Short Communication
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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 a Lauraceae family. It is one of the hardest timber species in Southeast Asia and unfortunately endangered in some part of Southeast Asia. The objective of this study was to determine the optimal culture medium for the induction of somatic embryogenesis from leaf explants of *E. zwageri*. Half strength of MS medium containing different concentrations of BAP with either NAA or 2,4-D were used for the induction of somatic embryos. It found that the globular somatic embryos were induced in half strength of MS medium with 1.0, 1.5, 2.0mg/L of BAP in combination with either 0.5mg/L of 2,4-D or NAA. The maturation of somatic embryos obtained in half strength of MS medium with BAP, NAA and GA3. The highest mean number of the induction of somatic embryos up to the cotyledonary phase was observed in culture medium containing 1.0mg/L of BAP, 0.5mg/L of NAA in combination with 1.0mg/L of GA3. This first reported preliminary study was useful for further plantlet regeneration of this species through induction of somatic embryogenesis. It was suggested that study on plantlet regeneration should be conducted.

Keywords: *eusideroxylon zwageri*, MS, timber, somatic embryogenesis

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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, Durabilty Class I with specific gravity of around 0.88–1.19g/cm^3.[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 of the rotting process for almost 40years and in the dry condition they can be up to a century.[2] Wong et al.[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 the optimal conditions.[5] According to Baekman,[6] it requires almost 200years 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 Cytokinin such as 6-benzylaminopurine (BAP).

Materials and methods

Plant material

In this study, the young leaf explants were collected from two 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 30minutes. The young leaf explants were further surface sterilized with 15% Sodium Hypochlorite solution with 3 drops of Tween 20 for 5minutes. After sterilization, these young leaf were thoroughly washed three times with sterile distilled water. The leaf explants were cut into 0.5 to 1.0cm 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 30g/L sucrose and solidified with 3.0g/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 121ºC for 20minutes. 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 2.0mg/L of BAP in combination with 0.5mg/L NAA. The induction of somatic embryogenesis of *E. zwageri* was tested in half strength of MS medium with BAP (1.0mg/L), NAA (0.5mg/L) and GA_3 (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0mg/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 0.5mg/L of NAA and 1.0mg/L of BAP in combination with different concentrations of GA_3. 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 0.5mg/L of NAA and 1.0mg/L of BAP in combination with 1.0mg/L of GA_3.

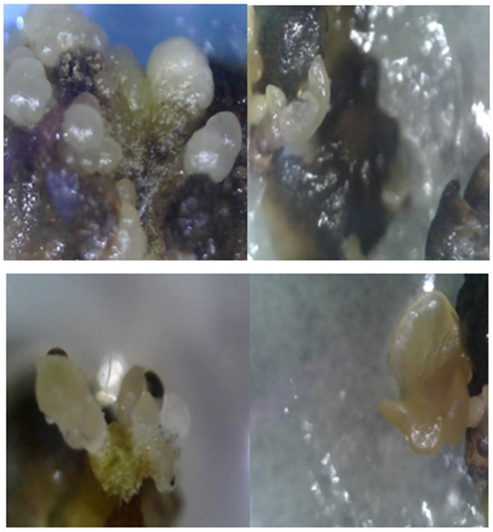

Figure 1 Stages of development of *E. zwageri*'s somatic embryos in half strength of MS medium with 0.5mg/L of NAA and 2.0mg/L of BAP in combination with 1.0 mg/L of GA_3 under compound microscope of 10x magnification. (A) Globular-shaped somatic embryo; (B) Heart-shaped somatic embryo; (C) Bunches of torpedo-shaped somatic embryos; (D) Cotyledonary-shaped somatic embryo (Bar =3mm).

Table 1 Mean (±SE) number of globular, heart, torpedo and cotyledonary that have been induced by using different concentrations of 1.0mg/L of BAP and 0.5mg/L of NAA in combination with different concentrations of GA

BAP+NAA+GA_3 (mg/L)	Mean (±SE) number of globular, heart, torpedo and cotyledonary somatic embryos			
	Globular	Heart	Torpedo	Cotyledonary
0	-	-	-	-
1.0+0.5+0.0	-	-	-	-
1.0+0.5+0.5	0.80±0.45	0.40±0.55	-	-
1.0+0.5+1.0	3.40±0.55	3.00±0.71	1.60±0.55	3.00±0.00
1.0+0.5+1.5	2.80±0.45	2.00±0.00	0.60±0.55	1.20±0.45
1.0+0.5+2.0	2.60±0.89	1.20±0.45	0.60±0.55	1.20±0.45
1.0+0.5+2.5	2.40±0.55	-	-	-
1.0+0.5+3.0	-	-	-	-

Mean along the column followed by the same alphabet are not significantly different at p≤0.05 (Tukey Test) Data represent mean of five replicates, each replicate consist of five explants.

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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 1.0mg/L of BAP and 0.5mg/L of NAA in combination with either1.0, 1.5 and 2.0mg/L of GA_3. This similar finding was also obtained during somatic embryogenesis of *C. kanehirae*[8] in which the addition of GA_3 of concentration of 1.0 and 2.0mg/L in combination with 1.0mg/L of BAP and 0.5mg/L of NAA into the culture medium have induced the highest percentage of somatic embryogenesis up to the cotyledonary-shaped. Another finding on Lauraceae species such as for *P. americana* by Sanchez *et al.*,[9] also revealed the same finding in which the addition of 1.0mg/L of GA_3 into the culture medium for the maturation of somatic embryos was necessary in order for the maturation of the somatic embryos of the species.

In this research of indirect somatic embryogenesis of *E. zwageri*, those medium that have been supplemented with 0.5 to 2.5mg/L of GA_3 were able to induce somatic embryos up to cotyledonary-shaped stages. This similar application of GA_3 was also used for the formation and germination of somatic embryos in *Cocus nucifera* by Ashton[10] and according to this author in the term of molecular study, GA_3 involved in the expression of KNOTTED like homeobox gene of *C. nucifera*. Ashton[10] stated that GA_3 at the concentration of 0.5 to 2.0mg/L successfully increased 1.5 fold the number of calli induced somatic embryos and two folds the number of somatic embryos per callus of *C. nucifera*. Li et al.,[11] in their research on somatic embryogenesis of Bermuda grass, mentioned that in the *in vitro* embryogenic culture system, the addition of GA_3 stimulates the regeneration process as well as the germination of the somatic embryos.

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 *E. zwageri*. The similar finding was also obtained in other Lauraceae species such as in *L. nobilis*,[12] *P. americana*[13] and in *C. camphora*.[14,15] In this present study, embryogenic cultures that have been treated with 1.0mg/L of BAP, 0.5mg/L of NAA and 1.0mg/L of GA_3 induced the highest mean numbers of globular-shaped, heart-shaped, torpedo-shaped as well cotyledonary-shaped somatic embryos.

Conclusion

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, 2.0mg/L of BAP in combination with either 0.5mg/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_3 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 1.0mg/L of BAP, 0.5mg/L of NAA in combination with 1.0mg/L of GA_3. Although the maturation of somatic embryos of this species was low, the result of this study still indicate that somatic embryogenesis in *E. zwageri* is feasible and the protocol described in this study will provide a source of somatic embryos production which will ensuring the optimization of a complete protocol for the regeneration of this species until plantlet regeneration.

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None.

Conflict of interest

The author declares no conflict of interest.

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