

RESEARCH

Open Access



Seed maturation, germination physiology, and storage behaviour of the tropical tree *Cochlospermum religiosum* (L.) Alston (Bixaceae)

Manish Kumar Vijay^{1*} , Neeraj Prajapati¹ and Neelu Singh¹

*Correspondence:
Manish Kumar Vijay
manishvijay89@gmail.com
¹ICFRE- Tropical Forest Research
Institute, Jabalpur,
Madhya Pradesh 482001, India

Abstract

Effective conservation of Rare, Endangered, and Threatened (RET) tree species requires a robust, species-specific understanding of seed biology. *Cochlospermum religiosum*, an ecologically and economically important dry-forest tree currently experiencing population decline, lacks comprehensive information on reproductive maturity, germination physiology, and storage behaviour. This study integrated carpological analysis, germination bioassays, and controlled storage experiments to generate evidence-based conservation guidelines. Sequential sampling (30–80 days after anthesis; DAA) revealed coordinated fruit–seed enlargement, biomass accumulation, dehydration, and physiological enhancement during maturation. The physiological maturity window occurred at 60–80 DAA, with 80 DAA representing the optimal harvest stage, characterized by maximum dry mass, minimum moisture content, and highest germination ($40.00 \pm 2.88\%$; Tukey HSD, $P \leq 0.05$). Seeds exhibited a rapid triphasic imbibition pattern ($\sim 80\%$ weight gain within 10 h; $\sim 97\%$ hydration by 30 h), indicating substantial water permeability under freshly harvested conditions, although the possibility of partial physical dormancy developing during drying and storage cannot be completely excluded. Pre-sowing treatment with gibberellic acid (GA_3) at 500 ppm significantly enhanced germination (26.8%) and seed vigour ($VI = 285.05$; ANOVA, $P < 0.001$), whereas higher GA_3 concentrations and other chemical treatments were ineffective. Storage experiments suggested intermediate to near-orthodox but chilling-sensitive seed behaviour, with germination performance strongly governed by moisture–temperature interactions. Seeds equilibrated to $\sim 11\%$ moisture content and stored at 15°C retained comparatively higher germination performance for six months, whereas excessive drying (4.9% MC) and room-temperature storage resulted in substantial reduction in germination. These findings provide practical guidance for seed harvesting, nursery propagation, and ex situ conservation of *C. religiosum* and comparable tropical dry-forest tree species.

Keywords Carpology, Germination ecology, Physiological seed maturity, Seed imbibition, Seed vigour, Intermediate storage behaviour, Ex situ conservation, Physical dormancy



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

1 Introduction

Global assessments of plant biodiversity increasingly highlight the vulnerability of tree species to accelerating environmental change. Recent analyses estimate that nearly one-third of the world's tree flora faces extinction risk due to habitat fragmentation, climate change, overexploitation, and declining natural regeneration [7]. These pressures are particularly pronounced in tropical and subtropical forest ecosystems, where anthropogenic disturbances disrupt reproductive cycles and regeneration dynamics of many woody species. In such contexts, a detailed understanding of seed biology, including carpology, fruit phenology, maturation dynamics, dormancy mechanisms, germination ecology, and storage behaviour, is widely regarded as fundamental for developing effective propagation strategies, restoration programmes, and ex situ conservation initiatives [11, 24]. Integrating optimized germination protocols with nursery propagation and seed banking has been shown to substantially improve reintroduction success and enhance long-term population resilience of threatened species [8, 25, 74].

Cochlospermum religiosum (L.) Alston (Bixaceae), commonly known as yellow silk cotton, is a deciduous tree species native to tropical Southeast Asia and the Indian subcontinent. The species typically occurs in tropical dry deciduous forests, rocky hill slopes, and open scrub forests across eastern, central, and southern India [54]. Although geographically widespread, natural populations are often sparse and highly fragmented. Regional conservation assessments have categorized the species as Vulnerable in Madhya Pradesh and Chhattisgarh and Critically Endangered in Rajasthan under regional IUCN criteria [60]. Beyond its ecological significance, *C. religiosum* possesses considerable socioeconomic importance. The species produces "Gum Katira," a natural bark exudate widely utilized in pharmaceutical formulations, confectionery products, and industrial applications, while the silky floss released from mature capsules has traditionally been used as a lightweight filling material for bedding and flotation devices [2, 31].

The decline of *C. religiosum* populations has been attributed to multiple interacting pressures, including unsustainable harvesting of plant parts, habitat degradation, overexploitation, and poor natural regeneration. These factors have resulted in reduced population size and severe habitat fragmentation, corresponding to threat categories defined under IUCN Criteria A2 (population reduction due to exploitation) and B2 (restricted area of occupancy with fragmentation) [32]. Despite increasing conservation concern, comprehensive information on seed maturation, dormancy behaviour, germination physiology, and storage ecology remains limited, thereby constraining the development of evidence-based propagation and conservation strategies.

Members of the family Bixaceae have frequently been associated with physical dormancy caused by water-impermeable seed coats and specialized water-gap structures regulating seed hydration [20, 35]. However, comprehensive information regarding maturation-linked changes in seed permeability, germination physiology, and storage ecology in *C. religiosum* remains limited. Furthermore, key processes underlying seed ecology, including seed production, dispersal mechanisms, viability dynamics, germination behaviour, and storage physiology, have received comparatively little scientific attention [4, 11, 24].

From a seed science perspective, identifying the physiological determinants of seed quality is central to improving regeneration and conservation outcomes. Studies have consistently demonstrated that fruit maturation stage strongly influences seed viability,

germination capacity, and storability. Similarly, imbibition kinetics, dormancy classification, and storage responses provide critical insights into seed physiological behaviour and determine the suitability of seeds for short- or long-term conservation [4, 24, 29]. For threatened woody species, integrating knowledge of maturation physiology with germination ecology and storage biology is therefore essential for designing reliable seed handling and conservation protocols. In particular, the development of optimized dormancy-breaking treatments, improved nursery practices, and appropriate seed banking strategies has proven instrumental in strengthening restoration and ex situ conservation programmes [8, 72].

Previous observations and preliminary germination trials indicated rapid seed hydration and comparatively fast imbibition behaviour in freshly harvested seeds of *C. religiosum*. Based on these observations and available literature, freshly harvested seeds were expected to exhibit limited evidence of strong physical dormancy. Nevertheless, the relatively low germination percentages reported in preliminary studies and related *Cochlospermum* Kunth species suggest that additional physiological or structural factors may regulate germination behaviour. Furthermore, drying and storage may alter seed permeability and dormancy expression, thereby influencing subsequent germination performance.

The present study therefore aimed to provide a comprehensive evaluation of seed biology in *C. religiosum* by integrating developmental, physiological, and storage-related perspectives. Specifically, the objectives were to (i) characterize fruit and seed maturation dynamics, (ii) quantify seed morphological attributes using image-based analytical techniques, (iii) evaluate germination responses under different pre-sowing treatments, and (iv) assess seed storage performance under controlled moisture and temperature regimes.

We hypothesized that (i) physiological seed maturity coincides with maximum dry matter accumulation and declining moisture content, (ii) seeds exhibit substantial water permeability and limited evidence of strong physical dormancy under freshly harvested conditions, and (iii) seed longevity is primarily governed by moisture-temperature interactions indicative of characteristic storage behaviour. By integrating these components, the study aims to generate robust, evidence-based guidelines to support the conservation, restoration, and large-scale propagation of *C. religiosum*.

2 Materials and methods

2.1 Study site and plant material

The study was conducted at the Tropical Forest Research Institute (TFRI), Jabalpur, Madhya Pradesh, India (21.959220° N, 79.944020° E) between January 2022 and November 2024. The geographic location of the study site in central India is illustrated in Fig. 1. Mature trees of *Cochlospermum religiosum* located in the Balaghat Forest Circle (South Balaghat Division, Lalbarra Range) were selected for monitoring reproductive phenology.

The study area lies within the tropical dry deciduous forest zone of central India and is characterized by seasonal temperature and rainfall fluctuations typical of dry tropical ecosystems. Associated vegetation in the study region includes *Tectona grandis*, *Terminalia tomentosa*, *Butea monosperma*, and *Lagerstroemia parviflora*. Flowering individuals were tagged at anthesis to accurately determine days after anthesis (DAA) for fruit

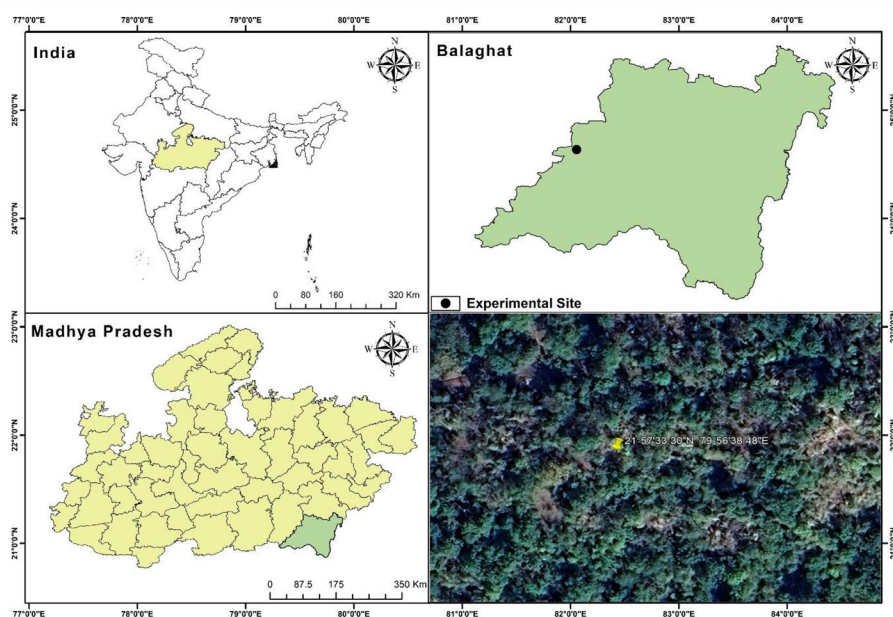


Fig. 1 Geographic location of the study site for *Cochlospermum religiosum* seed collection in central India. The map shows the sampling location in the Balaghat Forest Circle, Lalbarra Range, Madhya Pradesh, India (21.95922° N, 79.94402° E)

and seed sampling. Tagged fruits were monitored periodically to track developmental progression and identify appropriate harvest stages for subsequent analyses.

2.2 Fruit and seed maturity assessment

Fruit samples were collected at five developmental stages: 30, 40, 50, 60, and 80 days after anthesis (DAA). The 70 DAA stage was not evaluated due to limited fruit availability and asynchronous fruit retention among tagged trees during late maturation. Due to naturally low fruit set, six fruits were harvested from each of five randomly selected trees, resulting in a total of 30 fruits per developmental stage (five biological replicates, each consisting of six fruits collected from one tree). Harvested fruits were transported to the laboratory and air-dried under ambient conditions to facilitate capsule dehiscence. Mature capsules generally opened between 80 and 90 DAA. Seeds were manually separated from the floss, and only uniformly coloured, morphologically healthy seeds were selected for subsequent physiological and storage experiments.

2.3 Fruit and seed carpological analysis

Morphometric traits of fruits and seeds were measured following International Seed Testing Association [27] guidelines. Fruit length, width, and thickness were measured using a digital Vernier caliper (± 0.01 mm). Fresh and dry weights of fruits were recorded using a precision balance. Seed morphometric parameters were determined using a Biovis PSM-3000 seed scanner, which generated image-based measurements including seed area, perimeter, roundness, aspect ratio, and equivalent diameter. Mature capsules contained approximately 60–80 seeds per fruit. Seed moisture content was determined using the low-temperature oven-drying method (103 ± 2 °C for 17 ± 1 h) as recommended by ISTA. Fruit morphometric measurements were performed using five biological replicates (six fruits per replicate; $n = 30$ fruits per developmental stage). Seed

morphometric measurements were conducted using three biological replicates, with each replicate consisting of 100 randomly selected seeds drawn from the pooled seed lot of the corresponding developmental stage. All samples within each replicate were selected randomly to minimize measurement bias.

2.4 Seed Imbibition Assay

Seed water uptake was assessed using an imbibition experiment conducted under controlled laboratory conditions. For each replicate, 5 g of dry seeds (approximately 60–70 seeds) were immersed in 50 mL of distilled water at 25 ± 2 °C. Seeds were removed at predetermined time intervals (0–49 h), gently blotted to remove surface moisture, and weighed using an analytical balance. The percentage increase in seed mass was calculated relative to the initial dry weight following the method of Bewley and Black [5]:

$$\text{Seed mass increase (\%)} = \frac{W_i - W_d}{W_d} \times 100$$

where W_i and W_d represent the mass of imbibed and dry seeds, respectively.

The experiment followed a completely randomized design (CRD) with three biological replicates, and measurements were performed on randomly selected seed samples within each replicate.

2.5 Germination assays

Germination experiments were conducted using four replicates of 25 seeds ($n = 100$) per treatment. Seeds were placed on sterilized sand in germination trays and incubated at 25 ± 2 °C under a 16 h light / 8 h dark photoperiod. The experiment followed a completely randomized design (CRD). Radicle protrusion ≥ 1 cm was considered successful germination. Germination parameters calculated included:

- Final Germination Percentage (FGP) [53]
- Mean Germination Time (MGT) [39]
- Germination Value (GV) [14]
- Seed Vigour Index (SVI) [1]

To assess desiccation tolerance, subsets of seeds were gradually dried under ambient laboratory conditions with periodic moisture monitoring until approximately 5% moisture content was attained, after which seeds were subjected to the same germination protocol. Non-germinated seeds were recorded at the end of the germination test.

2.6 Seed pre-treatment experiments

Six pre-sowing treatments were evaluated to assess germination enhancement based on commonly used forestry seed treatment protocols and previous germination studies on tropical tree species [4, 6]:

- Untreated control
- Water soaking (48 h)
- Gibberellic acid (GA_3) 500 ppm for 48 h
- Gibberellic acid (GA_3) 1000 ppm for 48 h
- Thiourea [CH_4N_2S] (0.5%) for 48 h
- Potassium nitrate (KNO_3 ; 0.5%) for 48 h

Each treatment consisted of four replicates of 25 seeds ($n = 100$) arranged in a completely randomized design. After treatment, seeds were surface dried and subjected to germination tests as described above. Root and shoot lengths were measured from ten randomly selected normal seedlings per replicate to calculate seedling vigour indices.

2.7 Seed storage experiments

To evaluate storage behaviour, seeds were equilibrated to three moisture levels:

- 11.00%
- 6.23%
- 4.91%

Moisture equilibration was achieved through gradual ambient drying with periodic monitoring of seed moisture content. Seeds were stored at three temperature regimes:

- Room temperature (25–30 °C)
- 15 °C
- 5 °C

The storage experiment followed a factorial completely randomized design (3 moisture levels $\times$ 3 temperatures) with three biological replicates. Seed viability and vigour were evaluated after 0, 3, 6, 12, 15, and 18 months of storage. For each evaluation, four replicates of 25 seeds ($n = 100$) were tested for germination following ISTA protocols. Initial seed moisture content prior to storage was approximately 11–12% in freshly harvested control seeds.

2.8 Statistical analysis

All experiments followed a completely randomized design (CRD).. Statistical analyses were performed using R statistical software (version 4.3.2). Data normality and homogeneity of variance were evaluated using Shapiro–Wilk and Levene’s tests, respectively. Percentage data were arcsine square-root transformed prior to analysis to satisfy assumptions of normality; however, untransformed means are presented for clarity. Differences among treatments were assessed using analysis of variance (ANOVA). When significant differences were detected ($P \leq 0.05$), means were separated using Tukey’s Honest Significant Difference (HSD) test. For seed storage experiments, factorial ANOVA was used to examine the main effects and interactions among seed moisture content, storage temperature, and storage duration on germination percentage, seedling length, and seed vigour index.

3 Results and discussion

3.1 Fruit and seed maturity

Fruit and seed maturation in *C. religiosum* followed a coordinated progression involving structural enlargement, biomass accumulation, progressive dehydration, and improvement in physiological seed quality (Tables 1, 2, 3). Fruit dimensions and biomass increased rapidly from 30 to 60 days after anthesis (DAA), followed by stabilization toward 80 DAA (Table 1). This developmental transition was accompanied by a pronounced reduction in fruit moisture content and a distinct colour shift from yellow–green to deep brown, indicating the onset of physiological maturity and readiness

Table 1 Morphological and physiological changes in fruits of *C. religiosum* during maturation at different days after anthesis (DAA)

Days after anthesis (DAA)	Fruit length (mm) (Mean $\pm$ SE)	Fruit width (mm) (Mean $\pm$ SE)	Fresh weight of 10 fruits (g) (Mean $\pm$ SE)	Dry weight of 10 fruits (g) (Mean $\pm$ SE)	Initial moisture content (%) (Mean $\pm$ SE)	Fruit colour (RHS chart)
30 DAA	7.40 $\pm$ 0.40 c	4.18 $\pm$ 0.23 b	138.53 $\pm$ 7.62 d	38.50 $\pm$ 2.12 c	71.97 $\pm$ 3.96 a	Yellow Green Group 144 (Strong Yellow Green A)
40 DAA	10.24 $\pm$ 0.56 b	4.37 $\pm$ 0.24 b	356.80 $\pm$ 19.64 c	91.02 $\pm$ 5.01 c	74.24 $\pm$ 4.08 a	Greyed Orange Group 177 (Moderate Reddish Brown A)
50 DAA	11.56 $\pm$ 0.63 b	4.90 $\pm$ 0.27 b	492.85 $\pm$ 27.13 b	269.39 $\pm$ 14.83 b	45.19 $\pm$ 2.48 b	Greyed Purple Group 187 (Dark Red A)
60 DAA	13.05 $\pm$ 0.71 a	5.66 $\pm$ 0.31 a	699.16 $\pm$ 38.49 a	497.94 $\pm$ 27.41 a	28.68 $\pm$ 1.58 c	Brown Group 200 (Greyish Reddish-Brown B)
80 DAA	14.83 $\pm$ 0.81 a	6.75 $\pm$ 0.37 a	622.12 $\pm$ 34.25 a	532.85 $\pm$ 29.33 a	14.30 $\pm$ 0.78 d	Brown Group 200 (Greyish Reddish-Brown B)

*Values represent mean $\pm$ SE (n = 3). Means within a column followed by different letters differ significantly according to Tukey's HSD test ($P \leq 0.05$). ANOVA indicated significant treatment effects for all parameters ($P < 0.001$)

Table 2 Changes in seed morphological and physiological characteristics of *Cochlospermum religiosum* during maturation at different days after anthesis (DAA). Progressive increases in seed size and dry mass, together with declining moisture content, indicate the acquisition of physiological maturity

Days after anthesis (DAA)	Seed length (mm) (Mean $\pm$ SE)	Seed width (mm) (Mean $\pm$ SE)	Fresh weight of 100 seeds (g) (Mean $\pm$ SE)	Dry weight of 100 seeds (g) (Mean $\pm$ SE)	Moisture content (%) (Mean $\pm$ SE)	Seed colour (RHS chart)
30 DAA	5.39 $\pm$ 0.29 c	4.38 $\pm$ 0.24 b	11.16 $\pm$ 0.62 d	2.60 $\pm$ 0.14 d	76.49 $\pm$ 4.21 a	Orange White Group 159 (Light Yellowish Pink A)
40 DAA	7.50 $\pm$ 0.41 b	4.98 $\pm$ 0.27 b	14.21 $\pm$ 0.78 c	4.74 $\pm$ 0.26 c	66.38 $\pm$ 3.65 a	Greyed Orange Group 164 (Moderate Orange Yellow C)
50 DAA	8.53 $\pm$ 0.47 b	4.18 $\pm$ 0.23 b	18.19 $\pm$ 1.00 b	7.33 $\pm$ 0.40 b	59.52 $\pm$ 3.27 b	Brown Group 200 (Dark Greyish Reddish-Brown A)
60 DAA	10.96 $\pm$ 0.60 a	5.38 $\pm$ 0.29 a	23.53 $\pm$ 1.29 a	15.37 $\pm$ 0.84 a	34.59 $\pm$ 1.90 c	Brown Group 200 (Dark Greyish Reddish-Brown A)
80 DAA	10.36 $\pm$ 0.57 a	4.56 $\pm$ 0.25 b	22.22 $\pm$ 1.22 a	19.62 $\pm$ 1.08 a	11.65 $\pm$ 0.64 d	Brown Group 200 (Dark Greyish Reddish-Brown A)

*Values represent mean $\pm$ SE (n = 3). Means within a column followed by different letters differ significantly according to Tukey's HSD test ($P \leq 0.05$)

for capsule dehiscence. Mature capsules generally contained approximately 60–80 seeds per fruit, and natural capsule opening was commonly observed around 80–90 DAA. Such dehydration-driven maturation patterns are characteristic of dry, dehiscent fruits of tropical deciduous species, where late-stage drying facilitates synchronized capsule opening and efficient seed release [3, 38].

Table 3 Seed physiological parameters of *C. religiosum* at different developmental stages expressed as days after anthesis (DAA). Germination behaviour and seed vigour increased progressively with maturation, indicating acquisition of germinability and desiccation tolerance at later developmental stages

Days after anthesis (DAA)	Fresh seed germination (FSG) (%) (Mean $\pm$ SE)	Desiccated seed germination (DSG) (%) (Mean $\pm$ SE)	Seed vigour index-I (Mean $\pm$ SE)
30 DAA	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 c	0.00 d
40 DAA	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 c	0.00 d
50 DAA	21.66 $\pm$ 1.66 b	26.66 $\pm$ 1.66 b	175.41 $\pm$ 29.37 c
60 DAA	25.00 $\pm$ 2.88 b	31.66 $\pm$ 1.66 b	254.50 $\pm$ 9.29 b
80 DAA	40.00 $\pm$ 2.88 a	45.00 $\pm$ 2.88 a	395.16 $\pm$ 6.84 a

*Values represent mean $\pm$ SE (n = 3). Percentage data were arcsine square-root transformed prior to analysis; untransformed means are presented. Means within a column followed by different letters differ significantly according to Tukey's HSD test ($P \leq 0.05$)

Fruit length and width increased significantly during early developmental stages, reaching maximum values at 60–80 DAA. Fresh fruit weight exhibited a similar increasing trend up to 60 DAA, reflecting rapid tissue expansion and accumulation of structural biomass. Although fresh weight slightly declined at 80 DAA, dry weight continued to increase, suggesting continued reserve accumulation accompanied by moisture loss during late maturation. This pattern indicates the transition from active growth to desiccation-mediated maturation, a process commonly associated with physiological seed maturity in many tropical tree species.

The progressive accumulation of seed dry matter observed between 50 and 80 DAA likely reflects increased deposition of storage reserves and structural tissues; processes closely associated with acquisition of germination competence and desiccation tolerance (Table 2). Similar maturation-linked improvements in seed quality have been documented in several forestry species. For example, accurate identification of seed maturity stages significantly enhanced germination performance and responsiveness to pre-treatments in *Flacourtia indica* [64], while variations in seed size have been shown to influence germination behaviour and seedling vigour [67]. Likewise, maturity-related improvements in seed viability and germination have been reported in *Commiphora wightii* [66], *Cordia myxa* [68], *Gymnema sylvestre* [69], and *Butea monosperma* [70]. In these species, coordinated carpological development, reserve accumulation, and physiological maturation collectively enhanced germination capacity and storage performance.

Physiological parameters further corroborated the maturation trends observed in morphological traits. Fresh and desiccated seed germination remained negligible during early developmental stages (≤ 40 DAA), indicating that seeds harvested at these stages were physiologically immature and lacked germination competence (Table 3). Germination capacity increased markedly between 50 and 60 DAA and reached maximum values at 80 DAA, accompanied by significant improvements in seed vigour index. Although germination increased substantially during later maturation stages, approximately 60% of freshly harvested seeds and 55% of desiccated seeds remained ungerminated at the optimum maturity stage (80 DAA; Table 3). This may indicate variability in physiological maturity, partial dormancy induction during drying, or other physiological limitations affecting germination performance. These findings suggest that seeds attain maximum germinability and physiological stability during the later stages of maturation, when reserve accumulation and dehydration processes are largely complete. The 70 DAA

stage was not evaluated due to limited fruit availability and asynchronous fruit retention among tagged trees.

The compact morphology of mature seeds may contribute to efficient packing within the capsule and facilitate dispersal following capsule dehiscence, consistent with carpological adaptations reported in several wind-dispersed taxa [52]. Furthermore, rapid water uptake during imbibition indicated substantial seed permeability under freshly harvested conditions. However, considering reports of physical dormancy in members of Bixaceae, the possibility that some seeds may develop reduced permeability during maturation, drying, or storage cannot be completely excluded [20, 35].

Taken together, the convergence of morphological, physiological, and moisture-related indicators strongly suggests 60–80 DAA as the physiological maturity window for *C. religiosum*, with 80 DAA representing the optimal stage for harvesting seeds with maximum germination performance, seed vigour, and improved storage suitability.

3.2 Mature fruit and seed carpology

Fruits of *C. religiosum* are large, brown, oval capsules (approximately 7–10 × ~6 cm) that dehisce into five segments at maturity, releasing numerous seeds embedded within abundant yellow silky floss (Fig. 2). The floss imparts a cotton-like appearance and likely facilitates wind-mediated seed dispersal. Such fibrous appendages substantially increase aerodynamic drag and enhance buoyancy, representing a characteristic adaptation associated with anemochory in many wind-dispersed plant species [18, 21, 76].



Fig. 2 Carpological characteristics of *C. religiosum*: **a** flowering tree, **b** fruiting branch, **c** mature fruit capsule, **d** seeds with silky floss, and **e** microscopic image of seed morphology (scale bar = 2200 μm). High-resolution imaging illustrates the characteristic reniform seed shape and floss-associated dispersal structures facilitating anemochory

Seeds are small (~6 mm), black, curved, and reniform to cochleate in shape [78], with each seed surrounded by fine silky hairs that may further support aerial transport. Mature capsules contained approximately 60–80 seeds per fruit, while overall seed density ranged between 10,000–15,000 seeds kg⁻¹, indicating high reproductive output and substantial propagule production potential. Image-based morphometric analysis revealed the following seed characteristics: area 0.36 ± 0.01 cm², length 0.691–0.744 cm, width 0.294–0.617 cm, aspect ratio 1.18 ± 0.01 , mean diameter 0.65 ± 0.01 cm, perimeter 2.24 ± 0.05 cm, convex perimeter 2.14 ± 0.05 cm, and roundness 0.90 ± 0.01 . These morphological attributes indicate a relatively compact and aerodynamically efficient seed geometry that may facilitate dispersal efficiency and airborne transport stability. Similar relationships between seed morphology and dispersal trajectories have been widely documented in wind-dispersed species [47, 76].

The combined occurrence of a dehiscent capsule and numerous floss-covered seeds suggests a dispersal strategy adapted for effective aerial dissemination. Similar dispersal systems have been reported in other anemochorous taxa, where appendages such as hairs, plumes, or wings reduce terminal velocity and prolong airborne suspension, thereby enhancing dispersal range [9, 18, 46]. In *C. religiosum*, these structural adaptations may facilitate colonization of open and seasonally dry habitats and contribute to the species' broad distribution across tropical and subtropical regions [30].

In addition to ecological significance, characterization of fruit and seed carpological traits provides important baseline information for species identification, seed handling, nursery operations, and conservation-oriented propagation programmes. The morphological features supporting these dispersal adaptations are illustrated in Fig. 2.

3.3 Seed imbibition assay

The imbibition behaviour of *C. religiosum* seeds exhibited a characteristic triphasic water uptake pattern commonly observed in water-permeable seeds [22, 55]. This hydration behaviour reflects sequential physiological processes associated with germination, including rapid initial water uptake, metabolic activation, reserve mobilization, and subsequent embryo growth [42, 59].

During the initial imbibition phase (Phase I), seeds exhibited rapid water absorption, resulting in approximately 65–80% increase in fresh weight within the first 6–10 h (Fig. 3). Such rapid hydration indicates substantial seed coat permeability and efficient water uptake, facilitating activation of early metabolic processes required for germination [22, 29, 51, 77]. The absence of prolonged delay during initial hydration further suggests lack of strong water-impermeable seed coat barriers under freshly harvested conditions.

This was followed by Phase II, characterized by slower water uptake and stabilization of seed mass. Near-maximum hydration (~90–97% weight gain) was achieved between 24 and 30 h, after which seed weight remained relatively stable until 49 h. This plateau phase is generally associated with metabolic reorganization, activation of hydrolytic enzymes, repair of cellular structures, and mobilization of stored reserves prior to radicle emergence [19, 44, 62].

Importantly, *C. religiosum* seeds did not exhibit delayed hydration patterns typically associated with strongly water-impermeable seeds or pronounced physical dormancy. Instead, the smooth and continuous imbibition curve indicates unrestricted water entry

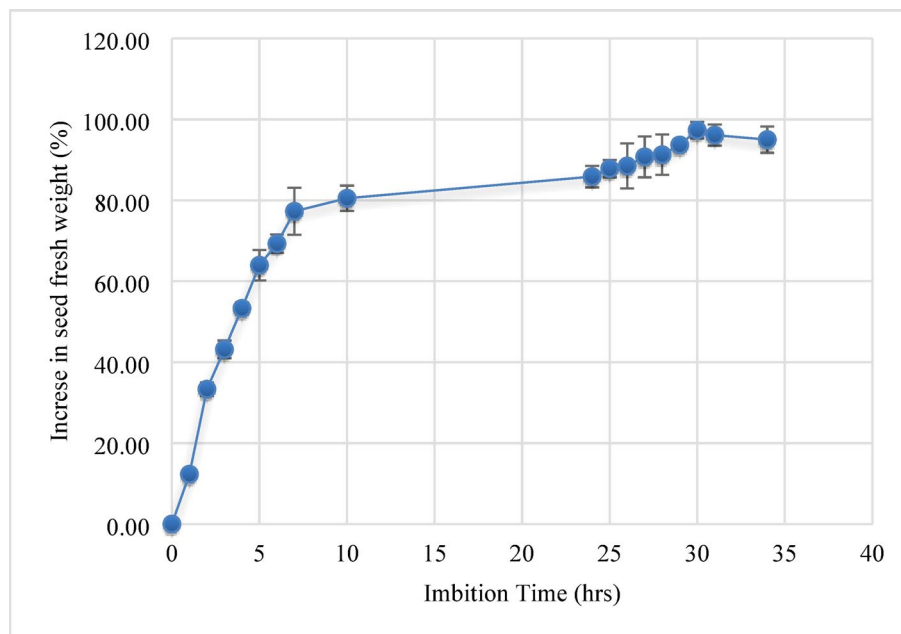


Fig. 3 Imbibition curve of *C. religiosum* seeds showing percentage increase in seed weight during water uptake over time. Rapid hydration during Phase I was followed by a stabilization phase (Phase II), indicating attainment of hydration equilibrium prior to germination. Data represent mean $\pm$ SE of three biological replicates

and relatively intact membrane organization. Similar triphasic hydration behaviour has been widely reported in permeable seeds lacking strong physical dormancy [12, 45]. In contrast, aged or physiologically deteriorated seeds often exhibit irregular hydration patterns resulting from membrane disorganization and impaired cellular integrity [58, 63].

Nevertheless, although freshly harvested seeds exhibited substantial water permeability, the possibility of variability in permeability among individual seeds or induction of partial dormancy during drying and storage cannot be completely excluded. Furthermore, because scarified and non-scarified seeds were not directly compared in the present study, additional investigations are required to conclusively evaluate the contribution of seed coat permeability and potential physical dormancy mechanisms in *C. religiosum*.

Overall, the rapid hydration, well-defined triphasic imbibition pattern, and stable hydration plateau indicate substantial seed permeability under freshly harvested conditions and favourable hydration dynamics that support germination and early seedling establishment in freshly harvested *C. religiosum* seeds.

3.4 Seed pre-treatment

Although the imbibition analysis demonstrated rapid water uptake and substantial seed permeability under freshly harvested conditions, the possibility of weak physiological dormancy or other physiological constraints regulating germination could not be completely excluded. Therefore, several hormonal and chemical pre-treatments were evaluated to assess their potential to improve germination performance of *C. religiosum* seeds (Table 4). Despite rapid imbibition, relatively low germination percentages observed in untreated seeds suggest that factors other than water uptake alone may regulate germination behaviour in this species, including physiological inhibition, incomplete embryo development, maturation heterogeneity, or variability in seed coat permeability.

Table 4 Effect of different seed pre-treatments on germination performance and seedling growth parameters of *C. religiosum*. Treatments were evaluated based on final germination percentage (FGP), mean germination time (MGT), seedling root length, shoot length, and seed vigour index (SVI-I)

Treatment	Final germination percentage (FGP) (%) (Mean $\pm$ SE)	Mean germination time (MGT) (days) (Mean $\pm$ SE)	Root length (cm) (Mean $\pm$ SE)	Shoot length (cm) (Mean $\pm$ SE)	Vigour Index-I (Mean $\pm$ SE)
Control	11.06 $\pm$ 0.91 c	15.83 $\pm$ 0.04 a	2.71 $\pm$ 0.17 b	7.24 $\pm$ 0.15 a	110.11 $\pm$ 9.60 c
Water soaking (48 h)	17.03 $\pm$ 0.87 b	18.00 $\pm$ 0.00 b	2.38 $\pm$ 0.25 b	5.16 $\pm$ 0.23 b	127.70 $\pm$ 4.77 c
GA ₃ 500 ppm (48 h)	26.80 $\pm$ 0.78 a	18.79 $\pm$ 0.51 b	3.42 $\pm$ 0.45 a	7.25 $\pm$ 0.29 a	285.05 $\pm$ 10.34 a
GA ₃ 1000 ppm (48 h)	13.28 $\pm$ 0.39 c	21.15 $\pm$ 0.25 c	2.06 $\pm$ 0.22 c	4.47 $\pm$ 0.39 b	87.02 $\pm$ 8.15 d
Thiourea 0.5% (48 h)	8.22 $\pm$ 0.47 d	22.88 $\pm$ 0.53 d	1.46 $\pm$ 0.11 d	2.32 $\pm$ 0.26 c	30.63 $\pm$ 1.11 e
KNO ₃ 0.5% (48 h)	7.04 $\pm$ 0.14 d	21.51 $\pm$ 0.13 c	1.15 $\pm$ 0.20 d	3.35 $\pm$ 0.68 c	31.63 $\pm$ 5.18 e

Values represent mean $\pm$ SE (n = 3). Percentage germination data were arcsine square-root transformed prior to ANOVA; untransformed means are presented for clarity. Means within a column followed by different letters differ significantly according to Tukey's HSD test ($P \leq 0.05$)

Among the treatments evaluated, gibberellic acid (GA₃) at 500 ppm produced the most pronounced improvement in germination behaviour. This treatment significantly increased final germination percentage (26.80%), enhanced root and shoot growth, and resulted in the highest seed vigour index compared with the untreated control. The stimulatory effect of gibberellic acid is commonly associated with enhanced embryo elongation, activation of hydrolytic enzymes, mobilization of stored reserves, and promotion of metabolic activity during early germination [13, 34, 50]. These findings suggest that hormonal stimulation can partially overcome physiological constraints limiting germination in *C. religiosum*.

In contrast, GA₃ at 1000 ppm reduced germination percentage and seedling vigour, indicating that supra-optimal concentrations of gibberellin may exert inhibitory effects on seed metabolism and seedling development. Similar inhibitory responses at elevated GA₃ concentrations have been reported in *Rhapis excelsa* [56] and *Withania somnifera* [61], suggesting that excessive hormonal stimulation may disrupt normal physiological regulation during germination.

Water soaking produced moderate improvement in germination compared with the untreated control, likely due to hydration-induced metabolic activation and enhanced enzymatic activity during early germination. Comparable effects of water soaking have been reported in *Rauvolfia serpentina*, where hydration facilitated metabolic reactivation of seeds and improved germination performance [26]. However, although water soaking improved germination percentage, it also resulted in relatively longer mean germination time (MGT) and reduced shoot growth compared with GA₃ 500 ppm treatment, indicating that hydration alone may be insufficient to fully optimize germination physiology in *C. religiosum*.

In contrast, thiourea and potassium nitrate (KNO₃) treatments showed little or no improvement in germination performance and significantly reduced seedling vigour. The inhibitory effects observed following thiourea and KNO₃ treatments suggest that these chemicals may adversely affect embryo metabolism and early seedling development in *C. religiosum*. Such limited responsiveness to chemical dormancy-breaking agents indicates limited evidence of strong physiological dormancy in freshly harvested seeds. Similar contrasting responses have been reported in highly dormant species such as *Aconitum* spp., where these treatments substantially enhance germination [41].

Overall, the results indicate limited evidence of strong physiological dormancy under freshly harvested conditions in *C. religiosum* seeds, consistent with the rapid imbibition behaviour observed earlier. Nevertheless, because viability testing and post-treatment permeability assessments were not conducted, the possibility of partial dormancy or variability in seed permeability among individual seeds cannot be completely excluded. Among the treatments evaluated, GA₃ at 500 ppm emerged as the most effective pre-sowing treatment, significantly improving germination and seedling vigour. These findings demonstrate that hormonal stimulation can substantially enhance germination performance and may facilitate nursery propagation and conservation programmes for *C. religiosum*.

3.5 Seed storage

Seed storage performance of *C. religiosum* was significantly influenced by seed moisture content, storage temperature, and storage duration ($P \leq 0.05$). Seeds equilibrated to 11% moisture content (MC) recorded the highest germination (37.05%), seedling length (8.67 cm), and seed vigour index (SVI-I = 342.48), followed by seeds at 6.23% MC, whereas seeds dried to 4.91% MC exhibited the lowest values for all parameters (Table 5). These results indicate that *C. religiosum* seeds are sensitive to excessive desiccation and exhibit tolerance to moderate drying only. However, since post-storage viability testing using tetrazolium staining was not conducted, the observed reduction in germination cannot be interpreted solely as complete viability loss, and the possibility of dormancy induction or reduced seed permeability during drying and storage cannot be completely excluded.

Unlike orthodox seeds, which can generally be dried to moisture levels below 5% without substantial reduction in germination performance [65, 71, 73], seeds of *C. religiosum* exhibited marked declines in germination and seedling vigour at lower moisture contents. Similar storage responses have been reported in species such as *Zelkova serrata* [75], *Psidium guineense* [37], and *Ulmus wallichiana* [43]. The pronounced reduction in germination observed at very low moisture levels may reflect oxidative stress and

Table 5 Effect of seed moisture content, storage temperature, and storage duration on germination percentage, seedling length, and seed vigour index (SVI-I)

Factor	Level	Germination (%) * mean	Seedling length (cm)* Mean	SVI-I * mean
Moisture (%)	11%	37.05 a	8.67 a	342.48 a
	6.23%	24.77 b	7.39 b	180.39 b
	4.91%	17.36 c	5.94 c	127.14 c
Storage temperature	15 °C	28.85 a	7.70 a	246.92 a
	Room temperature (RT)	26.34 b	7.09 b	214.03 b
	5 °C	23.99 c	7.21 b	189.06 c
Storage duration (MAS)	0 MAS	43.89 a	9.10 a	410.80 a
	3 MAS	42.78 b	7.28 b	307.22 b
	6 MAS	33.15 c	8.21 b	282.62 b
	12 MAS	23.15 d	7.57 c	185.50 c
	15 MAS	10.00 e	6.82 d	73.35 d
	18 MAS	5.40 f	5.02 e	40.52 e

*Values represent mean $\pm$ SE (n = 3) averaged across factorial levels. Data were analysed using factorial ANOVA (moisture $\times$ temperature $\times$ storage duration), and means were separated using Tukey's HSD test ($P \leq 0.05$). Means within each column followed by different letters differ significantly. Germination percentages were arcsine-transformed prior to analysis; untransformed means are presented for clarity

cellular damage associated with storage deterioration, including reactive oxygen species (ROS) accumulation, mitochondrial dysfunction, lipid peroxidation, and degradation of cellular macromolecules [33]. In addition, reduced seed permeability or induction of partial dormancy during drying and storage may also have contributed to the lower germination percentages observed after prolonged storage.

Storage temperature further influenced seed longevity and vigour. Seeds stored at 15 °C maintained significantly higher germination (28.85%), seedling length (7.70 cm), and vigour index (246.92) compared with seeds stored at room temperature. In contrast, storage at 5 °C resulted in substantial reduction in germination performance, indicating sensitivity to chilling temperatures. Comparable chilling-sensitive responses have been reported in several species exhibiting intermediate-like storage behaviour, including *Elaeis guineensis* [17], *Genipa americana* [36], and *Calophyllum inophyllum* [23]. These observations differ from orthodox seeds, where reduced storage temperature generally prolongs seed longevity under low moisture conditions [16, 40]. In chilling-sensitive seeds, excessively low temperatures may disrupt membrane integrity and cellular metabolism, thereby accelerating physiological deterioration [10, 15].

The progressive decline in germination with increasing storage duration further demonstrated the sensitivity of *C. religiosum* seeds to prolonged storage. Maximum germination and vigour were maintained during the initial 0–3 months after storage (MAS), followed by gradual reductions between 6 and 12 MAS and more pronounced declines thereafter. By 15 MAS, germination decreased to approximately 10%, accompanied by substantial reductions in seed vigour, while at 18 MAS germination declined to 5.40%. Nevertheless, because viability testing was not performed, these low germination values should be interpreted cautiously, as some non-germinated seeds may have remained viable but dormant. Similar deterioration patterns have been reported in other species exhibiting intermediate storage characteristics when optimal moisture–temperature combinations are not maintained [28, 49, 57].

Collectively, these results demonstrate that seed moisture content and storage temperature interact strongly to determine the storage performance of *C. religiosum* seeds. Seeds maintained at approximately 11% moisture content and stored at 15 °C exhibited comparatively better preservation of germination and vigour, whereas excessive drying ($\leq 5\%$ MC), room-temperature storage, and chilling conditions accelerated decline in germination performance (Fig. 4). The results therefore suggest intermediate to near-orthodox but chilling-sensitive storage behaviour in *C. religiosum* seeds. However, further investigations involving tetrazolium viability assays, permeability assessments, and dormancy evaluation following storage are required before definitive classification of storage behaviour can be confirmed.

Considering the ecological importance and medicinal value of *C. religiosum* [31, 48], the development of species-specific seed storage protocols is essential for effective ex situ conservation, nursery propagation, and long-term genetic resource preservation.

4 Conclusion

This study provides one of the first comprehensive evaluations of fruit maturation, seed physiology, germination behaviour, and storage ecology of *C. religiosum*, generating important insights for its propagation, conservation, and sustainable utilization. Sequential developmental analysis strongly suggested 60–80 days after anthesis (DAA)

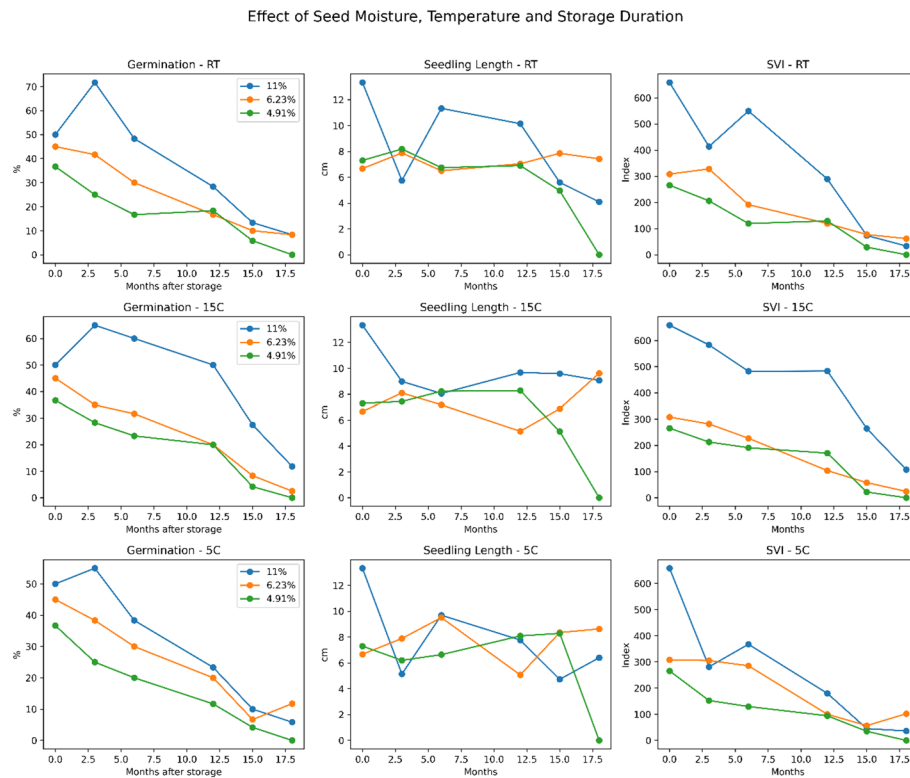


Fig. 4 Effect of seed moisture content, storage temperature, and storage duration on **A** germination percentage, **B** seedling length, and **C** seed vigour index–I of *C. religiosum*. Seeds stored at approximately 11% moisture content and 15 °C maintained comparatively higher germination and vigour during storage, whereas seeds dried to 4.91% moisture content exhibited substantial reduction in germination performance during prolonged storage. Data represent mean $\pm$ SE of three biological replicates

as the physiological maturity window, with 80 DAA representing the optimal harvest stage characterized by maximum seed dry mass, reduced moisture content, and highest germination and seed vigour. Carpological observations and imbibition analysis demonstrated rapid triphasic water uptake and substantial seed permeability under freshly harvested conditions, indicating limited evidence of strong physical dormancy. Among the pre-sowing treatments evaluated, GA₃ (500 ppm) significantly improved germination performance and seedling vigour.

Storage experiments further indicated intermediate to near-orthodox but chilling-sensitive storage behaviour, with seed longevity strongly influenced by moisture–temperature interactions. Seeds equilibrated to approximately 11% moisture content and stored at 15 °C maintained comparatively higher germination and vigour, whereas excessive desiccation ($\leq 5\%$ moisture content) and chilling conditions accelerated deterioration, particularly after 12–18 months of storage. However, because post-storage viability and permeability assessments were not conducted, the possibility of dormancy induction or persistence of viable but non-germinating seeds cannot be completely excluded.

These findings provide practical guidance for conservation and propagation programmes by emphasizing appropriate harvest timing, selective hormonal enhancement, and carefully regulated cool (but non-chilling) storage conditions. Such strategies can improve nursery propagation, seed-bank management, restoration programmes, and ex situ conservation initiatives for *C. religiosum* and similar tropical dry-forest tree species.

Nevertheless, certain limitations remain. Storage experiments were limited to 18 months, and long-term viability trends beyond this period remain unknown. In addition, the experiments were conducted under controlled storage conditions, whereas field-level storage environments may exhibit greater environmental variability. Although the findings strongly suggest intermediate storage behaviour, the physiological and biochemical mechanisms underlying desiccation tolerance and chilling sensitivity require further investigation. Population-level variation was also not examined and therefore the present findings represent species-level trends rather than geographic variation. In future studies, seed permeability to water should be tested after various periods of time for all treatments and storage conditions.

Future research should extend long-term storage studies and integrate physiological and molecular approaches, including reactive oxygen species (ROS) dynamics, membrane stability analysis, and moisture sorption studies to better understand seed deterioration processes. Integration of seed physiological studies with field emergence and restoration performance assessments will also be important for translating laboratory findings into practical conservation outcomes.

Overall, this work establishes a robust scientific foundation for improving seed management, conservation planning, and restoration strategies aimed at safeguarding the genetic resources and ecological resilience of *C. religiosum*.

Acknowledgements

The present work is the outcome of an on-going project focused on the Developing seed testing and seed storage protocols of selected forestry species from diverse forest types, generously sponsored by the CAMPA, Ministry of Environment, Forests & Climate Change, Government of India, New Delhi. Authors extend sincere gratitude to the funding agency for their invaluable support.

Author contributions

Manish K. Vijay: Conceptualization, methodology, experimental design, supervision, data interpretation, and manuscript writing (original draft preparation and finalization). Neeraj Prajapati: Experimental design support, field surveys, and data collection. Neelu Singh: Methodological refinement, data interpretation, and critical revision of the manuscript. All authors read and approved the final manuscript.

Data availability

The data generated and analysed during this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The plant material (*Cochlospermum religiosum* (L.) Alston) used in the present study was collected from natural populations located in the South Balaghat Forest Division, Balaghat Circle, Madhya Pradesh, India (21.95922° N, 79.94402° E; ~540 m asl). The collection of plant material was carried out in accordance with relevant national and state forest regulations and institutional guidelines. Prior permission for the collection of plant material was obtained from the concerned forest authorities of the Madhya Pradesh Forest Department. The plant species was identified by Manish Kumar Vijay (Seed Biologist, ICFRE–Tropical Forest Research Institute, Jabalpur, India). A voucher seed specimen has been prepared and deposited in the Ex-situ Seed Gene Bank of the Tropical Forest Research Institute (TFRI), Jabalpur, India, under voucher accession number TFRIO87. All procedures involving plant material complied with institutional, national, and international guidelines for plant research. Consent to participate: Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 17 March 2026 / Accepted: 16 September 2026

Published online: 21 September 2026

References

1. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. *Crop Sci.* 1973;13(6):630–3. <https://doi.org/10.2135/cropsci1973.0011183X001300060013x>.

2. Anonymous. Trees of Gujarat. Gandhinagar, Gujarat: Gujarat Forest Department; 2010. p. 50.
3. Anumula M, Mondli SR, Gottumukkula KM. A review on *Cochlospermum religiosum*. Int J Pharm Biol Sci. 2019;9(1):374–7.
4. Baskin CC, Baskin JM. Seeds: ecology, biogeography, and evolution of dormancy and germination. 2nd ed. Academic Press; 2014.
5. Bewley JD, Black M. Physiology and biochemistry of seeds, vol. Vol. 2. Springer-Verlag; 1982.
6. Bonner FT. Storage of Seeds. The Woody Plant Seed Manual edited by Franklin T. Bonner and Robert P. Karrfalt, Agriculture Handbook 727, U.S. Department of Agriculture, Forest Service, 2008, pp. 86–95.
7. Botanic Gardens Conservation International. State of the world's trees. BGCI Report. 2021.
8. Cochrane A, Crawford A, Monks L. The significance of ex situ seed conservation to reintroduction of threatened plants. Aust J Bot. 2007;55(3):356–61.
9. Collinson ME, van Bergen PF. Evolution of angiosperm fruit and seed dispersal biology and ecophysiology. Evol Plant Physiol 2004. <https://doi.org/10.1016/b978-012339552-8/50019-6>.
10. Corbineau F. The effects of storage conditions on seed deterioration and ageing: how to improve seed longevity. Seeds. 2024;3(1):56–75. <https://doi.org/10.3390/seeds3010005>.
11. De Vitis M, Hay FR, Dickie JB, Trivedi C, Choi J, Fiegenger R. Seed storage: maintaining seed viability and vigor for restoration use. Restor Ecol. 2020;28(3):S249–55. <https://doi.org/10.1111/rec.13174>.
12. Dekkers BJW, Pearce SP, van Bolderen-Veldkamp RPM, Holdsworth MJ, Bentsink L. Dormant and after-Ripened *Arabidopsis thaliana* seeds are distinguished by early transcriptional differences in the imbibed state. Front Plant Sci. 2016. <https://doi.org/10.3389/fpls.2016.01323>
13. Dhiman R, Gupta NK, Attri V, Sarvade S. Effect of soaking conditions with growth regulators on germination behaviour of Ratela. Environ Ecol. 2015;33(1):41–5.
14. Djavanshir K, Pourbeik H. Germination value—a new formula. Silvae Genet. 1976;25:79–83.
15. Dussert S, Serret J, Bastos-Siqueira A, Morcillo F, Déchamp E, Rofidal V, et al. Integrative analysis of the late maturation programme and desiccation tolerance mechanisms in intermediate coffee seeds. J Exp Bot. 2018;69(7):1583–97. <https://doi.org/10.1093/jxb/erx492>.
16. Ellis RH, Hong TD. Temperature sensitivity of the low-moisture-content limit to negative seed longevity—moisture content relationships in hermetic storage. Ann Bot. 2006;97(5):785–91. <https://doi.org/10.1093/aob/mcl035>.
17. Ellis RH, Hong TD, Roberts EH, Soetisna U. Seed storage behaviour in *Elaeis guineensis*. Seed Sci Res. 1991;1(2):99–104. <https://doi.org/10.1017/s0960258500000726>.
18. Faraji Dizaji F, Wang B, Sullivan LL, Kellogg EA. Drag coefficient of bent-awn plumegrass (*Saccharum contortum*) seeds in wind. Phys Fluids. 2024. <https://doi.org/10.1063/5.0231717>.
19. Fatokun K, Beckett RP, Varghese B. A comparison of water imbibition and controlled deterioration in five orthodox species. Agronomy. 2022;12(7):1486. <https://doi.org/10.3390/agronomy12071486>.
20. Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC. Water-gaps in physically dormant seeds. Ann Bot. 2013;112(1):69–84. <https://doi.org/10.1093/aob/mct094>.
21. Gan SR, Guo JC, Zhang YX, Wang XF, Huang LJ. “Phoenix in flight”: an unique fruit morphology ensures wind dispersal of seeds of the phoenix tree (*Firmiana simplex* (L.) W. Wight). BMC Plant Biol. 2022. <https://doi.org/10.1186/s12870-022-03494-z>.
22. Gruwel MLH, Chatson B, Yin X, Abrams S. A nuclear magnetic relaxation study of water uptake in Barley Kernels. Molecular Biology and Physiology of Water and Solute Transport. 2000:289–295. https://doi.org/10.1007/978-1-4615-1203-5_40.
23. Hathurusingha S, Ashwath N. *Calophyllum inophyllum*: recalcitrant or intermediate seed? J For Res. 2012;23(1):103–7. <https://doi.org/10.1007/s11676-012-0222-z>.
24. Hay FR, Probert RJ. Advances in seed conservation of wild species. Conserv Physiol. 2013;1(1):cot030. <https://doi.org/10.1093/conphys/cot030>.
25. Hoyle GL, Sommerville KD, Liyanage GS, Worboys S, Guja LK, Stevens AV, et al. Seed banking for tropical montane flora. Glob Ecol Conserv. 2023;47:e02627. <https://doi.org/10.1016/j.gecco.2023.e02627>.
26. Hussain A, Jha DK. Seed germination improvement in medicinal plants. Curr Agric Res J. 2014;2(2):10–17. <https://doi.org/10.12944/CARJ.2.2.10>.
27. International Seed Testing Association. International rules for seed testing. Bassersdorf, Switzerland. 2023.
28. Jaganathan GK, Li J, Yang Y, Han Y, Liu B. Complexities in identifying seed storage behavior of hard seed-coated species: a special focus on Lauraceae. Bot Lett. 2019;166(1):70–9. <https://doi.org/10.1080/23818107.2018.1563566>.
29. Jayasuriya KMG, Baskin JM, Baskin CC. Dormancy, germination requirements and storage behaviour of seeds of Convolvulaceae (Solanales) and evolutionary considerations. Seed Sci Res. 2008;18(4):223–37. <https://doi.org/10.1017/s0960258508094750>.
30. Johnson-Fulton SB, Watson LE. Phylogenetic systematics of Cochlospermaceae (Malvales) based on molecular and morphological evidence. Syst Bot. 2017;42(2):271–82. <https://doi.org/10.1600/036364417x695457>.
31. Kekuda TRP, Smruthi BS, Banu S, Swathi BG. A comprehensive review on the ethnobotanical uses and pharmacological activities of *Cochlospermum religiosum* (L.) Alston (Bixaceae). J Med Plants Stud. 2019;7(3):17–23.
32. Khanna KK, Dubey PC, Tiwari AP, Sikarwar RLS. Studies on threat status of tree species of Madhya Pradesh, India. 2021.
33. Kurek K, Plitta-Michalak B, Ratajczak E. Reactive oxygen species as potential drivers of the seed aging process. Plants. 2019;8(6):174. <https://doi.org/10.3390/plants8060174>.
34. Lavania SK, Singh RP, Singh V. Gibberellic acid and pH effects on *Pinus wallichiana*. Indian For. 2006;132(8):1024–8.
35. Louf JF, Zheng Y, Kumar A, Bohours T, Gundlach C, Harholt J, Poulsen HF, Jensen KH. Imbibition in plant seeds. Technical University of Denmark. 2018.
36. Magistrali PR, José AC, Faria JMR, Gasparin E. Physiological behavior of *Genipa americana* L. seeds regarding the capacity for desiccation and storage tolerance. J Seed Sci. 2013;35(4):495–500. <https://doi.org/10.1590/s2317-15372013000400011>.
37. Masetto TE, Silva Neves EM, QuintãoScalon SP, Dresch DM. Drying, Storage and Osmotic Conditioning of *Psidium guineense* Swartz Seeds. Am J Plant Sci. 2014;5(17):2591–8. <https://doi.org/10.4236/ajps.2014.517273>.
38. NParks. *Cochlospermum religiosum* (L.) Alston. Flora & Fauna Web. 2022.
39. Orchard T. Estimation of seedling emergence parameters. Seed Sci Technol. 1977;5:61–9.
40. Pandey CD, Pandey S. seed drying and packaging methods for long-term storage. Management of Plant Genetic Resources. 2015.

41. Pandey H, Nandi SK, Nadeem M, Palni LMS. Chemical stimulation of *Aconitum heterophyllum* seeds. *Seed Sci Technol*. 2000;2(1):39–48.
42. Penfield S. Seed dormancy and germination. *Curr Biol*. 2017;27(17):R874–8. <https://doi.org/10.1016/j.cub.2017.05.050>.
43. Phartyal SS, Thapliyal RC, Nayal JS, Joshi G. Seed storage physiology of Himalayan elm (*Ulmus wallichiana*): an endangered tree species of tropical highlands. *Seed Sci Technol*. 2003;31(3):651–8. <https://doi.org/10.15258/sst.2003.31.3.14>.
44. Prazeres CS, Coelho CMM. Hydration curve and physiological quality of maize seeds subjected to water deficit. *Semina Cienc Agrar*. 2017;38(3):1179. <https://doi.org/10.5433/1679-0359.2017v38n3p1179>.
45. Preston J, Tatematsu K, Kanno Y, Hobo T, Kimura M, Jikumaru Y, et al. Temporal expression patterns of hormone metabolism genes during imbibition of *Arabidopsis thaliana* seeds: a comparative study on dormant and non-dormant accessions. *Plant Cell Physiol*. 2009;50(10):1786–800. <https://doi.org/10.1093/pcp/pcp121>.
46. Qi-he Y, He-sheng Y, Hui-na L. The dispersal ways of plant seeds and their adaptability. *J Jiaying Univ*. 2013.
47. Qin X, Liang W, Liu M, Xin Z, Wang Z, Zhou Q, et al. Effects of plant crown characteristics on dispersal kernels of wind-dispersed seeds under low wind speeds. *J Plant Ecol*. 2024. <https://doi.org/10.1093/jpe/rtae106>.
48. Ramling GP, Omprakash GB. Pharmacognostic investigation of leaves and bark of *Cochlospermum Religiosum* Linn. *J Univ Shanghai Sci Technol*. 2021;23(2). <https://doi.org/10.51201/jusst12643>.
49. Rao ChS, Pandey V, Soni A, Naithani SC. Ex-Situ Seed Storage Behavior of *Dipcadi saxorum* Blatter, an Endangered Species from India. *J New Seeds*. 2009;10(4):282–92. <https://doi.org/10.1080/15228860903330455>.
50. Ratan PB, Reddy YN. GA₃ effects on *Annona squamosa*. *J Res ANGRAU*. 2004;32(2):93–5.
51. Rodrigues-Junior AG, Faria JMR, Vaz TAA, Nakamura AT, José AC. Physical dormancy in *Senna multijuga* (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. *Seed Sci Res*. 2014;24(2):147–57. <https://doi.org/10.1017/s0960258514000087>.
52. Sankara Rao K, Raja K Swamy, Deepak Kumar, Singh AR, Gopalakrishna Bhat K. Flora of Peninsular India. 2019.
53. Scott SJ, Jones RA, Williams WA. Review of data analysis for seed germination. *Crop Sci*. 1984;24:1192–9.
54. Singh J, Singh B. Gum Kondagogu A Natural Exudate from *Cochlospermum religiosum*. *The Botanica*. 2013;62–63:115–23.
55. Song SQ, Jiang CH, Chen RZ, Fu JR, Engelmann F. Dehydration damage and its repair in imbibed soybean (*Glycine max*) seeds. The biology of seeds: recent research advances. 2003:355–370. <https://doi.org/10.1079/9780851996530.0355>.
56. Tavares SL, Silva AR, Alves LF, Pimentel RD. GA₃ and growth of *Rhapis excelsa*. *Hortic Sci*. 2007;42(4):123–8. <https://doi.org/10.1080/14728028.2007.9752661>.
57. Thapliyal RC, Sood OP, Rawat MMS. Effect of moisture content and storage temperature on the viability of *Bambusa tulda* seed. *Int Tree Crops J*. 1991;7(1–2):67–75. <https://doi.org/10.1080/01435698.1991.9752903>.
58. Tilden RL, West SH. Reversal of the effects of aging in soybean seeds. *Plant Physiol*. 1985;77(3):584–6. <https://doi.org/10.1104/pp.77.3.584>.
59. Upreti P, Bandara MS, Tanino KK. The role of seed characteristics on water uptake preceding germination. *Seeds*. 2024;3(4):559–74. <https://doi.org/10.3390/seeds3040038>.
60. Ved DK, Sureshchandra ST, Barve V, Srinivas V, Sangeetha S, Ravikumar K, Kartikeyan R, Kulkarni V, Kumar AS, Venugopal SN, Somashekhar BS, Sumanth MV, Begum N, Rani S, Surekha KV, Desale N. FRLHT's ENVIS Centre on Medicinal Plants, Bengaluru. 2016.
61. Velmurugan S, Vadivel E, Paramaguru P. Seed germination in *Withania somnifera*. *National Seminar on Medicinal Plants*. 2003.
62. Veselova TV, Veselovsky VA. Possible involvement of aquaporins in water uptake by pea seeds differing in quality. *Russ J Plant Physiol*. 2006;53(1):96–101. <https://doi.org/10.1134/s1021443706010122>.
63. Veselova TV, Veselovskii VA, Usmanov PD, Usmanova OV, Kozar'VI. Hypoxia and Imbibition Injuries to Aging Seeds. *Russ J Plant Physiol*. 2003;50(6):835–842. <https://doi.org/10.1023/b:rupp.0000003283.24523.82>.
64. Vijay MK. Seed viability, imbibition dynamics and storability of *Aegle marmelos* (L.) Corrêa: Implications for propagation and conservation. *Seed Res*. 2025;53(2):157–163. <https://doi.org/10.56093/SR.v53i2.9>.
65. Vijay MK, Malviya D, Singh N. Seed viability dynamics and storage behavior of two important apocynaceae species *Holarhena pubescens* Wall. ex G. Don and *Wrightia tinctoria* (Roxb.) R.Br. *Discov Plants*. 2026;3:64. <https://doi.org/10.1007/s44372-026-00497-0>.
66. Vijay MK, Tiwari S, Kahar L, Singh N. Optimization of seed quality, germination and nursery performance of *Butea monosperma* (Lamk.) Taub. for sustainable propagation and conservation. *Asian J Environ Ecol*. 2025;24(10):1–13. <https://doi.org/10.9734/ajee/2025/v24i10798>.
67. Vijay MK, Tiwari S, Kahar L, Malviya D, Aharwar M, Singh N. Impact of seed size on germination parameters in *Flacourtia indica* (Burm.f.) Merr. *Int J Res Agron*. 2024;7(9):23–9. <https://doi.org/10.33545/2618060X.2024.v7.i9a.1432>.
68. Vijay MK, Malviya D, Berry N, Shirin F, Singh N. Assessment of seed physiological parameters in *Gymnema sylvestre* R. Br. for optimizing propagation and storage strategies. *Int J Plant Soil Sci*. 2025;37(8):383–393. <https://doi.org/10.9734/ijpss/2025/v37i85640>.
69. Vijay MK, Malviya D, Berry N, Shirin F, Singh N. Enhancing germination and storability in *Cordia myxa* Linn.: insights from seed carpology, viability, imbibition, pre-treatment and storage behaviour. *Asian Res J Agric*. 2025;18(3):121–31. <https://doi.org/10.9734/arja/2025/v18i3720>.
70. Vijay MK, Rathod D, Singh N, Tiwari S, Kahar L, Malviya D, Aharwar M. Assessment of germination dynamics through maturity and pre-treatment studies in *Flacourtia indica* (Burm.f.) Merr. *Int J Res Agron*. 2025;8(4):30–36. <https://doi.org/10.33545/2618060X.2025.v8.i4a.2738>.
71. Vijay MK, Tiwari S, Kahar L, Malviya D, Singh N. Fruit and seed development of critically endangered *Commiphora wightii* (Arn.) Bhandari: insights into physiological maturation and germination potential. *J Sci Res Rep*. 2025;31(7):374–84. <https://doi.org/10.9734/jsrr/2025/v31i73254>.
72. Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschold P. Climate change and seed regeneration. *Glob Change Biol*. 2011;17:2145–61. <https://doi.org/10.1111/j.1365-2486.2010.02368.x>.
73. Wang XF, Jing XM, Zheng GH. Effect of seed moisture content on seed storage longevity. *Acta Bot Sin*. 2001;43:551–7.
74. Werden L, Sugii N, Weisenberger L, Keir M, Koob G, Zahawi R. Ex situ conservation of Hawaiian plants. *Biol Conserv*. 2020;243:108435. <https://doi.org/10.1016/j.biocon.2020.108435>.
75. Yang JC, Kuo SR, Lin TP. Intermediate storage behaviour and the effect of prechilling on germination of Japanese zelkova (*Zelkova serrata*) seeds. *Seed Sci Technol*. 2007;35(1):99–110. <https://doi.org/10.15258/sst.2007.35.1.09>.

76. Zhu J, Buchmann CM, Schurr FM. Effects of seed morphology and orientation on secondary seed dispersal by wind. *J Plant Ecol.* 2022;15(6):1257–72. <https://doi.org/10.1093/jpe/rtac023>.
77. Liu F, Mallick S, O'Donnell TJ, Rouzimaimaiti R, Luo Y, Sun R, Wall M, Wongwiwatthananut S, Date A, Silva DK, Williams PG, Chang LC. Coumarinolignans with reactive oxygen species (ROS) and NF- κ B inhibitory activities from the roots of *Waltheria indica*. *Molecules.* 2022;27(10):3270. doi:10.3390/molecules27103270. <https://doi.org/10.3390/molecules27103270>
78. Montejo Valdés LA, Sánchez Rendón JA, Muñoz García BC. Caracterización morfológica de frutos, semillas y plántulas de *Talipariti elatum* (Malvaceae). *Acta Bot Cubana.* 2011;210:45–49. No DOI available. https://www.researchgate.net/publication/280935378_Caracterizacion_morfologica_de_frutos_semillas_y_plantulas_de_Talipariti_elatum_Malvaceae_Characterization_morphological_of_fruit_seeds_and_seedling_of_Talipariti_elatum_Malvaceae

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.