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Micropropagation of *Bauhinia variegata* L. Through Tissue Culture

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ABSTRACT: *Bauhinia variegata* L. locally known as "Koiralo" attains a medium sized tree with valuable flowers. The healthy seeds were surface sterilized by treating with 1% sodium hypochlorite solution (NaClO) for 20 minutes and removed the traces of NaClO by washing thoroughly with sterilized distilled water five times and then cultured on Murashige and Skoog (1962) (MS) medium. Multiple shoots were obtained from nodal segments of *in vitro* grown plantlets. Cultured nodal explants were first sub-cultured on MS plus 0.5 µM BAP(6-Benzylaminopurine) to multiply for the screening test of growth hormones. Nodal segments inoculated on the medium with various combinations of BAP and IAA (Indole-3-acetic acid) and separately showed varied results. Propagated plants were successfully acclimatized and rooted in the pot (6 cm diameter) in the 1:1 ratio soil and sand. The experiments were performed twice and the results obtained were calculated by SPSS, a system of analytical procedure.
Keywords: *Bauhinia variegata,* Murashige and Skoog (1962) medium, nodal culture, BAP and IAA

I. INTRODUCTIONS

Bauhinia variegata L. is economically important plant species in Nepal. The plant is locally known as "Koiralo", which is a medium sized tree with strong trunk. The leaves of the plant are deciduous and deeply cordate with prominent nerves. The flowers are large, fragrant with white and purplish petals. The tree plant generates flowers during March to April and fruits are formed in September to November. It is cultivated at an elevation of 100 m to 1600m on loamy soil. The bark is traditionally used in natural dyeing as well as the source of tannin and is reported to be applied in local textile dyeing. Traditional food items such as the Curry and Pickles are prepared from the flowers and the buds. The plant is accepted to yield a good fodder for the cattle. Decoction of young leaves after boiling is taken to cure the stomach trouble. The extracts of bark areapplied for the treatment of cancer. The wood is moderately hard and is used for making agricultural implements and also used for fuel. Leguminous trees possess importance in agro forestry due to their nitrogen fixing capability. They are also suitable plants for reforestation. Plants listed in CITIS (Convention on International Trade in Endangered Species) can be propagated *in vitro* and disseminate for cultivation. Tissue culture technique has been found in large scale application for the propagation of many agricultural and horticultural crops.However, in forestry it is not yet in wide application mainly because of the lack of protocols.

Micropropagation of plantlet from nodal culture have been reported by Kumar (1992) in *Bauhinia purpurea,* Silva and Sauza (1992) in *Ailanthus malabarica* D.C., Pradhan (1994) in *Eucalyptus tereticornis*, Beck *et al*. (1998) in *Acacia mearsii*.

This investigation is aimed to obtain maximum propagation of *Bauhinia variegata* throughthe culture of nodal explants. Huge cutting of this plant every year for its valuable flower is tending it to endangered condition. So, it is very necessary to protect and recultivate the plants. Therefore, the protocol developed for this plant will be an important aspect in forestry.

II. METHODOLOGY

The healthy seeds were selected and serially washed in running tap water for half an hour and washed with 100 ml of water with 3 drops of teepol and washed with distilled water for five times and surface sterilization was carried out by treating with 1% sodium hypochlorite solution (NaHOCl) for 20 minutes and removed the traces of NaHOCL by washing thoroughly with sterilized distilled water five times inside Laminar flow hood chamber. Finally, the seeds were again sterilized in 70 % alcohol for one minute and washed with sterilized distilled water for 5 times to remove the alcohol. The seeds were then inoculated in standard medium Murashige and Skoog's (1962) with 3 % sucrose. The medium was solidified with 0.8 % bacteriological agar and pH was adjusted to 5.8 before autoclaving and then sterilized at 15 lb. /sq. inch pressure for 15 minutes in

autoclave. Cultures were maintained in the culture room at 25°C (±2°C). Cool white fluorescent light of an intensity of approx. 40 µ mol.m^{-2} s^{-1} was supplied through OSRAM BIOLUX tubes at a 16 hr light period.

III. RESULT

Nodal explants obtained from germinated seedling were cultured on MS medium containing 0.5µM BAP produced multiple shoots. Nodal explants obtained from MS with 0.5µM BAP were transferred in the medium combination of IAA with the concentration of 0.1, 0.5, 1.0 and 2.0 µM and BAP with the concentration of 0.5, 1.0, 2.0 and 5.0 µM. Altogether 16 combinations of IAA and BAP were used and each compared with control medium. The experiments were repeated twice. All the results were recorded after 8 weeks of culture. For statistical reliability each of the experiments was performed twice and the Mean ± SE (standard error) was calculated by SPSS program, a system of analytical procedure.

Table-1: Effects of various concentrations of BAP and IAA in *Bauhinia variegata* L.

Treatments/ Media (µM)		**Number of Nodes/culture Mean ± SE**	**Shoot length(mm) Mean ± SE**	**Q Calli (mm) Mean ± SE**
BAP	IAA			
0.5	0.1	5.25 ± 0.4	34.60 ± 3.8	7.65 ± 0.4
1.0		5.35 ± 0.7	35.75 ± 5.4	7.00 ± 0.5
2.0		5.95 ± 0.6	53.20 ± 5.5	8.70 ± 0.4
5.0		5.50 ± 0.5	29.35 ± 3.2	10.90 ± 0.5
0.5	0.5	4.70 ± 0.4	22.40 ± 1.8	7.75 ± 0.4
1.0		3.85 ± 0.4	41.10 ± 5.3	7.90 ± 0.4
2.0		5.30 ± 0.4	38.65 ± 5.7	10.00 ± 0.5
5.0		4.55 ± 0.3	28.15 ± 2.8	10.45 ± 0.4
0.5	1.0	5.75 ± 0.6	41.10 ± 5.3	7.25 ± 0.6
1.0		4.60 ± 0.5	38.65 ± 5.7	8.95 ± 0.6
2.0		5.05 ± 0.4	28.15 ± 2.8	9.20 ± 0.5
5.0		4.85 ± 0.3	25.85 ± 2.2	10.65 ± 0.5
0.5	2.0	4.15 ± 0.4	24.50 ± 2.6	8.45 ± 0.5
1.0		4.10 ± 0.4	22.95 ± 2.9	8.45 ± 0.4
2.0		4.80 ± 0.3	31.25 ± 2.5	9.15 ± 0.3
5.0		6.30 ± 0.5	31.50 ± 2.4	12.65 ± 0.4
Control		3.85± 9.2	18.20 ± 1.3	0.00 ± 0.0

On the MS medium supplemented with 0.5, 1.0, 2.0 and 5.0 µM BAP each with 0.1 µM IAA resulted in increase in number of nodes and shoot elongation (Fig.1.). On MS medium supplemented with 2.0 µM BAP and 0.1 µM IAA the highest nodes 5.95 were propagated andshoot length also measured highest 53.20 mm.

Fig.1.MSwith BAP 0.5,1.0,2.0 and 5.0 each with 0.1 IAA

Fig.2.MSwith BAP 0.5,1.0,2.0 and 5.0 each with 0.5 IAA.

Fig.3.MS with BAP 0.5,1.0, 2.0 and 5.0 each with 1.0 IAA.   Fig.4.MSwith BAP 0.5,1.0,2.0 and 5.0 each with 2.0 IAA

Fig.5.Control medium.

On the medium with 0.5 µM IAA in combination with 0.5, 1.0, 2.0 and 5.0 µM BAP separately, nodal propagation was found highest in 2.0 µM BAP (5.30) and shoot length was 33.0 mm. (Fig.2.). On MS medium supplemented with 0.5, 1.0, 2.0 and 5.0 µM BAP each with 1.0 µM IAA varied shoot length 25-41mm were recorded (Fig.3). Similarly, MS medium supplemented with 0.5, 1.0, 2.0 and 5.0 µM BAP each with 2.0 µM IAA showed optimum growth of nodes and shoot elongation (Fig.4). On the medium with high concentrations of BAP ie.5.0 µM and 2.0 µM IAA were observed 6.30 nodes and shoot length elongation 31.50 mm. Leaves formed in all the cultures were very healthy and dark green in color. Plants produced were also healthy with rigid stem. The calli formation were maximum on MS medium supplemented with BAP 5.0 µM plus 2.0 µM IAA in comparison with control medium (Fig.5). In all the above mentioned factorial combinations of BAP and IAA calli formations were recorded slightly higher in higher concentrations of hormones.

Best propagated shoots on above mentioned medium were cut with nearly 3-4 nodes of 2-3 cm sized and transferred for rooting in 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.

IV. DISCUSSION

Relatively few scientificwork have been reported on *Bauhinia* species in tissue culture. Bhatta and Dhar (2000) regenerated *Bauhinia vahlii* using seedling explants on MS medium supplemented with thidizuron and kinetin (1.0 µM each). *In vitro* propagation for *Bauhiniavariegata* (Mathur and Mukunthakumar, 1992) have successfully conducted in the plants from nodal segments on MS medium with 13.3 µM BAP.Suwal *et al.*(1988) propagated *Dalbergia sissoo* Roxb. When cultured on MS medium supplemented with 1.0mg/l BAP and 0.1mg/l NAA. 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 saltssupplemented with 1.0 mg/l BA plus 0.01 mg/l IAA. However, in present study the shoot multiplication was found the best in 2.0 µM BAP and 0.1µM IAA combinations.

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