callus response and 2190 mg fresh weight of callus (Table No.2 and Graph 1 and 2). Higher callus fresh weight (2340 mg) in nodes are obtained on MS media fortified with BAP (2mg/l) with 70% culture response. The callus is firm and friable in texture with green creamy color.

In meristem explants higher callus growth (Fresh weight 2210 mg) with lower days for callus formation (10.93 ± 0.39) and higher culture response are reported in BAP (2 mg/l) supplemented media (Table No.1 and Graph No.1 and 2). But callus growth is moderate with green creamy firm and friable callus. In Kinetin plant growth regulator at 5 mg/l of concentration no callus formation is observed in nodal segmen and meristem explant.

V. Discussion:

Effective use of cytokinin in callus formation are studied out with using three types of explants where leaf explants shows better results followed by nodes and meristem. Its shows hard cuticle or epidermis of node and meristem may result less callus response than leaf. Leaf also gets more exposure due to its wider surface area.

In leaf and meristem explants MS media supplemented with lower concentration of BAP (2 mg/l) take lower days i.e. 9.90 and 10.93 days respectively for callus initiation. This shows that lower concentration (1-3 mg/l) of BAP is most effective than higher one (4-5 mg/l). Similar results is found in *D. insignis* where at lower concentration (0.01 to 2.0 mg/l) of BA report better callus formation [06]. This because higher concentration cause synergistic and toxic effect to the explants.

In nodal segment Kinetin at 2 mg/l takes lower days for callus (11.37 ± 0.39)-formation along with higher callus response (60%) and maximum fresh weight (2190 mg/l) after BAP (2mg/l) where fresh weight is higher i.e. 2340 mg/l. This shows that BAP is most effective to increase callus weight in all explants at lower concentration (1-3). Callus formation is depends on plant growth regulator, types of explants synergistic effect of hormones etc. [03] [13]. Higher concentration leads to the chromosomal instability of the regenerated plants [07] which is supported in our experiment where nodal segment and meristem explants supplied with Kinetin at 5 mg/l does not formed callus.

VI. Hardening:

Well rooted plants from callus organogenesis method are transplanted in various composition media. Initially the plants are supplied with Bavistin (1-2%) drenching to avoid fungal infection. Highest survival rate in in-vitro chamber, shade house and in open field are found in 2:1 proportion of cocopeat and soil i.e. 100%, 90% and 80 % respectively. This shows cocopeat and soil both are suitable for hardening where cocopeat takes higher role in plant survival.

VII Conclusion:

In vitro callus proliferation studies shows that among two cytokine (BAP and Kinetin) BAP at 2 mg/l and 3 mg/l are suitable for leaf and meristem segment while Kinetin (2mg/l) is suitable for Nodal segment. The higher concentration of (5mg/l) is ineffective for callus formation in nodal segment and meristem. Among three explants leaf are more suitable for callus formation than nodes and meristem. For hardening survival the rooted plats planted in 2:1 proportion of cocopeat and soil media are most effective with 80 % survival rate in open field.

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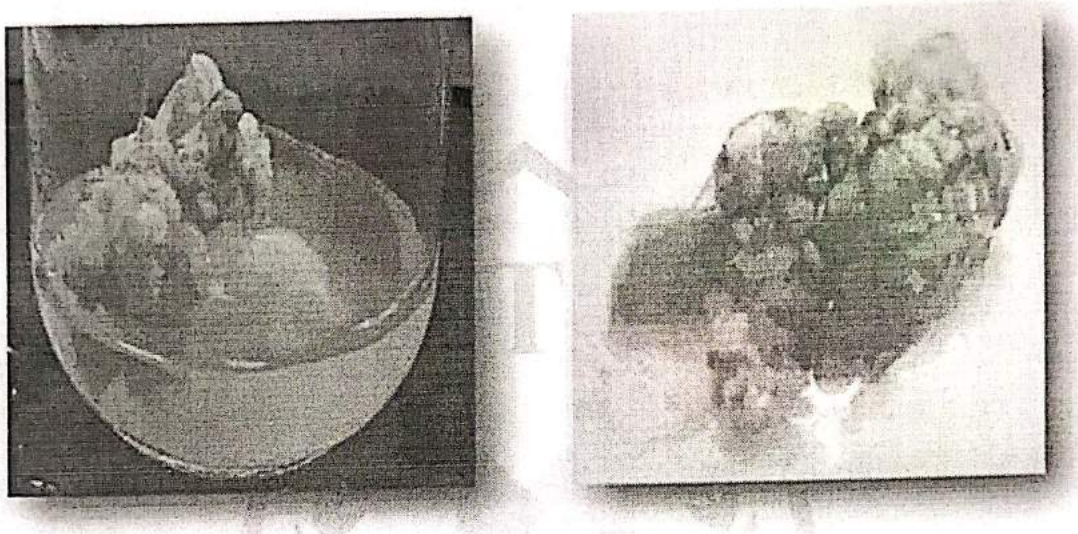

(a) **M.S. + BAP. 3.0 mg/l (Leaf)** (b) **M.S. + Kin. 2.0 mg/l (Meristem)**

Plate No.1 In-vitro Callus Formation Response.

Table No 1: Effect of BAP on Callus Formation

Explants	Conc. BAP	No of Days for Callus (Mean ± SE)	% of Callus Form.	Texture	Color	Growth	Weight of callus After 90 Days (Mean ± SE)
Leaf	1	10.87 ± 0.47	70	FF	GW	C^{+}	2100
	2	9.90 ± 0.40	80	FF	GC	C^{+++}	2300
	3	11.83 ± 0.55	60	FF	GYW	C^{+++}	2600
	4	14.43 ± 0.56	50	L	W	C^{+}	1900
	5	15.00 ± 0.56	50	L	B	C^{+}	1980
Nodal Segments	1	12.23 ± 0.61	60	FF	GC	C^{+}	2040
	2	11.80 ± 0.44	70	FF	GC	C^{+++}	2340
	3	13.28 ± 0.56	50	SF	YW	C^{++}	2190
	4	14.43 ± 0.72	50	L	W	C^{++}	1970
	5	16.13 ± 0.66	40	L	B	C^{+}	1890
Meristem	1	12.03 ± 0.30	60	FF	GW	C^{+}	2200
	2	10.53 ± 0.36	70	FF	GC	C^{+++}	2210
	3	12.83 ± 0.44	70	SF	GW	C^{++}	2120
	4	14.10 ± 0.61	60	L	YW	C^{++}	1940
	5	15.67 ± 0.54	50	L	W	C^{+}	1900

Table No. 2 Effect of Kinetin on Callus Formation

Explants	Conc. BAP	No of Days for Callus (Mean ± SE)	% of Callus Form.	Texture	Color	Growth	Weight of callus After 90 Days (Mean ± SE)
Leaf	1	13.87 ± 0.56	60	SF	GW	C^{+++}	2100
	2	12.70 ± 0.43	70	FF	GC	C^{+++}	2200
	3	10.23 ± 0.39	80	FF	GC	C^{+}	1980
	4	14.20 ± 0.53	60	SF	GW	C^{+}	1780
	5	15.44 ± 0.47	50	L	W	C^{+}	1670
Nodal Segments	1	12.87 ± 0.44	60	FF	GW	C^{+}	1840
	2	11.37 ± 0.39	60	FF	GC	C^{+++}	2190
	3	13.70 ± 0.63	50	SF	YW	C^{+}	1900
	4	14.87 ± 0.52	40	L	W	C^{+}	1890
	5	--	--	--	----	--	--
Meristem	1	11.77 ± 0.41	60	SF	GW	C^{+}	1780
	2	10.93 ± 0.39	70	FF	GC	C^{++}	1910
	3	13.60 ± 0.42	60	L	YW	C^{+}	1880
	4	14.60 ± 0.60	50	--	B	C^{+}	1840
	5	----	---	--	---	----	----

Growth of Callus: (--) No Callus, (C^{+}) Poor Callus, (C^{++}) Moderate Callus, (C^{+++}) Massive Callus. **Color of Callus:** (GC) - Greenish, Creamy, (GW) - Greenish, White, (GYW) - Greenish and Yellow with slight White,(YW) - Yellowish with Slight Whitish, (W) - Whitish, (CB) – Creamy, Browning. **Texture of Callus:** (FF) - Firm and Friable, (SF) - Slightly Firm and Friable, (L) – Loose