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RECALCITRANT SEED STORAGE BEHAVIOR OF TROPICAL FOREST TREE SPECIES

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Forest wealth plays an important role in a country's economic and ecological stability. Forests of tropical countries are declining at an alarming speed due to the interference of many factors like urbanization, industrialization etc. Each year 16 million hectares of virgin forests are cut and burnt. Tropical forest, a storehouse of Biodiversity accounts for 25% of the total forest area of the world. India possesses almost the entire range of tropical forest, from dry deciduous to wet evergreen. There is an imbalance in the environment due to deforestation and nudation. As a part of forestation program large quantities of forest seeds are being collected and used for raising seedlings in nurseries. Good quality seeds which are healthy are essential for raising plantations. Forest productivity and nursery efficiency depends on quality of seeds used. Unhealthy seeds cause diseases resulting in poor germination and seedling mortality.

In forestry practice, seed is the major source of regeneration. A large amount of seeds are being collected and stored for their future use. The information on flowering, fruiting and seedling behavior of forest tree species is also collected along with the documentation on fruit and seed characters.

World's agriculture and horticulture to a large extent depends on seeds and so the maintenance of seed viability in storage is of particular importance. The life span of seeds varies from a few days to few hundred years.

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The developmental stage of seed at harvest plays an important role in post harvest-storage longevity. This longevity also depends on plant species and external factors such as moisture content, temperature and composition of the gaseous atmosphere during storage. Seeds eventually deteriorate even under optimal storage conditions.

Roberts (1973) recognized three main categories of seed storage behavior.

1. Orthodox Seeds: These seeds survive long term dry storage. Most orthodox seeds (mostly Legumes) are shed in relatively dry condition and can further be dried to low moisture content (less than 5%) without losing viability. They can be stored successfully for many years at ambient temperature. Eg. *Bixa orellana* of Bixaceae.

2. Intermediate Seeds: These seeds can withstand dehydration to a certain extent but have reduced longevity. They survive drying up to 8 – 10% moisture content on fresh weight basis but get injured at low temperatures. Eg., *Citrus, Papaya* (Ellis *et al.*, 1990; Ellis, 1991).

3. Recalcitrant Seeds: These seeds cannot withstand dehydration. They are characterized by absence of maturation drying and are shed at moisture contents more than 50% on the fresh weight basis. They are readily damaged upon drying and cannot be stored at sub zero temperatures because they would be killed by freezing injury resulting from ice formation (Roberts, 1973; Roberts *et al.*, 1984). Most of the timber trees have recalcitrant seeds.

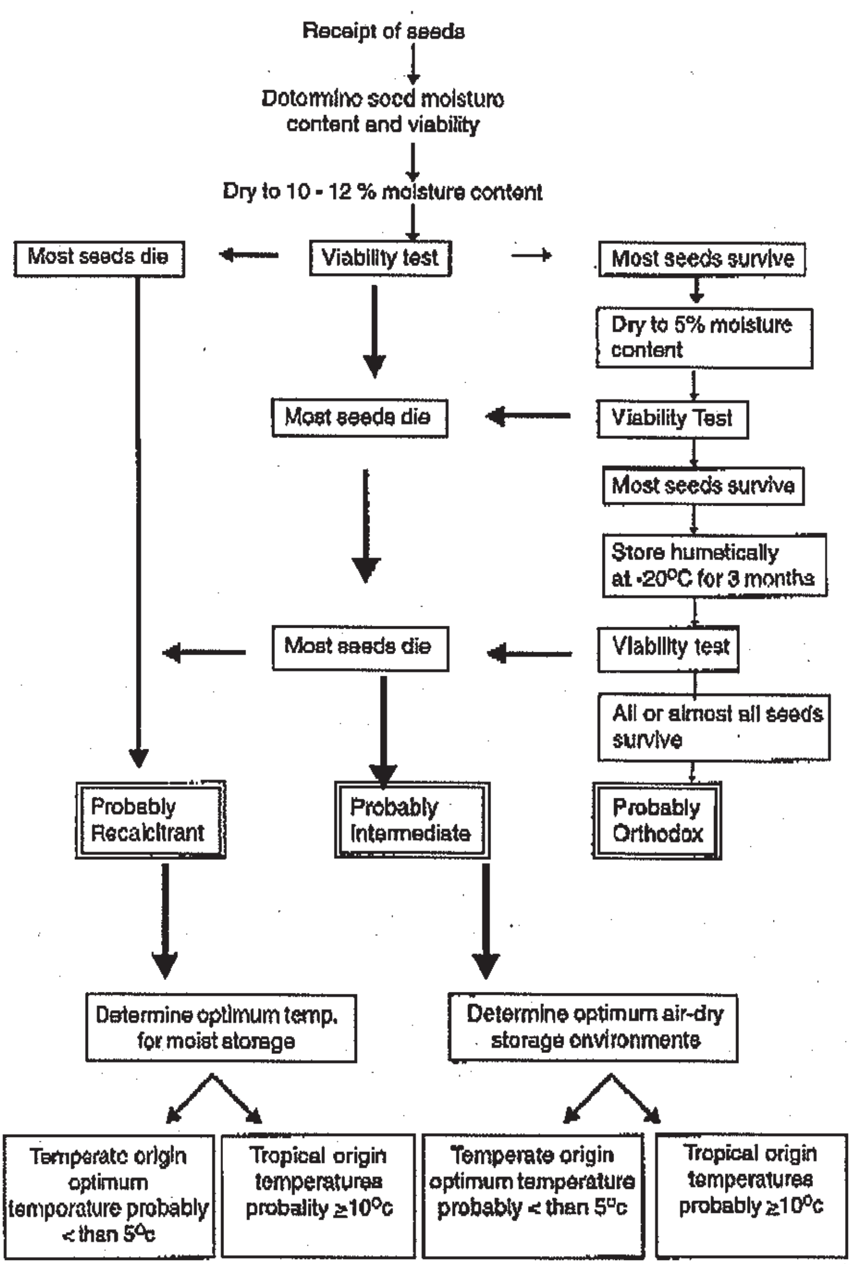
Due to their sensitivity to desiccation and low temperatures, recalcitrant and intermediate seeds are reported to have relatively short life span.

Recalcitrant seeds initiate germination - related metabolism shortly after shedding (Vertucci and Farrant, 1995). As germination events progress, the seeds become increasingly sensitive to drying and attempting to store these seeds is similar to air dry storage of germinated orthodox seeds (Farrant *et al.*, 1986; 1988). There is no clear cut event delineating the end of seed development and the start of germination. During both phases, recalcitrant seeds appear to remain metabolically active.

Longevity of Recalcitrant seeds in moist storage

There is no satisfactory method for maintaining the viability of intact recalcitrant seeds over long term, as they cannot be dried; neither can they be stored at sub zero temperatures, because they would then be killed by freeing injury sometime from ice formation. The longevity of recalcitrant seeds is generally short, particularly for species adapted to tropical environments; typically from few weeks to few months.

TO DETERMINE SEED STORAGE BEHAVIOUR



However, the longevity of seeds of species adapted to temperate environments can be maintained for much longer periods.

Classification of Recalcitrant Seed

Roberts *et al.* (1984) observed that species producing recalcitrant seeds were of two main types:

(1) Species from aquatic habitats. Eg: *Avicennia marina*.

(2) Tree species producing large seeds, including many important tropical plantation crops like Rubber, Cocoa, and Coconut; tropical fruit crops like Mango and Jackfruit and tropical timber species belonging to the families Dipterocarpaceae and Araucariaceae.

Morphology of Recalcitrant Seeds

Among tropical tree species 45 dicotyledonous families are characterized to have recalcitrant seeds. There is wide range of morphological and structural variations among recalcitrant seeds. Most of them are spherical or oval in shape and some are endospermic. The seeds or the nuts are often surrounded by thick endocarps in which case the structures are botanically true fruits. Many of these fruits are covered with a fleshy or juicy arilloid structure. The fleshy aril of rambutan and jackfruit are covered by thin papery membrane. In case of Jackfruit this membrane becomes impermeable to water on drying. Recalcitrant seeds show certain peculiarities. For eg., tiny embryos of rambutan seeds are attached at different positions between the two unevenly sized cotyledons (Chin, 1975). The seeds of a number of species like Mango and Mangosteen are polyembryonic.

Evolutionary status of Recalcitrant Seed storage behavior

According to the review made by Von-Tekhman-I and Van-Wyk-A-E (1994), 45 dicotyledonous families are associated with bitegmic and crassinucelate ovule and with nuclear endosperm development. All these are considered to be ancestral character states of the ovule. Most of these species are also known to have greater seed size, woody habit and tropical habitat; regarded as ancestral character states in Dicotyledons. In many species with recalcitrant seeds the predominant storage reserve is carbohydrate. Recalcitrance is significantly associated with exalbuminous type of reserve storage. It is proposed that in large Recalcitrant Seeds the transfer of main storage function from endosperm to embryo was probably an early development. In many species with Recalcitrance, the ovules/seeds are characterized by extensive vascularization of the integument/seed coat or by a patchy chalaza. Patchy chalaza is proposed to be significant functional adaptation for more efficient transfer of nutrients to the embryo/seed. Recalcitrance and some of the other character states proposed to be ancestral in Dicots are also present in some Gymnosperms. In relatively advanced dicotyledonous families with orthodox seed, recalcitrance persisted only in isolated and relict members. Available evidence supports the reviewer's view that the seed recalcitrance can be regarded as a relatively ancestral character state in Dicots.

Physiology and Biochemistry of Recalcitrant Seeds

Water in seeds is found as free water and bound water. The free water is necessary for the movement of molecules from one center of metabolism to another. On drying, the free water is removed and the loss in weight is expressed as the moisture content of the seeds. The moisture content of the seeds may be reported on fresh weight basis.

Free water is easily removed from seeds on drying while bound water, which is strongly held is not removed. The bound or subcellular water is closely associated with macromolecular surfaces and has structures imposed upon it. The structured water may possibly ensure the stability of macromolecular and sub cellular surfaces and may stabilize membranes in desiccated orthodox seeds. (Berjack *et al.*, 1984). Clegg (1979) suggested that structural water is involved in ensuring the precise functioning of multi enzyme systems. All metabolisms can, and probably does take place in structured water.

Loss of structured water results in disruption of metabolism. In the case of orthodox seeds this presumably does not occur because of their tolerance to desiccation. In recalcitrant seeds however, this does not appear to be the case (Berjak *et al.*, 1984).

It is also possible that a reduction in moisture content causes loss of membrane integrity and nuclear disintegration as has been shown with rubber seeds on sun drying (Chin *et al.*, 1981). The seeds of many tropical plants contain high concentration of phenolic compounds and phenolic oxidases. These compounds are normally compartmentalized within the cells; on desiccation the cell membranes are damaged and phenolic compounds are released. They are then oxidized and protein-phenol complexes are formed with a consequent loss of enzyme activity.

Farrant *et al.* (1988) hypothesized based on his work on seeds of *Avicennia marina* that recalcitrant seeds behaved like imbibed orthodox seeds when they are first shed and can withstand the loss of at least 18% of their initial content of water and still remain viable. The seed normally start to germinate on shedding. They become more sensitive to desiccation with the onset of cell division, vacuolization, and at the early stages of germination (Bewley, 1979). Dehydration of seeds stored for more than 4 days caused a marked decline on germination.

Sub ambient temperature susceptibility of recalcitrant seeds may be due to the declining fluidity of membrane lipids, which occur during chilling, and this can result in changes in membrane thickness and permeability effecting the membrane bound enzymes. In cocoa the fall in seed viability with declining temperature was abrupt. Possible reasons were:

1. The presence of some temperature – dependent, rate limiting reaction, the cessation of which causes metabolic disruption.
2. The absence of protective substances which are not susceptible to chilling.
3. Liberation of toxic material owing to cold induced changes in membrane permeability.
4. In cocoa Hor (1984), noticed a three-fold increase in leachate conductivity and major ultrastructural changes in the cell membrane systems.

LEA (Late Embryogensis Abundant) **Proteins**

LEA proteins are reported from most of the orthodox seeds and are not present in the recalcitrant species. Their m-RNA appears in mature seeds as desiccation commences, and become most abundant m-RNA species in the dry seed and disappears shortly after imbibition (Baker *et al.*, 1988). LEA proteins are reported to be transcriptionally regulated by ABA, cellular water loss and osmotic potential (Baker *et al.*, 1988). Detailed study of these proteins strongly implies that these have a fundamental role in desiccation tolerance, and also play a structural role as desiccation protectants. Lane (1991) speculated that these might function in water replacement. Sugars mainly sucrose and to a lesser extent lactose and raffinose preserve membrane integrity in dry organisms and afford protection to proteins (Farrant *et al.*, 1993; Leprince *et al.*, 1993).

Oxidative Stress and antioxidants

Several lines of evidence have suggested that in desiccation stressed-tissue highly reactive activated oxygen is produced. The concentration of this oxygen increases and results in low viability as the seeds age.

In *Quercus rubra* stable free radicals were reported to accumulate in the embryonic axis; coinciding with this there was loss of moisture and viability in the recalcitrant seeds. Membrane from desiccation tolerant seeds (Orthodox) are more resistant to attack of activated oxygen than those from sensitive seeds (Recalcitrant) suggesting that the cell regulates the compositions of the membrane, specifically its antioxidant content, during development and germination. Plants are endowed with numerous antioxidant defense systems that have been well-characterized particularly in photosynthetic tissues. Seeds contain large quantities of tocophenols, ascorbat and glutathione that may function as chain breaking antioxidants. Several studies have linked antioxidants with tolerance of oxidative stress from high light, low temperature or drought in photosynthetic tissues but in seeds their involvement is less resolved. The role of enzymatic scavengers, such as SOD, catalase and ascorbate peroxidase as protectants in a dry seed is probably negligible because of limitations in diffusion at low water contents. Protection of the dry seed against activated oxygen probably is restricted to the lipid soluble chemical antioxidants that are integral components of the membrane lipid matrix (Senaratna *et al.*, 1985). However the identity of these antioxidants is unknown, although tocophenols and phenolics may be candidates, (Leprince *et al.*, 1993).

The above review clearly shows the difficulty of storing the seeds of the species showing recalcitrant seed storage behaviour. From the literature we found that most of the tropical tree species which were economically and/or medicinally important show recalcitrant seed behaviour. Hence for the multiplication of these species we need to adopt other means like vegetative propagation.

In the literature very little information is available on the storage pattern of the recalcitrant seeds of the woody species especially those of the economically important forest tree species. A detailed study on the seed storage of the main tree species studied are the following:

Jack fruit	-	*Artocarpus heteterophyllus*
Jamum	-	Syzigium cumini
Bael	-	*Aegle marmelos*
Pongamia	-	Pongamia pinnata

List of Plants Showing Recalcitrant Seed Storage Behavior

	Name of the tree Species	Family	Duration of Viability at room temp	Time Taken for Germination at room temp
1	Artocarpus heteterophyllus	*Moraceae*	10 days	21 days
2	*Aegle marmelos*	*Rutaceae*	21 days	15 – 21 days
3	*Syzigium cumini*	*Myrtaceae*	10 days	10 days
4	*Pongamia Pinnata*	*Papilionaceae*	180 days	21 days
5	*Michelia champaka*	*Magnoliaceae*	30 days	15 days
6	*Terminalia Chebula*	Combertaceae	240days	60 days
7	*Terminalia belarica*	Combertaceae	150days	60 days
8	*Avicennia marina*	*Verbenaceae*	4- 6 days	10 days
9	*Hevea brasilensis*	*Euphorbiaceae*	120 days	6 months
10	*Shorea robusta*	*Dipterocarpeceae*	4- 8 days	10 days

Futures lines for Research

1. Genetic manipulation of these tree species to produce LEA proteins and desiccation protectants (Sucrose, Lactose & Raffinose) .
2. Standardisation of methodology for cryopreservation of embryos of such seeds.
3. Standardization of methodology for the Physiology of germination and seedling growth which is vital for understanding and developing strategies for the conservation of biodiversity and restoration of tropical and important forest tree species.

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