# ผลของผงถ่านและอาหารเหลวต่อการงอกและการพัฒนาเป็นพืชต้นใหม่จากโซมาติกเอ็มบริโอชุดที่สองของปาล์มน้ำมัน

# Effects of activated charcoal and liquid culture media on germination and plant regeneration from secondary somatic embryo of oil palm *(Elaeis guineensis* Jacq.)

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**บทคัดย่อ**: โซมาติกเอ็มบริโอของคู่ผสมที่ 7 และ 16 ในระยะสุดท้ายที่เรียกว่า haustorium embryos (HEs) วางเลี้ยงบนอาหารแข็งสูตร MS เติม ซอบิทอล 0.2 โมลาร์ และกรดแอสคอร์บิค 200 มก./ล. เพื่อชักนำโซมาติกเอ็มบริโอชุดที่สอง (secondary somatic embryo: SSE) จากนั้นย้าย SSE ของทั้งสองคู่ผสมไปเลี้ยงบนอาหาร 6 สูตร ดังนี้ 1-2) อาหารแข็งสูตร MS เติมซูโครส 3 % กรดแอสคอร์บิค 200 มก./ล. เติมหรือไม่เติมผงถ่าน 0.2 % 3-4) อาหารสูตร MS เติมซูโครส 3 % กรดแอสคอร์บิค 200 มก./ล. และเติมอาหารเหลวสูตร MS เติม benzylaminopurine (BA) 0.03 มก./ล. และ naphthalene acetic acid (NAA) 0.06 มก./ล. เติมหรือไม่เติมผงถ่าน 0.2% ปริมาตร 5 มล. และ 5-6) อาหารเหลวสูตร MS เติม BA 0.03 มก./ล. และ NAA 0.06 มก./ล. เติมหรือไม่เติมผงถ่าน 0.2% จากผลการทดลองพบว่า คู่ผสมที่ 7 งอกได้ดีบนอาหารแข็งสูตร MS ที่เติมอาหารเหลว 5 มล. ที่ไม่เติมผงถ่าน ในขณะที่คู่ผสมที่ 16 งอกได้ดีในอาหารเหลวสูตร MS ที่ไม่เติมผง ส่วนการพัฒนาเป็นพืชต้นใหม่พบว่า อาหารเหลวสูตร MS ที่ไม่เติมผงถ่านให้การสร้างยอดและการพัฒนาเป็นพืชต้นใหม่สูงสุดทั้งสองคู่ผสม

**คำสำคัญ**: ผงถ่าน, อาหารเหลว, การงอก, การพัฒนาเป็นพืชต้นใหม่, ปาล์มน้ำมัน

**ABSTRACT:** Somatic embryos (SEs) of cross number 7 and 16 at the final stages, called, haustorium embryos (HEs) were cultured on MS medium supplemented with 0.2 M sorbitol and 200 mg/l ascorbic acid for inducing secondary somatic embryo (SSE). SSEs of both crosses were transferred to six culture media could be distinguished: 1-2) solidifies MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% activated charcoal (AC), 3-4) solidifies MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and overly with 5 ml of liquid MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA with or without 0.2% AC and 5-6) liquid MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA, 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% AC. The results revealed that cross number 7 gave the highest germination of SSE were obtained from solidifies MS medium without AC, whereas were cross number 16 gave the highest germination of SSE were obtained from liquid MS medium. For plant regeneration, the results revealed that liquid MS medium without AC gave the highest number of shoot and complete plantlet formation of both crosses.

**Keywords:** Activated Charcoal, Liquid Culture Media, Germination, Plant Regeneration, Oil Palm

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## Introduction

Oil palm (*Elaeis guineensis* Jacq.) is three part of important in terms such as economic, industrial and biofuel. An oil palm tree is very important in terms of monetary value. In its productive life time of more than twenty years in the field, a palm produces about 150 kg (10 bunches x 15 kg) of fresh fruit bunches (FFB) per year and 3 tonnes of FFB over a twenty year period. The oil palm is also a crop species producing high quality oil, which can be obtained from the mesocarp of the fruit (palm oil) and the kernel of the nut (palm kernel oil). Interest in palm oil as a biofuel could eventually cause constraints on worldwide supply of edible palm oil and increase the pressure for higher yield and/or cultivatable areas (Biofuel. 2007). Accordingly a high yield plant is needed for fuel oil/biodiesel production. Indeed, the large amount of oil produced in the oil palm fruit is unique biological characteristic of this palm species. Plant regeneration of oil palm through *in vitro* culture has been reported by several researchers (Te-chato, 1998a). The oil palm has only a single growing point, and does not produce suckers like some other palm species, so clones cannot be produced by the common techniques such as cutting, grafting or layering (Corley and Tinker, 2003). So it is possible to enhance efficiency for propagation through somatic embryogenesis, especially *in vitro* culture through zygotic embryo (ZE) culture and also embryo explants are convenient because fruits are readily available, have a high degree of physiological uniformity, and can be shipped long distances. To ensure that the parents used for commercial hybrid seed production are not related, oil palm breeders rely on pedigrees (Te-chato, S. 1998b)

Somatic embryogenesis is the process whereby either a single somatic cell or clusters of cells develop into differentiated plants through characteristic embryological stages without fusion of gametes. Somatic embryos (SEs) can differentiate either indirectly from callus or directly from cells of an organized structure, without an intervening callus phase (Williamsand and Maheswaran, 1986; Uzelac *et al*., 2007). Various stages of ZE and genotypes of embryos were reported to be success by inducing somatic embryogenesis (Chehmalee and Te-chato, 2008). Regeneration of oil palm through secondary somatic embryos (SSEs) has also been reported using polyamines (Rajesh *et al*., 2003). However, percentage and number of new forming embryos were limited and germination of those embryos was not reported. Sorbitol were reported to induce SSE from haustorium embryo (HE) derived from culturing young leaf and ZE of oil palm subsequent to a high frequency of plantlet formation (Te-chato and Hilae, 2007). So far, there have no reports in the part of effects of activated charcoal and liquid culture media on germination and plant regeneration from SSEs. This project was to find that and the best way to increase the germination and plant regeneration of oil palm.

## Materials and Methods

SSE from HE-staged embryos at 2 months of cross number 7 and 16 after culture on MS medium supplemented with 0.2 M sorbitol and 200 mg/l ascorbic acid (AS) were transferred to germination medium, six culture media could be

distinguished: Hormone-free MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% activated charcoal (AC) (**Figure 1A, B**), Hormone-free MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and overly with 5 ml of MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA with or without 0.2% AC (**Figure 1C, D**) and liquid MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA, 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% AC (**Figure 1E, F**).

All cultures were carried out in bottle (60×110 mm containing 25 ml of medium) under the same conditions as described earlier. Factorial in completely randomized design (CRD) with 3 replicates (each containing 10 SSEs/replicate) was designed. Germination percentage in terms of shoot, root and complete plantlet production was recorded after 3 months of culture.

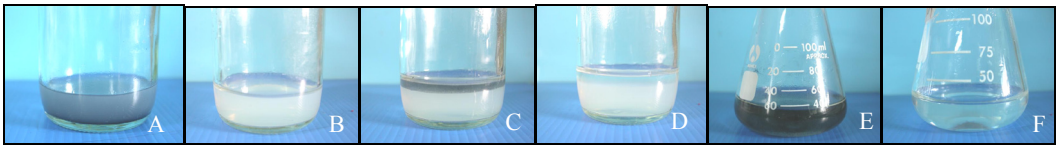


**Figure 1** Characteristics of culture media and culture method for germination of SSEs. (A,B) Hormone-free MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% AC. (C,D) Hormone-free MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and overly with MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA with or without 0.2% AC and (E,F) liquid MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA, 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% AC.

## Results and Discussion

SSEs were cultured on solid MS and overlay with 5 ml of liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA without AC and liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA gave the highest germination of SSE at 17.1 and 17 per explant, respectively. Similar result of Corley and Tinker (2003) reported that SE of oil palm gave the good way germination on liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA or hormone-free solid MS medium overlay with the same culture medium describe this above (Promchan et al., 2012). However germination of SSE depends on the culture media and genotype of oil palm. Cross number 16 gave the better germination of SSE than cross number 7 at 13.87 and 12.51, respectively (**Table 3**).

**Table 1** Effect of AC and liquid culture media on germination of SSE of oil palm after culture for 3 months.

| Cross No. / Culture media | Germination of SSE (SSE/explant) | | Average[2] culture media |
|---|---|---|---|
| | 7 | 16 | |
| 1 | 14.80bc[1/] | 16.40ab | 15.60A |
| 2 | 11.07d | 11.40d | 11.23B |
| 3 | 9.20e | 14.40c | 11.80B |
| 4 | 17.80a | 16.40ab | 17.10A |
| 5 | 5.60f | 7.20f | 6.40C |
| 6 | 16.60ab | 17.40a | 17.00A |
| Average[1] cross No. | 12.51B | 13.87A | ** |
| | | | C.V.(%) 21.74 |

** = Significant difference at P≤0.01 level.

[1/] = Value followed by different letter are significantly different according to DMRT

1: Solid MS medium without AC

2: Solid MS medium with 0.2% AC

3: Solid MS medium overlay with liquid MS medium supplemented with 0.06 mg/l NAA, 0.03 mg/l BA and 0.2% AC

4: Solid MS medium overlay with liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA without AC

5: Liquid MS medium supplemented with 0.06 mg/l NAA, 0.03 mg/l BA and 0.2% AC

6: Liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA without AC

For plant regeneration, SSE were cultured on solidifies MS medium overlay with liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA without AC gave the highest average number of shoot at 14.8 shoot/explant obtain from cross number 7 and 15 shoot/explant obtain from cross number 16 significant different with another method. However solidifies MS medium gave the highest number of complete plantlet of both crosses at 3.4 and 4.8 complete plantlet/explant, respectively (**Table 2** and **Figure 3**). That complete plantlet could be readily excised and transferred to soil. In case of embryos that germinated only shoots, it was necessary to induce root before transferring to soil. The present study successfully describes to find the effect of AC and liquid culture media for germination of SSE of oil palm via somatic embryogenesis. This result showed that AC and liquid culture media may have either beneficial or harmful effects on the culture, depending upon the medium, and tissue used like (Pan and Staden, 1999). We found that different genotype gave the different response germination and plantlets regeneration. Moreover, in our previous study, the larger seeds consisted of larger size of ZE of all crosses gave the higher percentage of germination (Te-chato and Hilae, 2007). Development of plant regeneration from HE as shown in **figure 3**.

**Table 2** Effect of AC and liquid MS medium on number of shoot, root and complete plantlet of SSE of oil palm after culture on germination medium for 3 months.

| Cross No. | Culture Media | No. of shoot | No. of Root | No. of Complete plantlet |
|---|---|---|---|---|
| 7 | 1 | 9.8[1/] | 1.6bc | 3.4b |
| | 2 | 7.2bc | 1.8ab | 2.0c |
| | 3 | 5.6c | 1.8ab | 1.8cd |
| | 4 | 14.8a | 1.6bc | 0.6de |
| | 5 | 5.8c | 0.0e | 0.0e |
| | 6 | 14.8a | 0.8cde | 1.0cde |
| 16 | 1 | 9.0b | 2.6a | 4.8a |
| | 2 | 7.8bc | 1.6bc | 2.0c |
| | 3 | 10.0b | 1.6bc | 2.0c |
| | 4 | 14.6a | 0.6de | 1.4cd |
| | 5 | 7.0bc | 0.0e | 0.0e |
| | 6 | 15.0a | 1.2bcd | 1.2cde |
| F-test | | ** | ** | ** |
| C.V.(%) | | 25.49 | 12.83 | 20.63 |

** = Significant difference at P≤0.01 level.

[1/] = Value followed by different letter are significantly different according to DMRT

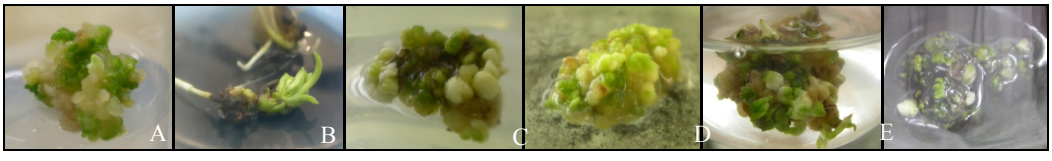


**Figure 2** Germination of SSEs on different culture media and culture method for 3 months. (A,B) Hormone-free MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% AC. (C,D) Hormone-free MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and overly with MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA with or without 0.2% AC and (E,F) liquid MS medium supplemented with 0.03 mg/l BA and 0.06 mg/l NAA, 3% sucrose, 200 mg/l ascorbic acid with or without 0.2% AC.

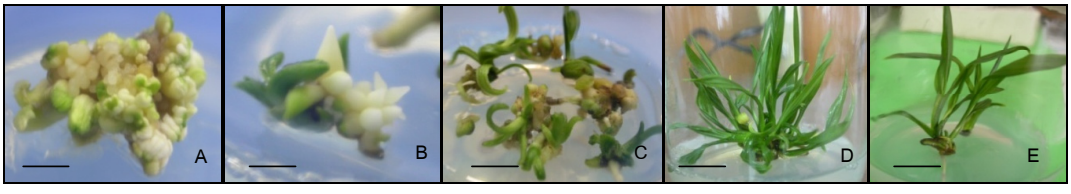


**Figure 3** Development of plant regeneration from HE (A) on 36.436 mg/l sorbitol containing MS medium for 1 month of culture (bar: 3 mm) subsequent to germination of SSE (B) on hormone-free MS medium (bar: 6 mm) for further 3 months. Multiple shoot (C,D). Complete plantlet (E).

## Conclusions

SSEs were cultured on solid MS and overlay with liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA without AC and liquid MS medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA gave the highest germination of SSE. Whereas solidifies MS medium gave the highest complete plantlet of both crosses. AC is improper for germination of SSE of oil palm and different genotype gave the different response on SSE formation and plantlets regeneration.

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