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

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Introduction

Paraserianthes falcataria (L.), Nielsen is a tree species belonging to the Leguminosae family that are naturally distributed in Indonesia, New Guinea, the Solomon Islands and Australia. Since the 1870s, this species has been widely planted and has spread vigorously in tropical countries especially south-east Asia. As a result the current distribution of the species is not known definitively (KAWAHARA, 1997).

P. falcataria is one of the fast growing trees and offers enormous economic potential as a source of pulpwood (SOERIANEGARA and LEMMENS, 1994). In Indonesia, it is one of the major species used for industrial forestation. Its cutting cycle is from 8 to 12 years and its annual growth rate is 25 to 30 m^3/ha (KAWAHARA, 1997). It is also used as a shade tree on tea and coffee plantations in social forestry programs. It is therefore important to develop a system of mass propagation and clonal propagation for this species.

There have been several reports on *Paraserianthes* tissue culture (TOMAR and GUPTA, 1988; SINHA and MALLICK, 1993; GHARYAL and MAHESWARI, 1983, UPADHYAYA and CANDRA, 1983), but relatively few reports on the *in vitro* regeneration of *P. falcataria*. SINHA and MALLICK (1993) reported *in vitro* regeneration and multiplication of the species through organogenesis from juvenile cotyledon explants, while ISHII *et al.* (1994) demonstrated a regeneration system for mature embryos of *P. falcataria*. Regeneration from cotyledon explant or embryo goes through adventitious bud or adventitious embryo. While regeneration from axillary bud goes through early branching. Therefore, the products from axillary bud are more genetically stable than those from cotyledon or embryo. There are no reports on *in vitro* regeneration using axillary bud explants.

In this study, we describe shoot regeneration and multiplication in *P. falcataria* using axillary bud culture and cotyledonary culture.

Materials and Methods

Plant materials

P. falcataria seeds were collected in Bogor, Indonesia. The seeds were soaked in tap water for one hour, dipped in boiling water for one minute, surface disinfected in 2.5% sodium hypochlorite solution for 5 minutes and then rinsed several times in sterile distilled water. Seeds were then placed aseptically on 1/5 MS (MURASHIGE and SKOOG) (1962) solidified agar (8 g/*l*) medium supplemented with 20 g/*l* sucrose for *in vitro* seed germination.

Induction of multiple shoots

Cotyledonary nodes and axillary buds obtained from 20-day-old seedlings *in vitro* were used as explants, and were cultured in test tubes containing WPM(Woody plant medium) (LLOYD *et al.*, 1981), B5 (GAMBORG, 1968) or MS medium supplemented with various concen-

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Table 1. Shoot development from *P. falcataris* axillary buds after 40 days of culture on WPM, B5 and MS medium containing various concentrations of BAP.

Medium	BAP (mg/*l*)	Percentage of explant which developed shoots	Number of shoots per explant	Mean length of shoot (mm)
			mean (range)	mean (range)
WPM	—	100 (r)	1.4 (1–2)	5.5 (3–10)
	0.11	100 (c)	1.9 (1–3)	5.7 (3–13)
	0.22	100 (c)	2.8 (1–4)	4.6 (3–13)
	0.33	100 (c)	2.5 (2–4)	3.8 (3–6)
	0.44	100 (c)	2.9 (1–5)	3.8 (3–7)
B5	—	100 (c, r)	1.2 (1–2)	11.0 (3–15)
	0.11	100 (c, r)	2.7 (1–5)	5.8 (3–12)
	0.22	100 (c)	4.2 (3–9)	6.6 (3–20)
	0.33	100 (c, r)	2.9 (2–6)	4.8 (3–15)
	0.44	100 (c, r)	3.7 (2–8)	4.9 (3–16)
MS	—	100 (c, r)	1.3 (1–2)	9.1 (3–22)
	0.11	100 (c)	2.6 (1–5)	5.2 (3–7)
	0.22	100 (c)	2.7 (2–4)	4.8 (3–7)
	0.33	100 (c)	2.9 (1–7)	6.0 (4–8)
	0.44	100 (c)	3.3 (2–8)	5.9 (3–8)

Notes; 'c' means the callus formation and 'r' means the root formation.

trations of BAP (6-benzylaminopurine). The pH of all media was adjusted to 5.8 prior to the addition of 0.8% (w/v) agar at final concentration, and then autoclaved at 121℃ for 20 minutes. The cultures were incubated at 25℃ under fluorescent lighting (5,000 lux) for a 16-hour photoperiod. Ten explants were used in each treatment and all experiments were repeated three times.

Rooting

To induce roots, shoots over 0.5 cm were excised from multiple shoots that were derived from either axillary buds or cotyledonary nodes. Shoots were then cultured on hormone-free MS medium supplemented with 20 g/*l* sucrose and 8 g/*l* agar. The pH was adjusted to 5.8 before agar addition, and media were autoclaved at 121℃ for 20 minutes. The cultures were then incubated in a growth chamber at 25℃ under fluorescent lighting (5,000 lux) for a 16-hour photoperiod.

Acclimatization of plantlets

Rooted plantlets of about 4 cm in length were washed in tap water, transplanted into vermiculite-filled plastic pots and raised in a polypropylene box that was covered with plastic film to maintain ambient humidity. The pots were maintained at 30℃ in a growth chamber with high relative humidity (70–90%) and were exposed to fluorescent lighting (5,000 lux) for a 16-hour photoperiod. Box covers were opened after three days of incubation and the relative humidity was gradually reduced to 50% to 60% after ten days. Plantlets were watered twice a week. The plantlets were kept in a growth chamber for 4 to 8 weeks, then transferred into a greenhouse.

Table 2. Shoot development from *P. falcataria* cotyledonary nodes after 40 days of culture on WPM, B5 and MS medium containing various concentrations of BAP.

Medium	BAP (mg/*l*)	Percentage of explant which developed shoots	Number of shoots per explant	Mean length of shoot (mm)
			mean (range)	mean (range)
WPM	—	100 (r)	1.9 (1–2)	5.8 (2–10)
	0.11	100 (c, r)	2.5 (1–5)	4.9 (2–9)
	0.22	100 (c)	2.2 (1–4)	5.1 (2–11)
	0.33	100 (c)	2.2 (1–4)	4.3 (2–11)
	0.44	100 (c)	2.9 (2–6)	3.3 (2–7)
B5	—	100 (r)	1.6 (1–2)	11.3 (4–25)
	0.11	100 (c)	4.3 (2–8)	5.0 (3–15)
	0.22	100 (c)	5.4 (4–9)	4.9 (3–16)
	0.33	100 (c)	5.7 (3–13)	4.6 (3–16)
	0.44	100 (c, r)	5.4 (3–9)	5.7 (3–15)
MS	—	100 (c, r)	1.9 (1–2)	16.1 (3–19)
	0.11	100 (c)	2.9 (1–5)	5.0 (3–10)
	0.22	100 (c)	3.5 (2–5)	5.1 (3–7)
	0.33	100 (c)	3.1 (1–5)	7.2 (4–13)
	0.44	100 (c)	4.3 (2–11)	5.9 (4–10)

Notes; 'c' means the callus formation and 'r' means the root formation.

Fig. 1. Multiple shoots of *P. falcataria* nodal explants developed on B5 medium containing 0.22 mg/*l* of BAP after 3 weeks of culture.
Bar=10 mm

Fig. 2. Shoots of *P. falcataria* rooted on hormone-free MS medium after one month of culture. Bar=10 mm

Results and Discussion

Induction of multiple shoots

Within ten days, shoot formation was observed in axillary buds and cotyledonary nodes of *P. falcataria* on all the media tested. The results of shoot induction from axillary bud explants after a month of culture on WPM, B5 and MS medium supplemented with various BAP concentrations are presented in Table 1, and the results from cotyledonary node explants are presented in Table 2.

Axillary bud explants appear to be suitable for shoot induction since all axillary buds sprouted and developed into shoots. The number of shoots per explant ranged from 1 to 9, and shoot length ranged from 3 to 22 mm. Among the combinations of media and BAP concentrations tested, B5 medium supplemented with 0.22 mg/*l* BAP produced the best shoot induction from axillary buds (4.2 shoots/explant). Although explants cultured on hormone-free medium produced the longest shoots, multiple shoot production was suppressed. Thus, hormonal conditions appear to be a determining factor for the successful enhancement of axillary bud proliferation. Among the combinations of media and BAP concentrations tested (except for hormone-free medium), B5 medium supplemented with 0.22 mg/*l* BAP produced the best shoot elongation (an average of 6.6 mm). This medium also produced the best shoot induction and elongation. Almost all explants formed calluses at the base of their shoots in all treatments, and direct rooting was also observed on some explants. Multiple shoots developing on B5 medium containing 0.22 mg/*l* BAP are shown in Fig. 1.

Cotyledonal explants expanded and shoots formed on cotyledonal nodes within ten days of culture. Almost all explants formed calluses at their cut ends, and direct rooting was also observed on some explants. It was also observed that cotyledonal node explants appear to be suitable for shoot induction, since shoots sprouted in all cotyledonal nodes on all tested media. The number of shoots per explant ranged from 1 to 13, and the shoot

Fig. 3. Regenerated *P. falcataria* plants 6 months after transplantation to pots. Bar=10 cm

length ranged from 2 to 25 mm.

Among the combinations of media and BAP concentrations tested, B5 media supplemented with BAP concentrations of 0.22 to 0.44 mg/*l* were better enhancers of axillary bud proliferation. On these media the average number of multiple shoots was from 5.4 to 5.7, with a range of 3 to 13 shoots per explant, while mean shoot length averaged 4.6 to 5.7 mm, with a range of 3 to 16 mm. However, only two shoots by average per explant elongated from 10 to 16 mm, which is the optimal size for rooting. Explants cultured on the hormone-free medium developed the longest shoots, although multiple shoots were rare. Thus, hormonal conditions seem to be determining factors for successful shoot induction from cotyledonal node explants. For multiple shoot induction, B5 media supplemented with BAP at concentrations of 0.22 to 0.44 mg/*l* were suitable. For shoot elongation, however, these media were not useful.

Thus, BAP was effective for multiple shoot induction in axillary bud and cotyledonary node explants of *P. falcataria*. SHINHA and MALLICK (1993) also found that BAP was effective for multiple shoot induction from cotyledonary node explants of this species. They reported that better shoot elongation was obtained with 0.5 mg/*l* or 1 mg/*l* BAP, and

suggested that a higher BAP concentration inhibited shoot elongation and callus formation in *P. falcataria* explants. This is in agreement with our finding. The optimal range of BAP concentration for shoot elongation is different between these two studies. It may originate from the difference of explants or medium. Moreover in the study of SHINHA and MALLICK, the tested concentrations of BAP were higher than 0.5 mg/*l*. If they studied on the medium containing lower concentration BAP, they may be able to obtain longer shoots.

Rooting

For rooting, explant shoots were isolated and subcultured on hormone-free MS medium. Using this procedure, 90% of shoots were successfully rooted. A rooted shoot on hormone-free MS medium is shown in Fig. 2. Excised shoots of this species develop roots readily.

Acclimatization of plantlets

For acclimatization, the rooted shoots (plantlets) were transferred into vermiculite-filled pots by the procedure described above. All plantlets were survived. Regenerated plants growing in soil are shown in Fig. 3.

In this study, the establishment of a *P. falcataria* regeneration system using nodal segments with axillary buds and cotyledonary nodes was conducted. These results show that plantlet formation can be successfully induced from nodal segments and cotyledonary node explants of *P. falcataria*. As *P. falcataria* is one of the major planting species for industrial forestation in Indonesia, it is important to produce high quality seedlings on a large scale. Therefore, tissue culture methods have been developed to facilitate the successful mass production of superior clones in this study. The regeneration system developed in this study is not only useful for mass propagation but will also be useful for the biotechnological application of this fast growing tree species.

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Summary

Plantlets were regenerated *in vitro* from seedling tissue of *Paraserianthes falcataria* (L.) Nielsen, which is a fast-growing, tropical tree of the Leguminosae family. Axillary buds and cotyledonary nodes of seedlings that were germinated under aseptic conditions were used as explants. Various concentrations of BAP were added to three kinds of basic media, WPM, B5 and MS, in order to find a suitable medium for shoot proliferation. Shoot induction was seen in all combinations of culture medium. When axillary buds were used as explants, the greatest number of shoots developed (4.2 per explant) when the explants were cultured on B5 medium with 0.22 mg/*l* BAP. When cotyledonary nodes were used as explants, many shoots were induced (5.4 to 5.7 per explant) on B5 medium with 0.22 mg/*l* to 0.44 mg/*l* of BAP. Although shoot elongation in axillary buds and cotyledonary nodes was most pronounced on hormone-free medium, few shoots per explant were induced. When shoots elongated to 0.5 cm or more were cultivated on a hormone-free MS medium, 90% of the shoots rooted. All plantlets cultivated for six weeks on rooting medium

survived transplantation and acclimatization to vermiculite-filled pots.

Key words: *Paraserianthes falcataria, In vitro,* tissue culture, regeneration

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Paraserianthes falcataria (L.) Nielsen の試験管内における植物体の再生

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要　旨

熱帯産マメ科早生樹である *Paraserianthes falcataria* (L.) Nielsen の芽生えの組織から，試験管内で植物体を再生させた。外植体として，無菌発芽させた芽生えの腋芽と子葉節を用いた。WPM, B5, MS の 3 種類の基本培地に BAP を様々な濃度で添加し，シュートの増殖に適した培地を検索した。すべての組み合わせの培地でシュートの発生が見られた。腋芽を外植体とした場合は，B5 培地に BAP を 0.22 mg/*l* 添加した培地でもっとも多くのシュート（外植体 1 つ当たり 4.2 本）が見られた。子葉節を外植体とした場合には，B5 培地に BAP を 0.22 mg/*l* から 0.44 mg/*l* 添加した培地で，多くのシュート（外植体 1 つ当たり 5.4 本から 5.7 本）ができた。外植体が腋芽と子葉節のどちらの時にも，3 種類の基本培地すべてで，ホルモンフリーの時にシュートの成長がもっともよかったが，外植体当たりのシュートの数は少なかった。0.5 cm 以上に伸長したシュートをホルモンフリーの MS 培地で培養したところ，90%のシュートが発根した。バーミキュライトを入れたポットに発根培地で 6 週間培養した幼植物体を移植し順化したところ，すべての植物体が生存した。

キーワード: *Paraserianthes falcataria*, 試験管内，組織培養，植物体再生

Abstract

Dynamics of Population and Basal Area in a Mixed Forest with Coniferous and Broad-leaved Species —A Case Study of Large-scale and Long-term Observation Plot in the Tokyo University Forest in Hokkaido—

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The dynamics of basal area and population in a large-scale permanent plot (36.25 ha) of mixed forest with coniferous and broad-leaved species was analyzed. A complete enumeration of d.b.h. has practiced in 1993 and 1998. In population, conifer increased and broad-leaved tree decreased. *Abies sachalinensis* and *Tilia japonica* increased in population and *Picea jezoensis* decreased. The basal area increased both in conifer and broad-leaved trees. The most part of the basal area increment occupies large sized conifers and the growth rate of large sized broad-leaved trees is low.

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

SUKARUTININGSIH, Yoko SAITO and Yuji IDE

Plantlets were regenerated *in vitro* from the axillary buds and cotyledonary nodes of *Paraserianthes falcataria* seedlings. Various concentrations of BAP were added to WPM, B5 and MS media. B5 medium containing 0.22 mg/*l* BAP induced the most shoots per explant from axillary buds. B5 medium containing 0.22 to 0.44 mg/*l* BAP induced the most shoots per explant from cotyledonary nodes. Following culture on hormone-free MS medium, 90% of shoots rooted. All plantlets survived transplantation into vermiculite-filled pots.