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In vitro Propagation of *Nyctanthes arbor-tristis*L. through Culture of Axillary bud and Shoot tip Explants from *In vitro* Germinated Seedlings

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Abstract: ***Nyctanthes arbor-tristis*** **L. is commonly known as Coral Jasmine and Parijat, belonging to the family Oleaceae (Nyctaginaceae). It is one of the most versatile medicinal plants having a wide spectrum of biological activities. The present study aimed to develop an improved protocol for *in vitro* propagation through the culture of axillary bud and shoot tip explants from *in vitro* germinated seedlings. Murashige and Skoog (MS) medium as basal medium and supplemented with different concentrations and combinations of 6-benzyl amino purine (BAP), α- naphalene acetic acid (NAA), Kinetin (Kn) and indole butyric acid (IBA) were used in order to shoot induction, multiplication and rooting. The medium without any plant growth regulators (PGRs) were employed for *in vitro* seed germination. The axillary bud and shoot tip were excised from *in vitro* germinated seedlings and inoculated on initiation medium. The maximum percentage of shoot induction with 6.10 ± 1.28 of shoots was produced on the medium fortified with 4.44 µM/L of BAP and 2.68 µM/L of NAA. The well initiated shoots were sub cultured on fresh medium composed of different concentration and combinations of PGRs for further multiplication of shoots. The medium enriched with 8.07 µM/L of BAP and 3.76 µM/L of NAA was found to be efficient for maximum percentage of shoot multiplication with the highest number of shoots (21.10 ± 1.85) and mean shoot length of 10.30 ± 1.41cm. The effective root induction with maximum number of roots was obtained on the half strength MS medium supplemented with 4.92 µM/L of IBA. The well rooted healthy plantlets were hardened and establish successfully with 95% of survival. This protocol may be useful for the large scale production of this plant and other medicinal plants.**

Keywords: ***Nyctanthesorbor-tirstis, in vitro*** **seed germination, axillary buds, shoot tip, *in vitro* propagation.**

1. INTRODUCTION

*Nyctanthes arbor-tristis*L. is an important medicinal plant belonging to the family Oleaceae (Nyctaginaceae), during the day the plant loses all its brightness and hence it is called as "Tree of sadness" (*arbor-tristis*). It is also commonly known as Harsinghar, Coral Jasmine, Parijat, Queen of the night and Night flowering jasmine and is well known in India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activities. It is usually a shrub or a small tree having brilliant, highly fragrant flowers, which bloom at night and fall off before sunrise, giving the ground underneath a pleasing blend of white and red. Folk people of Tripura predict the weather and rainfall variation through flowering phenology of night flowering jasmine which help them to plan agro forestry activities and disaster prevention [1]. Different parts of this plant are used in Indian medicine for various pharmacological actions. Phytochemical of leaf, fruit and seeds of *N. arbor-tristis*arephytosterols, phenolics, tannins, flavanoids, and saponins. The secondary metabolites such as glycosides and alkaloids are the largest groups of chemicals produced by this plant. The orange tubular calyx of the flower contains modified diterpenoidnyctanthin, anthocyanins, carotenoids and essential oils. Seed kernels yield 12-16% of the pale yellow brown fixed oil,which consists of glucosides of linoleic, oleic, lignoceric, stearic, palmitic acid and b-sitosterol. Its root contains glycosides, tannins and alkaloids [2].

The orange corolla tubes are used for dyeing silk and cotton, this practice was started with Buddhist monks whose orange robes were given their colour by this flower. The Parijata is regarded in Hindu mythology as one of the five wish-granting trees of Devaloka [3]. Different plant parts of this plant are known to possess various ailments by tribal people of India especially Orissa and Bihar along with its use in Ayurveda, Sidha and Unani systems of medicines.

The flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles.The stem bark pounded with *Zingiberofficinale*and *Piper longum*is boiled in water and the resultant liquid is taken for two days for the treatment of malaria in Orissa. The leaves are used for the treatment of chronic fever, rheumatism, and internal worm infections, and as laxative, diaphoretic and diuretic diseases. The seeds are used as anthelmintics and in alopecia. The root decoction is used for the enlargement of spleen [2]. Commercially useful products also obtained from *N. arbor-tristis*such as tannins of dyestuff have been used in traditional fabric dyeing and also used as timber andfuel. The essential oil obtained in the fragrant flowers is used as perfume [1].

Plant tissue culture refers to as *in vitro*, which is an important tool in both basic and applied studies as well as in commercial application [4], [5]. *In vitro* cultures are now being used as a tool for the study of various basic problems in plant sciences. *In vitro* propagation method offers highly efficient tool for germplasm conservation and mass multiplication of many threatened plant species [6], [7]. The *in vitro* regeneration of medicinal plants were carried out by various researchers with different types of explants such as axillary buds/nodal segments of *Ephedra gerardiana* [8]; *Artemisia annua* L.[9]; *Plumbagozeylanica* L. [10]; rhizome buds of *Zingiberzerumbet* (L.) Sm. [11] and shoot tip and nodal segments of *Punicagranatum* L. [12]; *Nyctanthes arbor-tristis* [13], [14], [15]. The aim of present study is to develop an improved protocol for *in vitro* propagation of *Nyctanthes arbor-tristis* L. through the culture of axillary bud and shoot tip explants from *in vitro* germinated seedlings.

2. MATERIALS AND METHODS

Source and preparation of explants

The healthy fruits of *Nyctanthes arbor-tristis*L. were collected from Tamilnadu Agriculture University, Information & Training Centre, Anna Nagar, Chennai, Tamilnadu and seeds were excised from the fruits (Fig:1a). The seeds were washed with running tap water and then soaked with soap solution and again washed thoroughly in running tap water and immersed in 70% ethanol for 5 minutes. The washed seeds were transferred in to Laminar Air Flow chamber (LAF) where the surface sterilization was carried out with the help of disinfectant mercuric chloride ($HgCl_2$). The seeds were subjected to surface sterilization with 0.1% of (w/v) of $HgCl_2$ for 5 minutes. After that, the seeds were rinsed three times with sterile water to remove the traces of disinfectant adhering on the surface of the seeds. The seed coat was removed with the help of sterile blade without damaging the cotyledons and embryos and then the seeds were used for *in vitro* germination (Fig: 1b). The axillary bud and shoot tips were excised from *in vitro* germinated seedlings and used as explants for *in vitro* propagation.

Nutrient medium

Growth and morphogenesis of plant tissues *in vitro* are largely governed by the composition of the culture media. In the present study, Murashige and Skoog [16] medium was used basal medium for all the experiments. The MS basal medium fortified with 3% of sucrose and without any plant growth regulators (PGRs) were used for *in vitro* seed germination. The MS basal medium fortified with different concentrations of 6-benzyl amino purine (BAP) (1.33 – 8.07µM/L) alone and in combination with α- naphalene acetic acid (NAA) (1.34 & 2.68 µM/L) and Kinetin (Kn) (2.32 – 9.29 µM/L) were employed for shoot induction and multiplication (Table-1&2). The full and half strength MS basal medium supplemented with indole butyric acid (IBA) (2.46 & 4.92 µM/L) were used for rooting (Table-3). The pH of the media was adjusted to 5.8 and all the media used were autoclaved for 20 minutes at 121°C and 15 psi of pressure.

Inoculation and culture condition

The excised seeds with embryo were subjected to *in vitro* seed germination on MS basal medium supplemented with 3% of sucrose and without any PGRs. The explants such as axillary buds and shoot tips from *in vitro* germinated seedlings were excised and size was about 0.5-1.0 cm length. The explants were used without surface sterilization and they were inoculated on MS basal medium fortified with different concentrations and combinations of PGRs. All the cultures were incubated under the temperature of 25 ± 2ºC and the light intensity of 2000 - 4000 Lux. The photoperiod regime for cultures was 16 hr light and 8 hr dark and the relative humidity was maintained between 50 - 60%.

Initiation of shoots and subculture

Axillary bud and shoot tip explants were inoculated in each culture bottles containing MS medium fortified with BAP (1.33 – 8.07µM/L) alone and in combination with NAA (1.34 & 2.68 µM/L) (MS:1-7) for initiation of shoots. The young multiple shoots initiated were removed and sub cultured on fresh culture bottles containing MS medium fortified different concentration and combinations of BAP (1.33 – 8.07µM/L), NAA (1.34 & 2.68 µM/L) and Kn (2.32 – 9.29 µM/L) for further multiplication of shoots. The cultures were observed regularly and the results were recorded for every sub culture. The number and the length of shoots were recorded in 4 weeks old culture to assess the best treatment.

Root induction and hardening

After the multiplication phase, the *in vitro* developed shoots were separated (5 - 6 cm in length) and transferred to the medium is constituted with half and full strength of MS basal medium supplemented with various concentrations of IBA (2.46 and 4.92µM/L) for root induction. The number and the length of roots were recorded to assess the best treatment. The developed and healthy plant lets with roots were taken out and washed thoroughly and then transferred to portrays containing sterile soil and vermiculite in the ratio of 1:1 for primary hardening. After primary hardening they are transfer to polythene bags containing sand, soil and vermiculite in the ration of 1:1:1secondary hardening under the shade house.

Statistical Analysis

The collected data were analyzed by analysis of variance (ANOVA) followed by Tuky's HSD test values to compare the significant differences among means at 5% level of significance. All the data were expressed in the Mean ± Standard Deviation.

3. RESULTS AND DISCUSSION

In vitro seed germination

The sterilized seeds of *Nyctanthes arbor-tristis*L. were inoculated on MS basal medium without any growth regulators. The seeds were started to break the cotyledon and looks pale yellow colour after four days of inoculation. Cotyledons were opened completely within seven days, and the cotyledons were turned in to green colour (Fig:1c). The induction of plumule and radical was noticed after seven days of inoculation. The plumule elongated with leaf primordium, and radical also elongated with root initials after 20 days of inoculation. The shoot grew to the length of 4.0 to 5.0 cm with 4-5 leaves within thirty days of incubation (Fig:1d). The well developed *in vitro* seedlings were obtained after 30 days of culture. The axillary bud and shoot tips were excised from these *in vitro* seedlings and used as explants for initiation of shoots. Paudel*et al.*, [17] reported that the protocol for *in vitro* seed germination and seedling development of *Esmeralda clarkei*Rchb.f. were achieved on the MS medium fortified with BAP and NAA alone or in combinations, but in the present study, the *in vitro* seed germination and seedling were developed efficiently on the MS basal medium supplemented without any plant growth regulators. This result coincides with the results reported by Gandhi *et al.*, [18] in micropropagation of *Aeglemarmelos* and Das *et al.*, [19] also reported that *in vitro* seed germination on MS medium without growth regulators.

Initiation and multiplication of shoots

The axillary bud and shoot tip explants were excised from the *in vitro* germinated seedlingsand used as an explant for direct regeneration. These explants were inoculated on MS basal medium supplemented with various concentrations of BAP (1.33, 2.66 and 4.44 µM/L) and in combination with NAA (1.34 and 2.68 µM/L) for initiation of shoots and the results are tabulated (Table-1).

The shoot induction was very less on the medium supplemented with only BAP 1.33µM/L (MS-1), which showed the induction percentage of 22.00 ± 3.36%. Among the various combinations used, MS-2 (BAP-2.66 µM/L) and MS-4 (BAP-2.66 + NAA-1.34 µM/L) was noticed moderate percentage (30.40 ± 2.67 and 39.60 ± 3.86) of shoot induction. They also produced moderate mean number of shoot buds per explant (3.10 ± 0.87 and 3.80 ± 0.78) with mean shoot length of 4.15 ± 0.91 and 4.80 ± 0.71cm. The highest percentage (77.10 ± 2.92%) of shoot induction was observed on the medium fortified with 4.44 µM/L of BAP and 2.68 µM/L of NAA (MS-7), with maximum shoot number of 6.10 ± 1.28, and with the mean length of 5.66 ± 1.15cm (Table-1; Fig:1e). This result is coinciding with the earlier report of Rout, *et al.*, [13]. They achieved maximum shoot number (6.65) on the medium augmented with combination of BAP-1.5mg/L, Adenine sulfate (Ads) 50 mg/L and Indole-3-acetic acid (IAA) 0.10 mg/L.

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In the present study, among these different concentrations and combinations of PGRs, the medium supplemented with the combination of BAP and NAA was comparatively more efficient than that of individual effect of BAP on maximum percentage of shoot induction with highest number of shoots per explants. Hence, the medium fortified with 4.44 µM/L of BAP and 2.68 µM/L of NAA (MS-7) was found to be suitable one for shoot initiation.The young multiple shoots initiated were sub cultured on fresh medium composed of different concentration and combinations of BAP, NAA and Kn for further multiplication of shoots. The multiplication of shoots was noticed in all media combinations but with varied response in terms of number and length of shoots (Table-2). The maximum percentage 83.00 ± 5.05 of shoot multiplication was recorded on the medium enriched with 8.07 µM/L of BAP and 3.76 µM/L of NAA (MS-9).

Figure:1 ***In vitro*** **Propagation of** ***Nyctanthes arbor-tristis*****L.; a.** Healthy seeds, **b.** Seed coat removed seeds, **c.** Initial stage of seed germination with opened cotyledon, **d.** Well developed *in vitro* germinated seedlings,**e.** Shoot initiation,**f.** Shoot multiplication, **g.** Profuse root induction, **h.** Hardened plant with vigorous growth.

Table - 1: Effect of plant growth regulators on shoot induction

Medium	Plant Growth Regulators		Percentage of Shoot induction (%)	Number of Shoots per node	Length of Shoots (cm)
	BAP (µM/L)	NAA (µM/L)			
MS-1	1.33	-	22.00±3.36^{a}	1.80±0.63^{a}	3.10±0.45^{c}
MS-2	2.66	-	30.40±2.67^{b}	3.10±0.87^{b}	4.15±0.91^{bc}
MS-3	4.44	-	50.50±3.65^{d}	4.10±0.87^{c}	3.85±0.74^{b}
MS-4	2.66	1.34	39.60±3.86^{c}	3.80±0.78^{bc}	4.80±0.71^{cd}
MS-5	4.44	1.34	55.60±4.76^{e}	5.00±0.81^{d}	5.05±0.64^{de}
MS-6	2.66	2.68	62.00±4.64^{f}	4.60±0.96^{cd}	5.30±0.67^{de}
MS-7	4.44	2.68	77.10±2.92^{g}	6.10±1.28^{e}	5.66±1.15^{f}
F-Value			253.299	22.956	8.098
P-Value			0.000	0.000	0.000

*The values represent the Mean ± SD of ten replicates and all experiments were repeated thrice. Means with different letter within column are significantly different from each other at $P \leq 0.05$.

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Table - 2: Effect of plant growth regulators on multiplication of shoots

Medium	Plant Growth Regulators			Percentage of Shoot Multiplication (%)	Number of Shoots	Length of Shoots (cm)
	BAP (µM/L)	NAA (µM/L)	Kn (µM/L)			
MS-1	1.33	-	-	21.20±3.22^{a}	2.60±1.07^{ab}	3.45±0.59^{a}
MS-2	2.66	-	-	31.90±2.37^{b}	5.00±1.15^{cd}	4.35±0.94^{b}
MS-3	4.44	-	-	45.70±5.25^{e}	7.70±0.94^{e}	4.60±0.80^{b}
MS-4	2.66	1.34	-	42.00±2.82^{d}	9.70±1.25^{f}	5.30±1.05^{b}
MS-5	4.44	1.34	-	49.40±5.12^{f}	11.60±1.83^{g}	8.85±0.94^{d}
MS-6	2.66	2.68	-	59.60±3.97^{h}	10.10±1.19^{f}	8.95±1.11^{d}
MS-7	4.44	2.68	-	70.10±2.96^{i}	15.30±2.05^{h}	13.10±0.85^{f}
MS-8	6.65	3.22	-	78.20±5.00^{j}	20.00±1.41^{i}	9.95±0.98^{e}
MS-9	8.07	3.76	-	83.00±5.05^{k}	21.10±1.85^{i}	10.30±1.41^{e}
MS-10	-	1.34	2.32	34.10±2.80^{bc}	2.00±0.66^{a}	5.10±0.65^{b}
MS-11	-	1.34	4.65	36.80±4.49^{c}	2.90±0.73^{ab}	4.85±0.94^{b}
MS-12	-	2.68	2.32	41.90±3.81^{d}	3.80±1.13^{bc}	4.55±1.14^{b}
MS-13	-	2.68	4.65	51.20±3.61^{fg}	4.80±0.91^{cd}	6.55±1.01^{c}
MS-14	-	3.22	6.97	54.00±3.39^{g}	5.90±1.10^{d}	6.45±0.98^{c}
MS-15	-	3.76	9.29	60.00±3.55^{h}	8.10±1.10^{e}	7.25±1.35^{c}
F-Value				194.287	220.580	76.156
P-Value				0.000	0.000	0.000

*The values represent the Mean ± SD of ten replicates and all experiments were repeated thrice. Means with different letter within column are significantly different from each other at $P \leq 0.05$.

This medium produced highest number of shoots (21.10 ± 1.85) with mean length of 10.30 ± 1.41cm (Table-2; Fig: 1f). This result is akin to the earlier report of Bansal*et al.*, [14]. They achieved the maximum number of shoot production (14.13) on MS basal medium with 22.2 µM/l of BAP. This result is also in line with earlier report of Siddique*et al.*, [15] in which the highest mean number of shoots (15.1) was achieved on the medium MS supplemented with BAP (2.5mg/L) and NAA (0.5mg/L). Hence, this combination is considered to be the most suitable one for the successful multiplication of shoots.In the present study, among the various media combinations, BAP + NAA was found to be the most favorable than the combination of Kn + NAA for multiplication of shoots because this combination was markedly enhance the number and length of the shoots.

Root induction and Hardening

After the multiplication phase, the *in vitro* developed shoots were separated (5 - 6cm in length) and transferred to rooting medium for root induction. The medium is constituted with half and full strength of MS supplemented with two concentrations of IBA (2.46 and 4.92 µM/L). The root induction was observed in all media combinations, but the response was varied based on the concentration (Table-3). The half strength MS medium supplemented with 4.92µM/L of IBA (MS-19) was responded to the maximum percentage of root induction (83.00 ± 6.30). This medium produced highest mean number of roots (21.60 ± 2.36) per shoot with mean length of 8.05 ± 1.53cm (Table-3; Fig: 1g). Rout *et al.*, [13] reported, the maximum percentage of rooting on half strength MS fortified with combination of IBA and IAA, but in this study we could produce the root induction with the medium containing IBA (4.92 µM/L) alone.

The earlier report by Bansal *et al.*, [14] showed maximum rooting (13.2 ± 1.80) on higher concentration of IBA (9.86 µM/L) but in this study we used minimal concentration of IBA (4.92 µM/L) and it has yielded maximum rooting response (21.60 ± 2.36). In root induction full and half strength of MS basal medium supplemented with various concentration of IBA were tried.

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Table - 3: Effect of IBA on Root induction

Medium	Plant Growth Regulators	Percentage of Root induction (%)	Number of Roots per Shoots	Length of Roots (cm)
	IBA (µM/L)			
MS-16	2.46	11.70±2.86[a]	5.60±1.95[a]	3.25±0.79[a]
MS-17*	2.46	40.60±4.37[c]	11.90±3.21[b]	4.95±1.01[b]
MS-18	4.92	31.90±6.50[b]	11.40±3.30[b]	5.63±0.86[b]
MS-19*	4.92	83.00±6.30[d]	21.60±2.36[c]	8.05±1.53[c]
F-Value		329.154	57.339	39.603
P-Value		0.000	0.000	0.000

Note: * Half strength MS medium.

The results clearly indicated that, the half strength MS was effective towards efficient root induction, when compare to full strength MS basal medium. The well developed healthy plantlets were subjected to primary hardening with hardening mixture under the green house condition for 4 weeks. After primary hardening they are transfer to polythene bags containing sand, soil and vermiculite in the ration of 1:1:1secondary hardening under the shade house for 4 weeks. Transferred plants showed 95% of survival rate after the hardening period of 8 weeks (Fig: 1h).

The plant growth regulator plays a vital role in controlling the growth and development of plants. Auxin and cytokinin are the two important plant growth regulators widely used for morphogenetic manifestation under *in vitro* condition. In the present investigation, the effect of growth regulators like BAP, NAA, Kn and IBA were tested. In general cytokinins stimulate shoot induction and bud formation in plant tissue culture. BAP has been considered to be one of the most active cytokinins in organogenic differentiation in plant tissue culture [20], [21]. Extensive work was carried out on the synergistic effect of auxin and cytokinin in plant regeneration by Skoog and Miller [22]. The cytokinin is required in optimal quantity for shoot proliferation in many genotypes but an inclusion of low concentration of auxin along with cytokinin increases the rate of shoot multiplication in several experiments [23], [24], [25], [26], [27].

In the present investigation, nodal segment of *in vitro* germinated seedlings were used as explants. The nodal segments with axillary buds were capable of directly producing multiple shoots on MS medium supplemented with various concentrations and combinations of cytokinins (BAP, Kn) and auxin (NAA). The nodal segment was found to be the suitable explant for the initiation of shoots. Ajithkumar*et al*., [28] reported that, the reason for the suitability of nodal segment is attributed due to the presence of protected axillary buds, which are not damaged during surface sterilization. A number of authors suggested the same type of explants for propagation of other medicinal plants, such as *Rouwolfiaserpentina* [29], *Embilicaofficinalis* [30], *Holarrhenaantidyseterica* [31] and *Enicostemmahyssopifolium* [32].

4. CONCLUSION

The aim of this study was achieved and the successful production of multiple shoots and *in vitro* root formation were dependent on the nutrient medium and the culture conditions. The standardized protocol of this study could be effective for rapid multiplication and reintroduction of important woody medicinal plant to the field. The explants axillary bud and shoot tip from *in vitro* germinated seedlings were found to be suitable for successful micropropagation. This protocol may helps to the large scale production of this plant and this study might provide new opportunities for clonal propagation and germplasam conservation of *Nyctanthes arbor-tristis* L.

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REFERENCES

[1] Champa Rani, SunainaChawla, ManishaMangal, A. K. Mangal, SubashKajla and A. K. Dhawan (2012). *Nyctanthes arbor- tristis*Linn. (Night Jasmine): A sacred ornamental plant with immense medicinal potentials. Indian Journal of Traditional Knowledge, 11 (3); 427-435.

[2] HarleenKaurSandhar, MohanjitKaur, Bimlesh Kumar and Sunil Prasher (2011). An update on *Nyctnthes arbor-tristis Linn.* International PharmaceuticaSciencia, 1(1).

[3] M.M. Meshram, B. SweteeRangari, B. Shashank, Kshirsagar, SurajGajbhiye, Madhuri R. Trivedi, Rajkumari S. Sahane (2012). *Nyctanthes arbor tristis*a herbal panacea. IJPSR, 3(8).

[4] T.A. Thorpe (1990). The current status of plant tissue culture. In S. S. Bhojwani (Ed.), Plant tissue culture: Applications and limitations (pp. 1–33). Amsterdam: Elsevier.

[5] H.E. Street (1977). Introduction. In H. E. Street (Ed.), Plant tissue and cell culture (pp. 1–10). Oxford, UK: Blackwell

[6] S. Murch, S.E. Peiris, C.Z. Liu, and P.K. Saxena (2004). *In vitro* conservation and propagation of medicinal plants. Biodiversity, 5: 19–24.

[7] Z.G. Pan, C.Z. Liu, and P.K. Saxena (2003). Efficient plant regeneration from protoplasts of Egyptian medicinal plants Artemisia judaica L. and EchinopsspinosissimusTurra. Plant Sci., 165: 681–687.

[8] Vandana Sharma, Gupta, S K; and ManjulDhiman (2012). Regeneration of plants from nodal and intermodal segment cultures of *Ephedra gerardiana*using thidiazuron. Plant Tissue Cult. & Biotech. 22(2): 153-161.

[9] S. Saravanan, B. Gopinath and K. Gandhi (2014). *In vitro* propagation of an important medicinal plant *Artemisia annua*L. from axillary bud explants. Advances in Applied Science Research, 5(1): 254-258.

[10] K. Gandhi, E. Rajesh, S. Saravanan, and M. Elamvaluthi (2018a). Micropropagation of *Aeglemarmelos*L. — A Medicinal Tree through Culture of Axillary bud and Shoot tip Explants from *In vitro* Germinated Seedlings. International journal of Scientific Research in Biological Sciences, 5(3): 65-71.

[11] K. Gandhi, S. Saravanan and B. Gopinath (2013). *In vitro* micropropagation of medicinal herb *Zingiberzerumbet*Sm. International Journal of Advanced Biotechnology Research. 3(1): 19-27.

[12] V. ValizadehKaji, A. Ershadi and M. Tohidfar (2013). *In vitro* propagation of pomegranate (*Punicagranatum* L.) Cv. 'Males Yazdi'. Albanian J. Agric. Sci.,12(1): 1-5.

[13] G.R. Rout, A. Mahato, S.K. Senapati (2007). *In vitro* clonal propagation of *Nyctanthes arbor-tristis*Linn. – a medicinal tree. Hort. Sci. (prague), 34,(2): 84-89.

[14] S. Bansal, A.J. Bharati, and Y.K. Bansal (2012). Efficient *in vitro* regeneration of a medicinal plant Harsinghar (*Nyctanthes arbor-tristis*L.*).* Plant Tissue Cult. & Biotech. 22(2): 137-142.

[15] I. Siddique, M. Anish and Anushi A. Jahan, (2006). Rapid multiplication of *Nyctanthes arbor-tristis*L. through *in vitro* axillary shoot proliferation. World Journal of Agricultural Sciences, 2(2): 188-192.

[16] T. Murashige, F. Skoog (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. PhysiologiaPlantarum, 15: 473-496.

[17] M. Paudel, S. Pradhan and B. Pant (2012). *In vitro* seed germination and seedling development of *Esmeralda clarkei*Rchb.f. (Orchidaceae). Plant Tissue Cult. & Biotech. 22(2):107-111.

[18] K. Gandhi, S. Saravanan and M. Elamvaluthi (2018b). *In Vitro* Regeneration of An Important Medicinal Plant *PlumbagoZeylanica*L. From Nodal Explants. Global Journal of Bio-Science and Biotechnology, 7(3): 375-378.

[19] R. Das, M.F. Hasan, M.S. Rahman, M.H. Rashid and M. Rahman (2008). Study on *in vitro* propagation through multiple shoot proliferation in wood apple (*Aeglemarmelos* L.) Int. J. Sustain. Crop prod. 3(6): 16-20.

[20] H.B. Gururaj, P. Giridhar and G.A. Ravishankar (2007). Micropropagation of *Tinosporacordifolia*(Willd.) Miers ex Hook.F&Thoms - a multipurpose medicinal plant.Curr.Scie. 9:23-26.

[21] F. Fracaro and S. Echeverrigaray (2004). Micropropagation of *Cunilagalioides*, a popular medicinal plant of south Brazil. Plant Cell, Tiss and Organ Cult. 64:1-4.

[22] F. Skoog, C.O. Miller (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*, Symp. Soc. Exp. Biol.11: 118-131.

[23] N. Sharma, K.P.S. Chandel, A. Paul, (1993). *In vitro* propagation of *Gentianakurroo*: an indigenous threatened plant of medicinal importance. Plant Cell, Tissue and Organ Culture, 34: 307-309.

[24] T.R. Sharma and B.M. Singh (1997). High frequency *in vitro* multiplication of disease-free *Zingiberofficinale*Rosc. Plant Cell Reports, 17: 68-72.

[25] A.K. Shashany, S.P.S. Khanuja, S. Dhavan, V. Yadav, S. Sharma and S. Kumar (1998). High regenerative nature of *Menthaarvensis* inter nodes. Journal of Biosciences, 23: 641-646.

[26] G.R. Rout, S. Samantaray and P. Das (2000). *In vitro* manipulation and propagation of medicinal plants. Biotechnology Advances, 18: 91-120.

[27] G.R. Rout (2005). Micropropagation of *Clitoriaternatea* Linn. (Fabaceae) – An important medicinal plant. *In vitro* Cellular and Developmental Biology – plant, 41: 516-519.

[28] D. Ajithkumar and S. Seeni (1998). Rapid clonal multiplication through *in vitro* axillary shoots proliferation of *Aeglemarmelos* (L). Corr., a medicinal tree. Plant cell Reports. 17:422-426.

[29] S.K. Roy P.K. Roy, M. Rahaman and Hossain; (1995). Clonal propagation of *Rouwolfia serpentine* through *in vitro*culture.ActaHortica. 390:141-145.

[30] M.M. Rahaman, P.K. Roy, M.A. Mannan and S.K. Roy (1999). Clonal propagation of *Emblicaofficinalis* through *in vitro* culture. Plant Cell Tiss. Org. 9:17-22.

[31] G. Ahmed, P.K. Roy and A.N.K. Mammu (2001). High frequency shoots regeneration from nodal and shoots tip explants of *Holarrhenaantidysenterica* L. Indian J. Exp. Biol. 39:1322-1324.

[32] Y.N. Seetharam, A. Barad, C. Gururaj, G. Joythishwaran and Ghanti (2002). *In vitro* shoot regeneration from leaf and nodal explants of *Enicostemmahyssopifolium* (Wild) Verd. A vulnerable medicinal plant. Indian J. Biotechnol. 1:401-404.