In Vitro Regeneration of *Paraserianthes falcataria* (L.) Nielsen

Dardjat Sasmitamihardja, Johannes S. Hadisutanto and Sri N.Widiyanto
Department of Biology, Institut Teknologi Bandung
Jl. Ganesha No. 10, Bandung - 40132
Indonesia

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**Abstract**

In vitro regeneration of *Paraserianthes falcataria* (L.) Nielsen was achieved through axillary shoot multiplication. Single-node segments from six-day-old seedlings were used as explants and cultured on modified MS (Murashige and Skoog, 1962) medium. The addition of 0.01 - 1.0 µM indole acetic acid (IAA) in combination with 0.1 - 1.0 µM benzyl adenine (BA) induced axillary shoot proliferation. During the first two-weeks in culture, 4-15 shoots per explant were proliferated. The highest number of shoots was obtained from single-node explants cultured on medium supplemented with 0.1 µM IAA and 0.5 µM BA. After three-weeks in culture, up to 23 shoots per explant were produced. Lengthening the period of culture produced no significant increase in shoot proliferation. In the last week of a 4-week period of culture, the shoot multiplication rate was very low. Regenerated shoots were elongated on MS medium without growth regulators. Shoots were rooted on soil during the period of acclimatization. One hundred percent of the plantlets survived and grew well in the green house.

**INTRODUCTION**

*Paraserianthes falcataria* (L.) Nielsen, formerly known as *Albizia falcataria* (L.) Fosberg, originally grew in the Eastern provinces of Indonesia extending to Papua New Guinea and northern Australia. It now grows widely in tropical areas and also in the humid tropical areas of South East Asia. Like many other legumes, *P. falcataria* is a fast-growing nitrogen-fixing species that has remarkable growth performances. It has potential for agroforestry and produces multipurpose softwood. It is a widely adaptive plant and is extensively planted in poor, dry or marginal lands (Zabala, 1993).

To meet the demand for saplings, micropropagation of *P. falcataria* is an alternative method that would be beneficial in accelerating large-scale plant regeneration, tree improvement and conservation. In vitro regeneration protocols have been developed for other species of *Albizia*. Seedling organs, leaf segments and axillary shoots are known to have potential as explant sources (Tomar and Gupta, 1988; Austin-Burns and Wetzstein, 1998; Kumar et al., 1998; Sinha et al., 2000). Bon et al. (1998) successfully applied micropropagation procedures to *P. falcataria*, using shoot formation. The influence of growth regulators in the culture medium proved to be the most critical factor. The successful application of in vitro culture depended primarily on the composition and combination of growth regulators that were used (Minocha, 1987; Bon et al., 1998; Scarpa et al., 2000).

Our research focused on the influence of growth regulators in the development of an in vitro regeneration procedure. The effect of benzyl adenine, either singly or in combination with indole acetic acid, on axillary shoot proliferation of *P. falcataria* was examined. Different explant sources were also investigated.

**MATERIALS AND METHODS**

**Explant Source**

Seeds of *Paraserianthes falcataria* (L.) Nielsen were used to obtain seedlings. Seeds were surface sterilized for 5-10 min. in 50% commercial bleach to which a drop of Tween 20 had been added. They were then rinsed three times in sterile distilled water.
before being placed on sterile germination medium. Six-day-old seedlings were used as the source of explants. Shoot stems were excised from seedlings and cut into 2-5 mm long segments with single-axillary nodes. All in vitro cultures were maintained in a controlled culture room under a continuous light condition provided by cool-white-fluorescent tubes at 23-25°C.

**Medium Composition**

All in vitro experiments used MS medium (Murashige and Skoog, 1962). Media were supplemented with various concentrations of indole acetic acid (IAA) (0.01 - 1.0 µM) alone or in combination with benzyl adenine (BA) (0.1 - 1.0 µM) for shoot induction. MS medium without added growth regulators was used for germination, inducing shoot elongation and root formation. Media were solidified with 0.8% agar, adjusted to pH 5.5 and autoclaved at 120°C for 15 min.

**Experimental Procedure**

Germination was induced in a week on MS medium without vitamins and growth regulators. About 5 mm long single-node segments were cut from seedling stems and placed on shoot induction medium. During a 4-week subculture, axillary shoots, which proliferated from shoot-tips, were counted every week. Excisable shoots were cut into single-node segments and subcultured on the best combination of shoot induction medium. Shoot multiplication rates were observed from 3 weeks until 3 periods of culture. Proliferated shoots were excised from clusters and subcultured individually on shoot elongation medium. Elongated shoots were rooted in a soil-sand mixture (soil:sand = 1:1) during a period of acclimatization and were grown naturally in the greenhouse. Experiments were arranged in a complete-randomized-block design. Each treatment consisted of 4 replicate culture vessels with 4-5 explants per vessel. Explants were randomly allocated in each vessel. Experiments were repeated twice. Data were analyzed using ANOVA and Duncan’s New Multiple Range Test (DNMRT).

**RESULTS AND DISCUSSION**

**Shoot Proliferation**

1. **Seedling Explants.** The result showed that axillary shoots proliferated from single node explants derived from seedlings on media containing BA in combination with or without IAA (Table 1). The addition of 0.01 - 1.0 µM IAA in combination with 0.1 - 1.0 µM BA induced axillary shoot proliferation. The highest number of shoots was obtained from single-node explants cultured on medium supplemented with 0.1 µM IAA and 0.5 µM BA.

   Shoot proliferation from each nodal segment was observed every week during the first 4-week period of culture. Observation of explants cultured on medium containing 0.1 µM IAA in combination with 0.1, 0.5 and 1.0 µM BA indicated that effectiveness of IAA-BA combinations in shoot proliferation was apparently dependent upon the concentration (Fig. 1). During the first 2 weeks in culture, 3-15 shoots were proliferated per explant. In the 3rd week, a remarkable increase of shoot proliferation was observed. After 3 weeks in culture, 19-23 shoots per explant were produced on medium with the addition of 0.1 µM IAA and 0.1-0.5 µM BA. Lengthening the period of culture showed no significant increase in shoot proliferation. In the last week of a 4-week period of culture, shoot proliferation capability of explants decreased. Based on this result, data was collected after 3 weeks of culture.

   On medium containing 0.1 µM IAA and 1.0 µM BA, no obvious increases in shoot proliferation occurred on seedling explants during the period of 1-4 weeks. It was observed that no additional shoots were produced after 2 weeks in culture. On the medium with 1.0 IAA µM and 1.0 BA µM, no shoots were observed and callus formed instead. However, the combination of 1.0 IAA µM with 0.1-0.5 BA µM induced both shoot and callus formation. Callus was swollen from the base of shoots and seemed to
inhibit the addition of new shoots. Tomar and Gupta (1988) also found that higher concentrations of auxin, such as IAA, enhanced root and callus formation on hypocotyl explants of some *Albizia* species.

### 2. Cultured Shoot Explants.

In contrast to single node explants from seedlings, axillary nodes from cultured shoots produced less shoots (Table 1). After 3 weeks, only 2-8 shoots had proliferated from a single node explant. However, medium containing 0.1 µM IAA and 0.5 µM BA promoted the highest rate of shoot proliferation (7.3 shoots from a single node in 3 weeks). Shoot numbers were much lower than those produced on seedling explants on the same medium (23.0 shoots per explant). It seemed that single node explants from seedlings were more regenerative than those of the cultured shoots.

High concentrations of IAA (1.0 µM) in combination with 0.1-0.5 µM BA promoted only 2.3-2.6 shoots per explant during the 3-week period. Although there was no indication of callus or root formation, shoot multiplication was inhibited. In some other *Albizia* species, embryogenic tissues of seedlings were found to be highly responsive to IAA-BA combinations. However, the IAA-BA combination could be either promoting or inhibiting shoot formation. Bon et al. (1998) reported that seedling explants responded well to IAA-BA combinations and showed a better result than those from the mature explants.

The successful application of growth regulators in in vitro regeneration depended mostly on the genotype and source of explants. The addition of cytokinins, such as kinetin or benzyl adenine enhanced shoot proliferation and root formation (Minocha, 1987; Scarpa et al., 2000). In many cases, cytokinins were used in combination with auxins, such as naphthalene acetic acid, indole butyric acid or indole acetic acid (Tomar and Gupta, 1988; Kumar et al., 1998; Sinha et al., 2000; Tawfik and Noga, 2001). Moreover, responsiveness of explants was also affected by the composition of micro and macronutrients in the medium (Minocha, 1987; Scarpa et al., 2000). Bon et al. (1998) showed the effectiveness of MS medium as the source of basic inorganic salts for in vitro regeneration of *P. falcataria*.

### Elongation and Root Induction

Further developmental observation indicated that regenerated shoots grew well, producing more branches and unfolded leaves. Shoots were elongated on MS medium without the addition of growth regulators, and rooted in a soil-sand mixture during a period of acclimatization. One hundred percent of the plantlets survived and grew naturally in the greenhouse. No morphological differences were observed.

Following this procedure, 23 shoots were produced in 3 weeks from one single node explant that originated from a seedling. Followed by three passages of culture inducing shoot multiplication of cultured shoots, more than 1000 young plants of *P. falcataria* were produced in about 4-5 months. Single node microcuttings have become a common method for plant production of some species (Borthakur et al., 2000; Scarpa et al., 2000; Jordan et al., 2001). Bon et al. (1998) suggested the use of single node explants, especially those originating from seedlings, in in vitro regeneration of *P. falcataria*.

### CONCLUSION

In vitro regeneration of *P. falcataria* has been achieved using single node microcuttings. The addition of 0.01 µM IAA in combination with 0.1-1.0 µM BA affected shoot formation and axillary shoot production. It was observed that the addition of IAA and BA could either stimulate or inhibit shoot proliferation. Medium containing 0.1 µM IAA and 0.5 µM BA promoted the highest rate of shoot proliferation on both seedlings and elongated shoot explants. Our results showed increase in shoot proliferation rates during the first 3 weeks of culture. After 3 weeks, shoot proliferation rates mostly decreased. We found that axillary node explants from seedlings were more regenerative than those from elongated shoots. Regenerated shoots grew normally and rooted in a soil-sand mixture during the period of acclimatization. Using this procedure, more than 1,000
Regenerated plants were produced in 4-5 months from one single node segment that had originated from a seedling.

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**Literature Cited**


Table 1. Effects of IAA and BA on shoot proliferation, callus induction and root formation occurred on single-node segments derived from seedlings and cultured shoots.

<table>
<thead>
<tr>
<th>Growth regulators</th>
<th>Sources of explants</th>
<th>Seedlings</th>
<th>Cultured shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave. shoots per explant</td>
<td>Callus formation</td>
<td>Root formation</td>
</tr>
<tr>
<td>IAA (μM)</td>
<td>BA (μM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>-</td>
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<tr>
<td>0.1</td>
<td>0.1</td>
<td>6.5 ± 3.5 g</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>16.8 ± 1.1 d</td>
<td>-</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>20.0 ± 4.3 b</td>
<td>-</td>
</tr>
<tr>
<td>0.01</td>
<td>0.1</td>
<td>18.6 ± 6.0 bc</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>14.8 ± 4.0 e</td>
<td>-</td>
</tr>
<tr>
<td>0.1</td>
<td>1.0</td>
<td>19.0 ± 3.0 b</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>23.0 ± 0.7 a</td>
<td>-</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1</td>
<td>3.6 ± 1.5 i</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>11.5 ± 0.7 f</td>
<td>+</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0</td>
<td>12.5 ± 0.7 f</td>
<td>+</td>
</tr>
</tbody>
</table>

1) Means ± SD of 20 replicates per treatment in two repeated experiments. Separately for each source of explants, means followed by different letter in the column are significantly different by LSD test (p=0.05).

2) + = callus or root formation was indicated
- = no-callus nor root formation was indicated
Fig. 1. Effects of 0.1 µM IAA in combination with 0.1 µM BA (■), 0.5 µM BA (♦) and 1.0 µM BA (▲) on numbers of shoots proliferated from every single node explant derived from seedlings during the period of 4 weeks.