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Effect of Storage Treatments on Physiological and Anatomical Changes in Desiccation Sensitive Curry Leaf, *Murraya koenigii* (L.) Sprengel Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. Author KA carried out the experiment and wrote the manuscript. Author KR designed the study, guided, interpreted the data and validated the manuscript. Author SS participated in the review of the work and manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Curry leaf, *Murraya koenigii* (L.) Sprengel is mainly propagated through seeds, which have poor storage potential due to recalcitrant in nature. The present study evaluated the suitable storage method for prolonging the viability of the seed. The results showed that the shelf life of curry leaf seed was extended upto 40 days by storing in 300 gauge polythene bags at 10°C. However, the seeds stored under environmental condition maintained the viability upto 10 days only. Also, the seeds stored along with moist media have not better way for extending the shelf life when compared with seed stored at 10°C. The maximum seedling vigour and least electrical conductivity and free sugars were found in the seeds stored at 10°C. Seed chlorophyll, total phenols, protein and α-amylase activity were reduced during seed storage and the reduction was lower when the seeds were stored at 10°C. The damage in cell structures was also found in the desiccated seeds that the results showed that the curry leaf seeds can be stored upto 40 days in 300 gauge polythene bag at 10°C.

Keywords: Curry leaf; recalcitrant seed; storage temperature; storage medium; viability; cellular changes.

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1. INTRODUCTION

Curry leaf, *Murraya koenigii* (L.) Sprengel is an important inevitable spice species in India. The origin of curry leaf is Indian subcontinent region and distribution is extended upto India, Pakistan, Sri Lanka, Bangladesh, Malaysia, Andaman Islands, Australia, Southeast Asia, South Africa, Pacific Islands and Reunion Islands. In India, it is spread from Eastwards Assam to Southwards Tamil Nadu. India is the largest producer and consumer of curry leaf in the world. The leaves are the major economic part of the plant and it is added to make flavour in Indian foods. The major constituents responsible for flavour and aroma have been identified as sabinene, pinene, cadinol, cadinene and caryophyllene. Also, the leaves contain the volatile oil and glycoside called "Koenin" which helps for easy digestion in human digestive system. In Indian ayurvedic and unani prescriptions, curry leaf plays an important role in curing many diseases [1].

Curry leaf is propagated through seeds and root stocks, but the root stocks are produced only in very few plants compare to seeds. So, the seeds are mainly used as the propagating material for better plant population. However, curry leaf seeds shows the recalcitrant behaviour and its viability decreases quickly [2]. The recalcitrant seeds cannot be stored for longer time with reduction of humidity or storage at low temperature. Generally, the recalcitrant seeds are containing high water level ranging from 30 to 70% [3]. So, the reduction in water content causes desiccation in cells and leads to death of the seeds. The majority of the recalcitrant seeds are from the humid warm tropical forest environment. So, they are not in a position to tolerate low temperatures and also the seeds of several species are killed even at sub-ambient temperatures. In recalcitrant seeds, sudden decrease in germination at certain water level is known as 'Critical Moisture Content' [4] or 'Lowest Safe Moisture Content' [5]. The researches in past 50 years have revealed that the recalcitrant seeds can only be stored for short periods of 1 - 12 months. The desiccation damage of recalcitrant seeds is due to absence or incomplete expression of physical characteristics of cells and intracellular constituents, metabolic 'switching off' and 'switching on' mechanism, accumulation of putative protective substances including Late Embryogenic Abundant (LEA) proteins, operation of repair systems, sucrose and other oligosaccharides as well as amphipathic molecules and presence of oleosins [6].

Generally, storage is the major problem for recalcitrant seeds. So, any development in short-term storage will ease the problem of field collection and transportation to gene banks. The current successful short-term storage methods are limited to moist or imbibed storage, controlled atmospheric techniques and partial drying methods. These methods are successful for many of the recalcitrant seeds. In imbibed storage, the most common storage media are damp charcoal, saw dust, moist sand and other moisture conserving materials and chemicals. Also, the storage of seeds in controlled atmosphere with various gases or in sealed containers or waxing has had some success [3]. In addition, it is difficult to maintain quality of recalcitrant seeds during storage as the seeds themselves are variable in their size, moisture and viability. Also, there is a lack in understanding of the fundamental mechanisms and behaviour in recalcitrant group of seeds. So, the information on physiology of seed deterioration, seed longevity, storage behaviour and storage methods are inadequate. Hence, the present study was carried out to find the better storage method to prolong the viability of the curry leaf seeds and the changes caused during the desiccation of the seeds.

2. MATERIALS AND METHODS

The experiment was conducted in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2018-19. The freshly collected curry leaf fruits were used to extract the seeds and the uniform size seeds were obtained by manual grading. Then, the seeds were submitted to different storage treatments *viz*., T_1 - storage of seed alone; T_2 - storage of seed with fruit; T_3 - storage of seed with 5% moist sand; T_4 - storage of seed with 10% moist sand; T_5 - storage of seed with 5% moist charcoal; T_6 - storage of seed with 10% moist charcoal; T_7 - storage of seed with PEG 6000 (-5 bar) treated sand at 5%; T_8 - storage of seed with PEG 6000 (-10 bar) treated sand at 5%; T_9 - storage of seed at 5°C; T_{10} - storage of seed at 10°C; T_{11} - storage of seed at 15°C; T_{12} - storage of seed at 20°C; T_{13} - storage of seed at 25°C; T_{14} - storage of seed in water at 10°C. The procedure described by Bhattacharyya and Basu [7] was followed for moist storage of curry leaf seeds. In case of moist sand incubation, medium ground building quality sand was used. The sand was thoroughly washed in running tap water, dried in the sun and again dried overnight in the hot air oven at 100°C followed by cooling to room temperature. In charcoal incubation, fine quality material was autoclaved at 15 lb pressure for 20 min followed by cooling to room temperature. After that, the incubation materials were mixed with carbendazim @ 4 g kg^{-1} and then moistened with respective solutions as per the treatments. The seeds were uniformly covered with the pre-moistened incubation materials at the ratio of 1:3 and stored in loosely bound 300 gauge polythene bags under ambient condition (30±2°C and 60% RH). Then, the physiological and biochemical observations were taken at once in 10 days interval.

The seed moisture content were determined by hot air oven method at 105°C for 16±1 h. Germination test was conducted in four replications with 100 seeds each in sterilized sand medium [8] and the final evaluation was done at 50 days after sowing. The vigour index was calculated by multiplying the germination percentage and seedling length [9]. Curry leaf seed possess chlorophyll and the chlorophyll content was measured with spectrophotometer at 652 nm wavelength [10]. Electrical conductivity [11] and free sugar [12] of such leachate were also determined. Alpha-amylase enzyme activity [13], protein [14] and total phenol content [15] of the seeds were analyzed at 620 and 650 nm wavelength respectively, using UV-VIS spectrophotometer. The cell changes were evaluated in fresh and desiccated seeds with the scanning electron microscope (SEM) (Model: QUANTA 250) during seed storage. Before placing the sample in SEM, sputter coating process was carried out. In which, the specimen was covered with a thin layer of conducting material such as gold. The seed sample was placed in a sputter coater (Model: EMITECH SC 7620) for 15 min with the electric current of 20 mA. After that, the longitudinally sectioned seed sample was placed on the double-sided conductive carbon tap fixed on the stub in sample chamber of SEM. Thereafter, to attain high vacuum in the chamber, the filament was switched on and adjusted the various parameters like electron beam, intensity, voltage, spot size and emission current. The controlled electron beam was passed through the seed sample with the help of aperture at different voltages. Based on preference, the images were focused at different focusing stages. Finally, the needed images were captured. The data collected were submitted to statistical analysis by ANOVA [16] and the standard error deviation (SEd) and critical difference (CD) values were calculated at 5 % probability level.

3. RESULTS AND DISCUSSION

The results showed that highly significant differences were observed in seed moisture due to the storage treatments, periods and their interactions. Initially, just after collection the curry leaf seed possessed higher moisture content (47.6%). However, the moisture content decreased drastically and reached 5.6% at 50^{th} day of storage under environmental condition. Whereas, reduction in moisture content was gradual in the seeds stored in polythene bags along with incubation materials stored at about 60% relative humidity. Also, the seeds stored at low temperature has showed similar trend. In which, the curry leaf seeds stored in sealed 300 gauge polythene bags at 10°C kept the maximum germination (18%) upto 40 days with moisture content of 21.7%. Whereas, the seeds stored at ambient condition drastically reduced its viability without germination upto 20 days due to drastic reduction in moisture content (Tables 1 and 2). All other incubation treatments have retained the viability upto 30 days and thereafter, it lost completely. Similar results of low temperature storage were recorded in cocoa in which, the seeds stored at 15°C maintained better viability compared with 17°C [17]. Song et al. [18] found that *Hopea hainanensis* seeds stored at 15 to 20°C maintained viability upto 6 months. Tompsett [19] found that the sudden loss of germination at 6°C was noticed in *Shorea robusta* seeds but the viability was maintained better at 11°C. The present findings also reveal similar trends that seeds stored at low temperature (5°C) recorded sudden reduction in germination compared with seeds stored at 10°C. This might be due to the formation of ice crystals in the cellular level of high moist curry leaf seeds which was stored at very low temperature like 5°C. Roberts [20] also revealed such freezing damage in moist seeds which leads to sudden loss of germination [21]. However, Barman [22] found that tea seeds are viable when stored in sealed polythene bags at 4°C for a period of one year. Similarly, low temperature storage was found in many crops *viz.,* jack at 10°C [23], *Calophyllum brasiliensis* at 15°C [24], *Madhuca indica* at 15°C [25], arecanut at 20°C [26] and *Embelia ribes* at 10°C [27].

In the present investigation, the higher seedling vigour index (1026) was found in the seeds stored at 10°C. Whereas, seeds stored at 5°C showed drastic reduction in the vigour (Table 3). The reason might be due to that the low temperature decreases the metabolic activity by the formation of ice crystals of seed moisture in the cellular system. Tamari [28] found that *Hopea* seeds stored at low temperature showed reduced radical emergence. Similar findings of very low temperature sensitivity on seed vigour were recorded in *Hopea ponga* and *H. parviflora* [29] and arecanut [26]. Also, the seeds stored at ambient condition have recorded lowest vigour index (374) due to the desiccation death of the seeds. The chlorophyll content in curry leaf gets reduced from 1.07 to 0.23 mg g^{-1} irrespective of the treatments. In which, seeds stored without any treatment has showed drastic reduction (0.08 mg g^{-1}) when compared with other treatments. Among the treatments, seeds stored at 10°C have recorded the maximum chlorophyll (0.34 mg g^{-1}) (Table 4). Similarly, maximum total phenols and protein content were recorded in the seeds stored at 10°C during the entire storage period when compared with other treatments (Fig. 1). The reduction of phenol and protein content might be due to denaturation of contents at low or high temperatures. Similar results have been observed at sub-ambient storage temperatures [30]. In *Quercus robur* and *Q. borealis* acorns, decreased protein content were recorded which is directly correlated with decline in germination [31]. Loomis and Battaile [32] reported that storage at high and low temperatures leads to loss of phenol and protein compounds in recalcitrant seeds. Similarly, arecanut seeds showed loss of phenol and protein contents during exposure to desiccation temperatures [26].

The least electrical conductivity (79 μSm^{-1}) in the seed leachate was recorded in the initial period of seed storage, whereas it increased drastically to 709 μSm^{-1} at 50th day of storage. The seeds stored in water at 10°C (1088 μSm^{-1})and at ambient conditions (972 μSm^{-1}) have recorded maximum electrical conductivity compared to the seeds stored at 10°C (566 μSm^{-1}) (Table 5). Similar results were observed in sub-ambient temperatures due to the changes in membrane thickness and permeability [33]. Ching [34] reported the increased permeability of cellular membrane would result in the leaching of organic and inorganic substances in imbibing medium.

Table 1. Effect of storage treatments on seed moisture (%) in curry leaf seed

Treatments	Days after storage						
	0	10	20	30	40	50	Mean
T_1 - Storage of seed alone	47.6	22.8	12.6	10.3	7.8	5.6	17.8
T_2 - Storage of seed with fruit	47.6	31.9	19.8	13.7	10.5	8.9	22.1
T_3 - Storage of seed with 5% moist sand	47.6	40.2	23.6	18.8	15.3	10.3	26.0
T_4 - Storage of seed with 10% moist sand	47.6	41.4	24.9	20.7	15.8	10.9	26.9
T_5 - Storage of seed with 5% moist charcoal	47.6	41.7	27.9	23.8	17.5	11.5	28.3
T_6 - Storage of seed with 10% moist charcoal	47.6	42.2	31.3	24.1	17.8	11.8	29.1
T_7 - Storage of seed with PEG 6000 (-5 bar) treated sand at 5%	47.6	40.6	24.4	20.9	16.4	13.5	27.2
T_8 - Storage of seed with PEG 6000 (-10 bar) treated sand at 5%	47.6	41.8	25.5	21.2	16.6	13.9	27.8
T_9 - Storage of seed at 5°C	47.6	44.6	36.4	31.8	22.1	17.4	33.3
T_{10} - Storage of seed at 10°C	47.6	43.9	35.8	31.5	21.7	17.1	32.9
T_{11} - Storage of seed at 15°C	47.6	43.7	33.9	30.2	21.3	16.8	32.3
T_{12} - Storage of seed at 20°C	47.6	44.4	33.5	29.7	20.5	15.9	31.9
T_{13} - Storage of seed at 25°C	47.6	42.8	24.7	20.1	17.9	15.3	28.1
T_{14} - Storage of seed in water at 10°C	47.6	44.9	40.6	35.9	31.5	27.8	38.1
Mean	47.6	40.5	28.2	23.8	18.1	14.1	

	Treatments (T)	Periods (P)	T×P
SEd	0.1	0.1	0.4
CD (P=0.05)	0.3	0.2	0.8

Table 2. Effect of storage treatments on germination (%) in curry leaf seed

Treatments	Days after storage						
	0	10	20	30	40	50	Mean
T_1 - Storage of seed alone	100 (87.1)	12 (20.3)	0 (2.8)	0 (2.8)	0 (2.8)	0 (2.8)	19 (25.8)
T_2 - Storage of seed with fruit	100 (87.1)	28 (32.0)	4 (11.5)	0 (2.8)	0 (2.8)	0 (2.8)	22 (27.9)
T_3 - Storage of seed with 5% moist sand	100 (87.1)	42 (40.4)	16 (23.6)	6 (14.2)	0 (2.8)	0 (2.8)	27 (31.3)
T_4 - Storage of seed with 10% moist sand	100 (87.1)	52 (46.2)	24 (29.3)	10 (18.4)	0 (2.8)	0 (2.8)	31 (33.8)
T_5 - Storage of seed with 5% moist charcoal	100 (87.1)	58 (49.6)	36 (36.9)	16 (23.6)	0 (2.8)	0 (2.8)	35 (36.3)
T_6 - Storage of seed with 10% moist charcoal	100 (87.1)	78 (62.0)	58 (49.6)	20 (26.6)	0 (2.8)	0 (2.8)	43 (41.0)
T_7 - Storage of seed with PEG 6000 (-5 bar) treated sand at 5%	100 (87.1)	44 (41.6)	22 (27.9)	10 (18.4)	0 (2.8)	0 (2.8)	29 (32.6)
T_8 - Storage of seed with PEG 6000 (-10 bar) treated sand at 5%	100 (87.1)	58 (49.6)	28 (32.0)	14 (22.0)	0 (2.8)	0 (2.8)	33 (35.1)
T_9 - Storage of seed at 5°C	100 (87.1)	76 (60.7)	68 (55.6)	32 (34.5)	12 (20.3)	0 (2.8)	48 (43.9)
T_{10} - Storage of seed at 10°C	100 (87.1)	88 (69.7)	80 (63.4)	48 (43.9)	18 (25.1)	0 (2.8)	56 (48.5)
T_{11} - Storage of seed at 15°C	100 (87.1)	78 (62.0)	70 (56.8)	36 (36.9)	14 (22.0)	0 (2.8)	50 (45.0)
T_{12} - Storage of seed at 20°C	100 (87.1)	72 (58.1)	68 (55.6)	28 (32.0)	8 (16.4)	0 (2.8)	46 (42.7)
T_{13} - Storage of seed at 25°C	100 (87.1)	64 (53.1)	18 (25.1)	8 (16.4)	0 (2.8)	0 (2.8)	32 (34.5)
T_{14} - Storage of seed in water at 10°C	100 (87.1)	44 (41.6)	10 (18.4)	0 (2.8)	0 (2.8)	0 (2.8)	26 (30.7)
Mean	100 (87.1)	57 (49.0)	36 (36.9)	16 (23.6)	4 (11.5)	0 (2.8)	

	Treatments (T)	Periods (P)	T×P
SEd	1.5	1.0	3.7
CD (P=0.05)	3.0	1.9	7.4

(Values in parenthesis indicate the arc sine transformed values)

Tamari [28] also reported the increased electrical conductivity at low temperature storage in *Hopea* seeds. Similar findings were reported in cocoa [17], *Hancornia speciosa* [35], jack [23] and arecanut [26]. In addition, free sugars of curry leaf seed leachate was increased with the advancement of storage period. The maximum free sugars (10.28 μSm^{-1}) were recorded in the curry leaf seeds stored in water at 10°C. However, the seeds stored at 10°C recorded minimum (5.21 mg g^{-1}) at 50 days because of maintenance of better membrane integrity (Table 6). Similar findings were reported in cocoa [17], *Shorea robusta* [36], jack [7], *Aquilaria agalocha* [37], avocado [2] and arecanut [26].

The anatomical studies revealed that the absence of epidermal fibre and cell wall damages occurred in the desiccated seeds rather the fresh seeds (Fig. 2). These cell changes might be the cause for electrolyte leakage and further viability loss in curry leaf seed. Chin *et al*. [38] found the ruptured cell walls with fragmented cytoplasmic content in the seeds dried below the critical moisture content in rubber seeds. Similar results on subcellular damages were reported in many crops *viz., Avicennia marina* [39], rubber [40, 41], cocoa [42], *Zizania palustris* [43], arecanut [26] and *Inga vera sub sp. affinis* [44]. In addition, Souza *et al*. [40] reported that the viability loss in rubber seeds showed with decreasing trend in starch granules, lipids and proteins. Similar trends were observed in curry leaf with reduced starch grains in the cells of the desiccated seeds (Fig. 2). Also, the embryonic axis particularly tip of the radicle was heavily damaged and showed with shrinkage of parenchymatic cells in the desiccated curry leaf seed. Also, the epidermal protrusions were more in the fresh seeds and it was meagre in the desiccated seed (Fig. 3). In addition, the absence of epidermal fibre and cell wall damage were found in the desiccated seeds rather the fresh seeds (Fig. 4). Therefore, these cell changes might be the cause for viability loss in curry leaf seed.

Table 3. Effect of storage treatments on vigour index in curry leaf seed

Treatments	**Days after storage**						
	0	**10**	**20**	**30**	**40**	**50**	**Mean**
T_1 - Storage of seed alone	2050	191	0	0	0	0	374
T_2 - Storage of seed with fruit	2050	473	48	0	0		514
T_3 - Storage of seed with 5% moist sand	2050	735	229	85	0	0	517
T_4 - Storage of seed with 10% moist sand	2050	822	324	152	0	0	558
T_5 - Storage of seed with 5% moist charcoal	2050	1073	446	234	0	0	634
T_6 - Storage of seed with 10% moist charcoal	2050	1303	748	312	0	0	736
T_7 - Storage of seed with PEG 6000 (-5 bar) treated sand at 5%	2050	726	277	130	0	0	531
T_8 - Storage of seed with PEG 6000 (-10 bar) treated sand at 5%	2050	1021	370	197	0	0	606
T_9 - Storage of seed at 5°C	2050	1490	1102	515	164	0	887
T_{10} - Storage of seed at 10°C	2050	1654	1408	713	263	0	1026
T_{11} - Storage of seed at 15°C	2050	1342	1113	551	196	0	875
T_{12} - Storage of seed at 20°C	2050	1339	1020	434	105	0	825
T_{13} - Storage of seed at 25°C	2050	1126	284	122	0	0	597
T_{14} - Storage of seed in water at 10°C	2050	827	140	0	0	0	503
Mean	2050	1009	536	251	52	0	
	Treatments (T)		**Periods (P)**			**T×P**	
SEd	8.2		5.3			20.0	
CD (P=0.05)	16.1		10.6			39.6	

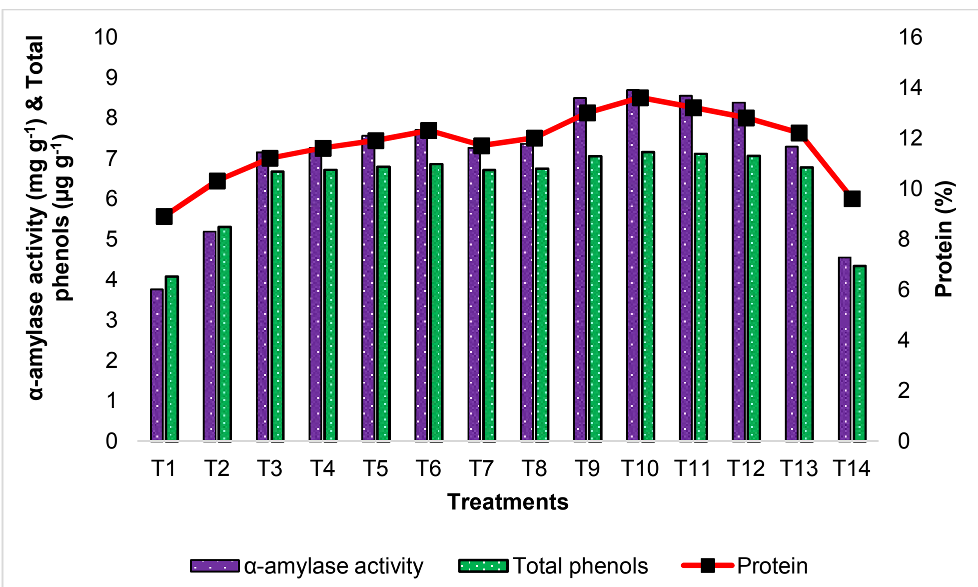

Fig. 1. Effect of storage treatments (for T_1 to T_{14} see text) on total phenol and protein and α-amylase activity in curry leaf seed

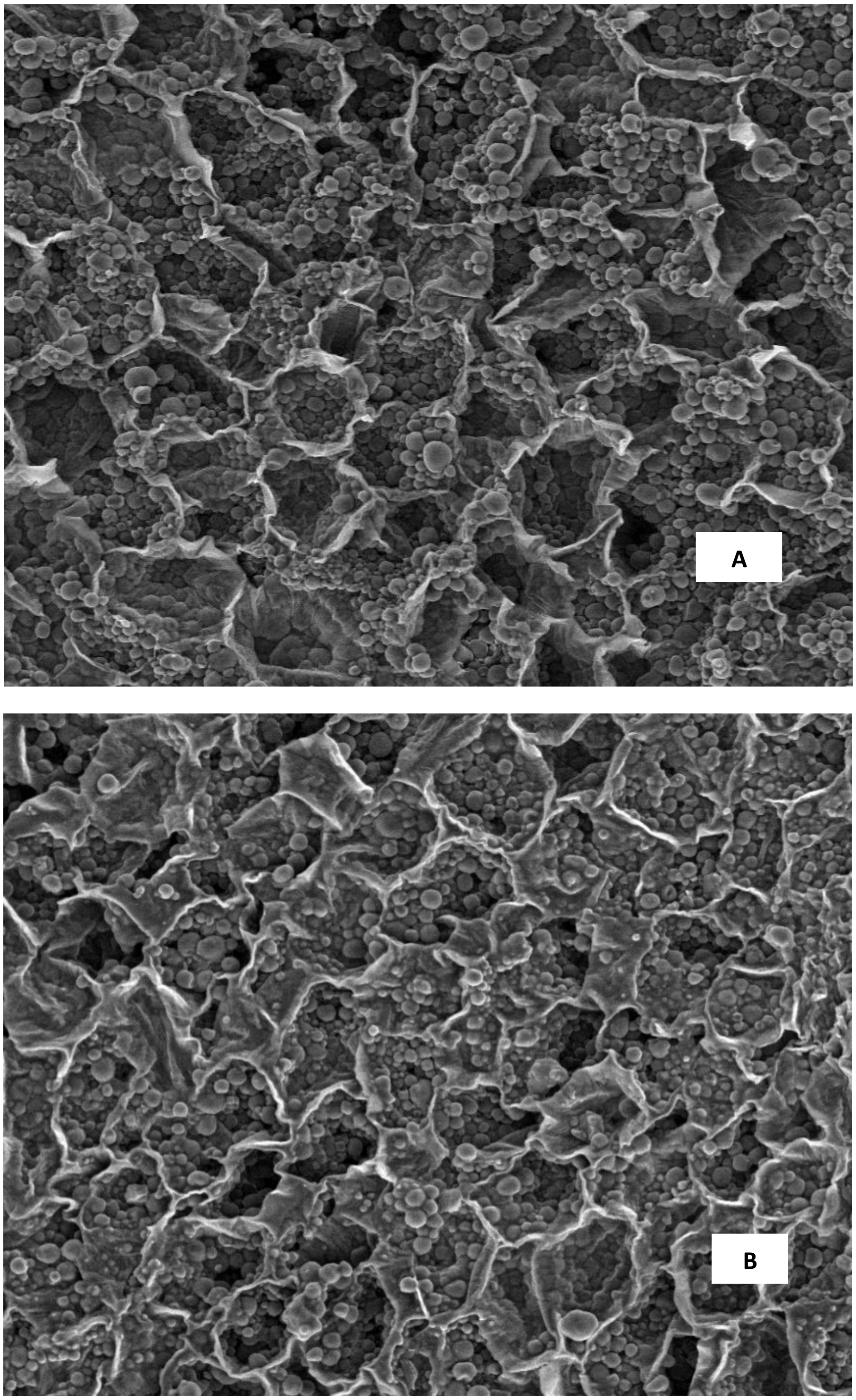

Fig. 2. Scanning electron micrographs of cell structure of curry leaf seed. (A) Fresh seed with more starch granules in the cells and intact cell wall (B) Desiccated seed with lesser aggregated starch granules in the cells and damaged cell walls (1000 X; 100 µm)

Table 4. Effect of storage treatments on seed chlorophyll (mg g^{-1}) in curry leaf seed

Treatments	Days after storage						
	0	10	20	30	40	50	Mean
T_1 - Storage of seed alone	1.07	0.40	0.28	0.20	0.13	0.08	0.36
T_2 - Storage of seed with fruit	1.07	0.42	0.37	0.26	0.16	0.12	0.40
T_3 - Storage of seed with 5% moist sand	1.07	0.44	0.41	0.34	0.31	0.22	0.47
T_4 - Storage of seed with 10% moist sand	1.07	0.47	0.44	0.36	0.32	0.24	0.48
T_5 - Storage of seed with 5% moist charcoal	1.07	0.50	0.48	0.40	0.35	0.27	0.51
T_6 - Storage of seed with 10% moist charcoal	1.07	0.55	0.51	0.41	0.36	0.29	0.53
T_7 - Storage of seed with PEG 6000 (-5 bar) treated sand at 5%	1.07	0.45	0.38	0.35	0.33	0.25	0.47
T_8 - Storage of seed with PEG 6000 (-10 bar) treated sand at 5%	1.07	0.47	0.41	0.37	0.37	0.26	0.49
T_9 - Storage of seed at 5°C	1.07	0.66	0.57	0.44	0.38	0.30	0.57
T_{10} - Storage of seed at 10°C	1.07	0.75	0.68	0.46	0.41	0.34	0.62
T_{11} - Storage of seed at 15°C	1.07	0.68	0.63	0.45	0.39	0.31	0.59
T_{12} - Storage of seed at 20°C	1.07	0.67	0.58	0.43	0.36	0.28	0.57
T_{13} - Storage of seed at 25°C	1.07	0.48	0.43	0.19	0.10	0.06	0.39
T_{14} - Storage of seed in water at 10°C	1.07	0.45	0.40	0.28	0.21	0.13	0.42
Mean	1.07	0.53	0.47	0.35	0.30	0.23	

	Treatments (T)	Periods (P)	T×P
SEd	0.004	0.002	0.01
CD (P=0.05)	0.009	0.005	0.02

Table 5. Effect of storage treatments on electrical conductivity (μSm^{-1}) of seed leachate in curry leaf seed

Treatments	Days after storage						
	0	10	20	30	40	50	Mean
T_1 - Storage of seed alone	79	321	582	741	830	972	588
T_2 - Storage of seed with fruit	79	298	355	548	729	897	484
T_3 - Storage of seed with 5% moist sand	79	282	327	486	577	680	405
T_4 - Storage of seed with 10% moist sand	79	243	272	471	563	671	383
T_5 - Storage of seed with 5% moist charcoal	79	236	263	453	558	662	375
T_6 - Storage of seed with 10% moist charcoal	79	230	245	446	542	659	367
T_7 - Storage of seed with PEG 6000 (-5 bar) treated sand at 5%	79	274	291	461	569	677	392
T_8 - Storage of seed with PEG 6000 (-10 bar) treated sand at 5%	79	239	284	478	553	673	384
T_9 - Storage of seed at 5°C	79	219	226	270	456	588	306
T_{10} - Storage of seed at 10°C	79	208	217	254	428	566	292
T_{11} - Storage of seed at 15°C	79	215	222	263	447	581	301
T_{12} - Storage of seed at 20°C	79	221	229	281	471	597	313
T_{13} - Storage of seed at 25°C	79	227	296	477	520	610	368
T_{14} - Storage of seed in water at 10°C	79	241	345	883	974	1088	602
Mean	79	247	297	465	587	709	

	Treatments (T)	Periods (P)	T×P
SEd	52.9	34.6	129.6
CD (P=0.05)	104.4	68.3	NS

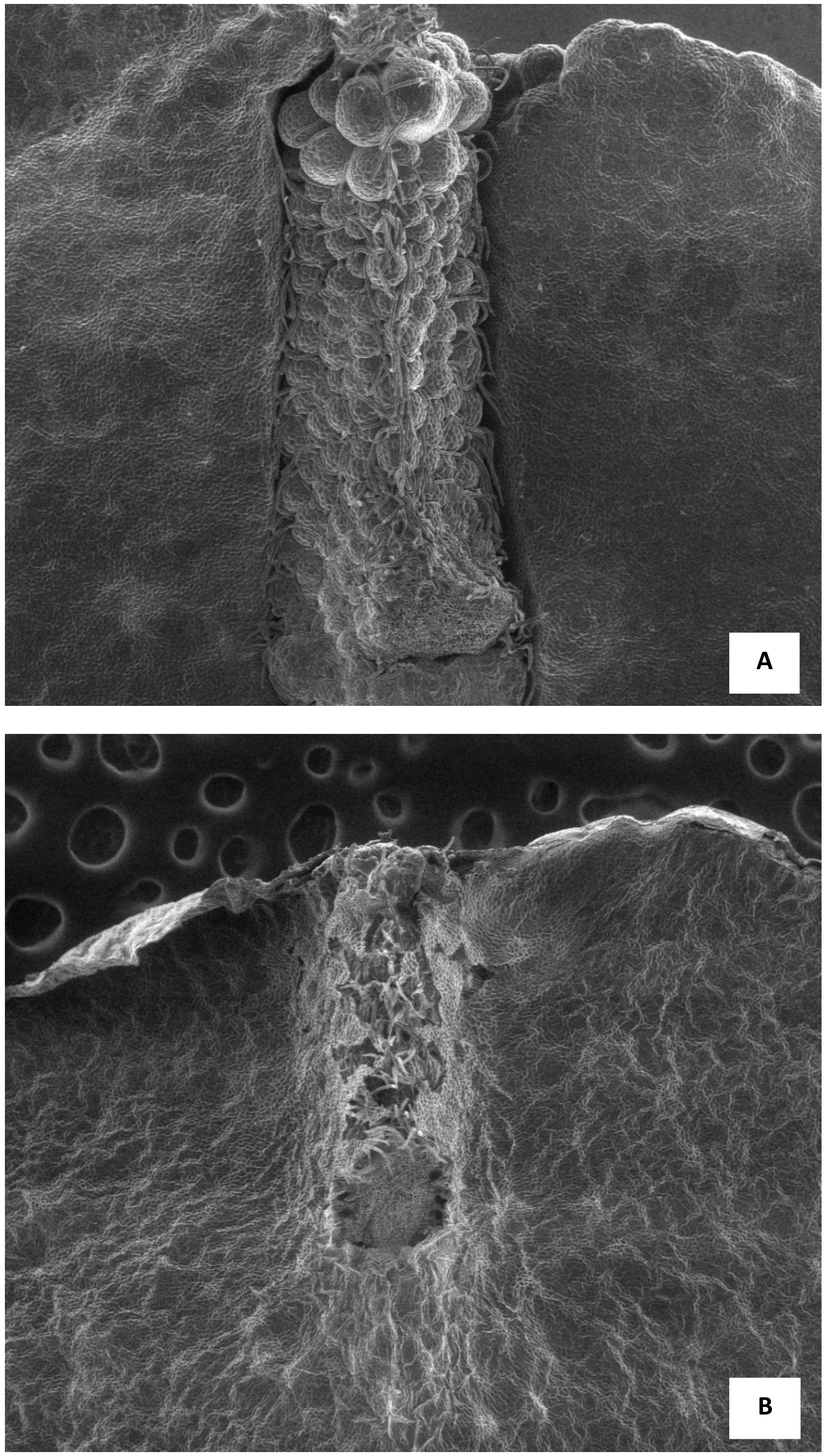

Fig. 3. Scanning electron micrographs of embryonic axis of curry leaf seed. (A) Fresh seed with integrated embryo (B) Desiccated seed with damaged embryo (100 X; 500 µm)

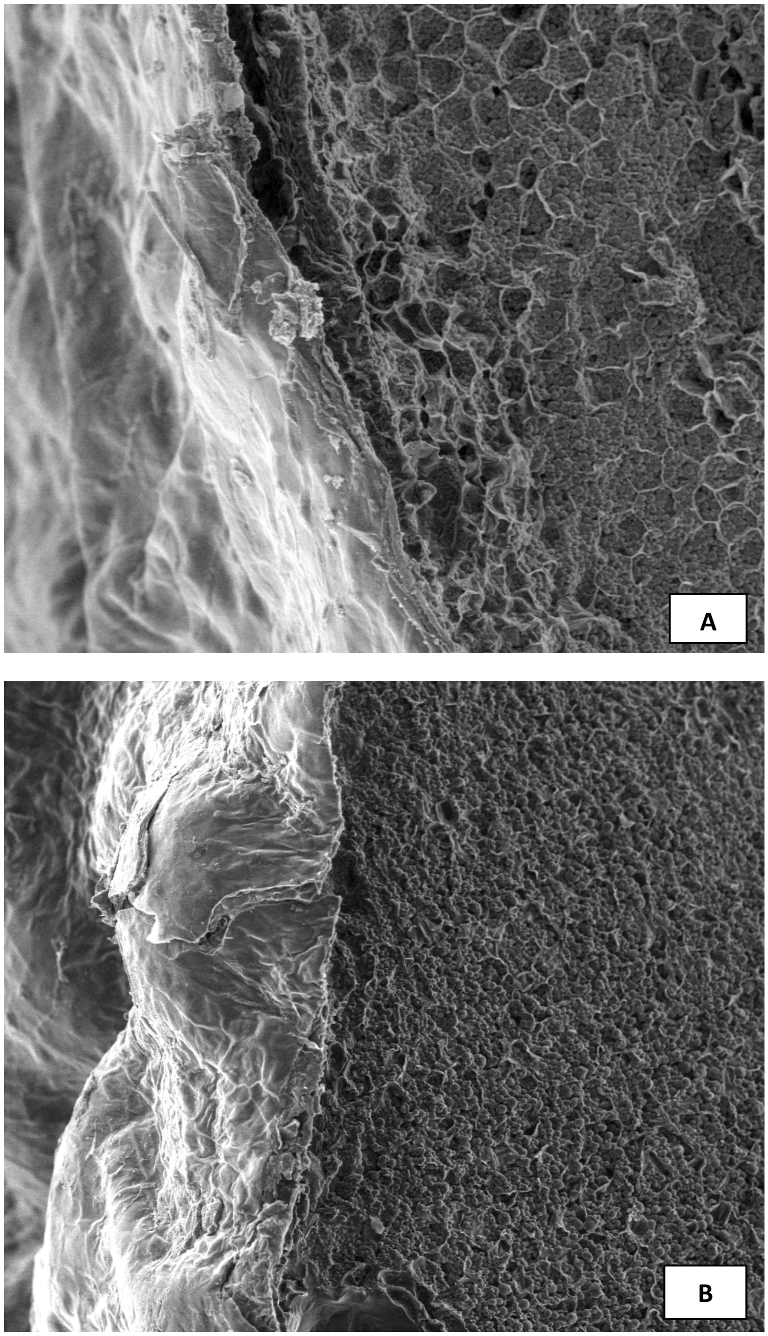

Fig. 4. Scanning electron micrographs of epidermal fibre, intact cell wall in curry leaf seed. (A) Fresh seed showing presence of epidermal fibre and intact cell wall (B) Desiccated seed with absence of epidermal fibre and ruptured cell wall (500 X; 200 µm)

In the present study, α-amylase activity was higher in the seeds stored at 10°C compared with other treatments (Fig. 1). Similar, results of reduction in amylolytic enzyme activity during desiccation of *Quercus robur* and *Q. borealis* seeds were studied earlier [45]. Disruption of metabolism due to loss of water in the cell structure might be the cause for the reduction in the enzymatic activities recalcitrant seeds [46].

Table 6. Effect of storage treatments on free sugars (mg g^{-1}) of seed leachate in curry leaf seed

Treatments	Days after storage						
	0	10	20	30	40	50	Mean
T_1 - Storage of seed alone	0.88	1.96	3.42	10.92	15.31	18.45	8.49
T_2 - Storage of seed with fruit	0.88	1.89	2.98	8.81	13.58	17.32	7.58
T_3 - Storage of seed with 5% moist sand	0.88	1.61	2.74	3.50	8.40	10.68	4.64
T_4 - Storage of seed with 10% moist sand	0.88	1.59	2.53	3.42	8.31	10.36	4.52
T_5 - Storage of seed with 5% moist charcoal	0.88	1.48	2.47	3.36	8.20	10.42	4.47
T_6 - Storage of seed with 10% moist charcoal	0.88	1.41	2.36	3.30	8.14	10.33	4.40
T_7 - Storage of seed with PEG 6000 (-5 bar) treated sand at 5%	0.88	1.57	2.41	3.44	8.38	10.47	4.53
T_8 - Storage of seed with PEG 6000 (-10 bar) treated sand at 5%	0.88	1.54	2.23	3.38	8.26	10.30	4.43
T_9 - Storage of seed at 5°C	0.88	1.41	1.48	1.69	1.92	5.48	2.14
T_{10} - Storage of seed at 10°C	0.88	1.12	1.23	1.56	1.84	5.21	1.97
T_{11} - Storage of seed at 15°C	0.88	1.47	1.55	1.64	1.89	5.39	2.14
T_{12} - Storage of seed at 20°C	0.88	1.51	1.62	1.78	1.96	5.73	2.25
T_{13} - Storage of seed at 25°C	0.88	1.54	1.76	1.98	2.50	7.29	2.66
T_{14} - Storage of seed in water at 10°C	0.88	1.61	3.08	14.12	21.38	24.08	10.86
Mean	0.88	1.55	2.28	4.49	7.86	10.82	

	Treatments (T)	Periods (P)	T×P
SEd	0.05	0.03	0.13
CD (P=0.05)	0.10	0.06	0.26

4. CONCLUSION

The curry leaf seeds possess very short storage life because of its recalcitrancy nature. The seeds are viable for about 10 days only if stored under ambient condition. However, the seeds can be stored upto 40 days by storing in 300 gauge polythene bag at the temperature of 10°C.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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