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# Effect of sizes of haustorium embryo on secondary somatic embryo formation and histological study in oil palm

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Sizes of haustorium embryo (HE) played an important role in secondary somatic embryo (SSE) production. HEs from various clone of oil palm were cultured on Murashige and Skoog (MS) medium supplemented with 0.2 M sorbitol and 1.136 mM ascorbic acid to initiate SSE. For Thepa clone, HE at size of 13 mm gave the highest number of SSE/HE formation at 45 SSEs /HE whereas Krabi clone gave the highest number of SSE/HE formation at 43 SSEs /HE from HE at size of 9 mm. In case of HE derived from immature zygotic embryo culture, a number of SSEs at 18/HE were obtained from HE at size of 9 mm which lower than those obtained from young leaves. Histological study revealed that SSE developed from 3 origins; epidermis, parenchyma and procambium or vascular cambium. Those cells were isodiametric, rich of cytoplasm with the large nucleus. The cells divided rapidly to form a cluster of globular embryos.

**Key words:** Oil palm, secondary somatic embryo, Elaeis guineensis, haustorium embryo, zygotic embryo

## Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a very important commercial crop in southern Thailand. The development of oil palm varieties with higher yields, modified to marginal zones or expressing other imperative agronomic characters are important for the improvement of oil palm sustainability in Thailand. Propagation through tissue culture is widely used as commercial scale. Although they could be induced directly or indirectly by the use of dicamba (Te-chato *et al*., 2002), the conversion rate of somatic embryos (SEs) to plantlets is quite low. Promoting germination of somatic embryo is of great importance in commercial plantation.

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Secondary somatic embryo (SSE) was reported to be induced directly from SE (Eudes *et al*., 2003) or indirectly from cotyledon of SE (Silva *et al*., 2005). In case of oil palm, there is only one author reported SSE induction from culturing zygotic embryo using polyamine (Rajesh *et al*., 2003). However, low efficiency of SSE induction obtained. Moreover, plantlet regeneration from SSE was not yet obtained. Hilae and Te–chato (2005) reported the great potential of clonal propagation of oil palm through SSE induced from haustorium embryos (HEs). They also reported that conversion of SSE to seedlings was significant higher than SE. Generally, micropropagation in many plants has been engaged with SE, e.g. cassava (*Manithot esculenta*). In case of cassava, a number of 30 SSEs/SE were developed after being cultured for 45 days. In one year, 6.6 million embryos could be produced from one SE (Raemakers *et al*., 1995). Origin of SSE has been reported to be from two main origins, epidermal and parenchymatous cells (Zegzouti *et al*., 2001). In case of oil palm, Te–chato (1998) reported that 2,4-D induced only epidermal cells to produced SE while dicamba induced from both origins. In this paper, we described the sizes of HE as an effect on SSE formation and origin of SSE from HE of oil palm.

## Materials and methods

### *Plant material*

In this experiment embryogenic callus derived from two sources of explants was used. First source was from young leave of clone from Thepa and Krabi) and second source was from immature zygotic embryo (IZE) from hybrid of DxP cross number 7 and 16. Those calluses were maintained on MS with 3% sucrose, 1.136 mM ascorbic acid and 4.5 μM dicamba known as embryogenic callus induction medium (ECIM). Subculture was carried out at monthly intervals for at least two years. During the next subculture HEs developed on callus in ECIM at various sizes were selected and transferred to SSE induction medium.

### *Culture medium and environments*

MS medium which was modified by supplementing with 3% sucrose, 1.136 mM ascorbic acid, 4.5 μM dicamba and 0.2 M sorbitol used for induction of SSEs (secondary somatic embryo induction medium (SSEIM). The culture medium was adjusted to pH 5.7 before autoclaving at 1.07 kg/cm$^2$ at 121$^o$C for 15 min. The cultures were maintained under 10 μmol/m$^2$/sec illuminations, 14 h photoperiod at 27±1$^o$C.

### *Effect of sizes of HE on SSE formation*

HEs induced from embryogenic callus in ECIM were divided into seven sizes ranging from 2-13 mm. The experiment was performed with four replicates. Each replicate consisted of 20 HE. The cultures were carried out in 25x150 mm glass test tube containing 10 ml of SSEIM and maintained under the above culture conditions. After culture for 4 months percentage and number of SSE were recorded.

### *Histological study*

SSEs developed from HE were collected, fixed in FAA (formalin: glacial acetic acid: 70 % ethanol 5:5:90 v/v/v), dehydrated using an ethanol-tertiary butanol series for 48 h and embedded in paraffin. Embedded tissue were sectioned at 6-8 µm using rotary microtome and mouthed on glass microscopic slides. Paraffin was removed in a xylene-ethanol series; tissues were stained with saffranin and fast green. All section were mouthed with Permount and viewed under bright field illumination on an inverted microscope.

## Results

### *Effect of sizes of HE on SSE formation*

After culture for 1 to 2 months first globular structure could be observed from the basal part of HE. They were creamy to pale yellow color and synchronous (Figure 1). For Thepa clone HE at size of 13 mm gave the highest number of SSE at 45SSEs /HE whereas HE at size of 9 mm from Krabi clone gave the number of SSE at 43 SSEs/HE (Figure 4). In case of HE derived from IZE of hybrid DxP, the number of SSEs was far lower than those obtained from young leaves. Between the two cross of hybrid cross number 16 gave the higher SSE formation at 18 SSEs/HE. Zygotic embryo-derived SSEs were creamy to green color (Figure 2).

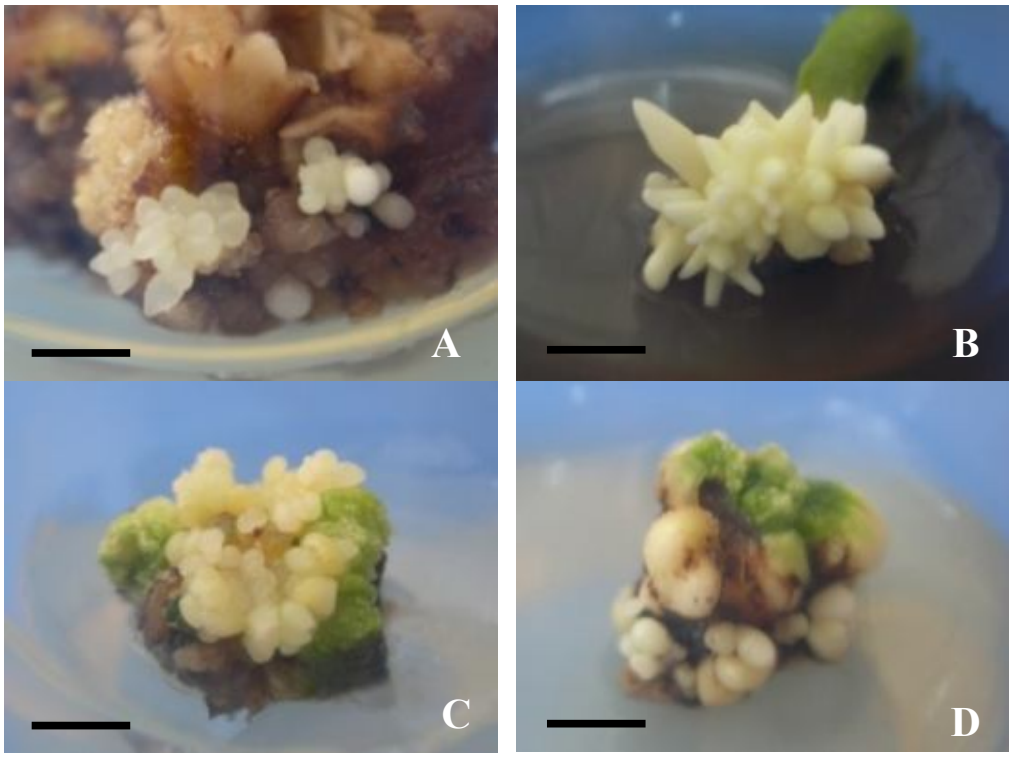
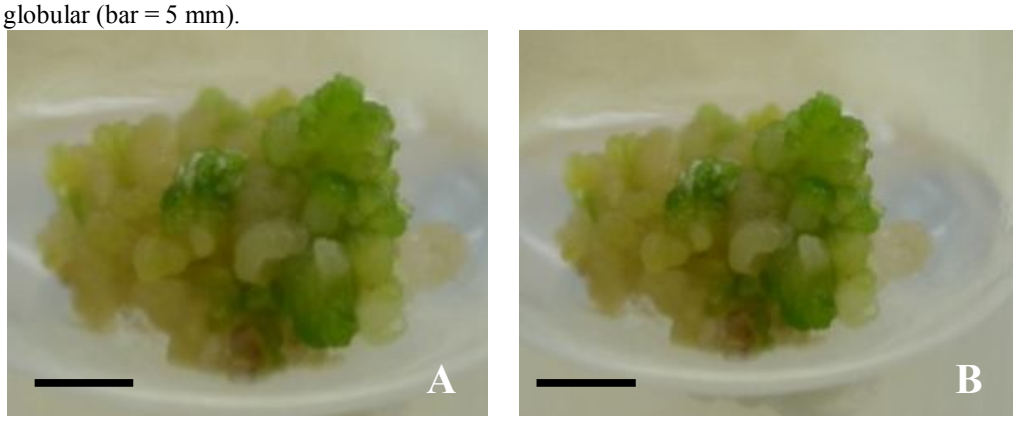



**Fig. 1.** Morphological characteristics of 2-month-old SSEs derived from culturing HE of young leave oil palm on MS medium supplemented with 0.2 M sorbitol, 1.136 mM ascorbic acid. (A) Clear torpedo. (B) Opaque torpedo. (C) Clear white globular. (D) Opaque white globular (bar = 5 mm).



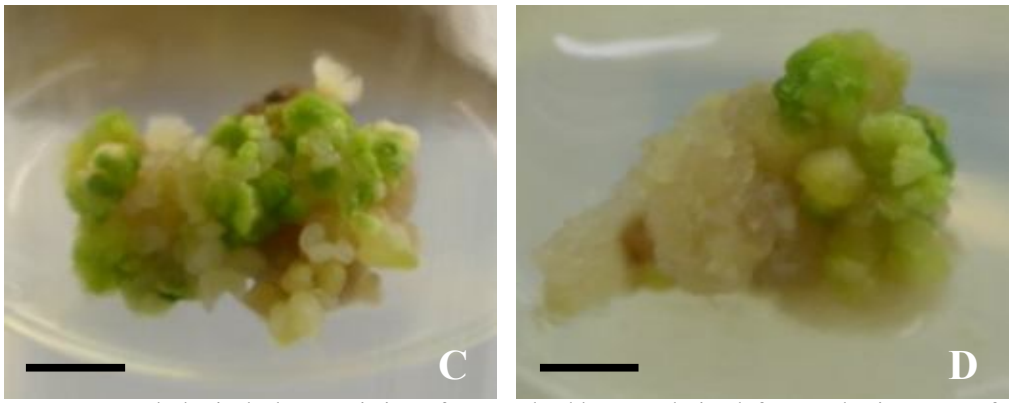



**Fig. 2.** Morphological characteristics of 2-month-old SSEs derived from culturing IZE of hybrid oil palm on MS medium supplemented with 0.2 M sorbitol, 1.136 mM ascorbic acid. (A) SSEs derived from HE of cross number 16 (bar = 0.5 mm). (B) HEs derived from GE of cross number 7 (bar = 0.3 mm). (C) SSEs derived from HE of cross number 16 (bar = 0.5 mm). (D) HEs derived from GE of cross number 16 (bar = 0.3 mm).

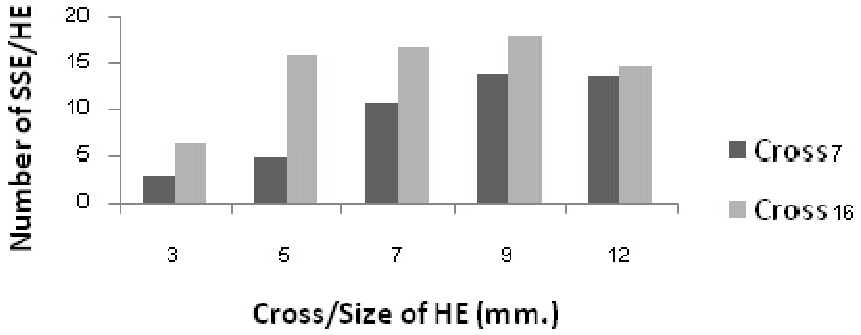



**Fig. 3.** Effect of genotype (cross number of hybrids oil palm) and sizes of HE on SSE formation on MS medium supplemented with 0.2 M sorbitol, 1.136 mM ascorbic acid after 2 months of culture.

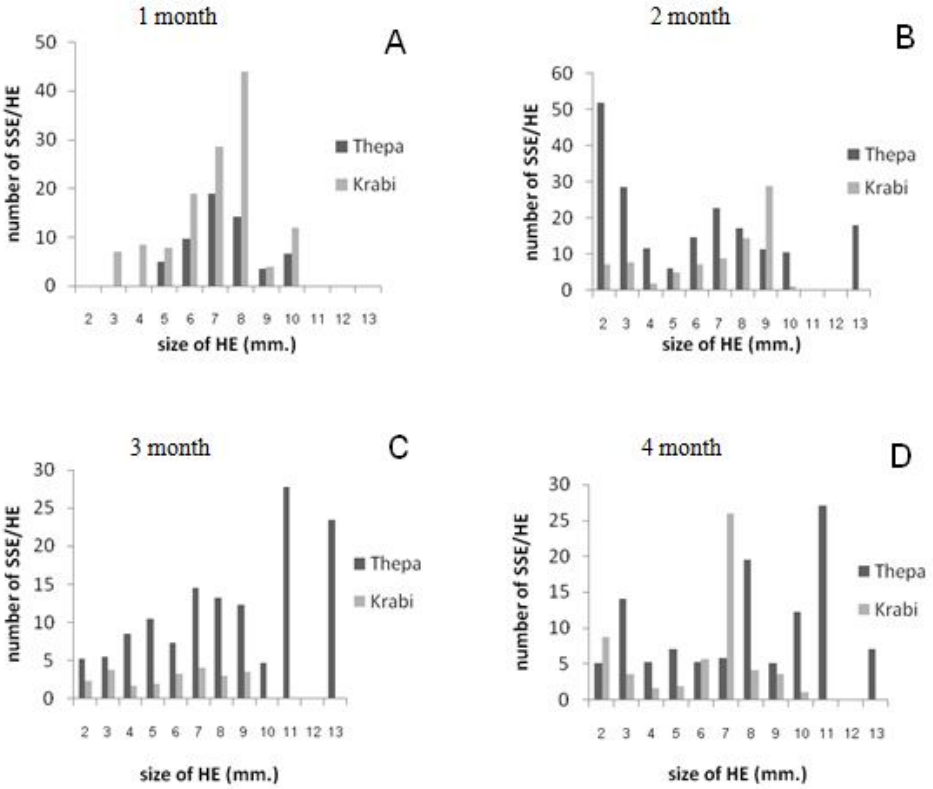



**Fig. 4.** Effect of source young leave and sizes of HE on SSE formation on MS medium supplemented with 0.2 M sorbitol, 1.136 mM ascorbic acid after 4 months of culture.

## *Histological study*

Histological studies revealed that SSE induced from HE on MS medium supplemented with 0.2 M sorbitol under light condition at 27±1°C developed from 3 origins; epidermal, parenchymatous and procambium or vascular cambium layer at the basal part of HE (Figure 5). Each cell in those layers was classified into embryogenic cell due to isodiametric, dense cytoplasm and having a large nucleus. Under certain conditions these embryo consisted almost entirely of meristematic cells which developed into embryogenic clumps.

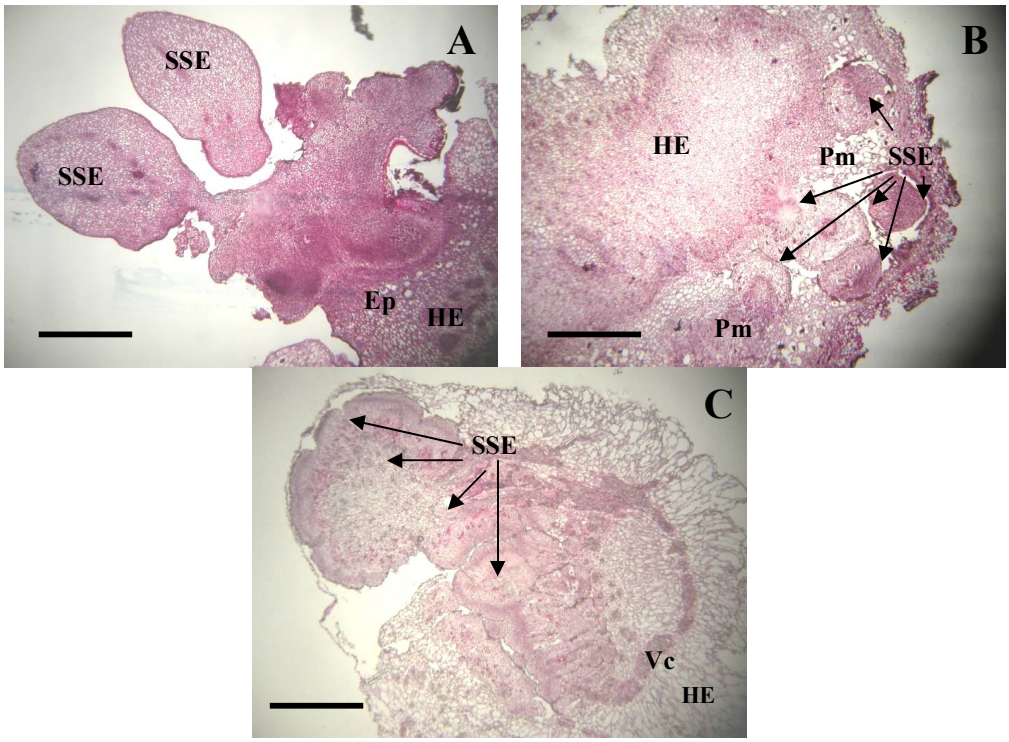



**Fig. 5.** Histological studies of SSE induced on MS medium supplemented with 0.2 M sorbitol under light condition at 27±1°C after 4 months of culture; epidermal (Ep) (A), parenchymatous (Pm) (B) and procambium or vascular cambium layers (Vc) (C). (bar = 1 mm)

## Discussion

Secondary somatic embryogenesis is a process whereby new somatic embryos are initiated from originally formed somatic embryos or primary somatic embryos. As an experimental system it has certain advantages compared to primary somatic embryogenesis such as very high multiplication rate, independence of an explant source and repeatability. Additionally, embryogenicity can be maintained for long period of time by repeated cycles of secondary embryogenesis. Furthermore, in many species the efficiency of explants in primary embryogenesis is lower than in secondary embryogenesis. This phenomenon has been described in at least 80 Gymnosperm and Angiosperm species (Raemakers *et al*., 1995).

This study has shown that oil palm can produce secondary somatic embryo from HEs, especially, the larger size (Te-chato *et al*., 2004). In some case, it is possible to use small size of HEs to induce secondary somatic embryo, however, small sizes (<5 cm) of HE were not reached full maturation and improper for induction secondary somatic embryo by exogenous hormonal

conditions. Independent of the size of cultured somatic embryos was demonstrated. A similar effect of somatic embryo size was also reported by Raj Bhansali *et al.* (1990) in peach and nectarine and Daigny *et al*. (1996) in *Malus* × *domestica* Borkh. (cv 'Gloster).

In case of histological study in SSE formation, it was reported to be induced directly from SE (Eudes *et al*., 2003) or indirectly from cotyledon of SE (Silva *et al*., 2005). In monocot plant such as *Alyssum borzaeanum*, SSE produced from different development pathway is observed on media containing NOA and BAP, simultaneously with the indirect secondary somatic embryogenesis involving the epidermal cells. Some epidermal cells, after several periclinally divisions, form an apical meristem-like structure, which develops shoots directly, without undergoing the embryonic stages, along a developmental route corresponding to a direct organogenesis pathway. The different morphogenetic response shown by the competent epidermal cells under apparently identical external conditions suggests that the endogenous regulation of organogenesis and embryogenesis pathways is interrelated (Păunescu, 2008). In oil palm, Te-chato *et al.* (2003) reported that SE was induced from both epidermal and parenchymatous cells of callus in dicamba containing medium, however, SSE induced from HE in this present study was from three origins. The different result might be due to the different between organelle (HE) and unorganize tissue (callus). Promchan and Te-chato (2007) reported that some single cell underwent proliferation rapidly to form the cambium-like zone, leading to protuberance of nodular structure. Under certain conditions these embryo consisted almost entirely of meristematic cells which developed into clumps of true SE.

## Conclusion

Sizes of HE played an important role in SSE production. HEs from embryogenic callus in various clone of oil palm could be induced SSEs on MS medium supplemented with 0.2 M sorbitol and 1.136 mM ascorbic acid. For Thepa clone HE at size of 13 mm gave the highest number of SSE/HE formation at 45 SSEs/HE whereas HE at size of 8 mm from Krabi clone gave the highest result at 43 SSEs/HE. In case of IZE cross 16, a maximum number of SSE at 18 /HE were obtained from HE at size of 9 mm which lower than those obtained from young leaves. Histological study revealed that SSE developed from 3 origins; epidermis, parenchyma and procambium or vascular cambium. Those cells were isodiametric, rich of cytoplasm with the large nucleus. The cells divided rapidly to form a cluster of globular embryos and passed through torpedo embryos.

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