

***IN VITRO* CALLUS FORMATION FROM MALE INFLORESCENCES OF OIL PALM (*Elaeis guineensis* Jacq.) BY THE APPLICATION OF PICLORAM**

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Abstract. This study aimed at studying the effect of different concentrations of Picloram on callus proliferation from young male inflorescences explants of oil palm. The inflorescences were isolated from 5 - 6 years old stock plants grown in the field. The concentration of Picloram tested were 10, 20, 30, 40, 50, 60, 70 and 80 ppm. Each treatment was repeated 3 times, and each replication consisted of 3 explants in one culture flask. Culture medium used was solid MS composition supplemented with 0.03% activated charcoal. Cultures were kept in light conditions at 27 ± 1 °C for 16 weeks without subculture. Observations were performed on the percentage of explants forming callus, time span to first callus proliferation, and the characteristics of callus (color and texture). Results showed that Picloram could increase the percentage of explants forming callus, where Picloram at 50 ppm was able to produce the highest percentage of callusing explants (77.78% on the average). In addition, the 50 ppm Picloram was also able to accelerate callus proliferation to an average of 10.23 days after culture initiation. The proliferated callus showed uniform characteristics, i.e. cream, yellowish cream and brownish cream in colour, with a friable texture.

Key words: tissue culture; Picloram; callus; oleaginous plant.

INTRODUCTION

Oil palm, known in Latin name as *Elaeis guineensis* Jacq., is an oil-producing plant originated from Africa. Its role as a source of vegetable oil for food industry and function as bio-fuel in automotive industry has made this plant economically very important. It was reported in 2017/2018 that palm oil production reached 34.27% of total world vegetable oil production, followed by soybean, canola and sunflower oil at 28.77%, 14.73% and 9.28%, respectively [25].

Indonesia is the largest palm oil producing country in the world with total production of 38.17 million tons Crude Palm Oil (CPO) in 2017. This production is targetted to increase and reach 40 million tons CPO in 2020. Most of Indonesian palm oil production is exported to China, Pakistan, Malaysia and Netherlands [11].

In order to reach the targetted production of 40 million tons of CPO as mentioned above, strategic and well-planned policies are needed. One important step is to encourage replanting of old trees. In addition, land expansion is another effort that can also be done to increase the production to reach the target.

Either replanting or area expansion, both require large amount of seeds as planting materials. Seed production through generative propagation is hampered by cross pollination among individuals resulting in genetic variability within progenies. On the other hand, vegetative propagation can not be applied because oil palm is a monocot that cannot be propagated vegetatively through stem cutting, grafting or other conventional methods.

Tissue culture technique is an alternative technology that can be applied in the propagation of oil palm seeds. This technique offers advantages of being able to regenerate large amount of uniform plants in a relatively short time. The success of plant propagation through tissue culture has been reported in many plantation crops such as cocoa [29], dates palm [3],

sugarcane [31] and coffee [1]. In oil palm, this technique has also been applied with varying degrees of success [5, 12, 33].

Tissue culture propagation of oil palm mostly applied has been through the induction of somatic embryogenesis as reported by many authors [4, 12, 18, 23, 24, 32]. The process of somatic embryogenesis is dependent on such factors as explant sources [26] and plant growth regulators employed in culture medium Zulkarnain [35]. The sources and developmental stage of explant are key factors that can change cellular competence [19, 26]. One source of explants in oil palm tissue culture is young male inflorescences. In principle, both male and female flowers can be used as explant materials, but the use of male flowers is preferable because it does not interfere with plant productivity since there are no fruits that are sacrificed.

In the present study we investigate the effect of Picloram on callus formation on explants from immature male inflorescences of oil palm. This is an effort of establishing a standard protocol for clonal propagation of oil palm through tissue culture technique.

MATERIALS AND METHODS

Plant materials

Plant tissue used as explant materials are young male inflorescences isolated from oil palm (*Elaeis guineensis* Jacq.) aged around 5 - 6 years old. Immature male inflorescences which are still enclosed in spathe were taken from leaf axil between 11th and 15th were taken (Figure 1), wrapped in polyethylene bag and brought to laboratory. In laboratory, the outer spathe was removed, and the inflorescences enclosed in inner spathe were soaked in 70% alcohol for 5 minutes, and air-dried aseptically. Thereafter, the inner spathe was carefully cut and removed, leaving the male inflorescences. The inflorescences were cut into pieces of approximately 1 mm and used as explant materials.

Explants were cultured on solid MS medium with a density of 3 explants per culture flask.

Culture Medium

Explants were cultured on MS [21] basal medium supplemented with the following additives: *Myo*-inositol (100 mg L⁻¹), Nicotinic acid (0.5 mg L⁻¹), Pyridoxine-HCl (0.5 mg L⁻¹), Thiamine-HCl (0.1 mg L⁻¹), Glycine (2 mg L⁻¹), and sucrose (30 g L⁻¹). The pH of the medium was adjusted to 5.8 ± 0.02 using either 0.1 N NaOH or 0.1 N HCl before solidified with 0.8% (w/v) agar. Activated charcoal (3 g L⁻¹) were added to the medium to anticipate media browning due to release of phenolic compounds that may inhibit explant growth and development. The medium was dispensed into culture flask of 10 mL each, and autoclaved at 1.06 kg cm⁻² and a temperature of 121 °C for 15 minutes.

Growth Regulator

Picloram (4-Amino-3,5,6-trichloropyridine-2-carboxylic acid) was used as auxin source for callus induction in this trial. Eight different levels of Picloram were prepared: 10, 20, 30, 40, 50, 60, 70 and 80 ppm. Each treatment consisted of three replications, and each replication consisted of three explants cultured in the same culture flask.

Culture Maintenance

Culture flasks with explants within them were kept in culture room at a temperature of 25 ± 1 °C, light intensity of 50 μmol m⁻² s⁻¹ and 16-hours photoperiod per day for 16 weeks without subculture. Observation was made on the following variables: time from culture initiation to first callus proliferation (days after culture), percentage of explant forming callus, and the characteristics of the callus (color and morphology).

Data analysis

Quantitative data were analyzed statistically by Descriptive Statistics method using Microsoft Excel computer applications [20] and the standard errors were calculated. In order to support quantitative data, qualitative information such as morphology of proliferated callus were presented visually in the images.

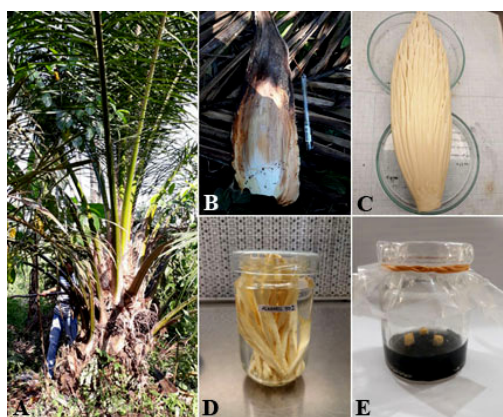


Figure 1. A. stock plant of oil palm (*Elaeis guineensis* Jacq.) aged of 5 – 6 years old used as explant sources; B. young male inflorescences enclosed in spathe; C. young male inflorescences after spathe removal; D. spikelet sterilized in 70% alcohol; E. explants cultured on solid medium with activated charcoal.

RESULTS

Callus proliferation is the first important step in oil palm tissue culture involving somatic embryogenesis. Only callus with embryogenic properties will produce somatic embryos through somatic embryogenesis. The manipulation of culture medium, particularly by the application of plant growth regulators, in some cases, resulted in embryogenic callus formation leading to the production of somatic embryos [2]. Thus, the production of embryogenic callus in oil palm tissue culture may be improved by altering Picloram content in culture medium.

It was found that the concentration of Picloram applied to culture medium was critical for callus proliferation on immature inflorescence explants of oil palm. Callus proliferation was first observed at the wounded edges following tissue swelling in all level of Picloram. The overall callus induction ranged from 33.33 to 77.78 percent (Table 1). The application of 50 ppm Picloram resulted in the highest percentage of explants forming callus (77.78%), and produced the fastest callus proliferation, that was 10.23 days from culture initiation (Table 2). Proliferated callus in all treatments showed relatively similar characteristics. They are yellowish-white or cream to brownish-yellow in colour, and dominated by friable texture.

Table 1. The effect of different concentrations of Picloram on the percentage of explants of immature male oil palm inflorescence that forming callus

2,4-D concentration (ppm)	Total explant forming callus (%)
10	55.56
20	66.67
30	44.44
40	33.33
50	77.78
60	33.33
70	44.44
80	44.44

Table 2. The effect of different concentrations of Picloram on the length of time needed for callus proliferation on explants of immature male oil palm inflorescence

2,4-D concentration (ppm)	Time needed for callus proliferation (day after culture initiation)
10	12.00 ± 1.50
20	11.33 ± 1.67
30	11.22 ± 1.75
40	11.75 ± 1.88
50	10.23 ± 0.30
60	12.00 ± 2.08
70	12.58 ± 2.55
80	13.43 ± 2.55

± standard error

DISCUSSION

The success rate of tissue culture protocol depends on various variables such as: explant materials, environmental conditions where explants were maintained, medium composition and plant growth regulators used. Formulating the combinations of these factors, particularly plant growth regulators involved in culture medium, were undoubtedly critical in obtaining the desired direction of explants growth and development under *in vitro* system. Laslo and Vicaș [17] suggested that explant growth and development were dependent on the balance of endogenous and exogenous growth regulating substances. Further, Winarto *et al.* [30] claimed that the performance of endogenous growth regulators in plant tissue might be affected by the incorporation of synthetic growth regulators into culture medium. As the consequence, it is important to seek for proper growth regulators (either types and/or concentrations) to be applied in culture medium to stimulate explants growth and development.

One of desired direction of explants growth and development in tissue culture system is the induction of callus proliferation on the surface of cultured materials. *In vitro* callus proliferation was a result of random and uneven development of unspecialized cells and the loss of structure of organized cells Gamborg and Shyluk [10]. Processes relating to the capacity of callus induction under *in vitro* system are a consequence of interaction of such factors as the combinations of growth regulators, explant origin, culture medium, and plant genotypes. In our experiment, the use of Picloram at different concentrations resulted in different level of callus proliferation. The application of 50 ppm Picloram was able to increase the number of explants forming callus up to 77.78% of total cultured explants. In addition, 50 ppm Picloram was also found to shorten the time needed for callus proliferation up 10 days after culture initiation.

The use of Picloram in oil palm tissue culture has been reported by many authors with various results. Scherwinski-Pereira *et al.* [24] found that on MS medium [21], Picloram application at 108.68 ppm was effective in inducing embryogenic callus formation on explants originated from basal part of young plants. Pádua *et al.* [22] noted an efficient callus induction from leaf segments of oil palm by the use of Y3 medium composition [7] supplemented with 2,4-D or Picloram at 1.0 - 9.0 mg L⁻¹. Both growth regulators efficiently induced cellular masses regardless of the concentrations applied. Jayanthi *et al.* [12] obtained maximum callus induction (82%) by culturing immature male inflorescence explants on Y3 medium with 72.45 ppm Picloram, and it was not significantly different compared to 66.0 ppm 2,4-D or a combination of 2,4-D (33 ppm) + Picloram (36.23 ppm). The highest embryogenesis (4.9%) was obtained on 2,4-D + Picloram followed by 3.4% on Picloram alone.

Callus proliferated in this investigation was friable and white-creamy (Figure 2). This indicates that callus proliferating on young male inflorescence explants of oil palm have the embryogenic potential, and if they are cultured on proper medium, somatic embryos could be produced. This in accordance with the statement of Kumar *et al.* [16] where colours and textures clearly indicate the effect of plant growth regulators on the morphology of callus.

In date palm, Aslam and Khan [3] reported compact and creamy callus were induced on shoot tip explants cultured on MS medium supplemented with 2,4-D. Initially, the callus appeared watery but then grew compact and globular, and their colour became milky with time. In *Jatropha curcas*, Zulkarnain and Lizawati [34] noted white and friable callus proliferated on hypocotyl and cotyledon sections treated with 2,4-D. Yadav *et al.* [31] found cream-coloured and compact callus with nodular somatic embryos on wounded edges of leaf explants of sugar cane cultured on MS medium supplemented with 2,4-D in combination with benzyladenine. Meanwhile Marbun *et al.* [18] reported transparent embryogenic callus with nodular structure in oil palm. Callus with bigger globular structure and whitish or yellowish colour were converted into somatic embryos using temporary immersion system.

Efforts to generate embryogenic callus can be done by modifying environmental factors, especially the composition of the culture medium, since somatic embryogenesis is chemically controlled by various growth regulators [28]. The importance of plant growth regulators in somatic embryogenesis had been intensively reviewed by Jiménez [13], Jiménez [14], Fehér *et al.* [9], Jiménez and Thomas [15] and Fehér [8]. Among those growth regulators, auxins and cytokinins are critical in determining the embryogenic response. This is presumably due to their role in cell cycling, division, and differentiation.

In addition, prolonged culture on the same medium will result in nutrient deficiency and water loss due to evapotranspiration. Therefore, it is important to carry out subculture to maintain the sustainability of callus growth. Dodds and Robert [6] recommended to subculture callus of 5 – 10 mm or 20 – 100 mg on to fresh medium to obtain better performance. Subcultures can also be done every 2 - 6 weeks, but the right time to transplant the culture depends on the speed of callus growth.

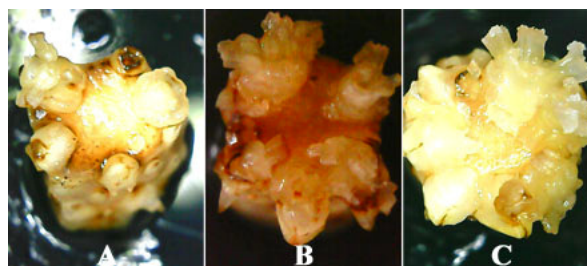


Figure 2. Morphology of callus proliferating on the surface of young male inflorescences of oil palm treated with Picloram 50 ppm (A), 60 ppm (B) and 70 ppm (C) at 60 days after culture initiation

Thus, the results of this study indicate the magnitude of the opportunity to obtain callus from young male flower explants of oil palm with the application of growth regulators, especially Picloram at a concentration of 50 ppm. It is expected that efforts to produce uniform, disease-free and large quantities of oil palm plants can be achieved via somatic embryogenesis pathway. Taji et al. [27] stated that somatic embryogenesis has an important meaning in plant propagation by tissue culture. However, this process is restricted by many factors since somatic embryos will only develop from embryogenic callus, and time needed to obtain callus with embryogenic properties is sometimes very long. In addition, other factors such as plant hormones, nutrients and environmental conditions must be optimized first so that embryogenesis can take place.

Though the characteristics of callus reported here indicate embryogenic properties, there has no somatic embryo formation until the end of trial period. This is presumably due to the limited time to observe explant development, in addition to the need for involvement of other factors to induce embryo formation.

In general, this study was among few contributions in the efforts of establishing modern clonal propagation of oil palm. The significant findings of this investigation are:

1. The addition of Picloram in culture medium was advantageous to stimulate callus induction from immature male inflorescences explants of oil palm in tissue culture system.
2. The effective concentration of Picloram for callus proliferation in immature male inflorescence of oil palm was 50 ppm.
3. The protocol reported here opens up the prospects of using Picloram as auxin source and immature male inflorescences as explant source for callus formation to support somatic embryogenesis technology for oil palm propagation.

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