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RAPID MICROPROPAGATION OF *Androgrphis paniculata* NEES, FROM NODAL EXPLANTS TO STUDY EFFECT OF VARIOUS PLANT GROWTH REGULATORS

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ABSTRACT

Andrographis paniculata **Nees, commonly called as 'Kalmegh' or 'Chiraita' is frequently used in traditional medicine. Due to extensive wild collection and lack of awareness for the conservation of medicinal plants, many medicinal plants of Chhattisgarh are falling under the category of endangered species. There is an urgent need for the conservation and commercial production of rare and medicinally important plants. The present work is to develop an efficient protocol for the rapid micropropogation of *Andrographis paniculata* from the nodal segments, with various plant growth regulators. When MS was medium supplemented with BAP, shoot/explants and bud break percentage at concentration of 1.5 mg/l of BAP was found to be 22.25 ± 2.99 and 91.75 ± 3.94 respectively. At 0.5 mg/l concentration of BAP average shoot length, nodes/shoot and leaves/shoot was observed to be 1.875 ± 0.25, 3.25 ± 0.95 and 6.50 ± 1.91 respectively. When MS medium was fortified with Kinetin, at concentration of 2.0 mg/l, shoot/explants and bud break percentage was 25 ± 6.25 and 72.25 ± 9.10 respectively. Similarly, average shoot length nodes/shoot and leaves/shoot at concentration 0.5 mg/l of Kinetin were 1.56 ± 2.49, 2.25 ± 0.95 and 4.5 ± 1.91 respectively. Effect of BAP in combination with Kinetin was further studied. The concentration of BAP was fixed at 1.0 mg/l with various concentration levels of Kinetin. 94.75 ± 2.21 was found to be the bud break percentage both at BAP 1.0 mg/l + Kn 2.0 mg/l and BAP 1.0 mg/l + 2.5 mg/l Kn. Shoot/explants, average shoot length, nodes/shoots and leaves/shoots was found to be 31.50 ± 2.64, 4.37 ± 0.40, 5.75 ± 0.50 and 11.50 ± 1.00 respectively. Among all the growth regulators tested BAP in combination with Kinetin gave best results at concentration of 1.0 mg/l BAP and .05 mg/l of Kinetin.**

KEYWORDS : Micropropagation, *Androgrphis paniculata,* Nodal Explants

Andrographis paniculata (Burn. f.) Wall. ex. Nees belongs to the family Acanthaceae. It is an erect annual herb found throughout India and Asian countries. In India it is commonly called as Kirayat, Mahatita, Chirayta or Kalmegh. The plant grows in waste grounds and prefers moist habitat. Highly bitter in taste and has a weak odor. Due to increase in demand plant the plant is cultivated also (Zhong, 1987).

Chemically the drug contains flavones and lactones. Among lactones Andrographolide is the main constituent. Andrographolide has been isolated in pure form as it has shown various pharmacological activities (Hu and Zhou, 2001). The herb also contains minerals and is a rich source of sodium chloride. The diterpene lactones have hepatoprotective, anti-pyretic, immunostimulant, anti-diarrhoeal, anti-malarial anti-thrombogenic, anti-snakevenom properties. It also shows antimicrobial activity against *Salmonella typhae* and *Helminthosporium sativum*.

The distribution of the plant in various regions of Chhattisgarh was found to be abundant, but past ten years the frequency with which it was found is reduced to a threshold limit. The basic reason for which this research has been conducted; firstly is to conserve the plant, secondly to standardize rapid regeneration method to meet the demand in the national and International market, as wild collection of various medicinal plants is posing a great threat to the natural repository of medicinal plants for which Chhattisgarh is known.

MATERIALS AND METHODS

Actively growing and healthy shoot material of *Andrographis paniculata* with 3 to 4 nodes were collected from the botanical garden of the Research Center. Er. Raghavendra Rao Autonomous PG. SC. College, Bilaspur (C.G.). The leaves were removed from the shoot and thoroughly washed under running tap water for 30-45 min. Later, shoots were washed with detergent Labolene for 5 min, followed by rinsing with double distilled water for 6-7 min. After thorough rinsing explants were cut onto 0.5-1.0 cm pieces and then soaked in double distilled water to avoid phenol exudation. After 20-25 min, cut explants were then treated with 0.1 % (w/v) mercuric chloride for 10 min, the liquid was stirred by swirling to give proper contact of chemical to the explants. The treated explants were rinsed with double distilled water for several times. Then again they were treated with 12% (w/v) hydrogen peroxide for 5

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Table 1 : Effect of MS Medium Supplemented With Different Levels of BAP on Shoots / Explants Raised from Nodal Explants of *Andrographis paniculata* after 30 Days of Incubation

S.No.	Conc. of BAP	Shoots / Explants				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	9	8	9	5	07.75	1.892	0.946
2.	1.0	11	9	14	12	11.50	2.081	1.040
3.	1.5	19	21	26	23	22.25	2.986	1.493
4.	2.0	19	12	17	19	16.75	3.304	1.652
5.	2.5	9	3	8	5	06.25	2.753	1.376

Table 2 : Effect of MS Medium Supplemented With Different Levels of BAP on Average Shoot Length, Raised from Nodal Explants of A*ndrographis paniculata* After 30 Days of Incubation

S.No.	Conc. Of BAP t/t	Average Shoot length				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	1.9	2.2	1.6	1.8	1.875	0.250	0.125
2.	1.0	1.6	1.8	1.3	1.8	1.625	0.236	0.118
3.	1.5	1.4	0.9	1.2	1.8	1.325	0.377	0.188
4.	2.0	1.4	0.6	0.9	1.3	1.05	0.369	0.184
5.	2.5	0.8	0.8	0.9	0.6	0.775	0.125	0.062

min and then again washed with double distilled water for several times, thereafter, explants were surface dried on pre-sterilized Petri dish with pre sterilized filter paper. The entire procedure after treatment with Labolene was conducted under Laminar Air Flow system. Finally the shoots containing a single node with dormant axillary buds were inoculated onto establishment medium.

RESULTS

Effect of Plant Growth Regulators

BAP in the micropropagation of *Andrographis paniculata* 1.1 Effect of BAP on shoot/explants of *Andrographis paniculata.*

The effect of BAP at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Shoot/explants ranged from 07.75 ± 1.892 to 22.25 ± 2.986. Maximum shoot/explants were obtained at the concentration of 1.5 mg/l of with the value of 22.25 ± 2.986. At this concentration the values of experimental replicates were 19, 21, 26 and 23.

In the statistical analysis, standard error of mean was 1.119, standard error of difference was 1.583 and coefficient of variance was found to be 17.363. F-calculated value for replicates was 3.043 and for treatment was 34.873** (Table 01).

Effect of BAP on average shoot length of *Andrographis paniculata*

The effect of BAP at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Average shoot length ranged from 1.875 ± 0.250 to 0.775 ± 0.125. Maximum average shoot length was observed at concentration of 0.5mg/l and minimum at concentration of 2.5mg/l. Maximum average shoot length at 0.5mg/l was 1.875 ± 0.250. A decreasing trend was observed for average shoot length at all concentration of BAP. The value for replicates at 0.5mg/l was 1.9, 2.2, 1.6 and 1.8.

Statically standard error of mean was 0.142, standard error of difference was 0.201, and coefficient of variance was 21.464. F-calculated value for replicates was 1.071 and for treatment it was 9.453 (Table 02).

Effect of BAP on nodes/shoot of *Andrographis paniculata*

The effect of BAP at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Nodes/shoot ranged from 3.25 ± 0.957 to 1.25 ± 0.500. Maximum nodes/shoot was observed at concentration of 0.5mg/l and minimum at concentration of 2.5mg/l. Maximum nodes/shoot at 0.5mg/l

Table 3 : Effect of MS Medium Supplemented With Different Levels of BAP on Nodes/ Shoot, Raised From Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S. No.	Conc. of BAP t/t	Nodes/ Shoot				Mean $\overline{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	4	4	2	3	3.25	0.957	0.478
2.	1.0	2	3	2	3	2.50	0.577	0.288
3.	1.5	3	1	2	3	2.25	0.954	0.478
4.	2.0	2	1	1	2	1.50	0.577	0.288
5.	2.5	1	1	2	1	1.25	0.500	0.250

Table 4 : Effect of MS Medium Supplemented With Different Levels of BAP on Leaves/ Shoot, Raised From Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. of BAP t/t	Leaves/ Shoot				Mean $\overline{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	8	8	4	6	6.5	1.914	0.957
2.	1.0	4	6	4	6	5.0	1.154	0.577
3.	1.5	6	2	4	6	4.5	1.914	0.9574
4.	2.0	4	2	2	4	3.0	1.154	0.577
5.	2.5	2	2	4	2	2.5	1.000	0.500

was found to be 3.25 ± 0.957. A decreasing trend was observed for nodes/shoot at all concentrations of BAP. The value for replicates at 0.5mg/l was 4, 4, 2 and 3.

Statically standard error of mean was 0.379, standard error of difference was 0.536, and coefficient of variance was 35.269. F-calculated value for replicates was 0.782 and for treatment it was 4.478* (Table 03).

Effect of BAP on leaves/shoot of *Andrographis paniculata*

The effect of BAP at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Leaves/shoot ranged from 6.5 ± 1.914 to 2.5 ± 1.000. Maximum value for leaves/shoot of *Andrographis paniculata* was observed at concentration of 0.5mg/l and minimum at concentration of 2.5mg/l. Maximum leaves/shoot at 0.5mg/l was found to be 6.5 ± 1.914. A decreasing trend was observed for leaves/shoot at all concentrations of BAP. The value for replicates at 0.5mg/l was 8, 8, 4 and 6.

On statistical analysis, standard error of mean was 0.758, standard error of difference was 1.072, and coefficient of variance was 35.269. F-calculated value for replicates was 0.782 and for treatment it was 4.478*. (Table 04)

Effect of BAP on Bud Break percentage of *Andrographis paniculata*

The effect of BAP at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Bud break percentage ranged from 91.75 ± 3.947 to 60.5 ± 7.187. Maximum value for bud break of *Andrographis paniculata* was observed at concentration of 1.5mg/l and minimum at concentration of 2.5mg/l. Maximum percentage of bud break at 0.5mg/l was found to be 91.75 ± 3.947. The value for replicates at 1.5mg/l was 87, 90, 95 and 95.

On statistical analysis, standard error of mean was 2.370, standard error of difference was 3.352, and coefficient of variance was 6.384. F-calculated value for replicates was 2.855 and for treatment it was 30.072** (Table 05).

Effect of Plant Growth Regulators- Kinetin (Kn) in the micropropagation of *Andrographis paniculata*

Effect of Kinetin (Kn) on shoot/explants of *Andrographis paniculata*

The effect of Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Shoot/explants ranged from 25 ± 6.25 to 11 ± 2.75. Maximum shoot/explants were obtained at the concentration of 2.0

Table 5 : Effect of MS Medium Supplemented with Different Levels of BAP on Bud Break Percentage, Raised From Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. of BAP t/t	Bud Break Percentage				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	66	72	76	77	72.75	4.991	2.495
2.	1.0	60	64	73	58	63.75	6.652	1.247
3.	1.5	87	90	95	95	91.75	3.947	1.973
4.	2	85	78	80	87	82.5	4.203	2.101
5.	2.5	50	64	66	62	60.5	7.187	3.593

Table 6 : Effect of MS Medium Supplemented With Different Levels of Kinetin on Shoots / Explants Raised from Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. of Kn t/t	Shoots / Explants				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	3	3	4	6	16	4.00	1.414
2.	1.0	4	6	4	7	21	5.25	1.500
3.	1.5	5	4	6	3	18	4.50	1.290
4.	2.0	5	5	7	8	25	6.25	1.500
5.	2.5	3	2	4	2	11	2.75	0.957

mg/l of with the value of 25 ± 6.25. At this concentration the values of experimental replicates at concentration of 2.0 mg/l were 5, 5, 7 and 8.

In the statistical analysis, standard error of mean was 0.663, standard error of difference was 0.937 and coefficient of variance was found to be 29.143. F-calculated value for replicates was 1.165 and for treatment was 3.938* (Table 06).

Effect of Kinetin (Kn) on Average shoot length of *Andrographis paniculata*

The effect of Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied for the average shoot length of *Andrographis paniculata*. Average shoot length ranged from 1.560 ± 2.499 to 0.425 ± 0.170. Maximum average shoot length was recorded at the concentration of 2.0 mg/l of with the value of 1.560 ± 2.499. At this concentration the values of experimental replicates at concentration of 2.0 mg/l were 0.2, 0.5, 0.4 and 0.6.

In the statistical analysis, standard error of mean was 0.109, standard error of difference was 0.154 and coefficient of variance was found to be 36.356. F-calculated value for replicates was 1.709 and for treatment was 4.124* (Table 07).

Effect of Kinetin (Kn) on Nodes/Shoot of *Andrographis paniculata*

The effect of Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied for the Nodes/shoot of *Andrographis paniculata*. Nodes/shoot ranged from 2.25 ± 0.957 to 1.25 ± 0.500. Maximum nodes/shoot was recorded at the concentration of 0.5 mg/l of with the value of 2.25 ± 0.957. At this concentration the values of experimental replicates at concentration of 0.5 mg/l were 3, 1, 2 and 3.

Table 7 : Effect of MS Medium Supplemented With Different Levels of Kinetin on Average Shoot length, Raised from Nodal Explants of Andrographis paniculata After 30 Days of Incubation

S.No.	Conc. of Kn t/t	Average Shoot length				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	1.2	0.5	0.9	1.3	0.975	0.359	0.179
2.	1.0	0.2	0.7	0.5	0.8	0.550	0.264	0.132
3.	1.5	0.4	0.5	0.3	0.6	1.260	2.101	1.050
4.	2.0	0.4	0.6	0.8	06	1.560	2.499	1.244
5.	2.5	0.2	0.5	0.4	0.6	0.425	0.170	0.085

Table 8 : Effect of MS Medium Supplemented With Different Levels of Kinetin on Nodes / Shoot, Raised from Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S. No.	Conc. of Kn t/t	Nodes/ Shoot				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	3	1	2	3	2.25	0.957	0.478
2.	1.0	1	2	1	2	1.50	0.577	0.288
3.	1.5	1	1	1	2	1.25	0.500	0.250
4.	2.0	1	1	2	1	1.25	0.500	0.250
5.	2.5	1	1	1	2	1.25	0.500	0.250

Table 9 : Effect of MS Medium Supplemented With Different Levels of Kinetin on Leaves/ Shoot, Raised From Nodal Explants of *Andrographis paniculata* After 30 days of Incubation

S.No.	Conc.of Kn t/t	Leaves/ Shoot				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	6	2	4	6	4.5	1.914	0.957
2.	1.0	2	4	2	4	3.0	1.154	0.577
3.	1.5	2	2	2	4	2.5	1.000	0.500
4.	2.0	2	2	4	2	2.5	1.000	0.500
5.	2.5	2	2	2	4	2.5	1.000	0.500

Minimum value of 1.25 ± 0.500 was found at the concentrations of 1.5, 2.0 and 2.5 mg/l.

When statistical analysis was done standard error of mean was 0.295, standard error of difference was 0.418 and coefficient of variance was found to be 39.440. F-calculated value for replicates was 1.714 and for treatment was 2.142 (Table 08).

Effect of Kinetin (Kn) on Leaves/Shoot of *Andrographis paniculata*

The effect of Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied for the leaves/shoot of *Andrographis paniculata*. Leaves/shoot ranged from 4.5 ± 1.914 to 2.5 ± 1.000. Maximum leaves/shoot was recorded at the concentration of 0.5 mg/l of with the value of 4.5 ± 1.914. At this concentration the values of experimental replicates at concentration of 0.5 mg/l were 6, 2, 4 and 6. Minimum value of 2.5 ± 1.000 was found at the concentrations of 1.5, 2.0 and 2.5 mg/l.

When statistical analysis was done standard error of mean was 0.295, standard error of difference was 0.418 and coefficient of variance was found to be 39.440. F-calculated value for replicates was 1.714 and for treatment was 2.142 (Table 09).

Effect of Kinetin (Kn) on Bud Break Percentage of *Andrographis paniculata*

The effect of Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied for the bud break percentage of *Andrographis paniculata*. Bud break percentage ranged from 72.25 ± 9.105 to 50.00 ± 8.524. Maximum bud break percentage was recorded at the concentration of 2.0 mg/l of with the value of 72.25 ± 9.105. At this concentration the values of experimental replicates at concentration of 2.0 mg/l were 65, 78, 82 and 64. Minimum value of 50.00 ± 8.524 was found at the concentration of 2.5 mg/l.

When statistical analysis was done standard error of mean was 3.493, standard error of difference was 4.940 and coefficient of variance was found to be 11.802. F-calculated value for replicates was 1.715 and for treatment was 6.914** (Table 10).

Effect of Plant Growth Regulators- BAP and Kinetin (Kn) in the micropropagation of *Andrographis paniculata*

Effect of BAP and Kinetin (Kn) on shoot/explants of *Andrographis paniculata*

The effect of BAP (1.0 mg/l) in combination with Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5

Table 10 : Effect of MS Medium Supplemented With Different Levels of Kinetin on Bud Break Percentage, Raised from Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. of Kn t/t	Average Shoot length				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	0.5	52	52	62	55	55.25	4.716	2.358
2.	1.0	52	48	60	48	52.00	5.656	2.828
3.	1.5	65	78	56	62	65.25	9.287	4.643
4.	2.0	65	78	82	64	72.25	9.105	4.552
5.	2.5	50	62	48	45	50.00	8.524	4.262

Table 11: Effect of MS Medium Supplemented With Different Levels of BAP and Kinetin on Shoots / Explants Raised from Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. of BAP+Kn t/t	Shoots / Explants				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	1.0+0.5	9	8	10	6	8.25	1.707	0.853
2.	1.0+1.0	15	9	13	12	12.25	2.500	1.250
3.	1.0+1.5	29	32	30	27	29.5	2.081	1.040
4.	1.0+2.0	35	29	32	30	31.5	2.645	1.322
5.	1.0+2.5	25	25	18	21	22.25	3.403	1.701

Table 12 : Effect of MS Medium Supplemented With Different Levels of BAP and Kinetin on Average Shoot Length, Raised From Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. Of BAP+Kn t/t	Average Shoot length				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	1.0+0.5	2.5	1.9	2.2	2.4	2.250	0.264	0.132
2.	1.0+1.0	2.8	2.5	3.3	2.6	2.800	0.355	0.177
3.	1.0+1.5	3.1	3.8	2.7	3.3	3.225	0.457	0.228
4.	1.0+2.0	4.2	3.9	4.8	4.6	4.375	0.403	0.457
5.	1.0+2.5	3.5	2.9	3.3	3.6	3.325	0.309	0.197

Table 13 : Effect of MS Medium Supplemented With Different Levels of BAP and Kinetin on Nodes/ Shoot, Raised from Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. of BAP+Kn t/t	Nodes/ Shoot				Mean $\bar{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	1.0+0.5	2	1	2	2	1.75	0.500	0.250
2.	1.0+1.0	3	2	3	2	2.5	0.577	0.288
3.	1.0+1.5	3	4	3	3	3.25	0.500	0.250
4.	1.0+2.0	6	5	6	6	5.75	0.500	0.250
5.	1.0+2.5	3	2	3	3	2.75	0.500	0.250

mg/l was studied. Shoot/explants ranged from 31.5 ± 2.645 to 8.25 ± 1.707. Maximum shoot/explants were obtained at 1.0mg/ml BAP in combination with 2.0mg/ml of kinetin with the value of 31.5 ± 2.645. At this concentration the values of experimental replicates are 35, 29, 32 and 30.

In the statistical analysis, standard error of mean was 1.180, standard error of difference was 1.669 and coefficient of variance was found to be 11.379. F-calculated value for replicates was 1.754 and for treatment was 75.852* * (Table 11).

Effect of BAP and Kinetin (Kn) on average shoot length of *Andrographis paniculata*

The effect of BAP (1.0 mg/l) in combination with Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5

mg/l was studied. Average shoot length ranged from 4.375 ± 0.403 to 2.250 ± 0.264. Maximum average shoot length was obtained at 1.0mg/ml BAP in combination with 2.0mg/ml of kinetin with the value of 4.375 ± 0.403. At this concentration the values of experimental replicates are 4.2, 3.9, 4.8, and 4.6.

In the statistical analysis, standard error of mean was 0.189, standard error of difference was 0.267 and coefficient of variance was found to be 11.859. F-calculated value for replicates was 0.625 and for treatment was 17.127* * (Table 12).

Effect of BAP and Kinetin (Kn) on node/shoot of *Andrographis paniculata*

The effect of BAP (1.0 mg/l) in combination with Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Nodes/shoot ranged from 5.750 ± 0.500 to 2.500 ± 0.577. Maximum nodes/shoot was obtained at 1.0mg/ml BAP in combination with 2.0mg/ml of kinetin with the value of 5.750 ± 0.500. At this concentration the values of experimental replicates are 6, 5, 6 and 6.

In the statistical analysis, standard error of mean was 0.241, standard error of difference was 0.341 and coefficient of variance was found to be 15.095. F-calculated value for replicates was 1.714 and for treatment was 39.857* * (Table 13).

Effect of BAP and Kinetin (Kn) on leaves/shoot of *Andrographis paniculata*

The effect of BAP (1.0 mg/l) in combination with Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Leaves/shoot ranged from 11.5 ± 1.00 to 3.5 ± 1.00. Maximum leaves/shoot was obtained at 1.0mg/ml BAP in combination with 2.0mg/ml of kinetin with the value of 11.5 ± 1.00. At this concentration the values of experimental replicates are 12, 10, 12 and 12.

In the statistical analysis, standard error of mean was 0.483, standard error of difference was 0.683 and coefficient of variance was found to be 15.059. F-calculated value for replicates was 1.714 and for treatment was 39.857* * (Table 14).

Effect of BAP and Kinetin (Kn) on Percentage of bud break of *Andrographis paniculata*

The effect of BAP (1.0 mg/l) in combination with Kn at various concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was studied. Bud break percentage ranged from 94.75 ± 2.217 to 73.5 ± 3.872. Maximum bud break percentage was obtained both at 1.0mg/ml BAP in combination with

Table 14 : Effect of MS Medium Supplemented With Different Levels of BAP and Kinetin on Leaves/ Shoot, Raised from Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc.of BAP+Kn t/t	Leaves/ Shoot				Mean $\overline{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	1.0+0.5	4	2	4	4	3.5	1.000	0.500
2.	1.0+1.0	6	4	6	4	5.0	1.154	0.577
3.	1.0+1.5	6	8	6	6	6.5	1.000	0.500
4.	1.0+2.0	12	10	12	12	11.5	1.000	0.500
5.	1.0+2.5	6	4	6	6	5.5	1.000	0.500

Table 15 : Effect of MS Medium Supplemented With Different Levels of BAP and Kinetin on Bud Break Percentage, Raised from Nodal Explants of *Andrographis paniculata* After 30 Days of Incubation

S.No.	Conc. of BAP+Kn t/t	Average Shoot length				Mean $\overline{X}$	SED	SEM
		Rep 1	Rep 2	Rep 3	Rep 4			
1.	1.0+0.5	69	75	78	72	73.5	3.872	1.936
2.	1.0+1.0	80	75	74	84	78.25	4.645	2.322
3.	1.0+1.5	89	86	88	90	88.25	1.707	0.853
4.	1.0+2.0	94	92	97	96	94.75	2.217	1.108
5.	1.0+2.5	96	97	92	94	94.75	2.217	1.108

2.0mg/ml of kinetin and with 2.5mg/ml of kinetin with the value of 94.75 ± 2.217.

In the statistical analysis, standard error of mean was 1.677, standard error of difference was 2.371 and coefficient of variance was found to be 3.904. F-calculated value for replicates was 0.385 and for treatment was 33.284* * (Table 15).

DISCUSSION

For the plant to grow via micropropagation, along with various nutritional requirements, some growth regulators (Hormones) are also required to support the luxuriant growth of tissues and organs. However required quantity varies depending on the endogenous levels of hormones of the tissue. Morphogenesis in vitro is regulated by the interaction between the exogenous plant growth substances. To obtain the morphogenetic response from the explants the culture medium was supplemented with different plant growth regulators (PGRs) viz. cytokinin and auxin in varied concentrations alone as well as in combination.

The regulatory role of hormones (PGRs) is most pronounced during the initial induction of proliferation in the primary explants, and most plant tissue required hormonal factors (auxin and cytokinin) for initial cell division (Dmitrieva 1985) Growth regulator concentration in the culture medium is critical to control the growth and morphogenesis (Skoog and Millar,1957). Supply of hormones in a proper sequence is important to achieve a particular response. Auxins stimulate root initiation on shoots but may inhibit or reduce subsequent growth of roots. Therefore, these sequences need to be standardized specially for various kinds of explants. It is well established fact that proper ratio of cytokinin and auxin is necessary for morphogenesis leading to the formation of complete plantlets (George and Sherrington, 1984).

The effects of BAP, BAP in combination of Kn and Kn alone was studied for the shoot multiplication of nodal explants. Nodal explants became swollen and developed shoots at the nodal region. The Percentage of response varied with various plant growth regulators, at various concentrations. The shoot forming capacity of the nodal explants was influenced by BAP and Kn as well. The positive effects of BAP on bud proliferation and multiple shoot formation were reported for the propagation of others plants including Helicteres isora L. (Shriram et al., 2008), *Trichodesma indicum* (Linn) R.Br. (Verma et al., 2008), and *Spilanthes acmella* Murr. (Singh et al., 2009) Among all the growth regulators tested BAP in combination with Kinetin gave best results at concentration of 1.0 mg/l BAP and .05 mg/l of Kinetin. The results were in accordance with the work of Bidari et al., (2012).

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REFERENCES

Bidari S.K., Anita S, Rajshekaran P.E, Shashidhara P, Tatti P.N.,2012. In *vitro* regeneration of *Andrographis paniculata* Nees- An important medicinal plant. An International Journal of Pharmaceutical Sciences. ISSN: 09767908, 2321-2330.

Dmitrieva N. N., 1985. Hormones, dedifferentiation and control of proliferation in cell and protoplast cultures. In: Plant cell culture R.G. Butenko (ed.) MIR Publishers, Moscow, 38-50.

George E. F. and P. D. Sherrington, 1984. In: Plant propagation by tissue culture- Handbook and Directory of Commercial Laboratories, George E.F. and Sherrington, P. D., eds, Exegetics Ltd, Eversly, Basingstone, UK, 1-38.

Hu C. Q. and Zhou B. N., 1982. Isolation and Structure of two new diterpenoid glucosides from *Andrographis paniculata* Nees. Yao Xue Xue Bao 1982 Jan ,**17**(6) : 435-440.

Shriram V., Kumar V. and Shitole M., 2008. Indirect organogenesis and plant regeneration in Helicteres isora L., and important medicinal plant. In vitro Cell Dev. Biol. Plant., **44**(3):186-193.

Singh S. K., Rai M. K, Asthana P., Pandey S., Jaiswal V. S. and Jaiswal U., 2009. Plant regeneration from alginate- encapsulated shoot tips of *Spilanthes acmella* (L.) Murr., A medicianally important herbal pesticidal plant sepecies. Acta Physiol. Plant. **31**(3):649-653.

Skoog F. and C.O. Miller, 1957. Chemical regulation of growth and organ formation in plant tissues cultivated in vitro, In: Biological Action of Growth Substances, Symp. Soc. Exp. Biol., **11**:118-131.

Verma N., Koche V., Tiwari K.L. and Mishra S. K., 2008. Plant regeneration through organogenesis and shoot proliferation in *Trichodesma indicum* (Linn.) R.Br. A medicinal herb. Afr. J. Bitechnol. **7** (20):3632-3637.

Zhong Yao, Cultivation of *Andrographis paniculata*. Tong Bao 1978 Jan; **12**(6) : 15-18.