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Research Article

The Effects of Plant Growth Regulators, Carbohydrate Substrates and Photoperiod on Shoot, Root and Callus Induction from Rhizome Shoot Bud Explants of 'Bentong' Ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

Zingiber officinale Roscoe from Bentong, also known as the 'Bentong' ginger, is a premium Malaysian-grown ginger recognised for its distinctive aroma, strong pungency, and elevated levels of 6-gingerol and 6-shogaol. *In vitro* platforms such as callus and cell suspension cultures represent a promising approach for the production of novel secondary metabolites from this valuable crop. This study aims to assess the effects of plant growth regulators, carbohydrate substrates and photoperiod in the induction of friable callus from rhizome bud explants of *Z. officinale* Roscoe from Bentong for subsequent studies on the establishment of cell suspension cultures. Explants were subjected to surface sterilisation and cultured in treatments of 2,4-D and kinetin, different carbohydrate substrates and photoperiods. Results in this study indicated that treatment with 2.5 mg/L 2,4-D resulted in 93.33% callus induction and 0.257 ± 0.06 g fresh weight, whereas treatment with 2.0 mg/L kinetin resulted in 85.71% callus formation, with 0.106 ± 0.02 g callus fresh weight. Both treatments of 2,4-D and kinetin produced yellowish white friable callus. The concentration of 30 g/L sucrose was considered the optimal carbohydrate level for callus induction in rhizome bud explants of 'Bentong' ginger. Additionally, explants cultured under continuous dark conditions achieved the highest percentage of callus induction (70.0%) with the highest callus fresh weight (0.270 ± 0.07 g). This study successfully induced friable callus from rhizome bud explants of 'Bentong' ginger (*Z. officinale* Roscoe), providing a suitable foundation for subsequent callus and cell suspension culture studies aimed at producing valuable secondary metabolites from this ginger cultivar.

Key words: Callus, Carbohydrates, Ginger, Photoperiod, *Zingiber officinale*

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INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is a herbaceous perennial belonging to the Zingiberaceae family and is native to Southeast Asia. It is known for its smooth green foliage, upright pseudo-stems, thick rhizomes, and fibrous, fleshy roots, as well as its yellow-green flowers. Due to its broad applications in cuisine and traditional medicine, ginger is extensively grown throughout tropical and subtropical areas. Its unique spicy aroma and sharp taste are primarily attributed to the rhizome's rich content of bioactive compounds or secondary metabolites, including monoterpene aldehydes and alcohols, sesquiterpenes, gingerols, shogaols, and other phenolics and flavonoids. These bioactive compounds underpin the diverse pharmacological properties of ginger, including its antioxidant, anti-inflammatory, antiemetic and antimicrobial activities.

Commercial cultivation of ginger in Malaysia includes several varieties, notably Bentong, Bara, China and Indonesian cultivars. Among these varieties, 'Bentong' ginger (*Z. officinale* Roscoe) exhibits a more intense aroma, pronounced spiciness and pungency, larger rhizomes with thinner epidermal layers, and a less fibrous, more succulent flesh compared to other local varieties. 'Bentong' ginger is regarded as distinctive due to the synergistic influence of its specific cultivation practices, including location, planting schedule, and agronomic techniques, alongside unique environmental conditions of its growing region, Bentong. These traits not only enhance its culinary appeal but also contribute to its higher market value. Phytochemical studies have demonstrated that 'Bentong' ginger possesses elevated levels of 6-shogaol and 6-gingerol, bioactive phenolics associated with significant therapeutic properties, thereby emphasising its medicinal and nutritional importance (Sharizan & Sahilah, 2021). Rhizomes are economically valuable plant organs widely utilised as a food ingredient and for various industrial applications, including the extraction of essential oils, the production of pharmaceuticals, and the preparation of traditional herbal remedies. Beyond this, rhizomes are also the primary propagation material in conventional cultivation systems whereby a

substantial proportion of harvested rhizomes must be reserved as planting material for subsequent growing seasons. This significantly reduces the quantity of rhizomes available for commercial processing and is often insufficient to satisfy increasing market demand.

Callus and cell suspension cultures, on the other hand, provide robust platforms for studying plant-based bioactive compounds and boosting the production of secondary metabolites with significant pharmaceutical and industrial value. The success of callus cultures is governed by several critical factors, including plant growth regulators (PGRs), carbohydrate substrates, and photoperiod, each of which exerts a substantial impact on callus initiation, maintenance, and morphogenic development (Mastuti *et al.*, 2017). An intermediate auxin-to-cytokinin ratio is generally optimal for callus induction (Dar *et al.*, 2021), as the synergistic interaction between these hormones stimulates cell division and proliferation, enhances protein synthesis, and thereby stimulates callus formation and boosts secondary metabolite production (Mayerni *et al.*, 2020). In addition, carbohydrate substrates serve not only as primary carbon and energy sources sustaining the photomixotrophic metabolism of *in vitro* plantlets but also act as osmotic agents and metabolic signalling molecules (Bhatia, 2015). Photoperiod, on the other hand, significantly influences biomass growth, morphogenetic responses, secondary metabolite biosynthesis and antioxidant activity in callus cultures (Ali *et al.*, 2018; Kumar *et al.*, 2020) as light signals perceived by plant cells modulate key molecular processes such as transcription, translation, and mRNA stability, thereby triggering diverse physiological and metabolic responses (Adil *et al.*, 2019).

The successful establishment of *Z. officinale* callus cultures from different cultivars and varieties has been previously achieved using a range of explant sources, including rhizome buds, leaf segments, and shoot tips (Guo & Zhang, 2005; Dehghani *et al.*, 2011; El-Nabarawy *et al.*, 2015; Ali *et al.*, 2016; El-Hameid *et al.*, 2020). Beyond simple callus induction, several reports have further optimised culture conditions to enhance biomass production and improve the accumulation of bioactive compounds. El-Nabarawy *et al.* (2015) generated callus cultures of *Z. officinale* aimed at boosting the accumulation of gingerols. However, despite these advancements, research focusing specifically on the local Malaysian 'Bentong' ginger remains scarce. Previous tissue culture studies on the 'Bentong' ginger have primarily focused on micropropagation (Sundram *et al.*, 2019; Zahid *et al.*, 2021a; Zahid *et al.*, 2021b). However, studies on callus culture development for the local Bentong variety remained limited. Although callus induction in Bentong ginger has previously been investigated (Farisi *et al.*, 2025), the study evaluated only a limited range of 2,4-D concentrations and did not comprehensively assess callus morphologies. In addition, the potential role of kinetin in callus induction of Bentong ginger has not yet been reported, thereby offering new insights into the tissue culture response of this species. In this study, a gradient concentration approach was employed to systematically evaluate and optimise the effects of selected plant growth regulators (2,4-D and kinetin), carbohydrate sources, and photoperiod conditions on friable callus induction in 'Bentong' ginger. The aim was to develop an optimised and reproducible protocol for the production of friable callus, providing a suitable foundation for the establishment of cell suspension cultures and the biosynthesis of pharmaceutically valuable secondary metabolites for 'Bentong' ginger.

MATERIALS AND METHODS

Source of explants

Mature and disease-free rhizomes of 'Bentong' ginger (*Z. officinale* Roscoe) were obtained from a commercial supplier specialising in Bentong ginger cultivation located in Bentong, Pahang, Malaysia. The rhizomes were identified as Bentong ginger by the supplier and authenticated based on characteristics and morphological features. The rhizomes were carefully washed under running tap water to remove any soil residues, then sun dried and stored in a dark, room temperature environment to encourage sprouting. Young sprouting rhizome buds (about 1.5 cm^3 in size), undamaged, and approximately two weeks old were carefully excised from the main rhizomes and were utilised as explants for further study (Figure 1).

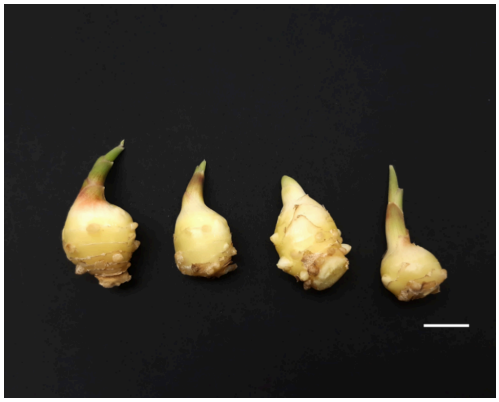

Fig. 1. Healthy and undamaged 'Bentong' ginger sprouting buds excised from the main rhizomes. The scale bar represents 1 cm.

Surface sterilisation of rhizome buds explants and culture initiation

The surface sterilisation methods utilised for this study were established with reference to previous protocols along with some modifications. Rhizome buds were carefully excised from the main rhizomes of 'Bentong' ginger, and their outer layers were removed. The bud explants were gently cleaned with Sunlight® dishwashing detergent and rinsed thoroughly under running tap water for at least 30 min. They were then immersed in a 20% (v/v) Dettol® solution with two drops of Tween-20 for 45 min, followed by treatment with 70% (v/v) ethanol for 10 min and immersion in a 40% (v/v) Clorox® solution containing two drops of Tween-20 for 10 min. The explants were then rinsed with sterile distilled water, and any bleached or damaged tissue was carefully trimmed away. They were then immersed again in 40% Clorox® solution with two drops of Tween-20 and gently swirled for one min. The explants were excised into approximately 1 cm^3 pieces, briefly swirled in 70% ethanol, and rinsed with sterile distilled water. The explants were then dried on sterile filter paper before inoculation onto Murashige and Skoog (MS) medium without plant PGRs, solidified with 0.8% (w/v) plant agar and adjusted to pH 5.8 before autoclaving. All cultures were incubated at 25 ± 2°C under cool white LED illumination (Philips TLD, 36 W; 150 µmol m^{-2} s^{-1}) with a 16 hr light / 8 hr dark photoperiod for a duration of two weeks.

Callus induction in single treatments of 2,4-D and kinetin

Sterile explants were inoculated onto MS medium supplemented with varying concentrations of 2,4-D (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L) and kinetin (0.5, 1.0, 1.5, 2.0 mg/L). Each treatment was conducted in triplicate, with a total of twenty explants used as replicates per treatment. Cultures were subcultured onto fresh treatment medium every three weeks.

Callus induction using different carbohydrate substrates

The concentration of 2.5 mg/L 2,4-D was identified as optimal for callus induction and was used for all subsequent experiments. Sterile explants were placed on full-strength MS medium supplemented with 2.5 mg/L 2,4-D and different carbohydrate sources and concentrations (30 g/L sucrose, 15 g/L sucrose, 30 g/L glucose, and 15 g/L glucose). The experiment was conducted in triplicate, with nine explant replicates per treatment. Cultures were subcultured onto fresh medium every three weeks.

Callus induction under different photoperiods

Sterile explants were inoculated into full-strength MS medium supplemented with an optimised concentration of carbohydrate substrates (30 g/L sucrose) and 2.5 mg/L 2,4-D. The explants were then cultured under three different photoperiod conditions: continuous darkness, 16-hr light / 8-hr dark, and continuous light. Explants grown under the 16 hr / 8 hr and continuous light conditions were exposed to the same white LED illumination (Philips TLD, 36 W, 150 μmol m^{-2} s^{-1}). The experiment was repeated three times, and each treatment group consisted of ten replicates. The cultures were subcultured into fresh culture medium every three weeks.

Data collection and statistical analysis

Parameters such as the percentage of callus formation, callus characteristics (including colour and texture), as well as the percentage of shoot and root induction were evaluated after six weeks of culture.

$$\text{Callus Induction } (\%) = \frac{\text{Number of Explants Producing Callus}}{\text{Total Number of Explants}} \text{ x } 100\%$$

$$\text{Shoot Induction } (\%) = \frac{\text{Number of Explants Producing Shoots}}{\text{Total Number of Explants}} \text{ x } 100\%$$

$$\text{Root Induction } (\%) = \frac{\text{Number of Explants Producing Roots}}{\text{Total Number of Explants}} \text{ x } 100\%$$

Callus was gently scraped from the explants and weighed using an analytical balance (Shimadzu Electronic Balance TX323L, Japan). The resulting data were analysed by one-way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test at $p \leq 0.05$, using IBM SPSS® Version 27 to assess differences among treatments.

RESULTS AND DISCUSSION

Callus induction using different Plant Growth Regulators (PGRs)

Callus induction in treatments of 2,4-D

In this study, rhizome buds of 'Bentong' ginger were treated with varying concentrations of 2,4-D to establish the optimal conditions for callus initiation. Based on Table 1, the maximum percentage of callus induction (100%) was achieved in MS medium containing 2.0 mg/L 2,4-D. Rhizome bud explants without the plant growth regulators (control) resulted in the lowest percentage of callus induction (70.59%), whereas the treatment of 3.0 mg/L of 2,4-D resulted in 84.62% of callus induction.

After six weeks of culture, the average callus weight ranged from 0.125 g to 0.257 g. As shown in Table 1, callus fresh weight increased with 2,4-D concentrations from 0 to 2.5 mg/L but declined at 3.0 mg/L. The maximum callus weight (0.257 ± 0.06 g) was obtained from rhizome buds treated with 2.5 mg/L 2,4-D. This callus weight was significantly higher than most other treatments, except for those treated with 2.0 mg/L and 3.0 mg/L 2,4-D. The callus produced across all 2,4-D treatments was friable and yellowish white in colour (Figure 2). The highest shoot induction percentage (20%) was observed in rhizome bud explants treated with 2.5 mg/L 2,4-D. Root formation was noted in the control as well as in explants treated with 0.5 mg/L and 1.0 mg/L 2,4-D.

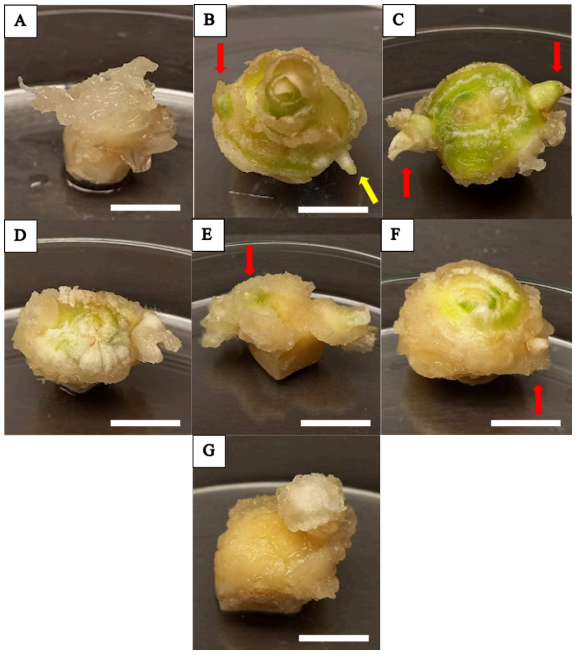

Fig. 2. The induction of callus from rhizome bud explants treated with different concentrations of 2,4-D after 6 weeks of culture. **(A)** Control; **(B)** 0.5 mg/L; **(C)** 1.0 mg/L; **(D)** 1.5 mg/L; **(E)** 2.0 mg/L; **(F)** 2.5 mg/L and **(G)** 3.0 mg/L. Red arrows indicate the initiation of shoot, whereas a yellow arrow indicates the initiation of root. Scale bar represents 1 cm.

The effect of exogenously applied plant growth regulators (PGRs) on callus formation is influenced by the endogenous hormonal balance within the explants (Sukweenadhi *et al.*, 2024). Accordingly, a straightforward dose-response relationship does not exist between the concentration of exogenously applied hormones and the physiological response of the explants and similarly, no clear association has been demonstrated between external hormone application and the timing of callus initiation (Sukweenadhi *et al.*, 2024). Auxins, particularly 2,4-D, are widely used to induce callus formation from various explants, as they promote cellular dedifferentiation and stimulate the re-entry of cells into the cell cycle, thereby initi-

Table 1. Percentage of callus induction, callus weight, morphology (colour & texture), percentage of shoot induction and percentage of root induction treated with different concentrations of 2,4-D.

2,4-D (mg/L)	Callus induction (%)	Callus fresh weight (g)#	Callus colour	Callus texture	Shoot induction (%)	Root induction (%)
0	70.59^{c}	0.125 ± 0.04^{bcd}	Yellowish white	Friable	5.88^{d}	11.76^{b}
0.5	72.22^{c}	0.127 ± 0.02^{bcd}	Yellowish white	Friable	11.11^{c}	22.22^{a}
1.0	81.25^{bc}	0.133 ± 0.02^{bcd}	Yellowish white	Friable	18.75^{a}	6.25^{bc}
1.5	87.50^{b}	0.135 ± 0.02^{bcd}	Yellowish white	Friable	12.50^{bc}	0^{c}
2.0	100^{a}	0.190 ± 0.04^{abc}	Yellowish white	Friable	13.33^{b}	0^{c}
2.5	93.33^{ab}	0.257 ± 0.06^{a}	Yellowish white	Friable	20^{a}	0^{c}
3.0	84.62^{bc}	0.218 ± 0.07^{ab}	Yellowish white	Friable	7.69^{d}	0^{c}

The average of callus fresh weight was expressed as the mean ± SE. Treatments labelled with different letters were significantly different in Duncan's Multiple Range Test ($p < 0.05$).

ating active cell division. The application of 2,4-D induces DNA hypermethylation that maintains cells in the active mitotic and dedifferentiation stage, maintaining the embryogenic potential of the cells. Moreover, 2,4-D increases cell plasticity by inducing cell wall loosening, facilitating water influx via osmosis, and hence promoting cell elongation and giving the characteristics of soft, watery, separable callus.

The high callus induction rates (>80%) in this study are consistent with previous findings on *Z. officinale*. Guo and Zhang (2005) reported optimal callus induction at 1.0 mg/L 2,4-D, producing yellow, friable, and granular callus from shoot-tip meristems of *Z. officinale* Roscoe from the Zhu Gen Jiang genotype. Similarly, Lincy *et al.* (2009) observed 90% callus formation in *Z. officinale* var. Jamaica at 2.0 mg/L 2,4-D and 100% in var. Varada at both 1.0 and 2.0 mg/L, resulting in hard, yellowish, nodular callus. These findings are consistent with the present study, in which 2.0 mg/L 2,4-D produced the highest callus initiation rate (100%). The increasing trend in callus fresh weight observed under 2,4-D treatments in the present study contrasts with the reports of Solanky *et al.* (2013) and Miri (2020), who reported enhanced callus induction between 0.5 and 1.0 mg/L, followed by a decline at 2.0 mg/L. In contrast, the current study recorded the highest callus induction (100%) at 2.0 mg/L 2,4-D and the highest callus fresh weight in the treatment of 2.5 mg/L 2,4-D (0.257 ± 0.06 g), with reduced induction observed at higher concentrations, likely due to the phytotoxic effects of excessive auxin on explant development (George *et al.*, 2008). Nonetheless, the morphological characteristics observed herein are consistent with the friable callus texture as reported in Solanky *et al.* (2013).

Spontaneous shoot and root formation was observed from the rhizome buds of 'Bentong' ginger in this study. Shoots were produced across all 2,4-D treatments, likely supported by the naturally sufficient levels of endogenous cytokinin and auxins present in the buds. Root induction was evident at lower 2,4-D concentrations (0.5 and 1.0 mg/L) but was inhibited beyond 1.0 mg/L, indicating that elevated levels of 2,4-D exert an inhibitory effect on root development. The presence of sufficient endogenous hormones in ginger rhizome buds suggests that excess 2,4-D disrupts hormonal balance and suppresses root formation. In contrast, low auxin levels promote apoplastic acidification and cell wall loosening, essential for root elongation. The absence of root formation alongside callus development is advantageous when establishing friable callus cultures intended for cell suspension systems, as root differentiation often indicates partial organogenesis and tissue specialisation. This could potentially reduce the uniformity and dispersibility in cell suspension cultures. Therefore, based on these observations, the optimal treatment for the induction of callus from the rhizome bud explants was 2.5 mg/L 2,4-D with a 93.33% callus induction rate and 0.257 ± 0.06 g callus fresh weight.

Callus induction in treatments of kinetin

In the present study, rhizome buds of 'Bentong ginger' (*Z. officinale* Roscoe) were subjected to different concentrations of kinetin for callus induction. The percentage of callus induction from rhizome buds ranged from 46.67% to 85.71% under kinetin treatments (Table 2). MS medium supplemented with 2.0 mg/L kinetin produced the highest callus induction rate (85.71%), whereas the control treatment resulted in the lowest rate (46.67%). This further demonstrated that kinetin treatments enhance callus formation.

The treatment of 0.5 mg/L kinetin resulted in the highest callus fresh weight (0.122 ± 0.03 g). However, this value is not significant compared to the rest of the treatments. Rhizome bud explants treated with 0, 1.5, and 2.0 mg/L kinetin produced yellowish white, friable callus, whereas those treated with 0.5 and 1.0 mg/L kinetin formed yellowish white, semi-friable callus. The highest shoot (28.57%) and root (35.71%) induction rates were recorded in explants exposed to 2.0 mg/L kinetin. In contrast, no shoots were observed for the control and root induction was absent in treatments with the control and 1.5 mg/L kinetin.

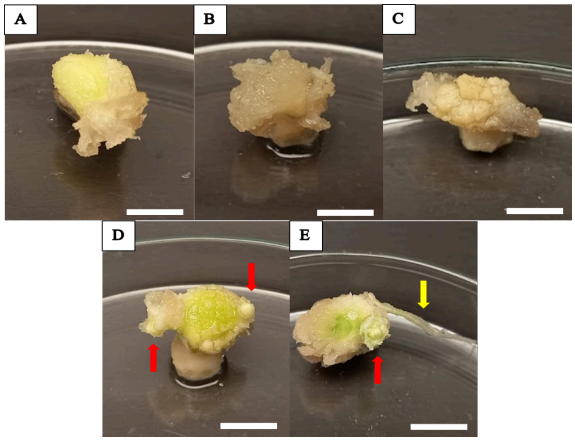

Fig. 3. The induction of callus from rhizome bud explants treated with different concentrations of kinetin after 6 weeks of culture. A) Control; B) 0.5 mg/L; C) 1.0 mg/L; D) 1.5 mg/L and E) 2.0 mg/L. Red arrows indicate the initiation of shoot, whereas the yellow arrow indicates the initiation of root. Scale bar represents 1 cm.

Kinetin is a cytokinin that plays a crucial role in stimulating cell division, a process essential for plant growth. Jamil *et al.* (2007) reported the formation of green, compact callus from shoot tips of *Z. officinale* L. cv. Wanju cultured on MS medium supplemented with 0.1 mg/L NAA and 1.0 mg/L kinetin. In contrast, Widyastuti *et al.* (2024) demonstrated that a balanced equal ratio of 2,4-D and kinetin produced the highest callus initiation rate (70%) in *Z. officinale* var. *Rubrum*. Throughout the present study, the 'Bentong' ginger rhizome buds demonstrated spontaneous induction of both

Table 2. Percentage of callus induction, weight of callus, callus morphology (colour and texture), percentage of shoot induction and percentage of root induction treated with different concentrations of kinetin.

Kinetin (mg/L)	Callus induction (%)	Callus fresh weight (g)#	Callus colour	Callus texture	Shoot induction (%)	Root induction (%)
0	46.67^{cd}	0.071 ± 0.02^{bc}	Yellowish white	Friable	0^{d}	0^{c}
0.5	68.75^{b}	0.122 ± 0.03^{abc}	Yellowish white	Semi-friable	12.5^{c}	6.25^{b}
1.0	57.14^{c}	0.061 ± 0.02^{c}	Yellowish white	Semi-friable	14.29^{c}	7.14^{b}
1.5	70.59^{ab}	0.066 ± 0.02^{c}	Yellowish white	Friable	17.65^{b}	0^{c}
2.0	85.71^{a}	0.106 ± 0.02^{abc}	Yellowish white	Friable	28.57^{a}	35.71^{a}

The average of callus fresh weight was expressed as the mean ± SE. Treatments labelled with different letters were significantly different in Duncan's Multiple Range Test (p<0.05).

shoot and root structures irrespective of the kinetin concentration applied. Zahid *et al.* (2021a, 2021b) previously reported that 2.22 mg/L (10 µM) kinetin achieved 86.67% shoot induction. In contrast, the current study recorded only 28.57% shoot induction at 2.0 mg/L kinetin. Kinetin is not commonly used to induce callus for *Z. officinale*. Rather, it is typically applied in combination with auxins to enhance callus formation, and at relatively lower auxin concentrations, to promote shoot regeneration from established callus tissues (Duszka *et al.*, 2009). For instance, a 1:1 ratio of kinetin and 2,4-D was reported to yield the highest callus induction rate (70%) in red ginger bud explants (Xuan *et al.*, 2004). In another study, embryogenic callus induction was achieved using a high concentration of 2,4-D (10 mg L^{-1}) in combination with a low level of kinetin (approximately 0.2 mg L^{-1}) in a shoot-tip culture system, indicating that elevated auxin levels can effectively drive embryogenesis even with minimal kinetin supplementation in red ginger (*Z. officinale*) (Widyastuti *et al.*, 2024). These studies have demonstrated that the type and concentration of auxin significantly influence callus characteristics such as friability, phenolic accumulation, and regeneration capacity; therefore, the effects of kinetin should be interpreted in the context of a co-applied auxin.

With reference to the results in the present study, the treatment of 2.0 mg/L kinetin was optimal for callus induction, with the highest callus induction percentage and callus fresh weight. However, based on our observation, the efficacy of kinetin in promoting callus formation was inferior to that of 2,4-D, as evidenced by lower induction rates and reduced average callus biomass. Despite these quantitative differences, no qualitative variation in callus colour was observed between the two treatments, with both producing yellowish white calli (Figures 2 and 3).

Callus induction using different carbohydrate substrates

The present experiment investigated the influence of different carbohydrate sources on callus induction from 'Bentong' ginger bud explants cultured on MS medium fortified with 2.5 mg/L 2,4-D. Six weeks post-inoculation, callus formation was recorded in all experimental groups (Figure 4). Sucrose demonstrated superior performance over glucose, achieving complete callus induction (100%) at both tested concentrations (15 g/L and 30 g/L), as shown in Table 3. Glucose-supplemented media exhibited comparatively lower induction frequencies of 88.89% (15 g/L) and 77.78% (30 g/L).

Among the four carbohydrate treatments, the application of 30 g/L glucose yielded the highest mean callus fresh weight (0.369 ± 0.10 g), while the lowest average (0.150 ± 0.02 g) was recorded in the 15 g/L glucose treatment, which was significantly different from the control. The mean weights of callus generated from both sucrose treatments and the 30 g/L glucose treatment exhibited no significant variance. Throughout all experimental conditions, the callus that developed was characterised by a friable texture and a yellowish white colouration. Furthermore, the emergence of shoots was documented within each treatment batch following a six week culture period. The maximum percentage of shoot induction (77.78%) was recorded in the 15 g/L glucose treatment; in contrast, the 15 g/L sucrose treatment yielded the minimum percentage (44.44%). Importantly, explants subjected to glucose treatment (both 15 g/L and 30 g/L) demonstrated an enhanced root induction rate (33.33%) when juxtaposed with those cultivated in sucrose-based media. No root formation was noted in the 30 g/L sucrose treatment.

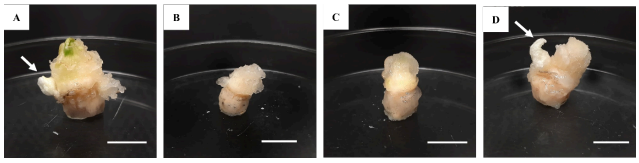

Fig. 4. The induction of callus from the rhizome bud explants after six weeks of culture in different carbohydrate substrate treatments. **(A)** 15 g/L sucrose; **(B)** 30 g/L sucrose (Control); **(C)** 15 g/L glucose; **(D)** 30 g/L glucose. The white arrow indicates shoot initiation. Scale bar represents 1 cm.

Carbohydrates, often referred to as carbon sources, serve an indispensable function in the formulation of plant tissue culture media by facilitating the photomixotrophic metabolic processes essential for the optimal development of *in vitro* plant specimens (Yaseen *et al.*, 2012; Puad *et al.*, 2020). In addition to serving as an energy source, sugars also function as osmotic agents, critically modulating the osmotic potential of the surrounding medium. This regulation is essential for controlling the influx of water and nutrients into plant tissues, thereby maintaining cellular turgor pressure. The mean fresh weight of callus observed in this study aligns with the findings of Prakoeswa and Suryaningsih (2020), who reported that *Z. officinale* "Jahe Gajah" explants cultured with glucose produced higher callus biomass compared to those cultured with sucrose. The relatively low callus induction percentage observed at 30 g/L glucose in the present study could be attributed to its high osmotic potential. Metabolizable sugars such as glucose may suppress growth or differentiation not only through osmotic effects, but also by participating in the metabolic and signalling pathways that influence sugar sensing, gene expression, and hormonal regulation (Roussos, 2013; Ćosić *et al.*, 2021).

On the other hand, sucrose showed a better callus induction rate than glucose. Previous studies on *Z. officinale* reported the applica-

Table 3. The percentage of callus induction, average callus fresh weight, callus morphology (colour and texture), percentage of shoot induction, and percentage of root induction under different carbohydrate substrate treatments.

Carbohydrate substrate	Conc. (g/L)	Callus induction (%)	Callus fresh weight (g)#	Callus colour	Callus texture	Shoot induction (%)	Root induction (%)
Sucrose	15	100^{a}	0.324 ± 0.03^{a}	Yellowish white	Friable	44.44^{bc}	11.11^{b}
Sucrose	30 (control)	100^{a}	0.302 ± 0.03^{a}	Yellowish white	Friable	55.56^{b}	0^{c}
Glucose	15	88.89^{b}	0.150 ± 0.02^{b}	Yellowish white	Friable	77.78^{a}	33.33^{a}
Glucose	30	77.78^{bc}	0.369 ± 0.10^{a}	Yellowish white	Friable	55.56^{b}	33.3^{a}

The average of callus fresh weight was expressed as the mean ± SE. Treatments labelled with different letters were significantly different in Duncan's Multiple Range Test ($p<0.05$).

tion of 30 g/L sucrose with PGRs leading to effective callus induction. Sucrose was also observed as the more efficient carbon source compared to glucose for callus induction, as observed in the species of *Aquilaria malaccensis* (Jayaraman *et al.*, 2014) and *Taraxacum officinale* (Martinez *et al.*, 2021). Yue *et al.* (2024) noted that sucrose influences regenerative capacity through its regulatory effects on glycometabolism and cellular osmoregulation, favouring callus differentiation by sustaining moderate reactive oxygen species (ROS) levels, conducive to morphogenesis. Sucrose is hydrolysed by extracellular and intracellular enzymes into metabolites that enter various anabolic and signalling pathways involved in callus formation (Chen & Dribnenki, 2004; Emiliani *et al.*, 2016). Acidic and neutral invertases hydrolyse sucrose into glucose and fructose, while sucrose synthase converts sucrose into UDP-glucose and fructose, thereby linking sucrose metabolism to cell wall formation and polysaccharide biosynthesis (Chen & Dribnenki, 2004). This further supports the effectiveness of sucrose as an efficient carbon source for callus induction in the present study.

Additionally, cells can take up sucrose through cell wall acid invertases, further hydrolysing it into the hexoses such as glucose and fructose, thereby generating sugar signals or supplying energy to support cell growth. Similarly, glucose in culture media may degrade into toxic compounds during the autoclaving process, reducing carbohydrate availability. Since media containing glucose solely lack an alternative carbon source, the limited sugar availability in MS media supplemented with 15 g/L in the current study could potentially result in the observation of lower callus fresh weight in the present study. The findings of this study noted that 15 g/L glucose may be more suitable for shoot and root induction but insufficient to sustain callogenesis. Beyond supporting callus formation, glucose serves as an energy source during shoot and root organogenesis, which may deplete the available supply and limit its capacity to sustain both energy-intensive processes simultaneously. The results also indicated that glucose is particularly favourable for root induction in ginger buds, likely by facilitating cell recruitment and promoting mitotic activity, thereby enhancing root initiation.

However, even though the use of 30 g/L glucose resulted in the highest average callus fresh weight, the findings indicated that full-strength MS medium supplemented with 2.5 mg/L 2,4-D and sucrose may be more generally appropriate for callus induction in 'Bentong' ginger rhizome buds based on its 100% callus induction rate. The differences in callus fresh weight between 15 g/L and 30 g/L sucrose were not evident, suggesting that both concentrations are suitable for callus induction. However, since 15 g/L sucrose was observed to induce roots via indirect organogenesis, 30 g/L sucrose is considered the optimal carbohydrate concentration for callogenesis for the bud explants of 'Bentong' ginger (*Z. officinale* Roscoe).

Callus induction under different photoperiod treatments

In this study, 'Bentong' ginger rhizome buds were exposed to various photoperiods to identify the most effective light condition for callus initiation. Based on Table 4, callus induction rates among the different photoperiod treatments ranged between 50.0% and 70.0%. The highest induction rate (70.0%) was recorded in explants cultured under continuous darkness and continuous light, whereas the lowest induction rate (50.0%) was observed in the control group exposed to a 16-hr light / 8-hr dark cycle. After six weeks of culture, the average callus weight ranged from 0.095 g to 0.270 g (Table 4). The highest callus weight was found in the continuous dark treatment (0.270 ± 0.07 g), while the lowest was under continuous light (0.095 ± 0.02 g). Both these values were statistically significant, and it was observed that callus weight decreased as light exposure increased, indicating that the presence of light could potentially inhibit callus formation for 'Bentong' ginger explants. All treatments produced friable, yellowish white callus (Figure 5). Shoots formed in all treatments, with the 16 hr / 8 hr light/dark condition showing the highest shoot induction (40.0%), and continuous light the lowest. Roots only developed under the 16h/8h light/dark and continuous dark treatments, both with a 10.0% root induction rate.

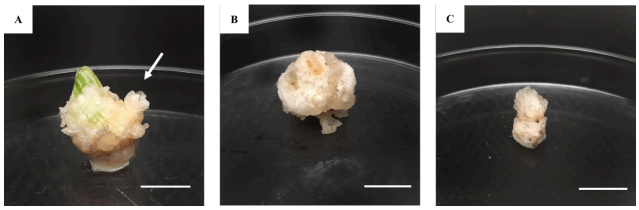

Fig. 5. The induction of callus from the rhizome bud explants in different photoperiod treatments for six weeks. **(A)** 16 hr / 8 hr (light/dark) (Control); **(B)** Continuous dark; **(C)** Continuous light. The white arrow indicates shoot initiation. The scale bar represents 1 cm.

Light is an essential environmental factor influencing *in vitro* plant growth and development, affecting callus initiation, differentiation, and secondary metabolite production. Factors such as light quality, intensity, and photoperiod play significant roles in determining secondary metabolite content. For instance, the composition and levels of secondary metabolites in the same plant species can vary markedly across different geographic regions due to differences in local light conditions (Huang & Liu, 2015). The light requirements under *in vitro* conditions vary according to the plant species, genotype, explant type, and cell type involved. For instance, some plant species require light to initiate callus formation, whereas others respond better under complete darkness. Light promotes callus formation by activating enzymes associated with cell division, such as cyclin-dependent kinases (I'anatushshoimah *et al.*, 2020). Light also affects callus development by altering the effectiveness of

Table 4. Percentage of callus induction, average callus fresh weight, callus morphology (colour and texture), percentage of shoot induction, and percentage of root induction under different photoperiod treatments.

Treatment	Photoperiod	Callus induction (%)	Callus fresh weight (g)[#]	Callus colour	Callus texture	Shoot induction (%)	Root induction (%)
Control	16 h/8 h (light/dark)	50.0^{b}	0.262 ± 0.07^{a}	Yellowish white	Friable	40.0^{a}	10.0^{a}
1	Continuous Dark	70.0^{a}	0.270 ± 0.09^{a}	Yellowish white	Friable	20.0^{b}	10.0^{a}
2	Continuous Light	70.0^{a}	0.095 ± 0.02^{b}	Yellowish white	Friable	10.0^{c}	0^{b}

The average of callus fresh weight was expressed as the mean ± SE. Means labelled with the same letters were not significantly different in Duncan's Multiple Range Test at $p<0.05$.

plant growth regulators (PGRs) in the medium and by modulating endogenous hormone levels, such as auxins and gibberellins.

Numerous reports have documented the application of continuous darkness for callus induction from the rhizome buds of various species belonging to the family Zingiberaceae. This includes *Z. officinale* Rosc. var. Sying Makhir, *Z. officinale* Rosc. var. Rubrum, *Curcuma kwangsiensis*, *Curcuma mangga*, *Boesenbergia rotunda*, from the inner core rhizome of *Elettaria cardamomum* Maton. This may be attributed to the enhancement of auxin activity under dark conditions, whereas exposure to light can lead to auxin degradation and hinder its translocation to the explants, thereby diminishing its effectiveness in promoting callus formation (I'anatushshoimah *et al.*, 2020). However, Chuengpanya *et al.* (2020) reported contrasting findings in *Hedychium longicornutum* Griff. ex Baker (Zingiberaceae), where optimal callus induction was achieved under light conditions. This indicated that the effect of light on callus induction may vary with species and explant type.

Reduced callus formation under continuous light conditions may be attributed to stress induced by prolonged exposure, which can suppress biomass accumulation while stimulating antioxidant activity and secondary metabolite production (Ali *et al.*, 2018). In addition, extended illumination may promote the photodegradation of certain culture medium components, thereby further limiting callus biomass (Hangarter & Stasinopoulos, 1991). However, in the present study, the callus produced under all photoperiod treatments was consistently yellowish white and friable, indicating that light conditions did not markedly influence callus morphology. This observation contrasts with the findings of Yaacob *et al.* (2014), who documented a progressive change in callus colour from white to yellow to green with increasing light intensity in *Citrus assamensis*.

In the present study, shoot formation was observed under all photoperiod conditions, whereas root development was absent under continuous light. Among the treatments, a 16 hr / 8 hr light/dark cycle was found to be optimal for the simultaneous induction of both shoots and roots. This finding is consistent with the report by Swarnathilaka *et al.* (2016), who demonstrated enhanced microrhizome development in *Zingiber officinale* rhizome buds of a Sri Lankan cultivar under the same photoperiod, resulting in the formation of multiple shoots and roots. In contrast, Kusumastuti *et al.* (2014) observed that continuous light promoted the highest number of shoots and roots for *Zingiber aromaticum* shoot cultures, highlighting the species- and genotype-specific responses to light conditions. The findings of this study suggested that shorter light exposure promotes greater callus biomass accumulation, with continuous darkness representing the most optimal condition for callus induction, likely due to the reduced differentiation of shoots from rhizome bud explants.

CONCLUSION

In conclusion, the optimal medium for inducing friable callus in 'Bentong' ginger (*Z. officinale* Roscoe) was MS medium supplemented with 2.5 mg/L 2,4-D, producing the highest callus induction rate (93.33%) and fresh weight (0.257 ± 0.06 g). Among all carbohydrate treatments, 30 g/L sucrose was identified as the most effective concentration for promoting callogenesis from bud explants. Additionally, continuous darkness resulted in the highest callus induction percentage (70.0%) and fresh weight (0.270 ± 0.07 g), highlighting the significant influence of photoperiod on callus development. The present study establishes a preliminary framework for the successful induction of friable callus from rhizome bud explants of 'Bentong' ginger (*Z. officinale* Roscoe), offering a foundation for developing cell suspension cultures and exploring novel secondary metabolites from this valuable cultivar.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adil, M., Abbasi, B.H. & ul Haq, I. 2019. Red light controlled callus morphogenetic patterns and secondary metabolites production in *Withania somnifera* L. Biotechnology Reports, 24: e00380. https://doi.org/10.1016/j.btre.2019.e00380

Ahmad, B., Rehman, M.U., Amin, I., Arif, A., Rasool, S., Bhat, S.A., Afzal, I., Hussain, I., Bilal, S. & Mir, M.U.R. 2015. A review on pharmacological properties of zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone). The Scientific World Journal, 2015: 1-9. https://doi.org/10.1155/2015/816364

Al Farisi, A., Utami, S. & Ar rahman, Z. 2025. The effect of growth regulatory hormones with various concentrations on the growth of Bentong ginger plants through tissue culture. Jurnal Agronomi Tanaman Tropika (Juatika), 7(2): 364. https://doi.org/10.36378/juatika.v7i2.4264

Ali, A.A.M., Mohamed El-Nour, M.E. & Mohamed Yagi, S. 2016. Callus induction, direct and indirect organogenesis of ginger (*Zingiber officinale* Rosc). African Journal of Biotechnology, 15(38): 2106-2114. https://doi.org/10.5897/AJB2016.15540

Ali, H., Khan, M.A., Ullah, N. & Khan, R.S. 2018. Impacts of hormonal elicitors and photoperiod regimes on elicitation of bioactive secondary volatiles in cell cultures of *Ajuga bracteosa*. Journal of Photochemistry and Photobiology B: Biology, 183: 242-250. https://doi.org/10.1016/j.jphotobiol.2018.04.044

Bapat, V.A., Kavi Kishor, P.B., Jalaja, N., Jain, S.M. & Penna, S. 2023. Plant cell cultures: Biofactories for the production of bioactive compounds. Agronomy, 13(3): 858. https://doi.org/10.3390/agronomy13030858

Beristain-Bauza, S.D.C., Hernández-Carranza, P., Cid-Pérez, T.S., Ávila-Sosa, R., Ruiz-López, I.I. & Ochoa-Velasco, C.E. 2019. Antimicrobial activity of ginger (*Zingiber officinale*) and its application in food products. Food Reviews International, 35(5): 407-426. https://doi.org/10.1080/87559129.2019.1573829

Bhatia, S. 2015. Plant tissue culture. In: Modern Applications of Plant Biotechnology in Pharmaceutical Sciences. Bhatia, S., Sharma, K., Dahiya, R. & Bera, T. (Eds.). Elsevier, Amsterdam. pp. 31-107. https://doi.org/10.1016/B978-0-12-802221-4.00002-9

Bhatt, N., Waly, M.I. & Ali, A. 2013. Ginger: A functional herb. In: Food as Medicine. Nova Science Publishers, New York. pp. 51-71.

Borisjuk, L., Walenta, S., Weber, H., Mueller-Klieser, W. & Wobus, U. 1998. High-resolution histographical mapping of glucose concentrations in developing cotyledons of *Vicia faba* in relation to mitotic activity and storage processes: Glucose as a possible developmental trigger. The Plant Journal, 15(4): 583-591. https://doi.org/10.1046/j.1365-313X.1998.00214.x

Chen, Y. & Dribnenki, P. 2004. Effect of medium osmotic potential on callus induction and shoot regeneration in flax anther culture. Plant Cell Reports, 23(5): 272-276. https://doi.org/10.1007/s00299-004-0831-x

Chen, Y.M., Huang, J.Z., Hou, T.W. & Pan, I.C. 2019. Effects of light intensity and plant growth regulators on callus proliferation and shoot regeneration in the ornamental succulent *Haworthia*. Botanical Studies, 60(1): 1-8. https://doi.org/10.1186/s40529-019-0257-y

Chuengpanya, R., Chuenboonngarm, N., Sakchaichanchol, K., Muangkroot, A. & Thammasiri, K. 2020. In vitro propagation and callus induction of *Hedychium longicornutum* Griff. ex Baker using different explants. Acta Horticulturae, 1298: 323-330. https://doi.org/10.17660/ActaHortic.2020.1298.44

Corrêa, L.D.R., Paim, D.C., Schwambach, J. & Fett-Neto, A.G. 2005. Carbohydrates as regulatory factors on the rooting of *Eucalyptus saligna* Smith and *Eucalyptus globulus* Labill. Plant Growth Regulation, 45(1): 63-73. https://doi.org/10.1007/s10725-004-6125-z

Ćosić, T., Motyka, V., Savić, J., Raspor, M., Marković, M., Dobrev, P.I. & Ninković, S. 2021. Sucrose interferes with endogenous cytokinin homeostasis and expression of organogenesis-related genes during *de novo* shoot organogenesis in kohlrabi. Scientific Reports, 11(1): 6494. https://doi.org/10.1038/s41598-021-85932-w

Dalila, Z.D., Jaafar, H. & Manaf, A.A. 2013. Effects of 2,4-D and kinetin on callus induction of *Barringtonia racemosa* leaf and endosperm explants in different types of basal media. Asian Journal of Plant Sciences, 12(1): 21-27. https://doi.org/10.3923/ajps.2013.21.27

Dantas, L.A., Faria, P.S.A., Dário, B.M.M., Arantes, A.L.M., Silva, F.G., Avila, R.G., Pereira, P.S. & Neto, A.R. 2021. The impact of carbon source on cell growth and the production of bioactive compounds in cell suspensions of *Hancornia speciosa* Gomes. Scientific Reports, 11(1): 1-14. https://doi.org/10.1038/s41598-021-03845-0

Dar, S.A., Nawchoo, I.A., Tyub, S. & Kamili, A.N. 2021. Effect of plant growth regulators on in vitro induction and maintenance of callus from leaf and root explants of *Atropa acuminata* Royle ex Lindl. Biotechnology Reports, 32: e00688. https://doi.org/10.1016/j.btre.2021.e00688

Dehghani, I., Mostajeran, A. & Gholamreza, A. 2011. In vitro and in vivo production of gingerols and zingiberene in ginger plant (*Zingiber officinale* Roscoe). Iranian Journal of Pharmaceutical Sciences, 7(2): 117-121.

Duszka, K., Clark, B.F.C., Massino, F. & Barciszewski, J. 2009. Biological activities of kinetin. In: Herbal Drugs: Ethnomedicine to Modern Medicine. Ramawat, K. (Ed.). Springer, Berlin. pp. 369-380. https://doi.org/10.1007/978-3-540-79116-4_20

El-Hameid, A.A.R., Abo El-kheir, Z.A., Abdel-Hady, M.S. & Helmy, W.A. 2020. Identification of DNA variation in callus derived from *Zingiber officinale* and anticoagulation activities of ginger rhizome and callus. Bulletin of the National Research Centre, 44(1): 28. https://doi.org/10.1186/s42269-020-0281-9

El-Nabarawy, M.A., El-Kafafi, S.H., Hamza, M.A. & Omar, M.A. 2015. The effect of some factors on stimulating the growth and production of active substances in *Zingiber officinale* callus cultures. Annals of Agricultural Sciences, 60(1): 1-9. https://doi.org/10.1016/j.aoas.2014.11.020

Emiliani, G., Traversari, S., Di Carlo, A., Traversi, M.L., Cantini, C. & Giovannelli, A. 2016. B-type cyclin modulation in response to carbon balance in callus of *Populus alba*. Plant Cell, Tissue and Organ Culture, 124(2): 283-293. https://doi.org/10.1007/s11240-015-0892-2

George, E.F., Hall, M.A. & De Klerk, G.J. 2008. Plant Propagation by Tissue Culture. 3rd Ed. Springer, Netherlands. 501 pp. https://doi.org/10.1007/978-1-4020-5005-3

Gnasekaran, P., Rahman, Z.A., Chew, B.L., Uddain, J., Solayappan, M., Chear, N.J.Y., Appalasamy, S., Mariappan, V., Wahyuni, D.K. & Subramaniam, S. 2023. Picloram enhanced the callus induction, growth kinetics, antioxidant potentials, and secondary metabolites production of *Zingiber officinale* var. *rubrum* callus cultures. Plant Cell, Tissue and Organ Culture, 155(3): 843-859. https://doi.org/10.1007/s11240-023-02603-6

Grossmann, K. 2010. Auxin herbicides: Current status of mechanism and mode of action. Pest Management Science, 66(2): 113-120. https://doi.org/10.1002/ps.1860

Gunasekaran, U. 2014. Callus induction and plant regeneration studies of *Clinacanthus nutans* (Sabah snake grass). Bachelor's Degree Thesis, Universiti Tunku Abdul Rahman, Malaysia.

Guo, Y. & Zhang, Z. 2005. Establishment and plant regeneration of somatic embryogenic cell suspension cultures of *Zingiber officinale* Rosc. Scientia Horticulturae, 107(1): 90-96. https://doi.org/10.1016/j.scienta.2005.07.003

Halliday, K.J., Martínez-García, J.F. & Josse, E.M. 2009. Integration of light and auxin signaling. Cold Spring Harbor Perspectives in Biology, 1(6): a001586. https://doi.org/10.1101/cshperspect.a001586

Hangarter, R.P. & Stasinopoulos, T.C. 1991. Repression of plant tissue culture growth by light is caused by photochemical change in the culture medium. Plant Science, 79(2): 253-257. https://doi.org/10.1016/0168-9452(91)90114-N

Hossain, M.A., Hossain, M.T., Ali, M.R. & Rahman, S.M.M. 2005. Effect of different carbon sources on in vitro regeneration of Indian pennywort (*Centella asiatica* L.). Pakistan Journal of Biological Sciences, 8(7): 963-965. https://doi.org/10.3923/pjbs.2005.963.965

Huang, L.Q. & Liu, C.X. 2015. Biosynthesis and regulation of secondary metabolites in medicinal plants. In: Molecular Pharmacognosy. Bao, Y. & Cao, L.Y. (Eds.). Science Press, Beijing. pp. 251-252.

I'anatushshoimah, I., Nurchayati, Y., Prihastanti, E. & Hastuti, R.B. 2020. Effects of light for callus induction of mangrove plant (*Rhizophora apiculata* Bi) by in vitro. Life Science, 9(2): 138-148.

Ibrahim, D., Danial, G., Mosa, V. & Khalil, B. 2015. Plant regeneration from shoot tips-derived callus of ginger (*Zingiber officinale* Rosc.). American Journal of Experimental Agriculture, 7(1): 55-61. https://doi.org/10.9734/AJEA/2015/13980

Jamil, M., Kim, J.K., Akram, Z., Ajmal, S.U. & Rha, E.S. 2007. Regeneration of ginger plant from callus culture through organogenesis and effect of CO_2 enrichment on the differentiation of regenerated plant. Biotechnology, 6(1): 101-104. https://doi.org/10.3923/biotech.2007.101.104

Jayaraman, S., Daud, N.H., Halis, R. & Mohamed, R. 2014. Effects of plant growth regulators, carbon sources and pH values on callus induction in *Aquilaria malaccensis* leaf explants and characteristics of the resultant calli. Journal of Forestry Research, 25(3): 535-540. https://doi.org/10.1007/s11676-014-0492-8

Kumar, S., Arya, M., Mahadevappa, P. & Giridhar, P. 2020. Influence of photoperiod on growth, bioactive compounds and antioxidant activity in callus cultures of *Basella rubra* L. Journal of Photochemistry and Photobiology B: Biology, 209: 111937. https://doi.org/10.1016/j.jphotobiol.2020.111937

Kusumastuti, M.Y., Bhatt, A., Indrayanto, G. & Lai Keng, C. 2014. Effect of sucrose, benzylaminopurine and culture condition on in vitro propagation of *Curcuma xanthorrhiza* Roxb. Pakistan Journal of Botany, 46(1): 279-288.

Lincy, A.K., Remashree, A.B. & Sasikumar, B. 2009. Indirect and direct somatic embryogenesis from aerial stem explants of ginger (*Zingiber officinale* Rosc.). Acta Botanica Croatica, 68(1): 93-103.

Lüthen, H. & Böttger, M. 1993. The role of protons in the auxin-induced root growth inhibition: A critical reexamination. Botanica Acta, 106(1): 58-63. https://doi.org/10.1111/j.1438-8677.1993.tb00338.x

Machakova, I., Zazimalova, E. & George, E.F. 2008. Plant growth regulators I: Introduction; Auxins, their analogues and inhibitors. In: Plant Propagation by Tissue Culture. George, E.F., Hall, M.A. & De Klerk, G.J. (Eds.). Springer, Netherlands. pp. 115-173.

Majda, M. & Robert, S. 2018. The role of auxin in cell wall expansion. International Journal of Molecular Sciences, 19(4): 951. https://doi.org/10.3390/ijms19040951

Manohari, C., Backiyarani, S., Jebasingh, T., Somanath, A. & Usha, R. 2008. Efficient plant regeneration in small cardamom (*Elettaria cardamomum* Maton.) through somatic embryogenesis. Indian Journal of Biotechnology, 7(3): 407-409.

Martinez, M.E., Jorquera, L., Poirrier, P., Díaz, K. & Chamy, R. 2021. Effect of the carbon source and plant growth regulators (PGRs) in the induction and maintenance of an in vitro callus culture of *Taraxacum officinale* (L.) Weber ex F.H. Wigg. Agronomy, 11(6): 1181. https://doi.org/10.3390/agronomy11061181

Mastuti, R., Munawarti, A. & Firdiana, E.R. 2017. The combination effect of auxin and cytokinin on in vitro callus formation of *Physalis angulata* L. - A medicinal plant. AIP Conference Proceedings, 1908: 040007. https://doi.org/10.1063/1.5012721

Mayerni, R., Satria, B., Wardhani, D. & Chan, S. 2020. Effect of auxin (2,4-D) and cytokinin (BAP) in callus induction of local patchouli plants (*Pogostemon cablin* Benth.). IOP Conference Series: Earth and Environmental Science, 583(1): 012003. https://doi.org/10.1088/1755-1315/583/1/012003

Miri, S.M. 2020. Micropropagation, callus induction and regeneration of ginger (*Zingiber officinale* Rosc.). Open Agriculture, 5(1): 75-84. https://doi.org/10.1515/opag-2020-0008

Mustafa, I. & Chin, N.L. 2023. Antioxidant properties of dried ginger (*Zingiber officinale* Roscoe) var. Bentong. Foods, 12(1): 178. https://doi.org/10.3390/foods12010178

Omidi, M. & Shahpiri, A. 2003. Callus induction and plant regeneration in vitro in potato. Acta Horticulturae, 619: 315-322. https://doi.org/10.17660/ActaHortic.2003.619.36

Prakoeswa, S.A. & Suryaningsih, D.R. 2020. Vegetative propagation in vitro with content analysis of ginger oil from calluses of "Jahe Gajah" (*Zingiber officinale*) on treatments of types of mediums and carbohydrates. IOP Conference Series: Earth and Environmental Science, 484(1): 012017. https://doi.org/10.1088/1755-1315/484/1/012017

Pramono, S. 2019. Utilisation and functional components evaluation of ginger. In: Ginger Cultivation and Its Antimicrobial and Pharmacological Potentials. Wang, H. (Ed.). IntechOpen, London. pp. 1-14. https://doi.org/10.5772/intechopen.88940

Puad, N.I.M., M. Fathil, N.A., Amid, A. & Azmi, A.S. 2020. Effects of carbon sources, plant growth regulators and inoculum size on *Citrus suhuiensis* cell suspension culture growth. Malaysian Applied Biology, 49(1): 87-93. https://doi.org/10.55230/mabjournal.v49i1.1658

Ramesh Babu, N., Zavith, M., Boopathi, R., Parvathy, S., Suresh, M. & Parvathy, S. 2020. Standardization of ginger callus culture and its comparison with ginger rhizome and callus treated with extrinsic molecule on their antioxidant activity. International Journal of Herbal Medicine, 8(4): 49-54.

Ravindran, P.N. & Babu, K.N. 2005. Ginger: The Genus *Zingiber*. 1st Ed. CRC Press, Boca Raton. 576 pp.

Rober-Kleber, N., Albrechtová, J.T.P., Fleig, S., Huck, N., Michalke, W., Wagner, E., Speth, V., Neuhaus, G. & Fischer-Iglesias, C. 2003. Plasma

membrane H^+-ATPase is involved in auxin-mediated cell elongation during wheat embryo development. Plant Physiology, 131(3): 1302-1312. https://doi.org/10.1104/pp.013466

Roussos, P.A. 2013. Growth and biochemical responses of jojoba (*Simmondsia chinensis* (Link) Schneid) explants cultured under mannitol-simulated drought stress in vitro. Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology, 147(2): 272-284. https://doi.org/10.1080/11263504.2013.768558

Sari, Y.P., Kusumawati, E., Saleh, C., Kustiawan, W. & Sukartingsih, S. 2018. Effect of sucrose and plant growth regulators on callogenesis and preliminary secondary metabolite production of different explants of *Myrmecodia tuberosa*. Nusantara Bioscience, 10(3): 183-192. https://doi.org/10.13057/nusbiosci/n100309

Sharizan, A. & Sahilah, A.M. 2021. Extraction and characterization of 6-shogaol and 6-gingerol from *Zingiber officinale* var. Bentong. Food Research, 5(5): 236-243. https://doi.org/10.26656/fr.2017.5(5).014

Shoaib, M., Shehzad, A., Butt, M.S., Saeed, M., Raza, H., Niazi, S., Khan, I.M. & Shakeel, A. 2016. An overview: Ginger, a tremendous herb. Journal of Global Innovations in Agricultural and Social Sciences, 4(4): 172-187. https://doi.org/10.22194/JGIASS/4.4.766

Solanky, R.U., Patel, S.R. & Patel, J.R. 2013. In vitro regeneration of ginger (*Zingiber officinale* Rosc.) through callus culture. AGRES - An International e-Journal, 2(2): 196-202.

Suhaimi, M.Y., Mohamad, A.M., Mahamud, S., Khadzir, D., Mohd, Y.S., Manas, M.A., Shahid, M. & Darus, K. 2012. Effects of substrates on growth and yield of ginger cultivated using soilless culture. Journal of Tropical Agriculture and Food Science, 40(2): 159-168.

Sukweenadhi, J., Setiabudi, J., Krisnawan, A.H. & Christanti, P. 2024. Initiation of red ginger callus (*Zingiber officinale* var. *rubrum* Rosc.) from various explants. BIO Web of Conferences, 91: 01014. https://doi.org/10.1051/bioconf/20249101014

Sulaiman, M. 2020. Effects and quantity ranges of some auxins on embryogenic callus induction from upland rice cultivars: An overview. International Journal of Life Sciences and Biotechnology, 3(2): 197-204. https://doi.org/10.38001/ijlsb.669914

Sundram, T.C.M., Suffian, M., Annuar, M. & Khalid, N. 2012. Optimization of culture condition for callus induction from shoot buds for establishment of rapid growing cell suspension cultures of mango ginger (*Curcuma mangga*). Australian Journal of Crop Science, 6(7): 1139-1146.

Swarnathilaka, D., Kottearachchi, N. & Weerakkody, W. 2016. Factors affecting on induction of microrhizomes in ginger (*Zingiber officinale* Rosc.), cultivar Local from Sri Lanka. British Biotechnology Journal, 12(2): 1-7. https://doi.org/10.9734/BBJ/2016/23903

Waghmare, P., Diwane, C., Jadhav, P., Patil, V. & Barhe, P. 2022. An overview on *Zingiber officinale* (ginger). World Journal of Pharmaceutical Research, 11(4): 625-646.

Wang, X.J. & Hsiao, K.C. 1995. Sugar degradation during autoclaving: Effects of duration and solution volume on breakdown of glucose. Physiologia Plantarum, 94(3): 415-418. https://doi.org/10.1111/j.1399-3054.1995.tb00947.x

Widyastuti, D.A., Santosa, D., Nuringtyas, T.R. & Rohman, A. 2024. Callus induction of red ginger (*Zingiber officinale* var. *rubrum*) with 2,4-D and kinetin combination to enhance total phenolic and flavonoid content. IOP Conference Series: Earth and Environmental Science, 1364(1): 012051. https://doi.org/10.1088/1755-1315/1364/1/012051

Xuan, P., Guo, Y., Yue, C. & Yin, C. 2004. Study on tissue culture and rapid propagation of ginger (*Zingiber officinale*). Southwest China Journal of Agricultural Sciences, 17(4): 484-486.

Yaacob, J.S., Mahmad, N., Mat Taha, R., Mohamed, N., Mad Yussof, A.I. & Saleh, A. 2014. Optimization of culture conditions (sucrose, pH, and photoperiod) for in vitro regeneration and early detection of somaclonal variation in ginger lime (*Citrus assamensis*). The Scientific World Journal, 2014: 262710. https://doi.org/10.1155/2014/262710

Yang, J., Zhang, Y., Gu, Y. & Wang, C. 2019. Studies on callus induction of *Valeriana officinalis* under different disinfection methods and photoperiod conditions. IOP Conference Series: Earth and Environmental Science, 371(4): 042042. https://doi.org/10.1088/1757-899X/677/4/042042

Yaseen, M., Ahmad, T., Sablok, G., Standardi, A. & Hafiz, I.A. 2012. Role of carbon sources for in vitro plant growth and development. Molecular Biology Reports, 40(4): 2837-2849. https://doi.org/10.1007/s11033-012-2299-z

Yu, Z., Zhang, F., Friml, J. & Ding, Z. 2022. Auxin signaling: Research advances over the past 30 years. Journal of Integrative Plant Biology, 64(2): 371-392. https://doi.org/10.1111/jipb.13225

Yue, J., Dong, Y., Du, C., Li, C., Wang, X. & Zhang, Y. 2024. Sucrose promotes the proliferation and differentiation of callus by regulating ROS intensity in *Agapanthus praecox*. Horticulturae, 10(12): 1350. https://doi.org/10.3390/horticulturae10121350

Yusuf, N.A., Annuar, M.S.M. & Khalid, N. 2011. Efficient propagation of an important medicinal plant *Boesenbergia rotunda* by shoot-derived callus. Journal of Medicinal Plants Research, 5(13): 2629-2636.

Zahid, N.A., Jaafar, H.Z.E. & Hakiman, M. 2021a. Micropropagation of ginger (*Zingiber officinale* Roscoe) 'Bentong' and evaluation of its secondary metabolites and antioxidant activities compared with the conventionally propagated plant. Plants, 10(4): 630. https://doi.org/10.3390/plants10040630

Zahid, N.A., Jaafar, H.Z.E. & Hakiman, M. 2021b. Alterations in microrhizome induction, shoot multiplication and rooting of ginger (*Zingiber officinale* Roscoe) var. Bentong with regards to sucrose and plant growth regulators application. Agronomy, 11(2): 320. https://doi.org/10.3390/agronomy11020320

Zhang, S., Liu, N., Sheng, A., Ma, G. & Wu, G. 2011. In vitro plant regeneration from organogenic callus of *Curcuma kwangsiensis* Lindl. (Zingiberaceae). Plant Growth Regulation, 64(2): 141-145. https://doi.org/10.1007/s10725-010-9548-8