



## PROMOTION OF SEED GERMINATION IN *PTEROCARPUS MARSUPIUM* ROXB.

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Received on 11 May, 2008

### SUMMARY

*Pterocarpus marsupium* Roxb. is a multipurpose leguminous tree valued internationally for its timber and pharmaceutical properties. Natural resurgence in this species is poor and the nursery germination is unpredictable. The species is recently included in the list of depleted plants. The present paper describes the effectiveness of wet heat, physical and acid scarification of seeds for inducing uniform and fast nursery germination in *P. marsupium*. The untreated seeds showed poor germination (28.2% in fifteen days, maximum of 44.3% in 30 days) and produced seedlings with least vigour index (1.1 – 1.4). The physically scarified seeds showed higher percentage germination (55.3%) within 15 days. Compared to the untreated seeds, the seeds treated with wet heat for 40 min produced 1.5 times more germination within 15 days. Among the seeds subjected to wet heat treatment and physical scarification and seeds scarified with concentrated HCl, H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub>, the scarification for 30 min with concentrated H<sub>2</sub>SO<sub>4</sub> was the most effective for induction of germination and production of uniform seedlings with higher vigour index. The maximum of 78% and 85% germination was observed within 15 and 30 days respectively with the maximum vigour index of 3.3. The seedlings attained the height of about 0.4 meters within 8-9 months after which they were transferred to the field. The 30 min scarification treatment of concentrated H<sub>2</sub>SO<sub>4</sub> can be used to induce uniform and fast nursery germination in *Pterocarpus marsupium* Roxb.

**Key words:** Acid scarification, nursery germination, physical scarification, *Pterocarpus marsupium*, wet heat treatment.

### INTRODUCTION

*Pterocarpus marsupium* Roxb., (Bija or Bijasar or Bijasal, Fabaceae), is a significant element of Indian forests and is commonly found in hilly regions throughout the Deccan Peninsula and extending to the states of Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar and Orissa (Krishnamurthi 1998). Its timber, which is rated next to that of teak in India, is highly valued in the international market for its quality (Kirtikar and Basu 1999, Husain and Shahzad 2007). The wood is used in the construction of railway carriages, wagons,

carts and boats and occasionally for shipbuilding. The gum exude 'Kino' obtained from this tree has antipyretic, antihelmintic properties and is used as an astringent (Singh *et al.* 1965). The bark is used for the treatment of stomachache, cholera, dysentery, urinary complaints, tongue diseases and toothache. The wood is of great importance as its infusion or decoction is taken for diabetes.

The overexploitation for commercial and pharmaceutical use is gradually widening the gap between demand and supply and thus putting further pressure on

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the species. Its indiscriminate exploitation has resulted in its inclusion in the list of depleted plant species (Chaudhari and Sarkar 2002). The depletion of this species from its natural habitat has brought it at the verge of extinction and will extinct soon if proper steps are not taken for its conservation (Tiwari *et al.* 2004). Recently, Anis *et al.* (2005) and Husain and Shahzad (2007) have reported a successful approach for *in vitro* propagation of *P. marsupium*. Such biotechnological approach requires trained manpower and higher economic and labour inputs. In view of this, a clear procedure to achieve quick and reliable germination would be a valuable asset.

The seed germination in *P. marsupium* Roxb. is very poor (Lakshmi *et al.* 1992, Das and Chatterjee 1993). Its fruit is an orbicular winged pod (samara) where the true seed remain enclosed inside a hard enclosure (Fig. 1a). Separating the seed from fruit is a tedious and labour intensive task. Owing to the hard and woody pericarp, the species seems to possess mechanical dormancy. Any method that would modify the permeability of enclosing structures should lead to improved seed germination. Wet heat treatment and physical scarification weakens the hard seed coat or pericarp leading to enhanced imbibition thereby improving percentage seed germination. Acid scarification leads to partial or complete removal of inhibitory substance(s) and weakening of the hard seed coat or pericarp (Mayer and Poljakoff-Mayber 1963). This process has been shown to give significant improvement in germination in many genera (Baskin and Baskin 1989). The present investigation describes the use of wet heat, physical scarification and acid scarification for enhanced and uniform nursery germination in *Pterocarpus marsupium* Roxb.

## MATERIALS AND METHODS

*Source of the seeds:* A naturally growing mature tree of *P. marsupium* located near the foothills of Sinhgad Fort, about 35 km South-West of Pune city, India was selected as a seed source. The pods were collected in the month of March 2006 from off the ground. A healthy, fully matured and disease-free pods were separated from the bulk. These pods were air dried under shade for one week in the laboratory and cleaned pods were stored in

polythene bags. The seed remain enclosed inside a hard and stony non-separable pericarp with orbicular wing. This orbicular wing of the fruit was excised with sharp scissors (Fig. 1b). Such dewinged pods were referred to as 'seeds' since the term refers both to true seeds and fruits and other dispersal units (Taylorson and Hendricks 1977).

*Seed viability test:* The seed viability was assessed by performing tetrazolium (TZ) test. The seeds were separated from pods. Hundred seeds were incubated in 50 ml of 1% (w/v) solution of 2, 4, 5-triphenyl tetrazolium chloride (TTC) prepared in 0.1 M Sorensen's buffer (pH 7.0) for 24 hours at 28 °C. After the incubation, the seed was longitudinally bisected and the embryo was observed. The seeds wherein embryos turned reddish-pink were scored as viable (Mitter 1993) and seeds that remained light yellow were scored as non-viable (Eplee and Norris 1987).

*Wet heat treatment:* The seeds were wrapped in a clean cotton cloth and then completely submerged in 500 ml hot distilled water set to 60 °C in 1000 ml beaker. The temperature was maintained at the set point during the course of the treatment by keeping the beaker containing seeds in a temperature-controlled water bath. The hot water treatment was given separately for 20, 40 and 60 min. The seeds were also subjected to boiling water treatment by immersing the seeds in 500 ml of distilled water brought to boiling temperature. The seeds were allowed to imbibe till the temperature of boiling water dropped to room temperature.

*Physical scarification of seeds:* The central seed-case was incised with sharp secateurs so as to cut the outside edge of seed-case by about 1-2 mm and thereby exposing the enclosed true seed. The incised seeds were allowed to imbibe for 60 min in distilled water. The control for physical scarification and wet heat treatment was maintained by immersing the seeds in distilled water for 60 min. After the specific treatments, the seeds were then blotted dry and immediately sown at the depth of about 2 cm in the plastic bags (6" height × 4" width) and cavity seeding trays containing moist garden soil.

*Acid scarification of seeds:* The hydrochloric acid (HCl), sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and nitric acid (HNO<sub>3</sub>)



**Fig. 1.** (a) Fruits of *P. marsupium* Roxb. (Bar = 5 cm), (b) Dewinged fruits (seeds), (Bar = 5 cm), (c) Viable seeds (TZ positive), (Bar = 1 cm), (d) Non-viable seeds (TZ negative), (e) Seeds scarified with concentrated  $H_2SO_4$  for 30 min, (f) One year old seedlings (Bar = 10 cm)

of analytical grade were used to scarify the seeds. All these chemicals were procured from Sisco Research Laboratory (SRL), India. The seeds were acid scarified in a 500 ml Borosil beaker by soaking the seeds in concentrated HCl (35%, 11 N), concentrated  $H_2SO_4$  (99%, 36 N), and concentrated  $HNO_3$  (70%, 15.6 N) separately for 15, 30 and 45 min. The seeds were exposed to acid in the ratio of two parts acid to one part seed (v/v) (Hartmann *et al.* 1997). The acid-seed mixture was sparingly stirred with glass rod. After the specified treatment, the acid was decanted and the seeds were washed under running tap water for 2 h. The control was maintained by submerging the seeds in distilled water for the same duration. The seeds were then sown in the plastic bags (4" × 6") containing moist garden soil. The plastic bags were watered and maintained in the shade-net house (temperature:  $26.8 \pm 5.8$  °C, relative humidity:  $81.53 \pm 15.43$ ).

*Collection of data and statistical analysis:* Fifty randomly selected seeds were allocated to each treatment. The seed germination was counted for 15 days at the interval of 5 days. The cumulative percentage

germination (CPG) was calculated using the method of Younsheng and Sziklai (1985) and the effectiveness of the treatments was judged by comparing the CPG on the 15<sup>th</sup> day (CPG 15D). Daily germination speed (DGS) was computed at each time interval by dividing CPG by number of days since beginning of the test (Muhammad and Amusa 2003). The effectiveness of the treatments was judged on the basis of mean DGS that was computed by taking average of DGSs computed at each time interval. The mean germination time (MGT) was calculated using the method of Younsheng and Sziklai (1985) and Hartmann *et al.* (1997).

Taking into account the importance of dry-matter accumulation in seedling health and low MGT as indication of seed vigour, a method was adopted (Butola and Badola 2004) to determine the seedling vigour index (SVI).

$$SVI = \text{Dry weight per seedling} / \text{MGT} \times 100.$$

Five randomly selected seedlings generated from each treatment were carefully uprooted from the soil after one month from the date of sowing the seeds. The root system was carefully washed to remove the associated soil particles. The fresh weight was noted and the seedlings were dried in an oven at 60 °C till constant weight was obtained (Sestak 1971). The average dry weight of seedling was used for the calculation of SVI. The experiments were concluded on the 30<sup>th</sup> day after sowing (DAS). The final germination percentage (FGP) was calculated from the total seeds that germinated on the 30<sup>th</sup> day out of those planted at the start.

Each experiment was performed in a completely randomized design and was repeated at least thrice. Germination percentages were normalized by arcsine transformation before performing statistical analysis (Zar 1996). The data were analyzed for variance by performing ANOVA test in MS Excel program. For acid scarification experiment the treatment means were compared with the best control mean by following Dunnett's (1964) test at  $p = 0.05$ . Where the difference between the best control mean and treatment mean was more than the critical difference (CD), the treatment mean was declared significantly different from the control mean. The treatment means were also compared

to identify relative effectiveness of the treatment and were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1955) at  $p = 0.05$ . The data were represented as mean  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

The TZ test revealed 98% viability in the seeds used (Fig.1c & d). However, separating the true seed from fruit was a tedious, labour intensive task. A high rate of seed injury during seed separation was noted. The presowing hydration of separated seeds resulted in high rate of imbibition injury thereby affecting germination and survival of seedlings. This clearly indicates that though the seed viability is very high, the surrounding fruit structure is imparting mechanical constraint for seed germination.

*Wet heat treatment:* The most widely used pre-germination treatments for damaging the coat or breaking dormancy in legumes are short lasting soaking of the seeds in hot water or mechanical scarification or piercing of the coat (Rolston 1978). The wet heat treatment given for 40 min to the seeds of *P. marsupium* seeds produced significantly better germination pattern compared to the control (Table 1). The 20 min treatment produced intermediate response whereas the longer exposure of 60 min was detrimental to seed germination in *P. marsupium*. In the seeds treated with wet heat for

40 min produced 1.8 times more germination within 15 days (CPG 15D) as compared to the germination in the control seeds in the same duration. The mean daily germination speed (mean DGS) was also significantly boosted by about 116%, which was coupled with about 11% reduction in mean germination time (MGT). The vigour in the seedlings obtained from this treatment was significantly improved over control. Kalimuthu and Lakshmanan (1995), however, observed contrasting results with the treatment of hot water and reported unimproved germination in the seeds of *P. marsupium* as well as *P. santalinus* soaked in hot water for 24 h. In this case, the prolonged exposure of seeds to the hot water might have damaged the embryo and reduced the chances of its germination. In the present investigation, the 60 min exposure to the wet heat (60 °C) might also have created similar effects on the seeds of *P. marsupium*.

The use of hot water treatment to improve seed germination has been reported in other species from Fabaceae. A 24-hour soak in water; a boiling water dip for 15 seconds or more followed by a 24-hour soak in cooler water; or immersion overnight in 80 °C hot water that gradually cools; has been generally used to overcome seed-coat dormancy in *Cercis* (Heit 1967, Raulston 1990). The seeds of *Parkia biglobosa* exposed to boiling water for 4 seconds gave maximum percentage germination (42.9%) whereas untreated seeds (control)

**Table 1.** Effect of wet heat treatment and physical scarification on seed germination in *Pterocarpus marsupium* Roxb.

Treatment	Duration (min)	CPG 15D (% $\pm$ SD)	Mean DGS (% seeds per day $\pm$ SD)	MGT (Days $\pm$ SD)	FGP (% $\pm$ SD)	SVI
Control (DW)	60	24.6 $\pm$ 3.3	1.2 $\pm$ 0.2	11.4 $\pm$ 1.7	43.3 $\pm$ 3.5	1.3 $\pm$ 0.2
Wet heat (60 °C)	20	40.0 $\pm$ 3.3 <sup>a</sup>	2.0 $\pm$ 0.3 <sup>b</sup>	11. $\pm$ 1.10 <sup>c</sup>	47.3 $\pm$ 2.5 <sup>b</sup>	1.3 $\pm$ 0.2 <sup>a</sup>
	40	44.6 $\pm$ 2.5 <sup>b</sup>	2.6 $\pm$ 0.3 <sup>c</sup>	10.2 $\pm$ 1.1 <sup>bc</sup>	50.6 $\pm$ 4.4 <sup>bc</sup>	2.2 $\pm$ 0.2 <sup>b</sup>
	60	26.7 $\pm$ 2.2 <sup>a</sup>	1.1 $\pm$ 0.1 <sup>a</sup>	12.3 $\pm$ 1.2 <sup>d</sup>	32. 6 $\pm$ 2.2 <sup>a</sup>	1.4 $\pm$ 0.3 <sup>a</sup>
Boiling water		52.0 $\pm$ 3.8 <sup>c</sup>	3.5 $\pm$ 0.4 <sup>d</sup>	9.5 $\pm$ 1.3 <sup>b</sup>	57.6 $\pm$ 4.0 <sup>cd</sup>	2.0 $\pm$ 0.3 <sup>b</sup>
PS		55.3 $\pm$ 3.3 <sup>c</sup>	4.4 $\pm$ 0.3 <sup>e</sup>	8.3 $\pm$ 1.0 <sup>a</sup>	67.3 $\pm$ 4.9 <sup>d</sup>	2.6 $\pm$ 0.2 <sup>c</sup>

CPG 15D = Cumulative percentage germination on 15<sup>th</sup> day, Mean DGS = Mean daily germination speed, MGT = Mean germination time, FGP = Final germination percentage, SVI = Seedling vigour index, PS = Physical scarification, The values represent (mean  $\pm$  SD) of three independent experiments each performed on 50 seeds, \* Values differ significantly from control as per Dunnett's Test at  $p=0.05$ , Treatment means with similar letters do not differ significantly as per DMRT at  $p=0.05$ .

did not germinate during the eight week experimentation period (Aliero 2004). Abdullah *et al.* (1989) reported that repeated brief (3-second) immersion in boiling water resulted in complete elimination of hard-seededness in *Cytisus scoparius*, but low germination percentage was indicative of some damage done to the embryo. The sudden exposure of dry seeds to hot or boiling water may rupture the seed coat, thereby allowing water to permeate the seed tissues causing physiological changes and subsequent germination of the embryo (Agboola and Adedire 1998).

*Physical scarification:* The physical scarification of the seeds was more advantageous to improve the seed germination pattern in *P. marsupium*. Germination pattern in physically scarified seeds was superior over the non-scarified seeds as well as seeds treated with wet heat for 40 min. The physical scarification resulted in significantly higher (by 124%) germination compared to control within 15 days (CPG 15D). The mean DGS was three times higher than recorded in the untreated control seeds. The germination time (MGT) was significantly reduced by about 27% coupled with about 1.5 times more germination after 30 days of sowing (FGP). The seedling obtained from physically scarified seeds had dramatically improved vigor compared to untreated control as well as seedlings from wet heat treated seeds.

The rapid germination of physically scarified seeds was probably due to water and gases entering the embryo early through the cracks that fired a sequence of biochemical reactions resulting in the transformation of the embryo into a seedling early enough than other physical treatments. These results are analogous to those reported by Dayanand and Lohidas (1988) in *Pterocarpus santalinus* wherein the seeds were scarified by rubbing them with sand. The untreated seeds showed only 36.5% germination whereas scarification improved the germination up to 58.5%. The rupturing of the seed coat is a mechanism which has triggered germination in many hard-seeded species with impermeable seed coats (Bewley and Black 1994, Tigabu and Oden 2001).

*Acid scarification:* The unscarified seeds of *P. marsupium* soaked in distilled water for 15 to 45 min showed up to 24.6% germination within 15 days and

reached the maximum value of 43.3% in 30 days (Table 2). Earlier studies have also reported very poor seed germination in this species (Lakshmi *et al.* 1992, Das and Chatterjee 1993).

The acid scarification of the seeds resulted in improved germination than unscarified seeds (Table 2). The scarification with concentrated HCl for 15 min significantly improved cumulative percentage germination on 15<sup>th</sup> day (CPG 15D) by 110% over unscarified seeds. Increased duration of scarification (30 min) slightly improved seed germination further, which did not differ significantly from the 15 min scarification with concentrated HCl. However, this treatment significantly reduced MGT and at the same time significantly improved the final percentage germination (FGP) over control (Table 2). Scarification for longer duration (45 min) with concentrated HCl resulted in physically damaged seedlings with high rate of mortality (~83%).

Improved seed germination pattern was observed in the seeds scarified with concentrated H<sub>2</sub>SO<sub>4</sub> as compared to unscarified seeds and seeds scarified with concentrated HCl for 15-45 min. The 15 min treatment of concentrated H<sub>2</sub>SO<sub>4</sub> increased the germination by about two fold within 15 days over unscarified seeds. This treatment was slightly better than similar treatment of concentrated HCl but there was no significant difference in their germination pattern as indicated by the insignificant difference in the values on mean DGS and FGP but significantly different mean germination time and seedling vigour index. This suggests that the scarification with concentrated H<sub>2</sub>SO<sub>4</sub> for 15 min is enhancing germination better than concentrated HCl used for the same duration.

To soften seed coat and/or remove chemical inhibitors from the testa, the use of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) scarification is well-known in literature on germination studies of many species (Baskin and Baskin 1989). Significant improvement in germination rate of *Pachycereus hollianus* (Weber) Buxb. seeds (Cactaceae) following sulphuric acid treatment have also been documented (Godínez-Alvarez and Valiente-Banuet 1998). Sixtus *et al.* (2003) treated the seeds of *Ulex europaeus* L. with concentrated sulphuric acid for 0 (control), 30, 60, 90, 120, 150, 180, 210, 240, 270 and

**Table 2.** Effect of acid scarification on seed germination in *Pterocarpus marsupium* Roxb.

Concentrated acid scarification	Duration (min)	CPG 15D (% $\pm$ SD)	Mean DGS (% seeds per day $\pm$ SD)	MGT (Days $\pm$ SD)	FGP (% $\pm$ SD)	SVI
Distilled water	15	20.0 $\pm$ 3.3	1.0 $\pm$ 0.2	12.4 $\pm$ 1.7	40.3 $\pm$ 2.5	1.1 $\pm$ 0.0
	30	23.2 $\pm$ 3.3	1.2 $\pm$ 0.5	11.6 $\pm$ 1.3	42.5 $\pm$ 2.6	1.2 $\pm$ 0.2
	45	24.6 $\pm$ 3.3	1.2 $\pm$ 0.2	11.4 $\pm$ 1.7	43.3 $\pm$ 3.5	1.3 $\pm$ 0.2
HCl	15	52.0 $\pm$ 4.2*c	3.8 $\pm$ 0.2*c	11.2 $\pm$ 1.3c	52.6 $\pm$ 4.5b	1.3 $\pm$ 0.1b
	30	52.6 $\pm$ 3.2*c	3.8 $\pm$ 0.2*c	9.1 $\pm$ 1.2*b	61.3 $\pm$ 3.6*bc	2.2 $\pm$ 0.1*c
	45	38.6 $\pm$ 2.5*b	2.1 $\pm$ 0.2*b	10.5 $\pm$ 1.2*b	56.0 $\pm$ 2.4b	1.7 $\pm$ 0.1*c
H <sub>2</sub> SO <sub>4</sub>	15	57.3 $\pm$ 3.2*c	4.4 $\pm$ 0.3*c	8.4 $\pm$ 0.8*ab	66.0 $\pm$ 3.5*bc	3.3 $\pm$ 0.2*d
	30	78.6 $\pm$ 3.7*d	6.2 $\pm$ 0.3*d	8.1 $\pm$ 0.5*a	85.3 $\pm$ 5.4*d	3.3 $\pm$ 0.1*d
	45	84.0 $\pm$ 3.6*d	6.8 $\pm$ 0.4d	8.2 $\pm$ 0.5*a	84.0 $\pm$ 3.6*d	2.1 $\pm$ 0.1*d
HNO <sub>3</sub>	15	22.0 $\pm$ 2.1a	1.0 $\pm$ 0.1a	12.1 $\pm$ 2.1c	24.6 $\pm$ 2.2*a	1.2 $\pm$ 0.1b
	30	22.0 $\pm$ 2.2a	1.0 $\pm$ 0.0a	12.8 $\pm$ 1.8c	22.0 $\pm$ 2.2*a	1.2 $\pm$ 0.1b
	45	17.3 $\pm$ 1.8a	0.6 $\pm$ 0.0a	12.9 $\pm$ 1.9*d	19.3 $\pm$ 2.0*a	1.0 $\pm$ 0.0a

CPG 15D = Cumulative percentage germination on 15<sup>th</sup> day, Mean DGS = Mean daily germination speed, MGT = Mean germination time, FGP = Final germination percentage, SVI = Seedling vigour index, The values represent (mean  $\pm$  SD) of three independent experiments each performed on 50 seeds, \* Values differ significantly from control as per Dunnett's Test at p=0.05, Treatment means with similar letters do not differ significantly as per DMRT at p=0.05.

300 min. The highest germination (81%) was observed in seeds treated with acid for 180 and 210 min.

However, Kalimuthu and Lakshmanan (1995) have reported that the treatment with concentrated H<sub>2</sub>SO<sub>4</sub> for 10 or 15 min failed to alleviate germination in *Pterocarpus santalinus* and *P. marsupium*. Dayanand and Lohidas (1988) treated the pods of *Pterocarpus santalinus* with 1% sulphuric acid for 4 days or dipped the pods in concentrated sulphuric acid for 5 min and reported indifferent germination in control and treated seeds.

Among the acid scarification treatments with concentrated HCl, H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> for 15-45 min durations, the treatment of concentrated H<sub>2</sub>SO<sub>4</sub> for 30 min was the most effective in enhancing the percentage germination as well as for improving germination pattern. The lowest MGT of 8.1 days was observed in the seeds scarified with H<sub>2</sub>SO<sub>4</sub> for 30 min indicating fastest germination as compared to all other treatment. This treatment resulted in the significantly higher CPG 15D which was 3 fold more than unscarified seeds and 1.5

fold more than observed in seeds scarified with concentrated HCl for the same duration. This was accompanied by about five time faster mean DGS compared to unscarified seeds. The FGP was significantly increased by about 96% over unscarified seeds and about 40% more over seeds scarified with concentrated HCl for the same duration. Although a slightly better germination was observed in the seeds scarified for 45 min with concentrated H<sub>2</sub>SO<sub>4</sub>, the seedlings produced were morphologically damaged, weak and slow growing. The cotyledonary leaves showed chlorosis and burnt area along the margins.

Acid scarification treatments improve the germination by making seed coats more permeable to water and gases (Bonner *et al.* 1974). In the seeds scarified for 30 min with concentrated H<sub>2</sub>SO<sub>4</sub>, hydrolysis of the organic structural components in the fruit coat was observed (Fig 1e). The strong mineral acids hydrolyze the organic structural components in the fruit or seed coat thereby making these structures weak and permeable and facilitate the seed germination by breaking the seed-coat-imposed dormancy (Gordon and Rowe 1982).

The scarification of seeds with concentrated  $\text{HNO}_3$  resulted in poor seed germination pattern as compared to unscarified seeds. With increase in the duration of acid treatment, there was gradual decrease in the CPG 15D, mean DGS and FGP. These seeds showed slowest germination as compared to unscarified seeds and seeds scarified with concentrated HCl and  $\text{H}_2\text{SO}_4$ . This was evident from the longer spread of germination (MGT) by about 4-5 days compared to the effective treatment of  $\text{H}_2\text{SO}_4$ . The seedlings produced after the  $\text{HNO}_3$  treatment showed reduced seedling vigour index as compared to unscarified seeds. The  $\text{HNO}_3$  treatments significantly decreased FGP as compared to unscarified seeds and seeds scarified with HCl and  $\text{H}_2\text{SO}_4$  for different durations, indicating inhibitory effect of  $\text{HNO}_3$  on seed germination and seedling vigour in *P. marsupium*. The results of the present investigation are on the similar line of results reported by Kalimuthu and Lakshmanan (1995), wherein the treatment of concentrated  $\text{HNO}_3$  was reported to be inhibitory for seed germination in *P. marsupium* as well as *P. santalinus*. The treatment of acid scarification was ineffective or harmful in some genera (Aduradola *et al.* 2005). The 8-9 months old seedlings of about 0.4 m height developed from seeds scarified with concentrated  $\text{H}_2\text{SO}_4$  for 30 min (Fig. 1f), were transferred to the field in the rainy season. All the seedlings transferred to field grew well and attained the height of about 1 m after one year of transfer of seedlings to the natural conditions.

The results on seed germination in the present investigation are suggestive of the existence of the pericarp-imposed dormancy in this species. The method reported here on scarifying the seeds with concentrated  $\text{H}_2\text{SO}_4$  for 30 min was the most effective in alleviating this pericarp-imposed dormancy. The method can be used to induce uniform and rapid seed germination and preparation of seedlings for plantation and conservation of *P. marsupium* Roxb.

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