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Direct multiple shoots proliferation of black night shade (S*olanum nigrum* l.) from shoot tip explants induced by thidiazuron

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Abbreviation: TDZ (1-Phenyl -3 - (1, 2, 3-Thiadiazo l-5-yl) IBA-Indole-3-butyric acid IAA-Indole-Acetic acid

ABSTRACT

The present investigation was undertaken in Black Night Shade (S*olanum nigrum* L.) which is an important medicinal plant. Direct multiple shoots proliferation was achieved from shoot tip. The shoot tips were cultured on MS medium fortified with Thidiazuron (TDZ) (1.0-7.0 mg/L) for multiple shoot induction. Multiple shoots proliferation was best observed at 3.0 mg/L TDZ from the shoot tip explants within three weeks of culture. Shoot number per explant ranged between 2 and 10. Individual shoots were aseptically excised and sub cultured in the same media for shoot elongation. The elongated shoots were transferred to Indole Acetic Acid/Indole Butyric Acid (IAA/IBA) (0.5mg/L–2.0mg/L) for root induction. Rooting was observed within two weeks of culture. Rooted plantlets were successfully hardened under culture conditions and subsequently established in the field conditions. The recorded survival rate of the plants was 86%. Plants looked healthy with no visually detectable phenotypic variations.

Introduction

Solanaceae A. L. Jussieu is one of the largest families of Angiosperms with 96 genera and approximately 2,300 species. The species of this family have great economic importance, because many plants are sources of chemical compounds of relevance in modern medicine and pharmacology, as well as important sources of human food. [1] The species that are widely cultivated as food include are the potato (*Solanum tuberosum* L.) [2] The tomato (*S. lycopersicon* L.), [3] and cayenne (*Capsicum frutescens* L.).[4] Plants of this family used in medicine include *Solanum paniculatum* (*jurubeba* true) to regulate intestinal functions, [5] and *lobeira* (*S. lycocarpum*) for hypertension, diabetes and high cholesterol.[6] For pharmacologically active drugs, species that produce alkaloids have been the most commonly used for therapeutic purposes, such as *Atropa belladonna* (atropine), *Hyosciamus niger* L. (hyoscyamine) and *Datura* spp. (hyoscine).[7]. The Solanaceae family also includes ornamental plants, such as *petúnias* (*Petunia* spp.) and jasmine (*S. jasminoides*), and tobacco (*Nicotiana tabacum*). Worldwide, these plants are economically significant along with other toxic and medicinal plants. [8].

Solanum nigrum L. is a medicinally important plant of the family Solanaceae. The plant has been traditionally used as hepatoprotective agent in India. Fruits make a delightful Jam [9, 10]. Fruit of plant is also used as a nervous tonic in the Mexican medicine. Chemically, solasodine, solasonine and solanidine have been identified from plant [11]. Fruits of plant have also been used as an antioxidant and cancer chemopreventive material [12]. Traditionally it is propagated through seed but seed propagation is season dependent. Considerable progress has been made in the propagation of this plant through plant cell, tissue and organ culture [13; 14; 15; 16 and 17).

Solanum nigrum proved to be resistant to the herbicide atrazine. In atrazine resistant *Solanum nigrum* biotypes, there is a single nucleotide; substitution of serine glycine at position 264 in the plastid genome encoded 32 Kd protein [18]. Some micropropagation works have been conducted from the various explants of *S. nigrum*. [19] Obtained plant regeneration from stem, leaf and root segments of *S. nigrum* through callus culture. They obtained callus on MS medium with NAA, regeneration of shoots on BAP and rooting on IBA. [17] cultured shoots from shoot cutting of germinated seeds of *S. nigrum* on different media (B5, MS or SH) and

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observed the best culture condition for shoot formation was the culture of stem internode segments on B5 medium supplemented with 0.5 mg dm-3 BAP at 16-h photoperiod (irradiance of 100 µmol m-2 s-1). Direct organogenesis and *in vitro* flowering was obtained in *S. nigrum* by [20]. High frequency plant regeneration from leaf explants obtained in *S. nigrum* by [21] the highest frequency and number of multiple shoots were obtained from leaf and nodal explants on MS medium supplemented with benzyladenine and IAA. Regenerated plants rooted and flowered on rooting medium supplemented with IBA or IAA. [22] Made a successful induction of callus from *S. nigrum* L. on MS basal medium supplemented with IAA and BAP. Regeneration shoots from callus and *in vitro* flowering were obtained on MS medium fortified with BAP and IAA or NAA or 2,4-D. The best rooting was obtained on MS containing 0.5 mg/l IBA. [23] Reported the *in vitro* regeneration of *S. nigrum* using different plant growth regulators and concluded that BAP 0.5 mg/l, 2, 4-D 1.0 mg/l and IBA gave the highest frequency of the well growing shoot. [24] Showed the accumulation of the alkaloid solasodine in the callus of *S. nigrum*. [25] Produced *S. nigrum* with a high power of alkaloid accumulation through *in vitro* regeneration trials followed by *in vivo* plant acclimatization. MS-basal medium containing BA and NAA (0.5 mg/ml each) was the best for both plants. A series of *in vitro* and *in vivo* plants were successfully produced and chemical analysis revealed contents of glycoalkaloids higher than those reported for intact field plants. [26] Obtained high frequency of shoots directly from the leaf explant of *S. nigrum* on MS medium supplemented with BAP and KIN without any callusing stage. Though some micropropagation studies have been conducted so far, this paper deals with the efficient plant regeneration system with large number of shoots within a short period from shoot tip explants of *Solanum nigrum* L induced by TDZ.

Material and methods

Mature seeds of *Solanum nigrum* were collected from a natural population showing Botanical Garden in SRR Govt Arts & Science College Karimnagar. The seeds were washed thoroughly in tap water 3–5 times and placed in 1% (v/v) Teepol solution (Reckitt Benckiser, India) which was kept under running tap water for 15 min. Then the seeds were disinfected with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 min. Finally the seeds were rinsed 3–4 times in sterile distilled water and inoculated on moist cotton in sterile test tubes. To assure uniform and rapid germination of seeds, test tubes were placed in dark at 28°C for 24–48 h. Then the germinated seeds were transferred to light intensity (15 µmol/s2/s), 16 h light per day photoperiod for another 4–7 days and maintained at 25 ± 2°C and 55–60% relative humidity.

Selection of explants

Shoot tips with one or two leaf primordia, of 15-d old *in vitro* raised seedlings were selected as explants for direct shoot multiplication. The shoot tips, segments of 5–8 mm in length were excised aseptically.

Culture media and culture conditions

MS media containing 3.0% sucrose and supplemented with various concentrations cytokinin such as TDZ (1.0 – 7.0 mg/L) were used. The initial pH of the culture media was adjusted to 5.8 before addition of 0.8% (w/v) agar- agar. The medium was dispensed into culture tubes (25 + 150 mm) each containing 15 ml of the culture medium capable with non-absorbent cotton and was autoclaved at 121^o C for 15 minutes. In each cultures tube one shoot tip explants was implanted. The cultures were maintained under 16h light provided with white fluorescent tubes (40 µ mol m-2s-2) at 25 ± 2^o C.

Results and discussion

Data on multiple shoot induction from shoot tip explants cultured on MS medium fortified with different concentrations of TDZ alone is presented in (Table-1). The important part of the present study was the preparation of contamination free explants. This was achieved by using *in vitro* germinated seedlings as an explant source. Sterilization of seeds required 0.1% (w/v) $HgCl_2$ 5 min treatment for maximum germination (98%) and minimum contamination[27]

Table 1: Effect of different concentration of TDZ on multiple shoot induction from shoot tip explants of *S. nigrum*

Growth regulators (mg/L)	% of explants showing response	No. of shoots per explants SE*	Average length of shoots SE*
TDZ			
1.0	60	5.0 ± 0.4	2.0 ± 0.4
2.0	65	6.0 ± 0.3	3.2 ± 0.3
3.0	85	15.0 ± 0.6	4.3 ± 0.2
4.0	80	10.0 ± 0.3	6.5 ± 0.5
5.0	70	8.0 ± 0.2	3.3 ± 0.5
6.0	68	5.0 ± 0.3	2.3 ± 0.5
7.0	53	4.0 ± 0.5	1.3 ± 0.5

***SE Standard Error**

Effect of TDZ

The meristem containing explants shoot tip were excised from the surface sterilized, *in vitro* grown, 30-d old seedlings and cultured on MS medium augmented with TDZ (1.0–7.0 mg/L) for multiple shoot induction of all the different concentrations of TDZ tested, (3.0 mg/L) TDZ was found to be more effective in inducing (15.0 ± 0.3 shoots/explants). But at high

concentration of TDZ (7.0 mg/L) considerably the number of shoot induction was found to be reduced. As the concentration of TDZ was increased up to 2.0mg/L the multiple shoots number was increased but as the concentration of TDZ (3.0mg/L) to (7.0 mg/L) TDZ resulted the number of shoots were reduced. (Fig-a, b, c&d).

The shoot induction and proliferation depend on plant growth regulators and types of explants [28 and 29]. In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or KIN [30, 31, 32 and 26]. In the Present study, a large number of shoots was produced from shoot tip explants *S. nigrum* on MS medium supplemented with both TDZ within a short period of 30-45 days.

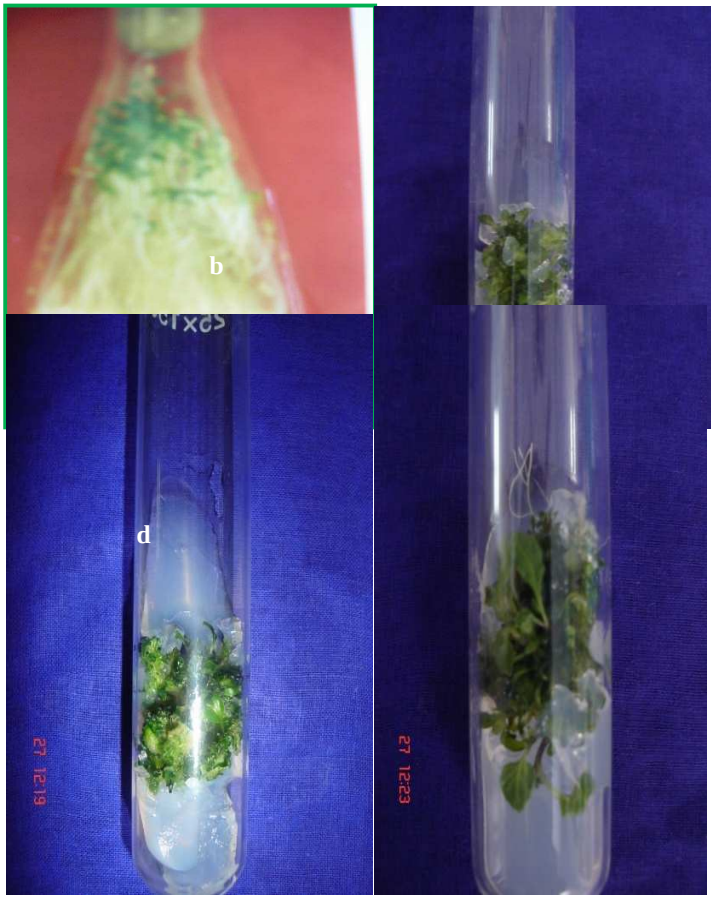

Fig 1: Direct *in vitro* shoots proliferation of *S. nigrum* L. (a) *In vitro* raised seedlings (30-d old), (b) Formation of multiple shoots on MS+TDZ (3.0) mg/L from shoot tip, (c) Proliferation of multiple shoots on MS+TDZ (4.0mg/L) from shoot tip, (d) Formation of multiple shoots with roots on MS+TDZ (5.0) mg/L from shoot tip.

***In vitro* rooting**

Fully elongated healthy shoots were transferred on to full strength MS root induction medium (RIM) [33] fortified with different concentration of IAA (0.5 – 2.0 mg/L) and IBA (0.5 – 2.0 mg/L).

Profuse rhizogenesis was observed on 1.5 mg/L IAA, compared to 0.5 -2.0 mg/L) IAA/ IBA on MS medium containing 1.5 mg/L IBA whereas 96% of plants produced roots with 14.3 ± 0.27 roots/ explant. (Table -2). (Fig-d).

In most of the studies IAA, IBA and NAA were used for root induction. High frequency of rooting was achieved by IAA in *Syzygium cuminii* [34], *Gossypium arboreum* and *G. hirsutum* [35] and IBA in *Aristolochia indica* [36], *Gymnema sylvestris* [37], *Avicennia marina* [38] and *Eclipta alba* [32]. Higher frequency of roots was observed in *Cichorium intybus* at 5 µM NAA [39], *Rubus chamoemorus* [40], *Plumbago zeylanica* at 3 µM NAA [41] and [42] also showed that NAA was found to induce more number of roots when compared to IAA and IBA in *Solanum nigrum*. In the present study also IBA was found to induce more number of roots when compared to IAA.

Table 2:Rooting ability of regenerated shoots from shoot tip explants culture of *S.nigrum* cultured on MS medium supplemented with IAA &.IBA

Growth Hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IAA	IBA		
00	00	23	1.0 ± 0.12
0.5	-	60	2.3 ± 0.37
1.0	-	70	3.2 ± 0.38
2.0	-	73	5.6 ± 0.38
-	0.5	54	4.3 ± 0.36
-	1.0	73	8.3 ± 0.87
-	2.0	70	6.3 ± 0.36

*** Mean ± Standard Error**

Acclimatization

Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to polypots containing pre- soaked vermiculite and maintained inside a growth chamber set at 28 ^{o}C and 70 – 80 % relative humidity. After three weeks they were transplanted to poly bags containing mixture of soil + s and + manure in 1: 1: 1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland's soulation every 3 days for a period of 3 weeks. The result of present investigation show that the shoot tip explants from mature plants of *Solanum nigrum* could be induced to produce multiple shoots *in vitro* maximum number of shoots was induced on MS medium fortified with various concentrations of TDZ. In recent years, shoot tip explants have been preferred to produce large number of genetically identical clones [43].

First time [44] reported the *in vitro* formation of plants from leave, of *Solanum nigrum* in which the elaimed cytokinins to induce shoot formation later [14] successfully from isolated protoplast. But true report is available on through investigation of *in vitro* shoot formation from leaf explants. Growth responses of *Solanum nigrum* explants were excellent and they produced cluster of shoots. The totipotency nature of *Solanum nigrum* cells are more pronounced by producing maximum number of shoots. Multiple shoot formation from shoot apices was obtained on MS medium supplemented with 20µM BA, 0.1µM NAA in pea [45]. Multiple shoot induction was also observed in *Ziziphus manritiana* [46] and *Vanilla plantifolia* [47] shoot tips cultured on MS + cytokinin alone as it was observed in the present studies. [48] Has studied the shoot meristem culture in 16 cultures of cotton using several media formation. They observed the best shoot developmentation MS media containing TDZ alone compared to other media with NAA / IAA in combination with TDZ. These results are to the present observation in *Solanum nigrum* which contain with cytokinins showed the increased number of shoots/ explants have also observed.

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Conflict of interest statement

We declare that we have no conflict of interest.

References

1. Wayne M. Watt, J.M. and M.G. Breyer-Brandwijk. 2011. *Solanum nigrum* L. Pp. 996-1000 *in* the Medicinal and Poisonous Plants of Southern and Eastern Africa. E. and S. Livingstone Ltd., Edinburgh and London.
2. Roy TS, Nishizawa T, Ali MH. Flower, berry and true potato seed productions in potato mother plants (*Solanum tuberosum* L.). 1. Effects of nitrogen and phosphorus fertilizers. *J Agron.* 2007;6:106–12.
3. Kaur C, Walia S, Nagal S, Walia S, Singh J, Singh BB, Saha S, Singh B, Kalia P, Jaggi S, Sarika S. Functional quality and antioxidant composition of selected tomato (*Solanum lycopersicon* L.) cultivars grown in Northern India. LWT – *Food Sci Techn.* 2013;50:139–45.
4. Kaouther Z, Nina H, Rezwan A, Cherif H. Evaluation of salt tolerance (NaCl) in Tunisian chili pepper (*Capsicum frutescens* L.) on growth, mineral analysis and solutes synthesis. *J Stress Physiol Biochem.* 2013;9: 209–28.
5. Mesia-Vela S, Santos MT, Souccar C, Lima-Landman MTR, Lapa AJ. *Solanum paniculatum* L. (jurubeba): potent inhibitor of gastric acid secretion in mice. *Phytomedicine.* 2002;9:508–14.
6. Dall'Agnol R,vonPoserGL. The use of complex polysaccharides in the management of metabolic diseases: the case of *Solanum lycocarpum* fruits. J Ethnopharmacol 2000; 71:337–41.

7. Silva TMS, Agra MF, Bhattachryya J.Studies on the alkaloids of *Solanum* of northeastern Brazil. Braz *J Pharmacogn.* 2005;15:292–3.

8. Edmonds JM, Chweya JA.(1997) Black night shades – *Solanum nigrum* L. and related species: Promoting the conservation and use underutilized and neglected crops. Institute of Plants Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute: Rome.

9. Chopra, R. N., Nayar, S. L., Chopra, I. C. (1986) Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi.

10. Watt JM, Breyer-Brandwijk MG, (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone, London.

11. Perez, R.M., Perez, J.A., Garcia, L.M., Sossa, H., (1998). Neuropharmacological activity of *Solanum nigrum* fruit. *J. Ethnopharmacol*. 62, 43–48.

12. Son, Y.O., Kim, J., Lim, J.C., Chung, Y., Chung, G.H., Lee, J.C. Ripe fruits of *Solanum nigrum* L. inhibits cell growth and induces apoptosis in MCF-7 cells.Food Chem. Toxicol. 2003;41, 1421–1428.

13. Zenkteler, M. *In vitro* formation of plants from leaves of several species of the Solanaceae family. Biochem. *Physiol. Pflanz*. 1972;163, 509–512.

14. Nehls, R. Isolation and regeneration of protoplasts from *Solanum nigrum* L. *Plant Sci. Lett*. 1978;12, 183–187.

15. Bhatt, P.N., Bhatt, D.P., Sussex, I.M. Organ regeneration from leaf disks of*Solanum nigrum*, *S. dulcamara*, and *S. khasianum*. *Z. Pflanzenphysiol*. 1979;95, 355– 362.

16. Shahzad, A., Hasan, H., Siddiqui, S.A. Callus induction and regeneration in Solanum nigrum L. *in vitro. Phytomorphology* 1999;49, 215–220.

17. Hassanein, A.M. and Soltan, D.M. *Solanum nigrum* is a Model System in Plant Tissue and Protoplast Cultures. *Biologia Plantarum* 2000;43(4) :501-509.

18. Gloubinoff P., Edelman ME. And Hallick RB. Chloroplast coded atrazine resistante in *Solanum nigrum* PSDA Loci from susceptible and resistant biotypes are isogenic except for a single condon change Nveleic acids *Res* 1984;12*:* 9489- 9596.

19. Akhter jahan, M. A. and Hadiuzzaman, S. Callus Induction and Plant Regeneration from Different Explants of *Solanum nigrum* L. Seedlings. *Plant Tissue Cult.* 1996;6(1): 57-62.

20. Venugopal, R. B., Kaviraj, C. P., Srinath Rao, Jabeen, F. T. Z., Kiran, G. Plant regeneration and *in vitro* flowering from leaf and naodal explants of *Solanum nigrum* (L.) – an important medicinal plant. *Plant Cell Biotechnology and Molecular Biology*,2005;6 (1/2): 17-22.

21. Ugandhar.T,Shekar GPV and Manjula P. High frequency plant regeneration from leaf explants of *Solanum nigrum Advances in Plant Sciences* 2010;23(1): 15-17

22. Basha, Amzad Kolar, L .Vivekanandan and Ghouse Basha M. *In vitro* Regeneration and Flower Induction on *Solanum nigrum* L. from Pachamalai hills of Eastern Ghats.*Plant Tissue Cult. & Biotech.* 2008;18(1): 43-48.

23. Kannan, T.M.S., S.M. Nagarajan and S. Kulothungan,. Micropropagation of *Solanum nigrum* L. a medicinal herb. *Plant Archieves:* 2005;609 305.

24. Yoganath, N., Bhakyaraj, R., Chanthuru, A., Parvathi, S. and Palanivel, S. Comparative Analysis of Solasodine from *in vitro* and *in vivo* cultures of *Solanum nigrum* Linn. Kathmandu University Journal of Science. Engineering and Technology 2009;5(1): 99-103.

25. Hanan Abd, Al-Hay and Al-Ashaal, A,. Regeneration, *in vitro* glycoalkaloids production and evaluation of bioactivity of callus methanolic extract of *Solanum tuberosum* L. *Fitoterapia* 2010:81(6): 600-606

26. Bhat, M.A., Mujib, A., Junaid, A. and Mohamooduafar, M. *In vitro* regeneration of *Solanum nigrum* with enhanced solasodine production. *Biologia Plantarum* 2010;54(4): 757-760.

27. Narasimhulu Reddy 1983

28. Patnaik, J. and Chand, P.K. Micropropagation of *Hemidesmus indicus* (L.) R. Br. through axillary bud culture. *Plant Cell Rep.*1996;15: 427-430.

29. Mohamed, S.V., Jawahar, M., Thiruvengadam, M., Jeyakumar, M. and Jayabalan, N. (1999).Effect of cytokinins on the proliferation of multiple shoots in horse gram (*Macrotyloma uniflorum* (L.) Verdc.). *Jour. Plant Biotech.*1999;1: 79-83.

30. Kumar, S., Chander, S., Gupta, H. and Sharma, D.R. (1998). Micropropagation of *Actinidia deliciosa* from axillary buds. *Phytomorphology* 1998;48(3): 303-307.

31. Velayutham, P. (2003). *In vitro* Regeneration, Pharmacognosy and Antimicrobial Activities of *Cichorium intybus* L. Ph.D. Thesis submitted to Bharathidasan University, Tiruchirappall.

32. Baskaran, P. and Jayabalan, N. An efficient micropropagation system for *Eclipta alba* a valuable medicinal herb. *in vitro* cell. Dev. *Biol.Plant* 2005;41:532-539.

33. Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plant*., 1962;15, 473-497.

34. Jain, N. and Babbar, S.B. Regeneration of 'juvenile' plants of black plum, *Syzygium cuminii* Skeels, from nodal exp of mature trees. *Plant Cell, Tissue and Organ Culture* 2003;73: 257-263.

35. Bajaj, Y.P.S. and Gill, M.S. Micropropagation and Germplasm. Preservation of cotton (*Gossypium* spp.) through shoot tip and meristem cultufre. *Ind. J. Exp. Biol.* 1986;24: 581-583.

36. Manjula, S., Anita, T., Benny, D. and Nair, C.M. *In vitro* plant regeneration of *Aristolachia indica* through axillary shoot multiplication and organogenesis. *Plant Cell Tiss. Org. Cult.* 1997;51: 145-148.

37. Komalavalli, N. and Rao, M.V. *In vitro* micropropagation of *Gymnema sylvestre* – A multipurpose medicinal plant. *Plant Cell Tiss. Org. Cult.* 2000;61:97-105.

38. Al-Bahrany, A .M. and Al-Khayri J. M. Micropropagation of grey mangrove Avicennia marina. *Plant Cell, Tissue and Organ Culture* 2003;72: 87-93.
39. Velayutham, P. and Ranjitha Kumari, B.D. Direct shoot regeneration from leaf explants of chicory (*Cichorium intybus* L.). *Plant Cell Biotech Mol Bio.* 2003;4(3&4): 125-130.
40. Martinussen, I., Nilsen, G., Svenson, L., Junttila, O. and Rapp, K.(2004). *In vitro* propagation of cloudberry (*Rubus chamaemorus*). *Plant Cell, Tissue and Organ Culture* 78: 43-49.Merck Index. 1989 Ed. S. Budavari, 11th Ed., NJ, USA. pp:1371.
41. Velayutham, P., Jahir Hussain, G. and Baskaran, P. *In vitro* plantlet formation from nodal explants of *Plumbago zeylanica* L. - an important medicinal plant. *J. Swamy Bot.* Cl. 2005;22 : 117-120.
42. Jabeen, F.T.Z., Venugopal, R.B., Kiran, G., Kaviraj, C.P. and Rao, S. Plant regeneration and in vitro flowering from leaf and nodal explants of *Solanum nigrum* (L). - An important medicinal plant. *Plant Cell Biotechnology and Molecular Biology* 2005;6(1&2):17-22.
43. Bajaj YPS and Dhanju MS. Regeneration of plants from apical meristem tips of some legumes*Curr.Sci.*1979;48: 906- 907.
44. Zenkteler, M., *In vitro* formation of plants from leaves of several species of the Solanaceae family. Biochem. Physiol. Pflanz. 1972;163: 509–512.
45. Griga M, Kubala Kovaj M and Tejk lovie E,. Somaticembryogenesis in *Vicia fablel* L. *Plant Cell Tissue and Organ Culture*, 1986;3: 319- 324.
46. Sudhershan L., Aboel M.N. and Hussian J. *In vitro* propagation of *Ziziphus mauritiana cultivar umrdn* by shoots tip and nodal multiplication. *Curr. Sci.*, 2000;80(2): 290-292.
47. Geetha N, Venkatachalam P and Lakshmisita G,. *Agrobacterium* mediated genetic transformation of pigeonpea (*Cajanus cajan* L.) and development of transgenic plants via direct organogenesis. *Plant Biotechnology*, 1999;16: 213-218
48. Nasir A Saeed, Yusuf Zafer and Kauser A Malik,. Simple procedures of Gossypium meristem shoot tip culture. *Plant Cell Tissue Org. Cult.*, 1997;51: 201- 207.

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