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INDIRECT ORGANOGENESIS OF *CLEOME GYNANDRA* L. USING LEAF EXPLANTS – AN IMPORTANT MEDICINAL PLANT

I. Sudan and A. V. P. Karthikeyan *

P.G. & Research Department of Botany, Government Arts College (Autonomous), Karur - 639005, Tamil Nadu, India.

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Correspondence to Author:
A. V. P. Karthikeyan

Assistant Professor,
P.G. & Research Department of Botany, Government Arts College (Autonomous), Karur - 639005, Tamil Nadu, India.

E-mail: avpkarthi1974@gmail.com

ABSTRACT: The present study was developed a suitable protocol for callus induction and plant regeneration of *Cleome gynandra* L. from leaf explants. Callus was initiated from leaf explants on Murashige and Skoog (MS) medium supplemented with different concentration and combinations of plant growth regulators. They are using (5-25 µM) of BAP (6-benzyladenine), KN (kinetin), and TDZ (thidiazuron) cytokinins along with (2-10 µM) different auxins [NAA (α-naphthalene acetic acid), IAA (indole-3 acetic acid), IBA (indole-3-butyric acid)]. Maximum regenerative callus (95%) was observed on TDZ + NAA (10 µM + 2 µM). Organogenic calli were transferred to shoot induction medium containing a different concentration of cytokinins (BAP, KIN, TDZ) ranging from 5-25 µM. Among the cytokinins tested BAP 10 µM produced number of shoots (35.2) with maximum shoot induction frequency (98%). Isolated shoots were carefully transferred to half-strength MS medium supplemented with auxins (IAA, IBA, and NAA) ranging from 2-10 µM. The maximum numbers of roots (8.8) were observed on 4 µM IBA. The rooted plantlets were successfully acclimatization.

INTRODUCTION: *Cleome gynandra* L. (*C. gynandra*) belongs to the family Cleomaceae and it is commonly called as "spider flower or Cat's whiskers." It is a well-known medicinal plant which is mainly used as a traditional medicine [1]. The seeds, leaves, and roots of the plant are widely used in traditional folk medicine as an analgesic, anthelmintic, anti-inflammatory, antimicrobial, hepatoprotective and immunomodulatory activities. The paste of plant has been used in rheumatism, neuralgia, headache, and stiff neck. The warm juice is a popular remedy for ear and skin disease, whereas fresh juice of leaves is applied externally during pyorrhea [2, 3].

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It has the metabolically active secondary metabolites such as alkaloids, carotenoids, flavonoids, phenols, saponins, tannins, *etc*. most of these chemicals posses scientifically proven free radical scavenging activity [4, 5, 6]. In most of the countries, there will be a drastic demand for the medicinally important plant material because of the exploitation and increasing urbanization. Therefore, *in-vitro* propagation is the only alternative way for the rapid regeneration of plants under aseptic culture conditions.

To grow the genetically identical copies of plants with desirable characteristics, a callus is used. The *in-vitro* propagation technique is found to be an alternative method because it offers a supply of biochemical independent of season and availability of plants [7, 8, 9]. Earlier, there have been few reports on the establishment of *in-vitro* propagation through nodal explants of *Cleome gynandra* [10]. The present communication deals with the development of a rapid regeneration system for indirect

organogenesis through leaf explants of *Cleome gynandra*.

MATERIALS AND METHODS:

Collection of Plant Material: Young leaves of *C. gynandra* from field plant were used as explants and were collected from the roadside of thanthonimalai, Karur. The leaves were washed in tap water for some time and disinfected with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 min followed by thorough rinsing in autoclaved distilled water for at least 7- 8 times.

Preparation of Culture: The leaves were thoroughly washed with running tap water to remove all the dust particles followed by three rinses with sterile double distilled water. To eliminate other contamination explants were rinsed with 70% alcohol for 1 min followed by distilled water washing twice. The explants were then treated with 0.1% (w/v) mercuric chloride for 3-5 min under aseptic conditions. After this explants were then thoroughly washed 4-5 times with sterilized double distilled water to remove the traces of mercuric chloride [11].

Preparation of Culture Media: MS medium was fortified with 30 g/l sucrose (Himedia, India) and gelled with 0.8% agar (Himedia, India), and the pH of the medium was adjusted to 5.8 by adding 0.1 N NaOH or 0.1N HCl [12]. The medium was dispensed in the culture tube and autoclaved at 121 °C, for 30 min. All the cultures were maintained in a sterilized culture room at 25 ± 2 °C, under 16 h photoperiods provided by cool white fluorescent with 55-60% relative humidity. The cultures were sub-cultured on the fresh medium after 15-20 days.

Callus Initiation: Explants of leaves (0.5 × 0.5 cm) were excised from prepared explant and placed horizontally on MS medium. In this experiment, we study the effects of cytokinins and auxins, both separately and in combination on callus initiation and proliferation.

They are 5-25 µM of BAP (6-benzyladenine), KN (kinetin), and TDZ (thidizauron) along with (2-10µM) different auxins [NAA (α-naphthalene acetic acid), IAA (indole-3 acetic acid), IBA (indole-3-butyric acid)]. These culture conditions were used in all the experiments mentioned below unless stated. Data of frequency (%) of callus formation and its fresh weight were recorded after 4 weeks of culture.

Shoot Organogenesis from Callus: Well-established, hard and compact callus (0.5 g fresh weight) were grown on MS medium supplemented with BAP (5-25 µM), KN (5-25 µM), and TDZ (5-25µM) for shoot organogenesis. Cultures were transferred on fresh media after 20th day of inoculation.

Formation of Adventitious Root: Regenerated shoots (3-5 cm long) obtained from micro-propagated plantlets were cultured on MS basal medium fortified with either of IBA, NAA, or IAA (2-10 µM) for adventitious rooting. Data were recorded on percentage of rooting, number, and length of the roots after 4 weeks of transfer onto the rooting media.

Statistical Analysis: The experimental data were recorded and subjected to the factorial design of analysis of variance (ANOVA) **Table 3** and **4** and Duncan multiple range tests calculated at the confidence level of $P < 0.05$. [13]

RESULTS AND DISCUSSION: Leaf explants of the *C. gynandra* were cultured on MS medium supplemented with different concentration (5-25 µM) of cytokinins and auxins **Fig. 1a-c**. Among the different concentration of cytokininis and auxins 92 % of green nodular callus was observed in 10 µM of TDZ **Table 1**. The growth-promoting activity of thidiazuron was accompanied by high acid-phosphate level [14] and is stable and biologically active at low concentration than other cytokinins [15]. The high efficiency of TDZ may be attributed to its ability to induce cytokinin accumulation or enhance the accumulation and translocation of auxin within the tissue [16, 17]. It was also recorded that TDZ combined with NAA (10 µM TDZ + 4 µM NAA) produced 95 % of green nodular callus **Table 2**. It is also reported that the combination of cytokinin and auxin stimulated the formation of regenerative callus in induction in teak [18, 19].

The regenerated calli were transferred to MS basal media supplemented with BAP, KIN, and TDZ (5 – 25µM) for shoot organogenesis **Fig. 1d-f**. The maximum number of shoots was observed from 10µM of BAP on the 4th week after transferring the callus on shoot organogenesis media and the

percentage was 98% of shoot induction with the maximum number of shoots (35.2 ± 0.42) and the average length of the shoot was 6.6 ± 1.66 cm was recorded. Similarly, 10µM of TDZ showed 90% percentage of shoot induction **Fig. 1g**, **h** with the maximum number of shoots (33.2 ± 0.41) and the average length of the shoot was 6.36 ± 1.66 cm **Table 3**.

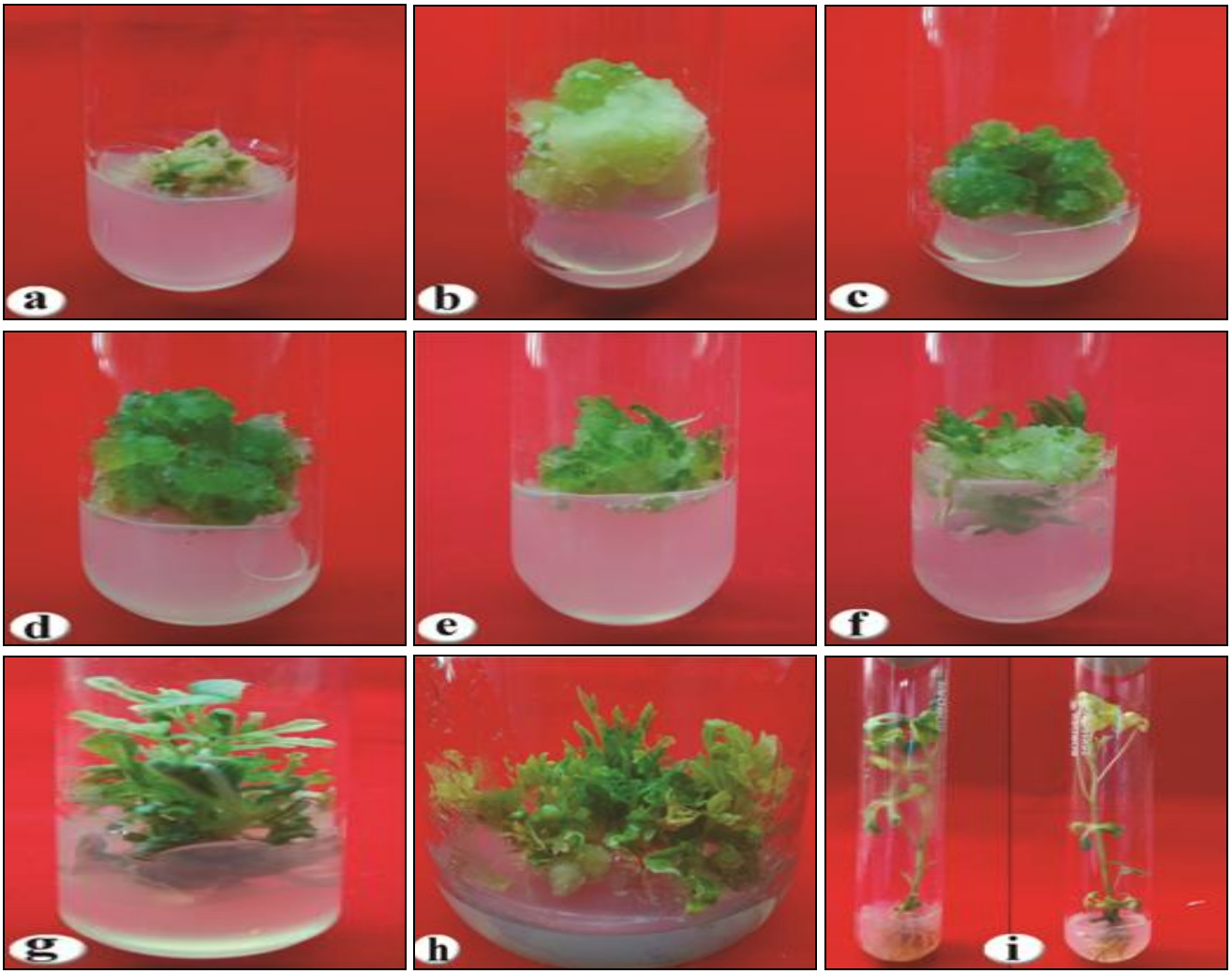

FIG. 1: INDIRECT ORGANOGENESIS OF *C. GYNANDRA* THROUGH LEAF EXPLANTS. a) Callus initiation; b – d) Callus proliferation; e & f) Shoot regeneration on callus; g & h) Shoot multiplication; i) Root initiation and multiplication

TABLE 1: EFFECT OF GROWTH REGULATORS ON CALLUS INDUCTION OF *C. GYNANDRA* FROM LEAF EXPLANTS

Growth regulators	% Callus Response	Callus Nature
5 µM TDZ	90.0	Green friable callus
10 µM TDZ	92.0	Green nodular callus
15 µM TDZ	86.6	Green compact
20 µM TDZ	73.3	Green compact nodular
25 µM TDZ	63.3	Green friable callus
5 µM KIN	20.6	White friable callus
10 µM KIN	33.3	White callus
15 µM KIN	14.0	White compact
20 µM KIN	18.0	White friable callus
25 µM KIN	15.6	Light green friable callus
5 µM BAP	65.0	Light Green friable callus
10 µM BAP	70.0	Yellow Green nodular callus
15 µM BAP	76.6	Light Green friable
20 µM BAP	72.3	Light Green compact nodular
25 µM BAP	69.3	Green friable callus

TABLE 2: EFFECT OF DIFFERENT COMBINATION OF AUXIN AND CYTOKININS ON CALLUS INDUCTION OF *C. GYNANDRA* THROUGH LEAF EXPLANTS

Growth regulators				% Callus response	Callus nature
TDZ	IAA	IBA	NAA		
10 µM	2 µM	-	-	88.5	Green friable callus
10 µM	4 µM	-	-	88.0	Green nodular callus
10 µM	6 µM	-	-	85.8	Green compact
10 µM	8 µM	-	-	80.5	Green compact nodular
10 µM	10 µM	-	-	76.3	Green friable callus
10 µM	-	2 µM	-	92.5	Green friable callus
10 µM	-	4 µM	-	90.6	Green nodular callus
10 µM	-	6 µM	-	77.0	Green compact
10 µM	-	8 µM	-	78.3	Green compact nodular
10 µM	-	10 µM	-	65.3	Green friable callus
10 µM	-	-	2 µM	93.5	Green friable callus
10 µM	-	-	4 µM	95.0	Green nodular callus
10 µM	-	-	6 µM	93.6	Green compact
10 µM	-	-	8 µM	89.3	Green compact nodular
10 µM	-	-	10 µM	77.3	Green friable callus

TABLE 3: EFFECT OF GROWTH REGULATORS ON SHOOT INDUCTION FROM REGENERATED CALLUS OF *C. GYNANDRA*

Growth regulators	% Shoot response	Mean no. of shoots/unit callus	Mean shoot length (cm)
5 µM BAP	80.0	28.0 ± 0.57^{cd}	5.86 ± 1.37^{c}
10 µM BAP	98.0	35.2 ± 0.41^{a}	6.6 ± 1.66^{a}
15 µM BAP	86.6	25.0 ± 0.40^{gh}	4.74 ± 0.65^{ef}
20 µM BAP	83.3	18.35 ± 0.47^{l}	4.68 ± 0.88^{fg}
25 µM BAP	73.3	13.8 ± 0.41^{m}	4.02 ± 1.21^{kl}
5 µM KIN	66.6	12.5 ± 0.28^{no}	4.14 ± 0.98^{jk}
10 µM KIN	83.3	26.75 ± 0.47^{de}	5.66 ± 1.50^{cd}
15 µM KIN	70.0	25.25 ± 0.75^{fg}	4.62 ± 0.62^{gh}
20 µM KIN	50.0	23.6 ± 0.57^{jk}	3.88 ± 1.06^{lm}
25 µM KIN	46.6	13.4 ± 0.44^{mn}	2.82 ± 1.02^{o}
5 µM TDZ	75.0	25.6 ± 0.57^{ef}	4.86 ± 1.37^{e}
10 µM TDZ	90.0	33.2 ± 0.41^{b}	6.36 ± 1.66^{ab}
15 µM TDZ	83.6	29.0 ± 0.40^{c}	4.24 ± 0.65^{ij}
20 µM TDZ	73.3	23.75 ± 0.47^{ij}	4.28 ± 0.88^{i}
25 µM TDZ	66.3	23.8 ± 0.41^{hi}	3.64 ± 1.21^{mn}

Values with the different letters indicate significant differences at 5% level.

TABLE 4: EFFECT OF GROWTH REGULATORS ON ROOT INDUCTION FROM REGENERATED SHOOTS OF *C. GYNANDRA*

Growth regulators	Root induction frequency (%)	Mean no. of roots/shoot	Mean root length (cm)
2 µM IBA	84.0	7.4 ± 1.60^{bc}	3.70 ± 1.12^{hi}
4 µM IBA	90.0	8.8 ± 1.85^{a}	7.32 ± 0.56^{a}
6 µM IBA	86.0	7.6 ± 1.48^{b}	6.7 ± 1.15^{b}
8 µM IBA	76.6	6.6 ± 2.01^{d}	5.84 ± 1.42^{bc}
10 µM IBA	60.0	5.2 ± 0.82^{e}	4.13 ± 0.45^{de}
2 µM IAA	44.0	2.4 ± 1.60^{mn}	2.70 ± 1.12^{lm}
4 µM IAA	60.0	2.8 ± 1.85^{jk}	4.32 ± 0.56^{d}
6 µM IAA	66.0	3.4 ± 1.48^{ij}	3.72 ± 1.15^{c}
8 µM IAA	66.6	3.7 ± 2.01^{gh}	3.84 ± 1.42^{ef}
10 µM IAA	50.0	2.7 ± 0.82^{kl}	3.13 ± 0.45^{j}
2 µM NAA	66.6	4.3 ± 1.78^{ef}	3.76 ± 0.65^{fg}
4 µM NAA	73.9	3.6 ± 1.91^{hi}	2.82 ± 0.49^{kl}
6 µM NAA	70.5	4.2 ± 0.96^{fg}	2.96 ± 0.77^{jk}
8 µM NAA	53.5	2.5 ± 1.29^{lm}	2.48 ± 0.74^{mn}
10 µM NAA	43.7	2.2 ± 0.65^{o}	2.20 ± 0.39^{o}

Values with the different letters indicate significant differences at 5% level.

Proliferative features of cytokinins TDZ and BAP play various roles in plant development during the formation and activity of shoot meristems [20-22]. These cytokinins improve proliferative rates in different species. BAP is involved in the improvement of shoot proliferation and elongation

[23]. The regenerated shoots (3-4 cm) were excised and transferred into half-strength MS medium supplemented with different concentrations of auxins (IAA, NAA and IBA) for rooting **Fig. 1i**. Among the different concentration of auxins 4 µM of IBA response as 90% of root induction, the number of roots per each medium as 8.8 ± 1.85 and the root length was recorded by the average of 7.32 ± 0.56 cm **Table 4**.

In the present study, the superiority of IBA over other auxins regarding *in-vitro* rooting is supported by the result of other studies in *Ephedra gerardiana* [24], *Commelina diffusa* [25] and *Dendrobium chrysotoxum* [26], *Cleome chelidonii* [27]. The present investigation suggests that the IAA showed the least response in root formation. In comparison with other auxins, a similar observation was reported [28]. Tissue culture techniques can play an important role in clonal propagation of elite genotypes of has diverse medicinal applications and eventually due to overexploitation and irregular concern this plant is facing local extinction. The result of this study shows a fruitful indirect organogenesis protocol for *C. gynandra* through callus formation, shoot regeneration and successful regeneration of new plantlets.

CONCLUSION: This study suggested an effective protocol for indirect regeneration system for *C. gynandra*. This protocol is simple, easy to carry out and produced a large number of callus and plants for mass propagation in a short period of time. The *in-vitro* regenerated plants were further subject to analysis the bioactive compounds, which can be used in the pharmaceutical industries.

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CONFLICT OF INTEREST: The authors do not have any conflict of interest to declare.

REFERENCES:

1. Partha PA and Satya BP: Medicinally important plant *Cleome gynandra*: a phytochemical and pharmacological explanation. Asian J of Pharm and Clin Res 2018; 11(1): 21.
2. Pillai LS and Nair BR: *In-vitro* anti-inflammatory studies in *C. viscosa* L. and *C. burmanni* W. & A. (Cleomaceae): Int J Pharm Sci & Res 2014; 5(11): 5000-05.
3. Shaika K, Shaika A, Kumar D and Kadirvel D: Evaluation of preliminary phytochemical properties and hypoglycemic activity of *Cleome gynandra* L. Int J Pharm Pharm Sci 2013; 5(3): 824-28.
4. Lokesha R: The African spider plant (*Gynandropsis pentaphylla* - DC. Syn. *Cleome gynandra* Linn.) (Capparaceae): Phytochemistry, pharmacological and biotechnological properties - A review. Int J Pharmacogn Chinese Med 2018; 2(4): 000144.
5. Rajaselvam J and Rose B: *In-vitro* antimicrobial activity and phytochemical analysis of *Cleome gynandra* Linn. leaf extracts against human pathogens. Int J for Res in App Sci & Engg Tech 2016; 4(8): 793-96.
6. Ahouansinkpo E, Atanasso J, Dansi A, Adjatin A, Azize O and Sanni A: Ethnobotany, phytochemical screening and toxicity risk of *Cleome gynandra* and *Cleome viscosa*, two traditional leafy vegetables consumed in Benin. Int J Curr Microbiol App Sci 2016; 5(2): 813-29.
7. Imanirampa L and Alele EP: Antifungal activity of *Cleome gynandra* L. aerial parts for topical treatment of Tinea capitis: an *in-vitro* evaluation. Imanirampa and Alele BMC Comp and Alt Med 2016; 16(194): 1-8.
8. Raza G, Singh BM and Bhalla LP: *In-vitro* plant regeneration from commercial cultivars of soybean. Bio Med Res Int 2017; 1-9.
9. Ikeuchi M, Ogawa Y, Iwase A and Sugimoto K: Plant regeneration: cellular origins and molecular mechanisms. Development 2016; 143: 1442-51.
10. Bhardwaj AK, Naryal A, Bhardwaj P, Warghat AR, Arora B and Saxena S: High-efficiency *in-vitro* plant regeneration and secondary metabolite quantification from leaf explants of *Rhodiola imbricata*. Pharmacog J 2018; 10(3): 470-75.
11. Karthikeyan AVP and Sudan I: GC-MS profile of *in-vivo* and *in-vitro* shoots of *Cleome gynandra* L. Int J Pharm Pharm Sci 2017; 9(11): 21-26.
12. Ishwarya M, Gayathri M, Ezhumalai M, Uthaya Prakash GK, Tamilarasan S and Ramakrishnan S: An efficient micropropagation of *Achyranthes aspera* L. using shoot tip explant. Int J Curr Res Biosci Plant Biol 2018; 5(2): 76-84.
13. Murashige T and Skoog F: A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plant 1962; 15: 473-97.
14. Kumar A, da Fayaz M, Bhat MH and Jain AK: An efficient protocol for *in-vitro* regeneration of *Vitex negundo* an important medicinal plant. Biosci Biotech Res Comm 2018; 11(2): 256-62.
15. Washimakar and Shende: Plant tissue culture in herbal medicinal plants. Euro J of Pharm and Med Res 2016; 3(11).
16. Bhujbal VDS, Sangoram A and Jadhav A: Use of tissue culture technique in the cultivation of medicinal plants. World J of Pharmacy and Pharmaceutical Sci 2018; 7(2): 1285-90.

17. Deb CR, Rout GR, Mao AA, Nandi SK, Nilasana Singha RK, Vijayan D, Langhu T, Kikon ZP, Pradhan S, Tariq M and Swain D: *In-vitro* propagation of some threatened plant species of India. Current Science 2018; 114(3): 567-75.
18. Murthy BNS, Murch SJ and Saxena PK: Thiadiazuron- A potent *in-vitro* plant morphogenesis. *In-vitro* Cell Dev Biol Plant 1998; 34: 267-75.
19. Victor JMR, Murthy BNS, Murch SJ, Krishnaraj S and Saxena PK: Role of endogenous purine metabolism in thidiazuron induced somatic embryogenesis of peanut (*Arachis hypogea* L.). Plant Growth Regul 1999; 28: 41-47.
20. Widiyanto SN, Erytrina D and Rahmania H: Adventitious shoot formation on teak (*Tectona grandis* L. f.) callus cultures derived from internodal segments. Acta Hort 2005; 692: 153-57.
21. Karunaratne MLWOM, Peries SE and Egodawatta WCP: Callus induction and organogenesis from leaf explants of *Tectona grandis*. Ann Biol Res 2014; 5: 74-82.
22. Gandhi K, Rajesh E, Saravanan S and Elamvaluthi M: Micropropagation of *Aegle marmelos* L. - A medicinal tree through culture of axillary bud and shoot tip explants from *in-vitro* germinated seedlings. Int. Jour of Sci Res in Bio Sci 2018; 5(3): 65-71.
23. Vandana CD, Shanti KN and Shantha SL: GC-MS analysis of callus and leaf extracts of *in-vitro* propagated plants of *Justicia wynaadensis* (nees) T. Anderson. Int J Pharm Sci & Res 2018; 9(2): 535-43.
24. Vijayakumar J, Rathi GS, Bhuvaneshwari SM, Kumari BR and Castantilde E: Thidiazuron - induced shoot organogenesis of *Cleome viscosa* L. through cotyledonary explants culture. African J Biotechnol 2014; 13: 1027-36.
25. Rautela I, Dhiman M, Sharma MD and Misra P: *In-vitro* regeneration of medicinal plant *Ephedra gerardiana*. Int J Pharm Sci & Res 2018; 9(3): 1183-88.
26. Karthikeyan AVP, Sudan I and Satheeshkumar R: *In-vitro* regeneration of *Commelina diffusa* Burm. F. Using nodal explants. World J of Pharm Res 2018; 7(3): 978 - 986.
27. Subhash Kumar and Sirangi: Induction of callus and shoot proliferation of a medicinally important herb *Cleome chelidonii* L. Int J Curr Res Biosci Plant Bio 2015; 2(7): 79-82.
28. Lata H, Chandra S, Techen N, Khan AI and ElSohl AM: *In-vitro* mass propagation of *Cannabis sativa* L. A protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical and genetic fidelity of micropropagated plants. J of App Res on Med and Aromatic Plants 2016; 3(1): 18-26.

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