A Preliminary Study on the Induction of Somatic Embryogenesis of *Eusideroxylon zwageri* Tesym. and Binned (Borneo Ironwood) from Leaf Explant

Abstract

*Eusideroxylon zwageri* is a tree of tropical rainforest zone and belongs to the Lauraceae family. It is one of the hardest timber species in Southeast Asia and economically very important for a source of hardwood timber. This species is commonly known as Belian (Sabah and Sarawak), Ulin (Indonesia) and Biliran (The Philippines). This hardwood timber is heavy and is classified into Class Of Strength I, Durability Class I with specific gravity of around 0.88 – 1.19 g/cm³ [1]. In Southeast Asia, *E. zwageri* is popularly known as the most durable timber and crucial for building material. According to Irawan and Gruber [1], this species possesses very dense, termite resistance silica and contain heartwood extractives known as *Eusiderin* which is the primary factor of its durability. Borneo Ironwood can survive under hazardous condition either in ground contact or submerged in the water without losing its strength because of its anatomical features and its contents of extractives. According to Martawijaya et al. [4], the heartwood of this species is yellow brown and turning reddish black brown when they are freshly sawn. The heartwood of Belian species is also very resistant to preservative treatment. *E. zwageri* is conventionally propagated by sexual reproduction which is by seed [1]. However, the recalcitrant characteristic of the seed makes it difficult to break the dormancy and therefore the natural propagation of this species are very slow. The germination takes around nine to twelve months in its natural habitat even under the optimal conditions [5]. According to Baekman [6], it requires almost 200 years or more for this species to reach their mature size. Vegetative propagation such as cutting can be used as the other method of propagation for replanting of Borneo Ironwood but the rooting rate of cutting is very low [1].

Realizing the economic importance of this hardwood timber species, it is necessary to regenerate and preserve this species through tissue culture. A micropropagation technique such as somatic embryogenesis has been reported in providing tools for cloning superior trees with similar adeptness that can be applied to other organisms [7]. Somatic embryogenesis in Lauraceae has been reported in *Persea americana*, *Ocotea catharinensis*, *Cinnamomum camphora* and *Cinnamomum verum*. In this study, the study on somatic embryogenesis of *E. zwageri* was conducted. The somatic embryo of *E. zwageri* were induced by the mean of tissue culture using somatic embryogenesis. The young leaves were used as the main source of explants and were cultured into half strength of the Murashige and Skoog (MS) under an aseptic condition. This medium was supplemented with various concentrations and combinations of different Auxin such as 1-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and Cytokin in such as 6-benzylaminopurine (BAP).

Materials and Methods

Plant material

In this study, the young leaf explants were collected from two

Introduction

*Eusideroxylon zwageri* is a tree of the tropical rainforest zone and economically very important for a source of hardwood timber. This species is commonly known as Belian (Sabah and Sarawak), Ulin (Indonesia) Borneo Ironwood (European Union) and Biliran (The Philippines). This hardwood timber is heavy and hard and is classified into Class Of Strength I, Durability Class I with specific gravity of around 0.88 – 1.19 g/cm³ [1]. In Southeast Asia, *E. zwageri* is popularly known as the most durable timber and crucial for building material. According to Irawan and Gruber [1], this species possesses very dense, termite resistance silica and contain heartwood extractives known as *Eusiderin* which is the primary factor of its durability. Borneo Ironwood can survive under the rotting process for almost 40 years and in the dry condition they can be up to a century [2]. Wong and Singh [3] claimed that the *E. zwageri*’s wood is naturally durable and can survive under hazardous condition either in ground contact or submerged in the water without losing its strength because of its anatomical features and its contents of extractives. According to Martawijaya et al. [4], the heartwood of this species is yellow brown and turning reddish black brown when they are freshly sawn. The heartwood of Belian species is also very resistant to preservative treatment. *E. zwageri* is conventionally propagated by sexual reproduction which is by seed [1]. However, the recalcitrant characteristic of the seed makes it difficult to break the dormancy and therefore the natural propagation of this species are very slow. The germination takes around nine to twelve months in its natural habitat even under

Keywords: *Eusideroxylon zwageri*; MS; Timber; Somatic embryogenesis
to three years old of *E. zwageri* seedlings originally from the forest and maintained in the pot culture outside Plant Tissue Culture Laboratory of Universiti Malaysia Sarawak (UNIMAS). These leaves explants were placed under running tap water for about one hour before soaked with 0.1% Benomyl for 30 minutes. The young leaf explants were further surface sterilized with 15% Sodium Hypochlorite solution with 3 drops of Tween 20 for 5 minutes. After sterilization, these young leaf were thoroughly washed three times with sterile distilled water. The leaf explants were cut into 0.5 to 1.0 cm before they were cultured into MS medium. The leaf explants were inoculated into the Petri dish containing MS media. All of these steps were carried out in the laminar flow cabinet. These cultures were incubated in the culture room at 25±2°C and kept in the dark.

**Culture Media and Conditions**

The preparation of MS basal media was based on the formulation and this MS media was added with 30 g/L sucrose and solidified with 3.0 g/L Gelrite. The pH of the medium was adjusted to 5.8 with 1 N KOH or 1 N HCl prior to sterilization by autoclaving at 121°C for 20 minutes. In each of the experiments, different concentrations and combination of various plant growth regulators were manipulated and added into sterilized medium and dispensed into the disposable Petri dishes in order to induce callus. For the establishment of the embryogenic cultures, the leaf explants were cultured into half strength of MS medium that have been fortified with 1.0, 1.5 and 2.0 mg/L of BAP in combination with 0.5 mg/L of NAA. The induction of somatic embryogenesis of *E. zwageri* was tested in half strength of MS medium with BAP (1.0 mg/L), NAA (0.5 mg/L) and GA₃ (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L).

**Statistical Analysis**

Each treatment consisted of five replicates with five explants per replicates. All collected data were analysed using one-way analysis of variance (ANOVA) followed by mean comparison carried out using Tukey Test at p<0.05 with SPSS Statistics Version 20.

**Results**

It took around four weeks for each of the somatic embryos developmental stages to occur from globular to heart, heart to torpedo and torpedo to cotyledonary stages (Figure 1). The developmental stages and the number of somatic embryos that have been induced from globular, heart, torpedo and cotyledonary were recorded in Table 1 and based on Tukey Test, there was no significance different among mean number of globular, heart, torpedo and cotyledonary somatic embryos that have been induced in this study by using different concentrations of 0.5 mg/L of NAA and 1.0 mg/L of BAP in combination with different concentrations of GA₃. The highest mean number of globular somatic embryos that have been induced in this study was 3.40±0.55, the highest mean number of heart somatic embryos was 3.00±0.71, the highest mean number of torpedo somatic embryos was 1.60±0.55 whereas the highest mean numbers of cotyledonary somatic embryos was 3.00±0.00. Based on this research, the optimal culture medium for the induction of indirect somatic embryogenesis *E. zwageri* was of half strength of MS medium with 0.5 mg/L of NAA and 1.0 mg/L of BAP in combination with 1.0 mg/L of GA₃.

**Discussion**

In this study on the induction of indirect somatic embryogenesis of *E. zwageri*, the maturation of somatic embryos up to the cotyledonary stage was successfully achieved in half strength MS medium with 1.0 mg/L of BAP and 0.5 mg/L of NAA in combination with either 1.0, 1.5 and 2.0 mg/L of GA₃. This similar finding was also obtained during somatic embryogenesis of *C. kanehirae*.
In conclusion, this is the first report on micropropagation of *E. zwageri* via induction of somatic embryogenesis by using leaf explants. In this study, the half strength of MS medium supplemented with 1.0, 1.5, 2.0 mg/L of BAP in combination with either 0.5 mg/L of 2,4-D or NAA successfully induced globular somatic embryos. The maturation of these globular somatic embryos were obtained in half strength of MS medium with BAP, NAA and GA, in which the highest mean number of the induction of somatic embryos up to the cotyledonary phase was observed in the half strength MS medium fortified with 1.0 mg/L of BAP, 0.5 mg/L of NAA and 1.0 mg/L of GA induced the highest mean numbers of globular-shaped, heart-shaped, torpedo-shaped as well cotyledonary-shaped stages. This similar application of GA stimulates the regeneration process as well as the germination of the somatic embryos.

**Conclusion**

In conclusion, this is the first report on micropropagation of *E. zwageri* via induction of somatic embryogenesis by using leaf explants. The maturation and the germination of somatic embryos of Lauraceae species was very difficult and the percentage was very low [8] and this was also observed in somatic embryogenesis of *Cinnamomum camphora* [10]. In this present study, embryogenic cultures that have been treated with 1.0 mg/L of BAP, 0.5 mg/L of NAA and 1.0 mg/L of GA induced the highest mean numbers of globular-shaped, heart-shaped, torpedo-shaped as well cotyledonary-shaped somatic embryos.

**References**

1. Irawan B, Gruber F (2003) A study on Tree Diversity in Association with Variability of Ironwood (*Eusideroxylon zwageri*) in Jambi, Indonesia. Indonesia: Jambi, p. 67.
2. MacKinnon (1986) The Conservation Status of Nonhuman Primates in Indonesia. In Primates the Road to Self-Sustaining Population. Springer Verlag, pp. 99-126.
3. WongAH, Singh AP (1995) Soft Rot Decay of Belian (*Eusideroxylon zwageri*) Wood. Bulletin of Tokyo University Forests 77: 119-126.
4. Martawijaya AI, Kartasudjana YI, Mandang SA, Prawira, Kadir K (1989) Atlas Kayu Indonesia. Badan Litbang Kehutanan Departemen Kehutanan: Jakarta, pp. 112.
5. Hidayat (2007) Induksi pertumbuhan eksplan endosperm ulin dengan IAA dan Kinetin. Agritrop 26(4): 147-152.
6. Baekman (1949) Houliet in Indonesia H Veenman and Zomen Wagjnegen, Terjemahan Wintrmoko Soekietjo. Fakultas Kehutanan Institut Pertanian Bogor, p. 33.
7. Mark SA, Dean JFD (2000) Forest Tree Biotechnology. Current Opinion in Biotechnology 11: 298-302.
8. Chen YC & Chang C (2009) Plant Regeneration through Somatic Embryogenesis from Young Leaves of *Cinnamomum kanehirae* Hayata. Taiwan Journal of Forest Science 24(2): 117-125.
9. Sanchez R, Quesada RP, Munoz AB, Alfaro FP (2005) Factor Affecting Maturation of Avocado Somatic Embryos. Scientia Horticulturae 102: 61-73.
10. Ashton P (2011) In vitro conservation of coconut (*Cocos Nucifera* L.) embryos in culture media. Biotropia 25: 11-21.
11. Li L, Qu R (2002) In vitro Somatic Embryogenesis in Turf-type Bermudagrass: Roles of Abscisic Acid and Gibberellic Acid and Occurrence of Secondary Somatic Embryogenesis. Plant Breeding 121(2): 155-158.
12. Canho JM, Lopes ML, Cruz GS (1999) Somatic embryogenesis induction on bay laurel (*Laurus nobilis*) L: Somatic embryogenesis in woody plants. Kluwer Academic Publishers: Dordrecht, Holland, pp. 341-348.
13. Witjaksono L, Litz RE (1999) Induction and growth characteristics of embryogenic avocado cultures. Plant Cell Tissue Organ and Culture 58(1): 19-29.
14. Cheng WH, Ma SS (1990) Somatic embryogenesis and plant regeneration in camphor tree (*Cinnamomum camphora*) (L) Presl. Journal of Chinese Society of Horticultural Sciences 36(2): 123-131.
15. Irawan B (2004) Ironwood (*Eusideroxylon zwageri*) Tet Binn.) and its varieties in Jambi, Indonesia. Indonesia: Jambi, p. 34.