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Effects of N-Benzyl-9-(2-tetrahydropyranyl) Adenine in Combination with Indole-3-butyric Acid on *in vitro* Culture of *Bauhinia variegata* L.

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Abstract
Firstly, the multiple shoots were obtained from the nodal segments *of Bauhinia variegata* L. cultured on Murashige and Skoog's (1962) (MS) medium containing 0.5 µM BAP (Benzylaminopurine) increased the high multiplication rate. Nodal segments inoculated on the MS medium with various combinations of 0.5, 1.0, 2.0 and 5.0 µM BPA (N-Benzyl-9-(2-tetrahydropyranyl) adenine with 0.1, 0.5, 1.0 and 2.0 µM concentrations of IBA (Indole-3-butyric acid) and separately showed drastically different results. Healthy propagated plants were acclimatized and transferred to the field. Data were worked out statistically with SPSS, a system of analytical procedure.

INTRODUCTION

Bauhinia variegate L. locally known as 'Koiralo' is a medium sized tree measuring 5-10 m in height. It is distributed throughout Nepal up to the elevation of 1800 m (Anonymous 1970). It is an important multipurpose agroforestry tree valued mainly for fodder, fuel, timber, medicine and dyeing agent. The flowers and buds are used as vegetable and pickle. Soup preparations from dried buds are given to the patient of diarrhoea, dysentery, piles and worms. The bark is described as an astringent, alternative tonic and useful in scrofula, skin diseases and ulcers. The bark is also reported to be used in dyeing purpose. Root is used as antidote to the snake poison and decoction of root is used in Dyspepsia (Anonymous 1970). Plant regeneration has been quite difficult among the tree legume (Mc Hughen and Swartz 1984, Hughe 1990). However, few species are regenerated *in vitro* like *Acacia koa* (Skolmen, 1986), *Albizia lebbeck, Sesbania sesban* (Ghariyal and Maheshori, 1980) and *Dalbergia sissoo* (Mukopadhya and Mohan Ram, 1981). *Dalbergia sissoo* has been successfully propagated from the cotyledonarynode culture on Murashige and Skoog's (MS) medium supplemented with 1 mg/l BAP and 0.1 mg/l NAA (Suwal *et al.* 1986).

Bauhinia variegata plants are decreasing day by day because of unmanaged cutting. So, it is very important to propagate and need to conserve the plants.

MATERIALS AND METHOD

The seeds of *B. variegata* L were procured from Aforestation Deparment Hattisar, Kathmandu, Nepal and were carried to the laboratory of Institute of Pharmacognosy, Vienna, Austria and were preserved at 4ºC until experimental use. The healthy seeds were washed with few drops of teepol

detergent solution and water. They were soaked in distilled water for an hour prior to sterilization. The soak seeds were washed with sterilized distilled water for 5 times and sterilized in 10% sodium hypochlorite for 10 minutes and washed with sterile water for 5 times. The seeds were again sterilized in 70% alcohol for one minute and washed with sterile distilled water for 3 times. The seeds were inoculated on Murashige and Skoog (1962) medium containing 3% sucrose and pH of the medium was adjusted to 5.8 ± 1 before autoclaving 15 lb/sqinch at 121ºC for 20 minutes. The media were solidified by adding 0.8% agar (bacteriological). The culture was maintained at 25ºC ± 2ºC under a photoperiod of 16h (40 µ mol.m–²s-1 supplied by OSRAM Biolux tubes) in the growth chamber with a level of 70% relative humidity was maintained.

The nodal explants (1cm) long were excised from the germinated seedlings and subcultured on MS medium plus 0.5 µM BAP to obtain sufficient explants for further experimentation. Such obtained explants were transferred on the medium with IBA alone with the concentration of 0.1, 0.5, 1.0 and 2.0 µM and BPA alone with the concentration of 0.5, 1.0, 2.0 and 5.0 µM and combination of both. Altogether twenty-five combinations of IBA and BPA and separately were used. The results were taken after 8-weeks of culture. For statistical reliability each of the experiments was performed twice and the mean ± (standard error) was calculated by SPSS, a system of analytical procedure.

Healthy propagated shoots were cut with nearly 3-4 nodes of 2-3 cm sized and transferred for rooting in plastic pots(diameter 6 inch) and were filled with 1:1 ratio of sand and soil (Humus- Ton substrate N8). The substrate was soaked with an aqueous solution of the fungicides Benlate and Previcure. The plants were kept at high humidity for two weeks, the humidity was reduced at second week. The well rooted and acclimatized plants were transferred to green house for further hardening.

RESULTS

The explants implanted on MS medium supplemented with 0.5 µM BPA showed 6.50 nodes, elongation of shoot 57.25 mm and calli proliferations 10.30 ø(mm). Medium with 1.0 to 2.0 µM BPA produced only optimum nodes as well as shoots and calli too. Cultures grown on MS medium with 5.0 µM BPA showed the best results with 6.15 nodes and 68 mm shoot length and 11.30 ø(mm) calli after eight weeks of cultures, (Fig.1).

Presence of IBA on the MS medium promoted little growth of nodes and little increase in shoot length. Higher number of nodes 3.90 and 18.62 mm shoot length was recorded on MS medium supplemented with 2.0 µM IBA. The calli formations were not recorded on 0.1 and 0.5 µM IBA but 1.0 µM showed slightly proliferation of calli ie. 0.05 (ø) mm and with 2.0 µM IBA showed 3.33 (ø) mm calli growth.

The nodal explants cultured on MS medium supplemented with BPA and IBA showed very poor growth of nodes as well as shoot length. Maximum node formation 5.0 was observed on MS medium supplemented with 1.0 µM BPA plus 0.1 µM IBA whereas, the highest shoot length 20.05 mm was observed on MS medium augmented with 0.5 µM BPA and 0.1 µM IBA as compared to control medium (Fig.2). The calli formations were found slightly increased as it increases the concentration of BPA. In remaining concentrations, the multiplication of shoot and the node formations was not satisfactory and the plants produced were unhealthy (Table-1).

Table 1: Effects of various concentrations of BPA and IBA on *in vitro* culture of *Bauhinia variegata* L

Additive/s in Media (µM)		Number of Nodes/culture Mean ± SE	Shoot length(mm) Mean ± SE	Q Calli (mm) Mean ± SE
BPA				
0.5		6.50±0.4	57.15±3.5	10.30±0.4
1.0		5.8 ± 0.5	43.45±3.4	8.75±0.4
2.0		4.40±0.3	38.35±2.2	10.20±0.3
5.0		6.15±0.4	68.00±7.0	11.30±0.4
IBA				
0.1		3.15±0.2	13.87±0.5	00.00±0.0
0.5		3.10 ± 0.2	15.25±0.6	0.00±0.0
1.0		2.62±0.2	13.30±0.6	0.05±0.0
2.0		3.90±0.2	18.62±1.1	3.33±0.3
BPA	**IBA**			
0.5	0.1	4.55 ± 0.3	20.05 ± 1.3	5.75 ± 0.4
1.0		5.00 ± 0.5	19.20 ± 1.8	7.30 ± 0.3
2.0		4.25 ± 0.4	15.65 ± 1.4	7.85 ± 0.4
5.0		3.50 ± 0.3	12.30 ± 1.0	8.95 ± 0.4
0.5	0.5	3.60 ± 0.3	15.25 ± 1.1	6.60 ± 0.4
1.0		3.25 ± 0.4	13.70 ± 1.2	7.00 ± 0.4
2.0		4.80 ± 0.4	17.65 ± 2.0	7.70 ± 0.4
5.0		3.25 ± 0.4	14.45 ± 1.9	7.00 ± 0.4
0.5	1.0	2.85 ± 0.4	17.10 ± 1.8	7.60 ± 0.4
1.0		3.20 ± 0.4	18.45 ± 2.0	6.55 ± 0.4
2.0		2.90 ± 0.4	14.15 ± 1.9	7.30 ± 0.4
5.0		2.65 ± 0.3	16.85 ± 1.8	9.50 ± 0.8
0.5	2.0	2.85 ± 0.3	15.45 ± 1.8	6.30 ± 0.3
1.0		2.80 ± 0.4	15.95 ± 1.8	8.10 ± 0.3
2.0		2.95 ± 0.4	14.45 ± 1.4	8.55 ± 0.4
5.0		3.00 ± 0.4	13.75 ± 1.8	8.75 ± 0.6
Control		3.85± 0.2	18.20± 1.3	0.00±0.0

Figure 1: Shoot proliferation on *B. variegata* on MS medium Containing 5 µM BPA

Figure 2: Shoot proliferation on *B. variegata* on MS medium containing 0.5 µM BPA +0.1 µM IBA.

DISCUSSION

The best results were observed on MS medium supplemented with 5.0 µM BPA. Where 6.15 nodes, 68 mm shoot length and 11.30 Φ (mm) calli were recorded. The plants grown on MS medium with BPA produced very healthy plants with appropriate sized leaves and rigid stem. They could be easily handed for propagation and subcultures. But IBA concentrations 0.1, 0.5, 1.0 and 2.0 µM showed less proliferation of shoots and node formations. Similarly, in all the treated combinations of BPA and IBA also did not lead to propagation of plants. Dhar and Uprety (1999) developed a protocol for good nodal culture of *Bauhinia vahlii* using MS medium supplemented with 2.5 µM Kn plus 100 mg/l adenine sulphate. On the other hand, Kumar (1992) propagated *Bauhinia purpurea* from stem cuttings of young branches of 15-18-year-old mature elite tree on MS medium supplemented with 5µM Kn. Similarly, Mathur and Mukunthakumar (1992) propagated *Bauhinia variegata* and *Parkinsonia aculeata* from nodal explants of mature (6-8-year tree) on the MS medium with 2.21- 31.1µM BAP. Beck *et al*. (1998) worked on *Acacia mearsii* and produced multiple shoots from nodal explants on MS medium supplemented with 2.0 mg/l BAP. Whereas, Sinha *et al*. (2000) multiplied *Albizia chinensis* from petiolar and distal cotyledonary segments on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l IAA. But Rout *et al*. (2001) encapsulated nodal explants excised from aseptic shoot cultures of *Plumbago zeylanica* in a sodium alginate matrix under aseptic conditions developed on MS basal salts supplemented with 1.0 mg/l BA plus 0.01 mg/l IAA. Singh (2019) propagated *Bauhinia purpurea* L. from nodal culture on MS medium supplemented with Benzylpyranyladenine and indole-3-acetic acid. Any way overall experiments the best protocol developed for the propagations of *Bauhinia variegata* was found 5.0 µM BPA alone.

CONCLUSION

In conclusion, the *in vitro* protocol developed in this study for regenerating plantlets of *Bauhinia variegata* using nodal culture is an important opportunity for large scale *in vitro* propagation and its conservation.

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