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RESEARCH ARTICLE

EFFECT OF NUTRITIONAL ENVIRONMENT, SUCROSE AND THIDIAZURON ON THE ABILITY OF SOMATIC EMBRYO REGENERATION THROUGH CULTURE OF VIETNAMESE GREEN DWARF COCONUT (COCOS NUCIFERA L.F.) PLUMULES

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ABSTRACT

Coconut palm (*Cocos nucifera* L.) is a globally significant perennial crop, providing substantial economic benefits and sustainable livelihoods for millions, particularly in tropical regions. However, global decline in coconut palm cultivation, led to the urgent development of efficient and rapid propagation strategies for high-quality plant. Somatic embryogenesis using plumule as explant was one of the most prominent methods for Dwarf coconut cultivar. In this study, several factors of coconut somatic embryogenesis were researched including effects of plumule maturity and sucrose concentration on the induction of primary embryogenic callus, as well as the impact of sucrose concentration during the subsequent callus multiplication stage. Furthermore, the effect of thidizuron (TDZ) on regeneration of coconut somatic embryo was studied. The results indicated that plumule maturity and sucrose concentration significantly influence primary callus induction, with the highest formation rates observed from 14-day preculture plumules, cultured in 45 gL^{-1} sucrose-supplemented medium. Sucrose concentration also affected significantly on embryogenic callus formation during callus multiplication stage. Crucially, this research demonstrated that TDZ, at concentrations ranging from 25 µM to 50 µM, effectively promoted development, germination, and plantlet regeneration of coconut somatic embryos. The result from the experiment provided valuable insights into optimizing the in vitro propagation protocols for coconut, paving the way for enhanced production and conservation efforts.

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INTRODUCTION

Coconut (*Cocos nucifera* L.) is a versatile and economically important crop, a sole member in Cocos genus, in Arecaceae family (Perera 2016) . This crop is cultivated worldwide across tropical and subtropical regions due to its cultural significance and economic contribution (Samosir and Adkins 2014). Beside its daily household consumption, coconut derivatives are integral to numerous industries, including food and beverage, medicinal, and cosmetics (Saat et al. 2002; Satheesh 2015; Pandiselvam et al. 2019; Samarajeewa 2024). The Asia-Pacific region accounts for over 80% of global coconut production, serving as a vital income source for numerous households (Beveridge et al. 2022). In 2024, global coconut products market was valued at more than 21 billion USD and was predicted to reach 53 billion USD in 2033 (IMARC group 2024). Consequently, this escalating annual demand necessitates a strategic expansion of coconut cultivation to ensure a stable supply of high-quality planting materials to sustain future production. Unfortunately, coconut cultivation faces significant challenges, including , palm senility, pest and diseases outbreak, and climate change (Eziashi and Amamor 2010; India 2013; Vadamalai et al. 2017; Silva et al. 2017). Concurrently, traditional propagation through breeding programs using seeds, which remains the predominant method, is notably inefficient for large-scale replanting due to its time- and space-consuming nature (Adkins et al. 2020; Kalaipandian et al. 2021) . Futhermore, the method is low seed-to-plantlet ratio, with each seed yielding only one plantlet yet while not all seed would germinate add another layer of complexity to the process (Ledo et al. 2001; Beveridge et al. 2022). Therefore, applying advanced techniques including tissue culture have been researched, contributed significantly to coconut propagation. The first attempt in *in vitro* coconut cultivation through zygotic embryo culture occurred in 1954 (Cutter, and Wilson 1954). Since then, many studies on coconut embryo culture have been conducted to provide the most optimal growing conditions and procedures (Sisunandar et al. 2018; Lédo et al. 2019). Even though embryo culture improved greatly coconut germination rate while requiring small working area, it is still 1:1 seed-to-plantlet ratio (Nguyen et al. 2015). To enhance both the quality and quantity of

coconut plantlets, plant tissue culture has been extensively applied. Somatic embryogenesis has emerged as the most promising technique for the large-scale production of new coconut palms. Somatic embryogenesis is an in vitro plant micropropagation technique that involves the formation of bipolar structures, or somatic embryos, from non-sexual (somatic) cells under specific culture conditions (Lédo et al. 2019). Since the first study of Somatic embryogenesis on coconut propagation in 1977, for almost five decades, somatic embryogenesis has been applied to numerous coconut varieties and retrieved prevalent remarkable accomplishment (Eeuwens and Blake 1977; Kalaipandian et al. 2021). In Vietnam, somatic embryogenesis has been studied and applied to high value Makapuno coconut, leading to the successful development of somatic plantlets (Phạm 2022; Nam 2024).

This study focused on the Vietnamese Xiem Green Dwarf (XGD) coconut variety, a well-established and widely cultivated cultivar in the Mekong Delta region (Lệ 2010). XGD coconut water , famous for its mild-sweetness and distinctive flavor, has widespread domestic popularity and serving as a major source of export revenue (VITIC 2018; An Hoà 2023). Until now, XGD cultivation has predominantly relied on conventional methods. In this experiment, somatic embryogenesis was applied to the XGD variety to gain a comprehensive understanding of this technique's efficacy on a specific Vietnamese coconut cultivar. Specifically, the effects of plumule maturity and sucrose concentration on callus induction were investigated. Additionally, TDZ (Thidiazuron) was employed to evaluate its role in plant regeneration.

MATERIALS AND METHODS

Explants: XGD coconut fruits, aged 10 to 12 months post-fertilization, were dehusked at the farm and transported to the IU plant biotechnology laboratory. The coconuts were chopped in halves to reveal the position of the embryos. Endosperm plugs containing embryos were extracted using a cork borer. These plugs were sterilized in 70% ethanol for 1 min and rinsed with distilled water three times. Subsequently, the plugs were transferred to a laminar airflow cabinet for aseptic embryo excision. Individual coconut embryos were carefully extracted from the plugs and immediately placed in sterile containers. The embryos were sterilized with 0.6% $Ca(ClO)_2$ for 10 minutes and then rinse with sterilized distilled water three times for 1 minute. Gentle shakes were required during this step.

Induction of primary embryogenic callus of XGD coconut variety: Following embryo sterilization, plumules were aseptically excised from the embryos under a light microscope. These excised plumules were then cultured on a medium composed of Y3 basal salts supplemented with 600µM 2,4-Dichlorophenoaxetic acid (2,4-D), 30 gL^{-1} sucrose, 2.5 gL^{-1} activated charcoal (AC) and 2.5 gL^{-1} phytagel (Pérez-Núñez et al. 2006). Cultures were incubated in complete darkness at a temperature of 27 ± 2°C for a period of 12 weeks. The induction and development of callus from XGD coconut explants were meticulously observed and recorded every four weeks.

Experiment 1: Effect of plumule maturity on development of initial and embryogenic callus in XGD coconut variety. Sterilized embryos were preculture media containing Y3 basal salt (Eeuwens 1976), supplemented with 5 µM of 6-Benzylaminopurine (BAP), 1 gL^{-1} AC and 2 gL^{-1} phytagel (Tran 2023) with three distinct sucrose concentrations – 30 gL^{-1}, 45 gL^{-1} and 60 gL^{-1}. Embryos were incubated in a completely dark environment at 27 ± 2 °C for 3 periods of time – 7 days, 10 days and 14. Following the preculture periods, germinated plumules were aseptically excised from the embryos. These plumules were transferred to a callus induction media containing Y3 basal salt, 600 µM 2,4-D, 2.5 gL^{-1} AC and 2.5 gL^{-1} phytagel with sucrose concentration similar to previous media. Cultures were maintained in a completely dark environment at 27 ± 2 °C for 12 weeks. Number of samples formed initial and embryogenic callus were recorded at checkpoint in week 12^{th}.

Experiment 2: Effect of callus multiplication progress on embryogenic callus induction: Primary embryogenic callus was aseptically dissected into smaller fragments, approximately 3 to 5 mm in diameter. These fragments were then transferred to new proliferation medium, which had a composition similar to the callus induction medium described previously. The sub-cultured callus was incubated in complete darkness at 27 ± 2 °C for a period of two months. Following this proliferation phase, the resulting embryogenic callus was subjected to a further subculture.

Experiment 3: Effect of Thidiazuron on somatic embryo regeneration: Coconut embryogenic callus was then transferred to culture medium containing Y3 basal salt, 45 gL^{-1} sucrose, 2.5g L^{-1} AC, and 7.5 gL^{-1} agar in the absence of plant growth regulator for 4 weeks. Cultures were maintained in darkness at 27 ± 2 °C. Subsequently, callus were transferred to the same Y3 basal medium containing varying concentrations of thidiazuron (TDZ) (0, 25, 50, 100, and 150 µM) combined with 300 µM BAP (Pérez-Núñez et al. 2006; Sáenz et al. 2018) as control for regeneration stages. The samples were kept in darkness for the initial four weeks, followed by a 16/8 h light/dark photoperiod. After two months, data were collected,and developing samples were subcultured onto fresh medium. Subculturing was performed every two months thereafter.

Data collection: Data were collected right before the samples were culture to new media. Each treatment of each experiment had 3 replicates. Data were collected and noted to excel and analyzed using SPSS. Ver 22.

RESULTS AND DISCUSSION

Development and induction of XGD embryogenic callus

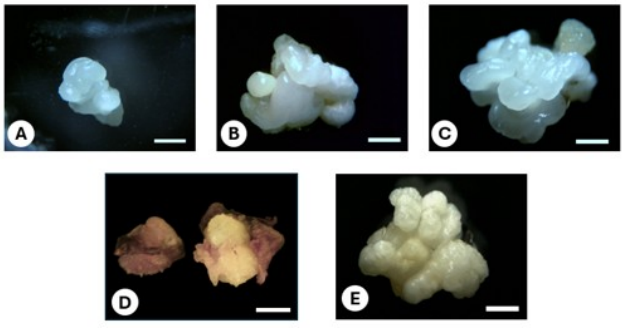

Figure 1. Formation of primary embryogenic callus at week 4 (A), week 8 (B), week 12 (C) in callus induction medium. Browning non-embryogenic callus (D) and spongy texture non-embryogenic callus (E). Scale bar = 3mm. Source: LH MAI (2020)

Plumules were cultured in callus induction medium and incubated in darkness, developmental changes were observed and noticed every 4 weeks. Initial callus formation was observed after four weeks, originating from the plumular tissues. Initial callus measured approximately 3 mm in size, exhibiting a smooth surface and a transparent appearance. This early development suggested a positive response of the plumular explants to auxin within the callus induction medium. After 8 weeks from initiation, transparent, ear-shaped structures with a smooth surface were observable within the culture. These morphological characteristics indicated the development of embryogenic callus, consistent with previous observations in somatic embryogenesis in other coconut cultivars (Pérez-Núñez et al. 2006; Jayaraj et al. 2015). At week 12th, coconut embryogenic callus were more clearly defined, reaching a size of 6-7 mm, while retaining their characteristic ear-shape, smooth surface, and transparent appearance. This development process was also reported in Malay Green Dwarf or Chowghat Orange Dwarf coconut varieties (Sáenz et al. 2006; Greeshma et al. 2018). Our observations align with findings indicating that embryogenic callus typically achieves optimal development and highest proliferation rates after 12 weeks in callus induction medium (Osorio-Montalvo et al. 2020). Alongside the developing embryogenic callus, which were characterized by their smooth, transparent morphology, were non-embryogenic callus mass. Non-embryogenic callus encompassed all callus tissues that did not exhibit these specific morphological characteristics. Fig. 1D and 1E demonstrated two types of non-embryogenic callus, browning color, rough surface or sponge like.

Effect of plumule maturity and sucrose concentration on induction of initial callus and primary embryogenic callus in XGD coconut variety: Coconut zygotic embryos were precultured in cultured media containing three different sucrose concentrations – 30 gL^{-1}, 45 gL^{-1} and 60 gL^{-1} for three periods of time – 7 days, 10 days and 14 days before plumules was excised and cultured in callus induction media. Results displayed in this experiment showed that plumule maturity and sucrose concentration significantly affected the formation of initial callus and primary embryogenic callus in XXD coconut variety.

After 12 weeks of dark incubation, primary embryogenic callus could be observed in all eight treatments. Treatment 7 (14 days preculture period – 45 gL^{-1} sucrose) yielded the highest embryogenic callus formation rate at 40.00 ± 2.87%. The initial three treatments, Treatment 1 (16.67 ± 4.81%), Treatment 2 (20.83 ± 2.08%), and Treatment 3 (18.52 ± 3.70%), exhibited comparably low embryogenic callus formation rates, with no significant statistical differences among them. Despite having the lowest initial callus formation rate, treatment 5 (34.48 ± 1.99%) did not significantly differ in its embryogenic callus formation rate from treatment 7 (35.35 ± 1.01%), which had the highest in initial callus induction category. Furthermore, both treatment 5 and treatment 7 were statistically similar to treatment 6 (36.67 ± 1.67%). Treatments 4 through 8 consistently demonstrated higher embryogenic callus formation rates compared to experiments where no preculture step was implemented (Le 2020).

Research across diverse coconut varieties suggests that the maturity of plumules, often achieved through embryo preculture stages, plays a crucial role in enhancing the primary embryogenic callus formation rate. For instance, research on the Yellow Malayan Dwarf variety demonstrated that plumules excised from 14-day precultured embryos yielded the highest embryogenic callus formation rate (Nguyen 2018). Meanwhile for the Aromatic coconut variety, optimal primary callus induction was achieved with a 7-day plumule maturity treatment (Pham 2019). The practice of preculturing zygotic embryos prior to plumule excision was not widely considered in coconut somatic embryogenesis studies, with many protocols advocating immediate plumule excision following sterilization (Chan et al. 1998; Sáenz et al. 2018). However, a 2005 study on the West Coast Tall coconut cultivar reported that over 74% of plumules pre-cultured in Y3 medium for one month produced embryogenic callus (Rajesh, M.K. 2005). This research group also applied this preculture practice to the Chowghat Green Dwarf and Malayan Yellow Dwarf cultivars in a later study (Rajesh et al. 2014). Furthermore, a significantly higher embryogenic callus formation rate was observed in Hainan Tall coconut variety when comparing plumules excised immediately from sterilized embryos with those from 15-day precultured plumules (Mu et al. 2024).

In addition to plumule maturity, sucrose concentration supplied in the culture medium plays a critical role in the induction of embryogenic callus in the XGD coconut cultivar. Sugars are fundamental components in tissue culture, and sucrose has consistently proven highly effective for plant somatic embryogenesis (Yaseen et al. 2012; Elmeer 2013). Despite its importance, the precise impact of differing sucrose levels on coconut somatic embryogenesis have not been thoroughly understood. In many cases, 30 gL^{-1} sucrose was the standard concentration utilized in coconut culture media (Nguyen et al. 2015). However, recent research, particularly from Mexico, reveal an adjustment in sucrose concentrations for callus induction media. Earlier studies in 2006 employed 30 gL^{-1} sucrose in culture medium (Pérez-Núñez et al. 2006; Sáenz et al. 2006), but later research in 2018, sucrose supplemented for callus induction media significantly increased to 50 gL^{-1} (Sáenz et al. 2018). Similar adjustments are seen in studies using immature inflorescence explants for callus induction (Sandoval-Cancino et al. 2016; Oropeza et al. 2018). Furthermore, remarkable embryogenic callus formation rates exceeding 90% have been reported for Aromatic and Makapuno coconut varieties when 50 gL^{-1} sucrose was included in the culture medium (Phạm 2022).

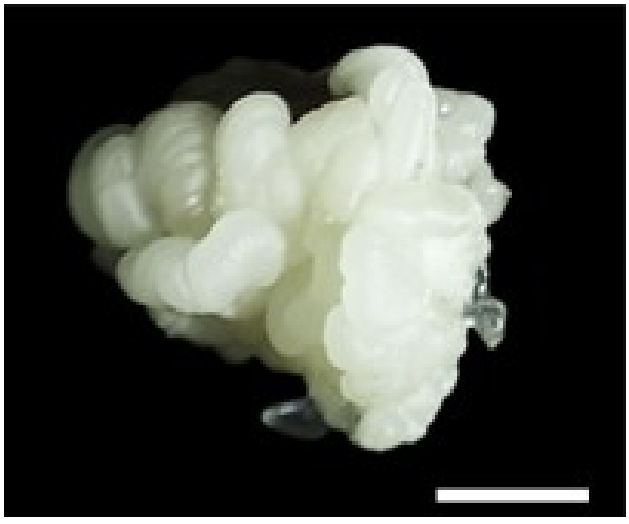

Figure 2. Secondary embryogenic callus after 8 weeks incubation. Scale bar = 3 mm

Table 1. Initial callus and embryogenic callus formation rate affected by plumule maturity and supplemented sucrose concentration

Treatment			Formation rate (%)	
No	Plumule maturity	Sucrose concentration	Initial callus	Embryogenic callus
1	7 days	45 g L^{-1}	83.33 ± 4.81ab	16.67 ± 4.81b
2	7 days	60 g L^{-1}	83.33 ± 2.08ab	20.83 ± 2.08b
3	10 days	30 g L^{-1}	74.07 ± 3.70b	18.52 ± 3.70b
4	10 days	45 g L^{-}	73.56 ± 1.15b	34.48 ± 1.99a
5	10 days	60 g L^{-1}	81.67 ± 1.67ab	36.67 ± 1.67a
6	14 days	30 g L^{-1}	91.92 ± 1.01a	35.35 ± 1.01a
7	14 days	45 g L^{-1}	86.67 ± 1.67ab	40.00 ± 2.87a
8	14 days	60 g L^{-1}	84.21 ± 3.04ab	29.82 ± 1.75ab

Table 2. Embryogenic callus formation rate from three subcultures

Treatments			Embryogenic callus formation rate (%)		
No	Plumule maturity	Sucrose concentrations	Subculture 1	Subculture 2	Subculture 3
1	7 days	30 g L^{-1}	12.28 ± 1.75f	8.33 ± 4.17e	0.00 ± 0.00g
2	7 days	45 g L^{-1}	36.67 ± 3.33bc	68.18 ± 2.62a	66.67 ± 0.57a
3	7 days	60 g L^{-1}	16.67 ± 3.33ef	50.00 ± 7.22b	19.61 ± 1.96e
4	10 days	30 g L^{-1}	63.89 ± 2.77a	51.61 ± 1.86b	35.51 ± 0.72c
5	10 days	45 g L^{-1}	42.95 ± 0.64b	20.39 ± 0.38de	24.50 ± 0.16d
6	10 days	60 g L^{-1}	31.43 ± 1.65cd	31.22 ± 0.42cd	46.26 ± 0.34b
7	14 days	30 g L^{-1}	15.32 ± 0.90ef	21.09 ± 0.68de	11.35 ± 0.24f
8	14 days	45 g L^{-1}	30.88 ± 0.85cd	30.85 ± 0.21cd	34.84 ± 0.13c
9	14 days	60 g L^{-1}	23.68 ± 1.52de	36.84 ± 1.01bc	42.82 ± 0.27b

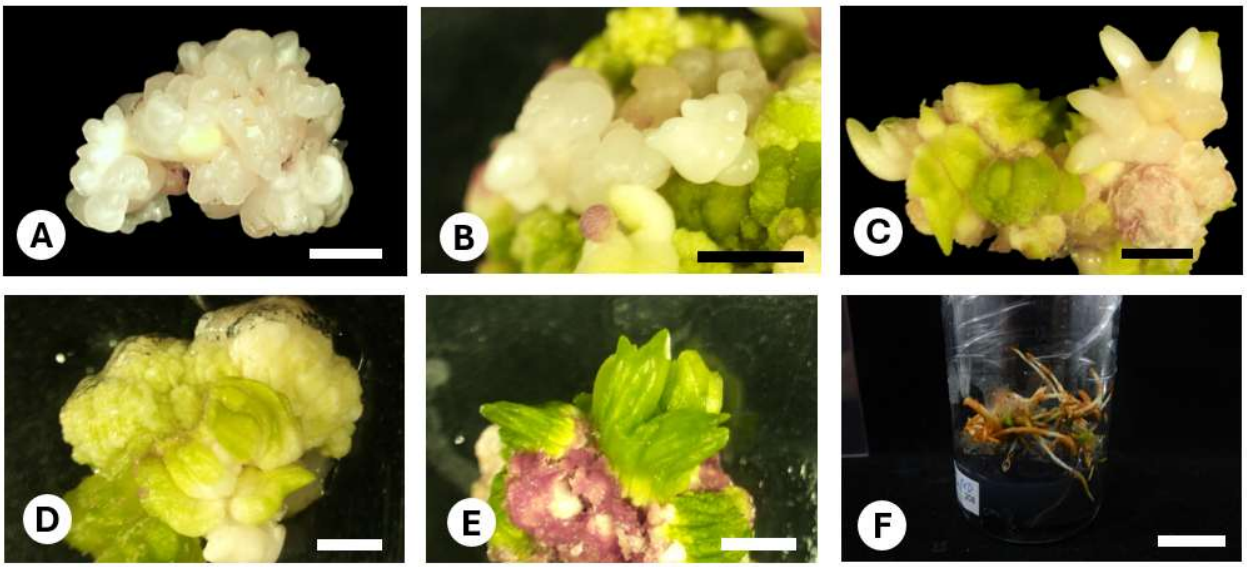

Figure 3. Development of XGD coconut somatic embryo in TDZ containing media at day 0 (A), after 2 months (B) and after 4 months (C). Haustorium-like structures (D, E) and adventitious roots (F). Scale bar of A, B, C, D, E = 3 mm, of F = 3 cm

Table 2. Development and germination of Somatic embryo under influence of TDZ and BAP

Treatment	2 months	4 months			Shoot formation
	Globular SE (%)	Germinating SE (%)	Globular SE (%)	Germinating SE (%)	
300 µM BAP	81.48 ± 2.14ab	50.62 ± 1.23ab	72.73 ± 5.25a	51.52 ± 3.03b	2/117
0 µM TDZ	46.46 ±3.84 c	22.22 ± 2.22c	6.67 ± 6.67b	0.00 ± 0.00c	0
25 µM TDZ	67.71 ±1.04ab	48.96 ± 1.04ab	70.18 ± 0.88a	47.37 ± 1.52b	2/249
50 µM TDZ	86.42 ±1.23a	54.32 ± 1.23a	86.11 ± 2.78a	63.89 ± 2.78ab	4/87
100 µM TDZ	79.17 ±2.08ab	64.58 ± 2.08a	85.71 ± 8.25a	71.43 ± 8.25a	0
150 µM TDZ	66.67 ± 8.33b	33.33 ± 8.33bc	N/A	N/A	0

Table 3. Formation of haustorium-like structures and adventitious roots during regeneration stage

Treatment	2 months		4 months	
	Haustorium-like structures (%)	Adventitious roots (%)	Haustorium-like structures (%)	Adventitious roots (%)
300 µM BAP	86.42 ± 1.23b	72.84 ± 1.23b	78.79 ± 3.03b	81.82 ± 5.24ab
0 µM TDZ	60.00 ± 3.85a	31.11 ± 2.22a	73.33 ± 6.67b	66.67 ± 6.67a
25 µM TDZ	89.58 ± 1.04b	68.75 ± 1.08b	71.05 ± 1.52ab	85.96 ± 0.87ab
50 µM TDZ	82.71 ± 1.23b	59.26 ± 2.14b	52.78 ± 2.78a	80.56 ± 2.78ab
100 µM TDZ	77.08 ± 2.08ab	70.83 ± 1.08b	61.90 ± 4.76ab	95.24 ± 4.76b
150 µM TDZ	91.67 ± 8.33b	66.67 ± 8.33b	N/A	N/A

Effect of sucrose concentration in culture media on secondary embryogenic callus induction during subculture process in XGD coconut cultivar: Primary embryogenic callus was dissected and subcultured onto fresh media with an identical composition to the previous stage, with each subculture lasting eight weeks. By the end of this eight-week period, the coconut embryogenic callus demonstrated complete development. It presented a transparent and smooth surface, increased significantly in size, and displayed a more distinct ear-shaped structure when compared to the initial callus masses obtained from plumular tissues. Occasionally, the formation of globular embryogenic callus was also noted. Analysis of embryogenic callus induction percentages revealed significant differences among the three subculture intervals. Following the first subculture, treatment 4 exhibited the highest embryogenic callus formation rate, reaching 63.89±2.77%. However, this percentage gradually declined with each subsequent subculture, dropping to 35.51 ± 0.72% after the third subculture. A similar degradation was observed in Treatment 1, which failed to produce any new embryogenic callus after three subcultures. In contrast, Treatment 2 showed a less prominent embryogenic callus formation rate during the first subculture, but its rate dramatically increased to a leading 68.18±2.62% after the second subculture and maintained this top rank in the third subculture (66.67±0.57%). A similar progressive increase was also found for treatment 9. Meanwhile, embryogenic callus formation rate from treatment 8 was seemly stable throughout the subculture times. The remaining treatments formation rates were either unstable or had decreasing tendency.

Analysis of the data reveals that a sucrose concentration of 30 gL^{-1} proved inadequate for optimal embryogenic callus induction during the multiplication stage, as evidenced by a consistent decline in formation rates across all three treatments. Conversely, treatments supplemented with 45 gL^{-1} and 60 gL^{-1} sucrose maintained stable or even increasing embryogenic callus formation rates. These findings indicated that sucrose concentrations exerted a significant influence on embryogenic callus induction during callus multiplication phase. Furthermore, the subculture process not only contributed to an increase in formation rate but also promoted the development of embryogenic callus with superior morphological characteristics, including larger size and a higher frequency of globular and ear-like structures. Furthermore, subculture process served as a selective mechanism for identifying callus lines that could tolerate and proliferate effectively in high-auxin conditions over extended periods. Subculture plays a critical role in the successful multiplication of embryogenic callus and, more broadly, in coconut somatic embryogenesis. Subculture helps maintain and enhanced callus embryogenic capacity concurrently providing a fresh supply of nutrients and plant growth regulator signals necessary for optimal callus survival and development (Pérez-Núñez et al. 2006). Benefit of subculture was also reported on oil palm embryogenic induction during its somatic embryogenesis protocol (Karyanti et al. 2021).

Effect of TDZ on regeneration of XGD coconut callus: Coconut embryogenic callus was transferred from callus induction media to PGR-free media and incubated in total darkness for 4 weeks for maturation. After 4 weeks, maturated callus masses were placed in new media containing different TDZ concentrations. After one month on a plant growth regulator free medium, coconut somatic embryos were observed with globular and smooth-surfaced. Most remained transparent, but some had transitioned to an off-white color (Figure 3A). Following the first two months on regeneration medium, somatic embryos developed further. In addition to their increased size, they were identified by their smooth surface and coleoptilar shape (Figure 3B signaling the initiation of the germination process. However, several structures retained their globular shape. After the next two months, somatic embryos were elongated to 4-5 mm. s the samples were exposed to a 16/8h photoperiod, the tips of the embryos turned green due to photosynthesis (Figure 3C). After first two months, somatic embryo development was observed across all treatments, though at varying rates. 50µM TDZ treatment had the highest rate of globular somatic embryo (86.42 ±1.23%) and and also the second-highest percentages of germinating somatic embryo (54.32 ± 1.23%). Statistically the rate of germinating somatic embryos for the 50 µM TDZ treatment was not significantly different from the highest-performing treatment, which contained 100 µM TDZ (64.56±2.08%). In contrast, the PGR-free regeneration medium yielded the lowest percentages of both globular and germinating somatic embryos. (46.46 ±3.84% and 22.22 ± 2.22%, respectively). This no-PGR treatment continued to rank lowest at the four-month checkpoint, with no germinating somatic embryos observed. Similar to the two-month results, highest formation The highest rates of globular somatic embryo formation were again found in the 50 µM TDZ and 100 µM TDZ treatments (86.11±2.78% and 85.71±8.25%, respectively), with no significant difference between them. However, 100 µM TDZ had the highest rate of germinating somatic embryo. When compared to the control treatment of 300 µM BAP, these two TDZ treatments yielded results that were equal to or higher.

Among the six experimental treatments, only three proved effective in promoting shoot formation and subsequent plantlet development: 300 µM BAP, 25 µM TDZ, and 50 µM TDZ. A notable difference was observed in the time required for this development. While the 300 µM BAP and 25 µM TDZ treatments needed 12 months to develop shoots, the 50 µM TDZ treatment achieved the same result in just 9 months. Alongside the desired development of somatic embryos, the presence of several unwanted structures, including haustorium-like structures and adventitious roots, was observed. Haustorium-liked structures had rough surface which had white or green color. Haustorium-like structures were characterized by a rough, white or green surface. These structures either appeared alongside developing somatic embryos or completely constituted the entire callus mass (Figure 3D). Another form of haustorium-like structure was identified as torpedo-shaped with a rough surface and a stiff, off-white or green texture (Figure 3E). Even though these structures shared a similar shape with germinating embryos, further cultivation and subculture only led to the growth of more unwanted haustoria. Based on table 4, haustorium-like structures were present in all treatments with high percentages at both the two- and four-month checkpoints. At the two-month checkpoint, the 25 µM TDZ treatment yielded the highest percentage of haustorium-like structures (89.59%), while the PGR-free treatment had the lowest (60.00 ± 3.85%). After four months, a general decline in haustorium formation rates was observed across all four treatments compared to the two-month results, except for the PGR-free treatment. At this stage, the highest was found in PGR free treatment while the lowest was in 100 µM TDZ treatment. Adventitious roots were

also observed to develop strongly during regeneration stages (Figure 3F), with the formation rates being consistently high. As detailed in Table 4, the lowest root formation rate at two months was in the PGR-free treatment. All other treatments displayed high rates, all exceeding 50% and showing no significant difference among them. After four months, unlike declining pace of haustorium formation, adventitious root formation in all treatments tended to increase noticeably. The highest rate was found in the 100 µM TDZ treatment, whereas the no-PGR treatment continued to show the lowest rate. The findings from this experiment suggested that 50 µM TDZ provided an optimal concentration for efficient somatic embryo regeneration in the Xiem Green Dwarf coconut cultivar. The low somatic embryo formation rate observed in the PGR-free treatment indicated the essential role of cytokinin in the regeneration process. When compared to the 300 µM BAP control, the 25 µM TDZ treatment showed no significant difference, while the 50 µM TDZ treatment was significantly higher. Furthermore, this concentration effectively balanced somatic embryo development with a moderate rate of unwanted haustorium-like structures and adventitious roots. Although the 100 µM TDZ treatment yielded a higher rate of germinating somatic embryos, it also led to a higher rate of adventitious root formation.

This suggests that the nutrient uptake of the explant was prioritized for root development over embryo maturation, which subsequently prevented any embryos from progressing to the shoot formation stage. The proliferation of these roots is likely a consequence of two factors: the high endogenous auxin levels from the initial long-term 2,4-D culture and the auxin-like properties of TDZ itself. It is worth noting that while TDZ was employed as a cytokinin in this research, its dual function as an auxin has been utilized for callus induction in numerous species, including coconut (Gairi and Rashid 2004; Zayed and Abdelbar 2017). The formation and germination of somatic embryos are processes initiated by cytokinin(Sáenz-Carbonell et al. 2020), and our findings confirm that TDZ is an effective regulator of this stage in coconut embryogenic callus. While BAP has long been the standard cytokinin for this stage in numerous plant tissue culture protocols, including those for coconut somatic embryogenesis (Oropeza et al. 2018; Sáenz et al. 2018), its use has often resulted in limited success in coconut embryo regeneration. Consequently, alternative cytokinins are being explored. TDZ has recently gained prominence for shoot organogenesis in many plants, including palm species(Graner et al. 2013; Ahmed 2014). A key advantage of TDZ is its superior hormonal activity compared to BAP, kinetin, and zeatin, allowing for successful regeneration at concentrations 10 to 1000 times lower than other plant growth regulators (Capelle et al. 1983; Guo et al. 2011).

Initially, coconut zygotic embryos were cultured on a BAP-supplemented medium for germination. The developed plumules were then excised and cultured on a high 2,4-D medium for callus induction (Figure 4A). Embryogenic callus was successfully induced from the plumular explants after an eight-week culture period, reaching full development after twelve weeks. To increase the quantity of embryogenic structures, the callus was subcultured a total of four times (Figure 4B). Following a four-week incubation period on a plant growth regulator free maturation medium, somatic embryos began to form (Figure 4C). A subsequent eight-week transfer to a TDZ-supplemented medium resulted in the formation of more globular somatic embryos (Figure 4D), which then began to elongate (Figures 4E, F). Complete somatic embryo development, including shoot induction and elongation, was observed after six months in regeneration medium (Figure 4G). Finally, after ten months, leaf formation was observed, and the resulting plantlets were transferred to a medium containing 5 mM BAP to promote root development.

CONCLUSION

This study presents significant findings that contribute to our understanding of coconut somatic embryogenesis, particularly for the high-value Xiem Green Dwarf cultivar in Vietnam. We have demonstrated that a 14-day preculture of plumules in a medium containing 45 g gL^{-1} sucrose yielded the highest embryogenic callus induction rate, reaching up to 40%. 45 gL^{-1} and 60 gL^{-1} sucrose concentration enhanced callus induction during subculture. Furthermore, this study found that TDZ can be used for coconut somatic embryo regeneration, with the optimal concentration being 50 µM. More research is needed to develop a more efficient protocol for coconut somatic embryogenesis.

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