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Innovative Approaches to Surface Sterilization and *In Vitro* Shoot Proliferation of *Curcuma longa* L.

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Abstract- Curcuma longa L. (Zingiberaceae) is a valuable cash crop in regions like Sri Lanka due to its medicinal and culinary uses. Micropropagation techniques are essential for the rapid year-round production of disease-free planting materials of this species to meet the increasing demand. This study aimed to optimize a surface sterilization procedure for establishing in vitro cultures and to explore the effect of plant growth regulator (PGR) combinations on efficient shoot proliferation using rhizome bud explants. Experiments were arranged as a Completely Randomized Design with 20 replicates per treatment. According to results revealed rhizome buds treated with 1% Topsin (70% Thiophanate-methyl) for 30 minutes, followed by 100% Clorox (5.25% sodium hypochlorite) for 10 minutes, showing the highest success (80% non-contamination and 100% non-browning rate) in establishing uncontaminated and non-browning cultures after four weeks among the evaluated five surface sterilization procedures. Regarding shoot multiplication, various hormone combinations were tested on liquid Murashige and Skoog (MS) medium. The most effective PGR combination for shoot proliferation (2.4 shoots/explant) was 4.0 mg/l Benzyl Amino Purine (BAP) combined with 0.25 mg/l Naphthalene Acetic Acid (NAA). The highest mean shoot length (6.56 cm) was achieved with 1.0 mg/l BAP and 0.5 mg/l NAA on liquid MS medium. The shoot proliferation rate can be improved further while identifying the correct duration and number of subcultures that could proceed. However, the rooting and acclimatization stages of micro propagation were yet to be developed. Finally, this research provides valuable insights for commercial propagation and sustainable cultivation of turmeric.

Keywords: Curcuma longa L, Explants, Surface sterilization, Micropropagation, Shoot proliferation

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1. Introduction

Turmeric, scientifically known as Curcuma longa L. and belonging to the Zingiberaceae family, is esteemed for its therapeutic properties, including anti-inflammatory, antioxidant, and anti-tumor effects (Ramadan, Al-Kahtani and El-Sayed (2011). Its applications span medicine, cosmetics, and the food industry, where it serves both medicinal and coloration purposes (Ghosh, Chatterjee and Ghosh (2013) . Additionally, turmeric finds use in landscaping and floral arrangements due to its vibrant flowers (Ferrari et al., 2019).

The C. longa is primarily cultivated as both a monocrop and an intercrop alongside coconut in Sri Lanka's wet and intermediate zones, with major cultivation areas including Kurunagala, Gampaha, Kalutara, Kandy, and Matale districts. Despite its importance, turmeric production in Sri Lanka lags than that of India. Propagation typically relies on rhizomes, which exhibit a dormancy period and sprout during the monsoon season (Mehaboob et al., 2019). To sustain future crops, a portion of the yield (10-20%) is stored annually (Abubakar and Pudake, 2019). However, challenges such as fungal and bacterial diseases, including *Ralstonia solanacearum* and *Pythium aphanidermatum,* pose significant threats to germplasm conservation (Sharma (2014); Sandhyarani et al., 2018).

Seed propagation of C. longa is challenging due to poor flowering and seed set, leading commercial growers to rely on rhizomes with viable buds. Yet, the multiplication rate of rhizomes is low, and prolonged propagation can lead to degeneration. Each growing season, a single rhizome produces only 10-15 lateral buds, complicating large-scale cultivation efforts today.

Advancements in tissue culture techniques, particularly through rhizome bud proliferation, offer promise for rapid and uniform production of disease-free planting materials year-round (Behar et al., 2014). This study aims to establish standardized protocols for surface sterilization and shoot proliferation of C. longa rhizome bud explants, pivotal steps in developing a reliable in vitro clonal propagation strategy to meet escalating demand.

2. Methodology

A. Maintenance of mother plants

The experiments were conducted over 15 weeks at the tissue culture laboratory of Greengrow Agriculture Private Ltd, Padukka. Mother plants of C. longa were cultivated in pots containing sandy loam soil mixed with organic matter within a controlled environment under protected house conditions. The plants were managed uniformly applying fertilizer (Albert solution), fungicide (Topsin, containing 70% Thiophanate-methyl), and insecticide (Imidacloprid, at 20% concentration) at two-week intervals. Irrigation was done in the morning to optimize plant hydration, while overhead watering was specifically avoided.

B. Identification of standardized surface sterilization procedure for rhizome bud explants

Sprouts (1-1.5 cm in length) emerging from rhizomes of the mother plants were excised using a sharp blade. Then the samples were thoroughly washed using soap and kept under running tap water for ½ an hour. Inside a laminar airflow cabinet, the sprouts were cut into small segments, each containing a bud (Sandhyarani et al., 2018), and then exposed to one of five different surface sterilization procedures (Table 01).

After exposure to each sterilizing procedure, explants were thoroughly washed using sterilized distilled water twice. Then they were cultured on a basal Murashige and Skoog (MS) medium

(Murashige and Skoog, 1962). Culture vessels were kept in a growth room and numbers of non-contaminated and non-browning cultures were recorded weekly up to 4 weeks.

Table 1

Tested surface sterilization procedures

Surface sterilization procedure	Description
S1	Dipping in 3% Topsin solution-30 min + 20% Clorox (10% Sodium hypoclorite-NaOCl) solution for 20 min
S2	Dipping in 1% Topsin solution-30 min + 30% Clorox solution for 30 min, 70% ethanol 2 min
S3	Dipping in 1% Topsin solution-30 min + 100% Clorox solution for 10 min
S4	100% Clorox solution for 10 min + 70% ethanol 2 min
S5	100% Clorox solution for 10 min

C. Identification of the best plant growth regulator combination to obtain the maximum number of shoots per explants

Non-contaminated explants were transferred to solid and liquid MS media supplemented with a combination of plant growth regulators (PGRs) and kept in a growth room. Four concentrations of BAP 1.0 ,2.0, 2.5, 4.0mg/l with three concentrations of NAA 0.0, 0.25 and 0.5 mg/l were tested to achieve the highest multiplication rate (Table 02). The number of shoots per explant and shoot length were recorded after 4 weeks of culture initiation and going to be repeated at each subculture.

The following growth room conditions were provided: 16/8 photoperiod, 3000 Lux light intensity from cool white, fluorescent lights, 26±20C temperature and 60% Relative Humidity

(RH). Liquid cultures were kept in an orbital shaker with slow agitation (speed 80 rpm) (Swarnathilaka and Nilantha, 2012).

The pH of the media was adjusted to 5.8 prior to the addition of 7 g/L agar for solidification. Then the media was autoclaved at 121°C and 1.05 kg/cm² pressure for 20 minutes to ensure sterilization.

Table 2

Tested plant growth regulator (PGR) combinations

PGR combination	BAP (mg/l)	NAA (mg/l)
P1	0.00	0.00
P2	1.00	0.00
P3	2.00	0.00
P4	2.50	0.00
P5	4.00	0.00
P6	1.00	0.25
P7	2.00	0.25
P8	2.50	0.25
P9	4.00	0.25
P10	1.00	0.50
P11	2.00	0.50
P12	2.50	0.50
P13	4.00	0.50

D. ***Statistical Analysis***

Completely Randomized Design was used for each experiment. Each treatment consisted of 20 replicates and experiments were repeated three times. The data were analyzed by using a One Way ANOVA at and significant means were separated by using Tukey's post hoc test at 5% significance level. All the analysis was done using Minitab (Version 17). statistical package

3. Results and discussion

A. Identification of standardized surface sterilization procedure for rhizome bud explants

In this experiment, C. Longa explants were subjected to five different surface sterilization procedures and established on basal MS medium. The effect of various concentrations of Topsin, Clorox, and ethanol for different exposure time durations was tested on contamination and browning of explants (Table 03). The highest contamination rate was observed with explants exposed to 100% Clorox solution for 10 min (S5). The cultures were highly contaminated by both bacteria and fungi. The highest number of non-contaminated cultures were obtained when explants were treated with 1% Topsin for 30 min followed by 100% Clorox for 10 min (S3). In presence of the fungicide, fungal contaminations were avoided. The second-highest non-contamination rate was recorded with S2. Since the concentration of Clorox was low in S2 than in S3, the contaminations were higher in S2 compared to S3.

The S1 resulted in the least non-contamination rate and it showed a high amount of bacterial contamination than S2, S3, and S4. In S1, a low concentration of Clorox was used and it was not effective in preventing bacterial contaminations. In S2 and S4, the presence of 70% ethanol was positively affected in avoiding bacterial contaminations (Sandhyarani et al., 2018, Talati, 2017).

Browning of explants was observed with S1 and S2. There were no browning incidents recorded with S3 and S4. Due to high contaminations non-browning cultures could not be observed with S5. Irrespective of concentration, long-time exposure to Clorox increased the browning of explants as S2 resulted in the lowest non-browning rate compared to S1 and S3. Both S2 and S4 treatments have 70% ethanol. Since browning was not observed with S4, 70% ethanol might not be the reason for the browning resulted with S2.

Most of the experiments related to the surface sterilization of C. longa explants used Mercuric chloride (HgCl2) as the surface sterilant (Sinchana et al.,2020, Ghosh et al., 2013, Sandhyarani et al., 2018). Even positive results were obtained, due to its toxic nature and problems with disposal, the current experiment was planned to identify low-cost alternatives by using a commercially available fungicide, Clorox, and ethanol as a surface sterilant. Based on the results the surface sterilization procedure could be further improved.

Table 3

Mean percentages of non-contaminated and non-browning cultures of different surface sterilization procedures

Surface terilization Procedure	Non-contaminated cultures (%) ± SE	Non-browning cultures (%) ± SE
S1	45.00 ± 0.10^{ab}	75.00 ± 0.10^{b}
S2	55.00 ± 0.10^{a}	25.00 ± 0.10^{c}
S3	80.00 ± 0.10^{a}	100.00 ± 0.00^{a}
S4	50.00 ± 0.10^{ab}	100.00 ± 0.00^{a}

S5	10.00±0.10[b]	0 .00±0.00[d]

The superscript letters above the values represents the statistically significant difference between the sterilization procedures by Tukey's post hoc test at 5% significant level

B. Identification of the best plant growth regulator combination to obtain the maximum number of shoots per explants

The effects of various plant growth regulators on producing new shoots in vitro and mean shoot lengths obtained by shoots are shown in Table 4.

Table 4

Mean no of shoots/explant and mean shoot lengths in liquid media (four weeks after culture initiation)

PGR combination	Mean no of hoots/explant ± SE	Mean shoot length (cm) ± SE
P1	0.00±0.00[e]	0.00±0.00[b]
P2	0.60±0.20[de]	0.90±0.40[b]
P3	0.80±0.20[cde]	1.08±0.30[ab]
P4	1.00±0.00[bcd]	2.14±0.10[ab]
P5	1.00±0.00[bcd]	2.66±0.10[ab]
P6	1.00±0.00[bcd]	3.30±0.10[ab]
P7	1.40±0.20[bcd]	3.41±0.10[ab]
P8	1.60±0.20[abc]	3.52±0.10[ab]
P9	2.40±0.20[a]	4.01±0.10[ab]
P10	1.00±0.20[bcd]	6.56±4.10[a]
P11	1.00±0.20[bcd]	2.60±0.10[ab]
P12	1.40±0.20[bcd]	3.45±0.10[ab]
P13	1.80±0.20[ab]	3.84±0.10[ab]

The superscript letters above the values represents the statistically significant difference between the sterilization procedures by Tukey's post hoc test at 5% significant level

PGR-free solid MS medium (P1) didn't support shoot proliferation. Supplementation of 4.0 mg/l BAP and 0.25 mg/l NAA (P9) was the most effective for initiating the highest mean number of new shoots (2.4 shoots/explant). When NAA concentration was increased up to 0.5 mg/l in presence of 4.00 mg/l BAP, the second-highest rate of multiplication was obtained (1.8 shoots/explant).

The medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA showed the highest mean shoot length (6.56 cm). This supports the earlier finding of cytokinins increase the number of proliferated shoots but retarded shoot elongation (Sharma, 2014). The medium supplemented only with BAP obtained low shoot height due to the absence of NAA. It was reported that auxins stimulate shoot elongation (Arghya et al., 2013).

Cell division, senescence, metabolic synthesis, and many other aspects of growth and development are functions of PGRs. Some studies have shown that high BAP concentrations can reduce the size of shoots during herbaceous plant micro propagation (Tan, 2016; Huda A et al.,2020) Similar studies on plants belong to family Zingiberaceae have shown that BAP concentrations less than 2.0 mg/l are better for the growth of plantlets (Tan, 2016; Huda A et al., 2020). However, the results of this study were similar to the results obtained by Swarnathilaka and Nilantha (2012) and Sharma (2014) where highest multiplication rates were recorded by turmeric rhizome explants in media incorporated with 4.0 mg/l BAP and 0.25 mg/l NAA.

It was reported that liquid media facilitate higher shoot multiplication in many herbaceous genera compared to solid media (Kuria et al., 2008; Vyas et al., 2015). The better aeration of cultures in the liquid medium might have been beneficial for shoot formation. It is also reported that the liquid media facilitate nutrient uptake by closer contact of the shoot base with the nutrient medium (Mehrotra et al., 2007). Hence, during the second experiment, attempts were taken to test both solid and liquid media with different PGR combinations. However, due to the short time duration allocated for the study and the availability of a limited no of explants, the solid media were prepared only for the P8, P9, P12 and P13 PGR combinations. As per the results, liquid MS media with all the tested PGR combinations showed higher proliferation rates and a higher shoot length than solid MS media with the same PGR combinations (Table 5). Rhizome buds in liquid MS media resulted considerable number of multiple shoots either with 0.25 mg/L or 0.5 mg/L NAA, in presence of 2.5 mg/l or 4.0 mg/l BAP. The explants in solid MS media required 0.5 mg/L NAA to produce multiple shoots satisfactorily with the same BAP concentrations. In the absence of NAA and with lower concentrations of BAP (1.0 and 2.0 mg/l) low multiplication rates were observed.

Continuous data collection within 4 weeks intervals at each subculture is required to analyse the growth pattern and to identify the number of subcultures that could be performed without minimizing the proliferation rate and vigour of the shoots. Further studies are required to optimize the liquid medium protocol towards increasing the shoot proliferation rate further and promoting in vitro rooting. It is beneficial to produce media protocol for micro rhizome production too.

Table 5

Mean no of shoots/explant and mean shoot lengths in liquid and solid MS media (four weeks after culture initiation)

PGRs	Medium	No of shoots/ Explant ± SE	Mean shoot length (cm) ± SE
P8	Solid	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	Liquid	1.60 ± 0.20^{a}	3.52 ± 0.10^{b}
P9	Solid	0.40 ± 0.20^{a}	2.38 ± 0.10^{a}
	Liquid	2.40 ± 0.20^{b}	2.60 ± 0.10^{a}
P12	Solid	1.20 ± 0.20^{a}	2.60 ± 0.10^{b}
	Liquid	1.40 ± 0.20^{a}	3.45 ± 0.00^{a}
P13	Solid	1.20 ± 0.20^{a}	2.74 ± 0.10^{b}
	Liquid	1.80 ± 0.20^{a}	3.84 ± 0.10^{a}

The superscript letters above the values represents the statistically significant difference between the sterilization procedures by Tukey's post hoc test at 5% significant level

4. Conclusion

In this study, the most effective method for achieving cultures free from contaminations and browning of C longa L involved treating rhizome bud explants with 1% Topsin for 30 minutes followed by immersion in 100% Clorox for 10 minutes. The optimal shoot proliferation rate was observed when the growth medium was supplemented with 4.0 mg/l BAP and 0.25 mg/l NAA. Furthermore, the longest shoots were obtained using a medium containing 1.0 mg/l BAP and 0.5 mg/l NAA. Liquid media outperformed solid media in promoting higher shoot numbers.

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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 - A Research Journal, 6(49)

Arghya, G., Padma, C. and Parthageb, G., 2013. A protocol for rapid propagation of genetically true to type Indian turmeric (Curcuma longa L.) through in vitro culture technique. Advances in Applied Science Research, 4(3), pp. 39–45.

Behar, N., Tiwari, K. and Jadhav, S., 2014. Effect of explant type in development of in vitro micropropagation protocol of an endangered medicinal plant: Curcuma caesia Roxb. Biotechnology, 13, pp. 22–27.

Ferrari, M.P.S., Queiroz, M.S., Andrade, M.M., Trettle, J.R. and Magalhães, H.M., 2019. Growth regulators affect the growth and biochemical activity of Curcuma longa plants grown in vitro. Journal of Agricultural Science, 11(10), p. 277.

Ferrari, M.P.S., Antoniazzi, D., Nascimento, A.B., Franz, L.F., Bezerra, C.S. and Magalhães, H.M., 2016. Evaluation of new protocols to Curcuma longa micropropagation: A medicinal and ornamental species. Journal of Medicinal Plants Research, 10(25), pp. 367–376.

Ghosh, A., Chatterjee, P. and Ghosh, P., 2013. A protocol for rapid propagation of genetically true to type Indian turmeric (Curcuma longa L.) through in vitro culture technique. Pelagia Research Library Advances in Applied Science Research, 4(3), pp. 39–45.

Huda, A.A., Al-Mayah, A.A. and Abd Al-Behadili, W.A., 2020. Efficient in vitro regeneration of Zingiber officinale Rosc. var. white through shoot tips culture. Plant Archives, 20, pp. 434–437.

Indrani, S., Deka, A.C., Sarma, S. and Sarma, T., 2011. High-frequency clonal propagation and microrhizome induction of Curcuma longa L. (cv lakadong) - a rich source of curcumin of Northeast India. An International Quarterly Journal of Life Science, 6(1), pp. 11–18.

Kuria, P., Demo, P., Nyende, A.B. and Kahangi, E.M., 2008. Cassava starch as an alternative cheap gelling agent for the in vitro micro-propagation of potato (Solanum tuberosum L.). African Journal of Biotechnology, 7(3), pp. 301–307.

Mehaboob, M., Faizal, K., Raja, P., Thiagu, G. and Aslam, A., 2019. Effect of nitrogen sources and 2,4-D treatment on indirect regeneration of ginger (Zingiber officinale Rosc.) using leaf base explants. Journal of Plant Biotechnology, 46(1), pp. 17–21.

Mehrotra, S., Goel, M.K., Kukreja, A.K. and Mishra, B.N., 2007. Efficiency of liquid culture systems over conventional micropropagation: A progress towards commercialization. African Journal of Biotechnology, 6(13), pp. 1484–1492.

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

Ramadan, G., Al-Kahtani, M.A. and El-Sayed, W., 2011. Anti-inflammatory and antioxidant properties of Curcuma longa (turmeric) versus Zingiber officinale (Ginger) rhizomes in rat adjuvant-induced arthritis. Inflammation, 34(4), pp. 291–301.

Rao, N.M.S., 2014. An in vitro evaluation of the antimicrobial activity of Curcuma longa against selected pathogenic microorganisms. Research Journal of Science and Technology, 6(2), pp. 71–74.

Sandhyarani, N.D., Imotomba, R. and Thokchom, R., 2018. Surface sterilization protocol for Curcuma angustifolia Roxb. micropropagation. Journal of Experimental Biology and Agricultural Sciences, 6(5), pp. 890–894.

Sharma, N.K., 2014. Effect of plant growth regulators on micropropagation of Curcuma longa L. Thai Journal of Botany, 2, pp. 135–142.

Shaw, P., 2015. Spices commonly consumed in West Bengal, India - an appraisal. International Journal of Life Sciences, 4(2), pp. 129–133.

Sinchana, N.S., Kattimani, K.N., Prabhuling, G. and Jagadeesha, N., 2020. Standardization of tissue culture protocol for turmeric (Curcuma longa L.) Cv, Salem. International Journal of Chemical Studies, 8(1), pp. 2721–2726.

Singh, W.R.C., Singh, H.B., Devi, S.S., Singh, W.N., Singh, N.M. and Devi, Y.P., 2015. Conservation of Curcuma caesia by in vitro techniques. Helix, 3(4), pp. 708–713.

Swarnathilaka, D.B.R. and Nilantha, K.A.R., 2012. Effect of plant growth regulators and liquid-solid nature of the media on in vitro propagation of Curcuma longa. Journal of Food and Agriculture, 5(1–2), p. 22.

Talati, A., 2017. Media preparation for plant tissue. ResearchGate. DOI: 10.13140/RG.2.2.16632.44802.

Tan, P.V., 2016. Micropropagation of Curcuma spp. a threatened medicinal plant. Advances in Bioscience and Biotechnology, 7(10), pp. 418–427.

Vyas, S., Singh, M., Kumar, R., Suthar, S. and Purohit, D., 2015. Liquid culture system stimulates in vitro growth and shoot multiplication in four medicinally important plants. ResearchGate, 20.