



Factors Important for Somatic Embryo Induction and Proliferation from Young Leaf Explant of the Biggest Gigantic Teak

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ต้นสักใหญ่พันธุ์สักทองที่วนอุทยานต้นสักใหญ่อำเภอน้ำป่าด จังหวัดอุดรดิตถ์ ประเทศไทย เป็นต้นสักซึ่งใหญ่ที่สุดในโลก ปัจจุบันต้นสักใหญ่นี้มีอายุชั้ยยืนยาวมากกว่า 1,500 ปี และกำลังเสื่อมสภาพ เพื่ออนุรักษ์ลักษณะต่างๆ ซึ่งมีคุณค่ายิ่ง เทคโนโลยีการเพาะเลี้ยงเนื้อเยื่อโดยเฉพาะการเกิดเอ็มบริโอจากเซลล์ร่างกายช่วยให้การอนุรักษ์และฟื้นฟูสภาพป่าได้ผลเร็วขึ้น แคลลัสต้นสักใหญ่ถูกชักนำจากใบอ่อนซึ่งนำมาเพาะเลี้ยงบนอาหารเลี้ยงเชื้อพื้นฐาน MS ที่เติม NAA และ BAP ความเข้มข้นแตกต่างกัน พบว่าอาหารสูตรสำหรับการชักนำและภาวะดีที่สุดสำหรับการชักนำเอ็มบริโอจากเซลล์ร่างกายคืออาหารเลี้ยงเชื้อพื้นฐาน MS ซึ่งเติม NAA 1 มิลลิกรัมต่อลิตร และ BAP 1 มิลลิกรัมต่อลิตร ภายใต้การให้แสง 1,500 ลักซ์ จากหลอดไฟ Gro-lux เป็นเวลา 16 ชั่วโมงที่อุณหภูมิ 30 องศาเซลเซียส และ 8 ชั่วโมง ที่อุณหภูมิ 25 องศาเซลเซียส ในช่วงสว่างและมีมืดตามลำดับ ที่ภาวะดังกล่าวให้ค่าดัชนีการเติบโตของแคลลัสเอ็มบริโอสูงที่สุด ภายหลังจากย้ายแคลลัสเอ็มบริโอลงในอาหารเหลวภายใต้ภาวะที่เหมาะสมที่สุด พบเอ็มบริโอรูปทรงกลมและรูปหัวใจเพิ่มจำนวนอย่างเห็นได้ชัดจนที่ผิวของกลุ่มเอ็มบริโอ นอกจากนี้ยังพบโครงสร้างดังกล่าวแขวนลอยอยู่ในอาหารเหลวอีกด้วย อาหารสูตรสำหรับการเจริญเต็มวัยและภาวะทางกายภาพที่ช่วยให้เอ็มบริโอจากเซลล์

ร่างกายมีการเจริญต่อไปอยู่ในช่วงระหว่างการศึกษาอยู่

คำสำคัญ: การอนุรักษ์ การเพาะเลี้ยงเนื้อเยื่อพืช การเกิดเอ็มบริโอจากเซลล์ร่างกาย ต้นสัก

Abstract

Giant teak (*Tectona grandis* L.f. var. *Sak Tong*) in Giant Teak Forest Reserve, Nam Pad district, Uttaradit province, Thailand, is the biggest teak in the world. Its longevity is over 1,500 years and is now being deteriorated. To conserve all of its beneficial characteristics, plant tissue culture technology, especially somatic embryogenesis, provides an opportunity for rapid conservation and reforestation. Giant teak callus was extracted from young leaf and cultured on half-strength MS basal medium supplemented with NAA and BAP in different concentrations. It was found that the induction medium and optimum condition for somatic embryo induction were half-strength MS basal medium containing 1 mg/l NAA and 1 mg/l BAP under 1,500 lux of Gro-lux fluorescence at 16 hour duration at 30°C and 8 hours at 25°C of light and dark periods, respectively. This condition gave the highest growth index of

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embryogenic callus. After those embryogenic calli were transferred into liquid medium under optimum condition, globular and heart-shape embryo clearly proliferated on the surface of embryogenic clumps. Moreover, these structures were also found suspending in the liquid medium. The maturation medium and physical conditions for further development of these somatic embryos are under investigation.

Keywords: Conservation, Plant Tissue Culture, Somatic Embryogenesis, Teak

1. Introduction

Teak (*Tectona grandis* L.f.), a large deciduous tree, is a member of the Verbenaceae family. It is considered to be one of the most valuable timber trees in the world. This plant is native to Thailand, Laos, Myanmar and India. It has been grown since ancient times in Asia and nowadays this species is still mostly cultivated in the tropical moist areas [1], [2]. Apart from being an extraordinarily durable wood with a worldwide reputation [2], parts of teak can be and have been utilized in various ways; leaf extract for wound healing [3], leaf powder as a biosorbent for removing Cu(II) from aqueous solutions [4], bark extract for bacterial inhibition [5] and reducing blood sugar level [6] and seed extract for hair growth initiation and promotion [7].

In Thailand, the natural distribution area of teak covers 2 million hectares [8]. Among this part, the most gigantic teak in the world, namely, giant teak was discovered in Giant Teak Forest Reserve, Nam Pad district, Uttaradit province, Thailand. Although this plant has a lot of good characteristics including very long life, disease resistance, large circumference and fine wood texture, it is currently quite old (over

1,500 years) and deteriorated. To conserve all of these worthwhile appearances, plant tissue culture, especially somatic embryogenesis, was found being an excellent way for fast conservation and reforestation.

Many investigations have been made on teak tissue culture so far. Gupta et al. [9] found that multiple shoot formation of teak was induced from seedling nodal stem explant in MS medium containing 0.1 mg/l kinetin (Kn) and 0.1 mg/l 6-Benzylamino purine (BAP). These shoots were excised and induced to root on White's medium consisted of Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and Indole-3-propionic acid (IPA) at 0.1 mg/l each. While Widiyanto et al. [10] induced teak callus from internode explant on woody plant medium supplemented with 0.22 mg/l thidiazuron (TDZ) and 0.02 mg/l IBA. Shoot organogenesis was subsequently induced on medium containing 2.25 mg/l BAP in combination with 0.347 mg/l gibberellic acid (GA). Regenerated shoots were then rooted ex vitro in a soil-sand mixture medium (1:1). Afterward, the in vitro rooting of teak shoot was obviously improved by de Gyves et al. [11]. It was demonstrated that 160 mg/l putrescine promoted both strong and highly ramified roots and fast growing shoots during the rooting period, conditioning the plantlets for a good survival and quality.

For Thai teak, the in vitro propagation was started in 1987 by Kaosa-ard et al. [12]. Thereafter, tissue culture technology in Thailand had successfully been applied for commercial production of improved teak planting stocks. The cost of in vitro production of plantlets was of the same level as the cost of production of seeds from clonal seed orchard and in vitro multiplication was found to be very useful for rapidly increasing the number of teak plant [13]. However, despite the fact that teak organogenesis was effectively



done [14], [15], researches on somatic embryogenesis of Thai teak are scanty. Hence, in this study, young leaf of giant teak was used to examine the possibility of this explant for somatic embryo induction and proliferation. Besides, some factors were also investigated whether they were necessary during somatic embryo development.

2. Materials and Methods

2.1 Plant Materials

Young leaves of giant teak (*Tectona grandis* L.f. var. *Sak Tong*) were collected from Giant Teak Forest Reserve, Nam Pad district, Uttaradit province, Thailand, and used as explants for somatic embryo induction

2.2 Surface Sterilization

Explants were washed repeatedly with 5% (v/v) Teepol detergent solution for 5 minutes and rinsed with tap water for 3 minutes to remove dust and other impurities. Then, they were soaked in 70% (v/v) ethanol for 3 minutes and surface sterilized for 20 minutes with 15% (v/v) Chlorox and 7 drops of Tween-20[®]. After rinsing with sterile distilled water for 5 minutes, explants were surface sterilized again for 10 minutes with 5% (v/v) Chlorox and 7 drops of Tween-20[®]. Consequently, explants were rinsed with sterile distilled water for 3 times of 5 minutes each and cut into 5 X 5 mm² before being transferred to the induction medium.

2.3 Somatic Embryo Induction Medium

Giant teak callus was initiated on half-strength Murashige and Skoog basal medium [16] supplemented with different concentrations and combinations of BAP and NAA (Table 1). Medium was adjusted to pH

5.6, gelled with 2% (w/v) bacto-agar, and autoclaved at 121°C and 15 psi for 20 minutes. All cultures were kept in a culturing room under 1,500 lux of Gro-lux fluorescence 16 and 8 hours light and dark condition, respectively, at 25°C.

2.4 Effects of Some Physical Factors on Teak Callus Growth

Callus grown on half-strength MS basal medium containing 1 mg/l NAA and 1 mg/l BAP was cut into small pieces (6-8 mm diameter) and transferred to different treatments under the following conditions.

1. Day/night temperature of 25/20°C, 30/25°C and 35/30°C,
2. Light source quality of Gro-lux, Cool white and Day light fluorescence,
3. Light intensities of 500, 1,000 and 1,500 lux from Gro-lux fluorescence,
4. Duration of light/dark exposure of 16/8, 14/10 and 12/12 hours

Callus growth was measured by using growth index below.

$$\text{Growth index} = \frac{\text{Callus growth in week I}}{\text{Callus growth in week O}}$$

I = current week

O = initial week

All of these factors were investigated for 6 weeks with 3 replications.

2.5 Somatic Embryo Proliferation Medium

After the optimum conditions were obtained, callus was transferred into liquid medium and cultured under optimum condition (half-strength MS basal medium containing 1 mg/l NAA and 1 mg/l BAP under 1,500 lux of Gro-lux fluorescence at duration

16 hrs /30°C and 8 hrs/25°C of light and dark period, respectively) on a shaker (110 rpm). Following 2 months, these calli were examined under a dissection microscope.

3. Results and Discussion

3.1 Somatic Embryo Induction

After young leaf of giant teak was transferred to half-strength MS basal medium consisted of BAP and NAA in different combination, it was found that explant swelled and forming callus at vein within 7 and 14 days, respectively. Callus could be observed only on the formulae 6 to 11 (Table 1) and the best result was seen on formula 9 that contained 1 mg/l NAA and 1 mg/l BAP (Figure 1).

Table 1 Combination of BAP and NAA in callus induction medium (half-strength MS basal medium)

Formulae	BAP (mg/l)	NAA (mg/l)
1	0	0
2	0.5	0
3	1	0
4	0	0.5
5	0.5	0.5
6	1	0.5
7	0	1
8	0.5	1
9	1	1
10	0	2
11	0.5	2
12	1	2

3.2 Effects of Some Physical Factors on Teak Callus Growth

During callus development, there were two types of calli forming in half-strength MS semi-solid induction medium: embryogenic (nodular greenish)



Figure 1 Characteristics of nodular greenish calli extracted from young leaf vein of giant teak on half-strength MS basal medium containing 1 mg/l NAA and 1 mg/l BAP.

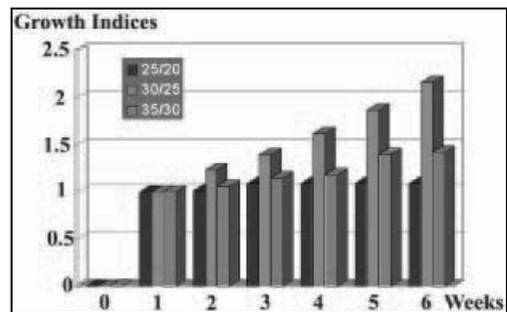


Figure 2 Relationship between growth indices of giant teak calli and temperature during day and night times.

and nonembryogenic (white-watery) calli. These embryogenic calli (Figure 1) were consequently used for investigation of some physical factors.

The results revealed that temperature at 30/25°C of day/night time obviously enhanced callus growth (Figure 2). The controlled temperature is in accordance with that of natural environment in teak forest, which is 30-32°C at daytime and about 25-27°C at nighttime. As far as light quality was concerned, Gro-lux fluorescence clearly gave the best result (Figure 3) while cool white fluorescence damaged

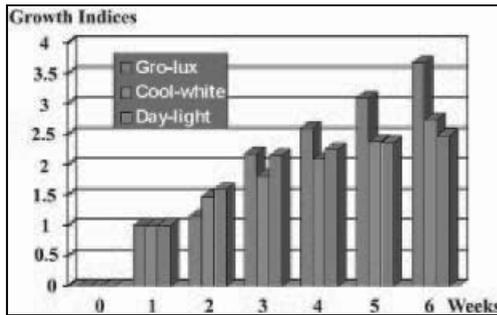


Figure 3 Relationship between growth indices of giant teak calli and light qualities.

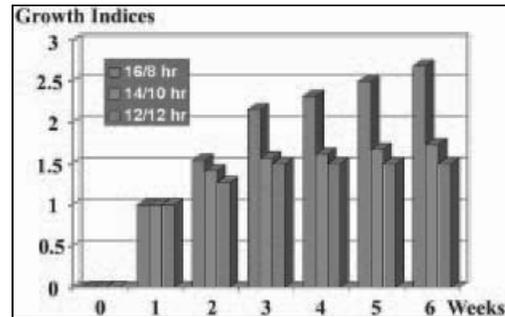


Figure 5 Relationship between growth indices of giant teak calli and duration of light and dark exposure.

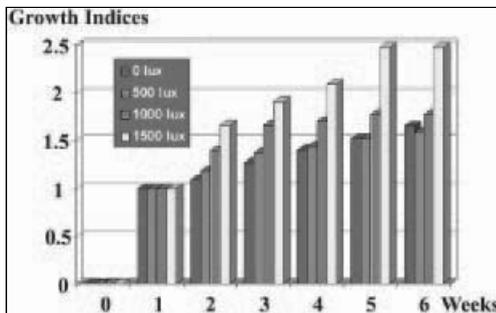


Figure 4 Relationship between growth indices of giant teak calli and light intensities.

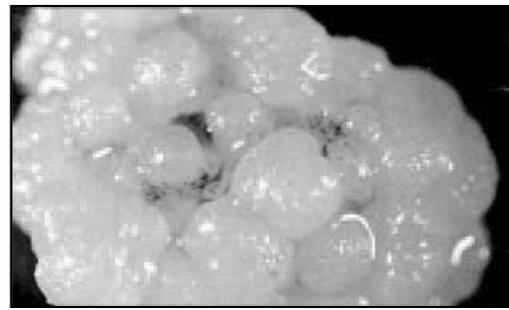


Figure 6 Embryogenic clumps of giant teak produced.

calli and day light fluorescence gave less number of nodular calli. Light intensity also played a key role in callus growth. If calli were grown under low light intensity, less development could have been seen. The optimum of light intensity was 1,500 lux (Figure 4). Duration of light and dark exposure was found to be essential as well. When the duration of light exposure decreased, growth index also declined. It was found that duration of light and dark exposure at 16/8 hours gave the highest growth (Figure 5).

3.3 Somatic Embryo Proliferation

When embryogenic calli were transferred into liquid medium and cultured under the optimum condition,

embryogenic clumps produced in half-strength MS liquid medium (Figure 6) showed greater development than in semi-solid medium. Globular and heart-shape embryo (Figure 7 and 8) were evidently observable proliferating on the surface of embryogenic clumps and thereafter found suspending in the liquid medium. Unfortunately, these embryoids were unable to develop further into torpedo-shaped and cotyledon-stage somatic embryos. It is possible that there should be dissimilar factors controlling each of giant teak embryoid developmental stages or these embryoids may need different types of plant growth regulators for the next stage development. In *Capsicum annum* L., there were many important factors concerning somatic embryogenesis from zygotic embryo explant, such

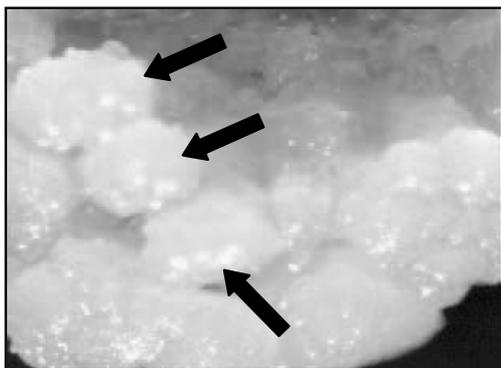


Figure 7 Globular embryos of giant teak (at arrows) evidently observed on surface of embryogenic clumps.

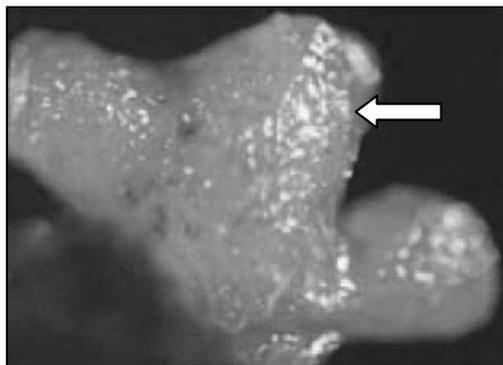


Figure 8 Heart-shape embryo of giant teak (at arrow) clearly appeared on the surface of embryogenic clumps.

as, explant source, size of explant, time held on initiation and maturation medium, requirement for coconut water, light and in liquid medium. dark conditions and sugar types [17]. As well as in papaya (*Carica papaya* L.) cv. ‘Kaekdum’, a well-known cultivar in Thailand, each stage of somatic embryo development required various supplements in the medium [18].

4. Conclusion

There were some important factors that influenced

giant teak globular and heart-shape embryo initiation. In this experiment, young leaf of giant teak could be an alternative explant for somatic embryo induction as it can easily be provided and is abundant. However, factors regulating the whole stages of somatic embryogenesis of giant teak from young leaf need further investigation. Thus, at the moment, maturation medium and physical conditions for the entire development of giant teak somatic embryos are under examination.

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