



REVIEW ARTICLE

# Current developments on taxonomy, agronomy, bioactivity and *in vitro* micropropagation of two Malaysian ginger varieties: *Zingiber officinale* Rosc cv. Bentong and *Zingiber officinale* Rosc cv. Rubrum

Noor Shaireen Shahira Khalid<sup>1\*</sup>, Lisa Amiera Rosli<sup>1</sup>, Muhamad Fahmi Yunus<sup>1</sup>, Mohd Syahmi Salleh<sup>1, 2</sup> & Tamil Chelvan Meenakshi Sundram<sup>\*1, 2\*</sup>

<sup>1</sup>Department of Plant Sciences, Kulliyah of Science, International Islamic University Malaysia, Kuantan 25200, Pahang, Malaysia

<sup>2</sup>Sustainable Agriculture and Green Technology (AG-Tech) Research Unit, International Islamic University Malaysia, Kuantan 25200, Pahang, Malaysia

\*Correspondence email - [tamil@iium.edu.my](mailto:tamil@iium.edu.my)

Both authors contributed equally

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## Abstract

*Zingiber officinale* Rosc., or ginger, is a widely cultivated tropical herb species belonging to the family Zingiberaceae. Ginger is renowned for its medicinal and culinary uses. In Malaysia, two prominent ginger varieties, *Z. officinale* Rosc. cv. Bentong (Bentong ginger) and *Z. officinale* Rosc. cv. Rubrum (red ginger), are highly valued for their distinctive properties and significant economic importance. Bentong ginger, recognised by its geographical indication associated with the Bentong district of Pahang, is characterised by elongated leafy stems up to 90 cm in height arising from large, horn-shaped rhizomes with a strong aroma and high phenolic contents. Owing to its high medicinal value, it is predominantly regarded as a superior option for promoting health and preventing disease. Meanwhile, red ginger, distinguished by its reddish-purple rhizomes, is well-known for its high levels of vanilloids, phenolics and flavonoids, contributing to its remarkable pharmacological properties. Both varieties are propagated through conventional vegetative methods, with Bentong ginger flourishing in highland areas and red ginger thriving in well-drained sandy loam soils. Recently, the increasing consumer demand for Bentong ginger and red ginger rhizomes has prompted researchers to adopt biotechnological approaches to enhance their production. *In vitro* micropropagation is one of the biotechnological alternatives, offering intriguing options for large-scale, disease-free ginger rhizomes. In this review, we highlight current knowledge into Bentong and red ginger, focusing on their taxonomy, morphology, cultivation practices, nutritional value and bioactive compounds, along with the incorporation of tissue culture techniques for their large-scale propagation.

**Keywords:** Bentong ginger; cultivation practices; *in vitro* micropropagation; nutrition; red ginger; secondary metabolites

## Introduction

*Zingiber officinale* Rosc. (common ginger) is a well-known tropical herbaceous perennial of the family Zingiberaceae that is widely distributed across tropical, subtropical and warm temperate regions worldwide (1–2). In addition to common ginger, this family also comprises more than 1300 species from 50 genera. In Southeast Asia, various ginger species are cultivated in Thailand, Indonesia and Malaysia (3). They are used as spices, as well as for production of medicines, dyes, perfume and ornamental plants (4–5).

In Malaysia, about 160 species of the Zingiberaceae family belonging to 18 genera have been recorded, as of 2019 (2). Some of these species, such as common ginger, turmeric, galangal, lemongrass and torch ginger are widely cultivated by local people (6–7). As for common ginger, Malaysia ranks among the leading ginger-producing countries in Southeast Asia (7). Approximately 10784.65 metric tonnes of ginger rhizomes were produced in 2023. Ginger is cultivated commercially in the state of Pahang, Johor,

Selangor, Kelantan, Sabah and Sarawak respectively (8). The most widely grown ginger cultivars, besides common ginger, are Bentong ginger (*Z. officinale* cv. Bentong) and red ginger (*Z. officinale* cv. Rubrum) (9, 10). These varieties are highly valued by locals and internationally due to their medicinal properties and strong aroma (10).

Bentong ginger is a unique ginger variety grown in the highlands of Bentong district, Pahang, Malaysia (11). It is locally known as the ‘Emperor of Ginger’ and is recognised as one of Pahang’s Geographical Indications by the Agriculture Bureau of Pahang state in 2015 (10). Bentong ginger is distinguished by its large rhizome and high pungency compared to common ginger. Bentong ginger is also well known for its significantly phenolic content and antioxidant activity that make it suitable for health care and disease prevention (12). Due to high consumer demand, Bentong ginger is priced between RM25–30 kg<sup>-1</sup>, compared to common ginger at about RM7 kg<sup>-1</sup>.

Halia Bara or red ginger is another unique ginger variety found in the forest of Malaysia, Indonesia and China. Red ginger is also known as Canton ginger, East Indian ginger and stem ginger in England; zenzabil and zingibil in Arabic; Chiang, ganjiang and kanchiang in China and jahe blasa or jahe merah in Indonesia (13). In Malaysia, red ginger is locally known as 'halia udang', 'halia merah' and 'halia bara' (14). This ginger variety is unique compared with the other varieties, mostly because of its small, reddish rhizomes, as well as its superior pharmacological properties, attributable to its high level of vanilloids, phenolics and flavonoids (10). Due to its unique properties and increasing market demand, red ginger is priced higher than common ginger at about RM19 kg<sup>-1</sup>.

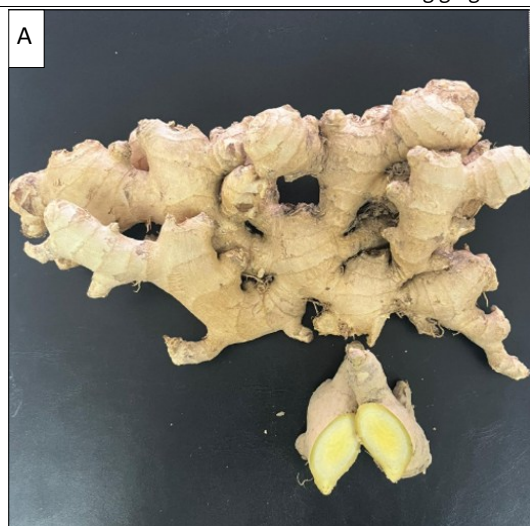
Overall, members of the Zingiberaceae family, especially *Z. officinale* and its cultivated varieties (Bentong ginger and red ginger) play vital roles in both agricultural and medicinal sectors across the tropical regions. Due to their unique morphological traits, potent bioactive compounds and exceptional market demand, these varieties hold significant cultural and economic importance. A clearer understanding of their taxonomy, morphology, nutritional value and propagation techniques is essential to support sustainable production and crop improvement. Thus, this review highlights the insights and explores the taxonomy, morphology, cultivation practices, nutritional properties and *in vitro* micropropagation of Bentong and red ginger.

### Taxonomy, morphology and botanical description of Bentong ginger and red ginger

The taxonomy and morphology of ginger are crucial for distinguishing its varieties and supporting breeding and cultivation efforts. Ginger is botanically described as a perennial herb with tuberous rhizomes that function in vegetative propagation and the storage of food reserves. Generally, in Southeast Asia, common ginger is generally classified ginger into three varieties based on

**Table 1.** Taxonomical classification of Bentong ginger

| Kingdom     | Plantae                                |
|-------------|----------------------------------------|
| Division    | Spermatophyta                          |
| Class       | Monocotyledoneae                       |
| Order       | Zingiberales                           |
| Family      | Zingiberaceae                          |
| Genus       | <i>Zingiber</i>                        |
| Species     | <i>Zingiber officinale</i>             |
| Variety     | <i>Zingiber officinale</i> cv. Bentong |
| Common name | Bentong ginger                         |



rhizome size and colour, mainly giant or white ginger (*Z. officinale* Rosc. var. *officinale*), small white or emprit ginger (*Z. officinale* Rosc. var. *amarum*) and red ginger (*Z. officinale* Rosc. cv. *Rubrum*) (15). These varieties share similarities but possess distinct morphological traits that influence their quality and commercial value. This section focuses exclusively on the taxonomy, morphology and botanical characteristics of Bentong and red ginger varieties to provide a foundation for subsequent discussions on their cultivation and bioactive potential.

The taxonomic classification of Bentong ginger is as mentioned in is presented in Table 1. Bentong ginger shares the same scientific name as the common ginger. However, it is referred to as the Bentong ginger variety as it originated and is largely cultivated in Bentong, Pahang, Malaysia. Morphologically, it has a pale golden exterior, light yellow colour internally and has a lower fibre content with unique sensory characteristics such as thinner and scaly skin on its surface compared with common ginger (Fig. 1A) (16). It is also characterised by a horn-shaped, irregular branched and relatively large rhizomes of around 3–16 cm long and 3–4 cm wide (12). This variety has a spicy, lemon-like flavour and pungent aroma (17). Other physical characteristics and features of Bentong ginger are almost similar to domestic ginger. Basically, closely resemble in terms of leaves, shoots and buds as to common ginger. As a rhizomatous plant, it is around 60–90 cm in height (Fig. 1B) (18). The leaves are alternate, sessile, distichous, linear, lanceolate, acuminate, lamina 15–20 cm long and 2–3 cm wide and extending into a transparent sheath on the stem (18). It has a stem of non-woody type, enclosed by leaf sheaths and digitately pale-yellow branched rhizome with small pale-yellow flowers. This variety can be harvested as young ginger after 3–6 months of cultivation or as mature ginger after 8–10 months of cultivation. The shoots usually emerge one to two weeks after planting through vegetative propagation using rhizomes (18, 19).

The taxonomic classification of red ginger is outlined in Table 2. Red ginger is an erect herbaceous perennial plant that grows to a height of 50–100 cm (Fig. 2A) (14). It is morphologically similar to common ginger but has smaller rhizomes and has a more pungent flavour (14). The local name "red ginger", refers to the reddish-purple surface of the rhizome (14). The rhizome is thick and reddish-brown (Fig. 2B), with a yellow to pink cross-section on the outside (Fig. 2C) (14). The reddish-purple colouration is partly attributed to



**Fig. 1.** Bentong ginger. A: Bentong ginger rhizomes and cross section of Bentong ginger rhizomes; B: Harvested Bentong ginger plant with young rhizomes.



**Fig. 2.** Red ginger. A: harvested red ginger plant with young rhizomes; B: red ginger rhizomes; C: cross-section of red ginger rhizomes.

**Table 2.** Taxonomical classification of red ginger

| Kingdom     | Plantae                                      |
|-------------|----------------------------------------------|
| Division    | Spermatophyta                                |
| Class       | Monocotyledoneae                             |
| Order       | Zingiberales                                 |
| Family      | Zingiberaceae                                |
| Genus       | <i>Zingiber</i>                              |
| Species     | <i>Zingiber officinale</i>                   |
| Variety     | <i>Zingiber officinale</i> cv. <i>Rubrum</i> |
| Common name | Red ginger                                   |

anthocyanin accumulation (20). It can be recognised readily identified by the yellow to pinkish cross-section rhizomes and red base of its leaf shoots. Unlike common ginger, young red ginger plant has a reddish petiole and the lip is scarlet red mottled with cream (21). The leafy stems of red ginger grow upright and are rounded and flat, measuring 10–25 cm in length, with small leaves at the base of the flower. The leaves are narrow, lance-shaped and measure 5–25 cm in length and 8–20 mm in width, tapering to a pointed tip and clasping the stem with long sheaths. The inflorescences are bear small, tubular bracts featuring a tridentate shape (20, 21). The corollas of red ginger are funnel-shaped, measuring 2–2.5 cm in length and exhibit a deep purple hue adorned with creamy yellow spots. The petals are small, tubular and tridentate in shape (22, 23). The flowers are borne in compact, ovoid inflorescences and supported by stems measuring 10–25 cm in length, with purple crowns approximately 2–2.5 cm in size. As the plant ages, the single root enlarges to form rhizomes and shoots, which will develop into new plants. The roots emerge from the underside of the rhizome, while buds sprout from its upper side (24).

In summary, both Bentong ginger and red ginger exhibit distinct morphological and anatomical characteristics that differentiate them from common ginger. Bentong ginger is recognised for its large, pale golden rhizomes, low fibre content and lemon-like aroma, whereas red ginger is characterised by its reddish-brown rhizomes, smaller size and higher pungency, partly due to anthocyanin accumulation. These variations not only contribute to their sensory and aesthetic appeal but also to differences in agronomic performance, phytochemical composition and market value. Therefore, understanding these distinctions provides a strong foundation for further understanding cultivation strategies and the sustainable improvement of these high-value Malaysian ginger varieties.

### Cultivation practices of Bentong ginger and red ginger

Most ginger varieties are propagated in areas with frequent rainfall and fertile soil. In Malaysia, ginger is mainly propagated in Pahang (Bentong district), Sabah (Keningau and Tambunan districts), Sarawak, Selangor and Johor. Ginger plants are generally sterile species that rarely flower and fail to set seed. However, they can be vegetatively propagated using mature rhizomes, known as seed rhizomes. Generally, the selection of rhizomes' size influences the ginger production, with larger seed rhizomes typically resulting in higher yields (25).

Bentong ginger and red ginger have similar planting methods as common ginger. Bentong ginger is mostly planted nomadically in the highland slopes especially at the highlands of Bentong district. The superior quality of Bentong ginger is influenced by the geographical conditions under which it is grown, with warm tropical climatic conditions and good agricultural practices (15, 26). Bentong ginger is usually cultivated during the slightly rainy season of tropical climates, equipped with a good drainage system and supplemented with rich in organic matter for optimum growth (26, 27). The cultivation practice starts with selecting healthy and disease-free seed rhizomes, preferably mature rhizomes that are approximately 10 months old. The seed rhizomes are treated with fungicides before planting to prevent diseases, with each rhizome having at least two to three viable shoot buds. The Bentong ginger seed rhizomes are planted under 4–5 inches of soil land, grown in a shade house covered with a 50 % black shade net and irrigated every two days (28, 29). The shoots will normally appear after 1–2 weeks of sowing through vegetative propagation of the seed rhizome. Fertilisers are applied after the first shoot emerges, mainly in water-soluble form to ensure complete dissolution (30). Insecticide and fungicide treatments are then applied once every two weeks as part of routine crop management. This variety is harvested as young ginger after 3–6 months or as mature ginger after 8–10 months (29). Proper harvesting techniques are used by carefully digging up the rhizomes to avoid the root damage. After harvesting, the land is generally rested for four years before replanting Bentong ginger, due to the depletion of trace elements. During this resting period, the land is used to grow other crops, such as legumes or vegetables. Additionally, Bentong ginger should not be grown in waterlogged soil as it is prone to rotting (29).

Moreover, the cultivation of Bentong ginger using modern fertigation system that address soil infertility, prevent soil-borne diseases and rotting of ginger due to waterlogged soil and potentially serve as an environmentally friendly alternative to shifting cultivation practices (29). The fertigation technique has been found to be most suitable organic growth substrate for growing ginger. Additionally, growing Bentong ginger in 100 % coir dust as the planting medium in fertigation technique produced the best growth performance and yield in a soilless culture system (30).

On the other hand, red ginger is cultivated in Asia (Indonesia, India and Malaysia) and a few tropical countries in Africa as this variety of ginger is well-suited to tropical climates (13, 31). It thrives best with an annual rainfall of 1000–2000 mm, though supplementary irrigation is necessary during drier periods. While the plant can grow in various soil types, it performs optimally in well-drained sandy loam soils enriched with high organic matter (32). Proper land preparation is essential for the successful cultivation of red ginger. The soil is generally ploughed at least twice using a disc plough, followed by a roto-ridge. Ground magnesium limestone (GML) are incorporated into the soil at least four weeks prior to planting red ginger in the field (32). Beds are also prepared after soil conditioning, particularly for areas with poor drainage. Usually, red ginger is not grown in the same field continuously but farmers practice shifting cultivation to allow the soil to recover and maintain its fertility (31, 32). Typically, each bed should be 1 m wide and 25 cm high, with 40 cm of space between them (32). Similar to other ginger varieties, red ginger is propagated using healthy and mature rhizomes, which are characterised by their dark red colour. The rhizomes are cut into segments approximately 5 cm long, each containing 2–3 growing buds. These cuttings are kept under moist conditions for a few days until sprouting begins to maximise germination. Before planting in the field, the cuttings will be soaked in a fungicide solution and allowed to dry (32). The cuttings are planted in small pits at a spacing of 20 × 20 cm to 25 × 25 cm and at a depth of 4–5 cm, ensuring that each rhizome cuttings has at least one healthy, viable bud positioned facing upwards (33). Fertilisation is carried out to achieve optimal growth of red ginger. For mineral soils, organic fertiliser, such as chicken manure are applied about one week prior to planting. Compound inorganic fertiliser (nitrogen, phosphorous and potassium (NPK)) is also applied periodically after planting (32–34).

Crop rotation and intercropping techniques are practiced in some countries to improve production quality (33–35). Another key technique is propagation through internodal cuttings. Additionally, aeroponic cultivation of ginger has been implemented, along with methods such as micropropagation and cytogenetic assessment (36, 37). Red ginger reaches full maturity 8–9 months after planting and ready for harvest, indicated by the drying of its leaves (33). Harvesting is usually done manually using a hoe, with the rhizomes carefully separated from the stems. After harvesting, the fibrous roots attached to the rhizomes are trimmed and the soil is removed to prepare them for commercial uses. Allowing the rhizomes to rest at room temperature under shade for 4–5 days promotes the formation of a protective outer layer, improving their storage potential (33).

Taken together, the cultivation success of Bentong ginger and red ginger is strongly influenced by environmental conditions, soil characteristics and agronomic management practices. Bentong ginger thrives in well-drained, organic-rich highland soils, whereas

red ginger performs best in sandy loam soils with adequate rainfall. Proper propagation, fertilisation and disease management are essential for maintaining plant vigour and enhancing yield quality. In addition to influencing plant growth and productivity, cultivation conditions may also influence the accumulation of phytochemicals and other bioactive compounds in ginger rhizomes. Therefore, a comprehensive understanding of these cultivation parameters is crucial for supporting sustainable production, improving agronomic performance, optimising phytochemical composition and enhancing the commercial value of these high-value ginger varieties

### **Nutritional values, biological activity and bioactive compounds of Bentong ginger and red ginger**

Ginger is a spice well known for its nutritional and medicinal value. It has been used for centuries as a versatile ingredient in culinary preparations and traditional medicine across many cultures. The rhizomes of the ginger plant are consumed raw, cooked as vegetables, or used as flavouring agents (38). Ginger contains numerous secondary metabolites such as oleoresins, gingerols and shogaols, which contribute to its characteristic pungency, flavour and aroma (37). In addition to its culinary uses, ginger is commercially available in several processed forms, including powder, oil and oleoresin, which are widely utilised in food, pharmaceutical and nutraceutical industries due to their health-promoting properties (38).

In recent years, Bentong ginger is found widely in the local market. It is used as a spice, health supplements and infusion in tea (11). Bentong ginger has a high demand in both domestic and international markets. This is due to its exceptional flavour, rich nutritional profile and health-promoting properties, making it a versatile ingredient with diverse applications ranging from culinary seasoning to functional food components. Bentong ginger-based products are currently available as ginger powder, ginger tea, ginger puree, ginger oil, ginger coffee, ginger soy milk and ginger cookies (39).

Bentong ginger generally consists of two main groups of bioactive compounds: volatile compounds and non-volatile compounds (40). Volatile compounds consist of essential oils that give a pleasant odour to ginger. They are made up of monoterpenoids and hydrocarbon intermediates such as  $\alpha$ -zingiberene,  $\alpha$ -curcumene,  $\alpha$ -farnesene,  $\beta$ -farnesene, linalool and  $\beta$ -sesquiphellandrene (40). Meanwhile, non-volatile compounds or oleoresins usually consist of various phenolic groups such as gingerol, shogaol and zingerone that exhibit the spicy flavour and pungency of Bentong ginger (15, 41). In terms of quality, the superiority of Bentong ginger is reportedly associated with high oleoresin content. Once extracted, the composition of oleoresins is usually in the range of 3–11 % (15, 42). Beyond its bioactive compounds, Bentong ginger is also a rich source of minerals, vitamins and dietary fibres, further solidifying its status as a health-promoting superfood (17).

The bioactive compounds found in Bentong ginger, are well-endowed with medicinal benefits including antidiarrheal, antimicrobial, antioxidant, anticancer and antibacterial activities (40, 43). For example, there is a correlation between antioxidant effects and antimicrobial efficacy of polyphenols and gingerols in Bentong ginger. These bioactive compounds may act synergistically to disrupt bacterial cell membranes, thereby inhibiting the growth of *Streptococcus mutans*, a major oral pathogen (44). Meanwhile, the phenolic compounds exhibit antioxidative properties that may mitigate the pathogenesis of oxidation-linked diseases, including

cancer progression, arthritis and diabetes mellitus (17). For example, gingerol, a pungent bioactive compound with woody aroma possesses therapeutic properties, including efficacy in mitigating gastrointestinal distress, nausea and respiratory ailments (15, 45). 6-, 8- and 10-gingerol were also found to ameliorate dextran sulphate sodium (DSS)-induced ulcerative colitis in mice by exerting antioxidant and anti-inflammatory effects. These compounds significantly reduced DSS-induced elevations of serum TNF- $\alpha$  and IL-1 $\beta$  levels in ulcerative colitis mice (42). The bioactivity and amount of some main bioactive compounds found in Bentong ginger is highlighted in Table 3.

In Malaysia, besides Bentong ginger, red ginger is also widely used as a key herb, functional food and traditional remedy among local populations due to its high nutritional value (49). Consequently, the demand for ginger rhizome including those of red ginger rhizomes has increased significantly as functional foods and nutraceuticals (50). This growing demand is driven by the incorporation of red ginger extracts into various commercial products, including topical formulations such as body lotions, creams and ointments as well as oral supplements in capsule form (14). Additionally, red ginger is utilised in multiple industries, including beverage production (51, 52), meat canning, confectionery, tobacco processing and soap manufacturing (43). For example, red ginger rhizome can be used in meat processing as it contains zingibain, a proteolytic enzyme with active properties (52). These proteolytic enzymes can break down peptide bonds in meat, enhancing its texture and quality. The incorporation of red ginger and chitosan films as packaging materials to store milkfish and increase its shelf life has also been studied (52).

In traditional medicine, red ginger is used to treat headaches, indigestion, nausea, vomiting and cancer (21). Numerous recent studies have confirmed the beneficial effects of red ginger, including its anti-inflammatory, antioxidant and antibacterial properties (21). A study reported that ginger suppressed platelet aggregation induced by arachidonic acid (AA), epinephrine, adenosine diphosphate (ADP) and collagen and contributing to the anti-inflammatory effect. This inhibition of platelet aggregation was strongest when AA served as the agonist, as lower concentrations were sufficient to achieve suppression compared with the other agonists (53). Due to its notable antimicrobial activity, red ginger has also been proposed as a natural food preservative (14).

Studies on the antibacterial activity of red ginger also have shown its potential to inhibit pathogenic bacteria that can damage body tissues and destroy healthy cells (54, 55). Moreover, combination of red ginger and nisin can be used in preserving milk and milkfish as they have a synergistic effect against *Bacillus cereus* (52). Red ginger extracts are also investigated for the management of autoimmune diseases, hypertension, hypercholesterolemia and hyperuricemia (14).

These bioactivities of red ginger are due to the presence of a unique bioactive compounds profile. Red ginger contains approximately 169 identified chemical constituents (55). The major components of red ginger rhizomes include carbohydrates (50–70 %), lipids (3–8 %), terpenes (such as zingiberene,  $\beta$ -bisabolene,  $\alpha$ -farnesene,  $\beta$ -sesquiphellandrene and  $\alpha$ -curcumene), phenolic compounds (including gingerol, paradols and shogaol), minerals and vitamins (13, 56). The bioactivity and amount of some main bioactive compounds found in red ginger is highlighted in Table 4.

**Table 3.** Bioactivities, mechanism of action and extracted quantities of essential secondary metabolites in Bentong ginger

| Main bioactive compounds | Bioactivities and mechanism of action                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | References           | Extracted quantities in Bentong ginger                                                                                                                                                                                                                                                                                 |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gingerols                | Gingerols in Bentong ginger are known for their potent antioxidant properties. Gingerols compound helps restore the balance of free radical production by disrupting the progression of oxidative stress.                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | (17)                 | i) 171.26 mg/g DW of gingerol content were extracted from dry Bentong ginger by using supercritical carbon dioxide extraction (12).                                                                                                                                                                                    |
|                          | 6-Gingerol in Bentong ginger is a potent antibacterial agent. Gingerol exhibits strong antibacterial effects through the formation of inhibition zones in the agar well diffusion assay. This indicates that the gingerol compound exhibited antibacterial activity against <i>Porphyromonas gingivalis</i> . This study shows that exposure to 6-gingerol at 0.5 $\times$ minimum inhibitory concentration (MIC) significantly suppressed the <i>rgpA</i> gene expression in <i>P. gingivalis</i> by 92 %. These results suggest that gingerol exerts its antibacterial effect by suppressing key virulence genes <i>rgpA</i> in <i>P. gingivalis</i> .                        | (43)                 | ii) Fresh ginger exhibited the highest 6-gingerol content (2.09 mg/g FW) at the seventh month of harvest, whereas dried ginger showed its maximum 6-gingerol concentration (0.66 mg/g DW) at the sixth month of harvest (40).                                                                                          |
| Zingerone                | Zingerone is an antioxidant compound that effectively scavenges free radicals generated during the radiolysis of food products, thereby mitigating oxidative damage.<br><br>Zingerone obtained from ginger exhibits anticancer properties against colon cancer, hepatocellular carcinoma and tumor development. A 2 mM dose of zingerone in dimethyl sulfoxide (DMSO) suppresses cancer cell proliferation by downregulating cyclin D1, a key regulator of the G1 phase of the cell cycle. In cancer cells, zingerone nanoparticles induce DNA damage, triggering apoptosis, particularly in hepatocellular carcinoma cells, through activation of caspase signalling pathways. | (16)<br><br>(46, 47) | Zingerone demonstrated the most potent antioxidant activity, with IC <sub>50</sub> values of 11.3 $\mu$ g/mL against the 2,2'-diphenyl-1-picrylhydrazyl radical and 19.0 $\mu$ g/mL against the 2,2'-amino-di(2-ethylbenzothiazoline-6-sulfonic acid) ammonium salt radical cation in <i>Z. officinale</i> Rosc. (46). |
| Shogaols                 | Rhizome extracts from Bentong ginger variety exhibited notable antibacterial activity against Gram-negative and Gram-positive bacteria. A study showed that fresh rhizomes and rhizomes stored at 5 °C for four months showed greater antibacterial activity against <i>Staphylococcus aureus</i> than the positive controls, gentamicin and ciprofloxacin.                                                                                                                                                                                                                                                                                                                     | (48)                 | The 6-shogaol content in Bentong ginger is reported to be approximately 1.85 mg/g DW in dried ginger rhizomes at the sixth month of harvest (40).                                                                                                                                                                      |

**Table 4.** Bioactivities, mechanism of action and extracted quantities of essential secondary metabolites in red ginger

| Major Bioactive compounds | Bioactivities and mechanism of action                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | References | Extracted quantities                                                                                                                                                                                                                        |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gingerols                 | Gingerols is one of the chemical compositions of red ginger rhizome that functions as an anti-cancer agent. 6-gingerol able to suppress MAPK/AP-1 signalling and leading to caspase-dependent apoptosis. This action inhibits the PMA-induced colon cancer growth. Similarly, 10-gingerol induces caspase-dependent apoptosis and causes an elevation in intracellular $Ca^{2+}$ levels. This can contribute to the cell cycle arrest in colon cancer cells. 10-gingerol also can cause an inhibition of AKT and p38 MAPK signalling to suppress invasion and proliferation of MDA-MB-231 breast cancer cells.                                                                                                                       | (57)       | The findings showed that under control conditions, the 6-gingerol content was 2.29 %, which increased to 6.02 % following treatment with 100 mg L <sup>-1</sup> NaN <sub>3</sub> through development of mutated red ginger (58).            |
| Camphene                  | Camphene (9.92 %) was identified as one of the predominant compounds in fresh red ginger, contributing to its fresh, citrusy and floral aroma. Additionally, camphene is significance due to their strong antibacterial activity, which is thought to occur through disruption of the bacterial cell membrane. For instance, a study reported that camphene present in ginger oil extracts exhibited broad-spectrum inhibitory activity against various microorganisms. In addition, volatile compounds particularly camphene in ginger essential oils were shown to reduce bacterial and yeast populations in shredded green papaya, indicating their potential application as antimicrobial agents in fresh-cut produce packaging. | (59, 60)   | The major constituents identified in fresh red ginger were $\beta$ -ocimene (31.33 %), $\beta$ -myrcene (8.79 %) and camphene (9.92 %), which are responsible for its fresh, citrus-like and floral aroma (61).                             |
| Shogaols                  | 6-shogaol is a bioactive compound with demonstrated anticancer potential. Head and neck squamous cell carcinomas (HNSCCs) represent one of the most aggressive and malignant cancer types affecting head and neck tissues and are primarily epithelial tumors involving the oral cavity, larynx and pharyngeal regions. Previous studies have shown that 6-shogaol suppresses the viability of HNSCC cells. It induces G2/M phase cell cycle arrest and enhances apoptosis in SCC-4 and SCC-25 cells through modulation of MAPK signaling pathways. As a natural compound, 6-shogaol is considered a promising, reliable and effective candidate for adjuvant therapy in HNSCC.                                                      | (48)       | One study reported that HPLC chromatogram analysis of red ginger extract identified the 6-shogaol compound with a resolution of $1.193 \pm 1.015$ , a tailing factor of $0.919 \pm 0.176$ and an asymmetry value of $0.907 \pm 0.136$ (34). |

When comparing Bentong ginger and red ginger in terms of antioxidant activity, a study reported that red ginger consistently exhibited higher DPPH radical-scavenging activity than Bentong ginger across all storage conditions, including fresh samples and those stored for 4 or 8 months at 5 °C or 15 °C. The antioxidant activity ranged from 16.9–41.7 %. The highest antioxidant activity was observed in fresh rhizomes and in samples stored at 5 °C for 4 months, with no significant difference between these two conditions. In contrast, prolonged storage and higher storage temperatures resulted in a marked decline in antioxidant activity in both ginger varieties. Storage at 15 °C caused a greater reduction in DPPH activity compared with extended storage duration, indicating that → storage temperature had a greater influence than storage time on antioxidant stability. Despite these reductions, red ginger maintained comparatively higher antioxidant activity, although both varieties exhibited substantially lower activity than standard antioxidants such as gallic acid and quercetin (62).

In summary, Bentong and red ginger can be distinguished by their rich phytochemical composition, potent bioactivities and high economic value. Their diverse range of bioactive compounds, including gingerols, shogaols and essential oils contributes to their distinctive aroma, flavour and broad spectrum of therapeutic properties. These varieties not only serve as important culinary and medicinal resources but also hold great potential for the development of functional foods, nutraceuticals and pharmaceutical products. Continued research into sustainable propagation methods, particularly through biotechnological approaches, may further enhance their utilisation and commercial potential in both domestic and international markets.

#### ***In vitro* micropropagation of Bentong ginger and red ginger**

Ginger rhizomes are the primary marketed part of the plant and are also used as propagules for vegetative propagation of ginger. Using a large proportion of ginger rhizome as starting material for cultivating the plant has been reported to negatively affect its market supply and causing economic losses to farmers (13, 29). In addition, handling ginger seed rhizomes as planting material is also costly and time-consuming due to their bulkiness. Therefore, *in vitro* micropropagation has been reported as an effective biotechnological approach for effective large-scale production of disease-free planting materials (39). Successful micropropagation of ginger relies on three key factors: suitable explant selection, effective surface sterilisation and optimised culture media composition. *In vitro* micropropagation of ginger can be done using various explants such as shoot tips or rhizome buds. Rhizome buds have been found to be the most effective as explants for *in vitro* cultures of plants within the Zingiberaceae family (55). Rhizome-sprouted buds of Bentong ginger are highly responsive explants to establish *in vitro* cultures of Bentong ginger plantlets (39). Similarly, rhizome buds were also used as explants for *in vitro* micropropagation of red ginger (63).

Surface sterilisation must be performed before culturing, with methods adjusted based on explant type and variety. Ginger is prone to bacterial wilt, leaf spot, soft rot and leaf yellowing, as these pathogens spread easily during rhizome propagation (10). Thus, proper sterilisation of explants is essential to remove these contaminants and to obtain disease-free plantlets. Mercuric chloride (HgCl<sub>2</sub>), Tween 20, ethanol and sodium hypochlorite (5.25 % NaOCl in commercial Clorox bleach) are among the most effective surface disinfectants for micropropagation of Bentong ginger rhizome buds

(39, 56). Meanwhile, the red ginger shoot buds have also been surface-sterilised using a combination of 70 % ethanol, sodium hypochlorite, povidone-iodine, along with fungicidal and antibacterial treatments (63).

Murashige and Skoog (MS) medium has been used as the basal media in all studies on Bentong ginger *in vitro* culture reported to date (10, 39, 55, 56). Supplementation of plant growth regulators (PGRs) is effective in enhancing shoot and root multiplication of Bentong ginger *in vitro* cultures. The major groups of PGRs that are used in culture media are auxins, cytokinins and gibberellins. In general, auxins such as indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid and naphthoxyacetic acid promote cell division, elongation and root differentiation. Meanwhile, cytokinins such as kinetin, zeatin, isopentenyl adenine, benzylaminopurine (BAP), 4-hydroxyl-3-methyl-trans-2-butenylaminopurine are important for shoot induction, development and proliferation (39). MS basal medium augmented with BAP and NAA recorded highest number of shootlets and root multiplication in Bentong ginger *in vitro* cultures (39, 56). Murashige and Skoog medium supplemented with zeatin also produced optimum shoot multiplication with highest number of shoots per explant (55). Furthermore, the inclusion of NAA not only improved *in vitro* microrhizome formation but also reduced the sucrose concentration required for effective *in vitro* microrhizome production. Application of 1.4 mg/L NAA reduced sucrose concentration requirements from 90 g/L to 75 g/L, while yielding the highest number of microrhizome inductions, outperforming the application of 0.46 mg/L NAA at the same sucrose concentration (75 g/L) (39).

Murashige and Skoog medium also serves as the basal medium in all *in vitro* cultures of red ginger reported to date.

Previous results reported that 0.5 mg/L NAA and 3.0 mg/L BAP augmented with MS basal medium produced the highest number of roots and shoots in red ginger *in vitro* culture (62). The shoot induction of red ginger using several types of cytokinins as PGRs found that 1mg/L of TDZ enhanced the shoot multiplication of *in vitro* red ginger (63).

In addition to PGRs, carbon sources such as sucrose are essential for organ development in Bentong and red ginger. Microrhizomes of Bentong ginger were successfully induced in MS medium supplemented with a high sucrose concentration (> 45 g/L) (55). Meanwhile, a study showed that 45 g/L of sucrose is the optimum concentration for red ginger as it resulted in the highest number of plantlets and roots (14). Previous research reported that the optimal shoots, roots and leaves development of red ginger *in vitro* cultures was obtained in 30 g/L sucrose. The most effective overall formulation for *in vitro* cultivation consisted of MS medium, 30 g/L sucrose and 1.0 mg/L BA (64). Table 5 summarises the successful studies on *in vitro* propagation of Bentong ginger and red ginger reported to date.

*In vitro* micropropagation offers an effective and sustainable method for the large-scale production of disease-free Bentong and red ginger plantlets. Using rhizome buds as explants with optimised sterilisation protocols ensure high culture success and minimal contamination. Murashige and Skoog medium supplemented with suitable concentrations of cytokinins (BAP or zeatin) and auxins (NAA) together with optimal sucrose concentrations promotes efficient shoot and root proliferation as well as microrhizome formation. Overall, this technique provides a reliable biotechnological approach to support continuous propagation, genetic improvement and commercial cultivation of these high-value ginger varieties.

**Table 5.** The successful *in vitro* micropropagation of Bentong ginger and red ginger

| Varieties                                          | Explants      | Surface sterilisation technique                                                                              | Best culture media                                                                               | Responses                                                                                                                                                                                                   | References |
|----------------------------------------------------|---------------|--------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Bentong ginger ( <i>Z. officinale</i> cv. Bentong) | Rhizome buds  | 70 % (v/v) of Clorox                                                                                         | MS media + 10 µM zeatin                                                                          | Produced highest number of shoots per explant ( $4.28 \pm 0.15$ )                                                                                                                                           | (39)       |
|                                                    |               |                                                                                                              | MS media + 7.5 µM NAA                                                                            | Produced highest number of roots per explant ( $15.44 \pm 0.80$ )                                                                                                                                           |            |
|                                                    | Microrhizomes | Tween20, 70 % (v/v) ethanol, 70 % (v/v) Clorox                                                               | MS media + 10 µM Zeatin + 2.5 µM NAA + 80 g/L sucrose                                            | Generated highest number of microrhizomes per explant ( $4.50 \pm 0.29$ )                                                                                                                                   | (55)       |
|                                                    |               |                                                                                                              | MS media + 10 µM BAP + 2.5 µM NAA + 80 g/L sucrose                                               | Produced highest number of shoots per explant ( $14.28 \pm 0.86$ )                                                                                                                                          |            |
|                                                    | Shoot buds    | Tween 20, 95 % (v/v) ethanol and 20 % (v/v) Clorox                                                           | MS media + 3 mg/L BAP + 1 mg/L NAA + 30g/L sucrose                                               | Recorded the highest number of shoots and roots multiplication.                                                                                                                                             | (56)       |
| Red ginger ( <i>Z. officinale</i> cv. Rubrum)      | Young buds    | Decon (5 % v/v), 20 % Clorox, 50 µL of Tween20                                                               | MS media + 3 mg/L BAP<br>MS media + 3 mg/L BAP + 0.5 mg/L NAA<br>MS media + 3 mg/L NAA + 1.0 BAP | Exhibited highest number of microshoots per explant ( $7.00 \pm 1.34$ ).<br>Produced highest number of shoots per explant ( $19.5 \pm 2.3$ ).<br>Produced highest mean number of roots ( $11.4 \pm 11.1$ ). | (62)       |
|                                                    | Shoot buds    | 70 % (v/v) alcohol solution, sodium hypochlorite, povidone iodine, fungicides, bactericides and antibiotics. | MS media + 1 mg/L thidiazuron (TDZ)                                                              | Increased the multiplication of shoots ( $2.8 \pm 0.00$ ) and highest shoot length ( $2.96 \pm 0.00$ ).                                                                                                     | (63)       |
|                                                    |               | Not stated                                                                                                   | MS medium + 1 mg/L IBA                                                                           | Produced highest percentage of explant rooted (70 %).                                                                                                                                                       | (65)       |
|                                                    |               |                                                                                                              |                                                                                                  |                                                                                                                                                                                                             |            |

MS: Murashige and Skoog; NAA: α-Naphthaleneacetic acid; BAP: 6-Benzylaminopurine.

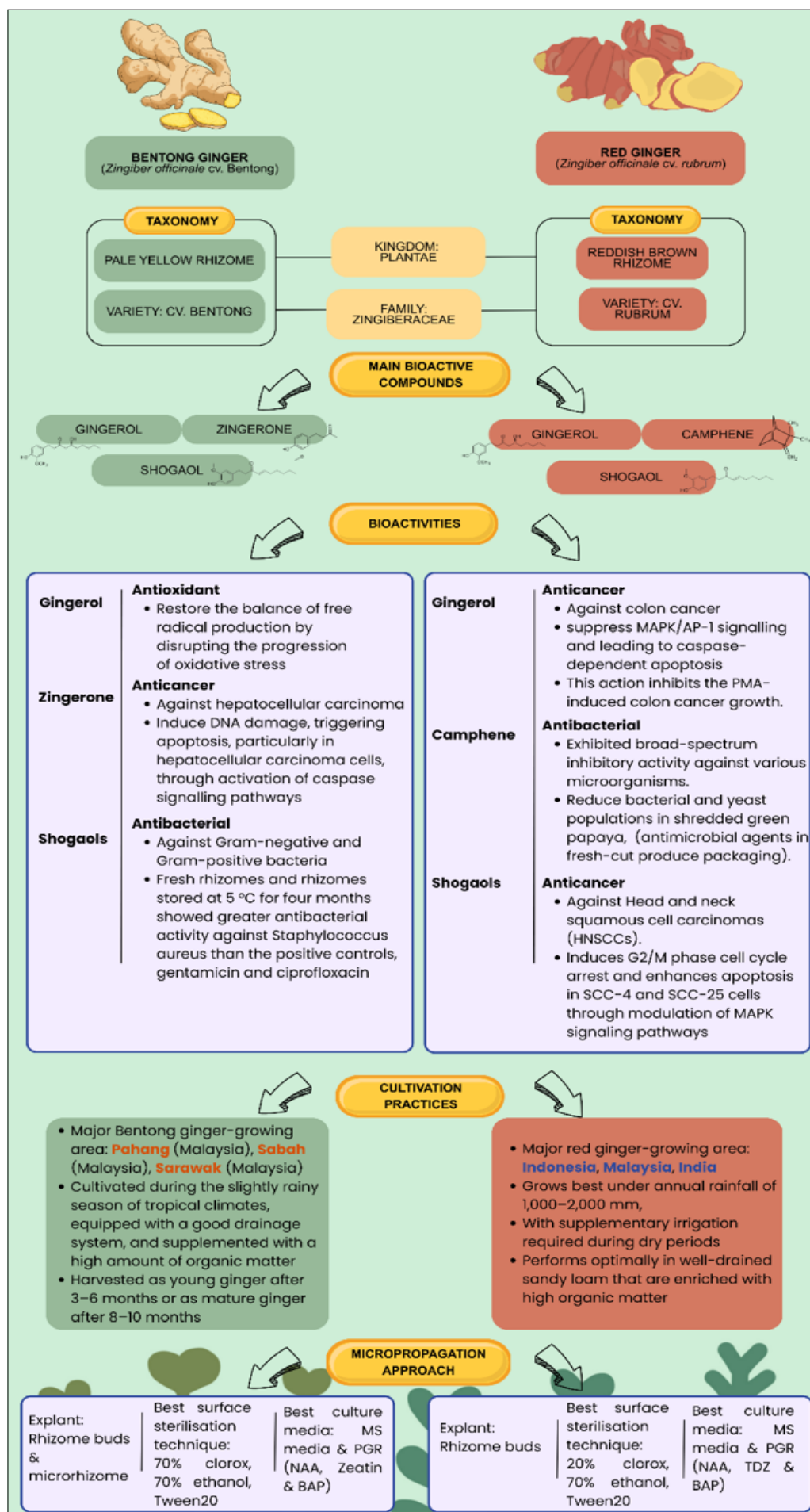


**Fig. 3.** Comparison of taxonomy, main bioactive compounds, bioactivities, cultivation practices and *in vitro* micropropagation techniques of two Malaysian ginger varieties: Bentong ginger and red ginger.

## Conclusion

Bentong ginger and red ginger are unique Malaysian varieties that hold significant agricultural, economic and medicinal value. These varieties are distinguished by their rich composition of bioactive compounds, including gingerols, shogaols and essential oils, which contribute to their antioxidant, anti-inflammatory, antimicrobial and anticancer properties. ginger, renowned as the "Emperor of Ginger," is prized for its high pungency, phenolic content and large rhizomes. In contrast red ginger is valued for its rich anthocyanins and strong pharmacological effects. The comparison between the two ginger varieties is illustrated in Fig. 3 to enhance understanding.

In recent years, the growing demand for these ginger varieties driven by their applications in food, pharmaceuticals and cosmetics highlights the need for advanced propagation techniques to ensure sustainable production. However, conventional cultivation of this ginger largely relies on vegetative propagation using rhizomes, which poses challenges such as disease transmission, high production costs and limited planting material. To address these issues, *in vitro* micropropagation has emerged as an efficient alternative, enabling mass production of disease-free planting material. Studies have successfully optimised sterilisation techniques and culture conditions, proving effective for the production of Bentong and red ginger plantlets for commercial cultivation.

Future research should focus on scaling up *in vitro* protocols, assessing the genetic uniformity of propagated Bentong ginger and red ginger plants and optimising bioactivity retention for commercial applications. Molecular marker analyses, such as random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) or simple sequence repeat (SSR) profiling, can be employed to confirm clonal uniformity and detect possible somaclonal variation induced during *in vitro* culture. Further optimisation of culture media composition and acclimatisation protocols may also enhance metabolite production and plant vigour. Additionally, comparative studies on the secondary metabolite profiles particularly the concentrations of key bioactive compounds such as gingerol, shogaol and zingerone are essential to ensure that micropropagated plants retain the phytochemical quality and therapeutic properties of field-grown plants. These efforts will strengthen the application of *in vitro* propagation techniques for sustainable mass propagation and crop improvement of high-value ginger varieties. By integrating biotechnological advancements with traditional knowledge, Malaysia can strengthen its position as a key producer of high-quality, medicinally potent ginger, meeting both domestic and global market needs.

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## Authors' contributions

TCMS conceived the review topic and designed its structure. TCMS, NSSK, and LAR conducted the literature search. LAR prepared the first draft of the manuscript. NSSK further developed the manuscript under the guidance of TCMS. TCMS critically reviewed and revised

the entire manuscript. MFY reviewed and revised the sections entitled "*In vitro* micropropagation of bentong ginger and red ginger" and the conclusion. MSS reviewed and revised the introduction and the section entitled "Conventional planting methods of bentong ginger and red ginger." All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

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During the preparation of this work the authors used ChatGPT in order for English language editing and paraphrasing. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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