

Effect of Seed Orientation and Medium Strength on *In vitro* Germination of *Pterocarpus marsupium* Roxb.

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Abstract

A method was developed for optimization of *in vitro* germination of an economically important timber-yielding multipurpose tree, *Pterocarpus marsupium* Roxb. The seeds inoculated in different orientation on different strengths of MS medium without any hormones showed varied response to the seed positions. The seeds inoculated on half strength medium in horizontal position recorded to produce a maximum germination (78.23%), shoot number (0.86) and root number (7.99). However, a maximum of shoot length of 3.67 cm was recorded in the quarter strength medium in the seeds inoculated in vertical down position, which was significantly higher than other media strength and positions. Our results indicate that the seed orientation including medium strength have tremendous effect on germination and seeds inoculated horizontally on half strength MS medium can be utilized to enhance *in vitro* seed germination of *Pterocarpus marsupium*.

Keywords: beejasal, germination, *Pterocarpus marsupium*, seedling, surface sterilization, seed orientation

Introduction

Pterocarpus marsupium (Beejasal, Indian Kino or Malabar Kino) commonly found in the Deccan Peninsula, central India and certain parts of northern India, is a deciduous and multipurpose leguminous tree and valued for excellent timber, which ranks next to teak and rosewood (Kirtikar and Basu, 1999). The Malabar Kino tree also yields gum kino, which is a powerful astringent, used for treatments of diarrhoea, dysentery, leucorrhoea, haemorrhages and toothache (Pullaiah, 1999). The water stored in vessels made of the wood is reputed to have antidiabetic properties due to transfer of glycosides into water (Handa *et al.*, 2000; Maurya *et al.*, 2004). In fact, two glycosides i.e. marsupin and pterostilben, present in heart wood of *Pterocarpus marsupium* are more effective than metformin, a potent medicine for diabetes (Manikam *et al.*, 1997). Supplementation in humans with 450 mg *Pterocarpus marsupium* extract is considered safe, based on a long history of use, the absence of abnormal blood cell counts and blood chemistry values and the absence of extract-related adverse events (Hougee *et al.*, 2005).

The mature tree harvested after 10-15 years has been estimated to produce approximately 500 kg of dry heartwood (NMPB, 2008). The natural stands of this tree are fast disappearing due to illicit felling for its significant multipurpose properties and current high market price of its dry heartwood ranging from Rs. 70-80/kg (NMPB, 2008). The winged pod is the only propagating material, but its germination is only 30% (Kalimuthu and Laksh-

manan, 1995). However, propagation and cultivation of forest tree species including *Pterocarpus marsupium* through seeds are difficult due to pathogenic infection (Ali and Sharma, 1996), which leads to low germination and causes its restricted natural regeneration. Sometimes, wet heat treatment, physical and acid scarification treatments are required for seed germination and considered as an essential to success (Barmukh and Nikam, 2008). Keeping in view low germination and difficulties in establishment of seedling due to pathogens, *in vitro* germination of *P. marsupium* can be utilized as an essential prerequisite for production en masse. Ahmad *et al.* (2012) used MS, B5 (Gamborg *et al.*, 1968) and White (1963) medium for *in vitro* seed germination of *Pterocarpus marsupium* without considering the seed orientation, which is an important and key factor for *in vitro* (Lee *et al.*, 2009) and *ex vitro* (Swaminathan *et al.*, 1993; Bhat, 2011) seed germination. Besides, they did not produce any plant through *in vitro* method.

In this study, we investigated *in vitro* seed germination of *P. marsupium* using different seed orientations and strengths of basal medium for optimization of seed germination and their successful establishment in nursery stage.

Materials and methods

Plant seed materials

The pods of *P. marsupium* were procured from Narayanpur range of Bastar forest division, Chhattisgarh, India. The seeds remain enclosed inside a hard and stony

pericarp with orbicular wing (Fig 1a). The wing of the pod was cut with scissors and the seed inside the pod was excised with the help of scalpel (Fig 1 b). The seeds were first washed with distilled water for five minutes followed by washing with aqueous solution of cetrimide (1:10 v/v) for five minutes. Finally seeds were washed with mercuric chloride solution for 5 minutes before inoculation.

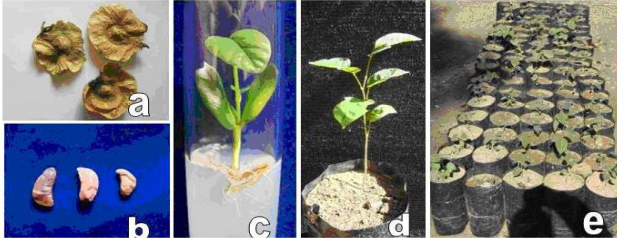


Fig. 1. In vitro seed germination of *Pterocarpus marsupium* (a) seed pods with orbicular wing; (b) true seeds excised from the pod; (c) seedling emerged from seed inoculated in horizontal orientation; (d) seedling transferred to polythene bag (e) seedlings maintained in polythene bags in the nursery

Medium composition

To optimize germination medium conditions, the surface-sterilized seeds were inoculated on three medium strengths of MS (Murashige and Skoog, 1965) medium, viz., full strength MS salts, half strength MS salts and quarter strength MS salts. In addition, to compare the effects of seed orientation, seeds were implanted horizontally, vertically up (embryo upward) and vertically down (embryo downward) on the medium. For the experiments, the medium contained 3% (w/v) sucrose as carbon source and 0.8% (w/v) bacto grade agar (Loba Chemie Ltd., India) as gelling agent. To investigate the effect of the seed sowing position and different strengths of MS medium, the seed germination percent, shoot number, shoot length and root number were recorded after 4 weeks of inoculation. The medium was not supplemented with any hormone.

Hardening and transplantation

The in vitro raised seedlings were removed from the test tubes and dipped for 30 second in 0.2% (w/v) bavistin solution and washed with tap water and transferred to root trainers comprising 25 cells each of 150cc (Neevedita Plastic Industries, Nagpur, India), filled with a mixture of soilrite: compost (1:1) and maintained at 25 ± 2 °C under 16 h photoperiod for in vitro hardening. After an acclimatization period of 2-3 weeks, the seedlings were transferred to polythene bags containing soil: sand: farmyard manure (1:1:1) in the shadehouse condition.

Culture conditions and analysis of data

The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving for 15 min at 1.06 kg cm^{-2} (121°C). The seeds were inoculated in test tubes ($25 \times 125 \text{ mm}$, Borosil, India) containing 10 ml of semi-

solid medium. The culture vials were covered with plastic caps. The cultures were incubated at a temperature of 25 ± 2 °C under 16 hrs illumination with fluorescent light (approx. $45 \mu\text{mol s}^{-2}$). The experiment was conducted twice and there were nine treatment combinations, each replicated three times, and each replicate was sown with twenty seeds. The seed germination rate was expressed as a percentage, which was calculated by using the following equation: (number of germinated seeds/total number of seeds per treatment) X 100. The data for germination, number of shoots, shoot length and root number were recorded after four weeks of inoculation. All experiments were conducted in complete randomized design (CRD) and data were analyzed by two-way analysis of variance employing Duncan's multiple range test for means comparisons at $p=0.05$ using SYSTAT-12 statistical package (SYSTAT analysis software, 1982, version 12, USA). The interactions of the treatments i.e, media strengths and orientations, were studied in their factorial combination. Arc sin transformation was used for the data expressed as percentages (Gomez and Gomez, 1984).

Results

Germination percent

The germination percent was significantly affected by the orientations in which seeds were inoculated. The highest seed germination (70.39%) was recorded on the medium with the seeds inoculated with horizontal position (Fig 1c), which was significantly higher than other two orientations (Tab. 1). Among the different strength of MS basal medium used, a non-significant difference was recorded for seed germination. However, in the interaction study a maximum of 78.23% germination was obtained in the seeds inoculated on half strength medium in horizontal orientation, which was significantly higher than other medium strengths and orientations.

Tab. 1. Effect of seed position and medium strength on seed germination and other parameters of *P. marsupium* under in vitro conditions after 30 days of inoculation

Position of inoculation	Seed germination % on different strength of MS media				Shoot number on different strength of MS media				Shoot length (cm) on different strength of MS media			
	Full	Half	Quarter	Mean	Full	Half	Quarter	Mean	Full	Half	Quarter	Mean
Horizontal	66.47	78.23	66.47	70.39	0.77	0.86	0.77	0.80	2.34	3.03	2.65	2.67
VU*	54.70	29.98	41.75	42.14	0.66	0.33	0.44	0.47	1.66	0.66	1.54	1.28
VD**	54.70	48.21	66.47	56.46	0.66	0.55	0.77	0.66	1.35	1.80	3.67	2.27
Mean	58.62	52.14	58.23		0.69	0.58	0.66		1.78	1.83	2.62	
Vertical Up*, Vertical Down**	LSD (0.05)				LSD (0.05)				LSD (0.05)			
Position of explants	NS				Position of explants 0.22				Position of explants 0.82			
Medium strength	22.62				Medium strength NS				Medium strength 0.82			
Position * Medium strength	22.76				Position * Medium strengths 0.27				Position * Medium strengths 1.37			

Shoot number

The highest shoot number (0.80) was recorded on medium with the seeds inoculated with horizontal orientation, which was significantly higher than other two positions (Tab. 1). Among the different strength of MS basal medium attempted, a non-significant difference was observed and a maximum 0.69 shoot number was recorded with full strength medium. However, in the interaction study a maximum of 0.86 shoot number was obtained in the seeds inoculated on half strength medium in horizontal position, which was significantly higher than other medium strengths and positions (Tab. 1).

Shoot length

From the perusal of data in the Tab. 1, it revealed that shoot length had significant effect on different medium strengths and orientations. A maximum of 2.67 cm length was recorded with horizontal orientation and 2.62 cm was obtained in the quarter strength medium. In the interaction study a maximum shoot length of 3.67 cm was recorded in the quarter strength medium in the seeds inoculated in vertical down, which was significantly higher than other medium strengths and orientations.

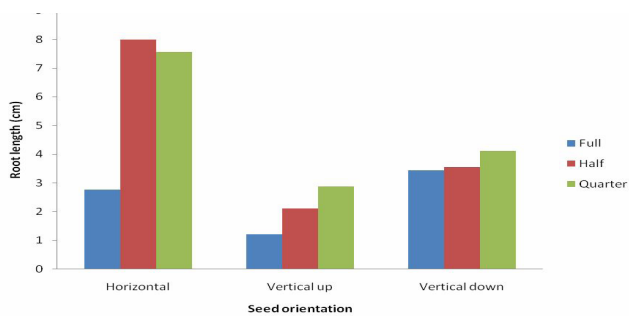


Fig. 2. Effect of seed orientation and medium strengths on root length (cm) of *Pterocarpus marsupium*

Root number

From the perusal of data depicted in the Fig. 2, it is revealed that root number had significant effect on different media strength. A maximum number of root (6.10) was recorded with horizontal orientation, which was significantly higher than other positions, whereas media strengths had no significant difference. However, in the interaction study a maximum of 7.99 root number was recorded in the half strength medium in the seeds inoculated in horizontal position, which was on par with the root number obtained in quarter strength of horizontal position (7.55) and significantly higher than other medium strengths and orientations.

Hardening and transplantation

After an acclimatization period of 2-3 weeks, the seedlings were transferred to polythene bags containing soil: sand: farmyard manure (1:1:1) in the shadehouse condi-

tion. The seedlings so obtained were successfully transferred in to polythene bags with 100 % survival (Fig. 1d) and maintained in the nursery of the division for field trial (Fig. 1e).

Discussion

The main purpose of this study was to optimize seed germination under in vitro conditions. The present data demonstrates the effects of medium strengths and seed orientations on germination and other related parameters of *P. marsupium*. The seeds were removed from the winged pods mechanically with the help of scissors and scalpels. The washing of seeds using cetrimide, 0.1% HgCl₂, and distilled water ensured removal of major microbial contaminants. The half strength MS media supplemented with 1% of sucrose significantly enhanced the seed germination rate compared to the other medium strengths, indicating that the reduced concentrations of MS salts and sucrose in the media increased the *P. marsupium* seed germination. Furthermore, the effect of seed orientations on germination rate was investigated. Regardless of the concentration of MS medium, the horizontally sown seeds had significantly enhanced germination rate, shoot number, shoot length and root number as compared to the vertical sown seeds under the in vitro conditions, indicating that the seed orientation affects *P. marsupium* seed germination. Out of three basal media, Ahmad *et al.* (2012) screened half MS as the best medium to optimize in vitro seed germination in *Pterocarpus marsupium* without reporting rooting success and failed to produce plantlet through in vitro method. Enhanced in vitro seed germination has been reported on reduced strengths of MS medium in other species like *Withania somnifera* (Sen and Sharma, 1991) and *Aloe polyphylla* (Bairu *et al.*, 2007). The role the germination media (MS medium) played on in vitro germination potential of the seedlings is of considerable importance. Generally, media high in salt and sugar content reduced germination efficacy. Maximum germination was obtained on lower strength of MS media (1/2 strength). This effect could partly be attributed to the role minerals play as osmotica. Because any germination process is preceded by imbibition, anything that affects this process is likely to affect germination.

Our results are consistent with a previous study where horizontally orientated seeds gave higher germination (81%) than vertical orientation (65%) in anjan (*Hardwickia binata*) (Masilmani *et al.*, 1999). Particularly, horizontal orientation of the micropyle of anjan seed significantly enhanced germination rate compared to vertical upward or downward orientations. These results are expected since the seed micropyle plays essential roles in water uptake during germination (Hamly, 1932). In our study also, the seeds inoculated in horizontal orientation enhanced the germination and other related parameters as compared to seeds inoculated on vertical down and up

position. The seedlings so obtained were successfully hardened and transferred in to polythene bags for field trial.

Conclusion

The current study demonstrates that the seed orientations and concentration of MS salts can be optimized to enhance in vitro seed germination of *P. marsupium*. It is concluded that the germination of *P. marsupium* seed can be increased to the tune of 78% in the seeds implanted horizontally on half MS basal medium under the in vitro culture conditions.

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