Original Research Article

Morpho-Physiological Analysis of *Artocarpus lakoocha* Roxb Seeds for Improved Storability

Abstract:

Artocarpus lakoocha Roxb., commonly known as Monkey Jack, is a tropical fruit tree native to India and valued for its diverse uses in food, fodder, and timber. This study investigates the seed storability of *A. lakoocha*, focusing on its morpho-physiological traits and desiccation sensitivity. Mature fruits, collected from New Delhi, were analyzed for morphological characteristics including fruit and seed size, shape, and moisture content. Seeds were found to be recalcitrant, with high moisture content (50-55%) and limited viability (7-15 days), reflecting their sensitivity to desiccation and chilling. Germination tests revealed that seeds without testa (seed coat) exhibited higher germination rates, with fresh seeds achieving 100% germination at 52% moisture content. Desiccation significantly impacted seed viability, with moisture levels reduced from 52% to 22% over 21 hours leading to decreased germination rates. Seeds desiccated to below 37% moisture content showed a notable decline in viability. Storage studies indicated that seeds stored at 15°C retained viability for up to 180 days, while those stored at 25°C, 5°C, -10°C, and -20°C lost viability more rapidly. The results underscore the recalcitrant nature of *A. lakoocha* seeds, characterized by high moisture content and sensitivity to desiccation. Effective storage conditions are crucial for maintaining seed viability, with 15°C proving optimal for extended storage. This research provides insights into the storage behavior of *A. lakoocha* seeds and emphasizes the need for conservation strategies to address the declining populations of this economically important species.

Key words: *Atrocarpus lakoocha*, desiccation, monkey jack, morpho-physiological, storage

Introduction:

Artocarpus lakoocha Roxb. (Monkey Jack) is an important, commercially uncultivated species under the genus Artocarpus, belongs to family Moraceae. It is known by many other names: dhau, dephal, badhal, lakuch, lakoocha etc. Lakoocha is a tropical fruit tree originated from India. It is a deciduous tree about 20-meter height. Its distribution occurs in wet, semi wet deciduous forests from northern India to southern China. Ripe fruits are found from the end of June to early August in most places but there can be considerable variations. In many places the populations are gradually decreasing due to extensive exploitation and poor seed viability. The genus Artocarpus is having nearly 50 species but two are very closely related species; *Artocarpus lakoocha* Roxb. and *A. gomezianus* subsp. zeylanicus. These two can be differentiated by leaves, flowers and fruits. The leaves of *A. lakoocha* are alternate, elliptic, oblong or ovate in shape, pointed and leathery and up to 25 cm length and 15 cm width. It is a monoecious plant, so the flowers (catkin) are of two types; male receptacles are yellow colored, light weighted, very soft but with hairy surface, spongy and depressed oblong shape and lakoocha fruit is obloid or irregular in shape and up to 9 cm length and 7 cm width. Fruit is a syncarp (the entire female inflorescence forming the fruit), irregularly rounded, green when young, and turning yellow at the time of maturity, later brown. Fruit weights vary from 50 to 250 gm. The number of seeds/fruits varies proportionally, but typically there are 10-30 seeds per fruit. Seeds are irregular in shape. At maturity, most seeds are about one cm long, more or less flattened and pointed at the embryo end; the seed-coat is thin and white. The seeds contain sticky white latex.

Lakoocha tree is used as food and timber. Timber and wood from this tree are used to made furniture, boats and cabinets. It also used for heavy construction, poles, furniture and plywood due to its hard and termite resistant capacities. The tender bark (containing 8-9% tannin) of the tree is used by several communities for masticatory purpose together with betel nut. The *A. lakoocha* is highly preferred as fodder tree for its deliciousness and healthful values. The leaves contain about 16% crude protein and one tree produces between 60 and 200 kg fresh fodder in a year. It is fed to lactating animals and considered one of the most important milk producing forages. In several studies it was revealed that *A. lakoocha* has many medicinal uses (Hossain *et al.*, 2016). The fruits are eaten raw, boiled, steamed or roasted. Fruits are highly nutritious and an important food item in the human diet. The fruits contain vitamins and most of the vitamins are excellent source of antioxidant. The plant of *A. lakoocha* has contained flavonoids and phenolic acids. The fruits are generally sweet-sour pulp eaten fresh but mostly made into curries. Different parts of these fruits and spike of male flower are used to prepare pickles and delicious sauce. Lakoocha seeds are recalcitrant as have short viability of 7-15 days (Joker and Adhikari, 2003; Hossain *et al.*, 2016). Mature seeds have moisture content of 50-55% and do not tolerate drying below 40% moisture content. Recalcitrant seeds are desiccation and chilling sensitive and are viable for a very short period (Ellis, 1984). Recalcitrant seeds are highly sensitive to desiccation because of their high moisture content at the time of shedding and due to the lack of maturation drying (desiccation) on the mother plant. Jackfruit (*Artocarpus heterophyllus* Lam.) seeds are highly recalcitrant as evident by studies of Chin *et al.* (1984), Fu *et al*. (1993), Chandel *et al*. (1995), Smith *et al*. (2001), Peran *et al*. (2004).

The exact causes of recalcitrant seed death and its relationship with moisture content are not fully understood (Fu *et al*., 1993). It is stated that loss of viability could be either due falling the moisture content below critical value or simply a general physiological deterioration with time (Chin *et al*., 1984). Several pre-harvest factors also determine the longevity, like cumulative effect of environment during seed maturation, harvesting, drying and the pre-storage environment, time of seed harvesting, duration of drying and the subsequent period before seed is placed in store (Hanson, 1984). Recalcitrant seeds do not undergo maturation drying and hence there are 3 known types of damages occurring in recalcitrant seeds at maturity and after drying and freezing: 1) Physical damage – membrane disruption, 2) Metabolism induced damage – Free radical generation and ineffective antioxidant system are instrumental in causing damages. Loss of viability has been reported due to production of ROS like superoxide and hydrogen peroxide leading to lipid peroxidation, 3) Macro-molecular denaturation – proper functioning of cell organelles is a prerequisite for retention of viability.

Materials and methods:

Seed sources:

Lakoocha (*Artocarpus lakoocha* Roxb.) fruits for the present study were collected from the trees located at NBPGR and IASRI, New Delhi in months of mid June to end of July. Fruits were collected at various intervals after flowering and brought to the laboratory. Fruits were cut open to extract the seeds. Seeds were thoroughly sterilized with 0.5% mercuric chloride for 10 min, washed three times with sterile distilled water and then kept in jam bottle.

Morphological characterization:

Some qualitative and quantitative traits are taken for estimation of the physiological maturity and studied the seed biology. The qualitative traits which were taken are fruit shape, fruit rind thickness, fruit rind colour, fruit surface, latex exudation, pulp firmness, pulp colour, seed shape, seed surface slimy, seed coat (testa) thickness, seed surface pattern, seed coat colour, adherence of seed coat to kernel and the quantitative traits which were recorded are fruit length (cm), fruit width (cm), fruit diameter (cm), fruit weight (gm)/fruit, seed length (cm), seed width (cm), seed diameter (cm), seed weight (gm)/seed, seed-coat ratio, number of seeds per fruit, moisture content (%) and germination percentage by using ten randomly selected seeds. The linear dimensions length and width were measured by vernier caliper. The morphological features were observed using magnifying lenses and a stereomicroscope.

Germination:

Seeds without their testa (papery parchment) were used for germination testing. Since the testa was found to act as a barrier for the uptake of moisture. Ten seeds each in duplicate were sampled and kept for germination in the rolled towel germination paper at 27±2°C with a 16/8h light/dark photoperiod under BOD. Protrusion of radicle and plumule was taken as the criterion of germination. The number of germinated seeds was noted at three days interval and the germination percentage were calculated as follows: $GP = (GS * 100)/TS$

Where GP = germination percentage, GS = number of germinated seeds and TS = total number of seeds kept in germination towel paper

Desiccation:

Seeds tied muslin cloth bags were kept over charged silica gel for desiccation in different batches in a desiccator for different durations from 3 hrs up to 21 hrs and determined the moisture content of each batch of seeds. The charged silica gel was replaced in a desiccator after every 24 h.

Moisture content determination:

Moisture content of seeds was determined using low constant temperature oven method by drying at 103±2˚C for 17hrs (ISTA, 1985). A part from 5 seeds in duplicate were taken for each determination and moisture content expressed on fresh weight basis. The moisture content in seeds was calculated by using the formula.

$$Moisture\ content\ =\ B - C\ \times\ 100\ /\ B - A$$

Where, A = weight of empty weighing bottle

B = weight of bottle + fresh seeds

C = weight of bottle + oven dried seeds

Seeds storage:

The lots of fixed number of fresh seeds containing 53 % moisture content were stored in jam bottle at different storage temperature like 25°C, 15°C, 5°C, -10°C and -20°C. The viability test was done at 30 days regular intervals along with moisture content determination of stored seeds up to 300 days (until the seeds become non-viable).

Predication of the likelihood of desiccation sensitivity:

Daws *et al*., (2006) used the following equation to predict the likelihood of desiccation sensitivity (P)

$$P = e3.269 - 9.974a + 2.156b/1 + e3.269 - 9.974a + 2.156b$$

Where, a is seed coat ratio (SCR) and b is $\log_{10}$ (seed dry weight) in grams. This calculation can easily be carried out by inserting the following formula into a spreadsheet such as Excel (typed on one line):

$$= EXP((3.269 + (-9.974 * B4) + (2.156 * LOG(B5))))/(1 + EXP((3.269 + (-9.974 * B4) + (2.156 * LOG(B5)))))$$

If P is greater than 0.5, species are likely to be desiccation sensitivity.

Results:

Lakoocha mature fruit shape is obloid and spheroid with an average size 6.4 cm length, 6.8 cm width, 21.6 cm diameter and 153.5 gm weight. Fruit rind become thin and fruit turn yellow at the time of maturity. Fruit surface is slightly hairy. Pulp firmness become soft and changes the color from light pink to yellow after ripening. Latex exudation is very low. Mature seeds shapes are varied; irregular, spheroid, ellipsoid, elongated more or less flattened and pointed at the embryo end with an average number of seeds per fruit 22 and average seed size 0.98 cm length, 0.77 cm width, 2.18 cm diameter and 0.32 gm weight. In mature seed, the testa become thin, papery and off- white in color with the seed coat ratio is an average 0.09. Seeds are easily separable from pulp of mature fruit. Germination was found 100 % with 52 % moisture content of fresh physiologically mature seeds. Moisture content of different category of air desiccated seeds and germination percentage shown in **Table 1** and the changes in moisture content with desiccation in seeds of lakoocha is shown in **Figure 1**. Standard deviation moisture content pooled from 5 seeds in three replications, data deficit due to exact counting was not possible. Desiccation sensitivity (P) value is found 0.82, which indicates that lakoocha seeds are most probably desiccation sensitive as P value is more than 0.5. The moisture content of freshly harvested seeds was 52% with 100% germination. Germination of fresh seeds was initiated at third day of being set up for germination. The rate of seed germination was more in seeds without testa (papery parchment). Moisture content of different category of silica gel desiccated seeds and germination percentage (**Table 2)** and Changes in germination percentage with desiccation of lakoocha seeds is shown in **Figure 2.** Moisture content reduced gradually with desiccation and resulted in significant reduction in seed moisture content from 52 to 22 % after 21 hrs. Seeds desiccated to 37 % moisture showed 80 % germination and started to decline viability and further desiccations up to 26 % and 22% moisture, seeds showed 10 % and zero percentage of germination. The relationship between moisture content and viability of Lakoocha seeds during desiccation is shown in **Table 3.**

Table 1: Moisture content of different category of air desiccated seeds and germination percentage

Air desiccation (hours)	Moisture content (%)	Germination percentage
0	51.97 (±1.24)	100
24	47.07 (±0.56)	100
48	41.34 (±0.25)	100
72	37.44 (±0.73)	80
96	26.68 (±0.08)	10

Table 2: Moisture content of different category of silica gel desiccated seeds and germination percentage

Silica gel desiccation (hrs)	Moisture content (%)	Germination percentage
Fresh	**51.97 (±1.24)**	100
3	47.07 (±0.56)	100
6	**41.34 (±0.25)**	100
9	37.44 (±0.73)	80
12	**34.61 (±0.08)**	55
15	30.32 (±0.08)	20
18	**26.17 (±0.23)**	10
21	**22.66 (±0.23)**	0

Table 3: Relationship between moisture content and viability of Lakoocha seeds during desiccation

Silica gel desiccation (hrs)	Moisture content (%)	Germination percentage
0	**51.97 (±1.24)**	100
3	47.07 (±0.56)	100
6	**41.34 (±0.25)**	100
9	37.44 (±0.73)	80
12	**34.61 (±0.08)**	55
15	30.32 (±0.08)	20
18	**26.17 (±0.23)**	10
21	**22.66 (±0.23)**	0

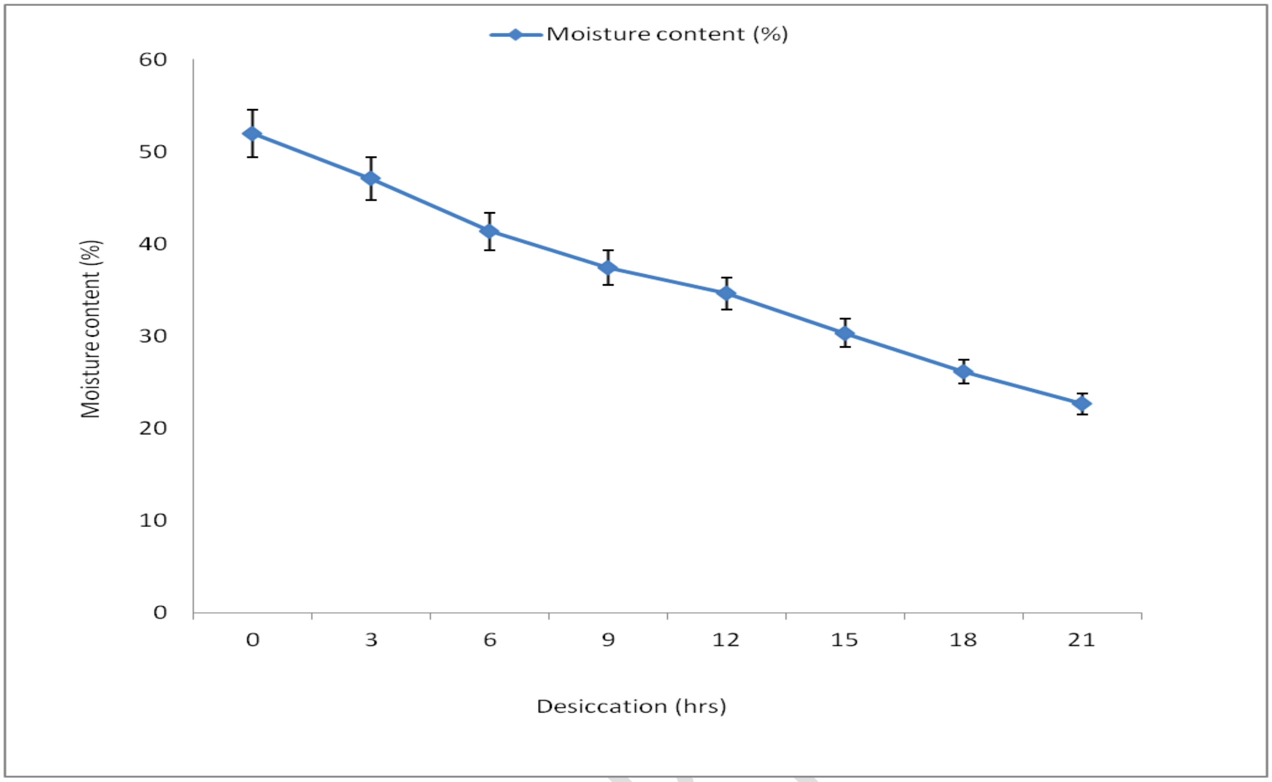

Figure 1: Changes in moisture content with desiccation in seeds of lakoocha

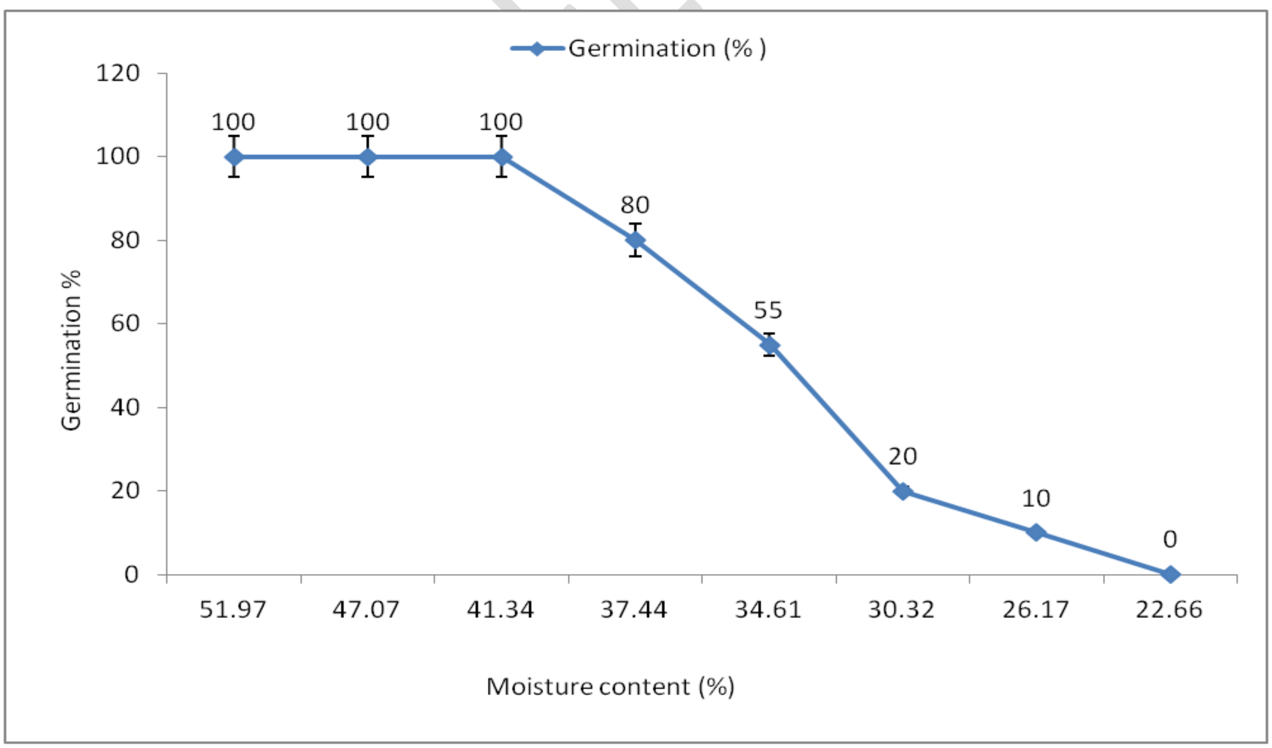

Figure 2: Changes in germination percentage with desiccation of lakoocha seeds

Table 4: Effect of different storage temperatures on longevity of Lakoocha seeds stored with 52% moisture content

Days after storage	**Germination (%)**				
	25°C stored seeds	15°C stored seeds	5°C stored seeds	-10°C stored seeds	-20°C stored seeds
0	100	100	100	100	100
30	100	100	100	0	0
60	100	100	80	0	0
90	90	100	35	0	0
120	40	100	0	0	0
150	0	100	0	0	0
180	0	100	0	0	0
210	0	75	0	0	0
240	0	20	0	0	0
270	0	0	0	0	0
300	0	0	0	0	0

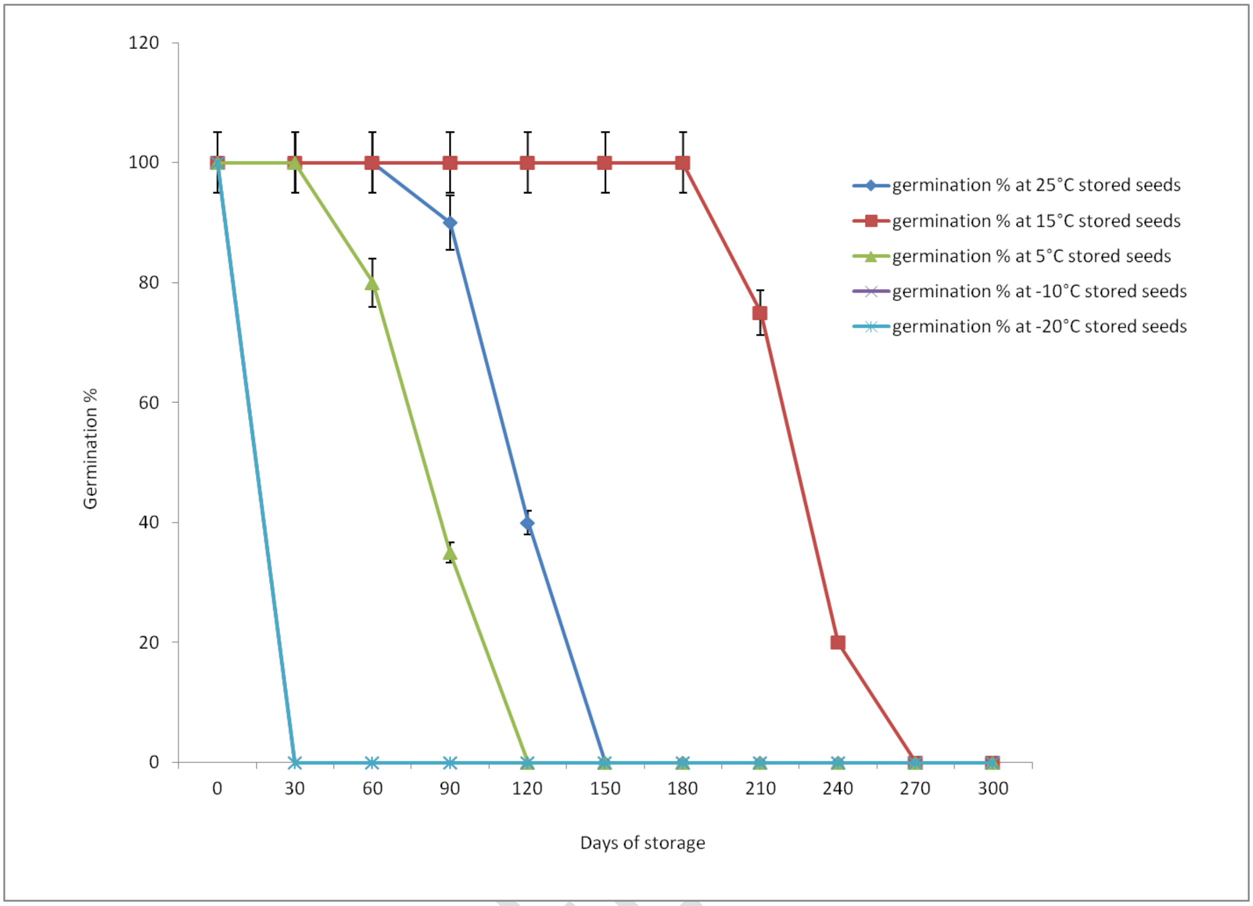

Figure 3: Effect of different storage temperature on longevity of lakoocha seeds

In longevity studies, the maximum longevity was found for 15°C temperature stored seeds and the viability was retained 100% up to 180 days of storage. After 180 days of storage, the longevity started to decline and lost viability completely after 240 days. At 25°C and 5°C storage temperatures, viability was lost completely after 120 and 90 days of storage. Seeds lost their viability very fast within 30 days, which were stored at the temperatures -10°C and -20°C. Effect of different storage temperatures on longevity of Lakoocha seeds stored with 52% moisture content is shown in **Table 4 and Figure 3**. Chin *et al*. (1984) reported that *Artocarpus heterophyllus* seeds lose viability when their MC decreases from 53 percent to 43 percent. This difference may be due to variation of environmental conditions of the area where the jackfruit tree grows or due to varietal difference. Moisture content was also calculated along with longevity testing and it was found that the moisture content gradually reduced with days of storage at both storage temperatures. Attempts to effective store of lakoocha seeds so far have been by manipulating the storage temperature. At 25°C storage temperature, the moisture reduced at faster rate as compare to 15°C storage temperature. An attempt was made to identify an optimum storage condition for a long time of storage. The variation in reduction rate of MC in 25°C and 15°C temperature stored seeds may be due to variation in temperature.

Discussion:

Jackfruit seeds have been included under recalcitrant category based on their storage behaviour (Chin et al., 1984; Fu et al., 1993; Chandel et al., 1995; Smith et al., 2001; Peran et al., 2004). Parameters like desiccation sensitivity, critical moisture content, chilling injury, assess the storage behaviour and recalcitrant nature of lakoocha seeds. Desiccation sensitivity has been reported in many recalcitrant seeds (Nautiyal and Purohit, 1985; Corbineau and Come, 1988; Finch-Savage, 1992; Chaitanya *et al*., 2000; Tompsett and Pritchard, 1993, 1998; Le Tam*et al*., 2004; Faria *et al*., 2004). Maintenance of high MC at ambient temperature is necessary for retention of viability in recalcitrant seeds. As recalcitrant seeds are shed from parent plant at high MC, they are highly sensitive to desiccation (Chin *et al*., 1984; Chin, 1988; Pammenter *et al*., 1994; Farrant *et al*., 1993; Chaitanya *et al*., 2000 a, b; Greggains *et al*., 2001).

Studies on critical moisture content (CMC) of recalcitrant seeds have shown that highly recalcitrant seeds which contain above 45-50 percent MC lose their viability when the MC reduces to 20-30 per cent (Danthu *et al*., 2000; Chaitanya *et al.*, 2000 a, b; Anil *et al*., 2000; Le Tam*et al*., 2004 and Malik *et al*., 2005). The CMC of lakoocha seeds was above 37 % which is more than the above given range, exhibiting their highly recalcitrant behaviour. It has been well established that recalcitrant seeds are metabolically active as they are shed with high moisture content (Chin *et al*., 1984; Pammenter *et al*., 1994) and desiccation causes damage these metabolic processes in highly hydrated conditions (Pammenter *et al*., 1994; Pammenter and Berjak, 1999). The response of recalcitrant seeds to desiccation depends not only on inherent characteristics of the species but also on the developmental status of the seeds and condition under which the seeds are dried.

According to Vertucci *et al.* (1994) desiccation sensitivity of recalcitrant seeds varies from species to species and on the physiological status of the seeds.

Moist storage of recalcitrant seeds at low temperature also has been suggested by many investigators (Chin *et al*., 1984; Anil *et al*., 1996, 2002; Danthu *et al*., 2000; Decruse and Seeni, 2003). Storage of lakoocha seeds at 15°C temperature in jam bottle, employed in the present study can be considered as a sort of moist storage and found to be an effective method of storage as viability was retained for about six months. During storage under this condition, only few percentages of moisture content reduction was observed throughout the storage period. Because of their high moisture content and desiccation sensitivity, moist or wet storage have been practiced in recalcitrant seeds (Bewley and Black, 1994; Copeland and McDonald, 1995; Baskin and Baskin, 2001). These seeds are found to be metabolically active and the seeds normally germinate within three days when retrieved from storage and subjected to germinate.

Hanson 1984 reported in Jackfruit that the longevity was found for 8 weeks at 53 % MC stored at room temperature. Imbibed seeds or seeds at moisture contents above 48% stored in polythene bags at 15°C with aeration can be maintained for 8-9 months whereas at temperatures below 10°C viability is rapidly lost after 2 weeks (Hanson & Suharto, 1984). Viability maintained for 2 years in moist storage at 15°C in polyethylene bags filled with perlite at 50% mc, storage temperatures of 5°C and 10°C are damaging (Fu et.al., 1993). *Lakoocha* Viability maintained for 1 month in open storage at room temperature (Campbell, 1980) ; There are two components of stress brought about during storage; water stress and temperature stress. At 15°C storage temperature, the longevity was found more because both stresses are very limited. Temperature was suitable for long term storage and due to less temperature moisture was also reduced very negligible. The losses occurring more rapidly at -20°C than at either 0°C or 15°C. Some tropical species of recalcitrant seeds are also killed by 'chilling' injury at temperatures less than 10- 15°C (King and Roberts, 1979; Chin, 1988). The term ‘recalcitrant’ was introduced by Roberts (1973) to describe seeds in which the storage life span was not increased in a predictable manner by reductions in water content and ambient temperature. Since then, the term has become descriptive for seeds that are desiccation-sensitive and of limited longevity.

The exact causes of recalcitrant seed death and its relationship with moisture content are not fully understood (Fu *et al*., 1993). It is stated that loss of viability could be either due to the moisture content falling below a certain critical value or simply a general physiological deterioration with time (Chin *et al*., 1984). It is also noteworthy that several pre-harvest factors determine the longevity, like cumulative effect of environment during seed maturation, harvesting, drying and the pre-storage environment, time of seed harvesting, duration of drying and the subsequent period before seed is placed in store (Hanson, 1984). The need for prolonging the viability of *A. lakoocha* seeds gains attention both on the basis of being a recalcitrant species and for the purpose of germplasm conservation. The seeds of *A. altilis* and *A. communis* are short-lived and optimum storability found for stored moist at 20°C temperature (Riley, 1981 Anon, 1993). In *A. hirsuta*, the viability is lost within 6 months in hermetic storage at room temperature with fresh moisture content of seeds (Kaul, 1979). Several of *Artocarpus* spp are not yet investigated for seed morphological and physiological features which determine the seed storage behaviour to facilitate their long-term storage. The development of strategies for the long-term storage of desiccation-sensitive germplasm remains a challenge for those involved in the conservation of endangered and commercially important species producing recalcitrant seeds. Qualitative and quantitative traits of fruits and seeds are used as maturity indices traits. These traits were helpful for differentiation of maturity stages and given the idea to choose right physiological maturity stage. But still, it is difficult because variation in plant to plant, asynchronous maturity, and different type of mature seeds within fruit etc. So, it is difficult to choose right physiological maturity stage of fruits and seeds but at some extent these traits are helpful. The ability of the seeds to tolerate desiccation appeared to relate closely to the native habitat in which the plants grow. *Lakoocha* grows in wet climates from south China to north India and hence exhibit characteristics of desiccation intolerance. Thus, the seeds may be considered to exhibit 'recalcitrant' seed storage behaviour based on their high moisture at shedding and sensitivity to desiccation and freezing. Lakoocha is under threatened due to less abundance of its population and commercially uncultivated although having so much economic importance. With reduction in moisture content (MC), a concomitant decline of viability was observed. The decline below the critical moisture content resulted to start in loss of viability. The moisture content reduced with a less percentage in stored seeds at 25°C and 15°C temperature.

Conclusion:

Seeds showed successful viability and germination before liquid nitrogen (LN) exposure of different moisture containing desiccated seeds but failed after LN exposure. The critical moisture content (CMC) was found near to 37 percentage. The longevity of seeds was retained up to 180 days of 15°C storage temperature as showed 100 % viability and germination. Lakoocha seeds are concluded as recalcitrant type basis on their seed's viability lost below CMC and if seeds stored at a freezing temperature. Cryopreservation success of seeds also in other experiments has not been found for different moisture containing of desiccated seeds. Seeds exhibit no dormancy and are physiologically sensitive to desiccation and freezing. So, it can ensure that lakoocha exhibit recalcitrant seed storage behaviour. For long-term conservation of indigenous germplasm of these species, germplasm conservation centers may be established besides maintaining *in situ* natural populations.

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