WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1135-1144. Research Article ISSN 2277– 7105

MICROPROPAGATION OF SAMANEA SAMAN (JACQ) MERR OF TIRUMALA HILLS

R. Vinolya Kumari[1]* and T. Pullaiah[2]

[1]Lecturer in Botany, K.V.R Govt College (w) Kurnool 518002.

[2]Dept. of Botany, SK.University, Ananthapur.

Article Received on
30 April 2015,

Revised on 25 May 2015,
Accepted on 14 June 2015

***Correspondence for Author**
R. Vinolya Kumari
Lecturer in Botany, K.V.R Govt College (w) Kurnool 518002.

ABSTRACT

Samanea saman (Jacq.) Merr. is important medicinal tree belongs to Mimosaceae. It could be micro propagated by Cotyledonary node explants on MS medium containing different hormones. But MS medium containing 2mg/l BAP+O.5mg/l NAA produce 6-7 multiple shoots. Regenerated shoots were excised and induce root on medium with MS+1.0 mg/l+0.5 mg/l BAP. The regenerated plants were transfer to the soil.

ABBREVATIONS: KN – Kinetin, BAP – N6 – Benzyl Aminopurine ; IAA – Indole 3 – acetic acid; IBA – Indole butyric acid; NAA – 1 - Naphthalene acetic acid; TDZ-Thidizuron.

INTRODUCTION

*Samanea saman (*Jacq.) Merr. is large tropical tree growing as much as 60m tall, with rough wrinkled bark and developing a symmetrical broad umbrella shaped crown. *Samnea saman* synonymous with and fromly known as *mimosa saman, Calliandra saman, Acasia propinquce, Inga cinerea, Pithcallobiumm saman and Enterlobium saman.* The common name for the species is rain tree, coco tamarind, Acasia preta, French tamarind, monkey pod etc. The most widely used common name for the species is rain tree, from the belief that the tree produce rain at night. The leaf lets close up at night or when under heavy cloud cover, allowing rain to pass easily trough crown. Apart from it offer excellent shade wood and produces and distributes great quality of fruits of high nutrition quality that are important cattle foliage supplement during the dry period. It incorporation to the diet, reveals increase in weight and milk production.[1] One of the most important uses is as a shade tree in cocoa coffee vanilla and in young nut Meg and teak plantation. It can be used as hedge tree, if

logged heavily. The mature tree is heavily valued as a host for the lac insect (*Laccifer lacca*). The plantation of the tree on either side will reduce the level of CO_2 and it is also our constituent effort to put a break on global warming. It is rich in alkaloids, glucosides and their extracts have been reported to possess various bioactivities,[2,3,4,5,6,7,8,9,10&11] It is antimicrobial, aflatoxigenic, antifungal, antifumonisin activities, antioxidants and antiulcer properties.[12&13] *Samanea saman* is rarely found in forest stands and requires high light intensities. A wound parasite *Ganoderma lucidum* may cause white soft rot in lower part of the stem. A powdery mildew is very common in nurseries and may cause complete defoliation of seedling or serious damage. So plant growth rate is less, but through *In Vitro* propagation we can avoid all there problem and grow for mass cultivation.

MATERIAL AND METHODS

Seeds were collected from the Tirumala hills of Sechachallum hills. Seed were surface sterilized for 10min in tween 80, then dipped in 70% alcohol for 3.5 min and subsequently in the 0.5% H_2O_2 for 2-3 min 3-4 times in the sterile distilled water. Before seed germination the seeds were soaked in concentrated H_2SO_4 for 10 minutes with this the germination rate was found to be the highest by breaking physical dormancy caused by the hard seed coat. Seedlings of *Samanea saman* were micropropagated on MS medium in the 2 mg/l BAP +0.5 IAA. Different explants hypocotyls; leaf, root and cotyledonary node explants were aseptically excised from 15-30 days old aseptic seedling. Of all explants tested cotyledonary node explants shows more response. The explants were transferred to the MS medium with various concentration and combinations of auxins, cytokines and sucrose 3% is as the carbon source. The PH of the medium was adjusted to 5-6 by adding 0.8% agar before sterilization was observed within 15 days and callus was subculture one in every fifteen days well developed shoots were excised and transferred to rooting media for rooting. All the experiment was repeated at least thrice.

RESULT AND DISCUSSION

Cotyledonary explants from seedlings of the earliest development stage(15-30 days) were most responsive to (2-5 mg/l) cytokinine and 0.5 mg/I auxine combination tested and produced a greater number of viable shoots on MS medium(Table1). Morphological and biochemical characteristic of *Samnea saman* changes with age and these changes may affect cytokinine uptake and competency of cell to initiate buds. Of all the explants tested, cotyledonary node of *In Vitro* grown seedlings responded most favorably in presence of the

lowest concentration of 1-2 mg/l BAP tested. The nodal explants responded by axillary bud enlargement and bursting within.

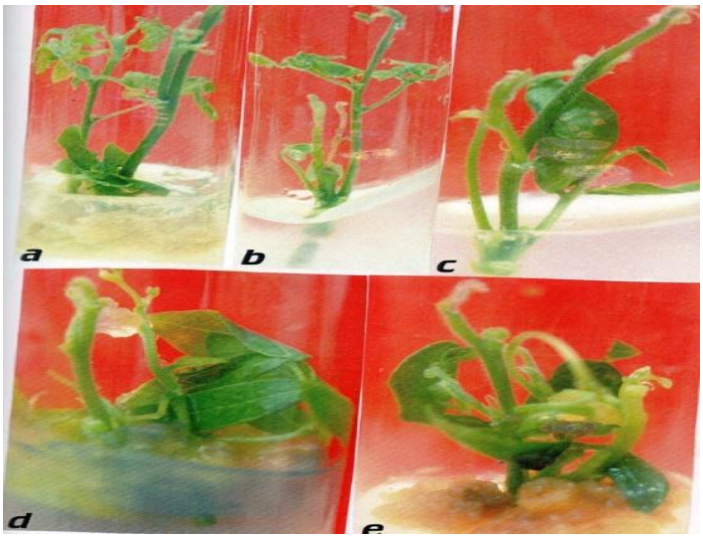

Figure: Multiple shoot induction from cotyledonary node explants on MS medium containing different concentrations of BAP with NAA, IBA&IAA.

A. MS + 5 mg/l BAP+0.5 mg/l NAA
B. MS + 2 mg/l BAP+0.5 mg/l IBA
C. MS + 2 mg/l BAP
D. MS + 2 mg/l BAP+0.5 mg/l IAA
E. MS + 2 mg/l BAP+0.5 mg/l NAA

3-6 weeks. The explants with proliferating shoots were cultured on the same media containing different concentrations of hormones. The hypocotyle, cotyledons and root explants are less responsive and formed less number of multiple shoots and more callus. Some multiple shoots further showed axillary branching as a result a dense mass of shoots were either cut back to stimulate further shoot formation for subsequent experimentation or these were excised and used as cutting for rooting . The effect of four different cytokinine on formation of multiple shoots in *Samanea saman* is variable. To test their ability to induce shoot regeneration, excised nodal explants were cultured directly on MS medium containing 2mg/l BAP. The presence of cytokinine at the basal medium resulted in the first viable sign of increased root diameter and promotion of shoot differentiation from the cotyledonary node. After 2-3 weeks of culture of nodal explants on MS medium containing 2 mg/l BAP produce only 3.9±0.9 shoots in 89% of the explants. Shoots after their initial proliferation on medium containing 2mg/l BAP were subculture on to medium containing 2mg/l BAP +

0.5mg/l NAA which produced high number of 6.6±0.6 multiple shoots in 82% of cotyledonaryno deexplants.

In this medium shoots were slightly longer when compared to those obtained during initial culture on medium containing 2mg/l BAP. This indicates that during culture establishment 2mg/l BAP was necessary to obtained an optimum response in terms of bud break and shoot yield per explants (figure1). Subsequently a lower concentration of BAP 1mg/l was also found to be optimum for multiplication rate and to obtain elongated shoots. Shoot proliferation was also achieved in the presence of 0.5% IAA or IBA with 2mg/l BAP and developed 4-5 shoots (Table-1). BAP was more effective cytokinin for shoot initiation in Leguminosae species,[14, 15&16] A synergistic effect of growth regulators in combination with BAP initiate shoot multiplication is well documented. Various successful combinations have been reported such as BAP+IAA of *Aegle marmalos*.[17] *Pentemon serrulantus*,[18] *Stryphnodendron polyphythum*,[19] *Alpinia galangal*,[20] and *Dubosia myoporoides*,[21] BAP+IBA for *Gardenia jasminoides*,[22] BAP+NAA for, *Acasia nilotica*,[23] *Kaempferia galangal*,[24] and *Feronia limonia* .[25]

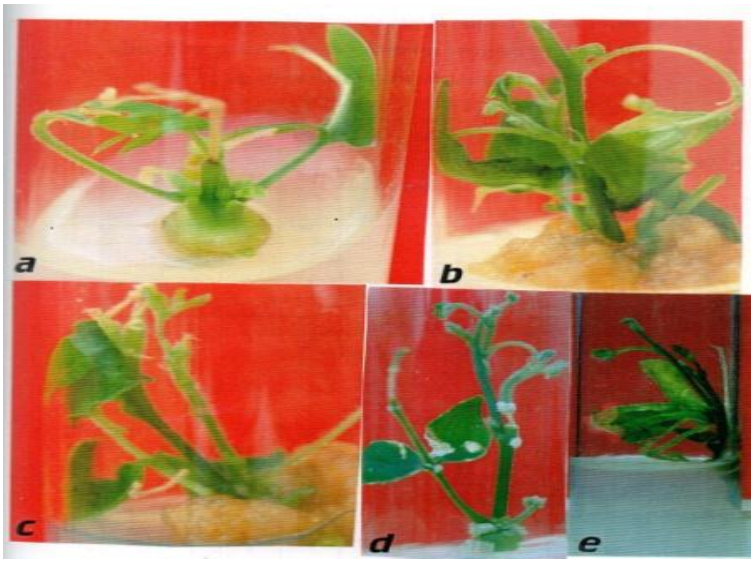

Figure2: Multiple shoot induction from cotyledonary node explants on MS medium containing different concentrations of TDZ with NAA, IBA&IAA.

A.MS + 2 mg/l TDZ + 0.5 mg/l NAA

B. MS + 5 mg/l TDZ + 0.5 mg/l NA

C. MS + 5 mg/l TDZ + 0.5 mg/l IA

D. MS +1.5 mg/l TDZ + 0.5 mg/l NAA

E. MS +1.5 mg/l TDZ + 0.5 mg/l NAA

TDZ in combination with auxin also produce multiple shoots when cotyledonary node explants were cultured on TDZ (1-5mg/l) with 0.5 mg/l IAA, IBA and NAA. The combinations of TDZ 2mg/l with 0.5mg/l NAA is more effective and developed 5-6 shoots (Figure2). In MS medium Containing TDZ 2-5 mg/l with IBA or IAA (0.5 mg/l) also developed 3-4shoots. But the percentage of response with TDZ (3mg/l) and 0.5mg/l NAA is more than (65%) with IAA (30%) and IBA (10%). TDZ is also a stimulant of shoot organogenesis in other woody species including Malus.[26] Populus,[27&28] *Prunus domestica*,[29] *Rhododendron*,[30] and Rubus,[31] In most of these TDZ was more potent and equal to amino purine cytokinine for stimulating shoot regeneration.

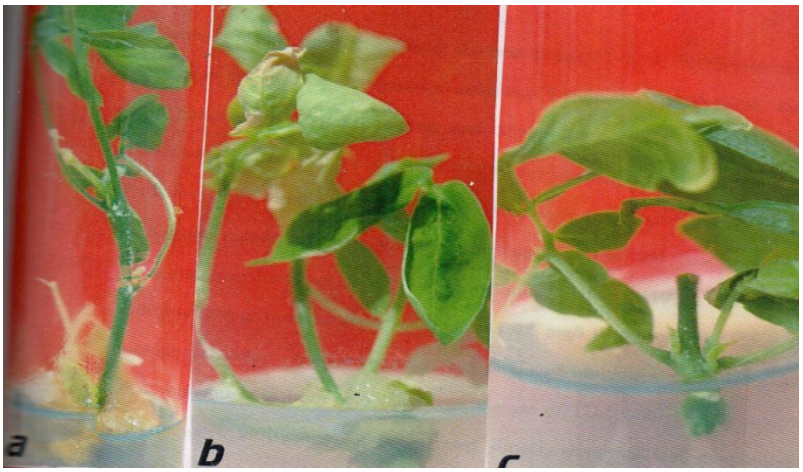

Figure 3: Multiple shoot induction from cotyledonary node explants on MS medium containing different concentrations of Cytokinine with Auxin

A. MS + 2 mg/l KN + 0.5 mg/l IBA

B. MS + 5 mg/l KN + 0.5 mg/l NAA

C. MS + 2 mg/l ip + 0.5 mg/l NAA

However BAP or KN in combination with TDZ also develops multiple shoots in Samanea saman. Maximum numbers of shoots of 4.3± 0.9 were also produced with the combination of 1 mg/l BAP + 2mg/l TDZ +0.5 mg/l NAA. Further increase in both plant growth regulators did not increase the number of shoots and the shoots were distorted and did not go further. At 2mg/l KN +0.5 mg/l NAA, 2-3 shoots developed from node with callus from the base of the explants. But at the concentration of 5mg/l KN with 0.5mg/l NAA, the degree of callusing was more and developed 3-4 multiple shoots from cotyledonary node (figu3). The combinations of 0.5 mg/l IAA + 2mg/l Kn supports callusing and induced multiple shoots (2-3). A degree of callusing without much difference in number of shoots was noticed at 0.5mg/l IBA+ 2mg/l KN.

TABLE.NO-1: EFFECT OF HORMONES ON MULTIPLE SHOOT INDUCTION OF NODAL EXPLANTS OF SAMANEA SAMAN ON MS MEDIUM

SNO	Hormones in mg/l							Percentage of response	No.of shoots ±S.E	shoot length (cm)
	2IP	KN	TDZ	BAP	IAA	IBA	NAA			
1				1				85%	3.6±0.9	3.8±0.7
2				2				89%	3.9±0.9	4.3±0.9
3				2	0.5			60	4.8±0.8	4.1±0.8
4				2		0.5		30	4.6±0.6	3.2±0.9
5				2			0.5	82	6.6±0.6	4.2±0.7
6				5			0.5	80	5.9±0.8	4.9±0.9
7			1				0.5	30	4.2±0.6	4.2±0.7
8			2				0.5	60	5.4±0.8	5.1±0.9
9			1.5				0.5	50	4.9±0.8	4.8±0.8
10			5				0.5	65	4.9±0.8	5.2±0.7
11			2		0.5			20	3.1±0.6	3.8±0.2
12			5		0.5			30	3.2±0.6	3.0±0.4
13			2			0.5		20	3.8±0.6	3.9±0.7
14			5			0.5		10	3.9±0.5	4.2±0.9
15		2					0.5	30	2.8±0.5	5.2±0.8
16		5					0.5	40	3.8±0.5	5.2±0.7
17		2				0.5		20	2.2±0.5	3.8±0.9
18		2			0.5			30	2.1±0.8	3.2±0.9
19	2						0.5	15	3.0±0.9	3.2±0.9

When cotyledonary node explants cultured on o.5 mg/l NAA + 2 mg/l 2-ip, induced both multiple shoot and callus. But combinations of 1 mg/l 2ip+ 2mg/l BAP induced more number of (3-4) multiple shoots. Shoots developed from explants produced compact callus at the base. Similar results were obtained in *Eucalyptus grandis*,[32] and *Pterocarpus santilinus,*[33] Initially the callus was yellowish green but it turned to dark within two months. Disadvantages have been associated with the use of TDZ with BAP in *InVitro*. This includes vitrification of regenerated shoots, abnormal leaf morphology, compact shoots and difficult in elongation and rooting. These results are in agreement with the observations of,[34] that, the explants could be induced with the lowest effective TDZ concentrations and kept on TDZ medium for minimum time required. The combination of BAP with 2-ip produce more shoots than KN or 2-ip. This is also reported by,[35] who found that KN and 2-ip had less promotive effect than BAP in producing shoots from hazelnut. In all the explants the presence of auxins in differentiation medium significantly improved shoot differentiation, shoot number and length. Similar observations were also observed in *Dalbergia latifolia*.[36] and *Madhuca longifolia,*[37] Micropropagation system has been linked by the frequently of

leaf abscission and death of shoots in culture. To avoid this, repeated sub culturing of nodes from shoot cultures helped to achieve continuous production of callus free and healthy shoots at least through five subculture cycles. A similar phenomenon was also observed in *Morus Australia*,[38] On the whole, the results suggested that a threshold level of endogenous growth regulators accumulated during culture initiation enabled the explants of shoot cultures to produce shoots optimally at reduced level of BAP with or without any auxin. The observed shoots forming ability of all the *InVitro* derived explants types and in particular, the high frequency formation of a large number of shoots in nodal explants greatly enhances the mass multiplication potential of the culture system.

Shoots developed from nodal cultures were transferred to MS medium containing 2mg/l IBA long tap root with number of lateral roots were developed. The high concentration of IBA resulted in heavy callus formation at the cut end of the shoots and reduced the percentage of root initials in nodal culture. During rooting experiment the explants were treated in 2 mg/l NAA treatments initiated vigours root system (fig-4). Roots developed from the NAA treated were larger and more vigorous than IBA. High concentration of IBA resulted in heavy callus formation at the cut end of the shoot and reduced the presentation of root initials in nodal culture. Better rooting system was observed, when shoots were treated with 1mg/l IBA + 0.5 mg/l BAP. Shoots when cultured on NAA along with BAP the rooting percentage and number of roots per explants increased significantly.

Figure4: Rhizogenesis from shoots on MS + 2 mg/l NAA and MS + 2mg/l IBA + 0.5mg/l BAP

Summary and Conclusion

This tree outstands among the promising agro forestry species, there poor information about natural seed propagation methods. But In Vitro propagation methods give favorable result for

mass multiplication. Seedlings are propagated on 2 mg/l BAP +0.5 IAA. Different explants are observed for shoot multiplication but cotyledonary node explants produce more number of shoots on 2mg/l BAP + 0.5mg/l NAA. Shoots produce form explants can initiate rooting on medium containing either on MS + 2 mg/l NAA or MS + 2mg/l IBA + 0.5mg/l BAP.

ACKNOWLEDGEMENTS

Author is thankful to Department of Botany S.K. University for providing facilities for research.

REFERENCES

1. Roncallo B Torres E and Sierra M. Production de vacas de dodle proposito supplementadas con frutos de Algarrobillo (*Pithecallobium saman*) durantle las llivias Available. 2009. http://WWW.fao.org/docrep/006/Y4435S/y4435s00.htm≠contents.
2. Tippeswamy S Mohana D Abhishek C and Manjunath K. Inhibitory effect of alkaloids of *Albizia amara* and *Alibis semen* on growth and fumonisin B1 production by *fusarium verticilloides*. International food research journal., 2014; 21(3): 947-952.
3. Kareru PG et al., Antimicrobial activity of some medicinal plants used by herbalist in Eastern province, Kenya. African Journal of Traditional, Complementary and alternative medicines., 2008; 5: 51-54.
4. Ragavendra MP Satish S and Raveesha KA. *InVitro* antimicrobial potential of alkaloids *Samanea saman* against *Xanthomonas* and human pathogenic bacteria. World Journal of Agriculture science., 2008; 4: 100-105.
5. Prasad RN et al., Preliminary phytochemical screening and antimicrobial activity of *Samanea saman* (Jacq.) Merr. Journal of medicinal Plant Research., 2008; 2: 268-270.
6. Azhar Ietal., Some biological evaluation on *Samanea saman* (Jacq.) Merr. Pakistan Journal of Pharmacology., 2009; 26: 47-53.
7. Nnamdi LO Anthony CCE Pius OU and Paul ME. Comparative phytochemical and antimicrobial screening of some solvent extract of *Samanea saman* pods. Africa journal of pure and applied Chemistry., 2010; 4: 206-2012.
8. Arulpriya P Lalitha P and Hemalatha S. In Vitro antioxidant testing of the extracts of *Samanea saman* (Jacq.) Merr. Der Chemica Sinica., 2010; 1: 73-79.
9. Fedruous A Iman MZ and Ahmed T. Antioxidant, Antimicrobial and cytotoxic activities of *Samanea saman* (Jacq.)Merr. Stanford Journal of Pharmaceutical Science, 2010; 3: 11-17.

10. Karmegam N Jayakumar M and Karuppuswamy S. Synergistic antibacterial activity of four medicinal plants collected from Dharmapuri Taluk of Tiruppur District, South India. Journal of Plant Sciences., 2012; 7: 32-38.
11. Ajam SMS Sallaeh B Al-Khalil S and Suleiman SF. Antimicrobial activity of spermine alkaloids from *Samanea saman* against microbes associated with sick buildings. International Conference on Environmental, Chemistry and Biology., 2012; 49: 150-155.
12. Anonymous. CAB International: Medicinal compendium, CAB International Wallingford. UK; 2003.
13. Suresh Armugum et al., Evaluation of anti –ulcer activity of *Samanea saman* (Jacq.) Merr. Bark on ethanol and stress induced gastric lesions in albino rats. Indian Journal of Pharmacology., 2011; 43(5): 585-590.
14. Kattar S and Mohan Ram HY. Organogenesis development of cultured cell organization culture growth from freely suspended cells. Am J Bot., 1982; 445: 705-708.
15. Kattar S and Mohan Ram HY. Organogenesis and plants formation *In Vitro* in *Sesbania grandifolia (*L.). Pers. Ind J Exp Biol., 1983; 21: 251-253.
16. Kapoor S and Gupta S. Rapid *InVitro* differentiation of *Sesbania bispinosa* plants a leguminous shrub. Plant Cell Tissue Organ Culture., 1986; 7: 263-268.
17. Ajithkumar D and Seeni S. Rapid clonal multiplications through *In Vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr. A. medicinal tree. Plant Cell Reports., 1998; 77: 422-426.
18. Halina Wysokinska. Micropropagation of *Pentemon Serrulatus* and *iridoid* and iridoida formation is regenerated plants. Plant Cell Tissue and organ culture. 1993; 33: 181-186.
19. Suzelei C et al,. Micropropagation of *Stryphnodendron polyphythum* (Barbatimao). Plant Cell Tiss. Org Culture., 1995; 291-293.
20. Anand PHM and Hariharan M. *In Vitro* Multiplication of greater galangal (*Alpinia galangal* L.) a medicinal plant. Phytomorphology, 1997; 47: 45-50.
21. Kukreja AK and Mathur AK. Tissue culture studies in *Dubosia myoporoides*. 1. Plant regeneration and clonal propagation by stem node cultures. Plant Med., 1985; 2: 93-96.
22. George PS Ravishankar GA and Venkataraman Lv. Clonal multiplication of *Gardenia jasminiodes Ellis* trough axillary bud culture. Plant Cell Rep., 193; 13: 59-62.
23. Debvan A Nanda K and Gupta SC. In Vitro micropropagation of *Acasia nilotica* subsp. Indica Brenan via cotyledonary node. Plant Cell Rep., 1992; 12: 18-21.
24. Vincent KA Mathew KM Hariharan M. Micropropagation of *Kaempferia galangal* L. A medicinal plant. Plant Cell Tissue Org Cult., 1992; 28: 229-230.

25. Hossain M et al,. *In Vitro* organogenesis of elephant alle (*Feronia limonia*). Plant Cell Reports., 1992; 11: 522-524.
26. Fasola F Zimmerman RH Fordham I. Adventitious shoot formation on excised leaves of *In Vitro* grown shoots of apple cultivars. Plant Cell Tissue org Cult., 1989; 16: 75-87.
27. Russell AJ and Mc Cown BH. Culture and regeneration of populous leaf protoplasts isolated from non-seedlings tissue. Plant Sci., 1986: 46: 133-142.
28. Russell AJ and Mc Cown BH. Recovery of plants from leaf protoplasts of hybrid-*Populus aspen* clones. Plant Cell Rep., 1988; 7: 59-62.
29. Mante S Scorza R and Cordts JM. Plant regeneration from cotyledons of *Prunus persica*, *Prunus domestica* and *Prunus cerasus*. Plant Cell Tissue Organ Cult., 1989; 19: 1-11.
30. Preece JE and Imel MR. Plant regeneration from leaf explants of *Rhododeron*. PJM Hybrids Sci I., 1991; 48: 159-170.
31. Fiola JA Hassam MH Swatz HJ Bors RW and MC Nicols R. Effect of Thidizuron, light, influence rates and Kanamycin on *In Vitro* shoot organogenesis from excised Rubus Cotyledons and leaves. Plant Cell Tissue Organ Culture., 1996; 20: 223-228.
32. Lakshmi Sita G and Shobha Rani B. *In Vitro* propagation of *Eucalyptus grandis* L. by tissue culture. Plant Cell Report., 1985; 4: 63-65.
33. Lakshmi Sita G Raghava Ram NV and Vaidyanathan CS. Differentiation of embryoids and plantlets from short callus of sandal wood. Plant Sci Lett., 1979; 15: 265-270.
34. Lu C. The use of thidiazuron in tissue culture. *In Vitro* Cell; Dev Biol., 1993; 29: 92-96.
35. Mesaaguer J and Mela E. *In Vitro* propagation of adult material and seedlings *Corylusa vellana*. Acta Hort., 1987; 212: 499-503.
36. Raghavaswamy BV Himabindu and Lakshmi Sita G. *In Vitro* micropropagation of elite rose wood (*Dalbergia latifolia* Roxb.). Plant cell Reports., 1992; 11: 126-129.
37. Rout GR and Das P. Micropropagation of *Maduca longhifolia* (Koening) Mac Bride var. *Latifolia* Rox. Plant Cell Rep., 1993; 12: 513-516.
38. Pattnaik SK Sahoo Y and Chand PK. Micropropagation of *Morus australis* Poir, syn, M. acidosa Griff. Plant Cell Rep., 1996; 15: 841-845.