**Enhancing Germination and Storability in *Cordia myxa* Linn.: Insights from Seed Carpology, Viability, Imbibition, Pre-treatment, and Storage Behaviour**

**Abstract**

### A comprehensive investigation was carried out on the seed biology, viability, and storability of *Cordia myxa* Linn., a valuable wild fruit species of central India. Carpological analysis revealed globular to ovoid drupes containing wrinkled ellipsoid seeds. Tetrazolium (TZ) viability testing highlighted that staining duration, particularly 24 hours significantly influenced viability results (p < 0.001), while TZ concentration had negligible effect. Water imbibition studies demonstrated a slow and steady hydration process with maximum water uptake (25.35%) observed at 27 hours. Among various pre-sowing treatments tested, hot water soaking (5 min) followed by 500 ppm GA₃ for 48 hours proved most effective, achieving the highest germination (70.70%), vigour index (1043.43), and a lower mean germination time (19.12 days). Acid scarification treatments, however, adversely affected germination. Seed storability trials confirmed the orthodox nature of *C. myxa* seeds with enhanced germination after desiccation (5.05% moisture) and sustained viability (35.44%) following three months at –20°C. These findings provide vital inputs for improving seed viability testing, germination efficiency, and storage strategies for the propagation and conservation of *C. myxa*, thereby contributing to its effective propagation, conservation, and future utilization in afforestation and agroforestry programs.

### ****Keywords:**** Seed germination, seed viability, tetrazolium test, seed storage, water imbibition, orthodox seeds, seed physiology, propagation techniques

**Introduction**

Underutilized fruit species native to the arid and semi-arid regions of India play a vital role in local nutrition, traditional medicine, and ecosystem stability. Despite their significance, these species remain neglected due to inadequate research, lack of standardized cultivation practices, and poor availability of quality planting material and improved varieties (Bhatnagar et al., 2016). Among these, *Cordia myxa* L. commonly known as lasoda, lehsua, or Indian cherry, is a drought-hardy, medium-sized deciduous tree of the family Boraginaceae, with wide adaptability in degraded soils and dry conditions (McCann, 1985; Arbonnier, 2002; Cordia myxa, 2016). It is distributed across India, Sri Lanka, Myanmar, Egypt, Iraq, and tropical Australia (McCann, 1985), and thrives particularly in arid and semi-arid climates.

Taxonomically, the genus *Cordia* includes over 300 species found in tropical and subtropical regions of America, Asia, and Oceania. Of these, *Cordia sinensis* and *C. myxa* are also found in Mediterranean climates and the Middle East (Davis, 1978; Greuter et al., 1984). *C. sinensis* is native to Egypt and the Arabian Peninsula, while *C. myxa* likely originated in tropical Asia or the Near East and has since become naturalized across southern Iran, northern and tropical Africa, and parts of the Mediterranean (Davis, 1978). Ecologically, *C. myxa* serves as a valuable agroforestry species. It has traditionally been planted for windbreaks, microclimate regulation, and erosion control. Once established, it thrives under rainfed conditions and requires minimal irrigation beyond the initial three years (Meghwal et al., 2014a; 2014b). Economically, its green fruits are widely used in pickles and vegetable preparations, particularly during lean agricultural seasons (Bhatnagar et al., 2016). The ripe fruits, though mucilaginous, are sweet and rich in carbohydrates and ascorbic acid (Pareek & Sharma, 1993), and are occasionally used in liquor preparation and folk remedies (McCann, 1985). Pharmacologically, various parts of the plant are used as astringent, anthelmintic, expectorant, and diuretic agents, and are effective in treating chest and urinary ailments (Abdallah et al., 2011; Kuppast & Vasudeva, 2006; Jamkhande et al., 2013). Its nutritional profile includes high levels of fiber, sugars (glucose, fructose, sucrose), proteins, fats, and essential minerals such as potassium, calcium, and iron (Karami et al., 2015).

Despite these benefits, *C. myxa* remains largely undomesticated and is found growing wild along farm boundaries and roadsides. Its propagation is constrained due to physical dormancy from a hard seed coat, and inconsistent germination—taking 40 to 60 days under natural conditions (Salami & Lawal, 2018). Studies have attempted to address these constraints using mechanical scarification, acid soaking, water soaking, and hormonal pre-treatments (Pundir, 1987; Meghwal, 2007; Ghaba et al., 2024). In addition to pre-sowing treatments, breeding programs have focused on selecting elite genotypes based on phenotypic and physicochemical characteristics. Genetic variability studies across 15 provenances in Rajasthan indicated high heritability and genetic advance in traits like fruit weight, pulp:seed ratio, TSS, and fruit diameter—suggesting good scope for selection and genetic improvement (Nagar, Fageria, & Pareek, 2013). Efforts at CAZRI and other institutions have led to the development of improved varieties like Maru Samridhi, Karan Lasoda, and Thar Bold (Meghwal et al., 2021, 2022). However, these advancements are hindered by the lack of robust nursery protocols and insufficient availability of quality planting material, which limits large-scale multiplication and commercialization.

Understanding natural variability, seed viability, dormancy-breaking treatments, and storage behavior is essential for the effective propagation, conservation, and utilization of this nutritionally and medicinally important species. The current study aims to evaluate the impacts of different pre-sowing treatments and storage conditions on seed germination and seedling performance of *Cordia myxa* L., thereby contributing to its propagation protocols and ex-situ conservation.

**Material and Methodology:**

**Study Area:** 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*.*

**Seed Extraction and Processing:** *Cordia myxa*, a perennial deciduous tree, fruits during summer and produces drupes ranging from small (2–8 g) to large (12–20 g). Fruits are harvested when fully ripe—identified by their color change—and seeds are manually or mechanically extracted from the pulp. Post-harvest, seeds are cleaned and shade-dried.

**Seed Viability Testing:** Seed viability was assessed using the tetrazolium (TZ) test following ISTA protocols. Three concentrations of 2,3,5-triphenyl tetrazolium chloride solution (1.0%, 0.5%, and 0.25%) were prepared. A random sample of 100 seeds per replication was soaked in water for hydration, and transverse cut were made to facilitate TZ solution penetration. Seeds were incubated in the TZ solution in the dark for up to 24 hours, with observations recorded at 1, 2, 4, and 24 hours. Viability was expressed as the percentage of viable seeds.

**Water Imbibition Test:** Fresh seeds were weighed and soaked in distilled water at room temperature. At specific intervals, seeds were removed, surface moisture was blotted off, and seeds were reweighed. The increase in fresh weight over time was used to analyse seed water uptake patterns.

**Pre-treatment Methods:** Six different pre-treatment methods were applied to break physical dormancy in *Cordia myxa* Linn.seeds:

1. Control (no treatment)
2. Soaking in distilled water at room temperature for 48 hours
3. Acid scarification using concentrated sulfuric acid (H₂SO₄) for 10 minutes, followed by water soaking for 48 hours
4. Hot water (80-100°C) for 5 minutes followed by soaking in water for 48 hours
5. Hot water (80-100°C) for 5 minutes followed by soaking in 500 ppm GA₃ solution for 48 hours
6. Acid scarification with H₂SO₄ for 10 minutes, followed by soaking in 500 ppm GA₃ solution for 48 hours

Prior to each treatment, seeds were first surface-sterilized by immersion in a **5% Captan solution for 5 minutes**. Following sterilization, three replicates of 25 seeds each were placed in sterilized sand trays lined with moistened filter paper using deionized water. The trays were incubated in a seed germinator at **25°C** under a **16/8 h light/dark cycle** (Silva et al., 2009). Germination was monitored daily for four weeks, with radicle protrusion of **1 cm** as the germination criterion. The following germination-related parameters were determined:

1. Final Germination Percentage (FGP) (Scott et al*.,* 1984)

FGP= G / T × 100

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

1. Mean germination time (MGT) (Orchard, 1977)

MGT = Gt × Dt / G

Where Gt = No. of germinated seeds at day-t, Dt = No. of days at “t” from the day of sowing and G = Total no. of germinated seeds.

1. Root Length (cm): Measured as the average length of 10 primary roots.
2. Shoot Length (cm): Measured as the average height of the 10-seedling shoot.
3. Seedling vigour index I = G% X Seedling length (cm) (Abdul-Baki, and Anderson 1973).

**Seed Desiccation trails:** Seeds were dried using silica gel at a 1:1 weight ratio (silica gel to seeds). At regular intervals, the seeds were removed to measure their moisture content. Once the moisture content dropped below 5%, the seeds were vacuum-packed for storage trials, with a portion set aside for seed germination assessments.

**Moisture Content Determination:** Seed moisture content was determined using the oven-drying method at **103 ±2°C for 17 ±1 hours**, following the International Seed Testing Association (ISTA, 2010) guidelines. Four replicates were used, and the calculation was performed on a fresh weight basis. Moisture content was expressed as a percentage.

**Seed Storage Behaviour:** The seed storage behavior was evaluated based on the simplified screening scheme for seed storage developed by Hong and Ellis (1996). Seeds were packaged in vacuum-sealed, three-layered aluminium foil pouches and stored at -20°C in a cold storage facility. A subset of seeds was also stored at +8°C for comparison. The moisture content of seeds was measured using the oven-drying method as per ISTA guidelines.

**Statistical Analysis:** Data were analysed using ANOVA to determine significant differences among treatments, with the critical difference (C.D.) set at a 5% significance level. Standard error (SEm) and coefficient of variation (C.V.) were calculated to assess variability in the data.

**Results**

**Seed Carpology:** The fruit of *Cordia myxa* is a globular to ovoid drupe (Fig 1) measuring approximately 2–3.5 cm in length. It begins as a pale brown or pink fruit and darkens as it ripens. The pulp is transparent, mucilaginous, and sweet in taste. The seed or pit is broadly ellipsoid to globose in shape, deeply wrinkled, and typically contains one to two seeds. Each kilogram contains approximately 3,500 to 4,500 seeds. The seed length ranges from 1.083 to 1.229 cm while the width varies between 0.9632 and 1.014 cm.

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**Fig 1.** Fruit and seed carpology of *Cordia myxa* (A) Follicles (B) Seed with silky hairs (C)Microscopic Seed Image (Scale 2900.00 µm)

**Seed viability:** Table 1 illustrates the impact of different tetrazolium (TZ) concentrations (1%, 0.5%, and 0.25%) and staining durations (1, 2, 4, and 24 hours) on the viability percentage of *Cordia myxa* seeds. The results indicate that staining duration significantly influences viability, with no staining observed at 1 hour across all concentrations, moderate viability at 2 hours (around 54–60%), and a marked increase at 4 and 24 hours, reaching up to 98.33%. While TZ concentration showed some variation, particularly with 0.25% performing slightly lower than 0.5% and 1%, statistical analysis revealed that staining duration was the most critical factor (F = 5.45, p < 0.001), whereas TZ concentration (F = 231.55) and the interaction between concentration and duration (F = 2.05) were not significant. Table 2 further supports these findings through topographic staining patterns: viable seeds exhibited dark staining in the embryonal axis and cotyledons, partially viable seeds showed light staining, and non-viable seeds were completely unstained. These results confirm that prolonged staining, particularly for 24 hours, is essential for accurate viability assessment of *Cordia myxa* seeds, and visual staining patterns offer a reliable diagnostic tool to differentiate between viable and non-viable seeds.

**Table 1.** Impact of TZ Concentration and Staining Duration on Viability Percentage of *Cordia myxa* Linn.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Staining duration (hrs.) | TZ Conc. (%) | | |  |
| **1** | **0.5** | **0.25** | **Mean** |
| 1 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 60.00 | 54.33 | 55.67 | 56.67 |
| 4 | 90.00 | 98.33 | 71.67 | 86.67 |
| 24 | 95.33 | 98.33 | 82.30 | 91.99 |
| Mean | 61.33 | 62.75 | 52.41 |  |
| Factors | **C.D.** | **SE(d)** | **SE(m)** | **F-Calculated** |
| TZ Conc. | 8.14 | 3.92 | 2.77 | 231.55 |
| Staining Duration | 7.05 | 3.40 | 2.40 | 5.45 |
| TZ Conc. X Staining Duration | N/A | 6.79 | 4.80 | 2.05 |

**Table 2.** TZ staining patterns of viable (1) and non- viable (2,3) seeds in *Cordia myxa* Linn.

|  |  |  |
| --- | --- | --- |
| **S. No.** | **Topographic description** | **Topographic pattern** |
|
| 1 | Embryonal axis and cotyledons darkly stained |  |
| 2 | Embryonal axis and cotyledons lightly stained |  |
| 3 | Embryonal axis and cotyledons unstained |  |

**Water Imbibition**

The water imbibition pattern of *Cordia myxa* Linn.seeds show a gradual increase in seed fresh weight over time (Fig 2), indicating a slow water absorption process. Initially, at 0 hours, there is no increase in fresh weight. By 2 hours, the weight increases to 15.80% (SE = 0.37), followed by a slight rise to 17.48% at 4 hours (SE = 0.38) and 18.88% at 8 hours (SE = 0.49). The absorption continues at a slow rate, reaching 19.55% (SE = 0.46) at 10 hours. A more noticeable increase occurs at 24 hours (22.44%, SE = 0.48) and peaks at 25.35% (SE = 0.60) at 27 hours, before slightly declining to 24.07% (SE = 0.57) at 30 hours, possibly indicating stabilization or minor water loss. This pattern suggests a slow and steady imbibition process with a prolonged stabilization phase.

**Fig 2.** Water Imbibition curve of *Cordia myxa*Seeds over Time period

**Seed Pre-treatment**

The effect of various seed pre-treatment methods on the germination and early growth characteristics of *Cordia myxa* was evaluated through multiple parameters, including Mean Germination Time (MGT), germination percentage, root length, shoot length, and Vigour Index I. In the control treatment, seeds exhibited a germination percentage of 44.55%, MGT of 17.82 days, root length of 5.34 cm, shoot length of 11.09 cm, and a vigour index of 732.48. Soaking seeds in water for 48 hours improved germination to 65.00%, increased root and shoot lengths to 5.50 cm and 11.88 cm, respectively and elevated the vigour index to 975.26, though MGT slightly rose to 19.54 days. In contrast, acid scarification for 10 minutes followed by 48 hours water soaking led to poor outcomes, with only 19.87% germination, MGT of 21.36 days, and a reduced vigour index of 246.83. Immersion in hot water (80–100°C) for 5 minutes followed by 48 hours soaking significantly enhanced germination to 55.37%, with a root length of 5.64 cm, shoot length of 9.50 cm, and vigour index of 970.08. The most effective treatment was hot water for 5 minutes combined with 500 ppm GA₃ soaking for 48 hours, which achieved the highest germination rate (70.70%), a relatively low MGT of 19.12 days, and the greatest vigour index of 1043.43. Conversely, acid scarification followed by GA₃ soaking showed limited success, with only 25.08% germination and a lower vigour index (387.89). Statistical analysis confirmed that differences among treatments (Table 3) were highly significant (p < 0.001), highlighting the hot water plus GA₃ treatment as the most promising approach for improving seedling emergence and growth (Fig 3) in *Cordia myxa*.

**Table 3.** Impact of seed pre-treatment methods on seed quality parameters of *Cordia myxa*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **MGT** | **Germination%** | **Root length (cm)** | **Shoot length (cm)** | **Vigour index I** |
| Control | 17.82±0.28 | 44.55±0.69 | 5.34±0.08 | 11.09±0.17 | 732.48±22.44 |
| 48 hrs water soaking | 19.54±0.23 | 65.00±0.75 | 5.50±0.06 | 11.88±0.14 | 975.26±22.52 |
| Acid 10 minute+48 hours soaking in water | 21.36±0.38 | 19.87±0.35 | 4.07±0.07 | 8.34±0.15 | 246.83±8.72 |
| Immersion in hot water (80-100°C) for 5 minutes followed by 48 hours soaking | 20.50±0.25 | 55.37±0.66 | 5.64±0.07 | 9.50±0.11 | 970.08±23.26 |
| Hot water 5 minute+ GA3 500 48 hours soaking | 19.12±0.39 | 70.70±1.46 | 4.95±0.10 | 9.80±0.20 | 1043.43±43.27 |
| Acid 10 minute + GA3 500 PPM 48 hours soaking | 24.88±0.50 | 25.08±0.51 | 4.92±0.10 | 10.54±0.21 | 387.89±15.70 |
| C.D. | 1.09 | 2.54 | 0.26 | 0.52 | 77.85 |
| SE(m) | 0.35 | 0.81 | 0.08 | 0.17 | 24.99 |
| C.V. | 2.96 | 3.02 | 2.85 | 2.86 | 5.96 |
| F-Calculated | 48.68\* | 657.08\* | 46.34\* | 55.27\* | 181.41\* |

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**Fig 3.** Seed germination pattern of *Cordia myxa* Linn.

**Seed Storability Behaviour**

Desiccation trials were conducted to assess the seed storability of *Cordia myxa* Linn**.** using silica gel in desiccators. The initial seed moisture content was 11.49%, with a germination rate of 31.15±1.25%. After desiccation to a safe moisture content of 5.05%, the germination percentage increased to 33.13±1.33%. Seeds stored at –20°C for three months maintained good viability, with a germination rate of 35.44±1.42 %. These findings suggest that *Cordia myxa* Linn**.** seeds are classified under the orthodox storage category, as they demonstrated high storability and maintained viability after prolonged storage under controlled conditions. In conclusion, the seeds of *Cordia myxa* Linn**.** exhibit good storability under orthodox conditions, with improved germination following desiccation and low-temperature storage.

**Table 4.** 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 OC storage germination % Mean±SE** | **Seed Storage Categories** |
| 11.49 | 31.15±1.25 | 5.05 | 33.13±1.33 | 35.44±1.42 | Orthodox |

**Discussion**

The present study provides valuable insights into the seed biology, viability, dormancy-breaking treatments, and storability behavior of *Cordia myxa* Linn., a multipurpose and underutilized fruit tree species of ecological and economic importance.

The morphological examination of *C. myxa* fruits and seeds confirms the drupe-like characteristics previously reported by McCann (1985) and Jasiem et al. (2016). The fruits are globular to ovoid (2–3.5 cm long) with mucilaginous, transparent pulp, enclosing one to two deeply wrinkled seeds. The seed dimensions (length: 1.083–1.229 cm; width: 0.9632–1.014 cm) and high seed count (3,500–4,500/kg) suggest that this species has strong propagation potential, provided quality seed sources are maintained.

Tetrazolium (TZ) staining tests revealed that staining duration was the most significant factor influencing viability while TZ concentration and its interaction with time showed no significant effects. A 24-hour staining period provided the highest viability values and clearest contrast between viable and non-viable tissues. These findings align with earlier studies (AOAC, 1990; Panse & Sukhatme, 1995) indicating that longer staining durations enhance TZ penetration and staining accuracy. Thus, a 24-hour staining duration is recommended for reliable viability testing of *C. myxa* seeds.

The imbibition behavior showed a gradual increase in seed moisture content, peaking at 25.35% after 27 hours. This slow hydration trend, followed by stabilization, suggests that the seeds possess a semi-permeable seed coat, characteristic of species with physical dormancy. Such a pattern is common in arid-region species where water conservation mechanisms are vital (Hendrickson & Veihmeyer, 1934; Salami & Lawal, 2018). This underlines the need for effective pre-treatment methods to break dormancy and enhance germination.

Significant improvements in germination were observed with pre-treatment protocols (p < 0.001). The control treatment showed the lowest germination (44.55%) and vigour index (732.48), confirming dormancy in untreated seeds. Among the tested treatments, immersion in hot water for 5 minutes followed by 48 hours soaking in 500 ppm GA₃ yielded the highest germination percentage (70.70%), vigour index (1043.43), and a shorter mean germination time (19.12 days). This indicates a synergistic effect of thermal shock and gibberellic acid in promoting germination and seedling vigor. In contrast, acid scarification treatments (alone or with GA₃) resulted in poor germination and vigor, possibly due to embryo damage from excessive chemical exposure. Supportively, Meghwal (2007) reported that GA₃ treatments (250–500 ppm) significantly enhanced germination in *C. myxa*, with the highest rate (66.66%) recorded with sandpaper scarification followed by 500 ppm GA₃ soaking. Likewise, Ghaba et al. (2024) found that soaking seeds in 4000 ppm GA₃ for 24 hours significantly improved germination and seedling growth in Egypt.

Storage trials confirmed that *C. myxa* seeds exhibit orthodox storage behavior. Desiccation using silica gel reduced seed moisture content from 11.49% to 5.05%, improving germination from 31.15% to 33.13%. After three months of –20°C storage, germination further increased to 35.44%, suggesting possible alleviation of residual dormancy. These results validate the classification of *C. myxa* as an orthodox species (Zobel & Talbert, 1984) and affirm findings from Kumar et al. (2022) that dryland trees often tolerate low-moisture storage well.

The integration of viability testing, imbibition analysis, pre-treatment efficacy, and storage potential offers a holistic approach to *C. myxa* propagation and conservation. The study affirms that a 24-hour TZ viability test, pre-treatment with hot water and GA₃, and cold storage under desiccated conditions are effective strategies for enhancing propagation success. This can facilitate large-scale multiplication efforts in nurseries and ex situ conservation programs. Further, genetic diversity and regional variability reported by Nagar et al. (2013) and Meghwal et al. (2014a, 2021) can be better harnessed by using scientifically validated seed handling techniques. These findings also complement earlier discussions on underutilized fruits by Pareek and Sharma (1993), positioning *C. myxa* as a viable candidate for dryland agroforestry, orchard establishment, and genetic resource conservation.

**Conclusion**

This study confirms that *Cordia myxa* benefits significantly from targeted pre-treatments and exhibits robust storability under orthodox seed storage conditions. The recommended protocol—hot water immersion followed by GA₃ treatment, coupled with 24-hour TZ viability testing and low-moisture cold storage—can serve as a model for propagating this valuable yet underutilized species. These findings have strong implications for sustainable nursery practices, afforestation efforts, and biodiversity conservation in arid and semi-arid regions.

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