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# Effect of Pre-treatments on Germination of *Pterocarpus marsupium* Roxb. for Developing Field Gene-Banks in Eastern India

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors YM and AS have contributed towards the conceptualization, designing and supervision of the study. Author RK and AM performed material collection and field experiments. Author AM prepared the first draft of the manuscript and performed statistical analyses. All authors edited and approved the final manuscript.*

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## ABSTRACT

**Aims:** *Pterocarpus marsupium* Roxb. is an important timber species with high medicinal properties belonging to the Fabaceae family. The species is severely threatened by anthropogenic and ecological pressures coupled with less natural regeneration. No results in vegetative propagation methods and very less germination is reported for the species under nursery conditions. This study investigates the germination potential of *P. marsupium* seeds under the prevailing climatic conditions of regions in eastern India.

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**Place and Duration of Study:** The study was carried out at the ICFRE - Institute of Forest Productivity (IFP), Lalgutwa, Ranchi, during the 24th to the 32nd Meteorological Standard Week (MSW) of 2022, spanning from June to August.
**Methodology:** Seeds were collected from three CPTs selected from natural forests of Jharkhand, Bihar and West Bengal and the experiment was carried out following split plot design. Observations on several germination parameters and seed vigour indices were recorded until eight weeks after sowing and subjected to factor multivariate analysis of variance (MANOVA) in SPSS 25.0.
**Results:** The experiment revealed that alternate wetting and drying of seeds significantly ($P$ <. 001) outperformed all other treatments with a highest germination percentage of 60±1.196%. Accessions from Jharkhand and West Bengal exhibited significantly ($P$ < .001) better vigour indices (1959.8, 1855.35 for SVI-I and 538.715, 527.742 for SVI-II, respectively) compared to the accession from Bihar (1796.49 and 532.618 for SVI-I and SVI-II). Although the study did not find any significant interaction between the treatments and accession, there was a statistically significant impact on the aggregate dependent variables ($F$ (126,503.279) = 1.579, $P$ <. 001; Wilks' $\Lambda$ = .077).
**Conclusion:** The results of this study report an economically feasible and rapid seed treatment method for establishing field gene banks of *P. marsupium* in eastern India to aid its *ex-situ* conservation practices.

*Keywords: Ex-situ conservation; germination; nursery technique; P. marsupium.*

## 1. INTRODUCTION

*Pterocarpus marsupium* Roxb., commonly known as ‘Bijasal’ or ‘Indian kino tree’, a member of the Fabaceae family, is an important forest tree with significant therapeutic potential. The species is indigenous to the Indian subcontinent, and is dispersed across India; including the states of Gujarat, Rajasthan, Uttar Pradesh, Madhya Pradesh, Jharkhand, Bihar and Orissa as well as the Western and Eastern Ghats where it typically grows on hills or hilly terrain at an average elevation of 150-500 m [1]. Due to its therapeutic applications in numerous ethnobotanical practices, *P. marsupium* is a vital species for local dwellers living in the species' natural habitat. Several parts of the plant, including leaves, barks, stems, heartwood, flowers, and 'Kino-gum', are used to treat different ailments [2]. *P. marsupium* has historically been used to cure leucoderma, elephantiasis, diarrhea, cough, hair discolouration, and rectalgia [3]. It is also beneficial for treating jaundice, fever, wounds, diabetes, stomach aches, and ulcers [4]. Nonetheless, anthropogenic overexploitation of genetic resources, along with poor natural regeneration, survival, and growth rates, has elevated the probability of species extinction [5]. A number of species from the same genus have already been red-flagged by the concerned authorities. In 2017, *P. santalinus* and *P. erinaceus* were listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) appendices, which prohibits the export of wood from these species for conserving their populations. *P. marsupium, P. santalinus, P. indicus*, and *P. angolensis* have been enlisted in the Red Data List of the International Union for Conservation of Nature (IUCN) [6].

According to the morphogenesis and reproductive biology of *P. marsupium*, apart from seed germination, there is no significant propagative method [7]. The seeds from freshly harvested ripe fruits in April-May are reported to be the best to be used to grow seedlings [8]. However, the germination rate is reported to be less than 30% due to the hard shell of the pods combined with short lifespan and poor pod formation [9]. Mishra et al. [10] reported an interesting positive correlation between seed size and mass with seed emergence and seedling development in the species. *P. marsupium* grows in a wide range of environmental conditions in India and shows a remarkable variation in growth parameters across different agro-climatic zones [11]. Besides marked variations in germination parameters have also been reported recently in plus trees of the species selected from different agro-climatic zones of Chhattisgarh [12].

Due to the high economic value of timber and numerous reported examples of its bioactivity, *Pterocarpus* species have attracted attention recently in experimental research [13]. Nevertheless, research on *Pterocarpus* is still primarily concentrated on improvement of the species for timber production [14], taxonomic identification [7,15], and cultivation for timber

[16]. As a corollary, it is imperative to uphold this valuable tree species and support the creation of massive *ex-situ* plantings for the conservation of this forest genetic resource. Comparing various protocols for increasing nursery produce and gratification of the requisite planting materials is one of the primary initiatives to be taken for *ex-situ* conservation of *P. marsupium*. Therefore, the purpose of this study was to investigate the germination potential of *P. marsupium* seeds with different pre-treatments under the prevailing climatic conditions of regions in eastern India with the long-term objectives of selecting premier seed sources for establishing seed orchards conserving the prevailing genetic diversity of *P. marsupium.*

## 2. MATERIALS AND METHODS

### 2.1 Experimental location

The experiment was conducted at the ICFRE – Institute of Forest Productivity (IFP), Lalgutwa, Ranchi in the nursery. The nursery is located with a mean height of 680 m above the mean sea level (MSL) at 23.353°N and 85.2438°E. The terrain is mostly flat with a little inclination toward the south-west. The study was carried out between the 24th to 32nd Meteorological Standard Week (MSW) of 2022, consisting of the months of June to August. The data referring to the climatic conditions prevalent in the institute during the experiment was obtained from the Agrometeorological station of Birsa Agricultural University, Ranchi and depicted in Fig. 1.

### 2.2 Experimental Material

Mature pods of the species were harvested in the month of April-May 2022 from three full-grown Candidate Plus Trees (CPTs) of *P. marsupium* situated at the forests of Hazaribag Division, Jharkhand (24.01638°N, 85.39410°E), Durgapur Division, West Bengal (23.59559°N, 87.43164°E) and Nawada Division, Bihar (24.65527°N, 85.63861°E). These CPTs were identified as potential seed trees during various field surveys conducted before seed collection by ICFRE-IFP in the aforementioned forests. The collected pods were brought to the nursery of the institute, sun-dried for fifteen days and seeds were extracted from the pods manually with scalpel. Seeds were graded for selecting quality seeds of a large seed size class as defined by Mishra et al. [10]. Accordingly, seeds with a minimum average diameter of 20 mm and test weight of 12 g were selected for this experiment. These selected seeds were fumigated with 0.2% Bavistin spray for 30 min to avoid the fungal infestation during the experiment.

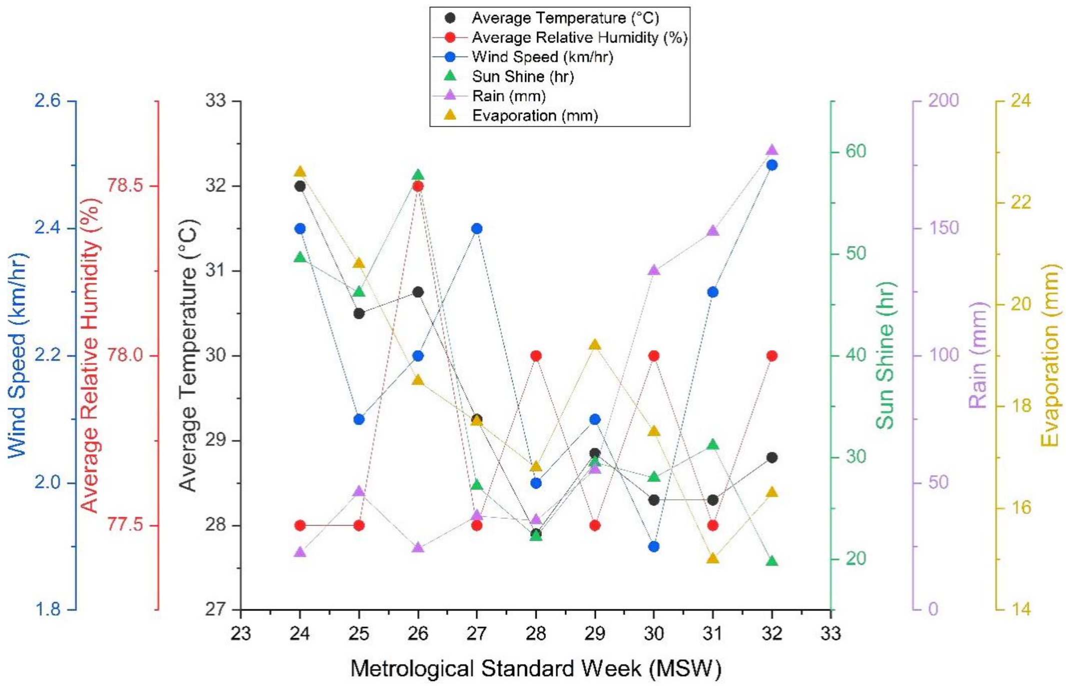


**Fig. 1. Meteorological conditions in ICFRE-IFP during the study (Authors' creation with Origin Pro v.22)**

## 2.3 Experimental Layout

The experiment was laid out following a Completely Randomized Block Design (CRBD) with eight treatments and four replications (Table 1). The seeds were sown at a spacing of 10X20 cm in the experimental field on 15th June 2022 after applying different treatments in raised sand bed. Throughout the trial period, the standard nursery procedures described by Mishra et al. [11] were followed (Fig. 2).

## 2.4 Experimental Parameters

The following germination parameters were recorded every week until 56 DAS (8 weeks) in this study –

### 2.4.1 Germination percentage (G)

The germination percentage or germinability was computed according to Reed et al. [17] using the following formula.

$$Germination\ \% = \frac{Total\ number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown}\ X\ 100$$

### 2.4.2 Mean germination time (MGT)

MGT determines the average time frame required for seeds to attain highest germination and is expressed using the same units as germination counts [18] and it was calculated as following.

$$MGT = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where, $t_i$ represents the period from the commencement of the experiment to the $i^{th}$ observation (in days); $n_i$ is the number of seeds that germinated on the observation day in $i^{th}$ time.

### 2.4.3 Germination rate index (GRI)

The GRI represents the percentage of seeds that germinates on each day of the germination phase [19] and it was computed as per the following formula.

$$GRI = \frac{\sum_{i=1}^{n} G_i}{T}$$

where, $G_i$ represents the percentage of seed germinated per day of observation, $T$ represents germination period.

### 2.4.4 Germination index (GI)

The GI was computed according to the updated following formula given by Yang et al. [20].

$$GI = \sum_{i=1}^{k} \frac{n_i}{t_i}$$

where, $n_i$ denotes the number of seeds germinated on the $i^{th}$ and $t_i$ denotes the cumulative number of days of the experiment on which the observation is taken.

### 2.4.5 Seed vigour indices (SVI)

International Seed Testing Association (ISTA) referred to the sum of the characteristics of the seed that impact the degree of performance and efficiency of the seed or seed lot during germination and seedling emergence as the seed vigour index [21]. Following two types of seed vigour indices were calculated according to Powell [22].

*Seed Vigour Index-I = Germination percentage × (Average shoot length of seedlings + Average root length of seedlings)*

*Seed Vigour Index-II = Germination percentage × Average seedling dry weight*

For each replication and treatment, one sample seedling was chosen at random to determine the average shoot-root length (Fig. 3) and dry weight of the seedlings. Shoot and root length was freshly taken and dry weight was taken after keeping the plants in a hot air oven at 37±2° C for 24 h.

## 2.5 Statistical Analysis

OPSTAT software was used to analyse the study's observations primarily for descriptive statistics. The arc sine transformation for the data of germination percentage was done in OPSTAT for further statistical analysis. Factorial Multivariate Analysis of Variance (MANOVA) with Duncan's Multiple Range Test (DMRT) was carried out in IBM SPSS 25.0 for finding the best treatment, accession, and their interaction with statistical significance.

## 3. RESULTS AND DISCUSSION

The findings demonstrated that the diverse physical and chemical treatments administered to the seeds resulted in considerable variances between the analysed parameters (Table 2). The

**Table 1. Details of treatments followed in the study**

| Treatments | Details of the treatments |
|---|---|
| T1 | Control |
| T2 | True seed (Without seed coat) |
| T3 | 5% $H_2SO_4$ soaking for 15 min |
| T4 | 10% $H_2SO_4$ soaking for 15 min |
| T5 | Hot and cold-water Treatment: Dipping in hot water (65°C) for 1h followed by normal cold water (20°C) for 12h |
| T6 | Alternate wetting and drying treatment: Dipping in normal water (20°C) for 24 h, followed by shade drying for 12 h and again dipping in normal water (20°C) for 24 h |
| T7 | 100 ppm $GA_3$ dipping for 1 h |
| T8 | 100 ppm IBA dipping for 1 h |


**Fig. 2. Standard nursery techniques followed during the study. (A) Quality seed of *P. marsupium* graded for the study; (B) Line sowing of seeds according to the design of experiment; (C) Sand bed with plastic mulching after sowing; (D) Shifting of seedlings to polybags after noting down all observations (after 56 DAS)**

highest germination was 60% in the Jharkhand accession (T6). The lowest germination of 12.5% was in the Bihar accession (T1). DMRT revealed that the germination percentage observed in T1 and T2 were at par whereas T3, T4, T5, T7 and T8 were at par across the two accessions of Jharkhand and West Bengal ($CD_{5\%}$ = 5.74). However, in Bihar accession, a different pattern of subsets was observed, where no critical difference among T1, T2, T3, T4, T8 were observed and T5, T7 were at par. T6 exhibited highest germinability across all three accessions. 60%, 56.25% and 53.25% germination had been achieved in Jharkhand, West Bengal and Bihar accession respectively, with the application of T6, which significantly differed from all other treatments ($P < .001$). The lowest mean germination time of 18.50 days was in T6 and highest of 34.42 days in T1 across the three accessions; no significant difference was observed due to different treatments.

The highest germination rate index of 32.958%$day^{-1}$ was observed with T6 in the Jharkhand accession and the lowest germination rate index of 2.604%$day^{-1}$ with T1 in the Bihar accession. The germination rate index observed in T1 and T2 were at par T3, T4, T5, T7 and T8 were at par ($CD_{5\%}$ = 6.55). Germination rate index in T6 significantly differed from all other treatments across all the accessions ($P < .001$). Similar results were obtained for the germination index, where the highest germination index of 0.942 with T6 in Jharkhand accession and the lowest of 0.074 with T1 in Bihar accession. Germination index with the treatment T6 significantly differed from all other treatments across all the accessions (*P* < .001). The highest seed vigour in both of the indices were in T6. The highest seed vigour index-I of 1959.8 and seed vigour index-II of 538.715 was in Jharkhand accession with treatment T6. The lowest seed vigour index-I of 337.455 and seed vigour index-II of 95.367 was in the Bihar accession with treatment T1. Both of the vigour indices observed in T1 and T2 were at par and T3, T4, T5, T7 and T8 were at par ($CD_{5\%,\ SV1}$ = 313.73, $CD_{5\%,\ SV2}$ = 78.86). Seed Vigour index-I and II significantly differed in T6 from all other treatments across all the accessions (*P* < .001).

No significant variation in all germination parameters except the germination index among the three accessions was observed statistically. However, significant variations in seed vigour indices among the studied accessions were revealed by MANOVA. Statistically significant variation among the highest germination indices across the accessions in T6 were observed (*P* = 0.05). Considering the highest seed vigour indices observed in T6, Jharkhand, West Bengal and Bihar accessions expressed vigour index-I of 1959.8, 1855.35 and 1796.49 with vigour index-II of 538.715, 527.742 and 532.618, respectively. Seed vigour index-I and vigour index-II significantly varied among the studied accessions at *P* = .05 and *P* = .001 respectively. Additionally, variation arising due to the interaction and intercept of treatments and accessions were also computed through multivariate tests. Although, no significant difference was observed due to the interaction of treatments and accessions, there was a statistically significant interaction effect between treatments and accessions on the combined dependent variables, *F* (126, 503.279) = 1.579, *P* <. 001; Wilks' Λ = .077 (Table 3). Other indices like Pillai's Trace, Hotelling's Trace and Roy's Largest Root in the multivariate test attested the same results.

Perusal of data obtained from statistical analysis performed on this experiment revealed that the alternate wetting and drying of seeds (T6) significantly outperformed all other treatments implied in the study. The control (T1) and the true seed without seed coat (T2) performed very poorly, which attests the poor natural regeneration of *P. marsupium*. When germination parameters were investigated, all other treatments failed to meet expectations. This might be because the treatments were unable to break the seeds' dormancy. Although germination percentage is the most popular and straightforward to compute metric to determine which treatment is doing the best, it offers no indication of the speed or uniformity of germination [23]. Given that this study was carried out in an environment that was uniform, it is quite likely that the mean germination time did not significantly change among the various treatments [18]. According to some authors, the Germination Index (GI) is the most complete measure for evaluating germination potential because it takes both germination percentage and rate into account [23].

**Fig. 3. Estimation of shoot and root length of sample *P. marsupium* seedling**

**Table 2. Statistics on different germination parameters as the effect of different treatments applied on different accessions in the experiment**

| Treatments | Germination % (G)[1] | | Mean germination time (MT) (days) | | Germination rate index (GRI) (% $day^{-1}$) | | Germination Index (GI) | | Seed Vigor Index – I | | Seed Vigor Index – II | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. |
| **ACCESSION 1: WEST BENGAL (Durgapur)** | | | | | | | | | | | | |
| T1 | 15 (22.634)$^{a}$ | 1.661 | 31.792$^{a}$ | 2.492 | 3.333$^{a}$ | 0.259 | 0.095$^{a}$ | 0.008 | 421.227$^{a}$ | 52.569 | 122.486$^{a}$ | 15.507 |
| T2 | 21.25 (27.327)$^{a}$ | 1.719 | 27.446$^{a}$ | 1.968 | 5.729$^{a}$ | 0.904 | 0.164$^{a}$ | 0.026 | 614.609$^{a}$ | 74.894 | 165.165$^{a}$ | 19.551 |
| T3 | 23.75 (28.98)$^{b}$ | 2.426 | 24.938$^{b}$ | 4.002 | 8.459$^{b}$ | 2.439 | 0.242$^{b}$ | 0.07 | 674.982$^{b}$ | 91.508 | 192.715$^{b}$ | 35.937 |
| T4 | 30 (33.097)$^{b}$ | 2.175 | 20.854$^{b}$ | 1.417 | 13.042$^{b}$ | 1.956 | 0.373$^{b}$ | 0.056 | 967.776$^{b}$ | 151.042 | 258.684$^{b}$ | 17.446 |
| T5 | 35 (36.207)$^{b}$ | 1.737 | 19.031$^{b}$ | 1.312 | 17.563$^{b}$ | 1.254 | 0.502$^{b}$ | 0.036 | 1,125.55$^{b}$ | 126.439 | 288.37$^{b}$ | 26.544 |
| T6 | 56.25 (48.582) | 1.381 | 21.043$^{b}$ | 0.361 | 26.875 | 1.275 | 0.768 | 0.036 | 1,855.35 | 107.43 | 527.742 | 20.836 |
| T7 | 27.5 (31.555)$^{b}$ | 1.567 | 21.45$^{b}$ | 2.175 | 11.584$^{b}$ | 0.485 | 0.331$^{b}$ | 0.014 | 890.509$^{b}$ | 91.664 | 221.639$^{b}$ | 34.023 |
| T8 | 30 (33.097)$^{b}$ | 2.175 | 20.388$^{b}$ | 2.735 | 13.979$^{b}$ | 2.386 | 0.4$^{b}$ | 0.068 | 927.259$^{b}$ | 127.343 | 243.499$^{b}$ | 35.073 |
| C.D. at 5% | 5.536 | | 6.741 | | 4.617 | | 0.132 | | 314.367 | | 78.683 | |
| SE(m) | 1.886 | | 2.296 | | 1.573 | | 0.045 | | 107.067 | | 26.798 | |
| SE(d) | 2.667 | | 3.247 | | 2.224 | | 0.064 | | 151.415 | | 37.898 | |
| C.V. | 11.538 | | 19.65 | | 25.02 | | 25.021 | | 22.91 | | 21.223 | |
| **ACCESSION 2: JHARKHAND (Hazaribag)** | | | | | | | | | | | | |
| T1 | 16.25 (23.722)$^{a}$ | 0.944 | 32.813$^{a}$ | 0.964 | 3.542$^{a}$ | 0.277 | 0.101$^{a}$ | 0.008 | 433.132$^{a}$ | 28.629 | 119.307$^{a}$ | 15.974 |
| T2 | 18.75 (25.524)$^{a}$ | 1.734 | 30.713$^{a}$ | 2.023 | 4.542$^{a}$ | 0.807 | 0.13$^{a}$ | 0.023 | 520.176$^{a}$ | 71.226 | 137.101$^{a}$ | 22.692 |
| T3 | 31.25 (33.962)$^{b}$ | 0.765 | 19.584$^{b}$ | 2.255 | 14.813$^{b}$ | 1.402 | 0.423$^{b}$ | 0.04 | 852.551$^{b}$ | 26.562 | 244.626$^{b}$ | 25.849 |
| T4 | 33.75 (35.404)$^{b}$ | 2.296 | 19.236$^{b}$ | 1.532 | 15.75$^{b}$ | 1.488 | 0.45$^{b}$ | 0.042 | 1,001.15$^{b}$ | 72.901 | 261.696$^{b}$ | 22.748 |
| T5 | 36.25 (36.996)$^{b}$ | 0.74 | 19.625$^{b}$ | 1.675 | 17.979$^{b}$ | 0.778 | 0.514$^{b}$ | 0.022 | 1,187.55$^{b}$ | 56.846 | 274.025$^{b}$ | 30.53 |
| T6 | 60 (50.763) | 1.196 | 18.495$^{b}$ | 0.812 | 32.958 | 1.838 | 0.942 | 0.053 | 1,959.80 | 52.861 | 538.715 | 19.598 |
| T7 | 33.75 (35.256)$^{b}$ | 4.266 | 18.879$^{b}$ | 1.812 | 16.271$^{b}$ | 2.495 | 0.465$^{b}$ | 0.071 | 1,058.90$^{b}$ | 260.621 | 245.958$^{b}$ | 46.266 |
| T8 | 28.75 (32.395)$^{b}$ | 0.802 | 20.884$^{b}$ | 1.54 | 13.396$^{b}$ | 2.065 | 0.383$^{b}$ | 0.059 | 885.287$^{b}$ | 88.429 | 238.291$^{b}$ | 10.149 |
| C.D. at 5% | 5.74 | | 4.822 | | 4.57 | | 0.131 | | 317.734 | | 77.095 | |
| SE(m) | 1.955 | | 1.642 | | 1.557 | | 0.044 | | 108.214 | | 26.257 | |
| SE(d) | 2.765 | | 2.322 | | 2.201 | | 0.063 | | 153.037 | | 37.133 | |
| C.V. | 11.414 | | 14.579 | | 20.884 | | 20.886 | | 21.921 | | 20.397 | |
| **ACCESSION 3: BIHAR (Nawada)** | | | | | | | | | | | | |
| T1 | 12.5 (20.602)$^{a}$ | 1.256 | 34.417$^{a}$ | 1.543 | 2.604$^{a}$ | 0.337 | 0.074$^{a}$ | 0.01 | 337.455$^{a}$ | 40.486 | 95.367$^{a}$ | 13.948 |
| T2 | 18.75 (25.524)$^{a}$ | 1.734 | 32.2$^{a}$ | 3.333 | 4.408$^{a}$ | 0.753 | 0.126$^{a}$ | 0.021 | 533.18$^{a}$ | 61.488 | 145.232$^{a}$ | 12.685 |
| T3 | 30 (33.097)$^{b}$ | 2.175 | 22.881$^{b}$ | 2.894 | 11.875$^{b}$ | 1.921 | 0.339$^{b}$ | 0.055 | 899.699$^{b}$ | 107.014 | 249.519$^{b}$ | 19.501 |
| T4 | 33.75 (35.389)$^{b}$ | 2.581 | 24.892$^{b}$ | 2.05 | 13.783$^{b}$ | 3.35 | 0.394$^{b}$ | 0.096 | 1,008.80$^{b}$ | 154.931 | 295.416$^{b}$ | 37.866 |
| T5 | 41.25 (39.94)$^{c}$ | 0.724 | 20.149$^{b}$ | 0.715 | 21.125$^{b}$ | 1.147 | 0.604$^{b}$ | 0.033 | 1,221.43$^{b}$ | 51.189 | 371.032$^{b}$ | 15.017 |
| T6 | 53.75 (47.141) | 1.81 | 21.805$^{b}$ | 2.514 | 25.741 | 4.269 | 0.735 | 0.122 | 1,796.49 | 111.865 | 532.618 | 38.155 |

| Treatments | Germination % (G)[1] | | Mean germination time (MT) (days) | | Germination rate index (GRI) (% day$^{-1}$) | | Germination Index (GI) | | Seed Vigor Index – I | | Seed Vigor Index – II | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. |
| T7 | 40 (39.2)$^c$ | 1.196 | 22.014$^b$ | 0.762 | 18.271$^b$ | 0.993 | 0.522$^b$ | 0.028 | 1,250.34$^b$ | 66.668 | 355.501$^b$ | 28.403 |
| T8 | 35 (36.232)$^b$ | 1.229 | 23.531$^b$ | 1.829 | 15.033$^b$ | 1.918 | 0.43$^b$ | 0.055 | 1,092.69$^b$ | 47.503 | 322.098$^b$ | 23.708 |
| C.D. at 5% | 4.948 | | 6.3 | | 6.549 | | 0.187 | | 259.961 | | 74.999 | |
| SE(m) | 1.685 | | 2.146 | | 2.23 | | 0.064 | | 88.537 | | 25.543 | |
| SE(d) | 2.383 | | 3.034 | | 3.154 | | 0.09 | | 125.211 | | 36.123 | |
| C.V. | 9.729 | | 17.004 | | 31.624 | | 31.633 | | 17.403 | | 17.268 | |

*[1] Arcsine transformation of data is performed before statistical analysis. Data outside parenthesis indicate the original values and inside parenthesis indicate the transformed data.*
*[a] Means followed by the same letters in each column are not significantly different based on the Duncan's Multiple Range Test (DMRT) at α = 0.05*
*C.D. stands for Critical Difference at α = 0.05; S.E.(m) stands for Standard Error for mean; S.E.(d) stands for Standard Error for difference, C.V. stands for Coefficient of Variation.*

**Table 3. Results from Multivariate Analysis of Variance (MANOVA)**

| Source of Variation | *d.f.* | Sum of square values | | | | | | Multivariate Tests [a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Germination % (G) [1] | Mean germination time (MT) (days) | Germination rate index (GRI) (% day$^{-1}$) | Germination Index (GI) | Seed Vigor Index - I | Seed Vigor Index - II | Pillai's Trace | Wilks' Lambda | Hotelling's Trace | Roy's Largest Root |
| Treatment | 7 | 1908.185** | 297.959 | 758.285** | 0.619** | 2380649.6** | 192024.2** | 2.11** | 0.0129** | 14.05** | 10.38** |
| Accession | 2 | 94.010 | 61.481 | 45.087 | 0.037* | 56262.4* | 17991** | 1.09 | 0.1466 | 4.19 | 3.76 |
| Treatment X Accession | 14 | 42.225 | 9.738 | 18.800 | 0.015 | 33961.6 | 4227.8 | 1.90** | 0.0765** | 3.84** | 2.18** |
| Intercept | 1 | 96901.042** | 53970.574** | 18442.868** | 15.055** | 92166269** | 6926867.6** | 0.99** | 0.0004** | 2337.47** | 2337.47** |
| Corrected Model (Type III Sum of Squares) | 23 | 14136.458** | 2345.012** | 5661.365** | 4.622** | 17252535** | 1439342.2** | | | | |
| | $R^2$ (#) | 0.849 (0.801) | 0.660 (0.552) | 0.857 (0.811) | 0.857 (0.811) | 0.853 (0.806) | 0.879 (0.841) | | | | |

** and ** represents significant at P<0.05 and P<0.001 respectively.*
*[1]Arcsine transformation of data is performed before statistical analysis.*
*[a] Design: Intercept + Treatment + Accession + Treatment X Accession.*
*[#] In parenthesis Adjusted $R^2$ Values are represented.*

For evaluating the germination ability of seeds, seed vigour indices are also regarded as equally significant [24]. The survival of the seedlings that emerged from the seeds was taken into consideration by both seed vigour indices. Improved seedling length (calculated using the Seed Vigor Index-I) and dry weight (calculated using the Seed Vigor Index-II) show that the seedlings are thriving well under the prevailing conditions. According to Kandasamy et al. [25], analysing germination characteristics alone without factoring in seedling survival yields skewed results and jeopardizes the study's potential for practical use. The feasibility of this experiment is thus assured by the assessment of both vigour indices. Accessions from Jharkhand and West Bengal showed noticeably superior seed vigour compared to accession from Bihar, albeit statistically showing no significant difference in germination potential among the accessions. This demonstrated the possibility of genetic diversity among the accessions, which must be investigated in future studies for the conservation of the species.

## 4. CONCLUSION

Conserving the depleting genetic resources of *Pterocarpus marsupium* is imperative for maintaining genetic diversity and enabling future tree improvement programs. By establishing *ex-situ* conservation efforts of selected superior genotypes, researchers can identify individuals with desirable traits such as faster growth rates, higher biomass production, resistance to pests and diseases, and superior wood quality. These superior genotypes can serve as valuable resources for future breeding programs for the species. In this study, the alternate wetting and drying proved to be the best seed treatment for growing *P. marsupium* under nursery conditions in eastern India. *P. marsupium* seeds are characterized by their very low germination percentage under natural conditions. Alternate wetting and drying treatment enhanced the imbibition process, which rapidly initiated germination. This treatment also exhibited the highest vigour, indicating that the seeds had a higher possibility of survival in the field and performed well under the prevailing conditions. Furthermore, no costly or harmful substances such as acids or growth regulators were utilized in this treatment, nor were any labour-intensive approaches used. Therefore, this specific treatment is considered more practical from an economic standpoint. This implies that the treatment may be used across all accessions and would produce better outcomes than any other treatment. In order to preserve the genetic resources of the species *ex-situ*, the results of this experiment may readily be employed to break the dormancy of *P. marsupium* seeds in eastern Indian nurseries for their mass multiplication. As future implications of the study, forest managers can also take proactive steps to establish seed orchards, develop improved varieties, and ensure the sustainable management and utilization of this valuable forest resource for future generations.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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