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Original Article

Effect of Zeatin 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 trifoliate leaves with beautiful flower. The seeds were surface sterilized and cultured on half strength Murashige & Skoog (1962) (MS) medium. Nodal explants obtained from cultured were subcultured on different concentrations of zeatin (Zin) and Indole-3-acetic acid (IAA). The best proliferation of nodes and shoots were observed on the MS medium supplemented with 5 µM Zin and 1.0 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.

INTRODUCTION

Butea buteiformis is a shrub tree with large trifoliate 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 reforestration mostly on slope areas.

Various parts of the plants are micropropagated which are reported from previous works. Micropropagations of plantlets from shoot tip have been reported by Aiya *et al*. (1982) in *Mallus prunifolia*, Paudyal and Haq (2000) in *Citrus grandis*, Lloyd G. and McCown (1980) in *Kalmia latifolia* and leaflet explants were carried out by Kumar *et al*. (1998) in *Albizia procera*. Legumes have traditionally been difficult to regenerate from cell culture. However, multiplications of *Albizia* have been conducted by Gharyal & Maheshori (1980).

Cotyledonary nodes were used for micropropagation by Suwal *et al*. (1988) in *Dalbergia sissoo*. However, a protocol for regeneration *in vitro* in *Butea buteiformis* was not known.
This investigation is aimed to obtain maximum propagation of *Butea buteiformis* through culture of nodal explants using cytokinin. The protocol obtained from this experiment will be an 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 4O°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 sterile distilled water. They were surface sterilized by rinsing in 30% ethanol for 10 min. followed by treatment of 10% sodium hypoclorite for 10 minutes and then rinsed three times with sterile distilled water. Then the sterilized seeds were implanted on half strength MS (Murashige & Skoog, 1962) medium. The pH of the medium was adjusted to 5.8± 1 before autoclaving at 121^{o}C for 20 minutes. The media was solidified by adding 0.8 % agar (bacteriological) and 3 % sucrose.

The cultures were maintained at 25 ^{o}C ± 4^{o}C under a photoperiod of 16 h (40mol. $M^{-2}s^{-1}$ 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. 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 µM of Zeatin and 0.1,0.5,1.0 and 2.0 µM Indole -3-Acetic Acid.

RESULTS AND DISCUSSION

Response of Zin concentrations 0.5, 1.0, 2.0 and 5.0 µM of each with 0.1 µM IAA showed good proliferation of nodes and shoot length elongation. Highest shoot length 33.0 mm was recorded on MS medium supplemented with 2.0 µM Zin and 0.1 0 µM IAA, the multiplication of nodes was 2.33 and shoot elongation 22.50 mm and few masses of calli were proliferated. On MS medium supplemented with 1.0 medium supplemented with 1.0 plus 1 0 µM IAA, healthy green shoots with 3.67 node multiplication and 32.50 mm shoot length proliferation were recorded. Whereas MS medium the supplemented with 5.0 µM BAP and 1 0 µM IAA, the nodes formation was 2.42 and shoot length formation was 24.50 mm.

Figure 1: Flowering plant of *Butea buteiformis*.

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

Additive/s in Media (µM)		Number of Nodes/culture Mean ± SE	Shoot length(mm) Mean ± SE	Q Calli (mm) Mean ± SE
Zin	**IAA**			
0.5	0.1	2.33 ± 0.3	22.50 ± 2.1	0.92 ± 3.0
1.0		3.67 ± 0.3	32.50 ± 4.5	12.08 ± 2.0
2.0		3.58 ± 0.4	33.00 ± 3.8	19.33 ± 2.1
5.0		2.42 ± 0.3	24.50 ± 3.0	17.75 ± 1.6
0.5	0.5	2.67 ± 0.1	24.75 ± 3.4	17.08 ± 2.8
1.0		2.17 ± 0.2	22.17 ± 3.5	10.42 ± 2.9
2.0		3.58 ± 0.4	29.17 ± 3.1	14.83 ± 3.0
5.0		2.83 ± 0.5	25.50 ± 3.9	21.75 ± 0.8
0.5	1.0	3.75 ± 0.3	23.50 ± 1.9	14.00 ± 1.4
1.0		1.67 ± 0.2	22.67 ± 2.9	20.50 ± 0.6
2.0		2.33 ± 0.4	15.75 ± 2.0	13.50 ± 4.2
5.0		3.42 ± 0.2	36.25 ± 5.5	19.75 ± 1.4
0.5	2.0	4.08 ± 0.5	29.92 ± 2.5	21.58 ± 1.4
1.0		3.00 ± 0.3	14.25 ± 1.2	15.58 ± 1.5
2.0		3.08 ± 0.5	17.33 ± 2.2	18.92 ± 1.8
5.0		3.42 ± 0.4	26.92 ± 3.3	18.33 ± 1.8
Control		2.63 ± 0.2	21.17 ± 2.9	13.08 ±2.0

Figure 2: showing propagation of plants on 5.0 µM of Zin with 1.0.µM IAA

On the next combination i.e. on MS medium supplemented with.0.5, 1.0, 2.0 and 5.0 µM of Zin each with 0.5 µM IAA showed optimum growth of the plants. Maximum nodes 3.58 with shoot length 29.17 mm and 14.83(ϕ) mm calli were observed on MS medium supplemented with 2.0 µM Zin 0.5 µM IAA. Among these combinations highest proliferations of calli i.e. 21.75 (ϕ) mm were observed on MS medium with 5.0 µM Zin and 0. 5 µM IAA. The nodal culture on MS medium in combination with 0 5 µM of Zin and 1.0 µM IAA showed 3.75 nodes and the shoot elongation 23.50 mm with 14.0(ϕ) mm calli proliferation. Whereas in combinations of 1.0 and 2.0 µM Zin each with 1.0 µM IAA, the multiplication of the nodes and the elongation of the shoot length were very poor and were not good for the proliferation of plants. But in combination with 5.0 µM of Zin with 1.0.µM IAA the node multiplication 3.42 and 36.25 mm of shoot length elongation were recorded. This is the highest number of proliferation occurred among all factorial combinations (Figure 2).

Next in combination of 0.5, 1.0, 2.0 and 5.0 µM of Zin each with 2.0 µM IAA highest nodes 4.08 and 29.92 shoot elongation and 21.58 (ϕ) mm calli were recorded (Table 1).

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 Previcure. 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.

Among all above factorial combinations of Zin and IAA, the proliferations of nodes and shoot length elongation was observed optimum growth .That means the response of Zin with IAA is not bad for the proliferation of shoots. On MS medium the supplemented with 5.0 µM of Zin and 1.0 µM IAA, the formation of nodes 3.42 and proliferation of shoot length36.25 were recorded. In this combination the plants produced were dark green healthy with good proliferation of shoots.

Wawrosch *et al.* (2001) propagated *Lilium nepalensis* D.Don, on MS medium the supplemented with, 2.0 µM Zin using longitudinally splited shoot halves. Mathur & Nadgauda (1999) in *Pinus wallichiana* A.B. Jack failed to induce buds in presence of Zin at the concentrations of 0.25, 0.5, 15.0 µM. However in *Vigna unguiculata* (L) Walp found that the presence of Zin was essential for conversion of cotyledonary stage embryos into plantlets. The germination of cotyledonary stage embryos into plantlets occurred in half strength MS semisolid medium containing Zin 2 µM, 3% manitol and 5.0 µMABA. Similarly, Maruyama & Isii (1998) regenerated *Guazuma ulmifolia* Lan. shoots taken from five month old potted seedlings on woody plant medium containing 1.0 mg/l Zin. Rashid *et al*. (2009) propagated important medicinal herb *Scoparia dulcis* L. from shoot and nodal segments.

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