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REVIEW

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Recalcitrant Seeds: A Review of Research on the Key Factors Affecting and some Important Management Strategies for Extending Longevity during Storage

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Recalcitrant seeds are susceptible to low temperatures and desiccation, which constitutes a significant problem for seed storage compared to orthodox seeds. In addition to carefully examining every aspect affecting the viability and longevity of recalcitrant seeds during storage, this review aims to explore management strategies to mitigate these challenges. The sensitivity of seeds to temperature changes, their moisture content, water activity or equilibrium relative humidity, pests, pathogens, drying rate, and oxidative damage are all significant factors that affect how quickly seeds deteriorate. The effectiveness of various management techniques, such as exercising precaution at the seed collection, choosing the ideal temperature and moisture-holding medium, promoting germination and seedling establishment, and cryopreservation, is assessed to increase the lifespan of seeds. The results highlight the necessity of an all-encompassing, diversified strategy to successfully handle recalcitrant seed storage and safeguard the preservation of plant species for upcoming generations.

Key words: Recalcitrant seeds, seed storage, desiccation sensitivity, cryopreservation, viability

Potential for storing seeds is strongly influenced by the physiological state of the seeds, the storage environment, and genetic variables. According to Shen-Miller *et al.* (2002) and Sallon *et al.* (2008), some plant species contain seeds that can survive for an exceptionally long time. For example, lotus (*Nelumbo nucifera*) seeds have been viable for around 1300 years. *Phoenix dactylifera* seeds have also been seen to germinate after 2000 years. It's possible that variations in the biochemical makeup of the seeds, which might be linked to their genetic makeup, are the cause of the variation in seed shelf life. Some plants have extremely short shelf lives for their seeds. For example, seeds from *Shorea robusta* and other Dipterocarpaceae trees rarely survive for 7–10 days (Saha *et al.*, 1992). Plant species differ in their seed storage behaviours, which has resulted in the division of seeds into two main categories: orthodox and recalcitrant seeds (Bewley *et al.*, 2013). Desiccation-sensitivity is a characteristic of the recalcitrant seeds. These seeds are shed from the trees with a relatively high moisture content (0.4–4.0 g water/g) and frequently high metabolic activity because they do not undergo drying during maturation, or just drying to a limited amount.

Deterioration processes start to take place during storage, which results in the loss of seed viability. According to McDonald (1999), the process of seed aging, also known as seed deterioration, is often characterized as irreversible, cumulative, and unstoppable. It can lead to the accumulation of cellular damage, which can postpone the emergence of seedlings, lessen their capacity to tolerate stress, and ultimately cause them to lose viability (Zhang *et al.*, 2021). Most of the time, limited damage can be fixed, but it will require time and energy, which will slow down the average germination rate and cause the radicle to protrude. Significant deterioration of the seeds may still permit this embryonic root protrusion, but it may also cause abnormalities in the seedlings. In the event of excessive deterioration, there is no radicle protrusion and no seedling formation. There are clear differences in how conventional and recalcitrant seeds deteriorate. Different species, genotypes, and populations exhibit wide variances in longevity (Clerkx *et al.*, 2004; Walters *et al.*, 2005; Probert *et al.*, 2009), which are largely ascribed to variations in their biochemical or biophysical properties (Walters *et al.*, 2010). All orthodox seeds exhibit prolonged viability under cool, dry storage, regardless of the seeds' natural capacity for storage; at higher temperatures and relative humidity, however, the storability of seeds is significantly decreased (Ellis and Hong., 2006).

Seeds exhibit behaviour that is unusual for living things: they are able to endure even in the absence of much water in their tissues. This is the same behavior as the so-called orthodox seeds, which may be stored for extended periods of time due to their ability to support desiccation up to 5% of their seed content (Barbedo., 2021). Because of their behavior, which is regarded as the norm for seeds, they are referred to as orthodox. Nonetheless, it has been noted that a certain class of seeds is desiccation resistant; they become sterile when dried out and cannot be kept at low temperatures for extended periods of time (Barbedo., 2018). Recalcitrant seeds are characterized by a "stubborn" behavior that deviates from the typical seed behavior (Barbedo., 2021). *Theobroma cacao*, *Araucaria angustifólia*, *Carapa guianensis*, *Persea americana*, *Mangifera indica*, and *Citrus spp*. Fonseca are among the seeds in this category (Freire, 2022). According to Subbiah *et al.* (2019), one explanation for the emergence of recalcitrant seeds is that they have evolved a metabolic "shortcut" that prevents desiccation because they live in a more humid environment.

When storing seeds, recalcitrant seeds are more challenging than other types of seed. This is because high moisture content storage is required because of the extreme susceptibility to water loss. The attack of microbes and germination during storage are encouraged by this interior dampness. Because recalcitrant seeds are harmed by temperatures near or below zero, the application of low temperatures is therefore restricted (King and Roberts., 2019).

KEY FACTORS INFLUENCING RECALCITRANT SEEDS

Three critical factors influence the decline in seed

quality and viability (i.e., vigor and germinability): seed moisture content in equilibrium with the atmosphere's relative humidity, storage temperature, and gaseous environment (Priestley, 1986; De *et al.*, 2020; Solberg *et al.*, 2020; Hay *et al.*, 2022; Nadarajan *et al.*, 2023; Walters *et al.*, 2010; 2005; Fu *et al.*, 2015). A study found that seed longevity increased when the temperature was lowered and the seed moisture content was decreased for most species classified as orthodox, or seeds that can withstand dehydration (Roberts, 1973). (Priestley, 1986; Murdoch and Ellis., 2000; Walters, 2005; Nadarajan *et al.*, 2023; Hong and Ellis., 1996; Pritchard and Dickie., 2003). However, the average seed lifespan for species categorized as recalcitrant—that is, seeds that cannot withstand dehydration—is only a few weeks to a few months (Roberts, 1973; Hong and Ellis, 1996; Wyse and Dickie, 2017). These seeds need to be kept in storage at high moisture content (20–70% fresh weight basis) and temperatures between 7 and 17 °C and −3 and 5 °C, respectively, for species that are native to tropical and temperate climates, respectively (Hong *et al.*, 1996). Few types of seeds survive for more than 100 years due to a variety of factors that affect their longevity (Ken *et al.* 1997). The lifespan of seeds in normal soils might be anywhere from a few hundred years to almost nonexistent. Carbon dating revealed some of the oldest still-viable seeds to be Lotus (Nelumbo nucifera) seeds discovered buried in pond soil, estimated to be 1,040 years old (Thomas *et al.* 2002). The seeds' life-span increases predictably with decreasing temperature and moisture content (Ellis *et al.* 1991). The seeds' lifespan under typical storage conditions is mostly governed by the seeds' moisture content and storage temperature. But there are also significant inherent variations in the lifespan of seeds amongst species. An important first step in ensuring the long-term conservation of plant genetic resources is ex-situ seed storage. It is crucial to sustain seed viability for an extended duration to safeguard the genetic integrity of samples that have been kept. In both domesticated and wild sources, simple procedures have been used to maintain the viability of the seeds (Ellis *et al.* 1991, Vertucci and Roos 1991).

The changes in seed physiology and shelf life of recalcitrant seeds under different storage environment circumstances need to be carefully considered, since they are the basis for increasing the shelf life of stored seeds. Numerous studies have shown that the conditions under which seeds are stored — temperature, moisture content, water activity, equilibrium relative humidity, and rate of drying — can significantly affect how long the seeds live. When there is moisture loss and/or low temperature, seeds that are recalcitrant or susceptible to desiccation have a lower chance of surviving (Roberts, 1973). According to Justice and Bass (1979), the time of storage, moisture content, and temperature all have an impact on how long seeds live. However, the extent to which each of the factors acts as the basal or critical cause for seed deterioration is debatable. The key factors influencing the preservation of resistant seeds will be enumerated in this review.

Initial viability

In comparison to seed lots with poor initial viability, those with high initial viability and germinative capability show greater lifespan in storage. Before storing a sample of every seed batch, germination tests should be performed, if required, to assess the length of time the seed would likely remain viable in storage. This can be done by administering the proper pretreatment to overcome dormancy. In the first test, the percentage of viable seeds that germinate is connected with the longevity of those seeds. Samples of two seed batches belonging to the same species, typically yielding 80% germination of fresh seeds, could produce initial germination findings of 50% and 90%, respectively. Not only would it take up room to store dead seed while storing the second seed lot, but even the 50% of initially viable seeds would probably become less viable in storage faster than the 90% viable seeds in the first seed lot. If the seeds are going to be sowed in a few weeks or months, then the initial viability may not be seriously compromised; however, only high-quality seed should be kept in storage for extended periods of time (Holmes and Buszewicz 1958, Magini 1962). It is advised that no seeds with an initial viability of less than 85% of what is thought to be normal for the species or variety in question should be accepted for storage when

storing agricultural seeds for an extended period of time in order to preserve genetic diversity (IBPGR 1976).

Content of Moisture, Water Activity, or Balance Comparison of relative humidity and drying rate

A first basic guideline for the quantitative effects of humidity on seed aging was created by Harrington (1972), and it indicates that for every 1% decrease in seed moisture, the shelf life of the seeds doubles when the moisture content is between 5% and 14%. According to Roberts (1973), some types of seeds are quickly killed by desiccation when the moisture content falls below a crucial value of 12.31% and are deemed recalcitrant, while others are deemed orthodox if they obey Harrington's rule and persist through cold conditions. When examining the effects of moisture on seed aging, it's critical to distinguish between seed moisture content and water activity, also known as storage relative humidity (RH). In the past, information about the moisture content of seeds was used to describe them in the seed trade. Moreover, seed technologists were accustomed to determining the seeds' moisture content and humidity level based on either fresh or dry weight. However, the non-oily portion of the seeds' water availability, and consequently the deterioration processes and rates at which these reactions are occurring, are not determined by the moisture content of the seeds.

The moisture content of seeds increases with increasing relative humidity (Justice and Bass, 1979). It is also known that the moisture content of the seeds reduces as the temperature rises when they are exposed to a constant relative humidity. *Citrus limonia* seeds were kept in both open and refrigerated conditions (10^{O}C; 45% RH) for six weeks while Kadam *et al.* (1994) observed their viability and germination. When the seeds were chilled at 10^{O}C with 45% RH, there was the least decrease in both parameters during storage. He claimed that even after 30 days of storage, seeds showed very strong germination capability (above 80%) at 20^{O}C, regardless of the RH, i.e., 60%, 66%, and 86%. The moisture content dropped and had an adverse effect on the germination capacity at both 27^{0}C and 20^{0}C when the relative humidity sharply dropped to 20%. The findings demonstrated that S. robusta's shelf life is negatively impacted by quick drying. The Seed Viability Equation states that seed survival increases up to a certain point as seed moisture content decreases. In actuality, seed degeneration may occur more quickly at extremely low moisture levels, or under "ultra-dry storage conditions," which correspond to eRH levels below roughly 15–20% (Chai *et al.*, 1998). Food science has described a quicker rate of lipid oxidation, which is most likely the source of this (Labuza and Dugan 1971).

According to Tamari (1976), there is a positive correlation between seed size and the storability of resistant seeds. According to King and Roberts (1979), large seeds may even be a factor in recalcitrant behavior because they have more difficulty internally transporting water than smaller seeds do, in addition to the fact that the moisture content of the seed varies depending on its part (Berjak *et al.*, 1984; Pritchard and Prendergast, 1986). The testa and the very little embryo are present in the big seeds. Large, resistant seeds hence experience early seed death. Finch-Savage (1992) reported that the cotyledons' water content appeared to have a higher impact on Quercus occur robur axes' ability to survive than the axes individually. Recalcitrant seeds are extremely susceptible to desiccation, especially the large-seeded species common in the tropics. A seed's moisture content might range from 30% to 70% when it sheds. The degree of drying determines how viable the seed becomes. The susceptibility of various species to desiccation varies. The moisture level below which the seeds are dead is known as the lowest safe moisture content (LSMC) of these seeds, according to Tippett (1987). This LSMC may vary from 13 to 35% depending on the species. A few of the resistant species' LSMC are displayed in Table 1.

Temperature

Higher temperatures cause chemical oxidation to occur more quickly, which causes seeds to decay more quickly. Because of this, gene banks are advised to dry and preserve their priceless germplasm at extremely low temperatures (Rao *et al.* 2006). Horticultural species seeds that are more costly are usually stored in storage

with temperature and humidity controls set to 15 °C and 30% relative humidity. According to Harrington's second thumb rule, the storage life nearly doubles for every 5 °C drop in temperature (Harrington 1972). According to Ellis *et al.* (1989), the moisture content and storage temperature of the seeds have a major impact on the chemical processes that occur in the seeds while they are being stored. Lowering the storage temperature has been demonstrated to increase seed longevity for many desiccation-tolerant varieties. For instance, Genes and Nyomora (2018) found that Escoecariabussei seeds kept its viability for nine months at 15 °C, but that three months at 30 °C resulted in the loss of germination capacity.

Table 1. Lowest safe moisture level (%) or critical moisture content (%) of certain species of recalcitrant seeds

Common Name of recalcitrant seeds	Binomial name	Critical moisture content or lowest safe moisture content (%)	Reference
Brazilian pine, candelabra tree, or Paraná pine	*Araucaria angustifolia (Bertol.) Kuntze*	30.0	Salmen *et al.* (1994)
Jackfruit	*Artocarpus heterophyllus Lam.*	26.0	Fu *et al.* (1993)
Lintah Bukit, Damar Mata Kuching	*Hopea helferi* (Dyer) Brandis	17.0	Tamari (1975)
Rubber tree	*Hevea brasiliensis* Müll.Arg.	15.2	Chin *et al.* (1981)
Nutmeg	*Myristica fragrans* Houtt	30.0	Gunasekaran (1997)
Malabar plum, Java plum, Black plum, Jamun, Jaman	*Syzygium cumini* (L.) Skeels.	20.0	Srimathi (1997)
Baboon wood, Ucuuba, Ucuhuba and Chalviande	*Virola surinamensis* (Rol. *ex* Rottb.) Warb.	11.0	Cunha *et al.* (1995)
Cacao tree or Cocoa tree	*Theobroma cacao* L	26.0	Hor *et al.* (1984)
Litchi	*Litchi chinensis*	27.0	Xia *et al.* (1992)
Clove	*Eugenia caryophyllata* Thunb	35.0	Gunasekaran and Krishnasamy (1998)
	Shorea roxburghii G. Don	17.0	Corbineau and Come (1988)

Table 2. The different techniques adopted for the short-term storage of recalcitrant seed

Name of (recalcitrant seed) crops	Binomial name	Storage condition	Longevity during storage (days)	Percentage of germination after storage duration	References
Mango	*Mangifera indica* L.	Sand +Sawdust or topsoil, or compost or sand +compost	29	60-100	Snxman (1981)
		Bags made of polythene at room temperature (25^{o}C)	90	40	Patin *et al.* (1986)
Litchi	*Litchi chinensis* Sonn.	15 ^{O}C	210	100	Corbineau and Come (1988)
		80%N_2O+20%O_2	84	92	Sowa *et al.* (1991)
Jackfruit	*Artocarpus heterophyllus* Lam.	Seeds are incubated in sand that contains 10% Jalsakthi or a 0.3 m KH2PO4 solution in a 1:3 or 1:4 ratio, and they are then packed in loosely bound polythene bags.	63	80-90	Bhattacharya and Basu, (1994)
Jamun	*Syzygium cumini* (L.) Skeels	Water – ambient temperature (25^{O}C -28^{O}C)	30	60	Srimathi (1997)
Nutmeg	*Myristica fragrans*	Water-10 ^{O}C	90	78	Srimathi (1997)
		Sealed plastic bag at 100%RH	45	-	Madhusudahan and Bau, (1994)

Table 3. Some examples of germplasm repositories for various plant species

Name of Germplasm Bank	Name of Place	Maintained genus and accessions	References
National Bureau of Plant Genetic Resources, New Delhi (NBPGR)	India	Legume crops , 67 274	ICAR-NBPGR, New Delhi., 2020
Potato Research Institute, Shimla	India	Solanaceae, 1500	Niino and Arizaga 2015; Gopal and Sukh Chauhan 2010
National Centre for Seeds and Seedlings	Japan	Solanaceae ,130	Panis *et al.*, 2001
National Institute of Aerobiological Sciences	Japan	Mulberry, approx. 1000); Juncus, 50	Panis *et al.*, 2001
The International Network for the Improvement of Banana and Plantain	France	Musa , 882	Panis *et al.*, 2001
Institute of Research for Development	France	Coffea, approx. 500	Niino Arizaga 2015
Centre for Research and Higher Learning in Tropical Agriculture	Costa Rica	Coffea, 80	Dulloo *et al.*, 2009

Oxygen

The free radical theory of ageing, founded with mammalian systems as the model by Deham Harman in 1956, explains the role played by oxygen in aging, disease, mortality, and degradation. According to the hypothesis, ageing is caused by a single, underlying process that is influenced by both genetic and environmental variables. This process involves the formation of free radicals during aerobic metabolism, which can cause random and harmful damage. Specifically, it is thought that oxygen, in all of its forms, is the primary molecular generator of harmful radical reactions. Unlike higher vertebrates, plants can manufacture a whole range of chemical defenses against oxidative stress. The most abundant source of antioxidants in plants is the chloroplast, which contains provitamin A, vitamin C, and tocopherols (Vitamin E). In contrast to most organisms, which require oxygen to thrive and for metabolic activity, seeds grown in dry conditions with glassy cytoplasm do not require oxygen (O_2) to survive.

Rate of drying:

The first study by Farrant *et al.* (1985) examined the impact of drying rate on the desiccation sensitivity of refractory A. marina seeds. After then, a number of research (Bhat, M. R., 1981; Pammenter *et al.*, 1991; Pritchard *et al.*, 1991; Normah *et al.*, 1986; Berjak *et al.*, 1990; Pammente *et al.*, 1998) have been conducted on the impact of drying procedures on desiccation tolerance and cryopreservation of recalcitrant seeds or cut axis. In comparison to slow drying, rapid dehydration has been shown to improve seed survival at lower water content (Farrant *et al.*, 1985; Berjak *et al.*, 1984; Pritchard *et al.*, 1991). According to Pammenter *et al.* (1998), slow dehydration may force the seeds to remain at "intermediate" water content for longer periods of time. At this point, aqueous-based metabolism-linked damage builds up and overwhelms, leading to the loss of seed viability even at relatively high water contents, such as 1.0 g g^{-1} for *Ekebergia capensis* axes. Liang and Sun (2001) report that when resistant seeds are dried for an extended period of time, a number of harmful events might occur, such as impaired antioxidant systems or disruptions in metabolic regulation. Additionally, it was postulated that the fast-dried seed axis' resistance to desiccation may be enhanced by an unequal distribution of water in the seed tissues (Tompset *et al.*, 1998; Pammente *et al.*, 1998). *Mangifera persiciformis* seed viability decreased more quickly with slow drying than with rapid drying, according to research by Tang *et al.* (2008). 94% of the seeds germinated in the rapid drying treatments after the seed moisture was lowered to 24.7% in under 24 hours. On the other hand, seed viability dropped steadily to 53.2% when dried to 18.2% moisture content, and it then dropped dramatically with more desiccation. After being dried for 96 hours, the non-viable seeds had 13.7% moisture content. In

contrast, viability was lost more quickly with slow drying. Furthermore, slow-dried seeds had a larger moisture content (approximately 24% versus 20%) than fast-dried seeds, which resulted in a 50% viability loss.

Pests and Pathogens

Apart from the direct impact of temperature, RH, and oxygen on seed deterioration, these factors are also crucial for the survival and growth of pests and pathogens, which in turn affects the reduction of seed viability. When seeds are stored, fungi can seriously damage them. Their survival rate is significantly decreased at temperatures below zero, but they can still develop inside and around the seeds at roughly 70% RH, growing faster at higher humidity levels (Roberts 1972). Fungi like Aspergillus species can still develop at 0.5% oxygen, albeit at a slower rate, despite the fact that storage at lower oxygen levels can significantly impede fungal growth (Hall and Denning, 1994).

STORING RECALCITRANT SEEDS: A CHALLENGE

When recalcitrant seeds of most tropical tree species are dispersing, their moisture content varies greatly, ranging from 23-25% to 46-53%. Many recalcitrant seeds of tropical species are not only susceptible to desiccation but also to cold, and they cannot be stored at temperatures lower than 15 °C. This places significant restrictions and difficulties on the long-term preservation of these seeds since they may be harmed by the methods commonly used to store orthodox seeds, which usually entail lowering their water content and keeping them in a refrigerated environment. irreversible, which causes viability to be lost. Conversely, retaining high water contents while storing recalcitrant seeds may encourage the growth of microbes that damage the seeds or cause them to germinate (Vieira *et al.*, 2018).

In this regard, any process designed to store recalcitrant seeds needs to minimize water loss, keep the seeds supplied with enough oxygen, and inhibit the growth of microbes and germination while the seeds are being stored. In situ conservation techniques for these species should also be taken into consideration, given the challenge of long-term storage of recalcitrant seeds, in order to ensure the preservation and protection of the genetic impact (Cruz, 2016).

MANAGEMENT STRATEGIES:

The following management practices are most important for the extension of storability of recalcitrant species.

Precaution during seed collection

The ideal period of seed collection determines the quality of the seed that is obtained. Since the amount of seed maturity is at its peak at that point, physiological seed maturity is the best indicator of when to harvest. The fruit's ability to change color indicates its ripeness, and this is frequently used to determine when to pick seeds. Ng (1983) states that the following precautions should be taken: (i) gather fruits in containers with enough ventilation and store them in an airtight environment. (ii) If seed fruit is present in huge quantities, it should not be packed closely to prevent suffocation and physiological disintegration, growth of fungi, and excessive heat. (iii) The collection containers' tops ought to be left ajar, and if using polythene bags, holes ought to be formed around the outside. (iv) Seeds should always be shared out of direct sunlight. (v) Covering containers with newsprint or cloth will lessen the desiccating effect of air movement and prevent fruit from becoming dry while in transportation. (vi) Prolonged collecting excursions have to be avoided. (vii) In the nursery, the seeds need to be planted as soon as possible.

Condition of storage room

Because the recalcitrant seeds have a limited capacity to store, under different storage room circumstances, their viability rapidly declines. Recalcitrant seeds need cold, damp storage conditions together with air exchange, or aeration, to prevent overheating from transpiring from seed moisture and respiration. Recalcitrant seed needs to be stored at a high moisture content, between 30% and 50%, or between 60% and 70% for certain species of genuine recalcitrant seed (Schmidt, 2000). Sadjad S. (1980) states that seeds that are difficult to germinate can be stored at room temperature (27–30°C, 70%–80% relative humidity) or in an air-conditioned room (temperature 18–20°C, 50%–60%).

Optimal temperature and moisture-holding media

According to Yuniarti and Zanzibar (2017), the seed should be combined with various kinds of moist materials to preserve its high moisture content, including sawdust, coconut fiber or cocopeat, perlites, and other materials. Some species are not tolerant of temperatures below 5^0C, although temperate species — like *Quercus spp*. — are more resilient to temperatures as low as 2^0C. *Nephelium lappaceum* (Chin, 1975), *Theobroma cacao*, *Dryopalanops aromatica* (Jensen, 1971), and *Shorea ovalis* (Sasaki, 1976). Nevertheless, depending on the species, the ideal temperature range that these organisms could withstand to maintain viability was 7–15^0C. Water-retaining media was found to improve seed storability at this low temperature when used for both ambient and low-temperature storage. According to Thompson (1950), Are (1964), Evanss (1953), Gunasekaran (1997), and others, common wet holding media include damp peat, sawdust, charcoal, vermiculite, sand, and coipith. For jamun seeds, water is also an excellent storing medium (Srimathi, 1997). According to Pyke *et al.* (1934) and Friend (1964), the dipping of cocoa pods in paraffin wax improves the seeds' ability to be stored longer than untreated. Cocoa can also be better preserved by putting it in a single polythene bag. The techniques used by different authors for extending the life span of recalcitrant seeds using different moisture holding media and temperature in short term storage are given in Table 2.

Using of suitable Container

The container used for storage must be porousor can allows air and water exchange. This means the container must be able to maintain humidity, such as cotton cloth, paper bags and gunny sacks. The kind of packaging that is used for storage can be extend the life of seeds by shielding them from harsh environments, preventing animals and insects from attacking them, making handling easier, and making the best use of available space (Freitas, 2019). Any procedure that is created will need to stop water loss, keep the seeds receiving enough oxygen, stop microbes from growing, and stop germination while the seeds are being stored. Partially dehydrating the seeds before storing them and encasing them in polyethylene packaging, which prevents water vapor from escaping the seeds and entering the surrounding air, has been suggested as an option (Ferreira; Gentil, 2023). The seeds may retain their high water content in these packages without obstructing the gaseous exchanges with the atmosphere, which are vigorous because of their high respiratory activity (Bonner, 2018).

Control of pests and diseases:

It is necessary to control insects, fungi, and other diseases because the seeds are kept at a level of moisture and temperature that promotes the growth and development of fungi. By administering CO_2 fumigation or just a short submersion in either warm or cold water, the infection cannot develop. Fungicides can be applied dry or moist, such as by soaking seeds in a solution. Treatments with hydration and dehydration can prolong the shelf life of intermediate seed storage. During the hydration stage, this treatment triggers the self-healing process (Schmidt, 2000).

Storage in the form of germination and seedling establishment for planting

According to Chacko (2009), 92 percent of the depulped seeds of A. hirsutus germinate cumulatively over the course of 60 days, beginning germination 12 DAS. For the species, 71–80% of seeds germination was recorded in earlier research (Gopikumar & Mahato, 1993; Kader *et al.*, 1999). For better germination, it is therefore advised to reduce the moisture content of A. hirsutus seeds by up to 40%. The germination test's objective is to gather data on seed viability that is responsible for the subsequent growth of seed lots in the field. Greenhouses and germinators can be used in the lab to perform germination studies. Paper tests are used for laboratory testing, whereas a mixture of sterile soil and sand is used in a greenhouse for testing (1: 1). According to Nurhasybi *et al.* (2007), the ideal way to test for germination of seeds in a greenhouse is to sow the exposed seed (wings above) with two thirds of the seeds submerged in soil medium. The findings of the study (Nurhasybi *et al.*, 2003) demonstrate that germination testing of various meranti species (*Shorea spp*.) was conducted in greenhouses with fluctuating

temperatures between 30° and 34°C, relative humidity between 47% and 78%, and light intensity between 5180 and 19400 lux. According to Tippett (1998), 26°–31ºC was the best temperature range for *Shorea roxburghii*, *Shorea robusta*, and *Shorea almon* to germinate. Sand + soil media (1:1 v/v) or rice husk charcoal + sand (1:1 v/v) can be used for germination in greenhouses, along with a germination bed or box wrapped in clear plastic (Widyani *et al.*, 2012). One of the forest species with recalcitrant seed characteristics, the agarwood species (*Aquilaria malaccensis*), had the best germination rate of 92% in a greenhouse covered with two layers of shade net (Tabin and Shristava, 2014).

Seedlings have to be kept growing slowly in order to predict the decline in its viability. A solution for obtaining seedlings from recalcitrant seed entails storage in the form of seedlings. Transporting seedlings is made easier by their continued growth. Many recalcitrant species, including meranti, bakau, cempaka, gaharu, agathis, kayu bawang, and mimba, have been stored as seedlings. (Kusmana *et al.*, 2011; Syamsuwida and Aminah, 2011; Syamsuwida *et al.* 2008; Syamsuwida and Amina;, 2010; Irawan *et al.*, 2018; Satjapradja *et al.* 2006). Several retardance agents, such as paclobutrazol or saline solution, are used in treatment to control the rate of growth of seedlings. These agents can slow down seedling growth, particularly in cases of excessive growth. Controlling the size of the seedlings until planting time is the goal of this treatment, and when the seedlings are prepared for planting, the substance augmentation must cease. Controlling the environment, such as the percentage of shading level or the seedling medium, is another treatment that might be used to impede the growth of seedlings. Numerous investigations revealed that there was no discernible variation in the growth of treated and untreated seedlings at the planting site. This implies that after being planted, the plants can develop to their usual size because the inhibitor's remnant is not kept indefinitely in the seedlings.

Cryopreservation

The procedure of conserving plant material in liquid nitrogen at -196 °C or in the vapor phase at -150 °C is known as cryopreservation. This method allows for the long-term preservation of the material by effectively paralyzing and latently storing all metabolic processes (Molina *et al.*, 2016). Pre-cooling, cooling, and reheating are the processes in this approach, and their effectiveness depends on how well they work together (Pieruzzi, 2013). The most important factor in determining whether the material to be cryopreserved will survive is its water content, and reducing humidity levels is crucial to prevent ice from forming when the item is frozen (Stegani *et al.*, 2017). This reduction must happen fast to lessen the stress that the chilling process causes and prevent damage to resistant seeds, which is usually disastrous (Pieruzzi, 2013).throughout the freezing process (Stegani *et al.*, 2017). Due to their inability to be frozen quickly enough to reach liquid nitrogen temperature, large recalcitrant seeds are very difficult to cryopreserve (Bonner, 1990). As such, an embryo, which preserves the genetic identity of the seeds, is the ideal cryopreservation explant. The act of separating the embryos/axes from the seeds causes the initial oxidative stress that arises during the processing and recovery of frozen materials. Two additional significant risks linked to cryopreservation are further dehydration and ice crystallization. Before being exposed to a cryogen, explants need to be partially dried to water content in order to minimize (but preferably avoid) ice formation in the tissues. This is frequently achieved by flash drying embryos or the embryonic axis (Ballesteros *et al.*, 2014). Conversely, recalcitrant embryos/axes are metabolically active forever and are unable to withstand the loss of non-freezable (structure-associated) water; these characteristics stand in the way of a successful cryopreservation process. Dimethyl sulfoxide (DMSO), a chemical cryoprotectant, is an additional choice for shielding such seeds from freezing damage (Naidoo *et al.*, 2011). In vitrification-based procedures, the process of dehydrating samples prior to freezing involves subjecting them to a concentrated cryoprotective solution or desiccating them by air. After then, there is a quick cooling phase. Consequently, all substances that have an impact on intracel1ular ice formation are avoided. Research by Pance (1990) and Engelmann *et al.* (1995) shown that zygotic embryonic axes of recalcitrant seeds could be cryopreserved

following partial desiccation. Furthermore, it has been shown that zygotic and somatic embryos can be better prepared for desiccation and/or freezing by using a sucrose pre-treatment, also known as a cryoprotectant (Dumet *et al.*, 1993; Assv-Bah and Engelmann, 1992; Engelmann *et al.*, 1995). Normah *et al.* (1996) suggested that desiccation sensitivity is related to the formation of embryonic axis. The numerous methods that various researchers have employed to improve the storability of recalcitrant seeds by the uses of embryonic axis (Table 3).

Certain species from temperate regions have vegetative and floral buds that go through a series of natural adaptations that allow them to become dormant and then become acclimated to the cold (i.e., cold hardy) (Hänninen and Tanino, 2011; Cooke *et al.*, 2012; Wisniewski *et al.*, 2018; Chang *et al.*, 2021). Vegetative and flowering buds can withstand the bitterly cold and icy winter weather by entering this dormant and cold-acclimated state. The 1960s saw the clever application of this innate ability to withstand cold and freezing temperatures to create a specific set of cryopreservation techniques (Sakai, 1960), which serve as the foundation for the long-term genetic resource preservation of numerous clonal temperate fruit and nut trees and shrubs (Tanner *et al.*, 2021).

Germoplasm Banks

To maintain genetic resources through germplasm banks and to build regulatory stocks, seed storage is a collection of practices designed to maintain the quality of the seed (Aguiar *et al.*, 2021). But as of right now, "in vitro" technologies play a significant role in keeping recalcitrant seeds viable. Isolated embryonic axes and cultures of zygotic embryos derived from mature seeds can be cryopreserved, and they can also withstand partial desiccation and freezing. (Towill, 2020). Due to the success of in vitro conservation techniques, many in vitro gene banks have been established nationally and internationally (Benson *et al.*, 2011) with several cryopreservation germplasm repositories set up for various plant species (Table 3).

CONCLUSION

A review of storage procedures for recalcitrant seeds revealed that extending the viable duration of seeds which usually expires in a few days requires appropriate storage conditions. Further scientific research is still required to fully understand the critical storage factors, such as seed viability at first, temperature sensitivity, water activity, and equilibrium relative humidity, oxidative damage, drying rate etc. The effectiveness of various management techniques, such as exercising precaution at the seed collection, choosing the ideal temperature and moisture-holding medium, promoting germination and seedling establishment, and cryopreservation, is assessed to increase the lifespan of seeds To find effective seed storage techniques for maintaining the viability of recalcitrant seeds during storage, the results highlight the necessity of an all-encompassing, diversified strategy to successfully handle recalcitrant seed storage and safeguard the preservation of plant species for upcoming generations.

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CONFLICTS OF INTEREST

The author declare no conflict of interest.

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