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The Effect of Culture Media Type and Plant Growth Regulators on Callus Induction of Oil Palm (*Elaeis guineensis* Jacq) Pisifera Type

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Abstract

Oil palm has a high economic value since it is one of the highest vegetable oil-producing plants compared to other oil-producing plants. The provision of good planting material for oil palm plantations is one of the determining factors to increase productivity. The parent Pisifera type of oil palm is important to develop the high-yielding oil palm Tenera varieties. The provision of oil palm material could be done through generative system with seeds and also could be done through vegetative approaches using tissue culture techniques. It aims to get plants that are genetically the same as their parents (true to type). The purpose of this study was to see the effect of different type of tissue culture media and plant growth regulators (PGR's) on the callus induction stage of Pisifera type oil palm. The results show that the treatment using Murasige and Skoog (MS) culture media is able to give a better effect when compared to the use of Eeuwens and Blake (Y3) basic media. While the use of PGR treatment (H6 = NAA 6 mg/l + 2.4-D 0.5 mg/l) showed a callus growth percentage of 76%, which was better than (H1 = Kin 0.1 mg/l + 2.4-D 100 mg/l) by 42%.

Keywords: Tissue Culture; Pisifera Type; Callus Induction, Culture Media, Plant Growth Regulators

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1. Introduction

The provision of genetic matrial to support oil palm breeding activities is very important. Pisifera type of oil palm is one of the most important parental materials and is needed in crossbreeding programs to produce superior seeds. Pisifera type oil palm has a recessive homozygous allele (sh-sh-) so it does not form a shell [1]. Meanwhile, from the condition that the potential of the oil produced is quite high, it can be further developed the best clone propagation [2] which is expected to inherit the same properties as the parent.

Propagation of oil palm clones, especially the Tenera type, has been widely carried out using tissue culture techniques [3] [4] with propagation material in the form of explants from immature leaves with the aim of inducing callus.

The use of basic media is one of the determinants of the successful implementation of oil palm tissue culture [2]. Several successful studies have been conducted on oil palm, especially on the Tenera type, using MS [4][5][6] and Y3 [4][7] as the base media. The use of Murashige and Skoog's (MS) basic media with the addition of PGR's from the auxin group 2,4-Dichlorophenoxyacetic acid (2,4-D) and Naphthaleneacetic acid (NAA) was able to induce callus[8].

In addition, the embryogenic callus of sago palm was induced by MS media with the addition of PGR's 2,4-D and kinetin [9]. The use of Eeuwens (Y3) media, compared to MS media, with the addition of PGR's 2,4-D and NAA was able to induce callus of Tenera type with young leaf explants [4]. Basal MS media containing a combination of NAA (107.41 µM) and 2,4-D (45.24 µM) was optimal for direct embryoid production of young leaves of Tenera-type oil palm explants [4].

The callus induction stage in oil palm mass is very important in starting the production process. Where in

particular the type of Pisifera as the male parent is used as a pollen donor for oil palm crossing activities. Based on the report of Almeida *et al.* for the propagation of Pisifera [6] by using the material in the form of immature leaf explants (grass), MS base media can induce callus.

This research is an initial study of vegetative propagation using tissue culture techniques for the preparation of pisifera clones, which aims not only to see the effect of the two culture media but also to see the effect of adding growth regulators (ZPT) to callus. induction. stage. Based on research conducted by Almaeda *et al.* wherein the callus induction medium consisted of MS salt and vitamins [10], supplemented with 30 g L-1 sucrose, 0.5 g L-1 glutamine, 0.5 g L-1 glutamine. g L-1 hydrolyzed casein, 2.5 g L-1 activated charcoal, 450 µM Picloram (4-amino 3, 5, 6-trichloropico-linic acid), and compacted with 2.5 g L-1 Phytag [6].

2. Materials and Methods

2.1 Planting Materials and Media Preparation

In this study, one Pisifera palm (318/81) was used as the source of explant. The immature leaf leaflets of the spear leaf were surface sterilized in 5% Chlorox solution and a few drops of Tween 20 twice for 10 minutes followed by three rinses in sterile distilled water. The culture media used were Murashige and Skoog's (MS) media, 1962 [10] and Eeuwens and Blake's (Y3) media, 1976 [11]. The composition consisted of mineral salts and vitamins plus 3% sucrose, and PGR'S treatment according to the experimental design, and compacted with 0.6% agar. The pH of the culture at 121°C, a pressure of 17.5 psi for 20 minutes.

2.2 Callus Induction

The 6 sterilized leaves were separated and cut with a width of 2 mm [12] and cultured 2 explant pieces in a vial containing 30 mL of media for callus induction.

Leaf explants were stored for six months in a dark room at a temperature of 27 ± 2°C and humidity of ± 45%. Subcultures on the same fresh medium were performed every 12 weeks and callus was observed from the explants every 2 weeks. The callus found from the induction stage were subcultured on the same medium with an interval of 8 weeks.

2.3 Experiment Design

This study aimed to examine the effect of using two types of culture media, namely M1 = MS [10] and M2 = Y3 [11] and the addition of PGR's during callus induction media (at the explant stage). The use of PGR's (PGR's) as treatment includes H0 = Control (without hormones); H1 = Kin 0.1 mg/l + 2,4-D 100 mg/l; H2 = Kine 0.5 mg/l + 2,4-D 90 mg/l; H3 = 2,4-D 100 mg/l; H4 =2,4-D 177 mg/l; H5 = NAA 10 mg/l + 2,4-D 0.5 mg/l; H6 = NAA 6 mg/l + 2,4-D 0.5 mg/l and H7 = NAA 10 mg/l at the callus induction stage.

2.4 Data Analysis

Data were collected from the induced callus formation during the incubation period. The data were statistically analyzed by one way ANOVA followed by Duncan's multiple distance test (DMRT) which was carried out at a p-value level of less than 0.05 ($p < 0.05$) using SPSS 20.0.

3. Results and Discussion

3.1 Callus induction in young leaf explants

Leaf explants began to swell in the second and third weeks on the induction medium. The first Pisifera type callus was induced in the third month in a dark room. In general, the types of callus induced in leaf explants included compact nodular, non-friable, non-nodular non-friable, and non-nodular compact, non-friable callus (Fig. 1). There types of callus, compact non-friable nodular callus was the most common.

The rate of callus induction increased in the first week to the fourth week in the third month of culture time (Fig. 2). In the first three months of culture, there were 8.91% of explants that formed callus. This indicated that the Pisifera type callus induction was better than the Tenera type where in the first three months of culture, only 0.78% of explants formed callus [8].

Figure 1. Type of compact, non-friable nodular callus (a); compact nodular callus, friable (b) and compact non nodular callus, non friable (c)

The frequency of Pisifera-type callus induction is highly dependent on the culture medium used and the use of PGR's. The first callus was induced on the 67th day after planting. And the percentage of callus induced in each treatment combination varies from 3% to 76%.

Note:** Means with the same letter are not.

The observed callus formation can be shown in (Fig.

3 and 4) where the callus color ranges from yellowish-white to brownish-yellow and the callus shape is irregular and tends to be hard. This callus appears on the surface of the explants where the leaf explants have started to change color from yellowish-white to brownish-yellow.

Table 1. Results of Callus Induction ANOVA

Source	df	Sum of Squares (SS)	Mean Square (MS)	F value	F Table 5%
Medium	1	2436.75	2436.75	64.02**	4.15
PGR's	7	12056.583	1722.369	45.25**	2.32
Medium x PGR's	7	4429.917	632.845	16.63**	2.32
Error	32	1218	38.062		
Total	48	33810			

Note: **Means with the same letter are not significantly different with Duncant-Multiple-Range-Test (P<0.05).

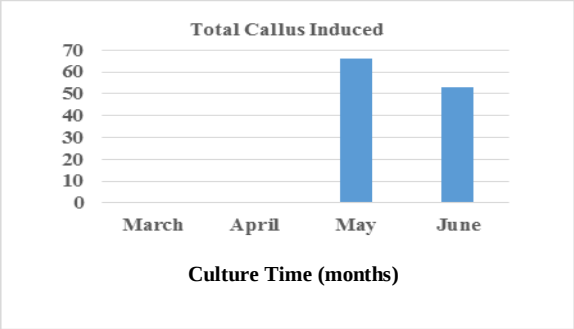

Figure 2. Callus induction rate for 3 months of culture time.

3.2 Effects of Culture Media and Growth Regulators

The results of the ANOVA analysis (Table 1) show that there is a significant effect of the type of media used to the induction of callus. M1 (MS) has a better effect than M2 (Y3). This result is in line with what was reported by Constantin *et al.* On the Tenera type [4]. Where the use of culture media both MS and Y3 were able to have an effect on callus induction, but in this study, the use of MS culture media had a significant effect on callus induction in the Pisifera type.

The use of M1 culture media (MS) had a higher average percentage of callus induction (22.50%) than the use of M2 culture media (Y3) with an average percentage of callus induction (8.96%).

The frequency of callus induction from the average of the best callus induction results obtained an average percentage of (48.75%) in treatment (H6) compared with treatment (H1) with a percentage (31.25%) and treatment (H5) with a percentage (25.42 %). However, the use of high concentrations of 2,4-D in the treatment of 2,4-D 100 mg/l (H3) and 2,4-D 177 mg/l (H4), is in line with what Reflini reported, wherein the presence of an increase in the concentration of PGR's 2.4-D will show a decrease in the rate of callus induction [8]. As for the use of 2,4-D with a combination of PGR's from the cytokinin group, Kin 0.1 mg/l + 2,4-D 100 mg/l (H1) and Kin 0.5 mg/l + 2,4-D 90 mg/l. (H2) callus can be induced but the percentage is not as good as that induced at low concentrations.

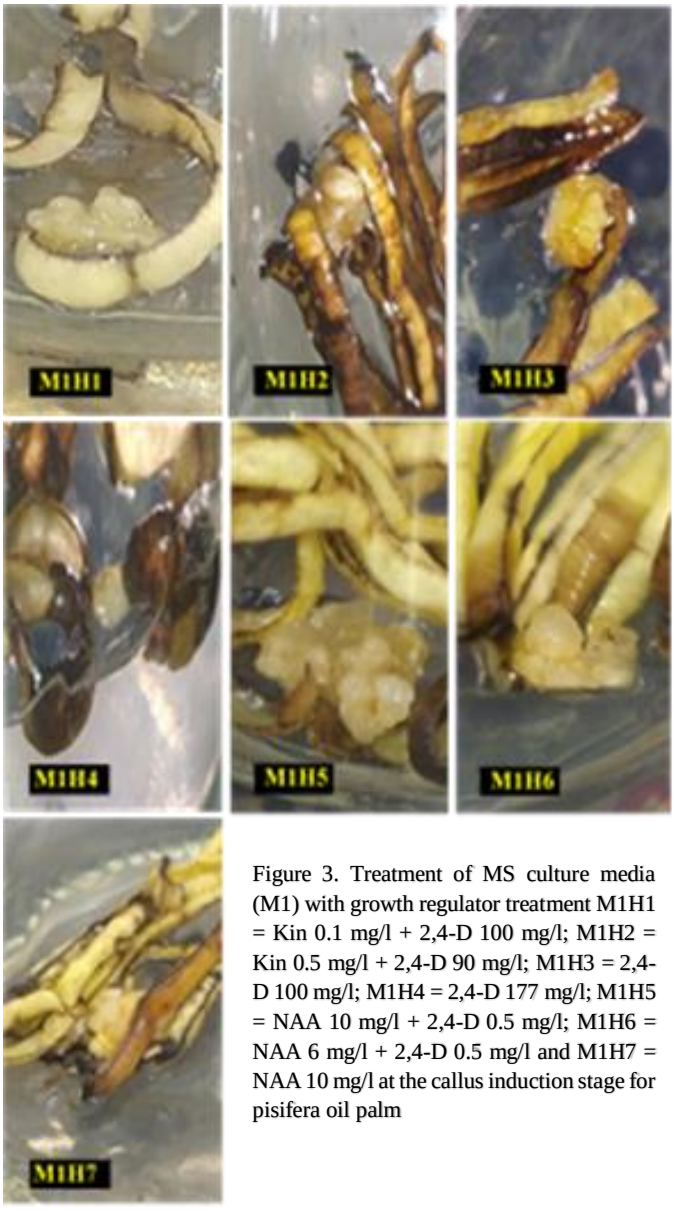

Figure 3. Treatment of MS culture media (M1) with growth regulator treatment M1H1 = Kin 0.1 mg/l + 2,4-D 100 mg/l; M1H2 = Kin 0.5 mg/l + 2,4-D 90 mg/l; M1H3 = 2,4-D 100 mg/l; M1H4 = 2,4-D 177 mg/l; M1H5 = NAA 10 mg/l + 2,4-D 0.5 mg/l; M1H6 = NAA 6 mg/l + 2,4-D 0.5 mg/l and M1H7 = NAA 10 mg/l at the callus induction stage for pisifera oil palm

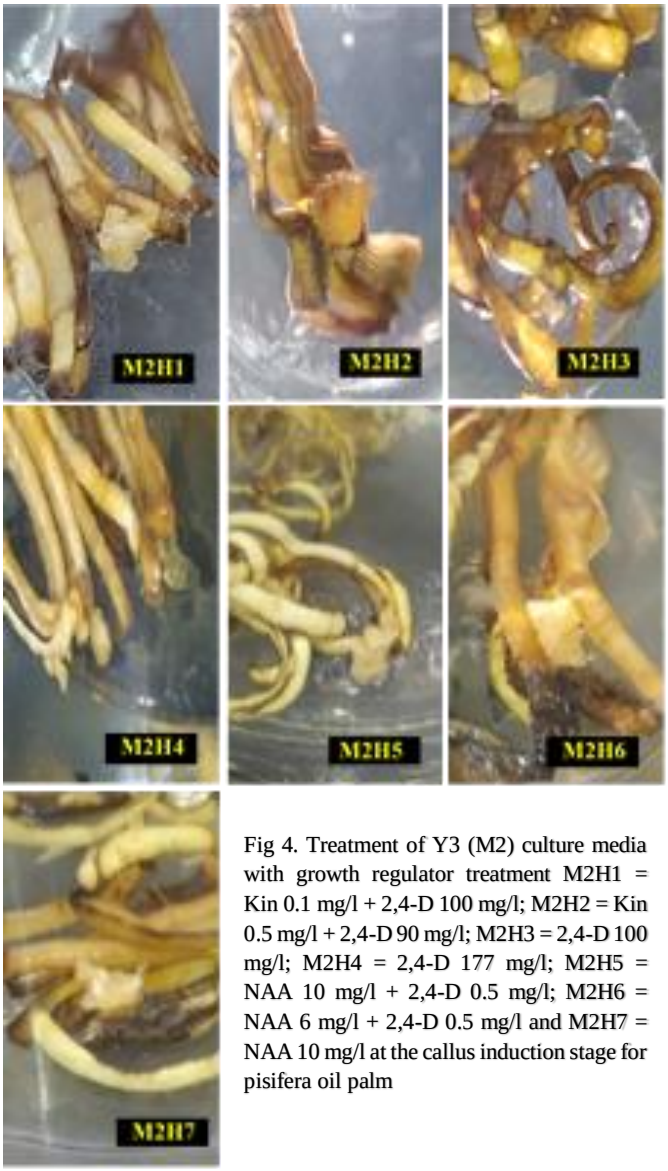

Fig 4. Treatment of Y3 (M2) culture media with growth regulator treatment M2H1 = Kin 0.1 mg/l + 2,4-D 100 mg/l; M2H2 = Kin 0.5 mg/l + 2,4-D 90 mg/l; M2H3 = 2,4-D 100 mg/l; M2H4 = 2,4-D 177 mg/l; M2H5 = NAA 10 mg/l + 2,4-D 0.5 mg/l; M2H6 = NAA 6 mg/l + 2,4-D 0.5 mg/l and M2H7 = NAA 10 mg/l at the callus induction stage for pisifera oil palm

In addition, the percentage of callus induction in the use of a single PGR where the use of PGR 2,4-D 100 mg/l (H4) was (5%) much better than the use of PGR 2,4-D 177 mg/l (H3) of (3.75%). The treatment of PGR either alone or in combination of two PGR on culture media give different effects on the percentage of callus induction (Fig. 5). Based on the results of observations and statistical analysis, it is known that there is an effect of the use of culture media and the PGR used (Table 1).

The use of M1 (MS) culture media with the addition of PGR's (H6) was able to give the highest average percentage of callus induction in the Pisifera type (Table 2). Meanwhile, the use of M2 culture media (Y3) in treatment (H6) also gave an average percentage of callus induction but not as good as M1 (MS) culture media (Fig.5). This is in line with what was reported by Constantin *et al*., (2015) [4].

Table 2. Callus Induction DMRT Test Results on Growth Regulatory Substances (PGR'S) Treatment

PGR's	Callus Induction Average (%)	
H6	49.17	a
H1	31.33	b
H5	25.50	b
H2	11.17	c
H4	8.17	c
H7	5.00	cd
H3	4.67	cd
H0	0.00	d

Note: The mean of callus induction followed by the same letter, is not significantly different at the 5% level with the DMRT test

The PGR's 2,4-D and NAA treatments were able to increase the percentage of callus induction when compared to the 2,4-D and Kinetin treatments. In addition, the use of 2,4-D at high concentrations can cause a decrease in the rate of callus formation. This is in line with what Constantin *et al.* (2015) [4] reported, increasing the concentration of 2,4-D can decrease the activity of meristematic cells. Auxin is able to affect callus production, cell elongation, and cell division in cambium tissue.

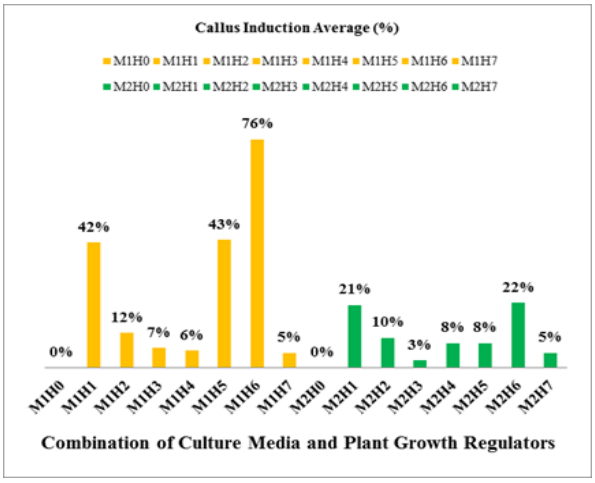

Figure 5. Effect of culture media and growth regulators on callus induction of pisifera type.

The effect of the type and concentration of synthetic ZPT used greatly affects the occurrence of somaclonal variations, especially from the auxin and cytokinin groups [13] which greatly affects the callus formation process in leaf explants, both at high and low concentrations. In line with the results of this study, it is necessary to evaluate the presence or absence of somaclonal variation in the regenerate [14].

This indicates that the use of ZPT from the auxin group can induce cells to differentiate into callus. However, prolonged exposure of explants to media containing high concentrations of auxin ZPT is not recommended. In line with what was described by Weckx *et al*., (2019) [13] where the occurrence of somaclonal variations due to leaf explants experiencing stress due to attachment of leaf explants for a long time on media containing PGRs such as synthetic auxin 2,4-D. In addition, Soh *et al*., (2011)[14] added that the high PGR auxin content in liquid media can also pose a potential risk of somaclonal variation in oil palm of less than 3% .

In addition to the effect of growth regulators auxin at high concentrations, abnormalities due to somaclonal variations can be caused by several factors including planting material in the form of explants, both leaves, flowers, and roots, which are very susceptible to somaclonal variations in addition to the number and length of the subculture period from the stages of the propagation method.[15].

4. Conclusion

This study succeeded in explaining the protocol of in vitro somatic embryogenesis with young explants of oil palm leaves of Pisifera species. MS culture media is a medium capable of influencing the percentage of callus induction. ZPT treatment containing 2.4-D 0.5 mg/l and 6 mg/l NAA was able to increase the average percentage of callus induction. Therefore, for the callus induction stage, the use of MS media with the addition of 0.5 mg/l PGR 2.4-D and 6 mg/l NAA was able to increase the percentage of callus induction compared to other treatments.

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