Research Article

Enhanced Effect of Additives on Direct and Adventitious Shoot Multiplication in *Helicteres isora* L.

Susmita Chawla, Yogendra Kumar Bansal*
Plant Tissue Culture Laboratory, Department of Biological Science, R. D. University, Jabalpur, M.P., India.
***Corresponding author's E-mail:** yogendrakbansal@rediffmail.com

Accepted on: 04-05-2014; **Finalized on:** 30-06-2014.

ABSTRACT

The present study reports an efficient protocol for micropropagation of *Helicteres isora* L. under the influence of additives for the enhancement of the number of shoots per explant. Among the different types of additives used, silver nitrate proved the most optimum for direct as well as indirect organogenesis. Maximum shoots (4.14 ± 0.25) in direct organogenesis was obtained on BAP ($2mgl^{-1}$) + KN ($1mgl^{-1}$) + $AgNO_3$ ($1mgl^{-1}$). Maximum indirect shoot multiplication (26.83 ± 2.38) was obtained on BAP ($1mgl^{-1}$) + $AgNO_3$ ($2mgl^{-1}$) in the III subculture passage. Plantlets showed maximum rooting (10.94 ± 0.88) and root length (5.75 ± 0.59) on IBA ($0.5mgl^{-1}$). The plantlets were successfully hardened with 75-80% survival.

Keywords: Additives, Axillary bud, IBA, Indirect organogenesis, Shoot multiplication.

INTRODUCTION

Helicteres isora L. commonly known as Indian screw Tree or Spiral bush belonging to the family Sterculiaceae is an important medicinal plant. It is a deciduous shrub or tree, 4-5m tall and occurs as an under-growth in forests. It is distributed throughout India, Nepal, Sri Lanka, Pakistan, Australia, Thailand, China and Brazil. It comprises of about 60 species.[1] The fruits and bark of the plant is used for treating diarrhoea and stomach ailments.[2,3] The fruit extracts has also been shown to possess anti-cancerous activity against melanoma cell line[4] and against HIV virus.[5] Root extract has been found to be very effective in controlling diabetes and reducing blood cholesterol levels.[6,7]

However, the plant is plagued with problems in natural regeneration like low seed germination[8], inadequacy of natural pollinators[9] and difficulties in vegetative propagation. The plant also suffers from destructive exploitation for its plant parts for commercial purposes. Consequently the plant has become endangered.[10]

In recent years, *in vitro* approaches have been used as an efficient tool for large-scale propagation of trees in short time.[11] In some cases, the supplementation of medium with plant growth regulators is not enough to induce multiple shoot formation and growth. Under these circumstances, *in vitro* shoot multiplication is enhanced with the use of additives in the medium. Various kinds of additives are being used for *in vitro* studies, but the present study was undertaken to study the effect of additives viz. casein hydrolysate, coconut milk, phloroglucinol and silver nitrate on direct and adventitious shoot formation of *Helicteres isora* L.

Casein hydrolysate (CH)

The promotory effect of casein hydrolysate has been attributed to various amino acids present in CH which positively affect nitrogen assimilation, alleviation of phosphate deficiency, replacement of toxic ammonium ions and chelation of divalent metal ions.[12] Some workers have successfully employed CH for direct as well as indirect shoot multiplication.[13,14]

Coconut milk (CM)

An undefined supplement whose composition can vary, the liquid endosperm of *Cocos nucifera* fruits has been known to induce plant cells to divide and grow rapidly. Unlike other undefined supplements to culture media (such as yeast extract, malt extract and casein hydrolysate) coconut milk has proved harder to replace by fully defined media morphogenesis.[15,16] The liquid has been found to be beneficial for inducing growth of both callus and suspension cultures and for the induction of morphogenesis. Varied type of regenerative responses have been elicited by various workers viz. shoot proliferation from axillary buds in *Ficus benghalensis*[17], shoot elongation in *Adhatoda vasica*[18] and somatic embryogenesis from cereal callus and suspension cultures.[19]

Silver nitrate

Silver ions in the form of nitrate, such as silver nitrate ($AgNO_3$), play a major role in influencing somatic embryogenesis, shoot formation and efficient root formation which are the prerequisites for successful genetic transformation.[20] $AgNO_3$ has been known to inhibit ethylene action by reducing the receptor capacity to bind ethylene.[21,22] In recent years, it has been employed in tissue culture studies because of its water solubility and lack of phytotoxicity at effective concentrations.[23] The use of silver nitrate has been reported to be beneficial for *in vitro* shoot multiplication of various plant species viz. *Coffea robusta*[24] *Prunus armeniaca*[25] and *Malaxis acuminata*.[26]

Phloroglucinol (PG)

A phenolic compound Phloroglucinol (1,3,5-trihydroxybenzene) is one of the degradation products of phloridzin known for its growth regulating property. It enhances growth and rate of auxiliary shoot proliferation from shoot tip cultures of several woody plants or acts as auxin synergist during auxin-sensitive phase of root initiation.[27] It also acts as a precursor in the lignin biosynthesis pathway and controls hyperhydricity thus maximizing the multiplication rate of woody species and other species that are difficult to propagate.[28]

It is therefore, interesting to understand the role of additives in triggering the endogenous levels of plant growth regulators in tissues and its subsequent effect on shoot multiplication. In the present communication, we report an improved direct and indirect *in vitro* shoot multiplication protocol of *Helicteres isora* L. under the influence of additives. Till date there have been no published reports on the use of additives for direct and indirect organogenesis in *Helicteres isora* L.

MATERIALS AND METHODS

Explant preparation and culture conditions

4 month old plant of *Helicteres isora* L. (Accession no. 22076) was procured from Jawaharlal Nehru Agricultural University (JNKVV), Jabalpur, Madhya Pradesh, India and planted in kitchen garden. Mature and dry pods used for the present study were collected from this plant after two years of plantation. Contrary to the earlier report[29], acid scarification failed to initiate germination in seeds in the present study. The dried pods were water soaked and seeds were sown in soil. The seed germination started within 8-10 days and mere 6-7% of seeds germinated.

Axillary bud (AxB) (0.5 -1cm) segments excised from 4-week-old germinating seedlings (4-5 cm in length) were used as explants for shoot bud proliferation and callus induction. The explants were washed under running tap water for an hour, treated with 0.1% (v/v) labolene (Qualigens, India) for 5 min, followed by washing with tap water for 20 min. It was surface sterilized with 70% ethanol for 30 sec, washed with 0.1% (w/v) mercuric chloride and rinsed with autoclaved distilled water 3-4 times

For direct shoot multiplication auxiliary bud explants were inoculated on Murashige and Skoog's medium[30] supplemented with 2mgl^{-1} of BAP (6-benzyl aminopurine) and 1mgl^{-1} of KN (6-Furturyl aminopurine) (Selected medium/SM) in combination with different concentrations (0.1, 0.5, 1.0 and 2.0 mgl-1) of additives viz. coconut milk, phloroglucinol and silver nitrate.

For indirect shoot multiplication, MS medium supplemented with 1mgl^{-1} of BAP (Selected medium Indirect/SMI) in combination with different concentrations (0.1, 0.5, 1.0 and 2.0 mgl^{-1}) of additives viz. Casein hydrolysate, coconut milk and silver nitrate. A piece of callus (2-3mm x 2-3mm) was used as an explant.

The shoots obtained from calli were studied up to three subculture passages at 4-week intervals. The pH of the media was adjusted (prior to inoculation) between 5.6-5.8 with 1 N NaOH or HCl. Cultures were maintained in a 16hr photoperiod at 25±1°C and 60-70% relative humidity.

Rooting of *in vitro* regenerated plantlets

The regenerated shoots (3-4 cm in length) were rooted on half-strength (1/2) MS medium (liquid) supplemented with IBA (Indole 3-butyric acid) (0.1, 0.5, 1.0 mgl^{-1}). The data for percentage of root formation, number of roots and root length per shoot was recorded periodically after 4 weeks of culture.

Hardening and acclimatization

The shoots with well developed roots were dipped in 1% (w/v) bavisitin solution for 1 min and then rinsed under tap water and transferred to small thermocol cups filled with sterilized garden soil, sand and vermiculite (1:1:1) for hardening These cups were covered with transparent polythene cover and after 1 week, covers were perforated with small holes to maintain humidity. After 3 weeks the covers were removed. Plants were transferred to garden soil in earthen pots after 2 months and kept under sunlight, initially for a short time and gradually, the time was increased. After 3–4 months the plants were transferred to field.

Data recording and Statistical analysis

All the experiments were conducted with minimum of 12 replicates per treatment. Each experiment was repeated thrice. The results are expressed as Mean ± SE of three experiments. Observations were recorded after 25-30 days of interval. Data were analysed for significance using analysis of variance (ANOVA) and means were separated at $p<0.05$ level of significance using Tukey's test. All statistical analyses were performed using the SPSS 20 statistical software package.

RESULTS AND DISCUSSION

Phloroglucinol

Phloroglucinol initially responded well with healthy shoot initiation. Maximum FSI (77.77) and MSN (1.77) were observed on SM + PG (0.5mgl^{-1}), however, after 2 weeks, leaves began to turn yellow with increased basal callusing which affected the further elongation and development of *in vitro* shoots (Table 1, Figure 1a).

Coconut milk

CM neither supported direct shoot regeneration (Table 1, Figure 1 b); nor did it improve the shoot regeneration from the callus when combined with Selected medium (Table 3, Figure 1c). Although a few shoot primordia were formed from calli, yet it failed to develop into well developed shoots in subsequent passages. Poor effect of CM supplementation has also been highlighted in several past reports.[31,32]

Casein hydrolysate

In the present study, CH exhibited a poor response both in terms of indirect shoot regeneration and elongation of regenerated shoots. Only lower concentrations of CH (0.1 & 0.5 mgl^{-1}) produced organogenetic calli. But the organogenesis was not frequent and in the subsequent subculture passages, the calli lost its regeneration capacity and generally produced non-organogenetic calli.

Table 1: Effect of additives on FSI, MSN and MSL in *Helicteres isora* L. on Selected Medium

SM + Additive Conc.	FSI	MSN	MSL
SM + CM (%)			
0.1	55.55 ± 2.45^{b}	0.94 ± 0.15^{b}	0.81 ± 0.03^{a}
0.5	72.22 ± 2.19^{c}	1.25 ± 0.12^{c}	1.03 ± 0.02^{a}
1.0	41.67 ± 3.86^{ab}	0.64 ± 0.15^{a}	0.74 ± 0.05^{a}
2.0	30.55 ± 3.50^{a}	0.47 ± 0.14^{a}	0.71 ± 0.06^{a}
SM + PG			
0.1	69.44 ± 3.78^{b}	1.50 ± 0.18^{c}	0.44 ± 0.16^{a}
0.5	77.77 ± 3.13^{b}	2.05 ± 0.21^{d}	1.05 ± 0.06^{d}
1.0	66.66 ± 2.98^{b}	1.17 ± 0.10^{b}	0.79 ± 0.05^{b}
2.0	38.89 ± 2.45^{a}	0.61 ± 0.15^{a}	0.54 ± 0.04^{a}
SM + $AgNO_3$			
0.1	63.89 ± 3.66^{a}	1.53 ± 0.18^{a}	2.12 ± 0.16^{c}
0.5	80.55 ± 3.92^{ab}	2.64 ± 0.19^{b}	2.40 ± 0.06^{d}
1.0	94.44 ± 3.93^{b}	4.14 ± 0.29^{c}	1.21 ± 0.05^{b}
2.0	77.78 ± 3.92^{ab}	2.67 ± 0.20^{b}	0.65 ± 0.04^{a}

Experiments were repeated thrice with 12 replicates. Values represent mean ± standard error. Mean values followed by different superscript letters within column is significantly different at P≤0.05, as determined by Tukey's test.

Very few shoots were regenerated from calli and the shoots formed were fragile and weak and the calli had a poor response for both shoot multiplication and elongation (Figure 1d, Table 3).

Silver nitrate

Maximum frequency of shoot initiation (94.44 ± 6.0) and shoot multiplication (4.14± 0.25) was observed on SM in combination with $AgNO_3$ ($1mgl^{-1}$). The shoots initiated better with green and healthy leaves within 2-3 days from AxB explants. Healthy shoots were formed in clusters and modulated meristemoids were observed which developed into healthy shoots (Table 1, Figure 1 e,f). $AgNO_3$ had a positive effect on shoot multiplication in subculture passages and significantly reduced basal callusing, with maximum MSN (6.0) being obtained on SM supplemented with $AgNO_3$ ($1mgl^{-1}$) in II passage (Table 2). Supplementation of $AgNO_3$ in medium enhanced *in vitro* shoot regeneration and growth of *Albizzia julibrissin*[33], *Coffea arabica*[34] and *Punica granatum.*[35]

Figure 1: Effect of additives on direct and indirect shoot formation and elongation. Shoot initiation on SM+PG ($0.5mgl^{-1}$) **(a)**, Basal callusing and shoot formation on SM+CM (0.5%) **(b)**, Adventitious shoot formation on SMI+CM (1%) **(c),** Adventitious shoot formation on SMI+CH ($0.1mgl^{-1}$) **(d)**, Multiple shoot formation on SM+$AgNO_3$ ($1mgl^{-1}$) **(e,f)**, Adventitious shoot formation on SMI+$AgNO_3$ ($2mgl^{-1}$) **(g,h)**, Root formation on ½ MS + IBA ($0.5mgl^{-1}$) **(i)**, Hardened plantlet after 2 months **(j)**.

Silver nitrate showed a enhanced effect on indirect organogenesis too. The shoot buds and meristemoids developing on SMI (BAP $1mgl^{-1}$) showed an increase in sprouting of new shoot buds and development of shoots when supplemented with $AgNO_3$ ($2mgl^{-1}$). Maximum shoot buds (26.83) were obtained in the III subculture passage which probably indicates the promotory effect of $AgNO_3$ on shoot multiplication (Table 3, Figure 1 g,h). In the past workers have successfully employed high concentrations ($20mgl^{-1}$) of $AgNO_3$ for micropropagation of several plant species.[36,37]

Rooting of regenerated shoots

Maximum frequency of roots (91.64), root number (10.94) and root length (5.75) was observed on IBA ($0.5mgl^{-1}$) (Table 4, Figure 1i) after 20-25 days of culture without the intervening basal callus. IBA is considered to be a potential auxin that induces rooting of in vitro regenerated shoots of several tree species.[38,39]

Hardening and acclimatization

75-80% of the plantlets survived during acclimatization (Figure 1j). The regenerated plants did not show any detectable variation in morphological or growth characteristics compared to the parent plant. The *in vitro* plants successfully flowered and fruited after two years of transplantation.

Table 2: Effect of $AgNO_3$ and subculture passages on MSN in *Helicteres isora* L. on Selected Medium

Conc. of $AgNO_3$	I passage		II passage		III passage	
	MSN	MSL	MSN	MSL	MSN	MSL
0.1	1.47±0.14^{a}	1.51±0.15^{b}	1.56±0.16^{a}	1.51±0.14^{b}	1.75±0.17^{a}	1.41±0.06^{b}
0.5	2.58±0.19^{b}	1.58±0.25^{b}	3.11±0.26^{b}	1.61±0.14^{b}	2.89±0.24^{b}	1.43±0.05^{b}
1.0	3.0±0.22^{b}	2.28±0.20^{c}	6.0±0.45^{c}	2.43±0.16^{c}	4.47±0.20^{c}	2.33±0.04^{c}
2.0	2.58±0.15^{b}	0.54±0.04^{a}	3.20±0.27^{b}	0.83±0.17^{a}	2.69±0.18^{b}	0.79±0.03^{a}

Experiments were repeated thrice with 12 replicates. Values represent mean ± standard error. Mean values followed by different superscript letters within column is significantly different at P≤0.05, as determined by Tukey's HSD.

Table 3: Effect of additives and subculture passages on MSN and MSL (Indirect) in *Helicteres isora* L. on Selected Medium Indirect (SMI)

Additive Conc.(mgl^{-1})	I passage		II passage		III passage	
	MSN	MSL	MSN	MSL	MSN	MSL
0.1	1.67±0.35^{a}	0.56±0.12^{a}	3.23±0.37^{a}	1.16±0.08^{ab}	6.44±0.96^{a}	1.23±0.19^{a}
0.5	3.06±0.71^{a}	0.62±0.11^{a}	4.89±0.50^{b}	1.25±0.17^{b}	9.83±0.98^{b}	1.28±0.17^{a}
1.0	7.0±1.27^{c}	1.93±0.31^{b}	10.67±1.04^{c}	2.35±0.24^{c}	17.80±1.87^{c}	2.20±0.24^{b}
2.0	14.58±2.39^{d}	0.46±0.08^{a}	20.39±1.71^{d}	0.69±0.07^{a}	26.83±2.38^{d}	0.92±0.09^{a}
CM						
0.1	-	-	0.39±0.17^{a}	0.14±0.07^{a}	0.55±0.18^{a}	0.15±0.7^{a}
0.5	0.80±0.21^{b}	0.27±0.07^{b}	1.25±0.32^{b}	0.30±0.15^{b}	1.0±0.30^{b}	0.22±0.32^{a}
1.0	1.72±0.52^{c}	0.42±0.10^{c}	2.22±0.50^{c}	0.29±0.32^{b}	1.97±0.58^{b}	0.29±0.53^{a}
2.0	0.64±0.15^{b}	0.27±0.07^{b}	0.75±0.17^{ab}	0.18±0.45^{a}	-	-
CH						
0.1	1.39±0.28^{c}	0.28±0.05^{b}	1.61±0.37^{c}	0.21±0.05^{b}	0.83±0.22^{b}	0.21±0.05^{b}
0.5	0.86±0.21^{b}	0.25±0.05^{b}	0.91±0.27^{b}	0.26±0.08^{b}	0.94±0.26^{b}	0.32±0.06^{b}
1.0	-	-	-	-	-	-
2.0	-	-	-	-	-	-

Experiments were repeated thrice with 12 replicates. Values represent mean ± standard error. Mean values followed by different superscript letters within column is significantly different at P≤0.05, as determined by Tukey's test.

Table 4: Effect of IBA on rooting of *in vitro* regenerated shoots of *Helicteres isora* L.

Conc. (mgl^{-1})	Frequency of root initiation	Mean root number	Mean Root length (cm)
½ MS (Control)	75.0 ± 3.40^{c}	6.50 ± 0.70^{b}	2.37 ± 0.23^{c}
0.1	30.55±1.96^{b}	1.92±0.54^{b}	1.88±0.49^{a}
0.5	91.64±3.40^{c}	10.94±0.88^{c}	5.75±0.59^{b}
1.0	13.88±1.96^{a}	0.97±0.40^{a}	0.30±0.13^{a}

Experiments were repeated thrice with 12 replicates. Values represent mean ± standard error. Mean values followed by different superscript letters within column is significantly different at P≤0.05, as determined by Tukey's test.

Acknowledgement: Authors would like to acknowledge the M.P. Biotechnology Council, Bhopal, Madhya Pradesh for their financial support to one of us (SC).

REFERENCES

1. Kubitzki K, Bayer C, Flowering plants – dicotyledons: Malvales, Capparales, and non-betalain Caryophyllales, In: The Families and Genera of Vascular Plants, K. Kubitzki, Springer-Verlag, Berlin, 5, 2003, 259–260.
2. Pohocha N, Grampurohit ND, Antispasmodic activity of the fruits of *Helicteres isora* L. Phytotherapy R, 15, 2001, 49-52.
3. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV, Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test), Int J Appl Sci Eng, 4, 2006, 115-125.
4. Pradhan M, Sribhuwaneswari S, Karthikeyan D, Minz S, Sure P, Chandu AN, Mishra U, Kamalakannan K, Saravanankumar A, Sivakumar T, *In-vitro* cytoprotection activity of *Foeniculum vulgare* and *Helicteres isora* in cultured human blood lymphocytes and anti-tumour activity against B16F10 melanoma cell line, Research J Pharm and Tech, **1**, 2008, 450-453.
5. Otake T, Mori H, Morimoto M, Ueba N, Sutardjo S, Kusomoto I, Hattori M, Namba T, Screening of Indonesian plant extracts for anti human immuno deficiency virus type1 (HIV-1) activity, Phytother Res, **9**, 1995, 6-10.
6. Jagadheshan H, Charkrabati R, Sharma VM, Vikramadithyan RK, Mullangi R, Rao YN, Sairam P, Rajagopalan R, Euglycemic and hypolipidemic activity of *Helicteres isora* extract, J Ethnopharmacol, 81, 2002, 343-349.

7. Venkatesh S, Sailaxmi K, Reddy BM, Ramesh M, Antimicrobial activity of *Helicteres isora* root, Indian J Pharm Sci, 69, 2007, 687-689.
8. Badave GN, Jadhav SC, Germination studies in local plants from Koyana Valley, I: Murudsheng- *Helicteres isora* L, Ayurveda Update, 1, 1998, 10.
9. Atluri JB, Rao SP, Reddi CS, Pollination ecology of *Helicteres isora* Linn. (Sterculiaceae), *Curr Sci*, 78, 2000, 713–718.
10. Kumar A, Major threats and vulnerability to the medicinal taxa located on the Rajgir hills, Bihar, Flora & Fauna, 11, 2005, 231-232.
11. Thomas TD, Philip B, Thidiazuron-induced high frequency shoot organogenesis from leaf-derived callus of a medicinal climber, *Tylophora indica* (Burm. F.) Merrill, *In Vitro* Cell Dev Biol-Plant, 41, 2005, 124-128.
12. George EF, Plant propagation by Tissue Culture, The Technology, Vol 1, Exegetics Ltd, Edington, Wiltshire, 1993.
13. Chaturvedi R, Razdan MK, Bhojwani SS, *In vitro* clonal propagation of an adult neem (*Azadirachta indica* A. Juss.) by forced auxiliary branching, Plant Sci, 166, 2004, 501-506.
14. Walia N, Kaur A, Babbar SB, An efficient *in vitro* cyclic production of shoots from adult trees of *Crataeva nurvala* Buch Ham, Plant Cell Rep, 26, 2007, 227-284.
15. George EF, Plant Tissue Culture Procedure-Background. In: Plant Propagation and Tissue Culture, Hall MA, De Klerk GJ, Eds., Springer, Dordrecht, 3, 2008, 1-28.
16. Molnar SJ, Nutrient modifications for improved growth of *Brassica nigra* cell suspension cultures, Plant Cell Tiss Organ Cult, 15, 1988, 257-267.
17. Munshi MK, Hakim L, Islam MR, Ahmed G, *In vitro* clonal propagation of banyan (*Ficus benghalensis* L.) through axillary bud culture, Int J Agri Biol, 6, 2004, 321-323.
18. Nath S, Buragohain AK, Micropropagation of *Adhatoda vasica* Nees-A woody medicinal plant by shoot tip culture, Indian J Biotechnol, 4, 2005, 369-399.
19. Vasil V, Vasil IK, Somatic embryogenesis and plant regeneration from tissue cultures of *Pennisetum americanum* and *P. americanum* × *P. purpureum* hybrid, Amer J Bot, 68, 1981, 864-872.
20. Bais HP, Sudha G, Suresh B, Ravishankar GA, Silver nitrate influences *in vitro* root formation in *Decalepis hamiltonii*, Curr Sci, 79, 2000, 594-598.
21. Pua EC, Sim GE, Chi GL, Kong LF, Synergistic effects of ethylene inhibitors and putrescine on shoot regeneration from hypocotyl explants of Chinese radish (*Raphanus sativus* L. var. longipinnatus Bailey) in vitro, Plant Cell Rep, 15, 1996, 685-690.
22. Kumar V, Parvatam G, Ravishankar GA, $AgNO_3$ - a potential regulator of ethylene activity and plant growth modulator, Electronic Journal of Biotechnology, 12, 2009, 1-15.
23. Beyer E Jr, Silver ion: a potent antiethylene agent in cucumber and tomato, Hort Sci, 11, 1976, 195-196.
24. Giridhar P, Indu EP, Vijaya Ramu D, Ravishankar GA, Effect of silver nitrate on in vitro shoot growth of coffee, Trop Sci, 43, 2003, 144-146.
25. Petri C, Alburquerque N, Perez-Tornero O, Burgos L, Auxin pulses and a synergistic interaction between polyamines and ethylene inhibitors improve adventitious regeneration from apricot leaves and *Agrobacterium*-mediated transformation of leaf tissues, Plant Cell, Tiss Organ Cult, 82, 2005, 105-111.
26. Meena K, Abraham CJ, Mani B, Thomas TD, Adventitious shoot induction from cultured internodal explants of *Malaxis acuminate* D. Don, a valuable terrestrial medicinal orchid, Plant Cell Tiss Organ Cult, 99, 2010, 199-208.
27. James DJ, Thurbon IJ, Shoot and root initiation *in vitro* in the apple rootstock M. 9 and the promotive effects of phloroglucinol, J Hortic Sci, 56, 1981, 15–20.
28. Teixeira da Silva JA, Dobránszki J, Ross S, Phloroglucinol in plant tissue culture, In Vitro Cell Dev Biol- Plant, 49, 2013, 1-16.
29. Shriram V, Kumar V, Shitole MG, Indirect organogenesis and plant regeneration in *Helicteres isora* L., an important medicinal plant, In Vitro Cell Dev Biol-Plant, 44, 2008, 186–193.
30. Murashige T, Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue culture, Physiol Plantarum, 15, 1962, 473-497.
31. Bachelard EP, Stowe BB, Growth *in vitro* of roots of *Acer rubrum* L. and *Eucalyptus camaldulensis* Dehnh, Physiol Plantarum, 16, 1963, 20-30.
32. Ammirato PV, Embryogenesis. In: Handbook of Plant Cell Culture, Evans DA, Sharp WR, P. Ammirato V, Yamada Y Eds, MacMillan, New York, 1, 1983, 82-123.
33. Sankhla D, Sankhla N, Davis DT, Promotion of *in vitro* shoot formation from excised roots of silktree (*Albizzia julibrissim*) by an oxime ether derivative and other ethylene inhibitors, Plant Cell Rep, 15, 1995, 143-146.
34. Fuentes SRL, Calheiros MBP, Manettifilho J, Vieira LGE, The effects of silver nitrate and different carbohydrate sources on somatic embryogenesis in *Coffea canephora,* Plant Cell Tiss Organ Cult, 60, 2000, 5-13.
35. Naik SK, Chand PK, Silver nitrate and aminoethoxy venyl glycene promotes *in vitro* adventitious shoot regeneration of Pomegranate (*Punica granatum* L), J Plant Physiol, 160**,** 2003, 423- 430.
36. Mallika VK, Mathew JP, Chacko D, Vijayakumar NK, Nair RV, Induction of multiple shoots in nodal explants of fully grown trees of cocoa, J Plantn Crops, 24, 1996, 503-510.
37. Sankar A, Libin MS, Vijaykumar A, Karthi RR, Raja SJ, Kohila R, Liby I, Vadivukarasi S, Ganesh D, Phloroglucinol enhances shoot proliferation in nodal explants of *Vanila planifolia* Andr, J Plantn Crops, 36, 2008, 127-131.
38. Rajore S, Batra A, Efficient plant regeneration via shoot tip explant in *Jatropha curcas* L, J Plant Biochem Biotechnol, 14, 2005, 73-75.
39. Rajeswari V, Paliwal K, In vitro adventitious shoot organogenesis and plant regeneration from seedling explants of *Albizia odoratissima* L.f. (Benth.), In Vitro Cell Dev Biol Plant, 44, 2008, 78-83.

Source of Support: Nil, **Conflict of Interest:** None.