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Micropropagation of an Indian Ginger (*Curcuma vamana* Sabu and Mangaly): A Wild Relative of Turmeric

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Abstract: *Curcuma vamana* Sabu and Mangaly, a wild relative of turmeric, is endemic to Kerala and its germplasm deserves special conservation measures. A micropropagation protocol has been outlined for the production of complete plantlets within 85 days. Nodal segments isolated from stoloniferous rhizomes achieved 60% bud break within 20 days. These explants responded in BAP (1.0 mg L^{-1}) supplemented medium with the sprouting of 1-3 shoots in 6-7 weeks of incubation. *In vitro* shoots obtained from primary cultures were used for further studies to minimise collection from the small wild populations of *C. vamana*. Efficient shoot multiplication has been obtained from *in vitro* shoot explants using thidiazuron (TDZ). The highest rate of shoot production was found in 0.5 mg L^{-1} TDZ which produced 9.6 shoots. Rhizogenesis was observed along with collagenesis in all treatments. Effect of various nutrient formulations was also compared. The best shoot multiplication response has been observed in Murashige and Skoog (MS) medium followed by Nitsch, Woody Plant (WPM), Gamborg (B5) and White's formulations. MS medium recorded about five-fold increase in the rate of shoot multiplication compared to that of the least responding medium under identical culture conditions. The regenerated plantlets were successfully established in the greenhouse condition with 90% survival.

Key words: *Curcuma vamana*, kunji-kuva, micropropagation, tissue culture, N^6-benzyladenine, thidiazuron, endemic species, rhizome, Zingiberaceae

INTRODUCTION

The genus *Curcuma* Linn. with around 120 species, distributed in tropical and subtropical Asia consists of rather homogenous group of rhizomatous perennials, belongs to the tribe Hedychieae of the family Zingiberaceae. The genus comprises of many economically important plants including *Curcuma longa*. Turmeric, the well renowned spice and medicinal herb (Sabu, 2006). In India, it represents 30 species and constitutes the largest genus of ginger family and mainly distributed in the Western Ghats. *Curcuma vamana* Sabu and Mangaly is endemic to Kerala, India, where it is mostly known as 'kunji-kuva', representing its smaller size of hardly 20 cm (Dan and Bejoy, 2007). The plant has also been reported as one among the 12 endemic *Curcuma* species to the forests of the Southern Western Ghats of peninsular India (Nayar, 1996). *C. vamana* is a delicate herb with conical stoloniferous rhizome, pseudo stem covered basally by leafless sheath. Flowers are pale yellow, on terminal short spike with prominent cup like bracts (Sabu, 2006; Sabu and Mangaly, 1988). This wild relative of turmeric in Western Ghats is a potential genetic resource worth conserving.

Several factors such as endemicity, restricted distribution, small populations in inaccessible areas and several anthropogenic pressures on its habitat, have made it necessary to take steps to conserve these species. Micropropagation offers a rapid means of producing a large number of clonal plants which can be used for restoration and conservation of rare and endemic germplasm. In *Curcuma*, plant propagation by tissue culture techniques have been reported in *C. zedoaria*, *C. aromatica* (Anisuzzaman *et al*., 2008; Yasuda *et al*., 1987), *C. aeruginosa*, *C. caesia* (Balachandran *et al*., 1990), *C. amada* (Barthakur and Bordoloi, 1992; Prakash *et al*., 2004), *C. longa* (Rahman *et al*., 2004), *C. haritha* (Bejoy *et al*., 2006), *C. angustifolia* (Shukla *et al*., 2007), *C. mangga* (Raihana *et al*., 2011) etc. The micropropagation of rare, endemic and threatened species offers an effective system causing least disturbance to the natural population as the regenerants can be used to replenish the wild population in its natural

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habitat or for *ex situ* conservation programme (Bejoy *et al*., 2010; Anish *et al*., 2008; Tandon *et al*., 2007). Though rhizomes are generally used in zingiberaceous plants as propagules, seed propagation is difficult due to prolonged dormancy up to 12 months. Conventional propagation of *C. vamana* is not yet established. Moreover, there is no report available on tissue culture studies in this endemic species. Considering the endemism and difficulty in conventional propagation of the species, the present study is drawn with an aim to develop an *in vitro* clonal multiplication system in *C. vamana*.

MATERIALS AND METHODS

Induction of multiple shoots: *C. vamana* was collected from Thenmala forest (Kerala, India) and the voucher specimens were deposited in the herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (No. TBGT- 47742), Palode, Thiruvananthapuram. Slender stolon cuttings from rhizome were washed thoroughly in running water and pre-sterilized with 1% (v/v) commercial bleach solution and 0.2% (v/v) labolene (Qualigens, Bombay) for 90 min. The surface sterilization was achieved in 15% (v/v) bleach solution for 12 min followed by 0.1% $HgCl_2$ for 7 min. These explants were rinsed four times with sterile distilled water before inoculating onto MS medium (Murashige and Skoog, 1962) supplemented with Plant Growth Regulators (PGR_S). In order to optimise shoot multiplication, PGRs such as N^6-benzyladenine (BAP), thidiazuron (TDZ) and kinetin (KN) at various concentrations were tested (Table 1). All the cultures were incubated at 24±2°C under 16 h light period provided by cool white fluorescent tubes (1000 lux).

Effect of nutrient media: Effect of different nutrient media was also studied after standardising PGR requirement. Five nutrient formulations such as MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1981), B5 (Gamborg *et al*., 1968), Nitsch (Nitsch, 1969) and White's (White, 1963) were tested to determine their effectiveness on *in vitro* morphogenesis. The basal media were supplemented with 30 g L^{-1} sucrose, 7 g L^{-1} agar and adjusted to pH 5.7 prior to autoclaving at 121°C for 20 min.

Deflasking and *in vivo* establishment: Plantlets showing well-developed roots and shoots were washed carefully in running water to remove traces of agar. The shoot clumps were immersed in 3% commercial fungicide (Indofil M-45) for 3 min and planted in earthen pots containing river sand and coarse charcoal. They were maintained in a greenhouse under semi-shade (50%) and high humid (RH 75-85%) conditions for hardening. After 30-35 days of hardening, individual plantlets were isolated and repotted in small poly bags containing river sand and top soil (3:1) for further establishment.

Statistical analysis: Responses of the cultures were observed and recorded over a period of five weeks. The shoot length and number of shoots and roots formed were observed. The data were analysed by single factor analysis of variance and the means were compared using Duncan's Multiple Range Test (DMRT) at p = 0.05.

RESULTS AND DISCUSSION

Culture initiation and multiplication: Establishment of aseptic cultures was laborious, since the explants were harvested from underground stem. Three level surface

Table 1: Effect of cytokinins on *in vitro* shoot explants of *C. vamana*

Treatment (mg L^{-1})*			*In vitro* response**		
BAP	KIN	TDZ	Rate of multiplication	Average shoot length (mm)	Average roots
0.5	-	-	1.5±0.27^{ab}	58.7±7.89^{e}	6.6±0.46^{c}
1.0	-	-	2.9±0.29^{bc}	68.7±3.40^{fg}	8.1±0.44^{c}
2.0	-	-	3.3±0.25^{bc}	79.9±2.25^{h}	15.8±0.73^{f}
-	0.5	-	2.4±0.18^{bc}	37.4±2.31^{ab}	7.0±0.33^{b}
-	1.0	-	1.8±0.25^{ab}	62.9±2.55^{ef}	11.1±0.55^{d}
-	2.0	-	1.1±0.13^{a}	74.6±2.75^{gh}	7.9±0.39^{b}
-	-	0.05	7.1±0.44^{e}	48.2±2.12^{c}	20.5±0.33^{k}
-	-	0.1	7.4±0.46^{e}	48.8±2.50^{cd}	17.5±0.63^{i}
-	-	0.5	9.6±0.26^{f}	58.5±1.90^{e}	18.5±0.50^{j}
0.5	0.5	-	3.3±0.31^{bc}	75.2±2.53^{gh}	16.8±0.87^{fg}
0.5	1.0	-	3.0±0.27^{bc}	75.8±2.42^{gh}	10.5±0.87^{d}
1.0	0.5	-	5.1±0.48^{d}	57.6±2.76^{e}	22.8±0.92^{l}
1.0	1.0	-	3.5±0.38^{c}	75.5±2.23^{gh}	14.0±0.42^{e}
1.0	-	0.1	3.3±0.37^{bc}	41.4±2.85^{bc}	3.6±0.26^{a}
1.0	-	0.5	3.6±0.26^{c}	29.6±1.43^{a}	3.4±0.32^{a}
-	1.0	0.1	2.8±0.30^{bc}	57.1±2.22^{de}	6.3±0.25^{b}
-	1.0	0.5	3.5±0.33^{c}	31.5±1.60^{a}	2.1±0.48^{a}

*Basal medium: MS+2% sucrose+0.7% agar and pH 5.7, **Values are Mean±SE, Means within column having different letters are significantly different according to DMRT at the 0.05 level of probability, BAP: N^6-benzyladenine, KN: Kinetin, TDZ: Thidiazuron

sterilization techniques were found effective with 70-75% survival as against 50-55% contamination in the trial experiment. Difficulty in eliminating infection from zingiberaceous explants of underground origin were reported from *C. longa* (Naz *et al*., 2009). Multilevel decontamination procedures were found beneficial to improve disinfection of explants of underground origin (Anish *et al*., 2008). The stoloniferous rhizome segments were initially established in BAP (1-2 mg L^{-1}) and KN (1-2 mg L^{-1}). The shoots appeared to be developing directly from dormant buds present on the explants and around 60% of the primary explants proliferated axillary buds in 20 days. The explants developed 1-3 shoots on basal medium supplemented with 1 mg L^{-1} of BAP in 6-7 weeks. The explants placed initially on KN medium did not develop as quickly as those placed on BAP, hence it seems to be less effective (data not given). BAP has been found suitable for initial bud brake in many zingiberaceous species such as *Amomum hypoleucum* (Bejoy *et al*., 2010), *Boesenbergia pulcherrima* (Anish *et al*., 2008) and *C. mangga* (Raihana *et al*., 2011). The present study was carried out with minimum samples as *C. vamana* is an endemic species and its wild population is narrow. Hence, *in vitro* shoots obtained from the primary explants were utilised for all further studies to avoid over collection of this threatened species from the wild.

The *in vitro* morphogenic responses of shoot explants showed visible difference according to PGR treatment (Table 1). About 85% of the *in vitro* shoot explants initiated more than two shoots in all the experiments tested. Even though, shoot multiplication was observed in all cytokinin, TDZ provided better support to develop multiple shoots followed by BAP. When BAP and KN were treated individually, the explants yielded only a maximum of 3.2 and 2.4 shoots per explant, respectively. Whereas under TDZ regime 5-12 shoots were achieved. At lower concentrations of 0.05 and 0.1 mg L^{-1} TDZ, the explants developed 7 and 7.4 shoots per explants, respectively. The *in vitro* shoot production was increased to average 9.6 shoots when inoculated onto MS medium supplemented with 0.5 mg L^{-1} TDZ in 35 days (Fig. 1). The present results showed that the rate of multiplication is directly proportional to the concentration of TDZ tested. Similar to our observations, enhanced *in vitro* shoot multiplication has been reported in species like *Aframomum corrorima* (Tefera and Wannakrairoj, 2004; Eyob, 2009) and *Curcuma longa* (Prathanturarug *et al*., 2003). Thidiazuron, a phenyl urea derivative (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) has been reported for its cytokinin-like activities in various explants (Mok *et al*., 1982; Huetteman and Preece, 1993). This could be due to

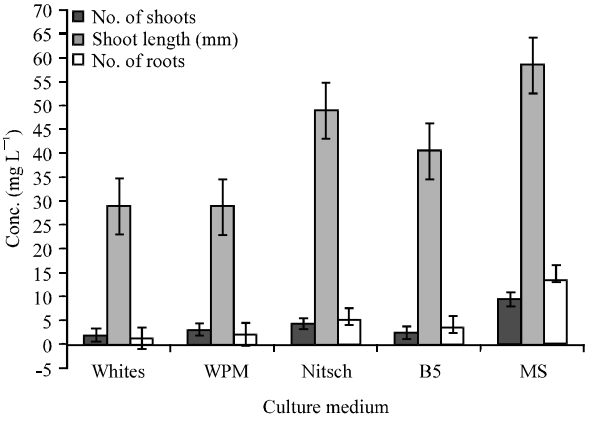

Fig. 1: Effect of nutrient formulations on *in vitro* morphogenic responses of *C. vamana* after 30 days: All basal media supplemented with 0.5 mg L^{-1} TDZ+30 g L^{-1} sucrose+0.7 g L^{-1} agar and pH 5.7, Vertical bars are Mean±SE

decreased dominance of the terminal meristem. Among other phenyl urea derivatives, TDZ is found to be the most potent cytokinin, since its biological activity at much lower concentration was relatively higher than that of aminopurine derivative group of cytokinins (Murthy *et al*., 1998). Possible explanation is that TDZ is prospective inhibitor of cytokinin oxidase and more stable than the adenine-type cytokinins, thereby enhancing the efficacy of available cytokinins (Kende and Zeevaart, 1997).

In order to further the multiple shoot development combinations, of the cytokinin treatments were tested. Combined use of cytokinins did not help to maximise shoot multiplication in *C. vamana*. However, the best production response (5.1 shoots) under joint action was recorded on BAP (1 mg L^{-1}) and KN (0.5 mg L^{-1}) combination, showing improvement over their individual use. Whereas, synergistic effect of TDZ with other cytokinins has been reported to increase shoot regeneration in Korarima (Tefera and Wannakrairoj, 2006), *Ochlandra wightii* (Bejoy *et al*., 2012), *A. hypoleucum* (Bejoy *et al*., 2010) etc. Even though, moderate growth response has been exhibited by the explants in all treatments, BAP and KN or their combinations showed better shoot growth than TDZ or its combinations (Table 1). Maximum shoot elongation of average 79.9 mm growth was obtained from explants inoculated onto MS medium containing 2 mg L^{-1} BAP in 35 days.

Rooting was readily observed in the multiplication medium and cytokinins did not inhibit rhizogenesis in all treatments. In 98% of the cultures roots developed simultaneously along with collagenesis. However,

different PGR treatments affected the root regeneration potential of *C. vamana*. Explants cultured onto TDZ resulted in good rooting whereas relatively less response has been noted when its combinations (BAP or KIN) were tried (Table 1). The best rooting response of average 22.7 roots was observed in a combination of BAP (1.0) with KIN (0.5) in 3 weeks. When treated individually, TDZ seems to be the best out of the three tested. According to Abdelmageed *et al*. (2011) a separate stage was necessary for rooting of *in vitro* shoots of *Etlingera elatior*. They also reported the induction of roots necessitates transfer of *in vitro* shoots from cytokinin (BAP) to auxin (IAA). The simultaneous root development observed in the present study is in agreement with previous reports on *A. hypoleucum* (Bejoy *et al*., 2010), *Alpinia galanga* (Barthakur *et al*., 1999), *Mantisia spathulata* (Tandon *et al*., 2007) *Kaempferia galanga* (Vincent *et al*., 1992) etc. Such instantaneous rhizogenic response obtained during shoot multiplication may be due to an inherent capacity of the tissue of rhizome origin (Anish *et al*., 2008). Thus, the present technique is simple and cost effective, avoiding a separate rooting stage.

Comparative study of nutrient formulation: Mineral nutrition has a pivotal role in the morphogenic responses of cultured explants (Preece, 1995; Ramage and Williams, 2002). The influences of nutrient formulations on *in vitro* responses of *C. vamana* were ascertained with the help of five media (Fig. 2). The effect of selected nutrient formulations revealed noticeable variability on culture behaviour, under identical conditions (0.5 mg L^{-1} TDZ). The best shoot multiplication response has been observed in MS medium followed by Nitsch, WPM and B5 formulations. MS medium recorded about five-fold increase in the rate of shoot multiplication compared to that of Whites medium. The Whites and B5 formulations registered least multiplication of 2 and 2.6 shoots per explant in 35 days, respectively while 80 % of the explants developed nearly 10 shoots or above on MS medium with same PGR. Apart from multiplication, MS seems to be the preferred basal medium for rooting and culture growth in *C. vamana* compared to other formulations (Fig. 2). Maximum shoot elongation (58.5 mm) and rooting (14.3) were achieved in MS whereas drastic reduction of the same was observed in White's and Woody Plant formulations. This is in line with our earlier reports on Zingiberaceae species, where MS medium was found the suitable nutrient formulation for optimum *in vitro* responses compared to other media (Bejoy *et al*., 2010; Anish *et al*., 2008; Bejoy *et al*., 2006). Very few reports showed the suitability of a different medium as reported in *Elettaria cardamomum*, where SH medium produced the highest multiplication (Bajaj *et al*., 1993). The relative

Fig. 2: Induction of multiple shoots on *Curcuma vamana* in MS+0.5 mg L^{-1} TDZ+3% sucrose+0.7% agar and pH 5.7 after 30 days

ratio of nitrate and ammonium is an important factor for active shoot development which is considerably higher in MS medium than that of other media tested. Nitrate and ammonium ionic ratio is an important aspect of nitrogen nutrition which strongly influences the pH of the media (Kirkby and Mengel, 1967), consequently the absorption of different nutrients. Perhaps, this is one of the reasons for the better performance of MS formulation.

Acclimatisation of microplants: After 35-40 days in culture, actively growing plantlets with profuse root system were washed thoroughly in running water to remove all adhering medium and transferred to greenhouse for acclimatisation. *In vitro* plants generally suffer from morphological abnormalities mainly of stomata and cuticle which in turn determine their *in vivo* survival. The deflasked *C. vamana* plantlets were planted in clay community pots containing 3:1 coarse river sand and charcoal and they registered 90% survival under greenhouse conditions (50% shade and 75-85% RH) in 4-5 weeks of initial acclimatisation. During the period they grew normally and developed new leaves (Fig. 3). In *C. longa*, transfer of potted plantlets to glass house with complete sunlight was found beneficial for 70-80 percent survival (Naz *et al*., 2009). In the present study, the young hardened plants repotted individually in poly-bags and kept under the same greenhouse conditions showed uniform healthy growth.

The present study could be highly significant, as this is the first report on successful *in vitro* propagation of the

Fig. 3: Microplants of *C. vamana* establishing in greenhouse conditions after 45 days

endemic *C. vamana*. The protocol offers the recovery of large number of plants in a short span of time.

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REFERENCES

Abdelmageed, A.H.A., Q.Z. Faridah, F.M.A. Norhana, A.A. Julia and B. Kadir, 2011. Micropropagation of *Etlingera elatior* (Zingiberaceae) by using axillary bud explants. J. Med. Plants Res., 5: 4465-4469.

Anish, N.P., M. Bejoy and M. Dan, 2008. Conservation using in vitro progenies of the threatened ginger-*Boesenbergia pulcherrima* (Wall.) Kuntze. Int. J. Bot., 4: 93-98.

Anisuzzaman, M., S.A. Sharmin, S.C. Mondal, R. Sultana, M. Khalekuzzaman, I. Alam and M.F. Alam, 2008. *In vitro* microrhizome induction in *Curcuma zedoaria* (Christm.) Roscoe-a conservation prioritized medicinal plant. J. Biol. Sci., 8: 1216-1220.

Bajaj, Y.P.S., B.R. Reghunath and P.K. Gopalakrishnan, 1993. *Elettaria cardamomum* Maton (Cardamom): Aromatic Compounds, *in vitro* Culture Studies and Clonal Propagation. In: Biotechnology in Agriculture and Forestry: Aromatic Plants-IV, Bajaj, Y.P.S. (Ed.). Vol. 21. Springer-Verlag, Berlin, Germany, ISBN 978-3-642-77006-7, pp: 132-147.

Balachandran, S.M., S.R. Bhat and K.P.S. Chandel, 1990. *In vitro* clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.). Plant Cell Rep., 8: 521-524.

Barthakur, M. and D.N. Bordoloi, 1992. Micropropagation of *Curcuma amada* (Roxb.). J. Spices Aromatic Crops, 1: 154-156.

Barthakur, M., J. Hazarika and R.S. Singh, 1999. A protocol for micropropagation of *Alipinia galanga*. Plant Cell Tissue Organ Cult., 55: 231-233.

Bejoy, M., M. Dan and N.P. Anish, 2006. Factors affecting the *in vitro* multiplication of the endemic zingiber-Curcuma haritha Mangaly and Sabu. Asian J. Plant Sci., 5: 847-853.

Bejoy, M., M. Dan, N.P. Anish, G.A. George and B.J. Radhika, 2010. Induction of multiple shoots in *Amomum hypoleucum* Thwaites: A threatened wild relative of large cardamom. J. Applied Hort., 12: 46-49.

Bejoy, M., N.P. Anish, B.J. Radhika and G.M. Nair, 2012. *In vitro* propagation of ochlandra wightii (Munro) fisch.: An endemic reed of Southern Western Ghats India. Biotechnology, 11: 67-73.

Dan, M. and M. Bejoy, 2007. Wild relatives of turmeric in Western Ghats. Natural Resource Management and Livelihood Support Systems-A Compendium of Research Papers, 5th March 2007, Western Ghats Cell, Planning and Economic affairs Department Government of Kerala, Pattom, pp: 143- 146.

Eyob, S., 2009. Promotion of seed germination, subsequent seedling growth and *in vitro* propagation of korarima *Aframomum corrorima* (Braun) P.C.M Jansen). J. Med. Plants Res., 3: 652-659.

Gamborg, O.L., R.A. Miller and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50: 151-158.

Huetteman, C.A. and J.E. Preece, 1993. Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant Cell Tissue Organ Cult., 33: 105-119.

Kende, H. and J.A. Zeevaart, 1997. The five classical hormones. Plant Cell, 9: 1197-1210.

Kirkby, A.E. and K. Mengel, 1967. Ionic balance in different tissues of the tomato plant in relation to nitrate, urea or ammonium nutrition. Plant Physiol., 42: 6-14.

Lloyd, G. and B. McCown, 1981. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Combined Proc. Int. Plant Prop. Soc., 30: 421-427.

Mok, M.C., D.W.S. Mok, D.J. Armstrong, K. Shudo, Y. Isogai and T. Okamoto, 1982. Cytokinins activity of N-phenyl-N`-1,2,3-thiadiazol-5-ylurea (Thidiazuron). Phytochem., 21: 1509-1511.

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

Murthy, B.N.S., S.J. Murch and P.K. Saxena, 1998. Thidiazuron: A potent regulator of *in vitro* plant morphogenesis. *In vitro* Cell. Dev. Biol. Plant, 34: 267-275.

Nayar, M.P., 1996. Hot Spots of Endemic Plants of India, Nepal and Bhutan. Tropical Botanic Garden and Research Institute, Kerala, India, ISBN: 9788190039710, Pages: 252.

Naz, S., S. Ilyas, S. Javad and A. Ali, 2009. *In vitro* clonal multiplication and acclimatisation of different varieties of Turmeric (*Curcuma longa* L.). Pak. J. Bot., 41: 2807-2816.

Nitsch, J.P., 1969. Experimental androgenesis in *Nicotiana*. Phytomorphology, 19: 389-404.

Prakash, S., R. Elongomathavan, S. Seshadri, K. Kathiravan and S. Ignacimuthu, 2004. Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. Plant Cell Tissue Organ Cult., 78: 159-165.

Prathanturarug, S., N. Soonthornchareonnon, W. Chuakul, Y. Phaidee and P. Saralamp, 2003. High-frequency shoot multiplication in *Curcuma longa* L. using thidiazuron. Plant Cell Rep., 11: 1054-1059.

Rahman, M.M., M.N. Amin, H.S. Jahan and R. Ahmed, 2004. *In vitro* regeneration of plantlets of *Curcuma longa* Linn. A valuable spice plant in Bangladesh. Asian J. Plant Sci., 3: 306-309.

Raihana, R., Q.Z. Faridah, A.A. Julia, A.H.A. Abdelmageed and M.A. Kadir, 2011. *In vitro* culture of *Curcuma mangga* from rhizome bud. J. Med. Plants Res., 5: 6418-6422.

Ramage, C.M. and R.R. Williams, 2002. Mineral nutrition and plant morphogenesis. *In vitro* Cell. Dev. Biol. Plant, 38: 116-124.

Sabu, M. and J.K. Mangaly, 1988. *Curcuma vamana* (Zingiberaceae): A new species from South India. J. Econ. Taxon. Bot., 10: 307-309.

Sabu, M., 2006. Zingiberaceae and Costaceae of South India. Indian Association for Angiosperm Taxonomy (IAAT), Kerala, India, ISBN-13: 9788190163705, Pages: 282.

Shukla, S.K., S. Shukla, V. Koche and S.K. Mishra, 2007. *In vitro* propagation of tikhur (*Curcuma angustifolia* Roxb.): A starch yielding plant. Indian J. Biotechnol., 6: 274-276.

Tandon, P., S.S.D. Bhowmik, A.A. Mao and S. Kumaria, 2007. Rapid *In vitro* clonal propagation of *Mantisia spathulata* Schult, a rare and endemic plant of northeastern India for recovery. Biotechnology, 6: 68-71.

Tefera, W. and S. Wannakrairoj, 2004. Micropropagation of Krawan (*Amomum krervanh* Pierre ex Gagnep). ScienceAsia, 30: 9-15.

Tefera, W. and S. Wannakrairoj, 2006. Synergistic effect of some plant growth regulators on *in vitro* shoot proliferation of korarima (*Aframomum corrorima* (Braun) Jansen). Afr. J. Biotech., 5: 1894-1901.

Vincent, K.A., K.M. Mathew and M. Hariharan, 1992. Micropropagation of *Kaempferia galanga* L. A medicinal plant. Plant Cell Tissue Organ Cult., 28: 229-230.

White, P.R., 1963. The Cultivation of Animal and Plant Cells. Ronald Press Co., New York, Pages: 228.

Yasuda, K., T. Tsuda, H. Shimizu and A. Sugaya, 1987. Multiplication of *Curcuma* species by tissue culture. Plant Med., 54: 75-78.