Oil palm (*Elaeis guineensis* Jacq.) micropropagation via somatic embryogenesis from female inflorescences explants

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**Abstract.** The oil palm (*Elaeis guineensis* Jacq.) is a perennial woody oil crop in the Arecaceae family. Oil palm is well known for a long regeneration time, therefore, in vitro propagation received great enthusiasm from oil palm industries. Somatic embryogenesis (SE) has become one of the most promising clonal propagation techniques in recent times. This study was aimed to determine a protocol for micropropagation somatic embryogenesis from female inflorescences explants of oil palm. The explant used was obtained from the female inflorescences of the oil palm 2.5 years old Tenera variety. The basal media used was Y3 with the addition of the hormone 2,4-D with different concentrations (33, 66, 99 and 132 mg/L). Callus initiation was formed in 99 mg/L and 132 mg/L 2,4-D concentration with the basal area and the percentage of callus formation is 31.25% and embryogenic callus was formed from primary callus development in the basal area of female florescence. Embryo somatic induction with cell suspension culture, because the liquid medium is more efficiently used in commercial-scale propagation. The embryogenic callus phase which is generally used in liquid medium is the nodular phase because it is still meristematic so that the potential for cell division is still high and can increase the percentage of embryogenic callus.

1. Introduction

The oil palm (*Elaeis guineensis* Jacq.) is a perennial woody oil crop and is a group of palm trees in the Arecaceae family [1,2]. The average annual production is as high as 4.27 tons per hectare Asia, Malaysia, Indonesia, West and Central Africa, South America, and North and Central America are the top producers of oil palm [2] and the leading producer and exporter of palm oil are Indonesia (more than 50 percent production and export) [3] if compared to other biofuel-producing plants such soybean, sunflower, and rapeseed, oil palm has stood out because of its high oil output [2,4-6].

Oil palm is well known for a long regeneration time of around 20 - 25 years [1] and in oil palm seed production, the problem of seed dormancy is still done conventionally. However, the latter is still constrained by factors such as the time it takes for seeds to germinate (up to 120 days) [7,8]. As a result, the oil palm industry has become quite enthusiastic about in vitro clonal propagation of seedlings [9]. As a result, SE has recently emerged as one of the most promising clonal propagation techniques, offering advantages such as large-scale multiplication of superior genotypes, accelerated
proliferation systems, and the ability to acquire a greater number of plantlets than other vegetative propagation methods [10,11]. In concept, if proper explants are employed, any plant species can develop somatic embryos in vitro if appropriate nutritional media, growth regulators, and culture conditions are provided. [11] Immature inflorescences were chosen as the main explants because of the high number of meristem flowers in inflorescence and they can be collected from parent plants without destroying to the mother plant [12,13]. Plant regeneration was achieved via somatic embryogenesis from immature female inflorescences of date palm (*Phoenix dactylifera* L.) cultivar Siwi (semi-dry cv.). So that, this study was aimed to identify a procedure for micropropagation somatic embryogenesis from oil palm female inflorescence explants.

2. Methods

Plant tissue culture techniques are of particular relevance for the collection, analysis, multiplication, and storage of germplasm, as well as the ability to produce a high number of true-to-type plants from elite genotypes in a relatively short period. Explants are kept in a sterile, pathogen-free environment throughout in vitro conservation [14,15]. Plant tissue culture has been employed as a primary platform for the generation of secondary metabolites due to its numerous advantages. Micropropagation, a technology for mass-producing propagules and plants, has satisfied industrial and other demands in a variety of situations and has been a useful tool in propagating medicinal and crop plants [16]. Somatic embryogenesis (SE) is the induction of somatic embryos (SE) from undifferentiated somatic cells into normal embryogenic cells and eventually into complete plants, this technique relies on the totipotential ability of somatic cells to be able to grow and develop into whole plant without gametic fusion process. The totipotent ability of somatic cells to generate a whole plant without gametic fusion allows for the induction of somatic embryos (SE) from the unicellular or multicellular origin [13,17,18]. Somatic embryogenesis has become a practical indication for the proliferation of oil palm elite genotypes. The production of somatic embryos can occur in two ways: directly or indirectly. In the first case, without progressing through the callus formation stage, determined cells that are already programmed for embryo differentiation acquire embryogenic competence and create somatic embryos [10]. The indirect somatic embryogenesis process consists of two differential steps they are: transition of an explant from primer callus to embryogenic callus and then the development of somatic embryo maturation (the globular, torpedo, and cotyledonary stages) [19].

In vitro propagation from monocotyledons, the source of explant used must contain meristematic cells, and the explant that best matches this requirement appears to be inflorescences because their tissues contain a large number of meristematic components [12] Immature inflorescences are a valuable source of explants for elite palm tree micropropagation [20].

3. Results and Discussion

The explant used was obtained from the female inflorescences of the oil palm (*Elaeis guineensis* Jacq.) 2.5-year-old Tenera variety. Sterilization technique in a laminar airflow hood under aseptic conditions. After gently opening the spathes with a sanitized knife, the spikelets were immersed in 0.1% mercuric chloride (HgCl$_2$) for 15 minutes. Inflorescence explants were washed three times in sterile distilled water. The basal media used was Y3 with the addition of the hormone 2,4-D with different concentrations (33, 66, 99, and 132 mg/L). Subcultures were performed on the same medium and growth conditions every 4 weeks.

3.1. Callus initiation

Callus began to form on the 62$^{nd}$ day after inoculation but there were some explants whose growth was very slow, namely on the 70$^{th}$ day after inoculation. This study showed that the given growth regulators influenced the time of callus formation, auxin 132 mg/L in Y3 was more effective in callus growth. High concentrations (100–150 mg/L) of 2,4-D and other plant growth regulators were employed in palm callus induction, especially during the early stages of multiplication, more
Specifically at the callus induction stage [21]. Callus was formed in 99 mg/L and 132 mg/L 2,4 – D concentration with the basal area and the percentage of callus formation is 31.25%. Auxin and cytokinin, two exogenously growth regulators, have a significant impact on callus development, either alone or in combination [22]. There is an interplay between exogenously applied auxins and endogenous hormones, most likely cytokinins, that leads to a condition of equilibrium that favors callus production [23].

3.2. Embryogenic Callus Initiation

Callus formed on day 134 after inoculation in solid medium, embryogenic callus was formed from primary callus development in the basal area of female florescence which had previously been subcultured using the same medium. Correlation between the formation of Embryogenic callus with a combination of 2,4 - D can be seen in Figure 1.

![Figure 1. Correlation between the formation of embryogenic callus with a combination of 2,4 – D the percentage of cultures that formed embryogenic callus was 23.33%. Based on the study, 2,4-D hormone affects the formation of embryogenic callus, the response to the emergence of embryogenic callus is also influenced by the endogenous hormone content of each plant [22].](image)

3.3. Cell suspension culture to improve somatic embryogenesis result

Cell suspension cultures are agitated on a regular basis to break up cell aggregates, maintain uniform cell distribution, and provide an adequate gaseous exchange to support cell respiration [23]. In this method, the liquid medium used in cell suspension culture is the same as that used in callus culture [22].

Regeneration of somatic embryos via cell suspension culture is divided into several stages, such as: 1) development of a friable embryogenic callus from primer callus; 2) establishment and transfer of an embryogenic cell suspension culture; 3) the differentiation of somatic embryos in the cell suspension culture; 4) the maturation and germination phase; 5) the elongation of shoots followed by induction of root; 6) the last step is acclimatization of the plantlets established.

The liquid medium is more efficiently used in commercial-scale propagation. The embryogenic callus phase which is generally used in liquid medium is the nodular phase because it is still meristematic so that the potential for cell division is still high and can increase the percentage of embryogenic callus [24]. Plant propagation by somatic embryogenesis utilizing traditional methods and cell suspension culture is depicted in this flow chart (Figure 2).
**Figure 2.** Flow diagram of the process from embryogenesis somatic with conventional propagation methods and combined with the suspension culture method [17].

### 4. Conclusion

For the multiplication of oil palm elite genotypes, somatic embryogenesis has become the preferred approach. A high concentration of 2,4 – D has an influence on callus development and embryogenic callus from female fluorescence in the basal area. Implementation of cell suspension culture for oil palm micropropagation is expected to have a high rate of multiplication and long-term embryogenic potential.

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