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RESEARCH ARTICLE

Dynamics of water relations and recalcitrant behaviour of jackfruit (*Artocarpus heterophyllus*) seeds

■ S. SHEELA AND NABEESA SALIM

SUMMARY

In order to study the desiccation sensitivity and water relations pertaining to recalcitrant behaviour of jackfruit seeds which contain very high MC (50%), the seeds were stored in different storage conditions like room-open (30±2°C), room-polythene (30±2°C) and refrigerator (4-8°C). The moisture content and germination percentage were determined at regular intervals of storage. During open air storage, these seeds remained viable only for 12 days and the moisture content was decreased resultantly the viability was lost when the MC reached below a critical level (32%). Room-polythene storage was found to be more effective since the seeds remained viable for about four months. In refrigerator-storage also viability was retained up to about four months. The data confirmed the recalcitrant nature of jackfruit seeds and interpreted in terms of distribution of moisture content superimposed with viability loss and metabolic changes that occur during storage.

Key Words : Desiccation, Jackfruit seeds, Moisture content, Storage, Viability

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Jackfruit (*Artocarpus heterophyllus* Lam.) is the largest among edible fruits and belongs to family Moraceae. 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). Recalcitrant seeds are highly sensitive or intolerant to desiccation because of their high moisture content at the time of shedding due to the lack of maturation drying (desiccation) on the mother plant. Desiccation sensitivity has been reported in the recalcitrant seeds of *Hevea braziliensis* (Chin *et al*., 1981), *Shorea* species (Nautiyal and Purohit, 1985 ; Corbineau and Come, 1988; Finch-Savage, 1992; Chaitanya *et al*., 2000 a b), *Aesculus* species (Tompsett and Pritchard, 1993, 1998) *Avicennia* species (Farrant *et al*., 1995; Greggains *et al*., 2001; Le Tam *et al*., 2004) *Inga* species (Pritchard *et al*., 1995; Faria *et al*., 2004) *Theobroma cacao* (Hor *et al*., 1984; Li and Sun, 1999) *Machilus* species (Lin and Chen, 1995; Chien and Lin, 1997), *Garcinia* species (Malik *et al*., 2005). Short life span or longevity and /or storability of recalcitrant seeds is another important characteristic which is intimately associated with desiccation and resultant impact on water relations.

Even though desiccation sensitivity has been studied in a large number of recalcitrant seeds, the distribution of moisture content and the dynamics of water relations during storage of *Artocarpus heterophyllus* seeds remain to be elucidated. So the present investigation was an attempt to study the relation between the distribution of moisture content, viability and germination pattern of jackfruit seeds during desiccation during storage under open air condition in closed polythene bags and refrigerator compared to open air storage.

MATERIAL AND METHODS

Jackfruits (*Artocarpus heterophyllus* Lam.) for the present study were collected from a specific (marked) tree

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
NABEESA SALIM, Department of Botany, Physiology and Biochemistry Division, University of Calicut, CALICUT (KERALA) INDIA
Email: nabeesasalim@ gmail. com

Address of the Co-authors:
S. SHEELA, Department of Botany, Govt. Arts and Science College, KOZHIKODE (KERALA) INDIA

growing at Chathannur Village in Kollam District, Kerala State. Such a particular tree was selected because mature fruits become available during November to February as and when the rainy season erases and hence, the fluctuations in the distribution of moisture content get minimized. Fruits ripened on the mother plant were collected manually and brought to the laboratory, cut open and seeds were collected, depulped washed and wiped with clean towel and were surface sterilized by wiping with a clean towel wetted with 80 per cent ethyl alcohol.

Storage :

Surface sterilized seeds (approximately 500) collected from pooled sample of 3-4 of ripened fruits at a time were kept for desiccation in open trays at room temperature (30±2°C) and designated as room-open.

Another lot consisting of 600 seeds were kept for storage in sealed polythene bags providing sufficient space for air, and kept at room temperature (30± 2ºC) designated as (Room-polythene). A third lot consisting of about 300 seeds were kept in sealed polythene bags in the lowest tray of refrigerator (4-8°C).

Sampling :

Seeds stored at open room condition were sampled for moisture content determination and viability studies at an interval of 4, 8, 12, 13, 14, 15 and 16 days after storage. Fresh seeds immediately after collection served as the control (0 sample). Seeds of other two types of storage (Room-polythene and refrigerator) were sampled for germination and moisture content (MC) determination at an interval of 10 days each up to 130 days (until the seeds become non-viable).

Viability studies :

Fifty seeds each in duplicate of control and stored at various conditions were sampled as described above and were kept for germination in the Petri plates lined with moist Whatman No. 1 filter paper (between paper). Protrusion of radicle was taken as the criterion of germination. The number of germinated seeds was noted daily and the germination percentage was calculated.

Moisture content determination :

Ten seeds in duplicate of control and stored seeds at each interval were taken and fresh weights were determined using Shimadsu Ax 120 Electronic balance. Then the weighed seeds were kept in hot air oven at 100°C for one hour and then at 60°C. Drying and weighing were repeated until concordant values were obtained. MC percentage was calculated as suggested by ISTA (1985). All experiments were repeated six times and the data were analyzed statistically and test of significance was done.

RESULTS AND DISCUSSION

The experimental findings obtained from the present study have been discussed in following heads:

Moisture content and viability :

Fresh (control) seeds contained 50.19 per cent MC and showed 100 per cent germination and cent per cent viability was maintained up to 10th day of desiccation under room-open condition (Table 1, Fig. 1). A gradual but negligible reduction of moisture content was observed up to 10th day and at this interval there was a 20 per cent reduction in initial moisture content resulting in sharp decline between 10th and 12th day of desiccation and during this period viability was reduced to 80 per cent when the MC was 33.58 per cent. With reduction in MC, a concomitant decline of viability was observed.

Table 1: Relationship between moisture content and viability of jackfruit seeds during desiccation (room-open storage)

Days of desiccation	MC %	Germination %
0	50.19±1.59	100
4	47.99±1.84	100
8	43.15±1.46	100
10	41.15±1.73	100
12	33.58±2.10	78.00±3.46
13	31.81±2.47	46.00±2.12
14	30.21±0.89	41.60±3.24
15	27.26±1.11	17.56±2.10
16	25.70±1.15	8.00±1.80

The decline below the critical moisture content (33%) resulted in loss of viability. Only 46 per cent of seeds remained viable on 13th day and the seeds were considered nonviable seed technologically.

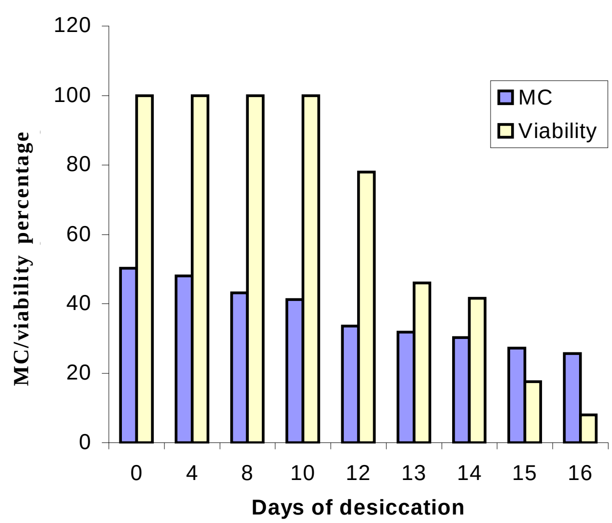

Fig. 1: Effect of room-open storage (desiccation) on moisture content and viability percentage in jackfruit seeds

In room-polythene condition, the seeds showed a slight increase in moisture content on 10th day of storage (Table 2, Fig. 2 and 3) and ,thereafter, the MC showed only insignificant changes (reduction/increase) up to110th day of storage. Cent per cent viability was shown by room-polythene seeds up to 100 days of storage (Table 2) and a significant decrease (P< 0.01) was observed in germination percentage. After 110 days of storage, the germination percentage declined sharply and on 130th day all seeds became non-viable.

In the case of seeds stored in refrigerator, negligible increase in MC was observed during the initial days up to 40th day (Table 2, Fig. 2 and 3). Thereafter, the moisture content was decreased and maintained the same up to 90th day of storage. Then these seeds showed a gradual decline in MC on 100th day and remained unchanged during the rest of the storage period. In these seeds the viability was100 per cent up to 90th day of storage (Fig. 3) and afterwards, the germination percentage was decreased significantly (P<0.01). After 110th day germination percentage declined and none of the seed was viable on 130th day of storage.

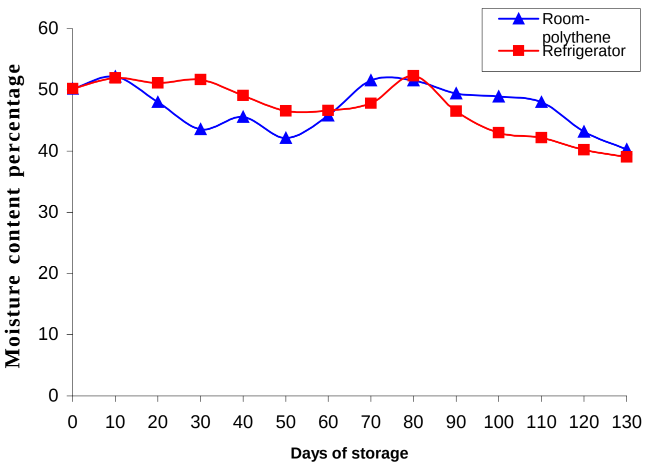

Fig. 2: Effect of storage conditions on moisture content per centage in jackfruit seeds under different conditions

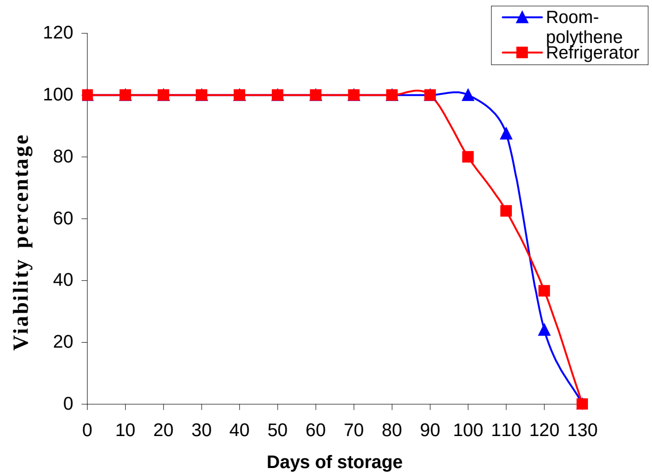

Fig. 3: Effect of storage conditions on viability percentage in jackfruit seeds under different conditions

The stored seeds in both the conditions maintained the same MC during the initial days up to 90 days. In room-polythene and refrigerator, the MC was declined significantly only after 110 and 90 days of storage, respectively.

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.*, 1981; 1984; Chin, 1988; Pammenter *et al.*, 1994; Farrant *et al.*, 1993; Chaitanya *et al.*, 2000 a, b; Greggains *et al.*, 2001). Hence, life span of

Table 2: Moisture content and viability of jackfruit seeds during storage under different conditions

Days of storage	Room polythene		Refrigerator	
	MC %	Germination %	MC %	Germination %
0	50.19±1.59	100	50.19±1.59	100
10	52.15±1.32	100	51.92±1.98	100
20	47.99±2.17	100	51.13±2.16	100
30	43.56±1.39	100	51.65±1.43	100
40	45.55±3.01	100	49.05±1.23	100
50	42.12±1.53	100	46.50±2.49	100
60	45.80±2.19	100	46.59±1.36	100
70	51.53±1.92	100	47.80±3.10	100
80	51.55±2.14	100	52.27±1.82	100
90	49.43±2.36	100	46.46±2.96	100
100	48.90±1.05	100	42.97±1.34	80± 2.56
110	48.00±3.21	87.5±2.15	42.14±2.17	62.5±3.12
120	43.15±1.86	24±1.89	40.18±1.54	36.66±2.16
130	40.24±1.94	0	39.04±3.06	0

recalcitrant seeds is very short which varies from a few days to few months.

Fresh seeds of ripenedjackfruit contain 50 per cent MC and exhibit cent per cent germination up to 12 days and the corresponding MC was in the range of 33 to 41 per cent (Table 1). These observations are suggestive of the highly recalcitrant nature of jackfruit seeds whereas Chin *et al*. (1984) reported that *Artocarpus heterophyllus* seeds lose viability when their MC decreases from 53 per cent to 43 per cent. This difference may be due to variation of environmental conditions of the area where thejackfruit tree grows or due to varietal difference. Smith *et al*. (2001) reported that rapid drying of embryonic axes of *Artocarpus heterophyllus* seeds to water content 0.4g g^{-1} dry weight can retain cent per cent viability and this view is almost in accordance with the findings of the present investigation because about 40 per cent MC was found to be essential for retaining viability irrespective of the mode of storage (Table 1 and 2).

Even though jackfruit seeds come under highly recalcitrant category due to their high MC and short life span (Table 1), about 10 per cent reduction in MC does not impose any adverse effect on viability since 100 per cent germination was retained upto 10 days as and when the MC was reduced to 41 per cent. But further 5 per cent reduction of MC within 2 days after 10^{th} day resulted in significant reduction of viability. Studies on critical MC of recalcitrant seeds have shown that highly recalcitrant seeds which contain above 45-50 per cent MC lose their viability when the MC reduces to 20-30 per cent (Tompsett and Pritchard, 1993; Lin and Chen, 1995; Connor and Bonner, 1998; Danthu *et al*., 2000; Chaitanya *et al*., 2000 a, b; Anilkumar *et al*., 2000; Le Tam *et al*., 2004 and Malik *et al*., 2005). The critical MC of jackfruit seeds comes under this 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 and germination is a continuum of development (Chin *et al*., 1981, 1984; Pammenter *et al*., 1994) and desiccation causes damage due to aberrant metabolic processes as the seeds are 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. Freshly harvested jackfruit seeds with 50 per cent MC show sensitivity towards desiccation only when the MC was reduced to 33 per cent during a period of 12 days under room open condition. So, the desiccation sensitivity is based on the water potential equivalents to 33 per cent MC *i.e.* the seeds become intolerant to desiccation at this level. According to Vertucci and Leopold (1986) and Vertucci *et al.* (1994) desiccation sensitivity of recalcitrant seeds varies from species to species and the principles of water relations are firmly based on the properties of water in the seeds at the various hydration levels corresponding to specific water potential ranges and on the physiological status of the seeds.

Moist storage of recalcitrant seeds at low temperature also (10-20ºC) has been suggested by many investigators (Chin *et al*., 1984; Anilkumar *et al*., 1996, 2002; Danthu *et al*., 2000; Decruse and Seeni, 2003). Storage of jackfruit seeds (room-polythene), employed in the present study can be considered as a sort of moist storage and found to be an effective method of storage because viability was retained for about four months (Table 2, Fig. 3). During storage under this condition, only negligible fluctuations in MC was observed maintaining more or less same MC throughout the storage period. Because of their high MC and desiccation sensitivity, moist or wet storage have been practiced in recalcitrant seeds (Chin *et al*., 1981; Bewley and Black, 1994; Copeland and McDonald, 1995; Baskin and Baskin, 2001). According to Danthu *et al*. (2000) in *Boscia senegalensis*, *Butyrospermum parkii*, *Cordyla pinnata* and *Saba senegalensis,* storage in wet-air tight containers resulted in maintenance of viability up to a few months retaining their MC above the critical level. More or less similar observations were obtained in jackfruit seeds under room-polythene storage.

Generally recalcitrant seeds which germinate when stored in air tight containers at ambient or low temperatures (Farrant *et al.*, 1988, 1995; Finch-Savage *et al*., 1993; Berjak *et al*.,1993). But jackfruit seeds never show visible sign of germination under room-polythene storage. Notwithstanding, these seeds are found to be metabolically active consisting of turgid cells with conspicuous nucleus, moderate amylase activity and the seeds normally germinate within 1-2 days when retrieved from storage and subjected to germinate (Sheela, 2007) without exhibiting any triphasic pattern of imbibition that occur in orthodox seeds (Bewely and Black, 1994). Pammenter *et al*. (1994) excellently reviewed the causes of recalcitrant behaviour of seeds and suggested that hydrated storage show degeneration process during long term storage but during short term storage, only non-degenerative changes occur since the seeds are metabolically active and show germination-associated changes.jackfruit seeds stored in room-polythene and refrigerator can be considered as storage under “hydrated” condition because MC registered only negligible reduction *vis-a vis* no sign of germination even after 100-110 days (Table 2, Fig. 2 and 3) and evidently no desiccation or chilling stress as the case may be is imposed. Nevertheless, prolonged storage is not possible under these conditions. Even though visible symptoms of germination are not seen, the most probable reason for seed death stored in closed polythene bags can be attributed to germination-associated changes initiated during earlier days of storage and later the metabolic changes associated with germination are impaired due to lack of sufficient oxygen or accumulation of stress induced metabolic products like free radicals or reactive oxygen species.

Distribution pattern of MC of jackfruit seeds during storage in room-polythene and refrigerator was almost similar and the values showed gradual insignificant reduction up to 110 and 100 days in room-polythene and refrigerator, respectively (Table 2,

Fig. 2). Thereafter, loss of viability is initiated and hence, some sort of water stress is seemed to be imposed on the seeds under storage. According to Pammenter *et al.* (1994) there are two components to the water stress brought about by germination-associated metabolism during hydrated storage of recalcitrant seeds *i.e.* the level of stress and the duration of stress. In the case of jackfruit seeds duration of stress is more prevalent because period of storage is critical in the maintenance of viability under room polythene and refrigerator storage. In the case of seeds stored at room open condition also the level and period of stress are important criteria because seeds undergo drastic dehydration (desiccation) and suffer a short term severe stress and lose viability within 13 days unlike the seeds that remain viable under 'hydrated storage'(both room-polythene and refrigerator) up to 100-110 days. Pammenter *et al*.(1994) opined also that while the intensity of water stress suffered by stored seeds is milder than that imposed by dehydration, the duration of the stress is considerably extended. Storage behaviour ofjackfruit seeds in both conditions, compared to the desiccation behaviour under room open is in consonance with the view of Pammenter *et al*.(1994) because only mild desiccation stress is found to be imposed in the seeds stored in room-polythene and refrigerator.

The processes of water relations occurring in seeds under 'hydrated storage' can be viewed in terms of the model proposed by Vertucci (1990, 1993) describing the thermodynamic states of water in seed tissues. Because the fresh seeds are able to initiate germination when shed and the osmoticum may be optimum for germination-associated metabolic activities and hence seeds probably possess 'type 4 and 5' water, which are the water with properties of dilute solutions (Vertucci *et al*., 1994). Jackfruit seeds under room-polythene storage, show no change in MC, although metabolic changes do occur resulting in starch depletion and concomitant sugar accumulation up to 110 days and consequent viability loss (Sheela, 2007). It can be presumed that the properties of 'type 4 and 5' water with the properties of dilute solutions get changed due to further dissolution of metabolites, particularly sugars resulting in the depletion of water potential. Even though viability loss is initiated when the MC exhibit only negligible reduction in the seeds stored in room polythene and refrigerator, significant reduction of water potential do occur owing to the increased concentration of sugars.

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