Research Article

# PIVOTAL ROLE OF PLANT GROWTH REGULATORS IN CLONAL PROPOGATION OF *MELIA AZEDARACH* L.

**Antara Sen*, Amla Batra and D.V. Rao**
Plant Biotechnology, lab. No.5, Department of Botany, University of Rajasthan, Jaipur.
***Corresponding author's E-mail:** antarasen_sible@yahoo.co.in

**Received on:** 25-09-2010; **Finalized on:** 27-11-2010.

**ABSTRACT**

A procedure has been standardized using a diverse array of plant growth regulators for *in vitro* regeneration of 'paradise tree' (*Melia azedarach* L., Meliaceae) from *in vitro* nodal explants. The immature seeds were allowed to germinate on filter bridges having sterilized distilled water. After 3 weeks, seeds were germinated and the *in vitro* grown seedlings were then used as source of explants. BAP (1.2 mg/l) has been found to be optimum for shoot initiation when *in vitro* nodal sections were inoculated. For further growth and multiplication, they were then transferred to fresh MS medium having optimized BAP (1.2 mg/l) along with IAA (0.1 mg/l), adenine sulphate (9.0mg/l), ammonium sulphate (200 mg/l) and potassium sulphate (100 mg/l). Repeated and periodic sub culturing yielded better growth and multiplication with 90% shoot formation. In vitro rooting was obtained on half strength MS medium supplemented with IBA (1.0 mg/l). Regenerated plants were successfully hardened and acclimatized, 80 % of plantlets survived under natural conditions after transplantation.

**Keywords:** Clonal Propagation, *Melia azedarach* L., immature seeds, multiple shoots, *in vitro* rooting.

## INTRODUCTION

Ayurveda is one of the Indian medicinal systems being practiced for thousands of years. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on ayurvedic medicinal plants. It is a time-tested science treating diseases with natural things like plant, animals and minerals.

*Melia azedarach* L. (*Meliaceae*), also known as Chinaberry or Persian lilac, is a deciduous tree. It is native to India and has long been recognized for its medicinal and insecticidal properties[1], [2]. It withstands cold climates better than its related tree "neem" (*Azidarachta indica* A. Juss) and it is a plant with long lasting wood, used as a component of agroforestry systems with inter cropping annual species[3]. Moreover, Chinaberry is resistant to locust and ants and its leaves, stems, flowers, seeds and fruits extract is a good source of natural compounds with potent insecticidal and antimicrobial actions[4-7].

Each and every part of the plant have traditional medicinal uses like stem is prescribed internally in asthma, bark is used in fever to relieve thirst, nausea, vomiting and general debility, loss of appetite and skin diseases[8,9]. Leaves also relieve from headache and cure the eruption on the scalp. Leaf juice is anthelmintic, diuretic, vermifuge and their decoction is astringent and stomachic. Flowers are astringent, refrigerent, anodyne, diuretic, resolvent, deobstruent and alexipharmic[8,10]. Fruits are used for the preparation of tonic which is purgative, emmolient and anthelmintic[11]. Seeds are bitter, expectorant, anthelmintic and aphrodisiac and are useful in helminthiasis, typhoid fever, pain in the pelvic region and scrofula[12]. Roots are bitter, astringent, anodyne, depurative, vulnerary, antiseptic, anthelmintic, constipating, expectorant, febrifuge, antiperiodic, and bitter tonic in low doses[13]. The tree yields valuable timber, is very useful for making furniture, plywood, toys, fuel wood and also as insect repellent[14].

Melia is considered as a multipurpose tree because of its multi directional and wide spread uses in medicine, therapeutics and other economics implications. It is conventionally propagated through seeds and has global importance. Unfortunately, the tree bears seed only during the summer and its natural rate of multiplication is dependent on season. Moreover, the population obtained often show great undesirable genetic variations [15]. It has also been indiscriminately cut resulting in a significant decline in the population. Its propagation is not sustaining the fast reproduction in the wild that can keep up with its increased demand. The use of modern *in vitro* techniques on woody trees has opened new possibilities for rapid mass multiplication of the existing stocks of germplasm[16-20] as well as for its ex situ conservation[21]. Therefore, this paper reports a protocol for its micropropagation from immature seeds on *M. azedarach* through aseptic nodal segments.

## MATERIALS AND METHODS

### Plant source and seed germination:

Immature fruits of *Melia azedarach* L. were collected in the month of April-June from the Nursery, University of Rajasthan, Jaipur. Seeds from immature fruits were carefully taken out by cracking the tough endocarp from presoaked fruits with the help of scalpel. Then, they washed under running tap water for about 5 minutes and dipped in teepol (1%) (v/v) and Bavisitin (2:1) for 2 minutes followed by proper rinsing with sterilized double

distilled water for 5-6 times, so as to remove all the traces of sterillant. They were then aseptically inoculated on filter bridges having double distilled water in culture tubes. From 3 weeks old seedlings, nodes were separated and cultured on various combinations of phytohormones in MS medium.

**Culture Media and Conditions:**

Murashige and Skoog medium[22] containing 3% sucrose and 0.8% agar along with cytokinin such as BAP, Kintein (0.0-2.0 mg/l) was prepared for shoot initiation. After that they were transferred on fresh MS medium with optimum BAP along with IAA (0.05-1.0 mg/l) for further growth and multiplication. For better morphogenic responses, AdS (5-13mg/l) was also added and to stop defoliation and leaf curling, 200 mg /l $(NH_4)_2SO_4$ and 100 mg /l $K_2SO_4$ were added to the medium as growth additives. The pH of the medium was adjusted to 5.8 ± 0.2 prior to autoclaving at 121° C for 15 minutes. For each experiment, 8 replicates were prepared and each protocol was repeated at least thrice.

After the solidification of media, the sterilized explants obtained from *in vitro* grown seedlings were then inoculated on the medium and the cultures were incubated at 25±2°C, 16/8 hours photo and dark period, respectively.

**Shoot initiation, Multiplication and Elongation:**

For in vitro shoot induction, *in vitro* grown seedlings were taken out from the culture tubes and nodal segments were separated, inoculated on MS medium with various concentrations of cytokinins (BAP and Kn) (0.0-2.0 mg/l) alone.

BAP at a concentration of (1.2 mg/l) initiated shoot buds after 1 week of inoculation. The shoots initiated on the BAP were sub cultured for further multiplication and elongation on MS medium supplemented with optimum concentration of BAP (1.2 mg/l) along with IAA (0.05-1.0mg/l). Moreover, addition of 200 mg/l ammonium sulphate, $(NH_4)_2SO_4$, and 100 mg/l $K_2SO_4$, prevent defoliation and tip burning without affecting the number of shoots. Also, to enhance shoot multiplication, addition of AdS (5-13 mg/l) gave maximum morphogenic response.

**Rooting and Hardening:**

The *in vitro* elongated shoots were then aseptically transferred on half strength MS medium supplemented with various concentration (1.0-4.0 mg/l) of different auxins (IAA, IBA) alone. For in vitro root induction, IBA at a concentration of 1.0 mg/l proved to be the best for rooting. The plantlets were then gently picked from culture vessel without damaging the delicate root system and then rinsed with distilled water to remove adhering agar. The plantlets were then transferred to polycups containing vermicompost and autoclaved soil (1:3). Plants were covered with inverted glass beakers to maintain high humidity and kept in culture chamber for their hardening where all physical condition was provided artificially. They were gradually exposed to natural conditions for their acclimatization.

**RESULTS AND DISCUSSIONS**

*Melia azedarach* L. is a multipurpose tree with immense medicinal value. Germination of immature seeds in *in vitro* condition was successfully done on filter bridges containing double distilled water after three weeks of culture (Figure 1). Filter bridge method was found to be effective for in vitro seed germination in many other plant species viz., *Macrotyloma uniflorum* (Lam.) verdc.[23], *Psidium guajava* L.[24]. However, different strength of MS salts (Full, half and quarter) and B5 medium without hormone was proved to be the best for in vitro seed germination in various plant species *Gossypium hirsutum* L.[25], *Hyoscyamus muticus* L.[26], *Hypericum retusum* Aucher.[27]. Moreover, gibberellic acid and kinetin reduced the effect of juglone stress on seed germination and growth of seedlings in many plants [28].

Amongst various concentrations of BAP and Kn tried, BAP (1.2 mg/l) was proved to be optimum for axillary bud breaking and shoot initiation (Table 1, Figure 2). The research findings carried out by other scientists also favoured the present results e.g. *Murrya Koengii* L. [29], *Phyllanthus amarus* Schum. and Thonn. [30] *Vitex negundo* L[31]. But in case of *Leucaena leucocephala,* TDZ was proved to be best for in vitro shoot initiation [32].

Auxin (IAA) was also added to nutrient medium along with optimized BAP (1.2 mg/l) for further multiplication and elongation of shoots. 10.47 ± 0.21 shoots /explants were obtained at IAA (0.1 mg/l), when previously initiated shoots on BAP (1.2 mg/l) were sub cultured (Table 2, Figure 3, 4). The combination of auxin and cytokinin for shoot multiplication and elongation was previously reported in many other plant species viz., *Polianthes tuberosa* L.[33], *Ruta graveolens* L. [34]. In contrast to the present results,[35] were successful for development of multiple shoots in *Hypercium mysorense* without auxin. However, the shoots regenerated singly on cytokinin showed premature leaf abscission, curling of leaves and burning of shoot tips. To check defoliation, the medium was added with growth additives viz. 200 mg/l $(NH_4)_2SO_4$ and 100 mg/l $K_2SO_4$. Their addition to the medium improved the vigor of shoots and falling of the leaves without affecting the number of shoots. [36] also reported that the addition of additives ($(NH_4)_2SO_4$) help to prevent defoliation of leaf in *Pterocarpus marsupium* Roxb. Moreover, gultamine was also found to be effective in controlling the necrosis of shoot tips in *Jatropha curcus* L [37].

The addition of adenine sulphate to MS medium with BAP (1.2 mg/l) and IAA (0.1 mg/l) significantly amended the regeneration response and multiple shoot formation. Out of all concentrations tried, 9.0 mg/l AdS significantly gave the maximum morphogenic response (90%), where the average number of nodes/shoot increased to 3.92 ± 0.02 with highest shoot length of 5.40 ± 0.20 cm. It is apparent

from the results that AdS reinforced the effect of other PGRs and also exhibited synergistic effect with other cytokinin and auxins. The strategy for using AdS as an adjuvant has also been adopted effectively for many other plant species such as *Thevetia peruviana* (Pers.) Schum.[38] *Curcuma angustifolia* Roxb.[39] and in *Holarrhena antidysenterica* Wall. [40]

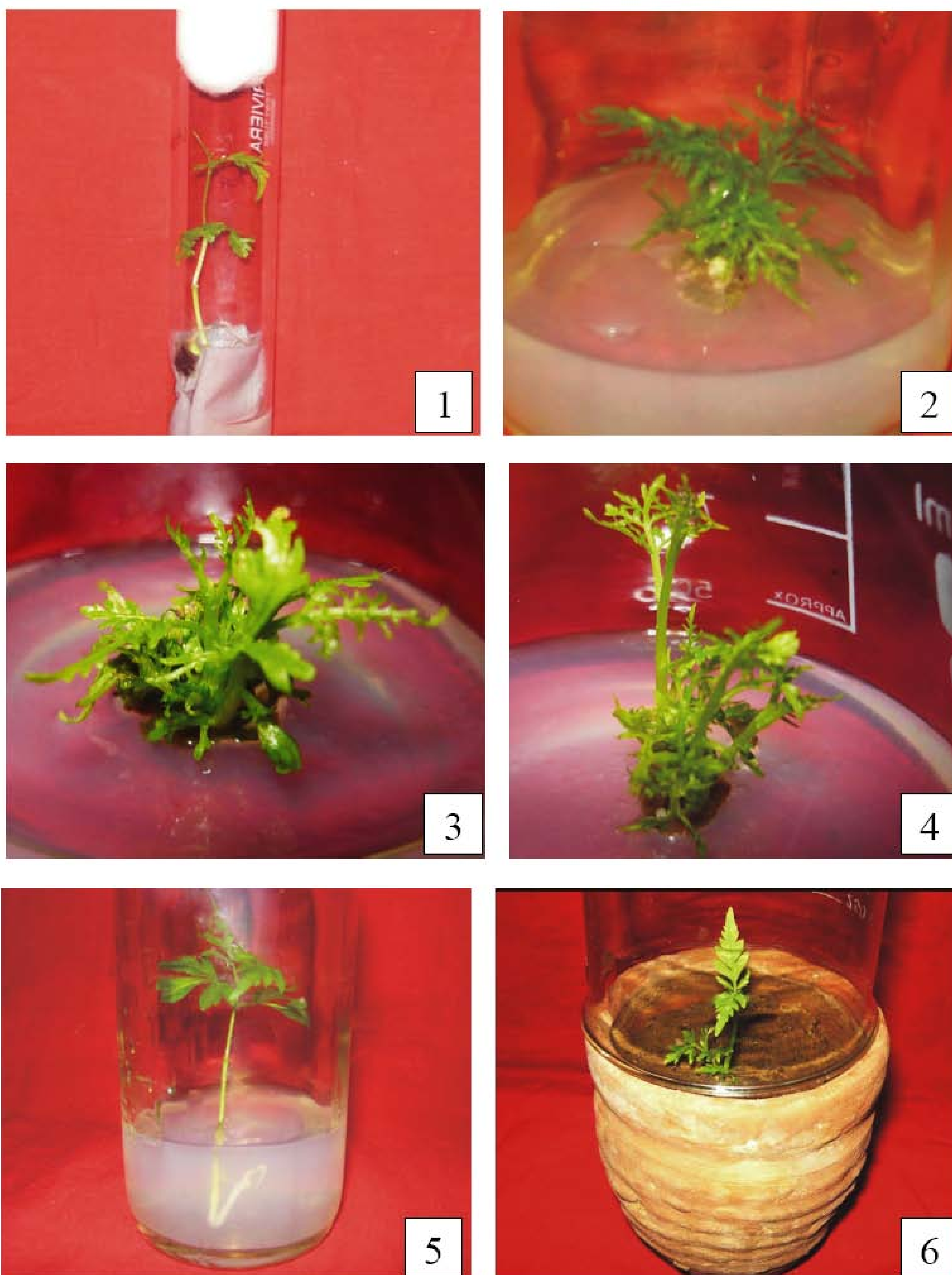



**Figure 1:** In vitro seed germination on Filter Paper Bridge after 3 weeks.
**Figure 2:** Initiation of from in vitro nodal segments on MS + BAP (1.2 mg/l).
**Figure 3-4:** Multiplication and elongation of shoots on MS + BAP (1.2 mg/l)+ IAA(0.1 mg/l)+ AdS (9.0 mg/l)+Ammonium Sulphate (200 mg/l)+Potassium Sulphate (100 mg/l).
**Figure 5:** Rooting of in vitro developed shoots on 1.2 MS + IBA (1.0 mg/l).
**Figure 6:** Hardening of in vitro raised plantlets in earthen pots.

**Table 1:** Effect of cytokinins (BA and KN) on shoot initiation from nodal stem segments of *M. azedarach* on MS medium after 1 weeks of culture.

| Growth regulators (mg/l) | % response of shoot formation | Mean number of shoots produced/explants (*Mean ± t $_{0.05}$S.E.) | No. of node / shoots (*Mean ± t $_{0.05}$S.E.) | Av. Length of shoots (cm.) (*Mean ± t $_{0.05}$S.E.) |
|---|---|---|---|---|
| **BAP** | | | | |
| 0.0 | 0 | - | - | - |
| 1.0 | 70 | 1.55± 0.01 | 1.38 ± 0.08 | 3. 24 ± 0.17 |
| 1.2 | 90 | 3.04 ± 0.15 | 2.95 ± 0.37 | 4.29 ± 0.08 |
| 1.4 | 62 | 5.27 ± 0.25 | 2.47 ± 0.18 | 3.90 ± 0.04 |
| 1.6 | 60 | 4.51 ± 0.30 | 2.85 ± 0.10 | 2.97 ± 0.13 |
| 1.8 | 60 | 2.36 ± 0.02 | 2.37 ± 0.21 | 2.70 ± 0.12 |
| 2.0 | 58 | 1.78 ± 0.01 | 1.28 ± 0.05 | 2.32 ± 0.21 |
| **Kn** | | | | |
| 0.0 | 0 | - | - | - |
| 1.0 | 47 | 1.57 ± 0.13 | 1.10 ± 0.35 | 3.26 ± 0.25 |
| 1.2 | 55 | 1.30 ± 0.05 | 1.80 ± 0.42 | 2.60 ± 0.35 |
| 1.4 | 60 | 2.00 ± 0.46 | 2.12 ± 0.16 | 3.23 ± 0.24 |
| 1.6 | 54 | 3.15 ± 0.11 | 1.58 ± 0.13 | 3.50 ± 0.12 |
| 1.8 | 48 | 2.27 ± 0.10 | 1.05 ± 0.31 | 2.10 ± 0.42 |
| 2.0 | 30 | 1.21± 0.02 | 1.01 ± 0.04 | 2.03±0.40 |

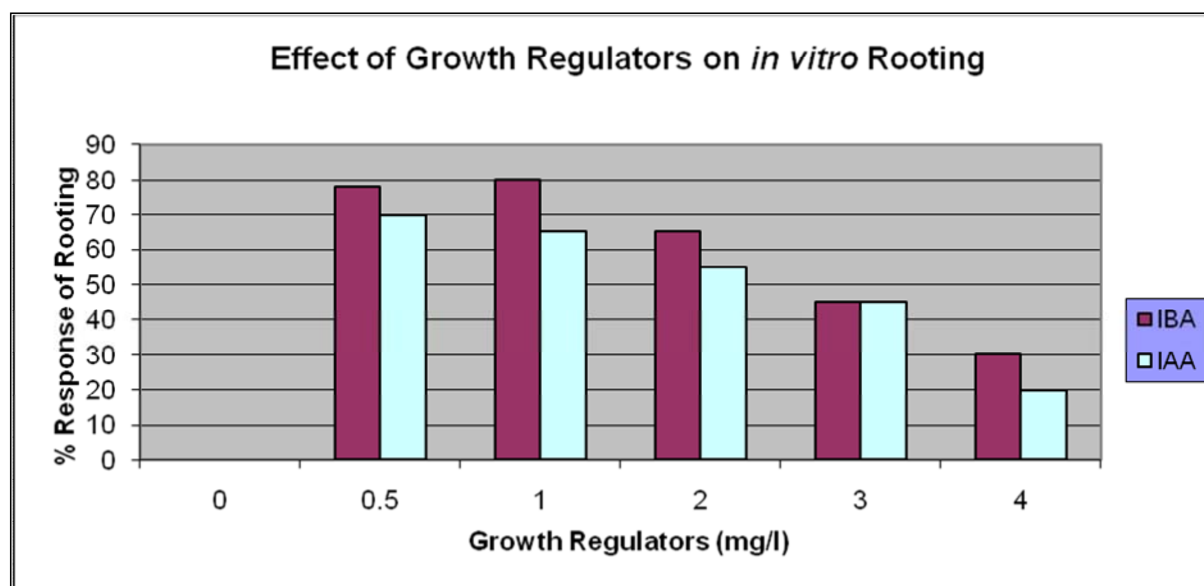
*Values are 95% confidence limits for Mean and mean values within the column followed by the same letter are not significantly different by the Tukey's test at 0.05% probability level

**Table 2:** Effect of IAA and AdS with optimal concentration of cytokinin (BA) and additives [200 mg/l $(NH_4)_2SO_4$; 100 mg/l $K_2SO_4$] on multiple shoot induction by regular subculturing from shoot initiation medium of *M. azedarach* on MS medium after 4 weeks of culture.

| Plant growth hormone | | | % response of shoot Formation (*Mean ± t $_{0.05}$S.E.) | Mean number of shoots produced/ explants (*Mean ± t $_{0.05}$S.E.) | No. of node per shoots (*Mean ± t $_{0.05}$S.E.) | Av. Length of shoots (cm.) (*Mean ± t $_{0.05}$S.E.) |
|---|---|---|---|---|---|---|
| BAP (mg/l) | IAA (mg/l) | Ads (mg/l) | | | | |
| 1.2 | 0.05 | 5 | 60 | 1.55± 0.21 | 1.61±0.04 | 0.40±0.12 |
| 1.2 | 0.05 | 9 | 72 | 2.4 ± 0.13 | 2.51±0.0 | 1.40±0.01 |
| 1.2 | 0.05 | 13 | 60 | 3.28 ± 0.05 | 1.56±0.02 | 2.42±0.19 |
| 1.2 | 0.1 | 5 | 75 | 5.30 ± 0.37 | 2.09±0.02 | 4.48±0.40 |
| 1.2 | 0.1 | 9 | 90 | 10.47 ± 0.21 | 3.92±0.02 | 5.40±0.20 |
| 1.2 | 0.1 | 13 | 80 | 6.85 ± 0.10 | 2.17±0.01 | 2.96±0.04 |
| 1.2 | 0.5 | 5 | 72 | 4.47 ± 0.18 | 2.14±0.01 | 1.80±0.11 |
| 1.2 | 0.5 | 9 | 70 | 3.38 ± 0.08 | 1.30±0.02 | 1.76±0.97 |
| 1.2 | 0.5 | 13 | 64 | 3.00 ± 0.42 | 1.21±0.07 | 1.70±0.08 |
| 1.2 | 1.0 | 5 | 64 | 2.12 ± 0.16 | 1.15±0.09 | 1.20±0.13 |
| 1.2 | 1.0 | 9 | 60 | 1.25 ± 0.13 | 1.12±0.07 | 0.80±0.05 |
| 1.2 | 1.0 | 13 | 59 | 1.22± 0.15 | 0.97±0.09 | 0.76±0.97 |

*Values are 95% confidence limits for Mean and mean values within the column followed by the same letter are not significantly different by the Tukey's test at 0.05% probability level

**Figure 7:** Effect of growth regulators on *in vitro* rooting




In vitro root induction was obtained when the elongated shoots were transferred on half strength of MS medium fortified with IBA (1.0 mg/l) after 3 weeks of transfer (Figure 5-7). IBA is more important plant growth hormone for the root induction in tissue culture of Chickpea [41] which is in consonance to the present research work. In contrast to this, [42] reported that NAA in combination with activated charcoal enhanced root proliferation in *Tigridia pavonia* (L. f.) DC.

*In vitro* raised plantlets were taken out from the culture vials and hardening and acclimatization (Figure 6) was done by the method described in "Materials and Methods".

The success of this protocol offers a highly efficient method for mass clonal propagation of *Melia azedarach* L., which would be beneficial for the plant tissue culturist, pharmaceutical industries and the nurseries where regular supply of the plants are more important.

**Acknowledgement:** Authors are thankful to UGC for providing financial assistance in the form of a major research project. Special thanks to Dr. M.M. Sharma for critical reading of the manuscript and Mr. Roop Narayan helping me in making photoplates.

## REFERENCES

1. Bohnenstengel FI, Wray V, Witte L, Srivastava RP & Proksch, P. Insecticidal meliacarpin (C- *seco* limonoids) from *Melia azedarach*. *Phytochem.* 1999;50:977-982.
2. Alche LE, Fereka GA, Meob M, Cotoa CE & Maier MS. An Antiviral Meliacarpin from Leaves of *Melia azedarach* L. *Z Naturforsch.* 2003; 58:215-219.
3. Saymaiya RK & Shukla KC. Biodiversity conservation through agro forestry system. *Advances Plant Sci.*1998; 11( 2):111-115.
4. Andreu J, Sans A & Riba M. Antifeedant activity of fruit and seed extract of *Melia azedarach* and *Azadirachta indica* on larvae of *Sesamia nonagrioides*. *Phytoparasitica* 2000; 28: 311-319.
5. Ursi VM & Ito M. Antifeedant activity of *Melia azedarach (L.)* extracts to *Diabrotica speciosa (Genn.) (Coleoptera: Chrysomelidae)* beetles. *Brazilian Archives of Bio & Tec.* 2000; 2: 215-219.
6. Wang JW, Wang QK, & Chiu S. Insecticidal compounds in *Meliaceae*. *Acta Entomologica Sinica.* 1994; 37 (1): 20-24.
7. Carpinella M, Herrero G, Alonso R & Palacios S. Actividad antifungica de extractos del fruto del paraiso (*Melia azederach* L.). *Fitoterapia.* 1999; 70: 296-298.
8. Dhiman AK. Sacred Plants and their Medicinal Uses. Daya publishing House,Delhi. 2003; pp: 125-127.
9. Venson I, Guzmán JAS, Talavera FJF & Richter HG. Biological, physical and mechanical wood Properties of paraíso (*Melia azedarach*) from a Roadside planting at huaxtla, Jalisco, Mexico. *J of Tropical Forest Sci.* 2008; 20(1): 38–47.
10. Sharma PC, Yelne MB & Dennis TJ. Data base on Medicinal plants used in Ayurveda. Documentation and Publication Division, Central Council for Research in Ayurveda and Siddha, New Delhi.(2001. pp. 389-406,
11. Rani M, Suhag P, Kumar R, Singh R & Kalidhar SB. Chemical component and biological efficacy of *Melia azedarach* stems. *J Med Aromatic Plant Sci.* 1999;21: 1043-1047.
12. Warrier PK, Nambiar VPK, & Ramankutty C. Indian medicinal plants, a compendium of 500 species.

Orient Longman Limited, Hyderabad. 1995; pp. 10-14,

13. Zhou H, Hamazaki A, Fontana JD, Takahashi H, Wandscheer CB & Fukuyama Y. Cytotoxic limonoids from Brazilian *Melia azedarach*. *Bio Pharm Bull.* 2005; 28(10): 1362-1365.
14. Tan QG, Li XN, Chen H, Feng T, Cai XH & Luo XD. Sterols and Terpenoids from *Melia azedarach*. *J. Nat. Prod.* 2010; 73:693–697.
15. Vila SK, Rey HY & Mroginski LA. Influence of genotype and explant source on indirect organogenesis by *in vitro* culture of leaves of *Melia azedarach* L. *Biocell.* 2004; 28(1): 35-41.
16. Domecq C. Cultivo *in vitro* de yemas axilares de paraíso gigante *(Melia azedarach* L. *var. gigantea). Phyton.* 1988; 48: 33- 42.
17. Ahmad Z, Zaidi N & Shah FH. Micropropagation of *Melia azedarach* from mature tissue. *Pak J Bot.* 1990; 22: 172-178.
18. Thakur R, Rao PS & Bapat VA. *In vitro* plant regeneration in *Melia azedarach L. Plant Cell Rep.* 1998; 18: 127-131.
19. Shahzad A & Siddiqui SA. Micropropagation of *Melia azedarach L. Phytomorph.* 2001; 51: 151-154.
20. Vila SK, Gonzalez AM, Rey HY& Mroginski LA. *In vitro* plant regeneration *of Melia azedarach* L.: shoot organogenesis from leaf explants. *Biol Plant.* 2003; 47:13–19.
21. Husain MK, Anis M & Shahzad A. *In vitro* propagation of a multipurpose leguminous tree (*Pterocarpus marsupium* Roxb.) using nodal explants. *Acta Physiol Plant.* 2008; 30:353–359.
22. Murashige T, Skoog F. A revised medium for rapid growth and bioassays in tobacco tissue.Physiol. Plant.1962;15:473-497.
23. Tejavathi DH, Devaraj VR, Murthy SM, Anitha P, & Nijagunaiah R. Regeneration of shoots from callus culture of *Macrotyloma uniflorum* (Lam.)verdc. *Indian J Biotech.*2010; 9:101-105.
24. Shah ST, Zamir R, Ahmad J, Ali H & Lutfullah G. *In vitro* regeneration of plantlets from seedlings explants of guava (*Psidium guajava* L..) Cv. *Safeda Pak* J Bot. 2008; 40(3): 1195-1200.
25. Khalafalla MM & Abdellatef E. *In vitro* multiple shoot induction and plant regeneration in elite sudanese cotton (*gossypium hirsutum* l.) cultivar (BARAC- B - 67).*J Innov Dev Strategy.*2008; 2(3): *77-82.*
26. Ibrahim A, Ibrahim AEK, Mostafa N, Ahmed,AMA & Asmaa AEA. Alkaloid Production and Organogenesis from Callus of *Hyoscyamus muticus* L. *In vitro J of App Sc Res* 2009; 5(1): 82-92.
27. Namli S, Akbas F, Iskalan C, Tilkat EA & Basaran D. The effect of different plant hormones (PGRs) on multiple shoots of *Hypericum retusum* Aucher. *Plant Omics J.* 2010; 3(1):12-17.
28. Terzi I & Kocacaliskan I. The effect of gibberellic acid and kinetin on overcoming the effects of juglone on seed germination oand seedling growth. *Turk J Bot.* 2010; 34:67-72.
29. Rani U, Sharma MM, Ismail N & Batra A. Quick *in vitro* plant regeneration from immature seeds of *Murrya Koengii* (L.) Spreng. *Global J of Biotech & biochem.* 2010; (In press).
30. Sen A, Sharma MM, Grover D & Batra A (2009). *In Vitro* Regeneration of *Phyllanthus amarus* Schum. and Thonn.: An Important Medicinal Plant. *Our Nature* 7: 110-115.
31. Sharma MM, Khanna P & Batra A. An efficient *in vitro* mass propagation of a medicinally potent plant species *Vitex negundo* L. via nodal segments. *Phytomorph.* 2006; 56(1&2):35-39.
32. Shaik NM, Arha M, Nookaraju A, Gupta SK, Srivastava S, Yadav AK, Kulkarni PS, Abhilash OU, Vishwakarma RK, Singh S, TatkareR, Chinnathambi K, Rawal SK & Khan BM Improved method of *in vitro* regeneration in *Leucaena leucocephala* – a leguminous pulpwood tree species. *Physiol Mol Biol Plants.* 2009; 15(4): 311-318.
33. Sangavai C & Chellapandi P. *In vitro* Propagation of a Tuberose Plant (*Polianthes tuberosa L.*). *Electronic J Bio.*2008; 4(3):98-101.
34. Bohidar S, Thriunavoukkarasu M & Rao T V. Effect of growth regulators on in vitro micropragation of 'Garden Rue' *Ruta graveolens* L. *Int J Integ Bio.* 2008;3(1):36-43.
35. Shiplashree HP & Rai R. *In vitro* plant regeneration and acclumation of Flavonoids in *Hypericum mysorense. Int J integrative bio.* 2009; 8(1):43-49.
36. Husain MK, Anis M & Shahzad A. *In vitro* control of shoot-tip necrosis (STN) in *Pterocarpus marsupium* Roxb.—a leguminous tree. *Physiol Mol Biol Plants.* 2006; 12:259–262.
37. Shrivastav S & Banerjee M. *In vitro* clonal propagation of physic nut (*Jatropha curcus* L.) influence of additives. *Int J integrative bio.* 2008; 3 (1) :73-79.
38. Zibbu G & Batra A. Effect of adenine sulphate on organogenesis via leaf culture in an ornamental plant: *Thevetia peruviana* (Pers.) Schum. *Int J Pharma Biosci.* 2010. 1(2):1-9.
39. Shukla SK, Shukla S, Koche V & Mishra SK. *In vitro* propagation of Tikhur *Curcuma angustifolia* Roxb.: a starch yielding plant. *Indian J biotech.* 2007; 6:274-276.

40. Raha S & Roy, SC. *In vitro* plant regeneration in *Holarrhena antidysenterica* Wall. through high frequency axillary shoot proliferation. *In Vitro Cell Dev Biol Plant.* 2001. 37:232–236.

41. Anwar F, Sharmila P & Saradhi PP. No More Hurdle: *In Vitro* Chickpea Rooting and Cent Percent Transplantation. *Aus J Basic App Sci.*2009; 3 (3): 2491-2496.

42. Escutia JLP, García LMV & Fernandez AMA. *In vitro* regeneration and genetic fidelity of *Tigridia pavonia* (L.f.) DC. *Electronic J of Biotech.*2010; 13(1):1-7.

**About Corresponding Author: Ms. Antara Sen**

Ms. Antara is Post graduated and graduated from Rajasthan University, Jaipur, India. She has taken specialization in Plant Biotechnology and Tissue culture. She is currently pursuing her Ph.D under the guidance of Prof. Amla Batra, University of Rajasthan, Jaipur, India.