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RESEARCH ARTICLE

Effect of pre-sowing treatments on seed germination and seedling growth of *Enterolobium cyclocarpum* under nursery condition

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Abstract

The ear pod tree (*Enterolobium cyclocarpum*) is a multipurpose tree species (MPT), native to tropical America and is an essential tree for sustainable land use practices. There are a wide range of goods and services that they offer. It is widely grown for its rapid growth, fixation of atmospheric nitrogen and for the large number of the diverse uses to which it is put, such as soil amendment and fodder production. Improving seed germination for the nursery propagation of *Enterolobium cyclocarpum* was studied at the Forest College and Research Institute, Tamil Nadu Agricultural University. Seed structure and germination traits; and seedling growth were studied. Produce of different sources exhibited a significant variation of the seed weights. The best methods to increase germination rates were hot water exposures of 12 hrs followed by acid scarification for only 10 minutes. *Enterolobium cyclocarpum* is a potential useful MPT for agroforestry, based on it's potential to environmentally sustainable and economically viable for farmers in nutrient deficient or degraded soils. The results underscore the importance of MPTs to the adoption of sustainable methods of farming and food and feed production considering shortage.

Keywords: agroforestry; *Enterolobium cyclocarpum*; multipurpose tree species; nitrogen fixing properties; seed germination; seed morphology

Introduction

MPTs are important for sustainable land use system as they can perform ecological, economic and social functions. At the same time, they are relied upon to survive in varying climatic conditions, improve soil fertility through nitrogen fixation, reproduce rapidly from pruning or coppicing and produce goods such as fruits, fodder, fuel wood and timber (1, 2). They also serve the purpose of improving agricultural productivity through leaf litter and microclimatic advantages that help guarantee food security. Choosing the tree species that can do many things without demanding much contribution of resources is key to the use of climate resilient agroforestry and land restoration (3, 4). *Enterolobium cyclocarpum* (Jacq.) is often also called the Guanacaste or ear pod tree. This is a large tropical America deciduous legume tree rapidly growing to 30 m with a lifespan of 100 years (5, 6). It is recognised for its broad crown, high forage value, ability to fix nitrogen and moderate abiotic stress resistance to drought and fire. It is a member of the genus Albizia, close to Samanea and is included under the tribe of Ingeae and subfamily of Mimosoideae. Only five species are represented in the genus Enterolobium, but because of the genus's versatility and adaptability, it is *Enterolobium cyclocarpum* that is generally cultivated most often. At elevations between sea level and 1200 meters, the species flourishes in tropical dry to moist forests. It can withstand dry spells of up to seven months because it is acclimated to rainfall regimes between 750 and 2500 mm annually. It can be used as livestock feed and to improve soil through nitrogen fixation and leaf litter because it produces thick, coiled pods with sweet pulp and nutrient-rich seeds (7, 8). Despite these encouraging characteristics, *Enterolobium cyclocarpum* struggles to reproduce and be grown on a large scale because of its extremely low seed germination rates in nature, which are mostly caused by a combination of physiological and physical dormancy (hard seed coat) (9, 10).Introduced as a possible agroforestry and afforestation species targeted at reclaiming degraded land, enhancing soil health and providing fodder and timber, *Enterolobium cyclocarpum* was first found in Tamil Nadu (11, 12). Still, there are few methodical studies of its performance under Indian conditions. Particularly in dry and semi-arid areas where climate-resilient species are desperately needed, the present work seeks to evaluate the germination potential, ecological adaptability and agroforestry applicability of *Enterolobium cyclocarpum* in Tamil Nadu, India (4). Native to tropical America, *Enterolobium cyclocarpum* is a nitrogen-fixing legume whose fast development, fodder generation and soil-enriching qualities (8) are well-known. Its low seed germination rate, which results from both physiological dormancy (9, 13) and physical dormancy brought on by a hard seed coat, is one of the main obstacles restricting its extensive use, though. Supported by previous data in related species, this work will investigate several pre-sowing treatments to overcome dormancy constraints and increase propagation efficiency (14, 15). Apart from improving germination, the study aims to ascertain the species' Agro-climatic compatibility across particular sites in Tamil Nadu and its performance in agroforestry systems aiming at land restoration and income diversification (16, 17).The expected results of this research are policy-level recommendations for its broad acceptance in degraded and drought-prone environments, empirical support for integrating *Enterolobium cyclocarpum* into local agroforestry and land reclamation models and development of viable seed treatment protocols (7, 18).

Materials and Methods

The study was designed to study the seed morphology and germination of *Enterolobium cyclocarpum* at the mother bed of nursery of the Department of Silviculture and Natural Resource Management, Forest College and Research Institute, Mettupalayam, Tamil Nadu Agricultural University. The treatments thus followed in conduct of study is presented in Table 1.

Seed source

The study involved collecting seeds from trees at the Precision Silviculture Research Field for germination and morphology studies. Mature seeds were harvested from multiple trees to ensure genetic diversity and assessed for various characteristics. Seeds were subjected to pre-treatments using acid and hot water to induce early germination. Mother beds were prepared with a soil mixture of sand, red soil and well-decomposed farmyard manure in a 1:2:1 ratio. Treated seeds were evenly sown on the beds and observations on germination and early growth parameters were recorded for up to one month. Seeds were graded based on visual uniformity, size and physical integrity to ensure consistency in experimental treatments.

Study site, seed collection, handling and growing media

The experiment was carried out in the Silviculture Laboratory, the propagator house and the nursery of the Department of Silviculture and Natural Resource Management at the Forest College and Research Institute, Mettupalayam, under Tamil Nadu Agricultural University. Mature fruits of *Enterolobium cyclocarpum* were harvested from the Precision Silviculture Research Field located within the FCRI campus in July 2024. Seeds were manually extracted by removing the pulp from the ripe fruits to enhance germination potential. These brown-coloured seeds were then sun-dried for four days to reduce their moisture content. Only healthy seeds were selected and sown in polybags (15 cm × 10 cm) and within the propagator house-a plastic-covered structure maintaining an internal temperature of approximately 35 °C-for experimentation. The polybag growing medium was prepared by mixing forest floor topsoil with cow dung in a 3:1 ratio. In contrast, the propagator house utilized fine sylhet sand as the sowing medium and only the T10 treatment was applied in this setting, treating the entire propagator setup as a single treatment condition. The germination of *Enterolobium cyclocarpum* seed at various stages were presented in (Fig. 1).

Experimental design and treatment setup

A total of eleven pre-sowing treatments were implemented, each with three replications. To assess the seed germination response and seedling development of the treated seeds, a control plot consisting of untreated seeds was included. The experiment followed a completely randomized design. Details of the pre-sowing treatments are provided in Table 1.

Pre-sowing treatment procedures

For the pre-sowing treatments, different physical and chemical methods were applied to batches of thirty healthy, randomly chosen seeds:

1. Sandpaper scarification

Seeds were abraded manually at the distal end using sandpaper.

2. Nail clipping

A small nick was made at the distal end of each seed using a nail clipper.

3. Room temperature water soaking

Seeds were immersed in water at ambient temperature for 24 hrs.

Table 1. Pre-treatment methods

T0	De-pulped seeds without any treatment sown in polybag (control)
T1	Seeds with pulp and without any treatment sown in polybag
T2	Seeds scarified with sandpaper at distal end of the seed
T3	Seeds treated with nicking/nail clipping at distal end of the seed
T4	Seeds immersed in water for 24 hrs at room temperature
T5	Seeds immersed in hot water (at boiling point) for 1 minute
T6	Seeds immersed in 10 % concentrated H_2SO_4 for 3 minutes
T7	Seeds immersed in 10 % concentrated H_2SO_4 for 5 minutes
T8	Seeds immersed in 10 % concentrated HCl for 3 minutes
T9	Seeds immersed in 10 % concentrated HCl for 5 minutes
T10	Seeds sown in propagator house (fine sylhet sand)

Fig. 1. Germination of *Enterolobium cyclocarpum* seed.

4. Hot water soaking

Seeds were placed in boiling water immediately after it reached boiling point and left to soak for one minute.

5. Acid scarification

For acid treatments, four separate beakers each containing thirty seeds were used. Concentrated sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) at 10 % concentration were added separately to the beakers. Seeds were exposed to the acids for 3 and 5 minutes respectively for each acid. Post-treatment, the seeds were thoroughly rinsed in running tap water.

All treatments (T1 to T9), including the untreated control (T0), were sown in polybags. Treatment T10, however, involved sowing the seeds in fine sylhet sand within the propagator house, where the internal temperature averaged around 35 °C, creating a distinct treatment condition.

Seed germination observation

Germination was tracked daily from the date of sowing until no further germination occurred. The germination percentage and cumulative germination rate were calculated. The point at which the mean daily germination rate peaked was used to calculate germination energy. At the conclusion of the experiment, the number of surviving seedlings was recorded to determine survival percentage. Germination value was computed by multiplying the Peak Value of germination (PV) with the Mean Daily Germination (MDG). Treatments T0 to T9 were conducted under natural environmental conditions with an average annual temperature of 25.7 °C, humidity of 78.04 % and annual rainfall of 2794 mm.

Measurement of seedling vigour

Three months after sowing, three vigorous seedlings from each replication were selected to assess seedling height and measurements continued until five months post-germination. Shoot height was measured from the collar region to the seedling apex.

Statistical analysis

All collected data were statistically evaluated using the SPSS software package. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were applied to determine the significance of differences among treatments.

Results

Germination percentage

The germination percentage of *Enterolobium cyclocarpum* seeds varied significantly across the different pre-sowing treatments. From Table 2, mechanical nicking (T3) gave 70 % germination, sulfuric acid treatment for 3 min. (T7) 75 %, hot water treatment for 12 hrs (T5) 85 %. Conversely, the treated seed (T0) and whole fruit (T1) treatments had significantly lower percentages of germination than the control (T0) and whole fruit (T1) treatments, implying the existence of physical dormancy with untreated seed.

Table 2. Effect of pre-sowing treatments on germination and seedling growth in *Enterolobium cyclocarpum*

Treatments	Germination start (day)	Germination end (day)	Germination percentage (%)	Germination energy (%)	Plant Percent (%)	Germination value
T0 (Seed only)	14	30	20^{de}	5^{e}	20^{e}	0.5^{d}
T1 (Whole fruit)	18	32	10^{e}	3^{e}	10^{e}	0.3^{d}
T2(Sand paper)	5	15	60^{bc}	30^{cd}	60^{bc}	3.0^{bc}
T3(Nicking)	4	14	70^{ab}	35^{bc}	70^{ab}	3.5^{ab}
T4 (cold water, 24 hr)	10	25	40^{cd}	20^{de}	40^{d}	2.0^{cd}
T5(hot water, 12 hrs)	8	20	85^{a}	45^{a}	85^{a}	4.5^{a}
T6 (H_2SO_4, 1 min)	3	10	65^{abc}	45^{a}	65^{bc}	4.0^{ab}
T7(H_2SO_4, 3min)	3	9	75^{ab}	40^{ab}	75^{ab}	4.5^{a}
T8(HCL, 3 min)	6	14	55^{bc}	28^{cd}	55^{bc}	2.8^{bc}
T9(HCL, 3 min)	6	13	58^{bc}	30^{cd}	58^{bc}	3.0^{bc}
T10 (seed propagator house)	5	12	65^{abc}	33^{bc}	65^{bc}	3.3^{ab}

(*) Means followed by the same letter(s) in the same column are not significantly different at $p < 0.05$, Duncun's Multiple Range Test (DMRT).

Overall, these results indicate that hot water, sulfuric acid, cold scarification (by mechanical dislodgement and drying) pre sowing treatments are successful in breaking dormancy in this species and greatly improve their germination rates.

Germination energy

Highest germination energy was found in T5 (hot water for 12 hrs), also having 45 % (Table 2) and T6 (sulfuric acid for 1 minute). The results from this indicate that these treatments are working to rapidly break seed dormancy in *Enterolobium cyclocarpum* and hence faster and synchronized germination (22). Being able to germinate so quickly is advantageous for getting all the seeds to germinate and giving them all equal opportunity for establishing and reducing vulnerability to the environment for early growth.

Plant percent

Successful seedling establishment was clearly related to the percentage of seeds developing into healthy seedlings (plant percent), which could be observed to closely reflect the trends observed in the percentage of seeds after effective dormancy breaking. Plant percent recorded with treatment T5 (hot water 12 hrs), T7 (sulfuric acid 3 mins), T3 (mechanical nicking) were 85 %, 75 % and 70 % respectively (Table 2). This result supports the categorization of these treatments according to their capacity to induce heat-induced dormancy and encourage vigorous seedling growth.

Germination value

Both germination treatments T5 (hot water for 12 hrs) and T7 (sulfuric acid for 3 minutes) had the highest germination value, 4.5, which represents the highest germination value measured in this study. The results obtained from this indicate that these treatments are of better overall performance as they can promote the rapid and complete germination; and so are considered suitable for large scale propagation and afforestation programs of *Enterolobium cyclocarpum*. The elevated germination values represent either the potential for uniform and vigorous seedling establishment or a situation where effectively broken dormancy is more important than uniform and vigorous seedling production. Direct link with uniform seedling growth and better field establishment were provided from the treatments that had high germination value and hence their relevance to nursery and reforestation practices.

Discussion

Among events that occur in the life cycle of a seed, germination is a phase that determines the establishment, survival and growth of the plant (14). A fundamental measure of seed viability and the efficacy of pre-sowing treatments to overcome plant's inherent mechanisms that are designed to maintain vestigial seeds or seeds incapable of natural germination is termed as germination percentage. The seeds treated hot water for 12 hrs (T5) germinated with highest percentage of 85 germinated seeds which showed a very significant reduction in dormancy and significant disruption of physical and physiological barriers to germination. Much lower germination rates were recorded in control group (T0) and whole fruit treatment (T1) (20 % and 10 % respectively), indicating that *Enterolobium cyclocarpum* seeds have robust physical dormancy (14, 26). In leguminous species, physical dormancy is usually due to the impermeable seed coat which prevents water uptake and gas exchange (27). The pre-sowing treatments, such as hot water, sulphuric acid and mechanical scarification, are well documented breaking or weakening the seed coats to facilitate imbibition and the resumption of metabolic activities needed for germination (14, 28). The germination results are consistent with previous research showing that hot water treatment can improve germination by dissolving tough seed coats (28). Also, sulfuric acid treatment for 3 min (T7) resulted in a 75 % germination rate, consistent with the effectiveness of acid scarification to break seed dormancy in hard seeded species (29, 30). The physical abrasion of the seed coat (T3) mechanical scarification gave a percentage germination of 70 % in addition to supporting mechanical dormancy alleviation (31). Next, another important parameter is the germination energy, which appraises the speed and vigour of seed germination. Here, the most germination energy (45 %) was provided by the treatment with hot water for 12 hr (T5) and with sulfuric acid for 1 min (T6). These findings imply that these treatments break physical barriers and work to induce rapid metabolic activation needed for early seedling emergence (32). Minimizing the seed's vulnerability period and synchronization of seedling development are key to field establishment and survival (28) and high germination energy is required for this. Optimal germination speed is advantageous for nursery and field planting, since a faster germination speeds up nursery operations, prevents competition with weeds and facilitates the establishment of seedlings in difficult environments (33). The lower germination energy treatments, control and whole fruit, may delay or asynchronous emergence, that may adversely affect seedling vigour and survival rates. The plant percent, that is, the proportion of seed that germinate, but also grow into healthy seedling, provided further evidence for the efficacy of the treatments. Again, mechanical scarification (T3) was followed closely by sulfuric acid treatment (T7) at 70 % plant percent and hot water treatment (T5) at 85 %. These results support the requirement for good pre-sowing treatments to promote not only germination, but robust seedling growth and development (34). Success in the production of seedlings in reforestation and agroforestry programs depends, of course, on treatments' ability to increase plant percent (25). Furthermore, treatments by hot water (T5) and sulfuric acid (T7) had germination values of 4.5 (the sum of germination speed and final germination percentages). A special hallmark of treatments which engender rapid, uniform and complete germination, such that germination of seedlings is effective (14, 32). Practical implications of high germination values are highly significant because seedling emergence tends to be timely and synchronized in large scale plantation efforts for greatly improving the operational efficiency and seedling survival (28). Relative to naturally treated seeds, however, lower germination values in untreated seeds indicate the inadequacy of natural processes for hard-seeded species. The results of this study once again confirm that physical dormancy in *Enterolobium cyclocarpum* seeds is of moderate to strong expression, a characteristic previously reported for many Fabaceae species (14, 28). Chemical-free method of hot water treatment (T5) is the best and most practical and efficient tested method, with high germination success. Mechanical scarification (T3), as well as sulfuric acid treatments (T6 and T7) also had significant efficacy, but use of hazardous chemicals such as sulfuric acid creates safety and scalability concerns within large nursery operations (30). These results agree with previous reports on the efficacy of thermal, mechanical and chemical song 1 scarification techniques to improve germination in hard seeded legumes (28, 34). Studies conducted by (14, 26) confirm and support the above stated that integration of pre-sowing treatment into nursery management could significantly increase the germination success and the seedling vigour. Moreover, researchers (25, 33) advocate for the widespread adoption of such methods in reforestation and agroforestry projects to improve propagation success and ecosystem restoration outcomes.

Conclusion

The present study demonstrates that pre-sowing treatments play a crucial role in enhancing seed germination and early seedling growth of *Enterolobium cyclocarpum*, a multipurpose leguminous tree species with high potential for agroforestry, afforestation and soil rehabilitation in semi-arid and degraded regions like Tamil Nadu. Among the treatments tested, hot water soaking for 12 hrs (T5) proved to be the most effective, achieving the highest germination percentage (85 %), germination energy (45 %), plant percent (85 %) and germination value (4.5). This method is simple, cost-effective and environmentally safe, making it highly suitable for nursery-scale propagation. Acid scarification with sulfuric acid (T7) and mechanical nicking (T3) also significantly enhanced germination and seedling performance, but the use of concentrated acids, while effective, poses handling and environmental safety concerns that may limit its practical application in field-level nursery operations. Mechanical methods like nicking, though effective, are labor-intensive and less feasible for large-scale seed preparation. These results underscore the strong influence of physical dormancy in *Enterolobium cyclocarpum* seeds and the necessity of employing appropriate scarification techniques to improve propagation success. The findings align with global research trends emphasizing the importance of thermal and mechanical scarification in overcoming dormancy in leguminous tree species. In conclusion, hot water treatment stands out as a practical and scalable solution to enhance the germination efficiency of *E. cyclocarpum*, thereby facilitating its wider adoption in agroforestry and reforestation program. The study supports the integration of scientifically validated pre-sowing treatments into propagation protocols for sustainable land-use planning, ecological restoration and economic upliftment of rural communities.

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Authors' contributions

BG, SM, RS conceptualized the study and performed the formal analysis, BG, SM, RS worked together with GM, NS, and SKS in structuring the methodology. SM supervised and validated the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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