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Effect of various plant growth regulators on in vitro root proliferation studies of *Spilanthes acmella L. (Murr.)* medicinal plant

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Abstract
In-vitro propagation studies are effective tool for the conserving rare medicinal plants. *Spilanthes acmella Mur.*, is a well-known medicinal plant which termed as antitoothache plant because of its major medicinal properties. Over exploitation of these plant will leads to use of *in-vitro* propagation studies in different plant growth regulators. Results revealed that in nodal segment explant the use of IBA hormone (3 mg/l) along with MS media are most effective in earlier root formation (16.50 days) along with 80 % vigorous root formation response. Same concentration gives 25.5 number of roots/per explant and maximum length (13.5 cm) within eight weeks. In auxin and cytokinin combinations studies maximum number of roots (21.2) and root length (11.8 cm) in addition with 80 % vigorous root formation has reported in IBA (1 mg/l) when combined with BAP (3 mg/l). The roots are thicker, longer, fibrous, healthy and vigorously grown in these combinations than any other combination. The hardening responses has recorded with different media combination and coco peat (80 %) + soil (20 %) are found to be the best suitable media for its 100 % survival.

Keywords: *Spilanthes acmella (Murr.)*, auxin, cytokinin, hardening, root proliferation etc

Introduction

In ancient times where no discovery of medicinal drugs and tablet the various kinds of herbal plants and their drugs are used to cure various diseases. India is one of those countries where various medicinal plants are used in Ayurveda the well-known treaties of medicinal plants and their uses. The ethnobotanical studies reported various importance of medicinal plants. These kinds of knowledge are stored and shared form one person to another in oral and in well written sources. *Spilanthes acmella* is one of the known species which recorded as akkarkarbha in Sanskrit text (Vaidya Bapalal G, 1968) [30]. As most the pharmaceutical drugs are prepared from herbal parts form the various plants. These drugs are widely exploited plant materials which leads to them in threatened or endangered category. Henceforth *in-vitro* propagation and multiplication can avoid overexploitation in in-situ condition and easily extracted various drugs in *in-vitro* condition. Extensive pharmaceutical studies on these plants revealed that it has various medicinal properties such as antibacterial, antimalarial, antiviral, antiseptic agent, antifungal treatment, and as a remedy for toothache, flu, cough, rabies diseases, (Sharma and Pegu, 2011, Revathi and Parimelazhghan, 2010) [26, 20]. The root of the plant are used to cure Tuberculosis (*Atiqur et al.*, 2007) [2], gargle for tooth pain and in dysentery (Balangcod and Balangcod, 2011) [4], Dental carries (Badgujar *et al.*, 2008) [3] and In Madhya Pradesh state root paste of the plant is used in throat problems in Chindwara and Betul district (Vijendra and Kumar, 2010) [13].

Whole plant has various phytochmeicals like spilanthol, N-isobutylamides such as undeca-2E,7Z,9E-trienoic acid isobutylamide 2 and undeca-2E-en-8,10-diyonic acid isobutylamide 3 (Raduner *et al.*, 2006, Ramsewak *et al.*, 1999) [21, 18], 2E-N-(2-methylbutyl)-2-undecene- 8,10-diynamide; 2E,7Z-N-isobutyl-2,7-tridecadiene-10,12-diynamide; and 7Z-N-isobutyl-7-tridecene-10,12-diynamide (Nakatani and Nagashima 1992) [14], Lauric, myristic, palmitic linoleic, and linolenic acids as their methyl esters (Molinatorres *et al.*, 1996) [7] etc., while root has triterpenoidal saponin olean-12-en-3-O-β-D-galactopyranosyl (1→4)-O-α-L-rhamnopyranoside was isolated from the roots of *S. acmella*. (Mukharya and Ansari, 1987) [17]. *Spilanthes acmella Murr.* plant unclearly reported from Brazilian acmella species. *Spilanthes* is a genus of African and South American plants. These comes in sunflower tribe within daisy family (Jansen, 1981) [8]. It also known as antitoothache plant, buzz butoottons, tingflowers (Due to its tingalness) and electric daisy. IT belongs to the asteraceae family contains more than 60 species in various regions like Africa, America, Borneo, India, Srilanka and Asia. (Sahu *et al.*, 2011; Tiwari *et al.*, 2011) [22, 29].

As this pharmaceutical importance of these plant and wide exploitation for these reasons we constraint to focus on *in-vitro* propagation studies of these plant where root formation response is studied out in various plant growth hormones.

Materials and Methods

The *in-vitro* studies are carried out in the Department of Botany, S. M. Dnyandeo Mohekar College Kalamb laboratory where the medicinal plant *Spilanthes acmela Murr.* are raised in our shade house which are collected from various location of farmers. The healthy nodal segments are selected from the selected well washed and sterilized plant part. These explants are again rinsed with normal water for 30 minutes followed by two times of

distilled water washing and Dettol (2%) detergent treatment each for five minutes. These explants are then excised in to 4-5 cm length and transferred in 2 % Bavistin and 0.03 % Streptomycin solution. For removal of excess chemical it rinsed with double distilled water for 10 minutes. The sterilized explants then transferred in laminar air flow (Previously UV treated for 5 minutes). The standardized MS media along with IAA, IBA and 2 4 D plant growth regulators with predetermined concentration and combination are autoclaved for 15-20 minutes under 15 lb pressure. These media then kept under laminar air flow for UV sterilization for 5 minutes. All explants are again washed with 70 % ethanol and 0.01 % $HgCl_2$ for 4 minutes followed with three times washing of double distilled water each for 10 minutes. These selected nodal segments are then inoculated in pre-sterilized MS media which contains different type of plant growth regulators concertation from 1-5 mg/l and along with different type of combinations. Those inoculated explants are then exposed under tissue culture lab with 25 ± 2 ^{0}C temperature. All observations of root formation and recorded till 8 weeks and well rooted explants are then shifted to the shifted to the (2:1) soil and cocopeat media and their hardening response are checked for 15 days.

Statistical analysis

Root proliferation observations are recorded manually frequently till 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 calculated with their mean ± SE. Each treatment is repeated three times with 20 bottles each.

Result

Studies on use of plant growth regulators with such as IBA, IAA and 2,4-D in individual and in combination with BAP and Kinetin with 1-5 mg/l as concentration are evaluated under various observations. The healthy nodal segment of 1 to 3 cm length is inoculated with these treatment combinations in well certified MS. media. Results proven that moderate concertation of IBA are most effective in root formation either on individual basis (3 mg/l) or in combination (1-2 mg/l) with BAP and Kinetin (Table and graph no 1 and 2).

The nodal segment is fortified with IBA (3 mg/l) gives 80 % root formation within 16.50 days and roots are lengthier (13.5 cm) and highest in number (25.5) within 8 weeks. The root growth is vigorous in character and having thick, long, fibrous and healthy in appearance. However, when MS media is inoculated with IAA (1-5 mg/l) the higher concentration (3-5 mg/l) are most suitable than lower concentration (1-2 mg/l). Among these concentration IAA (4 mg/l) gives vigorous root formation with 80 % root formation response within 17.10 days along with maximum number of roots (19.1) and root length (5.3 cm). The roots are thick, short, fibrous and healthier in character. In use of 2,4-D as media fortification in MS media where the lower concentration (1-3 mg/l) are most effective than higher concentration (4-5 mg/l) in nodal segments. The MS media augmented with 2,4-D gives earlier root formation (17.93), maximum number of roots (17.8) and root length (10.7 cm) with 70 % root formation response. Both concentrations of 2,4-D (1-2 mg/l) results in long, thin fibrous and healthy vigorous roots. Results also remarked under the combined use of IBA (1-2 mg/l) along with the (BAP/Kinetin at 1-5 mg/l) in nodal segment. The earlier root formation is reported in IBA (2 mg/l) + BAP (1 mg/l) but, maximum number of roots (21.2) and length (11.8) is reported in IBA (1 mg/l) + BAP (3 mg/l) within 8 weeks with 80 % root formation response. All roots are thicker, longer, fibrous, healthy and vigorous in nature. In Kinetin (1-5 mg/l) media in combination with IBA (1-2 mg/l) and MS media the lower concentration of Kinetin (1 mg/l) with IBA (2 mg/l) gives maximum number of root (17.8) and root length (10.8) and earlier root formation (17.50) than any other combination of Kinetin. The roots are vigorous, thick and healthy in nature with 70 % root formation response to the media.

Discussion

Among three hormones (IAA, IBA and 2,4 D) root promoting hormone, IBA which is found to be the best for root formation. These finding were supported by Nickell, 1982, and concludes that slow movement and degradation of IBA in MS media leads to localization at the applied regions which create root induction.

The use of high concentration of auxins can obstruct the earlier root formation and decline root number, root length, lower its characters and growth. The use of IBA at 3 mg/l gives earlier roots (16.50 days) along with 80 % root formation and highest number of root (25.5) and length (13.5 cm). These higher concentration leads to the thick long fibrous and healthy root which are supported by (Joshi *et al.*, 2015, Soni *et al.*, 2015, Niratkar *et al.*, 2014, Yadav and Singh, 2010 and 2011, Saritha and Naidu, 2008) [10, 28, 31, 32, 23] in *S. acmella*. Similarly in other plant like *Rhanterium epapposum* (Henzab *et al.*, 2018) [1], *Glinus lotoides L.* (Shiferaw and Tileye, 2015) [27], *Achyranthes aspera* L (Sen *et al.*, 2014) [25], *Operculina turpethum* (Sebastinraj *et al.*, 2013) [24], *Withania somnifera* (Chakraborty *et al.*, 2013) [6], *Solanum nigrum* (Rathore and Gupta, 2013) [19], *Psoralea corylifolia* (Pandey, *et al.*, 2013) [17], *Stevia rebaudiana* (Jitendra *et al.*, 2012) [9], *Origanum sipyleum* (Oluk *et al.*, *2009*) [16], *Chlorophytum borivilianum* (Bera *et al.*, 2009) [5], *Centella asiatica* (Mohapatra *et al.*, 2008) [44] plant IBA is found to be more responsive than any other auxin.

In combination response of IBA (1-2 mg/l) and BAP /Kinetin (1-5 mg/l)with the MS media supplemented with IBA (2 mg/l) and BAP (1 mg/l) start early root formation than other combinations and concentration but are quite effective than Individual response of use of Individual use of IBA. These combinations start early root formation but higher root formation response (80 %), number of a root (21.2), and root length (11.8 cm) has been reported in IBA (1 mg/l) and BAP (3 mg/l) after 8 weeks of inoculation. The roots are vigorously grown, thick, fibrous and healthy in character. These results supported by *Bryonopsis laciniosa* (Caroline and Mallaiah, 2011) in IBA and BAP and IBA and Kinetin.

Hardening

The plants are experimented with the hardening response after the 12 weeks and rooted sapling are when shifted in soil and cocopeat (2:1) media under shade house it gives more than 80 % survival rate. The plants initially supplemented with half strength MS media in order to avoid initial rooting culture nutrient media which later on

transferred with normal hardening media. Frequently along with irrigation water containing the Bavistin (2%) is sprayed and drenched on the plant to avoid soil born or air born pathogen entry.

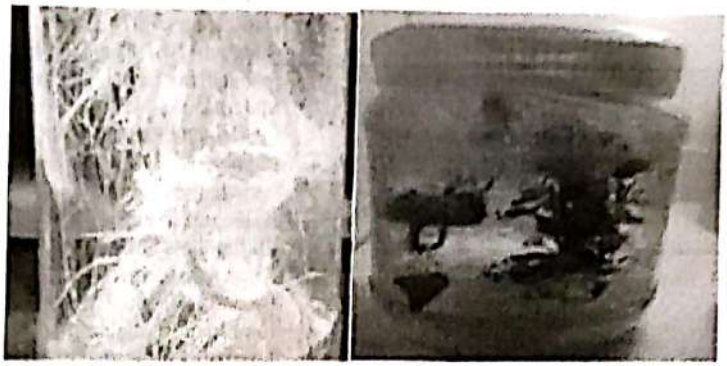


Fig 1: M S + IBA (3 mg/l) and (2) M S + IBA (1mg/l) + BAP (3 mg/l) in vitro root formation response.

Table 1: Effect of IAA, IBA and 2,4 D on Root Formation

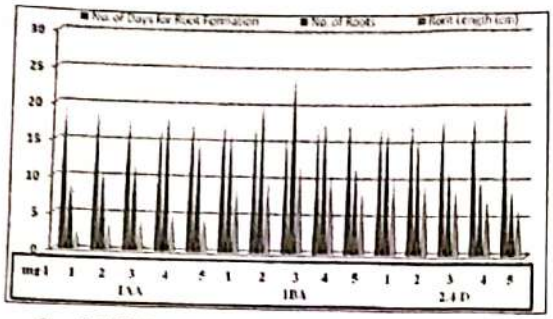
Name of Auxin	Conc. in mg/ l	No. of Days for Root Formation (Mean ± SE)	% of Root Formation	No. of Root (Mean ± SE)	Root Length in cm (Mean ± SE)	Root Characters	Root Growth
IAA	1	20.40 ± 0.94	50	09.2 ± 0.71	02.7 ± 0.15	Thin and Very Short	R^{+}
	2	19.17 ± 1.17	60	10.8 ± 0.55	03.6 ± 0.16	Thin and Short	R^{++}
	3	18.53 ± 1.27	70	12.6 ± 0.78	04.3 ± 0.15	Thin and Short	R^{++}
	4	17.10 ± 1.20	80	19.1 ± 1.16	05.3 ± 0.30	Thick, Short, Fibrous and Healthy	R^{+++}
	5	18.33 ± 0.97	60	15.7 ± 1.43	04.8 ± 0.29	Thin and Short and Healthy	R^{++}
IBA	1	18.83 ± 1.03	60	16.7 ± 1.27	09.1 ± 0.41	Thin and Short	R^{++}
	2	17.70 ± 0.87	70	20.9 ± 0.96	10.4 ± 0.43	Thick, Long and healthy	R^{++}
	3	16.50 ± 0.92	80	25.5 ± 1.13	13.5 ± 0.45	Thick, Long, Fibrous and Healthy	R^{+++}
	4	17.43 ± 1.00	70	18.6 ± 0.99	11.9 ± 0.72	Thick, Long Fibrous and Healthy	R^{+++}
	5	18.37 ± 0.85	70	12.1 ± 0.91	08.7 ± 0.37	Thin , Short and Healthy	R^{++}
2,4-D	1	17.93 ± 1.22	60	17.8 ± 1.11	10.7 ± 0.70	Thin, Long, Fibrous and Healthy	R^{+++}
	2	18.00 ± 1.27	70	15.8 ± 1.19	10.1 ± 0.48	Thin, Long, Fibrous and Healthy	R^{+++}
	3	18.57 ± 0.98	60	11.7 ± 1.03	09.4 ± 0.48	Thin, Short and Healthy	R^{++}
	4	19.50 ± 1.16	50	10.6 ± 0.62	08.3 ± 0.26	Thin, Short and Healthy	R^{++}
	5	20.83 ± 1.12	40	08.9 ± 0.53	06.7 ± 0.37	Thin and Short	R^{+}

Growth Reponses of Root Formation: R^{+} Poor Root, R^{++} Moderate root, R^{+++} Vigorous Root

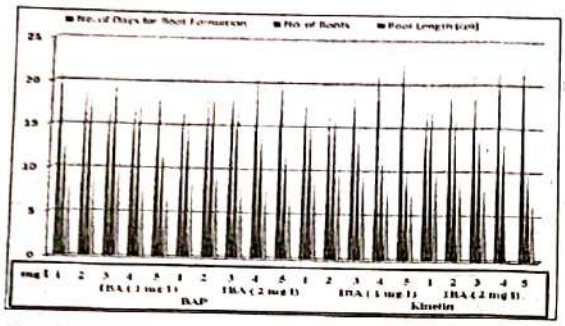
Table 2: Effect of Combination of IBA with BAP and Kinetin on Direct Root Initiation

Conc. Of IBA mg/ l	Conc. of Cyto. mg/ l	No. of Days for Root Form. (Mean ± SE)	% of Root Formation	No. of Root (Mean ± SE)	Root Length in cm (Mean ± SE)	Root Characters	Root Growth
Effect of Combination of IBA with BAP							
IBA 1 mg/ l	1	22.50 ± 0.90	40	13.6 ± 0.98	08.8 ± 0.39	Thin and Very Short,	R^{+}
	2	20.33 ± 1.18	70	18.6 ± 1.28	09.8 ± 0.49	Thick, Short and Fibrous Healthy	R^{+++}
	3	18.00 ± 1.22	80	21.2 ± 0.73	11.8 ± 0.39	Thick, Long , Fibrous and Healthy	R^{+++}
	4	18.27 ± 1.33	60	18.1 ± 1.69	09.2 ± 0.53	Thick, Short, Fibrous, Healthy	R^{++}
	5	19.43 ± 1.08	50	12.6 ± 1.28	08.1 ± 0.50	Thin and Very Short,	R^{+}
IBA 2 mg/ l	1	17.40 ± 1.12	70	16.0 ± 1.09	09.3 ± 0.50	Thick, Short, Fibrous	R^{++}
	2	19.07 ± 0.93	70	19.5 ± 1.25	10.7 ± 0.65	Thick, Long , Fibrous and Healthy	R^{+++}
	3	19.43 ± 1.14	60	17.6 ± 1.40	09.1 ± 0.62	Thick, Short, Fibrous, Healthy	R^{++}
	4	20.97 ± 1.20	50	15.2 ± 1.07	08.8 ± 0.63	Thick, Very Short Healthy	R^{++}
	5	22.03 ±0.86	40	12.5 ± 0.79	07.8 ± 0.33	Thin and Very Short	R^{+}
Effect of Combination of IBA with Kinetin							
IBA 1 mg/ l	1	18.27 ± 1.22	60	15.9 ± 1.18	09.8 ± 0.49	Thick and Short	R^{+++}
	2	17.67 ± 1.21	60	16.8 ± 0.92	11.1 ± 0.38	Thick, Long, Fibrous and Healthy	R^{+++}
	3	19.60 ± 0.96	50	14.2 ± 1.31	10.7 ± 0.87	Thin, Long, Fibrous and Healthy	R^{++}
	4	22.07 ± 0.95	50	12.6 ± 1.07	09.0 ± 0.30	Thin, Short and Healthy	R^{++}
	5	23.23 ± 1.09	40	10.5 ± 0.65	08.2 ± 0.47	Thin and Very Short	R^{+}
IBA 2 mg/ l	1	17.50 ± 0.97	70	17.8 ± 0.84	10.8 ± 0.68	Thick, Long, Fibrous and Healthy	R^{+++}
	2	19.87 ± 1.01	60	16.6 ± 1.18	09.5 ± 0.45	Thick, Short, Fibrous , Healthy	R^{+++}
	3	20.10 ± 1.03	50	14.3 ± 1.04	08.7 ± 0.33	Thin, Short Fibrous	R^{++}
	4	22.40 ± 0.87	50	13.9 ± 0.99	08. 2 ± 0.25	Thin and Short	R^{++}
	5	23.23 ± 1.17	40	10.3 ± 0.45	07.1 ± 0.43	Thin and Very Short	R^{+}

Growth Reponses of Root Formation: R^{+} Poor Root, R^{++} Moderate root, R^{+++} Vigorous Root



Graph 1: Effect of IAA, IBA and 2,4 D on Root Formation



Graph 2: Effect of Combination of IBA with BAP and Kinetin on Direct Root Initiation

Conclusion

Various plant hormones (Auxins) either in individual basis or in combination with the cytokinin's are reviewed under various observations.

The results show in *in-vitro* root formation studies use of IBA is most effective in overall root formation response with maximum 80 % response, root length (13. 5 cm) and number of roots (25.5) with healthy, thick vigorous and fibrous roots within 8 weeks. However, in auxin and cytokinin combination the IBA (1 mg/l) and BAP (3 mg/l) are best combination than other combination which results 80 % culture response for formation of thick, vigorous, fibrous and lengthy root (21.2 cm) and number of root (11.8). These *in-vitro* saplings are gives more than 80 % survival rate in (2: 1) proportion of soil and cocopeat media which initially supplied with half strength MS media and Bavistin (2 %) drenching.

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Conflict of interest statement: We declare that we have no conflict of interest

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