



Original Research Article

Dormancy and viability of *Litsea glutinosa* seeds

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Abstract

Litsea glutinosa, an important medicinal and economic plant, produces seeds that have low germination on shedding. The causes of low germination, the type of dormancy, and the effect of post-harvest storage on survivorship and dormancy also remain unexplored. The study on seed pre-treatments found that a combined treatment of hot water and gibberellic acid (500 ppm) resulted in a maximum germination of 67.8%. Fresh seeds were stored at four seed moisture contents and three temperatures. Seed germination and viability tests were conducted at intervals up to 21 months after harvest to assess dormancy loss and seed viability. Analyses of data indicated that both dormancy and viability were affected by temperature and seed moisture content in storage. Viability was completely lost or reduced in seeds stored with about 8 to 13% seed moisture content at 30 and 40°C within 8 months of storage. At 15°C, no seeds germinated with all seed moisture contents tested due to dormancy, and viability remained unaffected for up to 6 months of storage. Dormancy loss was optimized after 8 months of storage in the viable seeds of all treatments, where seeds stored with 3 to 6% moisture contents at 30 and 40°C and 3 to 13% moisture contents at 15°C showed maximum germination. Secondary dormancy was imposed after this period. These findings support the existence of physiological dormancy in the seeds of *L. glutinosa*. The results have significant implications for propagation and/or conservation, helping to overcome seed dormancy between collection and their use in nursery production or regeneration efforts.

Keywords: *Litsea glutinosa*, Dormancy, After-ripening, Storage, Germination, Viability.

Introduction

Dormant seeds are unable to germinate after dispersal under specific environmental conditions that typically allow germination after the dormant state has been terminated naturally or artificially (Nadella *et al.*, 2003). It is a natural defense mechanism of a plant to prevent germination in naturally unfavorable conditions for future seedlings without harming viability, and thus it helps the plant to wait for favorable conditions to germinate and grow. In plantation programs, removal of dormancy is essential to get uniform germination and establishment of seedlings in time. There are several ways to remove dormancy, such as scarification, cold stratification, treatment with chemicals, etc. On the other hand, there are reports on the effect of dry storage (i.e., after-ripening) in the alleviation of dormancy and here temperature and seed moisture content are the main storage conditions that affect dormancy (Cohn and Hughes, 1981; Foley, 1994), which is species-specific. It is also well known that temperature and seed moisture content are the factors that affect seed viability. Therefore, it is crucial to identify the proper storage conditions

that can remove dormancy but protect the seeds from reducing the viability of dormant seeds.

Litsea glutinosa Lour. C.B.Rob. is an evergreen, small to medium-sized, fast-growing tree belonging to the family Lauraceae (Chowdhury *et al.*, 2008). It is native to India, Southern China to Malaysia, Australia, and the Western Pacific islands, and introduced and established in several countries (Kostermans, 1995). The bark of this species is also popular in making local medicines and used for relieving pain, arousing sexual power, and is good for the stomach, in the treatment of diarrhea, dysentery, abdominal pain, fractured limbs, and some other diseases (Kritikar and Basu, 1981). Roots, leaves, and fruits are also used for treating wounds, bruises, sprains, and rheumatism (Agarwal *et al.*, 2013; Das *et al.*, 2013).

The bark is also used in the incense stick-making industry, which is valued at around Rupees 1000 crores with an annual growth rate of 10 to 15% and provides employment to around 1.5 million people in India (Verma *et al.*, 2017). In incense stick-making, the paste of the powdered bark is used as a binder for making incense sticks and cones due to its excellent viscosity and adhesive

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properties, which aid in continuous burning. Further, the odorless characteristic of the bark helps in retaining the original fragrance of the perfume in incense sticks (Ramana and Raju, 2017). Overexploitation of the stem bark made this species rare and critically endangered in India, Bangladesh, and the Philippines (Haque *et al.*, 2014; Reddy and Reddy, 2008; Rabena, 2010), though the species is reported to have high invasive potential (ISSG, 2015). The germination percentage of fresh seeds of *L. glutinosa* is very low, which may affect their regeneration. Ramana and Raju (2019) recorded a 17% germination percentage in their natural habitat. Bahar and Gopal (2020) showed 30.04% germination completed in 6 weeks, and treatment with gibberellin (500 ppm) increased the germination to 41.48%. Mohammad *et al.* (2016) recorded a maximum of 34% germination in *L. glutinosa* seeds after applying several treatments to 9-month-old seeds. Thapliyal *et al.* (2024) showed vermiculite was the best medium for the germination of seeds collected from Uttarakhand. However, there is a paucity of knowledge about the seed dormancy characteristics of this species, and insufficient research has been conducted on the factors affecting its dormancy and viability. To conserve and rehabilitate this species, it is crucial to thoroughly study the germination biology and longevity of the seeds. Because the seeds must not only be capable of germination (non-dormant) but also viable at the time of sowing. Therefore, to establish an effective propagation schedule, it is imperative to investigate the cause of non-germinability and develop appropriate dormancy-breaking treatments. The objective of this study was to investigate 1) the nature of dormancy, and 2) the effect of the factors (storage temperature and seed moisture content) on the loss of dormancy and viability of the seeds of *L. glutinosa* to improve germination.

Materials and Methods

The experiments were carried out in the laboratory of Seed Technology, Tropical Forest Research Institute (23°5'37"–23°6'10"N latitude and 79°59'49"–79°59'42"E longitude), Jabalpur in India. Fully mature black-colored seeds were collected from 5 trees of *L. glutinosa* growing in the Chhindwara (N22°24'28.2" E78°42'21.4") District of Madhya Pradesh in India. The climate of this region is with hot summers and mild winters with maximum shade temperature from 38 to 42°C, minimum 4 to 6°C, and mean annual precipitation of 1200 mm. Seeds from this mass collection were extracted from the fruits, dried, pooled, and used in experiments within 7 days of collection.

Determination of seed moisture content

Three replications of five seeds were weighed in an analytical balance with a precision of 0.001 g to get fresh weight. The dry weight was determined after drying at

103 ± 1°C for 17 ± 1 hours (ISTA, 1993). Seed moisture was calculated using the formula: Percentage of seed moisture content (fresh weight basis) = ((Fresh weight - dry weight)/ Fresh weight) 100.

Germination

A germination test was conducted by placing three replicates of 50 seeds sown in trays filled with sand and soil in 1: 2 ratio at 30°C with a 16:8 h alternating light/dark photoperiod in a seed germinator (Indosaw, India) having a temperature and light controller. Germinated seeds were counted daily. Seeds were considered germinated when the radicles were at least 1-cm in length. Final germination was recorded until no further germination occurred for at least one month. After completion of germination, the numbers of rotten, empty, and good seeds were determined by a cutting test. From this data, the percentage of germination was calculated.

To determine the nature of dormancy, two experiments were conducted:

Pre-treatment of seeds

Seeds were treated in the following ways for the enhancement of germination:

The experiment used a randomized complete block design with treatments including control and three replications. The different pre-sowing treatments used were:

Control (untreated seeds).

- *Cold water treatments*

Seeds were soaked in cold water overnight.

- *Boiling water treatment*

Seeds were poured into boiling water that was removed immediately from the heat source and allowed to cool.

- *Hot water treatments*

Seeds were soaked in hot water (80°C), allowed to cool and then left in it for 17 hours.

- *Treatments with plant growth regulators*

Seeds were soaked in the solution having the following plant growth regulators: GA₃ 250, 500, 750 ppm.

- *Combined treatment with hot water (80°C) and GA₃ 250 or 500 ppm*

Seeds were soaked in a hot solution (80°C) containing GA₃ 250 or 500 ppm.

- *Mechanical scarification*

Seeds were clipped individually with a single-edged razor blade at the distal end of the embryo.

- *Combined treatment of mechanical scarification and GA₃*

After clipping the seeds, they are soaked in a solution of GA₃ 250 or 500 ppm.

- *Sulfuric acid treatment*

Seeds were immersed in concentrated sulfuric acid (98% by mass) for 1 and 5 minutes, after which seeds were rinsed thoroughly in tap water six times and then soaked in cold water for 17 hours.

Combined treatment of sulfuric acid and GA₃ 250 or 500 ppm.

- *Removal of seed coat*

The seed coat was completely removed before sowing.

- *Combined treatment of removal of seed coat and treatment with GA₃ 500 or 750 ppm*

After removal of the seed coat, seeds were treated with GA₃ 500 or 750 ppm.

After treatment, seeds were allowed to germinate.

Water uptake by the seeds

The initial dry mass of three replicates of 25 seeds of fresh and after-ripened non-dormant viable seeds was measured. This was considered as 0 hours. Then the seeds were placed into dishes between two sheets of moistened blotting paper towels. The experiment was conducted at ambient room conditions (27–30°C) and sampling was done after fixed intervals. During each sampling, the surfaces of the seeds were blotted dry with a paper towel, weighed to the nearest 0.01 g, and returned to the moistened paper in the dish. Imbibition curves (increase in seed mass (fresh weight basis) over time) for each treatment were constructed and compared.

Effect of dry storage on germination and viability

Freshly collected seeds (moisture content $25.22 \pm 1.16\%$)

were dried over regularly regenerated silica gel in a desiccator for adjustment to four moisture contents, 13.45, 8.03, 6.38 and 3.12%. These moisture levels were taken within the range in which orthodox seed exhibits an inverse relationship between seed moisture content and viability (Hong *et al.*, 1996). Samples were then packed in thick sealed polythene bags for storage at three temperatures: 40, 30, and 15°C. Seeds were then sampled at intervals for germination and viability tests.

Determination of seed viability

Seed viability was determined by the tetrazolium test. Three replicates of 40 seeds were soaked in distilled water for 16 hours at a temperature of 25°C to activate seed enzymes as well as to soften the seeds for cutting. After soaking, the imbibed seeds were cut longitudinally. Half of the cut seed was immersed in a solution of 1% TTC (2,3,5-triphenyl tetrazolium chloride) and incubated in the dark at 27°C for 5 hours to allow staining. The seeds were then washed with distilled water and observed under a dissecting microscope. Seeds were counted as viable based on staining patterns. Fully stained embryos were recorded as viable seeds, whereas partially stained embryos or unstained whole embryos were considered non-viable. The percentage of viable seeds was recorded.

Statistical analyses

The experimental layout was a completely randomized design. Data were analyzed statistically using analysis of variance (ANOVA) using SPSS software. The LSD test was used to test for significant differences among means ($p \leq 0.05$).

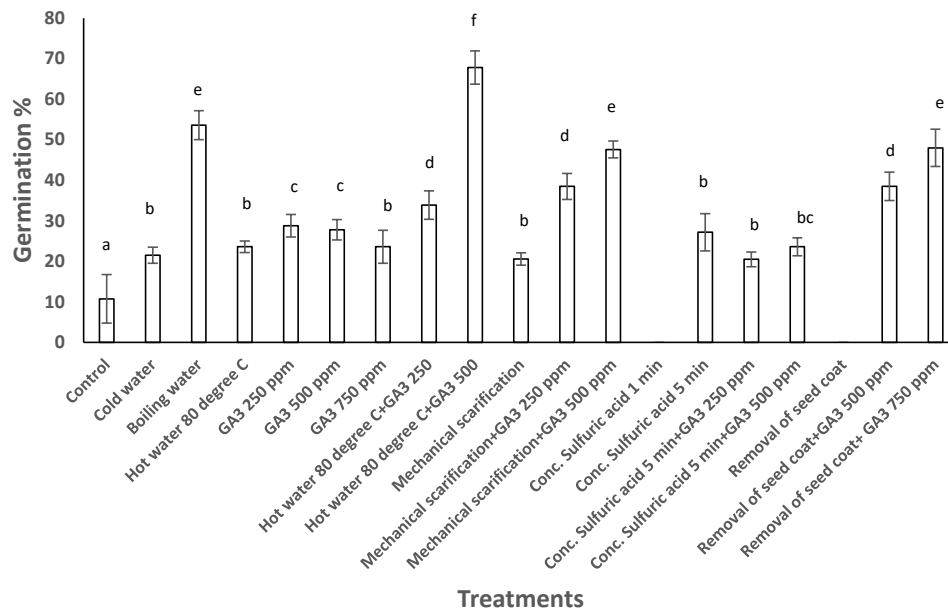


Figure 1: Mean germination percentage of untreated (control) and treated seeds of *Litsea glutinosa*. Error bars represent mean $\pm$ S.E. Different letter(s) represent significantly different means ($P \leq 0.05$).

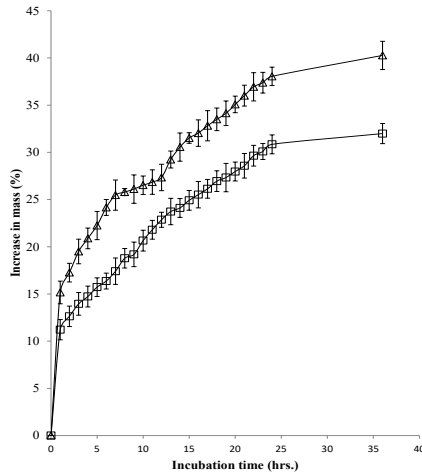


Figure 2: Percentage increase in fresh weight of freshly harvested and after-ripen seeds of *L. glutinosa* on germination blotter due to water uptake. -□- Freshly harvested seeds; -●- after-ripen seeds. Error bars represent mean $\pm$ S.E.

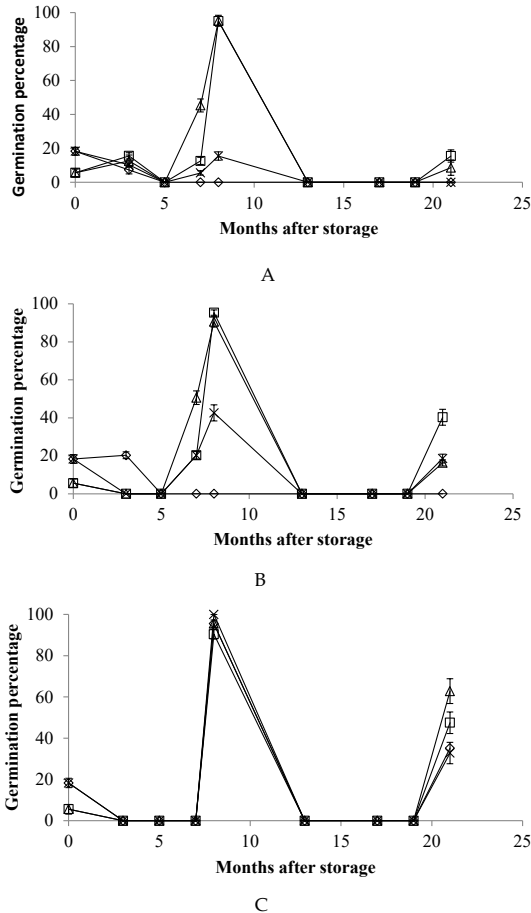


Figure 3: Germination percentage of *L. glutinosa* seeds stored with moisture content of 3.12, 6.38, 8.03 and 13.45% at 40, 30 and 15°C after different periods of storage. Seed moisture content percentage: -●- 3.12%; -□- 6.38%; -X- 8.03%; -Δ- 13.45%. A- 40°C, B- 30°C, C- 15°C. Error bars represent mean $\pm$ S.E.

Results

Pre-treatment of seeds

Among the various pre-treatments, the highest germination rate (67.8%) was observed with the combined treatment of hot water (80°C) and gibberellic acid (GA₃) at a concentration of 500 ppm. In comparison, GA₃ (500 ppm) alone resulted in 27.8% germination, while hot water (80°C) alone resulted in 23.6% germination. (Figure 1). Boiling water had a significant effect ($p \leq 0.05$) on increasing germination (53.6%) over the control (10.75%). Combined treatment of mechanical scarification and GA₃ (500 ppm) resulted in 47.6% germination and treatment of seed coat removal and GA₃ (750 ppm) gave 48%. No germination was found in seeds treated with concentrated sulfuric acid for 1-minute or complete removal of the seed coat.

Water uptake

In the water intake experiment, in the first hour, the weight of fresh seeds increased by about 15%, and after about 4 hours seed weight had increased to 21% of its initial weight (Figure 2). The imbibition curve for both fresh and after-ripen seeds were similar and in the first hour, water uptake of the fresh seeds was more than the after-ripen seeds. Maximum water uptake occurred within 24 hours of imbibition when about 38 and 31% increase in weight were observed, respectively, in fresh and after-ripened seeds.

Effect of dry storage on germination and viability

There was a significant difference in germination when seeds were sampled after different periods of storage. Differences were also observed in the germination of seeds stored at different temperatures with different moisture contents (Figure 3).

The seeds of *L. glutinosa* were harvested in the first week of November, at the onset of winter. All seeds from black-colored fruits were fully mature and viable, but the initial germination of fresh seeds was low. When the seeds, stored with about 3 to 13% moisture content at different temperatures, were sampled for germination after 3 MAS, no seeds stored at 15°C germinated. However, germination was observed in some seeds stored at 40°C with all moisture contents tested, as well as in those stored at 30°C with higher moisture content (13.45%). Dormancy was not fully broken among these seeds, but they were found to be viable. After 5 MAS, seeds stored at 30°C and 40°C with about 13% moisture content lost viability before the dormancy was fully overcome (Figure 4). Seed viability was also reduced in seeds stored with about 8% SMC at 40°C at this time of sampling, after 7 MAS, 45 to 50% of seeds germinated in seeds stored at higher temperatures with low SMC (6.3%). However, at this time, no seeds germinated in any treatments at

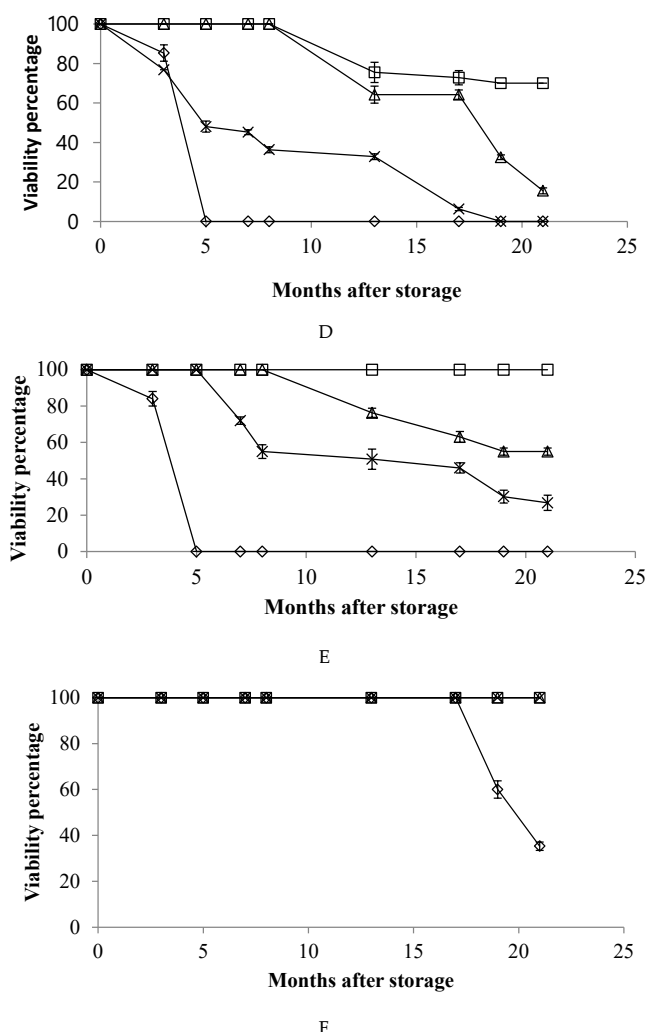


Figure 4: Viability percentage of *Litsea glutinosa* seeds stored with moisture content of 3.12, 6.38, 8.03 and 13.45% at 40, 30 and 15°C after different periods of storage. Seed moisture content percentage: -●- 3.12%; -D- 6.38%; -X- 8.03%; -à- 13.45%. D- 40°C, E- 30°C, F- 15°C. Error bars represent mean $\pm$ S.E.

15°C, and the seeds were found to be viable. The rate of after-ripening increased with the increase of moisture content among viable seeds. At 7 MAS, better germination was found in seeds stored with 6.38% than 3.12% at 30 and 40°C, although dormancy was not fully broken until this time.

It is also interesting to note that at 8 MAS, maximum germination was found in seeds with all moisture content tested at 15°C and those with lower moisture contents (6.3 and 3.12%) at 30 and 40 °C. There was no significant difference in germination among the seeds stored at 15 °C with 3 to 13% moisture content and the seeds stored at 30 and 40°C with 3 to 6% moisture content. Germination was comparatively lower in seeds with around 8% moisture content stored at 30 and 40°C at this time of sampling. The viability test also indicated that viability was reduced

in seeds stored at 30 and 40°C with about 8% moisture content, and it was completely lost in seeds with 13% moisture content at higher temperatures after 8 months of storage (Figure 4).

No germination was observed in all treatments after 13, 17, and 19 MAS. However, viable seeds began to germinate again at 21 MAS, although the germination percentage was lower than in the first year. Viability tests showed that in seeds with 8% moisture content, viability was lost or drastically reduced after 13 MAS at 30 and 40°C, respectively.

Discussion

The studies on pre-treatments on *L. glutinosa* seeds revealed that certain treatments were able to enhance germination, although they could not exceed 70%. GA₃ alone was not sufficient to improve germination above about 30%. Boiling water and a combination of hot water treatment (80°C) with GA₃ (500 ppm) increased germination capacity to above 50%. Mohammad *et al.* (2016) also demonstrated better germination (22.4%) in *L. glutinosa* seeds over the control (19.6%) when treated with a solution having GA₃ at the dose of 200 ppm with warm water. Although boiling water is used to break both physical and physiological dormancy, the positive effect of increasing germination by GA₃ with other treatments suggests that the seeds may have physiological dormancy. The experiment on water uptake showed that the seed coat of *L. glutinosa* seeds was permeable to water, and the fresh seed imbibed water without any disruption of the seed coat. A seed is considered water-permeable when 20% of its initial weight is increased by absorbing water within 48 hours. (Baskin and Baskin, 2003). Therefore, dormancy was not caused by the impermeability of the seed coat or pericarp. The effects of various pre-treatments on seed germination and water uptake indicated the presence of physiological dormancy in seeds of this species. This was further supported by the fact that dormancy loss was induced by dry storage (after-ripening) (Baskin and Baskin, 2014).

Temperature and seed moisture content are the major controlling factors to maintain seed viability in storage for all species. Again, these two factors are responsible for alleviating the dormancy of seeds of some species in dry storage (Foley, 2008; Iglesias-Fernandez *et al.*, 2011). Furthermore, the specific temperature and moisture conditions required for breaking seed dormancy vary widely from species to species (Commander *et al.*, 2009; Bazin *et al.*, 2011).

Seeds of *L. glutinosa* mature in October-November (at the onset of winter) in seasonally dry tropical forests. However, the germination of the fresh seeds was found to be low. Data showed that at a low temperature of 15°C, seeds stored with a range of moisture content (3–13%) stopped germinating when sampled after 3, 5, and 7 MAS.

Table 1: The dormancy and viability status of *L. glutinosa* seeds at different storage conditions.

Seed moisture content during Storage	Initial	After 5 months of storage			After 8 months of storage			After 17 months of storage			After 21 months of storage		
		40°C	30°C	15°C	40°C	30°C	15°C	40°C	30°C	15°C	40°C	30°C	15°C
↓	Storage temperature ↑												
13.45%	Viable dormant	Dead	Dead	Viable dormant	Dead	Dead	Viable non-dormant	Dead	Dead	Viable dormant	Dead	Dead	Low viability, non-dormant
8.03%	Viable dormant	Low viability, dormant	Viable dormant	Viable dormant	Low viability, non-dormant	Low viability, non-dormant	Viable non-dormant	Dead	Low viability, dormant	Viable dormant	Dead	Low viability, low germination	Viable, low germination
6.38%	Viable dormant	Viable dormant	Viable dormant	Viable dormant	Viable dormant	Viable non-dormant	Viable non-dormant	Low viability, dormant	Low viability, dormant	Viable dormant	Low viability, low germination	Low viability, low germination	Viable, low germination
3.12%	Viable dormant	Viable dormant	Viable dormant	Viable dormant	Viable dormant	Viable non-dormant	Viable non-dormant	Viable, dormant	Viable, dormant	Viable dormant	Viable, low germination	Viable, low germination	Viable, low germination
	Winter	Summer			Rain			Summer			Rain		

At 7 MAS, about 45 to 50% of seeds stored at 30 and 40°C germinated, if stored with 6.31% moisture content. It is also significant to note that at 7 MAS, dormancy began to be relieved as germination was found to be better in seeds with 6% moisture content compared to those with lower moisture content (3%) at 30 and 40°C. However, at these temperatures, moisture contents of 8 and 13% were too high for *L. glutinosa* seeds to remain viable. The seeds started to lose viability before breaking dormancy (Table 1).

Studies conducted on different species showed that storage at high temperatures and high seed moisture contents caused a decrease in seed viability, on the one hand, while on the other hand, it removed dormancy in some dormant seeds. In contrast, low temperatures and low moisture levels have been found to maintain viability while preventing dormancy breakage. According to Baskin and Baskin (2020), the most favorable conditions for breaking dormancy through dry storage are a seed moisture content of 5 to 10% on a fresh weight basis and a temperature range of 20 to 30°C. For example, red rice seeds maintained dormancy for one year when stored at -15°C (Cohn and Hughes, 1981). In the case of *Lolium rigidum*, after-ripening was inhibited at 9°C, and the rate of after-ripening increased as the temperature rose from 20 to 40°C (Steadman *et al.*, 2003). Foley (1994) showed that dormant wild oat (*Avena fatua*) seed converted to a non-dormant state at temperatures between 20 to 40°C with a seed moisture content of 5 to 20%. This study also found an inverse relationship between temperature and seed moisture content for after-ripening: as the temperature increases, the seed moisture content must decrease for maximum after-ripening to occur. At a particular temperature, loss of dormancy was found to be increased as SMC increased. In *Dioscorea hastifolia* at 30°C germination was achieved at 9.7% SMC in comparison to low SMC (5.3%) (Commander *et al.*, 2009).

An interesting observation was made regarding *L. glutinosa* eight months after storage. During this period, the majority of the viable seeds germinated across all treatments, regardless of the moisture content and temperature combinations tested. Notably, even the seeds stored at 15°C with a moisture content between 3 and 13% successfully germinated during this time. Previous studies showed that the time period required to break dormancy depended on the SMC and storage temperature. For tanglehead seeds, maximum germination was recorded after 9 and 12 months of storage at 30°C, when stored at 6 and 12% SMC, respectively, in October-harvested seeds (Baldos *et al.*, 2014). In *Oryza sativa*, after-ripening was faster at 20 or 30°C than at 5°C (Cohn and Hughes, 1981). But in *L. glutinosa*, dormancy was alleviated in all viable seeds of all treatments tested after 8 months of storage, which corresponds to the time gap between the seed maturation and the appearance of rain in natural conditions. It seems

the seeds are genetically programmed to germinate after a particular time period, unless the seeds lose viability due to aging before this period.

When the seeds were sampled beyond this period (13–17 MAS), no germination was observed among the viable seeds (in the corresponding winter to summer of the second year of storage). Secondary dormancy was imposed on the viable seeds stored at different temperatures. At 21 MAS (during rain of the second year of storage), seeds started germinating, though germination was less than the same time of the first year. In *Amaranthus tuberculatus* (Moq.) Sauer dormancy was completely removed due to after-ripening at room temperature with 30 to 50% relative humidity (RH) for 4 months, but germination was decreased again after 6 months of storage on account of secondary dormancy imposed on viable seeds (Wu and Owen, 2015).

This study can help to understand the dormancy loss of *L. glutinosa* seeds in natural conditions. It is well known that in nature, seed dormancy is the adaptive feature of the plant that restricts seeds from germinating in unfavorable environmental conditions. These specific unfavorable conditions also initiate the after-ripening process within the seeds, preparing them for germination in favorable conditions to facilitate seedling establishment. The seeds of this species mature in a seasonally dry tropical forest of India at the start of the dry winter period (November), which is not favorable for germination and seedling growth. Consecutive spring and early summer (March-June) are also dry seasons. These dry seasons contribute to dormancy loss so that during the arrival of monsoon rain (June-July), the plant can take advantage of the wet season to germinate and facilitate seedling growth. The study also indicates that intermittent rain at high temperatures (more than 30°C) in early summer may increase the seed moisture content and reduce seed viability and regeneration in its natural habitat.

Conclusion

The study revealed that *L. glutinosa* seeds have physiological dormancy and that temperature and seed moisture content during storage can impact both dormancy and viability. Optimal alleviation of dormancy can be achieved by storing the seeds at 30 to 40°C with 3 to 6% seed moisture content or at 15°C with 3 to 13% seed moisture content for approximately 8 months after harvest without losing viability. This information will be valuable for planning the propagation schedule of this important medicinal species and for its restoration to its natural habitat.

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Conflict of interest

The authors declare no conflict of interest.

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