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In vitro callus induction from a highly medicinal plant: *Pterocarpus marsupium* Roxb

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Abstract

The current study has focused on an efficient *in vitro* callus induction protocol for the *Pterocarpus marsupium* Roxb. plant. Explants from healthy plants, including leaves and stem nodes, were employed to induce callus formation. Murashige and Skoog medium supplemented with 2,4D resulted in the highest callus proliferation. Callus induction varies based on the type of explant and growth hormone concentration employed. Massive callus was achieved at 1.0 mg/L of 2, 4 D and BAP each utilizing a leaf explant.

Keywords: *Pterocarpus marsupium*; Micropropagation; *In vitro*; MS medium; Callus culture

1. Introduction

Pterocarpus marsupium Roxb. is a deciduous tree from the Fabaceae family, also known as the Beejasar, Indian Kino tree, Malabar Kino or 'Gum Kino' [1]. People commonly use it to treat diabetes, dysentery, stomachache, elephantiasis, leucoderma, cholera, urinary problems, and cough. The stem's gum is used as an astringent, in diarrhoea, and for toothache, while the leaves are used externally for boils, ulcers, and skin problems [2]. *P. marsupium* is naturally propagated through seeds. However, the germination rate is low (30%), and propagation through stem cutting is challenging. Due to poor propagation and overexploitation for pharmaceuticals and timber, *P. marsupium* has become threatened. *Pterocarpus marsupium* was most recently listed on The IUCN Red List of Threatened Species in 2017. It is classified as Near Threatened under the C1 category [3]. Therefore, *in vitro* procedures have become essential for conservation efforts [4]. Plant tissue culture is the technique of isolating small sections of a plant's living meristematic tissues (explants) and growing them aseptically on a semi-defined or defined nutritional medium. It is defined broadly as the numerous culture methods of plant organs and tissues that permit experimental approaches with the large goal of developmental biology and crop modification [5]. This study uses various explants to efficiently and quickly propagate and induce callus in threatened *P. marsupium*.

2. Material and methods

The explant-like leaf, stem, and node were collected from the mother plant located CIDCO, Chh. Sambhajinagar (Lat 20.011172^0 Long 75.392406^0). All of the explants were washed with running tap water for 5 minutes, then 70% ethanol for 2 minutes, and finally distilled water for 5 minutes. Surface sterilization of explants was performed by washing with sterile distilled water for 5 minutes, followed by different concentrations of mercuric chloride ($HgCl_2$), with leaf explants sterilized with 0.2% and stem nodes sterilized with 0.2% $HgCl_2$. Following that, two further rinses in laminar airflow with sterilized double-distilled water were performed. All of these explants were chopped into little pieces and inoculated on the appropriate medium. All experiments in this work were performed on MS media supplemented with varied amounts of growth regulators. The culture media was supplemented with 40 gm sucrose and 2.5 to 3 gm clerigar for solidification, and the pH was adjusted to 5.6-5.8. The medium was steam sterilized in an autoclave set to 15 psi and

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121 °C. After the inoculation, culture bottles were shifted to a culture room with a temperature of 25±2°C and a 16-hour photoperiod provided by cool white fluorescent cool tubes.

3. Results and discussion

Standard protocol for surface sterilization of explant was analyzed by trial and error method. Surface sterilization of leaf and stem node explant were tried with 0.1-0.5% of $HgCl_2$ for 2 to 3 minutes duration. The maximum microbe's free cultures and high regeneration percentage were recorded at 0.3% of $HgCl_2$ for leaf and stem node explant during the present study. All growth regulator combinations were shown to be more or less effective at inducing callus. It has been found that the optimal concentration of 2, 4-D has the capacity to generate profuse callus. Callus formed with these concentrations were pale to greenish in shade and friable to compact in texture. Maximum rate of callus induction was recorded on 1.0 mg/L of 2, 4-D using leaf and node as explant, 1.0 mg/L of 2, 4 D and 1.5 mg/L of KIN using leaf and node explant. However lower concentration of growth regulators was found less effective for induction of callus or poor type of callus induction was achieved. These induced calluses were sub-cultured on MS medium with different concentrations of hormones to achieve micropropagation. 2,4-D along with BAP having concentration 1.0 mg/L each shows promising results among different concentrations of other PGR (Table 1 and Fig 1).

Table 1 Impact of different concentrations of plant growth regulators on callus formation

Concentration of plant growth regulators (PGRs) (mg/L)		**Explant used**			
		Leaf		**Nodal segment**	
		Frequency of callus Induction	**Texture of callus**	**Frequency of callus Induction**	**Texture of callus**
2,4-D	0.5	+	Swelling of explant	+	Swelling of explant
	1.0	+++	Friable, Greenish	+++	Friable, Greenish
	1.5	++	Friable, Yellowish	+++	Friable, Yellowish
	2.0	++	Friable, Yellowish	++	Friable, Yellowish
	2.5	+	Compact, Whitish	++	Compact, Whitish
	3.0	--	--	--	--
2,4-D + KIN	1.0 + 0.5	++	Friable, Yellowish	+	Friable, Yellowish
	1.0 + 1.0	++	Friable, Greenish and white	++	Friable, Light Green
	1.0 + 1.5	+++	Friable, Greenish	+++	Friable, Greenish
	1.0 + 2.0	++	Friable, Yellowish	++	Friable, Yellowish
	1.0 + 2.5	++	Friable, Yellowish	+	Friable, Yellowish
	1.0 + 3.0	--	--	--	--
2,4-D + BAP	1.0 + 0.5	++	Friable, Greenish and white	++	Friable, Greenish and white
	1.0 + 1.0	+++	Friable, Greenish	+++	Friable, Greenish
	1.0 + 1.5	++	Friable, Yellowish	++	Friable, Yellowish
	1.0 + 2.0	++	Friable, Yellowish	++	Friable, Yellowish
	1.0 + 2.5	+	Brown	++	Compact, Brown
	1.0 + 3.0	--	--	--	--

--No Callus, +Poor Callus, ++Moderate Callus, +++Massive Callus, mean calculated by three separate experiment with five replicates.

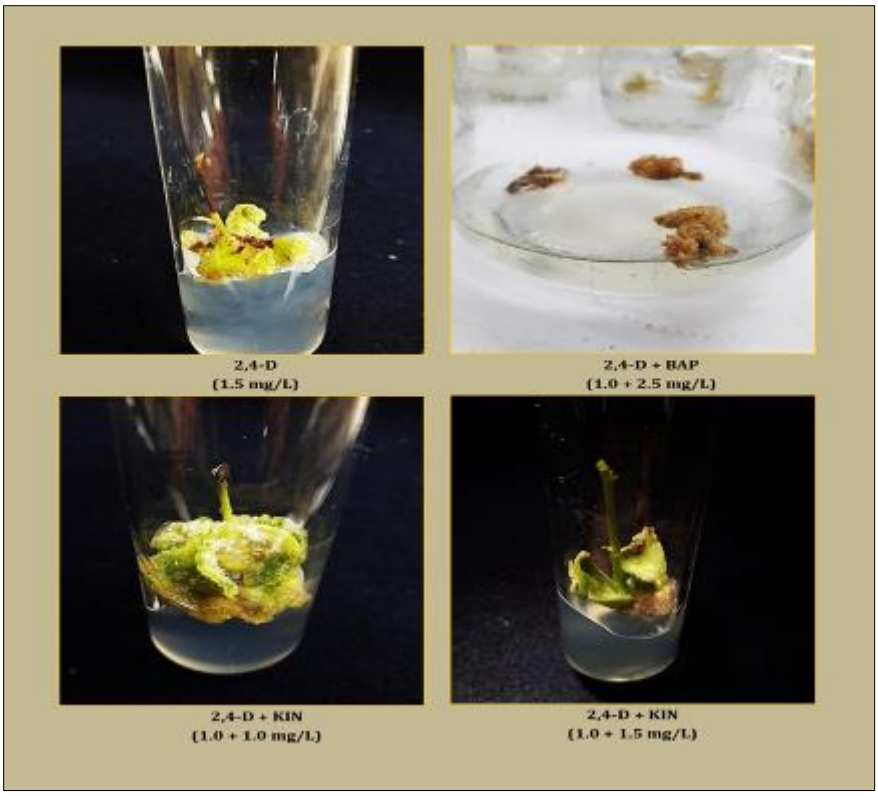

Figure 1 Impact of different combinations of 2,4-D, KIN and BAP on callus formation

4. Conclusion

People actively collect medicinal herbs to treat a variety of ailments. These plants function as bioreactors. If these plants were propagated using current methods such as tissue culture, the raw material could be used for therapeutic purposes. The current technique is useful for producing callus and extracting secondary metabolites as well. Present research work was carried out to standardize the protocol for *in vitro* regeneration and callus induction of *P. marsupium.* 2,4-D along with BAP having concentration 1.0 mg/L each shows promising results among different concentrations of other PGR.

Compliance with ethical standards

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Disclosure of conflict of interest

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