BIOTROPIA Vol. 31 No. 1, 2024: 87 - 95 DOI: 10.11598/btb.2024.31.1.2120

6-BENZYLAMINOPURINE INDUCES HIGH-FREQUENCY MULTIPLICATION IN VULNERABLE HILL TURMERIC (*Curcuma pseudomontana* J. Graham): A POTENTIAL IN VITRO CONSERVATION TOOL

GAYATRI V. VAZE[1*], PRAMOD J. HURKADALE[1] AND HARSHA V. HEGDE[2]

[1]*KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi 590010, India*
[2]*National Institute of Traditional Medicine, Indian Council of Medical Research, Belagavi 590 010, India*

Received 4 November 2023/ Revised 8 December 2023/ Accepted 8 January 2024

ABSTRACT

A rapid high-frequency multiplication protocol is designed for *Curcuma pseudomontana* J. Graham, belonging to the family Zingiberaceae, an endemic species to the Western Ghats of India. This species is listed as vulnerable by the International Union for Conservation of Nature's Red List of Threatened Taxa, highlighting the various underlying causes contributing to its vulnerability. Notably, the plant holds significant value in traditional and tribal medicine. The species has suffered habitat loss due to uncontrolled use in tribal medicine, leading to a 30% decline over the last decade. This study is strategically designed with the specific objective of conserving the species, marking the first-ever report on the micropropagation of *C. pseudomontana* using *in vitro* multiplication. An efficient and rapid protocol for micropropagation is developed using rhizome bud explants, which are transferred from the MS basal medium onto the MS medium fortified with 6-benzylaminopurine (BAP), kinetin (Kn), and thidiazuron (TDZ) at a varying concentration range. The maximum shoot induction is achieved in the MS medium enriched with BAP at a concentration of 2 mg L^{-1}, resulting in 9.66 ±2.08 shoots per explant with a shoot length of 6.40 ±0.36 cm. The response to root induction is investigated by aseptically transferring the shoots onto MS medium enriched with α-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA) at various concentrations. Noticeably, the maximum root length and root number are recorded in MS supplemented with NAA at 0.5 mg L^{-1}. Despite the variations in root induction treatments, a 100% root induction frequency is observed in all the samples under study. The rooted plantlets are carefully removed from the culture flasks and transferred into hardening media containing a 1:1:1 ratio of sand:soil:cocopeat. These hardened plants exhibit good health, are disease-free, and show a 92% survival rate after acclimatisation.

Keywords: conservation, *curcuma pseudomontana*, micropropagation, vulnerable, western ghats

INTRODUCTION

Curcuma pseudomontana J. Graham, popularly referred to as Hill turmeric, has been classified as a vulnerable species according to the International Union for Conservation of Nature's Red List of Threatened Taxa (Romand-Monnier & Contu 2013). *C. pseudomontana* is endemic to peninsular India, specifically found in states along the Western Ghats. It is an erect rhizomatous herb that attains a height of up to 75 cm, thriving at altitudes of 300–1500 m. Typically, it inhabits the moist and shaded areas of wet forests along sluggish grassy slopes. The plant bears almond-like sub-globose tubers at the end of its fibrous roots. The rhizomes of *C. pseudomontana* are small, measuring 3–6 cm in length and 1.25 cm in width, and exhibiting a slightly cylindrical shape with a yellow tinge at the center and a white periphery with a pleasant aroma. The tubers, initially presenting as white, fleshy, and aromatic when freshly cut, undergo a color transformation to a muddy shade over time. They are either fusiform or globose, ranging from 2 to 10 cm in length and 1–2.5 cm in width (Ravikumar *et al.* 2000; Sabu 2006). Further, the leafy shoots can reach a height

*Corresponding author, email: vaze.vgayatri@gmail.com

of 80–125 cm, featuring a pseudo stem that extends up to 35 cm. The leaves exhibit a green color with a pink/purple tinge, while the lamina, measuring (40–50 cm length × 6–9 cm width, is oblong-lanceolate in shape, with a hyaline margin and an acuminate or acute tip (Santapau 1945). In addition, *C. pseudomontana* bears both lateral and central inflorescence. Flowering spikes of around 10–25 cm emerge from the center of the tuft of leaves, carrying numerous compactly arranged flowers. The flowering season for *C. pseudomontana* spans from May to September.

The taxon holds significance in commercial trade as a medicinal plant (Molur & Walker 1997). Moreover, various tribes extensively use it in ethnomedicine for treating conditions such as jaundice, leprosy, dysentery, and general debility and for purposes such as increasing lactation and blood purification (Jagtap *et al.* 2006; Bisht *et al.* 2014; Hiremath & Kaliwal 2014). Additionally, *C. pseudomontana* has several reported applications in traditional and folk medicine, including its role in the production of arrowroot powder (Ravindran *et al.* 2007; Sasikumar 2005). The Korku tribes residing in the Amravati district of Maharashtra, India, consume the fresh tubers of *C. pseudomontana* (locally referred to as *kukus-mukus*) as a means of blood purification. In the Munchingiputtu mandal of Vishakhapatnam, Andhra Pradesh, India, various tribes employ the tubers (locally called *adavi pasupu*) for curing jaundice, whereas the Bagata and Valmiki tribes use these tubers for addressing diabetes and apply a warm paste on swellings (Hiremath & Kaliwal 2014). Additionally, the Savara tribes in Andhra Pradesh, India, use the boiled tubers of *C. pseudomontana* (locally called *Adavi jogra*) as a galactagogue. To administer, the boiled tubers are ground along with a pinch of salt and given orally (Prasad *et al.* 2006). Meanwhile, in the Srikakulam district of Andhra Pradesh, India, the rhizomes find applications for addressing stomach troubles and cough (Theanphing *et al.* 2010).

C. pseudomontana has undergone testing for its anticancer and antiproliferative/cytotoxic activity (Bisht *et al.* 2014), wherein the extracts of *C. pseudomontana* using distilled water, methanol, and ethanol were studied on the BHK-21 cell line. Further, the IC_{50} value for the plant extract was determined to be in the range of 2–20 mg for the BHK-21 cell line (Bisht *et al.* 2014). The antitubercular activity of the rhizome of *C. pseudomontana* is examined using microplate alamar blue assay (MABA), yielding promising results for the crude extract of *C. pseudomontana* for tuberculosis treatment. The antitubercular activity is tested for extract obtained via hexane, chloroform, ethyl acetate, and methanol on the mycobacterial tuberculosis strain (H37RV) at 100 and 50 mg mL^{-1} (Hiremath & Kaliwal 2013).

Despite its extensive and valuable applications, this species faces imminent threats to its survival due to several underlying challenges, including the invasion of exotic species in its natural habitats, forest fires, and the unrestricted use of agrochemicals near forests (Bawa *et al.* 2007). The unregulated exploitation of commercially traded medicinal plants solely for economic gain, without due consideration for conservation, has driven many species dangerously close to extinction (Molur & Walker 1997; Bawa *et al.* 2007). To counteract this issue, micropropagation emerges as a biotechnology application with the potential to contribute to the conservation of species by serving as an *ex-situ* conservation method, thereby aiding in the prevention of extinction (Barbara *et al.* 2011).

However, there is a lack of documented plausible conservation initiatives operating for *C. pseudomontana*. This study aims to address this gap by designing a method for *in vitro* mass propagation of *C. pseudomontana* using micropropagation by modifying the Murashige and Skoog media, drawing inspiration from successful *in vitro* propagation studies conducted on other *Curcuma* species (Salvi *et al.* 2001, Kou *et al.* 2013; Salvi *et al.* 2000; Mohanty *et al.* 2008; Prakash *et al.* 2004; Yasuda *et al.* 1988; Koarapatchaikol *et al.* 2017; Zhang *et al.* 2011; Sundaram *et al.* 2012). Thus, this approach serves as a valuable tool for the preservation of this vulnerable species, enabling rapid multiplication and potential reintroduction into its natural habitat to enhance the population.

MATERIALS AND METHODS

Collection of plant

The entire mother plant of *C. pseudomontana* was collected from the Western Ghats forests of Kolhapur District, Maharashtra, India, during the monsoon season. The complete plant, at its flowering stage, was collected, verified for

authenticity, transformed into a voucher specimen with the accession number RMRC-1346, and subsequently deposited at the Herbarium of the National Institute of Traditional Medicine, Indian Council of Medical Research, Belagavi, Karnataka.

Surface sterilisation

The rhizomes *of C. pseudomontana*, devoid of aerial parts and roots but containing active buds, were thoroughly washed under running tap water for 30 minutes. Thereafter, they were washed with 5% (*v/v*) Tween 20 solution, with continuous shaking on a rotary shaker set at 20 rpm. Any remaining soil particles were carefully brushed away. A final rinse under running tap water for 15 minutes ensured the complete cleaning of the rhizomes. The explants were prepared by carving the active buds out from the rhizome in a pyramid shape while leaving a small portion of the rhizome attached (Fig. 1). These were then immersed in a 0.2% carbendazim solution *(w/v)* for 5–6 minutes, with intermittent shaking. The as-prepared explants were shifted to the sterile bench floor of the laminar airflow (LAF) hood, followed by cleaning using distilled water and drying with the help of tissue paper. These surface sterilized explants were then transferred onto a sterile LAF bench and treated with 0.1% *(w/v)* mercuric chloride ($HgCl_2$) solution for 2 minutes. Subsequently, the explants (rhizome buds) were finally washed with distilled water, trimmed to remove the tissues exposed to mercuric chloride, and further inoculated onto the MS basal media.

Culture media and conditions

The study utilized Murashige and Skoog (Murashige & Skoog 1962) media as the base. The sterilized rhizome buds were employed as primary explants and inoculated onto the MS basal medium without any plant growth regulator. The explants were closely monitored for two weeks and observed for any change in morphology as well as contamination from bacterial and fungal origin. After two weeks, the explants were transferred from the initiation medium to the multiplication medium.

***In vitro* shoot multiplication**

The MS basal media was enriched with different concentrations of cytokinin and Auxin to induce multiple shooting and rooting, respectively. The cytokinins used in this study included 6-benzylaminopurine (BAP), kinetin (Kn), and thidiazuron (TDZ). The explants were inoculated onto MS supplemented with cytokinins at a working concentration ranging from 0.01 to 10.0 mg L^{-1}. The cytokinin concentrations showing a positive response were further utilized in shoot multiplication studies. Actively growing *in vitro* buds were aseptically transferred onto MS fortified with 1–5 mg L^{-1} BAP (Hi-media, India), 1.2–1.5 mg L^{-1} Kn (Hi-media, India), and 0.1–0.5 mg L^{-1} TDZ (Hi-media, India) supplemented with 30 g L^{-1} sucrose (Hi-media, India) with 0.8% (*w/v*) agar (Hi-media, India). The culture vessels were kept in a growth room at 25±2 °C, maintaining a relative humidity of 95–100% under a 16-hour photoperiod with a light intensity of 1000 Lux. Growth parameters were measured in terms of shoot number and shoot length per explant.

***In vitro* root induction**

The auxins employed for *in vitro* root induction studies included indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), and 1-naphthaleneacetic acid (NAA). The *in vitro* shoots were inoculated onto MS supplemented with auxins at a working concentration of 0.01–10.0 mg L^{-1}. The auxin concentrations demonstrating good response were further used in root induction studies. The *in vitro* raised individual shoots were relocated to the root induction media containing 1.1–1.5 mg L^{-1} of IBA and IAA and 0.1–1.0 mg L^{-1} of NAA. The MS medium served as the basal medium, which was fortified with auxins. Growth parameters were quantified in terms of root number and root length per explant. Each trial involving plant growth regulators in both shoot induction and root induction was conducted thrice with ten replicates each.

Hardening and acclimatization

The well-rooted plantlets bearing 3–5 healthy leaves were gently excised from the medium in LAF. The roots were washed with distilled water to remove the traces of agar medium adhered to

the roots. Subsequently, the rooted plantlets were placed safely in a hardening mixture composed of sand, soil, and cocopeat at a ratio of 1:1:1 (*v*/*v*) within a plastic cup and covered with another plastic cup gently to maintain essential humidity, retain moisture, and ensure sufficient light. The humidity levels were adjusted by removing the covering glass as per requirement and maintaining it under the same culture conditions for two weeks. Following the hardening period, the plantlets were transferred to the greenhouse, and their survival rates were recorded after four weeks (Loc *et al.* 2005).

Statistical analysis

The data obtained in this study was analyzed using one-way ANOVA (analysis of variance), and the results were compared for statistical significance using Duncan's multiple range test, with a significance level set at $p<0.05$. The data values are presented as mean ± standard deviation (M±SD).

Microrhizome induction

The induction of *in vitro* microrhizome was investigated by varying the sucrose concentration in the MS basal media, ranging from 30 g L^{-1} to 120 g L^{-1} in triplicates. Well-rooted shoots were extracted from the medium (2.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA), washed with distilled water to remove the agar traces, and blot-dried. The roots were carefully cut off from the plantlets, leaving only the individual shoots for inoculation back to the microrhizome induction media. This involved the use of PGR-free MS media with varying sucrose concentrations (30–120 g L^{-1}). The culture vessels were placed in the dark at 25±2 °C, and the observed response was recorded after a period of four weeks from the time of inoculation (Nayak & Naik 2006).

RESULTS AND DISCUSSION

The *in vitro* shoot growth parameters of *C. pseudomontana* were assessed in terms of the number of shoots and shoot length per explant after four weeks of culture initiation. Continuous daily observations were conducted to monitor any changes in the growth patterns. Similarly, the response to root induction was recorded in terms of root number and length per explant. The subculturing was carried out every 15 days or two weeks, or earlier if needed, to replenish the medium.

Initiation of culture and multiplication

The medium was standardized to yield multiple shoots per explant. The maximum number of shoots, i.e., 9.66 ±2.08, was achieved in MS+BAP (2 mg L^{-1}) + 3% sucrose at pH = 5.8.

Table 1 Shoot induction response in *C. pseudomontana* for different concentrations of BAP, Kn, and TDZ

Plant Growth Regulators (mg L^{-1})			Shoot length (in cm)	No. of shoots per explants
BAP	Kn	TDZ		
1	-	-	4.53±4.80^{a}	4.0 ±1.0^{b}
2		-	6.40 ±0.36^{b}	9.66 ±2.08^{f}
3		-	4.80 ±2.17^{a}	5.66± 1.52^{a}
4		-	3.10 ±1.83^{d}	3.66 ±0.50^{c}
5		-	2.76 ±1.90^{de}	2.0 ±1.0^{d}
-	1.1	-	1.96± 1.45^{e}	1.33± 0.57^{e}
-	1.2	-	1.73± 1.44^{e}	1.33± 0.57^{e}
-	1.3	-	1.83 ±0.41^{e}	2.66±0.57^{cd}
-	1.4	-	1.96± 1.32^{de}	1.66 ±0.57^{de}
-	1.5	-	2.06 ±1.56^{d}	1.66 ±1.15^{de}
-	-	0.1	0.86 ±0.72^{f}	1.33 ±0.57^{e}
-	-	0.2	1.56 ±0.32^{e}	1.33 ±0.57^{e}
-	-	0.3	1.06 ±0.41^{e}	1.0 ±0.0^{e}
-	-	0.4	-	-
-	-	0.5	-	-

The presented data shows mean values and corresponding standard deviations. "Mean" that share the same letter within each column of growth regulator combinations are not significantly different at a 5% level of significance, as determined by Duncan's multiple range test.

In the comparison of all three cytokinins for shoot multiplication (Table 1), BAP exhibited the highest potential of shoot induction and multiplication in *C. pseudomontana* rhizome bud explants. The number of shoots per explant increased with the increasing concentration of BAP, followed by a gradual decrease with a further rise in concentration, specifically from 3 mg L^{-1} to 5 mg L^{-1}. Within the tested range of BAP, the best response was registered at a concentration of 2 mg L^{-1}. On the other hand, a relatively consistent growth pattern and a stable number of shoots were observed with no recorded mortality in the studied concentration range of Kn. This suggested that Kn may be suitable as a component of maintenance media but not be ideal for the rapid multiplication of this species, which holds conservation value. These observations aligned with the findings in other studies on *Curcuma* species micropropagation, wherein the inefficiency of Kn in inducing multiple shoots is reported (Shanthala *et al.* 2021). The least favorable response was observed among the explants inoculated in MS fortified with TDZ at different concentrations. Specifically, TDZ at a concentration of 0.1–0.3 mg L^{-1} showed stagnant growth in the explant, accompanied by hyperhydricity in every culture vessel. The persistent hyperhydricity across all treated concentrations suggested that TDZ induced this undesirable condition in the growing shoots of *C. pseudomontana*, rendering it unsuitable for the *in vitro* multiplication of this species. Contrarily, the remaining concentrations resulted in explants that perished, displaying signs of survival only at lower concentrations. These observations deviate from other micropropagation studies within the genus *Curcuma*, which portrayed the positive effects of TDZ on micropropagation and successful multiple shoot induction (Toppoonyanont *et al.* 2004).

The superior responsiveness and the highest multiplication of explants observed with BAP confirmed its efficacy as the most effective cytokinin for *in vitro* shoot multiplication in *C. pseudomontana*. Furthermore, cultures treated with BAP and Kn demonstrated consistent morphological features and identity. Overall, concerning the highest multiplication induction rate, BAP is recommended as the most suitable cytokinin for this purpose. The effect of cytokinins on shoot multiplication is presented in Table 1. The notable efficiency of BAP in inducing effective multiplication aligned with findings from other micropropagation studies carried out on *Curcuma* species like *C. haritha* (Bejoy *et al.* 2006), *C. mangga* (Raihana *et al.* 2011), *C. caesia* (Bharalee *et al.* 2005), *C. zedoaria* (Loc *et al.* 2005), *C. vamana* (Bejoy *et al.* 2012), etc.

Effects of auxins on *in vitro* rooting and elongation

The effects of IBA, IAA, and NAA on root induction response were studied, with results listed in Table 2. The concentrations of auxins were selected based on their standard working concentration. The most favorable root induction response was recorded with the NAA treatment at 0.5 mg L^{-1} in MS medium containing 30 g L^{-1} sucrose at pH = 5.8. A 100% rooting success was observed in all the treated groups of auxins. Root number and length exhibited variations among the different treatments. The highest number of roots observed in NAA at 0.5 mg/L was 7.00±1.00 per explant, with the longest root length of 3.36±1.00 cm. A similar response to root induction was noted in other studies conducted on the genus *Curcuma*, including *C. mangga* (Raihana *et al.* 2011), *C. caesia* (Bharalee et al. 2005), *C. aromatica* (Mohanty et al. 2008), *C. longa* (Ugochukwu *et al.* 2013), and *C. zedoaria* (Bharalee *et al.* 2005). These results showed that NAA was suitable for root induction in C. *pseudomontana*. However, it is worth noting that both IBA and IAA also demonstrated satisfactory rooting responses.

Table 2 *In vitro* root induction response to different concentrations of IBA, IAA, and NAA

Plant Growth Regulators (mg L^{-1})			Root length (in cm)	No. of roots per explants
IBA	IAA	NAA		
1.1	-	-	0.16 ±0.19[a]	1.33 ±0.57[a]
1.2	-	-	0.16 ±0.05[a]	1.66±1.15[a]
1.3	-	-	0.26±0.05[a]	1.33±0.57[a]
1.4	-	-	0.23±0.05[a]	1.00±0.00[a]
1.5	-	-	0.30±0.26[ab]	1.00±0.00[a]
-	1.1	-	0.10±0.00[a]	1.00±0.00[a]
-	1.2	-	0.13±0.05[a]	1.33±0.57[a]
-	1.3	-	0.13±0.05[a]	2.00±1.00[b]
-	1.4	-	0.16±0.05[a]	1.66±0.57[a]
-	1.5	-	0.13±0.05[a]	2.00±1.00[b]
-	-	0.1	0.33±0.15[a]	4.00±1.00[c]
-	-	0.2	0.40±0.10[ab]	4.33±1.15[cd]
-	-	0.3	1.03±0.15[c]	4.65±1.15[cd]
-	-	0.4	1.86±0.23[c]	5.33±0.57[cd]
-	-	0.5	3.36±1.00[d]	7.00±1.00[e]
-	-	0.6	1.33±0.28[c]	4.00±1.00[c]
-	-	0.7	0.90±0.10[ab]	2.00±1.00[b]
-	-	0.8	0.53±0.90[ab]	1.00±0.00[a]
-	-	0.9	0.26±0.11[a]	0.66±0.57[c]
-	-	1.0	0.16±0.11[a]	0.66±0.57[c]

The presented data shows mean values and corresponding standard deviations. "Mean" that share the same letter within each column of growth regulator combinations are not significantly different at a 5% level of significance according to Duncan's multiple range test.

Hardening and acclimatization

Following a successful two-week hardening period with a 100% survival rate, the plantlets were carefully uprooted and replanted into pots with soil. Few plantlets were intentionally uprooted at this stage to assess root development. Notably, the formation of a small tuber was observed, measuring approximately 2.5 cm in length and 0.5 cm in thickness at the base of the roots, resembling the plants growing in their wild habitat. The *in vitro* regenerated plants exhibited a phenotype similar to the wild plants. Therefore, this high-frequency multiplication protocol was reproducible and highly reliable, yielding healthy plantlets. Consequently, it can serve as an effective tool for the *ex vivo* conservation of the vulnerable *C. pseudomontana* species. Importantly, a survival rate of 92% was recorded in the field (Fig. 1f).

Microrhizome formation

After four weeks, the media containing 30 g L^{-1} sucrose and the MS basal control displayed normal plant development, featuring 3–4 leaves and healthy root system. Similar positive results were obtained for media with 60 g L^{-1} of sucrose. In contrast, media with a triple dose of sucrose (90 g L^{-1}) exhibited lesser root growth but was accompanied by formation of microrhizomes (Fig.2). However, media with 120 g L^{-1} showed impaired root growth. Hence, it was concluded that the best response for microrhizome formation in *C. pseudomontana* was achieved with MS basal medium supplemented with 90 g L^{-1} sucrose without any plant growth regulators. Similar outcomes in microrhizome formation have been reported on other important *Curcuma* species, such as *C. longa* (Shirgurkar *et al.* 2001; Nayak *et al.* 2006) and *C. aromatica* (Nayak *et al.* 2000).

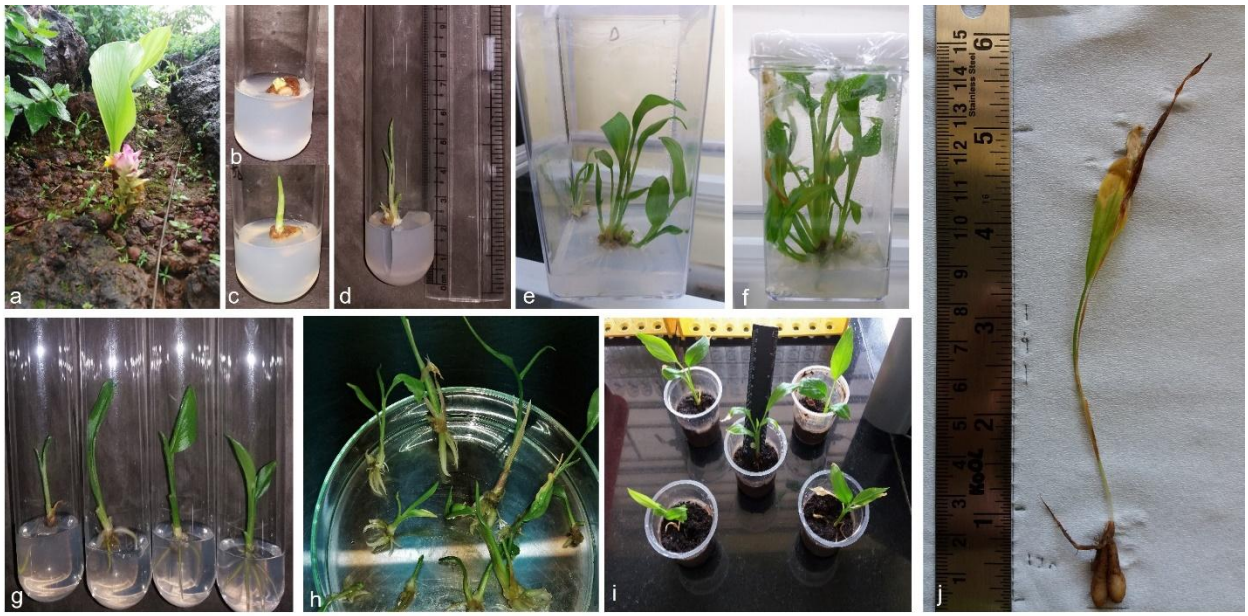

Figure 1 *In vitro* propagation of *C. pseudomontana* J. Graham using rhizome bud explants:(a) natural habitat of *C. pseudomontana*; (b) shoot initiation with rhizome bud; (c) initial shoot growth; (d) induction of multiple shoots; (e, f) multiple shoot proliferation; (g) induction of roots from *in vitro* raised shoots; (h) regenerated plants with a well-developed root system; (i) acclimatisation of micro-propagated plantlets into the hardening mixture, and (j) acclimatised plantlet after 60 days showing tuber formation at the base similar to that of the plants in wild habitat.

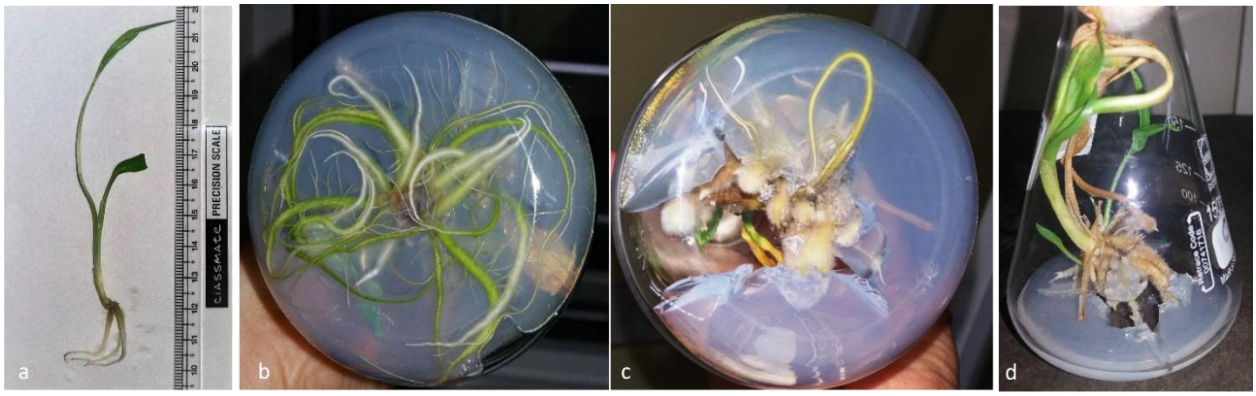

Figure 2 *In vitro* microrhizome induction: (a) well-rooted plantlet of *C. pseudomontana* developed *in vitro*; and root growth with MS supplemented with (b) 30 g L^{-1} and (c, d) 90 g L^{-1} sucrose.

CONCLUSION

To sum up, this study marks the first report on an efficient high-frequency multiplication protocol developed for the *in vitro* multiplication of *C. pseudomontana*, representing a significant step towards the conservation of this vulnerable species. The method yields healthy, disease-free, and uniform plantlets, demonstrating a consistently high multiplication rate with 100% rooting success and a commendable 92% survival rate after acclimatization. Hence, the as-devised protocol can be effectively employed for the mass multiplication of the species.

ACKNOWLEDGMENTS

The authors express their gratitude for the funding support received from the KLE Academy of Higher Education and Research, Belagavi, Karnataka, and the National Institute of Traditional Medicine, ICMR, Belagavi, Karnataka, for providing the necessary laboratory facilities.

REFERENCES

Barbara MR, Viswambharan S, Michaek K, Eric B & Valerie CP. 2011. Biodiversity conservation and conservation biotechnology tools. *In vitro* Cellular & Developmental Biology -Plant.47: 1-4.

Bawa KS, Das A, Krishnaswamy J, Karanth KU, Kumar NS and Rao M. 2007. Ecosystem Profile: Western Ghats and Sri Lanka Biodiversity Hotspot - Western Ghats Region. Critical Ecosystems Partnership Fund, Virginia.

Bejoy M, Dan M, Anish NP. 2006. Factors affecting the *in vitro* multiplication of the endemic zingiber *Curcuma haritha* Mangaly and Sabu. Asian J. Plant Sci.5(5): 847-53.

Bejoy M, Dan M, Anish NP, Nair AR, Radhika BJ, Manesh K.2012. Micropropagation of an Indian Ginger (*Curcuma vamana* Sabu and Mangaly): a wild relative of turmeric. Biotechnology. 11(6): 333-8.

Bharalee R, Das A, Kalita MC. *In vitro* clonal propagation of Curcuma caesia Roxb and *Curcuma zedoaria* Rosc from rhizome bud explants.2005. Journal of Plant Biochemistry and Biotechnology. 14: 61-3.

Bisht V.S., Kandwal S, Kanan D, Som D. 2014. Anticancerous and antiproliferative/cytotoxic activity of *Curcuma pseudomontana* (hill turmeric) collected from the sub-Himalayan region of Uttrakhand, India. Asian Journal of Plant Science and Research.4(6): 25-31.

Hiremath GB, and Kaliwal BB. 2013. Antitubercular activity of the rhizome of *Curcuma pseudomontana* J. Graham. International Journal of Pharmaceuticals and Health care Research, Int. J. Pharm. & H. Care Res.01 (04): 178-183.

Hiremath GB**,** Kaliwal BB. 2014. Pharmacognostic evaluation of rhizome of *Curcuma pseudomontana* j. Graham. International Journal of Pharma and Bio Sciences, Int J pharm bio sci. 5(2): 242-250.

Jagtap SD, Deokule SS, Bhosle SV. 2006. Some unique ethnomedicinal uses of plants used by the Korku tribe of Amravati district of Maharashtra, India. Journal of Ethnopharmacology. 107: 463-469.

Koarapatchaikol K, Kanmarangkol S, Kaewraksa J. 2017. *In vitro* Plant Regeneration via Callus Culture in Turmeric (*Curcuma longa* L.) Burapha Science Journal.22(1).

Kou Y, Ma G, Silva JA. 2013. Callus induction and shoot organogenesis from anther cultures of *Curcuma attenuata* Wall. Plant Cell and Organ Culture.112: 1-7.

Loc NH, Duc DT, Kwon TH, & Yang MS, 2005. Micropropagation of zedoary (*Curcuma zedoaria* Roscoe)–a valuable medicinal plant. Plant cell, tissue and organ culture.81: 119-122.

Mohanty S, Panda MK, Subudhi E, Nayak S. 2008. Plant regeneration from callus culture of *Curcuma aromatica* and *in vitro* detection of somaclonal variation through cytophotometric analysis. Biologia Plantarum.52(4): 783-786.

Molur S, Walker S. 1997. Conservation Assessment and Management Plan Workshop.

Murashige T, & Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3): 473-497.

Nayak S. 2000. *In vitro* multiplication and microrhizome induction in Curcuma aromatica Salisb. Plant Growth Regulation. 32(1): 41-7.

Nayak S, Naik PK. 2006. Factors effecting *in vitro* microrhizome formation and growth in *Curcuma longa* L. and improved field performance of micropropagated plants. Science Asia.32(1): 31-7.

Prasad M.N. V, Padmalatha, Jayaram K., Raju N.L. 2006. Healing plants of Andhra Pradesh-indigenous knowledge, trade, threats, and conservation strategies. Proc.A.P.Academi of Sciences. 10(2): 109-120.

Prakash S., Elangomathavan R., Seshadri S., Kathairavan K., Ignacimuthu. 2004. Efficient Regeneration of *Curcuma amada* Roxb. Plantlets from Rhizome and Leaf Sheath Explants. Plant Cell, Tissue and Organ Culture.78(2): 159-165.

Raihana R, Faridah QZ, Julia AA, Abdelmageed AH, Kadir MA.2011. *In vitro* culture of *Curcuma mangga* from rhizome bud. Journal of Medicinal Plant Research. 5(28): 6418-22.

Ravikumar K, Ved DK, assisted by Vijaya Sankar. 2000. 100 Red Listed Medicinal Plants of conservation concern in Southern India-Illustrated field guide.

Ravindran PN, Babu KN, Sivaraman K, editors. 2007. Turmeric: the genus *Curcuma*.CRC press.

Romand-Monnier, F. & Contu, S. 2013. *Curcuma pseudomontana. The IUCN Red List of Threatened Species* 2013: e.T22486190A44506743. DOI: 10.2305/IUCN.UK.2013-1.RLTS. T22486190A44506743.en.

Sabu M. 2006 Zingiberaceae and Costaceae of South India, Indian Association of Angiosperm taxonomy.

Salvi ND, George L, Eapen S. 2000. Direct regeneration of shoots from immature inflorescence cultures of turmeric. Plant Cell, Tissue and Organ Culture.62: 235-238.

Salvi ND, George L, Eapen S. 2001. Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. 2001.Plant Cell,Tissue and Organ Culture.66: 13-119.

Santapau H. 1945. *Curcuma pseudomontana* Grah. Journal of the Bombay Natural History Society 45: 618-623.

Sasikumar B. 2005. Genetic resources of *Curcuma*: diversity, characterization and utilization. Plant Genetic Resources. 3(2): 230-51.

Shanthala AA, Dilkalal A, Umesh TG. 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(3): 229-41.

Shirgurkar MV, John CK, Nadgauda RS. 2001. Factors affecting *in vitro* microrhizome production in turmeric. Plant Cell, Tissue and Organ Culture. 64: 5-11.

Sundram., Suffian M. Annuar M., Khalid N. 2012. Optimization of culture condition for callus induction from shoot buds for establishment of rapid growing cell suspension cultures of Mango ginger (*Curcuma mangga*) Tamil C.M. AJCS. 6(7): 1139-1146.

Theanphing O, Songsak T, Kerdmanee C. 2010. Effect of plant growth regulators on micropropagation of *Curcuma aeruginosa* Roxb.Thai journal of botany. 2: 135-142.

Toppoonyanont N, Chongsang S, Chujan S, Somsueb S, Nuamjaroen P. 2004. Micropropagation Scheme of *Curcuma alismatifolia* Gagnep. InIX International Symposium on Flower Bulbs. 673(98): 705-712.

Ugochukwu S., Bob E. 2013. Shoot Proliferation of *In vitro* Turmeric (*Curcuma longa* L.) Affected by Different Concentrations of Benzylaminopurine. World Journal of Agricultural. 9(3): 227-230.

Yasuda K, Tsuda T, Shimizu H, Sugaya A. 1988. Multiplication of *Curcuma* species by tissue culture. Planta Med. 54(1): 75-79.

Zhang S, Liu N, Sheng A, Ma G, Wu G. 2011. *In vitro* plant regeneration from organogenic callus of *Curcuma kwangsiensis* Lindl. (Zingiberaceae).Plant Growth Regulation. 64(2): 141-145.