



## Assessment of genetic fidelity *in vitro* regenerated plantlets of curcuma amada Roxb

Swapna Nareddy, S K Reddy\*

Department of Botany, Cytogenetics, Tissue Culture and Molecular Biology Lab, Osmania, University, Hyderabad, Telangana, India

### Abstract

A highly efficient and reproducible *in vitro* plant regeneration method has been developed from rhizome explants of *Curcuma amada* Roxb. The shoot bud explant developed from rhizome produced the highest number of shoots (16 shoots/ explant) on medium amended with 1 mg/L BAP, 1 mg/L TDZ and 0.2 mg/L NAA. Approximately 80% of shoots were induced spontaneous rooting on shoot induction media fortified with 0.2 mg/L NAA. The plantlets were acclimatized under greenhouse conditions, and the survival rate was recorded as 96%. The plantlets obtained from all explants were morphologically similar to mother plants. Random Amplified Polymorphic DNA (RAPD) molecular markers were employed to determine the genetic stability of plants obtained from the tissue culture of the *Curcuma amada* Roxb. The DNA markers validated the genetic uniformity of the plants obtained from all *in vitro* regenerants was similar to their mother plant. This plant regeneration procedure could be a helpful method for the genetic improvement of *Curcuma amada* Roxb.

**Keywords:** *Curcuma amada*, Genetic fidelity, medicinal plant, shoot bud, shoot regeneration, rhizome

### Introduction

India is home for the Zingiberaceae family and order Scitaminae, which includes the genus *Curcuma*. Turmeric is the common name for the plant *Curcuma*, which is grown for its underground rhizome and used in the textile, food, confectionary, cosmetics, and pharmaceutical sectors as a spice, condiment, and color. It is a crucial adjuvant in Indian cooking because it gives a variety of foods color and flavor. Additionally, "Amraharidra" herbal medication, which has a cooling and fragrant effect and aids in digestion, is used to treat illnesses. India has a long history of using turmeric to alleviate inflammation and sprains (Sasikumar 2005 [23]; Brijesh and Ajjappala 2022) [7].

Along with 60 other species of *Curcuma*, *Curcuma amada* is a member of the monocotyledonous Zingiberaceae family (Tyagi *et al.* 2007) [29]. Mango ginger, also known as *Curcuma amada*, has been grown in India since ancient times. The essential oil of mango ginger rhizome, which is beneficial as a spice or herbal medicine, has 68 volatile scents. According to Srivastava *et al.* (2001) [28] and Mustafa *et al.* (2005) [15], the presence of car-3-ene and cis-ocimene is mostly responsible for the odor. It has a number of therapeutic benefits, including laxative, antipyretic, aphrodisiac, stomachic, and carminative effects. It is also used to treat skin conditions, bronchitis, biliousness, asthma, hiccoughs, and inflammation brought on by wounds (Kirtikar and Basu 1984 [12]; Warriar *et al.* 1994) [30]. *Curcuma amada* may also have antibacterial, antioxidant, platelet aggregation inhibitory, and anticancer properties (Policegoudra *et al.* (2010) [16]. It has advantageous anti-hypercholesterolemic activity (Srinivasan *et al.* 2008) [27] and contains lebdane diterpenoid, an antitubercular drug (Singh *et al.* 2010) [25].

*Curcuma amada* is generally propagated vegetatively using portions of underground rhizomes. The major concern with *Curcuma amada* include its slow rate of proliferation, soilborne illness, rhizome degeneration brought on by bacteria and fungi, and insect attacks (Balachandran 1990

[2]; Prakash *et al.* 2004) [17]. Due of these issues, the approaches combining tissue culture and genetic engineering methods are required to support breeding programs for *Curcuma amada*.

Several plant regeneration studies were successfully attempted in *Curcuma* through direct and indirect organogenesis using leaf sheath (Raju *et al.* 2013) [20], leaf base (Raju *et al.* 2013) [20], rhizome buds (Jena *et al.* 2020) [10], leaf petiole (Jie *et al.* 2019) [11], and through callus induction using apical and lateral buds (Gurav *et al.* 2020) [9] explants. These results indicate that irrespective of the species studied, various explants cultured in MS medium supplemented with BAP and with or without other phytohormones or vitamins favored callus induction and multiple shoot production.

In any micro propagated plants, it was quite essential to assess genetic diversity and purity, *in vitro* propagated plants' genetic homogeneity could be validated at several levels, including genetic, morphological, physiological, and biochemical analysis. Because they are unaffected by environmental factors, DNA-based markers such as Random Amplified Polymorphic DNA (RAPD), semi-arbitrary (Inter-Simple Sequence Repeat, ISSR; Amplified Fragment Length Polymorphism, and AFLP), and sequence-based (Simple Sequence Repeat, SSR) techniques are used (Singh *et al.* 2013) [24] are promising tools for validating the clonal fidelity of micro propagated plants.

In the given study, we successfully established *in vitro* regeneration and assessed genetic fidelity of *in vitro* regenerated plantlets of *Curcuma amada* using RAPD markers.

### Materials and Methods

#### Explant and its sterilization

*Curcuma amada* rhizome was procured from local farmers and to identify embedded buds without causing damage, rhizome buds used as explants were meticulously cleaned, thoroughly rinsed with water, and dried. Those explants

were maintained submerged in flowing water while they were immersed in 5% bavistin overnight, cleaned for 10 to 15 minutes, and then had the bud sections removed and dipped in 1% bavistin for 30 minutes. The explants were placed in liquid detergent (tween 20) for 30 minutes after being washed with water. To get rid of every last trace of detergent, they were thoroughly rinsed with distilled water. 0.1% mercuric chloride solution was applied to the surface to sterilise it under aseptic circumstances for 8–10 minutes, followed by 15 minutes of 30% sodium hypochlorite. After sterilisation, the explants underwent numerous rounds of sterile distilled water washing in an aseptic environment before being inoculated.

### Plant multiplication and Culture conditions

Explants were inoculated into Murashige and Skoog's (MS) basal medium with different concentrations of kinetin and a consistent amount of NAA (0.2 mg/L). Cultures were kept alive in a culture chamber that had an ideal temperature of 25°C, a 16-hour photoperiod, and an 8-hour dark period that was kept alive by cool white fluorescent light with a light intensity of 2000–3000 lux.

Hormone-containing MS medium were autoclaved for 20 minutes at 121°C and 1.05 kg/cm<sup>2</sup> of pressure. The media's pH was adjusted to 5.7.

### Culture medium for multiple shoot induction

The base of the rhizome bud was cut off, and the sprouts were then sub cultured on MS media with different concentrations of BAP (1.0–5.0 mg/L), Kn (0.6 mg/L), and constant NAA (0.2 mg/L). The effect of TDZ is also examined for producing several shoots. As a result, various concentrations of TDZ (1–8 mg/L) were added to the MS medium, which was already saturated with 1 mg/L BAP and 0.2 mg/L NAA.

### Field transfer of plantlets

After ninety days of culture, *in vitro* grown plantlets with well-developed roots and shoots were transplanted into pots filled with a 1:1:1 mixture of soil, cow dung, and peat. These were transferred into greenhouse for acclimatization. The pots were moved to the typical field environment and allowed to mature for 30 days.

### Statistical analysis

Triplicates were maintained throughout the experiments, and the treatment was repeated thrice. Data regarding the number and length of shoots and roots per culture and their means were recorded after 8 weeks. Statistical analysis of data was done according to Duncan's Multiple Range Test (DMRT) for mean comparison ( $p < 0.05$ ).

### Molecular analysis

Young leaves were crushed in liquid nitrogen, and DNA was isolated using the CTAB method with a few minor adjustments. With the use of a Nanodrop spectrophotometer, DNA yield was calculated at A260/A280 ratio. DNA concentration was set to 25 ng/μl and preserved at –20 °C for molecular studies.

For DNA amplification, a set of ten decamer RAPD primers (AM1–10) were applied. With the use of a 25μL PCR mixture (GCC Biotech) made up of 2.5μL of buffer, 1μL of dNTPs (dCTP, dATP, dGTP, and dTTP), 0.2μL of Taq polymerase, 1μL of DNA (about 25 ng/L), 1μL of

primer (5 PM), and the remaining MilliQ water, amplification was carried out. The Thermal Cycler was used to perform the amplifications. PCR products were fed into a 0 electrophoresis equipment (GeNei™), operated at 50V for 2 hours, and then separated on a 1.2% agarose gel (Himedia, Mumbai, India) in 1.0% TE buffer (40mM Tris acetate, pH 8.2;

1mM EDTA). The molecular standard was a 100 bp DNA ladder. A gel documentation (BIORAD) was used to record gels. On the basis of presence and absence, only distinct and repeatable bands on gels were assessed. With each RAPD primer, there were two separate amplification processes.

## Results

### Establishment of Aseptic Cultures

In the current work, a new methodology for sterilizing *Curcuma amada* rhizomes is presented. *In vitro* cultivation of turmeric explants with mercuric chloride and sodium hypochlorite after pretreatment with bavistin, Tween 20, and sodium hypochlorite prevented bacterial and fungal infection. Rhizome germination was successful in 85% of cases, whereas the remaining 15% were contaminated (data not shown).

To encourage the development of explants in the appropriate direction, consideration must be given to the kind and dosage of the growth regulator added to the medium (Zulkarnain *et al.* 2019)<sup>[22]</sup>. Rhizome bud sprouting on enriched MS medium supplemented with KN and NAA has caused the rhizome bud to emerge from dormancy at KN(0.6 mg/L)+NAA(0.2 mg/L) (Fig. 1). Within 45 days of culture, *Curcuma amada* had the highest number of shoots 4.667±0.333 per rhizome bud with shoot lengths of 13.333±0.333cm. According to Jafrai *et al.* (2016), auxin has a stimulating influence on cell expansion, cell elongation, and the production of roots. The excellent root is stimulated by NAA's comparatively high auxin activity, high mobility, and slow rate of decay (Adriance and Brison, 1955)<sup>[1]</sup>. The length of the shoot appears to vary with Kn concentration. According to Brock and Kaufman (1991)<sup>[8]</sup>, cytokinin regulates crest induction and stimulates the growth of the lateral bud by promoting ribosome production in the cell cycle. Our findings also showed that NAA and BA work best combined to produce shoots in *Curcuma amada*.

Regenerated shoots were employed as explants and cultivated on various doses of BAP (1–5 mg/L) + Kn (0.6 mg/L) + NAA (0.2 mg/L) in order to enhance the number of multiple shoots (Fig. 1). Effective outcomes showed that 1mg/L of BAP has produced a maximum of 4.667±0.333 shoots per explant. In order to produce the greatest number of shoots 16.667±0.667 shoots with a length of 13.3±0.8820 cm. TDZ was shown to be most effective when combined with BAP and NAA at 1.0 mg/L and 0.2 mg/L, respectively. Plantlets in *Curcuma amada* were acclimated and maintained an 85% survival rate (Fig. 1).

### Molecular analysis of micro propagated plants using different molecular markers

Understanding the genetic heterogeneity in turmeric cultivars grown *in vitro* and *ex vitro* might benefit greatly from the use of molecular markers. The PCR settings for each RAPD and the primers used in the molecular analysis

of *Curcuma amada* were standardized. DNA markers such as RAPD have been widely employed to evaluate the genetic fidelity among several plants. A total of 10 random primers were used to analyze the tissue culture plants in comparison with the donor plant. Ten RAPD primers produced 35 bands i.e.; 3.5 bands per primer on an average. The band number varied from 1 to 6 and the band size was ranging from 50-2000 bp (Fig. 2 and 3). The primers AM2 and AM5 produced maximum number of bands (5), whereas the minimum number of bands (1) was produced by the primer Am6 for the plants analyzed. A total of 210 bands have resulted in all randomly selected micro propagated plantlets. All random primers produced identical bands in micro propagated and donor plants (Fig. 2 and 3).

**Table 1:** Details of RAPD primers employed for genetic homogeneity assessment in *Curcuma amada*.

S. No.	Primer code	Primer sequence (5'-3')
1	C12	GAGGCGTCGC
2	A6	TGGCCACCTG
3	AM7	CTTCGGCAGCATCTCTTCAT
4	AM8	CAGTGTGGAAGCCGATTATG
5	AM9	ATGTGTTGTCTGGCTTGGA
6	AM1	CTCTCTCTCTCTCTCTG
7	AM2	GGATGGAATAGTCTC
8.	AM3	GCATGGCAAGCTGCA
9.	AM4	GAGAGAGAGAGAGAGAC
10	AM5	ACACACACACACACACC

**Table 2:** Effect of KN and BAP and in combination with NAA on multiple shoot induction from shoot bud explants in *Curcuma amada*.

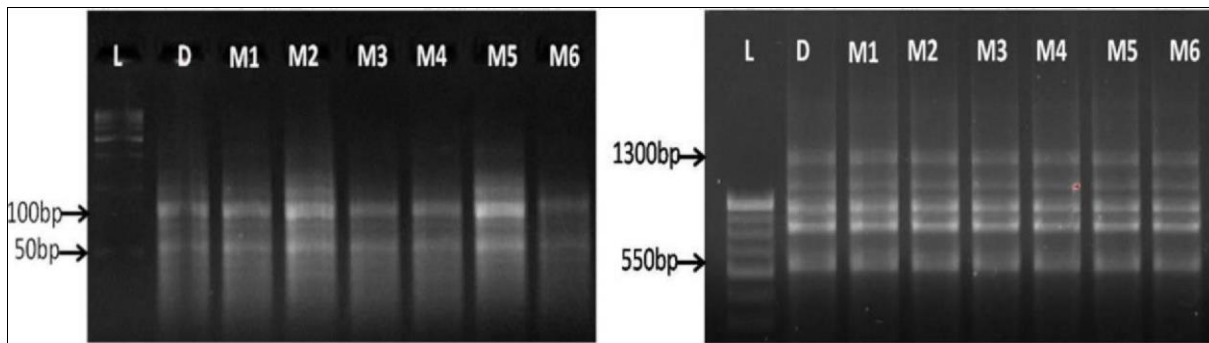
Hormones (mg/L)	Morphogenic Response	No. of shoots Mean $\pm$ SE	No. of roots Mean $\pm$ SE	Length of shoots Mean $\pm$ SE	Length of roots Mean $\pm$ SE
<b>KN+NAA</b>					
0.2+0.2	S+R	1.33 $\pm$ 0.33 <sup>a</sup>	4.33 $\pm$ 0.33 <sup>a</sup>	2.00 $\pm$ 0.57 <sup>a</sup>	2.00 $\pm$ 0.57
0.4+0.2	S+R	4.00 $\pm$ 0.00 <sup>a</sup>	7.33 $\pm$ 0.66 <sup>ab</sup>	5.00 $\pm$ 0.57 <sup>ab</sup>	2.00 $\pm$ 0.57
0.6+0.2	S+R	<b>4.66<math>\pm</math>0.33<sup>a</sup></b>	<b>9.00<math>\pm</math>0.00<sup>ac</sup></b>	<b>13.33<math>\pm</math>0.33<sup>ac</sup></b>	2.33 $\pm$ 0.33
0.8+0.2	S+R	4.00 $\pm$ 0.57 <sup>a</sup>	8.00 $\pm$ 0.57 <sup>ad</sup>	11.33 $\pm$ 0.88 <sup>ad</sup>	<b>2.63<math>\pm</math>0.33</b>
1.0+0.2	S+R	3.66 $\pm$ 0.33 <sup>a</sup>	8.00 $\pm$ 0.00 <sup>ae</sup>	4.66 $\pm$ 0.33 <sup>ae</sup>	1.66 $\pm$ 0.33
<b>BAP+KN +NA A</b>					
1.0+0.6+0.2	S+R	<b>4.66<math>\pm</math>0.33<sup>a</sup></b>	<b>14.66<math>\pm</math>0.33<sup>a</sup></b>	<b>4.66<math>\pm</math>0.33<sup>a</sup></b>	<b>1.66<math>\pm</math>0.33</b>
2.0+0.6+0.2	S+R	3.66 $\pm$ 0.33 <sup>a</sup>	12.66 $\pm$ 0.66 <sup>a</sup>	2.33 $\pm$ 0.33 <sup>b</sup>	1.33 $\pm$ 0.33
3.0+0.6+0.2	S+R	4.00 $\pm$ 0.00 <sup>a</sup>	11.00 $\pm$ 1.00 <sup>d</sup>	2.66 $\pm$ 0.66 <sup>ab</sup>	1.00 $\pm$ 0.00
4.0+0.6+0.2	S+R	2.66 $\pm$ 0.33 <sup>c</sup>	9.00 $\pm$ 0.00 <sup>c</sup>	3.33 $\pm$ 0.33 <sup>ab</sup>	1.33 $\pm$ 0.33
5.0+0.6+0.2	S+R	1.66 $\pm$ 0.33 <sup>b</sup>	6.66 $\pm$ 0.33 <sup>b</sup>	3.66 $\pm$ 0.33 <sup>ab</sup>	1.00 $\pm$ 0.00
<b>BAP+TDZ +N AA</b>					
1.0+1.0+0.2	S+R+C	12.66 $\pm$ 0.66 <sup>a</sup>	13.00 $\pm$ 1.00	10.00 $\pm$ 0.57 <sup>a</sup>	2.33 $\pm$ 0.33 <sup>ab</sup>
1.0+2.0+0.2	S+R+C	<b>16.66<math>\pm</math>0.66<sup>a</sup></b>	<b>20.33<math>\pm</math>1.20</b>	<b>13.33<math>\pm</math>0.88<sup>b</sup></b>	<b>3.33<math>\pm</math>0.33<sup>a</sup></b>
1.0+3.0+0.2	S+R+C	15.33 $\pm$ 1.33 <sup>a</sup>	11.66 $\pm$ 0.66	11.66 $\pm$ 0.66 <sup>a</sup>	3.00 $\pm$ 0.00 <sup>ab</sup>
1.0+4.0+0.2	S+R+C	12.66 $\pm$ 1.33 <sup>a</sup>	12.66 $\pm$ 0.66	9.66 $\pm$ 0.33 <sup>ac</sup>	2.66 $\pm$ 0.33 <sup>ab</sup>
1.0+5.0+0.2	S+R+C	11.00 $\pm$ 0.57	11.00 $\pm$ 1.00	8.33 $\pm$ 0.33 <sup>ad</sup>	1.66 $\pm$ 0.33 <sup>b</sup>

BAP, 6-benzylaminopurine; KN, Kinetin; NAA, 1-naphthaleneacetic acid. Each experiment had 20 replicates and was repeated three times. Values represent the mean  $\pm$  standard error.

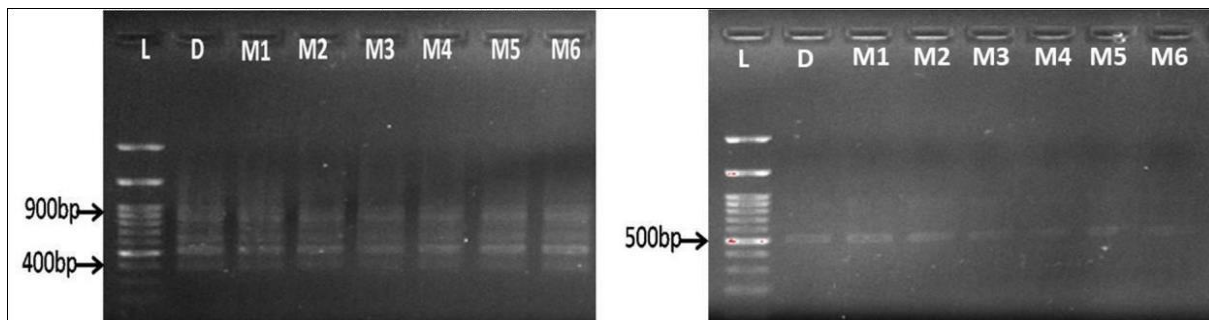
According to Duncan's new multiple range test at 5% level, mean values with the same letter within columns are not significantly different.



**Fig 1:** Different stages of the *in vitro* regeneration from rhizome explants of *Curcuma amada* Roxb. (a) Inoculation of rhizome bud explants on MS medium containing different concentrations of Kn (0.2-1.0mg/L) + NAA (0.2 mg/L); (b) Sprouted explant; (c) Sprout elongation, (d) Subculture on to the medium containing BAP (1-5mg/L) + Kn (0.6 mg/L) + NAA(0.2 mg/L). (e) Responding with 3-4 shoots per explant. (f) Sub-culture on to the medium containing TDZ (1-5mg/L) + BAP (1mg/L) + NAA(0.2 mg/L). (g) Elongation of the subcultured shoot buds. (h) Maximum number of 16 shoots per explant resulted on 2mg/L TDZ containing medium and elongation of shoots and roots. (i) Hardening of *in vitro* raised plantlet of *Curcuma amada* Roxb.



**Fig 2:** RAPD banding pattern with primers (a) C12 and (b) AM2 in both micro propagated and field grown mother plant of *Curcuma amada* Roxb. (lane 1: ladder, donor plant and lane 38 micro propagated plants).



**Fig 3:** RAPD banding pattern with primers (a) AM5 and (b) AM6 in both micro propagated and field grown mother plant of *Curcuma amada* (lane 1: ladder, donor plant and lane 3-8 micro propagated plants).

## Discussion

A plant *in vitro* regeneration system of *Curcuma amada* was effectively disclosed, Factors such as basal medium concentration and combination of PGRs controlled regeneration frequency in *Curcuma amada* According to research done by Faridah *et al.* (2011), Yusuf *et al.* (2011), and Mohanty *et al.* (2012), the highest explant contamination was found in the buds from ginger rhizomes, Chinese ginger, and mango ginger. The present study suggested that usage of different sterilants in various concentrations resulted decreased contamination in *Curcuma amada* Factors including basal media concentration and the combination of PGRs affected the induction of the shoot and the roots in *Curcuma amada* In this study the various concentrations of cytokinins were used which resulted in to increased shooting and rooting. In ginger tissue culture, similar results were found demonstrating a steady increase and decrease in the number of shoots after a given quantity of cytokinins (Balachandran *et al.* 1990 [2]; Sit *et al.* 2005) [26]. On subculturing on the medium containing BAP the explants produced multiple shoots this was on the same line with both ginger and *Kaempferia galanga* tissue cultures, produced using BAP (Sit *et al.* 2005 [26]; Bhattacharya and Sen, 2006 [6]; Bhattacharya and Sen, 2013) [5].

According to Barthakur and Bordoloi's (1992) [3] observations on multiple shoot regeneration in BAP and NAA, NAA and BAP have a synergistic effect on promoting shoot multiplication in *Curcuma amada* Our work predicted the same on combining BAP with NAA resulted in to increased shoot number. The result of multiple shoots in medium with is in agreement with the results of Kuruvnashetti and Iyer (1982) [13], Barthakur and Bordoloi (1992) [3] with *Curcuma amada* and Balachandran *et al.* (1990) with *C. longa*. In *C. domestica* and *Z. officinale*, respectively, Mukhri and Yamaguchi (1986) [14] and Babu *et*

*al.* (1992) found concurrent development of shoots and roots. In the current investigation, it was discovered that TDZ promoted multiple shoot regeneration better than BA. Accordingly, TDZ has recently been demonstrated to have good impacts on *in vitro* development for several species (Yucescan *et al.* 2007 [31]; Basalma *et al.* 2008 [4]; Zhihui *et al.* 2009) [32]. Notably, Salvi *et al.* (2000) [21] showed that TDZ caused a high average number of shoots (8.9 per explant) in turmeric, whilst Prathanturug *et al.* (2003, 2005) [18, 19] discovered that TDZ stimulated shoots from *in vitro* cultures of *C. longa* more successfully than the cytokinins BA, KIN, and 2iP. In the current work, the impact of TDZ on *Curcuma amada in vitro* regeneration was evaluated for the first time.

## References

1. Adriance GW, Brison FR. Propagation of Horticultural Plants. New York: McGraw Hill Book Company Inc., 1955, 298.
2. Balachandran SM, Bhat SR, Chandal KPS. *In vitro* clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.) Plant Cell Rep,1990:8:521–524.
3. Barthakur MP, Bordoloi DN. Micropropagation of *Curcuma amada* Roxb. J. Spice. Arom. Crops,1992:1(2):154–156.
4. Basalma D, Uranbey S, Gurlek D, Ozcan S. TDZ-induced plant regeneration in *Astragalus cicer* L. Afr. J. Biotechnol,2008:7:955–959.
5. Bhattacharya M, Sen A. *In vitro* regeneration of pathogen free *Kaempferia galanga* L. -a rare medicinal plant. Res. Plant Bio,2013:3(3):24-30.
6. Bhattacharya M, Sen A. Rapid *in vitro* multiplication of disease-free *Zingiber officinale* Rosc. Indian J. Plant Physiol,2006:11(4):379-384.

7. Brijesh, Hemavathi, and Brijesh Ajjappala. "Micropropagation strategies in medicinally important turmeric (*Curcuma* sp): Current research and future challenges." *Journal of Applied Biology and Biotechnology*,2023;11(3): 1-8.
8. Brock TC, Kaufman PB. Growth regulators: an account of hormone and growth regulation. London: Growth and Development. Academic Press Inc., 1991, 277340. In F.C. steward (ed.). *Plant Physiology: A Treatise*. Vol 10.
9. Gurav SS, Gurav N, Patil AT, Duragkar NJ. Effect of explant source, culture media, and growth regulators on callogenesis and expression of secondary metabolites of *Curcuma longa*. *J Herbs Spices Med Plant*,2020;26:172-90.
10. Jena S, Ray A, Sahoo A, Sahoo S, Dash B, Kar B, *et al*. Rapid plant regeneration in industrially important *Curcuma zedoaria* revealing genetic and biochemical fidelity of the regenerants,2020;10:17.
11. Jie EY, Ahn MS, Lee J, Cheon YI, Kim CY, Kim SW. Establishment of a high-frequency plant regeneration system from rhizome-derived embryogenic cell-suspension cultures of *Curcuma longa* L. *Plant Biotechnol Rep*,2019;13:123-9.
12. Kirtikar KR, Basu BD. *Indian medicinal plants*, vol 4. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1984.
13. Kuruvinashetti MS, Iyer RD. An evaluation of tissue culture technique in coconut and turmeric. In: Vishreshwara E (ed) *Proc. PLACROMSYM-IV*, 1982, 101–105.
14. Mukhri T, Yamaguchi H. *In vitro* plant multiplication from rhizomes of turmeric *Curcuma domestica* Val.) and Ternoe Lawak (*C. xanthorrhiza* Roxb.). *Plant Tiss. Cult. Lett*,1986;3(1):28–30.
15. Mustafa A, Ali M, Khan NZ. Volatile oil constituents of the fresh rhizome of *Curcuma amada* Roxb. *J Ess Oil Res*,2005;17:490–491.
16. Policegoudra RS, Rehna K, Rao LJ, Aradhya SM. Antimicrobial, antioxidant, cytotoxicity and platelet aggregation inhibitory activity of a novel molecule isolated and characterized from mango ginger (*Curcuma amada* Roxb.) rhizome. *J Biosci*,2010;35:231–240.
17. Prakash S, Elangomathavan R, Seshadri S, Kathiravan K, Ignacimuthu S. Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. *Plant Cell Tiss Org Cult*,2004;78:159–165.
18. Prathanturug S, Soonthornchareonnon N, Chuakul W, Phaidee Y, Saralamp P. High-frequency shoot multiplication in *Curcuma longa* L using thidiazuron. *Plant Cell Rep*,2003;21:1054–1059.
19. Prathanturug S, Soonthornchareonnon N, Chuakul W, Phaidee Y, Saralamp P. Rapid micropropagation of *Curcuma longa* using bud explants pre-cultured in thidiazuron-supplemented liquid medium. *Plant Cell Tissue Organ Cult*,2005;80:347–351.
20. Raju CS, Kathiravan K, Aslam A, Shajahan A. An efficient regeneration system via somatic embryogenesis in mango ginger (*Curcuma amada* Roxb.). *Plant Cell Tiss Org Cul*,2013;112:387-93
21. Salvi ND, George L, Eapen S. Direct regeneration of shoots from immature inflorescence cultures of turmeric. *Plant Cell Tissue Organ Cult*,2000;62:235–238.
22. Zulkarnain Z, Kartika E, Lizawati L. Growth and development of young mail inflorescences of oil palm (*Elaeis guineensis* Jacq.) in tissue culture system: the effect of 2,4-Dichlorophenoxyacetic acid. Annual conference on environmental science, Society and its application. IOP Publishing, 2019, 1-7.
23. Sasikumar B. Genetic resources of *Curcuma*: Diversity, characterization and utilization. *Plant Gen Res*,2005;3:230-51.
24. Singh S, Dalal S, Singh R, Dhawan AK, Kalia R. Evaluation of genetic fidelity of *in vitro* raised plants of *Dendrocalamus asper* (Schult. & Schult. F.) Backer ex K. Heyne using DNAbased markers. *Acta Physiol Plant*,2013;35:419-30
25. Singh S, Kumar JK, Saikia D, Shanker K, Thakur JP, Negi AS, Benerjee S. A bioactive labdane diterpenoid from *Curcuma amada* and its semisynthetic analogues as antitubercular agents. *Eur J Med Che*,2010;45:4379–4382.
26. Sit AK, Bhattacharya M, Chenchiah KC. Effect of benzyl amino purine on *in vitro* shoot multiplication of ginger (*Zingiber officiale* Rosc.) cv Garubathan. *J. Plantation. Crops*,2005;33(3):184-186.
27. Srinivasan MR, Chandrasekhara N, Srinivasan K. Cholesterol lowering activity of mango ginger (*Curcuma amada* Roxb.) in induced hypercholesterolemic rats. *Eur Food Res Technol*,2008;227:1159–116.
28. Srivastava V, Khan SA, Banerjee S. Anevaluation of genetic fidelity of encapsulated microshoots of the medicinal plant: *Cineraria maritima* following six months of storage. *Plant Cell Tissue Org. Cult*,2009;62:235–238
29. Tyagi RK, Agrawal A, Mahalakshmi C, Hussain Z, Tyagi H. Low-cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers. *In vitro Cell Dev. Biol. Plant*,2007;43:51–58.
30. Warriar PK, Nambiar VPK, Ramankutty C. *Indian medicinal plants: a compendium of 500 species*, vol 2. Orient Longman, Chennai, India, 1994.
31. Yucescan B, Turker AU, Gurel E. TDZ-induced high frequency plant regeneration through multiple shoot formation in witloof chicory (*Cichorium intybus* L.). *Plant Cell Tissue Organ Cult*,2007;91:243–250.
32. Zhihui S, Tzitzikas M, Raemakers K, Zhengqiang M, Visser R. Effect of TDZ on plant regeneration from mature seeds in pea (*Pisum sativum*). *In vitro Cell Dev. Biol. Plant*,2009;45:776–782.