

In vitro propagation of *Pterocarpus marsupium* Roxb.: An endangered medicinal tree

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Nodal segments of *Pterocarpus marsupium* Roxb. were inoculated on seven different media compositions, viz. MS, B5 and White's without growth hormones (MS₀₀, B5₀₀ and WH₀₀), each supplemented with 3.0 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA (MSBN, B5BN, WHBN) and MS media supplemented with 0.2 mg l⁻¹ IBA (MSIB). Seed germination improved in all the media studied, however, MS combinations were the best (95-100%). Maximum number of shoot induction per explant was in MS₀₀ (3.25) followed by MSIB (2.26). Maximum nodes per shootlet were observed in medium MSIB (4.95), while shoot length was maximum in MSIB (2.92 cm) followed by MS₀₀ (2.41). Regenerated plants were acclimatized and successfully transferred under field conditions.

Keywords: *in vitro* culture, nodal segments, plant regeneration, *Pterocarpus marsupium*

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Introduction

Pterocarpus marsupium Roxb., commonly known as *Beejasar*, is one of the most important tree species of medicinal value belonging to family *Fabaceae*. Its wood is of immense importance as infusion or decoction of it is taken for diabetes. The bark is used for the treatment of stomachache, cholera, dysentery, urinary complaints, tongue diseases and toothache. The Gum exude 'Kino', derived from this tree, is used as an astringent¹. The gum is bitter with a bad taste. However, it is antipyretic, anthelmintic and tonic to liver; useful in all diseases of body and styptic vulnerant; and good for griping and biliousness, ophthalmia, boils and urinary discharges. The flowers are bitter, improve the appetite and cause flatulence. The timber is also highly valued in the international market for its quality².

The natural resurgence of *P. marsupium* is through seeds. However, their germination rate has been reported very poor, which was attributed in part to the impervious seed coat^{3,4}. Furthermore, its propagation through stem cuttings poses difficulties. The poor propagation coupled with over-exploitation for pharmaceutical use has depleted the species from its natu-

ral habitat. Thereby, widening the gap between demand and supply and thus putting further pressure on the species. Owing to these factors, the species is at the verge of extinction and will extinct soon if proper steps are not taken for its conservation. Among the 645 medicinal plant species listed in Madhya Pradesh, 121 species including *P. marsupium* are trees, and many of them have been categorized either as endangered or threatened⁵.

It is essential for the conservation of *P. marsupium*, to encourage the *ex situ* plantations, which require large-scale planting materials. In view of the problems of conventional propagation and high demand of planting material, the large-scale multiplication of this tree species can only be met efficiently and economically, in a short span of time, by *in vitro* propagation. However, sufficient information has not been available on *in vitro* propagation of *Pterocarpus* species and *in vitro* response of various culture media. Therefore, the present investigation has been carried out to ascertain the most appropriate basal culture media and growth hormones for *in vitro* regeneration of *P. marsupium*.

Materials and Methods

Plant Material

The seeds of *P. marsupium* were used as initial explants. The mature seeds were collected from the

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Botanical Garden, Department of Plant Physiology, JNKVV, Jabalpur. The seeds were placed in gunny bags and stored under shade at a dry place.

Preparation of Explants

The hard fruit coat of seeds was cut open mechanically with the help of secateur after removal of wings. These seeds, soaked in sterilized water, were kept overnight and used for culture on the following day. Before culture, they were surface sterilized using 10% (v/v) NaOCl solution for 10 min, followed by three times rinsing with doubly distilled sterilized water. Soft seed coat was removed and seeds were cultured under aseptic conditions on MS medium⁶ without growth hormones (MS₀₀). Seed cultures were incubated separately under light (8 hrs photoperiod regime of 55 $\mu\text{mol m}^{-2} \text{S}^{-1}$) and dark conditions (provided by covering the cultures with a black cloth). Germinated plantlets attained a height of 4-5 cm with 2-3 nodes in 35-40 days.

Nodal Segment Culture

The nodal segments from the 35-40-day grown *in vitro* germinated plantlets were used for initial culture. The nodal segments of 1.5-2.0 cm length with a single node were excised under aseptic conditions and used for culture on seven previously defined culture media.

Culture Media and Condition

Three basal media, viz. MS, B5⁷ and White's⁸ (WH) were used for initial culture of nodal segments. Each of them without growth hormones were used as control (denoted as MS₀₀, B5₀₀ and WH₀₀). Further, these basal culture media were supplemented with 3.0 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA (MSBN, B5BN and WHBN). In addition, MS media supplemented with 0.2 mg l⁻¹ IBA (MSIB) was also used for initial culture. All the culture media were supplemented with 30 g l⁻¹ sucrose and 8 g l⁻¹ agar, and the pH was adjusted to 5.8. The media were autoclaved at 1.2 kg/cm² at 121°C temperature for 20 min. Culture tubes containing one nodal segment with an axillary bud were incubated under 8 hrs photoperiod at 25±2°C temperature. The experiment was replicated twice with 20-culture tubes in each treatment.

Hardening Regenerants

The rooted plants were thoroughly washed with running tap water to remove the adhering agar and planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. The transplanted plants were then transferred under 30±2°C and 60±5%

RH for 30 days in a glass house for acclimatization, prior to transfer to the net house for further establishment and growth. Only well established 25-30 cm tall plants were transferred to the field.

The data on number of shoots per explant, number of nodes per shootlet and shoot length was recorded after 45 days of inoculation of the nodal explants and was subjected to analysis using Completely Randomized Design⁹.

Results and Discussion

In the present study, cultural conditions were found to influence the pattern of growth during *in vitro* germination of seeds. A dark treatment at the time of seed imbibition brought about cotyledon enlargement coupled with callus proliferation and a stunted shoot (Fig. 1a). This may perhaps be the outcome of endogenous stimulation of growth factor(s), most likely a cytokinin, in the period of complete darkness when

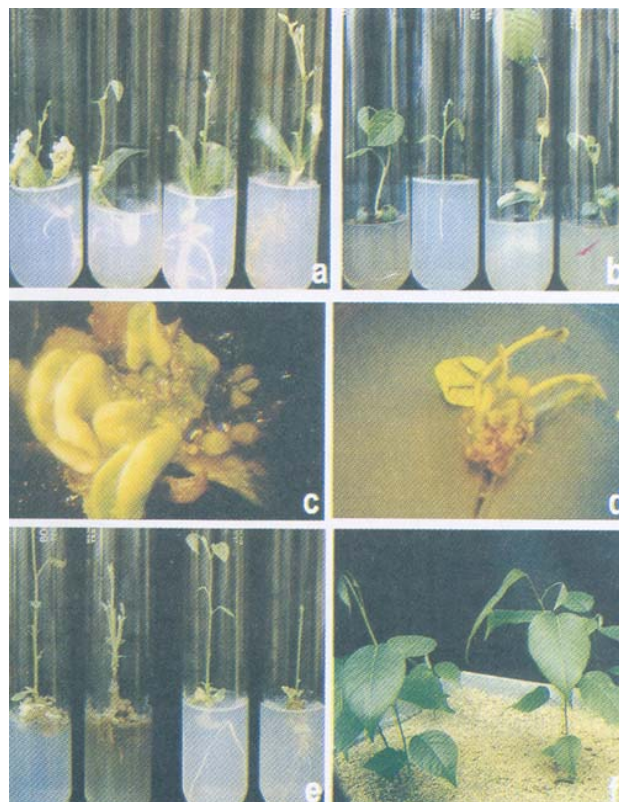


Fig. 1— *In vitro* regeneration of *P. marsupium*: Germination from seeds incubated (a) under dark conditions, and (b) under 8 hrs photoperiod; (c) Shoot initiation from callus; (d) Plant regeneration from callus; (e) Plantlet formation from callus cultures; and (f) Regenerated plants under hardening.

the stored carbohydrates are being gradually mobilised. On the contrary, the seeds incubated under 8 hrs photoperiod regimen exhibited a rapid elongation of the shoot and a diminutive expansion of the eophylls (Fig. 1b). This seems to be illustrative of the exhaustion of carbohydrates stored in the eophyll and their translation into brisk shoot growth following photosynthesis. Further, the nodal explants isolated from light germinated plantlets exhibited better *in vitro* response for mass propagation as compared to those isolated from dark germinated plantlets.

The callus proliferation started within one week from the set up of nodal explant cultures on medium free of plant growth regulators. Shootlet initiation occurred from the callus within a fortnight (Fig. 1c & d). Shoot and root formation also occurred on the same medium. *In vitro* developed plantlets were complete with a shoot as well as a root; however, branching was normally not observed (Fig. 1e). Each plantlet carried between 3 to 6 nodes that are now being used either for repeated subculture onto the medium as above or being subjected to hardening for multiple plant production.

The results on the effects of three basal media, viz. WH, MS and B5, with and without growth hormones on the micropropagation of *P. marsupium* are presented in Table 1. In the present investigation, the explants cultured on MS₀₀ produced more shoots per

explant as compared to rest of the media studied. This can be attributed to the different nitrate ammonium ratio of MS medium, which is considered to be an important factor for nitrogen uptake and pH regulation during culture¹⁰. However, the number of nodes per shootlet was found higher in all MS combinations and B5₀₀ medium. The mean length of the shoots produced on MSIB, MS₀₀ and B5BN media was significantly higher than those obtained on other media. Thus, the best results on micropropagation of *P. marsupium* were obtained with MS medium. The present findings have been found in agreement with previous reports on tissue culture of *Pterocarpus*^{11,12}. Whereas, Lakshmi *et al*⁴ suggested that B5 medium was superior to MS for the plant regeneration of *Pterocarpus*.

The inclusion of cytokinins and auxins to the culture media stimulated the *in vitro* multiplication and the growth of shoots in several plant species¹⁰. However, in the present study, the addition of BA and NAA to the basal media (MSBN, B5BN, WHBN) significantly reduced the number of shoots per explant and number of nodes per shootlet. This phenomenon may be attributed to the presence of endogenous hormones in sufficient quantity to proliferate under congenial conditions. On the other hand, culture medium MSIB, formulated to produce roots, exhibited sizeable results for micropropagation of *P. marsupium*, especially for stimulating more nodes per shootlet and

Table 1—*In vitro* shoot formation and growth of *P. marsupium* nodal segment on various culture media

Culture media	Seed germination (%)	No. of shoots per explant [†]	No. of nodes per shootlet [†]	Shoot length (cm) [†]
WH ₀₀ White's media w/o growth hormones	64	1.28 ± 0.15 ^a	1.46 ± 0.27 ^a	0.68 ± 0.09 ^a
WHBN White's media + 3.0 mg l ⁻¹ BAP + 0.5 mg l ⁻¹ NAA	71	1.20 ± 0.23 ^a	1.02 ± 0.18 ^a	0.63 ± 0.11 ^a
MS ₀₀ MS media w/o growth hormones	100	3.25 ± 0.63 ^c	4.12 ± 0.48 ^{bc}	2.41 ± 0.32 ^c
MSBN MS media + 3.0 mg l ⁻¹ BAP + 0.5 mg l ⁻¹ NAA	97	1.80 ± 0.53 ^{ab}	3.40 ± 0.57 ^b	1.30 ± 0.27 ^b
MSIB MS + 0.2 mg l ⁻¹ IBA	95	2.26 ± 0.21 ^b	4.95 ± 0.53 ^c	2.92 ± 0.29 ^c
B5 ₀₀ Gamborg's media w/o growth hormones	78	2.60 ± 0.50 ^{bc}	4.28 ± 0.58 ^{bc}	1.54 ± 0.25 ^b
B5 BN Gamborg's media + 3.0 mg l ⁻¹ BAP + 0.5 mg l ⁻¹ NAA	80	2.20 ± 0.38 ^b	1.50 ± 0.28 ^a	2.20 ± 0.21 ^c

[†]Evaluation was made after 45 days in culture

^{a-c}Means followed by the same letters are not significantly different (p<0.05) using Tukey's test

higher shoots length. However, the maximum number of plantlets was formed when MS medium was devoid of growth hormone.

All plantlets acclimatized well in the green house (Fig. 1f) and then under field conditions. The establishment of micropropagated plants was >68%. All the 86 regenerated plants transferred to the field showed the high homogeneity without obvious morphological evidences of somaclonal variations. The induction of multiple shoot through axillary branching is now being practiced as a useful technique for propagation. The present work demonstrates a simple procedure for rapid clonal multiplication of *P. marsupium* through nodal segment culture.

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