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Characterization and morphological development of oil palm transformed callus on modified culture media

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Abstract

Genome editing through cisgenesis develops into scientific breakthroughs in accelerating oil palm breeding programs. However, one remaining problem is the low success of transformed calli regeneration, while its scientific explanation is still underexplored. This study aimed to characterize and regenerate transformed calli using various amino acids and antioxidants. Transformed callus that did not regenerate (un-regenerated transformed callus or UTC) after the transformation process was taken, then T-DNA integration was detected using the *NPTII* gene. Furthermore, the UTC was divided into four types based on morphological characteristics. The four types of UTCs were regenerated on media enriched with glutamine (for Type-1 callus), cysteine and putrescine (for Type-2 callus), and a combination of cysteine and ascorbic acid (for Type-3 and Type-4 callus). The research results obtained *NPTII* successfully amplified with a band size of 700bp. The results showed that on Type-1 callus, enrichment media with 10 mg L^{-1} L-glutamine could induce the formation of new nodular structures on UTC Type-1. On Type-2, media enriched with 5 mg L^{-1} L-cysteine + 20 mg L^{-1} putrescine increased the density of callus structures. Media enriched with 25 mg L^{-1} ascorbic acid + 25 mg L^{-1} L-cysteine could prevent the spread of brown callus on Type-3 callus, while Type-4 callus did not show any response and became dry. Our new findings will facilitate the basic research and unregenerated transformed callus and morphological callus development behavior in oil palm.

[Keywords: oil palm, plant development, transformed callus]

Introduction

Oil palm is a versatile industrial plant worldwide because it is the key to vegetable oil and biofuel resources. As the highest-yielding oil crop in the world, oil palm can produce 81 million tons of oil from 19 million hectares. This production is eight-fold higher than vegetable oil crops such as soybean and rapeseed (Murphy et al., 2021). In Mahlia et al. (2019) found that oil palms and their wastes are viable as a fuel source, especially as fossil fuel substitutes that are not environmentally friendly. Developing oil palm planting material with high-value traits has become a recent challenge for breeding researchers.

In addition, a breeding program for oil palm takes more than ten years. As a result, classical breeding programs and biotechnology approaches have to collaborate to find a market gap in the oil palm industries, such as developing superior oil palm planting material. Moreover, genome editing can be one of the efficient techniques in the oil palm breeding program. This technology has opened the possibility of enhancing oil palm's future traits. Some genes in oil palm have been successfully edited using this approach, such as *early methionine-labeled polypeptide* (*EgEMLP*), *phytoene desaturase (EgPDS*), *brassinosteroid-insensitive 1* (*EgBRI1*), *palmitoyl-acyl carrier protein thioesterase* (*EgPAT*), *fatty-acid desaturase 2* (*FAD2*) (Bahariah et al., 2023). Furthermore, the IOPRI cisgenic research team from 2017 successfully inserted some oil palm genes that are involved in fatty acid biosynthesis i.e. palmitoyl-ACP thioesterase (*EgPTE*) (Minarsih et al., 2023) and genes that are responsible for disease attack i.e. early methionine-labeled polypeptide (*EgEMLP*) (Budiani et al., 2019).

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However, some calli failed to regenerate and transform and still encountered several issues, such as underdevelopment, browning, and necrosis. Efforts to improve the quality of transformed calli are necessary for the success of biotechnology breeding programs, and one way to achieve this is by modifying the culture media. Some methods for regenerating callus into healthy and complete plantlets have been reported, such as adding ascorbic acid, hydrolyzed casein, glutamine, asparagine, and arginine at variable concentrations. Moreover, amino acids such as glutamine and cysteine have been used as an organic nitrogen source. Organic nitrogen enhances plant regeneration by making cells use energy more efficiently because the cells can directly uptake and use the nitrogen source (Dellero, 2020). Polyamine is a small amine that promotes cell growth with stable DNA (Chen et al., 2019). There are some types of polyamines namely, putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm). Each polyamine could be found abundantly in different plant organs. Putrescine was higher in leaves, while Cad, SPd, and Spm in shoot apical meristem (Sakhanokho et al., 2005). Moreover, some reports found that polyamines could affect the embryogenesis process in plants (Sivanandhan et al., 2011). Another obstacle in oil palm regeneration is the browning phenomenon since oil palm is a recalcitrant plant. Browning handling in plant tissue culture is carried out by modifying the medium with an anti-browning compound, manipulating cultural practice, and inhibiting the enzyme that caused the activity of phenol oxidase. In addition, some research suggests that antioxidant additions like ascorbic acid can prevent lethal browning in plants in vitro micropropagation (Ndakidemi et al., 2014; Bhat et al., 2022; Sinta et al., 2024).

This study aimed to characterize the morphology of transformed callus in oil palm and regenerate transformed callus on a medium supplemented with amino acid (AC) (glutamine and cysteine), polyamines (PA) (putrescine), and antioxidants (AO) (ascorbic acid).

Materials and Methods

Plant material

The six-month-old transformed oil palm calli (TOPC), which showed no growth and development character, were picked as samples. The selected samples were distinguished into the unregenerated transformed callus (UTC), and the T-DNA integration was checked using PCR amplification. The positive transformant (UTC) was then characterized based on its appearance such as structure, texture, color, and water content. UTC was classified into four types of callus based on morphology character (Table 1, Figure 1). Each type of transformed callus was cultured into the modified medium.

Medium modification

The basal medium for regeneration calli was deFossard (DF) (de Fossard et al., 1974) supplemented with 30 g L^{-1} sucrose, 1 g L^{-1} activated charcoal, and pH media was set at 5.6-5.8 before autoclave sterilization. The medium was sterilized using an autoclave at 120°C and pressure 101 Pa for 20 minutes. Four types of UTC were treated in different modified media (Table 1, Figure 1).

Table 1. Morphological characteristics of oil palm transformed callus

Type	Characteristic	Callus structure	Callus surface texture	Dominant callus color	Callus water composition
1	Non-embryogenic	Compact	Nodular – dried	Creamy-White	Anhydrous
2	Non-embryogenic	Not compact	Spongy - watery	Creamy-Translucent	Hydrous
3	Non-embryogenic	Compact	Nodular – dried	Creamy – Brown	Anhydrous
4	Non-embryogenic	Compact	Nodular – dried	Brownish – Black	Anhydrous

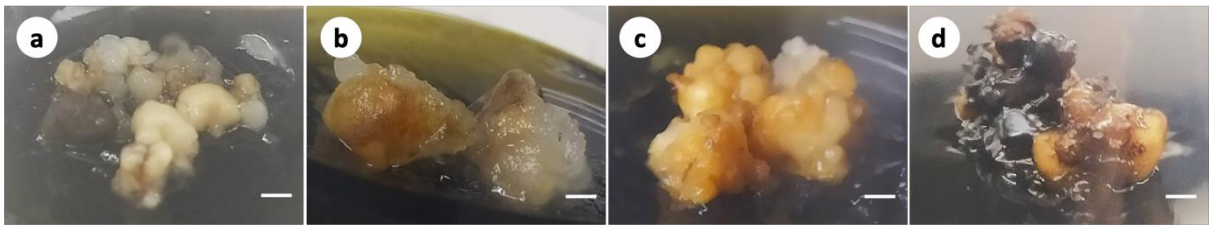

Figure 1. Type of oil palm transformed callus (a) Type-1; (b) Type-2; (c) Type-3; (d) Type-4 (1 bar = 25 mm)

Type-1 callus was cultured in a medium enriched with 0, 5, and 10 mg L^{-1} of glutamine (medium was coded as G0 (control), G5, and G10). For Type-2, the media was enriched with 5 mg L^{-1} cysteine combined with putrescine at 0, 10, and 20 mg L^{-1} (medium was coded as C5P0 (control), C5P10, and C5P20). Meanwhile, for Type-3 and Type-4 callus, the media was enriched with cysteine combined with ascorbic acid at 0, 10, and 25 mg L^{-1} (coded as C0A0 (control), C10A10, and C25A25). The medium was placed in jar bottles, with each jar containing 30 mL of medium. Every treatment consisted of four replications. The cultures were then incubated in the dark condition at 27±2°C. Afterwards, the callus weight was measured using a digital balance aseptically every four weeks, representing the culture cycles.

Molecular detection

All samples were ground using micro-pestle and DNA from callus was extracted using the CTAB method (*Doyle & Doyle, 1990*). Polymerase chain reaction (PCR) detection was performed using *ACT* primers specific to β-actin (ACT) as a housekeeping gene (F: 5'-CCC-ACC-TGA-ACG-GAA-ATA-CA-3'; R: 5'-CGG-ATG-GCA-CCT-CAG-TCT-TA-3') and *NPTII* primer to amplify the kanamycin resistance gene (F: 5'-ATC-GGG-AGC-GGC-GAT-ACC-GT-3'; R: 5'-TAG-CCG-TCG-CCG-CTA-TGG-CA-3'). PCR was conducted in 35 cycles with the condition: pre-denaturation (95 °C; 1 min), denaturation (95 °C; 15 s), annealing (55 °C; 15 s), extension (72 °C; 10 s), and post-extension (72 °C; 10 min). The electrophoresis was conducted using 0.8% agarose gel in 0.5x TBE buffer.

Statistical analysis

The experiments were performed with four replications. A one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used to establish the significance ($P<0.05$) of differences. Data were analyzed using the SPSS v.2 software (IBM, USA).

Results and Discussion

Molecular detection of un-regenerated transformed callus (UTC)

The presence of transgene construct in UTC was carried out using PCR targeting the marker gene, *NPTII*. Amplification of *NPTII* gene was observed presence only in samples C#1, C#2, and C#4 (Figure 2). This means these clumps were expected to be positive transformants and can continue regenerating to be fully planted. In plant transformation using *Agrobacterium tumefaciens*, T-DNA integration into plant chromosomes is the mark that the system is successfully carried out, and we will get a stable plant. According to Gelvin (2021), integrating T-DNA from *Agrobacterium* into plant cells involves DNA repair process. This is because the process contains invasion and ligation of DNA. In addition, samples C#3, C#5, and C#6 did not show any band in the electropherogram. This result means there is a failure in the T-DNA integration system. In Edwards et al., (2022), the integration of T-DNA via *Agrobacterium*-mediated transformation is still random in the plant genome. Recent studies also reveal that the T-DNA integration may relate to epigenetic phenomenon. So, it is difficult to detect the precise location of the integration. Furthermore, as internal control, *ACT* was also amplified to convince that PCR was obtained successfully. In Figure 2, *ACT* fragment could be amplified in samples except for the non-template control (C(-)) with a band size 400 bp.

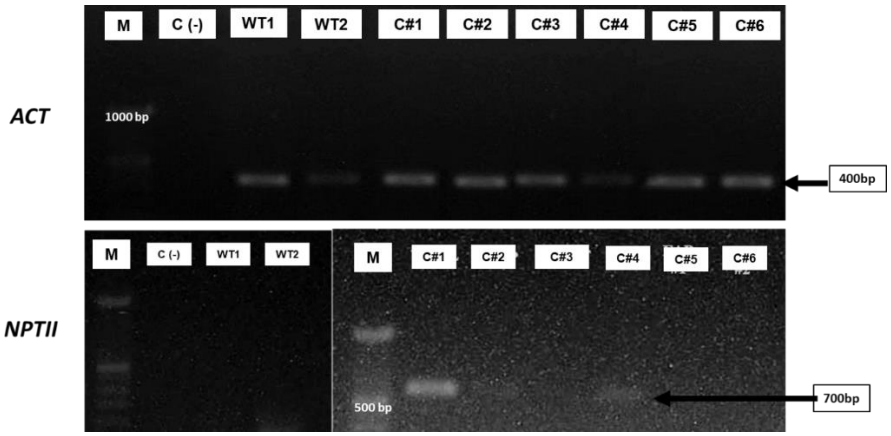

Figure 2. Electrophoregram T-DNA detection in UTC. Marker (M); non-template control (C(-)); Wild Type (WT); Callus (C)

Regeneration of UTC on enrichment medium

Four types of UTC used in this research are classified into non-embryogenic calli. According to Rajesh et al. (2003), embryogenic calli in oil palms had a whitish-yellow and friable appearance. Meanwhile, the non-embryogenic callus looked translucent and spongy. Regeneration in plants includes cell repair and organ reconstruction after injury. The process of plant regeneration is divided into two cellular strategies: reactivation of undifferentiated cells and reprogramming of differentiated somatic cells (Ikeuchi et al., 2019). Efforts to improve the health of transformed calli are necessary for the success of biotechnology breeding programs, and one way to achieve this is by modifying the culture media using ascorbic acid, hydrolyzed casein, glutamine, asparagine, and arginine at variable concentrations.

Data showed that each callus type had a different response (Table 2). Glutamine enrichment for Type-1 callus did not significantly affect callus weight. The combination of additive chemical cysteine and putrescine in Type-2 callus also did not show a significant result depending on its control. However, adding cysteine and ascorbic acid for callus Type-3 and Type-4 can provide statistically different results compared to the control after three culture cycles. In oil palm, a suitable protocol for micropropagation was carried out, and the result showed that the response varies depending on the type of explant, genotype, and age of the explant. Regeneration rates in oil palm somatic embryogenesis still become an obstacle. Usually, somatic embryos fail to develop a shoot or root system (Gomes et al., 2016).

Effect of L-glutamine in UTC Type-1

Based on the result, medium G10 enhanced callus growth rate by 2.13 mg until the 3rd cycle (Table 2). In Elmeer (2013), glutamine is one of the amino acids that are most available in the cell. Abundant glutamine in the cell could be a source of energy when the cell is dividing rapidly. According to Mariani et al. (2018), optimizing callus regeneration in oil palms using amino acids, specifically glutamine 10 mM, affected callus size. Allegedly, adding glutamine affects cell division due to the enlargement of oil palm callus meristematic zone.

In the callus biomass, every treatment of L-Glutamine rises in each cycle and remains stagnant in the 3rd cycle until 5th cycle (Figure 3). Callus stagnancy after 3rd cycle may be caused by the decreased concentration of L-Glutamine. According to Pawar et al. (2015) in rice, L-Glutamine 500 mg L^{-1} can enhance callus growth rate and it is a relatively non-toxic additive chemical that can maintain callus health and growth. According to Velmurugan & Sivakumar (2020), the best concentration of L-Glutamine for callus growth and callus phenotype in *Stevia rebaudiana* was 15 mg L^{-1}.

*T*able 2. Effect of medium enrichment on Un-regenerated Transformed Callus (UTC) after three cycles of culture

Callus type	Medium treatment	Average callus growth rate (mg)
Type-1	G0 (Control)	1.52 [a*)]
	G5	1.75 [a]
	G10	2.13 [a]
	P-Value	**NS**
Type-2	C5P0 (Control)	1.26 [a]
	C5P10	1.19 [a]
	C5P20	1.97 [a]
	P-Value	**NS**
Type-3 and Type-4	C0A0 (Control)	0.73 [a]
	C10A10	0.89 [a]
	C25A25	1.50 [b]
	P-Value	**0.0043**

*) *Means in the same column of the same callus type followed by the same letters are not significantly different according to Duncan's multiple range test at α = 0.05.*

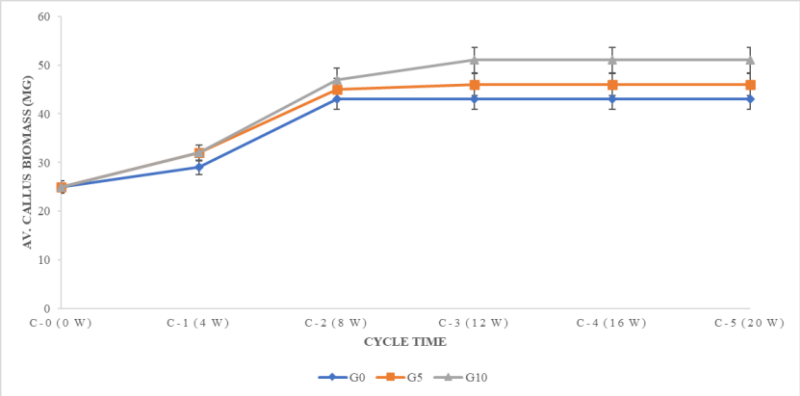

Figure 3. Biomass of UTC Type-1 in regeneration medium enriched with L-Glutamine

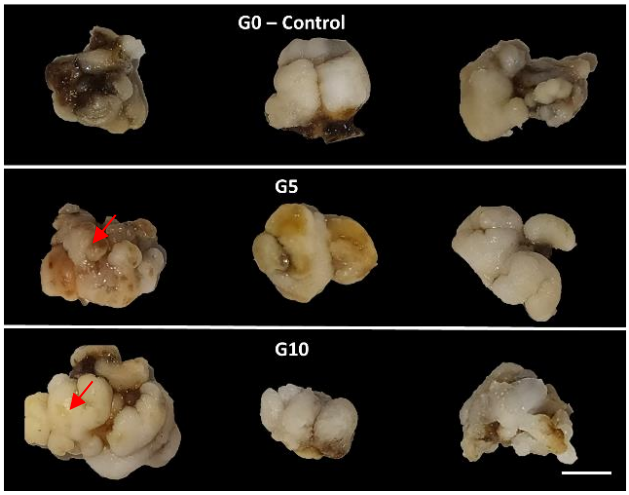

Figure 4. UTC Type-1 callus at 20 weeks old on medium with L-Glutamine enrichment (1 bar = 100mm)

The morphology in 20-week-old UTC showed that control (G0) did not show any additional formation of cells; meanwhile, G5 and G10 could give additional cell growth phenotypes (red arrow) (Figure 4). Adding glutamine into the medium show positive result in the way some friable callus structures are expected as a meristematic callus. This structure perhaps could develop into a new oil palm-transformed callus. According to Karyanti et al. (2021), the best time for callus proliferation in a liquid medium occurs from the 1st to 4th subculture cycle and decreases after 5th to 7th.

Effect of putrescine in the UTC Type-2

Based on Table 2, the average callus growth rate in each treatment is not significantly different depending on the control. In Figure 5, the biomass of UTC Type-2 increased in 1st and 2nd cycles, but C5P20 gave the best enhancement, although the former weight of the Type-2 callus was lighter than others. Interestingly, we also found the stagnancy pattern in Type-2 transformed callus. Stagnancy occurs from the 3rd cycle until the 5th cycle.

UTC Type-2 was the callus with a watery and soft structure (Figure 1). Polyamines are reported to play a role in the various physiological and developmental processes in plants. This research found that the medium combination of C5P20 was the best concentration to develop a watery callus into dense callus (red arrow). The morphology of callus in 20 weeks old depicted in Figure 6. Medium C5P20 provides callus with a solid texture (red arrow) compared to C5P0 and C5P10. The embryogenic callus and its regeneration ability in oil palms could be projected from its structure. Usually, calli with soft and watery structures lose 20% of the ability to regenerate (Karyanti et al., 2021). In oil palm, 1 mM putrescine-containing medium enhances the formation of somatic embryos and secondary somatic embryo shoot meristemoid (Rajesh et al., 2003). Report from El-Dawayati et al. (2018) in date palm, 100 mg L^{-1} putrescine can induce more than 30 somatic embryos from fresh callus.

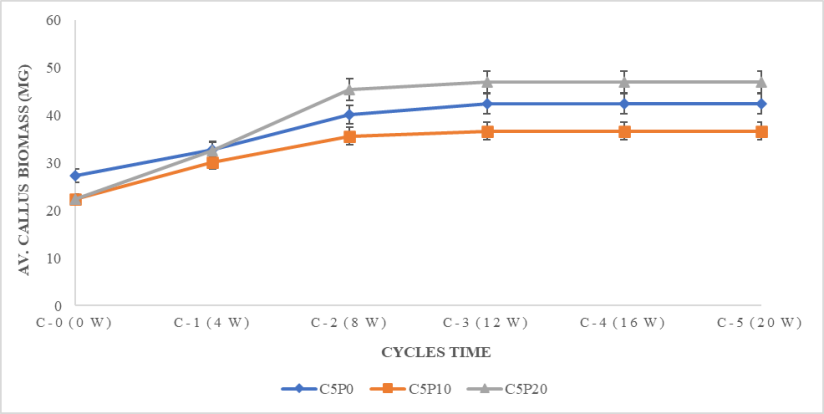

Figure 5. Biomass of UTC Type-2 in regeneration medium enrichment with L-Cysteine and Putrescine

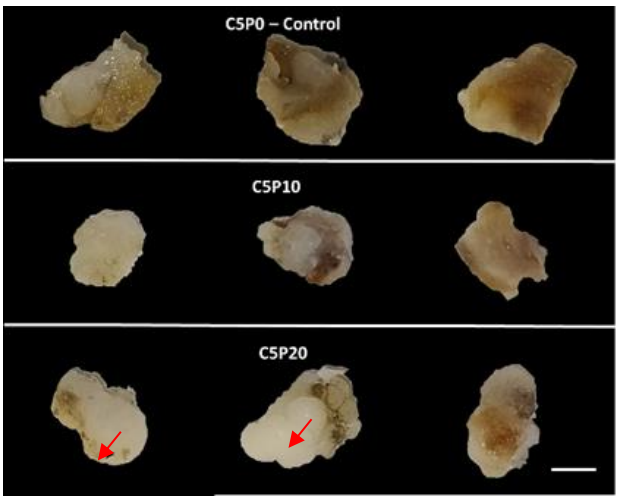

Figure 6. Type-2 callus at 20 weeks old on medium with Putrescine and L-Cysteine enrichment (1 bar = 100 mm)

Effect of L-Cysteine and ascorbic acid on the UTC Type-3 and Type-4

The most significant difference between Type-3 and Type-4 was in the severity level of browning. The main objective of this treatment was to give the best combination of antioxidants to generate a healthy secondary callus. The best medium to enhance callus biomass was in the C25A25, either in Type-3 or Type-4 (Figure 7). Callus morphology after 20 weeks of incubation in medium treatment showed that Type-3 still could regenerate a secondary callus. Meanwhile, Type-4 did not show any formation of fresh callus (Figure 8). Browning of plant tissue culture is usually caused by a chemical process reaction such as lipid oxidation, enzymatic oxidation, carbonyl amine reaction, and phenylalanine deamination (Wu et al., 2018). The severe browning can cause the failure of regeneration into a plantlet and the death of the explant.

Phenolic compound secretions in the tissue culture process have been associated with a low conversion rate in the regeneration stage. According to Alemanno et al. (2003), only a small amount of polyphenol could be found in the regenerated somatic embryos of cocoa, while polyphenols were distributed randomly across all tissues in non-regeneration plants. Consistent with Khosroushahi et al. (2011), phenolic compounds are a phenomenon that involves many toxic compounds that can cause cell necrosis. Adding ascorbic acid and cysteine in oil palms could prevent browning during callus induction and lead to embryo maturation. The dosage that is usually used varies depending on genotype, culture stages, and explant type. According to Romero et al. (2013), Cysteine plays an important role as a precursor to a large number of essential biomolecules. Cysteine possesses a thiol group that is usually located in the active sites. This group can catalyze the enzymatic reaction related to numerous plant cell processes (Richau et al., 2012).

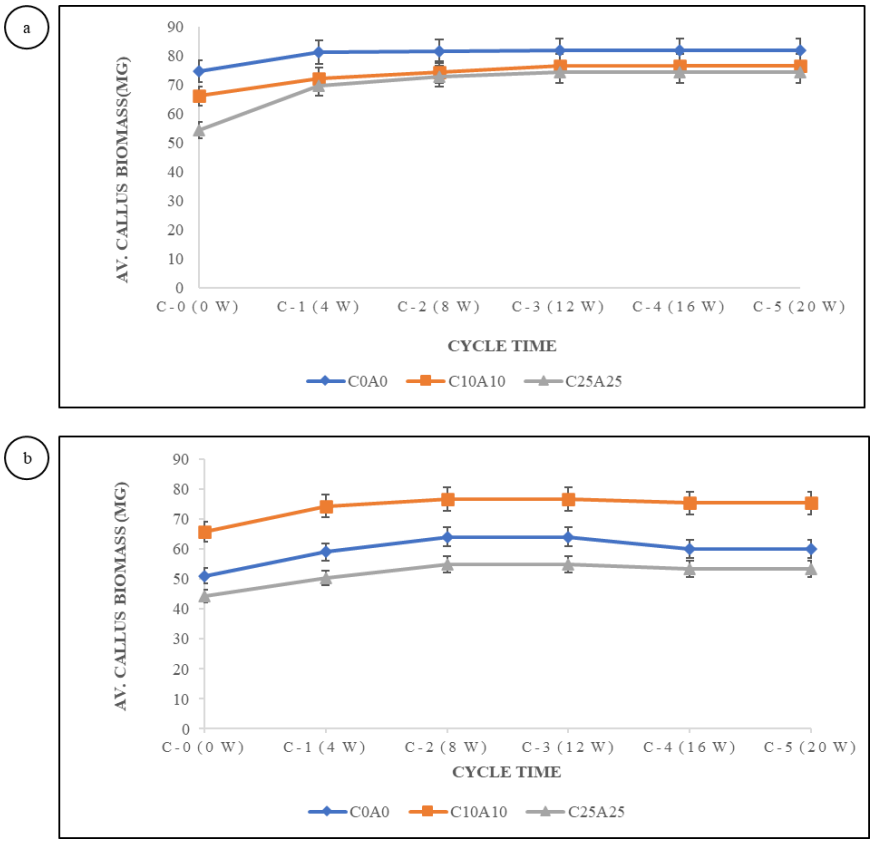

Figure 7. Biomass of UTC Type-3 (a) and Type-4 (b) in regeneration medium enriched with L-Cysteine and ascorbic acid

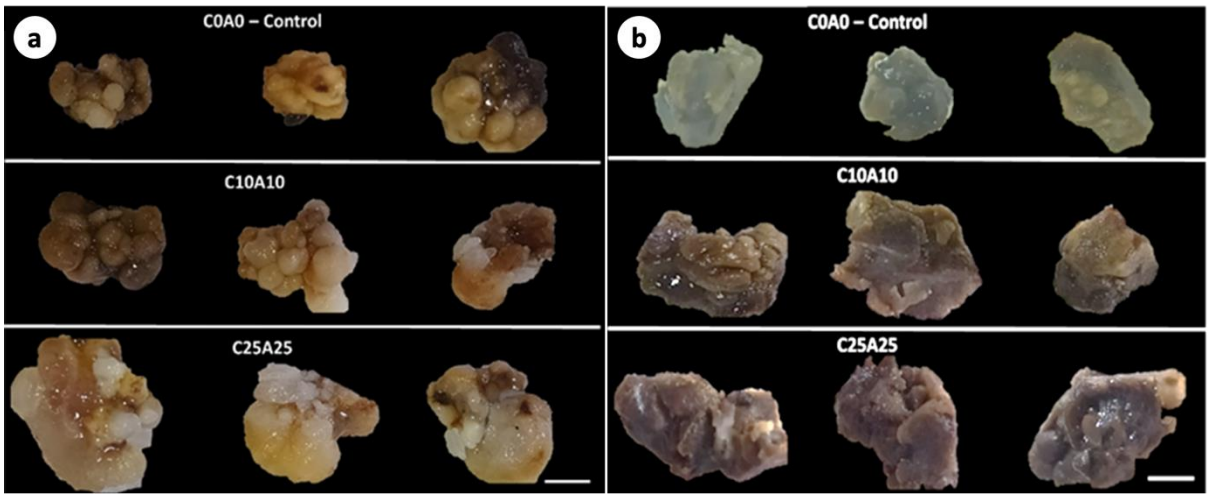

Figure 8. Type-3 (a) and Type-4 (b) calli at 20 weeks old on medium with L-Cysteine and ascorbic acid enrichment (1 bar = 100 mm)

The stagnancy phenomenon was also observed in Type-3 and Type-4 calli. This could be caused by the severity of browning in callus and the dosage range being unsuitable for oil palms. According to Permadi et al. (2023), in *Musa spp*. the addition of ascorbic acid at 10 – 150 mg L^{-1} has been reported to decrease tissue darkening. Therefore, ascorbic acid is thermolabile, so adding it into the medium may become a determining factor in minimizing lethal browning.

Conclusion

Four types of transformed callus were characterized as non-embryogenic calli. These types of calli have not been successfully regenerated into plantlets. Each type of callus has a stagnancy phenomenon after 3rd cycle of subculture. In Type-1

callus, L-Glutamine can enhance callus biomass. The best treatment was found in 10 mg L^{-1} L-Glutamine, which indicates positive callus development. Type-2 callus on medium with L-Cysteine and Putrescine (C5P20) show morphological development with higher callus density. A combination of L-Cysteine and ascorbic acid (C25A25) can develop a new secondary structure in Type-3 callus, but there was no morphological development in Type-4 callus.

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References

Alemanno, L., Ramos, T., Gargadenec, A., Andary, C., & Ferriere, N. (2003). Localization and identification of phenolic compounds in Theobroma cacao L. somatic embryogenesis. An Bot. 92 (4), 613-623. doi: 10.1093/aob/mcg177

Bahariah, B., Masani, M. Y. A., Fizree, M. P. M. A. A., Rasid, O. A., & Parveez, G. K. A. (2023). Multiplex CRISPR/Cas9 gene-editing platform in oil palm targeting mutations in EgFAD2 and EgPAT genes. Journal of Genetic Engineering and Biotechnology, 21(1). https://doi.org/10.1186/s43141-022-00459-5

Budiani, A., Nugroho, I. B., Sari, D. A., Palupi, I., & Putranto, R. A. (2019). CRISPR / Cas9 - mediated knockout of an oil palm defense - related gene to the pathogenic fungus Ganoderma boninense. 24(2), 0–4. https://doi.org/10.22146/ijbiotech.52170

Bhat, S.N., Khalil. A., Nazir, N., Mir, M.A., Khan, I., Mubashir, S.S., Dar, M.S., Wani, S.H., & Hossain, M.A. (2022). In vitro prevention of browning in Persian Walnut (Juglans regia L.) cv. Sulaiman. International Journal of Plant Biology 13(3),330-342. https://doi.org/10.3390/ijpb13030027

de Fossard, R.A., Myint, A., & Lee, E.C.M. 1974. A broad spectrum tissue culture experiment with tobacco (Nicotiana tabacum L.) pith tissue callus. Physiol Plant 30, 125-130. https://doi.org/10.1111/j.13993054.1974.tb03116.x

Chen, D., Shao, Q., Yin, L., & Younis, A. (2019). Polyamine Function in Plants : Metabolism , Regulation on Development , and Roles in Abiotic Stress Responses. 9(January), 1–13. https://doi.org/10.3389/fpls.2018.01945

Dellero, Y. (2020). Manipulating Amino Acid Metabolism to Improve Crop Nitrogen Use Efficiency for a Sustainable Agriculture. 11.https://doi.org/10.3389/fpls.2020.602548

Doyle, J.J., & Doyle, J.J. (1990). Isolation of Plant DNA From Fresh Tissue. FOCUS, 12(1).

Edwards, B., Hornstein, E. D., Wilson, N. J., & Sederoff, H. (2022). High - throughput detection of T - DNA insertion sites for multiple transgenes in complex genomes. BMC Genomics, 1–20. https://doi.org/10.1186/s12864-022-08918-6

El-Dawayati, M., H.S, Ghazzawy., M, Munir. 2018. Somatic embryogenesis enhancement of date palm cultivar Sewi using different types of polyamines and glutamine amino acid concentration under in-vitro solid and liquid media conditions. Int. J. Biosci. 12(1): 10.12692/ijb/12.1.149-159

Gelvin, S. B. (2021). Plant DNA Repair and Agrobacterium T − DNA Integration. International Journal of Molecular Sciences Review 22 (8458). https://doi.org/10.3390/ijms22168458

Gomes, T. H., Monah, P., Bartos, C., Aparecida, T., & Scherwinski-pereira, J. E. (2016). Regeneration of somatic embryos of oil palm (Elaeis guineensis) using temporary immersion bioreactors. Industrial Crops & Products, 89, 244–249. https://doi.org/10.1016/j.indcrop.2016. 05.021

Ikeuchi, M., Favero, D. S., Sakamoto, Y., Iwase, A., Coleman, D., Rymen, B., & Sugimoto, K. (2019). Molecular Mechanisms of Plant Regeneration. Annu. Rev. Plant Biol, 70. https://doi.org/10.1146/annurev-arplant050718

Karyanti., T, Tajudin., H, Khairiyah., D, Purwoko., T, Sukarnih., G, Rahmadara., N.F, Hanifah., Y, Rudiyana., S, Kitagawa., F.R, Mira., & H.Saga. (2021). Proliferation of oil palm (Elaeis guineensis Jacq) Embrogenic Callus with Repeated Subculture in Liquid Medium. Jurnal Bioteknologi & Biosains Indonesia. 1 (8): 1- 13. Doi: 10.29122/jbbi.v8i1.4715

Khosroushahi, AY., Naderi-Manesh H & Simonsen HT. (2011). Effect of antioxidants and carbohydrates in callus cultures of Taxus brevifolia: Evaluation of browning, callus growth, total phenolics and paclitaxel production. BioImpacts 1(1): 37-45. doi: 10.5681/bi.2011.006

Mahlia, T. M. I., Ismail, N., Hossain, N., Silitonga, A. S., & Shamsuddin, A. H. (2019). Palm oil and its wastes as bioenergy sources: a comprehensive

review. In Environmental Science and Pollution Research. Springer Verlag. https://doi.org/ 10.1007/ s11356-019-04563-x

Mariani, T.S., Purnaning, A.S, & Latif, S. (2018) Effect of Glutamine Addition in Maturation Stage on the Germination and Plantlet Conversion of Oil Palm (Elaeis guineensis Jacq.) Somatic Embryo. American Journal of Applied Sciences 9(1)

Minarsih, H., Permatasari, G. W., Setiawati, Y., Mardhika, L. D., & Saptari, R. T. (2023). Transformation of CRISPR / Cas9 expression construct to enhance saturated fatty acid synthesis in oil palm for biofuel production. 1–7. IOP Conf. Series: Earth and Environmental Science 1255 (012058) doi:10.1088/1755-1315/1255/1/012058

Murphy, D. J., Goggin, K., & Paterson, R. R. M. (2021). Oil palm in the 2020s and beyond: challenges and solutions. In CABI Agriculture and Bioscience (Vol. 2, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s43170-021-00058-3

Ndakidemi, C., Mneney, E. & Ndakidemi, P. (2014). Effects of ascorbic acid in controlling lethal browning in in vitro culture of Brahylaena huillensis using nodal segments. American Journal of Plant Sciences, 5(1), 187-191. doi: 10.4236/ajps.2014.51024.

Pawar, B., Kale, P., Bahurupe, J., Jadhav, A. Kale, A., & Pawar, S.. (2015). Proline and Glutamine Improve in vitro Callus Induction and Subsequent Shooting in Rice. Rice Science 22(6): 283-289. https://doi.org/10.1016/j.rsci. 2015. 11. 001

Permadi, N., Nurzaman, M., Alhasnawi, A.N., F, Doni, F., & Julaeha, E. (2023). Managing Lethal Browning & Microbial Contamination in Musa spp. Tissue Culture: Synthesis and Perspectives. Horticulturae, 9(4). https://doi.org/10.3390/ horticulturae 9040453

Richau, K.H., Kaschani, F., Verdoes, M., Pansuriya, T.C., niessen, S., Stuber, K., Colby, T., Overkleeft, H.S., Bogyo, M., & Van der Hoorn, R.A. (2012). Subclassification and biochemical analysis of plant papain-like cysteine proteases displays subfamily-specific characteristics. Plant Physiol. 158, 1583–1599. https://doi.org/ 10.1104/pp.112.194001

Romero, L.C., Garcia, I., & Gotor, C. (2013). L-Cysteine Desulfhydrase 1 modulates the generation of the signaling molecule sulfide in plant cytosol. Plant Signal. Behav. 8, e24007. doi: 10.4161/psb.24007

Rajesh, M. K., Radha, E., Karun, A., & Parthasarathy, V. A. (2003). Plant regeneration from embryo-derived callus of oil palm-the effect of exogenous polyamines. In Plant Cell, Tissue and Organ Culture (Vol. 75).

Sakhanokho, H. F., Ozias-Akins, P., May, O. L., & Chee, P. W. (2005). Putrescine enhances somatic embryogenesis and plant regeneration in upland cotton. Plant Cell, Tissue and Organ Culture, 81(1), 91–95. https://doi.org/10.1007/s11240-004-1541-3

Sinta, M. M. Saptari, R.T, Riyadi., I & Sumaryono (2024). Browning prevention of oil palm somatic embryos in the temporary immersion system with antioxidant mixture and modification of basal media. IOP Conf. Ser.: Earth Environ. Sci. 1308 012002. Doi: 10.1088/1755-1315/1308/1/012002

Sivanandhan G, Mariashibu TS, Arun M, Rajesh M, Kasthurirengan S, Selvaraj N, G. A. (2011). The effect of polyamines on the efficiency of multiplication and rooting of Withania somnifera (L.) Dunal and content of some withanolides in obtained plants. Acta Physiol Plant, 33, 2279.

Velmurugan, R and S.R, Sivakumar. 2020. Effect of L: Glutamine on in vitro callus induction and embryogenic potential of Stevia rebaudiana Bert. International Journal of Botany Studies 5(6): 355 – 357. doi: 10.24247/ijbtrapr20181

Wu, T. Li, J. Zhang, J., Lin, M., Wu, Z., Cai, X., Xiang, W., Tan, S., Zhang, Z. (2018). Graphene oxide inhibits the lethal browning of Cymbidium sinense by reducing activities of enzymes. J. Plant Biotechnol. Microbiol. 1: 11–20.