Biotechnological Communication

Biosci. Biotech. Res. Comm. 11(2): 256-262 (2018)

BBRC Bioscience Biotechnology Research Communications

An efficient protocol for *in-vitro* regeneration of *Vitex negundo* an important medicinal plant

Amit Kumar*, Mufida Fayaz, Musadiq Hussain Bhat and Ashok Kumar Jain

School of Studies in Botany, Jiwaji University, Gwalior (M.P.)-474011, India

ABSTRACT

An efficient in vitro protocol has been established for propagation of elite plant of *Vitex negundo* L. (Verbenaceae) commonly known as Nirgundi. It is a large woody aromatic and multipurpose medicinal shrub. It is used medicinally throughout the greater part of India. This species is widely used in Chinese herbal medicine and is the second most important for treatment of chronic bronchitis. Leaf extract of this plant possess antibacterial and antitumor activity. In the present study, nodal segments of *Vitex negundo* were taken as source of explants and grown on MS media with 3% Sucrose and 0.8% agar-agar, supplemented with different concentrations of BAP, KIN (0.5 – 3.5 mgl^{-1}) and TDZ (0.5-2.0), with various auxins (NAA, IBA, TIBA), incubated under a photoperiod of 16h illumination of light and 8h dark at 25±2°C. MS + 1 mgl^{-1} BAP was found to be the best concentration for shoot regeneration (90%). The regenerated shoots were sub-cultured for rooting, using different concentrations of IBA and NAA. Present optimized micropropagation protocol offers the possibility of germplasm conservation and mass cultivation of this important medicinal plant.

KEY WORDS: *VITEX NEGUNDO, REGENERATION, NODAL EXPLANT, CALLOGENESIS*

INTRODUCTION

Medicinal plants have been the subject of curiosity since times immemorial (Constable, 1990). Almost every civilization has a history of medicinal plant uses. About 80% of the people living in developing countries depend on indigenous medicines to meet their primary health care needs. About 85% of these traditional medicines involve the consumption of plant extracts. Out of 250 species of the genus *Vitex*, near about 14 species have been found to occur in India. *Vitex negundo* L. (Verbenaceae) is a perennial aromatic, large woody shrub, tri or

ARTICLE INFORMATION:

**Corresponding Author: tclabju17@gmail.com*
Received 29th March, 2018
Accepted after revision 26th June, 2018
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007 CODEN: USA BBRCBA
Thomson Reuters ISI ESC / Clarivate Analytics USA and Crossref Indexed Journal
NAAS Journal Score 2018: 4.31 SJIF 2017: 4.196

Online Contents Available at: http//www.bbrc.in/
DOI: 10.21786/bbrc/11.1/10

penta-foliate leaves with purple color flower in branched tomentose cymes. It is commonly called as Chaste tree, Nirgundi (Hindi) and Monk's pepper. It is an important agro-forestry tree (200–300 cm high) found throughout the greater part of India and has been included in the list of valuable plant species due to its wide use in the Indian system of medicine (Kapur et al., 1994). It posseses various medicinal properties (Muthuswamy et al., 2012; Basri et al., 2014; Bano et al, 2015; Lad et al., 2016).

The plant possesses anti-arthritic, hepatoprotective, anti-inflammatory, anti-allergic, insecticidal, antioxidant, antibacterial, immunomodulatory, antifungal as well as mosquito repellant activities (Islam et al., 2013; Zheng et al., 2014; Singh et al., 2015; Lad et al., 2015; Lad et al., 2016). Leaves are aromatic, used as an antifertility drug (Bhargava, 1986) and possess snake neutralizing activities, (Minu et al., 2012) (Muthuswamy et al., 2012; Durairaj et al., 2014) Dharmadasa et al., (2016) also reported the anti-snake venom properties. Leaves are antiparasitic and used as alternative vermifuge and anodyne. They are also very effective to reduce inflammatory swellings of joints in rheumatism and relieve catarrh and headache. Root is used as tonic, diuretic and expectorant. It regulates hormones, enhances breast milk production and possesses progesterogenic properties as well (Au et al., 2008; Arora et al., 2011; Basri et al., 2014; Haider et al; 2017).

Betulinic acid, ursolic acid and β-sitosterol are some of its active constituents, isolated from its leaves which have been found to possess anti-cancer, anti-HIV and angiogenic properties, respectively (Basri et al., 2014). In nature the species propagates through stem cutting and seeds. Based on our preliminary investigations propagation with vegetative cuttings is very slow and the survival rate is very limited. Propagation through seeds is hindered due to poor germination. Thus conventional propagation through seeds and vegetative cutting is not an adequate solution to meet the demand for this rare medicinal plant. Hence this study was carried out to develop an efficient protocol for its mass cultivation.

MATERIAL AND METHODS

Nodal explants were excised from elite plants of *Vitex negundo* growing in medicinal plants garden, School of Studies in Botany, Jiwaji University, Gwalior (M.P). The excised nodal explants of *V. negundo* were washed for 10 min under continuous stream of running tap water. Surface sterilization was done by treating the explants with 4% (v/v) Tween-20 (detergent; SRL, Pvt. Ltd, Mumbai, India) and rinsed with distilled water. These explants were then treated with 2% (w/v) bavistin solution (Systemic fungicide; BASP India Ltd., Mumbai India) for 5 min and followed by treatment with freshly prepared 0.1% $HgCl_2$ (SRL, Mumbai, India) for 3 min with continuous shaking under a laminar flow cabinet. These explants were finally washed 2-3 times by sterile distilled water prior to implantation in semisolid media.

The MS (Murashige and Skoog, 1962) basal medium was supplemented with 6-Benzylaminopurine (BAP), 6-Furfuryl-aminopurine (KIN), Thidiazuron (TDZ), Indole-3-butyric acid (IBA), 2,3,5-triiodobenzoic acid (TIBA), α-naphthalene acetic acid (NAA), at various concentrations and in various combinations for rhizogenesis. Full and half strength MS basal medium with IBA and NAA at different concentration was employed. All the plant growth regulators were procured from SRL and Himedia-Qualigens, SRL, Glaxo, CDH, Titan biotech and Himedia. 3% (w/v) sucrose (SRL, Mumbai, India) was used as Carbon source, solidified with 0.8% agar-agar and pH was adjusted to 5.75 using 0.1 N NaOH or 0.1 N HCl. 20 ml media (aprox.) was dispended in each 150×25 cm test tube (Borosil, India), tightly covered with air tight plastic test tube caps and sterilized by autoclaving at 1.06 $kgcm^{-2}$ at 121°C for 15 min. The explants were cultured in vertical orientation in test tubes containing semisolid medium. Cultures were maintained at 25±2 °C temperature with a relative humidity of 55±5 % under regular cycle of light (450-460 µW cm^{-2}) by cool day light emitted from fluorescent incandescent tubes (40 W, Philips &tFinolex, India) of 16 hr light followed by 8 hr dark period.

After root formation, healthy plantlets with well developed root system were removed from medium and washed under running tap water to remove the medium. These are then transferred to plastic pots (5 cm diameter) containing autoclaved mixture of soil, sand and vermicompost (1:1:1). Subsequently acclimatization was achieved by covering the plastic pots with polythene bags to maintain humidity. Plants were irrigated with $1/10^{th}$ of major salts of MS media. After 1 week, 3-5 holes are made in the poly bags. Plants were irrigated after every 5 days. The potted plants were maintained in the culture room. After 30 days the plantlets were potted in earthen pots with garden soil.

The shoot response of explants was evaluated after 35 days of culture in terms of percentage of explants producing shoots, average number of shoots per explant and average shoot length per explant. For root response, percentage of shoot producing roots, average number of roots per explant and average root length was recorded. All the values have been reported as mean value along with standard error (Mean ± SE).

RESULTS AND DISCUSSION

An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is

Table 1. Effect of growth regulators on shooting during in-vitro culture of *Vitex negundo* L. on MS media.

Cytokinins (mgl1)		% of shoot induction	Average number of shoots per explant (Mean ± SE)	Shoot Length (cm) (Mean ± SE)
Control	0	10	0.12 ± 0.01	0.72 ± 0.01
BAP	0.5	80	3.48 ± 0.34	2.64 ± 0.59
	1.0	90	4.29 ± 0.07	3.28 ± 0.31
	1.5	80	2.59 ± 0.37	2.01 ± 0.27
	2.0	70	3.11 ± 0.82	1. 81 ± 0.14
	2.5	50	2.40 ± 0.30	1.78 ± 0.07
	3.0	40	2.10 ± 0.15	1. 62 ± 0.14
	3.5	30	2.00 ± 0.03	1.50 ± 0.18
KIN	0.5	70	2.43 ± 0.03	1.86 ± 0.18
	1.0	60	2.87 ± 0.24	3.02 ± 0.24
	1.5	80	3.47 ± 0.14	3.33 ±0.08
	2.0	70	2.8 ± 0.2	2.17 ± 0.20
	2.5	60	2.62 ± 0.18	1.81 ± 0.34
	3.0	50	1.92 ± 0.20	1.61 ± 0.16
	3.5	40	1.64 ± 0.07	1.63 ± 0.16
TDZ	0.5	60	2.16 ± 0.16	1.63 ± 0.19
	1.0	50	2.4 ±0.28	1.46 ± 0.06
	1.5	30	1.75 ± 0.25	1.7 ± 0.09
	2.0	20	2.33 ± 0.33	1.43 ± 0.12

potent and has opened extensive areas of research for biodiversity conservation. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species. (Sharma *et al.*, 2010). Conventional propagation methods are unable to meet the demand of the pharmaceutical industries and drug research. Therefore, it is necessary to develop a non-conventional method for propagation to fulfill the demands of the drug market (Rathore et al. 2008). *In vitro* propagation methods offer a powerful tool for conservation of germplasm and mass-multiplication of threatened plant species (Murch et al. 2000). It helps in micropropagation of large number of plant in shorter time period, irrespective of season and serves as an alternative source of plant propagation (Yadav and Singh, 2012; Yadav et al., 2013; Groach et al., 2014). This method can be employed in multiplying important endangered plant species which are difficult to propagate by conventional means and saves the plant from the extinction.

In order to establish an efficient in vitro micropropagation protocol for commercial exploitation of this plant, nodal explants of *V. negundo* were inoculated on MS medium supplemented with varied concentration (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mgl^{-1}) of cytokinins (BAP, KIN) and TDZ (0.5-2.0 mgl^{-1}). The nodal segments cultured on growth regulator free MS medium showed minimum signs of bud break even after 15 days of inoculation. The average number of shoots induced on MS basal medium was 10% with an average shoot length of 0.72 ± 0.01 cm after 35 days of culture (Table 1).

However, addition of cytokinin was essential for differentiation of multiple shoots from the nodal explants. Of the three cytokinins tried, BAP was most effective over the other two for induction of multiple shoots. Similar effect have already been reported in various taxa like *Cassia angustifolia* (Agrawal et al., 2002), *Spilanthes acmella* (Pandey and Agrawal 2009), *Aegle marmelos* (Yadav and Singh 2011), *Tylophora indica* (Faisal et al., 2007), *Achyranthes aspera* (Ishwarya et al., 2018), *Vitex trifolia* (Ahmad and Anis, 2014).The nodal segments responded by initial enlargement of dormant axilary buds followed by bud break within a week and multiple shoot induction and proliferation within 15 days of culture on BAP containing media. 1 mgl^{-1}BAP was optimum in inducing 90% morphogenic culture with an average of 4.29±0.07 shoots per explant having an average shoot length of 3.28±0.31 cm after 35 days of culture (Table 1, Fig.1A). BAP at 3.5 mgl^{-1} displayed poor morphogenic response both in terms of average number of shoots and average

FIGURE 1. In vitro plant regeneration through nodal segment and establishment of *Vitex negundo* L. (A) Cultures showing shoots on MS medium with BAP (1.0 mgl^{-1}), (B & C) Multiple shoot development from nodal explants on MS with BAP + IBA (1+0.50 mgl^{-1}) (D) Formation of light creamy callus on TDZ (E) In vitro rooted shootlet with half MS medium + 0.75 ml^{-1} IBA (F) Acclimatized plantlets, 35 days old (G) Plants in pots.

Table 2. Effect of BAP 1 mgl1 and 1.5 mgl1 KIN in combination with different concentrations of auxins on shoot bud regeneration from nodal explants of *Vitex negundo*

Concentration of growth regulators in mgl1	IBA	TIBA	Percentage of explants producing shoots (%)	Number of shoots per explant (Mean ± SE)	Shoot Length (cm) (Mean ± SE)
BAP 1	0.25		60	2.66 ± 0.33	3.23 ± 0.09
	0.50		90	6.12 ± 0.63	3.85 ± 0.21
	0.75		70	3.14 ± 0.40	3.78 ± 0.39
		0.25	50	2.8 ± 0.37	3.02 ± 0.17
		0.50	70	3.5 ± 0.42	3.53 ± 0.08
		0.75	60	2.5 ± 0.34	3.28 ± 0.10
KIN 1.5	0.25		70	4.37 ± 0.41	2.83 ± 0.26
	0.50		80	5.88 ± 0.38	4.13 ± 0.43
	0.75		60	4.16 ± 0.60	3.18 ± 0.17
		0.25	60	4.33 ± 0.42	3.21 ± 0.20
		0.50	80	5.2 ± 0.53	3.71 ± 0.15
		0.75	70	4.38 ± 0.47	3.32 ± 0.12

Results were recorded after 35 days and are presented as Mean ± Std. Error.

Table 3. Effect of growth regulators on rooting pattern of *Vitex negundo* L. during *in-vitro* culture (full and ½ strength of MS media)

Growth regulator (mgl1)		Percentage of explants producing roots (%)	Average number of roots per explants	Average root length (cm)
MS Full + IBA	0.50	70	5.71 ± 0.71	1.88 ± 1.20
	0.75	80	6.75 ± 0.52	2.33 ± 2.21
	1.0	60	4.33 ± 0.42	1.86 ± 2.10
MS half + IBA	0.50	80	7.57 ± 0.92	3.25 ± 3.76
	0.75	90	12.12 ±0.83	4.98 ± 2.50
	1.0	70	8.9 ± 0.89	4.53 ±3.92
MS full + NAA	0.50	70	4.85 ± 0.76	1.32 ± 0.77
	0.75	80	6.25 ± 0.59	2.23 ± 2.62
	1.0	60	4.33 ± 0.42	1.71 ± 3.37
MS half + NAA	0.50	70	6.8 ± 0.86	1.74 ± 0.07
	0.75	90	8.42 ± 0.89	2.66 ± 0.30
	1.0	60	7.33 ± 0.66	1.95 ± 0.13

shoot length (Table 1). On increasing the concentration of BAP, induction of multiple shoots was comparatively low and average shoot length too decreased (Table 1). Except BAP, all the tried concentrations of KIN and TDZ showed poor morphogenic response in term of average number of induced shoots and shoot length (Table 1). Considerable callusing at the basal cut end of nodal segment along with formation of multiple shoots was also reported in the present study which agrees with the study on *Azadirachta indica* (Arora et al., 2010) which showed similar results (Fig.1 D). The formation of callusing at the basal cut ends of nodal segment due to the action of accumulated auxins at the basal cut proliferation, especially in the presence of cytokinins (Marks and Simpson, 1994). The present study also revealed the synergistic effect of BAP in combination of auxin for effect shoot regeneration which has also been reported in studies of *Celastrus paniculatus* (Lal et al., 2010).

The highest number of shoots (6.12±0.63) developed was observed in MS with BAP 1 + IBA 0.50 mgl^{-1} (Table 2 Fig.1 B & C). The highest proliferation rate (90%) was also found at the same combination of plant growth regulators in the medium.

The best results were observed on a medium containing BAP and IBA which is supported by earlier studies in *Chonemorpha grandiflora* (Nishitha et al., 2006), *Vitex negundo* (Ahmad and Anis, 2011), *Launaea cornuta* (Ambajo and Matheka, 2016). *Mimosa pudica* (Bianchetti et al., 2017) *Tylophora indica* (Najar et al., 2018), *Ceropegia juncea* (Binish, 2018), In these studies also synergitic effects were observed when Cytokinin was used in combination with auxin. Among the two different types of auxins employed for root induction on in vitro excised shoots of *V. negundo*, IBA was found to be most effective. A maximum of 90% shoots induced an average of 12.12±0.83 roots with an average root length of 4.98±2.50cm after 3 weeks on half strength MS medium augmented with 0.75 mgl^{-1} IBA (Table 3). The roots were induced directly from the shoot base without callus formation at this concentration. (Table 3, Fig.1 E). Similar responses have been already reported in *Spilanthes acmella* (Pandey and Agrawal 2009, Yadav and Singh 2010), (Reddy et al., 2014), *Ceropegia juncea* (Binish, 2018),.However, at higher concentration of IBA, the number of roots and root length showed decline. Compared to IBA, poor rooting response was observed at the concentration of IBA + full MS and NAA + full and half MS. The tissue culture derived plantlets (Fig.1 F & G) were acclimatized in the field condition with 90% survival. Such micropropagated plants were found to be morphologically similar to the mother plant.

An efficient protocol has been developed for regeneration of *Vitex negundo* which offers a great potential to cater the needs of different pharmaceutical industries. In the present study, enhanced *in vitro* regeneration of plants with combination of plant growth regulators such as BAP, KIN, TDZ, IBA and TIBA was observed. This will be helpful in understanding the callogenesis and organogenesis through the nodal explants and to facilitate the mass propagation of *Vitex negundo*.

ACKNOWLEDGEMENTS

The authors are thankful to Director, Institute of Ethnobiology and Head, School of Studies in Botany, Jiwaji University Gwalior (M.P.) India, for providing necessary facilities to carry out this work.

REFERENCES

Constable F. (1990). Medicinal plant biotechnology. Planta Med. 56, 421-425.

Kapur V, Pillai KK, Hussain SZ and Balani DK. (1994). Hepatoprotective activity of Jigrine (An Unani polypharmaceutical herbal formulation) on liver damage and lipid peroxidation caused by alcohol-carbon tetrachloride and paracetamol in rats. Indian J. Pharm. Sci.5, 160.

Muthuswamy U, Kuppusamy A, Nandagopi U, Thirumalaisamy S and Varadharajan S. (2012). Protective effect of the leaves of *Vitex negundo* against ethanol-induced cerebral oxidative stress in rats. Tanzania J. Health Res. 14(1), 1-11.

Basri F, Sharma HP, Firdaus S, Jain P and Ranjan A. (2014). A Review of Ethnomedicinal Plant - *Vitex negundo* Linn. International Journal of Advanced Research. 2(3), 882-894.

Bano, Jabeen A, Ahmed A and Siddiqui MA. (2015). Therapeutic Uses of *Vitex nigundo*. World Journal of Pharmaceutical Research. 4(12), 589-606.

Lad H, Joshi A, Dixit D, Sharma H and Bhatnagar D. (2016). Antioxidant, genoprotective and immunomodulatory potential of *Vitex negundo* leaves in experimental arthritis. Oriental Pharmacy and Experimental Medicine. 16(3), 217-224.

Islam S, Akhtar M, Parvez S, Alam J and Alam FM. (2013). Antitumor and antibacterial activity of a crude methanol leaf extract of *Vitex negundo* L. Archives of Biological Sciences. 65(1), 229-238.

Zheng CJ, Zhao XX, Ai HW, Lin B, Han T, Jiang YP, Xing X and Qin LP. (2014) Therapeutic effects of standardized *Vitex negundo* seeds extract on complete Freund›s adjuvant induced arthritis in rats. Phytomedicine. 21(6), 838–846.

Singh H, Dixit A, et al. (2015) Comparative evaluation of total phenolic content, total flavonoid content and DPPH free radical scavenging activity of different plant parts of *Vitex negundo*. Int. J. Pharm. Pharm. Sci. 7, 144–147.

Lad H, Dixit D, Joshi A and Bhatnagar D. (2015). Antioxidant and antiinflammatory effects of *Vitex negundo* on Freund's complete adjuvant induced arthritis. Int. J. Pharma. Pharmaceutical Sci. 7, 81–85.

Bhargava SK. (1986). Antifertility effects of the flavanoids of *Vitex negundo* L., seeds in dogs, Planta Med. Phytother. 20, 188-198.

Minu V, Harsh V, Ravikant T, Paridhi J and Noopur S. (2012). Medicinal plants of Chhattisgarh with anti-snake venom property. Int. J. Curr. Pharm. Rev. Res. 3(2), 1-10.

Durairaj B, Muthu S and Shreedhar K. (2014). *In vitro* antivenom and antioxidant potential of *Vitex negundo* leaves (green and blue) against Russell's viper (*Daboia russelli*) and Indian cobra (*Naja naja*) venom. Eur. J. Exp. Biol.4 Suppl 4, 207-19.

Dharmadasa RM, Akalanka GC, Muthukumarana PRM, Wijesekara RGS. (2016). Ethnopharmacological survey on medicinal plants used in snakebite treatments in Western and Sabaragamuwa provinces in Sri Lanka. Journal of Ethnopharmacology. 179, 110–127.

Au DT, Wu J, Jiang Z, Chen H, Lu G and Zhao Z. (2008). Ethnobotanical study of medicinal plants used by Hakka in Guangdong, China. Journal of Ethnopharmacology. 117, 41-50.

Arora V, Lohar V, Singhal S and Bhandari A. (2011). *Vitex negundo* - A Chinese Chaste Tree. International Journal of Pharmaceutical Innovations. 1(5), 9- 20.

Haider A, Spellman C and Mok M. (2017). Prolactin-secreting Microadenoma masked by a herbal product, Vitex Agnus-Castus. AACE Clinical Case Reports. 3(1), e51-e53.

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

Sharma S, Rathi N, Kamal B, Pundir D, Kaur B and Arya S. (2010). Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology-a review. Agriculture and Biology Journal of North America. 1(5), 827-833.

Rathore MS, Dagla HR, Singh M and Shekhawat NS. (2008). Rational development of in vitro methods for conservation, propagation and characterization of *Caralluma edulis*. World Journal of Agricultural Science. 4, 121-124.

Murch SJ, Choffe KL, Victor JMR, Slimmon TY, Raj K, Saxena PK (2000). Thiazuron-induced plant regeneration from hypocotyls cultures of St. John's wort (*Hypericum perforatum* L. cv. Anthos). Plant Cell Reports. 19, 576-581.

Yadav K and Singh N. (2012). Factor influencing in vitro regeneration of Liquorice (*Glycyrrhiza glabra* L.). Iranian Journal of Biotechnology. 10, 161-167.

Yadav K, Aggarwal A and Singh N. (2013). Arbuscular mycorrhizal fungi (AMF) induced acclimatization, growth enhancement and colchicine content of micropropagated *Gloriosa superba* L. plantlets. Industrial Crops and Products. 45, 88–93.

Agrawal V, Prakash S and Gupta SC. (2002). Effective protocol for in vitro shoot production through nodal explants of *Simmondsia chinensis*. Biol. Plant. 45, 449–453.

Pandey V and Agrawal V. (2009). Efficient micropropagation protocol of *Spilanthes acmella* L. possessing strong antimalarial activity. In Vitro Cell Dev. Biol. Plant. 45, 491–499.

Yadav K and Singh N. (2011). In vitro propagation and biochemical analysis of field established wood apple (*Aegle marmelos* L.), AnaleleUniversitatii din Oradea, Fascicula Biologis. 18, 23-28.

Faisal M, Ahmad N and Anis M. (2007). An efficient micropropagation system for *Tylophora indica*: an endangered medicinally important plant, Plant Biotechnology Reports.1(3), 155-161.

Ishwarya M, Gayathri M, Ezhumalai M, Prakash GKU, Tamilarasan S and Ramakrishnan S. (2018). An Efficient Micropropagation of *Achyranthes aspera* L. using shoot tip explant. Int. J. Curr. Res. Biosci. Plant Biol. 5(2), 76-84.

Ahmed R and Anis M. (2014). Rapid in vitro propagation system through shoot tip cultures of *Vitex trifolia* L.–an important multipurpose plant of the Pacific traditional Medicine. Physiology and Molecular Biology of Plants. 20(3), 385-392.

Arora K, Sharma M, Srivastava J, Ranade SA and Sharma AK. (2010). Rapid in vitro cloning of a 40-year-old tree of *Azadirachtaindica* A. Juss. (Neem) employing nodal stem segments. Agroforest. Syst. 78, 53-63.

Marks TR and Simpson SE. (1994). Factor affecting shoots development in apically dominant Acer cultivars in vitro. J. Hortic. Sci. 69, 543–551.

Lal D, Singh N, Yadav K. (2010). *In vitro* studies on *Celastrus paniculatus*.J. Trop. Med. Plant. 11, 169–174.

Nishitha IK, Martin KP, Ligimol, Beegum AS, Madhusoodanan PV. (2006). Micropropagation and encapsulation of medicinally important *Chonemorpha grandiflora*. In Vitro Cell Dev. Biol. Plant. 42, 385–388.

Ahmad N and Anis M. (2011). An efficient in vitro process for recurrent production of cloned plants of *Vitex negundo* L. Eur. J. Forest Res. 130, 135–144.

Ambajo F and Matheka JM. (2016). Micropropagation of *Launaea cornuta*-an important indigenous vegetable and medicinal plant. African Journal of Biotechnology. 15(32), 1726-1730.

Najar RA, Fayaz M, Bhat MH, Bashir M, Kumar A and Jain AK. (2018). An efficient micropropagation protocol for direct organogenesis from nodal explants of medicinal climber, *Tylophora indica*. Biosci. Biotech. Res. Comm. 11(1), 144-153

Binish T. (2018). Micropropagation of traditional medicinal plant *Ceropegia juncea*. Annals of Plant Sciences. 7(2), 1992-1996.