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RESEARCH ARTICLE

EFFECT OF THIDIAZURON ON CALLOGENESIS IN MATURE LEAF EXPLANTS OF *COSCINIUM FENESTRATUM* (GAERTN.) COLEBR., A CRITICALLY ENDANGERED, MEDICINAL PLANT

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ABSTRACT

Coscinium fenestratum (Gaertn.) Colebr., (Menispermaceae) is a critically endangered, medicinal plant. Mature leaf were used as the explants for the initiation of callus and cultured into three different media, Murashige and Skoog (MS), Woody plant medium (WPM) and B_5 medium with varying concentrations of Thidiazuron (TDZ). MS medium was found to have a superior proliferation rate. The frequency of callus formation reached 95% with a mean fresh weight of 2854.76 ± 0.30 mg for explants cultured on MS basal medium supplemented with 0.75 mg/l TDZ. The highest frequency 75% of organogenic callus induction was observed when the calli were subcultured in MS medium containing 0.25 mg/l BAP, which induced an average of 6.71 ± 0.12 adventitious shoot buds.

INTRODUCTION

Coscinium fenestratum (Gaertn.) Colebr., (Menispermaceae) is a critically endangered, dioecious medicinal liana (Ravikumar and Ved 2000) a more or less primitive group, indigenous to the Indo-Malayan region. In India, it is restricted to the few habitats of Western Ghats, mostly in the high rainfall receiving wet evergreen forests, moist evergreen, semi-evergreen and semi-deciduous forests at an altitude of 500 to 750 m (Mohanan and Sivadasan 2002). 1997 IUCN Red list of threatened plants recorded the status of *Coscinium fenestratum* as highly endangered in India, vulnerable in Vietnam, rare in Singapore and indeterminate in Srilanka (Walter and Gillet 1998). The stem and roots of this species is used in a variety of indigenous medicinal preparations in the treatment of fever, skin infections, snake bite, diarrhea, tetanus, anorexia, chronic dyspepsia, anaemia and psiolosis and can be used with good results in the form of tincture and infusion (Kritikar and Basu 1935; Warrier *et al*., 1994). It is used in over 62 ayurvedic preparations (Nambiar *et al*., 2000).

The active principle of this plant was identified as berberine (Siwon *et al*., 1980; Malhotra *et al*. 1989) $C_{20}H_{18}NO_4Cl$ an isoquinoline alkaloid having numerous biological activities (Birdsall and Kelly 1997). The other major components in wood and root of *C.fenestratum* include palmatine, tetrahydropalmatine, crebanine and jatrorhizine (Keawpradub 1992; Pinho *et al*., 1992). *Coscinium fenestratum* has been reported to possess immense pharmacological actions such as antioxidant (Venukumar and Latha 2002; Shirwaikar *et al*., 2007), antiproliferative (Ueda *et al*., 2002; Narasimhan and Nair 2005), antidiabetic (Jittaprasatsin *et al*., 2005; Punitha *et al*.,2005; Shirwaikar *et al*., 2005), antiplasmodial(Tran *et al*., 2003), anti inflammatory (Sudharshan *et al*., 2010), antifeeding (Javasinghe *et al*., 2003), hypotensive (Singh *et al*., 1990; Wongcome *et al*., 2007), hepatoprotective (Venukumar and Latha 2004), neurotoxicity (Wattanathorn *et al*., 2006), antibacterial (Nair *et al*., 2005) and wound healing (Anitha *et al*., 2011) activities. Combination of the rampant destruction of the forests along with over exploitation of the species for the raw drug market and very slow rate of regeneration has seriously depleted its population in the wild, making conservational measures very urgent (Tushar and Udayan 2005). *Coscinium fenestratum* are considered to be recalcitrant to regeneration via callus and pose various problems during in vitro culture. In previous reports, protocols for berberine production by cell suspension cultures from *Coscinium fenestratum* were established in petiole and leaf segments but these explants were not able to form organogenic callus and no shoot bud plant regeneration was observed (Nair *et al*., 1992; Khan *et al*., 2008; Narasimhan and Nair 2004). It appears from the existing reports that induction of organogenic callus in *Coscinium fenestratum* remains a rare event. In the present investigation, an attempt has been made to establish an efficient protocol for production of rapid high regenerative callus from mature leaf explants using TDZ in order to induce *in vitro* shoot bud organogenesis. To our knowledge, this is the first report of indirect shoot bud organogenesis in *Coscinium fenestratum*.

MATERIALS AND METHODS

Plant material and sterilization of explants

The healthy mature plants were brought from the natural habitat in Thrissur, Kerala, India. The fully developed mature leaves from were harvested and used as explants. The explants were washed with distilled water containing a few drops of

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detergent (Tween 20) for 5 min and rinsed 2–3 times with sterile distilled water and then soaked in fungicide (Bavistin 1%) for 5 min followed by rinsing with sterile distilled water. Thereafter, the explants were surface disinfected with 70% ethanol for 30 s and rinsed 2–3 times with sterile distilled water, treated with 0.1% aqueous mercuric chloride (HgCl2) for 3 min and thoroughly washed 4–5 times with sterile distilled water under aseptic condition.

Culture conditions

The pH of the medium was adjusted to 5.8 before solidifying with 0.8% w/v Difco bacto agar. The chemicals used in this study were of analytical grade (Hi-media, Qualigens, SD fine chemicals, India). Molten medium (10 ml) was dispensed into 50 ml test tubes (Borosil) and plugged with non absorbent cotton plugs. The culture tubes containing the media were autoclaved at 121˚C for 15 min. All the cultures were maintained at 22 ± 2˚C under a 16 h photoperiod at a photosynthetic flux of 35-50 µmol/ (m^{-2} s^{-1}), provided by cool daylight fluorescent lamps.

Callus induction

Mature leaf explants were used for the callus induction. The explants were cut into small pieces and inoculated into different media viz., MS (Murashige and Skoog, 1962), Woody plant (Lloyd and Mc Cown, 1980) and B_5 (Gamborg *et al*., 1966). These media were further supplemented with 3% sucrose and various concentrations of Thidiazuron (TDZ), a substituted phenylurea (N-phenyl-1,2,3-thidiazol-5-ylurea), at 0.0, 0.05, 0.1, 0.2, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. The developed calluses were separated from the initial explants and healthy masses subsequently sub-cultured at regular intervals. The number of explants forming callus was scored to calculate callus formation frequency and their fresh and dry weight were recorded.

Adventitious shoot bud induction

Calli were transferred onto MS media containing 3% sucrose plus different concentrations of BAP (0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 mg/l) for indirect shoot bud organogenesis. The number of originated adventitious shoot buds were recorded 5 weeks after culture.

RESULTS AND DISCUSSION

Callus induction and growth of callus

In order to explore the possibility of TDZ in callus induction of *Coscinium fenestratum* , mature leaf explants measuring about 5mm in length were cultured horizontally on three different media with various concentrations of TDZ. Murashige and Skoog (MS), Woody Plant Media (WPM), B_5 (Gamborg *et al*. 1966), were tested to determine their suitability for in vitro cultures of *Coscinium fenestratum.* Medium formulation displayed a strong effect on the fresh and dry weight of the callus. Comparing the three formulations reveals that the proliferation rate of the MS medium was superior to the WPM or B5 medium. The highest percentage of callus formation from mature leaf explants reached 95% with a highest callus growth in terms of mean fresh weight (2854.76 ± 0.30 mg) (Table 1) was observed in MS medium fortified with 0.75 mg/l TDZ. Whereas in WPM, highest callus growth in terms of mean fresh weight was (2762.65 ± 0.58 mg) (Table 2) and in B5 medium the highest mean fresh weight (2616.19 ± 0.19 mg) (Table 3) was observed. This reactivity difference of MS medium was seems to be in relation to the calcium and nitrogen concentrations. In effect, the MS have eight and four fold higher calcium and nitrogen levels than the B5 medium (Zouzou *et al*. 2000). The nitrogen and potassium content of the MS medium was approximately two- to four-fold higher than that of the WPM. Inorganic nitrogen has a determining action on callogenesis (Trolinder and Goodin 1987; Grimes and Hodges 1990) and this probably explains the differences of callus fresh weight on MS and other media in *Coscinium fenestratum*. Consequently, MS medium was used for the following studies. Leaf explants showed no response on MS hormone – free medium, and TDZ was a pre requisite for their callus induction. Leaf explants grown on MS medium supplemented with various concentrations of TDZ formed calli from the cut ends within 2 weeks of culture (Fig. 1A), and at the end of fifth week, the entire surface of explants was covered with callus(Fig. 1B). The calli were slow growing, creamish yellow and compact in texture. During incubation on medium containing TDZ, expansion, swelling and thickening of leaf explants were observed.

This could probably due to intense cell division as in the work reported on *Pelargonium capitatum* (Arshad *et al*., 2011). The first stage in the morphogenic process is the development of an undifferentiated cell mass commonly known as callus (Murthy *et al*., 1998) and the process of callus formation is called callogenesis, which is the primary step in the stimulation of shoot reproduction via indirect mode and adventitious organs regeneration. Thidiazuron is a potent cytokinin for promoting callus formation from woody explants, especially when used at ≥0.1 µM (Huetteman and Preece 1993). N-phenyl-N'-1,2,3-thidiazol-5-ylurea or thidiazuron is a substituted phenylurea which was first developed as a cotton defoliant and now is used as a potent growth regulator in diverse plant species for eliciting a wide spectrum of *in vitro* responses (Murthy *et al*. 1998) However, the utilization of TDZ for callus induction in *Coscinium fenestratum* had never been employed before. There have been several reports of significant TDZ effects on callus formation in other species (karami *et al*., 2009; Gill *et al*., 1993; Gill&Saxena 1993; Capelle *et al*.,1983; Murthy&Saxena 1998; Chand&Roy 1980; Sahai *et al*. 2010; Jones *et al*., 2007).

The growth of callus initiated on 0.75 mg/l TDZ could not be sustained during subsequent subcultures on the same medium, but remained prolific only at 0.2 mg/l TDZ. Whereas repeated subcultures on 0.5 to 1.5 mg/l TDZ concentrations triggered a shift to a static state and ceased repetitive growth within 2-3 wk. Callus induced in 0.2 mg/l TDZ were morphologically similar to those raised in 0.75 and 1.0 mg/l TDZ supplemented medium. Since TDZ at 0.2 mg/l was optimum for maintenance and proliferation of callus, it was subsequently used for regular maintenance. Progressive browning of the callus as well as culture medium was the major constraint encountered in establishing calli cultures of *Coscinium fenestartum*, which is probably due to oxidation and production of phenolic compounds. Browning of excised explants and the resultant discolouration of culture media is a major challenge in plant tissue culture systems (Huang *et al*., 2002). (Nair *et al*., 1992) proposed that in media with high PGR concentrations, the production and release of berberine into the culture media could result in browning. However, (Figueiredo *et al*., 2000),

Table 1. Effect of different concentrations of TDZ on induction of callus grown on MS medium from mature leaf explants of *Coscinium fenestratum* after 45 days of incubation.

TDZ con (mgL-1)	% of callus formation	Fresh weight of callus (mg) mean ± SE	Dry weight of callus (mg) mean ± SE	Callus colour
0.00	-	-	-	-
0.05	70	410.05 ± 0.45	17.29 ± 0.29	Creamy yellow
0.1	65	726.85 ± 0.20	35.00 ± 0.17	Creamy yellow
0.2	90	1510.59 ± 0.18	75.00 ± 0.11	Yellow green
0.5	80	2329.21 ± 0.32	116.23 ± 0.11	Yellow green
0.75	95	2854.76 ± 0.30	142.85 ± 0.15	Yellow green
1.0	60	1375.67 ± 0.30	68.00 ± 0.20	Creamy yellow
1.5	55	966.55 ± 0.27	46.85 ± 0.20	Light yellow
2.0	50	512.37 ± 0.21	24.77 ± 0.13	Yellow brown
2.5	45	369.74 ± 0.28	17.37 ± 0.11	brownish
3.0	40	245.25 ± 0.55	11.33 ± 0.10	Brownish black

Table 2. Effect of different concentrations of TDZ on induction of callus grown on WPM medium from mature leaf explants of *Coscinium fenestratum* after 45 days of incubation.

TDZ con (mgL-1)	% of callus formation	Fresh weight of callus (mg) mean ± SE	Dry weight of callus (mg) mean ± SE	Callus colour
0.00	-	-	-	-
0.05	55	323.00 ± 0.29	14.79 ± 0.14	Creamy yellow
0.1	60	645.75 ± 0.46	31.56 ± 0.15	Creamy yellow
0.2	65	1423.95 ± 0.47	70.87 ± 0.13	Creamy yellow
0.5	55	2246.27 ± 0.72	112.70 ± 0.15	Creamy yellow
0.75	80	2762.65 ± 0.58	136.15 ± 0.19	Creamy yellow
1.0	50	1283.40 ± 0.38	63.20 ± 0.15	Creamy yellow
1.5	50	886.07 ± 0.91	44.10 ± 0.15	Light yellow
2.0	40	425.29 ± 0.64	20.25 ±0.14	Yellow brown
2.5	35	285.81 ± 0.58	13.29 ± 0.14	brownish
3.0	25	168.47 ± 0.61	7.53 ± 0.13	Brownish black

Table 3. Effect of different concentrations of TDZ on induction of callus grown on B5 medium from mature leaf explants of *Coscinium fenestratum* after 45 days of incubation.

TDZ con (mgL-1)	% of callus formation	Fresh weight of callus (mg) mean ± SE	Dry weight of callus (mg) mean ± SE	Callus colour
0.00	-	-	-	-
0.05	50	211.60 ± 0.37	9.03 ± 0.26	Creamy yellow
0.1	50	504.27 ± 0.38	23.87 ± 0.16	Creamy yellow
0.2	60	1304.75 ± 0.54	64.94 ± 0.15	Yellow green
0.5	50	2105.37 ± 0.53	105.87 ± 0.16	Yellow green
0.75	60	2616.19 ± 21	130.94 ± 0.13	Yellow green
1.0	40	1151.83 ± 0.83	56.63 ± 0.25	Creamy yellow
1.5	35	766.43 ± 0.47	36.86 ± 0.19	Light yellow
2.0	40	314.04 ± 0.92	15.25 ± 0.14	Yellow brown
2.5	30	159.50 ± 0.66	7.50 ± 0.12	brownish
3.0	25	99.33 ± 0.73	4.60 ± 0.13	Brownish black

Table 4. Effect of BAP on Adventitious Shoot Bud differentiation of *Coscinium fenestratum*

BAP con (mgL-1)	Shoot bud differentiation %	Number of shoot bud per culture mean ± SE	Callus growth pattern
0.00	-	-	Greenish, compact
0.05	40	3.38 ± 0.1	Yellowish green
0.1	50	5.33 ± 0.14	Creamish yellow; fasicled buds
0.25	75	6.71 ± 0.12	Brownish calli; fasicled and vigorous buds
0.5	60	4.39 ± 0.11	Yellowish green fasicled buds
0.75	-	-	Greenish, compact
1.0	-	-	Greenish, compact

who observed a similar phenomenon in cell suspension cultures of *Rollinia mucosa* with 2,4-D, suggested that the PGR itself could be involved in the formation of phenols. In the present study, frequent subculture of the calli to the fresh medium for every five days was found to be effective in reducing the browning and maintainence of the callus. Similar results was reported by (prakash *et al*.,1999). This practice was used to control the blackening of the cultures in a considerable number of species such as *Euphorbia lathyris* (Ripley and Preece 1986) and *Pisonia alba* (Jagadish *et al*., 1999).

Regeneration of adventitious shoot bud from callus

The leaf callus maintained on medium with 0.2 mg/l TDZ when subcultured onto MS medium without PGRs degenerated slowly and did not promote any further growth even after 5 weeks of incubation, but when subcultured onto MS medium supplemented with different concentrations of BAP alone,

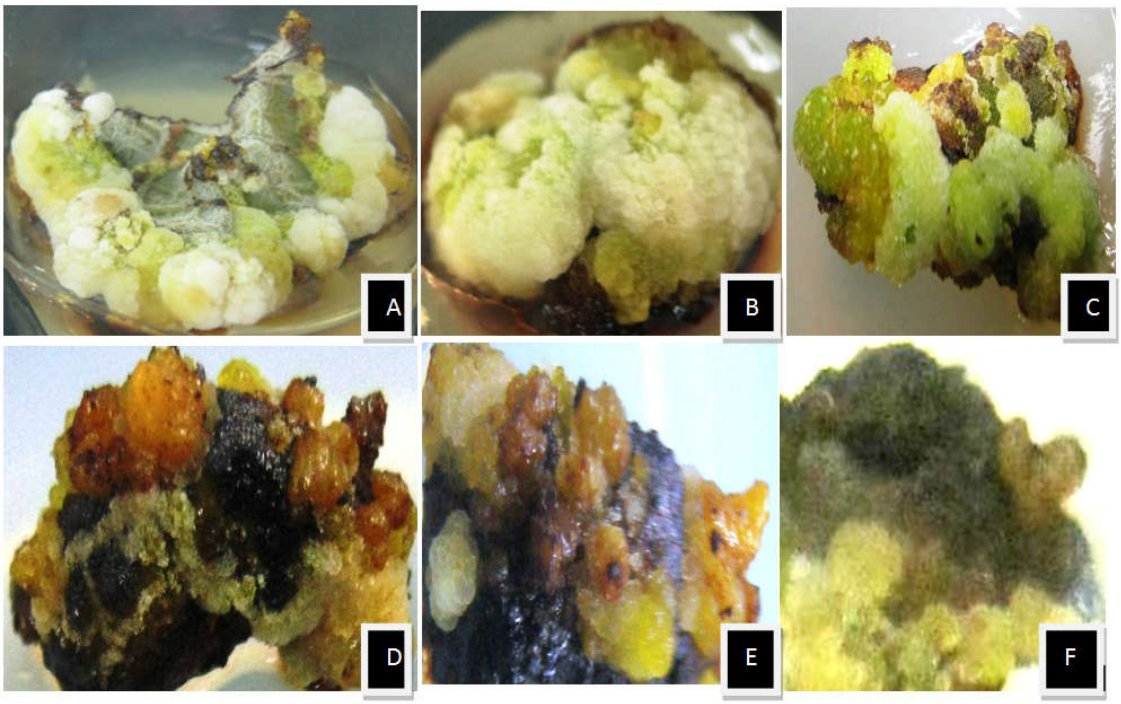

Fig. 1. Thidiazuron induced callogenesis in Coscinium fenestratum (Gaertn.) Colebr., A – initiation of callus from mature leaf explants after 12 days of culture on MS medium containing 0.75mg/l TDZ; B –proliferation of callus after 3 weeks; C –organogenic calli showing green points; D&E – adventitious shoot bud induction in medium containing 0.25 mg/l BAP; F-Prominent tiny shoot primordia visible.

responded for shoot bud regeneration. BAP at 0.25 mg/l induced highest number of adventitious shoot buds (6.71 ± 0.12) per calli. The regenerative callus first turned dark green and nodular, and then these nodular structures further developed into well organized small shoot buds. The developed adventitious shoot buds were chocolate brown in colour (FIG.1D). De novo shoot buds differentiated from the surface of callus with distinct tiny leaf primordia (Fig. 1F) within 3 weeks. However, these structures did not differentiate any further into leafy shoots. The cytokinin BAP promotes cell division, shoot multiplication and axillary bud formation while inhibiting root development (Sutter 1996).The effectiveness of BA on multiple shoot induction has already been reported in other species (Ebida and Hu 1993; Singh and Shukla 2001; Sobhakumari and Lalithakumari 2003).

The fresh as well as sub-cultured calluses derived from leaf explants cultured on all the media except MS medium with BAP, when transferred onto the medium with different levels of (KIN) Kinetin and TDZ, alone or together with auxins IAA or NAA, did not show shoot bud regeneration. Addition of TDZ, KIN and NAA (0.05 to 2.0 mg/l) to medium containing 0.25 mg/l BAP completely inhibited shoot regeneration (data not presented). The absence of shoot development from callus placed on regeneration media containing TDZ (data not shown) may be due to carry-over effects of the relatively high concentration of TDZ used during callus induction (Mohamed *et al*., 1992). (Angelini and Allavena 1989) found an increase in the regeneration frequency of *P. coccineus* on medium containing BA and *N*-(3-methyl-2-butenyl)-1 *H*- purin- 6-amine (2iP), suggesting beneficial interaction between the different cytokinins. Several attempts to elongate shoot buds have been unsuccessful. Plant regeneration via shoot organogenesis is severely limited due to inefficient development of induced buds into whole plants (Szasz *et al*., 1995). Our observations indicated that *Coscinium fenestartum*, in general, was recalcitrant in regard to shoot bud organogenesis. This may, in part, explain why in vitro regeneration via organogenesis in *Coscinium* has not been previously reported. Recalcitrance occurs when plant cells, tissues or organs do not respond to in vitro manipulations. A myriad of factors trigger recalcitrant responses in woody plant species, namely, whole plant physiology of the donor, in vitro manipulations and in vitro plant stress physiology. Correct manipulation of the in vitro environment can reduce or overcome the problem of recalcitrance (Benson 2000).

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REFERENCES

Angelini R. R, Allavena A (1989) Plant regeneration from immature cotyledon explant cultures of bean (*P. coccineus* L.). Plant Cell Tissue Organ Cult 19:167-174.

Anitha S, Suresh GS, Ramaiah M, Vaidya VP (2011) Extraction, Isolation and Wound Healing Activity of Flavonoid from *Coscinium fenestratum*. Res J Pharmaceutical Biological Chemical Sciences 2(3):1090-1095.

Arshad M, Silvestre J, Merlina G, Dumat C, Pinelli E, Kallerhoff J (2011) Thidiazuron-induced shoot organogenesis from mature leaf explants of scented *Pelargonium capitatum* cultivars. Plant Cell Tissue Organ Cult DOI 10.1007/s11240-011-0045-1.

Benson EE (2000) *In vitro* recalcitrance: an introduction. In Vitro Cell Dev Biol Plant 36:141–148.

Birdsall TC, Kelly GS (1997) Berberine, Therapeutic potential of an alkaloid found in several medicinal plants. *Altern Med Rev* 2:94-103.

Chen H. R, Galston AW (1967) Growth and development of pelargonium pith cells in vitro.II. initiation of organized development. Physiol plant 20:533-539.

Figueiredo SFL, Simoes C, Albarello N *et al* (2000) *Rollinia mucosa* cell suspension cultures: establishment and growth conditions. Plant Cell Tissue Organ Cult 63:85–92.

Gamborg OL, Millar RA, Ojima K (1966) Nutrient experiment of suspension cultures of soybean root cells. Expt. Cell Res. 50: 151-158.

Gill R, Gerrath JM, Saxena PK (1993) High-frequeney direct embryogenesis in thin layer cultures of hybrid seed geranium *(Pelorgonium × hortorum).* Can. J. Bot 71:408-413.

Gill R, Saxena, R K (1993) Somatie embryogenesis in *Nicotiana tabacum:* induction by thidiazuron of direct embryo differentiation from leaf disc. Plant Cell Rep 12:154-159.

Grimes H. D, Hodges T. K (1990) The inorganic NO_3^- : NH_4^+ ratio influences plant regeneration and auxin sensibility in primary callus derived from immature embryos of indica rice (*Oryza sativa* L.). J. Plant Physiol 136: 362-367.

Huang LC, Lee YL, Huang BL, Kuo C, Shaw JE (2002). High polyphenol oxidase activity and low titratable acidity in browning bamboo tissue culture. In Vitro Cell Dev Biol Plant 38:358–365.

Huetteman C. A, Preece JE (1993) Thidiazuron-A potent cytokinin for woody plant-tissue culture. Plant Cell Tissue Org Cult 33(2): 105- 119.

Jagadish Chandra KS, Rachappaji S, Gowda KRD, Thara Saraswathi KJ (1999) In vitro propagation of *Pisonia alba* (Linn.) Sapanogae (Lettuce Tree), A threatened species. Phytomorphol 49: 43-47.

Javasinghe U.L, Kumarihamy BM, Bandara AG, Waiblinger J, Kraus W (2003) Antifeeding activity of some Sri Lankan plants. Nat Prod Res 17 (1): 5–8.

Jones MPA, Cao J, O'Brien R, Murch SJ, Saxena PK (2007) The mode of action of thidiazuron: auxins, indoleamines, and ion channels in the regeneration of *Echinacea purpurea* L. Plant Cell Rep 26(9): 1481- 1490.

Karami O, Piri K, Bahmani R (2009) Plant regeneration through callus cultures derived from immature-cotyledon explants of oleaster (*Elaeagnus angustifolia* L.) Trees 23:335-338.

Keawpradub S (1992) The Alkaloids from the Stems of *Coscinium fenestratum* (Gaertn.) Colebr.; Master's thesis Graduate School, Chulalongkorn University.

Khan T, Krupadanam D, Anwar SY (2008) The role of phytohormone on the production of berberine in the calli cultures of an endangered medicinal plant, turmeric (*Coscinium fenestratum* I.). African J Biotechnology 7(18): 3244-3246.

Kritikar K. R, Basu B.D (1935) Indian Medicinal plants.Vol I, M/s. Bishen Singh Mahendrapal Singh, New Connaught place, Dehradun, pp 84-86.

Lloyd G. B, McCown BH (1980) Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. Proc Int Plant Propagators Soc 30:421-437.

Malhotra S, Taneja S. C, Dhar KL (1989) Minor alkaloid from *Coscinium fenestratum*. Phytochem 28: 1998-1999.

Mohamed M. F, Read PE, Coyne DP (1992) Dark preconditioning, CPPU, and thidiazuron promote shoot organogenesis on seedling node explants of common and faba beans. J Am Soc Hortic Sci 117:668-672.

Mohanan N, M Sivadasan (2002) Flora of Agasthyamala. Bishen Singh Mahendra Pal Singh Publishers, Dehra Dun, India. p. 65.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15(3): 473-497.

Murthy BNS, Saxena PK (1998) Somatic embryogenesis and plant regeneration of Neem *(Azadirachta indica* A. Juss). Plant Cell Rep 17:469-475.

Murthy BNS, Murch SJ, Saxena PK (1998) Thidiazuron: A potent regulator of *in vitro* plant morphogenesis. *In Vitro Cell Dev Pl* 34: 267-275.

Nair AJ, Sudhakaran PR, Madhusudana JR, Ramakrishna SV, (1992) Berberine synthesis by callus and cell suspension cultures of *Coscinium fenestratum*. Plant Cell Tissue org cult 29 : 7-10.

Nair GM, Narasimhan S, Shiburaj S, Abraham TK (2005) Antibacterial effects of *Coscinium fenestratum*. Fitoterapia 76: 585–587.

Nambiar VPK, Warrier PK, Ganapathy PM (2000) Some Important Medicinal Plants of the Western Ghats, India: A profile. AVS Publications, IDRC, Artstock, New Delhi, India, pp 105-120.

Narasimhan S, Nair GM (2004) Effect of auxins on berberine synthesis in cell suspension culture of *Coscinium fenestratum* (Gaertn.) Colebr- A critically endangered medicinal liana of Western Ghats. Indian J Exp Biol 42: 616-619.

Narasimhan S, Nair GM (2005) Cytotoxic effect of *Coscinium fenestratum* (Gaertn.) Colebr. and its active principle berberine on L929 cells. Med. Chem. Res 14: 118-124.

Pinho PMM, Pinto MMM, Kijjoa A, Pharadai K, Diaz JG, Herz W (1992) Protoberberine alkaloids from *Coscinium fenestratum*. Phytochemistry 31: 1403–1407.

Prakash E, Sha Vallikhan PS, Sairam Reddy P, Rao KR (1999) Regeneration of plants from seed-derived callus of *Hybanthus enneaspermus* (L) Muell., a rare ethnbotanical herb. Plant Cell Rep 18(10): 873-878.

Ravikumar K, Ved DK (2000) Hundred red listed medicinal plants of conservation concern in South India. 1st ed. Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore, India, pp 99-102.

Ripley KP, Preece JE (1986) Micropropagation of *Euphorbia lathyris* L. Plant Cell Tissue and Org Cult 5: 213-218.

Sahai A, Shahzad A, Anis M (2010) High frequency plant production via shoot organogenesis and somatic embryogenesis from callus in *Tylophora indica*, an endangered plant species. Turk J Bot 34(1): 11-20.

Shirwaikar A, Rajendran K, Punitha ISR (2005) Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin nicotinamide induced type 2 diabetic rats. J Ethnopharmacol 97: 369–375.

Shirwaikar A, Shirwaikar A, Punitha ISR (2007) Antioxidant studies on the methanol stem extract of *Coscinium fenestratum*. J Nat Prod Sci 13(1): 40–45.

Siwon J, Verpoorte R, Van Essen GFA, Svendsen AB (1980) Studies on Indonesian Medicinal Plants III. The Alkaloids of *Coscinium fenestratum*. Planta Med 38: 24-32.

Sudharshan S. J, Prashith Kekuda TR, Sujatha ML (2010) Antiinflammatory activity of *Curcuma aromatic* Salisb and *Coscinium fenestratum* Colebr: A comparative study. J Pharm Res 3(1): 24-25.

Sujatha R, Luckin CB, Nazeem PA (2003) Histology of organogenesis from callus cultures of Black pepper (*piper nigrum* L.) J Trop Agri 41: 16-19.

Sutter EG (1996) General laboratory requirements, media and sterilization methods. In: Trigiano, R.N., Gray, D.J. (Eds.), Plant Tissue Culture Concepts and Laboratory Exercises. CRC Press, New York, pp 11-25.

Szasz A, Nervo G, Miklos F (1995) Screening for *in vitro* shoot-forming capacity of seedling explants in bell pepper (*Capsicum annuum* L.) genotypes and efficient plant. regeneration using thidiazuron. Plant Cell Rep 14:666–669.

Tran Q. L, Tezuka Y, Ueda JY, Nguyen NT, Maruyama Y, Begum K, Kim HS, Wataya Y, Tran QK, Kadota S (2003) *In vitro* antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine. J Ethnopharmacol 86: 249–252.

Trolinder N. L, Goodin JR (1987) Somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep 6: 231-234.

Tushar K. V., Udayan PS (2005) Ex situ conservation of ayurvedic medicinal plants at Arya Vaidya Sala, Kottakkal. In Proceedings of XVIIth Kerala Science Congress, 29-31 January, Kerala Forest Research Institute (KFRI) Peechi, Thrissur, Kerala, India, pp: 311.

Venukumar MR, Latha MS (2004) Effect of *Coscinium fenestratum* on hepatotoxicity in rats. Indian J Exp Biol 42:792–797.

Walter K. S, Gillett HJ (ed.) (1997) IUCN Red List of Threatened Plants.- World Conservation Monitoring Centre, The World Conservation Union, Gland - Cambridge 1998.

Warrier PK, Nambiar VCK, Ramankutty C (1994) Indian medicinal plants: A compendium of 500 species. Vol I, Orient Logman; New Delhi, pp 47-53.

Wattanathorn J, Uabundit N, Itarat W, Mucimapura S, Laopatarakasem P, Sripanidkulchai B (2006) Neurotoxicity of *Coscinium fenestratum* stem, a medicinal plant used in traditional medicine. Food Chem.Toxicol 44: 1327-1333.

Wongcome T, Panthong A, Jesadanont S, Kanjanapothi D, Taesotikul T, Lertprasertsuke N (2007) Hypotensive effect and toxicology of the extract from *Coscinium fenestratum* (Gaertn.) Colebr. J Ethnopharmacol 111: 468-475.

Zouzou M, Kouadio YJ, Kone M, Kouakou TH, Denezon DO (2000) Callogenese chez *Gossypium hirsutum* L.: effets cultivar, conditions de culture et type de materiel. Biot Rev Int. Sci. Vie. Terre 1: 48-56.

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