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Seed Viability, Imbibition Dynamics and Storability of *Aegle marmelos* (L.) Corrêa: Implications for Propagation and Conservation

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ABSTRACT: The present investigation assessed seed viability, water imbibition behavior, pre-treatment requirements, and storability of *Aegle marmelos* (L.) Corrêa. Seed viability, determined through the Tetrazolium (TZ) test, showed significant variation depending on the TZ concentration and staining duration. The highest viability (100%) was observed using 0.5% TZ solution after 4 hours of staining, establishing this combination as optimal for viability assessment. Imbibition studies revealed a progressive increase in seed fresh weight, following a characteristic triphasic pattern indicative of physiological readiness for germination. Germination experiments confirmed the absence of dormancy in *A. marmelos* seeds, indicating that no pre-treatment is necessary for successful germination. Storage behavior studies demonstrated that seeds dried to a moisture content of 4.58% and stored at –20°C retained viability above 86% after three months, confirming orthodox storage behavior. This finding contrasts with earlier reports suggesting intermediate seed storage characteristics. Overall, *Aegle marmelos* seeds exhibit high viability, favorable imbibition dynamics, and excellent storability, supporting their effective utilization in propagation and long-term conservation efforts.

Keywords: Seed viability, tetrazolium, water imbibition, seed dormancy, seed storability, conservation

INTRODUCTION

Aegle marmelos is a slow-growing, deciduous tree belonging to the family Rutaceae. Indigenous to the Indian subcontinent, it is widely distributed across India, Sri Lanka, Bangladesh, Myanmar, and Southeast Asia, where it is valued for its nutritional, medicinal, religious, and ecological importance [1, 2]. In India, the species is considered sacred in Hinduism and frequently planted near temples due to its association with Lord Shiva, to whom its trifoliate leaves are ritually offered [3]. Bael exhibits remarkable ecological adaptability, thriving from lowlands up to 1200 m elevation, tolerating extreme temperatures from –6°C to over 48°C, and growing in diverse soil types, including alkaline, stony, and marshy substrates with pH ranging between 5.0 and 10.0 [4, 5].

The species is botanically characterized by a scaly bark, thorny branches, aromatic trifoliate leaves, and greenish-white fragrant flowers. The fruit is large, woody, and takes about ten to eleven months to mature. It contains a mucilaginous pulp rich in bioactive compounds such as marmelosin, tannins, coumarins, and polysaccharides [6, 7]. These confer several therapeutic properties including antimicrobial, antipyretic, anti-inflammatory, anti-ulcer, antidiabetic, and immunomodulatory effects, making *A. marmelos* an integral part of traditional medicinal systems like Ayurveda, Siddha, and Unani [8, 9]. Various parts of the plant, including roots, leaves, bark, and fruit, are used in the treatment of digestive ailments, respiratory disorders, and metabolic syndromes [10].

Despite its importance, *A. marmelos* remains an underutilized and semi-domesticated species, with significant constraints in propagation and genetic improvement. The species is highly heterozygous, and most cultivation is seed-based, leading to substantial genetic variability and inconsistency in fruit quality and yield [11, 12]. In addition, the seeds are known for erratic germination behaviour, which is often attributed to factors like hard seed coat, immature embryos, or the presence of endogenous inhibitors [13]. Moreover, the seeds exhibit short-lived viability and are sensitive to moisture loss, posing a serious challenge for long-term storage and large-scale nursery production [14].

To overcome these challenges, various physiological and biochemical seed enhancement techniques have been explored. Pre-sowing treatments using plant growth regulators such as gibberellic acid (GA$_f$) and chemical

agents like potassium nitrate (KNO_3) have been reported to improve seed germination, uniformity, and early seedling vigour in tropical fruit species [15, 16]. Rapid viability assessment methods, particularly the Tetrazolium (TZ) test, offer a reliable alternative to traditional germination tests. The TZ test detects the activity of dehydrogenase enzymes in living cells, helping to identify viable seeds in a shorter timeframe [17].

The growing interest in *A. marmelos* for its agroforestry potential, medicinal value, and commercial viability, along with increasing cultivation across India, underscores the urgency to develop reliable and efficient propagation strategies. However, existing literature on seed viability, water uptake behaviour, and storability remains fragmented. The present study aims to evaluate seed viability using the Tetrazolium test, assess water imbibition dynamics essential for understanding germination physiology, and investigate storage behaviour under low temperature and desiccation conditions. This research seeks to contribute toward refining nursery protocols, supporting conservation efforts, and enhancing the propagation potential of this valuable tree species.

MATERIALS AND METHODS

Study area and sample collection

The research was conducted at Jabalpur, Madhya Pradesh, India (21°17′ to 26°52′ N latitude; 74°08′ to 82°49′ E longitude), characterized by a subtropical climate with distinct seasons: hot summers (April–June), monsoon rains (July–September), and cool winters (October–February). The region receives an average annual rainfall of ~1370 mm, with temperatures ranging from 10°C in winter to 48°C in summer.

The seeds of *Aegle marmelos* (L.) Corrêa were sourced from mature, healthy trees across multiple locations in Central India. Mature fruits were collected at physiological maturity to ensure seed quality and uniformity. Collection techniques involved climbing trees to directly access mature fruits, along with additional methods like branch cutting, fruit pickers, and beating with sticks to dislodge fruits.

Seed extraction and processing

After harvesting, the fruits were spread out in a single layer and sun-dried for 4-5 days. The drupes were turned regularly during drying to ensure uniformity. Once the drupes became brittle and dry, the seeds were mechanically extracted by cracking the hard seed coat using a hammer. The processed seeds were stored in well-ventilated, controlled environments to maintain their viability [18].

Seed carpology

Seed carpological traits were assessed by measuring fruit and seed dimensions (length and width) using a digital caliper. Data were recorded from three replicates, each consisting of ten fruits and fifty seeds. Seed and fruit color were evaluated using the RHS Colour Chart (2015 edition; 2019 reprint) on 20 samples.

Viability testing (Tetrazolium test)

Viability was assessed using the tetrazolium (TZ) test in accordance with ISTA guidelines (ISTA, 2020). Seeds were preconditioned by soaking in distilled water and then dissected to expose the embryo. They were immersed in 1.0%, 0.5%, and 0.25% aqueous 2,3,5-triphenyl tetrazolium chloride (TZ) and incubated in the dark at 25°C. Staining durations were recorded at 1, 2, 4, and 24 hours, and viability was determined based on the presence of uniform red staining in the embryo, indicating dehydrogenase activity [18].

Water imbibition test

To evaluate imbibition behavior, fresh seeds were initially weighed, then soaked in distilled water at room temperature (25°C). After specific intervals (2, 4, 8, 16, 24, 36, and 48 hours), surface moisture was blotted, and seeds were reweighed. The rate and pattern of water uptake were calculated and plotted to assess the triphasic imbibition curve [19].

Seed germination

Aegle marmelos (L.) Corrêa seeds did not exhibit physical or physiological dormancy, and therefore no pre-treatment was required prior to sowing. 4 replicates of 25 seeds per treatment were sown in sterilized sand trays lined with moist filter paper and incubated at 25°C in a germination chamber under a 16/8 h light/dark cycle. Germination was monitored daily for four weeks. A seed was considered germinated when the radicle exceeded 1 cm in length [18]. The following parameters were calculated:

1. Final Germination Percentage (FGP) [20].

   $FGP = (G / T) \times 100$

   Where G = No. of germinated seeds; T = No. of seeds sown

2. Mean Germination Time (MGT) [21].

   MGT = Σ (Gt × Dt) / G

   Where Gt = No. of seeds germinated at day t; Dt = Days from sowing; G = Total seeds germinated

3. Root Length (cm): Average length of 10 primary roots

4. Shoot Length (cm): Average height of 10 seedling shoots

5. Seedling Vigour Index-I [22].

   SVI = FGP × (Root + Shoot Length)

Desiccation trials

To assess desiccation tolerance, seeds were dried using silica gel (1:1 w/w, silica: seed) in airtight containers. Moisture content was monitored periodically until it reached below 5%, after which seeds were vacuum-sealed for storage trials [23].

Moisture content determination

Moisture content was estimated by the oven-drying method at 103 ±2°C for 17 ±1 hours using four replicates, as per [18]. Results were reported on a fresh weight basis.

Storage behavior evaluation

Seed storage behavior was assessed following the protocol [23]. Seeds were packed in tri-layered aluminium foil pouches and stored at two temperatures: –18°C and +8°C. Germination and moisture content were periodically recorded to evaluate seed viability and storability over time.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA). Means were separated using critical difference (C.D.) at a 5% significance level ($p < 0.05$). Standard error of the mean (SEm) and coefficient of variation (C.V.) were also computed using MS Excel and SPSS software.

RESULT AND DISCUSSION

Seed carpology

The fruit of *Aegle marmelos* is classified as a berry, typically globose in shape and measuring between 5 to 15/ cm in diameter. Its pericarp is woody and yellow-orange in color, often displaying morphological variability with shapes ranging from round and pyriform to oval or oblong. Internally, the fruit comprises 8 to 20 indistinctly triangular segments, filled with a pale-orange, mucilaginous pulp that is aromatic, resinous, sweet, and mildly astringent. Numerous aromatic oil glands are observable as minute dots on the fruit surface, contributing to its distinctive fragrance.

Seeds are numerous and embedded within the pulp. Each seed is oblong, flat, and approximately 1/ cm in length, enclosed in a transparent, adhesive mucilage sac that solidifies upon drying—an adaptation likely aiding seed dispersal and moisture retention. Structurally, seeds possess a white testa, dense woolly hairs, a large embryo with conspicuous cotyledons, and a short superior radicle. Notably, *A. marmelos* seeds lack endosperm. Some seeds may abort during development, reducing the viable seed count.

Viable seeds are non-dormant and exhibit rapid germination, typically sprouting within two to three weeks under favorable conditions. A single mature fruit can yield anywhere from 10 to 50 seeds, with an estimated seed count ranging from 5,000 to 15,600 seeds per kilogram. Seed size analysis revealed a variation in length from 0.6490 to 1.0105/ cm and in width from 0.559 to 0.8954/ cm, indicating considerable morphometric diversity.

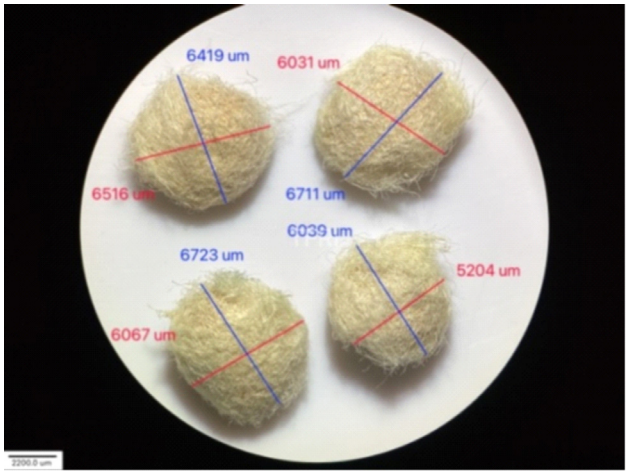

Figure 1. Seeds of *Aegle marmelos*

These findings suggest that *A. marmelos* exhibits seed traits favorable for natural regeneration and propagation, such as high seed yield per fruit, absence of dormancy, and viable embryo structure. However, seed variability and occasional abortion may impact germination uniformity, which could be critical in large-scale propagation or conservation programs.

Table 1. Impact of TZ Concentration and Staining Duration on Viability Percentage of *Aegle marmelos*

Staining duration (hrs.)	TZ Conc. (%)			Mean
	1	0.5	0.25	
1	0.00	0.00	0.00	0.00
2	89.15	98.68	61.82	83.22
4	96.14	100.00	66.66	87.60
24	97.15	100.00	77.88	91.68
Mean	70.61	74.67	51.59	
Factors	C.D.	SE(d)	SE(m)	F-Calculated
TZ Conc.	3.58	1.73	1.22	1293.18*
Staining duration	3.10	1.50	1.06	135.95*
TZ Conc. × Staining duration	6.21	2.99	2.11	17.62*

Seed viability

The observation (Table 1&2) demonstrate that both Tetrazolium (TZ) concentration and staining duration significantly influence the viability of *Aegle marmelos* seeds. No staining was observed at any concentration with a 1-hour exposure, indicating that this duration is inadequate for viability assessment. Viability increased notably at 2 hours, especially with 0.5% TZ (98.68%) and 1% TZ (89.15%), while 0.25% remained low (61.82%). At 4 hours, viability peaked at 100.00% with 0.5% TZ, followed by 96.14% with 1% and 66.66% with 0.25%. The highest overall viability was observed after 24 hours, with 0.5% and 1% TZ maintaining high levels (100.00% and 97.15%, respectively), while 0.25% improved moderately to 77.88%.

Among all treatments, 0.5% TZ for 4 hours proved to be the most effective, yielding 100% viability with reduced

Table 2. Examples of TZ staining patterns of viable (1-2) and non- viable seeds (3-6) in *Aegle marmelos*

S. No.	Staining Pattern	
1	Embryonal axis and cotyledons completely stained	
2	Embryonal axis and cotyledons stained except periphery of cotyledon	
3	Embryonal axis & peripheral cotyledons stained except radicle tip	
4	Radical tip and less than 25% cotyledons stained	
5	Embryonal axis and cotyledons unstained	
6	Embryonal axis lightly stained with cotyledons unstained	

chemical concentration. Statistical analysis confirmed that TZ concentration, staining duration, and their interaction significantly affected viability ($p < 0.01$). The findings suggest that a 0.5% TZ solution applied for 4 hours offers a reliable, efficient, and cost-effective method for viability testing in *Aegle marmelos* seeds.

Water imbibition

The water imbibition curve of *Aegle marmelos* seeds reveals a gradual and steady increase in fresh weight, indicative of the classic triphasic water uptake pattern. No change is observed initially, but by 2 hours, seeds gain 13.23% in weight, rising to 19.06% at 4 hours. Absorption progresses steadily, reaching 24.67% at 10 hours and significantly increasing to 36.27% after 24 hours. The uptake peaks at 47.27% by 28 hours and stabilizes around 49.44% by 32 hours, indicating saturation (Figure 2).

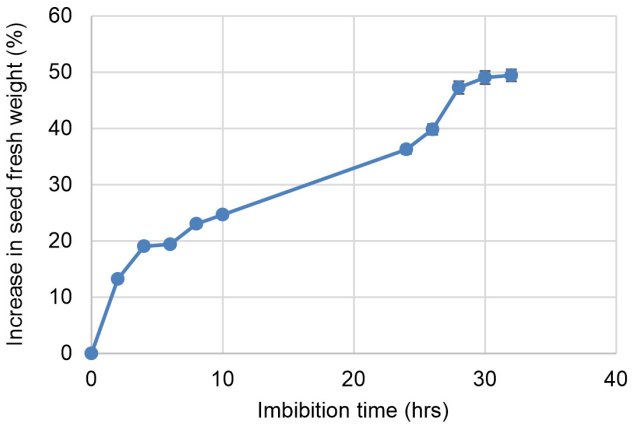

Figure 2. Water imbibition curve of *Aegle marmelos* seeds

This progressive hydration pattern reflects typical seed metabolic activation phases, where initial rapid imbibition is followed by metabolic reactivation and plateau, preparing the seed for germination. The findings confirm that *A. marmelos* seeds exhibit efficient water uptake, a key prerequisite for successful germination without any pretreatment need.

Seed storability behaviour

Desiccation and cold storage trials indicate that *Aegle marmelos* seeds exhibit orthodox storage behaviour. Initial germination at 10.57% moisture was 82.93%, which slightly improved to 81.17% after drying to 4.58% moisture (Table 3). Seeds stored at –20°C for three months maintained high viability (86.08%). These results confirm that the seeds tolerate desiccation and low temperatures well, making them suitable for long-term conservation under standard orthodox storage conditions.

The study revealed that optimal viability differentiation in seeds was achieved using a 0.5% tetrazolium (TZ) solution after 4 hours of exposure, which is consistent with the findings of [24] who emphasized that precise viability assessments depend heavily on the correct balance of staining concentration and duration. The successful visualization of viable tissues under these conditions suggests effective dehydrogenase activity, an indicator of metabolic integrity in living seeds.

Additionally, the classical triphasic water uptake pattern observed during imbibition strongly supports the notion of successful dormancy release and initiation of germination, in alignment with the model described [25]. This triphasic pattern is typical of metabolically active, viable seeds transitioning from a quiescent to an active growth phase, further validating the seed quality and readiness for germination under the applied treatments.

The species exhibited characteristics consistent with orthodox seed behavior, as demonstrated by their ability to tolerate desiccation to a moisture content as low as approximately 4.58% and retain viability above 86% following storage at –20°C (Table 3). These findings align with the criteria established by [23] who defined orthodox seeds as those capable of withstanding both low moisture levels and cold storage conditions without a significant loss in viability. This supports the species' suitability for seed banking and long-term ex situ conservation, presenting valuable opportunities for species restoration and the preservation of genetic resources. However, our

Table 3. Different desiccation stages and respective germination percentage after desiccation

Initial seed moisture content%	Initial seed germination% Mean±SE	Desiccated safe moisture content%	Germination% Mean±SE	3 months after -20 °C storage germination % Mean±SE	Seed storage categories
10.57	82.93±3.76	4.58	81.17±3.68	86.08±3.91	Orthodox

Figure 3. Seed germination of *Aegle marmelos* after 3 months of storage at –20 °C

results contrast with earlier reports [26-34] that classified the species as having intermediate seed storage behavior. This discrepancy may be attributed to the lack of proper control over seed moisture content during the initial phases of those storage behavior studies. Further research is necessary to validate these findings and resolve the contradictions observed in long-term seed storage outcomes.

CONCLUSION

The study on *Aegle marmelos* seeds demonstrates their strong potential for both propagation and conservation. The species exhibits favorable carpological and seed traits, including high seed yield, structural viability, and the absence of dormancy, which collectively support efficient natural regeneration. The tetrazolium test proved effective in determining seed viability when optimized for concentration and duration, ensuring accurate assessment of seed health. The observed water uptake pattern confirmed active metabolic reactivation during germination, reflecting the readiness of seeds to germinate under suitable conditions without the need for pretreatment. Additionally, the seeds displayed characteristics of orthodox storage behavior, indicating their ability to withstand desiccation and low-temperature storage. This positions *Aegle marmelos* as a promising candidate for seed banking and long-term ex situ conservation efforts aimed at species preservation and restoration initiatives.

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