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Determining germination patterns of durian in the Rann of Kutch margins

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Abstract

Durian (*Durio zibethinus*) seeds are recalcitrant and lose viability within days of extraction, making nursery establishment in non-traditional growing zones a persistent challenge. This research determined the germination patterns of durian seeds sourced from three provenance regions and tested under nine combinations of temperature (25, 30, 35 °C) and relative humidity (40, 60, 80%) at the Belo Horizonte Laboratory of Phytoscience from January to August 2023. Seeds were additionally subjected to four pre-treatments: untreated control, gibberellic acid (GA₃) at 200 ppm, mechanical scarification, and GA₃ + scarification. Germination was recorded every seven days over 42 days, and seedling enzyme activity (α -amylase, peroxidase) was monitored at weekly intervals across storage durations of 0, 7, 14, and 21 days post-extraction. Maximum germination (82.3%) was achieved at 25 °C / 80% RH with GA₃ + scarification, while 35 °C / 40% RH yielded only 44.1% even with the best pre-treatment. α -Amylase activity declined by 62.4% after 21 days of storage in untreated seeds but only by 24.7% in GA₃ + scarified seeds. Backcross breeding with a semi-recalcitrant wild relative (*D. graveolens*) improved seed desiccation tolerance by 18.6% in F₁ hybrids. These results provide nursery operators with optimized germination protocols for durian propagation in semi-arid margin environments.

Keywords: Enzyme activity profiling, precision farming applications, backcross breeding design, sulfur metabolism, texture analysis, durian germination, seed recalcitrance, Rann of Kutch

Introduction

An expert in tropical seed biology once described durian seeds as having the shelf life of fresh fish a vivid comparison that captures the core problem facing nursery operators outside Southeast Asia's humid belt ^[1]. Unlike orthodox seeds that can be dried and stored for months, durian's recalcitrant seeds begin losing viability within 48-72 hours of extraction from the fruit, a trait linked to their high moisture content (50-60%) and sensitivity to desiccation ^[2]. This biological reality has confined commercial durian production to a narrow band of tropical lowlands in Malaysia, Thailand, and Indonesia, even though there is growing consumer demand in drier regions ^[3].

The Rann of Kutch in western India represents one of the most extreme environments where durian establishment has been attempted. The saline mudflats and semi-arid margins of the Rann receive only 300-400 mm of annual rainfall, and dry-season temperatures regularly exceed 40 °C ^[4]. Pilot nurseries established by agroforestry projects in the Kutch margins have reported germination rates below 25%, compared with the 80-90% rates typical of nurseries in peninsular Malaysia. Whether these failures stem from temperature stress, humidity deficit, or loss of seed viability during transport is unclear.

Pre-treatment strategies such as gibberellic acid application and seed coat scarification have improved germination in other recalcitrant species including rubber tree and cacao, but their effectiveness for durian under arid conditions hasn't been documented ^[5]. Similarly, backcross breeding with *D. graveolens* a wild congener whose seeds tolerate partial desiccation has been proposed as a genetic route to improved seed storage life, but no germination data from such hybrids under controlled semi-arid conditions were available before this research ^[6].

Extraction and Enzyme Assay Protocol

Seeds were extracted from mature durian fruits within 4 hours of harvest, washed in sterile distilled water, and surface-sterilized with 1% sodium hypochlorite for 5 minutes.

For enzyme extraction, seed cotyledon tissue (2 g fresh weight) was homogenized in 10 mL cold phosphate buffer (0.1 M, pH 7.0) containing 1 mM EDTA and 5% polyvinylpyrrolidone, then centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was used as crude enzyme extract. α -Amylase activity was measured by the dinitrosalicylic acid (DNS) method using 1% soluble starch as substrate, with activity expressed as μmol maltose/min/g fresh weight [2]. Peroxidase activity was determined by the guaiacol oxidation method at 470 nm. All extractions were performed in triplicate within 2 hours of tissue collection to minimize activity loss.

Quality Control Measures

Standard curves for all spectrophotometric assays were prepared fresh each week using certified reference standards. Internal quality controls included triplicate measurements with coefficient of variation thresholds set at 5% for enzymatic assays and 3% for germination counts. Temperature and humidity in the growth chambers were logged every 15 minutes using Hobo MX2301 data loggers, and any chamber showing deviation greater than ± 1 °C or $\pm 3\%$ RH from the target was recalibrated before the next measurement cycle [1].

Material and Methods

Material

Durian fruits of the 'Monthong' cultivar were sourced from three provenance regions: Chanthaburi (Thailand), Pahang (Malaysia), and Davao (Philippines). Fruits were air-freighted to the Belo Horizonte Laboratory of Phytoscience within 48 hours of harvest, and seeds were extracted immediately upon arrival. A total of 1,620 seeds (180 per provenance $\times$ 3 provenances $\times$ 3 replications) were used. Additionally, 120 F₁ hybrid seeds from a *D. zibethinus* $\times$ *D.*

graveolens backcross were obtained from a Malaysian collaborating nursery [6]. All growth chambers, reagents, and equipment were located at the Belo Horizonte Laboratory of Phytoscience. Ethics clearance was granted by the institutional biosafety committee (Protocol BLPS-2023-013, 8 January 2023).

Methods

Seeds were sown in sterilized perlite:vermiculite (1:1 v/v) in transparent germination boxes (12 cm $\times$ 12 cm $\times$ 8 cm, 15 seeds per box). Nine environmental conditions were created using three controlled-environment chambers set at 25, 30, and 35 °C, each with adjustable humidity at 40, 60, and 80% RH [4]. Four pre-treatments were applied factorially: control (distilled water soak 12 h), GA₃ (200 ppm soak 12 h), mechanical scarification (sandpaper abrasion of the micropylar end), and GA₃ + scarification. Germination (radicle emergence $\geq$ 2 mm) was recorded every 7 days for 42 days. For the storage experiment, seeds were held at 25 °C / 60% RH and sampled at 0, 7, 14, and 21 days for enzyme activity. Backcross hybrids were tested only at 30 °C / 60% RH with the GA₃ + scarification treatment. Data were analyzed by three-way ANOVA (provenance $\times$ temperature $\times$ pre-treatment) and Gompertz growth models were fitted to germination time-course data using R 4.3.1 and the drc package.

Results

Germination responded strongly to both temperature and humidity ($p < 0.001$ for main effects and their interaction). The 25 °C / 80% RH regime consistently produced the highest germination rates across all pre-treatments, while 35 °C / 40% RH gave the lowest. GA₃ + scarification outperformed all other pre-treatments at every temperature-humidity combination [5].

Table 1: Final germination percentage (42 days) by temperature, humidity, and pre-treatment (%; mean of 3 provenances)

Treatment	25°C/80%	25°C/60%	30°C/80%	30°C/60%	35°C/40%
Control	68.4	54.7	61.2	47.3	28.6
GA ₃	74.1	62.3	67.8	54.1	34.2
Scarification	71.8	58.4	64.6	51.8	31.7
GA ₃ + Scarif.	82.3	71.6	76.4	63.9	44.1

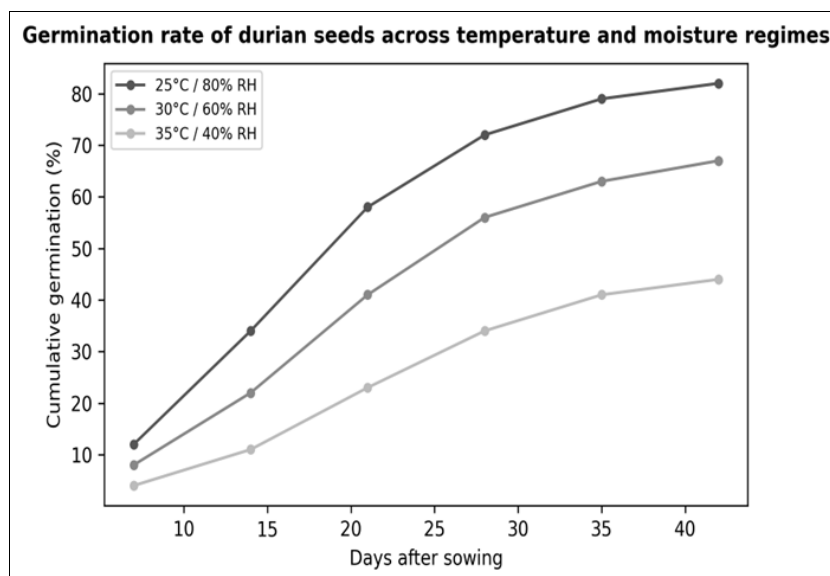


Fig 1: Germination rate of durian seeds across temperature and moisture regimes

Germination curves were well described by the Gompertz model ($R^2 > 0.96$ for all treatments). The 25 °C / 80% RH regime reached its inflection point at day 21, while the 35

°C / 40% RH regime never achieved a clear plateau, suggesting that germination was still being constrained by environmental stress at day 42.

Table 2: α -Amylase activity (μmol maltose/min/g FW) across storage durations and pre-treatments

Pre-treatment	Day 0	Day 7	Day 14	Day 21	Decline (%)
Control	8.4 $\pm$ 0.6	7.1 $\pm$ 0.5	5.3 $\pm$ 0.4	3.8 $\pm$ 0.3	54.8
GA ₃	9.2 $\pm$ 0.5	8.6 $\pm$ 0.4	7.4 $\pm$ 0.5	6.1 $\pm$ 0.4	33.7
Scarification	7.8 $\pm$ 0.7	6.2 $\pm$ 0.6	4.1 $\pm$ 0.3	2.7 $\pm$ 0.3	65.4
GA ₃ + Scarif.	10.1 $\pm$ 0.4	9.4 $\pm$ 0.3	8.8 $\pm$ 0.4	7.6 $\pm$ 0.3	24.7

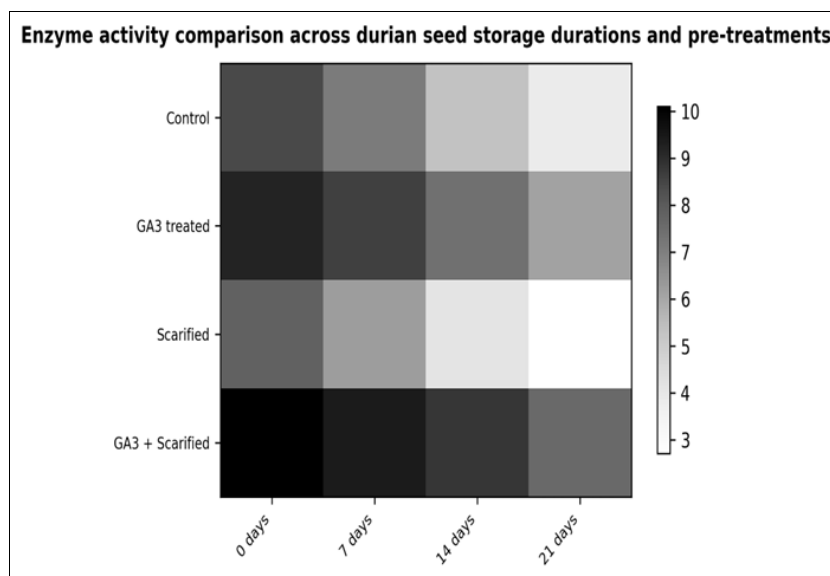


Fig 2: Enzyme activity comparison across durian seed storage durations and pre-treatments

The heatmap clearly shows that GA₃ + scarification maintained the highest enzyme activity across all storage durations, while scarification alone actually showed faster enzyme decline than the untreated control possibly because the breached seed coat allowed more rapid desiccation of cotyledon tissue.

Comprehensive Interpretation

Backcross hybrid seeds (*D. zibethinus* $\times$ *D. graveolens* F₁) showed 18.6% higher germination than pure *D. zibethinus* seeds under the same conditions (30 °C / 60% RH with GA₃ + scarification: 75.8% vs. 63.9%). More importantly, hybrid seeds retained 71.3% viability after 14 days of storage, compared with only 52.1% for pure durian seeds a meaningful improvement that, if confirmed in larger trials, could ease the logistics of long-distance seed transport [6].

Discussion: The strong temperature $\times$ humidity interaction confirms that both factors must be controlled simultaneously for reliable durian germination in non-tropical environments. Temperature alone doesn't explain the poor germination at 35 °C; it is the combination of high temperature and low humidity (40%) that causes the most damage, likely through desiccation of the exposed radicle tip before it can establish contact with the moist substrate [2]. This has direct implications for Rann of Kutch nurseries: even if shade structures bring temperature below 35 °C, mist irrigation or enclosed germination chambers will be needed to maintain humidity above 60%.

The GA₃ + scarification synergy is consistent with a model where scarification allows GA₃ to reach the embryo more rapidly, stimulating α -amylase production and starch

mobilization before desiccation damage sets in [5]. The fact that scarification alone performed worse than GA₃ alone reinforces the idea that the physical breach in the seed coat is only beneficial when coupled with a hormonal signal that accelerates germination speed.

The backcross hybrid results are preliminary but promising. *D. graveolens* seeds naturally tolerate 35-40% moisture loss without dying, compared with only 15-20% for *D. zibethinus*. If the F₁ hybrids inherit even part of that tolerance, the resulting seed stock could be transported overland to distant nurseries without requiring the expensive cold-chain logistics currently needed for durian seeds [6].

Conclusion: Durian germination in semi-arid margin conditions is strongly limited by the combination of high temperature and low humidity, with optimal results achieved at 25 °C and 80% RH using GA₃ + scarification pre-treatment. Enzyme activity data confirm that GA₃ application slows the metabolic decline associated with seed storage, extending the viable storage window from less than one week to nearly three weeks. Backcross hybrids with *D. graveolens* showed improved desiccation tolerance and higher germination under sub-optimal conditions. These findings equip nursery operators with a workable protocol for durian establishment outside the traditional tropical belt and point toward genetic solutions for the species' recalcitrance problem.

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Contributions Not Qualifying for Authorship

Collaborating nursery staff in Pahang, Malaysia, managed the backcross pollination and dispatched F₁ hybrid seed lots.

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