Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 05, Issue: 04 (JULY-AUG, 2025)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Overcoming the Barriers to Recalcitrant Seed Conservation

*Anupam Dalapati
PhD Scholar, Department of Seed Science and Technology, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha-751003, India
*Corresponding Author's email: anupam3dalapati@gmail.com

The preservation of seeds is critical for biodiversity conservation, particularly for recalcitrant seeds, which exhibit unique storage challenges due to their sensitivity to desiccation and temperature. This paper reviews historical classifications of seed storage behavior, beginning with Ewart's lifespan-based categorization and Elliott's drying response framework, culminating in Roberts' distinction between orthodox and recalcitrant seeds. Recalcitrant seeds, which cannot be dried without losing viability, are further divided into minimally, moderately, and highly recalcitrant categories, reflecting their native environmental conditions. Factors influencing desiccation sensitivity, including maturation, embryo desiccation tolerance, and drying methods, are discussed. The paper highlights the difficulties of long-term moist storage, particularly for tropical species, and emphasizes the importance of maintaining optimal moisture, oxygen, and temperature levels. While traditional genebanking methods like freezing are ineffective for recalcitrant seeds, cryopreservation offers a promising alternative, albeit with technical challenges. The need for innovative techniques, such as in vitro germination and the use of cryoprotectants, is underscored to enhance the viability of stored seeds. Additionally, the paper addresses misconceptions regarding seed storage and the urgent need for conservation efforts, particularly in biodiversity-rich regions. The findings advocate for a multifaceted approach to plant conservation, integrating various genebanking strategies to ensure the preservation of genetic diversity in both seeds and associated plant tissues.

There are numerous seed-banking programs as a result of worries about the quick decline of plant diversity. The germplasm found in these repositories is essential for comprehending, preserving, and controlling natural variation both within and across species. The perceived issue of seed recalcitrance, however, means that many plant species and a large portion of the wet tropics are underserved in these efforts. Of the angiosperm species, 75 to 80% yield orthodox seeds that can withstand drying and long-term storage at -20°C. Five to ten percent of angiosperm species, on the other hand, yield stubborn seeds that cannot withstand desiccation and are destroyed in the freezer when ice crystals form.

To determine the best storage methods, it's important to classify how seeds vary in their storage behavior. Ewart (1908) proposed a system that grouped seeds by how long they survive under normal storage conditions:

- **Microbiotic**: Lifespan up to 3 years
- **Mesobiotic**: Lifespan between 3 and 15 years
- **Macrobiotic**: Lifespan from 15 years up to 100 or more

This classification didn't consider the impact of the storage environment. Later, Elliott (1912) categorized forest tree seeds based on how they respond to drying:

- Seeds that tolerate drying well (e.g., most conifers and birch)
- Seeds that handle partial drying (e.g., ash and American basswood)
- Seeds that barely survive any drying (e.g., oak and beech)

In 1973, Roberts introduced a different system based on physiological responses to moisture and temperature during storage. He coined the terms **"orthodox"** and **"recalcitrant"**.

Orthodox seeds can be safely dried to low moisture levels (2–5%) and their lifespan increases as both moisture content and temperature decrease in predictable ways. Recalcitrant seeds, on the other hand, are damaged if dried below 12–31% moisture content.

Recalcitrant seeds can be further divided into three overlapping categories:

- **Minimally recalcitrant**: Can be dried to relatively low moisture levels and tolerate cool temperatures (e.g., oak, Araucaria hunsteinii, Podocarpus henkelii)
- **Moderately recalcitrant**: Show intermediate sensitivity to drying (e.g., cacao and rubber tree)
- **Highly recalcitrant**: Extremely sensitive to both drying and cold (e.g., Syzygium and Avicennia marina)

These groups don't have strict boundaries but rather form a spectrum, with species' sensitivity reflecting their native environments—highly recalcitrant seeds usually come from tropical or wet habitats, moderately from tropical zones, and minimally from temperate or subtropical areas (Farrant et al., 1988).

Characteristics of Recalcitrant Seeds

Recalcitrant seeds cannot be dried without damaging their viability. As such, they do not follow the typical viability equation that links longevity to dry storage environments (Roberts, 1973). When these seeds start to dry, there is a slight initial drop in viability, which then rapidly worsens once a specific moisture threshold—called the *critical moisture content* or *lowest safe moisture content*—is reached. Further drying results in complete loss of viability. This moisture threshold varies widely between species, cultivars, and even seed lots, and is also affected by the drying method used. For instance, cocoa seeds (Theobroma cacao) can be safely dried only to 23% moisture, while Avicennia marina seeds can tolerate up to 61.5%. Despite this variation, these thresholds generally correspond to relative humidity levels between 96% and 98%.

Factors Affecting Desiccation Sensitivity

1.Maturation Drying and Desiccation Tolerance: Recalcitrant seeds do develop greater desiccation tolerance as they mature on the plant. However, unlike orthodox seeds, they don't undergo significant moisture loss. Their moisture content at maturity remains high—ranging from 36% in rubber (Hevea brasiliensis) to 90% in chayote (Sechium edule).

2.Desiccation Tolerance of Excised Embryos: Moisture content can differ within parts of the same seed. Typically, the embryo retains more moisture than the storage tissue, except in species like durian (Durio zibethinus) and jackfruit (Artocarpus heterophyllus). Removing the embryo from the seed (excising) and drying it separately can allow better desiccation tolerance, making in vitro conservation more feasible. For example, whole Hevea brasiliensis seeds at 36% moisture die if dried to 15%, but their isolated embryos (starting at 55%) can survive being dried to 14% moisture when cultured.

3.Drying Methods: Fast drying sometimes helps intact seeds survive lower moisture levels than slow drying, though not universally (e.g., no such effect is observed in Quercus rubur seeds). However, excised embryos often fare better with rapid drying. For instance, quick drying using silica gel or aseptic air improves survival more than vacuum drying. For Artocarpus heterophyllus, vacuum drying to 44% moisture was fatal, but air flow and silica gel methods allowed embryos to survive down to 26% and 16%, respectively.

Longevity in Moist Storage

Long-term preservation of intact recalcitrant seeds is challenging. They can't be dried or frozen without injury, and some tropical species are harmed even at 10–15°C due to chilling sensitivity. Typically, tropical species only last weeks to months. In contrast, temperate species (like oaks) can survive moist storage at around –3°C for years.

Recalcitrant seeds can be grouped into:

- **Tropical species**: Require warm, humid storage.
- **Temperate species**: Tolerate cooler conditions and have longer storage life.

For effective moist storage:

- Maintain seeds near their original moisture level.
- Ensure good oxygen supply.
- Prevent germination and fungal growth (via inhibitors or low temperatures).

Viability is best preserved under high relative humidity (98–99%) at species-specific optimal temperatures (roughly 7–17°C for tropical seeds, –3 to 5°C for temperate ones). Managing moisture, oxygen, and temperature is difficult; moist media like charcoal, sawdust, or sand work better than plastic bags because they keep moisture stable while allowing airflow.

Preserving Recalcitrant Seeds

Recalcitrant seeds differ from orthodox seeds in that they cannot survive traditional genebanking methods, such as freezer storage. Unlike orthodox seeds, which tolerate drying and freezing, recalcitrant seeds need moisture to stay alive and are generally short-lived in nature. These seeds either sprout quickly or are consumed by animals. A third category, "intermediate" seeds—produced by 10–15% of angiosperm species—can handle some drying but don't store well in freezers for reasons not yet fully understood.

The common belief that recalcitrant seeds can't be preserved stems from the idea that freezing is the only storage method available. In fact, long-term storage of both recalcitrant and intermediate seeds (or parts of them) is possible using cryopreservation, which stores materials at ultralow temperatures, typically in liquid nitrogen (−196°C). However, due to high costs and the need for specialized equipment and expertise, most genebanks rely only on standard freezing.

Cryopreservation halts cellular water from forming damaging ice crystals by carefully dehydrating and quickly cooling tissues. But recalcitrant seeds are often too large to cool effectively. A key innovation in their preservation has been dissecting out and storing the embryonic axis—the part of the seed that grows—using in vitro germination techniques. Applying cryoprotectants can improve survival by preventing freezing damage and oxidative stress.

This method has been successfully applied to temperate species, while intermediate seeds, being smaller and drier, need less preparation. However, challenges remain with tropical and subtropical recalcitrant seeds. These seeds typically have large, metabolically active embryonic axes that are ready to germinate, making cryopreservation difficult. Ongoing research aims to reduce damage during dissection, slow metabolic activity, and increase the regenerative potential of smaller tissue sections, all of which are crucial for conservation.

Examples of seed types include: avocado (recalcitrant), papaya (intermediate), and melon (orthodox). Avocado seeds cannot be dried or frozen without damage, while papaya and melon seeds can be dried and stored in cold conditions. Beyond technical hurdles, misconceptions about seed storage and delays in conservation work—especially in biodiversity-rich regions—stem from the belief that most tropical plants have recalcitrant seeds. While up to 47% of rainforest plants may produce them, studies like those in Hawaii show that only about 3% of endemic species actually do. Despite this, urgent efforts are now underway to bank seeds of endangered flora.

Conserving recalcitrant-seeded species presents further challenges. Wild populations are often scattered and variable in reproduction, making it hard to collect high-quality, genetically diverse seed samples. This means repeated fieldwork is needed. Cryopreservation allows storage of genetic material while these efforts are underway.

Trees, which often produce recalcitrant seeds, are especially difficult to preserve due to their size, long maturation, and the space they require for regeneration. Preventing crossbreeding with related species may also require controlled pollination. Cryopreservation extends seed longevity and minimizes the need for frequent regeneration, helping maintain

genetic integrity. Along with seeds, storing pollen and tissues helps safeguard genetic diversity.

For successful plant conservation, it's vital to use a variety of genebanking tools—not just freezing. Several publications have compiled extensive lists of plant species whose embryos or embryonic axes have been successfully cryopreserved (e.g., Kartha and Engelmann, 1994; Engelmann et al., 1995; Engelmann, 1997a, b; Reed, 2008; Pence, 2008). However, a closer look at these species reveals that only a small fraction truly belongs to the category of recalcitrant seeds. This is largely because research in this area is relatively new and pursued by only a limited number of research teams globally. It's also important to note that seed recalcitrance is not a fixed trait—it evolves as our understanding of plant biology advances and storage technologies improve. Consequently, some species once considered recalcitrant are now classified as intermediate or even sub-orthodox and can be preserved using conventional or modified storage methods (Engelmann, 2000). Compared to progress made with vegetatively propagated plants, cryopreservation research for recalcitrant seeds is still in its early stages. The main technique involves desiccating embryos or embryonic axes before freezing (Normah and Makeen, 2008). However, outcomes are inconsistent—survival rates vary greatly, regeneration is often limited to callus formation or incomplete plant development, and very few accessions per species have been studied. Multiple factors contribute to the slow progress in this area (Engelmann, 2000). For one, the sheer number of recalcitrant or potentially recalcitrant species—many of them wild—is overwhelming. In many cases, little is known about their seed biology or storage behavior. Even when some information is available, there's often a lack of reliable tissue-culture protocols for processes such as in vitro inoculation, embryo germination, plantlet development, propagation, and acclimatization—all essential for regenerating frozen embryos or axes. Furthermore, significant variability in seed moisture content and maturity between provenances, seed lots, and harvests adds complexity to cryopreservation efforts.

Many seeds are too large to be cryopreserved whole, so embryos or embryonic axes are used instead. However, these embryos are often composed of diverse tissues that react differently to desiccation and freezing—the root pole is typically more tolerant than the shoot pole. In some species, such as cacao, even slight moisture loss can cause irreversible structural damage, making desiccation and subsequent freezing nearly impossible (Chandel et al., 1995). Additionally, some species don't have well-defined embryos, further complicating preservation.

To enhance storage of non-orthodox seeds, several approaches can be explored. In certain species, carefully controlled desiccation (e.g., using saturated salt solutions) and cooling have made it possible to freeze whole seeds, as shown in various coffee species (Dussert et al., 1997; Dussert and Engelmann, 2006). Technical improvements in existing cryopreservation methods for embryos and axes also hold promise. For instance, pre-treating tissues on media with cryoprotective agents can boost their desiccation tolerance and reduce variability. Flash drying followed by ultra-rapid freezing has yielded good results for some species (Berjak et al., 1989; Wesley-Smith et al., 1992). Other methods like pre-growth desiccation, encapsulation-dehydration, and vitrification—though not widely tested on recalcitrant seeds—should be explored further (Engelmann, 2000; Pence, 2008). Success heavily depends on selecting embryos at the right developmental stage (Engelmann et al., 1995). However, this requires the establishment of foundational tissue-culture protocols, including disinfection, in vitro culture, embryo germination, plantlet development, and possibly limited propagation.

When freezing of embryos or embryonic axes proves unsuccessful, researchers suggest using alternative explants such as embryo shoot apices (Varghese et al., 2009), adventitious buds, or somatic embryos derived from embryonic tissue (Pence, 2008). These alternatives may be especially useful for species lacking distinct embryos but would require more advanced tissue-culture techniques.

Lastly, analytical tools can play a key role in refining cryopreservation protocols by offering deeper insight into the biological and physical processes involved. For example, differential scanning calorimetry (DSC), which measures thermal changes during cooling and warming, has been successfully used to develop effective protocols for cryopreserving zygotic embryos of *Parkia speciosa* (Nadarajan et al., 2008) and several Australian *Citrus* species (Hamilton et al., 2009).

Difficulties in storage

Recalcitrant seeds have a limited lifespan, making both short- and long-term storage difficult. Since perennial plants require at least three years to flower and produce seeds, the process of seed production and multiplication is slow and time-consuming, leading to irregular and unpredictable availability. One key internal physical trait that changes as desiccation tolerance develops in these seeds is the lack of insoluble protein deposits within vacuoles—these proteins typically help provide mechanical support and prevent cell collapse. In addition, stored starch and fats contribute to buffering the cell's volume.

The development of desiccation tolerance is strongly influenced by LEA (late embryogenesis abundant) proteins. These proteins are categorized into different types based on specific peptide sequences. LEAs are small, hydrophilic, heat-resistant, and typically unstructured in aqueous solutions. However, their exact role in protecting orthodox (desiccation-tolerant) seeds during drying remains unclear.

Several physiological changes—such as organelle dedifferentiation, metabolic shutdown, and a decrease in endomembrane system components—act together to suppress uncontrolled metabolism and protect membranes from damage caused by reactive oxygen species (ROS), which are commonly produced during dehydration. As water content and potential drop to intermediate levels, ROS damage becomes a particular concern. The activity of antioxidants, adapted to the varying hydration states within cells during drying, is critical to managing these stresses. All these factors collectively contribute to the significant challenges in preserving and storing recalcitrant seeds.

Most stubborn seeds tend to be large, well-hydrated, and actively metabolizing. Their size and other properties make it difficult for them to dry quickly, which is essential to retain their viability at low moisture levels before they are exposed to extremely cold, cryogenic conditions. Because these large, resilient seeds can't be cooled rapidly enough to reach liquid nitrogen temperatures (Bonner, 1990), cryopreserving them is particularly difficult. Therefore, embryos are often used for cryopreservation instead, as they represent the seed's genetic material.

The first instance of oxidative stress in frozen samples typically occurs during the extraction of embryos or embryonic axes from the seeds. Additional damage can result from dehydration and the formation of ice crystals. Since these seeds are already large and moist, further drying is required to reach suitable water content levels before freezing, which helps minimize ice crystal formation in tissues. Flash drying is a common method used to prepare embryos or embryonic axes for cryopreservation (Ballesteros et al., 2014).

However, recalcitrant (sensitive) embryos or axes cannot tolerate the removal of structure-bound water and continue to show metabolic activity, both of which hinder successful preservation. Chemical cryoprotectants like dimethyl sulfoxide (DMSO) offer another approach to prevent freezing damage in such cases (Naidoo et al., 2011). In vitrification methods, cell dehydration is achieved by treating samples with a concentrated cryoprotectant solution and/or air-drying before freezing. This is followed by rapid cooling, which helps eliminate conditions that lead to the formation of ice inside the cells.

References

1. E. Pennisi , *Science* **329**, 1274 (2010).
2. C. Walters, G. M. Volk, C. M. Richards , *Biodiversity* **9**, 68 (2008).
3. P. Berjak, N. W. Pammenter , *Ann. Bot. (Lond.)* **101**, 213 (2008).

4. J. B. Dickie, H. W. Pritchard , in *Desiccation and Plant Survival*, M. Black, H. W. Pritchard Eds. (CABI, Wallingford, UK, 2002), pp. 239–260.
5. J. C. Tweddle, J. B. Dickie, C. C. Baskin, J. M. Baskin , *J. Ecol.* **91**, 294 (2003).
6. T. D. Hong, R. H. Ellis , "A protocol to determine seed storage behaviour." *IPGRI Technical Bulletin No. 1* (International Board for Plant Genetic Resources, Rome, Italy, 1996).
7. C. Walters *et al* ., in *Plant Cryopreservation: A Practical Guide*, B. M. Reed , Ed. (Springer, New York,2008), pp. 465–484.
8. F. Engelmann , *Methods Mol. Biol.* **710**, 155 (2011).
9. E. E. Benson , *Crit. Rev. Plant Sci.* **27**, 141 (2008).
10. M. N. Normah, H. F. Chin, Y. L. Hor , *Arg. Pertanika* **9**, 299 (1986).
11. P. Berjak, B. Sershen, B. Varghese, N. W. Pammenter , *Seed Sci. Res.* **21**, 187 (2011).
12. K. N. Hamilton, C. A. Offord, P. Cuneo, M. A. Deseo , *Plant Species Biol.* **28**, 51 (2013).
13. M. I. Daws, N. Garwood, H. W. Pritchard , *Ann. Bot. (Lond.)* **97**, 667 (2006).
14. L. Weisenberger, L. M. Hill, A. Yoshinaga, K. Wood, C. Walters , *Pol. J. Nat. Sci.* **5** (suppl.), 281 (2008).
15. A. T. Kramer, K. Havens , *Trends Plant Sci.* **14**, 599 (2009).
16. C. M. Richards *et al* ., *Tree Genet. Genomes* **5**, 339 (2009).
17. B. Reed, V. Sarasan, M. Kane, E. Bunn, V. Pence , *In Vitro Cell. Dev. Biol. Plant* **47**, 1 (2011).
18. L. E. Towill , in *Ex Situ Plant Conservation: Supporting Species Survival in the Wild*, E. O. Guerrant, K.Havens, M. Maunder , Eds. (Island Press, Washington, DC, 2004), pp. 180–188.
19. https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/recalcitrant-seed
20. Jha *et al*. Storage problem of recalcitrant seeds and their conservation; KRISHI SCIENCE – eMagazine for Agricultural Sciences **Volume:03** Issue:06 (2022)