J. Hortl. Sci.
Vol. 16(1) : 69-76, 2021

Original Research Paper

In vitro multiplication protocol for *Curcuma mangga* : Studies on carbon, cytokinin source and explant size

Waman A.A., Bohra P.*, Karthika Devi R. and Pixy J.
ICAR – Central Island Agricultural Research Institute,
Port Blair, Andaman and Nicobar Islands, India
*Corresponding author e-mail : pooja.bohra@icar.gov.in

ABSTRACT

Mango ginger (*Curcuma mangga* Valeton & Zijp.) is an underutilized rhizomatous species that has been valued in the tropical Asian countries as a source of vegetable, spice, salad, medicine and essential oil. This species is hardy and requires less care for obtaining good yields. Rhizomes are the commonly used propagules for the species, which are also the economic part of the crop. Huge quantity of seed rhizomes is required to promote this crop in larger area. Efficient *in vitro* multiplication protocol is one of the options to meet the planting material requirement. Effects of carbon source (glucose, fructose and sucrose) and concentration (1 and 3%, w/v), cytokinins (BAP and *meta* topolin) and concentration (1 mg/ L and 2 mg/L), size of explants (one/ two/ three bud) and IBA treatment (0, 250, 500 and 1,000 mg/L) for concurrent *ex vitro* rooting cum hardening were studied. Results revealed that for facilitating efficient multiplication, medium should be supplemented with glucose (3%) as carbon source and *meta* topolin (1 mg/L) as cytokinin. Two-bud explant should be used for subculture as it promoted superior shoot proliferation. Concurrent *ex vitro* rooting cum hardening was possible even without auxin treatment. The present protocol could be useful for large scale production of quality planting material of this underexploited tropical species.

Keywords: *Ex vitro* rooting, Glucose, Hardening, Inoculum size, Mango ginger and Tropical

INTRODUCTION

Curcuma mangga Valeton & Van Zijp. is an important species of Zingiberaceae family, which is found distributed in Asian countries including Java, India, Thailand, Malaysia and Indonesia (Sirirugsa *et al.*, 2007; Leong-Škorničková *et al.*, 2010; Singh, 2017). In most of the native regions, young shoots and rhizomes of this underutilized species are used as vegetable, salad or medicine. It is known to be a source of curcumin, a key component in drug industries due to its numerous uses (Aggarwal *et al.*, 2007). Curcumin is found in all rhizome parts including mother, primary and secondary rhizomes in the range of 0.34 to 0.45% (Waman *et al.*, 2018). Studies suggested that the species possesses antioxidant, anticancer and anti-inflammatory activities (Liu and Nair, 2012; Malek *et al.*, 2011). Recently, a cream formulation containing *C. mangga* was found to have wound healing and anti-inflammatory activities (Suthasinee and Supinya, 2019). Further, it has been found to be a source of essential oil, which contains β-myrcene (52.4 to 65.3%) as dominant compound (Wahab *et al.*, 2011, Waman *et al.*, 2018). Considering raw mango like aroma of rhizomes, the species has good scope for use as a flavouring agent in processing industries as well.

Mango ginger, like other rhizomatous species, is propagated by using rhizome pieces. During earlier studies, seed rhizomes (15 to 25 g size) have been recommended for obtaining produce suitable for fragrance, pharmaceutical and value addition industries (Waman *et al.*, 2018). However, mango ginger is generally planted at a closer spacing of 25 to 30 cm, thereby requiring higher quantities of planting material. Micropropagation is an efficient means for large scale production of plantlets.

Earlier studies on *in vitro* culture in the species have primarily dealt with optimization of plant growth regulators for direct shoot regeneration and somatic embryogenesis (Hutami and Purnamaningsih, 2003; Raihana *et al.*, 2011; Pikulthong *et al.*, 2016). However, a number of factors are known to determine the ultimate success of a micropropagation protocol (Waman and Bohra, 2018). Carbon sources are generally added in the culture medium for providing necessary energy and to act as an osmoticum (Lipavska and Konradova, 2004). As both kind and concentration of carbon source influence *in vitro* culture response, these aspects were studied in present investigation. Further, new sources of cytokinins such as *meta* topolins have been found to be superior in several species (Escalona *et al.*, 2003; Bairu *et al.*, 2008; Woztania, 2010) and hence, its efficacy was tested in *C. mangga*. Optimization of explant size is required for obtaining maximum shoots per unit inputs (Elboullani *et al.*, 2017) and hence, was also studied here. Process of concurrent *ex vitro* rooting cum hardening (CEVRH, method in which *in vitro* derived shoots are subjected to rooting and hardening simultaneously) has been found to be superior method than *in vitro* rooting and hardening in various species (Bohra *et al.*, 2016). Hence, the present investigation was undertaken for standardization of carbon source, cytokinin, size of inoculum and CEVRH for *in vitro* culture of *C. mangga*.

MATERIALS AND METHODS

Present investigation was carried out in the Plant Tissue Culture Laboratory, Division of Horticulture and Forestry, ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India during 2018-19. Chemicals including salts of MS medium, plant growth regulators, carbon sources, gelling agents *etc*. were procured from M/s. HiMedia Laboratories, Mumbai, India. Borosilicate culture bottles of 200 ml capacity with polypropylene screw caps were used for culture incubation.

Media preparation and culture incubation

For all the experiments, MS medium was used and other inputs were added as per the experiment. pH of the medium was adjusted to 5.7 using digital pH meter (Hanna, HI2211). Medium was gelled using Clerigar (5 g/L) and 30 ml of it was dispensed into each culture bottle prior to sterilizing in autoclave (Optics Technology, Delhi) at 121 °C for 15 min. Culture bottles were wrapped with cling film and were incubated at 24 °C with photoperiod of 16:8 (light: dark) cycle. For maintaining photoperiod, 40 W fluorescent lights (Crompton Greaves, Mumbai) were used.

Collection of explants and aseptic culture establishment

Growing shoots of *C. mangga* (IC-0628643) were collected from experimental fields of the Institute. Shoots were separated after washing of clumps in running tap water to remove adhering soil. Rhizome blocks containing shoot tips were excised from the clumps and used for establishing aseptic cultures. For this, excised tissue blocks were soaked in 0.2% carbendazim (Bavistin) and streptocyclin sulphate (200 mg/L) for 20 min. It was followed by removal of thin layer of tissues from the blocks. In laminar air flow, the excised explants were washed with sterile deionized water, followed by surface sterilization using 0.1% mercuric chloride (12 min.). Such explants were rinsed thoroughly for four times with sterilized deionized water to remove traces of mercuric chloride. Explants were initiated onto basal Murashige and Skoog's (MS) medium supplemented with ascorbic acid (50 mg/L).

Effect of carbon sources and their concentrations on shoot multiplication

Effect of three types of carbon sources (glucose, fructose and sucrose) at 1 and 3% concentration was studied on culture multiplication. Medium was supplemented with 1 mg/L BAP and 0.2 mg/L NAA. Cultures obtained after first subculture were used for the experiment. Besides culture growth parameters, chlorophyll content in leaves of micro-shoots obtained from various treatments was determined by procedure described earlier (Lichtenthaler and Wellburn 1983).

Effect of cytokinins on shoot multiplication

Effect of two cytokinin *viz*. 6- benzylamino purine (BAP) and *meta* topolin (*m*T) at 1 mg/L and 2 mg/L was studied during subculture 4 and 5. Growth regulator free MS medium was used as control. During subculture 6, both levels of *m*T were compared with MS basal medium. Media also contained 0.2 mg/L naphthalene acetic acid (NAA) and glucose (3%) as carbon source. Experiment was

laid in CRD with six replications each during subculture 4 and 5 and nine replications during subculture 6.

Effect of size of explant on culture multiplication

Effect of one, two and three bud explant was studied on culture multiplication. MS medium supplemented with *m*T (2mg/L) + NAA (0.2 mg/L) + glucose (3%) was used for the experiment. Experiment was laid in CRD with ten replications each.

Effect of auxin dipping on CEVRH

Concurrent *ex vitro* rooting cum hardening was studied in which effect of three concentrations of indole-3- butyric acid (IBA, 250, 500 and 1,000 mg/ L) was compared with de-ionized water as control. Micro-shoots of *ca*. 3 cm were excised and washed with water to remove adhering agar. Basal parts of the micro-shoots were dipped in the solution (30 s) and planted in cups of 200 ml capacity filled with coir pith as a substrate. Plantlets were covered with polythene bags to maintain humidity during hardening. Experiment was laid in CRD. Survival and growth parameters were recorded after 7 weeks of transplanting. For establishment, two replications of ten plants each were used, while for growth observations, ten plants were used.

Experimental design, data collection and statistical analysis

All the experiments were laid out in completely randomized design (CRD). All the values were presented as mean ± standard error of mean. Data collected from all the experiments was subjected to analysis of variance using Web Agri Statistical Package (WASP v. 2.0, ICAR- CCARI, Goa, India). Mean separation was done using least significant difference.

RESULTS AND DISCUSSION

Effect of carbon sources and their concentrations

Influence of three carbon sources at two levels was studied on *in vitro* shoot multiplication in mango ginger (Table 1), which revealed significant differences among the treatments. Sucrose is mainly employed as a source of energy in culture medium. However, kind and concentration vary depending upon the species (Sharmin *et al.*, 2013). During the present investigation, glucose (3%) was found to support significantly superior multiplication (6.3 shoots/ culture). Though higher number of shoots was produced in media supplemented with 3% concentration than those with 1% sugars; except for glucose, none of the treatments were statistically significant. Positive effects of glucose on culture multiplication have been reported in *C. longa* (Salvi *et al.*, 2001) and banana (Bohra *et al.*, 2016).

Alteration of carbon source or its content had no influence on number of leaves per micro-shoot (3.8 to 5.8) and mean shoot length (3.6 cm to 5.5 cm). Interestingly, despite producing highest number of shoots, medium supplemented with glucose (3%) could not improve leaf production and shoot length. It was probably due to utilization of energy for shoot bud proliferation rather than for leaf production. Previous studies on banana have also suggested significant role of carbon source (sucrose/dextrose/ fructose, when used alone) on shoot multiplication,

Table 1: Effect of carbon source and their concentration on shoot multiplication in *C. mangga*.

Carbon source	No. of shoots/ explant	No. of leaves/ shoot	Mean shoot length (cm)
Fructose (1%)	1.8 ± 0.48 b	5.8 ± 0.25 a	3.8 ± 0.90 a
Fructose (3%)	3.0 ± 0.58 b	5.0 ± 0.34 a	4.8 ± 0.38 a
Glucose (1%)	2.3 ± 0.63 b	4.4 ± 1.20 a	3.6 ± 0.94 a
Glucose (3%)	6.3 ± 1.25 a	3.8 ± 0.25 a	4.5 ± 0.39 a
Sucrose (1%)	2.0 ± 0.00 b	5.0 ± 0.20 a	4.0 ± 0.27 a
Sucrose (3%)	2.8 ± 0.25 b	4.6 ± 0.31 a	5.5 ± 0.40 a

Values presented as mean ± SE. Values followed by similar alphabet in a column do not differ significantly at 5% level of significance using least significant difference

while there was no significant difference in mean shoot length (Madhulatha *et al.*, 2006).

Differences in chlorophyll content in micro-shoots were influenced by treatments (Fig. 1). Highest values of photosynthetic pigments *viz*. Chl a (1.77 mg/g), Chl b (0.54 mg/g) and total chlorophyll content (2.31 mg/g) were reported from shoots cultured on medium containing 3% glucose. This means, treatment D_3 not only produced a greater number of shoots, but also had higher accumulation of photosynthetic pigments in them. Such improvement in chlorophyll concentration with increment in quantity of carbon source in the medium has been reported by Capellades *et al.* (1991) in rose. On the contrary, the lowest values for photosynthetic pigments (0.58 mg/g, 0.15 mg/g and 0.73 mg/g) were observed in shoots derived from cultures grown on F_3. This suggested specificity of carbon source required for *C. mangga* in the present study.

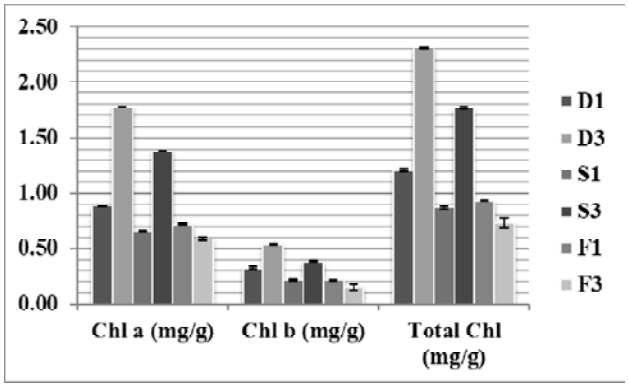

Fig. 1: Chlorophyll components (mg/g) in leaves of micro-shoots cultured on media supplemented with different carbon sources (D_1: Glucose 10 gL^{-1}; D_3: Glucose 30 gL^{-1}; S_1: Sucrose 10 gL^{-1}; S_3: Sucrose 30 gL^{-1}; F_1: Fructose 10 gL^{-1}; F_3: Fructose 30 gL^{-1}). Values are expressed as mean ± SE.

Effect of cytokinins on shoot multiplication

Cytokinins are added in culture medium to facilitate shoot proliferation and its requirement needs to be standardized for each species. In present study, performance of BAP and *m*T was tested in comparison with growth regulator free medium as control during fourth and fifth culture cycles. Even without cytokinins, culture multiplication of 4.0 and 8.2 shoots/ inoculum was possible (Table 2). However, Raihana *et al.* (2011) reported mere 1.2 to 2.3 shoots per explant in growth regulator free medium. This variation could be attributed to differences in the genotypes used in both studies, probably indicating higher concentration of endogenous cytokinins in genotype used in present study.

Though, addition of BAP supported shoot multiplication up to 7.0 (1 mg/L) and 7.7 (2 mg/L) shoots/ culture during fourth subculture, the cytokinin was not effective in inducing multiple shoot buds in subsequent cycle and values remained on par with MS basal medium (Table 2). Similar observations have been reported by Raihana *et al.* (2011) in *C. mangga*, who observed non-significant differences among cultures grown on basal medium and those grown on media supplemented with up to 11 mg/L BAP. Hence, from findings of both present and earlier report, considering the low efficacy of BAP, it should be avoided for obtaining mass multiplication in *C. mangga*. Similarly, Wojtania (2011) had encountered similar conditions in which BAP did not improve,

Table 2: Effect of cytokinins on shoot multiplication in *C. mangga* during subculture

Treatment	No. of shoots/ inoculum		No. of leaves/ shoot	
	SC 4	SC 5	SC 4	SC 5
Basal	4.0 ± 0.68 b	8.2 ± 1.30 b	2.8 ± 0.37 a	4.1 ± 0.18 a
*m*T (1 mg/l)	8.0 ± 0.73 a	13.3 ± 0.84 a	0.8 ± 0.11 c	2.4 ± 0.31 b
*m*T (2 mg/l)	9.3 ± 1.05 a	15.2 ± 1.56 a	1.0 ± 0.11 c	2.4 ± 0.16 b
BAP (1 mg/l)	7.0 ± 0.45 a	7.0 ± 0.45 b	2.1 ± 0.18 b	4.0 ± 0.29 a
BAP (2 mg/l)	7.7 ± 1.15 a	9.2 ± 1.40 b	2.0 ± 0.25 b	3.9 ± 0.35 a

SC – Sub culture, Values presented as mean ± SE. Values followed by similar alphabet in a column do not differ significantly at 5% level of significance using least significant difference

Table 3: Effect of *meta* topolin on shoot multiplication in *Curcuma mangga* during sub-culture 6

Treatment	No. of shoots/ inoculum	No. of leaves/ shoot	Mean shoot length (cm)
Basal	7.9 ± 1.05 b	4.5 ± 0.38 a	8.1 ± 0.31 a
*m*T (1 mg/l)	11.9 ± 0.79 a	3.9 ± 0.26 ab	6.4 ± 0.56 b
*m*T (2 mg/l)	13.7 ± 1.39 a	3.4 ± 0.14 b	5.9 ± 0.11 b

Values presented as mean ± SE. Values followed by similar alphabet in a column do not differ significantly at 5% level of significance using least significant difference

rather hampered the shoot proliferation process in *Pelargonium* hybrids.

Meta topolin was found to be highly efficient cytokinin in the present study. As high as 9.6 (subculture 4) and 15.2 (subculture 5) shoots/ inoculum were reported in cultures grown on medium supplemented with 2 mg/L *m*T. These values remained statistically similar with shoot multiplication observed on medium supplemented with 1 mg/L *m*T. Present findings suggested the efficacy of *m*T in mango ginger even at low concentrations. The results are in conformity with studies in *Pelargonium* hybrids, wherein *m*T was found to be more promising cytokinin whereas BAP did not show positive signs on multiplication (Wojtania 2011). Interestingly, number of leaves/ shoots showed reducing trend as shoot multiplication increased. This was more prominent in case of *m*T as these treatments induced higher bud proliferation.

During subculture 6 also, similar trend was maintained in which 13.7 and 11.9 shoots/ culture were obtained in medium supplemented with 2 mg/ L and 1 mg/L *m*T as against 7.9 shoots/culture in MS basal medium (Table 3). Superiority of *m*T in long term culture has previously been reported (Wojtania 2011), thereby suggesting the superiority of *m*T observed in present study. About 4.5 leaves/ shoot were produced in control, which remained on par with shoots obtained from 1 mg/L of *m*T (3.9 leaves/ shoot). Longest shoots (8.1 cm) were noticed in cultures grown onto basal medium and increase in *m*T concentration resulted in decrease in shoot length to 6.4 cm and 5.9 cm.

Effect of explant size on culture multiplication

Optimum explant density is one of the key determinants for success of a micropropagation protocol. In the present study, culture multiplication improved from 7.1 (one bud) to 12.9 (two bud) and 12.8 (three bud) (Fig. 2 and 3). As there was no significant difference between two and three bud explant, it is recommended to use two bud inoculum as it would reduce the explant requirement without compromising the multiplication ratio. Influence of explant density on culture multiplication has been reported in turmeric and globe artichoke (El-Hawaz *et al.*, 2015; El Boullani *et al.*, 2017).

Fig. 2: Effect of inoculum density on *in vitro* culture multiplication in *C. mangga*;
(one bud, two bud and three bud - from left to right)

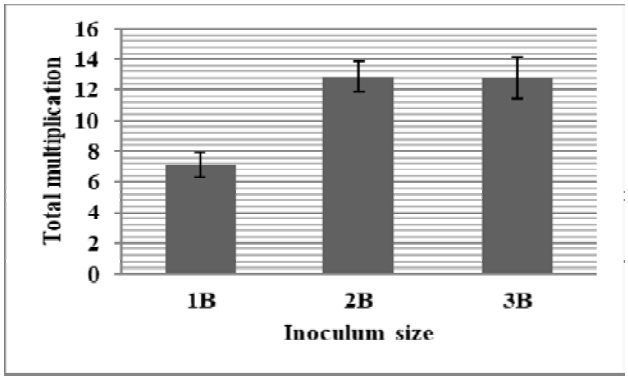

Fig. 3: Effect of explant size (1B: one bud; 2B: two bud and 3B: three bud) on culture multiplication in *C. mangga*. Values are expressed as mean ± SE.

Effect of auxin dipping on CEVRH

Single step *ex vitro* rooting cum hardening has been considered as an efficient method to reduce time, labour and other operational costs (Ranaweera *et al.*, 2013). This is particularly relevant in cases wherein planting material requirement per unit area is higher as in case of present species. During present study, root induction and hardening was possible without external auxin application (Fig. 4). In fact, auxin dip had adverse effect on these parameters. All the micro-shoots treated with deionized water (control) showed 100% establishment and rooting percentage got reduced to 60% at the highest (1,000 mg/L) concentration of IBA (Fig. 5). Being a rhizomatous species, each micro-shoot carried a small part of rhizome at the base and when transferred to the substrate *ex vitro*, it might have given the stimulus for root induction as seen in case of rhizome sett grown plants.

Fig. 4: Concurrent *ex vitro* rooting cum hardening (CEVRH): multiplied cultures (a), isolated micro-shoots (b) and micro-shoots planted in cups for hardening

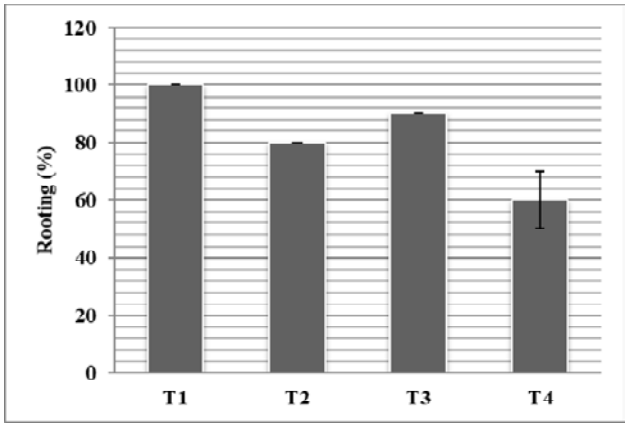

Fig. 5: Root induction percentage as influenced by auxin (IBA) dipping treatments (T_1: 0 mgL^{-1}; T_2: 250 mgL^{-1}; T_3: 500 mgL^{-1}; T_4: 1000 mgL^{-1}) during concurrent *ex vitro* rooting cum hardening (CEVRH) in *C. mangga*

Growth parameters as influenced by CEVRH have been presented in Table 4. Values amongst the treatments varied between 4.0 ± 0.46 cm and 5.0 ± 0.70 cm (shoot length), 0.50 ± 0.03 cm and 0.50 ± 0.05 cm (collar thickness), 4.0 ± 0.57 and 4.9 ± 0.55 (number of primary roots), 2.6 ± 0.21 cm and 3.2 ± 0.45 cm (mean root length), 5.2 ± 0.70 and 5.5 ± 0.71 (number of leaves per plantlet). As none of the treatments could improve the plant growth over control, it could be recommended to use *in vitro* grown micro-shoots for *ex vitro* rooting and hardening without any auxin dip. Hardened plants were transplanted in pots and all the plants grew normally.

CONCLUSION

In the present study, an efficient micropropagation protocol was developed for *C. mangga*. Various factors studied showed profound influence on the *in vitro* multiplication of the species. Use of MS medium supplemented with glucose (3%) as carbon source and *meta* topolin (1 mg/L) as cytokinin was recommended to promote superior shoot proliferation. Single step *ex vitro* rooting cum hardening was successful even in the absence of auxin treatment.

ACKNOWLEDGEMENTS

Authors are thankful to the Director of the Institute for providing necessary facilities for conducting the studies.

Table 4: Shoot and root growth parameters in mango ginger plantlets as influenced by auxin dipping during CEVRH

Auxin (IBA) concentration	Shoot Length (cm)	Collar thickness (cm)	No. of Primary roots	Mean root length (cm)	No. of leaves/ plantlet
0 mg/L	4.5 ± 0.73	0.5 ± 0.04	4.9 ± 0.55	3.2 ± 0.45	5.5 ± 0.43
250 mg/L	4.0 ± 0.46	0.5 ± 0.04	4.0 ± 0.57	2.6 ± 0.21	5.5 ± 0.71
500 mg/L	5.0 ± 0.70	0.5 ± 0.05	4.8 ± 0.70	2.6 ± 0.42	5.6 ± 0.56
1,000 mg/L	4.2 ± 0.61	0.5 ± 0.03	4.5 ± 0.43	3.1 ± 0.20	5.2 ± 0.70

Values presented as mean ± SE.

REFERENCES

Aggarwal, B.B., Sundaram, C., Malani, N. and Ichikawa, H.2007. Curcumin: the Indian solid gold. *Adv. Exp. Med. Biol.* **595:**1-75.

Bairu, M.W., Stirk, W.A., Dolezal, K. and Staden, J.V. 2008. The role of topolins in micropropagation and somaclonal variation of banana cultivars 'Williams' and 'Grand Naine' (*Musa* spp. AAA). *Plant Cell Tiss. Org. Cult.***95:**373-379.

Bohra, P., Waman, A.A., Sathyanarayana, B.N. and Umesha, K. 2016. Concurrent *ex vitro* rooting and hardening in Ney Poovan banana (*Musa* AB): effect of carbon sources and their interactions. *Erwerbs-Obstbau* **58:**193-198.

Capellades, M., Lemeur, R. and Debergh, P. 1991. Effects of sucrose on starch accumulation and rate of photosynthesis in *Rosa* cultured *in vitro*. *Plant Cell, Tissue and Organ Culture*, *25*(1): 21-26.

Elboullani, R., Lagram, K., Al Mousadik, A. and Serghini, M.A. 2017. Effect of explant density and size on the *in vitro* proliferation and growth of separated shoots of globe artichoke (*Cynara cardunculus* var. *scolymus* L.). *J. Mat. Environ. Sci.***8**:2469-2473.

El-Hawaz, R.F., Bridges, W.C. and Adelberg, J.W. 2015. *In vitro* growth of *Curcuma longa* L. in response to five mineral elements and plant density in fed-batch culture systems. *PLoS One* **10:**e0118912.

Escalona, M., Cejas, I., Gonzalez-Olmedo, J., Capote, I., Roels, S., Canal, M.J., Rodriguez, R., Sandoval, J. and Debergh, P. 2003. The effect of *meta*-topolin on plantain propagation using a temporary immersion bioreactor. *InfoMusa***12:**28-30.

Hutami, S. and Purnamaningsih, R. 2003. Augment clonal Appointment Mangga (*Curcuma mangga*) through cultured *in vitro*. *Bulletin Plasma Nutfah* **9**: 39-44.

Leong-Škorničková, J., Otakar, Š. and Karol, M. 2010. Back to types! Towards stability of names in Indian *Curcuma* L. (Zingiberaceae). *Taxon* **59:**269–282.

Liu, Y.and Nair, M.G. 2012.*Curcuma longa* and *Curcuma mangga* leaves exhibit functional food property. *Food Chemistry***135:**634–640.

Lichtenthaler, H. K., Wellburn, A. 1983. Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans*. **603**: 591-592.

Madhulatha P., Kirubakaran S.I., Sakthivel N. 2006. Effects of carbon sources and auxins on *in vitro* propagation of banana. *Biol. Plant.* **50:** 782–784

Malek, S.N.A., Lee, G.S., Hong, S.L., Yaacob, H., Wahab, N.A., Weber, J.F.and Shah, S.A.A. 2011. Phytochemical and cytotoxic investigations of *Curcuma mangga* rhizomes. *Molecules* **16:**4539–4548.

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

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

Ranaweera, K.K., Gunasekara, M.T.K. and Eeswara, J.P. 2013. *Ex vitro* rooting: A low cost micropropagation technique for Tea (*Camellia sinensis* (L.) O. Kuntz) hybrids. *Sci. Hort.* **155:**8-14.

Salvi, N., George, L. and Eapen, S. 2001. Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. *Plant Cell Tiss. Org. Cult.* **66:** 113-119.

Sharmin, S.A., Alam, M.J., Sheikh, M.M.I., Zaman, R., Khalekuzzaman, M., Mondal, S.C., Haque, M.A., Alam, M.F. and Alam, I. 2013. Micropropagation and antimicrobial activity of *Curcuma aromatica* Salisb., a threatened aromatic medicinal plant. *Turkish J. Biol.* **37:**698-708.

Singh, A.K. 2017. Revisiting the status of cultivated plant species agrobiodiversity in India: an overview. *Proc. Indian Nat. Sci. Acad.* **83:**151–174.

Sirirugsa, P., Larsen, K. and Maknoi, C.2007.The genus *Curcuma* L. (Zingiberaceae): distribution and classification with reference to species diversity in Thailand. *Gardens' Bull. Singapore* **59:**203–220.

Suthasinee, S. and Supinya, T. 2019. Anti-inûammatory and wound healing eûects of cream containing *Curcuma mangga* extract. *J.Ethnopharmacol.***238:**111828.

Wahab, I.R.A., Blagojevic, P.D., Radulovic, N.S. and Boylan, F. 2011. Volatiles of *Curcuma mangga* Val. & Zijp (Zingiberaceae) from Malaysia. *Chem. Biodiv.* **8**: 2005–2014.

Waman, A.A., Bohra, P. and Aarthi, S. 2018. Propagule size affects yield and quality of *Curcuma mangga* Val. et Zijp.: An important medicinal spice. *Ind. Crops Prod.***124:** 36-43.

Waman, A.A. and Bohra, P. 2018. Factors governing success in shoot tip culture of bananas with special reference to mixed genomic groups: an overview. *Erwerbs-Obstbau* **61:**9-21.

Woztania A. 2010. Effect of *meta*-topolin on *in vitro* propagation of *Pelargonium x hortorum* and *Pelargonium x hederaefolium* cultivars. *Acta Societatis Botanicorum Poloniae* **79:** 101-106.

(Received 19.03.2021, Revised on 21.05.2021, Accepted on 19.06.2021)