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Research Paper

In Vitro Shoot Proliferation Studies of *Spilanthes acmella* L. (MURR.) in Different Plant Growth Regulators

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ABSTRACT

Spilanthes acmella Mur., is a popularly called antitoothache plant. These are propagated in vitro condition with various plant growth regulators to avoid ex situ exploitation. Results reported that maximum shoot proliferations are observed in BAP (3 mg/l). In MS medium fortified with BAP (3 mg/l) takes 10.07 days for shoot formation along with 80% shoot formation. It also revealed that BAP (3 mg/l) produce the highest length of shoot (3.7 cm) and leaf length (2. 8 cm) after 8 weeks. It also gets maximum number of a shoot (4.6) and leaf (9.9) per explants. In auxin and cytokinin combination IBA (1 mg/l) together with BAP (3 mg/l) takes the lowest day for shoot proliferation (10.03) along with 4.3 number of shoot and 80% shoot formation. While, IBA (1 mg/l) + BAP (2 mg/l) gives lengthy shoot (3.3 cm) than any other combination.

Key words: Auxin, Cytokinin, Hardening, Shoot proliferation, *Spilanthes acmella* (Murr.)

India is lands of knowledge and culture where Ayurveda is one of the ancient knowledges transferred and distributed to other peoples and country from one generation to other. Various medicinal plants with their uses are recorded in Ayurvedic books such as Charaksamhita, Sushrutsamhita etc. Akkalkara is mentioned as akkarkarbha in Sanskrit text [1]. It has remedial properties against various sexual disorders hence now medicinally overexploited for pharmaceutical drugs and medicines like in Viagra [2]. Basically, these plants belong to the Asteraceae family and grow up to 2 to 3 feet in height. It has flower buds in golden or yellow color with pink to purple tinge at the top [3]. In India near around 9 species are reported which in the stage of threatened species which is similar to Acmella [4-5].

It contains *Spilanthol* ($C_{14}H_{23}NO$) [6-7], stigmasterol, alkylamides, saponine, β-Sitosterol, α and β- Amyrin [8], Myricyl alcohol and pentacyclic triterpene [9-10] etc. Hence it widely used to cure wounds and cuts [11], scabies, scurvy, psoriasis, paralysis of the tongue, high cough, nausea, digestion problem etc. [12-13]. It also used to minimize labor pain [14], tuberculosis and pneumonia [15]. Due to this pharmaceutical importance and requirements in drugs formulations, it is widely exploited in forest area however it has slow germination problem to maintain its diversity. Keeping in the above constraint we derived in vitro shoot production (Biomass) by using various plant growth regulators.

MATERIALS AND METHODS

The plants are procured from the local farmers filed and cultivated in Shade House, Department of Botany, S. M. Dnyandeo Mohekar College Kalamb. Nodal segments with 6 to 7 weeks old are selected from healthy plants. These explants are rinsed with running tap water for 30 minutes and again washed 2 times with distilled water each for 5 minutes after 5 minutes of Dettol detergent treatment (2%). These explants are then cut into 4-5 cm length and transferred in 2% Bavistin and 0.03% Streptomycin solution. After each treatment, it rinsed with using distilled water each for 10 minutes. These explants are then transferred in laminar air flow (Previously UV treated for 5 minutes). The MS media along with predetermined plant growth regulators are autoclaved for 15 lb pressure for 15 -20 minutes. These autoclaved media are again keeping in laminar air flow for UV sterilization. All explants are treated with 70% ethanol and 0.01% $HgCl_2$ for 4 minutes. These sterilized explants are repeatedly washed for three times with autoclaved distilled water each for 10 minutes. The explant ends are cut with a sharp scissor to get increased surface area and exposure to the MS media. All inoculated explants are kept in tissue culture lab with 25 ± 2°C temperature. After three and half month when proper rooting is formed it shifted to the (2:1) soil and cocopeat media and their hardening response are checked for 15 days.

Statistical analysis

Shoot proliferation reading is recorded manually after 8 weeks and 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

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calculated with their mean ± SE. Each treatment is repeated three times with 20 bottles each.

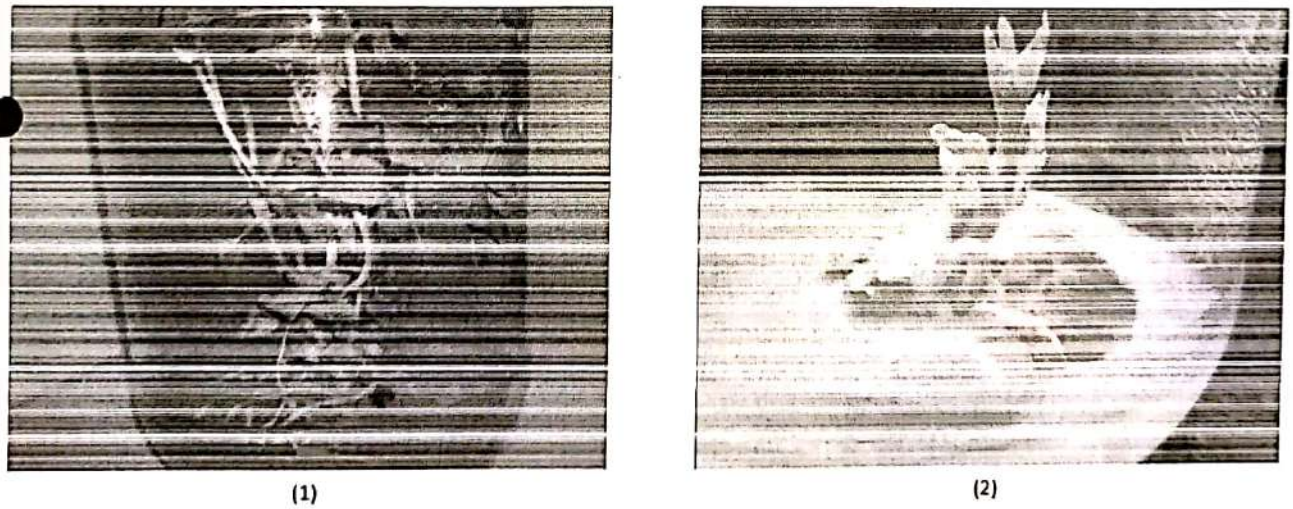
RESULTS AND DISCUSSION

An experiment carried out in *Spilanthes acmella Murr.*, using different types and combination of auxins (IAA, IBA and 2,4, D) and Cytokinin (BAP, Kinetin). Results revealed that MS medium together with BAP (1-3 mg/l) is best among other cytokinins in shoot proliferation. It takes the lowest day for shoot formation (10.07), with the highest number of a shoot (4.6) and leaf (9.9) when MS medium fortified with BAP (3 mg/l). While it also found that highest shoot length (3.7 cm) and leaf length (2.8 cm) is reported in BAP (3 mg/l) followed by (BAP 4 mg/l).

When the medium is fortified with using Kinetin (2 mg/l) lowest day for shoot formation (11.17) along with the highest number of a shoot (3.5) and leaf (8.7). But highest shoot length (3.1 cm) and leaf length (2.5 cm) is reported in 2 mg/l of Kinetin along with MS medium. In GA supplemented MS media lowest day (14.47) taken by 4 mg/l of GA with 80% shoot formation. The maximum number of a shoot (3.5) and leaf (7.8) along with maximum shoot length (3.2 cm) and leaf length (2.3 cm) are observed in the same concentration of GA. In auxins and cytokinin combination IBA (2 mg/l) in addition with (BAP 3mg/l) are takes the lowest day (10.03) for shoot formation along with 80% shoot formation response and highest shoot number (4.3). While IBA (1 mg/l) together with BAP (2 mg/l) gives lengthy shoot (3.3 cm). In auxin and Kinetin combination IBA (2 mg/l) supplied with Kinetin (2 mg/l) are gives 80% culture response with lowest day for shoot formation (10.43). When 2, 4 D (1 mg/l) is supplied with Kinetin (3 mg/l) it gives a maximum number of a shoot (4.2) and shoots length (3.0 cm).

Table 1 Effect of BAP on Shoot Initiation and Multiplication at Various Concentrations

Treatment	No of days for shoot formation (Mean ± SE)	% of shoot formation	No. of shoot / explants (Mean ± SE	Length of shoot / explants cm (Mean ± SE)	No of leaf / explants (Mean ± SE)	Length of shoot / explants cm (Mean ± SE)
M.S. + BAP 1	15.73 ± 0.23	50	2.1 ± 0.10	2.3 ± 0.15	6.5 ± 0.43	2.1 ± 0.28
M.S. + BAP 2	12.93 ± 0.30	60	3.5 ± 0.17	2.7 ± 0.15	7.8 ± 0.51	2.3 ± 0.21
M.S. + BAP 3	10.07 ± 0.21	80	4.6 ± 0.22	3.7± 0.15	9.9 ± 0.28	2.8 ± 0.13
M.S. + BAP 4	10.17 ± 0.24	70	4.5 ± 0.22	3.4 ± 0.16	8.7 ± 0.15	2.5 ± 0.17
M.S. + BAP 5	12.47 ± 0.32	70	4.4 ± 0.22	2.9 ± 0.10	8.5 ± 0.31	1.8 ± 0.13
M.S. + Kin 1	15.67 ± 0.58	50	2.4 ± 0.34	2.1 ± 0.23	6.1 ± 0.18	1.9 ± 0.10
M.S. + Kin 2	14.77 ± 0.66	70	3.1 ± 0.28	3.1 ± 0.31	7.0 ± 0.21	2.5 ± 0.17
M.S. + Kin. 3	11.17 ± 0.63	80	3.5 ± 0.17	2.8 ± 0.33	8.7 ± 0.42	2.4 ± 0.22
M.S. + Kin. 4	12.57 ± 0.78	80	3 ± 0.15	2.4 ± 0.22	7.3 ± 0.26	2.1 ± 0.10
M.S. + Kin. 5	13.97 ± 0.74	80	2.4 ± 0.22	2.1 ± 0.23	6.5 ± 0.31	1.8 ± 0.29
M.S. + GA 1	18.87 ± 0.65	50	2.1 ± 0.18	2.9 ± 0.10	6.6 ± 0.31	1.4 ± 0.22
M.S. + GA 2	18.10 ± 0.76	70	2.4 ± 0.22	2.9 ± 0.10	7 ± 0.30	2.0 ± 0.15
M.S. + GA 3	15.93 ± 1.01	80	3.0 ± 0.21	3.0 ± 0.21	7.1 ± 0.31	2.2 ± 0.25
M.S. + GA 4	14.47 ± 1.02	80	3.3 ± 0.21	3.2 ± 0.20	7.8 ± 0.33	2.3 ± 0.15
M.S. + GA 5	15.57 ± 0.93	70	3.1 ± 0.18	3.1 ± 0.10	7.7 ± 0.30	1.8 ± 0.13



(1) (2)

Plate (1) M S + BAP (3 mg/l) and (2) M S + IBA (2mg/l) + BAP (2 mg/l) in vitro shoot formation response

In our in vitro shoot proliferation studies, it is found that alone use of cytokinin is better than use with the combination of auxin where BAP (3 mg/l) is the most effective for shoot proliferation. The results show that the role of cytokinin specifically BAP is the most effective for the maximum number of a shoot, leaf, shoot and leaf length with highest shoot proliferation response than use with the combination of auxins. Similar results reported in whereas when IBA with BAP is in combination can result in early shoot formation.

Higher concentration does not give any better response [16], where they found that higher concentration of BA could induce the formation of callus tissue but leads to chromosomal

instability along with necrosis [17]. Even in papaya explants [18] and *Tectona grandis* plants [19] the same observations recorded for a higher dose of BA. However higher concentration (5 mg/l) of BAP also reported in *S. acmella* plant for a higher multiple shoot (31.5 /explants) with shoot formation (97%) [20]. It also reported that combination of BAP, Kinetin or NAA is not so much effective than individual BAP. Increasing the concentration of BAP leads to the various problems which are reported in *Artmesia vulgaris* [21]. The superiority of BAP over Kinetin is reported in *Stevia rahaudiana* [22] and *Coleus blumei* [23] emphasized results of BAP. Synergistic effect recorded in auxin and cytokinin combination but a lower concentration of combination leads positive impact than higher concentration. However, IBA or IAA and BA combination is not much responsive than NAA with BA [24].

Table 2 Effect of combination of Auxins with BAP and Kinetin on direct shoot initiation

Conc. of	Effect of combination of Auxins with BAP					Effect of combination of Auxins with Kinetin				
	Conc. of BAP mg/l	No. of days for shoot formation (Mean±SE)	Percent of shoot formation	No. of shoot (Mean±SE)	Shoot length cm (Mean±SE)	Conc. of BAP mg/l	No. of days for shoot formation (Mean±SE)	Percent of shoot formation	No. of shoot (Mean ± SE)	Shoot length cm (Mean±SE)
IAA 1 mg/l	1	14.13 ± 0.60	60	3.5 ± 0.31	3.0 ± 0.15	1	13.17 ± 0.25	70	2.9 ± 0.23	2.4 ± 0.16
	2	10.13 ± 0.34	80	4.0 ± 0.21	3.1 ± 0.18	2	12.07 ± 0.49	80	3.1 ± 0.18	2.6 ± 0.16
	3	11.13 ± 0.48	70	3.1 ± 0.10	2.8 ± 0.13	3	12.10 ± 0.66	60	3.2 ± 0.13	2.0 ± 0.26
	4	12.13 ± 0.49	60	3.0 ± 0.15	2.3 ± 0.15	4	14.13 ± 0.54	60	2.8 ± 0.13	1.9 ± 0.21
	5	15.17 ± 0.45	60	2.7 ± 0.15	2.0 ± 0.15	5	14.83 ± 0.93	50	2.4 ± 0.16	2.3 ± 0.15
IAA 2 mg/l	1	15.10 ± 0.38	70	2.8 ± 0.13	2.6 ± 0.16	1	14.13 ± 0.57	80	2.5 ± 0.22	2.5 ±0.17
	2	12.20 ± 0.42	80	3.4 ± 0.16	2.9 ± 0.10	2	11.10 ± 0.49	80	3.2 ± 0.20	2.7 ±0.26
	3	12.07 ± 0.55	80	3.8 ± 0.13	2.7 ± 0.26	3	13.10 ± 0.46	80	2.9 ± 0.10	2.3 ± 0.15
	4	14.20 ± 0.58	60	3.7 ± 0.15	2.1 ± 0.10	4	14.87 ± 0.61	70	2.4 ± 0.16	2.1 ± 0.10
	5	16.13 ± 0.51	50	3.2 ± 0.20	2.0 ± 0.26	5	15.57 ± 0.46	70	2.3 ± 0.15	1.9 ± 0.23
IBA 1 mg/l	1	14.97 ± 0.53	60	3.6 ± 0.37	3.1 ± 0.18	1	11.97 ± 0.37	70	3.8 ± 0.36	2.7 ± 0.26
	2	12.67 ± 0.55	70	4.1 ± 0.18	3.3 ± 0.15	2	10.73 ± 0.34	80	4.1 ± 0.18	2.9 ± 0.23
	3	10.03 ± 0.49	80	4.3 ± 0.26	3.0 ± 0.15	3	12.37 ± 0.66	70	3.6 ± 0.16	2.5 ± 0.27
	4	11.63 ± 0.48	70	3.8 ± 0.25	2.6 ± 0.16	4	13.37 ± 0.56	60	3.1 ± 0.10	2.1 ± 0.28
	5	15.40 ± 0.48	60	3.3 ± 0.26	2.2 ± 0.20	5	15.37 ± 0.94	50	2.6 ± 0.16	1.9 ± 0.10
IBA 2 mg/l	1	11.77 ± 0.67	70	3.9 ± 0.18	3.1 ± 0.23	1	12.00 ± 0.62	70	2.3 ± 0.15	2.8 ± 0.25
	2	12.67 ± 0.40	80	3.7 ± 0.21	2.9 ± 0.18	2	10.43 ± 0.49	80	3.9 ± 0.38	2.5 ± 0.22
	3	13.07 ± 0.50	60	2.9 ± 0.23	2.4 ± 0.22	3	12.77 ± 0.47	70	4.0 ± 0.26	2.2 ± 0.29
	4	14.50 ± 0.60	60	2.5 ± 0.27	2.2 ± 0.13	4	13.10 ± 0.67	60	3.6 ± 0.30	2.0 ± 0.15
	5	15.87 ± 0.57	40	2.1 ± 0.10	2.0 ± 0.82	5	15.00 ± 0.47	50	2.8 ± 0.29	2.0 ± 0.30
2,4-D 1 mg/l	1	14.03 ± 0.61	60	2.9 ±0.35	2.8 ± 0.13	1	13.17 ± 0.25	70	2.6 ± 0.22	2.3 ± 0.21
	2	12.87 ± 0.68	60	3.7 ± 0.26	2.9 ± 0.18	2	10.57 ± 0.33	80	3.6 ± 0.22	2.5 ± 0.17
	3	10.87 ± 0.48	80	3.9 ± 0.18	3.5 ± 0.22	3	12.10 ± 0.66	70	4.2 ±0.29	3.0 ± 0.30
	4	11.80 ± 0.60	70	3.4 ± 0.16	3.2 ± 0.25	4	14.13 ± 0.54	70	4.1 ± 0.23	2.7 ± 0.33
	5	14.87 ± 0.53	60	3.1 ± 0.23	2.8 ± 0.29	5	14.83 ± 0.93	60	3.5 ± 0.22	2.4 ± 0.16
2,4-D 2 mg/l	1	13.50 ± 0.62	60	2.9 ± 0.23	2.7 ± 0.15	1	12.60 ± 0.55	70	2.9 ± 0.28	2.2 ± 0.25
	2	11.67 ± 0.45	80	3.7 ± 0.15	3.4 ± 0.22	2	11.17 ± 0.48	70	3.1 ± 0.18	2.6 ± 0.27
	3	12.43 ± 0.54	70	3.4 ± 0.16	3.0 ± 0.26	3	10.97 ± 0.50	60	3.9 ± 0.23	2.9 ± 0.28
	4	12.77 ± 0.60	60	2.9 ± 0.28	2.8 ± 0.29	4	12.80 ± 0.73	50	3.2 ± 0.25	2.4 ± 0.16
	5	14.70 ± 0.68	50	2.5 ± 0.17	2.4 ± 0.22	5	13.67 ± 0.66	50	2.8 ± 0.29	2.1 ± 0.28

Hardening

Hardening is carried out in different types of media. The rooted explants are shifted in shade house with proper care and practice. The hardening media soil and cocopeat (2:1) are most effective with more than 80% survival rate are obtained in cultured bottles. This carry bag is initially supplied with ½ strength M.S. media to maintain changing nutrient media. Later all cultured pants are irrigated with normal water with frequent application of Bavistin (1-2%) for avoiding soil borne fungus problem.

CONCLUSIONS

Experiment on in vitro shoot proliferation is revealed that individual uses of cytokinin are better for shoot proliferation as compare to auxin and cytokinin combination. The maximum number of a shoot (4.6) and leaf (9.9) are reported in BAP (3 mg/l) along with the highest length of shoot (3.7 cm) and leaf (2.8 cm). However early shoot formations are effectively observed in auxin (IBA 2 mg/l) and cytokinin (BAP 2 mg/l) combination. Hardening response is best (more than 80%) in (2:1) proportion of soil and cocopeat media.

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