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Storage behaviour of forest orthodox and recalcitrant seeds

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ABSTRACT

Ex situ seed bank conservation of forest genetic resources requires knowledge on the seed storage behaviour in terms of either orthodox or recalcitrant type. Orthodox seeds of many forest species including *Pinus* and *Tectona* possess physiological and biochemical mechanisms enabling them to endure desiccation to 2-5% moisture content, while the desiccation sensitivity of recalcitrant seeds like *Quercus* and *Shorea* restricts their moisture content at high level (30-70%). Hence, while orthodox seeds can be conserved ex situ for decades to centuries, the recalcitrant species are unable to form persistent soil seed banks, threatening their long-term conservation in the backdrop of forest degradation and climate change. Thus, special efforts including tissue culture, maintaining live gene banks, and cryopreservation of embryonic axes at ultra-low temperatures are necessary for conservation of recalcitrant species.

Keywords: Orthodox seeds, Recalcitrant seeds, Tissue culture, Cryopreservation

Introduction

Forest ecosystems are the foundation of biodiversity, climate regulation, and ecological integrity. In this regard, ex situ conservation of forest tree genetic resources in the form of seed banks, as a measure of species conservation, reforestation, and restoration, has become a compelling practice. Not all forest tree species have seeds that are amenable to seed banking. There is a need to understand the basic science of forest tree seed storage behaviour to adopt appropriate conservation strategies.

The categorization of seeds according to their tolerance of desiccation and response to storage was proposed by Roberts (1973) and modified by Ellis *et al*. (1990). Seeds are classified into different categories based on their physiological responses to drying and low temperature during storage. Knowing if a particular forest tree species has orthodox or recalcitrant seeds is the first step towards developing conservation strategies.

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Orthodox seeds

Orthodox seeds are capable of withstanding desiccation (drying) and exhibit an increase in desiccation tolerance during the late stages of development. They usually lose viability rapidly upon storage at ambient temperatures, but this process can be slowed down by lowering the temperature and moisture content. Orthodox seeds can endure desiccation down to a moisture content (MC) of 2-5%. According to Harrington's rule, for orthodox seeds, the lifespan can be predicted for a given level of MC or temperature; for every 1% reduction in MC or 5ºC reduction in storage temperature, the lifespan of the seeds doubles. The Orthodox seeds can be stored in conventional gene banks at –18 to –20ºC for long-term conservation. The few forest tree species that are orthodox include the pines (*Pinus spp*.), teak (*Tectona grandis*), acacia (*Acacia spp.),* and eucalyptus (*Eucalyptus spp.).*

Recalcitrant seeds

Recalcitrant seeds are exceptionally sensitive to desiccation and cannot tolerate exposure to low temperatures. Compared to orthodox seeds, they do not tolerate desiccation and are typically shed at a relatively high moisture content (30-70%). Desiccation sensitivity occurs during the later stages of their development. A major proportion of recalcitrant forest tree species including *Shorea* and *Quercus* exhibit extreme sensitivity to low temperatures especially below 10-15ºC. For example, the seeds of *Shorea* species contain very little moisture and cannot be conserved in gene banks. The longevity of recalcitrant forest tree species is usually short, ranging from days to months.

Parameter	Orthodox Seeds	Recalcitrant Seeds
Moisture content at shedding	Low to moderate (10-20%)	High (30-70%)
Desiccation tolerance	High	Low
Optimum storage temperature	–18 to –20ºC	15ºC (tropical) and 0-5ºC (temperate)
Lifespan/Longevity	Decades to centuries	Days to months
Primary damage mechanism	Aging Desiccation, DNA degradation	Desiccation damage, chilling injury, metabolic arrest
Common forest tree species	Pines (Pinus), Teak (Tectona)	Oaks (*Quercus*), Sal (*Shorea*)

Physiological mechanisms of storage behaviour

Mechanisms in orthodox seeds

Orthodox seeds exhibit unique physiological adaptions such as the synthesis of Late Embryogenesis Abundant (LEA) proteins and accumulation of sugars during the maturation phase so that they normally tolerate desiccation down to a MC of 2-5%. At low moisture and temperature, the cytoplasm of orthodox seeds is vitrified into a glass-like consistency that prevents any reaction occurring in the cells. Orthodox seeds also have antioxidant defense mechanisms that help in detoxifying reactive oxygen species that may accumulate during desiccation.

Mechanisms in recalcitrant seeds

Recalcitrant seeds normally do not undergo the maturation drying process on the mother plant. Due to their inability to adjust membrane lipid phase transitions, water removal from these seeds causes cessation of metabolic processes, resulting in the accumulation of toxic metabolites such as reactive oxygen species. The seeds also have decreased production of LEA proteins and oligosaccharides, and their cellular membranes transition to a liquid state when dehydrated, leading to loss of cellular content upon rewetting. Additionally, in tropical regions, the cellular membranes of the recalcitrant seeds are vulnerable to cold temperatures and undergo phase transition which causes significant cellular damage.

Forest seed examples

Orthodox forest tree species: Orthodox forest tree species are predominantly found in temperate conifers and the dry tropical region. For example, the seeds of Pinus spp. can be desiccated to a very low moisture content and subsequently stored in gene banks for long-term conservation. The seeds of *Acacia* and *Eucalyptus* species are also highly tolerant to desiccation. Many *Acacia* species have hard seed coats that induce physical dormancy. Another good example of a forest tree species that produces orthodox seeds is *Tectona grandis*. Being highly valuable tropical timber, Tectona provides an excellent opportunity for international trade of its seeds.

Recalcitrant forest tree species: Recalcitrant forest tree species are largely confined to the canopy of tropical rainforests, but some species also occur in temperate climax forests. For example, *Quercus* and *Castanea* spp. are temperate trees that produce recalcitrant seeds. The majority of their seeds mature in late summer or autumn and should be stored in a moist forest for winter. These seeds are sensitive to desiccation but resistant to near-freezing temperatures.

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Meanwhile, in the tropics, trees of the genus *Shorea* are keystones species of dipterocarps. The seeds of *Shorea* spp. are short-lived and germinate soon after falling from the tree to the ground. Moreover, they die at temperatures below 15ºC and a moisture content of less than 35%. By contrast, the genus *Theobroma* is a representative of Amazonian understory species with recalcitrant seeds.

Management, practical storage techniques & conservation strategies

Strategies for orthodox seeds

Due to the predictable nature of orthodox seed physiology and their ability to withstand desiccation, they can be stored in conventional gene banks. The first step to this process is appropriate seed processing. This involves drying the cleaned seed lots at a controlled environment (15ºC, 15% RH) to reduce the moisture content (MC) to 3-5%. Seeds with stabilized moisture can then be stored in moisture-proof containers (glass jars or laminated bags) in gene banks. To store such seeds for a longer period, the containers can be placed in a deep freeze (walk-in freezer) set at –20ºC.

Strategies for recalcitrant seeds

Since recalcitrant seeds cannot be stored in gene banks, special measures such as short-term moist storage, cryopreservation, and live gene banks (ex situ arboreta) are needed for their conservation. For short-term conservation, recalcitrant seeds may be stored at an optimal temperature and moisture content mixed with appropriate media (peat or sawdust) for a few weeks to months. As far as long-term conservation is concerned, cryopreservation of embryos or embryonic axes appears to be the most promising technique for the conservation of recalcitrant forest tree seeds. In this process, the embryonic axis is cut out from the seed and subjected to rapid drying to a specified moisture content followed by cryoprotection and ultra-rapid freezing by plunging them into liquid nitrogen at –196ºC. Another conservation option includes the maintenance of live gene banks. Here, the gene bank curator maintains a collection of plants of desired species in botanical gardens under appropriate environmental conditions. Alternatively, tissue culture can be undertaken to maintain plantlets of desired species in vitro under aseptic conditions.

Ecological and conservation implications

Seed storage behaviour plays a critical role in ecology and conservation. For instance, dipterocarps are termed ‘recalcitrant’ species because they ‘mast seed’ to guarantee that enough seeds reach the ground to produce the required number of seedlings for new forest growth to occur. This is an

adaptation that makes them highly vulnerable to anthropogenic disturbances such as logging; it means that any human activity that affects the forest cover will affect their ability to regenerate. Moreover, climate change poses a serious threat to these species because changes in rainfall patterns can affect germination and establishment of these species. It has been postulated that the majority of critically endangered plant species are recalcitrant and thus not amenable to conventional seed banking. In fact, it is estimated that 36% of endangered species and over 90% of climax forest tree species are recalcitrant and thus require specialized downstream conservation approaches. Therefore, more research is needed to bridge the gap between conservation needs and the actual practice of ex situ seed bank conservation for recalcitrant forest tree species.

Conclusion

The concept of orthodox and recalcitrant seeds and their conservation approaches are of utmost importance to forest genetic resources conservation. Due to the presence of LEA proteins and physiological adaptations enabling cells to withstand desiccation, orthodox seeds can be stored in gene banks at ultra-low temperatures for long-term conservation. Recalcitrant seeds, on the other hand, require special approaches such as tissue culture, live gene banks, and cryopreservation techniques to overcome desiccation and low-temperature injuries. With the current threat to forest ecosystems across the globe due to climate change, conservation of recalcitrant forest tree species appears to be a major challenge for the seed technologists and conservation biologists in the near future.

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