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In vitro Callus Development in *Pterospermum reticulatum* Wight and Arn. -A Rare and Threatened Plant

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Pterospermum reticulatum is a rare and threatened medicinally important plant belonging to the family Sterculiaceae. The present work was carried out to develop a standard method for callus induction from the nodal explant. The air dried stem powder of the plant was extracted with solvents of varied polarity like petroleum ether, benzene, chloroform, methanol and distilled water. The results showed that the plant contains chemical compounds like steroids, triterpenes, phenolic groups, saponin, tannin, sugar, catachin, amino acids and reducing sugars. Maximum callusing was obtained in the explant inoculated in MS medium supplemented with Bap 1.5 mg/l in combination with NAA 0.2 mg/l. *In vitro* generated callus can be used as a source for the isolation of secondary metabolites from the plant.

Pterospermum reticulatum / Phytochemistry

***P**terospermum reticulatum* Wight and Arn. (Sterculiaceae) is reported to be a rare and threatened species (Shetty and Kaveriappa, 2001, Nayar & Sastry 1990, Oldfield *et al.*, 1998). It comes under the IUCN red data book as vulnerable (IUCN, 2000). It is commonly known as Malavuram in Malayalam and in Tamil Muli polavu or Thopuli. *Pterospermum reticulatum* was used by the tribal peoples of Kodayar to treat ulcer, wounds, inflammation etc. Various phytochemicals present in the plant are responsible for the medicinal activity of the plant. The chemical constituents in medicinal plant usually explain the use of the plants to prepare drugs (Farnsworth, 1984).

The plant is a tree, up to 25 m tall. Leaves elliptic-obovate, up to 12 x 9 cm, cuneate to obliquely subcordate at base, acuminate at apex, entire or coarsely toothed near apex, underside covered with cream-coloured mealy tomentum dotted with darker minute stellate hairs, 3-nerved at base. Flowers 1-3, axillary, ca 3 cm across, yellowish. Bracteoles laceriate. Sepals 5, linear - lanceolate, connate at base, rusty stillate-hairy outside. Petals 5, obovate - oblong, recurved. Stamens 15 in 5 groups of 3 between the 5 filiform staminodes; connective produced into a terminal point. Ovary inserted on top of the staminal column, 5-locular. Capsules ovoid or oblong, 5-7 x 3 cm, obtusely 5 - angled, stightly contracted at base, stellate-pubescent, loculicidally 5-valved. Seeds 4 in each locule, winged above. Correct botanical identity based on the external morphology is possible when a complete plant specimen is available. Anatomical characters can also help in the identification when morphological features are indistinct (David *et al*., 2008).

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This species is under threat due to their exploitation from natural habitat by Tribals, residential and commercial development, housing and urban areas, commercial and industrial areas, biological resource use, logging and wood harvesting. This tree being medicinally important needs conservation and propagation both *in vivo* and *in vitro*. Since there are no reports on *in vitro* propagation, the present work is based on to develop an efficient protocol for the *in vitro* production of callus from stem explants of the plant. The tissue culture technique is a best approach for *in vitro* propagation and assures the availability of callus as a source of secondary metabolites for pharmaceutical uses. In consideration of the role that callus plays in medicinal purposes, as well as to estimate the potential of the usage of callus to extract secondary metabolites the present study was undertaken to develop a standard *in vitro* technology for callus formation.

Materials and Methods

In vitro Callus Development

Pterospermum reticulatum plant were collected from the hills of Upper Kodayar, Kanyakumari district, Tamilnadu, South India and grown in green house condition. The nodal part of the plant was used as the explant. The fresh plant materials were collected from the green house and washed with running tap water for 30 minutes and were soaked in an aqueous solution containing 0.2 % bavistin for 15 minutes. This was followed by gentle wash in running tap water for 5 minutes. Then the explants were immersed in aqueous solutions of tween 20 for 10 minutes and were shaken regularly. Then the explants were washed thoroughly with running tap water for 5 minutes. After this treatment, the explants were sterilized with 0.1% Mercuric chloride aqueous solution for 7 minutes within the chamber. Then the explants were removed from the sterilizing solution and rinsed thoroughly thrice with sterile double distilled water to remove the traces of $HgCl_2$ and inoculated in Murashige and Skoog, (1962) medium fortified with 3% sucrose. The pH of medium was adjusted 5.8 and solidified with 0.5% agar. The medium was then autoclaved at 121°C for 20 minutes. The cultures were maintained under sterile control conditions at 25 ± 1°C with a photoperiod of 16 h light and 8 h darkness at 2000 lux light intensity of cool white fluorescent light.

Data Collection

Data were taken after 5-45 days by visual observation of the culture. At the end of the observation period the percentage of response, the day of callus initiation and the nature as well as the color of the callus to different concentrations of plant growth regulators were recorded.

Phytochemical Studies

Mature and healthy stem part of the plant was collected and dried at room temperature for about one month. The dried plants were ground to powder. 5 grams of the powdered plant was put in to a bottle and shaken with a mechanical shaker for 12 hours. Then filtered with Whatman No. 1 filter paper to obtain petroleum ether, benzene, chloroform, methanol and distilled water extracts. The qualitative phytochemical analysis was carried out on the extracts to determine the presence or absence of reducing sugar, protein, phenolic groups, alkaloids, steroid, triterpene, flavone, catachin, tannin and anthraquinone (Brindha *et al.*, 1981).

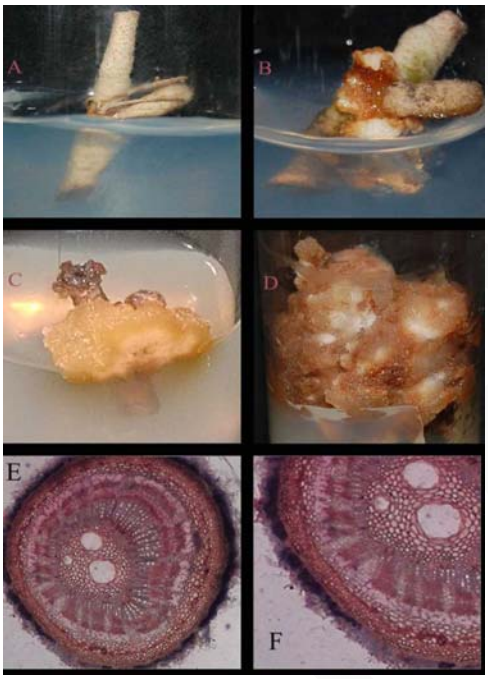
Microscopic (Anatomical) Studies

Fresh plant of *P. reticulatum* was collected and fixed in FAA (Formalin Acetic acid and alcohol mixtures). Free hand section of stem, leaf and root were taken and kept in 70% ethanol. The sections were stained with saffranin and mounted according to the methods described by (Johansen, 1940). The photomicrographs were taken using Motic digital camera, and Phase Contrast Microscope, Japan.

Results and discussion

Surface sterilized nodal explants cultured on MS medium supplemented with different combinations of BAP and NAA showed callusing from nodal part (Fig-2:A-C). It was observed that nodal explants cultured on 1.5 mg/l BAP+0.2 mg/l NAA showed highest response and the maximum callusing was obtained in this concentration after one month from the date of inoculation. Similar results was reported in *Stevia rebaudiana* where also

maximum callus production was obtained from the nodal explants culture in MS medium with 13.56 µM 2,4-D (Uddin *et al.,* 2006). But the rate of callus production was decreased at lower and higher concentrations. The nodal explants cultured on higher conc. of BAP 2.5mg/l + NAA 0.3 mg/l showed only 10% callusing after six weeks of culture initiation. The callus obtained from the nodal part was brown and soft. (Table-1, Fig-2:C-D). Explants inoculated in kinetin, TDZ, IAA or IBA have no any response. Explants inoculated in other mediums such as ½ MS, woody plant medium and nistch medium also have no response.



A-Explant, B-Callus Initiation, C-More Callus, D-Maximum Callus, E- Mature Stem Transverse Section, F-Stem Anatomy-a Portion Enlarged.

Plate-1: Callus induction and anatomy of the stem part of *P. reticualtum.*

Phytochemical screening of the stem part of the plant showed the presence of secondary metabolites like steroids, triterpenes, phenolic groups, flavone groups, saponin, tannin, sugar, catachin, amino acids and reducing sugars. Alkaloids and anthraquinones are absent in all the extracts. (Table-2). Among all the extracts used most of the compounds are separated in the methanol extract. Phenolic compound and sugar are found in all the solvents tested except petroleum ether. The presence of secondary metabolites suggests that the plant might have some medicinal properties. For instance, the presence of flavonoids suggest that the plant might have an anti-oxidant, antiallergic, anti-inflammatory, anti- microbial, anticancer activity (Kunle and Egharevba, 2009). The presence of tannins shows that the plant is astringent as documented and suggests that it might have anti-viral and anti-bacterial activities and can aid in wound healing and burns (Haslem, 1989).

The anatomical feature of a plant is important for its correct identification. Transverse section of the stem appears as a complete circle. (Fig-1:E). The outer region is the bark covered with tannins. The epidermis consists of a double layered barrel shaped cells, followed by the hypodermis which consists of few layers of collenchyma cells forming a continuous layer. Next to the hypodermis a few layers of chlorenchyma cells with conspicuous intercellular spaces present. Inner to the hypodermis consists of 8-10 layers of thin walled parenchyma cells. The innermost layer of the cortex consists of a single layer of barrel shaped cells known as starch sheath and is wavy in outline. The vascular bundles are collateral and are arranged in the form of a ring. Phloem lies towards the peripheral region. A layer of fascicular cambium is present in between the xylem and phloem whose cells are rectangular. Xylem is endarch. Few layers of parenchyma cells form the pith at the centre. Pith consists of three large central vacuoles. (Fig-1:E-F).

Conclusion

The plant *Pterospermum reticulatum* is a rare and threatened plant. The stem part of the plant was used by the tribal peoples of Kodayar to treat ulcer, wounds, inflammation etc. No any reports were found in literature regarding the chemical constituents of the plant and *in vitro* callus production. Although the plant is under threatened category, it must require a standard method for conservation and high yield of similar secondary metabolites as in the natural plant. Above all in view and consideration the current work was undertaken to produce a standard procedure for the *in vitro* induction of callus.

Table-1: Effect of Plant Growth Regulators for Callus Induction in *P.reticulatum*

Sl.No	Explant used	Growth regulators		Degree of Callusing	% of response	Callus initiation in days	Type of callus	Colour of callus
		BAP	NAA					
1	NODE	0.5	0.1	Good	20	6	Friable soft	Brown
2		1.0	0.1	Good	25	6	Friable soft	Brown
3		1.5	0.2	Maximum	50	4	Friable soft	Brown
4		2.0	0.2	Good	20	6	Friable soft	Brown
5		2.5	0.3	Good	10	7	Friable soft	Brown

Table-2: Preliminary Phytochemical Screening of the Stem part of *P.reticulatum*

Sl. No	Solvent	Steroids	Triterpenes	Alkaloids	Phenolic groups	Flavone groups	Saponin	Tannin	Anthroquinone	Sugar	Catachin	Amino acids	Reducing sugar
1	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-
2	Benzene	-	-	-	+	-	-	-	-	+	-	-	-
3	Chloroform	+	+	-	+	+	-	-	-	+	-	-	+
4	Methanol	+	+	-	+	+	+	+	-	+	+	+	+
5	Distilled water	-	+	-	+	+	-	-	-	+	-	+	-

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