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In Vitro Regeneration of the Medicinal Herb, *Merremia Tridenteta* L. From Shoot Tip and Flower Explants

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Indirect shoot regeneration of Merremia tridentata was established from shoot tip and flower by culturing on MS medium supplemented with auxins (IAA and 2, 4-D) and Cytokinins (BA and Kn). Present protocols shows effective sterilization methods in treatment of explants with 70% ethanol for 2 to 3 minutes, followed by rinsing thrice with double distilled water, transferred to 1% sodium hypochlorite containing 4 drops of tween-60 for 15 to 20 minutes. Weekly subculturing to the media with same composition produced better results. Enormous amount of calli having regenerative potential were formed from the explants when cultured on MS medium supplemented with 1 mg/l 2,4-D and 1 mg/l BA. Of different concentrations and combinations tested, MS medium containing 3 mg/l BA + 0.5 mg/l IAA produced highest number of shoots per gram calli. Rooting was more at concentration of 1 mg/l IAA. The plantlets shown 80% survival rate while transferring from in vitro to natural environment.

Keywords: *Merremia tridentata, in vitro, shoot tip and flower explants.*

1. INTRODUCTION

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine [1]. About eighty percent of the world population depends on herbal based alternative systems of medicine. Herbal medicine is now expanding its base at a faster rate due to the great inputs from ethno medicinal practices being pooled from all over the world [2]. Medicinal herbs are moving from fringe to mainstream use as a greater number of people endeavor to opt for herbal formulations over the allopathic compounds, since these are devoid of side effects and cost effective [3].

Donald and Kent, 1991 proposed that nearly 700 species of plants might become extinct by the year 2000 [4]. The number faced with this overwhelming prospect, plant conservationists must take advantage of every technique available. Development of micropropagation protocol is a pre-requisite for *in vitro* conservation [5]. Due to large demand of phytomedicines, plant tissue culture techniques are now being used to obtain large number of diverse medicinal plants monitoring their respective secondary metabolites [6, 7]. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties [8].

Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry [9]. Studies on plant secondary metabolites have been increasing over the last 50 years. The molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of pharmaceuticals [10]. The great chemical diversity and intraspecific variability of secondary metabolism is the result of processes of natural selection that act upon highly variable chemical structures [11].

Merremia tridentata is an important medicinal plant, belonging to Convolvulaceae family, used for curing bone fracture and piles. The aerial parts of

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the plant were also having wound healing activity [12]. *Merremia tridentata* is a climbing weed with slim, grayish leaves, white flowers and seed capsules. Herbs perennial, prostrate or tips ± twining [13,14].

2. MATERIALS AND METHODS

Collection of Plant Materials: The plant material is largely found in Gajuwaka region of Visakhapatnam District, India. Whole plant was collected during rainy season and the experiments are conducted.

Preparation of Culture Media: Double distilled water was used for the preparation of culture media. After addition of all constituents of media, the pH was adjusted to 5.8 using 0.1 N KOH or 0.1 N HCl. Gelling agent (agar-agar) was added as per requirement and the medium was steamed to melt the gelling agent. It was then dispensed into test tubes (20 ml per tube) or Erlenmeyer flasks (100 ml per 250 ml flask) or screw capped bottles (50 ml per bottle) and was autoclaved at 121 °C at a pressure of 15 lbs for 20 min. When no gelling agent was added, each tube contained liquid medium with a filter paper bridge of Whatman No. 1 filter paper. Heat labile constituents like hormones were filter-sterilized by passing through a Millipore membrane (0.22 µm pore size) ("Millipore Corporation", USA) and added aseptically to the autoclaved medium just before gelling. All the plant growth regulators used during the course of the present work were added to the medium. 15 to 20ml medium were poured in 85mm sterile glass petriplates in a laminar flow hood.

Explants: Healthy explants such as shoot tips and young flowers of *Merremia tridentata* were selected for tissue culture.

Surface Sterilization: The explants were washed thoroughly under running tap water, followed thrice with distilled water and submerged for 3 min in 70% ethanol. The explants were again rinsed thrice in sterile, double distilled water and are then submerged in 5% sodium hypochlorite for 10 minutes. Finally the explants are rinsed thrice in sterile, double distilled water. After the surface sterilization the explants were cultured on different nutrient media under aseptic conditions.

Control of Phenolic Exudation: To control the phenolic exudation in the cultures of *Merremia tridentata*, activated charcoal (1%) is used in culture media. Periodic subculturing (at weekly intervals) to fresh media with same compositions were also tested to overcome the problem.

Indirect Shoot Regeneration: Shoot regeneration potential via callus phase of different explants (shoot tips and flower) of *Merremia tridentata* were studied by culturing on MS medium fortified with different combinations of auxins and cytokinins.

Growth and Maintenance of Plant Tissue and Cell Cultures: The callus and shoot cultures on solid media were subcultured at regular intervals of 1-2 weeks. Observations were taken at the same time.

Rooting of *in vitro* Shoots: For root induction in *Merremia tridentata*, the shoots (> 3 cm) were excised from primary cultures and cultured on semi solid MS medium supplemented with NAA (0.1 - 3mg/l) and IAA (0.1 - 3mg/l) individually.

Acclimatization and Transfer of Plantlets to Field: The plantlets, regenerated through various *in vitro* techniques, with healthy root and shoot systems were taken out from the culture medium and washed gently with sterile distilled water for removing all traces of medium from the plantlet. The washed plantlets were transferred to small plastic cups containing sterile sand. The pots were then covered with polythene bags to maintain high humidity and kept in plant growth chamber. The plantlets were moistened with water 2 times/day. After fifteen to twenty days, the polythene bags were removed and transferred to larger pots containing sterile sand and soil (1:1 ratio) and kept under shade in the net house for another two weeks before transferring to field.

3. RESULTS

Tissue culture methods have been employed as an important aid to conventional methods of plant improvement [15]. The explants viz. shoot tips and flower of *M.tridentata* are induced to organogenesis through callus formation on different media supplemented with various hormonal combinations (Figure 1 and 2). These are Efficient and suitable protocols for micropropagation and regeneration developed by *in vitro* techniques.

The sterilization was carried out by dipping the plant material into 70% [v/v] ethanol for 2-3

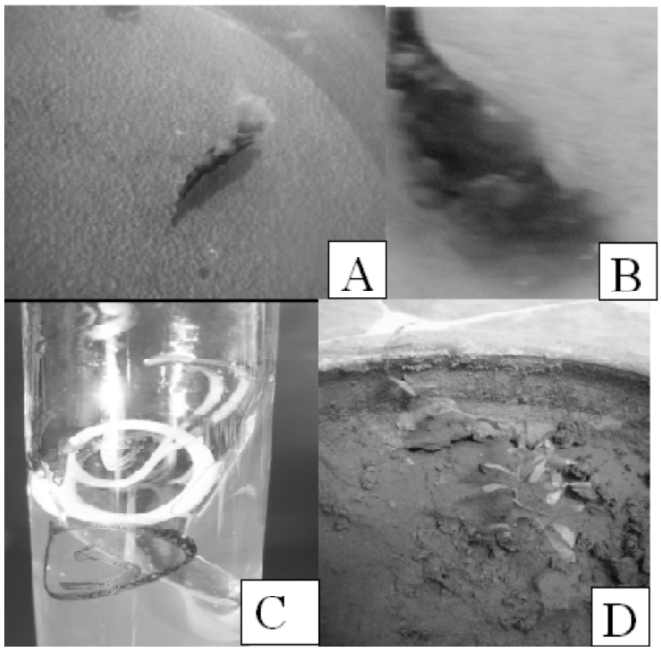

Figure 1: Micropropagation through indirect plant regeneration from flower of M. tridentata

(A) Flower on MS+2, 4-D (1mg/l) +BA (1mg/l) for callus induction.
(B) Growth of callus forming embroides on MS+2, 4-D (1mg/l) +BA (1mg/l). (40 days old)
(C) Indirect shoot regeneration on MS+ BA (2.0 mg/l) + IAA (0.5 mg/ml).
(D) Hardened plant under field conditions in pot.

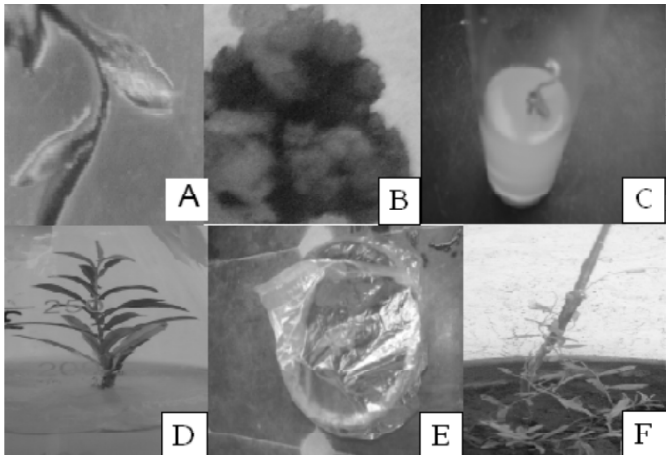

Figure 2: Micropropagation through indirect plant regeneration from shoot tip of M.tridentata

(A) Shoot tip on MS+2, 4-D (1mg/l) +BA (1mg/l) for callus induction.
(B) Pale green friable callus induced on MS+2, 4-D (1mg/l) +BA (1mg/l). (20 days old)
(C, D) Indirect shoot regeneration on MS+ BA (2.0 mg/l) + IAA (0.5 mg/ml).
(E) Plantlet under hardening.
(F) Hardened plant under field conditions in pot.

minutes, whereupon it was transferred to a solution of 1% sodium hypochlorite containing 4 drops of Tween-60 for 20 minutes was shown most effective.

Addition of 1% activated charcoal was an effective method to overcome the problem of phenolic exudation but the addition retarded growth in cultures; hence it was not used in subsequent experiments. Weekly sub culturing to the medium of same composition produced best results.

The shoot regeneration potential through callus phase from shoot tip and flower to various concentrations and combinations of growth regulators in MS medium was studied.

Callus Induction

For callus induction, the explants were cultured on MS medium supplemented with IAA, 2, 4-D, BA, Kn either alone or in combinations (Table 1 and 2).

Table 1
Effect of Growth Regulators (alone) on Callus Induction from Various Explants of Merremia Tridentata

Growth regulators (mg/l)		*Explants*	
		Node	*Immature flower*
IAA			
	0.5	+	+
	1.0	+	+
	2.0	++	++
	3.0	+++	+++
2,4-D			
	0.5	+	+
	1.0	++	++
	2.0	++	++
	3.0	+++	+++
BA			
	0.5	-	-
	1.0	+	-
	2.0	++	+
	3.0	++	++
Kn			
	0.5	-	-
	1.0	-	-
	2.0	+	-
	3.0	+	+

Table 2
Effect of Growth Regulators (combinations) on Callus Induction from Various Explants of *Merremia tridentata*

Growth regulators (mg/l)		*Explants*	
		Node	*Immature flower*
2,4-D+BA			
0.5	0.5	+	+
1.0	0.5	+	+
0.5	1.0	++	++
1.0	1.0	+++	+++
2,4-D+kn			
0.5	0.5	+	+
1.0	0.5	++	+
0.5	1.0	++	++
1.0	1.0	++	++
IAA+kn			
0.5	0.5	-	-
1.0	0.5	-	-
0.5	1.0	+	-
1.0	1.0	+	+

-= no callus + = little ++ = Moderate +++ = profuse
Data from 20 replicates in two experiments
Growth period 60 days

Effect of IAA

MS medium supplemented with IAA at the range of 0.5 to 3.0 mg/l induced the callus formation in explants within 15 days. Higher concentrations of IAA (upto 3 mg/l) was effective for callus induction and concentrations beyond 3mg/l did not yield higher amount of callus induction. The calli were green and friable. Leaving the calli in the medium turned brown and dried within 4 months.

Effect of 2, 4-D

MS medium supplemented with 2,4-D at the range of 0.5 to 3.0 mg/l induced the callus formation in shoot tips and flower explants within 15 days. Higher concentrations of 2, 4-D (≥ 3 mg/l) was effective for callus induction on shoot tips and flower explants. The calli were green and friable. Leaving the calli in the medium turned brown and dried within 5 months.

Effect of BA

MS medium supplemented with BA at the range of 0.5 to 3.0 mg/l was tested for the induction of callus formation in shoot tips and flower explants. Lesser concentrations of BA (0.5 mg/l in shoot tip and upto 1 mg/l in flower) had not induced callus. Little and moderated induction of callus was observed upto 3 mg/l and did not show any progressive change in callus proliferation beyond 3 mg/l on shoot tip and flower explants. The calli were green and friable.

Effect of Kn

When explants were treated with varying concentrations of Kn 0.5 to 3 mg/l, calli were not induced. Very little induction of callus was observed at 2-3mg/l in shoot tip and 3mg/l for flower explants.

Effect of 2,4-D+BA

Synergistic effects of growth regulators on explants were cultured with 2,4-D (1.0 mg/l) and BA (1.0 mg/l) for callus induction of *M.tridentata*. It was noticed that enormous amount of calli was produced from explants with 1mg/l of 2,4-D and 1mg/l of BA. The calli were green in flower, pale green in shoot tip, friable and meristematic. These calli were used for regeneration on shoot induction medium.

Effect of 2,4-D+Kn

MS media with growth regulators 2, 4-D (0.5-1.0 mg/l) and Kn (0.5-1.0 mg/l)) on shoot tips and flower explants were cultured for callus induction of *M.tridentata*. Moderate amount of Calli were produced from explants when treated with 0.5mg/l of 2, 4-D + 1mg/l of BA and 1mg/l of 2, 4-D + 1mg/l of BA. The calli were pale green, friable and meristematic.

Effect of IAA+Kn

MS medium containing the combinations of IAA and Kn was found low effect in callus induction.

Callus Regeneration

Calli obtained from shoot tips and flower on MS medium supplemented with 1mg/l of 2, 4-D and 1mg/l of BA were selected for regeneration and different concentrations of 2,4-D and BA were tried to find out optimum growth regulator combination on MS medium for callus regeneration. A combination of BA (3.0 mg/l) + IAA (0.5 mg/l) on MS medium produced highest number of shoots (2) per gram calli. The tests made on two cytokinins (BA and Kn), showed that BA (1 mg/l) effectively regenerated shoots from calli while Kn with MS

medium showed ineffectiveness for regeneration of shoots from the calli.

Only the callus induced growth regulators (BA + 2, 4-D and BA) on MS medium were found to be regenerative on subsequent cultures. The regeneration capacities of various calli were tested by subculturing on MS medium supplemented with BA (3 mg/l) and IAA (0.5 mg/l) and was listed in Table 3. Weekly transfer of the callus to medium with the same composition can retain Morphogenic potential of the calli. Green colored calli, which turned dark brown, and explants turning dark brown color never showed regeneration.

Table 3
Effect of Growth Regulators on Callus Regeneration of *Merremia Tridentata*

Growth regulators (mg/l)		*% Response*	*Mean±SE*
BA			
0.5		65	1.40±0.27
1		75	1.75±0.27
2		75	1.90±0.29
3		80	1.55±0.23
NAA	Kn		
0.5	3.0	No shoot formation was observed	
BA	IAA		
0.5	1.0	70	1.65±0.30
1.0	0.5	70	1.60±0.30
2.0	0.5	80	1.95±0.29
3.0	0.5	85	2.05±0.30

Rooting *in Vitro*

Individual shoots with ≥3 cm were excised and cultured on MS medium supplemented with IAA (0.1 - 3.0 mg/l). Rooting was more at IAA concentration of 1 mg/l and subsequently decreased with an increase in the concentration of IAA. Response percentage was also more at 1mg/l of IAA and the results are given in Table 4.

Table 4
Effect of Auxin (IAA) on in Vitro Rooting of Merremia Tridentata

Growth regulators (mg/l)	*% Response*	*Mean±SE*
IAA		
0.1	60	1.45±0.31
0.3	65	1.60±0.31
0.5	80	2.05±0.30
1.0	85	2.25±0.30
2.0	70	1.75±0.31
3.0	75	1.75±0.30

Acclimatization and Transfer of *in Vitro* Grown Plants to Field Conditions

Healthy plantlets that are regenerated through *in vitro* technique were washed with sterile distilled water and transferred to small plastic pots containing sterile sand. The pots were covered with glass bottles/polythene bag for the maintenance of high humidity. The plantlets were watered daily. Polythene bag/glass bottles were removed after 30 days. The plantlets produces new leaves within 10 days were transferred to large pots containing sand and soil in 1: 1 ratio. Morphologically there was no variation between the *in vivo* plants and *in vitro* grown plants. The plantlets shown 80% survival rate while transferring from *in vitro* to natural environment.

4. DISCUSSION

Merremia tridentata is a perennial wiry herb with woody rootstock and slender glabrous angular elongate stems. The plant founds throughout India, on hedges and open wastelands. To define and describe the future tasks of phytomedicinal research in the new millennium, an analysis not only of the current state of development of phytomedicinal research but also of chemosynthetic pharmaceutical research [16].

Tejavathi and Purushothama (2004) developed a protocol on *Evolvulus alsinoides* L.. Stem, leaf, pedicel and flower buds were cultured on MS medium supplemented with various growth regulators (including IAA, IBA, NAA, 2, 4-D, kinetin, benzyladenine and isopentenyladenine) either alone or in combination [17]. The efficiency of ethanol, sodium hypochlorite, and Tween in surface sterilization was previously reported in some medicinal plants like - *Citrus aurantifolia* (lime) [18], *Polygonum multiflorum* [19], *Scrophularia yoshimurae* [20] and *Aframomum corrorima* [21].

Addition of activated charcoal to overcome the problem of phenolic exudation was effective to get best results. The use of activated charcoal to overcome phenolic exudation was previously reported in *Tinospora cordifolia* [22], *Gloriosa superba* [23] and *Dioscorea bulbifera* [24].

For callus induction, the explants were cultured on MS medium supplemented with IAA, 2, 4-D, BA, Kn either alone or in combinations. Profuse and

higher amount of calli was produced from explants with 1mg/l of 2, 4-D and 1mg/l of BA. Previous reports have been made with 1mg/l of 2, 4-D and 1mg/l of BA for the regeneration from inflorescence pieces of *Bowiea volubilis* [25].

Calli obtained from shoot tip and flower of *M.tridentata* selected for regeneration studies to find out optimum growth regulator combination on MS medium for callus regeneration. A combination of BA (2.0 mg/l) + IAA (0.5 mg/l) on MS medium produced highest number of shoots per gram calli. BA. Previous reports has been made with 1mg/l of 2, 4-D and/or 1mg/l of BA for the regeneration in *Acacia nilotica* subspecies *indica* Brenan [26], *Acacia mearnsii* [27], MS + 0.1 mg/l BA + 0.2 mg/l indole-3-acetic acid in *Bacopa monniera* (L.) Wettst [28], *Ricinus communis* L. [29] and *Melissa officinalis* L. with 5.71 µM indole-3-acetic acid + 6.66 µM 6-benzyladenine [30].

Rooting in *M.tridentata* was more at IAA concentration of 1 mg/l. The reports from previous protocols has been made in various plants such as *Aegle marmelos* (L.) Corr. -0.5 mg l^{-1} IAA [31], *Coleus forskahlii* Briq- IAA (1.0 mg/l) [32], *Acacia catechu* - IAA at 3.0 mg/l [33], *Ocimum gratissimum* L. [34] and *Spilanthes mauritiana* DC. [35].

The plantlets shown 80% survival rate while transferring from *in vitro* to natural environment. Survival rate was relatively low (20 to 30%) from axillary buds of *Prunus mume* Sieb. et Zucc. [36], *Alpinia purpurata*- 95% survival rate [37], *Cymbidium ensifolium* var. *Misericors* -survival rate to 80% [38], *Citrus aurantifolia* - Over ninety per cent of plantlets (76 out of 83 plantlets) survived acclimatization [18].

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References

[1] Lucy Hoareau and Edgar J. DaSilva, Medicinal Plants: a Re-emerging Health Aid, *Electronic Journal of Biotechnology*, 2(2), (1999), 56-70.

[2] Daniel M., Medicinal Plants: Chemistry and Properties, Science Publishers, USA, 1, (2006), 9-10.

[3] Dubey N. K., Rajesh Kumar and Pramila Tripathi, Global Promotion of Herbal Medicine: India's Opportunity, Current Science, 86(1), (2004), 37-41.

[4] Donald A. Falk and Kent E. Holsinger, Genetics and Conservation of Rare Plants, Oxford University Press US, (1991). 195.

[5] Tyagi R. K. and Prakash S., Clonal Propagation and in Vitro Conservation of Jojoba (*Simmondsia chinenesis* (Link) Schneider), *Indian J. Plant Genet. Resour.*, 14, (2001), 298-300.

[6] Merkle S. A. and Nairn C. J., Hardwood Tree Biotechnology, *In vitro* Cellular and Developmental Biology – Plant, 41(5), (2005), 602–619.

[7] Rajasekharan P. E., Ganeshan S. and Bhaskaran S., Conservation of Endangered Medicinal Plants; Challenges and Optiona, *Indian J. Pl, Genet. Resour.*, 14, (2001), 296-297.

[8] Marjorie Murphy Cowan, Plant Products as Antimicrobial Agents, *Clinical Microbiology Reviews*, 12(4), (1999), 564-582.

[9] Hostettmann K. and Hamburger M., Bioactivity in Plants: the Link between Phyto-chemistry and Medicine, Phytochem., 30(12), (1991), 3864-3874.

[10] Ramachandra Rao S. and Ravishankar G. A, Plant Cell Cultures: Chemical Factories of Secondary Metabolites, *Biotechnology Advances*, 20, (2002), 101-153.

[11] Thomas Hartmann, Diversity and Variability of Plant Secondary Metabolism: A Mechanistic View, Entomologia Experimentalis et Applicata, 80(1), (1996), 177-188.

[12] Hatapakki, B. C., V. Hukkeri, D. N. Patil and M. J. Chavan, Wound Healing Activity of Aerial Parts of *Merremia Tridentate*, Indian Drugs, 41, (2004), 532.

[13] Fang Rhui-cheng and George Staples, Convolvulaceae. In: *Flora of China* z—Wu ZY, Raven PH, eds. Beijing: Science Press. Gentianaceae through Boraginaceae. 16, (1995), 271–325.

[14] Skinneria Choisy and Spiranthera Bojer, *Merremia dennstedt ex Endlicher*, Gen. Pl. 1: 1403. 1841, nom. cons. FOC, 16, (1995), 291-299.

[15] George E. F., Plant Propagation by Tissue Culture. Part 1, The Technology, 2nd edn, *Exegetics Ltd*. England, (1993), 574.

[16] Zohara Yaniv and Uriel Bachrach (2005) Handbook of Medicinal Plants. *Haworth Press*, Binghamton, NY, 3-4.

[17] Tejavathi D. H. and Purushothama L., *In vitro* Propagation of *Evolvulus alsinoides, Journal of Tropical Medicinal Plants*, 5(1), (2004), 83-88.

[18] Jameel M. Al-Khayri and Abdulaziz M. Al-Bahrany, *In Vitro* Micropropagation of *Citrus aurantifolia* (lime), Current Science, 81(9), (2001), 1242-1246.

[19] Li-Chang Lin, Satish Manohar Nalawade, Vanisree Mulabagal, Mau-Shing Yeh, and Hsin-Sheng Tsay, Micropropagation of *Polygonum Multiflorum* THUNB and Quantitative Analysis of the Anthraquinones Emodin and Physcion Formed in *in Vitro* Propagated Shoots and Plants, Biol. Pharm. Bull, *26*(10), (2003), 1467—1471.

[20] Abhay P. Sagare, Chao-Lin Kuo, Fu-Shin Chueh, and Hsin-Sheng Tsay, *De Novo* Regeneration of *Scrophularia*

yoshimurae YAMAZAKI (Scrophulariaceae) and Quantitative Analysis of Harpagoside, an Iridoid Glucoside, Formed in Aerial and Underground Parts of i*n vitro* propagated and i*n vivo* plants by HPLC, Biol. Pharm. Bull, 24(11), (2001), 1311—1315.

[21] Wondyifraw Tefera and Surawit Wannakrairoj, A Micropropagation Method for Korarima (*Aframomum corrorima* (Braun) Jansen), ScienceAsia, 30, (2004), 1-7.

[22] Gururaj H.B,Giridhar P and Ravishankar G.A, Micropropagation of *Tinospora cordifolia* (Willd.) Miers ex Hook. F & Thoms – A Multipurpose Medicinal Plant, Current Science, 92(1), (2007), 23-26.

[23] Sayeed Hassan A. K. M. and Shyamal K. Roy, Micropropagation of *Gloriosa superba* L. Through High Frequency Shoot Proliferation, Plant Tissue Cult., 15(1), (2005), 67-74.

[24] Alka Narula, Sanjeev Kumar, Bansal K.C and Srivastava P.S, *In Vitro* Micropropagation, Differentiation of Aerial Bulbils and Tubers and Diosgenin Content in *Dioscorea bulbifera,* Planta Med., 69, (2003), 778-779.

[25] Afolayan A. J. and Adebola P. O, i*n Vitro* Propagation: A Biotechnological Tool Capable of Solving the Problem of Medicinal Plants Decimation in South Africa, *African Journal of Biotechnology*, 3(12), **(**2004) 683-687.

[26] Anita Dewan, Kanan Nanda and Shrish C. Gupta, *in Vitro* Micropropagation of *Acacia Nilotica* Subsp. *indica* Brenan via Cotyledonary Nodes, *Plant Cell Reports*, 12(1), (1992), 18-21.

[27] Sascha L. Beck, Dunlop R. and Van Staden J., Micropropagation of *Acacia Mearnsii* from *ex vitro* Material, Plant Growth Regulation**,** 26(3), (1998), 143-148.

[28] Tiwari V, Deo Singh B and Nath Tiwari K, Shoot Regeneration and Somatic Embryogenesis from Different Explants of Brahmi [*Bacopa monniera* (L.) Wettst.], Plant Cell Reports, 17(6-7), (1998), 538-543.

[29] Sujatha M. and Reddy T. P., Differential Cytokinin Effects on the Stimulation of in Vitro Shoot Proliferation from Meristematic Explants of Castor (*Ricinus communis* L.), Plant Cell Reports, 17(6-7), (1998), 561-566.

[30] Annamária Mészáros, Andrea Bellon, Éva Pintér and Gábor Horváth, Micropropagation of lemon balm, Plant Cell, Tissue and Organ Culture, 57(2), (1999), 149-152.

[31] Ajith kumar D and Seeni S, Rapid Clonal Multiplication through *in Vitro* Axillary Shoot Proliferation of *Aegle marmelos* (L) Corr., a Medicinal Tree, Pl. Cell. Rep., 17(5), **(**1998), 422-426.

[32] Neelam Sharma, Chandel K. P. S and Srivastava V. K, i*n Vitro* Propagation of *Coleus Forskohlii* Briq., a Threatened Medicinal Plant, *Plant Cell Reports*, 10(2), (1991), 67-70.

[33] Kaur K., B. Verma and U. Kant, Plants Obtained from the Khair Tree (*Acacia catechu* Willd.) using Mature Nodal Segments, *Plant Cell Reports*, 17(5), (1998), 427-429.

[34] Gopi C., Nataraja Sekhar Y. and Ponmurugan P., *in Vitro* Multiplication of *Ocimum Gratissimum* L. through Direct Regeneration, *African Journal of Biotechnology*, 5(9), (2006), 723-726.

[35] Harsh Pal Bais, Julie B. Green, Travis S. Walker, Paul O. Okemo, and Jorge M. Vivanco, *In Vitro* Propagation of *Spilanthes Mauritiana* DC., an Endangered Medicinal Herb, through Axillary Bud Cultures, *In Vitro Cell. Dev. Biol. – Plant*, 38, (2002), 598–601.

[36] Hisashi Harada and Yasuhiro Murai, Micropropagation of *Prunus mume,* Plant Cell, Tissue and Organ Culture, 46(3), (1996), 265-267

[37] Rolf D. Illg and Ricardo T. Faria, Micropropagation of *Alpinia Purpurata* from Inflorescence Buds, Plant Cell, Tissue and Organ Culture, 40(2), (1995), 183-185.

[38] Chen Chang and Wei-Chin Chang, Micropropagation of *Cymbidium Ensifolium* var. *Misericors* through Callus-derived Rhizomes, In Vitro Cellular & Developmental Biology – Plant, 36(6), (2000), 517-520.

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