WALAILAK JOURNAL
http://wjst.wu.ac.th Agricultural Technology and Biological Sciences

Callus Induction and Somatic Embryogenesis from Cultured Zygotic Embryo of *Eleiodoxa conferta* (Griff.) Burr., an Edible Native Plant Species in Southern Thailand

Duangkhaetita KANJANASOPA*, Benjamas SOMWONG, Theera SRISAWAT, Suraphon THITITHANAKUL, Yoawaphan SONTIKUL and Suchart CHOENGTHONG

Faculty of Science and Industrial Technology, Prince of Songkla University, Surat Thani 84000, Thailand

(*Corresponding author's e-mail: duangkhae_kanjanasopa@yahoo.com)

Received: 5 June 2016, Revised: 5 December 2016, Accepted: 5 January 2017

Abstract

This research aimed to study the *in vitro* culturing of *Eleiodoxa conferta* (Griff.) Burr., collected from the Natural Study Center of Khan Thuli Peat Swamp Forest, located at Amphor Tha Chana, Surat Thani province. Primarily, the explant types for callus induction were investigated, and it was found that zygotic embryo is a suitable explant source, with a high potential of responsive tissue, and lacking browning secretions during culturing. The callus induction process was investigated by culturing zygotic embryos on MS medium supplemented with dicamba concentrations of 1.0, 2.5, and 5.0 $mg.L^{-1}$ combined with 200 $mg.L^{-1}$ ascorbic acid. The best 74.16 % callus response was obtained with 2.5 $mg.L^{-1}$ dicamba. Callus proliferation was good on a medium with the reduced dicamba concentration of 0.5 $mg.L^{-1}$, giving the largest 0.41 cm callus size, and the highest 0.141 g callus fresh weight. Embryogenic callus competence was successfully induced at 38.33 % when culturing with 0.1 $mg.L^{-1}$ dicamba and 1.0 $g.L^{-1}$ casein hydrolysate. Embryogenic calli clump cultivated on MN6 medium developed to somatic embryos in the globular stage at 95.23 %. This suggests that the NO3/NO3+NH4 ratio and vitamin contents in MN6 could promote somatic embryogenesis.

Keywords: Callus, embryo, *in vitro* propagation, tissue culture, *Eleiodoxa conferta* (Griff.) Burr.

Introduction

Eleiodoxa conferta (Griff.) Burr. is a palm species within the Arecaceae family, and mostly grows in the tropical regions of Southeast Asia, such as Indonesia, Malaysia, and Thailand. In Thailand, it is originally found in the southern part, and has the common names Lumphi, Kra-Lu-Bi, or Lu-Bi. It is a monocotyledonous, dioecious (with male and female spadices on separate plants), and grows in peat swamp forests throughout the tropical zone [1]. This is a strictly tropical species that thrives in warm, sheltered, shady, and very moist swamp forests. It prefers organic-rich, fertile, acidic soil, and grows well in like company, forming large colonies. It is, therefore, a major component of the agro-ecosystems in these localities with constantly high humidity, and is a source in the food supply chain. The utilization is, in many ways, affected by properties specific to this case; for example, the fruit is extremely sour, so it is often pickled and used as a substitute for lemon or tamarind, and the leaves are used in thatching. The medicinal uses include plant extracts from the pounded stem or from scaly fruit husks, and these have been used in mixtures to treat cough and hoarseness [1]. Unfortunately, these plants might face losses in population and in genetic diversity, caused by environmental changes, increased cultivation of industrial crops, and residential land use.

Plant regeneration through tissue culture is an attractive method of utilizing the genetic resources of a plant, both as a source of bioactive compounds and in the conservation of a plant species [2]. While *in vitro* techniques allow rapid propagation and large numbers of plants, some significant problems can impede efficient plantlet production, such as unsuitable medium or hormones. In practice, inducing the explants indirectly through somatic embryogenesis produces the greatest number of plantlets, but is applicable to the least number of plant species. This process is strongly affected by the culture conditions, including the choice of culture medium composition, the subculture interval, and the type and concentration of growth regulators [3].

Over the past 5 decades, *in vitro* technology has been used in germplasm storage and in the clonal propagation of critically endanger plants [4,5]. Numerous investigations have demonstrated *in vitro* regeneration of economic palm plants, such as oil palm (*Elaeis guineensis*) [6], date palm (*Phoenix dactylifera* L.) [7], *Salacca glabrescens* [8], and coconut (*Cocos nucifera* L.) [9]. However, *in vitro* cultures of *E. conferta* (Griff.) Burr. have not been reported. Therefore, the present study was a preliminary investigation into the *in vitro* culture conditions for the best source of *E. conferta* explants, induction, and proliferation of calli by culturing zygotic embryos, with the medium compositions ranked by embryogenesis and somatic embryogenesis competence.

Materials and methods

Explant preparation and culture establishment

Suitable explant type was initially investigated for the best source of *in vitro* culture. Samples of *E. conferta* (Griff.) Burr. (age about 5 years, of 3 - 4 m height) were collected from Khan Thuli Peat Swamp forest at the Natural Study Center in Tambon Khan Thuli, Amphor Tha Chana, Surat Thani, from August-December, 2014. The samples collected from the field were transported to the laboratory within 4 h prior to *in vitro* culturing. For explant preparation, immature male and female flowers were collected, followed by the cutting off of spikelets from the axis, cleaning under running tap water, soaking in Teepol® solution for 10 min, and finishing with several rinses with running tap water. Subsequently, each female flower was excised from the spikelet and immediately soaked in 70 % alcohol for 2 - 3 min, followed by surface sterilization with 20 % (v/v) Clorox® with Tween-20 for 20 min, and rinsing with sterile distilled water 3 times. Otherwise, the cleaned male spikelets were soaked in 95 % ethanol, followed by burning to sterilize their surfaces. Ovaries and anthers were aseptically separated from flowers and cultured on MS medium [10] supplemented with 0.5 $mg.L^{-1}$ BA, 1.5 $mg.L^{-1}$ 2,4-D, and 1 $mg.L^{-1}$ NAA. Both surface sterilization and culture steps took place under laminar air flow. For immature inflorescence, the spadix was cut from the plant and sheaths were removed from immature inflorescence, which was surface sterilized by Clorox® as mentioned before, cut to approximately 1×1 cm^2 size, and subsequently cultured on the same medium. Individual immature/mature fruits were carefully cut off and washed under running tap water to remove dirt and debris. The fruits were soaked in Teepol® solution for 10 min and rinsed with running tap water several times. The cleaned fruits were further processed under laminar air flow by removing scaly fruit husks, and were soaked in 95 % ethanol, followed by burning. Zygotic embryos were aseptically excised from the seeds using secateurs, and were cultured on medium. The cultures were incubated at 27±2 °C under 1,000 - 3,000 Lux illumination for 16 h daily photoperiod. These same conditions were used throughout all culturing in this work.

Zygotic callogenesis induction and callus proliferation

Callus induction was carried out by culturing mature zygotic embryos (collected approximately 90 to 100 days after pollination) on basal MS medium supplemented with 200 $mg.L^{-1}$ ascorbic acid. The culture medium was supplemented with alternative dicamba concentrations of 1.0, 2.5, and 5.0 $mg.L^{-1}$. Each treatment group consisted of 120 embryos (12 bottles; 10 embryos per bottle). The percentage of callus formation was recorded after culturing for one month. Primary calli were transferred to proliferate on medium with identical composition to the callus induction state, except for reducing dicamba concentrations to 0.25, 0.5, or 1.0 $mg.L^{-1}$. Each treatment contained 100 primary calli (20 bottles; 5 calli per bottle). Culture conditions were similar as previously described. Twenty-five calli per treatment were

randomly selected to measure the increments in callus size and fresh weight, after culturing for one month, as differences between the final and initial observations.

Embryogenic callus induction and somatic embryogenesis competence

Embryogenic callus initiation was studied by transferring calli to MS medium containing 0.1 $mg.L^{-1}$ dicamba supplemented with 1.0 $g.L^{-1}$ casein hydrolysate. All the media were supplemented with 200 $mg.L^{-1}$ ascorbic acid. Each treatment group had 60 calli (12 bottles; 5 calli per bottle). The percentage of embryogenic development was recorded after 1 month of culture. Plant regeneration was assessed by transferring embryogenic clumps to fresh MS and Modified N6 (MN6) media [11]. Each treatment was performed to 45 embryogenic clumps (15 bottles; 3 embryogenic calli per bottle). The percentage of somatic embryogenesis was recorded after 1 month of culture. All culture conditions were as described previously.

Experimental design and data analysis

The experiments followed a completely randomized design. The observed data included percent of callus formation, callus size and callus fresh weight, percent of embryogenic calli, and percent of somatic embryogenesis; these data were subjected to analysis of variance (ANOVA), and differences in means of treatments were subjected to Tukey's test for statistical significance ($p < 0.05$).

Results and discussion

Callus induction and proliferation

Various factors influence plant regeneration under *in vitro* conditions, such as the type of explants, basal medium, growth regulators, genotype, and culture conditions. For initiation of the culture, the source materials used in the *in vitro* culture of *E. conferta* were preliminarily tested. The explant types tested were immature anther, immature ovary, immature male/female inflorescence, and immature/mature zygotic embryo. All these explants were cultured on the callus induction medium containing a combination of BA, 2,4-D, and NAA. We found that immature female inflorescence and immature or mature embryos gave better callus induction than the other types that showed browning exudation (data not shown). However, in prolonged culture on this medium, the immature female inflorescence had browning exudates, except for the zygotic embryo tissues. Apparently, the combination of wounding, the type of tissue, and the growth regulator, induced phenolic compound production [12]. The immature female inflorescence gives some trouble in the collection of sufficient sample quantity, which must be done during the period of inflorescence formation. Zygotic embryos are the best choice for tissue culture experiments, providing sufficient sample sizes, easy shipping (even over long distances), aseptic processes, and in being tremendously responsive. Zygotic embryos are young tissues, which are usually the preferred choice for tissue culture. They have higher potential to produce embryos and organs than the more differentiated and more mature tissues [13]. Rapid multiplication of oil palm was successful from cultured zygotic embryos as the starting material [11]. Various stages of zygotic embryos and various genotypes of oil palm have been reported in the successful induction of somatic embryogenesis [14]. Likewise, a high rate clonal propagation of the nipa palm (*Nypa frutican* Wurmb.) was first reported using zygotic embryos as the initial explants [15]. Micropropagation of *Heliconia bihai* L. was also established using zygotic embryos, excised from either immature or from mature fruit [16].

After 2 weeks of culture on the callus induction medium, almost mature zygotic embryos were swelled, and the calli were developed a week later, at the ridge of tissue contacting the medium. The callus induction was strongest by the media with 2.5 and 5.0 $mg.L^{-1}$ dicamba, as shown in **Table 1** and in **Figure 1(a)**. While the zygotic embryo is the best tissue for culturing, the physical wounding during embryo excision greatly affected the callus induction capacity by causing secretion of phenolic compounds into the medium, and these turned from clear to brownish in color. The phenolic compounds revealed toxicity and made the tissues unresponsive, ultimately causing death, even if ascorbic acid and antioxidants were supplemented to the medium [17]. In all media cultures, the compact calli were developed, with white, yellowish, and pale green colors, as shown in **Figure 1**. The calli were transferred

to the callus multiplication medium with reduced dicamba concentration. The results showed that the size and fresh weight of callus were not significant factors in any media, but culturing calli with 0.5 mg.L^{-1} dicamba gave the best callus proliferation, as shown in **Table 2**. Friable calli were observed in all media, showing white, yellow, and green colors.

Dicamba is an effective auxin, and is usually used at low concentrations (0.2 - 10 mg.L^{-1}) for callus induction, embryogenic callus, and somatic embryogenesis induction in various monocotyledons. It preferentially induces division in epidermal cells, parenchyma cells, and vascular tissue, while the 2,4-D stimulates only epidermal cell division [18]. Micropropagation of oil palm *Elaeis guineensis* Jacq. *var.* Tenera gave stronger callus induction and embryogenic callus proliferation in cell suspension with 0.3 mg.L^{-1} dicamba than with 2,4-D [19]. Moreover, the 2,4-D induces considerable production of phenolic compounds in plant cells, and causes mutations in *in vitro* cultures [20]. Also, dicamba at the high 20 mg.L^{-1} concentration has successfully promoted callus induction and multiplication from the young leaves of *Vatica diospyroides* [21].

Table 1 Callus induction rates of zygotic embryos cultured on various media.

Concentration of dicamba (mg.L^{-1})	Percent of callus formation
1	61.67
2.5	74.16
5	71.67

MS was used as the basal medium, with 3 % sucrose and with 200 mg.L^{-1} ascorbic acid. Tukey's test did not indicate statistically significant differences.

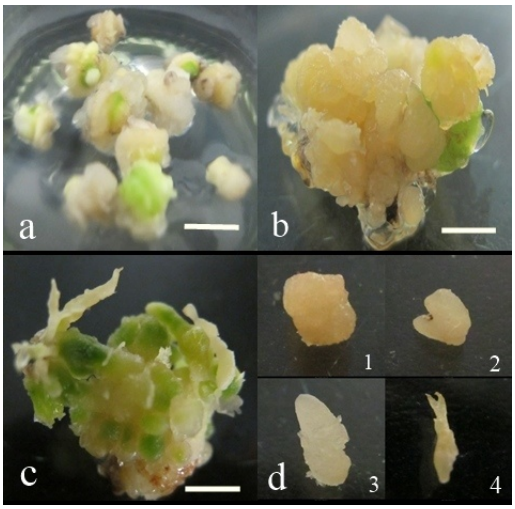

Figure 1 *In vitro* zygotic culture of *E. conferta* through somatic embryogenesis. (a) zygotic embryo at 1 month of culturing on callus induction medium (bar 1 cm). (b) embryogenic callus developed on 0.1 mg.L^{-1} dicamba and 1 g.L^{-1} casein hydrolysate in the medium (bar 1 cm). (c,d) somatic embryogenesis on MN6 medium showed globular, heart, torpedo, and cotyledonary stages, here labeled as d1, d2, d3, and d4, respectively (bar 1 cm).

Table 2 Callus proliferation.

Concentration of dicamba (mg.L^{-1})	Increment in		Callus characterization
	size (cm)	fresh weight (g)	
0.25	0.25	0.009	compact and friable callus, white and green
0.5	0.41	0.141	compact and friable callus, yellow and green
1	0.24	0.06	compact and friable callus, yellow and green

MS was used as the basal medium, with 3 % sucrose and with 200 mg.L^{-1} ascorbic acid. Tukey's test did not indicate statistically significant differences.

Embryogenic callus induction and somatic embryogenesis

Development of the primary embryogenic calli was carried out by culturing the calli on medium having 0.1 mg.L^{-1} dicamba with or without 1.0 g.L^{-1} casein hydrolysate. The observed embryogenic callus formation did not differ significantly between the alternative media. The medium containing 0.1 mg.L^{-1} dicamba showed 23.33 % embryogenic calli, while 38.33 % was reached when casein hydrolysate was added to the medium (**Table 3**). This experiment induced embryoid formation in callus transferred to fresh medium, with dicamba concentration reduced to 0.1 mg.L^{-1}. The decreased dicamba concentration stimulated the proliferation rate of embryogenic calli, and also promoted the formation of large numbers of embryoids [22]. This is a typical rule of embryogenesis induction. Similar approaches have been used to promote the somatic embryogenesis from immature inflorescence of *Areca catechu* [23], and to multiply embryogenic and somatic embryo formation from immature zygotic embryos of various genotypes of oil palm [24]. However, embryoid formation also occurred on MS basal medium, most likely due to the influence of dicamba accumulation in the tissue during the callus multiplication step, which affected the embryogenesis process. The addition of casein hydrolysate at 1.0 g.L^{-1} enhanced embryogenic formation from calli because of its organic substance composition. Casein hydrolysate is a complex organic additive and is an excellent source of amino nitrogen and free amino acids [25]. It has been shown to improve and expedite responses of *in vitro* cultures of various plant species, for example, enhancing the callus growth in Indian mulberry *Morinda citrifolia* [26], supporting the callus production in pea *Pisum sativum* [27], and producing greenish healthy nodular calli with improved embryogenic capacity in the medicinal plant *Stevia rebaudiana* [28]. In the palm family, the best callus growth and somatic embryo formation of date palm (*P. dactylifera* L.) were achieved either with yeast extract or with casein hydrolysate in the culture medium [7].

In the experiments, somatic embryogenesis was observed in the globular stage on some embryogenic clumps on the embryogenic induction medium. Consequently, the capacity of tissue differentiation was evaluated by transferring embryogenic clumps to MS and MN6 media. The somatic embryogenesis in the globular stage became stronger in both these media. The MN6 gave the higher 95.23 % somatic embryogenesis, while the basal MS medium gave 17.77 %, and the embryos became greenish from the chlorophyll pigment produced (**Table 4** and **Figure 1(c)**). Somatic embryo shapes found included heart and torpedo shapes in the MN6 medium, and some somatic embryogenic capable cases differentiated to the cotyledonary stage, shown in **Figure 1(d)**. Comparison of medium components between MS and MN6 showed a difference in nitrogen content ratio and in various vitamin contents. Nitrogen is a major nutrient component influencing *in vitro* morphogenesis by the ratio between NO3 and NH4 ions, and this ratio needs to be adjusted for each plant species [29]. In the current experiments, increasing the nitrogen NO3/NO3+NH4 ratio from 1.91 (MS) to 8.68 (MN6) improved tissue morphogenesis. This is similar to observations about the embryo/plantlet regeneration frequencies of *Coffea* species, cultured on primary culture medium, when increasing the NO3/NO3+NH4 ratio from 0.66 to 0.75 and with a 2-fold lower growth regulator concentration [30]. Growth and morphogenesis of plant tissue culture can also be enhanced by some vitamins [31]. Several vitamin contents in MN6 were higher than in MS, with 5-fold nicotinic acid, 5-fold pyridoxine, and 25-fold thiamine. In a prior study, increased concentrations of nicotinic acid (4 μM to 30 μM) and/or thiamine (0.3 μM to 5 μM) in MS enhanced the

embryogenesis frequency of zygotic embryos and somatic embryos of *Glycine max* [32]. This suggests that the nutrients, especially nitrogen content and/or vitamin contents in MN6, were more suited for tissue regeneration than the composition of MS. This is in agreement with Thuzar *et al.* [11], where the highest plant regeneration through somatic embryogenesis of zygotic embryo culture of oil palm was productive on N6 and on MN6 media without growth regulator. Plant regeneration on medium without plant regulator is an advantage to the *in vitro* culture, because prolonged cultivation in a cytokinin supplemented medium during the regeneration usually leads to somaclonal variations [33] and a shorter transfer interval, and low auxin plus high cytokinin levels in the medium increase the incidence of abnormalities [34]. The experimental results of the current study suggest that MN6 could be better suited for somatic embryogenesis and plant regeneration than MS. However, the type of medium for callus acclimatization and the subculture interval should be studied further, to obtain most efficient plant regeneration through the embryogenesis and somatic embryogenesis of *E. conferta*.

Table 3 Embryogenic callus induction.

Medium type	Embryogenic callus induction (%)
MS	30
MS+0.1dicamba	23.33
MS+0.1 dicamba + CH	38.33

MS was used as the basal medium with 3 % sucrose, and with 200 $mg.L^{-1}$ ascorbic acid. Observed differences were not statistically significant according to Tukey's test. CH is casein hydrolysate added in medium at 1.0 $g.L^{-1}$.

Table 4 Somatic embryogenesis with relative proportions of different stages.

Medium type	Somatic embryogenesis (%)	globular	heart	torpedo
MS	17.77^{b}	+++	+	+
MN6	95.23^{a}	++++	++	+++

Different superscripts indicate highly significant differences ($p < 0.01$) according to Tukey's test.

Conclusions

Effective callus induction and callus proliferation of *E. conferta* were achieved when cultured with MS with 2.5 $mg.L^{-1}$ dicamba and subsequently multiplied with MS containing 0.5 $mg.L^{-1}$ dicamba. Reducing the dicamba concentration and supplementing the medium with casein hydrolysate effectively improved embryoid formation. The N6 medium was superior to the MS basal medium in promoting somatic embryogenesis.

Acknowledgements

This research was financially supported by Prince of Songkla University annual government statement of expenditure under the Plant Genetic Conservation Project under the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn Year 2012 - 2014. The authors would like to thank Mr. Junthachoti Pusin, for assistance with collecting samples, and Assoc. Prof. Dr. Seppo Karrila, for assistance with manuscript preparation.

References

[1] TK Lim. *Edible Medicinal and Non-Medicinal Plants*. Vol I. Springer, New York, 2012, p. 396-8.
[2] TA Thorpe. History of plant tissue culture. *Mol. Biotechnol.* 2007; **37**, 169-80.
[3] A Feher TP Pasternak and D Dudits. Transition of somatic plant cells to an embryogenic state. *Plant Cell Tiss. Organ. Cult.* 2003; **74**, 201-28.
[4] AK Patel, T Agarwal, M Phulwaria, V Kataria and NS Shekhawat. An efficient *in vitro* plant regeneration system from leaf of mature plant of *Leptadenia reticulate* (Jeewanti): A life giving endangered woody climber. *Ind. Crop. Prod.* 2014; **52**, 499-505.
[5] C Sudhersan, M AboEl-Nil and J Hussain. Tissue culture technology for the conservation and propagation of certain native plants. *J. Arid. Environ.* 2003; **54**, 133-47.
[6] S Chehmalee and S Te-chato. Induction of somatic embryogenesis and plantlet regeneration from cultured zygotic embryo of oil palm. *J. Agric. Tech*. 2008; **4**, 137-46.
[7] JM Al-Khayri. Influence of yeast extract and casein hydrolysate on callus multiplication and somatic embryogenesis of date palm (*Phonix dactylifera* L.). *Sci. Hortic*. 2011; **130**, 531-5.
[8] AZ Zulkepli, H Jaafar and AHA Rusni. Optimization of sterilization method and callus induction of *Salacca glabrescens*. *In*: Proceedings of the International Conference on Biology, Environment and Chemistry IPCBEE, Singapore, 2011.
[9] SC Fernando and CKA Gamage. Abscisic acid induced somatic embryogenesis in immature embryo explants of coconut (*Cocos nucifera* L.). *Plant Sci*. 2000; **151**, 193-8.
[10] T Murashige and F Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum*. 1962; **15**, 473-97.
[11] M Thuzar, A Vanavichit, S Tragoonrung and C Jantasuriyarat. Efficient and rapid plant regeneration of oil palm zygotic embryos cv. 'Tenera' through somatic embryogenesis. *Acta Physiol. Plant*. 2011; **33**, 123-8.
[12] V Sarasan, MM Ramsay and AV Roberts. *In vitro* germination and induction of direct somatic embryogenesis in 'Bottle Palm' [*Hyophorbe lagenicaulis* (L. Bailey) H. E. Moore], a critically endangered Mauritian palm. *Plant Cell Rep*. 2002; **20**, 1107-11.
[13] M Elhiti and C Stasolla. *The Use of Zygotic Embryos as Explants for in vitro Propagation*: *An Overview*. *In*: TA Thorpe and EC Yeung (eds.). Plant Embryo Culture: Methods and Protocols, Methods in Molecular Biology. Springer Science Business, 2011, p 229-55.
[14] S Chehmalee and S Te-chato. Genotypes, physiological ages of zygotic embryos and auxin as affect on germination and callus formation of oil palm. *In*: Proceeding of the International Conference on Integration of Science & Technology for Sustainable Development, Bangkok, Thailand, 2007, p. 166-9.
[15] VC Lapitan, KLC Nicolas and ET Rasco. Tissue culture technique for clonal propagation of nipa palm (*Nypa fruticans* Wurmb., Arecaceae) from embryo culture. *Asia Life Sci.* 2015; **24**, 111-25.
[16] C Ulisses, GF Melo-de-Pinna, L Willadino, CC de Albuquerque and TR Camara. *In vitro* propagation of *Heliconia bihai* (L.) L. from zygotic embryos. *Acta Bot. Bras.* 2010; **24**, 184-92.
[17] AM Taji and RP Williams. *Overview of Plant Tissue Culture*. *In*: AM Taji and RP Williams (eds.). Tissue Culture of Australia. University of New England Press, Australia, 1996, p. 1-5.
[18] S Te-chato and A Hilae. High-frequency plant regeneration through secondary somatic embryogenesis in oil palm (*Elaeis guineensis* Jacq. *var*. tenera). *J. Agric. Tech*. 2007; **3**, 345-57.
[19] T Niyakid and S Te-chato. Induction and proliferation of embryogenic cell suspension of oil palm (*Elaeis guineensis* Jacq. *var*. tenera) and evaluation of genetic variation by RAPD marker. *J. Agric*. 2012; **28**, 273-83.
[20] B de Touchet, Y Duval and C Pannetier. Plant regeneration from embryogenic suspension culture of oil palm (*Elaeis guineensis* Jacq.). *Plant Cell Rep*. 1991; **10**, 529-32.
[21] T Srisawat and J Nathinan. Preparation of *Vatica diospyroides* Symington: An endangered medicinal Dipterocarp of peninsular Thailand by cultures of embryonic axes and leaf-derived calli. *Pak. J. Biol. Sci.* 2013; **16**, 396-400.

[22] HC Wang, JT Chen and WC Chang. Somatic embryogenesis and plant regeneration from leaf, root and stem-derived callus cultures of *Areca catechu*. *Biol. Plantanum* 2006; **50**, 279-82.
[23] DA Steinmacher, CR Clement and MP Guerra. Somatic embryogenesis from immature peach palm inflorescence explants: Towards development of an efficient protocol. *Plant Cell Tiss. Organ. Cult*. 2007; **89**, 15-22.
[24] S Sanputawong and S Te-chato. Effect of genotypes of oil palm on callus, embryogenic callus and somatic embryo formation. *In*: Proceeding of the 1st Mae Fah Luang University International Conference. Chiang Rai, Thailand, 2012.
[25] F Bister-Miel, JL Guignard and C Agier. Glutamine as an active component of casein hydrolysate: its balancing effect on plant cells cultured in phosphorus deficient medium. *Plant Cell Rep*. 1985; **4**, 161-3.
[26] MH Zenk, H El-Shagi and U Schulte. Anthraquinone production by cell suspension cultures of *Marinda citrifolia*. *Planta Med*. 1975; **28**, 79-101.
[27] T Cardiand and LM Monti. Optimization of callus culture in pea (*Pisum sativu*m). *Ann. Fac. Agric*. *Portici*. 1990; **24**, 11-8.
[28] A Das and N Mandal. Enhanced development of embryogenic callus in *Stevia rebaudiana* Bert. by additive and amino acids. *Biotechnology* 2010; **9**, 368-72.
[29] EF George and GJ de Klerk. *The Components of Plant Tissue Culture Media I: Macro- and Micro-Nutrients*. *In*: EF George, MA Hall and GJ de Klerk (eds.). Plant Preparation by Tissue Culture. 3rd ed. Springer, 2008, p. 65-113.
[30] NP Samson, C Campa, LL Gal, M Noirot, G Thomas, TS Lokeswari and A de Kochko. Effect of primary culture medium composition on high frequency somatic embryogenesis in different *Coffea* species. *Plant Cell Tiss. Organ. Cult*. 2006; **38**, 37-45.
[31] T Thorpe, C Stasolla, EC Yeung, GJ de Klerk, A Roberts and EF George. *The Components of Plant Tissue Culture Media II: Organic Additions, Osmotic and pH Effects, and Support Systems*. *In*: EF George, MA Hall and GJ de Klerk (eds.). Plant Preparation by Tissue Culture. 3rd ed. Springer, 2008, p. 115-73.
[32] UB Barwale, HR Kerns and JM Widholm. Plant regeneration from callus cultures of several soybean genotypes via embryogenesis and organogenesis. *Planta* 1986; **167**, 473-81.
[33] I Besse, JL Verdeil, Y Duval, B Sotta, R Maldiney and E Miginia. Oil palm (*Elaeis guineensis* Jacq.) clonal fidelity: endogenous cytokinins and indolacetic acid in embryogenic callus cultures. *J. Exp. Bot.* 1992; **43**, 983-9.
[34] CJ Eeuwens, S Lord, CR Donough, V Rao, G Vallejo and S Nelson. Effects of tissue culture conditions during embryoid multiplication on the incidence of ‘‘mantled’’ flowering in clonally propagated oil palm. *Plant Cell Tiss. Organ. Cult.* 2002; **70**, 311-23.