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Full Length Research Paper

Role of auxins in the *in vitro* rooting and micropropagation of *Holarrhena antidysenterica* Wall., a woody aromatic medicinal plant, through nodal explants from mature trees

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An economic and efficient procedure has been outlined for plant regeneration of an important medicinal shrub, *Holarrhena antidysenterica* W. using nodal explants obtained from about 20-year-old mature trees growing in the field, belonging to the family of Apocynaceae. Shoot development was maximum (90%) on Murashige and Skoog's (MS) basal medium supplemented with α-naphthalene acetic acid (NAA, 2.0 mg/L), indole-3-acetic acid (IAA, 1.0 mg/L), and KIN (1.0 mg/L) with (10.06 ± 0.24) mean number of shoots per explants and the maximum shoot length was found to be 4.01 ± 0.37. The role of auxins were instrumental as rooting of the differentiated shoots was best in MS medium with combination of indole-3-butyric acid (IBA, 1.5 mg/L) and IAA (1.5 mg/L) with 13.14 ± 0.08 mean number of roots per shoots and the mean root length was found to be 5.61 ± 0.03. Regenerated plantlets were successfully acclimated in the green house and after a hardening period of 4 weeks, 90% transplantation success was achieved under the natural condition. The established *in vitro* propagated plants were identical and uniform on the basis of the morphology and growth characteristics to the donor plants used in the study.

Key words: *Holarrhena antidysenterica*, medicinal shrub, rooting, plant regeneration, nodal explants.

INTRODUCTION

The plant, *Holarrhena antidysenterica* W. commonly known as telicherry bark in English is a lactiferous medicinal shrub or a tree which may attain a height of 9 to 10 m; it has a good distribution in eastern part of India (Satyavati et al., 1987). Medicinal plants are been extensively used globally for the preparation of herbal medicine, these are been investigated or micropropagated gated to know the active principles available in optimum quantities at the requisite time for standardization of herbal preparations (Chand et al., 1997). The wild stock of this important plant species has been markedly depleted. The shrub bears seeds only during the onset of the monsoon rains and its natural rate of multiplication is limited (Raha and Roy, 2003).

This lactiferous medicinal shrub was been used in the formulation of herbal traditional medicines from ancient time, such as, the bark is considered to be stomachic, anti-helminthic, and the dried bark was rubbed over the body in dropsy disease. Seeds are also used as an astringent, febrifuge, anti-dysenteric, carminative, anti-bronchitic and used for boils and ulcers (Chopra et al., 1956). The bark and the roots have been found to be an

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Abbreviations: IAA, indole-3-acetic acid; **IBA,** indole-3-butyric acid; **NAA,** α-naphthalene acetic acid; **MS,** Murashige and Skoog's (1962) medium.

excellent remedy for both acute and chronic dysentery, especially in cases where there is excessive blood with mucus and colic pain associated with stool (Ghose, 1984)

The regeneration ability of this plant is poor, and limited to the monsoons, seed viability is also low. Therefore, *in vitro* culture seems to be a useful tool for conservation and propagation of such rare, endangered, aromatic medicinal plant (Raha and Roy, 2003). Previously, several attempts have been made to conserve some of the rare medicinal plants (Arora and Bhojwani, 1989; Sharma et al., 1991; Bera and Roy, 1993; Sudha and Seeni, 1994; Sahoo and Chand, 1998; Wawrosch et al., 1999). *In vitro* conservation can be possible through micropropagation which allows the making numerous clones of the plant by the application of tissue culture techniques (Kanungo and Sahoo, 2011). Research works on a valuable medicinal plant also suggest that the medicinal plant need to have some urgent attention unless they will be threatened. Biochemical research work was reported by Heble et al. (1976) on the phytochemical studies involving cholesterol metabolism from callus cultures of *H. antidysenterica*. Although, some attempts on tissue culture studies of *H. antidysenterica* has been carried out (Panda et al., 1991; Ahmed et al., 2001; Raha and Roy, 2001, 2003; Agrawal et al., 2005; Mallikarjuna and Rajendrudu, 2007); in most of this papers, rapid *in vitro* propagation and mass propagation was not achieved and in few of them additional growth adjuvant are used for better response.

The present study describes the formulation of suitable media for shooting, rooting, and hardening of the plant in order to achieve quality transplants without the addition of growth adjuvant like casein hydrolysate, coconut milk, and adenine sulphate. Until now, there is no report on the development of an economic protocol which can minimise the experimental cost. The subsequent successful *ex vitro* establishment of the regenerated plants may help in the preservation of the plants for their rich therapeutic values and pharmacological importance.

MATERIALS AND METHODS

Plant and surface sterilization

The nodal explants were collected from about 20 years old healthy plants of *H. antidysenterica* found near the botanical garden of P.G. Department of Botany, Utkal University, Bhubaneswar, Odisha, India. For the purpose of micropropagation, young shoots were harvested during the month of January to April. The nodal explants were washed thoroughly under running tap water for 30 min, followed by immersing in a 5% (v/v) solution of detergent (Labolene, Qualigens, India) for 20 min, and were subsequently agitated in 0.1% Bavistin (fungicide by BASF India Ltd.) for 15 min, followed by rinsing with distilled water to remove the traces of Bavistin. After washing, the explants were taken to the laminar air flow chamber, where the explants were sterilised in 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for another 2 min, followed by rinsing with sterile double distilled water and the step was repeated for three times. Afterwards, the surface-decontaminated explants were implanted in MS (Murashige and Skoog, 1962) medium containing conical flasks.

Culture media and conditions

MS medium was used as the basal medium for shoot and root proliferation. The medium was supplemented with 3% (w/v) sucrose and was gelled with 0.8% (w/v) agar bacteriological grade (Himedia, Mumbai, India). The pH of the medium was adjusted to 5.8 before being autoclaved at 121°C for 15 min. All the cultures were maintained at 24 ± 2°C under 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 50 µmol $m^{-2}s^{-1}$ provided by cool white fluorescent lamps of (2 × 40 W, Phillips, India) with 60 to 65% relative humidity. The cultures were subcultured twice, at fortnightly intervals, by transferring to fresh medium.

Shoot proliferation and rooting

The induction of shoot development from the nodal bud explants was attempted with various concentrations and combinations of auxins and cytokinin such as α-naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), and KIN with a concentration not exceeding 3 mg/L. MS medium devoid of growth regulator served as control. For root induction, the *in vitro* developed shoots from the original nodal segments were implanted in MS medium supplemented with auxins, such as indole-3-butyric acid (IBA) and IAA. The culture flasks were maintained under the same conditions as described earlier.

Hardening

The rooted shoots ware removed from the culture medium and the roots were washed thoroughly in sterile distilled water to remove the media. The plantlets were transferred to thermocoal pots (5 cm diameter) containing autoclaved mixture of soil, sand, and vermi compost (1:1:1). The pots were kept in the culture room for 2 weeks with a supplementation of liquid MS (without agar) media for the survival of the small plants. The plants are subsequently transferred to the green house and were kept for another 4 weeks before transferring them to the garden soil under normal condition.

Statistical analysis

All the experiments were conducted with a minimum of 10 replicate per treatment, and were repeated three times. Data was analysed statistically and the significance of differences among means was carried out using Duncan's multiple range test (DMRT) at P = 0.05. The results are expressed as the mean ± standard error of mean (SEM) of three experiments.

RESULTS AND DISCUSSION

Initially, higher level of contamination (40 to 50%) was observed in the cultures, which must have probably risen from the explants, since they were collected from the natural condition from the garden. Nevertheless, treatment of the nodal segments with Bavistin remarkably reduces the contamination level to less than 10%. Shoot initiation was tried with different combination of auxins

Table 1. Role of auxins and cytokinin on shoot induction in *H. antidysenterica* W. from nodal explants in MS medium after 4 weeks of culture.

Conc. (mg/L) NAA + IAA+ KIN			Regeneration (%)	Mean no. of shoots/explants	Mean shoot length (cm)
Hormone-free control			NR	NR	NR
0.5	0.5	0.5	40	2.31 ± 0.19^{c}	1.02 ± 0.13^{d}
0.5	0.5	1	45	2.46 ± 0.39^{a}	1.19 ± 0.07^{c}
1	1.5	1.5	50	3.23 ± 0.23^{ab}	2.16 ± 0.26^{ab}
1.5	0.5	2.5	60	4.08 ± 0.19^{a}	2.49 ± 0.06^{d}
1.5	2	0.5	65	6.51 ± 0.19^{bc}	2.69 ± 0.03^{bc}
1.5	1	2	70	7.26 ± 0.22^{b}	3.04 ± 0.58^{a}
2	2	2	70	9.36 ± 0.18^{a}	3.16 ± 0.18^{d}
2	1	1	90	10.06 ± 0.24^{b}	4.01 ± 0.37^{b}
2.5	2.5	2.5	75	9.68 ± 0.12^{e}	3.38 ± 0.26^{d}
2.5	1	2.5	70	8.86 ± 0.09^{c}	3.15 ± 0.21^{b}
F value			-	1.006*	11.31*

Values represent mean ± SEM. Means sharing the same letter are not significantly different (P = 0.05) using Duncan's multiple range test. *Significant at P = 0.05, NR: indicates no response. Data represents the mean of 10 replicates for each treatment and recorded after 4 weeks of culture.

and cytokinin as presented in Table 1. Each nodal segment implanted differentiated in to a single shoot and all the nodal explants responded with bud breaks within a period of 2 week (Figure 1A) when tested with the combination of auxins and cytokinin.

Shooting percentage was increased (90%) with increase in the addition of NAA up to (2 mg/L). The best result was obtained when NAA (2.0 mg/L) was added to the medium along with IAA (1.0 mg/L) and KIN (1.0 mg/L). Maximum number of shoots 10.06 ± 0.24 per explants and the corresponding percentage of shoot length of 4.01 ± 0.37 cm were obtained in the aforementioned combination (Figure 1D). Increase in shoot number may be due to the suppression of apical dominance during subculture that induced basal dormant maristematic cells to form new shoots (Shukla et al., 2008).

Further, it was observed that shoot bud proliferation was suppressed a little bit when the aforementioned combination was applied with a higher concentration. A reduction in the number of shoots higher than the optimal level was earlier reported in other medicinal plants including *Capsicum annum* L. (Ahmad et al., 2006). Higher concentration of cytokinin that elicited inhibitory effects on shoot elongation was also earlier reported by Pattnaik et al. (1996). In the present study, it was observed that the combination of auxins and cytokines gives better yielding of shoots and there multiplication. Similar observations were also found (Kanungo et al., 2012; Rout et al., 2000; Sharma et al., 1993; Sharma and Singh, 1997; Shasany et al., 1998; Tsay et al., 1989) for other medicinal plants.

The newly formed leafy shoots derived from the nodal segments are introduced to MS media containing individual and combinations of auxins for rooting. In general, IBA is documented for its *in vitro* rooting promotion attributes. Previous investigators also obtained comparable results related to rooting with IBA in *Cleistanthus collinus* (Quraishi et al., 1996), *Rauwolfia micrantha* (Sudha and Seeni, 1996), *Holostemma adakodien* (Martin, 2002), *Ocimum basillicum* (Siddique and Anis, 2007), *Vitex negundu* (Chandramu et al., 2003), and *Solanum trilobatum* (Arockiasamy et al., 2002). Based on the earlier reports, *H. antidysenterica* rooting was tried with only auxins (Raha and Roy, 2003). Individual concentration of IBA or IAA, however, been able to initiate roots, but fails to give appreciable percentage of root induction (50%) each; hence, combination of both the hormones were used for better response. MS medium containing IBA and IAA (1.5 mg/L) each proved to be the most effective for rooting of micro shoots than that of any other concentration (Table 2) as the rooting percentage was 100%. Medium containing the aforementioned concentration showed the highest percentage of 13.14 ± 0.08 mean number of roots per shoots and the mean root length was found to be 5.61 ± 0.03 (Figure 2B). However, it was observed that increasing the concentration of the aforementioned hormones more than 1.5 mg/L had a negative impact as it affect growth of roots and ultimately on the rooting percentage (Table 2). The results of rooting experiment coincide with the observation of Karthikeyan et al. (2009), who obtained maximum root growth in MS medium supplemented with 1.5 mg/L IBA, while working on *Scoparia dulcis*.

Plantlets with 8 to 10 leaves and well developed roots were successfully transferred to the thermocole pot containing sterile soil, sand, and vermi compost in the ratio of 1:1:1 (Figure 2C and D). The pots were kept inside the tissue culture room for few days with the

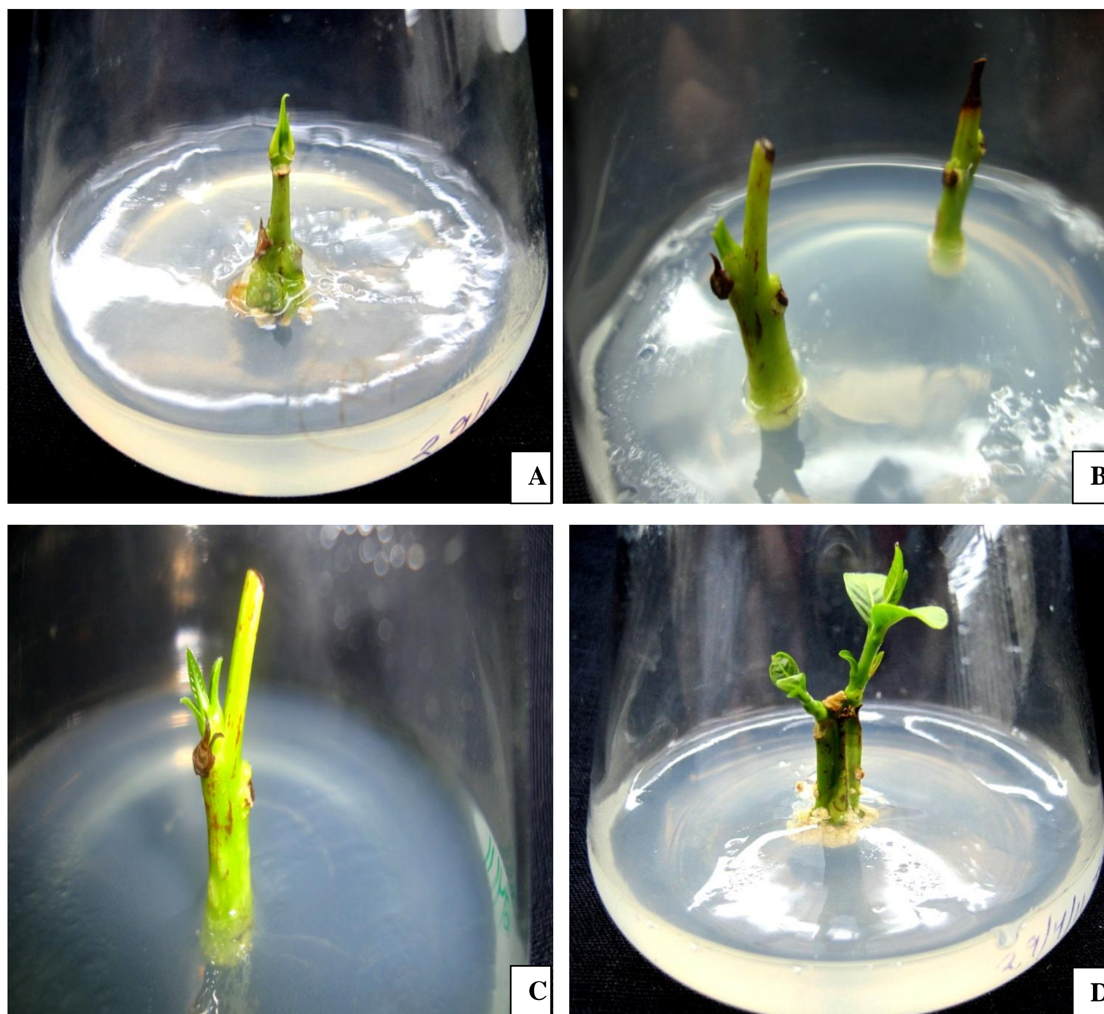

Figure 1. (A) Inoculated *H. antidysenterica* apical bud explant showing bud break; (B) and (C) Nodal explants of *H. antidysenterica* showing shoot initiation; (D) Shoot multiplication in *H. antidysenterica*.

Table 2. Influence of individual and combinations of auxins on rooting of *in vitro* raised shoots of *H. antidysenterica* W. in MS medium.

Conc. (mg/L) IBA + IAA		Rooting	Mean no. of root/explants	Mean root length (cm)
Hormone-free control		NR	NR	NR
0.5	0.5	50	3.18 ± 0.43^{a}	1.16 ± 0.15^{e}
0.5	1	55	3.36 ± 0.51^{bc}	2.09 ± 0.03^{c}
1	0	50	4.19 ± 0.73^{b}	2.56 ± 0.21^{cd}
0	1	50	5.08 ± 0.09^{bc}	2.92 ± 0.09^{b}
1.5	0.5	70	5.63 ± 0.09^{e}	2.98 ± 0.33^{d}
1.5	2.5	80	8.40 ± 0.23^{c}	3.16 ± 0.53^{b}
1.5	1.5	100	13.14 ± 0.08^{a}	5.61 ± 0.03^{a}
2.5	1	80	7.12 ± 0.09^{b}	3.82 ± 0.39^{c}
3	2	80	8.39 ± 0.17^{b}	4.19 ± 0.15^{a}
2.5	3	75	9.33 ± 0.14^{f}	4.47 ± 0.58^{e}
F value			1.871*	131.9*

Values represent mean ± SEM. Means sharing the same letter are not significantly different (P = 0.05) using Duncan's multiple range test. *Significant at P = 0.05, NR: indicates no response. Data represents the mean of 10 replicates for each treatment and recorded after 4 weeks of culture.

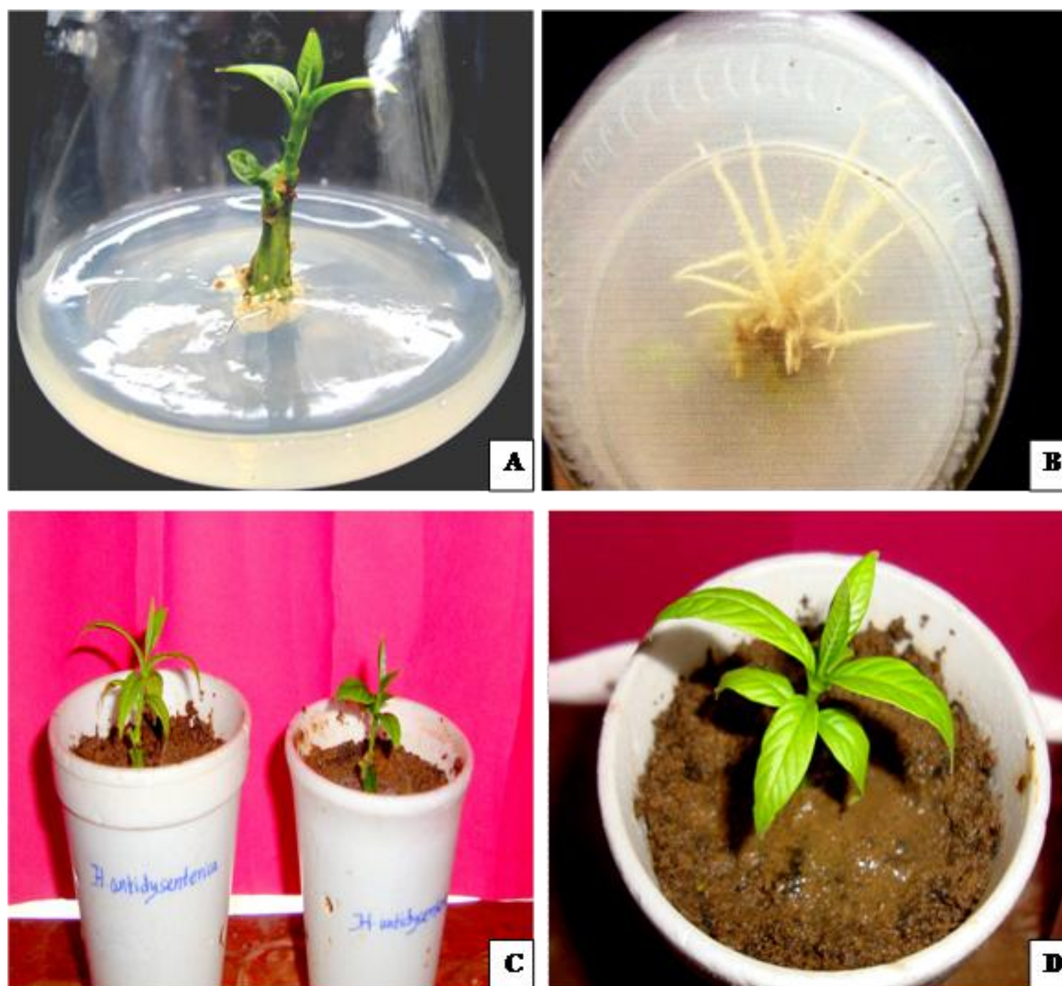

Figure 2. (A) and (B) Rooting initiation and profuse rooting of *H. antidysenterica*; (C) and (D) Transfer of newly formed explants of *H. antidysenterica* to sterile soil.

supplementation of liquid MS media on requirement and subsequently transferred to the green house.

MS media was used throughout the present investigation, as earlier, it was frequently used by several researchers in the micropropagation of *H. antidysenterica*, the only exception was woody plant medium (WPM) and B5 media used by Mallikarjuna et al. (2007), but he has also concluded that MS medium was more effective in inducing multiple shoots than the other medium. In this investigation, growth adjuvant like coconut milk, casein hydrolysate, and adenine sulphate are not been used, but appropriate response in terms of shoot initiation and rooting was observed, hence, the present protocol can become economic and it can minimize the experimental cost and time. Formation of basal callus is a common observation in tissue culture (Bhattacharya and Bhattacharya, 2001), perhaps due to the action of accumulated auxin at the basal cut end as on the cell proliferation, especially in the presence of cytokinins (Marks and Simpson, 1994). Basal callus formation is often observed in plants showing strong apical dominance (Preece and Sutter, 1991). Initial branching and multiplication was usually not found resulting in unbranched solitary shoot formation in tree species like *Holarrhena*. The same observation was reported in *Ulmus punila* (Corchete et al., 1993). In such cases, more buds were induced to develop by removal of existing shoots and reculture of entire plant; the same procedure was followed in the present study.

Efficient rooting of micropropagated shoots and their field establishment are the critical stages for growing plants using tissue culture technology. In the present study, root induction was achieved on the presence of auxins in MS medium containing individual and combinations of IAA and IBA. Similar results were reported by Shrivastava and Manerjee (2008) in *Jatropha curcas.* The same process was also followed in case of *Plumbago zeylanica* by Saxena et al. (2000). Although, many of the protocols standardised earlier for the *in vitro* propagation of *Holarrhena* suggest that IBA is the common

hormone which can induce rooting reported for other plant species, such as cherry species (Kris et al., 2005) and *Lagerstroemia parviflora* (Tiwari et al., 2002). However, the survival rate in the present investigation (90%) and the rooting was achieved in all the explants in the combination of auxins; hence, it can be considered as evidence that it is better to take the combination of IAA and IBA to get profuse rooting and ultimately good survival rate.

Conclusion

The main aim of the present investigation was to develop an economic protocol to minimize the experimental cost and time and the high survival rate in the present study indicates that this procedure could be easily established for *in vitro* culture. This method can be adopted for large scale propagation which may help in the conservation study of *H. antidysenterica*; a valuable medicinal shrub. The development of easy tissue culture protocol will help the nursery owners to invest more money in the cultivation of this endangered medicinal plant.

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REFERENCES

Agrawal V, Kumar R, Sharma K (2005). *In vitro* clonal propagation of *Holarrhena antidysenterica* (L.) Wall. through nodal expalnts from mature trees. *In vitro* Cell. Dev. Biol. Plant. 41:137-144.

Ahmad N, Siddique I, Anis M (2006). Improved plant regeneration in *Capsicum annum* L. from nodal segments. Biol. Plant. 50:701-704.

Ahmed G, Roy PK, Mamun ANK (2001). High frequency shoot regeneration from nodal and shoot tip explants in *Holarrhena antidysenterica* L. Indian J. Exp. Biol. 39:1322-1324.

Arockiasamy DI, Muthukumar B, Natrajan E, Britto SJ (2002). Plant regeneration from node and internode explants of *Solanum trilobatum* L. Plant Tissue Cult. 12(2):93-97.

Arora R, Bhojwani, SS (1989). *In vitro* propagation and low temperature storage of *Sausurea lappa* C.B. Clarke- an endangered medicinal plant. Plant Cell Rep. 8:44-47.

Bera TK, Roy SC (1993). Micropropagation of *Tylophora indica* (Burman f.) merr. by multiple bud formation from mature leaf explants without callus intervention. Bot. Bull. Acad. Sin. 34:83-87

Bhattacharya R, Bhattacharya S (2001). High frequency *in vitro* propagation of *Phyllanthus amarus* Schum. & Thom. by shoot tip culture. Indian J. Exp. Biol. 39:1184-1187.

Chand S, Sahrawat AK, Prakash DVSSR (1997). *In vitro* culture of *Pimpinella anisum* L. (Anise). J. Plant Biochem. Biotechnol. 6:1-5.

Chandramu C, Rao DM, Reddy VD (2003). High frequency induction of multiple shoots from nodal explants of *Vitex negundu* L. using sodium suphate. J. Plant Biotechnol. 5(2):107-113.

Chopra RN, Nayar SL, Chopra IC (1956). Glossary of Indian medicinal plants. New Delhi: Publication and Information Directorate, CSIR, pp. 134-135.

Corchete MP, Diez JJ, Valle T (1993). Micropropagation of *Ulmus punila* L. from mature trees. Plant Cell Rep. 12:534-536.

Ghose SC (1984). *Holarrhena antidysenterica* and *Wrightia tinctoria.* In: Drugs of Hindustan. Calcutta: Hahnemann Publishing Co. Pvt. Ltd., pp. 309-320.

Heble MR, Narayanswamy S, Chadha MS (1976). Metabolism of cholesterol by callus culture of *Holarrhena antidysenterica*. Phytochemistry 15:1911-1912.

Kanungo S, Sahoo SL (2011). Direct organogenesis of *Withania somnifera* L. from apical bud. Int. Res. J. Biotechnol. 2(3):58-61.

Kanungo S, Sahoo SL, Sahu RK (2012). Development of a simplified protocol for *in vitro* propagation of a valuable medicinal plant *Plumbago zeylanica* Linn. through nodal explants found in Odisha, India. J. Med. Plants Res. 6(13):2627-2632.

Karthikeyan S, Prasad R, Mahendran TS, Rajgopal K, Ravendran V (2009). Direct regeneration and *in vitro* flowering of *Scoparia dulcis* L. Indian J. Sci. Technol. 2(5):55-57.

Kris P, Tess A, Jerry N (2005). Tissue culture propagation of mangolian cherry (*Prunus fruticosa)* and nanking cherry (*Prunus tomentosa*). Plant Cell Tissue Organ Cult. 82:207-211.

Mallikarjuna K, Rajendrudu G (2007). High frequency *in vitro* propagation of *Holarrhena antidysenterica* from nodal buds of mature tree. Biol. Plant. 51(3):525-529.

Marks TR, Simpson SE (1994). Factors affecting shoot development in apically dominant *Acer* cultivars *in vitro*. J. Hort. Sci. 60:543-551.

Martin KP (2002). Rapid propagation of *Holostema adakodien,* Schult, a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. Plant Cell Rep. 21:112-117.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Panda AK, Bisaria VS, Mishra S, Bhojwani SS (1991). Cell culture of *Holarrhena antidysenterica*: growth and alkaloid production. Phytochemistry 30:833-836.

Pattnaik SK, Sahoo Y, Chand PK (1996). Micropropagation of a fruit tree *Morus australis* Poir. Syn. M. Acidosa Griff. Plant Cell Rep. 15:841-845.

Preece JC, Sutter EG (1991). Acclimatization of micropropagation plants to the greenhouse and the fields. In: Debergh, P.C., Zimmerman, R.H. (ed.): Micropropagation, Kluwer Academic Publishers, Dordrecht. pp. 71-93.

Quraishi A, Koche V, Mishra SK (1996). *In vitro* micropropagation from nodal segments of *Cleistanthus collinus*. Plant Cell Tiss. Organ. Cult. 45:87-91.

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

Raha S, Roy SC (2003). Efficient plant regeneration in *Holarrhena antidysenterica* Wall. from shoot segment derived callus. *In vitro* Cell. Dev. Biol. Plant., 39: 151-155.

Rout GR, Samantray S, Das P (2000*). In vitro* manipulation and propagation of medicinal plants. Biotechnol. Adv. 18: 91-120.

Sahoo Y, Chand PK (1998). Micropropagation of Vitex negundo L., a woody aromatic medicinal shrub, through high frequency axillary shoot proliferation. Plant Cell Rep. 18: 301-307.

Satyavati GV, Gupta AK, Tandon N (nee) Bhatia, eds. (1987) Medicinal plants of India, Vol. 2. New Delhi: Indian Council Med. Res. pp. 41-48.

Saxena C, Samantaray S, Rout GR, Das P (2000). Effect of auxins on *in vitro* rooting of *Plumbago zeylanica*: peroxidise activity as a marker for root induction. Biol. Plant. 43(1):121-124.

Sharma N, Chandel KPS, Paul A (1993). *In vitro* propagation of *Gentiana kurroo*: an indigenous threatened plant of medicinal importance. Plant Cell Tiss. Org. Cult. 34:307-309.

Sharma N, Chandel KPS, Srivastava VK (1991). *In vitro* propagation of *Coleus forskohlii* Briq., a threatened medicinal plant. Plant Cell Rep. 10: 67-70.

Sharma TR, Singh BM (1997). High frequency *in vitro* multiplication of disease free *Zingiber officinale* Rosc. Plant Cell Rep. 17:68-72.

Shasany AK, Khanuja SPS, Dhawan S, Yadav V, Sharma S, Kumar S (1998). High regenerative nature of *Mentha arvensis* internodes. J. Biosci. 23: 641-646.

Shrivastava S, Manerjee M (2008). *In vitro* regeneration of physic nut (*Jatropha curcas* L.): Influence of additives. Int. J. Integr. Biol. 3(1):73-79

Shukla S, Shukla SK, Mishra SK (2008). *In vitro* plant regeneration from seedling explants of *Stereospermum personatum* DC: a medicinal tree. Trees Struct. Funct. 23:409-413.

Siddique I, Anis M (2007). Rapid micropropagation of *Ocimum basilicum* using shoot tip explants pre-cultured in thidiazuron supplemented medium. Biol. Plant, 51: 787-790.

Sudha GC, Seeni S (1996). *In vitro* propagation of *Rauwolfia micrantha*, a rare medicinal plant. Plant Cell Tiss. Organ Cult. 44:243-248.

Sudha GC, Seeni S (1994). *In vitro* multiplication and field establishment of *Adhatoda beddomei* CB Clarke, a rare medicinal plant. Plant Cell Rep. 13:203-207.

Tiwari SK, Kashyap MK, Ujjaini MM, Agarwal AP (2002). *In vitro* propagation of *Lagerstroemia parviflora* Roxb. from adult tree. Indian J. Exp. Biol. 40:212-215.

Tsay HS, Gau TG, Chen CC (1989). Rapid clonal propagation of *Pinellia ternate* by tissue culture. Plant Cell Rep. 8:450-454.

Wawrosch C, Maskay N, Koop B (1999). Micropropagation of the threatened Nepalese medicinal plant *Swertia Chirata* Buch.- Ham ex. Wall. Plant Cell Rep. 18:997-1001.