View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Update Publishing (E-Journals)

Research in Plant Biology, 2(4): 08-15, 2012
ISSN : 2231-5101
www.resplantbiol.com

Regular Article

In vitro propagation of *Solidago virgaurea* L. through nodal culture

John Peter Paul J, Revathy I and Johnson, M*

Department of Plant Biology and Plant Biotechnology,
St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India
*Corresponding author E-mail : ptcjohnson@gmail.com

A reliable *in vitro* propagation protocol has been established from nodal segments of a highly valuable medicinal plant *Solidago virgaurea* L. The explants were cultured on Murashige and Skoog's medium augmented with different concentrations and combinations of plant growth regulators for shoot bud initiation and multiplications. Highest frequency of shoot proliferation (91.8 ± 0.64) and maximum number (12.1 ± 0.20) per node was observed in Murashige and Skoog's medium augmented with4.5 mg/l of Kinetin in combination with 0.5 mg/l of Indole -3- Acetic acid. Highest frequency (91.7 ± 1.43) of rooting and maximum number (3.8 ± 0.28) of rootlet per shoot let was achieved on Murashige and Skoog's medium fortified with 4.5 mg/l of Indole -3- Acetic acid in combination with 0.5 mg/l of Benzyl -6- amino purine. Regenerated plants were successfully transferred to field (78%).

Keywords: *in vitro*; Clonal; Nodal segments; Medicinal plants

Abbreviations: MS – Murashige and Skoog's medium; PGRs – Plant Growth Regulators; BAP – Benzyl Amino Purine; IAA – Indole -3-Acetic Acid; IBA – Indole -3- Butyric Acid; NAA – **α** Naphthalene Acetic Acid; Kin – Kinetin

Plants are the main source of medicines and play a key role in world health and global economy (Constable 1990; Srivastava *et al.,* 1995). In the past few decades there has been a resurgence of interest in the study and use of medicinal plants in health care and in recognition of the importance of medicinal plants to the health system (Lewington 1993; Mendelsohn and Balick 1994; Hoareau and DaSilva 1999). This awakening has led to a sudden rise in demand for herbal medicines, followed by a belated growth in international awareness about the dwindling supply of the world's medicinal plants (Bodeker 2002). Most of the pharmaceutical industry is highly dependent on wild populations for the supply of raw materials for extraction of medicinally important compounds. The genetic diversity of medicinal plants in the world is getting endangered at an alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines, with little or no regard to the future (Nawalde and Tsay 2004). Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization, etc. are other factors. Hence there is a strong need for proactive understanding in the conservation, cultivation, and sustainable usage of important medicinal plant species for future

use (Nawalde and Tsay 2004). During the last few years, *in vitro* culture techniques have been developed into a successful and rapid mean of asexually propagating a number of plant species. Clonally propagating by tissue culture is highly desirable to regenerate sufficient populations of plants with similar characteristics, decreasing or eliminate the possibility of anomaly what occurring with others methods (Bajaj *et al.,* 1988). Also, plant tissue culture is useful for conservation and rapid propagation of rare and endangered medicinal plants. Regeneration through micropropagation has been obtained in many medicinal species (Johnson *et al.,* 2002; Johnson and Manickam 2003; Johnson *et al.,* 2004; Benniamin *et al.,* 2004; Johnson *et al.,* 2005; Baskaran and Jayabalan 2005; Johnson 2006; Johnson *et al.,* 2007; Johnson 2007; Rout *et al.,* 2007; Johsnon *et al.,* 2009; Vijayakumar *et al.,* 2010; Chandra Prabha Rama Subbu 2010; Johnson *et al.,* 2010; Shukla *et al.,* 2011; Wesely *et al.,* 2010; Pandeya *et al.,* 2010; Okere and Adegeye 2011; Wesely *et al.,* 2011). There are no previous reports on micropropagation of any species of *Solidago virgaurea*. So, the objective of this investigation was to develop efficient systems for the *in vitro* propagation of this medicinal species *Solidago virgaurea* L. by apical and nodal segment proliferation.

Plants of *Solidago* (fam. Asteraceae) genus contain terpenoids, saponins, phenolic acids, phenolic glycosides and high amounts of flavonoids, mainly quercetin, kaempferol, and rutin (Lorenzi and Matos, 2002; Reznicek *et al.,* 1991). *Solidago* species have been used in folk medicine for the treatment of various inflammatory and infectious processes. Studies using in vitro and in vivo models have demonstrated that plants of this genus have important antimicrobial, analgesic, antineoplasic and antioxidant effects. It is recommended as a diuretic, analgesic and anti-inflammatory to treat burns and rheumatic disease, among other conditions. From the chemical point of view, flavonoids and their derived compounds have been isolated from the *Solidago* sps. Besides their anti-inflammatory activity effect, flavonoids have been demonstrated to have important antimicrobial and antiplatelet aggregation properties *Solidago virgaurea* L. is an herbaceous perennial plant of the family Asteraceae. It has been traditionally used to treat urinary tract, nephrolithiasis and prostate. The plant contains triterpene saponins, flavonoids (derivatives of quercetin, kaempferol and apigenin), polyphenolic acids (ferulic and chlorogenic), tannins, essential oil and polysaccharides. In addition, plant possesses antimicrobial, antimycotic, anti-oxidant, anti-inflammatory, analgetic, anticancerogenic, sedative, and hypotensive activities (Ivancheva and Stancheva, 2000; Thiem and Goslinska, 2002; Demir *et al.,* 2009). The crude ethanolic and methanolic extracts of *S. virgaurea* showed a moderate bactericidal activity (Gross *et al.,* 2002).

The Indian system of medicine is predominantly dependent upon the use of plant based raw materials in most of their preparations and formulations, thereby, widening the gap between demand and supply and thus putting further pressure on the species. In view of the problems of conventional propagation and high demand of planting material the large scale multiplication of this species can only be met efficiently and economically in a sort span of time by *in vitro* propagation. Therefore, an efficient *in vitro* propagation system for producing this plant *S. virgaurea* is required to further clarify its potential medicinal values and germplasm conservation. Thus the present study has been intended to develop a trustworthy and reproducible protocol of this important medicinal plant *S. virgaurea* using nodal segments as explants which could be used for mass multiplication

of this plant species to meet the increasing requirement of the pharmaceutical industry as well as for the conservation of *S. virgaurea*.

Materials and Methods

Plants of *Solidago virgaurea* L. (Asteraceae) collected from the native habitats at Tirunelveli, Tamil Nadu, India and grown in the Botanical garden of Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India. Young shoots were harvested and washed with running tap water and surface sterilized in 0.05 and 0.1% mercuric chloride for 2, 3 and 5 min. After rinsing 3-4 times with sterile distilled water, nodal segments were separated and cut into smaller segments (0.5 to 1.0 cm) used as the explants. The explants were placed vertically (nodal segments) on solid basal Murashige and Skoog (1962) medium supplemented with 3% sucrose, 0.7% (w/v) agar (Hi-Media, Mumbai) and different concentration (0.5-2.0 mg/l) and combination of BAP, Kin and IAA for *in vitro* shootlets and rootlets regeneration. The pH of the medium was adjusted to 5.8 before autoclaving at 121˚C for 15 min. The cultures were incubated at 25 ± 2˚C under cool fluorescent light (2250 lux 12 hr/d photoperiod). Each and every experiment was performed with 20 replicates and repeated twice. For hardening, the *in vitro* raised plantlets were removed from culture, washed thoroughly with tap water planted in small polycups filled with sterile garden soil (3:1), covered by unperforated polybags, and hardened for 4 weeks in a mist chamber before transfer to field.

Results and Discussion

The surface sterilization of *S. virgaurea* was carried with different concentration of mercuric chloride such as 0.05%. 0.1% and 0.15% for different time duration. Among them, 0.1% mercuric chloride for 3 min showed low percentage contamination and highest (92.3%) percentage of microbes / contaminants free explants. The explants treated with 0.05% and 0.15% of mercuric chloride for 3 min showed 55- 60% of microbes free explants. The explants treated with 0.1% for above 3 min and 0.15% for 2½ min and above obtained hundred percentages of microbes free explants with high percentage of explants mortality, high concentration of mercuric chlorides leads the death of the explants (lethal effect). Singh *et al.* (2009) observed that 5.5 ± 2.12 percentage of contamination when the explants were treated with 0.1 % (w/v) mercuric chloride for 5 min and 40 % (v/v) Sodium hypochlorite for 20 min. Wesely *et al.,* (2011) observed that the explants treated with 0.1% mercuric chloride for 3½ min showed 4% microbial contamination. In the present study we observed that 3 min sterilization with 0.1% $HgCl_2$ showed 7.7% of contamination with less percentage of explants mortality rate. The medium (MS) augmented with different concentrations and combinations of plant growth regulators (BAP (0.5- 5 mg/l), Kin (0.5- 5 mg/l) and IAA (0.5- 5.0 mg/l)) were used for multiple shoots emergence from the shoot tip and nodal segments. The explants (nodal segments) of *S. virgaurea* started growing in MS medium supplemented with BAP and Kin in combination with IAA within a week. Highest percentage (91.8 ± 0.64%) and maximum number (12.1 ± 0.20) of shoot induction from nodal segment was observed on MS medium supplemented with 4.5 mg/l of Kin in combination with 0.5 mg/l of IAA (Fig. 1. A & B) (Table 1). The MS medium augmented with cytokinin alone or in combination with auxin (Kin + IAA) induced maximum number of multiple shoots with maximum percentage. The nodal explants cultured on MS medium augmented with

cytokinin (BAP) alone induced multiple shoot formation was observed in *Baliospermum montanum* (Johnson and Manickam 2003), *Adenia hondala* (Johnson *et al.*, 2004), *Passiflora mollussima* (Johsnon *et al.*, 2007), *Stevia rebaudiana* (Janarthanam *et al.*, 2009), *Vitex negundo* (Islam *et al.*, 2009), *Marsdenia brunoniana* (Ugriah *et al.*, 2010) and *Alternanthera sessilis* (Wesely *et al.*, 2011). The *in vitro*-raised shootlets were transferred to half-strength MS medium with different concentrations of BAP, Kin, IBA, IAA and NAA for rooting (Table 1). Highest percentage (91.7 ± 1.43%) and maximum number (3.8 ± 0.28) rootlets were observed on MS medium augmented with 4.5 mg/l of IAA in combination with 0.5 mg/l of BAP (Fig. 1. C). In the present study, we observed the highest percentage and maximum number of rootlets per shootlets on half strength medium supplemented with 3 mg/l IBA. The cytokinin in combination with auxin promoted shoot formation in various plant species as observed by Yasmeen and Rao (2005), Guo *et al.*, (2007) Jawahar *et al.*, (2008), Nisha *et al.*, (2009), Ioan Băcilă *et al.*, (2010) and Yadev and Singh (2010).

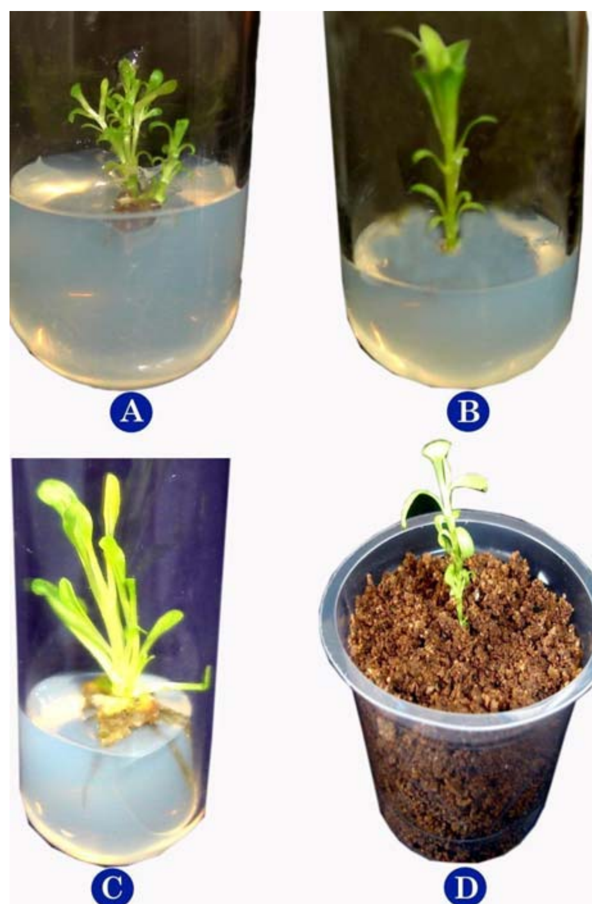

Fig.1. ***In vitro*** **propagation of** ***Solidago virgaurea*** **L**

A.Multiple Shootlets formation from the nodal segment of *S. virgaurea* cultured on MS+Kin 4.5 mg/l + IAA 0.5 mg/l; B. Shoot Elongation of *S. virgaurea* cultured on MS+Kin 4.5 mg/l + IAA 0.5 mg/l; C. *In vitro* rooltets formation from the *in vitro* derived shootlet of *S. virgaurea* cultured on MS+BAP 0.5 mg/l + IAA 4.5 mg/l; D. Hardened Plantlet of *S. virgaurea*

Table 1: Influence of Plant Growth Regulators on multiple shootlets and rootlets formation of *Solidago virgaurea* L.

Plant Growth Regulators Concentration in mg/l			Mean % of Shootlets formation ± S.E.	Mean No. of shootlets /nodal segments ± S.E.	Mean % of Rootlets formation ± S.E.	Multiple Rootlets /Shootlet ± S.E.	Degree of Callus formation
IAA	Kin	BAP					
0.5	4.5	0.0	91.8 ± 0.64	12.1 ± 0.20	-	-	-
1.0	4.0	0.0	83.8 ± 0.53	9.4 ± 0.36	-	-	-
1.5	3.5	0.0	72.4 ± 0.46	2.3 ± 0.24	-	-	+
2.0	3.0	0.0	63.7 ± 0.39	1.4 ± 0.18	-	-	++
2.5	2.5	0.0	45.6 ± 0.54	1.1 ± 0.31	-	-	++
4.5	0.0	0.5	48.4 ± 0.29	1.2 ± 0.23	91.7 ± 1.43	3.8 ± 0.28	-
4.0	0.0	1.0	54.4 ± 0.62	1.5 ± 0.36	83.8 ± 1.27	3.6 ± 0.33	-
3.5	0.0	1.5	61.3 ± 0.43	1.6 ± 0.53	78.3 ± 0.96	2.8 ± 0.44	++
3.0	0.0	2.0	63.8 ± 0.46	1.7 ± 0.42	65.8 ± 0.48	2.1 ± 0.52	++
2.5	0.0	2.5	65.3 ± 0.56	2.3 ± 0.56	56.9 ± 1.12	1.8 ± 0.63	++

+ - sign indicates average callus induction; ++ - sign indicates high amount of callus induction

In the present study the auxin in combination with cytokinin (BAP) accelerated the rootlets formation. Similar to the present study observation Marta *et al.*, 2009; Taware *et al.*, 2010; Kalidass *et al.*, 2010 also were observed the BAP in combination with IAA promoted rootlets formation in different plants. After 30 days, *in vitro*-raised plantlets were hardened in polycups containing a mixture of sterile garden soil: sand (3:1), covered with polypropylene bags and irrigated with 10 X diluted MS liquid medium. The plants were kept in a culture room for 15 days. 63% of plants were successfully established in polycups (Fig. 1. D). After 15 days the polycups hardened plants were transferred to pots and kept in green house. Seventy four percent of plants were well established in the green house condition. After one month, the plants were transferred to the field. About 67% of plants were established in the field. The present study has established reliable and repeatable protocol for large scale multiplication of *S. virgaurea* through nodal segments. This protocol could be used as a tool for the large scale multiplication.

References

Bajaj YPS, Furmanowa M and Olszowska O (1988) Biotechnology of the micropropagation of medicinal and aromatic plants. In: Bajaj YPS (Ed). Biotechnology in agriculture and forest: medicinal and aromatic plants, New York: *Springer Verlag.* PP.60-103

Baskaran P and Jayabalan N (2005) An efficient micropropagation system for *Eclipta alba* – a valuable medicinal herb. In Vitro Cell. Dev. Biol. Plant. 41: 532–539.

Benniamin A, Manickam VS, Johnson M and Jospeh LH (2004) Micropropagation of *Crataeva magna* (Lour.) DC. – A Medicinal Plant. Ind J Biotech. 3: 136-138.

Bodeker G (2002) Medicinal plants: towards sustainability and security. Discussion

paper for MEDPLANT. http://source.bellanet.org/medplant/docs/ssong/MEDPLANT_Discussion_Paper1.doc.

Chandra Prabha A and Rama Subbu R (2010) Micropropagation of *Aristolochia tagala* Cham. - a Rare and Endemic Medicinal plant from Western Ghats. J. Bio sci. Res. 1(2): 70-73.

Constabel F (1990) Medicinal plant biotechnology. Planta Med. 56: 421–425.

Demir H, Leyla Açık, Burcu Bali E, Yasemin Koç L and Gönül Kaynak (2009) Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts. African Journal of Biotechnology 8(2): 274-279.

Gross S, Goodarzi G, Watabe M, Bandyopadhyay S, Pai S and Watabe K (2002). Nutr. Cancer, 43:76.

Guo B, Gao M and Liu CZ (2007) *In vitro* propagation of an endangered medicinal plant *Saussurea involucrata* Kar. et Kir. Plant Cell Rep. 26: 261-265

Hoareau L and DaSilva E (1999) Medicinal plants: a re-emerging health aid. Electronic J. Biotech. 2: 56–70.

Ioan Băcilă, Ana Coste, Adela Halmagyi and Constantin Deliu (2010) Micropropagation of *Hypericum maculatum* Cranz - an important medicinal plant. Romanian Biotechnological Letters 15(1): 86-91.

Islam S, Banik H, Alam S, Tarek M and Rahman M (2009) *In vitro* Propagation of *Holarrhena antidysenterica* Wall., *Wedelia chinensis* (Osb.) Merr. and *Woodfordia fruticosa* (L.) Kurz. Plant Tissue Cult. & Biotech. 19(2): 253-255.

Ivancheva S and Stantcheva B (2000) Ethnobotanical inventory of medicinal plants in Bulgaria. J. Ethnopharmacol. 69: 165-172.

Janarthanam B, Gopalakrishnan M, Lakshmi Sai G and Sekar T (2009) Plant Regeneration from Leaf Derived Callus of *Stevia rebaudiana* Bertoni. Plant Tissue Cult. & Biotech. 19(2): 133-141.

Jawahar M, Ravipaul S and Jeyaseelan M (2008) *In vitro* regeneration of *Vitex negundo* L.- A Multipurpose woody aromatic medicinal shrub. Plant Tiss. Cult. Biotech.18: 37-42.

Johnson M (2007) Somoclonal Variation studies on *Phyllanthus amarus* Schum & Thonn. Iranian Journal of Biotechnology 3(4): 240-245.

Johnson M and Manickam VS (2003) *In vitro* multiplication of *Baliospermum montanum* (Willde) Muell-Arg.- a threatened medicinal plant. Indian Journal of Experimental Biology 41:1349-1351.

Johnson M, Usha A and Babu A (2010) Rapid Multiplication of *Plumbago auriculata* L. A Medicinally Important Plant. Journal of Basic and Applied Biology 4 (1&2): 135-141.

Johnson M, Berahanu AT, Eyayu M, Mulugeta K, and Manickam VS (2005) Regeneration from callus cultures of *Rhinacanthus nasutus* L. Kurtz. Ethiopian Journal of Science and Technology 3: 17-24

Johnson M, Manickam VS, Nikhat Y, Sonali D and Andal N (2004) *In vitro* multiplication of two economically important and endangered medicinal plants – *Justicia gebdarussa* Brum and *Adenia hondala* (Gaertn) De Wilde. Malaysian Journal of science 23(2): 49-53.

Johnson M, Nikhat Yasmin, Sonali Das and Raja Sekara Pandian M (2007) The role of cytokinin and auxin in organogenesis of *Passiflora mollissima* and Evaluation of Biochemical changes using isozyme and protein profiles. Ethiopian Journal of Science and Technology 4(2): 27-36.

Johnson M, Sonali D, Yasmin N and Babu A (2009) Impacts of Cytokinins and Auxins

on organogenesis and somatic embryogenesis of *Vitex negundo* var. *negundo* L. and evaluation of genetic fidelity. Biotechnology An Indian Journal 3(4): 236-241.

Johnson M, Vallinayagam S and Manickam VS (2002) Micropropagation of *Rhinacanthus nasutus* (L.) Kurt- A medicinal important plant. Phytomorphology 52(4): 331-336.

Johnson M (2006) *In vitro* multiplication of *Solanum anguivi* Lam, *Phyllanthus amarus* Schum and Thonn, *Jasminum calophyllum* Wall – Ethano medicinal Plants. Ethiopian Journal of Science and Technology 3(2): 78-84

Kalidass C, Daniel A and Mohan VR (2010) Rapid Micropropagation of *Plumbago zeylanica* L. An Important Medicinal Plant. Journal of American Science 6(10): 1027-1031.

Lewington A (1993) Medicinal plants and plant extracts: a review of their importation into Europe. A traffic network report. Cambridge, Traffic International.

Lorenzi H and Matos FJA (2002) Plantas Medicinais no Brasil. In: Nova O, Sao P, eds. Nativase Exóticas Cultivadas. Brazil, Instituto Plantarum de Estudos da Flora Ltda, 1-170.

Marta VM, Andrés M and Gatica Arias (2009) Effect of BAP and IAA on shoot regeneration in cotyledonary explants of *Costa rican* melon genotypes Agronomía Costarricense 33(1): 125-131.

Mendelsohn R and Balick M (1994) The value of undiscovered pharmaceuticals in tropical forests. Econ. Bot. 49: 223-228.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with Tobacco tissue culture. Plant physiology 15:475-497.

Nalawade SN and Tsay HS (2004) *In vitro* propagation of some important Chinese medicinal plants and their sustainable usage. In vitro Cell. Dev. Biol. Plant. 40:143-154.

Nisha MC, Rajeshkumar S, Selvaraj T and Subramanian MSA (2009) Valued Indian medicinal plant – *Begonia malabarica* Lam. Successful plant regeneration through various explants and field performance. Maejo Int. J. Sci. Technol. 3(2):261-268

Okere AU and Adegeye A (2010) *In vitro* propagation of an endangered medicinal timber species *Khaya grandifoliola* C. Dc. African Journal of Biotechnology 10(17): 3335-3339.

Pandeya K, Tiwari KN, Singh J, Verma JP and Dubey SD (2010) *In vitro* propagation of *Clitoria ternatea* L.: A rare medicinal plant. Journal of Medicinal Plants Research 4(8): 664-668.

Reznicek G, Johann Jurenitsch and Michaela Plasun (1991) Four major saponins from *Solidago canadensis*. Phytochem. 30: 1629-1633.

Rout AGR, Mahato SK and Senapati (2007) *In vitro* clonal propagation of *Nyctanthes arbortristis* Linn. – a medicinal tree. Hort. Sci. 34(2): 84-89.

Shukla P, Singh A, Gawri S, Alexander A and Sonwane S (2011) *In vitro* propagation of *Barleria prionitis* Linn and its antibacterial activity. International Journal of Pharma Professional's Research 2(1): 198-200.

Singh A, Kandasamy T and Odhav B (2009) *In vitro* propagation of *Alternanthera sessilis* (sessile joyweed), a famine food plant. Afr. J Biotechnol. 8(21): 5691-5695.

Srivastava J, Lambert J and Vietmeyer N (1995) Medicinal plants: an expanding role in development. World Bank technical paper no. 320. Washington, DC: World Bank Agriculture and Forestry Systems.

Taware AS, Mukadam DS, Chavan AM and Tware SD (2010) Comparative studies of *in vitro* and *in vivo* grown plants and callus of *Stevia rebaudiana* (Bertoni). International Journal of Integative Biology 9(1): 10-15.

Thiem B and Goslinska O (2002) Antimicrobial activity of *Solidago virgaurea* L. from *in vitro* cultures Fitoterapia. 73(6):514-516.

Ugraiah A, Karuppusamy S and Pullaiah T (2010). Micropropagation of *Marsdenia brunoniana* Wight and Arn. A rare antidiabetic plant. Plant Tiss Cult Biotech. 20: 7-12.

Vijayakumar RM, Vijayakumar and Stephen R (2010) *In vitro* propagation *of Bacopa monnieri* L. - a multipurpose medicinal plant. Indian Journal of Science and Technology 3(7): 781-786.

Wesely EG, Johnson M, Regha P and Kavitha MS (2010) *In vitro* propagation of *Boerhaavia diffusa* L. Through Direct and Indirect Organogenesis. J. Chem. Pharm. Res. 2(5): 339-347.

Wesely EG, Johnson M, Kavitha MS and Selvan N (2011) Micropropagation of *Alternanthera sessilis* (L.) using Shoot tip and Nodal segments. Iranian Journal of biotechnology 9(3): 206-212.

Yadav K and Singh N (2010) Micro-propagation of *Spilanthes acmella* Murr. – An Important Medicinal Plant. Nature and Science 8(9): 5-11.

Yasmeen A and Rao S (2005) Regeneration of multiple shoots from cotyledons of *Vigna radiata* (L.) Wilczek. J. Indian Bot. Soc. 84: 141-143.