INTRECTIVE EFFECTS OF INDOLE 3-ACETIC ACID& BENZYL ADENINE ALONG WITH MS MEDIA IN DEVELOPMENT OF ROOT AND SHOOT REGENERATION BY MICROPROPAGATION METHODS IN CRITICALLY ENDANGERED MEDICINAL PLANT *AJUGA BRACTEOSEA* WALL EX. BENTH (RATHPATHA)

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ABSTRACT:

*Ajuga bracteosa*Wall ex Benth. is broadly used as a traditional medicine but the plant is now considered as critically endangered plant. In the present study Leaf explant of *Ajuga bracteosa*Wall ex Benth. was chosen which disinfected with 0.2% Solution of cetrimide, 0.25% solution of Streptomycin and 0.1% solution of mercuric chlorideand washing thoroughly with distilled water. The leaf explants were placed on MS medium supplemented with Indole-3-acetic acid (lAA) and Benzyladenine (BA) with various concentrations were 0.2 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L and 5 mg/L. In 28 days and 42 days 37.8 and 41.2 shoot per culture regenerate respectively**.** With increase the concentration of BA the no of shoot regeneration increases but at higher concentration it decline. Thus, this study could be ideal for rapid micropropagation of elite plants of *Ajuga bracteosa*Wall ex Benth.

KEYWORDS: *Ajuga bracteosa*; IAA; BA; in vitro culture; micropropagation; leaf explant.

INTRODUCTION:

Since a long time, medicinal plants with identification of their numerous properties that enable them to be used to treat various ailments, have attracted the attention of many scientists and researchers worldwide. Their effectiveness has attracted enormous awareness in the context of the concern on the protection of biological resources, related traditional knowledge available in developing countries, and their furtive use by MNCs and other companies of developed nations. Biotechnological development and the advancements being made in genome research have opened up new possibilities for the use of medicinal plants for the needs of mankind[1].

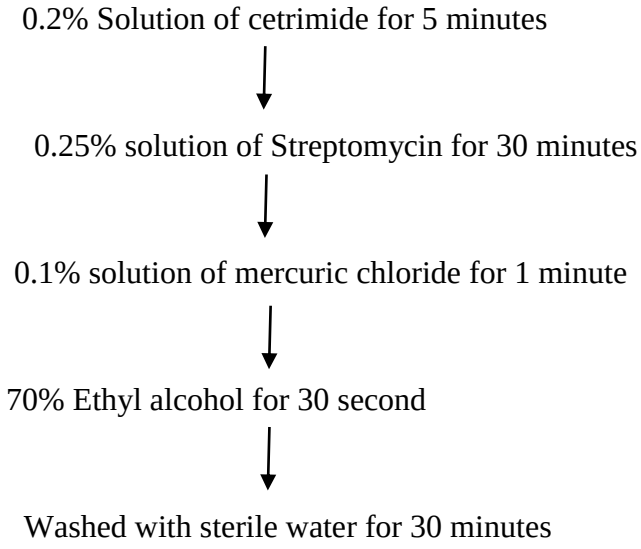
*Ajuga bracteosa*Wall ex Benth. is a perennial, erect to ascending herbaceous, flowering plant of Lamiaceae family that grows up to 5-50 cm tall with its prostrate oblanceolate leaves. It is found along the road sides open slops and rock crevices. Among almost 300 species of the genus Ajuga, *Ajuga bracteosa*Wall ex Benth. is broadly used as a traditional medicine in number of ailments like fever, toothache, dysentery, malaria, high blood pressure, diabetes, inflammation, fungal and helminthic infections, kidney and liver diseases (as diuretics) and in various gastrointestinal disorders [2,3]. The plant has its origin from Europe, Asia and Africa. It also grows in Australia and North America, subtropical areas of Bhutan, Afghanistan, Pakistan, China and Malaysia. In India, It is widely distributed in moderate and subtropical areas of Kashmir and upper region of India from western Himalayas at an altitude of 1300 meter currently being considered as endangered plant [4,5]. It has been revealed from the previous researches that the plant *Ajuga bracteosa*Wallex Benth. exhibits various pharmacological activities such as antispasmodic, anticancer, analgesic, anti-coagulant and anti-depressant etc. due to the presence of different chemical constituents like tannins, glycosides, volatile oils, esters, alkaloids and fatty acids. It is widely used to cure hypertension, leprosy, blood purification and fever [6,7].

MATERIAL &METHODS:

*Ajuga bracteosa*was collected in the month of April from ‘Chamoli District of Uttarakhand. And introduced in the Herbal Garden at Uttaranchal University, Dehradun, Uttarakhand. The herbs were plantedin the beds in rows with plant to plant distance at 20 cm each. The plant used to raise the cultures was planted in a pot as mother plant. The plants were maintained under shade conditions and irrigated when required to keep the soil moist. The plants were covered with green nylon net.

Sterilization of Explant:

The young leaves and petiole explants of*Ajuga bracteosea*wall.Ex.benth From In-situ plants were wiped with 70% of Alcohol and washed under running tap water for 30 minutes. The sterilization of explants was done by following protocol



Inoculation and Culture condition:All the experimental work was performed inside the laminar air flow chamber. The sterile explants were cut into the small segments and inoculated on MS medium supplemented with different concentrations of auxins and cytokinins. The forceps,scalpels, petriplates and other components used for inoculation were sterilized by autoclaving and subsequently flamed. All the cultures were maintained in a culture room Callusinduction, differentiation and regeneration response of each explants under various treatments were studied and the observations such as percent callusing, nature of the callus, percent regeneration, number and height of shoots and rooting were recorded weekly. Optimal conditions were determined for the maintenance of the cultures.

Callus induction and shoot regeneration:

MS medium supplemented with Indole-3-acetic acid (lAA) and Benzyladenine (BA) in the concentration range of(0.2 to 5.0 mg/L) the PH of all the media was adjusted to PH5.6 was used to induce callus and shoot regeneration in *Ajuga bracteosa*from leaf and petioleexplants. The degree of callus.5.8 ± 0.1 prior to gelling with 0.8% (w/v) agar-agar (bacteriological grade), dispensed (10 ml) into culture tubes and moist sterilization was done by autoclaving at (121_C for 15 min). The cultures were maintained in the culture room under a regime of 16 h photoperiod (intensity - 40 μEcm-2/min/secs) at 25_C. All experiments were conducted at least three times with 15 replicates each. The developed shoots were excised, subjected to vertical cuts (two to three) and then transferred to MS with BA (2.21 μM) for multiple shoot induction. The multiple shoots were isolated and transferred (after three weeks) to MS liquid medium containing different concentrations of NAA (0.53 - 5.3 μM) for root induction. After two weeks, the rooted shoots were transferred to the plastic netpots (3 cm diam.) in protrays containing a mixture of soil and vermiculite (1:1). Prior to their transfer, the plants were treated (20 min) with bavistin (0.01%). The plants were irrigated with one-fourth strength of MS (5 - 6 ml/pot) and also sprayed with 0.01% bavistin to control the fungal growth. A high humidity condition was maintained by regular spray of distilled water at an hourly interval. Following hardening, the plantlets were transplanted to earthen pots containing a mixture of garden soil and compost (1 : 1).

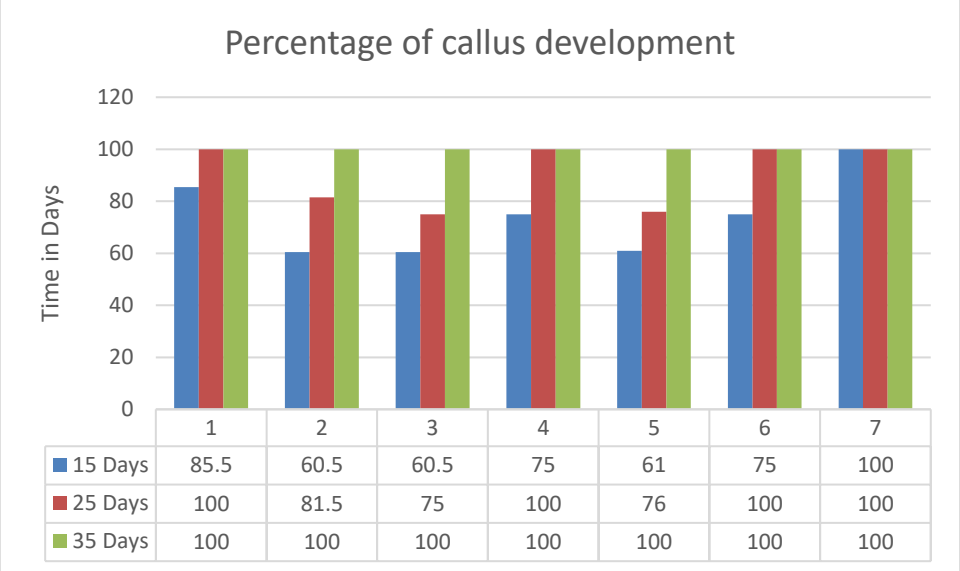
RESULT:

Percentage of callus development in MS media supplemented with IAA&BA in different concentration shown in table No 01.

MS (MEDIUM)		**TIMES IN DAYS**		
IAA(mg/ L)	**BA(mg/L)**	**15**	**25**	**35**
1	0.5	85.5	100	100
1	1	60.5	81.5	100
1	2	60.5	75	100
1	5	75.0	100	100
2	1	61.0	76	100
2	2	75	100	100
2	5	**100**	**100**	**100**

Table No 01.Percentage of callus development

Among all the combination of Auxins and cytokinin tried MS+BA+IAA proved for best media for callus development as well for regeneration of shoot.

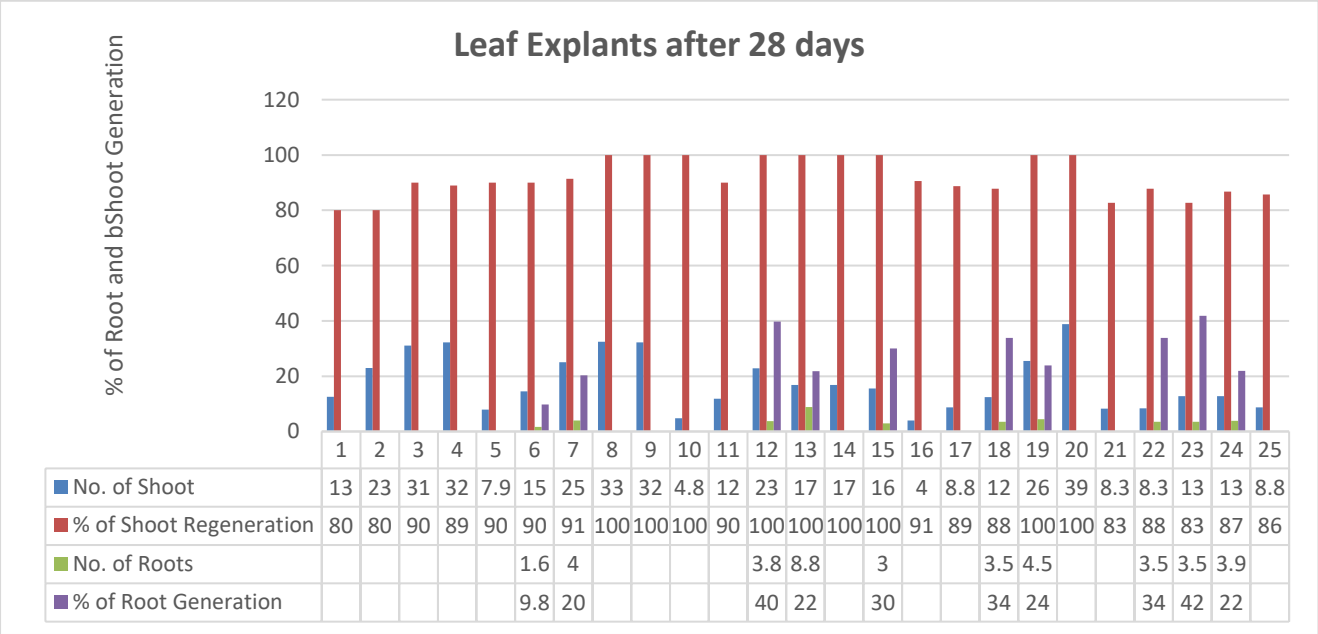


Graph No 01. Percentage of callus development

Medium MS in (mg/L)		NO of shoots	% of shoot regeneration	No of roots	% of root regeneration
IAA	BA				
0.2	0.2	12.5±0.5	80±2.5	……	……
0.2	0.5	23.0 ± 0.55	80±0.0	……	……
0.2	1	31.1± 3.1	90±2.5	……	……
0.2	2	32.2±0.125	89±5.5	……	……
0.2	5	7.9±0.31	90±5.9	……	……..
0.5	0.2	14.5±1.125	90±5.1	1.63±0.38	**9.77**±1.42
0.5	0.5	25.0±0.521	91.33±4.5	4.0±0.35	20.33±2.5
0.5	1	32.5±0.50	100±0	……..	……

0.5	2	32.2±0.31	100±0	…….	…….
0.5	5	4.8 ±0.55	100±0	……	……..
1	0.2	11.8 ±0.50	90±1.4	…….	…….
1	0.5	22.8 ±0.50	100.0±0.0	3.8 ±0.150	39.8 ±6.50
1	1	16.8 ±0.30	100.0±0.00	8.8 ±0.30	21.8 ±1.50
1	2	16.8 ±0.50	100.0±0.00	…….	…….
1	5	15.5±0. 61	100.0 ±0.00	2.98±0.33	30±1.43
2	0.2	4±0.16	90.55±5.5	……	……
2	0.5	8.77±0.224	88.76±6.63	…….	…….
2	1	12.38±0.266	87.76±8.63	3.48±0.30	33.89±8.5
2	2	25.52±0.124	100±0	4.48±0.29	23.89±3.5
2	5	38.87±0.324	100±0	……	…….
5	0.2	8.27±0.28	82.76±5.63	…….	…….
5	0.5	8.33±0.22	87.76±8.69	3.48±0.26	33.89±8.9
5	1	12.77±0.245	82.76±8.67	3.48±0.30	41.89±8.4
5	2	12.77±0.224	86.74±8.63	3.88±0.35	21.89±8.5
5	5	8.77±0.224	85.76±7.63	……..	……

Table-02 Effect of IAA and BA on leaf explants after 28 days

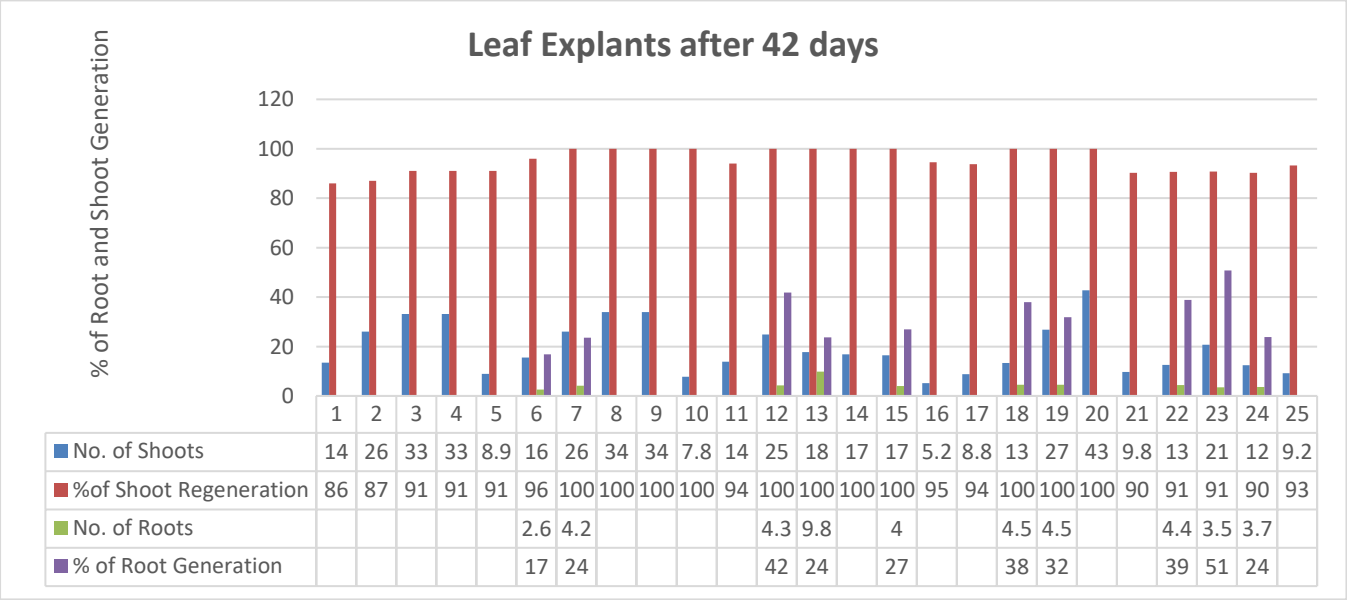


Graph 02: Effect of IAA and BA on leaf explants after 28 days

Medium MS in (mg/L)		NO of shoots	% of shoot regeneration	No of roots	% of root regeneration
IAA	BA				
0.2	0.2	13.5±0.66	86±2.42	……	…….
0.2	0.5	26.0 ± 0.55	87±5.87	…….	…….
0.2	1	33.1± 3.14	91±2.76	…….	…….
0.2	2	33.2±0.125	91±5.49	……	…….

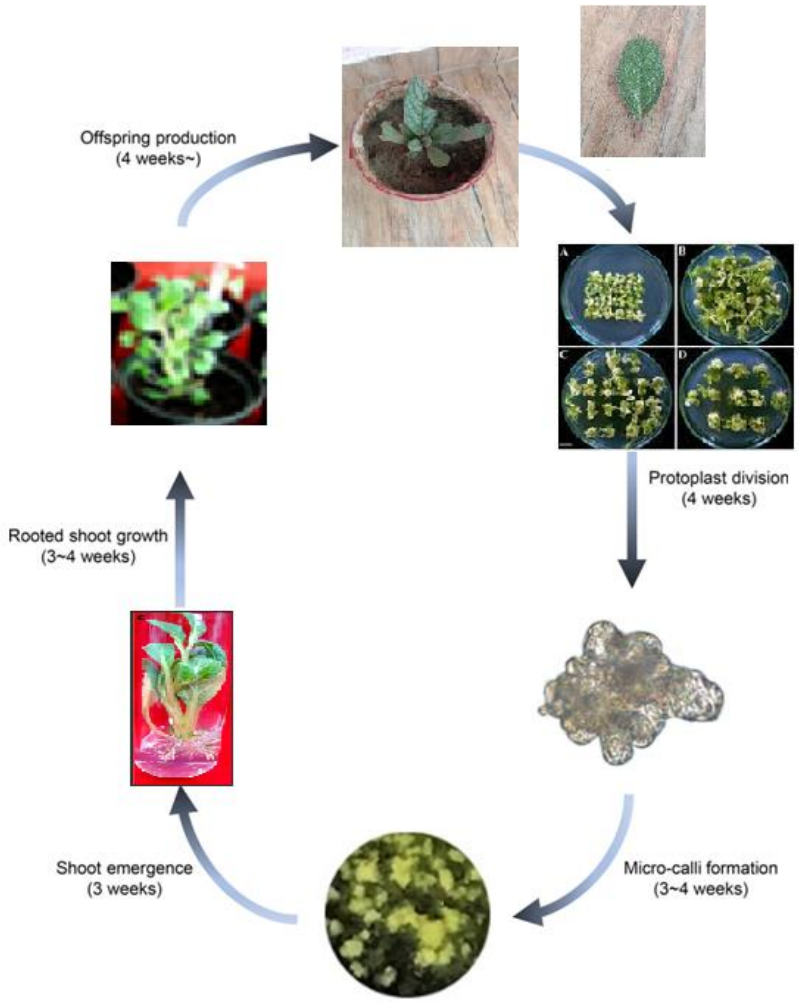
0.2	5	8.9±0.33	91±6.6	……	……..
0.5	0.2	15.5±1.125	96±4.99	2.63±0.58	**16.77**±0.42
0.5	0.5	26.0±0.521	100 ±0	4.2±0.35	23.63±2.5
0.5	1	33.9±0.50	100±0	……..	……
0.5	2	33.9±0.31	100±0	……	……..
0.5	5	7.8 ±0.58	100±0	……	……..
1	0.2	13.8 ±0.50	94±1.4	……	……
1	0.5	24.8 ±0.55	100±0	4.3±0.150	41.8 ±6.50
1	1	17.8 ±0.30	100±0	9.8 ±0.30	23.66 ±1.50
1	2	16.8 ±0.53	100±0	……..	……
1	5	16.5±0. 61	100±0	3.98±0.33	27±1.43
2	0.2	5.22±0.17	94.55±5.8	……	……
2	0.5	8.75±0.223	93.76±6.63	……	……
2	1	13.38±0.266	100±0	4.48±0.30	37.89±8.7
2	2	26.77±0.124	100±0	4.48±0.29	31.89±3.5
2	5	42.77±0.324	100±0	……	……
5	0.2	9.76±0.224	90.26±8.63	……..	……
5	0.5	12.52±0.221	90.72±8.21	4.41±0.32	38.89±8.2
5	1	20.73±0.224	90.76±8.63	3.48±0.30	50.79±7.5
5	2	12.37±0.212	90.21±8.06	3.68±0.39	23.79±7.5
5	5	9.27±0.212	93.21±8.06	……	……..

Table-03 Effect of IAA and BA on leaf explants after 42 days



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
No. of Shoots	14	26	33	33	8.9	16	26	34	34	7.8	14	25	18	17	17	5.2	8.8	13	27	43	9.8	13	21	12	9.2
%of Shoot Regeneration	86	87	91	91	91	96	100	100	100	100	94	100	100	100	100	95	94	100	100	100	90	91	91	90	93
No. of Roots						2.6	4.2					4.3	9.8		4			4.5	4.5			4.4	3.5	3.7	
% of Root Generation						17	24					42	24		27			38	32			39	51	24	

Graph 03 Effect of IAA and BA on leaf explants after 42 days



Stages of Callus induction for Root and Shoot regeneration

Discussion:

After 35 days the green regenerating callus was induced from the leaf explants of Ajuga bracteosea. In all culture fastest callusing was observed.

The response of 25 combination of MS media+IAA+BA used for leaf explants the MS+IAA(2mg/ml) + BA(5mg/ml) proved best media for root and shoots regeneration. In 28 days and 42 days 37.8 and 41.2 shoot per culture regenerate respectively**.** With increase the concentration of BA the no of shoot regeneration increases but at higher concentration it declines.

Conclusion:

Micropropagation is the technique which used to conserve the endangered plant species. In the present study, we observed an encouraging result of hormone effects for Ajuga bracteosea commercial micropropagation. In order to achieve particular results for future research, more hormone combinations in treatment doses can be administered with shorter intervals. The present study described an efficient protocol for shoot and root proliferation of Ajuga

bracteoseawith 100% development of shoots and roots per explants with better quality plantlets in terms of growth. Higher shoot number reported in the present study in the shoot induction media could be attributed to the beneficial effect of IAA+BA in culture media. Hence, the shoot regeneration procedure described in the present study could be ideal for rapid micropropagation of elite plants of Ajuga bracteoseafor its beneficial purpose.

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