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Studies on *in vitro* callus induction from a medicinal plant: *Pterocarpus marsupium*

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Abstract
Pterocarpus marsupium belongs to family Fabaceae and its role as anti-diabetic has been very well established. *Pterocarpus marsupium* extract also shows promising results in cataract and hypertriglyceridaemia. This plant also finds its use as cardiotonic and hepatoprotective agent. The effective protocol of *in vitro* callus induction would aid secondary metabolite and its commercial production. In the present study, callus culture was raised for *Pterocarpus marsupium* from *In vitro* raised leaves and cotyledonary explants which were taken from seedlings germinated on MS supplemented with auxin. The objective of the research is to induction of callus and maintains the callus culture. Results illustrated that leaves obtained from *in vitro* raised shoots were not responsive in any treatment and only the cotyledons showed callus induction. Callus induction was maximum 62.22% at 9.04µM 2,4- D.

Keywords: Callus culture, Pterocarpus marsupium, cotyledonary, auxin secondary metabolite

Introduction
Pterocarpus marsupium (Roxb.) is a deciduous tree, commonly called as Indian Kino tree or Malabar Kino, belonging to the family fabaceae. The bark exudes a red gummy substance called 'Gum Kino'. The gum Kino is externally applied to leucorrhoea (Rahman *et. al*, 20184) [19]. Gum Kino is used in the treatment of polyurea and inordinate night sweat and *Phthisis plumonalis*. Bark is useful in vitiated condition of *kapha* and *pitta*, elephantiasis, erysipelas, urethrorrhea, rectalgia, opthalmopathy, hemorrhages, dysentery, cough and grayness of hair. Aqueous infusions of the bark possess antidiabetic potential (Anonymous, 1968) [2]. Bark is useful in urinary discharge and piles. Its extract has been prepared using many methods like infusion, maceration, decoction and percolation. Several chemical constituents like pterostilbene, (-)-epicatechin, pterosupin, marsupsin, etc., have been identified and isolated, which has several medicinal properties (Dhayaney and Sibi, 2019) [9].

Materials and Method
Source of plant material and explant preparation
In vitro shoots and cotyledons (from *in vitro* raised seedlings) were used as explants material for callus induction. Leaves were dissected from these *in vitro* shoots and were inoculated on MS medium. Explants were inoculated in horizontal position on the medium and were pre injured (cuts were made) before they were inoculated on MS medium supplemented with auxins alone and in combination of cytokinin.

Basal nutrient medium for callus induction and proliferation
The explants were inoculated on MS medium with various concentrations of auxin for callus induction studies on MS medium with sucrose (3%) were used throughout the experiment. To find out the effect of auxin on induction of callus induction leaf base and cotyledons were cultured on semisolid MS medium supplemented with different concentration of 2,4-D (2.26, 4.52, 9.04 & 13.57 µM) or NAA (0.54, 1.34, 2.69 & 5.37 µM).
The effect of auxin - cytokinin interaction on callus induction leaf base and cotyledons were cultured on semisolid MS medium supplemented with different concentration of 2,4-D (2.26, 4.52 & 9.04 µM) alone and in combination with Kn (0.88, 2.22 µM) or BAP (0.92, 2.32 µM) were also studied.
Different sets of experiments were performed to study the proliferation of callus. To study the effect of plant growth regulators on callus supplemented with auxin 2,4-D alone or in

combination with cytokinins. The effect of plant growth regulators on proliferation of callus on MS medium supplemented with different concentration of auxin 2,4-D (2.26, 4.52,9.04, 13.57 µM) alone or in combination with Kn (0.88, 2.22 µM) were studied.

The pH of the medium was adjusted to 5.8 with 1N NaOH or 1N HCL and the medium were solidified with 0.8% agar. The medium was then sterilized by autoclaving for 20 min. at 121 ºC.

Culture conditions

The cultures were maintained at 26 ± 2 °C under 16 h light photoperiod with light intensity of 1600 lux, obtained by cool white fluorescent tubes of 40 watts [Philips, India].

Experimental design, data collection and statistical analysis

MS medium without hormone was treated as control in all experiments. All experiments were repeated three times. Observations were recorded after 4 weeks of interval. The results are expressed as mean ± SE of three experiments. The data was analyzed statistically using SPSS version 17 and significant difference between means were assessed by Duncan's multiple range test (DMRT) at P = 0.05.

Results and Discussion

Callus induction

Effect of plant growth regulators

Results illustrated that leaves obtained from *in vitro* raised shoots were not responsive in any treatment and only the cotyledons showed callus induction. Callus induction response was 35.56% at 2.26 µM 2, 4- D, which increased to 62.22% at 9.04µM 2, 4- D. Further increase of 2, 4- D a decreased response of (42%) callus induction was obtained at 13.57 µM 2, 4- D. Callus induction was maximum (62.66%) at 9.04µM,2,4- D. A similar promoting effect of 2, 4-D on callusing was earlier reported in *Dracaena sanderiana* species (Ilah *et al*., 2002) [13]. Additionally, in many other plants profuse callusing was observed on 2, 4-D added medium (Khan *et al*., 2002) [15].

Effect of Basal medium

Among the three basal media MS medium was found to be best for *in vitro* callus induction, as compared to B5 and White's media. In B5 medium, optimal callus induction (46.22%) was obtained and White's medium 48.89% callus induction was obtained. Maximum *in vitro* callus induction (62.22%) was obtained on MS medium supplemented with 9.04µM 2, 4- D.

Callus Proliferation

Among different combination of auxin 2, 4 –D supplemented with MS medium callus proliferation and its culture were maintained on MS medium supplemented with 9.04µM,2, 4-D. Cells obtained were irregular in shape and nodular or globular growth was observed. Few cells elongated and gave outgrowths.

Table 1: Effect of 2, 4-D in MS medium on callus induction of *Pterocarpus marsupium*. Data recorded after 4 weeks of incubation.

2,4-D (µM)	Callus Induction%
0.00	0.00 ±0.00^{c}
2.26	35.56 ±0.07^{b}
4.52	46.67 ±0.0.7^{ab}
9.04	62.22±0.07^{a}
13.57	42.22±0.07^{b}
df	4
F- value	12.22
P- value	0.00

Value within the columm with similar superscrits are not significantly different at p ≤ 0.05 level as determined using Duncun's multiple range test. A value represents ± standard error.

Table 2: Effect of 2, 4-D+ Kn in MS medium on callus induction from cotyledons of *Pterocarpus marsupium*. Data recorded after 4 weeks of incubation.

2-4,D (µM)	Kn (µM)	Callus Induction %	
0.00	0.00	0.00±0.00^{b}	
4.52	0.93	26.66 ±0.07^{a}	
4.52	2.32	31.11 ±0.07^{a}	
9.04	0.93	46.67 ±0.06^{a}	
9.04	2.32	37.78 ±0.06^{a}	
Analysis of variance			
PGR	df	F value	P value
2,4 –D	1	0.31	0.55
Kn	1	0.02	0.99
2,4- D* Kn	1	0.26	0.87

Value within the columm with similar superscrits are not significantly different at $p \leq 0.05$ level as determined using Duncun's multiple range test. A value represents ± standard error.

Table 3: Effect of basal medium on *in vitro* callus induction from cotyledons of *Pterocarpus marsupium.* Media supplemented with 9.04 µM 2-4, D. Data recorded after 4 weeks of incubation.

Basal medium	Callus Induction %
MS	62.22^{a}
B_5	48.89^{b}
White's	46.22^{b}
df	2
F value	9.08
P value	0.00

Value within the columm with similar superscrits are not significantly different at $p \leq 0.05$ level as determined using Duncun's multiple range test. A value represents ± standard error.

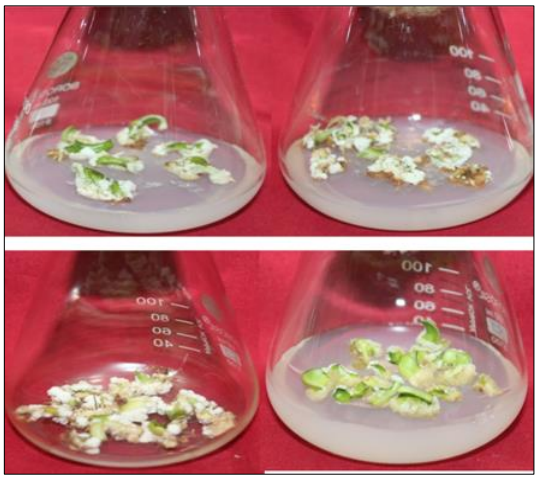

Fig 1: Effect of 2, 4-D in MS medium on callus induction of *Pterocarpus marsupium*. (A) 2.26µM (B) 4.52µM (C) 9.04µM (D) 13.57µM.

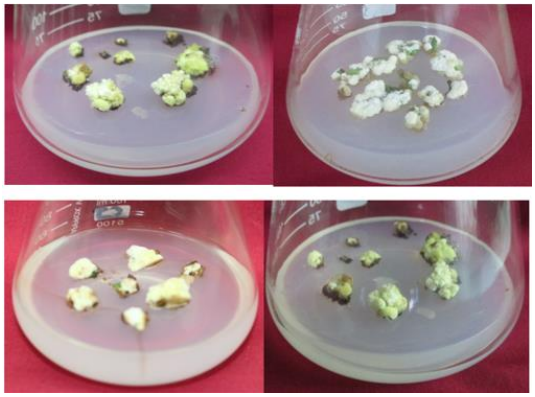

Fig 2: Effect of 2, 4-D in MS medium on callus induction of *Pterocarpus marsupium*. (A) 2.26µM (B) 4.52µM (C) 9.04µM (D) 13.57µM.

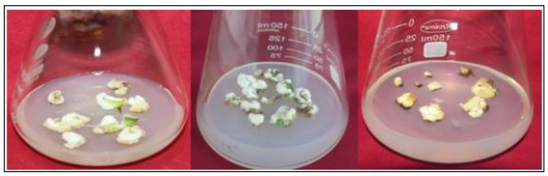

Fig 3: Effect of basal medium on *in vitro* callus induction of *Pterocarpus marsupium.* (A) MS (B) B_5 (C) White's.

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Conclusion
The good frequency of callus indicated potential of *Pterocarpus marsupium* for exploring secondary metabolite production useful in Pharmaceautical industry. Additionally, the establishment of callus culture was recommended as it could be a source of bioactive molecule. This finding is a starting point for further studies. Studies on callus culture can further be utilized for organogenesis, genetic manipulation and molecular studies of *Pterocarpus marsupium*. Further it would facilitate its use for future tree improvement programme.

References
1. Anis M, Husain MK, Shahzad A. *In vitro* plantlet regeneration of *Pterocarpus marsupium* Roxb., an endangered leguminous tree. Current Science. 2005;88:861-861.
2. Anonymous. The wealth of India: A dictionary of raw material and Industrial products. Council of scientific and Industrial Research, New Delhi. 1968;(7):303-305.
3. Anuradha M, Pullai T. *In vitro* seed culture and induction of enhanced axillary branching in *Pterocarpus santalium* and *Pterocarpus marsupium*: A Method for rapid multiplication. Phytomorphology. 1999;49:157-163.
4. Bari MA, Ferdaus KMKB, Hossain MJ. Callus induction and plantlet regeneration from *in vivo* nodal and internodal segments and shoot tip of *Dalbergia sissoo* Roxb. Journal of Biological Science. 2008;16:41-48.
5. Bunn E, Turner SR, Panaia M, Dixon KW. The contribution of *in vitro* biotechnology and cryogenic storage to conservation of indigenous plants. Australian Journal of Botany. 200;755:345-355.
6. Chand S, Singh AK. *In vitro* shoot regeneration of *Pterocarpus marsupium* Roxb., an endangerd Leguminous tree. *In vitro* Cellular & Developmental Biology- Plant. 2004;40:464-466.
7. Das T, Chatterjee A. *In vitro* studies of *Pterocarpus marsupium*- an endangered tree. Indian Journal of Plant Physiology. 1993;36:262-272.
8. Devgun M, Nanda A, Ansari SH. *Pterocarpus marsupium* Roxb. - A Comprehensive Review. Phramacognosy Review. 2009;3(6):359-363.
9. Dhayaney Vijayan, Sibi G. Pterocarpus Marsupium for the Treatment of Diabetes and Other Disorders Journal of Complementary Medicine & Alternative Healthcare, 2018, 9.
10. Gairola S, Gupta V, Singh B, Maithani M, Bansal P. Phtytochemistry and Pharmacological Activity of *Pterocarpus marsupium* - A review. International Research Journal of Pharmacy. 2010;1(1):100-104.
11. Husain MK, Anis M, Shahzad A. Somatic embryogenesis and plant regeneration in *Pterocarpus marsupium* Roxb. Trees. 2010;24:781-787.
12. Hussain A, Qarshi IA Nazir H, Ullah I. Plant Tissue Culture: Current Status and Opportunities. 2012, 2.
13. Ilah A, Junaid A, Mujib A. Biotechnology and future of an underused medicinal plant *Dracaena*. In: Khan, I. and Khanum, A. (Eds.). Role of Biotechnology in Medicinal and Aromatic Plants. Ukkaz Publication, Hyderabad, 2002, 192-206.
14. Khan MK, Misra P, Sharma T, Shukla PK, Ramteke PW. Effect of adenine sulphate on *in vitro* mass propagation of *Stevia rebaudiana* Bertoni. Journal of Medicinal Plant Research. 2014;8(13):543-549.
15. Khan MR, Rashid H, Quraishi A. Effect of various growth regulators on callus formation and regeneration in *Brassica napus* Cv. Oscar. Pakistan Journal of Biological Science. 2002;5(6):693-695.
16. Mankani KL, Krishna V, Manjunatha BK, Vidya SM, Singh SD, Manohara YN, *et al*. Evaluation of hepatoprotective activity of stem bark of *Pterocarpus marsupium.* Indian Journal of Pharmacology. 2005;37(3):165-168.
17. Patil UH, Gaikwad DK. Biochemical standardization of stem bark of *Pterocarpus marsupium* (Roxb.).

Pharmacophore. 2011;2(1):65-71.
18. Pua EC, Mehra-Palta A, Nagy F, Chua NH. Transgenic plants of *Brassica napus* L. Biotechnology. 1989;5:815-817.
19. Rahman MS, Mujahid M, Siddiqui MA, Rahman MA, Arif M, Eram S, *et al*. Ethnobotanical Uses, Phytochemistry and Pharmacological Activities of Pterocarpus marsupium: A Review. Pharmacognosy Journal. 2018;10:1-8.
20. Vinterhalter DV. *In vitro* propagation of green foliage *Dracaena fragrans* Ker- gwal. Plant Cell Tissue and Organ Culture. 1989;17:13-20.