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A comparison of callus induction in 4 *Garcinia* species

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ABSTRACT

Background: This research is intended to determine suitable types and concentrations of plant growth regulators (PGRs) to induce callus on stem and leaf sections of 4 species of the genus *Garcinia*, namely, *Garcinia mangostana, Garcinia schomburgkiana*, *Garcinia cowa,* and *Garcinia celebica.* The base medium was MS medium containing 30 g l^{-1} sucrose, 0.5 g l^{-1} polyvinylpyrrolidone (PVP), and 7 g l^{-1} agar, and for the different treatments, PGRs were added to the medium as follows: thidiazuron (TDZ) at concentrations of 0, 0.1, 0.5, 1, and 2 mg l^{-1}; 6-(3-hydroxybenzylamino) purine (*meta*-topolin) at concentrations of 0, 0.5, 2.5, and 5 mg l^{-1}; 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram) at concentrations of 0, 0.5, 2.5, and 5 mg l^{-1}; and 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations of 0, 0.5, 1, 2, and 4 mg l^{-1}. The occurrence of callus was observed after 4 weeks.
Results: A maximum of 100% and 93% of *G. mangostana* leaf explants formed callus in the 0.5 mg l^{-1} and 1 mg l^{-1} TDZ treatments, respectively, while 100% of *G. schomburgkiana* stem explants formed callus in the 1 mg l^{-1} TDZ treatment and 89% of *G. schomburgkiana* leaf explants formed callus in the 0.5 mg l^{-1} picloram treatment. The highest callus induction rate for *G. cowa* was 62% in the 1 mg l^{-1} TDZ treatment and for *G. celebica* was 56% in the 0.5 mg $l^{-1} \cdot mT^{-1}$ treatment.
Conclusions: For all 4 species, the greatest amount of large nodular callus was observed in the TDZ treatments. White, friable callus was observed on most of the 2,4-D and picloram treatment groups. Most *meta*-topolin treatments resulted in minimal callus formation.
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1. Introduction

Garcinia is a genus in the family Clusiaceae (formerly Guttiferae) comprising evergreen tropical trees and shrubs that originated mainly in Asia, as well as some species from Australia, Africa, and Polynesia. There are 397 species in the genus *Garcinia* that are accepted by The Plant List [1]. At least 22 *Garcinia* species are found in Thailand [2]. The most well-known member of the genus is *Garcinia mangostana* L., commonly known as mangosteen, an important fruit crop in Southeast Asia. Thailand is the world's largest exporter of mangosteen, and the export value reached US$ 234,496,785 in 2017 (Office of Agricultural Economics). With many traditional uses in folk medicine, *Garcinia* species have been found to be a rich source of secondary metabolites including xanthones (such as alpha-mangostin and gamma-mangostin (Fig. 1)), flavonoids, benzophenones, lactones, triterpenes, and phenolic acids [3]. Many of these phytochemicals have been shown to have antioxidant, antiprotozoal, antifungal, antibacterial, anti-inflammatory, and anti-immunosuppressive properties [3]. For instance, recent research has illuminated the antibiotic and cytotoxic potential of garcinol found in *Garcinia cambogia, Garcinia indica, Garcinia atroviridis,* and several other *Garcinia* species [4,5,6].

Garcinia species that are related to mangosteen were investigated with a view to future breeding applications because mangosteen is an obligate apomict. Conventional hybridization has not yet been achieved because mangosteen flowers do not produce viable pollen [7]. Gene transfer or protoplast fusion offers exciting alternatives for crop improvement, and callus induction in species genetically related to mangosteen is a step in this direction.

This study was conducted on 4 species, namely, *G. mangostana* L., or mangosteen, *Garcinia celebica,* or "seashore mangosteen," *Garcinia cowa* ("chamuang" in Thai), and *Garcinia schomburgkiana* Pierre ("madan" in Thai).

Mangosteen is valued for its delicious fruit, and the juice is promoted as a healthy beverage. Several studies have also investigated the

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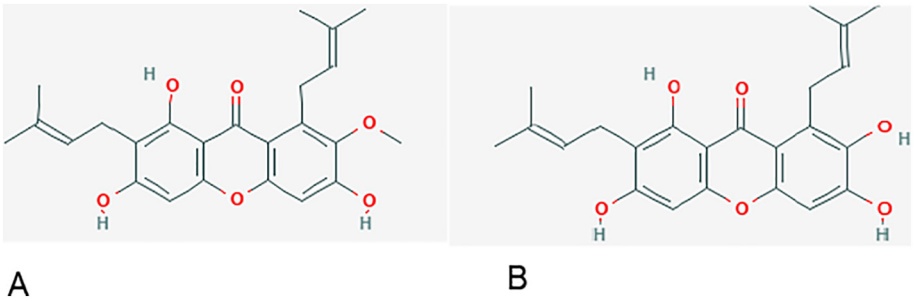

Fig. 1. (A) Alpha-mangostin; (B) gamma-mangostin; Credit: PubChem.

medicinal properties of crude extract of mangosteen rind and isolated active compounds such as xanthones and alpha-, beta-, and gamma-mangostin. For instance, alpha-mangostin exhibited an MIC value of 1.95 μg/ml against methicillin-resistant *Staphylococcus aureus* [8,9]. A study conducted by Jang et al. [10] in mouse indicated that alpha- and gamma-mangostin may have therapeutic potential for the treatment of allergic asthma. Similarly, alpha- and gamma-mangostin were shown to inhibit allergic inflammatory responses in the lab [11]. Xanthones were demonstrated to significantly suppress the degranulation in Ag-mediated activation of a high-affinity IgE receptor, a primary event in several allergic responses [12]. In an interesting product development study, researchers at Silpakorn University demonstrated that chitosan-based nanofiber mats loaded with *G. mangostana* extracts exhibited antioxidant and antibacterial activities and could accelerate the rate of wound healing when compared to the control in a rat study [13]. There is evidence that alpha-mangostin can be effective in killing cancer cells as well [4,14,15].

Less research has been carried out on other *Garcinia* species that are not cultivated on a commercial scale; however, they also contain phytochemicals with good potential for further development. Benzophenones and biflavonoids isolated from *G. celebica* were found to have moderate to high antioxidant activity [16]. Garcihombronane D (Fig. 2), a xanthone found in *G. celebica*, was found to inhibit the growth of the malaria-causing protozoan parasite *Plasmodium falciparum* [17]. Five xanthones extracted from the bark of *G. cowa* were also found to possess antimalarial activity against *P. falciparum* in laboratory tests [18]. Extract from the rind of *G. cowa* fruits was reported to be effective in inhibiting the growth of 4 common forms of gram-positive bacteria [19]. In addition, the strong antioxidant properties of phytochemicals from rinds of the *G. cowa* fruit may have a useful application for suppressing the synthesis of aflatoxin by *Aspergillus* sp. in peanuts and animal feed [20]. *G. cowa* is cultivated on a small scale in Thailand, where its leaves, rich in Vitamin A, are harvested for the preparation of soup or curry [2]. *G. schomburgkiana* fruit, also rich in Vitamin A and calcium, is sold in local markets and

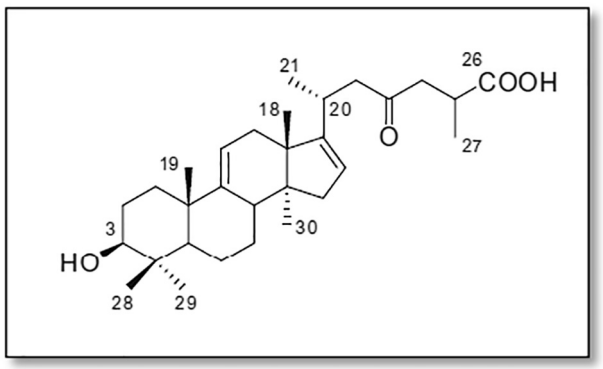

Fig. 2. 3b-Hydroxy-23-oxo-9,16-lanostadien-26-oic acid or garcihombronane D, a triterpene found in *G. celebica* by Elfita et al. [17].

may be used as pickle or candy or prepared as a dipping sauce [2]. All these other *Garcinia* species have edible fruit, but it is not favored by consumers as much as mangosteen. They may be considered underutilized tropical fruit trees.

The focus of this research was to induce callus *in vitro* from leaf tissue of the selected *Garcinia* species. Aseptic callus tissue is useful for starting cell suspensions that can be scaled up for the efficient collection of useful phytochemicals from these plants in a way that saves space compared to growing them outdoors in plantations [21]. In addition, the callus tissue has important applications for advanced plant breeding techniques such as genetic manipulation through *Agrobacterium*-mediated gene transfer, particle bombardment, or protoplast fusion.

We tested varying concentrations of thidiazuron (TDZ), 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram), 2,4-dichlorophenoxyacetic acid (2,4-D), and 6-(3-hydroxybenzylamino) purine (*meta*-topolin) for inducing callus on young leaf sections of *G. cowa* and *G. celebica* and (due to greater availability of plant material) on both leaf sections and immature stem sections from *G. mangostana* and *G. schomburgkiana* Pierre *in vitro* seedlings.

Thidiazuron (TDZ) is a cytokinin-like urea derivative that has been found to facilitate the efficient micropropagation of many recalcitrant woody species. Huetteman and Preece [22] wrote in a review that at concentrations of higher than 1 μM, TDZ can stimulate the formation of callus, adventitious shoots, or somatic embryos. In woody plant species, TDZ has successfully been utilized in micropropagation systems for *Semecarpus anacardium* L. [23] and sandalwood (*Santalum album* L.) [24].

The auxin derivative 2,4-D has been used together with kinetin to induce callus in at least 3 species of woody plants grown *in vitro*: *Dalbergia sissoo* [25], *Ulex europaeus* [26], and *Prunus persica* [27].

Picloram, an auxin-like synthetic PGR, was used to induce embryogenic callus on cashew [28] and coffee [29]. Picloram was able to induce large amounts of callus on *Rudgea jasminoides* petioles as a way to obtain phytoalexin, a defensive metabolite [30].

Meta-topolin is a relatively newly discovered PGR; hence, not as much research has been carried out on its use in tissue culture. *Meta*-topolin is a naturally occurring cytokinin first isolated from poplar [31]. It was reported to induce callus on the herbaceous species *Spathiphyllum floribundum* Schott cv. Petite [32] and the woody species *Sclerocarya birrea* [33].

The objective of this study was to test varying concentrations of TDZ, picloram, 2,4-D, and mT to induce callus and/or shoots on leaf explants of *G. mangostana, G. schomburgkiana, G. celebica,* and *G. cowa* to gain more information that can be of use for biotechnological applications in the future.

2. Material and methods

For *G. mangostana* and *G. schomburgkiana,* seeds were removed from the flesh of fresh fruit obtained from the local market. The seeds were washed in dishwashing detergent for 2 min and then immersed in 1000 ppm antibacterial solution (Kanker-X) plus 1000 ppm fungicide

(Captan orthocide) for 2 h (shaken on an Innova 2300 orbital shaker at 105 rpm). Subsequently, they were surface sterilized in 70% ethanol for 1 min, 20% Clorox solution for 15 min, and 10% Clorox solution for 10 min and then rinsed with autoclaved distilled water 3 times. The seeds were then placed on 1/2-macro nutrient MS medium supplemented with 0.5 g l^{-1} polyvinylpyrrolidone (PVP) to prevent browning, 3% sucrose, and 0.7% agar in 8-ounce glass jars with 25–35 ml of medium per jar. The jars were kept in the tissue culture lab at 25 ± 2°C with an 8:16 h photoperiod. After the seedlings were large enough, they were taken out and sectioned under aseptic conditions into leaf sections (5–7 mm × 10–15 mm pieces containing midrib) and stem sections (approximately 0.5–1 cm in length), and placed in separate test tubes, each containing 10 ml of the different treatment media. The basal medium (control) was 1/2-macro nutrient MS medium supplemented with 0.5 g l^{-1} PVP, 3% sucrose, and 0.7% agar. The experimental treatments were basal medium with the following PGRs added: 0, 0.5, 2.5, or 5.0 mg l^{-1} picloram; 0, 0.5, 1, 2.5, or 5.0 mg l^{-1} *meta*-topolin; 0, 0.5, 1.0, 2.0, or 4.0 mg l^{-1} 2,4-D; or 0, 0.1, 0.5, 1.0, or 2.0 mg l^{-1} TDZ. Twenty or more leaf sections and at least 10 stem sections were used for each treatment group.

G. celebica seeds were obtained from Bogor Botanic Gardens, Indonesia. *G. cowa* seeds were obtained from The Park Adventure, Rayong Province, Thailand. Seeds of these 2 species were grown in potting soil in seedling trays outdoors. Young leaves were cut from the seedlings and surface sterilized by first washing in dishwashing detergent for 2 min and then immersing in 1000 ppm Kanker-X plus 1000 ppm Captan orthocide for 2 h on an orbital shaker. Next, the leaves were immersed in 70% ethanol for 1 min, 10% Clorox solution for 15 min, and 5% Clorox solution for 10 min. Finally, they were rinsed with autoclaved distilled water 3 times in a laminar flow hood. Following surface sterilization, each leaf was cut into leaf sections measuring approximately 5–7 mm × 8–12 mm, each piece containing midrib. Each leaf section was randomly placed (adaxial side up) in a test tube containing 10 ml of one of the different treatment media listed above or control (basal medium with no PGRs added), with a minimum of 12 leaf segments per treatment.

Test tubes were kept in the tissue culture lab at 25 ± 2°C with an 8:16 h photoperiod. The percentage and appearance of callus or shoots were recorded after 4 weeks. The size of callus was visually determined and recorded as "-" = no callus, "+" = minimal or slight callus, "++" = small callus, "+++" = medium callus, "++++" = large callus) and color and nature of callus (friable or compact) was observed and photographed.

Fig. 3 shows a graphical illustration of the process.

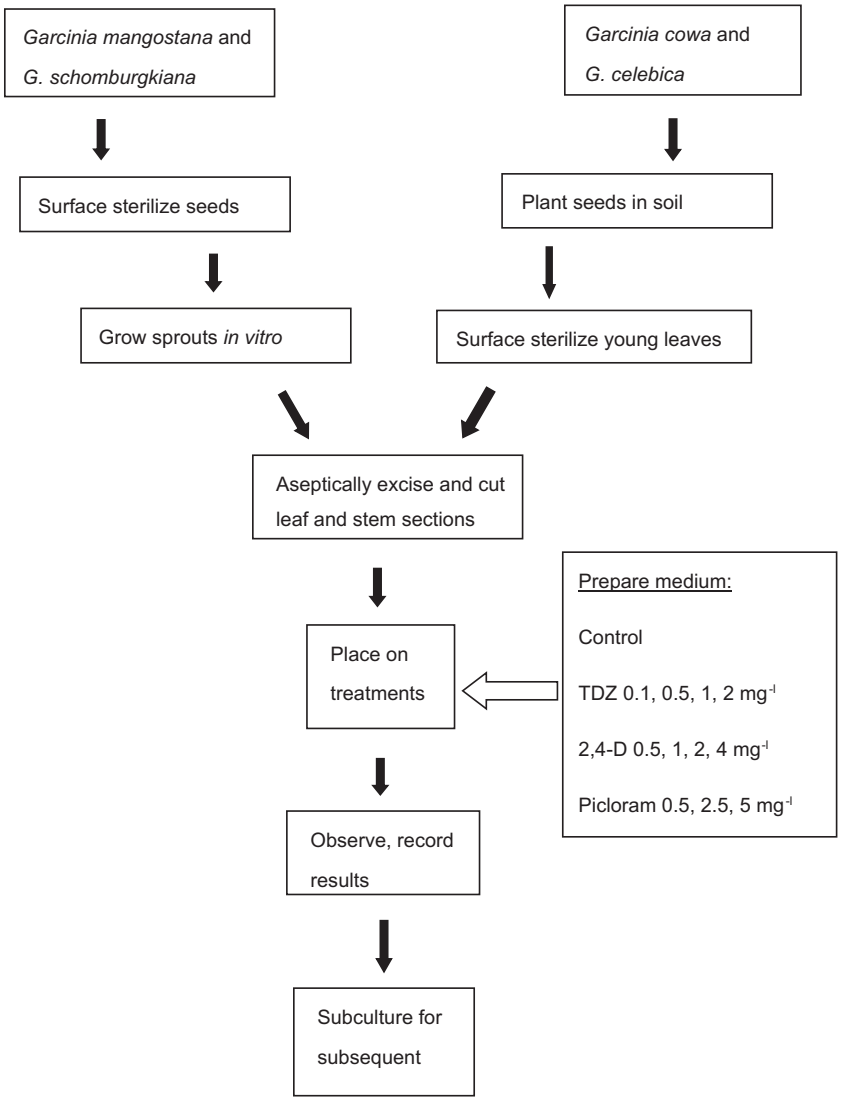

Fig. 3. Workflow diagram.

3. Results and discussion

Callus was induced on explants of all 4 species tested, with all 4 PGRs tested, but the nature and amount of callus differed with species and concentration of PGR. The highest callus induction rate at 4 weeks was 100%, observed both in *G. mangostana* leaf sections cultured in medium with 0.5 mg l^{-1} TDZ and in *G. schomburgkiana* stem sections cultured in medium with 1 mg l^{-1} TDZ (Table 1 and Table 2). The second highest callus induction rate observed was 93%, from the 0.1 mg l^{-1} TDZ treatment on *G. mangostana* leaf sections and the 0.5 mg $l^{-1} \cdot mT^{-1}$ treatment on *G. mangostana* stem sections (Table 1 and Table 3). Other treatments that resulted in a high percentage of callus formation were 2 mg l^{-1} TDZ on *G. mangostana* stem sections (90.5%) and 0.5 mg l^{-1} picloram on *G. schomburgkiana* leaf sections (89%) (Table 3 and Table 4).

G mangostana young stem sections from *in vitro* seedlings (height approximately 5–10 cm) appeared to readily form callus even on basal medium with no PGRs added because the mean callus induction rate for *G. mangostana* stem sections in the control group was 55.36% (Table 3). This may be because the cambium tissue is actively growing in small seedlings. However, *G. schomburgkiana* green stem tissue, although it appeared softer and less woody than stems of *G. mangostana* seedlings, was less prone to forming callus, with a mean callus induction rate of only 8.33% in the control group (Table 2).

On the control leaf sections with no PGRs added to the medium, the mean callus induction rate was 8.5% for *G. mangostana,* 0% for *G. schomburgkiana,* 7.6% for *G. cowa,* and 16% for *G. celebica* (Table 1 and Table 4, Table 5, Table 6). The formation of small amounts of callus on the control leaf sections was likely a stress response to the wound caused when the leaves were cut into sections. The amount and size of callus induced on the control leaf sections were very small compared to most of the treatments with PGRs added.

There were some exceptions, however, because some PGR treatments appeared to be toxic, as the leaf sections tended to turn brown and die more quickly than the control, especially when PGRs were added at a high concentration. For example, in the case of *G. mangostana* stem sections, picloram tested at all concentrations and 2,4-D tested at all concentrations failed to induce callus or induced callus at a lower rate than the control (Table 2). In *G. mangostana* leaf sections, picloram at the higher concentrations of 2.5 mg l^{-1} and 5 mg l^{-1} and 2,4-D at concentrations of 2 mg l^{-1} and 4 mg l^{-1} resulted in a lower callus induction rate than the control (Table 1). Additionally, for *G. celebica* leaf sections, the percent callus formation at 4 weeks was less than that of the control with the 1 mg l^{-1}, 2 mg l^{-1}, and 4 mg l^{-1} 2,4-D treatments and with the 0.5 mg l^{-1} and 5 mg l^{-1} picloram treatments (Table 6). For *G. cowa*, the percent callus formation at 4 weeks was less than that of the control with the 4 mg l^{-1} 2,4-D treatment and the 5 mg $l^{-1} \cdot mT^{-1}$ treatment (Table 5). 2,4-D is used as a herbicide in agriculture; hence, it is not surprising that it would be harmful to plants at high concentrations.

Table 1
Callus induction in *Garcinia mangostana* leaf sections at 4 weeks.

Plant growth regulators added to MS media (mg l^{-1})	Percentage of callus induction	Callus size	Nature of callus
Control			
0 ($n = 62$)	8.5	+	White/yellow
TDZ			
0.1 ($n = 24$)	74.5	++	Green/yellow, embryogenic
0.5 ($n = 26$)	100	+++	
1 ($n = 26$)	93	++++	
2 ($n = 24$)	78	+++	
2,4-D			
0.5 ($n = 71$)	59	++	White, friable
1 ($n = 25$)	12	++	
2 ($n = 25$)	0	-	
4 ($n = 25$)	0	-	
Meta-topolin			
0.5 ($n = 28$)	29	+	Yellow, compact
2.5 ($n = 28$)	46	+	
5 ($n = 28$)	18	+	
Picloram			
0.5 ($n = 22$)	18.5	+	White to brown, nodular
2.5 ($n = 22$)	0	-	
5 ($n = 22$)	0	-	

Mean callus induction rate for *G. mangostana* leaf: 35.8%, S.D. 36.27. - = no callus; + minimal or slight; ++ small; +++ medium; ++++ large.

Table 2
Callus induction in *Garcinia schomburgkiana* stem sections at 4 weeks.

Plant growth regulators added to MS media (mg l^{-1})	Percentage of callus induction	Callus size	Nature of callus
Control			
0 ($n = 54$)	8.33	+	Yellow, compact
TDZ			
0.1 ($n = 10$)	70	++++	Green/yellow, embryogenic
0.5 ($n = 10$)	70	+++	
1 ($n = 8$)	100	+++	
2 ($n = 10$)	70	+++	
2,4-D			
0.5 ($n = 24$)	50	+++	Yellow, compact
1 ($n = 24$)	33.33	++	
2 ($n = 24$)	8.33	+	
4 ($n = 24$)	8.33	++	
Meta-topolin			
0.5 ($n = 21$)	0	-	Yellow, compact
2.5 ($n = 21$)	14.29	+	
5 ($n = 21$)	12.5	+	
Picloram			
0.5 ($n = 24$)	41.67	+	White to brown, nodular
2.5 ($n = 24$)	0	-	
5 ($n = 24$)	8.33	++	

Mean callus induction rate for *G. schomburgkiana* stem: 34.77%, S.D. 32.01; - = no callus; + minimal or slight; ++ small; +++ medium; ++++ large.

Table 3
Callus induction in *Garcinia mangostana* stem sections at 4 weeks.

Plant growth regulators added to MS media (mg l^{-1})	Percentage of callus induction	Callus size	Nature of callus
Control			
0 ($n = 46$)	55.36	++	Yellow, embryogenic
TDZ			
0.1 ($n = 20$)	80	++++	Green/yellow, embryogenic
0.5 ($n = 23$)	72	++++	
1 ($n = 20$)	85	++++	
2 ($n = 21$)	90.5	++++	
2,4-D			
0.5 ($n = 33$)	36.36	++	White to yellow-brown, compact
1 ($n = 22$)	27.27	+++	
2 ($n = 22$)	18.18	++	
4 ($n = 23$)	28.57	++	
Meta-topolin			
0.5 ($n = 14$)	93	+	Yellow, compact
2.5 ($n = 14$)	64	+++	
5 ($n = 14$)	64	++	
Picloram			
0.5 ($n = 21$)	0	-	n/a
2.5 ($n = 21$)	0	-	
5 ($n = 21$)	0	-	

Mean callus induction rate for *G. mangostana* stem: 47.06%, S.D. 33.92. - = no callus; + minimal or slight; ++ small; +++ medium; ++++ large.

Table 4
Callus induction in *Garcinia schomburgkiana* leaf sections at 4 weeks.

Plant growth regulators added to MS media (mg l^{-1})	Percentage of callus induction	Callus size	Nature of callus
Control			
0 ($n = 69$)	0	-	na
TDZ			
0.1 ($n = 24$)	0	-	Green/yellow,
0.5 ($n = 24$)	12.5	++	embryogenic
1 ($n = 24$)	20.83	++	
2 ($n = 24$)	37.5	++	
2,4-D			
0.5 ($n = 24$)	79.17	+++	Yellow, compact
1 ($n = 20$)	79.17	++	
2 ($n = 24$)	79.17	+	
4 ($n = 24$)	37.5	++	
Meta-topolin			
0.5 ($n = 19$)	0	-	Yellow, compact
2.5 ($n = 19$)	0	-	
5 ($n = 19$)	8.33	+	
Picloram			
0.5 ($n = 24$)	88.89	+	White to brown,
2.5 ($n = 24$)	75	+	nodular
5 ($n = 24$)	37.5	++	

Mean callus induction rate for *G. schomburgkiana* leaf: 39.68%, S.D. 34.47; - = no callus; + minimal or slight; ++ small; +++ medium; ++++ large.

Table 6
Callus induction in *Garcinia celebica* leaf sections at 4 weeks.

Plant growth regulators added to MS media (mg l^{-1})	Percentage of callus induction	Callus size	Nature of callus
Control			
0 ($n = 47$)	16.45	+	Yellow, compact
TDZ			
0.1 ($n = 12$)	41.67	+++	Green/yellow,
0.5 ($n = 20$)	18.18	++	embryogenic
1 ($n = 12$)	0	-	
2 ($n = 12$)	16.67	++	
2,4-D			
0.5 ($n = 19$)	41.18	+++	Yellow, compact
1 ($n = 23$)	13.64	++	
2 ($n = 24$)	9.52	+	
4 ($n = 24$)	4.17	++	
Meta-topolin			
0.5 ($n = 25$)	55.56	++	Yellow, compact
2.5 ($n = 23$)	29.41	+	
5 ($n = 28$)	20	+	
Picloram			
0.5 ($n = 18$)	11.11	+	White, nodular
2.5 ($n = 18$)	17.65	+	
5 ($n = 17$)	5.89	++	

Mean callus induction rate for *G. celebica* leaf: 34.5%, S.D. 15.54; - = no callus; + minimal or slight; ++ small; +++ medium; ++++ large.

Looking at all treatments together, the range of callus induction rates were 0–100% for *G. mangostana* leaf sections, 0–93% for *G. mangostana* stem sections, 0–89% for *G. schomburgkiana* leaf sections, 0–100% for *G. schomburgkiana* stem sections, 0–86% for *G. cowa* leaf sections, and 6–82% for *G. celebica* leaf sections. The mean callus induction rate across all PGRs at all concentrations and control was 35.8% for *G. mangostana* leaf sections (S.D. 36.27), 47.06% for *G. mangostana* stem sections (S.D. 33.92), 39.68% for *G. schomburgkiana* leaf sections (S.D. 34.47), 34.77% for *G. schomburgkiana* stem sections (S.D. 32.01), 42.71% for *G. cowa* leaf sections (S.D. 16.73), and 34.5% for *G. celebica* leaf sections (S.D. 15.54).

Table 5
Callus induction in *Garcinia cowa* leaf sections at 4 weeks.

Plant growth regulators added to MS media (mg l^{-1})	Percentage of callus induction	Callus size	Nature of callus
Control			
0 ($n = 109$)	7.61	+	Yellow, dry
TDZ			
0.1 ($n = 29$)	48.27	+++	Yellow, compact
0.5 ($n = 29$)	24.14	++++	
1 ($n = 29$)	62.07	+++	
2 ($n = 29$)	31.03	++	
2,4-D			
0.5 ($n = 22$)	22.72	+++	White, fuzzy
1 ($n = 24$)	20.83	+++	
2 ($n = 26$)	7.69	++	
4 ($n = 24$)	0	-	
Meta-topolin			
0.5 ($n = 29$)	8.33	+	Yellow, compact
1 ($n = 29$)	8.33	+	
2.5 ($n = 29$)	20.83	+	
5 ($n = 29$)	0	-	
Picloram			
0.5 ($n = 25$)	24	++++	White, fuzzy
2.5 ($n = 26$)	24	++++	
5 ($n = 26$)	11.54	++	

Mean callus induction rate for *G. cowa* leaf: 42.71%, S.D. 16.73; = no callus; + minimal or slight; ++ small; +++ medium; ++++ large.

Looking at each PGR separately, the mean callus induction rate across species and across concentrations was 52.53% for TDZ (S.D. 23.69), 28.16% for 2,4-D (S.D. 24.79), 27.31% for mT (S.D. 25.84) and 20.23% for picloram (S.D. 25.27). Qualitatively, the PGR that resulted in the greatest amount of large-sized callus growth was TDZ, followed by 2,4-D. The nature of the callus differed noticeably between different PGRs. Callus induced by TDZ was generally compact, nodular, yellow to green, and, especially in the case of *G. celebica*, embryogenic (Fig. 4 and Fig. 5). Callus induced by 2,4-D was mostly white, with a fluffy appearance on *G. cowa* leaf sections but yellower and more compact on the other species (Fig. 4). Large amounts of white callus with a fuzzy appearance were induced by picloram on *G. cowa* leaf sections but only smaller amounts on the other species.

Some research has been carried out on tissue culture of other species in the genus *Garcinia*. For example, Te-chato [34] reported the induction of callus from leaf explants of *G. speciosa* on MS medium supplemented with BA at 3 different concentrations (0.1, 0.5, and 2.5 mg l^{-1}) in combination with 0.5 or 1.0 mg l^{-1} TDZ or 0.1, 0.5, or 0.25 mg l^{-1} NAA. Meristem nodular callus developed under all these treatments after 4–6 weeks, but the highest percentage of callus (90%) was formed in the treatment of 2.5 mg l^{-1} BA and 0.25 mg l^{-1} NAA, followed by the treatment with 0.1 mg l^{-1} BA and 0.1 mg l^{-1} NAA (60% callus). In the same report, Te-chato also tested 3 different PGR treatments on *G. atroviridis* leaf explants and observed that no callus emerged in the treatment with 0.5 mg l^{-1} BA and 0.5 mg l^{-1} TDZ, but friable callus developed on 15% of the explants in the treatment with 0.5 mg l^{-1} BA and 0.5 mg l^{-1} thiourea (TU) and 35% of the explants in the treatment with 1.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA. This is comparable to a meristem nodular callus formation rate of 69% reported for *G. mangostana* on Te-chato's callus induction medium containing 0.5 mg l^{-1} BA and 0.5 mg l^{-1} TDZ. [34]. Together with our results from the present study, this indicates that TDZ may be the most effective PGR for inducing callus in *Garcinia* species.

Kuo et al. [21] reported that callus culture can be an important method for harvesting metabolites from plants such as the medicinal alkaloids Fangchinoline and Tetrandrine synthesized in the tree *Stephania tetrandra* S. Moore. Their experimental results showed that MS medium supplemented with 1.0 mg l^{-1} BA and 0.5 mg l^{-1} TDZ was

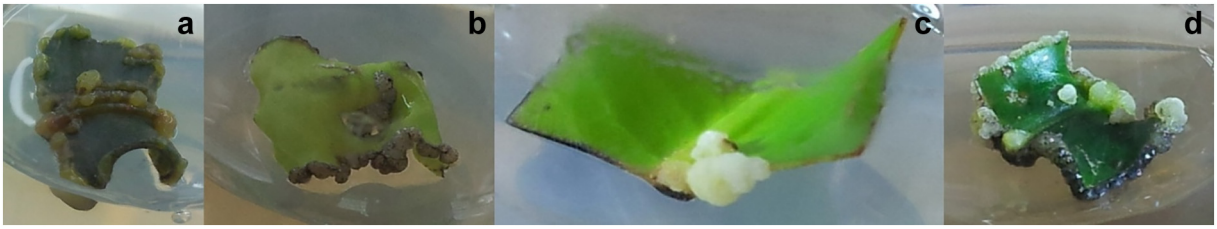

Fig. 4. Examples of medium- to large-sized callus on leaf explants of 4 species in the genus *Garcinia.* (a) *Garcinia mangostana* leaf section from *in vitro* seedling 4 weeks after culture on MS medium with 1 mg l^{-1} TDZ; (b) *G. schomburgkiana* leaf section from *in vitro* seedling 4 weeks after culture on MS medium with 0.5 mg l^{-1} picloram; (c) *G. celebica* leaf section from outdoor grown seedling 4 weeks after culture on MS medium with 1 mg l^{-1} 2,4-D; (d) *G. cowa* leaf section from outdoor grown seedling 4 weeks after culture on MS medium with 0.1 mg l^{-1} TDZ;

the best medium for inducing callus growth and its proliferation in that species. Similarly, our results indicate that TDZ could be used to induce callus from *Garcinia* species for the extraction of beneficial phytochemicals.

Singh et al. [24] reported *in vitro* plant regeneration by indirect organogenesis from callus cultures of sandalwood (*Santalum album* L.). They tested culturing leaf explants on Woody Plant Medium (WPM) supplemented with either TDZ or 2,4-D. The highest callus frequency (100%) was obtained when leaf tissue was cultured in the medium with 0.4 mg l^{-1} TDZ and the highest amounts of fresh weight and dry weight of leaf-derived callus were observed in the medium supplemented with 0.8 mg l^{-1} TDZ [24]. Our results were similar because the largest amount of compact callus obtained (measured by visual observation) was from the 0.5 mg l^{-1} TDZ treatment (Fig. 4 and Fig. 5).

Collado et al. [35] reported that 2,4-D at the concentration of 4.0 mg l^{-1} combined with 1.0 mg l^{-1} kinetin was more effective for inducing callus on immature cotyledons of *Swietenia macrophylla* King (mahogany) than 2,4-D at the concentrations of 2.0 or 6.0 mg l^{-1} [35]. This was in contrast to the results of the present study, in which we found 2,4-D at the concentrations of 2 and 4 mg l^{-1} to have a negative effect on callus formation on leaf sections of *G. mangostana, G. cowa,* and *G. celebica.* For *Garcinia* species, it appears that only lower concentrations of 2,4-D are useful for callus induction.

Kiong et al. [36] obtained a callus induction rate of 100% on leaf explants of *Melaleuca alternifolia* (tea tree) with 2,4-D added to MS medium at the rates of 1, 3, and 5 mg l^{-1}. They described the callus as greenish yellow, both friable and nodular. In the same report, Kiong et al. [36] also reported callus induction rates of 93.3%, 96.7%, and 100% when picloram was added to the medium at the concentrations of 1, 3, and 5 mg l^{-1}, respectively. The callus from the picloram treatments was noted to be yellowish brown and more friable [36]. We observed large amounts of white to green, fuzzy-looking callus on *G. cowa* in all three picloram treatments (0.5, 2.5, and 5 mg l^{-1}). In an experiment on another woody species, *Anacardium occidentale* (cashew), Cardoza and Souza [28] found picloram at concentrations of 0.5 and 1 mg l^{-1} to be effective in inducing callus.

Most of the research on mT has been done on herbaceous plants. For instance, it was found to be effective for shoot induction in the endangered African plant *Aloe polyphylla* [37] and the ornamental plant *Spathiphyllum floribundum* Schott cv. Petite [32]. We were interested in testing the effect of mT on callus formation in *Garcinia* species to contribute to the body of knowledge for culturing woody species. Thus far, to our knowledge, only one other study has been done using mT on woody plants. Moyo et al. [33] induced shoots from shoots, hypocotyls, and epicotyls excised from 30- to 60-day-old seedlings of the drought-tolerant multipurpose African tree with edible fruit, marula (*Sclerocarya birrea*), a member of the family Anacardiaceae. They reported a shoot induction rate of 63% in the treatment with 8 μM mT [33]. In the present study, mT did not have as strong an effect on callus induction in *Garcinia* species as the other PGRs studied. Although the callus induction rate was as high as 56% on *G. celebica* leaf sections at the concentration of 0.5 mg l^{-1}, the size of callus formed with mT was minimal compared to most TDZ treatments.

The interactions between endogenous PGRs in different plant organs at different developmental stages are very complex, and the manner in which a given explant will react to different concentrations of different exogenous PGRs *in vitro* can vary greatly depending on the species, variety, age, and source of the explant [38]. There were some important limitations to this study. The exact age and size of the leaves that were used as explants were not completely uniform. Variation in the extent of callus formation may have been partly due to the age of the leaf from which each section was taken. In addition, individual leaves were not all the same size; hence, some of the leaf sections that were cut were slightly larger and thicker than the other sections. This may have had an influence on the amount of callus tissue that developed. Finally, for this preliminary study, we tested each PGR separately in different concentrations but did not test combinations of PGRs. In future research, it would be interesting to test combinations of auxins and cytokinins for callus formation.

4. Conclusions

In this research, we induced callus *in vitro* from young leaf and stem tissue from *G. mangostana* and *G. schomburgkiana,* and leaf tissue of two underutilized woody plant species, *G. celebica* and *G. cowa,* using TDZ, 2,4-D, picloram, and mT. The largest amount of compact, nodular

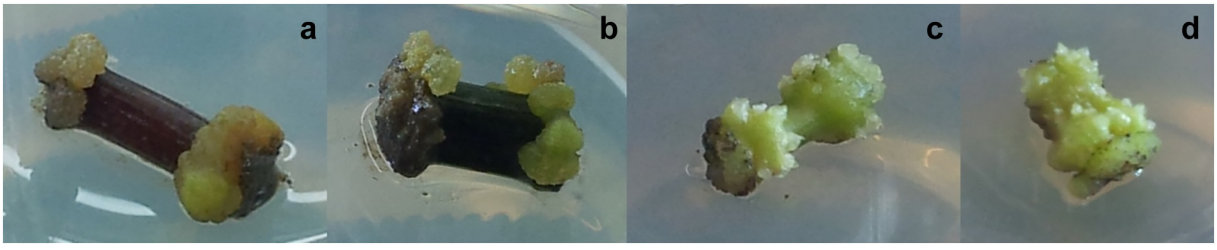

Fig. 5. Examples of medium- to large-sized callus on stem explants of 2 species in genus *Garcinia.* (a) *Garcinia mangostana* stem section from *in vitro* seedling 4 weeks after culture on MS medium with 0.5 mg l^{-1} TDZ; (b) *Garcinia mangostana* stem section from *in vitro* seedling 4 weeks after culture on MS medium with 0.1 mg l^{-1} TDZ; (c) *Garcinia schomburgkiana* stem section from *in vitro* seedling 4 weeks after culture on MS medium with 0.1 mg l^{-1} TDZ; (d) *G. schomburgkiana* stem section from *in vitro* seedling 4 weeks after culture on MS medium with 0.5 mg l^{-1} TDZ;

callus was obtained from the 0.5 mg l^{-1} TDZ treatment for *G. mangostana, G. schomburgkiana,* and *G. cowa* and from the 0.1 mg l^{-1} TDZ treatment in the case of *G. celebica.* The results may be useful for biopharming and plant breeding efforts in the future.

Conflict of interest

The authors declare that they have no conflict of interest.

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