

Original Research Article

Comparative Evaluation of Scarification Techniques on Seed Germination and Seedling Performance of Lotus (*Nelumbo nucifera*)

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Abstract

Seed propagation of *Nelumbo nucifera* is limited by strong physical dormancy due to its hard, impermeable seed coat. This study evaluated the efficacy of four scarification treatments-mechanical nicking, sand abrasion, hot water immersion and concentrated sulphuric acid (98%, 1 h)-compared with an untreated control, on germination, biochemical activity and early seedling growth under ambient conditions of Ananthapuramu, India (latitude 14°41 N, longitude 77°36 E) during June 2025. Scarification significantly enhanced imbibition, seed coat permeability, α -amylase activity, germination percentage and seedling vigour ($p < 0.05$). Among treatments, acid scarification was most effective, achieving 78.4% germination, the shortest mean germination time (4.6 days), highest enzymatic activity (5.76 mol maltose $\text{min}^{-1} \text{g}^{-1} \text{FW}$) and superior seedling growth (plumule: 9.4 cm; radicle: 12.6 cm). Mechanical nicking and sand abrasion moderately improved germination, whereas hot water immersion showed limited effectiveness. Root-to-shoot ratios remained relatively stable across treatments, indicating balanced biomass allocation. These results demonstrate that optimised acid scarification efficiently overcomes physical dormancy, accelerates metabolic activation and enhances seedling establishment in *N. nucifera*, providing a reliable, cost-effective method for large-scale propagation and wetland restoration programs.

Keywords: *Dormancy, Germination, Nelumbo nucifera and Seed scarification*

Introduction

The sacred lotus (*Nelumbo nucifera* Gaertn.), belonging to the family Nelumbonaceae, is an ecologically significant aquatic perennial widely distributed across tropical and subtropical Asia, including India, China, Japan, Korea and Australia. It is one of the most culturally revered plants in human civilisation, holding deep symbolic importance in Hinduism, Buddhism and ancient Egyptian culture, where lotus motifs were prominently employed in the decoration of temples, palaces, tombs and ceremonial architecture (1,2). In India, *N. nucifera* holds the distinction of being the national flower and its representation in religious iconography, art and literature has persisted for millennia. The lotus flower is regarded as a symbol of purity, prosperity and spiritual enlightenment and its aesthetic appeal continues to make it a preferred ornamental plant in water gardens, ponds and landscape horticulture worldwide (3). Beyond its cultural and ornamental value, the lotus is a plant of remarkable economic and pharmaceutical importance. All parts of the plant - flowers, leaves, seeds, seed pods, rhizomes, stamens and embryos - are utilised across food, medicine and cosmetic industries. Lotus seeds (*Nelumbinis semen*) are nutritionally rich, containing proteins, carbohydrates, dietary fibre, alkaloids, flavonoids, polyphenols and proanthocyanidins and have been extensively reviewed as an emerging therapeutic food ingredient with antioxidant, anti-obesity and anti-inflammatory properties. The seeds have also demonstrated the ability to protect against oxidative stress-induced DNA damage in human lymphocytes, reinforcing their nutraceutical significance. Lotus seed starch exhibits unique structural and physicochemical properties of relevance to food processing and the chemical composition of seeds varies significantly with maturity and harvesting time, influencing both nutritional quality and propagation potential. The metabolomics of lotus seeds has emerged as an active area of research, with studies documenting a wide array of bioactive secondary metabolites of pharmaceutical interest (4,5). The flowers of *N. nucifera* are equally valued in the cosmetics and pharmaceutical sectors. Alkaloid-rich flower bud extracts have been shown to possess melanogenesis inhibitory and hyaluronidase inhibitory activities, supporting their application in skin-whitening and anti-ageing formulations. The floral scent of lotus, composed of complex volatile compounds, is also exploited in the perfumery industry. Reviews on the use of lotus in herbal cosmetics confirm its growing acceptance as a multi-functional botanical ingredient (3,6,7). From an ecological standpoint, the lotus contributes meaningfully to wetland ecosystem health by stabilising sediments, reducing turbidity

and providing structural habitat for aquatic organisms. The thermogenic capacity of its flowers, wherein floral temperature is actively regulated above ambient levels during anthesis, is a unique physiological trait that facilitates pollinator attraction and successful fertilisation. A thorough understanding of lotus floral biology, including flowering, pollination and seed formation processes, is therefore essential for optimising seed production and propagation strategies (8,9,10). The lotus is increasingly being recognised as an emerging horticultural model plant, with advances in molecular biology, metabolomics and tissue culture - including efficient callus induction systems - expanding the scope of genetic research and improvement in this species (2,4,5). In natural and cultivated systems, lotus is primarily propagated through vegetative means, particularly rhizome division, which ensures uniformity and rapid establishment. However, dependence on vegetative propagation imposes practical constraints on large-scale production, including limited availability of healthy rhizome planting material, high labour inputs and risk of pathogen transmission through clonal propagules. Seed-based propagation, by contrast, offers several distinct advantages - it enables the generation of genetic variability, facilitates ease of storage and long-distance transport and is particularly suited to mass multiplication programmes for wetland restoration and biodiversity conservation. Notably, ancient sacred lotus seeds recovered from sediment deposits in China, estimated to be several centuries old, have been successfully germinated under controlled laboratory conditions, demonstrating the extraordinary seed longevity characteristic of this species and its inherent potential for long-term ex situ conservation (11,12). Despite these propagation advantages, seed germination in *N. nucifera* is severely constrained by the presence of strong physical dormancy. The seed coat of lotus is extremely hard, thick and impermeable, composed of tightly interlocked sclerenchymatous cells that restrict water absorption, impede gaseous exchange and thereby delay or completely inhibit germination under natural conditions. Without deliberate dormancy-breaking intervention, germination rates in untreated lotus seeds remain characteristically low, erratic and unpredictable, limiting the reliability of direct sowing for commercial or restoration purposes (1,3). Various pre-sowing scarification treatments have been evaluated to overcome this physical dormancy by mechanically or chemically disrupting the seed coat and enabling water uptake and embryo activation. Among chemical approaches, sulphuric acid scarification has been studied in white lotus and found to produce uniform testa erosion, facilitating rapid imbibition and

metabolic activation. Comparative studies on chemical versus mechanical scarification in *N. nucifera* have confirmed the superiority of acid treatment for improving germination speed and uniformity. Mechanical scarification through nicking, filing or sand abrasion has also been explored, with results varying depending on the precision and extent of abrasion applied [?, ?].

Hot water immersion, another physical approach, has demonstrated inconsistent outcomes, as excessive thermal exposure can damage the embryo rather than promote germination. The efficacy of scarification has further been linked to measurable changes in seed membrane permeability, electrolyte leakage and the activation of α -amylase, a key enzyme responsible for starch hydrolysis and energy mobilisation during germination. Studies on hard-seeded aquatic macrophytes and tropical tree species—including *Acacia nilotica*, *Prosopis cineraria*, *Pongamia pinnata*, *Luffa cylindrica* and *Albizia lebbek*—have consistently demonstrated that appropriate scarification treatments, whether chemical, mechanical or thermal, significantly enhance germination percentage, germination speed and subsequent seedling vigour.

Investigations on seed coat scarification, priming and cold stratification in tropical waterlily hybrids have similarly underlined the species-specific and condition-specific nature of dormancy-breaking responses, reinforcing the need for locally validated protocols [?, ?]. The longevity of scarified seeds under different storage conditions is also an important practical consideration, particularly for conservation seed banks and large-scale nursery operations. Recent work on enhancing lotus seed germination through mechanical and chemical treatments has reaffirmed the relevance of standardised scarification protocols for reliable seedling production. Drone-based field monitoring technologies have also been applied to count lotus flowers and seedpods at the canopy level, reflecting the expanding precision horticulture interface with lotus crop management (8,17,18). Although considerable research has been conducted on lotus biology and seed dormancy globally, optimised and regionally validated scarification protocols for *N. nucifera* under Indian agroclimatic conditions, particularly in semi-arid environments, remain insufficiently documented. Given that temperature, humidity, soil properties and water quality significantly influence the outcome of pre-sowing treatments, standardisation of dormancy-breaking methods under local conditions is essential for the successful deployment of seed-based lotus propagation at a commercial and ecological scale (19,20). The present study was therefore undertaken to comparatively evaluate the efficacy of four scarification techniques - mechanical

nicking, sand abrasion, hot water immersion and sulphuric acid treatment - against an untreated control, with respect to their influence on seed germination and seedling performance of *Nelumbo nucifera* under the conditions of Ananthapuramu, Andhra Pradesh.

Material and Methods

The experiment was conducted in the Floriculture and Landscape Architecture Laboratory at Sri Krishnadevaraya College of Horticultural Sciences, Ananthapuramu (14°41' N, 77°36' E; elevation \approx 460 m above mean sea level) during June 2025. Freshly matured lotus (*Nelumbo nucifera* Gaertn.) seeds were collected from a well-maintained cultivated pond within the college campus. The seeds were hand-harvested on 10 June 2025, thoroughly cleaned to remove adhering pericarp tissues and air-dried under shaded conditions for two days before the initiation of treatments. Five



Figure 1. Lotus pond at SKCHS serving as a seed source

different treatments were imposed in a completely randomized design (CRD) with five replications, each consisting of 50 seeds. The treatments were as follows: T₁ (Control): seeds were rinsed with clean tap water and soaked in ambient water ($\approx 28 \pm 2^\circ\text{C}$) for 24 h without any scarification; T₂ (Mechanical Nicking): seeds were firmly held in vise grips and a small nick was made carefully at the micropyle end using a metal file until the whitish inner tissue was exposed, followed by gentle rinsing in tap water; T₃ (Sand Abrasion): seeds were rolled slowly and uniformly over coarse sandpaper until a lighter inner surface appeared, ensuring that the seed coat was weakened without injuring the embryo, then rinsed thoroughly; T₄ (Hot Water Immersion): seeds were immersed in hot water maintained at 80°C for 2 min, after which they were immediately transferred to room-temperature water and soaked for 24 h to cool and hydrate; and T₅ (Acid Scarification): seeds were soaked in concentrated sulphuric acid (H₂SO₄, 98%) for 1 hour with gentle and continuous stirring to etch the seed coat uniformly, then re-

peatedly washed under running tap water for 10 min to remove acid residues and soaked in ambient water for an additional 1 hour before further incubation.

After treatment, all seeds were placed in 500 ml transparent glass beakers containing 300 ml of dechlorinated tap water maintained at approximately 30°C under ambient laboratory conditions. The beakers were loosely covered with perforated lids to minimize contamination and evaporation. Water was replaced daily to maintain oxygenation and prevent microbial growth. Germination was monitored daily for 15 days and a seed was considered germinated when the radicle protruded at least 2 mm from the seed coat.

Mean Germination Time (MGT) was calculated using the formula:

$$MGT = \frac{\sum(n_i \times t_i)}{\sum n_i}$$

where n_i represents the number of seeds germinated on day t_i .

Germination percentage and MGT were computed for each replicate to assess treatment effects. The data were subjected to one-way analysis of variance (ANOVA) and treatment means were compared using Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. All statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

Scarification treatments significantly influenced germination, biochemical activity and seedling growth of *Nelumbo nucifera* ($p < 0.05$) (Tables 1 and 2). The untreated control (T_1) exhibited low imbibition (18.6%), poor seed coat permeability (0.21), reduced α -amylase activity ($2.36 \mu\text{mol maltose min}^{-1} \text{g}^{-1} \text{FW}$), delayed germination (10.4 days) and low germination percentage (22.4%), confirming strong physical dormancy imposed by the hard and impermeable seed coat. Similar dormancy-related constraints in lotus and other hard-seeded aquatic species have been reported by earlier workers (21,22).

All scarification treatments significantly enhanced water uptake, metabolic activation and germination compared to the control. Among them, sulphuric acid scarification (T_5) was the most effective, recording the highest imbibition rate (68.9%), seed coat permeability index (0.79), germination percentage (78.4%) and the shortest time to 50% germination (4.9 days). The superior response of acid-treated seeds is attributed to uniform erosion of the seed coat, which facilitated rapid

water uptake and embryo activation. Similar effectiveness of acid scarification in overcoming physical dormancy has been documented in Indian studies on hard-seeded species as well as in lotus (23,24,25).

Mechanical nicking (T_2) and sand abrasion (T_3) resulted in moderate improvement in germination, while hot water immersion (T_4) showed limited effectiveness. These findings are consistent with earlier reports who observed variable success of physical and thermal scarification due to uneven weakening of the seed coat or potential thermal injury (26,27).

Electrical conductivity of seed leachates increased significantly in scarified treatments, particularly under sand abrasion and hot water immersion, indicating enhanced membrane permeability following testa disruption. Such increases in conductivity are commonly associated with rapid hydration and dormancy release in hard-seeded species (28,29). Correspondingly, α -amylase activity increased significantly under scarification, with the highest value recorded in acid-treated seeds ($5.76 \mu\text{mol maltose min}^{-1} \text{g}^{-1} \text{FW}$), followed by sand abrasion ($4.32 \mu\text{mol maltose min}^{-1} \text{g}^{-1} \text{FW}$), while untreated seeds showed the lowest activity. Elevated α -amylase activity reflects enhanced starch mobilisation and energy supply necessary for rapid germination and early seedling growth, in agreement with earlier researchers (5,6,30).

Seedling growth and vigour parameters closely paralleled germination responses. Acid-scarified seeds (T_5) produced the highest seedling vigour index (1724.5), germination index (7.86) and maximum plumule (9.4 cm) and radicle lengths (12.6 cm), indicating rapid and uniform seedling establishment. Comparable improvements in seedling growth following scarification have been reported by earlier workers. Root-to-shoot ratio varied only slightly among treatments (1.23–1.44), with the control showing a marginally higher ratio due to restricted shoot elongation, while scarified treatments—particularly acid scarification—maintained balanced biomass allocation, indicative of healthy seedling development (31,32).

Overall, sulphuric acid scarification (98%, 1 h) proved to be the most efficient method for breaking physical dormancy in *N. nucifera*, resulting in enhanced imbibition, enzymatic activation, rapid germination and superior seedling vigour under Ananthapuramu conditions. Mechanical nicking and sand abrasion were moderately effective, while hot water immersion showed inconsistent results. The findings corroborate earlier Indian and international studies and highlight the potential of optimised acid scarification as a reliable, rapid and scalable method for large-scale lotus propagation,

wetland restoration and conservation programmes (33,34,35).

Table 1. Effect of scarification treatments on seed coat and germination parameters

Treatments	Imbibition Rate	Seed Coat Permeability Index	Electrical Conductivity of Seed Leachates	Amylase Activity	Time to 50% Germination	Mean Germination %
T ₁	18.6 ± 1.4 ^a	0.21 ± 0.02 ^a	92.4 ± 4.6 ^a	2.36 ± 0.12 ^a	10.4 ± 0.5 ^a	22.4 ± 2.1 ^a
T ₂	42.8 ± 2.1 ^b	0.48 ± 0.03 ^b	138.6 ± 6.2 ^b	3.84 ± 0.18 ^b	7.6 ± 0.4 ^b	45.2 ± 3.0 ^b
T ₃	51.4 ± 2.4 ^c	0.56 ± 0.04 ^c	152.3 ± 5.8 ^c	4.32 ± 0.20 ^c	6.8 ± 0.3 ^c	53.6 ± 2.8 ^c
T ₄	36.2 ± 1.9 ^b	0.41 ± 0.03 ^b	165.4 ± 7.1 ^c	3.42 ± 0.16 ^b	8.3 ± 0.5 ^b	39.2 ± 2.5 ^b
T ₅	68.9 ± 2.8 ^d	0.79 ± 0.05 ^d	128.7 ± 5.4 ^b	5.79 ± 0.22 ^d	4.9 ± 0.2 ^d	78.4 ± 3.4 ^d
CD (P = 0.05)	3.01	0.066	10.84	0.38	0.55	4.52
SEd	1.42	0.031	5.12	0.18	0.26	2.14

Treatments details: T₁: Control, T₂: Mechanical Nicking, T₃: Sand Abrasion, T₄: Hot Water Immersion and T₅: Acid Scarification

Table 2. Effect of scarification treatments on seed coat and germination parameters

Treatments	Seedling Vigour Index	Germination Index	Germination Time (days)	Plumule Length (cm)	Radicle Length (cm)	Root:Shoot Ratio
T ₁	174.1 ± 15.2 ^a	2.14 ± 0.18 ^a	9.8 ± 0.4 ^a	3.2 ± 0.3 ^a	4.6 ± 0.4 ^a	1.44 ± 0.08 ^a
T ₂	592.6 ± 28.4 ^b	4.36 ± 0.26 ^b	7.2 ± 0.3 ^b	5.8 ± 0.4 ^b	7.3 ± 0.5 ^b	1.26 ± 0.06 ^b
T ₃	827.5 ± 35.6 ^c	5.18 ± 0.31 ^c	6.4 ± 0.3 ^c	6.9 ± 0.5 ^c	8.5 ± 0.6 ^c	1.23 ± 0.05 ^b
T ₄	472.3 ± 22.7 ^b	3.82 ± 0.21 ^b	7.8 ± 0.5 ^b	5.2 ± 0.4 ^b	6.8 ± 0.5 ^b	1.31 ± 0.07 ^b
T ₅	1724.5 ± 68.3 ^d	7.86 ± 0.42 ^d	4.6 ± 0.2 ^d	9.4 ± 0.6 ^d	12.6 ± 0.7 ^d	1.34 ± 0.06 ^b
CD (P = 0.05)	73.2	0.51	0.60	0.78	0.88	0.09
SEd	34.6	0.24	0.28	0.37	0.42	0.04

Treatments details: T₁: Control, T₂: Mechanical Nicking, T₃: Sand Abrasion, T₄: Hot Water Immersion and T₅: Acid Scarification.

CONCLUSION

Seed coat scarification significantly improves germination performance, enzymatic activation and initial seedling growth in *Nelumbo nucifera*. Among the methods evaluated, concentrated sulphuric acid (98%, 1 hr) was the most effective in overcoming physical dormancy, resulting in the highest imbibition rate, seed coat permeability, α -amylase activity, germination percentage and seedling vigour. Mechanical nicking and sand abrasion provided moderate improvements, whereas hot water immersion showed limited efficacy. The enhanced enzymatic activity and balanced root-to-shoot growth observed under acid scarification indicate efficient mobilisation of stored reserves and accelerated metabolic activation. These findings establish sulphuric acid scarification as a reliable, rapid and scalable strategy for large-scale propagation, wetland restoration and conservation of *N. nucifera* under semi-arid Indian conditions, with potential for optimisation

in terms of exposure duration and concentration to enhance safety and sustainability.

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