International Journal of Agriculture Innovations and Research
Volume 4, Issue 6, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 25/05/2016 | Accepted on : 03/06/2016 | Published : 09/06/2016

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

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***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. The objective of this study was to determine the optimal culture medium for the induction of somatic embryogenesis from leaf explants of *E. zwageri*. It was found that the globular somatic embryos were induced in half strength of MS medium 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. The maturation of somatic embryos obtained in half strength of MS medium with BAP, NAA and GA_3. The highest mean number of the induction of somatic embryos up to the cotyledonary phase was observed in culture medium containing 1.0 mg/L of BAP, 0.5 mg/L of NAA in combination with 1.0 mg/L of GA_3. This first reported preliminary study was useful for further plantlet regeneration of this species through induction of somatic embryogenesis.**

***Keywords* – *Eusideroxylon Zwageri*, MS, Timber, Somatic Embryogenesis.**

I. Introduction

Eusideroxylon zwageri or Borneo Ironwood is a tree of the tropical rainforest zone and economically very important for a source of hardwood timber. 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 40 years and in the dry condition they can be up to a century [2]. *E. zwageri* is conventionally propagated by sexual reproduction which is by seed [3]. 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 [4].

According to Baekman[5], 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 [6]. Realizing the economic importance of this hardwood timber species, it is necessary to regenerate and preserve this species through tissue culture.

II. Materials & Methods

A. 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 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.

B. 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 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 GA_3 (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L).

C. 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.

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III. Results & Discussion

In this study on the induction of indirect somatic embryogenesis of *E. zwageri*, the maturation of somatic embryos (**Figure 1**) 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 GA_3 (**Table 1**). This similar finding was also obtained during somatic embryogenesis of *C. kanehirae* [6] in which the addition of GA_3 of concentration of 1.0 and 2.0 mg/L in combination with 1.0 mg/L of BAP and 0.5 mg/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 [7] also revealed the same finding in which the addition of 1.0 mg/L of GA_3 into the culture mediumfor 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*, medium that have been supplemented with 0.5 to 2.5 mg/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 *Cocusnucifera* by Ashton [8]in which GA_3 at the concentration of 0.5 to 2.0 mg/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 and Qu [9] 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 [6] 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*[10], *P. americana*[11] and in *C. camphora* [12].

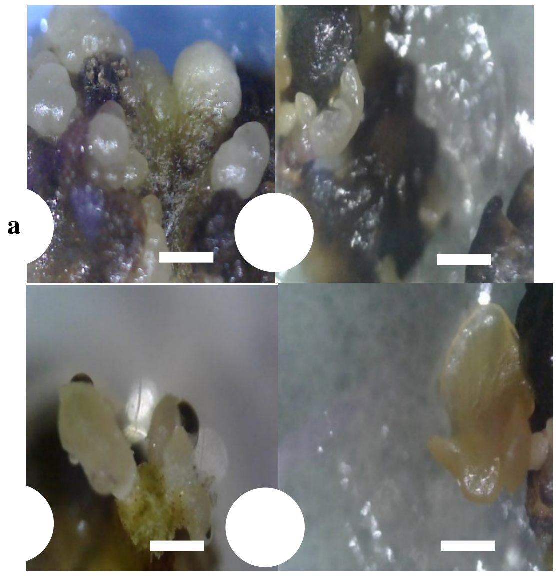

Fig. 1. Stages of development of E. zwageri's somatic embryos in half strength of MS medium with 0.5 mg/L of NAA and 2.0 mg/L of BAP in combination with 1.0 mg/L of GA_3 under compound microscope of 10x magnification. (A) Globular-shaped somatic embryo; (B) Heart-shaped somatic embryo; (C) Bunches of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

Table 1. Mean (±SE) number of globular, heart, torpedo and cotyledonary that have been induced by using different concentrations of 1.0 mg/L of BAP and 0.5 mg/L of NAA in combination with different concentrations of GA_3.

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 explant

IV. 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, 2.0 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_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 1.0 mg/L of BAP, 0.5 mg/L of NAA in combination with 1.0 mg/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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