Effect of sucrose and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on callus induction and proliferation of Coconut cv MATAG from Plumule Explants

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Abstract: The coconut palm, also known as Cocos nucifera L is one of the world's most important annual tropical plantation crops. This tropical crop makes a big difference in terms of food security, nutrition, employment, and income production. The plumule explant was used for this research in order to determine the effect of different concentrations of sucrose with 2,4-Dichlorophenoxyacetic acid (2,4-D) as the plant growth regulator and to perceive the most suitable concentration of sucrose towards callus induction and growth of plumule coconut cv MATAG. Various concentrations of sucrose (15, 30, 45, 60 g/L) with a combination of plant growth regulators of 2,4-D (1, 10, 20, 40, 60 mg/L) were used for callus formation and proliferation. The fastest formation of callus was at 20, 40, 60 mg/L 2,4-D that took 1-2 months. The percentage of explants that produced callus, with the highest percentage (5%) observed at 20-60 mg/L 2, 4-D combined with 30 g/L sucrose treatment. During the 5-6 months of culture, 60% of callus proliferation was obtained at 20 and 40 mg/L of 2, 4-D, respectively. The percentage of callus proliferation continued to increase for another 4 months. It increased up to 90%. Thus, it can be concluded that sucrose at 30 g/L combined with 40 mg/L of 2, 4-D was ideal and gave the best results for callus induction and proliferation.

Keywords:

Introduction

One of the world's most important annual tropical plantation crops is the coconut palm (Cocos nucifera L.). Coconut is monoecious, with a huge axillary spadix with a great number of staminate blooms and a few pistillate flowers (Perera et al., 2010). This tropical crop makes a big difference in terms of food security, nutrition, employment, and income production (Sáenz-Carbonell et al., 2016). Its commercial importance has risen at a rapid rate in recent years for various high-value items around the world, such as packed coconut water, causing a high demand from society. In order to support biotechnological research, tissue culture systems have been created to generate specific plants in huge quantities. The availability of this resource in nature has been impacted as a result of rising demand. To get only the desired product, tissue culture can be developed into a full plant or cell suspensions (Wahyuni et al., 2020). Nonetheless, the genotype and variety of the plant, the culture media, growth-controlling chemicals, growth environment, carbon source, and light all play a role in the success of callus culture (Wahyuni et al., 2020). In addition, changes in light conditions and sucrose concentration could affect the production of such molecules (Hogewoning et al., 2010; Kapoor et al., 2018).

Plant growth regulators are artificial chemicals that mimic natural plant hormones and are frequently used in tissue culture experiments (Jimenez, 2001). Auxins, abscisic acid, cytokinin, ethylene, and gibberellins are the five primary types of naturally occurring plant hormones. In plant tissue and organ cultures, auxin, cytokinin, and auxin-cytokinin interactions are commonly thought to be the most significant and are normally necessary to promote

growth and organise development. (Vasil & Thorpe, 1994). The aim for this research was to determine the effect of different concentrations of sucrose with 2, 4-Dichlorophenoxyacetic acid (2,4-D) as the plant growth regulator and to perceive the most suitable concentration of sucrose towards callus induction and growth of plumule coconut cv MATAG.

Material and methods

Sources of explants and sterilization.

The experiments of this study were carried out to investigate the response of the plumule of coconut zygotic embryos to varied sucrose and 2,4-D concentrations for callus induction and proliferation. The coconut zygotic embryos were obtained from old nuts harvested from the coconut mother-plants of MATAG Variety from MARDI Bagan Datok farm in Perak, Malaysia. Zygotic embryos were removed from the kernel (Figure 1a,b). A cylinder of solid endosperm containing the embryo was excised from the open nuts using a cork borer, then surface sterilized. A zygotic embryo (Figure 1c) was conducted in the laminar flow chamber to reduce the risk of contamination. The laminar flow was cleaned with 70% ethanol before commencing work. The zygotic embryos were removed from the seeds using scalpel blades and forceps. Briefly, the embryos were immersed in 50% ethanol for 2 min, then in 1% Virkon disinfectant for 30 min. This was followed by immersing the embryos in 50%Clorox (sodium hypochlorite, 5.2%) for 40 min, followed by immersion in 10% or 20% Clorox for 20 min. Two drops of Tween 20 (a polysorbate surfactant that serves as a detergent and emulsifier) were added. After rinsing three times in sterile distilled water, the embryos were dried for 1 hour. Remove the plumule (Figure 1d, e) from these embryos using a stereoscopic microscope and place them on treatment medium at 27 °C in complete darkness.

Primary callus induction and proliferation

Plumules were cultured individually under aseptic conditions on basal Y3 medium containing 0.1 g arginine, 0.1 asparagine, and 0.1 g glutamine. The treatment medium consists of 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations of 0, 10.0, 20.0, 40.0, and 60.0 mg/L and is combined with sucrose at 15, 30, 45, and 60 g/L, respectively. The medium was adjusted to pH 5.8 and then solidified with 3 g/L gelrite before autoclaving at 121°C for 20 min. The cultures were maintained in a dark culture room at 25±2. The media that could induce primary callus was designated as producing callus medium. The percentage of callus that grew in all treatments was checked every month for the first 5 months of culture and recorded.

Clumps of primary callus initiated from the primary callus induction experiment were utilized to induce additional callus or boost callus proliferation. The callus was cultured in the same medium, where it was subcultured with an interval of 8 weeks until embryogenic callus formation occurred for 8 months. The percentage of proliferation was recorded for 6 and 8 months of culture was calculated as below:

$$\text{Proliferation (\%)} = \frac{\text{FW of proliferated callus} - \text{FW of initial callus}}{\text{FW of initial callus}} \times 100$$

Result and discussion

This study was focused on the effect of different concentrations of sucrose (15.0, 30.0, 45.0,60.0 g/L) with a combination of plant growth regulator 2,4-D (1.0, 10.0, 20.0, 40.0, 60.0 mg/L) on callus formation, the percentage of explant producing callus, and percentage of callus proliferation up to 4 months and 6-8 months. 2, 4-D is a plant growth regulator that is commonly employed in callus culture due to its influence on cell dedifferentiation, organogenesis, and callus growth (Mayerni et al., 2020). Due to the separation of carboxyl groups by carbon or carbon and oxygen, 2, 4-D showed stronger and more optimum activity than other auxins (Nasution & Nasution, 2019). Table 1 shows a variety of responses towards the explant, producing callus (%) and callus proliferation (%).

A poor result was observed at a high sucrose concentration (15.0 g/L). In addition, there was no callus formation, resulting in no explant producing callus and no callus proliferation being produced. The ability to produce calluses was strongly influenced by the type and concentration of the growth regulator and combinations in the growth media (Sandoval-Cancino et al., 2016). According to a previous study conducted by Nasution and Nasution (2019),

stem explants of Garcinia mangostana L. were cultured on MS medium with different combinations of plant growth regulators 2,4-D and coconut water for callus induction, resulting in a variety of growth rates.

Moreover, there was an increasing pattern when the concentration of sucrose was doubled up from the previous concentration. Numerous values are shown for sucrose (30g/L). The fastest formation of callus (Figure 1f,g,h,) was at 20.0, 40.0, 60.0 mg/L 2,4-D concentrations that took 1-2 months to form, while at the lowest concentration, 1.0 mg/L, there was no formation of callus, but at treatment of 10.0 mg/L, the callus formation took a longer time, which was 2-3 months. By referring to the parameters of callus formation, the goal was to see if using plant growth regulators in combination with a higher concentration of growth regulators was faster at generating callus. Hence, explant cultivated cells grew at a different rate when plant growth regulators were added to the tissue culture media. Due to the availability of nutrients and better oxygen, the rapid growth of callus production happens in the region's periphery. Nasution and Nasution (2019) found that the amount of callus production shows how the explant responded to the use of plant growth regulators.

The percentage of explants producing callus was the highest percentage (5%) observed at treatment of 20.0, 40.0, 60.0 mg/L 2,4-D concentration combined with 30.0 g/L sucrose. Despite having the same percentage, the callus proliferation was different for each concentration. During the 4 months of culture, 60% of callus proliferation was obtained at 20.0 and 40.0 mg/L of 2,4-D while 20% at a concentration of 60.0 mg/L (Figure 1i,j). However, these plumule callus Coconut cv MATAG were further cultured to another 6-8 months for observation, resulting in another rapid growth. Figure 1k showed the rapid callus growth under a light microscope. From 60% of callus proliferation for 5 months, it increased to 80% and 90% respectively, after 6-8 months (Figure 1l, m). Meanwhile, the 20% of callus proliferation at 60 mg/L gives a slightly increasing result with only 40%. The sucrose concentration was then increased at 45 and 60, causing the callus formation to take a longer time to develop, which was 3-4 months, and a poor result was obtained. The range of explants producing callus was 1-2% respectively. This results in a low percentage of callus proliferation, with only 10% for the first 5 months. It was then further investigated for another growth at 6-8 months, but the result was also not good. The range of callus proliferation obtained was 20-40%. This can be supported by a study by Kumar et al. (2015) found that increasing the sucrose concentration in the culture medium caused an increase in the production of phenolics, which resulted in cell death.

Nevertheless, the addition of sucrose accelerated callus growth by increasing cell respiration, which resulted in an increase in callus biomass but with an ideal amount of sucrose must be fulfilled. The rate of respiration and nitrogen absorption were both slowed by low sugar concentrations. As a result, the energy supply was reduced, and protein synthesis was slowed as the nitrogen supply decreased (Zheng & Konzak, 1999). Thus, it can be concluded that sucrose (30) was ideal and gives the best result for callus proliferation growth at a concentration of 40 mg/L of 2,4-D.

Conclusion

This study focused on using sucrose and 2,4-D as plant growth regulators to observe the effects and growth of plumule explant Coconut cv MATAG. The process was carried out in the tissue culture system by giving attention to the callus induction of these plumule explants. Various results were obtained after undergoing observation for up to 6-8 months. The concentration of sucrose and 2,4-D played an important role in the growth of the sample. Furthermore, the best results can be seen at sucrose (30) and at a concentration of 40mg/L of 2,4-D, which gives the highest callus proliferation (90%) after 6-8 months compared to others. Hence, with a good percentage callus proliferation rate, it helps to support biotechnological research in order to generate specific plants in huge quantities to fulfil the rising demand towards the availability of this resource in nature.

Table 1: Effects of various 2,4-D and sucrose on callus development and proliferation in coconut cv MATAG from Plumule Explants.

Sucrose (g/L)	2,4-D (mg/L)	Day of callus formation (months)	Explant producing callus (%)	Callus proliferation (%) (5-6 months)	Callus proliferation (%) (7-8 months)
15	1	-	0	0	0
	10	-	0	0	0
	20	-	0	0	0
	40	-	0	0	0
	60	-	0	0	0
30	1	-	0	0	0
	10	2-3	1±0.5	20±5.5	30±2.1
	20	1-2	5±0.9	60±8.1	80±14.5
	40	1-2	5±1.0	60±11.4	90±21.8
	60	1-2	5±0.5	20±3.6	40±5.9
45	1	-	0	0	0
	10	-	0	0	0
	20	-	0	0	0
	40	3-4	1±0.05	10±2.7	20±3.1
	60	3-4	1±0.1	10±2.1	20±7.6
60	1	-	0	0	0
	10	-	0	0	0
	20	-	0	0	0
	40	3-4	2±0.7	10±3.1	40±9.6
	60	-	0	0	0

Figure 1: Callus induction and proliferation of in Coconut cv MATAG from plumule explants. Kernel of cococnut cv MATAG (a), zygotic embryos extracted form kernel (b), a zygotic embryo (c), plumule under stereoscopic microscope (d,e), formation of callus after 1-2 months of culture (f,g,h,), callus proliferation (i,j), rapid callus growth under a light microscope (k), callus proliferation after 6-8 months of culture (l, m).

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