International Journal of Design & Nature and Ecodynamics
Vol. 21, No. 2, February, 2026, pp. 375-388
Journal homepage: http://iieta.org/journals/ijdne

Breaking Physical Dormancy in *Helicteres isora* Seeds: Integrating Scarification, Morphology, and Phytochemical Profiling to Improve Germination

Diana Prameswari[1], Dede J. Sudrajat[1], Intani Quarta Lailaty[1], Aris Sudomo[2], Dani Pamungkas[3], Sudarmono Sudarmono[4*], Surya Diantina[1], Ratna Uli Damayanti Sianturi[1], Yosephin Martha Maria Anita Nugraheni[1], Popi Aprilianti[1], Elizabeth Handini[1], Dwi Murti Puspitaningtyas[1]

[1] Research Center for Applied Botany, National Research and Innovation Agency (BRIN), Bogor 16911, Indonesia
[2] Research Center for Ecology, National Research and Innovation Agency (BRIN), Bogor 16911, Indonesia
[3] Center for Socio-Economic Development of Forest Community Bogor, Bogor 16110, Indonesia
[4] Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Bogor 16911, Indonesia

Corresponding Author Email: paksudarmono154@gmail.com

https://doi.org/10.18280/ijdne.210207

Received: 26 December 2025
Revised: 10 February 2026
Accepted: 18 February 2026
Available online: 28 February 2026

Keywords:
scarification, medicinal plants, seed dormancy, physical dormancy, hot water treatment, phytochemical compounds

ABSTRACT

Helicteres isora L. is a medicinal plant from eastern Indonesia whose natural regeneration is limited by poor seed germination. This study evaluated the effects of scarification treatments and germination media, and examined how seed morphology and phytochemical composition influence germination behavior. Germination was tested using a factorial Completely Randomized Design (CRD) with seven dormancy-breaking treatments (hot water at 80°C for 5–15 minutes, 1% H_2SO_4 for 4–6 hours, and a control) and two germination media. Scanning Electron Microscopy (SEM) analyses showed that *Helicteres isora* seeds have a thick, multilayered seed coat that restricts water uptake, confirming the presence of inherent physical dormancy. Germination experiments demonstrated that scarification significantly affected all germination parameters, whereas the germination media had no significant effect. Soaking seeds in hot water at 80°C for 5 minutes produced the highest germination capacity (78%), germination velocity (5.33), and maximum growth potential (98%). In contrast, prolonged acid scarification caused severe embryo damage. Gas Chromatography–Mass Spectrometry (GC-MS) analysis identified phenolic compounds, aldehydes, organic acids, and fatty acids, suggesting potential roles in maintaining oxidative balance and inhibiting germination. At the same time, lipid reserves support seedling establishment after dormancy release. Overall, combining morphological observations with germination assays and seed phytochemical profiling provides additional insight into seed dormancy in *H. isora* beyond treatment-based approaches. These findings demonstrate that germination in *H. isora* is governed by physical seed-coat constraints interacting with endogenous phytochemical composition and that appropriate scarification provides an effective strategy to improve the propagation of this medicinal species.

1. INTRODUCTION

Medicinal plants have been utilized since ancient times to treat various diseases, with therapeutic knowledge initially developed through empirical and instinctive practices due to limited scientific understanding. Over time, accumulated experience and documentation improved the identification and utilization of medicinal plants for specific ailments, resulting in extensive written evidence that can be referenced today [1]. Currently, the use of medicinal plants has expanded to an industrial scale, increasing demand for raw materials predominantly harvested from natural ecosystems. The continuous extraction of plant-based medicinal resources from the wild has contributed to the decline and scarcity of several medicinal plant species [2]. Therefore, sustainable propagation strategies are essential to ensure a stable supply of raw materials for the herbal industry while conserving natural populations.

Helicteres isora L. (screw tree), locally known as *kayu ules*, is a medicinal shrub species naturally distributed in several regions of Indonesia, including East Nusa Tenggara (Nusa Tenggara Timur, NTT). According to the IUCN Red List (2024), *H. isora* is currently listed as Least Concern. Morphologically, *H. isora* belongs to the family Sterculiaceae and grows as an erect shrub reaching 1–2.5 m in height, characterized by red flowers and spirally twisted fruits, which give rise to its common name "screw tree" [3, 4]. Various plant parts, including bark, roots, leaves, seeds, and fruits, have been traditionally reported to possess medicinal properties, and these claims have been experimentally confirmed. These include treatments for diarrhea (bark), diabetes, neonatal weakness, and ear sores (fruit), dysentery (seed), and scabies (leaves). Phytochemical analyses have revealed that *H. isora* contains bioactive compounds, including betulinic acid, β-tocopherol, coumarins, β-sitosterol, saponins, flavonoids, tannins, and alkaloids, which support its medicinal significance [2, 3, 5].

Due to its high medicinal value, *H. isora* has long been recognized as a commercially important species. Its fruits are widely used as a key ingredient in traditional herbal medicine (*jamu*) produced by local industries. At the regional level, farmers commonly harvest fruits and trade them through collectors or traditional markets. However, fruit production from natural populations remains low and inconsistent. To meet increasing demand, a substantial proportion of *H. isora* fruits used in Indonesia are imported from India, despite the presence of wild populations within the country [6]. This reliance on natural harvesting and imports highlights the urgent need to develop effective cultivation and propagation methods for *H. isora*.

Propagation of *H. isora* through vegetative methods has been investigated, with previous studies reporting successful multiplication using stem cuttings [7]. However, vegetative propagation alone may limit genetic diversity and the production of large numbers of seedlings. Generative propagation through seeds has also been attempted [3, 8], yet these studies indicate that seed germination remains inconsistent and generally low, suggesting the presence of seed dormancy constraints. A recent study by Qadir and Khan [1] reported that hot water soaking was among the most effective pre-treatments for improving germination, although optimal temperature and soaking duration were not clearly defined. Furthermore, seed viability of *H. isora* declines with prolonged storage. According to Muthukumar et al. [9], seed viability remains relatively stable during the first six months after harvest but decreases significantly thereafter, reaching a reduction of up to 63.3% after 24 months of storage.

Physical dormancy is a widespread seed dormancy class characterized by water-impermeable layers in the seed coat that prevent imbibition and delay germination until specific environmental cues are encountered [10]. Physical dormancy is controlled by the physical and structural properties of the seed coat [11] and by its chemical composition during maturation [12]. Physical dormancy is primarily associated with specialized palisade cell layers in the testa, often reinforced by lignin, suberin, or phenolic compounds that form a hydrophobic barrier [13]. The ecological function of physical dormancy is to regulate germination timing, ensuring that seeds germinate only under favorable environmental conditions, thus enhancing survival and persistence in natural habitats [14-16].

Mechanistically, dormancy break in physically dormant seeds typically occurs when environmental signals—such as temperature fluctuations, fire-related heat pulses, microbial action, or mechanical abrasion—disrupt the integrity of the water-impermeable layer and open specialized structures such as the lens or chalazal plug [13, 17, 18]. Artificial scarification methods, including mechanical abrasion, acid treatment, and hot-water immersion, mimic natural processes and are widely used to enhance germination in species with hard seed coats. Recent reviews emphasize that hot water treatment and mechanical scarification are among the most efficient and environmentally practical approaches for breaking physical dormancy in tropical and medicinal species [19, 20]. Furthermore, advances in seed coat microscopy and phytochemical profiling have demonstrated that variations in lignin deposition, phenolic content, and cuticular thickness directly influence the degree of impermeability and responsiveness to dormancy-breaking treatments [11, 21].

Improving germination techniques through appropriate seed scarification and suitable germination media may provide a cost-effective, practical solution to enhance generative propagation of *H. isora*. Such approaches are particularly relevant for smallholder farmers, who often face technical and economic constraints in adopting advanced propagation methods such as tissue culture [22]. However, information on the structural and phytochemical mechanisms underlying seed dormancy in *H. isora* remains limited, as previous studies have emphasized mainly treatment effectiveness over biological explanations. Therefore, the present study aims to evaluate the effects of different seed scarification treatments and germination media, and to examine how fruit and seed morphology, as well as seed phytochemical composition, influence germination performance in *H. isora*. In addition, this study investigates fruit and seed morphology, antioxidant activity, and seed metabolite profiles to support bioprospecting efforts and contribute to a more comprehensive understanding of the biological and phytochemical characteristics associated with seed dormancy and germination of this essential medicinal species.

2. MATERIALS AND METHODS

2.1 Plant material and research location

Fruits of *H. isora* were collected from its natural habitat in Bosen Village, North Mollo Subdistrict, Timor Tengah Selatan Regency, NTT Province, Indonesia (09°42′34.1″ S, 124°18′03.5″ E; altitude 691 m above sea level) (Figure 1). The study area represents a natural distribution zone of *H. isora* in eastern Indonesia. Fruits were collected during the peak fruiting season to ensure optimal physiological maturity.

2.2 Observation of seed morphology

Morphological observations of *H. isora* seeds were conducted using Scanning Electron Microscopy (SEM). SEM observations were performed using a Dual-Beam FIB Aquilos 2 microscope (Thermo Fisher Scientific), equipped with a field emission gun (FEG), at room temperature (± 25 °C) at the Cryo-EM Laboratory, National Research and Innovation Agency (BRIN), Indonesia. This analysis focused on the detailed characterization of seeds and fruit surface structures.

Seed samples were obtained from natural populations in East Nusa Tenggara, and plants were grown from germinated seedlings under controlled conditions in Bogor to allow comparative observation. Whole seeds and transversely cut seed sections were observed without prior chemical treatment to examine both external and internal seed structures. The dried samples were mounted onto aluminum SEM stubs using carbon double-sided adhesive tape.

All samples were transferred under high-vacuum conditions from the preparation chamber to the main microscope chamber. To improve surface conductivity and image resolution, samples were coated with a thin platinum layer (~5 nm thickness) using an integrated retractable sputter coater operated at 30 mA for 20 seconds. SEM imaging was performed at an accelerating voltage of 4 kV, a beam current of 25 pA, and working distances of 9-16 mm. The microscope stage provided full 360° rotation and tilting, enabling optimal imaging from multiple angles without repositioning the samples.

Observations of fruits and seeds were conducted at magnifications of 150×, 200×, and 1000×.

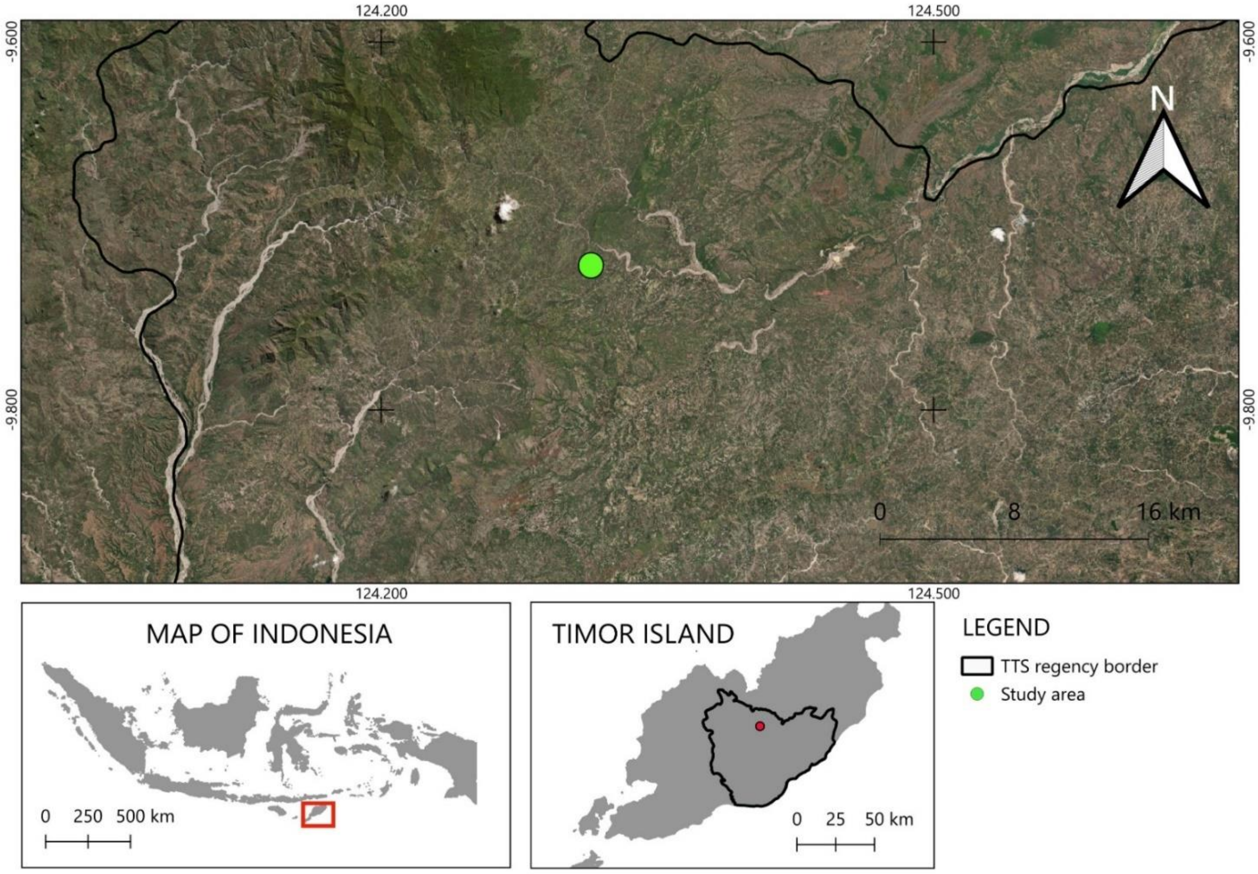


Figure 1. Location of seed collection at Bosen Village, North Mollo Subdistrict, Timor Tengah Selatan Regency, East Nusa Tenggara (NTT) Province, Indonesia

2.3 Experimental design of the seed germination test

Seed moisture content was measured before germination treatments, following procedures outlined by the International Seed Testing Association (ISTA) [23, 24] to ensure uniform physiological status of the initial seeds. Germination experiments were arranged in a factorial design involving two main factors. The first factor consisted of seed dormancy-breaking treatments, including:

(1) control (untreated seeds), (2) soaking in hot water at 80°C for 5 minutes, (3) soaking in hot water at 80°C for 10 minutes, (4) soaking in hot water at 80°C for 15 minutes, (5) soaking in concentrated 1% H_2SO_4 for 4 hours, (6) soaking in 1% H_2SO_4 for 5 hours, and (7) soaking in 1% H_2SO_4 for 6 hours.

These treatments were selected to evaluate both physical and chemical scarification methods commonly applied to hard-coated seeds. The second factor was germination media, consisting of (1) a mixture of river sand and topsoil (1:1, v/v), and (2) a mixture of cocopeat, rice husk char, and river sand (1:1:1, v/v/v).

The experiment was laid out using a Completely Randomized Design (CRD) with a factorial arrangement. Each treatment combination was replicated three times, with each replicate consisting of 50 seeds, yielding a robust sample size for statistical analysis.

2.4 Media preparation and seed sowing

Only healthy, undamaged fruits with a uniform brown color—indicating full maturity—were manually selected and harvested (Figure 2). Seed extraction was carried out destructively by opening the fruits, and the seeds were separated manually. Seed selection was conducted under a stereomicroscope by spreading all extracted seeds onto Petri dishes to eliminate malformed, damaged, or immature seeds. Only visually uniform and intact seeds were used for subsequent analyses.

Figure 2. Flowers, fruits, and seeds of *Helicteres isora* L.

Before sowing, the germination media were carefully prepared to ensure uniform physical properties. River sand was sieved using a mesh with openings smaller than 1 mm to remove coarse particles and debris. The sand was subsequently sterilized using an autoclave at 121 °C under 1 atm pressure for 60 minutes to eliminate potential microbial contamination. Other media components were air-dried and homogenized before mixing according to the designated ratios.

Seeds subjected to soaking treatments were drained immediately and briefly air-dried at room temperature to remove excess surface moisture. Germination containers were prepared by creating planting holes approximately 1 cm deep in the respective media. One seed was placed in each hole and gently covered with the surrounding medium to ensure adequate seed–soil contact.

2.5 Germination measurement

Seed germination was monitored every two days throughout the observation period. A seed was considered germinated when the radicle protrusion was clearly visible. Germination parameters evaluated included maximum growth potential (MGP), germination capacity (GC), mean germination time (MGT), and time required to reach 80% germination (T80%), calculated according to standard seed testing procedures [19]. These parameters were used to comprehensively assess both germination speed and uniformity.

2.6 Antioxidant activity analysis

Seeds of *H. isora* and fruits pericarp (100 g each) were finely ground into a fine and homogenous powder and then extracted using the maceration method with methanol (500 mL) for 24 hours at room temperature. The extracts were filtered to remove solid residues and concentrated to dryness under reduced pressure at 38 °C using a rotary evaporator. The resulting crude extracts were stored at 4 °C until further analysis of their antioxidant properties. The antioxidant activity of seed extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Extract samples and vitamin C (positive control) were prepared at various concentrations ranging from 3.25 to 100 ppm. A volume of 100 µL of each sample solution was added to a microplate well, followed by 100 µL of DPPH solution (125 µM in ethanol). Negative control wells contained 200 µL of ethanol p.a., while blank wells contained 100 µL ethanol p.a. and 100 µL DPPH. The reaction mixtures were incubated for 30 minutes at room temperature in the dark to prevent photodegradation. Absorbance was measured at 517 nm using a spectrophotometer. Each measurement was conducted in duplicate to ensure reproducibility. Radical scavenging activity (%) was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = (A-B)/A \times 100$$

where, A is the absorbance of the negative control (DPPH + ethanol), and B is the absorbance of the sample (DPPH + ethanol + extract). The relationship between extract concentration and scavenging activity was plotted, and IC_{50} values (concentration required to inhibit 50% of DPPH radicals) were determined by interpolation.

Gas Chromatography–Mass Spectrometry (GC-MS) was used to analyze and identify complex, insoluble, high-molecular-weight compounds by heating them to decompose into smaller, volatile components, then separating the resulting components by gas chromatography (GC) and identifying them by mass spectrometry (MS). Seed samples were ground into a fine, homogeneous powder using a laboratory mill and passed through a 60-mesh sieve to ensure uniform particle size. The powdered samples were oven-dried at 40 °C to constant weight to remove residual moisture, then stored in airtight containers in a desiccator before analysis. Approximately 1–2 mg of the dried powder was accurately weighed and transferred into a quartz pyrolysis cup for direct analysis without solvent extraction, allowing characterization of thermally derived marker compounds representing the macromolecular seed matrix.

The resulting pyrolysates were transported into the GC-MS system using helium as the carrier gas. The GC-MS operated under low-vacuum (< 15 Pa) and high-vacuum ($< 1.5 \times 10^{-3}$ Pa) conditions, achieved after approximately 2 hours of system stabilization. Chromatograms obtained from Py-GCMS analysis were processed, and compound identification was performed using the Wiley 7 mass spectral library.

2.7 Data analysis

All quantitative germination data were subjected to analysis of variance (ANOVA) using SAS software version 9.0 to determine the significance of treatment effects and interactions. When significant differences were detected, mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Morphological observations of seeds were analyzed descriptively. Metabolite screening was conducted using Py-GCMS results by selecting compounds with a similarity index greater than 85%. Identified compounds were categorized by chemical class and reported biological activity, using standardized reference literature.

3. RESULTS AND DISCUSSION

3.1 Seed morphology

H. isora bears dry, dehiscent, capsule-type fruits composed of five helically twisted follicles, with the direction of torsion occurring either sinistrally or dextrally and terminating in an acuminate apex. Fruits measure 3.1–7.8 cm in length and 5.0–9.1 mm in width, with individual fruit mass ranging from 0.28 to 1.4 g. Fruit coloration varies with ontogenetic stage, being light green at the immature stage and progressively changing to grayish brown or dark brown upon full maturity. The seeds are dark brown to blackish, exhibiting a smooth, lustrous (highly polished) testa. Seed shape ranges from triangular to rectangular, generally rhomboid in outline, with well-defined edges and a compact seed body (Figure 3).

Seeds of *H. isora* were blackish-brown in color and varied in shape from rectangular to triangular. Macroscopically, seed length ranged from 1.3 to 2.8 mm, while seed width ranged from 0.3 to 1.4 mm. Individual seed weight ranged from 0.17 to 0.40 g. The number of seeds per follicle ranged from 9 to 36, resulting in approximately 68–177 seeds per fruit. The average weight of 100 seeds was recorded as 0.17 g, consistent with previous reports [20].

Micromorphological analysis showed that seeds collected from natural populations in NTT and seeds obtained from germinated seedlings exhibited similar morphological characteristics (Figure 4 and Figure 5). Seed length ranged from 2178.78 to 2215.87 µm, while seed width ranged from

1393.93 to 1556.21 µm (Figure 4(a)). These results indicate minimal variation in seed size between seeds from natural habitats (NTT) and those produced under controlled germination conditions (Bogor) (Figure 5(a)). Also, the seed coat (testa) exhibits a consistent morphology across samples (Figure 4(b) and 5(b)).

Detailed SEM observations revealed distinct structural components of the seeds. The seed apex (plumula) represented the region from which shoot emergence occurs during germination (Figures 4(c) and 5(c)). The basal part of the seed contained the hilum, which functions as the point of attachment between the seed and the fruit tissue (Figures 4(d) and 5(d)). The seed coat consisted of multiple layers, including the exocarp, endocarp, and endosperm, which were clearly distinguishable in transverse sections (Figures 4(e)-(f)) and 5(e)-(f)). Microscopic observation of the fruit surface revealed that the fruits of *H. isora* were elongated and spirally twisted. The outer fruit surface (exocarp) exhibited considerable structural variation when observed at different magnification levels. These micromorphological features were consistently observed across the examined fruit samples (Figure 6).

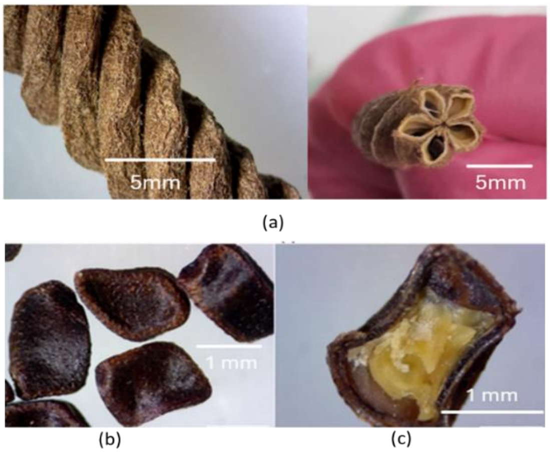


Figure 3. *Helicteres isora* (a) fruit, (b) seeds, (c) cross-sectioned seed

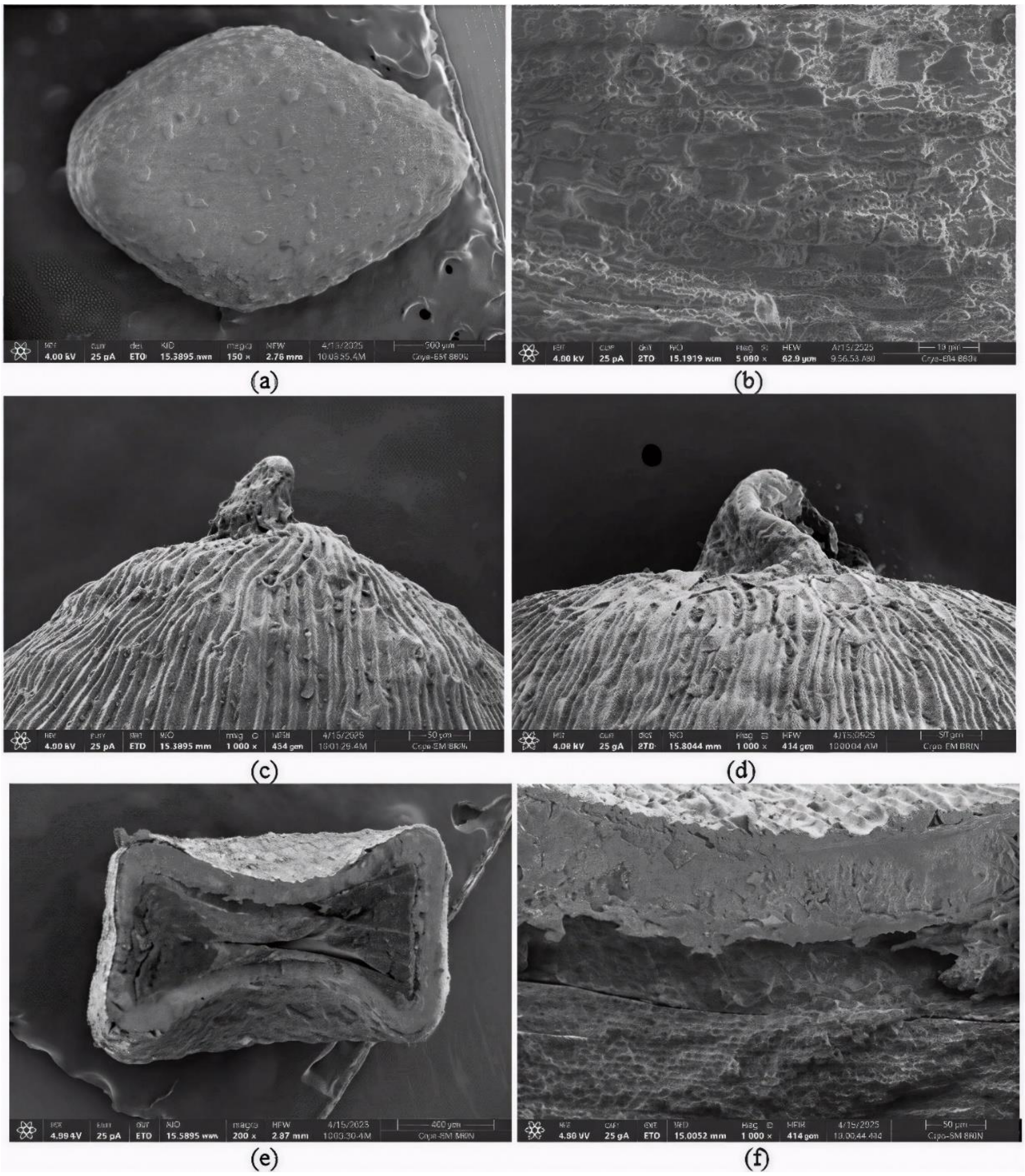


Figure 4. The seed of *Helicteres isora* originated from East Nusa Tenggara (NTT): (a) whole seed, (b) testa, (c) apex/ plumula, (d) hilum, (e-f) layers part of the seed

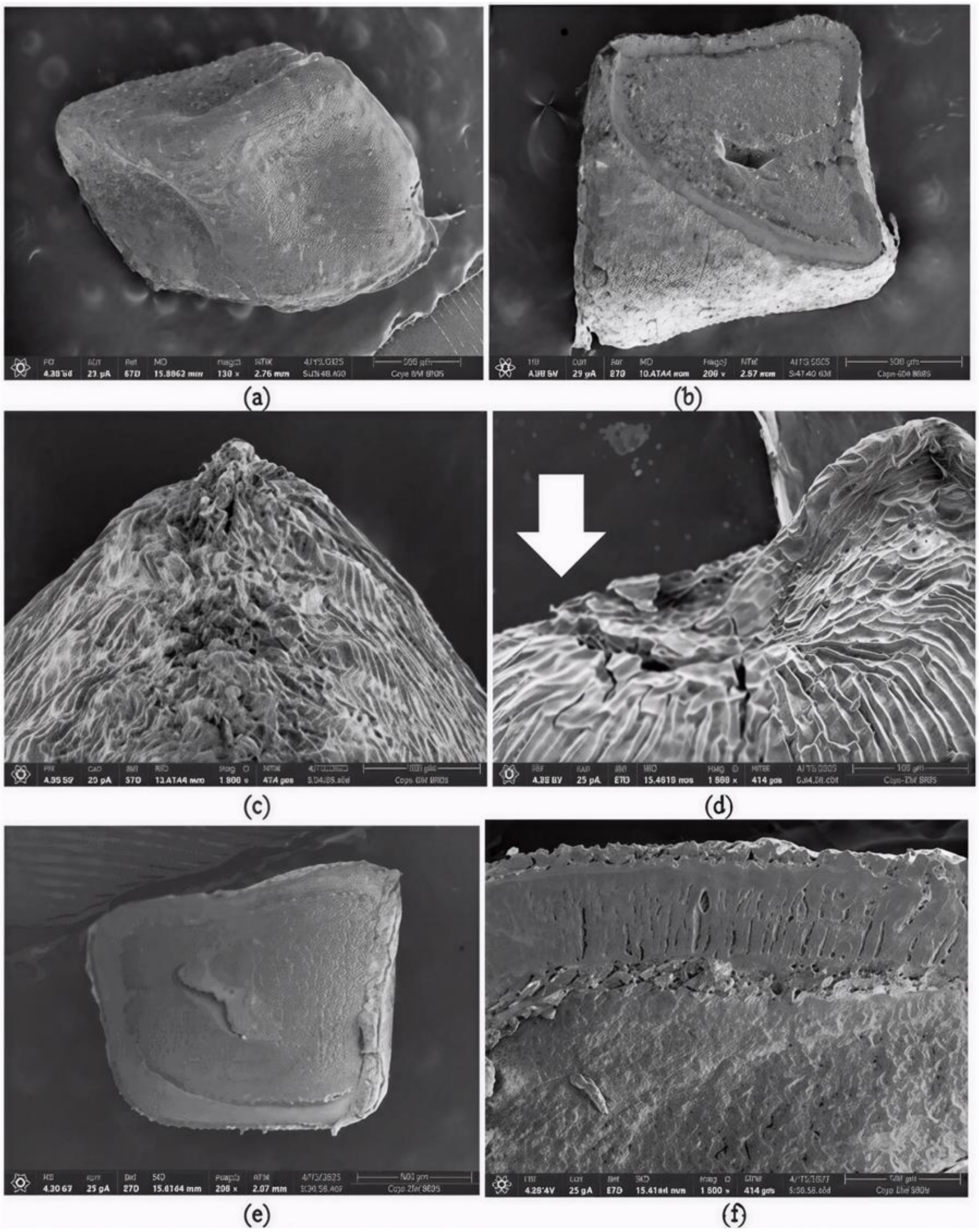
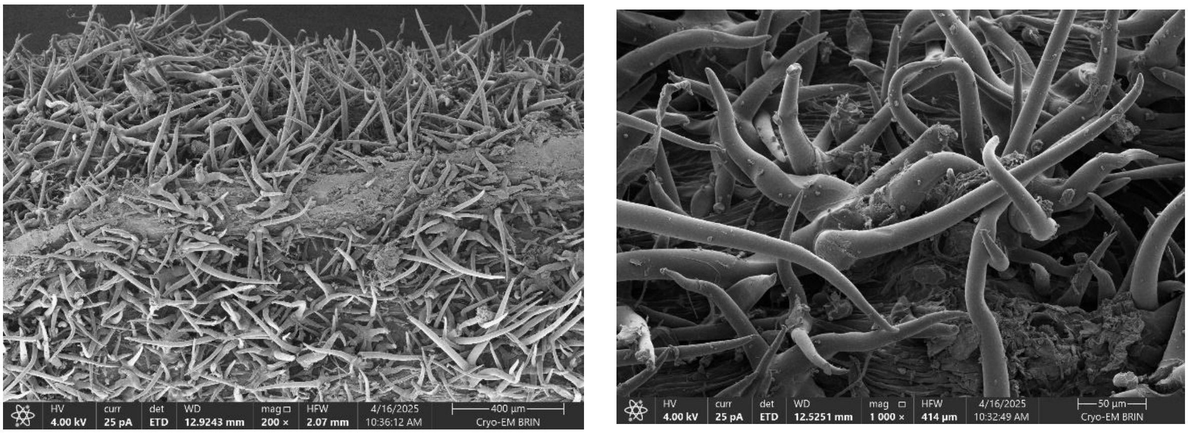
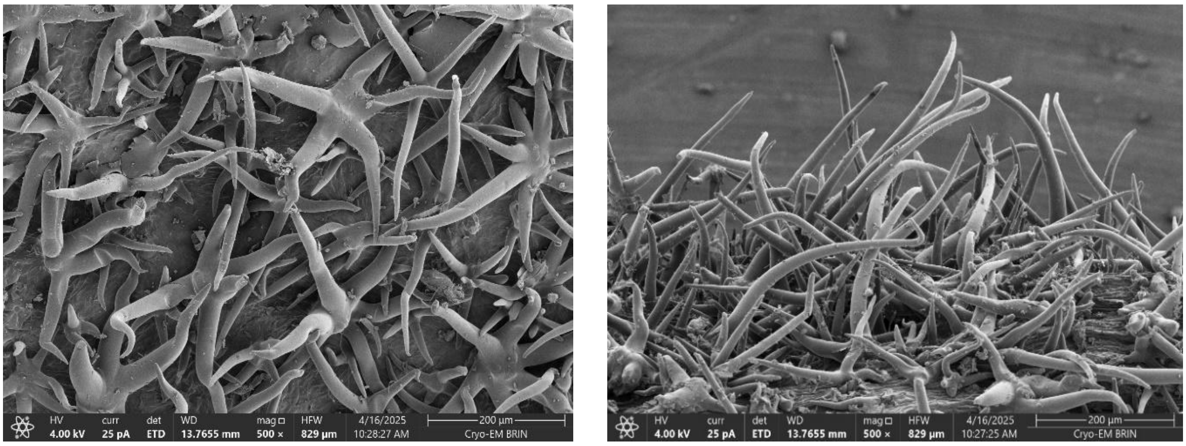


Figure 5. The seed of *Helicteres isora* results from seed germination (a) whole seed, (b) testa, (c) apex/ plumula, (d) hilum, (e-f) layers part of the seed



(a) (b)



(c) (d)

Figure 6. Exocarp of *Helicteres isora* fruit in various magnifications: (a) 200×; (b) 1000×; (c-d) 500×, showing different viewing angles

Seed morphological observations revealed that *H. isora* seeds possess a smooth, glossy, highly polished testa with a compact, rigid structure. SEM analysis further demonstrated that the seed coat is thick and continuous, with minimal surface ornamentation. These features are characteristic of seeds exhibiting physical dormancy, in which the seed coat restricts water imbibition and gas exchange, thereby delaying germination [13, 21]. The presence of multiple seed coat layers observed in transverse sections supports the hypothesis that dormancy in *H. isora* is primarily imposed by structural barriers rather than physiological limitations of the embryo.

Fruit morphology may further contribute to the regulation of dormancy in *H. isora*. The seeds are enclosed within dry, dehiscent capsules composed of five helically twisted follicles. This tightly coiled fruit structure may limit rapid seed release and reduce direct exposure to environmental moisture. Similar fruit–seed structural interactions have been reported to enhance seed protection and prolong dormancy in woody and medicinal plant species [19]. The combined effects of hardened fruit tissue and an impermeable seed coat likely explain the low and irregular germination rates reported for *H. isora* under natural conditions.

The similarity in micromorphological characteristics between seeds collected from natural habitats and those obtained from germinated seedlings indicates that dormancy-related traits are inherent to the species rather than environmentally induced. Such structural consistency suggests that seed dormancy in *H. isora* is genetically regulated, as commonly observed in species with physically dormant seeds [13]. Consequently, dormancy-breaking treatments are required to overcome these inherent morphological constraints.

The effectiveness of scarification treatments reported in previous studies can be mechanistically explained by their ability to disrupt or weaken the seed coat, thereby facilitating water uptake and oxygen diffusion to the embryo [25]. Water and chemical scarification are widely applied to medicinal and forest species with hard-coated seeds, representing practical, low-cost techniques suitable for smallholder propagation systems [10]. In this context, the present morphological evidence provides a structural basis for the observed improvement in germination following scarification treatments.

3.2 Effect of scarification treatments and germination media on seed germination

The results indicated that dormancy-breaking treatments had a significant effect ($p < 0.05$) on maximum growth potential (MGP), germination capacity (GC), germination rate, and the time required to reach 80% germination (T80%). In contrast, germination media and the interaction between dormancy-breaking treatments and media showed no significant effects on these parameters ($P > 0.05$). The average values of MGP and GC differed significantly among scarification treatments (Table 1). Germination begins on the 5th day and continues through the 10th day (Figure 7). The structure of a normal seedling, which is characterized by the growth of a pair of perfectly developed leaves, can be seen in Figure 8. Soaking seeds in hot water at 80 °C for 5 minutes (A2) resulted in the highest MGP (94%) and GC (78%), whereas prolonged soaking in concentrated H_2SO_4 for 6 hours (A7) resulted in the lowest MGP (3%) and GC (1.5%). The control treatment (A1) also showed very low germination, indicating strong dormancy in untreated seeds.

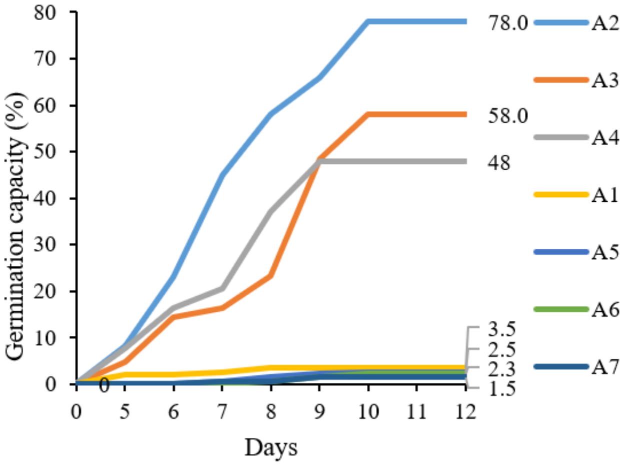


Figure 7. Cumulative germination (%) of *Helicteres isora* seeds treated by scarification treatments

Notes: A1 = control, A2 = soaking in hot water at 80 °C for 5 minutes; A3 = soaking the seeds in 80°C hot water for 10 minutes, A4 = soaking the seeds in 80°C hot water for 15 minutes, A5 = soaking the seeds with 1% H2SO4 for 4 hours, A6 = soaking the seeds with 1% H_2SO_4 for 5 hours, A7 = soaking the seeds with 1% H_2SO_4 for 6 hours.

The effects of dormancy-breaking treatments on mean germination time and germination speed are shown in Table 2. Seeds treated with hot water at 80 °C for 5–15 minutes (A2–A4) exhibited higher germination speed than acid-treated seeds. In contrast, prolonged H_2SO_4 treatment (A6 and A7) significantly reduced germination rate and delayed germination onset, resulting in a higher mean germination time.

The germination media did not significantly affect any germination parameters ($P > 0.05$). However, seeds sown in a mixture of soil and sand (M1) showed higher mean MGP (83.33%) and GC (68.67%) compared to those planted in a mix of sand, cocopeat, and rice husk charcoal (M2), which exhibited MGP and GC values of 70.33% and 66.36%, respectively (Table 3). Although the interaction between dormancy-breaking treatments and germination media was not statistically significant ($P > 0.05$), differences in mean values were observed. The combination of hot-water soaking at 80 °C for 5 minutes with a sand, cocopeat, and rice husk charcoal medium (A2M2) produced the highest germination performance, with an MGP of 94.0%, a GC of 78.07%, a faster germination rate, and a shorter mean germination time.

The differential effects of hot water and sulfuric acid treatments can be interpreted in relation to seed coat microstructure. Hot water exposure at 80 °C likely induces localized thermal stress, leading to microcracks or disruption of the palisade layer, thereby enhancing water permeability while largely preserving the underlying embryonic tissues. SEM observations support this interpretation by showing structural discontinuities in the testa without extensive tissue collapse. In contrast, concentrated sulfuric acid treatment may cause more aggressive chemical corrosion, leading not only to erosion of the seed coat but also potential degradation of subepidermal tissues. Prolonged acid exposure may therefore compromise membrane integrity and embryo viability, explaining the reduced or inconsistent germination response under longer acid treatments.

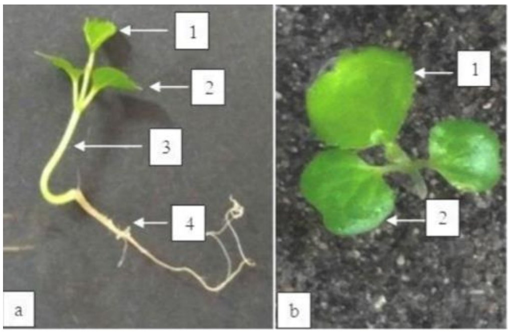


Figure 8. Normal seedling of *Helicteres isora* (a1: leaf, a2: cotyledon, a3: stem, a4: Root, b1: leaf, and b2: cotyledon)

Table 1. Duncan test results of the effect of breaking dormancy on the average maximum growth potential (MGP) and average germination capability (GC) in the treatment of breaking dormancy of *Helicteres isora* seeds

Parameter	Treatment							Significance
	A1	A2	A3	A4	A5	A6	A7	
MGP (%)	7c	94.0a	67b	55b	5c	4c	3c	P < 0.05 (*)
GC (%)	3.5c	78.0a	58b	48b	2.5c	2c	1.5c	P < 0.05 (*)

Remarks: * = Values within a row followed by different lowercase letters are significantly different according to Duncan's multiple range test ($p < 0.05$), A1 = control, A2 = soaking in hot water at 80 °C for 5 minutes; A3 = soaking the seeds in 80 °C hot water for 10 minutes, A4 = soaking the seeds in 80 °C hot water for 15 minutes, A5 = soaking the seeds with 1% H_2SO_4 for 4 hours, A6 = soaking the seeds with 1% H_2SO_4 for 5 hours, A7 = soaking the seeds with 1% H_2SO_4 for 6 hours.

Table 2. Effect of dormancy breaking on germination day and growth rate of *Helicteres isora* seeds

Parameter	Treatment							Significance
	A1	A2	A3	A4	A5	A6	A7	
Germination day	4.833ab	6.333a	6.167a	5.500a	5.167a	3.667ab	1.333b	P < 0.05 (*)
Growing speed	5.667a	5.333a	5.167a	5.000a	5.000a	3.667ab	1.333b	P < 0.05 (*)

Remarks: * = Significantly different at 95% level, and values within a row followed by different lowercase letters are significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 3. Effect of media on maximum growth potential and germination of *Helicteres isora* tree seeds

Treatment	Media		Significance
	Mix of Soil and Sand (M1)	A Mixture of Sand with Cocopeat and Rice Husk Charcoal (M2)	
MGP (%)	83.33a	70.33a	P > 0.05 (ns)
GC (%)	68.67a	66.36a	P > 0.05 (ns)

Remarks: ns = No significantly different at 95% level; * = Values within a row followed by different lowercase letters are significantly different according to Duncan's multiple range test ($p < 0.05$)

The measured seed moisture content of *H. isora* ranged from 8.6% to 12.0%, with an average of 9.6%. This moisture range suggests that the seeds can tolerate desiccation and are therefore likely to be orthodox. However, confirmation through critical moisture content testing is still required. Orthodox seeds are known to tolerate drying to low moisture levels while maintaining viability [26, 27]. However, previous studies reported a rapid decline in *H. isora* seed viability during storage, with viability decreasing by more than 50% after 1 year and to approximately 24.9% after 2 years [9]. This discrepancy between desiccation tolerance and storage longevity suggests that *H. isora* seeds may exhibit intermediate storage behavior rather than strictly orthodox characteristics, as described by Hong et al. [27].

The low germination observed in untreated seeds confirms the presence of dormancy in *H. isora*. Seed morphology observations revealed a smooth, glossy, and compact seed coat, which likely acts as a physical barrier restricting water uptake and gas exchange. Physical dormancy caused by an impermeable seed coat is common among tropical medicinal plant species [13].

Hot water scarification at 80 °C for 5 minutes proved to be the most effective treatment, significantly increasing both MGP and GC. This treatment softened the seed coat, increased permeability, and facilitated imbibition, thereby activating metabolic processes required for germination. Similar findings have been reported for other physically dormant species, in which short-duration hot-water treatments effectively break dormancy without damaging the embryo [28, 29].

In contrast, prolonged immersion in concentrated 1% H_2SO_4 resulted in very low germination rates. Although acid scarification is commonly used to weaken hard seed coats, excessive exposure can damage the testa and embryo tissues, thereby reducing viability [17, 30]. The extremely low germination observed after 5–6 hours of acid treatment showed chemical injury rather than the release of adequate dormancy.

The improved germination speed observed in hot-water-treated seeds further indicates enhanced seed vigor, as rapid germination is closely associated with higher metabolic efficiency and uniform seedling emergence [31]. Although germination media did not significantly affect germination parameters, seeds grown in soil: sand media showed higher MGP and GC values. Sand-dominated substrates provide adequate aeration and moderate water retention, which are critical for early seedling establishment [25]. Conversely, the lighter texture of the sand-cocopeat-rice husk charcoal mixture facilitated faster emergence and shorter germination time, likely due to reduced mechanical resistance and improved oxygen diffusion.

The absence of a significant interaction between scarification and media indicates that dormancy-breaking treatment plays a more decisive role in controlling germination success than substrate composition. Nonetheless, the superior performance of the A2M2 combination suggests that optimal physical seed treatment, combined with a well-aerated medium, can enhance early seedling performance under nursery conditions.

3.3 Antioxidant activity of *Helicteres isora* seeds

The antioxidant activity of *H. isora* seed and fruit coat extracts was evaluated using the DPPH radical scavenging assay, and the results are presented in Table 4. The IC_{50} values varied among plant parts, indicating differences in antioxidant capacity. The fruit coat extract exhibited the most potent antioxidant activity, with an IC_{50} of 45.11 ppm, followed by the seed extract, with an IC_{50} of 89.89 ppm. Vitamin C, used as a positive control, showed the highest antioxidant activity, with an IC_{50} value of 5.08 ppm. According to commonly accepted classifications, extracts with IC_{50} values below 50 ppm are considered potent antioxidants, while those with IC_{50} values between 50 and 100 ppm are categorized as moderate antioxidants. Based on this classification, the fruit coat of *H. isora* showed vigorous antioxidant activity, whereas the seed extract exhibited moderate antioxidant activity.

Table 4. Antioxidant activity of *Helicteres isora* seeds

Samples	IC_{50} (ppm)
Fruit's pericarp	45.11
Seed	89.89
Vitamin C	5.08

The moderate antioxidant activity observed in *H. isora* seeds suggests the presence of bioactive compounds capable of scavenging free radicals. Previous phytochemical studies have reported that *H. isora* seeds contain flavonoids, tannins, phenolic acids, and alkaloids, all of which are known contributors to antioxidant capacity [32, 33]. Although the antioxidant activity of the seed extract was lower than that of the fruit coat, its IC_{50} value still reflects biologically relevant radical scavenging potential.

Antioxidant compounds play a crucial role in seed germination by regulating reactive oxygen species (ROS) levels. During the early stages of imbibition and germination, ROS are rapidly produced due to increased metabolic activity. At controlled concentrations, ROS act as signaling molecules that promote endosperm weakening, radicle protrusion, and improve germination [34, 35]; however, excessive ROS accumulation can cause oxidative damage to membranes, proteins, and nucleic acids, ultimately reducing seed vigor and germination capacity [25, 36, 37].

The moderate antioxidant activity of *H. isora* seeds suggests a protective role against oxidative stress during germination. Seeds with sufficient endogenous antioxidant capacity are better at maintaining cellular integrity and metabolic balance, which contributes to faster and more uniform germination once physical dormancy barriers are removed [38]. This mechanism is particularly relevant for *H. isora*, where scarification treatments facilitate water uptake and oxygen diffusion, potentially increasing ROS production during early imbibition.

The more potent antioxidant activity observed in the fruit coat may be ecologically significant, as fruit protects seeds and often serves as a protective barrier against environmental stressors such as ultraviolet radiation, microbial attack, and oxidative degradation during seed development and dispersal [39]. However, once the seed is separated from the fruit and germinates, the seed's antioxidant capacity becomes more critical in determining seed vigor and germination success.

The integration of antioxidant activity data with germination performance suggests that successful germination in *H. isora* depends on a balance between dormancy-breaking treatments and the seed's internal oxidative defense system. While scarification effectively overcomes physical dormancy by weakening the seed coat, endogenous antioxidants in the seed likely help protect embryonic tissues from oxidative damage during rapid metabolic activation. Similar relationships between antioxidant capacity and germination performance have been reported in other medicinal and forest species [40, 41].

3.4 Metabolite profiling of *Helicteres isora* seeds by Gas Chromatography–Mass Spectrometry and its relation to germination capacity

GC-MS analysis of *Helicteres isora* seeds revealed a complex metabolite profile comprising amines, organic acids, aldehydes, phenolic compounds, alcohols, fatty acids, and fatty acid derivatives, with similarity indices (SI) greater than 85, indicating reliable compound identification (Table 5). The detected metabolites represent diverse biochemical classes known to influence seed dormancy, oxidative balance, and early germination processes. Among low-molecular-weight metabolites, carbamaldehyde (5.67%), lactic acid (3.83%), acetic acid (3.06%), and furfural (4.43%) were prominent. These compounds are commonly associated with anaerobic metabolism, reserve mobilization, and stress-related biochemical pathways during seed imbibition. Additionally, nitrogen-containing compounds such as 2-aminopyrimidine (4.89%) and tetramethylethane-1,2-diamine (TEMED/TMDAE) (0.85%) were detected, suggesting the presence of metabolites involved in nucleic acid metabolism and enzymatic activation.

Table 5. Metabolite screening of *Helicteres isora* seeds by Gas Chromatography–Mass Spectrometry (GC-MS) analysis (SI > 85)

R. Time	SI	Concentration (%)	Compounds	Category
4.717	96	5.67	Carbamaldehyde	Amide
5.025	95	3.06	2-BENZYLOXYETHYLAMINE	Amine
5.212	96	3.83	L(+)MILCHSAEURE	Organic Acid
6.792	88	0.28	Hydroperoxide, 1-methylethyl (CAS) Isopropyl hydroperoxide	Hydroperoxide
8.442	93	3.06	Acetic acid (CAS) Ethylic acid	Organic Acid
10.174	94	4.43	2-Furancarboxaldehyde (CAS) Furfural	Aldehyde
10.665	88	0.85	1,2-Ethanediamine, N, N, N', N'-tetramethyl- (CAS) Tetramethylethane-1,2-diamine (TEMED/TMDAE)	Amine
10.996	91	1.97	1,2-Ethanediol, diacetate (CAS) Ethylene diacetate	Ester
11.758	90	0.89	Acetamide, N, N-dimethyl- (CAS) DMA	Amide
12.025	89	1.01	2(3H)-Furanone, dihydro- (CAS) Butyrolactone	Lactone
13.642	91	0.16	2-Cyclopenten-1-one, 2-hydroxy-3-methyl- (CAS) Corylon	Ketone
14.177	89	0.99	4-Hexen-3-one, 4,5-dimethyl- (CAS) 4,5-DIMETHYL-4-HEXEN-3-ONE	Ketone
14.342	85	1.07	Phenol, 4-methyl- (CAS) p-Cresol	Phenol
14.575	89	1.72	Cyclopropyl carbinol	Alcohol
15.714	86	4.89	2-Pyrimidinamine (CAS) 2-Aminopyrimidine	Amine
15.942	85	0.80	2,3-DIHYDRO-BENZOFURAN	Heterocyclic
16.846	92	3.84	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol	Phenol
17.697	90	4.11	Phenol, 2-methoxy-3-(2-propenyl)- (CAS) Phenol, 3-allyl-2-methoxy- (CAS) 3-	Phenol
19.708	91	1.96	Phenol, 2,6-dimethoxy-4-(2-propenyl)- (CAS) 4-Allyl-2,6-dimethoxyphenol	Phenol
20.229	91	1.06	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol	Terpenoid
20.538	89	1.06	Cyclohexanepropanoic acid, 3,4-dihydroxy-	Organic Acid
20.858	96	0.60	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	Fatty Acid (ester)
21.340	96	3.64	Hexadecanoic acid (CAS) Palmitic acid	Fatty Acid
22.107	92	1.23	10,13-Octadecadienoic acid, methyl ester (CAS)	Fatty Acid (ester)
22.635	96	3.63	9,12-Octadecadienoic acid (Z, Z)- (CAS) Linoleic acid	Fatty Acid
23.058	96	0.51	Hexadecanamide (CAS) Amide 16	Amide
23.475	94	0.24	N, N-DIMETHYL PALMITAMIDE	Amide

Table 6. Impact of metabolite profile on seed dormancy/germination

No.	Category	Impact on Seed Dormancy/Germination
1	Aldehyde	Can interact with metabolic pathways and alter seed coat structure, facilitating dormancy breakage and initiating germination processes [42, 43]
2	Alcohol	Alcohols such as ethanol and methanol can induce osmotic stress when added to the germination medium, delaying or inhibiting seed germination by reducing the water available for activating metabolic processes in seeds. Higher concentrations of alcohol can create toxic environments that prevent water absorption and damage seed structures [44, 45]
3	Amide	Influence metabolic processes and protein synthesis in seeds, potentially affecting dormancy regulation and germination [46]
4	Amine	Polyamines and abiotic stress in plants: a complex relationship [47, 48]
5	Ester	Influence seed coat permeability and may interact with organic acids to break dormancy or stimulate germination [43]
6	Fatty Acid	Play a role in membrane fluidity, energy storage, and influencing hormone production, thereby aiding dormancy breakage [49, 50]
7	Fatty Acid (ester)	Influence seed coat permeability and may interact with organic acids to break dormancy or stimulate germination [51]
8	Heterocyclic	Can have bioactive properties that influence metabolic regulation and hormone production, potentially affecting dormancy and germination processes [52]
9	Hydroxyde	Hydroxide ions can affect cell membrane stability by modulating ion transport. This can enhance nutrient uptake and promote hydration, which is a crucial step in seed germination. Proper hydration activates metabolic processes that break down stored food reserves in seeds. Hydroxides can promote faster water absorption and encourage germination [53]
10	Ketone	Alter metabolic pathways, influence hormone synthesis, and aid dormancy breakage by promoting water absorption [54]
11	Lactone	Influence plant hormones like gibberellins, involved in dormancy regulation and breaking dormancy [55]
12	Organic Acid	Alter pH and seed coat permeability, promoting dormancy breakage and aiding germination [51, 56]
13	Phenol	Can inhibit seed germination by interfering with metabolic enzyme activity and altering hormonal balances [57]
14	Terpenoid	Influence hormone synthesis and play a role in seedling growth, helping to break dormancy [58]

Phenolic compounds constituted a substantial fraction of the seed metabolome, including p-cresol (1.07%), 2,6-dimethoxyphenol (3.84%), 3-allyl-2-methoxyphenol (4.11%), and 4-allyl-2,6-dimethoxyphenol (1.96%). These phenolics are well known for their antioxidant and allelopathic properties, which may help maintain seed dormancy and regulate ROS. Lipid-related metabolites were also abundant, particularly palmitic acid (3.64%), linoleic acid (3.63%), phytol (1.06%), and their methyl esters.

The presence of these fatty acids indicates substantial energy reserves and membrane structural components, which are essential for seed germination once the dormancy constraints are alleviated. Overall, the metabolite composition of *H. isora* seeds suggests a biochemical balance between dormancy-maintaining compounds (phenolics, aldehydes) and germination-supporting metabolites (fatty acids, organic acids), which likely interacts with scarification treatments and germination media to determine final germination performance (Table 6).

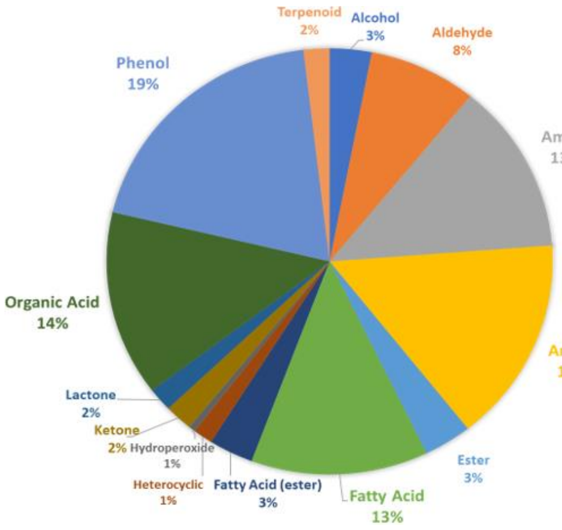


Figure 9. Sum of concentration (%) metabolite screening of *Helicteres isora* seeds by category

The presence of these compounds is consistent with a potential role in regulating chemical dormancy (Figure 9). Phenolic derivatives detected by GC-MS may help maintain dormancy by suppressing metabolic activation, modulating redox balance, and restricting premature radicle emergence. In physically dormant seeds, such compounds can be retained within the impermeable seed coat, reinforcing dormancy through combined structural and biochemical constraints. Conversely, the detection of fatty acids such as palmitic and linoleic acid indicates the availability of lipid reserves necessary for membrane reorganization and energy supply during germination. Scarification likely enhances permeability and facilitates partial leaching or redistribution of inhibitory metabolites, thereby shifting the internal biochemical balance toward germination. Most allelochemicals are classified as secondary metabolites of the plant [59], alkaloids, phenols, and flavonoids, which are known to be associated with allelopathic interactions [60].

GC-MS metabolite profile of *H. isora* seeds suggests that physiological dormancy is strongly associated with the endogenous phytochemical composition, particularly phenolic compounds and aldehydes. Phenolics such as p-cresol, dimethoxyphenols, and allyl-substituted phenols have been widely reported to exert inhibitory effects on seed germination by suppressing enzymatic activity, restricting cell elongation, and modulating hormonal balance [13, 25]. These phenolic compounds may act as natural germination inhibitors, stabilizing dormancy by maintaining low metabolic activity and preventing premature radicle protrusion in hard-coated seeds such as *H. isora*. The impermeable seed coat likely retains these inhibitory metabolites, reinforcing dormancy through both physical and chemical constraints. This dual dormancy mechanism is commonly observed in woody and medicinal plant species [19]. Scarification treatments applied in this study likely enhanced germination by reducing seed coat resistance and facilitating leaching or redistribution of inhibitory metabolites, particularly phenolics and aldehydes. Mechanical or chemical scarification can disrupt seed coat integrity, allowing oxygen diffusion, water uptake, and metabolite exchange**,** thereby shifting the internal biochemical environment toward germination [14]. A decrease in phenolic concentration or activity following scarification may alleviate their suppressive effects on α-amylase activation, respiration, and cell wall loosening**,** processes essential for radicle emergence. Similar interactions between scarification and phenolic metabolism have been reported in leguminous and medicinal species exhibiting coat-imposed dormancy [61].

GC-MS profiling revealed several chemical categories potentially involved in dormancy regulation and germination. Phenolic compounds likely contribute to dormancy maintenance by inhibiting enzymatic activity and modulating ABA-related pathways, whereas organic acids and esters may enhance seed coat permeability and facilitate water uptake. Aldehydes and ketones, often associated with lipid oxidation, can function as metabolic and redox signaling molecules during early imbibition. Lactones and heterocyclic compounds may exhibit hormone-related activities, suggesting a role in promoting germination once physical constraints are alleviated.

Fatty acids and their esters indicate the presence of essential lipid reserves required for membrane restructuring and energy production during radicle emergence. Amide-containing compounds may further support nitrogen metabolism and protein synthesis during embryo activation. Together, these findings suggest that dormancy in *Helicteres isora* is regulated by an interaction between physical seed-coat barriers and endogenous phytochemical balance, which collectively determine the efficiency of dormancy release following scarification.

The detection of lactic acid, acetic acid, carbamaldehyde, and furfural suggests active pathways related to carbohydrate breakdown and anaerobic respiration, particularly during early imbibition. Organic acids can serve as metabolic intermediates and contribute to cytoplasmic pH regulation, enzyme activation, and energy supply during the transition from dormancy to germination [62]. Furfural and related aldehydes, although potentially inhibitory at high concentrations, may also act as signals of oxidative stress, triggering antioxidant defense responses. Their effects on germination are therefore concentration-dependent and strongly influenced by seed coat permeability and germination environment. The presence of substantial amounts of palmitic acid, linoleic acid, phytol, and fatty acid derivatives indicates that *H. isora* seeds possess adequate lipid reserves to support seedling establishment once dormancy barriers are removed. Unsaturated fatty acids, such as linoleic acid, are critical for maintaining membrane fluidity, facilitating signal transduction, and enabling reserve mobilization, particularly under favorable germination conditions [25]. On the other hand, the existence of unsaturated fatty acids may be involved in low seed storability due to peroxidation stress. Taken together, the GC-MS data indicate that seed dormancy in *H. isora* is regulated by a complex phytochemical network, in which phenolics and aldehydes maintain dormancy. At the same time, fatty acids and organic acids support germination once physical barriers are disrupted. Scarification acts as a critical trigger, shifting this balance and enabling metabolic reactivation and successful germination.

4. CONCLUSIONS

This study confirms that seed dormancy in *Helicteres isora* is primarily physical and can be effectively overcome using a simple and practical propagation protocol. For optimal seedling production, mature fruits should be harvested, seeds extracted and soaked in hot water at 80 °C for 5 minutes, and then sown in a sandy soil mixed substrate. This procedure provides an efficient, low-cost, and reproducible method for enhancing germination and supporting large-scale propagation of the species.

The morphological and SEM analyses confirm that *Helicteres isora* seed exhibits clear structural traits of physical dormancy. The smooth, thick, and multilayered testa forms an effective barrier to water uptake and gas exchange, while the helically twisted capsule may further delay seed exposure under natural conditions.

GC-MS analysis revealed that *H. isora* seeds contain substantial levels of phenolic compounds, aldehydes, and organic acids, which are known to regulate oxidative balance and suppress premature germination. Scarification likely facilitates the leaching or redistribution of these inhibitory metabolites while enabling the mobilization of lipid reserves such as palmitic and linoleic acids, thereby supporting early seedling growth.

ACKNOWLEDGMENT

We acknowledge fruit supply from Nusa Tenggara Barat, Indonesia, collected by the team from BRIN, the Indonesian Ministry of Environment and Forestry (KLHK), and local villagers. The experiment was funded by LPDP grants through the RIIM Kompetisi 2025 (Contract No B-3864/II.7.5/KS.00/4/2025), a competitive grants program of the National Research and Innovation Agency (BRIN).

REFERENCES

[1] Qadir, M., Khan, F. (2019). Effect of various pre-treatments for breaking the dormancy of *Helicteres isora* Linn. Indian Journal of Applied and Pure Biology, 34(2): 84-89.

[2] Meena, R.S., Das, A., Yadav, G.S., Lal, R. (2018). Legumes for Soil Health and Sustainable Management (p. 541). Singapore: Springer. https://doi.org/10.1007/978-981-13-0253-4

[3] Kanthale, P.R., Biradar, S. (2017). Pharmacognostic study of *Helicteres isora* L. Pharmaceutical and Biological Evaluations, 4(1): 47-51.

[4] Kumbhani, N.R., Kuvad, R.P., Thaker, V.S. (2017). Development of linear model for leaf area measurement of two medicinally important plants: *Helicteres isora* L. and Vitex negundo L. Journal of Applied Biology & Biotechnology, 5(3): 57-60. https://doi.org/10.7324/JABB.2017.50310

[5] Dayal, R., Singh, A., Ojha, R.P., Mishra, K.P. (2015). Possible therapeutic potential of *Helicteres isora* (L.) and it's mechanism of action in diseases. Journal of Medicinal Plants Studies, 3(2): 95-100.

[6] Cunningham, A.B., Ingram, W., Brinckmann, J.A., Nesbitt, M. (2018). Twists, turns and trade: A new look at the Indian Screw tree (*Helicteres isora*). Journal of Ethnopharmacology, 225: 128-135. https://doi.org/10.1016/j.jep.2018.06.032

[7] Pamungkas, D., Siswadi, S., Manurung, G.E.S. (2019). A study of vegetative propagation on medicinal plant of Kayu Ules (*Helicteres isora* Linn.) through stem cuttings. Journal of Forestry Research Faloak, 3(1): 29-42. https://www.researchgate.net/publication/332751925_A_Study_of_Vegetative_Propagation_on_Medicinal_plant_of_Kayu_Ules_Helicteres_isora_Linn_Through_Stem_Cuttings.

[8] Ferdousi, A., Rahman, M.O., Hassan, M.A. (2014). Seed germination behaviour of six medicinal plants from Bangladesh. Bangladesh Journal of Plant Taxonomy, 21(1): 71-76. https://doi.org/10.3329/bjpt.v21i1.19270

[9] Muthukumar, M., Kumar, T.S., Rao, M.V. (2017). Phenology and seed germination of the Indian screw tree *Helicteres isora* L.(Malvales: Malvaceae). Journal of Threatened Taxa, 9(12): 11040-11044. https://doi.org/10.11609/jott.3058.9.12.11040-11044

[10] Paulsen, T.R., Colville, L., Kranner, I., Daws, M.I., Högstedt, G., Vandvik, V., Thompson, K. (2013). Physical dormancy in seeds: A game of hide and seek? New Phytologist, 198(2): 496-503. https://doi.org/10.1111/nph.12191

[11] Willis, C.G., Baskin, C.C., Baskin, J.M., Auld, J.R., et al. (2014). The evolution of seed dormancy: Environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytologist, 203(1): 300-309. https://doi.org/10.1111/nph.12782

[12] Jaganathan, G.K., Wu, G.R., Han, Y.Y., Liu, B.L. (2017). Role of the lens in controlling physical dormancy break and germination of *Delonix regia* (Fabaceae: Caesalpinioideae). Plant Biology, 19(1): 53-60. https://doi.org/10.1111/plb.12451

[13] Baskin, C.C. (2003). Breaking physical dormancy in seeds: Focusing on the lens. New Phytologist, 158(2): 229-232. http://www.jstor.org/stable/1513979

[14] Baskin, J.M., Baskin, C.C. (2014). What kind of seed dormancy might palms have? Seed Science Research, 24(1): 17-22. https://doi.org/10.1017/S0960258513000342

[15] Baskin, C.C., Baskin, J.M. (2000). Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination. Academic Press.

[16] Allen, P.S., Meyer, S.E. (1998). Ecological aspects of seed dormancy loss. Seed Science Research, 8(2): 183-192. https://doi.org/10.1017/S0960258500004098

[17] Bhardwaj, A.K., Kapoor, S., Naryal, A., Bhardwaj, P., Warghat, A.R., Kumar, B., Chaurasia, O.P. (2016). Effect of various dormancy breaking treatments on seed

germination, seedling growth and seed vigour of medicinal plants. Tropical Plant Research, 3(3): 508-516. https://doi.org/10.22271/tpr.2016.v3.i3.067

[18] Yulia, N.F., Supriyanto, S., Prameswari, D. (2021). Seed breaking dormancy technique and growth of screw tree (*Helicteres isora* Linn.) seedlings. Jurnal Penelitian Sosial dan Ekonomi Kehutanan, 9(1): 43-58. https://doi.org/10.20886/bptpth.2021.9.1.43-58

[19] Finch-Savage, W.E., Footitt, S. (2017). Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. Journal of Experimental Botany, 68(4): 843-856. https://doi.org/10.1093/jxb/erw477

[20] Penfield, S. (2017). Seed dormancy and germination. Current Biology, 27(17): R874-R878. https://doi.org/10.1016/j.cub.2017.05.050

[21] Smýkal, P., Vernoud, V., Blair, M.W., Soukup, A., Thompson, R.D. (2014). The role of the testa during development and in establishment of dormancy of the legume seed. Frontiers in Plant Science, 5: 351. https://doi.org/10.3389/fpls.2014.00351

[22] Muthukumar, M., Muthukrishnan, S., Kumar, T.S., Rao, M.V. (2020). Direct regeneration, microshoot recovery and assessment of genetic fidelity in *Helicteres isora* L., a medicinally important tree. Biocatalysis and Agricultural Biotechnology, 23: 101415. https://doi.org/10.1016/j.bcab.2019.101415

[23] Pompelli, M.F., Castaño, S.G.G., Bettin, A.M.V., Montaño, M.J.P., Ramírez, A.M.J., Violeth, J.L.B. (2024) Desert species: Seed germination, ecological achievement, and land area protection. Brazilian Journal of Development, 10(12): e76092. https://doi.org/10.34117/bjdv10n12-060

[24] Sudrajat, D.J., Nurhasybi, B.Y., Bramasto, Y. (2015). Standards for Testing and Quality of Forest Tree Seeds. IPB Press. https://muspera.kehutanan.go.id/perpusadikusumo/index.php?p=fstream&fid=617&bid=21310. Accessed 11/04/2026.

[25] Bewley, J.D., Black, M. (2013). Seeds: Physiology of Development and Germination. Springer Science & Business Media. http://ceiba.agro.uba.ar/cgi-bin/koha/opac-detail.pl?biblionumber=345.

[26] Roberts, E.H. (1973). Predicting the storage life of seeds. Seed Science and Technology, 1: 499-514.

[27] Hong, T.D., Linington, S., Ellis, R.H. (1996). Seed Storage Behavior: A Compendium. International Plant Genetic Resources Institute (IPGRI). Rome, Italy. https://hdl.handle.net/10568/105158.

[28] Hartmann, K.M., Grundy, A.C., Market, R. (2005). Phytochrome-mediated long-term memory of seeds. Protoplasma, 227(1): 47-52. https://doi.org/10.1007/s00709-005-0130-6

[29] Nazara, R.V., Harefa, K.S.E., Nazari, A.P.D., Ninasari, A., Arini, N., Syafi, S., A, S.E., Murrinie, E.D., Anwar, K., Larosa, Y.M., Sriwahyuni, I. (2024). Seed Science and Technology. CV HEI Publishing Indonesia. Padang, West Sumatra. https://www.researchgate.net/publication/390893271_ILMU_DAN_TEKNOLOGI_BENIH#:~:text=ketentuan%20ISTA%3B%20dan%205)%20Jumlah,benih%20yang%20berlaku%20pada%20suatu&text=Fakultas%20Pertanian%20Universitas%20Lampung.,E.%202024.%20Teknologi%20Benih%20Tanaman.

[30] Salisbury, F.B., Ross, C.W. (1992). Plant Physiology., (Wadsworth Publishing Company: Belmont, CA). Plant Physiology.

[31] Copeland, L.O., McDonald, M.F. (2012). Principles of Seed Science and Technology. Springer Science & Business Media.

[32] Singh, R., Tripathi, R.D., Dwivedi, S., Kumar, A., Trivedi, P.K., Chakrabarty, D. (2010). Lead bioaccumulation potential of an aquatic macrophyte Najas indica are related to antioxidant system. Bioresource Technology, 101(9): 3025-3032. https://doi.org/10.1016/j.biortech.2009.12.031

[33] Kumar, R.S., Rajkapoor, B., Perumal, P. (2012). Antioxidant activities of *Indigofera cassioides* Rottl. Ex. DC. using various in vitro assay models. Asian Pacific Journal of Tropical Biomedicine, 2(4): 256-261. https://doi.org/10.1016/S2221-1691(12)60019-7

[34] Sudrajat, D.J., Putri, A., Purwanto, Y.A., Siregar, I.Z. (2022). Effectiveness of ultrafine bubbles and gamma irradiation treatments to improve seed viability and vigor of *Albizia chinensis* (Osbeck) Merr. Journal of Forestry Research, 33(5): 1607-1613. https://doi.org/10.1007/s11676-021-01442-5

[35] Siregar, I.Z., Muharam, K.F., Purwanto, Y.A., Sudrajat, D.J. (2020). Seed germination characteristics in different storage time of *Gmelina arborea* treated with ultrafine bubbles priming. Biodiversitas, 21(10): 4558-4564. https://doi.org/10.13057/biodiv/d211013

[36] Bailly, C. (2004). Active oxygen species and antioxidants in seed biology. Seed Science Research, 14(2): 93-107. https://doi.org/10.1079/SSR2004159

[37] Gill, S.S., Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48(12): 909-930. https://doi.org/10.1016/j.plaphy.2010.08.016

[38] Kranner, I., Minibayeva, F.V., Beckett, R.P., Seal, C.E. (2010). What is stress? Concepts, definitions and applications in seed science. New Phytologist, 188(3): 655-673. https://doi.org/10.1111/j.1469-8137.2010.03461.x

[39] Sreelatha, S., Padma, P.R. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods for Human Nutrition, 64(4): 303-311. https://doi.org/10.1007/s11130-009-0141-0

[40] Kibinza, S., Bazin, J., Bailly, C., Farrant, J.M., Corbineau, F., El-Maarouf-Bouteau, H. (2011). Catalase is a key enzyme in seed recovery from ageing during priming. Plant Science, 181(3): 309-315. https://doi.org/10.1016/j.plantsci.2011.06.003

[41] El-Maarouf-Bouteau, H., Bailly, C. (2008). Oxidative signaling in seed germination and dormancy. Plant Signaling & Behavior, 3(3): 175-182. https://doi.org/10.4161/psb.3.3.5539

[42] Liang, X., Qian, R., Wang, D., Liu, L., Sun, C., Lin, X. (2022). Lipid-derived aldehydes: New key mediators of plant growth and stress responses. Biology, 11(11): 1590. https://doi.org/10.3390/biology11111590

[43] Oliveira, W.S., Chen, Q., Edleman, D., Annor, G.A., Dias, F.F. (2024). Unraveling the impacts of germination on the volatile and fatty acid profile of intermediate wheatgrass (Thinopyrum intermedium) seeds. Molecules, 29(17): 4268. https://doi.org/10.3390/molecules29174268

[44] Chen, Y., Almasaud, R.A., Carrie, E., Desbrosses, G., Binder, B.M., Chervin, C. (2020). Ethanol, at physiological concentrations, affects ethylene sensing in tomato germinating seeds and seedlings. Plant Science, 291: 110368. https://doi.org/10.1016/j.plantsci.2019.110368

[45] Der Vartanian, D. (2020). The effects of alcohol toxicity on seed germination. Cantaurus, 28: 2-4.

[46] Sultanova, J. (2024). Synthesis of Amides and salts of oxalic acid and their use as plant growth regulators. Бюллетень Науки и Практики, 10(3): 51-59. https://doi.org/10.33619/2414-2948/100/05

[47] Minocha, R., Majumdar, R., Minocha, S.C. (2014). Polyamines and abiotic stress in plants: A complex relationship. Frontiers in Plant Science, 5: 175. https://doi.org/10.3389/fpls.2014.00175

[48] Tyagi, A., Ali, S., Ramakrishna, G., Singh, A., Park, S., Mahmoudi, H., Bae, H. (2023). Revisiting the role of polyamines in plant growth and abiotic stress resilience: Mechanisms, crosstalk, and future perspectives. Journal of Plant Growth Regulation, 42(8): 5074-5098. https://doi.org/10.1007/s00344-022-10847-3

[49] Kaymak, H.Ç. (2014). Potential effect of seed fatty acid profile of pepper (Capsicum annuum L.) cultivars on germination at various temperatures. Zemdirbyste-Agriculture, 101(3): 321-326. https://doi.org/10.13080/z-a.2014.101.041

[50] Mendes, A., Kelly, A.A., van Erp, H., Shaw, E., Powers, S.J., Kurup, S., Eastmond, P.J. (2013). bZIP67 regulates the omega-3 fatty acid content of Arabidopsis seed oil by activating fatty acid desaturase3. The Plant Cell, 25(8): 3104-3116. https://doi.org/10.1105/tpc.113.116343

[51] Tang, Q., Chen, Y., Jiang, L., Chen, J., et al. (2024). Characterization and mechanism of seed dormancy in Symplocos paniculata. Frontiers in Plant Science, 14: 1322238. https://doi.org/10.3389/fpls.2023.1322238

[52] Solomyanny, R., Mrug, G., Frasinyuk, M., Shablykin, O., Brovarets, V. (2016). Study of auxin, cytokinin and gibberellin-like activity of heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole and isoflavones. European Journal of Biotechnology and Bioscience, 4: 29-44.

[53] Masilamani, P., Annadurai, K., Balamurugan, J., Bhaskaran, M. (2012). Effect of different hydroxides on germination and seedling vigour of teak (Tectona grandis Lf). Indian Journal of Forestry, 35(2): 167-170.

[54] Fincheira, P., Parada, M., Quiroz, A. (2017). Volatile organic compounds stimulate plant growing and seed germination of Lactuca sativa. Journal of Soil Science and Plant Nutrition, 17(4): 853-867. http://doi.org/10.4067/S0718-95162017000400002

[55] Ueno, K., Furumoto, T., Umeda, S., Mizutani, M., Takikawa, H., Batchvarova, R., Sugimoto, Y. (2014). Heliolactone, a non-sesquiterpene lactone germination stimulant for root parasitic weeds from sunflower. Phytochemistry, 108: 122-128. https://doi.org/10.1016/j.phytochem.2014.09.018

[56] Lynch, J.M. (1980). Effects of organic acids on the germination of seeds and growth of seedlings. Plant, Cell & Environment, 3(4): 255-259. https://doi.org/10.1111/1365-3040.ep11581824

[57] Wang, Z.Y., Yan, K.L., Qin, Y.M., Zhang, N.C., Chen, X.C., Chen, M. (2025). Effects of allelochemicals from plants on seed germination. Seed Biology, 4(1): e023. https://doi.org/10.48130/seedbio-0025-0022

[58] Pouresmaeil, M., Movafeghi, A., Sabzi-Nojadeh, M., Kosari-Nasab, M., Maggi, F. (2025). Monoterpenoids from Artemisia austriaca essential oil disrupt hormone-mediated reserve mobilization to suppress Avena fatua seed germination. Pest Management Science, 81(10): 6911-6927. https://doi.org/10.1002/ps.70043

[59] Kruse, M., Strandberg, M., Strandberg, B. (2000). Ecological effects of allelopathic plants - A review. NERI Technical Report, 315.

[60] Sobola, O.O., Maiguru, A.A., Olusola, J., Mch-mcha, B.D., (2022). Assessment of allelopathy effects of agroforestry trees leachates on seed germination and growth of Vigna unguiculata L.Walp. Annal of Plant Sciences, 11(5): 5128-5138. https://www.annalsofplantsciences.com/index.php/aps/article/view/941/pdf. Accessed 10/02/2026.

[61] Nonogaki, H., Bassel, G.W., Bewley, J.D. (2010). Germination—Still a mystery. Plant Science, 179(6): 574-581. https://doi.org/10.1016/j.plantsci.2010.02.010

[62] Weitbrecht, K., Müller, K., Leubner-Metzger, G. (2011). First off the mark: Early seed germination. Journal of Experimental Botany, 62(10): 3289-3309. https://doi.org/10.1093/jxb/err030