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Evaluation of Seed Germination of Chironji (*Buchanania cochinchinensis* (Lour.) M. R. Almedia) under Nursery Conditions

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Abstract

The study was carried out during the month of June to August in the year, 2022 in the FGR field in the nursery of Institute of Forest Productivity (IFP), Lalgutwa, Ranchi, Jharkhand (835303), India to find out effective germination techniques to expedite nursery propagation of Chironji, an economically significant non-timber forest produce (NTFP) in red and lateritic zone (RLZ) of eastern India. The research evaluated seven pre-treatments to enhance germination potential using seeds harvested from a Candidate Plus Tree (CPT) in the natural forest of Burdwan Forest Division, West Bengal. The study employed a completely randomized design with four replications, evaluating various germination parameters over a 50-day period. The combination of hot water treatment and mechanical scarification (T_6) exhibited highest germination of 87.5% and also outperformed all other pre-treatments in every germination parameter evaluated. It also yielded the highest seed vigour indices (SVI) of 1303.53 (SVI-I) and 109.155 (SVI-II). This method proved to be the most effective for breaking seed dormancy and promoting robust seedling development of Chironji for the prevailing agroclimatic conditions in RLZ of eastern India. The outcomes of this study demonstrate an affordable and swift seed treatment method without relying on costly growth hormones or chemicals contributing to conservation efforts. Given the challenges of seed dormancy in this species, marginal farmers and state forest departments (SFDs) can employ the pre-treatment reported in the study for commercial cultivation of Chironji as well as contributing to conservation efforts of the species.

Keywords: Chironji, germination, NTFP, nursery technique, seed dormancy

1. Introduction

Chironji (*Buchanania cochinchinensis* (Lour.) M.R. Almedia), a native species of Indian subcontinent often found as wild in the forests, belongs to the family Anacardiaceae. It has enormous potential to improve the socioeconomic condition of tribal and rural residents as Non-timber Forest Produce (NTFP). Fruits, fuel, food for animals, and medications are just a few of the uses for it. The species has significant possibilities for cultivation as commercial crop and can bring in considerable economic benefits (Srivastava et al., 2021). However, its production sector is not very much organized and there are a very few reports on commercial cultivation procedure of the species (Malakar et al., 2022). Chironji is threatened by several anthropogenic pressures like destructive over-harvesting and illegal felling which have endangered the species habitat significantly (Singh et al., 2022). Chironji exhibits exceptional tolerance to challenging climatic and soil conditions (Agrawal and Quraishi, 2023). The species can flourish practically everywhere like nutrient-lacking soil including gravelly red soils, salty soils, and sodic soils (Garai et al., 2023). Additionally, it can survive well even in the crevices of barren rocks. Its deep taproots enable it to sustain increased water potentials when moisture is lacking and it can easily tolerate severe drought conditions (Zhang et al., 2007). Chironji is frequently found to be growing spontaneously in degraded forest areas and wastelands, however it cannot thrive in waterlogged environments (Avani et al., 2019). Additionally, it can tolerate a variety of temperatures. The species favours a dry, sub-humid region and can endure temperatures as high as 45 °C and low to moderate annual rainfall of 750–1200 mm (Chauhan et al., 2012). Hence the species can prove to be very much important for reclamation of wastelands and eroded forest areas (Rajkumar et al., 2023). Seeds are the preferred propagule for Chironji, as vegetative propagation is found to be much complicated and cumbersome with less success rate in the species (Malakar et al., 2023).

Physical seed dormancy has been documented as a significant issue in Chironji (Vijay et al., 2022). The freshly extracted seeds show very poor germination potential due to the hard seed coat on the kernels (Sharma, 2012). According to Singh et al. (2019), Chironji seeds should be harvested from matured fruits in April-May and put in polybags or on raised beds in June-July for optimal seed propagation. Several authors have applied different mechanical, physical and chemical seed treatments to achieve the highest germination percentage from Chironji seeds (Shukla and Solanki, 2000; Narayan et al., 2014; Joshi et al., 2017; Nimbalkar et al., 2017; Ajith et al., 2018). All these studies imply the same concept that elevated level of imbibition in seeds aids in the hard seed coat breaking considerably faster, and it proved to be the most efficient and cost-effective approach to improve seed germination percentage in Chironji (Agrawal and Quraishi, 2024). The authors reported a wide variation in germination percentages ranging from 31% to 85% while applying different pre-treatments, raising the question of what is the optimal seed treatment for Chironji that can be utilized without any confusion. The germination potential of the seeds plays the key regulatory role in the regeneration of natural Chironji populations in forests (Malakar et al., 2023). Therefore, for conserving the depleting genetic resources of the species, agroclimatic-zone specific germination study should be carried out. The present study had been taken up to investigate the germination of Chironji seeds under the prevailing climatic conditions of red and lateritic zone of eastern India.

2. Materials and Methods

2.1. Experimental location

The study was carried out during the month of June to August in the year 2022. in the FGR field in the nursery of Institute of Forest Productivity (IFP), Lalgutwa, Ranchi, Jharkhand (835303), India. The field is situated at 23.353°N, 85.2438°E with an average altitude of 680 m above mean sea level (MSL). The site is almost plain with a mild slope towards south-west. The study was conducted from the month of June to August in the year 2022. The data pertaining to the climatic conditions prevailing in the institute during the experiment (24^{th}–31^{st} Meteorological Standard Week) was collected from the agrometeorological station of Birsa Agricultural University, Ranchi, Jharkhand. Maximum temperatures exhibited a gradual decline from 37.7°C in the 24^{th} MSW to 32.0°C in the 31st MSW, while minimum temperatures fluctuated between 23.4°C and 26.3°C. Relative humidity remained consistently high, with morning readings between 85-88% and afternoon readings steady at 69–70%. Rainfall patterns were irregular, with the highest precipitation of 37 mm recorded in early July (2^{nd} to 8^{th}). Sunshine hours varied significantly, peaking at 57.7 hours in late June and dropping to a low of 22.2 hours in mid-July. Evaporation rates generally decreased over the period, from 22.6 mm to 15 mm, potentially correlating with declining temperatures and increased rainfall in later weeks.

2.2. Experimental material

A full-grown Candidate Plus Tree (CPT) of Chironji situated at the forest of Khandari beat under Panagarh Range of Burdwan forest division, West Bengal (23.49512° N, 87.53935° E) was selected for the study. The tree bloomed in early February, 2022, and the fruits began to form in mid-March, 2022. By late April 2022, the fruits reached maturity and fully ripened fruits of good quality were harvested in the month of May, 2022. The collected fruits were brought to the nursery of IFP, Ranchi and seeds were extracted by de-pulping the fruits through washing in running water. Washed seeds were shade-dried for 15 days. Then seeds were graded and sorted for selecting quality seeds good of size bearing an average diameter of 30 mm to 40 mm (Kumar et al., 2012). These selected seeds were treated with 0.2% Bavistin for 30 minutes to avoid fungal infestation during the experiment. After that the seed treatments were carried out. The details of the seed treatments are enumerated in Table 1.

Table 1: Details of treatments followed in the study

Treat-ments	Details of the treatments
T_1	Control: No treatments were given
T_2	Mechanical scarification: Treatment was done by using 100 grade sand paper in a uniform way
T_3	5% H_2SO_4 Treatment for 5 minutes
T_4	5% H_2SO_4 Treatment for 15 minutes
T_5	Hot and cold-water Treatment: Seeds were dipped in hot water (65°C) for 1 hour and then dipped into normal cold water (20°C) for 12 hours
T_6	Mechanical scarification and hot-water Treatment: Seeds were given mechanical scarification using 100 grade sand paper and then the seeds were dipped in hot water (65°C) for 1 hour
T_7	Alternate wetting-drying treatment: Seeds were dipped into normal water (20°C) for 24 hours, then shade dried for 12 hours and again dipped into water in normal water (20°C) for 24 hours.

2.3. Experimental layout

The experiment was laid out following a Completely Randomized Design (CRD) with seven treatments and four replications in each treatment. The seeds were sown in the experimental field on 13^{th} June 2022 after applying the pre-treatments. The seeds were then line sown in raised sand bed of 10×1 m^2 dimension. In each line 20 seeds were sown, representing one replication of each treatment. Plant-to-plant distance and row-to-row distance was maintained 5 cm and 10 cm respectively. Standard nursery practices reported by Malakar et al. (2022) were followed during the experimental period (Figure 1).

Figure 1: Standard nursery techniques of Chironji followed in the experiment: (a) Different pre-treatments given to seeds; (b) Seedlings germinated in sand bed; (c) Sand bed shaded with Agro-Net (50%) after sowing; (d) Estimation of shoot and root length of sample Chironji seedling; (e) Shifting of seedlings to polybags after noting down all observations (50 DAS)

2.4. Experimental parameters

As reported by Sharma (2012), germination should be recorded till the 35^{th} days after sowing (DAS) to express germination percentage of seed properly. However, observations were taken weekly till 50 DAS (7 weeks) in this experiment for recording germination parameters. Observations on the following germination parameters were recorded in this study –

2.4.1. Germination % (G)

Germination % was calculated with the following formula according to Reed et al. (2022):

Germination %= (Total number of seeds germinated)/(Total number of seeds sown)×100 ………………………………………. (1)

2.4.2. Mean germination time (MGT)

MGT measures the mean duration of time necessary for seeds to achieve maximum germination, and it is represented in terms of the same units used in germination counts (days for this experiment). Statistically, it is computed as the weighted mean of the germination time where the number of seeds germinated in certain intervals of time is used as weight (Ranal et al., 2009). Because a variable number of seeds germinates in each interval, the weighted mean is essential in this scenario. Mean germination time was calculated by the following formula:

$MGT=\sum_{i=1}^{k} n_i t_i/(\sum_{i=1}^{k} n_i)$………………………………………………………. (2)

Where, t_i is the duration from the start of the study to the i^{th} observation (days for this experiment); n_i is the number of seeds germinated in the day corresponding to the observation in i^{th} time and k denotes last time of germination.

2.4.3. Coefficient of variation of germination time (CV_t)

CV_t is a relative dispersion measurement which independently compares the magnitude of the mean germination time and essential to measure the accuracy of the computation of the mean germination time (Czabator, 1962). It was calculated as per the following formula –

$CV_t=(St/t)\times100$ ……………………………………………………………. (3)

Where, S_t stands for the standard deviation of the germination time and denotes the mean germination time. Here, S_t is calculated by using the formula, $S_t=\sqrt{(\sum_{i=1}^{k} n_i\ (ti-\bar{t})^2/\sum_{i=1}^{k} n_i-1)}$ (notations are already denoted in formula 2).

2.4.4. Mean germination rate (MR)

MR represents the mean number of seeds germinating per day. Maguire (1962) gave a generic mathematical expression for MR and mathematically it can be calculated as the reciprocal of the mean germination time. Hence, MR is expressed in $days^{-1}$ and it was calculated as:

$MR=1/MGT=(\sum_{i=1}^{k} n_i)/(\sum_{i=1}^{k} n_i t_i)$ ………………………………………… (4)

Where, t_i is the duration from the start of the study to the i^{th} observation (days for this experiment); n_i is the number of seeds germinated in the day corresponding to the observation in i^{th} time and k denotes last time of germination.

2.4.5. Germination rate index (GRI)

The computation of GRI was proposed by Timson (1965) and hence it is also called as Timson's germination index. The GRI denotes the germination percentage of seeds on each day of across the germination period. It is represented with the unit % day^{-1}. It was mathematically calculated in the study according to the following formula given by Shah et al. (2021):

GRI= $(\sum^{n}_{i=1}G_i)/T$ .... (5)

Where, G_i denotes the percentage of seed germinated per day of observation, *T* represents germination period in which observation is taken and *n* denotes the numeric count of observations.

2.4.6. Germination index (GI)

The calculation of GI was first proposed by Throneberry and Smith (1955) and they mathematically denoted it as and it is computed as one of the measures of seedling vigour. Recently, Yang et al. (2021) modified the mathematical expression for calculating GI which was used in this study as per:

GI=$\sum^{k}_{i=1}(n_i/t_i)$ .... (6)

Where, n_1, n_2, n_3, ..., n_k number of seeds germinated on the first day, second, third, ... and k^{th} days of observations, and t_i denotes the cumulative number of days of the experiment on which the observation is taken.

2.4.7. Germination energy (GE)

Germination energy is expressed as the percentage by number of seeds in a given sample that germinate within a certain time frame of the experiment (Gupta et al., 2022). The total number of seeds germinated up to the day when the rate of germination reached its highest is used to determine germination energy. This parameter is used to determine the rate and consistency of seed germination in the early stage, which measures the seeds' vitality. It was calculated with the following formula:

GE=(No. of seeds germinated on the particular day of observation)/(Total number of seeds sown)×100 .... (7)

In this study, for calculating GE, observations were taken on the 28 DAS as it represented peak germination.

2.4.8. Uncertainty of germination process (U)

The coefficient of uniformity of germination expresses the variation among seeds in respect to the sample's mean germination time (Bewley et al., 2013). It was calculated with the following formula:

U=$\sum^{k}_{i=1}f_i.\log_2.f_i$ .... (8)

Where, fi is calculated as $n_i/\sum^{k}_{i=1}n_i$, ni represents number of seeds germinated on the i^{th} time and *k* denotes last day of observation.

2.4.9. Synchrony of germination process (Z)

The Z index was designed to assess the degree of overlapping across individuals in a same group. Using Primack's (1980) concept, the synchronization of one seed with another included in the same replication of one treatment is calculated using the following formula:

Z=$\sum^{k}_{i=1}C_{ni,2}/C\sum n_{i,2}$ .... (9)

Where, $C_{ni,2}$ denotes the combination of the seeds germinated in the i^{th} time, two by two, and n_i is the number of seeds germinated in the i^{th} time.

2.4.10. Seed vigour indices (SVI)

Seed vigour indices are defined as "the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence" (Powell, 2022). Two types of Vigour indices were calculated according to the following formulae –

Vigour Index-I=Germination prectange×(Average shoot length of seedlings+Average root lenth of seedlings) .... (10)

Vigour Index-II= Germination prectange×Average seedling dry weight .... (11)

For calculating average shoot-root length and dry weight of seedlings, one sample seedling was selected randomly per replication for each treatment. Shoot and root length was taken freshly and dry weight was taken after keeping the plants in hot air oven at 37±2° C for 24 h.

2.5. Statistical analysis

The observations recorded during the study were primarily analysed in OPSTAT software (Sheoran et al., 1998) for descriptive statistics. The arcsine transformation on the data of germination percentage, coefficient of variation of germination time and germination energy was carried out in OPSTAT for further statistical analysis. Levene test for homogeneity of variances at p>0.01, Duncan's Multiple Range Test (DMRT) (Post Hoc Analysis) for homogenous subsets at α=0.05 and Analysis of variance (ANOVA) was performed in SPSS v.25.0 software for finding the best treatment for germination with statistical significance.

3. Results and Discussion

In this experiment, variation among several germination parameters of Chironji were studied significant differences between the studied parameters were observed due to different physical and chemical treatments applied to the seeds (Figure 2 and Figure 3). The ANOVA for significant differences among the germination parameters studied is presented in Table 2. The highest G of 87.5% was observed in T_6. Conversely, T_1 displayed the lowest G at 16.25%. MGT varied among treatments, with T_6 demonstrating the shortest MGT of 19.77 days and T_3 exhibiting the longest MGT at 29.575 days. CV_t was most pronounced in T_6 (51.261%), while T_2 displayed the lowest CV_t at 22.88%. Furthermore, T_6 demonstrated the highest GRI at 25.729% day^{-1}, whereas T_3 displayed the lowest GRI at 5.708% day^{-1}. Treatment T_6 also yielded the GI of 0.735, contrasting with T_3, which exhibited the lowest GI at 0.163. Further, T_6 displayed the highest GE at 45%, while T_2 exhibited the lowest GE at 10%. U was maximal in T_6 at 2.083 bit, in contrast to T_2 with the lowest U at 1.256 bit. At 0.175, Z peaked in T_6, while T_5 displayed the lowest Z at 0.068. The

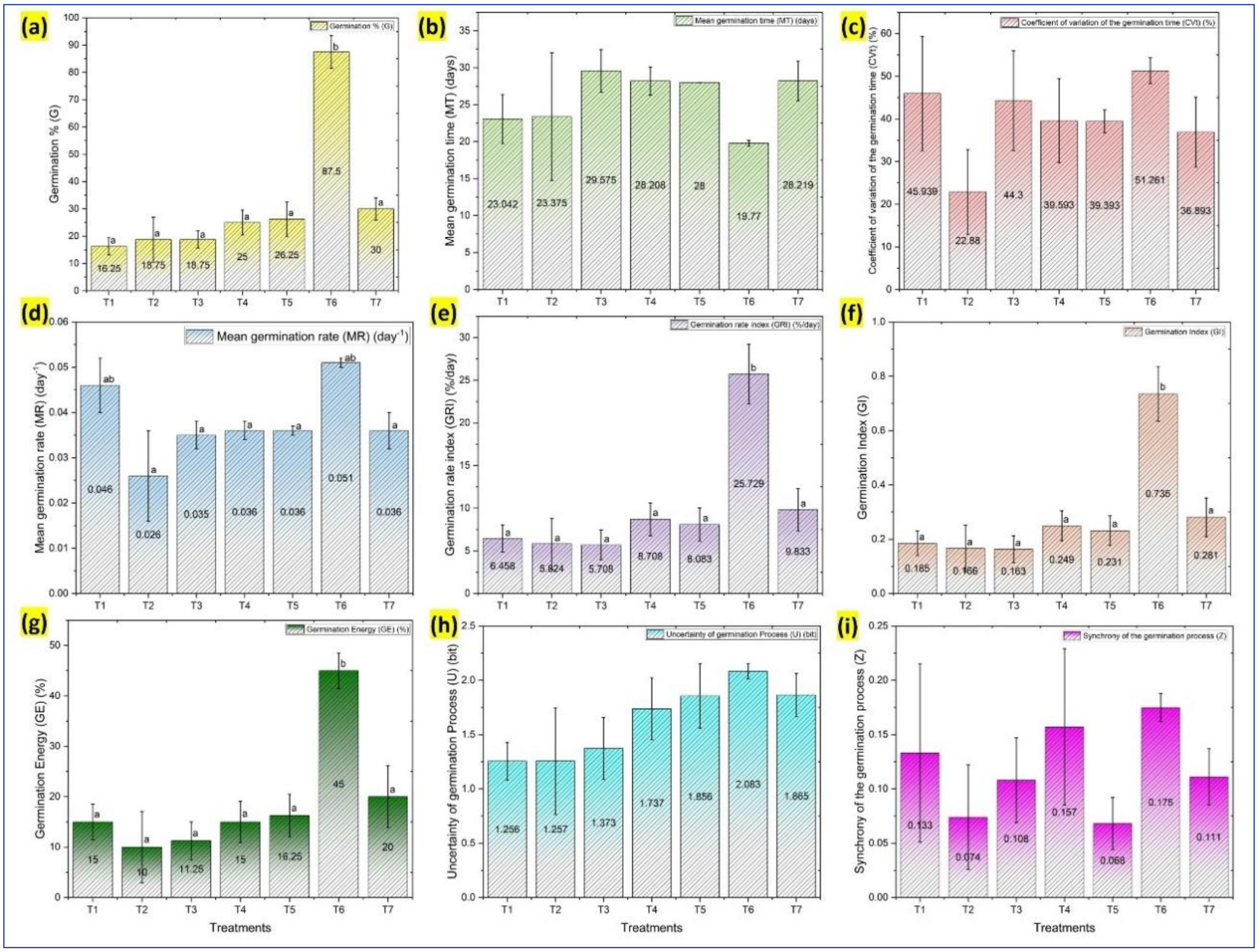

Figure 2: Evaluation of performance of Chironji seeds after implementing different treatments: (a) Germination percentage; (b) Mean germination time (MGT); (c) Coefficient of variation of the germination time (CVt); (d) Mean germination rate (MR); (e) Germination rate Index (GRI); (f) Germination Index (GI); (g) Germination energy (GE); (h)Uncertainty of germination process (U); (i) Synchrony of germination process. Treatments corresponding to same letters are not significantly different based on the Duncan's Multiple Range Test (DMRT) at α=0.05. Graphs with no corresponding letters (b, c, h and i) to treatments imply that there was no significant difference observed (at *p*<0.5) among the treatments

greatest SVI–I and SVI–II, 1303.530 and 109.155, respectively, were displayed by T_6. With SVI–I and SVI–II values of 352.35 and 20.781, respectively, T_1 displayed the lowest values. ANOVA indicated that T_6 significantly differs from all other treatments in several germination parameters like G ($p<.01$), CV_t ($p<.05$), GRI ($p<.001$), GI ($p<.001$), GE ($p<.001$), SVI-I ($p<.001$) and SVI-II ($p<.001$). Nonetheless, we were unable to find significant difference among the treatments in four germination parameters studied, i.e., mean germination time, coefficient of variation of the germination time, uncertainty of germination process and synchrony of the germination process.

Perusal of the results obtained from the statistical analysis on this experiment reveals that the treatment T_6 (Mechanical

Table 2: Sum of square values from analysis of variance (ANOVA)

Source of variation	d.f.	Germination % (G)[1]	Mean germination time (MT) (days)	Coefficient of variation of the germination time (CV_t) (%)[1]	Mean germination rate (MR) (day^{-1})	Germination rate index (GRI) (% day^{-1})
Treatments	6	3660.72**	322.28	1935.34	0.0016*	1204.63***
Error	21	2400.00	1256.63	7137.01	0.0019	483.48
Total	27	6060.72	1578.91	9072.35	0.0034	1688.11

Table 2: Continue...

Source of variation	Germination index (GI)	Germination energy (GE) (%)[1]	Uncertainty of germination Process (U) (bit)	synchrony of the germination process (Z)	Seed Vigour Index - I	Seed Vigour Index - II
Treatments	0.98***	3430.36***	2.67	0.04	2518749.75***	21281.11***
Error	0.39	1937.50	6.79	0.20	1257568.47	5921.73
Total	1.38	5367.86	9.46	0.24	3776318.22	27202.84

[1]Arcsine transformation of data is performed before statistical analysis; *, **, *** Significant at the (p=0.05), (p=0.01) and (p=0.001) probability levels, respectively

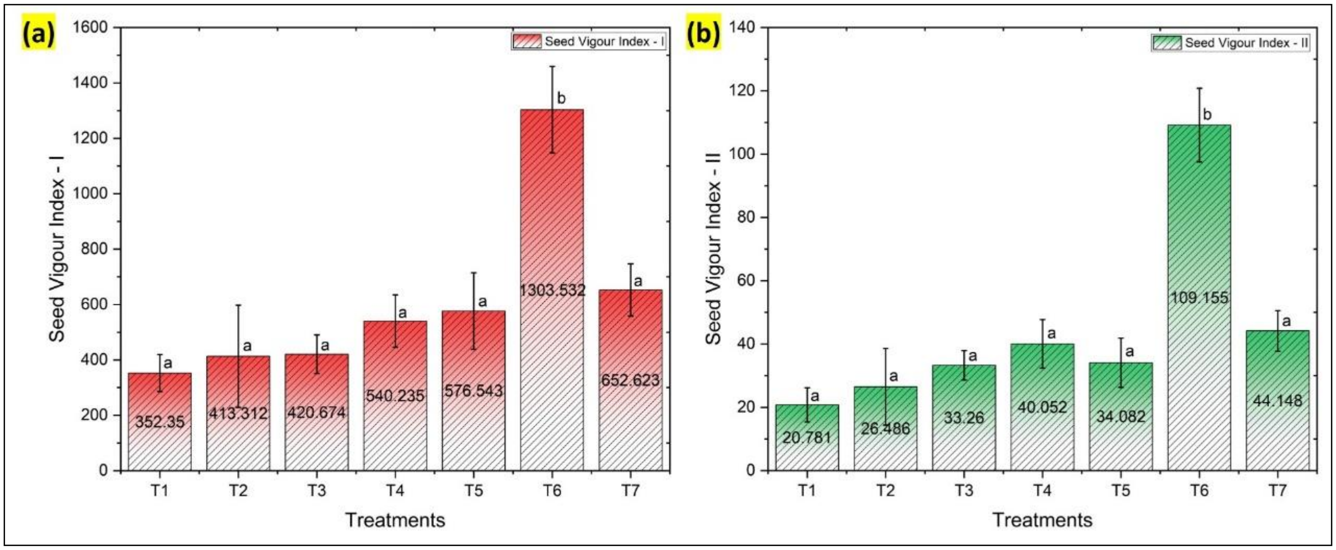

Figure 3: Evaluation of performance of Chironji seeds after implementing different treatments: (a) Seed Vigour Index – I; (b) Seed Vigour Index – II. Treatments corresponding to same letters are not significantly different based on the Duncan's Multiple Range Test (DMRT) at α=0.05

scarification and hot-water treatment) out-performed all the treatments in every single germination parameter studied. Following T_6, the treatment T_7 (Alternate wetting-drying treatment) performed well in all germination parameters. However, it was proven in the study that T_6 significantly differs and performed a lot better than T_7, which required nearly 2.5 days to treat the seeds, indicating a highly time-consuming process that increases the risk of fungal infestations during the prolonged treatment. In the present study T_2 (Mechanical scarification) did not perform well, although it was earlier reported to achieve the highest germination percentage (Shukla and Solnaki, 2000; Khobragade and Rai, 2022). This may be the outcome of the treatments' inability to penetrate the physiological barriers of Chironji seeds. While Narayan et al. (2014) proposed that acid scarification using H_2SO_4 was useful for overcoming dormancy, it's possible that the H_2SO_4 treatments (T_3 and T_4) had an impact on the embryo in this investigation, as seen by the reduced germination percentage.

It is very much crucial to study all other germination parameters depicted in this experiment along the germination percentage. Kader (2005) reported GI to be the most comprehensive parameter for measuring germination potential combining both germination percentage and rate. The variation among the evaluated treatments, that can be compared using a straightforward numerical assessment, is also exacerbated by this parameter (Ranal and Santana, 2006). Higher GI value indicates good potential of germination for the seeds. GE measures the germination percentage when germination reaches its peak. Hence, higher GE signifies better germination. Uncertainty of germination process measures the degree of spreading of germination through the time and this measure is used to infer the synchrony of germination (Ranal et al., 2009). On the other hand, synchrony of germination process measures the degree of germination overlapping and produces results only if there is more than one seed is germinating on the same observation day or time (Ranal and Sanatana, 2006). These mathematical equations are utilized to compensate for the inadequacies that cannot be inferred from the calculation of mean germination time. These two are also used to illustrate the relationship and correlation between the other germination parameters (Ranal et al., 2009).

Apart from these, Seed Vigour Indices are also considered equally important for assessing the germination potential of seeds (Abdul-baki and Anderson, 1973). Both of the seed vigour indices take the survivability of the seedlings germinated from the seeds in account. Better seedling length (calculated in SVI–I) and dry weight (calculated in SVI–II) indicates that the seedlings are performing well under prevailing conditions. Kandasamy et al. (2020) reported that assessing mere germination parameters without evaluating the survivability of seedlings raised indicates biased results and practical implications of the study is jeopardised. Hence, evaluation of Seed Vigour Index – I and II ensures the practicality of this experiment.

4. Conclusion

This investigation sought to elucidate optimal techniques for surmounting seed dormancy and enhancing germination in Chironji, specifically under nursery conditions in the red and lateritic soils of eastern India. Among the seven pre-treatments evaluated, the synergistic application of hot water treatment and mechanical scarification demonstrated superior efficacy, yielding 87.5% germination and maximum seed vigour indices.

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