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Callus Formation of *Curcuma mangga* in MS Media Supplemented by 2.4-Dichlorophenoxyacetic Acid

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Abstract Callus induction in *Curcuma mangga* was investigated in three different explant sources. At the concentrations of 0.5 to 2.5 ppm, it was shown that 2.4-D significantly affected on callus induction of *C. mangga*. Supplementation of 0.5 to 1 ppm of 2.4-D on MS media produced larger of callus diameter than higher concentration of 2.4-D. Increasing 2.4-D concentration tended to reduce the size and weight of callus. There was no callus formation in the MS media without 2.4-D. Apical bud was the better source of explant compared to lateral bud or leaf lamina indicated by the earlier callus initiation, larger callus diameter and higher callus fresh weight. The calli formed from apical bud were friable and white in color. Apical bud culture in MS media without 2.4-D produced a considerably high number of shoots. All source of explants showed similar response to the supplementation of 2.4-D on MS media.

Keywords: Auxin, Indirect propagation, Mango ginger, Rhizome bud

Introduction

In Indonesia, Zingiberaceae, often known as curcumas, is a family of plants that is widely distributed throughout the tropics, notably in Southeast Asia. The Curcuma genus is frequently utilized as a source of raw materials for herbs and spices. This is due to the plant's presence of the antioxidant and antitumor compound curcumin (Putri, 2014). The antioxidant is any substance or sample capable of inhibiting free radical reactions in the oxidation reaction. The curcumin found in *Curcuma* species is also primarily employed as a supplement in cancer treatment because of its antiinflammatory characteristics (Yuandani *et al*., 2021). Additionally, Curcuma plants can be utilized as food supplements. *Curcuma* species like *C. longa, C. heyneana, C. mangga, and C. xanthorriza* were frequently used as herbal ingredients in some traditional medicine because of the presence of curcuminoids (Rohman *et al*., 2020).

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Curcuma mangga in Indonesia is known as mango ginger and belongs to the family Zingiberaceae. This is called mango ginger because when the rhizome is cut it smells like mango. Indonesian people eat fresh young rhizomes as a salad mix. The old rhizomes are consumed as a drink or extract in capsules. In addition, the existence of curcumin, demethoxycurcumin, curcumanggoside, calcaratarin A, zerumin B, and difurocumenonol from *C. mangga* could be used for anti-diabetes (Awin *et al*., 2020). Because of its many benefits, mango ginger is widely studied either in cultivation or its secondary metabolite content.

The cultivation of mango ginger is constrained by the availability of planting materials. *Curcuma* sp generally takes about 9 months to produce good quality planting materials. Alternative propagation of *Curcuma* sp. is by tissue culture. Tan (2016) reported direct regeneration of *Curcuma* sp using the lateral bud sprout and apical sprout of the rhizome as explants. However, organogenesis of *C. karnatakensis* from the callus resulted in more shoots per explant than direct regeneration (Shanthala *et al*., 2021). Indirect regeneration from aseptic shoots of *C. mangga* produced 95.83% of somatic embryos (Pikulthong *et al*., 2016). Jose and Thomas (2015) used callus to indirectly propagate ginger. Bud and leaf segment explants were cultivated on Murashige and Skoog (MS) media supplemented with 6.7 M 2.4-D and 2.7 M naphthalene acetic acid (NAA) to get the best response for callus induction. On MS medium that had been added with 6.8 M thidiazuron (TDZ) and 1.6 M NAA for shoot induction, the white, friable, organogenic calli were subcultured. Ninety percent of the bud-derived calli reacted on this medium with an average of 16.2 shoots per culture.

Callus induction requires high concentrations of auxin. Pikulthong *et al*. (2016) used 5 mg/L 2.4-D and 5 mg/L NAA to induce embryogenic callus of *C mangga*. According to a research by Al-Taha *et al.*, (2020), the medium of MS containing 1.0 mg/L 2.4-D, 0.5 mg/L BA, and 500 mg/L PVP promoted primary callus production (100%) with large diameter, fresh weight, and callus weight in white ginger (*Zingiber officinale* var. Roscoe). The greatest callus induction results were shown for 83 days when Chowdhury *et al*. (2020) utilized 2.4-D at a concentration of 2.0 mg/L in *C. caesia*.

The ability to form callus or production of secondary metabolites is influenced by the source of explants (Gurav *et al.*, 2020). In general, rhizome buds are used as a source of explants on *Curcuma* sp plants (Tan, 2016, Sundram *et al.*, 2012b). However, the lateral buds are often stunted due to the dominance of the apical buds. Cutting the apical bud stimulates rhizome lateral sprouting (De Souza *et al*., 2016). On MS medium enriched with 1 mg/L 2.4-D, explants from the shoot of *C. mangga* could also develop a friable callus

(Sundram *et al*., 2012a). Information on the callus-forming ability of different shoot types in *C mangga* has not been reported. Differences in endogenous hormone content of lateral shoots and apical shoots are thought to affect the success of callus formation.

Besides the rhizome buds, the leaf lamina of mango ginger is also reported to be able to form callus. Embryogenic callus induction was obtained from leaf lamina explants of *C. amada* Roxb. (Raju *et al*, 2013) and *C. mangga* (Pikulthong *et al.,* 2016). Several buds commonly on Zingiberaceae rhizome have different potential to be used as sources of in vitro vegetative propagation. Similarly do the leaf lamina which grows from the onset of sprouting. The objective of this study was to evaluate the response of the source of explant to various concentrations of 2.4-D on *C. mangga*.

Materials and methods

Establishment of in vitro culture

The experiment was conducted at the *In Vitro* Laboratory of The Department of Crop Production, Faculty of Agriculture, The University of Bengkulu, in the year of 2021. Young rhizome buds were used as explants. The explants were washed thoroughly under running tap water for 30 minutes by adding detergent as a surfactant. This prewash was useful to remove surface contaminants such as dirt or dust. The leaf lamina and 1.5 cm-long buds explants were surfaces sterilized for 20 minutes with 20% clorox®, a commercial bleach solution that contains 5.3% of sodium hypochlorite. Bacteria are known to be particularly sensitive to hypochlorite. When diluted in water, the hypochlorite salt used (NaOCl) produces hypochlorous acid (HOCl) which has bactericidal activity. The sterilized explants were rinsed three times with sterile distilled water (Romeida *et al*., 2016). The sterilized explants were diluted for a second in betadine before being transplanted on the MS basal medium (Murashige and Skoog, 1962). The explants were maintained for two weeks and the aseptic explants were subsequently used for treatments.

Six concentrations of 2.4-D were added in accordance with the treatment to create the culture medium according to the MS formula. The pH of the medium was adjusted to 5.8 once the nutrients had been prepared. If the medium's pH exceeded 5.8, 0.1N HCl was supplied. On the other hand, 0.1 N KOH was added if the pH of the medium was lower than 5.8. The pH was adjusted gradually while being stirred with a magnetic stirrer until it was steady. Agar is then added to the medium and heated until it boils. Approximately 20 cc of the medium per culture vial were used for the pouring

process. The culture bottles were then securely covered with plastic and kept in the culture chamber.

Experimental design and statistical analysis

Factorial in Randomized Complete Block Design (RCBD) was performed with three replications. The first factor was 2.4-D concentration consisting of control (without 2.4-D), 0.5 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm, and 2.5 ppm of 2.4-D. The second one was explant sources i.e. apical bud, lateral bud, and leaf lamina. Three explants were used for each experimental unit. Therefore, there were seventy-two experimental units which were the combination of 2.4-D concentration and explant sources. The explant sources used are shown in Figure 1.

The percentage of live explants, callus frequency, days to callus initiation, callus diameter, callus fresh weight, callus morphology, and callus nature from each explant were determined after twelve weeks of culture.

The data were analyzed by the Analysis of Variance (ANOVA) and the mean comparisons were performed by the Duncan Multiple Range Test (DMRT) at $p \leq 0.05$ using Costat version 6.311.

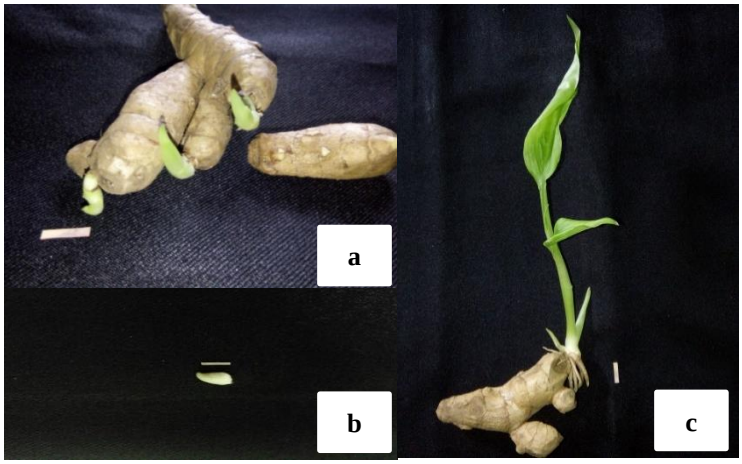

Figure 1. Rhizome bud and leaf lamina of *C. mangga* collected as explants (a) Apical bud and lateral bud, (b) The bud as explant (c) The leaf lamina (Bar represent 1 cm)

Results

The results showed that there was no interaction between the explant source and the concentration of 2.4-D. The treatment of 2.4-D concentration

significantly affected the percentage of callus frequency and callus diameter. While the treatment of explant sources had a significant effect on all observed variables. The obtained data had a large variation distribution because some explants, especially leaf lamina, did not produce callus. Therefore, the percentage of live explants and the days to callus initiation were transformed by $\sqrt{(X+1)}$, while the percentage of callus frequency, callus diameter, and callus fresh weight were transformed by $\sqrt{(X+0.5)}$ (Table 1).

Table 1. Analysis of variance (ANOVA) on all variables

Variables	F-value		
	2.4-D	**Explant sources**	**Interaction**
Persentage of live explants^{T}	0.22 ^{ns}	31.61**	0.45 ^{ns}
Persentage of callus frequency$^{\infty}$	2.40 *	7.85**	0.43 ^{ns}
Days to callus initiation^{T}	2.24 ^{ns}	4.87*	0.69 ^{ns}
Callus diameter$^{\infty}$	2.39 *	19.64**	1.47 ^{ns}
Callus fresh weight$^{\infty}$	1.06 *	16.84**	0.62 ^{ns}

Note: ^{T}and $^{\infty}$= Data were transformed by $\sqrt{(X+1)}$ and $\sqrt{(X+0{,}5)}$, respectively. *, **, and ^{ns} were significant at α= 5%, 1% and non-significant, respectively.

The percentage of live explants was not affected by the concentration of 2.4-D. In all 2.4-D treatments, the highest live explants were only up to 36.11%. The addition of 2.4-D succeeded in inducing callus formation. However, the percentage of callus formation was not significantly different with the addition of a concentration of 2.4-D up to 2.5 ppm. The days to callus initiation were also not affected by the concentration of 2.4-D treatment. Treatment with 2.4-D above 2.0 ppm tended to faster callus appearance than the concentration below. In the control treatment without 2.4-D, all living explants did not form a callus (Table 2).

The size of the explants increased with the formation of the callus. Callus was not formed on explants grown on MS media without the addition of 2.4-D. Without the addition of 2.4-D, the apical bud formed shoots while the lateral bud and leaf lamina explants stay stagnate. The addition of 2.4-D induced callus formation on the apical bud and lateral bud explants resulted in an increase in the size of the explants (Table 2). There was no significant difference between callus diameter and callus fresh weight with the addition of 2.4-D concentration. At a low 2.4-D concentration, the callus diameter tended to be larger.

The same response as callus diameter was obtained for callus fresh weight. The addition of 2.4-D significantly increased the callus fresh weight. The increase is smaller with an increasing concentration of 2.4-D. Observation on callus morphology and nature, concentrations of 0.5 to 2 ppm 2.4-D were better at producing calli with fragile morphology and white color.

Table 2. The response of explant to the application of 2.4-D

Concentration of 2.4-D (ppm)	Live explants (%)	Callus frequency (%)	Days to callus initiation	Callus diameter (mm)	Callus fresh weight (g)	Callus morphology and nature
0.0	36.11	0.00 b	na	0.00 b	0.00 b	na
0.5	36.11	44.44 a	14.58	10.49 a	1.59 a	F, W
1,0	22.22	41.67 a	11.50	9.70 a	1.03 ab	F, W
1.5	27.78	45.83 a	16.75	8.54 ab	0.95 ab	F, W
2.0	30.56	20.83 ab	5.00	9.10 ab	0.85 ab	F, W
2.5	36.11	44.44 a	10.92	7.50 ab	0.61 ab	C, Y

Remarks: numbers in the same column followed by the same letters were non-significant difference based on DMRT at α=5%. W=white, Y=yellow, F=friable, C= compact, na = not available.

Callus morphology and nature were analyzed at the age of 12 MST. Observation on callus color was performed by a Munsell Plant Tissue Color Chart. Most of the calluses were white, but there were also yellowish color. The texture of the calluses was commonly friable, but some were compact. The best callus was derived from the apical bud, with a white friable callus. However, at 2.5 ppm 2.4-D the callus was yellowish and had a compact texture (Figure 2). Lateral bud explants found several compact calluses at all concentrations of 2.4-D.

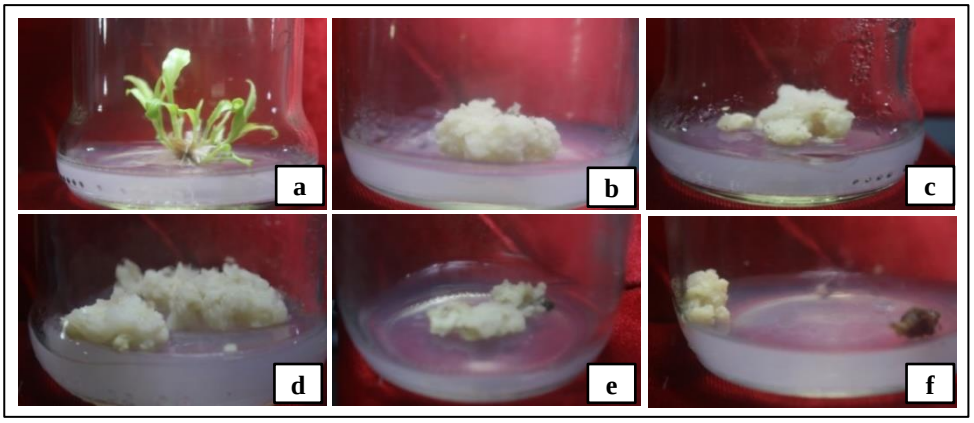

Figure 2. The growth of apical bud explants on MS media supplemented by 1 ppm 2.4-D. a) control, b) 0.5 ppm, c) 1.0 ppm, d) 1.5 ppm, e) 2.0 ppm, f) 2.5 ppm

Shoot growth was observed periodically every 2 weeks until 12 weeks. In the MS medium without the addition of 2.4-D, each explant was able to form shoots ranging from 1 to 5 with an average of 2.3 shoots/explant. Shoots begin to form in the second week after planting. Rapid growth occurs at the age of 4 to 8 weeks Up to the twelve weeks, the average shoot height reached 28.5 mm (Figure 3).

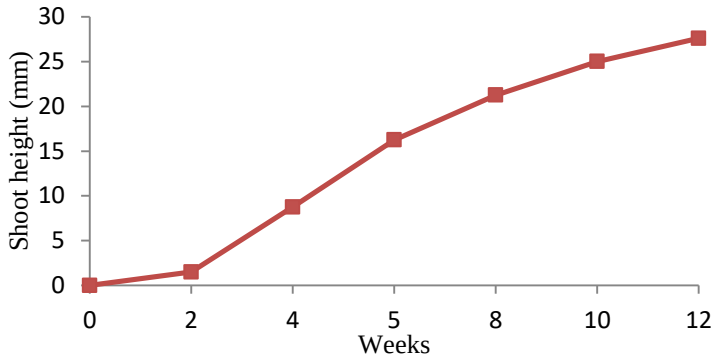

Figure 3. The shoot height of *C mangga* from the apical-bud explants on MS media without 2.4-D

A different source of explants showed different performances in *in vitro* culture. In general, apical bud explants were able to form the callus better. The three explant sources formed callus from 6 to 14 days after planting. In the variable percentage of living explants, apical and lateral bud explants were significantly higher than leaf lamina. The callus frequency of lateral bud explants was only 27.78% not significantly different from the leaf lamina explant. Apical bud explants also produced the largest callus diameter and callus fresh weight compared to lateral buds and leaf lamina (Table 3). Callus from apical bud explants increased significantly from 2 weeks to 10 weeks after planted with friable callus quality (Figure 4).

Table 3. The growh of explants from diferent sources *in vitro*

Source of explants	Live explants (%)	Callus frequency (%)	Days to callus initiation	Callus diameter (mm)	Callus fresh weight (g)
Apical bud	48.61 a	58.33 a	5.63 a	13.98 a	1.77 a
Lateral bud	45.83 a	27.78 b	11.75 a	6.46 b	0.81 b
Leaf lamina	18.06 b	12.50 b	12.00 a	4.27 b	0.04 b

Remarks: numbers in the same column followed by the same letters were nonsignificant difference based on DMRT at α=5%

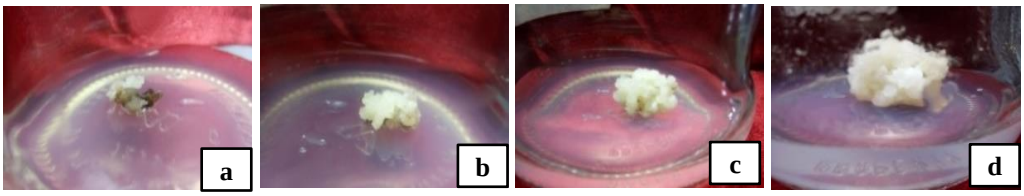

Figure 4. The growth of apical bud cullus on MS media supplemented by 1 ppm 2.4-D (a) 4 weeks, (b) 6 weeks, (c) 8 weeks and (d) 10 weeks after planted

Discussion

Explants for in vitro propagation of *C mangga* are usually rhizomes. The direct contact of explants with bacteria and fungi in the field during their lifetime made it difficult to obtain sterile explants using the usual sterilization techniques. Therefore, the percentage of live explants in the in vitro culture of this study was relatively low. Other researchers used $HgCl_2$ to suppress contamination of *Curcuma* sp. Abubakar and Pudake (2019) obtained 85% *C. cesia* free of contaminants using 15% $NaHClO_3$ + 70% Ethanol + 0.1% $HgCl_2$. In the treatment without $HgCl_2$, sterile explants were not obtained on day 16. In this study, less than 50% of mango ginger explants survived in in vitro culture. Even in leaf lamina explants treated with 1.0 to 2.5 ppm 2.4-D, the explants were unable to survive. Explants undergo browning, bleaching, and contamination by fungi or bacteria. Bacterial contamination is known by the symptoms of the growth of white mucus around the explants. Meanwhile, the symptom of fungal contamination in culture is the growth of hyphae/mycelium which produces blackish spores. Contamination by bacteria is more difficult to control because bacteria live in cells. This is because the rhizome explants came from the field without going through the quarantine process. Sterilization that has been carried out in 3 stages (before the laboratory, in the laboratory, in laminar air flow) has not been able to produce sterile explants above 50%.

In vitro callus initiation requires the balance of growth regulators, especially the presence of auxin. Auxin affects both cell division and cellular expansion (Xu *et al.*, 2018). One of the auxins commonly used in in vitro culture is 2.4-D. At the appropriate concentration, 2.4-D can be used to induce callus formation (Romeida *et al*., 2016, Wang and Ruan 2013). Gomes-D ás *et al.* (2014) stated that the addition of 1 to 2 ppm 2.4-D was effective in inducing callus on torch ginger (*Etlingera elatior*). Increasing the concentration of 2.4-D to 4 ppm reduced callus formation by up to 60%. The role of 2.4-D in inducing callus formation can also be seen in this study. The best callus formation was obtained at 0.5 to 1 ppm 2.4-D. A callus was formed beginning with swelling in

the explant wound area. The swelling is caused by excessive cell division to close the injured tissue. This phenomenon occurs naturally in plants. At the beginning of growth, the callus texture is compact with white color. According to Pikulthong *et al.* (2016), this phenomenon is a process of mucilaginous callus formation at the cut end from the explants. The calli had not undergone differentiation. Of the three explants tested, the fastest callus formation was from the apical bud. Meristem cells in the apical bud are more responsive to dividing to form callus. The active response of the apical buds can be seen from the highest callus fresh weight.

Leaf-lamina explants had lower growth rates than buds. Most of the explants (57%) had browning from the edges. Some others lost chlorophyll so that the explants became transparent (32%). The remaining explants that remained green were not able to form callus until the end of the observation. Browning due to 2.4-D treatment was also reported by Baharan *et al*. (2015) on leaf explants of *Phoenix dactylifera* L. Explants that changed color to brown were due to the release of phenolic compounds from injured tissues. As a result of this browning causes inhibition of tissue development including callus formation. Browning can also be caused by the destruction of chlorophyll due to a mismatch of nutrient content and PGR media. The leaves did not respond to the treatment, possibly because the size of the explants was too small. In this study, leaf lamina explants were prepared with a length of ±3 mm. Young leaves do not have a sufficient amount of hormone to stimulate callus formation. Kurup *et al*. (2014) used 3 to 5-mm lengths from the base of differentiated tender leaves as explants and succeeded in forming a callus. While the leaf lamina explants of *C mangga* became transparent due to the loss of cell compounds such as carbohydrates, proteins, and lipids. The addition of exogenous auxin was not able to stimulate callus formation of leaf lamina in this study. Sundram *et al*. (2012b) used MS liquid medium enriched with 0.3 ppm 2.4-D coupled with 0.1 ppm NAA to get rapidly developing calli from leaf lamina explants of *C. mangga*. The culture media and, in particular, the exogenous application of PGR like 2.4-D, IAA, and NAA are crucial for the success of *C. mangga* in vitro culture.

The increasing concentration of 2.4-D improved the diameter and weight of the callus. In this study, concentrations of 0.5 ppm to 1.0 ppm 2.4-D were quite significant in inducing the callus of *C. mangga*. The addition of the concentration of 2.4-D up to 2.5 ppm decreased the diameter and fresh weight of the callus. However, the addition of 2.4-D more than 1.5 ppm was faster to form callus. Similar results were obtained by Sundram *et al*. (2012a) who reported that 1 ppm 2.4-D could induce callus *C. mangga.*

Faster callus formation is not followed by good callus quality. The callus quality of the *C. mangga* can be seen from the callus texture and nature. There are several natures of callus that are usually obtained from in vitro culture. Jie *et al*. (2019) obtained that the calli of *C. longa* L. were white and yellowish. The white, friable callus supported somatic embryos, whereas the yellowish callus did not. This white and friable callus is actively dividing. The yellowish callus is a further development toward the final phase of active division. While the brownish callus is a mass of cells that are heading to the aging phase (Rasud and Bustaman, 2020). In this study, apical bud treatment produced white calli with fast growth. The best growth and callus quality (white and friable) was obtained at a concentration of 0.5-1.0 ppm 2.4-D. The same finding was also made by Gomes-D ás *et al*. (2014) that a more friable callus was found at the addition of 1.0 to 2.0 ppm 2.4-D on MS medium. Until the end of the observation, the callus was white or greenish white which indicated that callus division activity was still occurring. This is also indicated by the callus texture. Callus produced from apical bud explants were mostly friable, while those from lateral bud explants produced more compact. Both types of callus can be used according to research objectives. According to Sarma and Deka (2020), the friable callus of *C. caesia* showed embryogenic responses after subcultures. While the compact callus texture is good for use as secondary metabolite production.

In the control treatment (without 2.4-D), mango ginger explants did not form calli but formed shoots. Shoots begin to grow at the age of 3 weeks. Apical bud culture in MS media without 2.4-D produced a considerably high number of shoots, with an average of 2.3 shoots/explant. Lateral and apical buds are clusters of cells that naturally develop into shoots under suitable conditions. Therefore, without a plant growth regulator, the lateral and apical buds will develop into shoots.

Apical bud explant was the best explant for callus formation of *C mangga* compared to lateral bud and leaf lamina. Apical bud explants produced calli with friable texture and white color There was no interaction between the source of explants and concentrations of 2.4-D. The addition of 0.5 ppm to 1 ppm 2.4-D in MS medium was sufficient to initiate callus formation of *C. mangga*. Increasing the concentration of 2.4-D tended to decrease the size and quality of the callus.

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References

Abubakar, A. S. and Pudake, R. N. (2019). Sterilization procedure and callus regeneration in black turmeric (*Curcuma caesia*). Agricultural Science Digest, D-4714:1-6.

Al-Taha, H. A., Al-Mayah, A. A. and Al-Behadili, W. A. A. (2020). Effect of auxin 2,4-D and NAA on induction callus and adventitious shoots and roots regeneration from half shoot tips culture of white ginger (*Zingiber officinale* var. Roscoe). Joint University Programmes Admissions System, 28:160-171.

Awin, T., Mediani, A., Faudzi, S. M., Maulidiani, M., Leong, S. W., Shaari, K. and Abas, F. (2020). Identification of α-glucosidase inhibitory compounds from *Curcuma mangga* fractions, International Journal of Food Properties, 23:154-166.

Baharan, E., Mohammadi, P. P., Shahbazi, E. and Hosseini, S. Z. (2015). Effects of some plant growth regulators and light on callus induction and explants browning in date palm (*Phoenix dactylifera* L.) in vitro leaves culture. Iranian Journal of Plant Physiology, 5:1473-1481.

Chowdhury, S., Pal, K., Chakraborty, M., Chakraborty, S., Mandal, S., Kr.Pandit, G., Maitra, S. and Sahana, N.. (2020). Conservation and in vitro propagation of an endangered wild turmeric (*Curcuma caesia* Roxb.) species from Sub - Himalayan Terai Region of West Bengal. International Journal of Current Microbiology and Applied Sciences, 9:2132-2140.

De Souza, C. S., Ferreira, A. P. S. and Finger, F. L. (2016). Growth regulators and carving on breakage of apical dominance in tannia (*Xanthosoma sagittifolium* L. Schott) rhizomes. Australian Journal of Crop Science, 10:434-437.

Gomes-Dás, G. M., Almendagna-Rodrigues, F., Rodrigues-Soares, J. D., Pasqual M. and Portugal-Pinto de Carvalho, A. C. (2014). Embryogenic induction of torch ginger (*Etlingera elatior*) callus. Agrociencia, 48:173-184.

Gurav, S. S., Gurav, N., Patil, A. T. and Duragkar, N. J. (2020). Effect of explant source, culture media, and growth regulators on callogenesis and expression of secondary metabolites of *Curcuma longa*, Journal of Herbs, Spices & Medicinal Plants, 26:172-190.

Jie, E. Y., Ahn, M. S., Lee, J., · Cheon, Y. I., · Kim, C. Y. and · Kim, S. W. (2019). Establishment of a high-frequency plant regeneration system from rhizome-derived embryogenic cell-suspension cultures of *Curcuma longa* L. Plant Biotechnology Reports, 13:123-129.

Jose, S. and Thomas, T. D. (2015). High-frequency callus organogenesis, large-scale cultivation and assessment of clonal fidelity of regenerated plants of *Curcuma caesia* Roxb., an important source of camphor. Agroforestry Systems, 89:779-788.

Kurup, S. S., Aly, M. A. M,, Lekshmi, G. and Tawfik, N. H. (2014). Rapid in vitro regeneration of date palm (*Phoenix dactyliferaL*.) cv. Kheneizi using tender leaf explant. Emirates Journal of Food and Agriculture, 26:539-544.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15:473-497.

Pikulthong, V., Teerakathiti, T., Thamchaipenet, A. and Peyachoknagul, S. (2016). Development of somatic embryos for genetic transformation in *Curcuma longa* L. and *Curcuma mangga* Valeton & Zijp. Agriculture and Natural Resources, 50:276-285.

Putri, M. S. (2014). White turmeric (*Curcuma zedoaria*): its chemical substance and the pharmacological benefit. Jurnal Majory, 3:88-93.

Raju, C. S., Kathiravan, K., Aslam, A. and Shajahan, A. (2013). An efficient regeneration system via somatic embryogenesis in mango ginger (*Curcuma amada* Roxb.). Plant Cell Tiss. Organ Cult., 112:387-393. https://doi.org/10.1007/s11240-012-0244-4

Rasud, Y. and Bustaman, B. (2020). In vitro callus induction from clove (*Syzigium aromaticum* L.) leaves on medium containing various auxin concentrations. Jurnal Ilmu Pertanian Indonesia (JIPI). 25:67-72. http://journal.ipb.ac.id/index.php/JIPI.

Rohman, A., Widodo, H., Lukitaningsih, E., Rafi, M., Nurrulhidayah, A. F. and Windarsih, A. (2020). Review on in vitro antioxidant activities of *Curcuma* species commonly used as herbal components in Indonesia. Food Research, 4:286-293.

Romeida, A., Ganefianti, D. W. and Rustikawati R. (2016). Embryogenic callus induction of pencil orchid (*Papilionanthe hookeriana* Rchb.f.) through in vitro culture. International Journal on Advanced Science Engineering Information Technology, 6:196-200.

Sarma, I. and Deka, A. C. (2020). Conservation of *Curcuma caesia* Roxb.- A critically endangered species via in vitro plant regeneration from organogenic callus. Asian Journal of Conservation Biology, 9:151-155.

Shanthala, A. A., Dilkalal, A. and Umesh, T. G. (2021). An efficient invitro approach for direct and callus mediated regeneration of *Curcuma karnatakensis* – An endemic plant of Karnataka. Journal of Herbs, Spices & Medicinal Plants, 27:229-241.

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

Sundram, T. C. M. (2012b). In vitro cultures of *Curcuma mangga* Val. for the production of (E)-Labda-8(17), 12-Diene-15, 16-Dial. (Masters Thesis). University of Malaya.

Tan, P.V. (2016). Micropropagation of *Curcuma* sp., a threa- tened medicinal plant. Advances in Bioscience and Biotechnology, 7:418-427.

Wang, l. and Ruan, Y. (2013). Regulation of cell division and expansion by sugar and auxin signaling. Frontiers in Plant Science, 4:1-9.

Xu, C., Cao, H., Xu, E., Zhang, S. and Hu, Y. (2018). Genome-wide identification of arabidopsis LBD29 target genes reveals the molecular events behind auxin-induced cell reprogramming during callus formation. Plant and Cell Physiology, 59:749-760.

Yuandani, Jantan, I., Rohani, A. S. and Sumantri, I. B. (2021). Immunomodulatory effects and mechanisms of curcuma species and their bioactive compounds: A review. Frontiers in Pharmacology, 12:643119.

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