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HAPLOID PLANT PRODUCTION FROM A POTENTIAL MEDICINAL PLANTS, *CALOTROPIS GIGANTEA* (LINN)

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ABSTRACT

Calotropis gigantea Linn. belongs to the family Asclepiadaceae, is commonly known as giant milkweed which has significant medicinal properties. Tissue culture is a part of our economy, pharmaceutical, food and emerging Biotechnology industries. The aim of this study is to standardize haploid plant production from pollinium and callus production from leaf, node explants cultured on MS medium supplemented with various combinations and concentrations of plant growth hormone such as 2,4 Dichlorophenoxy acetate (2,4 D) and Indole 3- acetic acid (IAA). Callus induction of the leaf explants of IAA of (0.5 mg/mL) showed the maximum 94.2% of callus. When compared to the other hormones such as 2, 4-D showed 94.2% and callus induction of 2,4-D + IAA showed 94.3%. Nodal explant showed maximum 81.7% on MS medium supplemented with 2,4-D + IAA (0.5mg/mL) when compared to the IAA and 2,4-D and it showed 77.2% and 77.0% of callus induction respectively. Pollinium explants showed 92.2% maximum growth on MS medium supplemented with 2,4-D (0.5mg/mL). IAA and 2,4-D + IAA showed 84.6% and 92% of callus induction respectively.

KEYWORDS: *Calotropis gigantea*, Callus induction, Haploid plant production, Pollinium.

INTRODUCTION

Tissue culture can be a powerful technique if conducted correctly, and a great waste of time and money when done sloppily. Nowadays Tissue culture is not only for cell biologists, but also for adjunct to a virtually every discipline of the biological sciences. In Ancient times,

plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in cytodifferentiation and organogenesis.

The field of plant tissue culture is based on the premise that plants can be separated into their parts, which can be manipulated *in vitro* and then grown back into complete plants.[1] Gottlieb Haberlandt discovered the Plant tissue culture in 1902. He made several valuable predictions about the nutrient requirement for *in vitro* conditions, which would possibly induce cell division, proliferation and embryo induction.[2]

Narges *et al.*,[3] determined the optimum level of plant growth regulator in different concentration of NAA and BA (mg/l) were used in MS medium for callus induction of stem and leaf explant.[4] Callus culture can be used to study the stress physiology and genetic improvement at the cellular level and also to select mutants *in vitro.*[5] Plant tissue culture is an important tool in both basic and applied studies as well as in commercial application.[6]

In *in vitro* culture, microspore can be deviated to a proliferation process that can leads to embryogenesis.[7] Analysis of climatic parameters (temperature, sunshine hours and rainfall) during two growing seasons indicated that anther donor plants grown under better environmental conditions.[8] Anther culture is a best method to induce gametic embryogenesis.[9] A high genotype dependency was recorded for callus induction, callus regenerating of green double haploid plantlets.[10] Nowadays, the demand of floriculture product has increased. In Indian commercial floriculture is a developing area both domestic as well export market.[11] Physical presence is necessary to allow for inspection, quality control and break bulk activities.[12]

Floriculture is one of the essential components in agri-business. The floriculture industry itself is responsible to ensure that all flowers and potted plants meet acceptable levels set by social and environmental criteria.[13] It may affect the soil pH and resulting solubility of micronutrients in the root zone during commercial production.[14]

Somatic embryos were successfully regenerated from callus tissue of anthers and ovaries excised from inflorescences of grapevines.[15] Callus induction and somatic embryogenesis was significantly influenced by the size, physiological age, and orientation of leaf explants on the culture medium and plant growth regulators.[16] The sterilized nodal explants were inoculated in MS medium containing various concentrations.[17] Seyed *et al.*,[18] examined the

influence of the growth regulators thidiazuron (TDZ) and naphthalene acetic acid (NAA) on adventitious shoot formation in these cultivars.

Hardening is simply means of gradual exposure of plantlets to an environmental conditions.[19] Hardening induced the increase in the frost tolerance of all cereals under study, and the resistance of rye and soft wheat was found to be significantly higher than that of durum wheat and barley.[20] The *in vitro* rooted plantlet were hardened and acclimatized by using different treatments. The maximum survival during hardening was observed by covering the plantlets with glass beaker individually and kept in culture room.[21]

PLANT COLLECTION

The healthy and fresh plant of *Calotropis gigantea* were collected in Coimbatore district, Tamilnadu, India. The explants such as leaves, nodes and pollinium were used. Care should be taken while collecting the plant to avoid the damage of the cell.

MATERIALS AND METHODS

General laboratory techniques recommend by Purvis *et al*[22], Tuite[23] and Booth[24] were followed. The cleaned glassware were dried in hot air oven and stored. Dried glassware and media were sterilized in an autoclave for 15 min at 15lb /sq inch pressure.

SURFACE STERILIZATION OF EXPLANTS

Healthy plant leaves, node and pollinium were collected and washed thoroughly under running tap water for 25 min without damage to the tissue. The explants were washed with sodium hypochlorite (1.0%) and mercuric chloride (0.1-0.3 %) for different time duration. The explants were cut into small pieces (0.5-1.0 cm) barring the cut ends and transferred to semi-solid culture medium under aseptic conditions.

PREPARATION OF TISSUE CULTURE MEDIA

Murshige and Skoog[25] medium were used for this investigation. Sucrose 3% (30g /L) 0.1% meso-inositol (100 mg /L) and required plant growth hormones were added to the medium and buffered by 1N HCl or NaOH to adjust pH of the medium (5.6 to 5.8) before autoclaving. The medium was solidified by addition 0.8% agar (8 g/L) and autoclaved at 15 Ib pressure for 15 min at 121˚C. After inoculated with explants, all culture vials were kept under 16 / 8 h (light/ dark) photoperiod at 25± 2˚C.

CALLUS INDUCTION: Explants were inoculated on MS basal medium supplemented with different growth hormone such as IAA, 2, 4-D and combination of both the hormone.

HAPLOID CULTURE

Sterilized anthers were used for dissection of pollinium and inoculated on MS basal medium supplemented with different growth hormone such as IAA, 2, 4-D and combination of both the hormone.

STATISTICAL ANALYSIS

Data are presented as mean ± standard deviation and were analyzed using one – way analysis of variance (ANOVA). The different among means were tested by dunnet's test at $P < 0.05$ level of significance. The analyses were carried out with the statistical software graphprism (USA).

RESULT AND DISCUSSION

Surface sterilized leaf, node and pollinium explants of *Calotropis gigantea* L. with sodium hypochlorite (1%) (For 20 min) and mercuric chloride (0.1%) (For 2 min) yielded 98% contamination free explants (Table 1- 3).

Table 1: Effect of Sodium hypochlorite 1% and Mercuric chloride 0.1% *Calotropis gigantea* L. Leaf.

S. no	$NaHCl_3$/Min	$HgCl_2$/Min	Germination%
1.	10	1	95.8±0.5
2.	15	2	94.9±0.7
3.	20	3	94.5±0.1

Table 2: Effect of Sodium hypochlorite 1% and Mercuric chloride 0.1% *Calotropis gigantea* L. Node

S.no	$NaHCl_3$/ Min	$HgCl_2$/Min	Germination%
1.	10	1	94.5±0.1
2.	15	2	94.7±0.6
3.	20	3	94.2±1.0

Table 3: Effect of Sodium hypochlorite 1% and Mercuric chloride 0.1% *Calotropis gigantea* L. Pollinium.

S.no	$NaHCl_3$/Min	$HgCl_2$/Min	Germination%
1.	10	1	94.7±0.7
2.	15	2	93.5±0.2
3.	20	3	94.3±0.3

Similarly the same result were obtained by Raaman *et al.*,[26] surface sterilization of leaf explants of *Hibisicus sabdariffa* with sodium hypochlorite (1%) for 20 min and mercuric chloride (0.3%) 5 min yielded 96% of contamination free explants.

Interestingly in our study showed 98% of contamination free explants were obtained sodium hypochlorite (1%) for 20 min and mercuric chloride (0.1%) for 2min. In the internodal *Hibisicus sabdariffa* with sodium hypochlorite (1%) for 20 min and mercuric chloride (0.1%) 5 min yielded 94% of contamination free explants. Similarly in our study showed 98% of contamination free explants were obtained sodium hypochlorite (1%) for 20 min and mercuric chloride (0.1%) 2min. Whereas in the anther explants of *Hibiscus sabdariffa* with sodium hypochlorite (1%) for 20 min and mercuric chloride (0.3%) 5 min yielded 92% of contamination free explants. Interestingly in our study also showed 98% of contamination free explants with 2 min of surface sterilization with sodium hypochlorite (1%) for 20 min and mercuric chloride (0.1%) 2min.

CALLUS INDUCTION

Callus induction of the leaf explants of *Calotropis gigantea* was observed in the 7th day of inoculation. Among the various growth regulators of IAA of (0.5 mg/mL) showed the maximum 94.2% of callus observed (Table. 4.4). When compared to the other hormones such as 2, 4-D showed 94.2% (Table. 4.5) and callus induction of 2,4-D + IAA showed 94.3% (Table 4-6, Fig.1 and 2).

Table 4: Effect of different concentration of IAA on callus induction from leaf, nodal and pollinium explants of *Calotropis gigantea* L.

Hormone	Concentration	Leaf	Node	Pollinium
IAA	**0.1**	67.1±1.6^{e}	63.3±1.1^{e}	44.9±0.1^{e}
IAA	**0.2**	74.8±0.2^{d}	72.3±0.4^{d}	55.4±0.5^{d}
IAA	**0.3**	83.5±0.8^{c}	75.4±0.4^{c}	65.5±0.3^{c}
IAA	**0.4**	86.6±0.3^{b}	82.5±0.6^{b}	66.8±0.2^{b}
IAA	**0.5**	**93.4±0.5^{a}**	**85.8±0.3^{a}**	**75.8±0.7^{a}**

Values represent mean ± standard deviation of three replicates per treatment. Mean followed same letter in same column did not differ significantly at $p < 0.05$ according to Dunnet's HSD test.

Table 5: Effect of different concentration of 2-4 D on callus induction from leaf, nodal and pollinium explants of *Calotropis gigantea* L.

Hormone	Concentration	Leaf	Node	Pollinium
2,4-D	**0.1**	65.1±0.7^{e}	66.6±1.3^{e}	58.0±1.9^{e}
2,4-D	**0.2**	75±0.1^{d}	73.9±0.3^{d}	61.2±3.7^{d}
2,4-D	**0.3**	76.8±0.8^{c}	77.7±1.6^{c}	68.5±0.7^{c}
2,4-D	**0.4**	84.9±0.3^{b}	85±0.3^{b}	75.4±0.05^{b}
2,4-D	**0.5**	**90.2±2.6^{a}**	**89.4±3.9^{a}**	**80.0±1.1^{a}**

Values represent mean ± standard deviation of three replicates per treatment. Mean followed same letter in same column did not differ significantly at $p < 0.05$ according to Dunnet's HSD test.

Table 6: Effect of different concentration of IAA, 2-4 D on callus induction from leaf, nodal and pollinium explants of *Calotropis gigantea* L.

Hormone	Concentration	Leaf	Node	Pollinium
IAA, 2-4 D	**0.1**	73.3±2.6^{e}	60.2±0.5^{e}	45.7±0.4^{e}
IAA, 2-4 D	**0.2**	77.4±0.4^{d}	65.5±0.7^{d}	54.5±1.1^{d}
IAA, 2-4 D	**0.3**	83.9±1.2^{c}	73.6±0.6^{c}	58.8±1.9^{c}
IAA, 2-4 D	**0.4**	83±3.8^{b}	77.5±0.6^{b}	65.1±0.2^{b}
IAA, 2-4 D	**0.5**	**91.5±0.7^{a}**	**82.5±0.6^{a}**	77.2±1.9^{a}

Values represent mean ± standard deviation of three replicates per treatment. Mean followed same letter in same column did not differ significantly at $p < 0.05$ according to Dunnet's HSD test.

Fig 1: *Calotropis gigantea* L. node explants inoculated on MS medium supplemented with IAA and 2,4 D.

Nodal explant showed maximum 81.7% on MS medium supplemented with 2,4-D + IAA (0.5mg/mL) when compared to the IAA and 2,4-D and it showed 77.2% and 77.0% of callus induction respectively (Table. 4-6; Fig. 3).

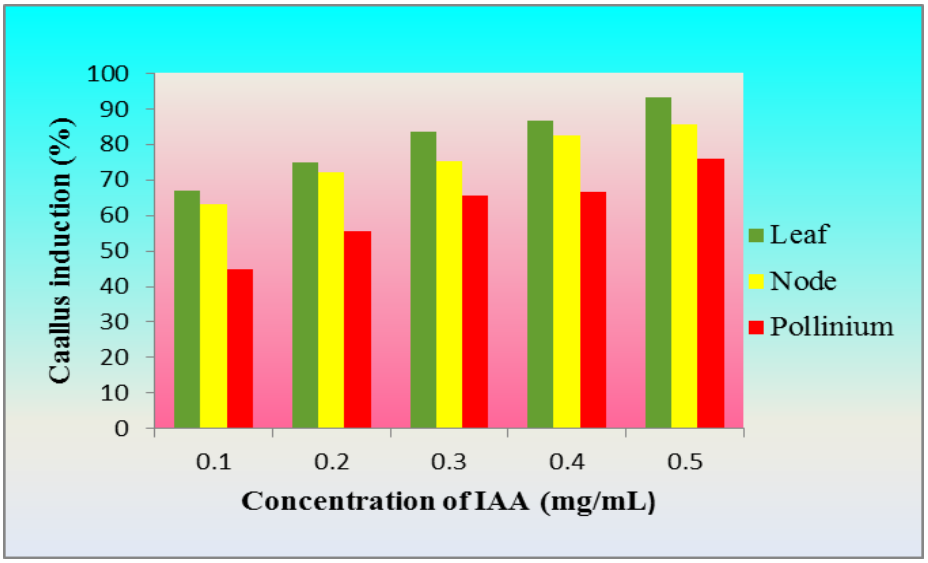


Fig 2: Effect of different concentration of IAA on callus induction from leaf, nodal and pollinium explants of *Calotropis gigantea* L.

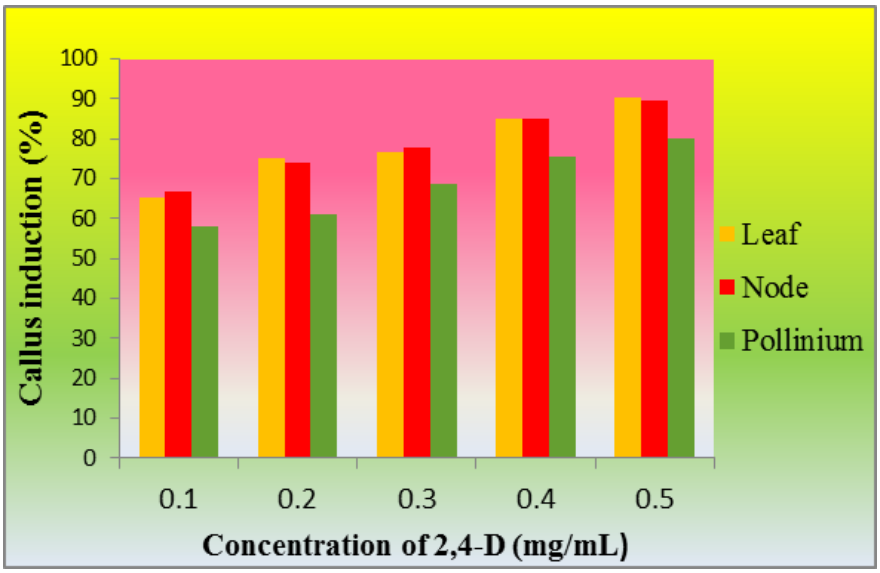


Fig 3: Effect of different concentration of 2,4D on callus induction from leaf, node and pollinium explants of *Calotropis gigantea* L.

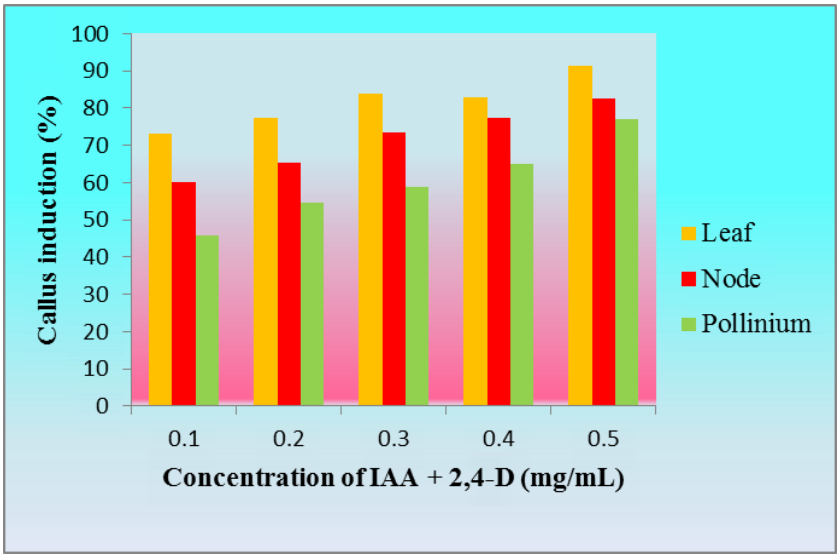
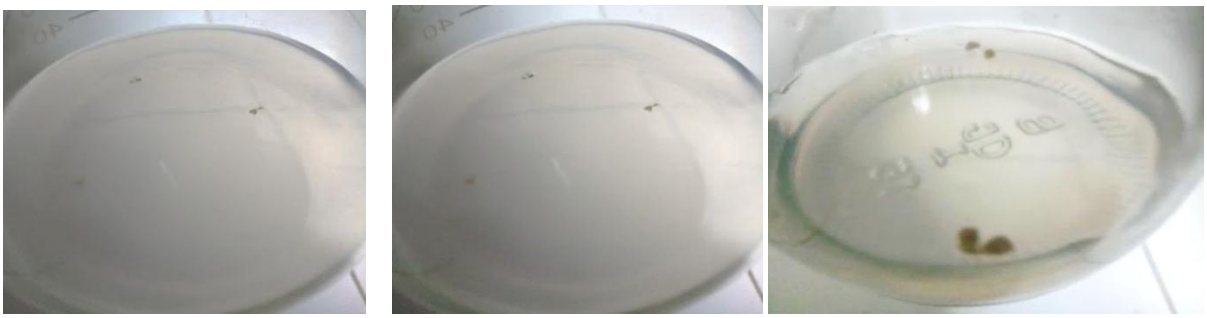


Fig 4: Effect of different concentration of 2,4 D and IAA on callus induction from leaf, node and pollinium explants of *Calotropis gigantea* L.

Manonmani and Francisca[27] studied the *in vitro* nodal multiplication of *Gymnema sylvestre* inoculated on MS medium supplemented with BA and NAA. Due to various concentrations the better result were observed in 1.0 mg/l of BA for the growth of root and 1.0 mg/l of NAA for shoot growth.

Pollinium explants showed the callus induction on 10th day of culture and showed 92.2% maximum growth on MS medium supplemented with 2,4-D (0.5mg/mL). IAA and 2,4-D + IAA (Table 4-6 and Fig. 4 and 5) showed 84.6 % and 92% of callus induction respectively.



a. On 1st day of inoculation

b. b. On 10th day of inoculation

c. On 30th day of inoculation

Fig 5: Calotropis gigantea L. pollinium explants inoculated on MS medium supplemented with 0.5 µL of IAA, 2,4 D

Ashis and Deepesh[28] evaluated the immature embryo of *Calotropis gigangea.* It were inoculated on basal MS Medium supplemented with 2, 4-D, NAA, IAA, IBA and BAP. As a result it showed the better response of higher growth in 0.1 mg/l^{-1} of NAA for shoot formation and auxin for root formation in the form half-strength Basal MS medium.

According to Raaman *et al*.,[26] the callus induction of leaf explants of *Hibiscus sabdariffa* yields a 25% in IAA and IBA and 2, 4-D showed the 35% and 80% at (0.5mg/mL). Interestingly in our studies IAA + 2, 4-D showed 92.3% of callus induction and IAA showed 92.2% whereas 2, 4-D showed 92.2% respectively. The callus induction of inter-nodal region of *Hibiscus sabdariffa* showed the 28% and IBA and 2, 4-D showed the 26% and 75% of callus induction in MS supplemented with IBA and 2-4D (0.5mg/mL). Interestingly we also got maximum 81.7% of callus growth on MS supplemented with IAA +2, 4-D (0.5mg/ mL).

Alina *et al.*,[29] evaluated the *in vitro* micropropagation of *Senecio macrophyllus*. The seed were inoculated on MS medium supplemented with Gibberlic acid (GA_3) and different kinds of Cytokinins (BA, KN and ZEA). The highest response of shoot is observed in MS medium supplemented with 4.4 µM of BA in combined with 0.54 µM of NAA but the root have higher growth in half or full strength MS medium.

Amuthapriya and Ravichandran[30] analyzed the micropropagation technique by using a seed of *Calotropis gigantea.* It was inoculated on MS medium supplemented with the growth hormone BAP and IAA. As a result it showed a better growth response in MS medium supplemented with the 10µM BAP and IAA.

CONCLUSION

Plants are used as source of traditional medicine from ancient time. Medicinal plants plays an alternative role to therapeutic medicine. *Calotropis gigantea* is easily available in agriculture and non-agriculture fields and several researchers reported the usage of medicinal properties. It contains antibacterial, antimicrobial, antifungal, anti-diuretic, anticancer properties and so on. In current scenario plants and their derivatives produce a new compounds which is useful to obtain drug discovery. Due to this over consumption and exploitation of the plant which become rare and vulnerable. To overcome this, plant can be produced with the help of Plant tissue culture technique. The explants (leaves and node) of *Calotropis gigantea* is used to propagate in a suitable solid medium supplemented with suitable growth hormone. Haploid plant production is one of the significant method in tissue culture. By using the pollinium, the

development and production of haploid plant *in vitro* can be carried out, which is important of fundamental and applied studies. This *in vitro* study is used to conserve the medicinal plant and produces large number of plants. As a result, the percentage of growth and cell viability can be calculated by using mean and standard deviation.

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REFERENCES

1. James D Caponetti, Dennis J Gray, Robert N Trigiano. History of plant tissue and cell culture, Plant Tissue Culture Concepts and Laboratory Exercises, 2018; 11-17.
2. Anwar Shahzad, Shiwali Sharma, Shahinapraveen, Taiba Saeed, Ariumend Shaheen, Historical perspective and basic principles of plant tissue culture. Plant biotechnology: principles and applications, 2017; 1-36.
3. Narges Khanpour-Ardestani, Mozafar Sharifi, Mehrdad Behmanesh,. Establishment of callus and cell suspension culture of *S. Striata* Boiss: "An *in vitro* approach for acetoside production". Cytotechnology, 2015; 67(3): 475-485.
4. Ehsamdar S, Majd A, Choukam R. Callus formation and regeneration of the first modified Iranian potato cultivar (Salavalan). Advanced crop science, 2013; 3: 201-208.
5. Laboney U Z, Biswas G C, Abdullah-Al-Shoeb M. Callus induction and regeneration of potato shoot tip culture. International journal of agricultural science, 2013; 3: 40-45.
6. Trevor Thorpe. History of plant tissue culture. Plant cell culture Protocols, 2012; 877: 9-27.
7. Pilar S Testillano, Beatriz Pintos, Aranzazu Gomez-Garay, Maria C Risueno. Stress-Induced Microspore Embyogenesis by Anther Culture of *Quercussuber*. Step Wise Protocols for Important Woody Plants, 2018; 84: 93-105.
8. Mayakaduwa D M R G, Silva T D. Seasonal variation in anther response and the effect of Nitrogen stresses on *in vitro* anther culture of Indica rice. Asian Journey of Botany, 2018; 1(1): 47-56.
9. Giuseppe Cimo, Annalisa Marchese, Maria Antonietta Germania. Microspore embryogenesis induced through *in vitro* anther culture of almond. Plant cell, Tissue and organ culture, 2017; 128(1): 85-95.

10. Isidre Hooghvorst, Eduardo Ramos-Fuentes, Camilo Lopez-Cristofannini, Mirari Ortega. Antimitotic and hormone effects on green double haploid plant production through anther culture of Mediterranean japonica rice. Plant cell, Tissue and Organ culture, 2018; 1-11.
11. Ninama A P, Sipal S A, Khadayata K G, Patel P C. Floriculture in India : Problems and prospect. Advances in life Sciences, 2016; 5(4): 1150-1153.
12. Jack GAJ Van Der Vorst, Robert Ossevoort, Marlies De Keizer, Tom Van Woensel, Cor N. DAVINC3I: Towards Collaborative Responsive Logestics Networks in Floriculture. Logistics and Supply chain Innovation, 2016; 37-53.
13. Coetzee J H, Hoffman E W. Social and environmental responsibility of the floriculture industry in South Africa. Acta Horticultururae, 2017; 1201(64): 479-484.
14. Ryan W Dickson. Solution ammonium: Nitrate ratio and cation/anion uptake affect acidity or basicity with floriculture species in hydroponics. Scientia horticulturae, 2016; 200: 36-44.
15. Goussard P G, Wiid J. The elimination of fan leaf virus from grapevines using *in vitro* somatic embryogenesis combined with heat therapy. South African journal of Enology and viticulture, 2017; 13(2): 81-83.
16. Reetika Singh, Manoj Kumar Rai, Nishi Kumari, Somatic Embryogenesis and Plant regeneration in *Sapindus mukorossi* Gaertn. from Leaf Derived callus Induced with 6-Benzylaminopurine. Applied Biochemistry and Biotechnology, 2015; 177(2): 498-510.
17. Balakrishnan subbaiyan, Ponnusamy samydurai, Rajendran venkatesh. *In vitro* Multiple shoot induction of selected *Ceropegia* species - Medicinally important plants. International journal of Conservation Science, 2015; 6(2): 253-260.
18. Seyed Ali Ravanfar, Maheran Abdul Aziz, Azmi Abdul Rashid, Shahida S. *In vitro* adventitious shoot regeneration from cotyledon explant of *Brassica oleracea* subsp. italica and *Brassica oleracea* subsp. capitata using TDZ and NAA. Pakistan Journal of Botany, 2014; 46(1): 329-335.
19. Sujoy Dey. Plant tissue culture: environmental condition, methods, types and application, 2017.
20. Kolupaev Y U E, Ryabchun N I, Vayner A A, Yastreb T O, Oboznyi A I. Antioxidant enzyme activity and osmolyte content in winter cereal seedlings under hardening and cryostress. Russian journal of plant physiology, 2015; 62(4): 499-506.
21. Shahnawaz Ahmed, Akash Sharma. Studies on hardening and acclimatization of micro-propagated plantlets of banana. Studies, 2014; 9(3): 965-967.

22. Purvis M J, Collier D C, Walls D. Laboratory techniques in Botany. CABI Direct, 1966; 1-439.
23. Tuite J. Plant pathological methods: fungi and bacteria. Burgess Publishing Company, 1969; 1-239.
24. Booth C. The genus fusarium. C.A.B. Publishing Company, 1971; 1-237.
25. Murashige T, Skoog F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. An International journal Plant Physiology, 1962; 15(3): 473-497.
26. Raaman N, Balan Leeba, Swaminathan Amutha, Mahalingam Amsaveni and Chandrasekaran Sriram. *In vitro* callus induction and comparative antimicrobial activity of wild plant and callus of a medicinal plant *Mukia maderaspatana* (L.) Cogn. Medicinal Plants, 2013; 5(3): 126-134.
27. Manonmani R, Francisca P. *In vitro* multiplication of *Gymnema sylvestre* R. Br. Through nodal explants. International Journal of Pharma and Bio Sciences, 2012; 3(2): 49-53.
28. Ashis Taru Roy, Deepesh N. Tissue culture and plant regeneration from immature embryo explants of *Calotropis gigantea* (Linn.) R. Br. Plant Cell, Tissue and Organ Culture, 1990; 20: 229-233.
29. Alina trejgell, Magdalena michalska, Andrzej tretyn. Micropropagation of *Senecio macrophyllus* M. Bieb. Acta Biologica Cracoviensia Series Botanica, 2010; 52(1): 67–72.
30. Amuthapriya T, Ravichandran P. Rapid *in vitro* shoot multiplication through hypocotyl segment culture in *Calotropis gigantea* R. BR. International Journal of Bioassays, 2014: 3(11): 3523-3525.