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REGENERATION OF *Dura* PALM FROM RAMET VIA SOLID AND LIQUID TISSUE CULTURE SYSTEMS

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ABSTRACT

Oil palm tissue culture via clonal propagation provides an efficient means for mass propagation. In this study, we explored the viability of cabbage from young ramets (two years old) of dura *as an alternative to mature ortet cabbages for* in-vitro *regeneration. We hypothesised that cabbage from young ramet has similar potential in terms of embryogenic and regeneration efficiency over mature ortet. During the callus induction phase, the explants developed three distinct forms of callus, namely primary, friable and nodular. We used nodular callus and friable callus for regeneration of* in vitro *plantlets, using solid and liquid systems, respectively. The callusing, embryogenic and friability rates over explants are 78.3%, 67.3% and 9.3%, respectively. The nodular callus successfully regenerated into 5,074 plantlets in the solid culture system, while 136 plantlets were regenerated from the friable callus in the liquid culture system. Our findings indicate that young ramet demonstrates regenerative potential, offering other advantages such as easier handling, reduced risk of palm death and minimised injury during sampling. These initial findings can be used to maintain and preserve the elite* dura *palm as an essential source of germplasm for breeding.*

Keywords: *dura*, embryogenic calli, oil palm tissue culture, ramets.

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INTRODUCTION

In the breeding of commercial palms, *dura* is an essential source of germplasm (Kushairi & Amiruddin, 2022). *Dura* functions as a pure parental line (mother palm) and is often used to cross with another parent in the oil palm breeding programme. It can be used for biclonal programmes, crossing and backcrossing to generate hybrids, including transgenic lines, which is impossible with *tenera* (Zulkifli et al., 2017). Seeds are the sole means by which oil palm naturally reproduces, but the constraints involved include the relatively low seed germination rate (Green et al., 2013), genetic variability, prolonged juvenile phases and low uniformity in elite planting materials (Corley & Tinker, 2016). Due to these limitations, maintaining or preserving *dura* palms through seeds is not a practical approach. Moreover, for the production of transgenic oil palm lines, the optimal starting material for DNA transformation is embryogenic calli (Masani et al., 2018). Therefore, the establishment of a tissue culture protocol for *dura* palms is essential to enable genetic improvements and the development of transgenic varieties.

Since oil palm has only one growing apex, vegetative propagation is not viable (Weckx

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et al., 2019), thus tissue culture technique via micropropagation or clonal propagation provides an efficient means for mass propagation of genetically uniform oil palm clones (Hashim et al., 2018). This approach allows for the multiplication of elite planting materials while maintaining genetic stability and high-yield traits. The aim was not only focused on producing high-yielding oil palm materials but also on producing clones with outstanding traits, such as virescens, dwarf, high bunch index and compactness (Zulkifli et al., 2017). Efforts to improve the oil palm tissue culture protocols were extensively carried out and were reported by Gomes et al. (2016), Wan Nur Syuhada et al. (2016) , Monteiro et al. (2017) and Hashim et al. (2018).

Most of tissue culture laboratories use young spear leaves from ortets originated from mature oil palms as the explant to initiate callogenesis and somatic embryogenesis (Ooi et al., 2013). Although ramets have significant advantages, not many laboratories use them as explants for oil palm clonal propagation. This may be due to the preference for ortets, which become a standard approach for oil palm tissue culture (Hashim et al., 2018). However, using ramets as explants offers several advantages. For example, ramets ensure genetic uniformity, as they are clonal materials that maintain the genetic identity of the parent oil palm, leading to consistent and predictable results in the resulting tissue cultures (Martin et al., 2022). This genetic stability is crucial for producing homogeneous oil palms with desired traits, such as disease resistance and high oil yield.

Despite the common use of cabbages from mature ortet as explants, their sampling process poses challenges, including high labour intensity, risk of palm injury, and limited availability of elite germplasm. Young ramet cabbages offer a promising alternative to mature palm, yet their potential remains underexplored. This study evaluates the potential of cabbage explants from two years old *dura* clonal ramets, to produce callus and somatic embryos and subsequently regenerate into plantlets via solid and liquid culture systems.

Recent studies highlight significant advancements in somatic embryogenesis and clonal propagation, improving regeneration efficiency and reducing genetic variability (Karim, 2021; Weckx et al., 2019). Additionally, study from the Malaysian Palm Oil Board (MPOB) has refined techniques for liquid culture systems, optimising callus proliferation and embryogenic development for large-scale propagation (MPOB, 2025). Further innovations in bioreactor-based culture systems have enhanced multiplication rates while maintaining genetic stability (Yarra et al., 2019).

We hypothesised that young ramet cabbage would exhibit similar or better regeneration potential compared to mature ortet. The nodular callus was used for the solid culture system because of its compact and organised structure, which supports somatic embryogenesis and facilitates differentiation during plantlet regeneration. Conversely, friable callus is more suitable for the liquid culture system because it consists of rapidly growing cells and loosely attached cells, which promotes rapid multiplication of undifferentiated cells and uniform formation of embryos due to better absorption of nutrients and hormones in the media. The embryogenic states of the callus were confirmed through double staining and histological analysis. The embryogenic callus development at different stages through solid or liquid culture systems was observed and the callusing, friability, embryogenesis and regeneration rate were calculated.

MATERIALS AND METHODS

Plant Material, Explant Preparation and Callus Induction

Two years old ramets (D614) from Pamol *dura* background were used to provide explants for callus initiation (*Figure 1a*). The ramets originated from the ortet of *dura* mature palms and had been generated through the solid culture system. The ramets were removed from the polybag, and the shoots and roots were cut and trimmed to approximately 15 cm in length, for easy handling (*Figure 1b*). The trimmed ramets were thoroughly washed under running tap water until clean. For surface sterilisation, the trimmed ramets were then transferred into a laminar flow and were washed a few times with sterile distilled water, followed by 70% (v/v) ethanol and then air dried.

The harder outer leaf layers covering the cabbage were carefully removed layer by layer with a sterile scalpel and forceps (*Figure 1c*). The cabbage basal and medial parts, approximately 6 cm in length, were cut and transferred into a petri dish to avoid drying out (*Figure 1d* and *1e*). The 6 cm explants were then cut into smaller pieces of approximately 1 cm in length and sliced into five small strips with widths ranging from 0.10-0.25 cm. Five strips (*Figure 1f*) were transferred onto a petri dish containing 30 mL of solidified callus induction medium (CIM) (Hashim et al., 2018). The cultures were incubated in dark conditions at 28 ± 1ºC with ±60% relative humidity. Explants were observed monthly, and the formation of the primary callus was recorded. The cultures were subcultured every three months on fresh CIM. The formation of primary callus was continuously monitored for up to 12 months before the explants were discarded.

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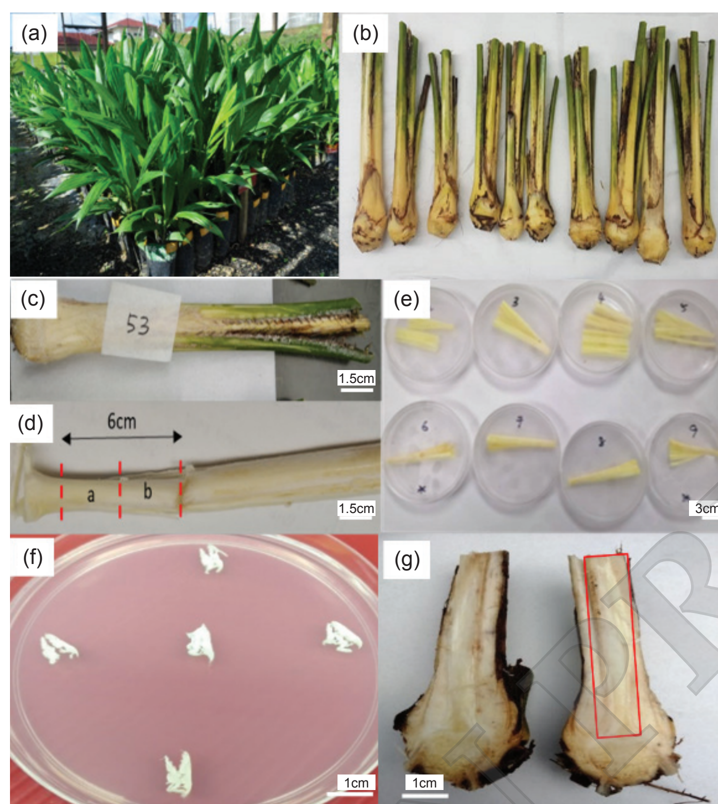

Figure 1. Preparation of cabbage or leaf explants from oil palm dura *ramets. (a) Two years old oil palm ramets in polybags. (b) Washed and trimmed ramets for surface sterilisation. (c) Surface sterile ramet before the outer layers are removed. The basal and medial parts (a, b) of cabbage, (d) approximately 6 cm in length. (e) Leaf explants were set aside in petri dishes. (f) Thinly sliced leaf explants were cultured onto callus initiation medium. (g) The vertical section of cabbage is located at the centre part of the ramet (red box).*

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Regeneration of Nodular Calli in Solid Culture System

The nodular calli (*Figure 2m* and *2n*) with their attached leaf explant strips were cultured onto the embryoid induction medium. This medium is similar to the callus induction media (CIM) but contains half the amount of hormones. The compact nodular callus was continuously subcultured in dark conditions at three-month intervals for embryoid initiation. Explant strips without any callus formation were continued to be subcultured onto fresh CIM at three-month intervals and maintained for a total of one year before discarding. The embryoids obtained were further regenerated into polyembryoids, shoots and roots. All media used for the regeneration process were referred to Hashim et al. (2018).

Initiation of Cell Suspension in Liquid Culture System

Friable calli (*Figure 2n*) derived from the explants were used to initiate cell suspension culture for the liquid culture system. A basic protocol for the liquid culture system developed by MPOB was followed (Hashim et al., 2018). A portion of friable calli (approximately 0.05 g) was transferred into a 100 mL flask containing 20 mL liquid medium (L-1). The flasks were incubated in dark conditions at 28 ± 1°C and were shaken on a rotary shaker at 100 rpm. The suspension cultures were filtered using a 100 mm sieve, and approximately 0.5 g of the calli were collected and transferred into fresh L-1 medium every 3 weeks for a total of nine subcultures. The number of flasks and calli weight were recorded, and graphs were plotted. Calli at each of the nine subculture stages were collected and transferred onto solid media for regeneration through the solid culture system. The cultures were incubated in dark conditions for a month before transferring to light (16 hr) and dark (8 hr) conditions at 28 ± 1°C. Regeneration of the calli into complete plantlets was observed, and data were recorded.

Double Staining and Histological Analysis

Double staining was performed according to the method described by Mohajer et al. (2012). The stained samples were examined under a light

microscope (Nikon AZ100, Japan). For histological analysis, the calli were sampled, embedded and stained with Periodic acid-Schiff and Naphthol Blue Black solutions according to the method used by Fakhrana et al. (2019). The stained samples were viewed under a light microscope (Leica DM600, Germany).

RESULTS AND DISCUSSION

Callus Initiation from Cabbage Explants of Two Years Old *Dura* Ramets

A total of 30 ramets were used, yielding 1,775 explants (*Figure 2a*) for callus initiation. After one month, a total of 1,390 primary calli were produced. The primary callus had a soft texture with yellowish to brownish colour and morphological variations were observed, including uneven, globular and elongated shapes (*Figure 2b-2d*). Less than 1% of the primary callus exhibited abnormal morphology, which resembles organ-like features, namely cotton- and root-like with pneumatodes (*Figure 2e-2l*). The normal primary callus further developed into nodular callus and friable callus, while some remained dormant without further morphological changes (*Figure 2m-2n*).

All data collected from this study were summarised in *Table 1*. It was observed that cabbage from each ramet produced primary calli (100.0%). A total of 165 friable calli and 1,194 embryogenic calli were obtained. The callusing rate for primary calli over total explants was 78.3%. Meanwhile, friable rates over explants and primary calli were calculated as 9.3% and 11.9%, respectively. However, out of 30 ramets, only 26 could produce friable calli. Meanwhile, the embryogenic rates calculated for explants and primary calli are 67.0% and 86.0%, respectively.

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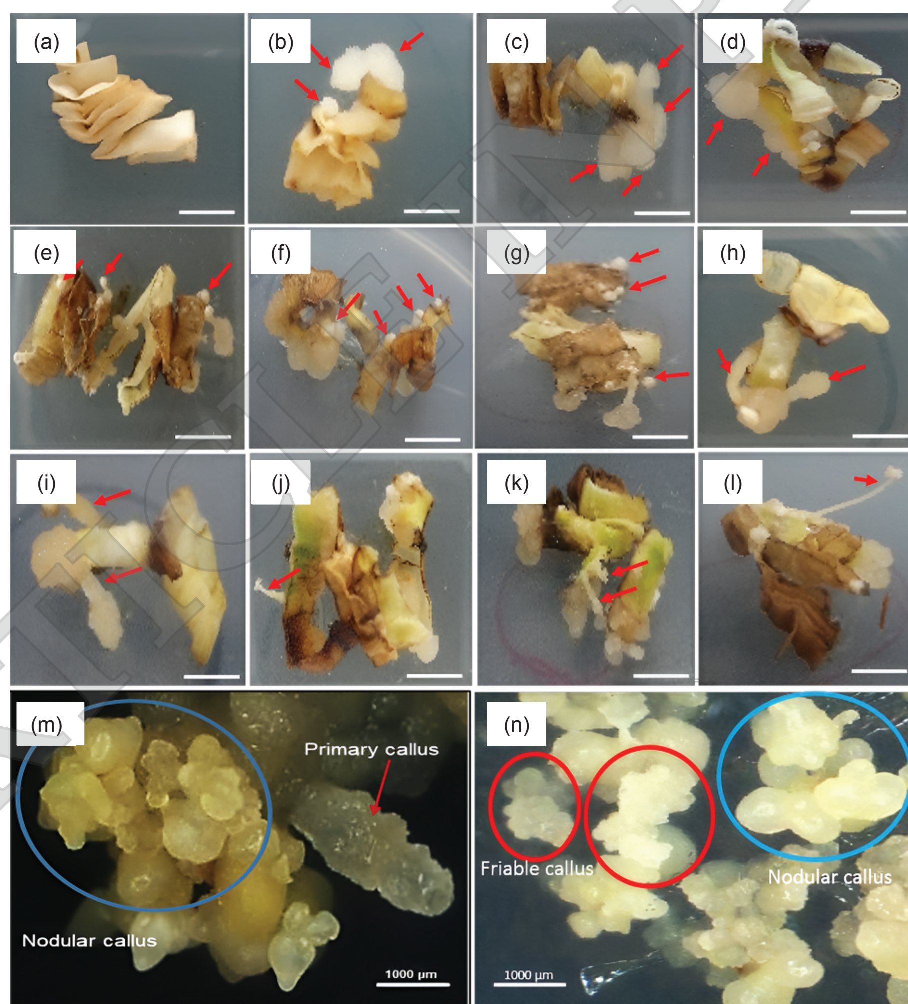

Note: Bar in (a-l) is 0.25 cm, and (m-n) is 1,000 µm.

Figure 2. Formation of different types (indicated by red arrows) of oil palm primary calli observed on (a) cultured leaf explants from two-year-old ramets. (b, c, d) Formation of normal primary callus and (e, f, g) abnormal callus types such as cotton-like callus, (h, i) root-like callus and (j, k, l) root-like callus with pneumatodes. (m, n) Development of oil palm primary callus into nodular callus (circled in blue) and friable callus (circled in red). A dormant or unchanged callus is shown by a red arrow in (m).

TABLE 1. CULTURE PRODUCTION RATES AT DIFFERENT STAGES OF SOLID CULTURE SYSTEM

Stages	Yield/rates
Number of initial ramets	30 ramets
Number of explants	1,775 strips
Primary callus	1,390
Embryogenic callus (EC)	1,194
Friable callus	165
Shoots	12,427
Plantlets	5,074
Callusing rate over ramet (%)	100
Callusing rate over explant (%)	78.3 ± 16.94
Embryogenic rate over explant (%)	67.3 ± 16.56
Embryogenic rate over primary callus (%)	85.9 ± 19.60
Friable rate over explant (%)	9.3 ± 6.09
Friable rate over primary callus (%)	11.9 ± 12.02

The production of embryogenic calli is important to ensure success in oil palm clonal propagation through somatic embryogenesis. Therefore, it is important to induce as many primary calli as possible because producing embryogenic calli depends on the induced callus. Gomes et al. (2017) reported that the oil palm primary callus has an elongated shape, a compact appearance and is slightly watery with a brown-beige colour. Constantin et al. (2015) reported that a similar structure of the primary callus was able to develop into a whitish-yellow colour and friable structure that are characteristics of oil palm embryogenic calli. The transition from primary callus to nodular and friable callus is indicative of enhanced embryogenic potential, as nodular callus is often associated with somatic embryo initiation, while friable callus facilitates large-scale propagation due to its loosely packed cellular structure (Weckx et al., 2019). Oil palm embryogenic calli that exhibit compact and nodular structures, will undergo somatic embryogenesis (Yusnita & Hapsoro, 2011). We observed that all ramets successfully produced primary callus, which was yellowish and of various shapes (*Figure 2*). The primary calli obtained in this study were almost similar to the callus described by those studies. The primary calli obtained in this study were also almost similar to the callus types of coffee described by Pádua et al. (2014), which are TYPE 2 (uneven and translucent) and TYPE 3 (globular and beige). The primary callus was further developed into three different types of secondary callus: Nodular, friable and dormant or unchanged callus (*Figure 2m-2n*). Similarly, primary callus derived from *Swietenia macrophylla* also developed into three different callus types such as friable white, compact cream-coloured and spongy brown calli (Gatica-Arias et al., 2019).

The emergence of abnormal primary callus (*Figure 2e-2l*) suggests irregular patterns during the differentiation process. Excessive proliferation of non-embryogenic cells may produce cotton-like calli, while spontaneous organogenesis triggered by auxin dominance in the culture medium can produce a root-like structure (Reflini, 2017). The root-like structure with pneumatodes callus can be associated with abnormal vascular differentiation, which resembles the aeration structures of wetland plants (Sadguna & Mustafa, 2014).

Ho et al. (2009) and Gomes et al. (2017) reported that oil palm callusing rates per ortet from spear leaf tissues of mature palms in the plantation are between 11.0%-20.0% and 22.3%, respectively. Roowi et al. (2010) and Babu et al. (2025) reported that the average oil palm embryogenesis rate per ortet of mature palm, which is genotype dependent, is at 1.0%-6.0%. Approximately 5.0% of the explants regenerate into somatic embryos (Gomes et al., 2017; Jayanthi et al., 2011, 2015). Our results, therefore, suggest that higher callusing (78.3%) and embryogenic rates (85.9%) can be obtained from the cabbage of young ramets, as compared to mature ortet palms in the field.

Differences Between Embryogenic and Non-embryogenic Calli

Initial assessment of embryogenic status was done using double staining with acetocarmine and Evans blue. The friable callus showed bright red stains indicating high cell viability and embryogenic potential (*Figure 3a*). In contrast, the dormant callus stained dark blue, suggesting non-viable or non-embryogenic characteristics (*Figure 3b*). Histological analysis was performed using Periodic acid-Schiff (PAS) and Naphthol Blue Black staining to further confirm the embryogenic status. The result shows the embryogenic cells such as friable callus (*Figure 3c*) exhibited pro-embryos characteristics, which are isodiametric cells with a large nucleus, clear nucleolus and dense cytoplasm indicating an actively dividing tissue. In contrast, the non-embryogenic callus such as dormant callus (*Figure 3d*) showed unsymmetrical large cells with large vacuoles, and sparse cytoplasm with a small nucleus size, indicating metabolic inactivity. Similar observation (non-embryogenic) was also found for primary callus (*Figure 3e-3f*) and abnormal primary calluses, namely the cotton-like calli (*Figure 3g-3h*) and root-like calli with pneumatodes (*Figure 3i-3k*).

A double staining technique and histological analysis can distinguish between embryogenic callus (EC) and non-embryogenic callus (NEC). Both are reliable methods to evaluate the cells viability and can help to distinguish between EC and NEC. The result from friable callus (*Figure 3a*)

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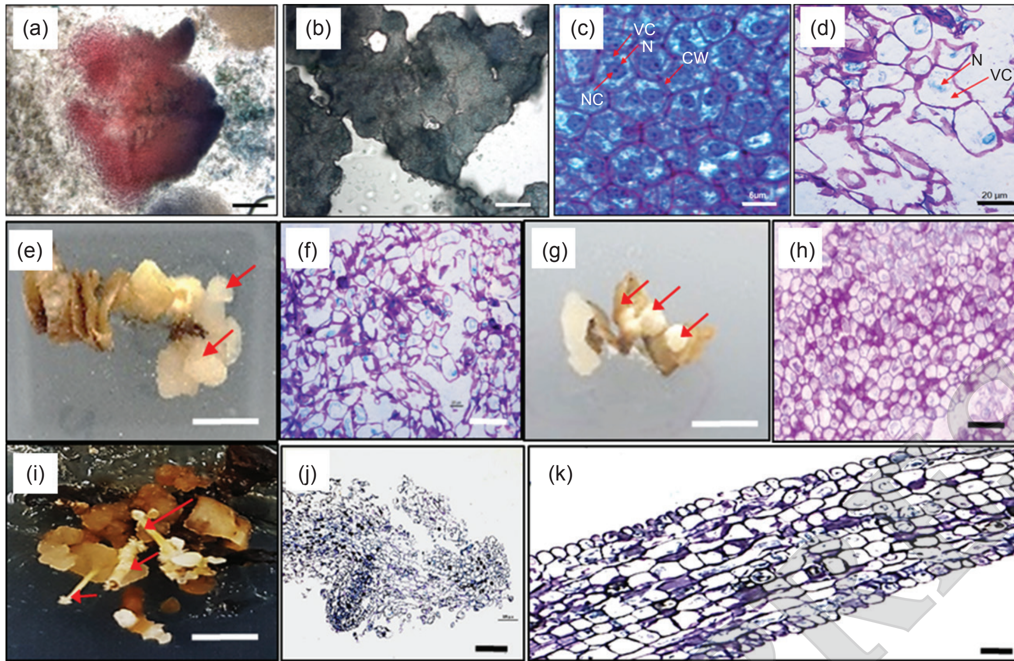

Note: Bar in (a), (b) is 500 µm, (c) is 6 µm, (d) is 20 µm, (e), (g), (i) is 0.25 cm, (f), (h), (j) is 50 µm and (k) is 250 µm.

Figure 3. (a) Double staining of friable callus stained bright red, and (b) non-embryogenic callus stained dark blue, (c) histological analysis of friable callus with the pro-embryos characteristic and (d) non-embryogenic callus characteristic in dormant callus. The red arrows with capital letters in (c) and (d) are VC - Vacuole; CW - Cell wall; N - Nucleus and NC - Nucleolus. Morpho-histological analysis of (e, f) primary callus, (g, h) cotton-like callus and (i, j, k) root-like callus with pneumatodes. Red arrows in (e, g) and (i) indicate the primary callus, cotton-like callus, and root-like callus with pneumatodes, respectively.

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showed bright red stains due to the absorption of acetocarmine into the nucleus and cytoplasm representing high cell viability of the actively dividing cells which are usually found in EC. This may indicate a better potential for embryogenesis. Mohajer et al. (2012) reported that embryogenic cells induced from different explants (leaf, root and stem) of Sainfoin (*Onobrychis sativa*), stained intensely bright red with acetocarmine which is similar to embryogenic cells in the suspension culture of *Brassica oleracea* var. Botrytis (Siong et al., 2011). Histological analysis showed that the pro-embryos characteristic can only be found in embryogenic calli (*Figure 3c*), which indicates the embryogenic potential for plant regeneration (Klimek-Chodacka et al., 2020; Nandhakumar et al., 2018).

As a comparison, NEC (dormant callus, *Figure 3b*) stained dark blue due to the reaction with Evan's blue, indicating lower viability and lack of embryogenic characteristics of the cells (Pádua et al., 2014). The same technique has been used to distinguish between viable and non-viable cells of *Plasmodiophora brassicae* (Harding et al., 2019). Histological analysis also showed that the abnormal primary callus consists of large, vacuolated cells with poorly defined nuclei and loose cellular organisation (*Figure 3f, 3h, 3j, 3k*); therefore, it will not further develop into somatic embryos or plantlets. The result was similar to pineapple, whereby the NEC is self-incompatible, highly heterozygous and incapable of producing uniform pineapple plantlets at the end (Bukhori et al., 2013).

Plant Regeneration from Nodular Calli in Solid Culture System

The nodular calli were successfully developed into white embryoids, green polyembryoids and finally regenerated into shoots and whole plantlets (*Figure 4*). It was observed that within three months of cultivation, white embryoids developed from nodular callus (*Figure 4a*) and turned into green embryoids after being transferred into light (16 hr) and dark (8 hr) conditions for one month (*Figure 4b*). After two months, the green embryoids were multiplied into polyembryoids (*Figure 4c*) and developed into shoots after another two months (*Figure 4d*). Normal healthy shoots were selected and transferred to shoot regeneration media (*Figure 4e*) and allowed to grow further for about three months. Then, the mature shoots were transferred to the rooting media (*Figure 4f*). After about three months, normal strong roots were developed (*Figure 4g-4h*), and these plantlets were deemed ready for transfer to jiffy peat and maintained in a pre-nursery.

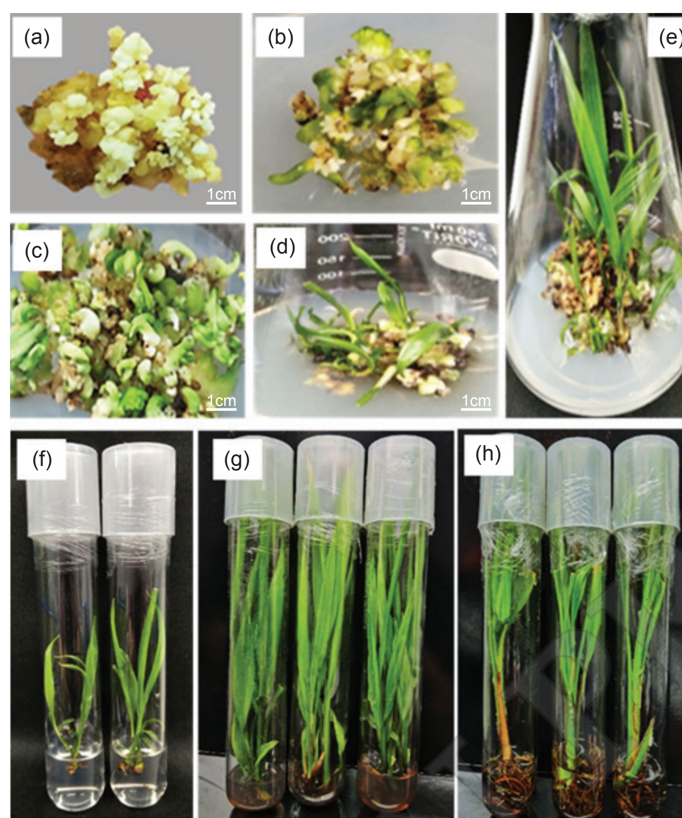

Figure 4. Development of (a) white embryoids and (b) green polyembryoids . Multiplication of (c) green polyembryoids. Development of (d) shoots from polyembryoids. (e) Elongation of shoots. (f) Individual shoots cultured in rooting medium. (g) After three months of shoot development. (h) Complete root development with primary and lateral roots.

Around 12,427 shoots were produced, but only about 5,047 plantlets with normal roots were regenerated (*Table 1*). Our results agree with those of Weckx et al. (2019), whereby conversion of somatic embryos into plantlets is often problematic. The regeneration rates were low, and many incomplete plantlets lacked well-developed shoot and root systems. In *in-vitro* oil palm clonal propagation, it is not surprising that some abnormal shoots and root systems are obtained. *In-vitro* plantlets with healthy shoots and complete root systems are crucial as these have higher survivability rates once transferred into jiffy peat for cultivation in the pre-nursery. In summary, the production of plantlets from explants of young ramets took approximately 1.5 years via the solid culture process.

Plant Regeneration from Friable Calli in Liquid Culture System

In this study, friable calli developed from the primary calli (*Figure 5a*) were selected and transferred into a liquid medium to initiate a liquid culture system (*Figure 5b*). Callus proliferation was observed after the second subculture (*Figure 5c*), and suspension cultures were established after the third subculture (*Figure 5d*). The number of cell suspension flasks and callus weight from 23 ramets generally showed an upward trend until it peaked at the seventh subculture and started to decline at the eighth and ninth subcultures (*Figure 5e*). However, some suspension cultures behave differently. For example, cultures from RD69 did not proliferate, while RD75 was established late (7 subcultures) and RD57, RD60, RD61, RD65, RD74 and RD77 failed to establish after more than three subcultures with less than 10 flasks (*Table 2*).

The liquid culture system involves cell suspensions, in which a single cell or small aggregates of cells are allowed to multiply in an agitated growth medium to form a suspension. Cell suspension cultures are established due to the rapid growth of undifferentiated cells grown in a liquid medium. Since oil palm somatic embryogenesis rates are generally low, implementing a liquid culture system can enhance mass propagation and proliferation (Weckx et al., 2019). Friable calli are the most suitable starting inoculum for a liquid culture system (Hashim et al., 2018) because they consist of small, rapidly growing, isodiametric cells with a high frequency of cell divisions (Souza et al., 2014). These cells are easily breakable and loosely attached (friable), exhibiting fast and uniform growth (Dewanti et al., 2016).

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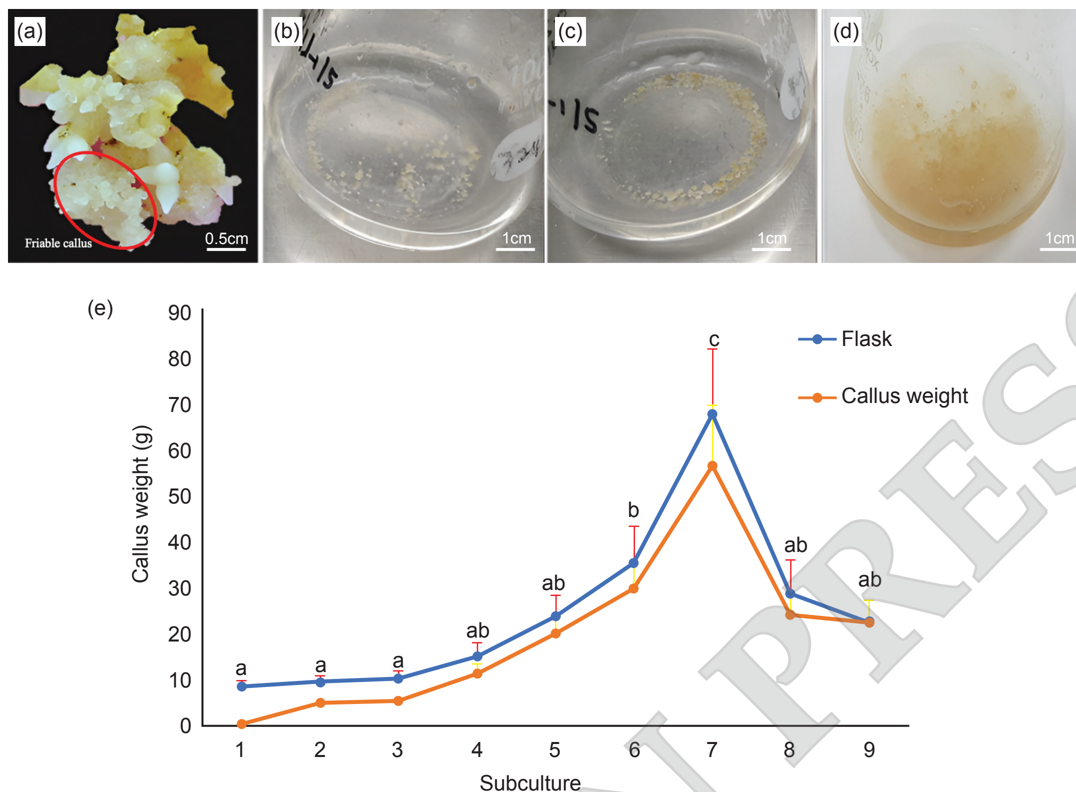

Note: Different letters (a, b, ab, c) represent statistically significant differences at p = 0.05 according to Duncan's multiple range test.

Figure 5. Initiation and establishment of suspension cultures. (a) Selected friable callus (circled in red), (b) friable callus transferred into a liquid medium for suspension culture initiation, (c) suspension culture development after second subculture and (d) established suspension culture after third subculture. (e) Production of cultures (tabulated by number of flasks) and callus weight in suspension cultures at each subculture for all ramets. The data represent the means of 23 ramets. The error bars represent the standard deviations from 23 ramets.

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Previously, oil palm suspension cultures were successfully established after over three subcultures, with about a 2- to 4-fold weight increase from approximately 0.50 g of friable calli inoculum per flask (Hashim et al., 2018). In this study, starting from 0.05 g of friable calli, a weight increase of 18- to 21-fold was observed after 2-4 subcultures, which dramatically rose to 225-fold at seven subcultures. However, in the eighth subculture, a 1.5-fold weight decrease occurred. The decrease in callus production after the seventh subculture could be due to saturation in the amount of callus in the flask or progression into the next development stage, as most of the calli have initiated the somatic embryogenesis stage rather than continued multiplying.

Calli suspensions at the 9th subculture were cultured on solid media for plant regeneration. However, only 38 embryoids and, subsequently, 22 polyembryoids were produced, suggesting that the embryogenesis capability for calli collected at the 9th subculture was poorer. Therefore, the high multiplication of suspension calli to produce thousands of flasks is likely not associated with high regeneration into shoots and plantlets. In addition, only 136 shoots were produced from the total cell suspensions derived from the 23 ramets, and approximately 46 plantlets were finally regenerated.

Although friable calli were originally embryogenic, after going through the propagation process in the liquid culture system, most calli failed to develop into embryoids when transferred and cultured on solid media. The embryogenesis process may be hindered due to being in suspension media for too long (nine subcultures), resulting in lower production of embryoids and poorer regeneration of shoots (Karyanti et al., 2021). It is impossible to compare the regeneration rates between the solid and the liquid culture systems because embryoids from the solid culture system are derived from nodular callus, and embryogenesis and regeneration are straightforward in this system. In contrast, embryoids derived from friable calli collected from liquid culture have different characteristics and have to undergo a dual system for (i) proliferation phase (liquid system) and (ii) embryogenesis and regeneration phase (solid system). These systems may involve different routes or pathways that influence the subsequent embryogenesis or regeneration processes.

TABLE 2. PRODUCTION OF CULTURES (FLASKS) PER RAMET AT EACH SUBCULTURE

No.	Ramet	Subculture								
		1	2	3	4	5	6	7	8	9
1	RD 48	17	19	25	39	59	107	205	75	51
2	RD 49	16	19	20	38	52	70	135	32	12
3	RD 50	9	9	11	15	17	20	40	5	4
4	RD 51	14	18	19	25	57	93	129	88	51
5	RD 53	5	5	3	2	3	7	11	5	0
6	RD 55	5	7	7	10	14	37	76	39	30
7	RD 56	11	10	13	18	26	48	101	59	36
8	RD 57	3	2	4	4	3	5	8	8	0
9	RD 58	6	5	3	3	15	15	29	12	12
10	RD 59	5	5	5	10	10	14	16	2	4
11	RD 60	5	5	6	7	9	8	1	3	0
12	RD 61	3	3	2	2	4	10	4	0	0
13	RD 62	4	5	5	8	9	18	40	12	12
14	RD 63	8	11	11	17	30	42	92	55	47
15	RD 64	13	12	11	17	24	16	56	25	16
16	RD 65	2	1	1	2	1	3	1	0	0
17	RD 66	8	8	8	10	14	19	47	33	34
18	RD 67	2	2	2	4	5	6	17	8	0
19	RD 68	4	4	5	5	14	14	30	3	0
20	RD 69	2	1	0	0	0	0	0	0	0
21	RD 74	2	2	2	2	3	5	3	0	0
22	RD 75	3	3	2	3	6	8	23	15	8
23	RD77	1	1	1	2	2	4	1	0	0
Total flasks		**148**	**157**	**166**	**243**	**377**	**575**	**1,055**	**452**	**336**

CONCLUSION

The selection of explants plays a crucial role in the success of clonal propagation, influencing callus formation and regeneration efficiency. In this study, the use of cabbage from young *dura* ramets resulted in the formation of three distinct callus types: Primary, nodular and friable. Among these, nodular calli demonstrated notable regeneration potential, yielding over 5,000 plantlets through the solid culture system. In contrast, friable calli, which served as the efficient starting inoculum for the liquid culture system, multiplied from 148 initial flasks to approximately 1,000 suspension cultures, yet only regenerated into 46 plantlets. These findings strengthen the utility of young ramet cabbage as an explant source while highlighting the difference in regeneration efficiency between solid and liquid systems. Given the low conversion rate in the liquid culture, further optimisation studies are necessary to refine subculture conditions, improve embryogenic responsiveness and enhance plantlet recovery. Future research should explore media composition adjustments, hormonal fine-tuning and nanotechnology integration to maximise regeneration efficiency. By addressing these challenges, this study contributes to advancing tissue culture methodologies for oil palm propagation and broader applications in sustainable clonal expansion strategies.

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