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A REVIEW ON RECALCITRANT SEEDS

K. RAJA*, V. PALANISAMY and P. SELVARAJU****

Department of Seed Science & Technology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India.

*Present address : Central Institute for Cotton Research, Regional Station, Coimbatore 641 003, Tamil Nadu, India.

**Agricultural College & Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli 620 009, Tamil Nadu, India.

In general the seeds are grouped into two categories based on their storage behaviour viz., "orthodox" and "recalcitrant". In which the orthodox seeds can be dried and stored for many years without any problems. However, many forest and fruit tree species from temperate and especially humid tropical regions produce problematic seeds that are damaged by desiccation and are often sensitive to higher and lower temperature. Normally these seeds have the moisture content of 50 to 60% during maturity and they could not tolerate the desiccation below the critical moisture level of 30 to 35%. Accordingly, these so-called recalcitrant seeds have a short life. These seeds can only be stored for short periods of a few months to a year. At present short-term storage methods like moist storage, partial drying and controlled atmospheric storage techniques are used to store the seeds. These methods were successful for many of the recalcitrant seed species. Even though, these conventional methods of short-term storage of recalcitrant seeds will ever be used for germplasm conservation. Hence, long-term storage of recalcitrant seed is achieved through the *in vitro* technique.

Keywords : Desiccation sensitive; Freezing sensitive; Recalcitrant seed; Storage; Viability; Vigour.

Introduction

Generally seeds are classified into "orthodox" and "recalcitrant" based on their sensitivity to desiccation and temperature. Most of the agricultural and horticultural crop seeds come under othodox class. These seeds have the ability to tolerate reduction in moisture content and temperature, which increase the life span of seeds. This group of seeds remain viable for longer periods even upto hundreds of years when they are dried and stored properly. They can be even stored at very low temperature of -196^{0}C. While recalcitrant seeds are killed if, their moisture content is reduced below certain relatively high critical value of 20 to 35%. It is estimated that 15% of the world's flora posses recalcitrant seeds i.e., approximately 37,500 species. Generally, recalcitrant seeds occur in humid forest environment. They may also occur in tropical, subtropical or temperate regions. In horticultural crops, except vegetables, few spices and fruits, most of the seeds have the recalcitrant behaviour. The fruit crops viz., mango, few species in citrus, avocado, jack, jamun, litchi, mangosteen, durian and rumbutan are coming under the recalcitrant seeds. The plantation crops like arecanut, coconut, cocoa, coffee, clove, nutmeg, rubber, and tea and the spices like pepper, cardamom, curryleaf, cinnamon and cassia are also some of the examples of recalcitrant seeds. Forest tree species belonging to the Araucariaceae and Dipterocarpaceae have recalcitrant behaviour.

Seed recalcitrancy - Recalcitrancy is the behaviour of seeds to desiccation-sensitivity. These seeds are well known for their sensitivity to desiccation and freezing temperatures. Harrington[1] proposed a basic rule related to the effect of seed moisture content and storage temperature in which the reduction in moisture content and temperature increases the life span of seeds. Obeying the above rule, most seeds remain viable for longer periods when they are dried and stored at low temperatures, even as low as -196^{0}C. But, Roberts[2] clarified the situation by introducing the terms 'orthodox' and 'recalcitrant' to describe the storage behaviour of seeds. He referred to seeds obeying Harrington's rule that is seeds which can tolerate desiccation and freezing

temperatures as orthodox, while the many other types of seed which are readily killed by desiccation if the moisture content falls below a critical value between 12 to 31% and cannot tolerate freezing temperatures were classified as recalcitrant. Later, Hanson[3] has suggested more accurate and descriptive terms, by which orthodox seeds are called *desiccation-tolerant* and recalcitrant seeds are referred to as *desiccation-sensitive.*

Characteristics of recalcitrant seeds - Generally recalcitrant seeds are larger in size and weigh more as compared to orthodox seeds because of their high moisture content. Many recalcitrant seeds are covered with a fleshy layer, which is often edible, as in case of avocado, durian, jackfruit, jamun, litchi, mango, mangosteen, and rambutan. Similarly some recalcitrant seeds are large and are found in a single-seeded, simple fruit such as arecanut and coconut or as seeds in a composite fruit such as jackfruit. The shape and size of recalcitrant seeds of different species are differing greatly even within species or even within the same fruit[4].

The other important property of recalcitrant seeds is their high moisture content, even after they have been shed from the mother plant. Unlike orthodox seeds, they do not undergo maturation drying. These recalcitrant seeds generally have high moisture content, ranging from 30 to 70%[4]. For example the freshly collected arecanut fruit and seed has 63.6% and 50.1% moisture content, respectively. However, unlike orthodox seeds these arecanut seeds are highly intolerant of further desiccation and make them as recalcitrant[5,6]. The main characteristics of orthodox and recalcitrant seeds are as follows[4].

Characteristics	Orthodox	Recalcitrant
1. Tolerance to drying	Can be dried to low moisture content	Can not be dried
2. Tolerance to desiccation	Tolerant	Sensitive
3. Tolerance to low temperature	Tolerant	Sensitive
4. Size of the seeds	Small to medium	Large
5. Storage life	Many years	Few days to few months
6. Storage methods	Ordinary storage is enough	Special methods required
7. Seed moisture content at harvest	Low	High
8. Examples	Cereals, Millets, Pulses, Oil seeds, Vegetables.	Mango, Jack, Jamun Arecanut, Coconut.

Desiccation sensitivity - As discussed earlier recalcitrant seeds are well known for their sensitivity to desiccation, especially large seeded species generally found in the tropics. In recalcitrant seeds, decline in viability occurs abruptly below a certain moisture level, which is called "Critical Moisture Content (CMC)"[7]. Tompsett[8] coined the term "Lowest Safe Moisture Content (LSMC)" which is defined as the moisture content below which freshly collected seeds died when the seed lot is dried. Again, Tompsett[9] stated the critical moisture content as the lowest safe moisture content. The degree of sensitivity varies between species[2] but generally the critical moisture content is 20-35% below which the seeds will be killed. For example, the critical moisture content of few recalcitrant seeds is given below :

Crop seeds	Critical Moisture Content (%)	References
1. *Quercus robur*	38	Vlase[10]
2. *Nephelium lappaceum*	20	Chin[11]
3. *Shorea talura*	17	Sasaki[12]
4. *Hopea helferi*	35	Tamari[13]
5. *Hevea brasiliensis*	15-20	Chin *et al.*[14]
6. *Theobroma cacao*	26	Hor *et al.*[15]
7. *Litchi sinensis*	40	Fu *et al*[16]
8. *Hancornia sp.*	25	Oliveira & Valio[17]
9. *Myristiga fragrans*	45	Sangakkara[18]
10. *Artocarpus heterophyllus*	39	Shylla Merlin & Palanisamy[19]
11. *Euterpe edulis*	39	Martins *et al.*[20]
12. *Persia americana*	49	Raja *et al.*[21]
13. *Murraya koeniigii*	34	Raja *et al.*[22]
14. *Areca catechu*	33	Raja *et al.*[23]

Intra-varietal differences in critical moisture have also been found in number of species. For example, the critical moisture content for mango varieties are 25% in Alphonso, 32% in Totapuri[24], 28% in Neelum and 34% in Goa[25]. The extent of damage that occurs on the removal of water from desiccation sensitive seeds and thus the water content at which they lose viability, depends upon number of factors like the rate at which the seeds are dried and their metabolic activity when they are subjected to drying (which affect the 'metabolism-induced damage') as well as the extent to which any of the protective mechanisms are expressed[26]. Pritchard and Manger[27] also stated that the truly recalcitrant seeds couldn't survive the removal of any structure-associated water. Drying of desiccation sensitive seeds induced the damage in cell membrane that could not be revive after imbibition and finally results in loss of viability[28]. Sudden loss of water, gradual deterioration of cellular organization, breakdown of cellular storage components[29], depletion of food reserves and accumulation of toxic substances are some of the causes of desiccation induced seed deterioration[30].

Chilling sensitivity - We can store the orthodox seeds even up to the temperature of-196^{0}C without loss of viability. But the recalcitrant seeds cannot be stored at lower temperatures; even some species do not survive the temperatures of 10-15^{0}C. It is because of chilling injury to the seeds, which varies according to species. Stanwood[31] has stated that there is a high moisture freezing limit (HMFL) which is the threshold, and if it is exceeded the viability of a seed sample will be reduced during liquid nitrogen storage. Thus the chilling sensitivity in most recalcitrant seeds is mainly due to the formation of ice crystals in between the cells when the moisture contnet is higher than 14 to 20%[32]. Chin and Roberts [33] stated that even short periods below 10 to 15^{0}C would cause loss of viability for many tropical recalcitrant species. For example, in cocoa a sharp reduction in storability was observed at 15^{0}C compared to 17^{0}C[34]. The seeds of many tropical species are killed at sub-ambient temperatures. For example, the *species like Theobroma cacao*[15], *Nephelium*

lappaceum[35], *Dryobalanops aromatica*[36], *Hopea odorata*[37], *Shorea ovalis*[12] and Garcinia mangostana[38] were very sensitive to chilling temperatures. Song *et al.*[39] found that at 5°C almost all seeds were dead after 6 months, but at 15 to 20°C no loss of viability had occurred in *Hopea hainanensis*. Similarly, Tompsett[40] found sudden loss of germination ability at 6°C than at 11°C in *Shorea robusta*. This sudden loss of germination was due to chilling injury to the seeds at lower temperatures[41]. Tompsett[42] also reviewed that the reduced germination following exposure to temperatures in the range of 0 to 16°C (chilling damage) occurs in all moist dipterocarp seeds. In case of *Calophyllum brasiliensis*, the seeds are very sensitive to 5°C but best results are achieved for seeds with a moisture content around 30% at ambient temperature and 15°C[43]. Rajeswari Dayal and Kaveriappa[44] found that the *Hopea parviflora* and *H. ponga* seeds subjected to freezing (0±2°C) showed a rapid decline in the subsequent germination percentage at 30±2°C, as well as vigour, even though the moisture content of these seeds did not vary much. In Hopea, Tamari[13] observed a decline in the development of radicle with decrease in temperature. The reason might be the decreased metabolic activity of the seedlings at lower temperatures.

Basis for recalcitrancy - The absence of sufficient water to stimulate germination is the basic feature of recalcitrant seeds appears to be continued embryo development (increasing dry weight) following shedding. For example this occurs in *Cycas revoluta*[45], some dipterocarps[9] and *Podocarpus henkelii seeds*[46]. The declining fluidity of the membrane lipids during chilling is also one of the reasons for recalcitrancy[47]. King and Roberts[41] suggested the following two hypothesis by which death occurs in these seed; either death occurs rapidly at or below some critical moisture content (critical moisture content hypothesis), or loss of viability occurs at a rate which is negatively related to moisture content over a wide range of moisture contents (non critical moisture content hypothesis). Similarly, Pammenter and Berjak[26] opined that the damage of recalcitrant seeds were due to absence or incomplete expression of physical characteristics of cells and intracellular constituents, metabolic 'switching off' and 'switching on' mechanism, efficient operation of antioxidant systems, accumulation of putatively protective substances including the Late Embryogenic Abundant (LEA) proteins, the presence and operation of repair systems, sucrose and other oligosaccharides as well as amphipathic melecules and the presence of role of oleosins. Similarly, scientists have suggested the possible reasons for fall in viability of recalcitrant cocoa seeds which include (i) the presence of some temperature dependent, rate limiting reaction, the cessation of which causes lethal metabolic disruption; (ii) the absence of some protective substance which is present in those seeds not susceptible to chilling; and (iii) the liberation of some toxic material owing to cold induced changes in membrane permeability[48].

Desiccation and vigour - Germination rate may provide the first indication of desiccation stress in recalcitrant seeds; thus mango exhibits a reduced vigour index before there is any noticeable fall in germination *per se*[16]. The freshly harvested clove seeds are the best suited for sowing in order to get maximum germination and vigorous seedlings[49]. Normah and Chin[50] observed decreased germination, seedling height, seedling dry weight and respiration rate with increased storage of rubber seeds. Raja *et al.*[21] observed decreased viability and seedling vigour during the desiccation of recalcitrant avocado seeds. In another study Anderson[51] stated that reduction in seedling growth precede or accompany loss of germinability need not occur in every case of seed deterioration.

Biochemical changes during desiccation- The dehydration-induced deterioration of the cell membrane in recalcitrant seeds is

indicated by high increase in leakage of solutes[33]. Heydecker[52] suggested that weakening of cell membrane might be the cause of leaching metabolite like electrolytes and other cell soluble compounds into the imbibing medium. This loss in mebrane integrity results in the enhanced efflux of cellular constituents like sugar, amino acid, phenol and phosphates[53]. Also the membrane conductance depends upon the concentration of the carrier for electrolytes in the membrane, the density of the membrane surface charge, the quantity of permanent low and the ionic strenght on both the sides of the membrane[54].

The seed vigour is more closely related to the integrity of the *protein synthesizing system* than to the protein content of the seed[55]. Szezotka[56] reported that both decreased protein synthesis and oxygen uptake are parallel to the decline in *germination of Quercus robur* and *Q. borealis*. Also protein synthesis is essential for germination to be completed and for radicle emergence to occur[57]. Nautiyal et al.[58] noticed lower amount of soluble proteins in non-viable seeds of *Shorea robusta* than in viable seeds. It was observed that the desiccation of developing seeds was characterized by the accumulation of a partcular set of m RNA and related proteins called 'Late Embryogenic Abundant (LEA) proteins' in the desiccated state[59]. Further, Farrant *et al.*[60] reported that *Avicennia marina* seeds do not produce LEA proteins, which supported the suggestion that production of such proteins might facilitate desiccation tolerance. The group of LEA's that has received the most attention is the LEA D_{11} family, also known as the dehydrins, which is responsible for dehydration stress and in recalcitrant seeds appears to be anomalous[26]. The accumulation of a class of LEA proteins[61-63], often called 'dehydrins' during development is thought to protect against desiccation damage, particularly to membranes. Desiccation sensitivity in seeds has therefore been suggested to result from the absence of dehydrin proteins[64, 56]. Farrent et al.[65] reported that no such new proteins are produced during the late stages of development in the highly desiccation-sensitive seeds of *Avicennia marina.*

Phenols are aromatic compounds which include an array of components like tannins, flavonoids etc. The seeds of many tropical plants contain high concentrations of phenolic compounds and phenolic oxidases. These compounds are normally compartmentalized within cells. On desiccation, the cell membranes are damaged and the phenolic compounds are released. They are then oxidizd and *protein - phenol complexes* are formed leading to loss of enzyme activity[66]. In arecanut seeds reduction in phenol content during desiccation was evident due to the destruction of the tanniferous cells and release of phenolic acids to the surrounding cells[67].

The fat content of recalcitrant seeds declines with steady increase in free fatty acids due to desiccation of seeds[67]. Clatterbuck and Bonner[68] observed a steady decrease in crude fats of *Quercus robur.* Georgi *et al.*[69] found that the untreated rubber seeds deteriorated rapidly in storage with a considerable development of acidity in the oil. The oxidation of fat contributed to the accumulation of free fatty acids might be the reason. Accumulation of free fatty acids in the seeds are subjected to slow and constant attack by oxygen resulting in the production of hydrogen peroxide and other oxygenated free fatty acids and free radicals. Due to the accumulated oxygenated fatty acids, death occurs through the destruction of the respiratory pathway by the toxic aldehydes and free radicals formed by peroxide and epoxide decomposition[70]. Koostra and Harrington[71] stated the Millard reaction in which the degradation of lipo-protein cell membranes by free radical induced lipid peroxidation reaction has been suggested to be a basic reason of senescence and ageing. Free radical activity has been associated with viability loss in several recalcitrant seeds during

desiccation[72-74]. Many authors[67, 75-78] have also supported that lipid peroxidation is a cause of membrane deterioration during desiccation and that the damage would result in loss of semi-permeability, increase in free fatty acids and accumulation of thiobarbituric acid- reactive substances such as melondialdehyde. Results obtained by Senaratna *et al.*[79] with microsomal membranes seem to show that free radicals induce de-esterification of membrane phospholipids rather than change in fatty acid saturation. But according to Leprince *et al.*[80] mitochondria might be the primary source of electrons leading to the production of stable free radicals in desiccation intolerant radicles.

Volatile aldehydes constitute an important component of the gaseous emanations of deteriorating seeds[81]. Besides volatile aldehydes, other growth inhibiting substances in the gaseous emanations were also observed by Woodstock and Taylorson[82]. Bailey et al.[83] found various volatile compounds such as isovaleraldehyde, isobutyraldehyde, propionaldehyde, methanol, acetaldehyde, methyl acetate and diacetyl in cocoa bean seeds. Bhattacharyya and Basu[77] has found that vigour biossay of gaseous emanation of jackfruit seeds and demonstrated that deteriorated seeds produced larger quantities of volatile growth inhibitory substances during germination.

Protective mechanisms- Generally in the seeds, the multiple protective mechanisms exist against the highly reactive oxygen radicals. They involve free radical and peroxide-scavenging enzymes such as catalase, peroxidase and superoxide dismutase. Recalcitrant seeds (or their embryos) do appear to posse's antioxidant mechanisms[72]. However, these protective mechanisms may become impaired under conditions of water stress[84], certainly, they are ineffectual in terms of protecting against desiccation damage. For example, during desiccation of arecanut seeds the activities of scavenging enzymes such as catalase and peroxidase were reported to decrease and the cells lose the protective mechanism of tolerance to desiccation[67]. This indicates that oxygen free radicals are continuously produced in the plant system. When exposed to stress they produce the enzymes in large amounts to eliminate the free radicals. As they exceed the eliminating ability, the plant system is damaged. The peroxidase enzyme is also involved in the dehydrogenation of a large number of organic compounds like phenols and aromatic amines. The destruction of the enzyme can lead to the accumulation of toxic substance in the seed. Hendry *et al.*[72] found a rapid accumulation of free radicals accompanied by decreasing activity of antioxidant enzymes and declining α-tocopherol content. In case of *Quercus robur and Q. borealis* a very high amylolytic enzyme activity was noticed at the beginning of storage. However, it was quickly declined to non-detectable levels by 8 months[85]. Polyphenol oxidase, another group of enzymes capable of degrading hydrogen peroxide and using as a electron acceptor is capable of catalyzing the same type of reactions as peroxidase[86]. Nevertheless, some recalcitrant seeds have retained active metabolism and thus showed an enhanced level of polyphenol oxidase activity during initial drying period. However the suppression of polyphenol oxidase activity during later desiccation was noticed with loss of seed viability[67,87].

The structured water in the seed is involed in ensuring the precise functioning of these multi-enzyme systems[88]. All metabolic activities probably do, take place in structured water. Loss of structured water results in the disruption of metabolism. In case of orthodox seeds, this presumably does not occur, as shown by their tolerance to desiccation. But in recalcitrant seeds, the situation is quite different[89].

Anatomical changes during desiccation- The desiccation of recalcitrant seeds to below the critical moisture content may result in the cellular and sub-cellular changes

particularly cell wall rupturing and cytoplasmic fragmentation[14]. The sub-cellular deterioration accompanies with desiccation was reported by several workers in many recalcitrant seed crops like *Avicennia marina*[29], rubber[90], cocoa[91], *zizania palustris,* and *Ekebergia capensis*[93]. Chin et al.[14] observed that the nucleus, which was irregularly shaped and sometimes the membranes surrounding the nucleus were hardly recognizable and a nucleolus was missing in dried rubber cells. Similarly, Ruhl and Dambroth[91] observed complete disintegration of the cell constituents in desiccated cocoa seeds. The ergastic substances are metabolites such as fats, starch or crystals, which are the passive products of cellular activity. The differential ergastic build up in the necrotic cells of the dried samples had been attributed to the result of deranged metabolic processes in the plant system[94]. The highly vacuolated cell structure of the seed may lead to a situation where the vacuoles lose their turgidity[95]. It is also possible that the water requirement for the developing embryonic axes may be met by the withdrawal of water from the other tissues in the seed. Such a withdrawal of water from the vacuolated cells contributes to water stress in these cells, which ultimately leads to cellular damage[96, 97].

Storage of recalcitrant seeds- The longevity of recalcitrant seed is very short; it varies from a few days to a few months or a year under proper storage conditions[4]. Therefore recalcitrant seeds are to be sown immediately after collection. Then only they will give the maximum germination. For example the arecanut seed lose its viability within 24 days under ambient open condition[98]. Some seeds may be kept within the fruit itself for few days. But due to the high moisture content it may enhance the pathogen entry and create the germination loss. There can be no doubt in the mind of any investigator working on desiccation - sensitive (recalcitrant) seeds that microorganisms, more particularly fungi, play a significant role in post harvest deterioration[99]. For example, the fungal species viz., Fusarium spp., Penicillium spp., Aspergillus flavus, A. niger, Rhizopus stolonifer, Trichoderma spp. and Botryodiplodia theobromae were found associated with the arecanut seed[100]. The relatively high moisture contents and temperatures that tropical recalcitrant behaviour demands also favour profuse growth of pathogenic (and nonpathogenic) microorganisms[101]. The composition of the microflora on and in recalcitrant seeds has been found to narrow with increasing storage period[102].

Storage methods- Recalcitrant seeds are named as such because of their difficulties in handling and storage. There seeds do not tolerate desiccation and low temperatures, as detailed earlier, both pre requisites for optimal storage. By maintaining high seed moisture content and storing seeds in the containers that allow some gas exchange is important to preserve the viability. This method, involving the storage of seeds in various gases or in sealed containers or by waxing, has had some success[4].

Recalcitrant seeds can be stored by the following methods :

a) Moist or imbibed storage
b) Partial dehydration
c) Controlled atmospheric storage
d) Cryopreservation and *in vitro* conservation

Amoung the four methods first three methods are short-term storage methods. Now a day the cryopreservation and *in vitro* conservation has gaining importance for long-term conservation of recalcitrant seeds.

a) Moist or imbibed storage-At present, successful short-term storage method is limited to moist storage[4]. Moist storage has been practiced for many years for a number of crops, including rubber[103], rambutan[11] and cocoa[104]. To avoid the desiccation sensitivity, the recalcitrant seeds are to be stored in most media like damp charcoal, saw dust, moist sand and other moisture conserving materials and chemicals. This is a short-term storage method and the viability can be

maintained up to three month or little bit more. Many workers had the successful storage with moist incubation treatments. For example, Bernard[105] in Shorea spp., Chacko and Singh[106] in mango, Ang[103] in rubber, Patil et al.[107] in mango, Gunasekaran and Krishnasamy[108] in rubber and Shylla Merlin and Palanisamy[19] in jack. In arecanut storage of fresh seeds with 5% moist sand or 0.2 M potassium dihydrogen phosphate premixed with sand at 5% level (seed: sand ration 1:3) and packing loosely in 350 gauge polythene bags and stored in zero energy cool chamber recorded higher germination (85%) after four months of storage[109]. The moist storage treatments are found very effective in counteracting physiological deterioration of seeds[77]. In imbibed storage, the seeds are stored under water but only for a short period, for example the rubber seeds stored under water recorded only 60% germination, after one month of storage[110]. But in this method the problem is a fungal growth during storage. Therefore it is necessary to give some chemical treatment to control the pathogens during storage.

Also the seeds incubated with moist media may result some *in situ* germination. Nevertheless, these sprouted seed can also be used for sowing because it will produce good seedlings as had been reported in *Synphonia globulifera*[111], *Artocarpus heterophyllus*[77] and *Hevea brasiliensis*[108].

b) Partial dehydration- In partial dehydration, the recalcitrant seeds are dried to certain critical moisture content by air at a temperature of 20°C and then stored. Rubber seeds can be stored for one year with 50% germination if they are cleaned, soaked in 0.3% benlate, surface air dried and stored in perforated polythene begs at an ambient temperature of 25°C[4]. Similarly, the cocoa seeds can be stored up to 24 weeks with more than 50% viability[34].

c) Controlled atmospheric storage- The recalcitrant seeds can be strored in controlled atmosphere of carbon dioxide or sealed containers. For example, cocoa seeds can be stored up to 45 days in carbon dioxide atmosphere[112] and durian seeds in sealed containers for about 32 days[113]. Wills *et al.*[114] stated that the wax formulations on fruits generally inhibit senescence. For example, the most successful storage method for cocoa could be storing the seeds within pods coated with paraffin wax. Similarly, Friend[115] stored cocoa seeds within pods coated with paraffin wax. Raja *et al.*[98] reported that the wax-coated arecanut seeds stored in gunny bag under ambient conditon were successfully extended the storage life up to 50 days with 60% viability. While, uncoated seeds stored in ambient condtion loses its complete viability within 24 days. The litchi seed retained with fruits treated with benomyl (0.05%) and wax emulsion (6%) and sealed in polythene bags maintained 42% viability for 24 days[116]. Shivarama Reddy[117] found similar result in coffee seeds and stored the seeds up to 300 days with 49% viability. The viability retained due to wax coating might be due to the reduced rate of respiration and moisture loss. Chin[4] stated that this type of controlled atmosphere storage would have much practical application in the storage of recalcitrant seeds. Coating had little effect on internal CO_2, O_2 and C_2H_4 levels and great effect on reduced water loss[118-121]. Controversly, coating of seeds with substances such as paraffin wax might be expected to restrict oxygen access to the seed and reduce storage life in *Shorea javanica*[122]. Therefore the ventilation of recalcitrant seed is needed to remove the excess toxic gases and to prevent anoxia[42]. The acorns of *Quercus alba* be stored more successfully if palstic bags with a wall thickness of only 0.04 mm are used to provide more aeration[123].

d) Cryopreservation and in vitro conservation- Reports over the past fifty years have shown that recalcitrant seeds can only be stored for short periods of few days or months to a year. Hence, long-term storage of recalcitrant seed is rapidly gaining momentum simultaneously with the development of *in vitro* technique[124]. The recalcitrant embryos are tolerant to

desiccation and low temperatures than whole seeds and together with their smaller size are amenable and practical for conservation[125]. The stategy adopted for long-term genetic conservation of these recalcitrant seeds is to cryopreserve the embryos, which are more resistant to adverse conditions. Bajaj[126] suggested that the germplasm of recalcitrant seeds could possibly be conserved through cryopreservation of their excised embryos. For successful cryopreservation, at very low temperature, excised embryos must be dried to suitably low moisture content to avoid ice formation by ultra low temperature[127]. A number of species of recalcitrant type, both temperate and tropical clones have been known to survive after cryopreservation[128], which includes *Juglans, Carya, Fagus, Corylus, Castanea*[129-130], litchi[131], coffee[132] and *Areca*[133]. The damage to viability of axes exposed to liquid nitrogen at high moisture content results from the presence of freezable water in tissues, which contributed to the formation of ice crystals in the intercellular space within the axes[134-136]. Similarly desiccation of axes progresses beyond certain moisture content, the reduction in survival will be there due to severe dehydration injury[135-137].

Conclusion- The short storage of recalcitrant seeds is a problem, as these seeds can't tolerate the desiccation and freezing temperatures. It is also important to maintain the viability of these seeds for long-term for the genetic conservation to create genetic diversity. Hence, the optimum storage conditions for recalcitrant seeds should be determined. Besides the physiological and biochemical basis for the desiccation non-tolerance of the recalcitrant seeds should also be investigated deeply.

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