AcademicPres

Available online at www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2013, 5(4):476-479

Notulae Scientia Biologicae

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

Yogeshwar MISHRA*, Rimi RAWAT, Brajesh NEMA, Fatima SHIRIN

Division Genetics and Plant Propagation, Tropical Forest Research Institte
*P.O.- R.F.R.C., Mandla Road, Jabalpur, 482021, India; m_yogiraj@yahoo.com (*corresponding author)*

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 Lakshmanan, 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

Received 28 July 2013; accepted 28 August 2013

pericarp with orbicular wing (Fig 1a). The wing of the pod was cut with sciossors and the seed inside the pod was excised with the help of scalpel (Fig 1 b). The seeds were first washed with distlled 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) seedling 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 × 125 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 µmol 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. 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.

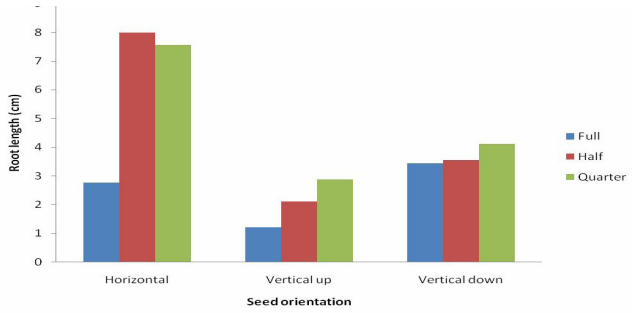

Fig. 2. Effect of seed orientation and medium strengths on root length (cm) of *Pterocarpus marsupium*

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 condition. The seedlings so obtained were successfully transferred in to poylthene 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 poylthene 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.

Acknowledgement

The authors gratefully acknowledge the receipt of research grant received from Compensatory Afforestation Fund Management and Planning Authority (CAMPA), State Forest Department, Chhattisgarh, INDIA for funding the research project on genetic improvement of *Pterocarpus marsupium* through germplasm selection (Sanction No. CAMPA/26/2011/1584, dated 23.12.2011) and for the work reported herein.

References

Ahmad N, Alia A, Khan F, Kour A, Khan S (2012). In vitro seed germination and shoot multiplication of *Pterocarpus marsupium* Roxb-An endangered medicinal tree. Researcher 4: 20-24.

Ali MMI, Sharma JK (1996). Impact of seed microflora on seed germination and seedling vigour of some indegenous tree species of Kerala, Proc. IUFRO symposium. Impact of disease and insect pest in tropical forest, KFRI, Pecchi, India, 33-51.

Bairu MW, Stirk WA, Doležal K, Van Staden J (2007). Optimizing the micropropagation protocol for the endangered Aloe polyphylla: Can meta-topolin and its derivatives serve as replacement for benzyladenine and zeatin? Plant Cell Tiss Org Cult 90:15–23.

Barmukh RB, Nikum TD (2008). Promotion of seed germination in *Pterocarpus marsupium* Roxb. Ind J Plant Physiol 13:143-150.

Bhat, RP (2011) Effect of orientation of seed placement on seedling emergence in some species of Calamus. Advances Bioresearch 2:86 – 89.

Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements of suspension culture of soyabean root cells. Exp Cells Res 50:15-158.

Gomez KA, Gomez AA (1984). Statistical Procedure for Agricultural Research, John Wiley and Sons, New York, 207-208

Hamly DH (1932). Softening of the seeds of *Melilotus alba*. Bot Gaz 93(4):345-375.

Handa SS, Singh R, Maurya R, Satti NK, Suri KA, Suri OP (2000). Pterocarposide, an isoaurone C-glucoside from *Pterocarpus marsupium*. Tetrahedron Letters 41:1579-1581.

Hougee S, Faber J, Sanders A, de Jong RB, van den Berg WB, Garssen J, Hoijer MA, Smit HF (2005). Selective COX-2 ihibition by a *Petrocarpus marsupium* extract characterized by petrostilbene, and its activity in healthy human volunteers. Plant Med 71(5):387-392.

Kalimuthu K, Lakshmanan KK (1995). Preliminary investigation on micropropagation of *Pterocarpus marsupium* Roxb. Ind J For 18:104–106.

Kirtikar KR, Basu BD (1999). Indian Medicinal Plants, Vol. I (Naya Prakashan, Kolkata) 828-829.

Lee JH, Young KK, Eun YO, Kuk YJ, Kisung K (2009). Optimization of in vitro seed germination of *Taraxacum platycarpum*. Korean J Environ Agri 28: 403-408.

Manikam M, Ramanathan M, Jahromi MA, Chansouria JP, Ray AB (1997). Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. J Nat Prod 60: 609-10.

Masilamani P, Gurudev Singh B, Chinnusamy C, Annadurai K (1999). Influence of seed orientation and depth of sowing on germination and vigour of anjan (*Hardwickia binata* Roxb.). Tropi Agri Res and Exten 2: 76-78.

Maurya RS, Mundkinajeddu D, Handa SS, Prem PY, Mishra K, Pushpesh (2004). Constituents of *Pterocarpus marsupium*: An ayurvedic crude drug. Phytochem 65: 915-920.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15(3):473- 497.

NMPB (2008). National Medicinal Plant Board, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, Agro techniques of selected medicinal plants, published by TERI press, New Delhi, Vol I: 155-158.

Price J, Li TC, Kang SG, Na JK, Yang JC (2003). Mechanisms of glucose signaling during germination of Arabidopsis. Plant Physiol 132:1424-1438

Pullaiah T (1999). Medicinal plants of Andhra Pradesh (India). New Delhi: Regency Publications.

Swaminathan C, Vinaya Rai R S, Suresh KK, Sivaganam K (1993). Improving seed germination of *Derris indica* by vertical sowing. J Trop For Sci 6:152-158.

Sen J, Sharma AK (1991) Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. Plant Cell Tiss Org Cult 26:71-73.

White PR (1963). The cultivation of animal and plant cells. The Ronald Press, New York, p. 228.