



## Effects of N-benzyl-9-(2-tetrahydropyranyl) and indole-3-acetic acid *in vitro* culture of *Butea buteiformis* Voigt

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### Abstract

*Butea buteiformis* is a valuable tree with large trifoliolate leaves with beautiful flower (Fig.1). It is distributed in tropical and subtropical region of Nepal. The seeds are bitter in taste and used for an anthelmintic. The plant possesses importance in agroforestry due to their nitrogen fixing capability. Plants are used for reforestation mostly on slope areas. The seeds were surface sterilized and cultured on half strength Murashige and Skoog (1962) (MS) medium. Nodal explants obtained from cultured were subcultured on different concentrations of N-Benzyl-9-(2-tetrahydropyranyl) (BPA) and Indole-3-acetic acid (IAA). The best proliferation of nodes and shoots were observed on the MS medium supplemented with 0.5  $\mu\text{M}$  BPA and 0.5  $\mu\text{M}$  IAA. After 8 weeks of culture the propagated plants were acclimatized and transferred to the sand box containing 1:1 soil and sand. Well rooted plants were then established in the field. All the data collected were worked out statistically with SPSS, a system of analytical procedure.

**Keywords:** *Butea buteiformis*, micropropagation, N-benzyl-9-(2-tetrahydropyranyl), Indole-3-acetic acid (IAA), acclimatization

### Introduction

*Butea buteiformis* is a shrub tree with large trifoliolate leaves with beautiful flower (Fig.1). It is distributed in tropical and subtropical region of Nepal. The seeds are bitter in taste and used for an anthelmintic. The flower possesses importance in agroforestry due to their nitrogen fixing capability. Plants are used for reforestation mostly on slope areas. Micropropagation of plantlets from shoot tip have been reported in *Mallus prunifolia*<sup>[1, 2]</sup> in *Citrus grandis*<sup>[3]</sup>, in *Kalmia latifolia* and leaflet explants were carried in *Albizia procera*<sup>[4]</sup>. Leguminous plants have traditionally been difficult to regenerate from cell culture. However, multiplications of *Albizia* have been successfully reported<sup>[5]</sup>. Cotyledonary nodes were used for micropropagation in *Dalbergia sissoo*<sup>[6]</sup>. However, a protocol for regeneration *in vitro* in *Butea buteiformis* was not known.

This investigation is aimed to see the effects of BPA and IAA and to obtain maximum propagation of *Butea buteiformis* through culture of nodal explants. If the good protocol obtained from this experiment will be important aspect in forestry.

### Material and Methods

Mature seeds were collected from the plants grown in the garden of Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal and were brought to the Laboratory of Institute of Pharmacognosy, University of Vienna, Austria, where they were stored in the refrigerator at 4°C until use. First of all, the seeds were soaked in distilled water with few drops of tween 20 for an hour and washed three times with sterilized distilled water. They were surface sterilized by ringing in 30% ethanolic for 10 min followed by treatment of 10% sodium hypochlorite for 10 minutes and then rinsed three times with sterilized distilled water.

Then the sterilized seeds were implanted on half strength MS (Murashige and Skoog 1962), medium<sup>[7]</sup>. The pH of the medium was adjusted to 5.8 $\pm$  1 before autoclaving at 121°C for 20 minutes. The media was solidified by adding 0.8% agar (bacteriological) and 3% sucrose. The cultures were maintained at 25°C $\pm$  4°C under a photoperiod of 16 h (40mol. m<sup>-2</sup>s<sup>-1</sup> supplied by OSRAM Biolux tubes) in the growth chamber with a level of 70% relative humidity

Under this condition, the seeds germinated after 10-12 days (Fig. 2). After two weeks, the nodes (1 cm long) from germinated seedlings were excised and cultured on MS media containing 3% sucrose with different concentrations 0.5, 1.0, 2.0 and 5.0  $\mu\text{M}$  of BPA and 0.1, 0.5, 1.0 and 2.0  $\mu\text{M}$  IAA.

### Results

All the results were taken only after eight weeks of culture. On the MS medium supplemented with 0.5, 1.0, 2.0 and 5.0  $\mu\text{M}$  BPA each with 0.1  $\mu\text{M}$  IAA, the number of node proliferation was observed 2.0-2.5, the shoot length elongation was recorded 11-14.5 mm and the calli formation 11-14  $\theta$  mm calli was recorded. Here the growth was suppressed rather than stimulating the proliferation of shoots.

On the MS medium supplemented with 0.5, 1.0, 2.0 and 5.0  $\mu\text{M}$  BPA each with 0.5  $\mu\text{M}$  IAA, the number of node proliferation was observed 1.7-3.5, the shoot length elongation was recorded 11.0-24.9 mm and the calli formation 12-15  $\theta$  mm calli were recorded. Here the growth of nodes and the shoot length elongation was found better (Fig.3).

On the MS medium supplemented with 0.5, 1.0, 2.0 and 5.0  $\mu\text{M}$  BPA each with 1.0  $\mu\text{M}$  IAA, the number of node proliferation

was observed 2.0-2.7, the shoot length elongation was recorded 10.0-13.0 mm and the calli formation 7-15  $\theta$  mm calli were recorded. Here the growth was found not satisfactory for the proliferation of nodes as well as shoots.

On the MS medium supplemented with 0.5, 1.0, 2.0 and 5.0  $\mu$ M BPA each with 2.0  $\mu$ M IAA, the number of node proliferation was observed 2.0-2.6, the shoot length elongation was recorded 10.0-21.0 mm. The plants obtained were moderate sized.

On the MS medium without hormone (control medium), the number of node proliferation 2.63 was recorded, the shoot length elongation was recorded 21.17 mm. and up to 13.08  $\theta$  mm calli were recorded. The plants obtained were moderate sized (Table-1).

**Table 1:** Effects of BPA in combination with IAA in *Butea buteiformis* Voigt.

Additive/s in Media ( $\mu$ M)		Number of Nodes/culture Mean $\pm$ SE	Shoot length (mm) Mean $\pm$ SE	$\theta$ Calli (mm) Mean $\pm$ SE
BPA	IAA			
0.5	0.1	2.50 $\pm$ 0.3	14.00 $\pm$ 2.0	10.75 $\pm$ 1.4
1.0		2.08 $\pm$ 0.3	14.50 $\pm$ 3.7	11.17 $\pm$ 2.3
2.0		2.25 $\pm$ 0.1	13.08 $\pm$ 1.4	14.92 $\pm$ 0.7
5.0		2.25 $\pm$ 0.1	11.17 $\pm$ 0.8	13.67 $\pm$ 1.8
0.5		3.50 $\pm$ 0.1	24.92 $\pm$ 2.3	15.67 $\pm$ 0.9
1.0	0.5	2.83 $\pm$ 0.3	13.92 $\pm$ 1.6	14.17 $\pm$ 1.3
2.0		1.92 $\pm$ 0.2	11.00 $\pm$ 0.7	12.83 $\pm$ 0.8
5.0		1.75 $\pm$ 0.1	11.58 $\pm$ 1.0	13.25 $\pm$ 1.1
0.5		2.75 $\pm$ 0.3	13.50 $\pm$ 1.8	7.25 $\pm$ 1.6
1.0	1.0	2.33 $\pm$ 0.2	12.17 $\pm$ 1.3	10.75 $\pm$ 1.5
2.0		2.67 $\pm$ 0.3	17.25 $\pm$ 1.5	15.58 $\pm$ 1.1
5.0		2.08 $\pm$ 0.3	10.25 $\pm$ 0.7	14.67 $\pm$ 1.3
0.5	2.0	2.67 $\pm$ 0.3	14.17 $\pm$ 1.5	11.67 $\pm$ 1.6
1.0		2.00 $\pm$ 0.2	10.42 $\pm$ 1.0	15.50 $\pm$ 1.1
2.0		2.67 $\pm$ 0.2	11.67 $\pm$ 1.2	13.83 $\pm$ 0.8
5.0		2.33 $\pm$ 0.3	10.75 $\pm$ 0.8	13.83 $\pm$ 0.9
Control			2.63 $\pm$ 0.2	21.17 $\pm$ 2.9



**Fig 1:** Flowering plant of *Butea buteiformis*.



**Fig 2:** Seedling grown *in vitro* culture.



**Fig 3:** Left to right, 8 weeks old plants, on MS medium supplemented with 0.5, 1.0, 2.0 and 5.0  $\mu$ M BPA each with 0.5  $\mu$ M IAA.

For acclimatization the eight weeks old healthy plants grown *in vitro* were removed from the culture and washed thoroughly in tap water to remove traces of nutrient medium and agar. The plastic pots (diameter 6 cm) were filled with soil (Humus-Ton substrate N8) with sand in 1:1 ratio and hardened in mist chamber. The substrate was disinfected by using Benlate and Pevicure. The plants were kept at high humidity (80 %) for two weeks; the humidity was reduced to (60 %) and the acclimatization process continued for two weeks. The well rooted and acclimatized plants were transferred to green house for further hardening.

### Discussion

Among all above factorial combinations of BPA and IAA, the

proliferations of nodes and shoot length elongation was observed optimum growth. That means the response of BPA with IAA is not good for the proliferation of shoots. On MS medium the supplemented with 0.5  $\mu\text{M}$  BPA and 0.5  $\mu\text{M}$  IAA, the formation of nodes 3.50 and proliferation of shoot length 24.92 and the calli formation 15.67  $\theta$  mm were recorded. In this combination the plants produced were dark green healthy with good proliferation of shoots compared with MS basal medium i.e. control medium. *Lilium nepalensis* D. Don, was propagated on MS medium supplemented with, 2.0  $\mu\text{M}$  Zin using longitudinally splited shoot halves <sup>[8]</sup>. The results in *Pinus wallichiana* A.B. Jack was failed to induce buds in presence of Zin at the concentrations of 0.25, 0.5, 15.0  $\mu\text{M}$  <sup>[9]</sup>. Similarly, regeneration was successful in *Guazuma ulmifolia* Lan. From shoots taken from five month old potted seedlings on woody plant medium containing 1.0 mg/l Zin <sup>[10]</sup>. Important medicinal herb *Scoparia dulcis* L. was propagated from shoot and nodal segments <sup>[11]</sup>. Whereas, *Dalbergia sissoo* Roxb. Was propagated when cultured on MS medium supplemented with 1.0 mg/l BAP and 0.1mg/l NAA <sup>[6]</sup>. In the same way, the multiple shoots of *Bauhinia purpurea* was propagated from nodal explants obtained from MS medium with 0.5  $\mu\text{M}$  N-Benzyl-9-tetrahydropyranyl and 0.1  $\mu\text{M}$  Indole -3-acetic acid <sup>[12]</sup>. Again, *Bauhinia purpurea* was propagated on MS medium with 0.5  $\mu\text{M}$  N-Benzyl-9-tetrahydropyranyl for multiplication of shoots and 5.0  $\mu\text{M}$  Zeatin for multiplication of nodes <sup>[13]</sup>.

In the present investigation, the results obtained in all the concentrations of BPA and IAA are not satisfactory and cannot be applicable for the protocol development for the micropropagation of *B. variegata* as compared to former research work.

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