Embryogenic callus initiation from leaf explants of *Elaeis oleifera* x *Elaeis guineensis* (OxG) hybrids

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**Abstract.** *Elaeis oleifera* x *Elaeis guineensis* (OxG) hybrid is an oil palm interspecific crossbreed to produce a variety with better oil quality, slow stem growth, and resistance to bud rot disease. However, one of the problems in OxG hybrid breeding program is an inefficiency in the conventional propagation method, while the protocol for OxG hybrid tissue culture has not been established yet in Indonesia. Somatic embryogenesis (SE) using a temporary immersion system (TIS) will be used for OxG clonal propagation as it has been established successfully in *E. guineensis*. Explants were young leaves derived from genotypes of selected F1 and backcross (BC) of OxG hybrids where the *E. oleifera* was originated in Brazil and Suriname. Callus initiation used eight media with various compositions of minerals, hormones, and active charcoal. Initial callus emerged in 8-10 weeks after culture. The average of callogenesis frequency was 3.49% in F1 and 5.59% in BC hybrid explants, with the highest at 10.73% occurred in BC Brazil. Callogenesis of OxG hybrid showed a high potency as callogenesis of *E. guineensis*. The callus had nodular structure which was potentially embryogenic. After 3 cultures, some of the embryogenic calli were transferred to TIS for somatic embryo induction.

1. Introduction

Oil palm belongs to the Arecaceae family and is the largest and the most efficient source of vegetable oil in the world. Palm oil is mainly used as an edible product, but also has many other uses such as non-food derivatives, oleochemicals, and biofuels. The demand for palm oil has increased considerably especially due to the increasing demand for biofuels and for edible oils especially in Asia [1]. In order to meet this demand, the oil palm plantation areas in many oil palm producing countries, including Indonesia are expanding rapidly.

There are two commercial oil palms, African oil palm (*Elaeis guineensis* Jacq.) and American oil palm (*Elaeis oleifera* HBK Cortes). Due to its higher yield, African oil palm, *E. guineensis*, is overwhelmingly used for commercial cultivation. The fast expansion of *E. guineensis* as monoculture plantations in very large areas is dangerously fragile to climate changes and diseases attacks; therefore crossbreed with *E. oleifera* that has useful genetic traits is very important in oil palm breeding program. *E. oleifera* has a low oil yield, but it has valuable characters such as a low shoot growth rate, a high content of unsaturated fatty acids, substantial levels of carotenes and vitamin E, and more...
resistance to bud rot disease. An interspecific crossbreed of *E. oleifera* and *E. guineensis* (OxG) is intended to combine both valuable traits of the parents and its backcross (BC) with *E. guineensis* is expected to maintain higher yields.

OxG hybrids have been developed mostly in the Latin American countries such as Ecuador, Colombia and Brazil [2] to overcome yellowing disease which *E. guineensis* is susceptible while *E. oleifera* is resistance but with lower oil yield. OxG hybrids have also been developed in Southeast Asian countries for slower growth and better oil quality. The physiological and morphological characteristics of *E. oleifera* and their hybrids (OxG) have been determined to select the best female parents of OxG hybrids for breeding program purposes and the best hybrids for agronomic performance [3]. The best progenies of OxG hybrids and BC should be clonal propagated to maintain their characteristics. These OxG hybrids have a problem regarding the development of embryos, resulting occasionally in seed abortion. Thus, propagation of OxG hybrids by seeds is limited. Since oil palm belongs to monocotyledonous plant and does not produce any suckers, its clonal propagation can be conducted only by tissue culture.

*In vitro* propagation of OxG hybrids through somatic embryogenesis (SE) has been reported mostly using zygotic embryos as explants [4, 5, 6], but SE in OxG hybrids from young leaf explants has been reported only by Ginting et al. [7] using one OxG hybrid on solid media. However, SE with young leaves as explants has been widely used in *E. guineensis* [8]. Furthermore, SE using a temporary immersion system (TIS) was reported to improve somatic embryo production and uniformity [9] and decrease floral abnormality in *E. guineensis* [10]. Therefore, in this research SE-TIS was applied for clonal propagation of F1 and BC of OxG hybrids developed by the Indonesian Oil Palm Research Institute (IOPRI) where the *E. oleifera* was originated from Brazil and Suriname. At the present time, we report the progress of the callus initiation and embryogenic calli formation from young leaf explants of OxG hybrids.

2. Materials and Methods

2.1. Plant material and culture conditions

The research was conducted at the Laboratory of Plant Cell Culture and Micropropagation, the Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), Bogor, West Java. Explants used were young leaves of selected genotypes of F1 and BC of OxG hybrids that have been developed by IOPRI since the 1970’s where the *E. oleifera* plants were originated from Brazil and Suriname. The OxG genotypes were planted at Marihat and Bah Jambi experimental gardens, Pemantang Siantar, North Sumatra, Indonesia. The explant sources were selected from the best progenies of F1 and BC OxG hybrids of Brazil and Suriname origins. Selection of the progenies was based on fruit productivity, oil quality and vegetative growth. The explants were cut from mature trees, cleaned, washed with alcohol, packed, and sent to Bogor by air. Young leaves used were from leaf phyllotaxis number from -3 to -8. Approximately 3000 explant cuts could be generated from one young leaf explant of oil palm.

All the cultures, otherwise stated, were placed in a dark culture room at a constant temperature of 26±2 °C.

2.2. Treatments and observation

Every leaf explant cut (1 cm x 2 cm) having a mid-vein with two blades was placed on a small culture tube (diameter 2.5 cm, height 15 cm) containing a solid medium with various compositions of minerals, hormones, and active charcoal. Eight different media (M1 to M8) were used as treatments for callus initiation of selected genotypes of F1 and BC of OxG hybrids from Brazil and Suriname (F1 Brazil, F1 Suriname, BC Brazil, BC Suriname). Each genotype consisted of at least 90 explant cuts (tubes) of each medium treatment. The pH of the medium was adjusted to 5.8 before autoclaved at 121 °C and 1.0 kg/cm² for 20 min.

The observation was conducted every 2 weeks in the first two months on contamination and callus emergence, and then every month until 8 months on callus formation. The cultures without callus
formation were discarded after 8 months of culture. Primary calli that have been formed were transferred to solid media with a reduced concentration of auxin for callus proliferation and differentiation. After 3 subcultures, the embryogenic calli were transferred to liquid culture in a temporary immersion system (TIS) using 250 mL Nalgene flasks for somatic embryo induction. The embryogenic calli were immersed for 3 min every 6 h [10]. The cultures were incubated in the culture room at 10 μmol/m²/s light intensity over a 12-h photoperiod.

3. Results and Discussion

3.1. Callus initiation

Leaf explants folded along the vein and became green brownish. Initial calli emerged in 8 weeks, quicker than those of *E. guineensis* where usually emerged in 10-12 weeks [8, 11]. The primary callus together with its leaf explant was sub cultured to a new media after 4, 6, and 8 months of culture. The callus was white brownish in colour and its texture was mostly soft and watery (Figure 1).

The average of callogenesis was 3.49% in F1 and 5.59% in BC hybrid explants on eight different media, after 8 months of culture. BC Brazil explants had the highest callogenesis frequency on average at 10.73%, followed by F1 Suriname at 5.41%, F1 Brazil at 1.73% and BC Suriname at 0.39% (Table 1). Hence, the best media for callogenesis of OxG hybrids were M6 and M3 at 8.87% and 8.11%, respectively. Leaf explants of F1 Brazil and BC Suriname cultured on several media did not form any calli at all. On the contrary, leaf explants of BC Brazil on M6 and M3 produced callus at the frequency of 18.89% and 18.21%, respectively. M6 media also gave the high frequency of callus initiation on F1 Suriname at 16.23%. This callogenesis frequency of OxG hybrid was comparable to that of *E. guineensis* at 15% [8] or 11-20% [12] and another OxG hybrid at 11.25% [7]. Callogenesis frequency of OxG hybrids using zygotic embryos was very high at 77.9-95.3% after 90 days on Brazilian OxG hybrids [4] and at 10-60% after 6 months on other Brazilian OxG hybrids [6]. Activated charcoal at 0.25% was used to reduce oxidation in the culture of zygotic embryos of OxG hybrids [13].

Callogenesis levels can be improved by selecting oil palms as a source of explants (ortets) that are predicted by molecular markers to be amenable to tissue culture process. Gene expression markers for callogenesis of oil palm have been identified to be associated with cytokinin and brassinosteroid such as putative cytokinin dehydrogenase (*EgCKX*), brassinosteroid responsive gene (*EgBrRK*), and putative response regulator type A gene (*EgRR1*) [14]. These markers were significantly correlated to callogenesis rates of oil palm, therefore can be used as an early assessment of the callogenesis potential of oil palms [14].

| Culture media | F1 Brazil | F1 Suriname | BC Brazil | BC Suriname | Average |
|---------------|-----------|-------------|-----------|-------------|---------|
| M1            | 3.94      | 7.34        | 14.68     | 0.34        | 6.58    |
| M2            | 4.52      | 1.59        | 7.25      | 0.00        | 3.34    |
| M3            | 3.04      | 9.17        | 18.21     | 2.00        | 8.11    |
| M4            | 0.00      | 1.56        | 6.06      | 0.00        | 1.90    |
| M5            | 0.68      | 0.96        | 1.52      | 0.00        | 0.79    |
| M6            | 0.35      | 16.23       | 18.89     | 0.00        | 8.87    |
| M7            | 0.00      | 1.05        | 6.96      | 0.34        | 2.09    |
| M8            | 0.41      | 4.92        | 12.81     | 0.33        | 4.62    |
| Average       | 1.73      | 5.41        | 10.73     | 0.39        | 4.54    |
3.2. Primary callus culture

The primary calli formed after 4 months, 6 months and 8 months were transferred to solid media containing a lower concentration of auxin. After 3 cultures, there was no significant increase in the callus biomass in term of the number of callus clumps, but the callus was growing bigger. Some calli were contaminated or dead, reducing the number of callus clumps. After 8 months, OxG hybrid BC Brazil had 308 callus clumps (culture tubes), F1 Suriname had 111 clumps, F1 Brazil had 37 clumps, and BC Suriname had only 7 callus clumps (Table 2). Many of the calli become more friable (Figure 2A) and some calli turned to embryogenic and nodular in structure (Figure 2B) after being subcultured several times.

The calli with nodular structure (Figure 2B) were very potential to form somatic embryos. These calli also grew relatively fast. Ginting et al. [7] restricted to use only calli formed within the first 5 months of OxG hybrid culture. Primary callus culture was conducted for 4 months to form somatic embryos. However, only a limited number of somatic embryos were obtained [7].

Table 2. Number of callus clumps of OxG hybrids on different culture media after 8 months.

| Culture media | F1 Brazil | F1 Suriname | BC Brazil | BC Suriname | Total |
|---------------|-----------|-------------|-----------|-------------|-------|
| M1            | 12        | 17          | 54        | 0           | 83    |
| M2            | 13        | 5           | 26        | 0           | 44    |
| M3            | 8         | 26          | 63        | 5           | 102   |
| M4            | 0         | 4           | 25        | 0           | 29    |
| M5            | 2         | 2           | 3         | 0           | 7     |
| M6            | 1         | 47          | 71        | 0           | 119   |
| M7            | 0         | 2           | 23        | 1           | 26    |
| M8            | 1         | 8           | 43        | 1           | 53    |
| Total         | 37        | 111         | 308       | 7           | 463   |
Figure 2. Embryogenic calli of OxG hybrid after the second (A) and the third (B) primary callus culture. Bar = 1 cm.

3.3. Somatic embryo induction in TIS
After 4 cultures, the embryogenic calli were transferred to TIS. Somatic embryo induction, maturation, and germination will be conducted in TIS. Liquid culture using TIS has been utilised successfully in *E. guineensis* [9, 10] but has not been applied either in *E. oleifera* or OxG hybrids. In *E. guineensis*, SE using TIS was stated to improve somatic embryo production and uniformity [9] and to decrease floral abnormality [10]. The embryogenic calli were in TIS for the first passage and have not been converted to somatic embryos yet.

Figure 3. Induction of somatic embryos in TIS. Bar = 1 cm.

4. Conclusions
Calli have been successfully initiated from F1 and BC of OxG hybrids of oil palm. Different OxG hybrids responded differently to different media of callus initiation. The best calllogenesis was BC OxG hybrid Brazil at 18.9% on M6 medium and at 18.2% on M3 medium.

After being sub cultured on solid media with a lower concentration of auxin, the primary callus has become embryogenic and nodular in structure. These embryogenic calli have been transferred into a liquid culture of TIS. The embryogenic calli on TIS have not produced somatic embryos yet.
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6. References

[1] Murphy D J 2014 J. Oil Palm Res. 26 1
[2] Kushairi A, Tarmizi A H, Zamzuri I, Ong-Abdullah M, Samsul K R, Ooi S E and Rajanaidu N 2010 International Seminar on Advances in Oil Palm Tissue Culture, Yogyakarta, Indonesia p 1
[3] Rivera Y D. Cayón D G and López J E 2013 Agro. Colombiana 31 314
[4] Silva Angelo P C, Lopes R, Moraes L A C and da Cunha R N V 2009 Crop Breed. Appl. Biotechnol. 9 274
[5] Silva Angelo P C, Steinmacher D A, Lopes R, da Cunha R N V and Guerra M P 2013 Agric. Sci. 4 1
[6] Bonetti K A P, Nesi J, Quisen R C and Quoirin M 2016 African J. Biotechnol. 15 2028
[7] Ginting G, Fatmawati and Purba A R 1994 Bulletin PPKS 2 17
[8] Soh A C, Wong G, Tan C C, Chew P S, Chong S P, Ho Y W, Wong C K, Choo C N, Nor Azura H and Kumar K 2011 J. Oil Palm Res. 23 935
[9] Tahardi J S 1998 Proc. Internat. Oil Palm Conf. Bali: Indonesia p 595
[10] Sumaryono, Riyadi I, Kasi P D and Ginling G 2008 Indonesian J. Agric. 1 109
[11] Yusnita and Hapsoro D 2011 Hayati J. Biosci. 18 61
[12] Ho Y W, Tan C C, Soh A C, Wong G, Chong S P, Choo C N and Norazura A 2009 International J. Oil Palm 6 86
[13] Alves S A O, de Lemos O F, dos Santos Filho B G and da Silva A L L 2011 J. Biotechnol. Biodivers. 2 1
[14] Ooi S E, Choo C N, Zamzuri I and Ong-Abdullah M 2013 J. Oil Palm Res. 25 9