TOWARDS THE PRODUCTION OF *PARASERIANTHES FALCATARIA* (L.) (BATAI) PLANTLETS FOR COMMERCIAL PLANTATION

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ABSTRACT

Paraserianthes falcataria (L.) Nielsen locally known as Batai is a native tree in South East Asia. In Malaysia it has been widely planted because P. falcataria is a pioneer and fast-growing tree. The wood of P. falcataria has been used for furniture, pulp, veneer, matches and light weight packing materials. The main objective of this study is to establish the micropropagation protocol of P. falcataria for commercial plantation purposes. In this study, we used seeds as explants. Surface sterilized seeds were cultured on MS basal medium with two methods applied. After 2 weeks in culture, it was observed that seeds soaked with hot water obtained 87% clean culture. After 4 weeks in culture, 97% from clean culture obtained were germinated. They were cultured on the MS basal media containing a different concentration of 6-benzylaminopurine (BAP) for shoot multiplication. The most suitable medium for shoot multiplication was the MS basal medium containing 0.25 mg/L BAP. For the in vitro rooting, shoots were rooted on half strength MS basal medium containing different concentration of Indole-butyric acid (IBA). After 4 weeks in culture, the root multiplication rate with different IBA concentration was too low not as expected. To encounter the problem, ex vitro rooting for P. falcataria was carried out together with acclimatization stage under nursery condition.

Keywords: *Paraserianthes falcataria, Batai, micropropagation.*

INTRODUCTION

Paraserianthes falcataria also locally known as batai has been widely planted in Malaysia because it is a pioneer and fast-growing tree. The woods of *P. falcataria* has been used for light-construction materials, veneer, furniture, particle board, matches and also pulp. The growth performance and wood qualities should be enhanced by tree breeders to promote the establishment of commercial plantation in Malaysia. According to Chigira *et al.* (2007), the growth characteristics of *P. falcataria* such as height and diameter breast height (DBH) may vary from different seed sources or seedlings and these growth characteristics can be improved by genetic manipulation. In fact, breeding for growth and wood quality of *P. falcataria* show a strong potential.

The important of tissue culture methods because it's can produce the similar properties as their parents, can produce a large number of plantlets and the plantlets will be free of viruses, may produce with a large number at one time with not limited by times, places and season (Nurhanis *et al.* 2019). According to Hussain *et al.* (2012), recent years, it is become a major industrial importance in plant propagation, plant improvement, plant and disease control and a production of secondary metabolites on tissue culture techniques.

Le Roux & Van Staden, (1991) reported that the benefit of micropropagation is reduces the high risk of genetic variation due to chromosomal changes occurs in callus culture but it's potentially useful in increasing the gene pool for some of species. The establishment of *P. falcataria* propagation protocol is needed to cater the needs from the industry for commercial plantation. Therefore, this study provides information on the establishment of the efficient micropropagation protocol of *P. falcataria* using seed explants.

MATERIALS AND METHODS

Plant materials and surface sterilization

P. falcataria seeds were obtained given from Sabah. Those seeds were surface sterilized in laboratory. Two surface sterilization methods were applied which were soaked with distilled water and another one with hot water for 5 minutes. The germinated plantlets were transferred into MS basal media for shoot multiplication.

Shoot multiplication

In vitro shoots from the basal media were used as explants for shoot multiplication experiment. Shoots were cultured into the Murashige and Skoog (MS) basal media supplemented with five different concentrations of 6-benzylaminopurine (BAP) i.e. (0 mg/L, 0.1 mg/L, 0.25 mg/L, 0.5 mg/L and 1.0 mg/L). The experiment was conducted with ten bottles of each treatment and containing three shoots per bottles. The height of shoots is about 1.0 cm. The data on number of shoots induced per explants, number of leaves and leaves length were observed and recorded after eight weeks of cultured.

In vitro rooting

Individual shoots about 1.5 cm were used for *in vitro* rooting experiment. Half strength of Murashige and Skoog (MS) basal medium supplemented with different concentrations of Indole-3-Butyric Acid (IBA) from 0 mg/L to 4.0 mg/L were used.

Ex vitro rooting and acclimatization in the nursery

Ex vitro rooting were carried out to speed up the process of propagation and reduce the cost for commercial purposes. Individual shoots about 3-4 cm were used for *ex vitro* rooting. Shoots were sprayed with 1-Naphthaleneacetic acid (NAA) and dipped in Seradix® (rooting powder) as a plant growth regulator (PGR) and potting in Jiffy 7 for acclimatization process in weaning chamber.

Statistical Analysis

Data from shoot multiplication experiment were subjected to analysis of variance (ANOVA) using SAS ver. 9.1 software and Tukey's post-hoc test was used to determine significant differences between treatments ($p<0.05$).

RESULTS AND DISCUSSION

Surface sterilization and clean culture

The method which soaked with hot water gave 84% clean culture and germinated after four weeks in culture. The reason seeds were soaked into the hot water because it's to break down their dormancy (Chujo *et al.* 2010). The germinated plantlets were transferred into MS basal media for shoot multiplication.

Shoot multiplication

The growth of *P. falcataria* plantlets were essential for shoot multiplication experiment. Shoot multiplication were observed eight weeks of culture and analysed using SAS ver. 9.1. Table 1 showed the mean number of shoots, leaves length and number of leaves respectively. There were significantly different among treatments which MS basal added with plant growth regulators (PGR) which is MS basal medium with 0.1 mg/L BAP produced the highest shoots numbers which is 5.96 ± 0.41 followed by MS with 0.25 mg/L BAP (5.38 ± 0.53) and MS with 0.5 mg/L BAP (3.76 ± 0.36).

Plant growth regulators (PGR) play an essential role in determining the development pathway of plant cells and tissues in culture medium. Those cytokinins, auxins and gibberellins are most commonly used for micropropagation. The concentration of hormones used depending on the species, the tissue or organ cultured in the certain experiment (Ting, 1982). There were also significant different on number of leaves produced. *P. falcataria* cultured in MS basal medium with 0.1 mg/L BAP obtained the highest mean number of leaves (10.44 ± 1.01) followed with MS basal added with 0.25 mg/L BAP (10.31 ± 1.39) and MS basal with 1.0 mg/L BAP (6.27 ± 1.12). For leaves length, the results showed that MS basal media with no addition of BAP produced the highest mean leaves length which is 0.32 ± 0.04 followed by MS basal with 1.0 mg/L BAP (0.19 ± 0.02) and MS basal with 0.25 mg/L BAP (0.14 ± 0.04). In conclusion, MS basal media with 0.1 mg/L BAP and 0.25 mg/l can be used for shoot regeneration and shoot multiplication of *P. falcataria*. In contrast, Sukendah *et al.* (2020) reported that MS plus with 2.0 mg/l BAP gave the highest mean shoot number (7 shoots). Used of lower BAP concentration can reduce the propagation cost for commercial purposes of *P. falcataria*.

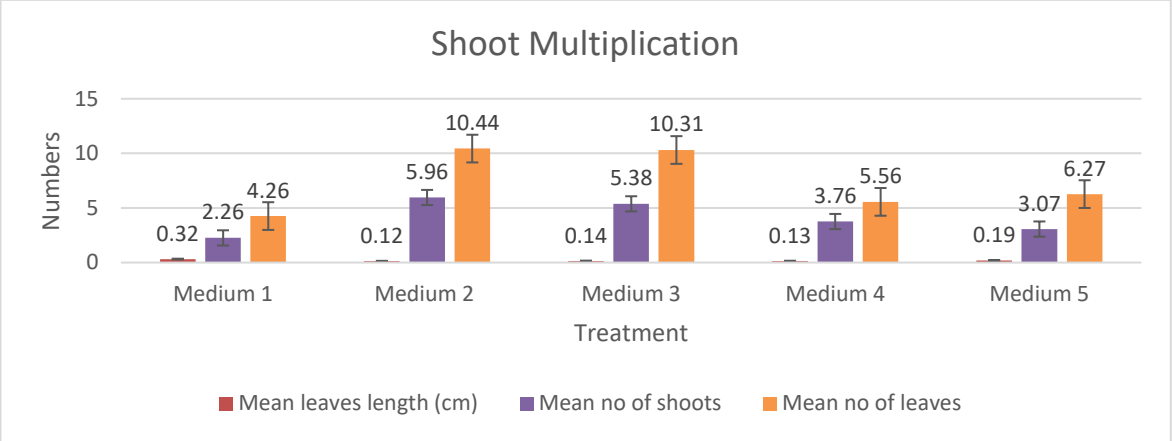
Table 1: Effect of different BAP concentration in MS basal medium on *P. falcataria* shoot multiplication, no. of leaves and leaves length

Media	BAP conc. (mg/L)	Mean no. of shoots	Mean no. of leaves	Mean leaves length (cm)
M1	0.0	2.26 ± 0.23 [b]	4.26 ± 0.72 [c]	0.32 ± 0.04 a
M2	0.1	5.96 ± 0.41 [a]	10.44 ± 1.01 [a]	0.12 ± 0.01 b
M3	0.25	5.38 ± 0.53 [a]	10.31 ± 1.39 [a b]	0.14 ± 0.04 b

M4	0.5	3.76 ± 0.36 [b]	5.56 ± 0.79 [c]	0.13 ± 0.02 b
M5	1.0	3.07 ± 0.36 [b]	6.27 ± 1.12 [b c]	0.19 ± 0.02 b

Note: Values (average ± standard deviation) with a different alphabet are statistically significant according to Tukey post-hoc test.

Figure 1: Graph of *P. falcataria* shoot multiplication



***In vitro* rooting**

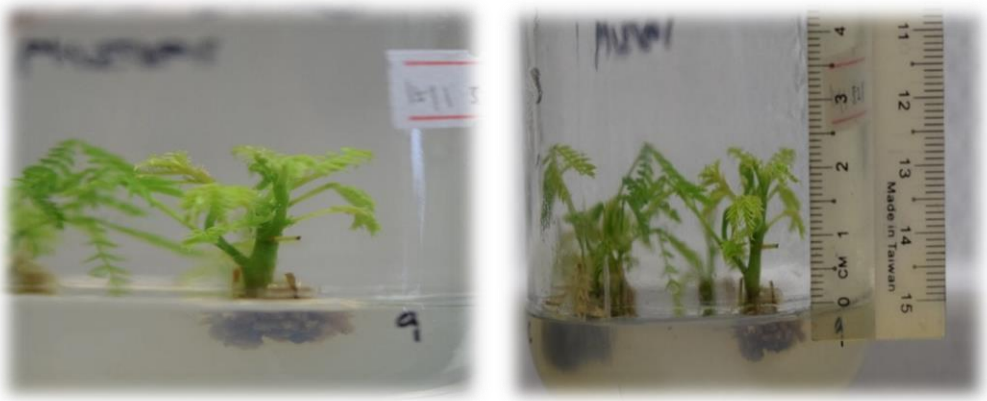
For *in vitro* rooting experiment, half strength MS basal medium was added with Indole-3-Butyric Acid (IBA) to obtained roots. According to Murashige & Skoog (1962), auxin is very influential to stimulate the growth formation of roots and root length therefore it causing plants to absorb more water and nutrients.

In this study, the formation of roots from half strength MS basal media contained with different concentration of IBA was not successful. Even though it took eight weeks of culture there were still less root formed.

***Ex vitro* rooting and acclimatization in the nursery**

Plantlets obtained from *ex vitro* rooting were surviving and rooted in the acclimatization chamber. 70% of the *P. falcataria* plantlets were survived and rooted within two weeks. Those plantlets rooted *ex vitro* was transferred into polybag consist of burnt soil and peat moss (1:1) and placed on the bench in the nursery and watering three times a day using a sprinkler (10 min per watering).

Figure 2: (i) Shoot multiplication of *P. falcataria* in MS basal medium with 0.1 mg/L BAP, (ii) *P. falcataria* plantlets were observed and measured.



(i) (ii)

Figure 3: (i) *P. falcataria* plantlets through *ex vitro* rooting were rooted within 2 weeks, (ii) *P. falcataria* plantlets were survived and rooted ready to transfer into a polybag.

(i) (ii)

CONCLUSION

P. falcataria tissue culture protocol was successfully developed especially for promoting and commercialize for commercial plantation to cater the needs of timber products in Malaysia. Pilot study on planting tissue culture plantlets of *P. falcataria* on their growth performance, wood quality and pest and disease control need to be done to support this research for industries and stakeholders.

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REFERENCES

Chigira. O., K. Matsune., Y. Ogawa., & S. Kurinobu. (2007). Family Variation in Two Years Old Seedling Seed Orchard of *Paraserianthes falcataria* in East Java, Indonesia. *Kyushu Journal of Forest Research* 60: 106-108.

Chujo. M., Eizawa. J., Yokota. S., Ishiguri. F., Lizuka. K., Priadi. D., Sumiasri. N. & Yoshizawa. N. (2010). Micropropagation and Protoplast Culture in *Paraserianthes falcataria*. *Wood Research Journal* Vol. 1. No. 2: 108-114.

Hussain. A., Qarshi. I. A., Nazir. H. & Ullah. I. (2012). Plant Tissue Culture: Current Status and Opportunities. *Recent Advances in Plant in vitro Culture*. DOI: 10.5772/50568.

Le Roux. J. J & Staden. J. V. (1991). Micropropagation of *Eucalyptus* species. *Horticultural Science* 26. P. 199-200.

Murashige. T. & Skoog F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarium* 15: 473-497.

Nurhanis. S. E., Wulandari. R. S & Suryantini. R. (2019). Variations of IAA Concentration to the Growth of Sengon Tissue Culture. *IOP Conf. Series: Earth and Environmental Science 394 (2019) 012024.* DOI: 10.1088/1755-1315/394/1/012024.

Sekendah, Hidayat R., & Widjajani B. W. (2020). Direct and indirect plantlet regeneration from explants of *Albizia* (*Albizia falcataria*). *Eurasia J Biosci*. 14: 7211-7216.

Ting. I.P. (1982). Plant Tissue Culture: *Current Status and Opportunities; Addison-Wesleyn Reading, Massachusetts.* 642. Retrieved from https://www.intechopen.com/books/recent-advances-in-plant-in-vitro-culture/plant-tissue-culture-current-status-and-opportunities.