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Critical Role of Rate of Seed Drying in Maintaining the Seed Viability Potential of Recalcitrant Seeds of *Ligustrum perrottetii*

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ABSTRACT: Changes in physiological potential of the recalcitrant seeds of *Ligustrum perrottetii*, were examined after subjecting the seeds to differential rate of drying by exposing the fruits to variable relative humidity and silica gel ratios. Periodic observations on fruit and seed characteristics and topographical tetrazolium staining was conducted to observe the pattern of staining in the regions of embryonic axis and cotyledon. The experiments revealed that highest rate of drying caused by highest seed : silica gel ratio and lowest RH had resulted in higher electrical conductivity (dSm^{-1}) and *vice versa.* The higher rate of drying, had caused maximum damage to membrane integrity as revealed by high electrical conductivity of seeds with corresponding decrease in seed viability potential. Detailed observation of staining patterns in seeds stored in conditions causing differential rates of drying envisaged that embryonic axis tissues were more sensitive to drying compared to the cotyledons. Among the three temperatures *viz.,* ambient (Max 30.9 ^{o}C, Min 18.2 ^{o}C; 65% RH), 10^{o}C (41% RH) and – 3.3^{o}C (27% RH), the rate of drying was more gradual in 10^{o}C (41% RH), and it resulted in higher viability potential (20 percent of fully stained seeds) even after four weeks of storage. It is phenomenal be-cause, within four weeks of storage, all the seeds had become nonviable, in all other storage conditions. In future, recalcitrant seed storage should be experimented in a constant temperature of 10^{o}C at levels of relative humidity still higher than 41%, so as to further reduce the rate of drying which is crucial to reduce mechanical damage and metabolism induced damage which are characteristic of recalcitrant seed which are shed from the tree at very high seed moisture content.

Keywords: *Ligustrum perrottetii,* Membrane integrity Recalcitrant seeds, Rate of drying, Relative humidity, Seed viability, Temperature

Ligustrum perrottetii (Nilgiri Privet) is a small tree growing up to 5 m tall. It belongs to the family of Oleaceae. The species was named *perrottetii* in the honour of George Samuel Perrottet (1793-1870), a Swiss-born, French botanist and horticulturist, who spent the later part of his life working as a Botanist in Pondicherry, the erstwhile French colony in South India. Nilgiri Privet is important to the Nilgiri ecosystem. The tree is found along the margin of semi-evergreen forests up to 2300 m MSL, all along the Western Ghats, and is endemic to the region. The trees produce recalcitrant seeds, which are shed with high moisture content from the trees during the month of December.

Recalcitrant seeds are characteristic of remarkably short life, particularly when stored in open air. Seeds of Dipterocarpaceae and Lauraceae survive only for few days or months e.g. *Quercus, Populus* etc. Most of the Malaysian dipterocarp species exhibit reduction in germination rate within one week after dispersal [1]. Legumes of Central American and South-East Asian rainforests which produce fleshy seeds must germinate within few weeks or else they became nonviable. Seeds of *Shorea robusta*, which are common in moist and dry tropical forests, remain viable for only 7-10 days [2]. [3] studied the seed viability of *Syzygium cumini* by holding the seeds in equal volume of moist vermiculite in inflated polythene bags, ventilated at 26 ^{o}C. The initial germination of 100 per cent decreased to 75 per cent within 35 days of storage. Since the desiccation-sensitive, recalcitrant seeds continue to be metabolically active and are mostly short lived, the prevailing problem with respect to recalcitrant seeds is how to successfully store them in *ex situ* conditions [4-8]. *Ex situ* conservation of recalcitrant seeds is very important to ensure continuous supply of seeds for artificial regeneration.

Understanding of the physiological changes that occur in recalcitrant seeds during storage is imperative for developing a storage technique which will enable better maintenance of seed viability at least for a short period. Seed deterioration physiology of orthodox seeds which are known for long storage life is largely attributed to lipid peroxidation and free radical generation which affect membrane integrity as well as formation of toxic metabolites [9]. However, recalcitrant storage physiology has not been fully understood [10]. The short post harvest life of high moisture recalcitrant seeds have been mainly related to eventualities such as mechanical damage, metabolism induced damage and macromolecular denaturation [6]. The dehydration of seeds shed with high moisture leads to structural damgage of i) vacuoles ii) cytoskeleton and iii) cellular membranes, culminating in loss of seed viability. [7] suggested that dehydration of recalcitrant seeds leads to volume reduction and associated mechanical damage to cells by way of collapse of the vacuoles causing damage to embryos and embryonic axes. [6] put forth that when the seeds of high moisture levels dry, the membrane systems become irreversibly disrupted. Upon seed imbibitions, recalcitrant seeds are not capable of reinstating the original structure of the cellular membranes and reform completely [12].

In the desiccation sensitive recalcitrant seeds, with gradual loss in seed moisture content, the aqueous-based metabolism continues unabatedly and becomes unbalanced, owing to persistent generation of ROS even as the anti-oxidant systems collapse [13,14]. This is termed as metabolism linked damage [15]. [16] further envisaged that recalcitrant seeds are highly predisposed to ultrastructural damages *viz.,* initiation of subcellular changes such as vacuolation and withdrawal of plasma lemma as the days after harvesting advanced. Within a week of storage, mitochondria in the cells of plumule and radical apical meristem were less organized and there was marked increase in vacuolation.

Many storage studies on recalcitrant seeds have also brought out the strong effects of storage temperature and rate of drying on the post harvest life of recalcitrant seeds. [17] reported that the viability of recalcitrant or desiccation – sensitive seeds is affected by moisture loss and/or cold temperatures. [18] stated that the interrelations among temperature, moisture content and storage time impacts the shelf life of seeds. However, the extent to which each of the factors act as the critical cause for seed deterioration is debatable. The optimum temperature for safe storage life has been found to vary with species. Seeds of *Hopea* species could tolerate 4ºC [19]; *Trichilia emeria* seeds were damaged at 6ºC (20) and *Theobroma cacao* succumbed to temperatures below 10°C (21). Umboh (22) concluded that germination capability of *Shorea javanica* was greatly influenced by temperature. He reported that unlike 27± 2°C or 20± 2°C, < 10°C, registered zero germination percentage after 30 days after collection from the trees. [23] compared the viability loss of *Garcinia kola* when subjected to drying over silica gel (relatively fast drying) and in shade (slower drying). They observed that in both cases, whether seed or seed parts were dried in silica gel or under shade, viability was drastically reduced below this critical moisture content (30-32%). [24] concluded that under very slow-drying conditions, seed axes may be damaged by various deleterious processes that take place during the period of prolonged dehydration, ranging from the disruption of metabolic regulation to the failure of antioxidant systems. Certain other studies have proposed that fast drying actually enabled recalcitrant seeds or excised axes to survive at lower water contents and to improve the survival after cryopreservation (25, 26).

The objective of the present research was to examine the changes in physiological potential of the recalcitrant seeds of *Ligustrum perrottetii*, when subjected to differential rate of drying by exposing the fruits to varied storage temperatures, relative humidities and silica gel ratios .

MATERIALS AND METHODS

Seed collection

The fruits of *L. perrottetii* were collected from Vandisolai area of The Nilgiris situated at 11Ú22’23"N latitude and 76Ú49’6"S longitude. The maximum temperature ranged between 25 and 10°C while minimum temperature ranged between 20 and 0°C, with slight variations; the relative humidity ranged between 58 and 100%. From the identified trees, fruits were harvested during the first week of December, when they turned to brown colour. The fruits bunches collected were brought to the laboratory on the same day and the fruits were separated from the bunch.

Effect of relative humidity on fruit and seed characteristics of *L. perrottetii* during storage

Fruits of *L. perrottetii* were packed in 60 perforated butter paper covers @ 60 fruits per cover. Three desiccators were filled with saturated solutions of magnesium

chloride, calcium nitrate, and sodium nitrate to keep the relative humidity at around 32.4 per cent, 46.6 per cent and 63.3 per cent respectively [27]. In each of the desiccator, 20 packets of fruits packed in perforated moisture pervious butter paper covers were stored and maintained at room temperature (Max. 30.9°C; Min. 18.2°C; 47% RH). Packets were drawn @ 5 packets week^{-1} from each treatment for a period of four weeks and subjected to analyses of fruit and seed characteristics.

Effect of silica gel on fruit and seed characteristics of *L. perrottetii* during storage

Silica gel is a desiccant which can bring about rapid desiccation of seeds. Each of the species was packed in 60 zip lock polythene covers @ 60 fruits per cover. Fruits in zip lock covers were mixed with silica gel @ 1:1, 1:1/2 and 1: 1/4, by allotting 20 covers for each seed : silica gel ratio treatments. The covers were stored at room temperature (Max. 30.9°C; Min. 18.2°C; 47% RH). for four weeks. The silica gel was renewed, whenever it had changed into blue colour on absorbing sufficient moisture from the fruits. Fruit packets were drawn @ 5 packets week^{-1} from each treatment for a period of four weeks and subjected to analyses of fruit and seed characteristics.

Effect of storage temperature on fruit and seed characteristics of *L. perrottetii* during storage

Fruits were packed in 60 zip lock polythene covers @ 60 fruits per cover. 20 fruit covers were stored at three temperature levels *viz.,* room temperature (Max. 30.9°C; Min. 18.2°C; 65% RH) 10°C (41% RH) and -3.3°C (27% RH) for four weeks. Packets were drawn @ 5 packets week^{-1} from each treatment for a period of four weeks and subjected to analyses of fruit and seed characteristics. At the end of each week, five replicates of 30 fruits from each treatment were weighed to the nearest 0.0001 g in an electronic balance and expressed as fresh weight (g). Later, the rate of drying (%) was calculated using the following formula.

$$\text{Rate of drying (\%)} = \frac{\text{Fresh weight of fruits (Previous week)} - \text{Fresh weight of fruits (Current week)}}{\text{Fresh weight of fruits (Current week)}} \times 100$$

In order to measure the electrical conductivity of the seeds, five replicates of 30 seeds extracted from the fruits were immersed in 30 ml distilled water in 100 ml beakers. The beakers were covered and left in the laboratory at room temperature (Max. 30.9°C; Min. 18.2°C; 47% RH) for 16 h. After 16 h, the seeds were strained and the electrical conductivity of the steep water was measured in 'Deep Vision Model 601 E' Conductance Meter. The mean conductivity of the blanks was subtracted from each sample reading and the result was expressed as dSm^{-1} of 30 seeds.

Seed viability (%) was assessed by employing quick viability test [28]. Five replicates of 10 seeds extracted from the fruits were subjected 16 h soaking, and seed coats were removed carefully. Such seeds were subjected to incubation for 4 h in a solution of 1 per cent (w/v) 2, 3, 5 triphenyl tetrazolium chloride (TTC) prepared in phosphate buffer 0.05 M, at pH 7.3. After 4 h, seeds were washed and observed for staining pattern. The development of red colour was considered as indication of tissue viability. The pattern of staining of individual seed was observed using a dissection microscope and recorded. The seeds were categorized as viable and non viable by using the following assessment categories.

S. No.	Viable seeds	Non viable seeds
1	Complete staining of seed	Complete non-staining of seed
2	Staining in more than ½ the area + complete staining of embryonic axes	Staining in less than ½ the area + complete non-staining of embryonic axes
3		Embryonic axes fully non-stained

Statistical procedures: The experiments were carried out using a completely randomized experimental design (CRD). Result data (in percentage) were transformed to arcsine values before statistical analysis in order to unify the variance of the data [29]. For rest of the variable, non-transformed data were statistically analyzed using analysis of variance and treatment means were compared using LSD test at 0.05 level of probability, when the F-values were significant [30].

RESULTS AND DISCUSSION

In the present experiment, fruits of *L. perrottetii* were subjected to storage under varied levels of relative humidity, silica gel and temperature. Observations were made on fruit characteristics such as fresh weight and rate of drying and seed characteristics such as electrical conductivity and seed viability percent. The results obtained are presented hereunder:

i) Relative humidity

The initial fruit fresh weight recorded was 7.4150 g. Among the three different levels of relative humidity *viz.,* 32.4%, 46.6%, 63.3%, a steep reduction in fresh weight (48.1 per cent) was noted in the lowest RH of 32.4%, even in the first week of storage. Subsequently the reduction in fresh weight became gradual *viz.,* 21.4, 8.54 and 3.27 per cent in second, third and fourth week of storage. In the 46.6% RH, the reduction in fresh weight was 27.6, 19.4, 7.30 and 3.1 per cent, respectively and in 63.3% RH, the corresponding values were 26.9, 10.4, 7.10 and 5.31, respectively (Table 1).

Table 1. Effect of relative humidity on characteristics of stored fruits and seeds of *L. perrottetei*

Fresh Weight (g) of fruits

Relative Humidity (%)	Storage period (weeks)					
	Initial	1	2	3	4	Mean
32.4	7.4150	3.8442	3.1668	2.9443	2.8509	4.0442
46.6	7.4150	5.3670	4.3254	4.0090	3.8808	4.9994
63.3	7.4150	5.4169	4.8516	4.5070	4.2675	5.2916
Mean	7.4150	4.8760	4.1146	3.8201	3.6664	4.7784
	T	P	T*P			
SE d	0.09076	0.11718	0.20296			
CD (0.05)	0.18281	0.23601	0.40879			

Rate of drying (%) of fruits

Relative Humidity (%)	Storage period (weeks)				
	1	2	3	4	Mean
32.4	48.15	17.62	7.02	3.17	18.99
46.6	27.61	18.63	7.31	3.19	14.18
63.3	26.94	10.43	7.10	5.31	12.44
Mean	34.23	15.56	7.14	3.89	15.20
	T	P	T*P		
SE d	0.403	0.465	0.806		
CD (0.05)	0.817	0.944	1.635		

Electrical Conductivity (dSm^{-1}) of seeds

Relative Humidity (%)	Storage period (weeks)					
	Initial	1	2	3	4	Mean
32.4	1.90	2.67	2.73	2.95	3.01	2.65
46.6	1.90	2.30	2.53	2.88	3.00	2.52
63.3	1.90	2.06	2.50	2.63	2.91	2.4
Mean	1.90	2.34	2.58	2.82	2.97	2.52
	T	P	T*P			
SE d	0.045	0.059	0.102			
CD (0.05)	0.092	0.119	0.207			

Seed Viability (%)

Relative Humidity (%)	Storage period (weeks)					
	Initial	1	2	3	4	Mean
32.4	100	50	30	5	0	37
46.6	100	60	30	10	0	40
63.3	100	70	50	10	0	46
Mean	100	60	36.6	8.3	0	41
	T	P	T*P			
SE d	1.2	0.9	2.2			
CD (0.05)	2.5	1.9	4.4			

Eventually, the rate of drying was also found to be highest in 32.4% RH by the end of one week storage (48.15%), while in 46.6% and 63.3% RH, it was significantly lower *viz.,* 27.61 and 26.94 per cent, respectively. In the second week, however, rate of drying was highest in 46.6% RH (18.63 per cent) followed by 32.4% (17.62 per cent) and 63.3% (10.43 per cent). In the third week of storage, the differences in rate of drying among the three different levels of relative humidity narrowed to reach 7.02, 7.31 and 7.10 per cent in 32.4%, 46.6% and 63.3% RH, respectively. In the fourth week, 32.4% and 46.6% RH recorded lower rate of drying while the highest RH of 63.3% continued to cause significantly highest rate of drying (5.31 per cent) (Table 1).

Corresponding to the higher levels of drying, steep increase in seed electrical conductivity was recorded in the lowest relative humidity of 32.4%, within one week of storage (2.67 dSm^{-1}). At a higher RH of 46.6 and 63.3%, the seeds recorded lower values of 2.30 dSm^{-1} and 2.06 dSm^{-1}, respectively. After four weeks of storage, when the fruits had dried to a lower fresh weight irrespective of the level of relative humidity the electrical conductivity of seed recorded in all the three RH levels *viz.,* 32.4, 46.6 and 63.3% were found to be at par with each other by recording 3.01, 3.00 and 2.91 dSm^{-1}, respectively (Table 1).

The seed viability as assessed through quick viability test proved that in recalcitrant seeds of *L. perrotteiei,* high seed viability was found to be correlated with high fresh weight, and low rate of drying as well as low electrical conductivity (dSm^{-1}) of seeds. Within one week of storage, the seed viability reached the lowest level (50 per cent), in the lowest RH of 32.4% and highest rate of drying (17.62%) a higher electrical conductivity (2.67 dSm^{-1}) was noted (Table 1). In 46.6% and 63.3% RH, where comparatively lower rate of drying and electrical conductivity were recorded, the seed viability observed was also relatively higher *viz.,* 60 and 70 per cent, respectively.

The observation on tetrazolium staining pattern of *L. perrotteiei* seeds exposed the differential vulnerability of embryonic axis and cotyledon to the rate of drying (Figure 1). When the rate of drying was highest (32.4% RH), in the first week of storage, in almost 30 per cent of seeds complete loss of viability was among them, more importantly noted the embryonic axis of 20 per cent of seeds had lost viability. On the contrary, when seeds were stored in the higher RH of 63.3%, which recorded lowest rate of drying, none of the seeds showed complete loss of viability. However, in almost 30 per cent of seeds, embryonic axis alone was found to be totally unstained, although rest of the embryo was stained. The differential impact of rate of drying was more explicit in the fourth week after of storage, wherein at 32.4% RH, 40 per cent of the seeds had completely lost their viability, while, in 63.3% RH again only 10 per cent of the seeds showed total loss in seed viability.

ii) Silica gel

The influence of rapid drying on seeds of *L. perrotteiei* was also confirmed by storing the fruits in three different levels of silica gel ratios at ambient relative humidity and temperature. Absorption of moisture from fruits by the surrounding silica gel leads to drying of fruits. As the seed: silica gel ratio is increased the rate of drying of fruits is also expected to increase. The initial rate of drying was the highest (46.66 percent) in 1:1 seed: silica gel ratio, during the first week of storage compared to 32.85 and 17.88 per cent in 1:1/2 and 1:1/4 seed: silica gel ratio, respectively. The same trend continued for four weeks of storage, except in the second week when the 1:1/2 ratio recorded slightly higher level of rate of drying (13.02 per cent) compared to 1:1, 1:1/4 seed: silica gel ratios (Table 2). The lowest rate of drying in the 1:1/4 silica gel ratio had resulted in lower electrical conductivity (dSm^{-1}) and *vice versa*. The decrease in seed viability of *L. perrotteiei,* over the period of storage was found to be gradual in lowest silica gel ratio of 1:1/4, wherein the initial seed viability of 100 percent reduced to 70, 56.6, 16.6 and 0 percent in the first, second, third and fourth week after storage, respectively. However, in 1:1 ratio, the corresponding decrease in seed viability was 80, 70, 20 and 0 per cent, respectively. The results established that lower rate of drying and better maintenance of membrane integrity were fundamental to high seed viability potential of the seeds. As observed in low RH conditions, higher rate of drying observed in higher seed : silica gel ratio had led to rapid loss in seed viability. After one week of storage, in 1:1 ratio, the seed viability percent had reduced to 40 per cent while at 1:1/4 ratio, 90 per cent of seeds had retained the viability (Figure 2). Similarly, the higher rate of drying (1:1 ratio) had caused rapid damage to embryo (40 per cent) compared to 1:1/4 ratio, were only 10 per cent of embryo had lost viability.

iii) Temperature

In the third experiment, *L. perrotteiei* seeds were

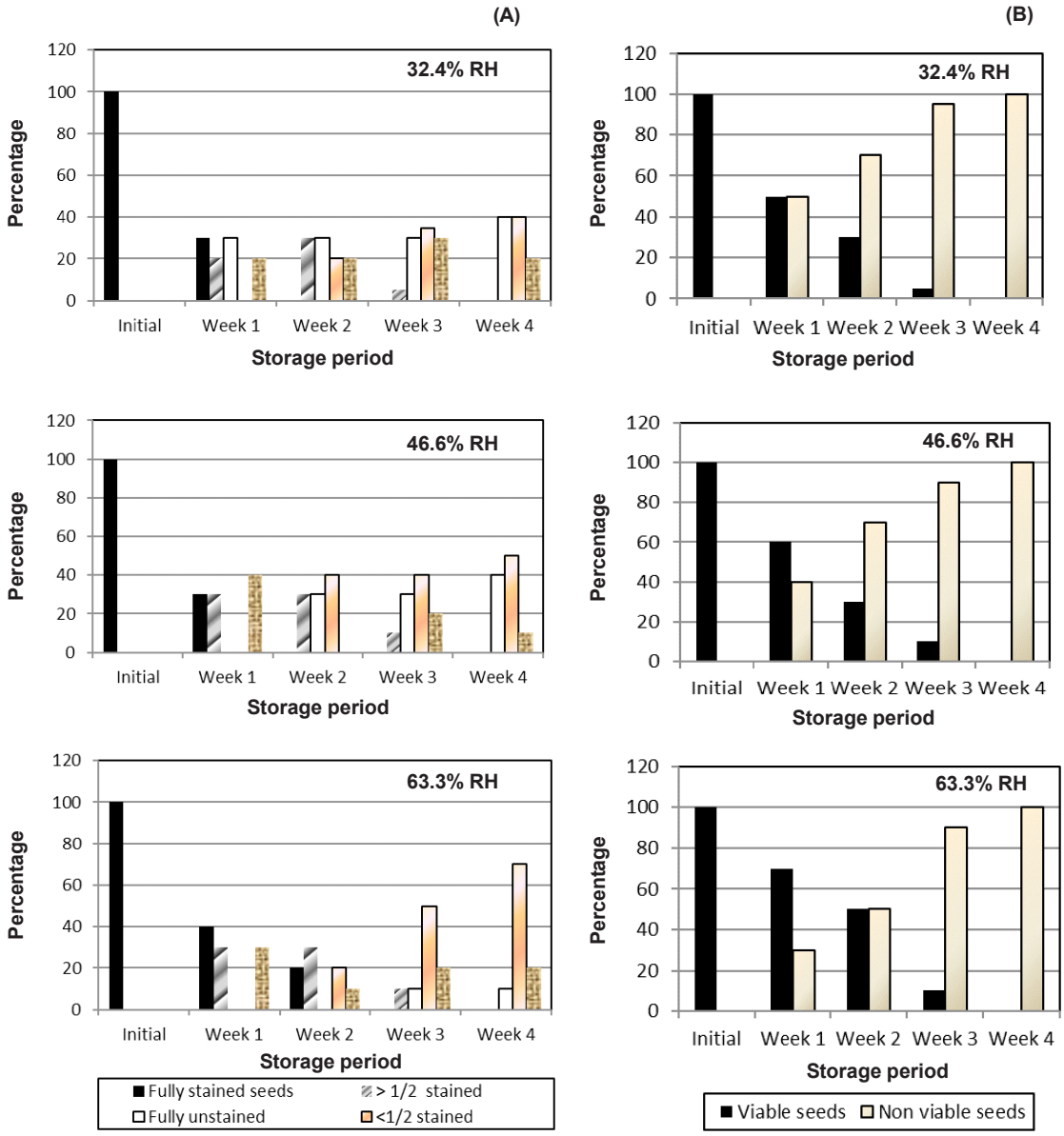

Figure 1. Effect of different relative humidity levels on (A) staining pattern of seed tissues and (B) total viable and non viable seed percentage

subjected to storage in three different temperatures viz., ambient (Max 30.9°C, Min 18.2°C; 65% RH), 10°C (41% RH) and – 3.3°C (27% RH). The fresh weight of the fruits decreased gradually in all the three temperatures of storage. However, the initial rate of drying was found to be very rapid in ambient conditions, followed by – 3.3°C and 10°C by recording 53.59, 5.07 and 3.30, respectively. Between the –– 3.3°C and 10°C, rate of drying was found to be steep in – 3.3°C, throughout the four weeks of storage. Although the rate of drying in ambient conditions aided by higher temperature, yet the seed electrical conductivity (dSm^{-1}) was much higher only in fruits stored in – 3.3°C, in the first, second and fourth weeks after storage (Table 3). This observation proves that nature of drying varies between ambient conditions and freezing conditions due to differences in relative humidity levels. The data brings out that under freezing conditions (-3.3°C) the damage to seed tissues is greater when compared to ambient conditions due to lower RH of 27%. The seed viability as estimated through quick viability test also revealed that the fruits stored in ambient conditions and 10°C recorded 100 per cent seed viability, while fruits

Table 2. Effect of seed : silica gel ratio on characteristics of stored fruits and seeds of *L. perrottete*

Fresh Weight (g) of fruits						
Seed : Silica gel (v/v)	Storage period (weeks)					
	Initial	1	2	3	4	Mean
1:1	7.4150	3.9549	3.5063	3.0632	2.9867	4.1852
1:1/2	7.4150	4.9787	4.3295	4.2449	4.1858	5.0307
1:1/4	7.4150	6.0890	5.7551	5.6564	5.5966	6.1024
Mean	7.415	5.0075	4.5303	4.3215	4.2563	5.1061
	T	P	T*P			
SE d	0.09638	0.12443	0.21552			
CD (0.05)	0.19413	0.25062	0.43409			
Rate of drying (%) of fruits						
Seed : Silica gel (v/v)	Storage period (weeks)					
	Initial	1	2	3		Mean
1:1	46.66	11.34	4.80	2.40		16.3
1:1/2	32.85	13.03	1.95	1.39		12.30
1:1/4	17.88	5.30	1.70	1.05		6.48
Mean	32.46	9.89	2.81	1.61		11.69
	T	P	T*P			
SE d	0.366	0.422	0.732			
CD (0.05)	0.742	0.857	1.484			
Electrical Conductivity (dSm^{-1}) of seeds						
Seed : Silica gel (v/v)	Storage period (weeks)					
	Initial	1	2	3	4	Mean
1:1	1.90	2.21	2.53	2.81	2.98	2.48
1:1/2	1.90	2.16	2.49	2.70	2.86	2.42
1:1/4	1.90	2.10	2.41	2.63	2.74	2.35
Mean	1.9	2.15	2.47	2.71	2.86	2.42
	T	P	T*P			
SE d	0.044	0.056	0.098			
CD (0.05)	0.088	0.114	0.198			
Seed Viability (%)						
Seed : Silica gel (v/v)	Storage period (weeks)					
	Initial	1	2	3	4	Mean
1:1	100	40	30	10	0	36
1:1/2	100	80	70	20	0	54
1:1/4	100	90	70	20	0	56
Mean	100	70	56.6	16.6	0	48.6
	T	P	T*P			
SE d	1.4	1.0	2.4			
CD (0.05)	2.8	2.2	4.9			

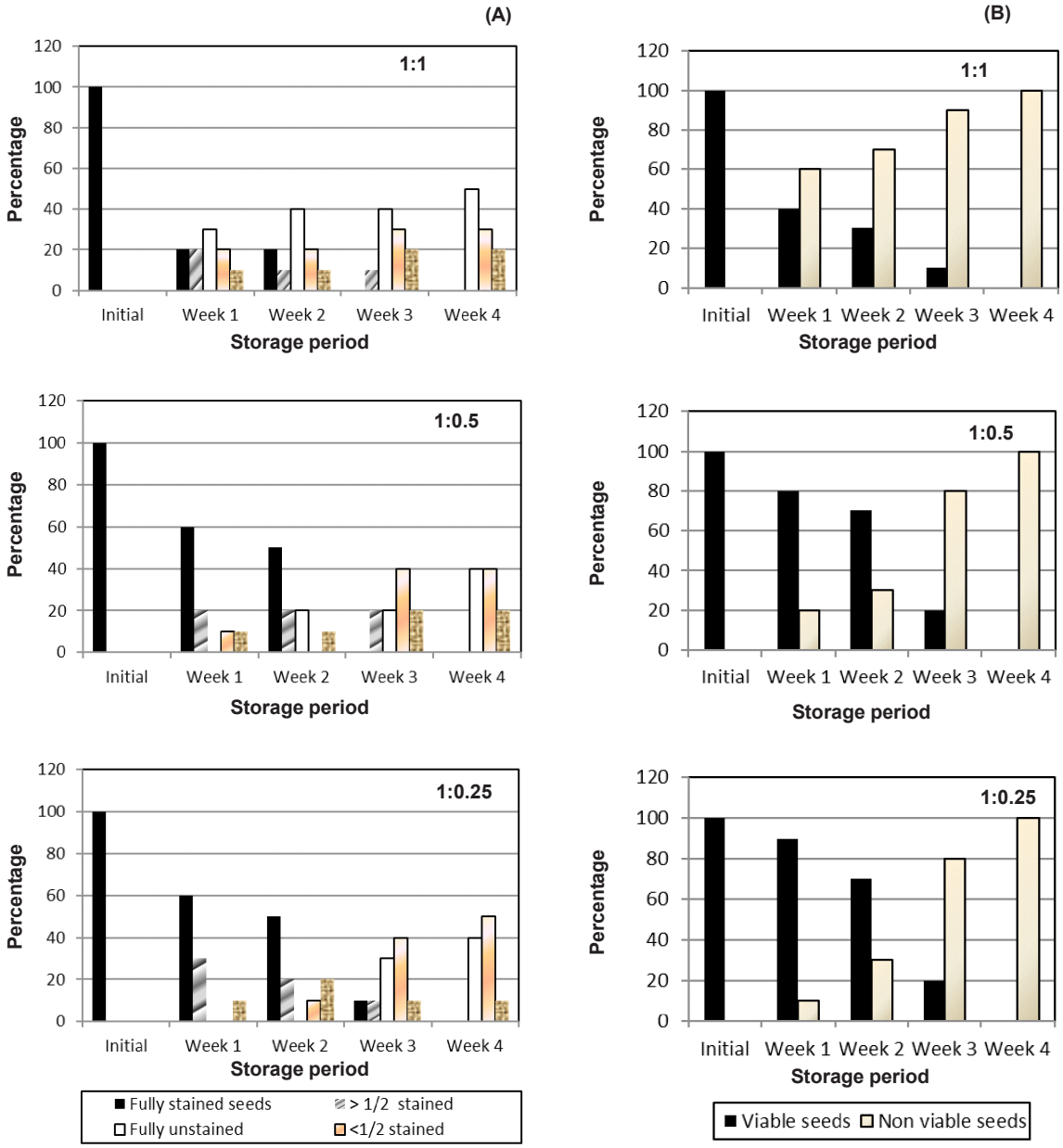

Figure 2. Effect of different seed : silica gel ratio levels on (A) staining pattern of seed tissues and (B) total viable and non viable seed percentage

stored in – 3.3°C recorded only 70 per cent viability, after one week of storage. In the consequent weeks also lower levels of viability was recorded in – 3.3°C followed by ambient temperatures while seeds stored in 10°C recorded higher levels of viability was observed even up to four weeks (Figure 3). In the final week of observation, although under ambient conditions total loss of viability was noted yet at – 3.3°C and 10°C, up to 10 and 20 per cent viability, respectively was noted.

The study on the storage behavior of the recalcitrant seeds of *L. perrottetii* under different levels of relative humidity, silica gel: seed ratio and temperatures, have put forth many important revelations *viz.,* increased susceptibility of embryonic axis tissues to dehydration, negative impact of drying on cell membrane integrity and seed viability potential, irrelevance of 'low safe seed moisture content' in extending the seed storage life and the critical effect of optimum low temperature in improving seed viability potential.

Table 3. Effect of temperature on characteristics of stored fruits and seeds of .*L perrottetei*

Fresh Weight (g) of fruits						
Temperature	Storage period (weeks)					
	Initial	1	2	3	4	Mean
Ambient	7.4150	3.4413	3.1049	2.9922	2.9359	3.9778
10° C	7.4150	7.1630	7.1213	7.1012	7.0913	7.1783
-3.3° C	7.4150	7.0390	6.7227	6.5670	6.4500	6.8387
Mean	7.415	5.8811	5.6496	5.5534	5.4924	5.9983
	T	P	T*P			
SE d	0.11305	0.14595	0.25279			
CD (0.05)	0.22770	0.29396	0.50916			
Rate of drying (%) of fruits						
Temperature	Storage period (weeks)					
	Initial	1	2	3		Mean
Ambient	53.59	9.77	3.50	1.00		16.96
10° C	3.30	0.50	0.28	0.13		1.05
-3.3° C	5.07	4.49	2.31	1.78		3.41
Mean	20.65	4.92	2.03	0.97		7.14
	T	P	T*P			
SE d	0.322	0.371	0.644			
CD (0.05)	0.653	0.754	1.306			
Electrical Conductivity (dSm^{-1}) of seeds						
Temperature	Storage period (weeks)					
	Initial	1	2	3	4	Mean
Ambient	1.90	2.12	2.35	2.88	2.91	2.43
10° C	1.90	2.12	2.31	2.34	2.47	2.22
-3.3° C	1.90	2.37	2.57	2.64	2.73	2.44
Mean	1.9	2.20	2.41	2.62	2.70	2.36
	T	P	T*P			
SE d	0.043	0.055	0.096			
CD (0.05)	0.087	0.112	0.194			
Seed Viability (%)						
Temperature	Storage period (weeks)					
	Initial	1	2	3	4	Mean
Ambient	100	100	70	20	0	58
10° C	100	100	80	40	20	68
-3.3° C	100	70	40	20	10	48
Mean	100	90	63.3	26.6	10	58
	T	P	T*P			
SE d	1.5	1.2	2.7			
CD (0.05)	3.1	2.4	5.4			

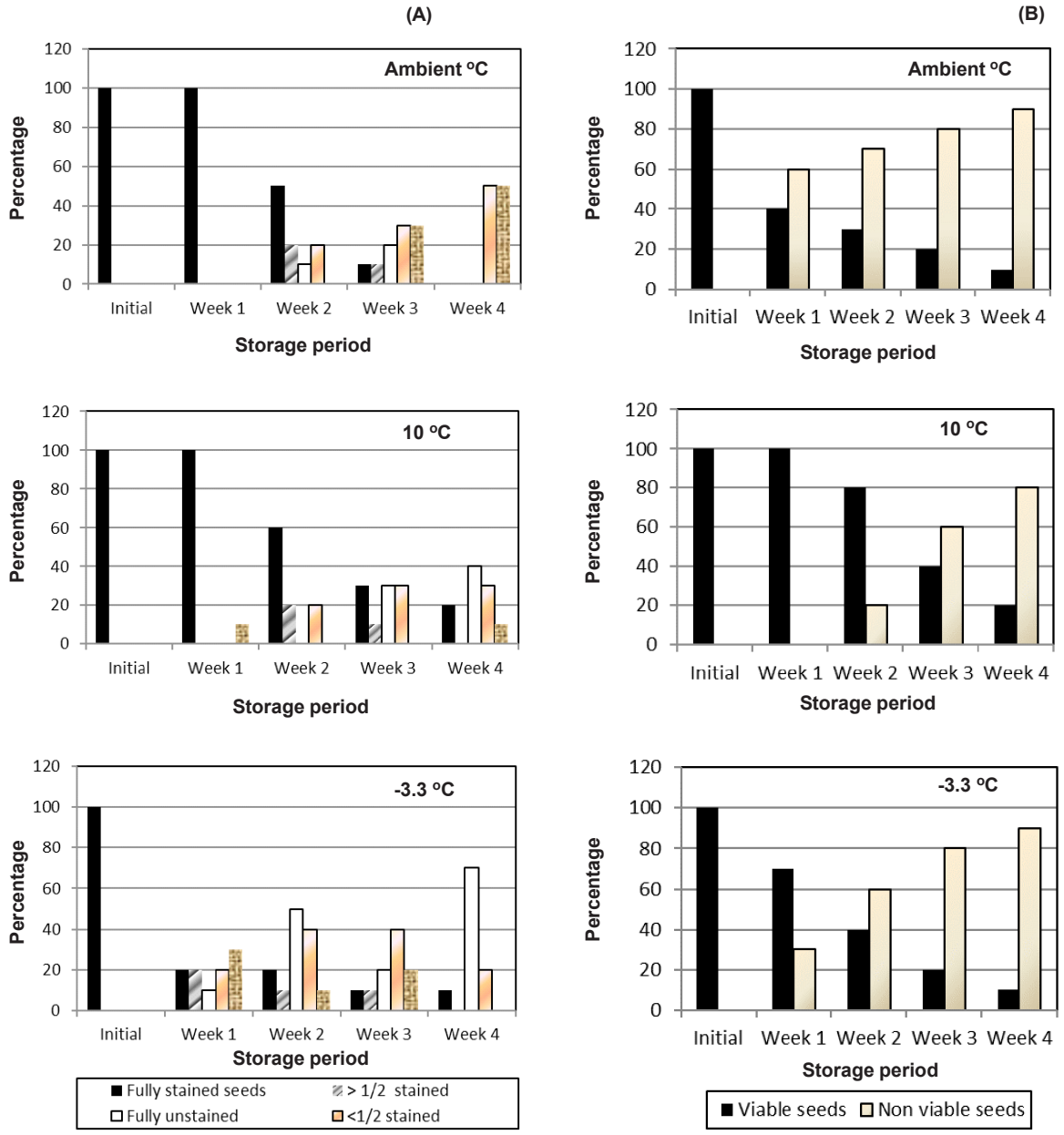

Figure 3. Effect of different temperature levels on (A) staining pattern of seed tissues and (B) total viable and non-viable seed percentage

The first significant revelation is that the embryonic axis and cotyledons of *L. perrotteiei* were differentially susceptible to dehydration. A detailed observation on tissue staining pattern of *L. perrotteiei* seeds, revealed that in the RH levels of 32.4%, after four weeks of storage, the categories of non viable seeds was found to be 20, 60 and 20 per cent in the categories of 'fully non-stained seeds, <½ the seed stained and axis fully non-stained seeds, respectively, while it was to the level of 40, 50 and 10 per cent in 46.6% RH and 40, 40 and 20 per cent, in 63.3% RH. The data revealed that, irrespective of the rate of drying, percentage of 'fully non-stained seeds' and '< ½ the seed stained' was comparatively higher to per cent of 'axis fully non-stained'. This was probably because, the 'embryonic axis' succumbed to higher rate of drying earlier than the 'cotyledons'. Further, in the higher RH of 63.3%, which caused lower rate of drying, during the fourth week of storage, none of the seeds showed 'fully non-stained seeds', whereas 80 per cent of seeds showed < ½ the seed staining, while 'axis

fully non-stained' seeds was noted only in 20 per cent of seeds (Figure 1). The storage of seeds in varied seed: silica gel ratio also revealed that of the three ratios, highest rate of drying was recorded in 1:1 ratio followed by 1/ ½ and 1:1/4 ratio. In the 1:1 ratio, the highest per cent of seeds (50 per cent) showed complete loss of viability (fully non-stained seeds) followed by < ½ the seed stained (30 per cent) while only in 20 per cent of seeds, 'axis fully unstained' category was noted (Figure 2). It is therefore, envisaged that loss of tissue viability due to seed dehydration was first initiated in the embryonic axis and later proceeded towards the cotyledons, envisaging that embryonic axis was more susceptible to dehydration compared to the cotyledons. The findings are in contrast with [31] who reported that rapid drying favoured viability retention in excised embryos of *Avicennia marina* Firsk.

The second significant finding of the present study is about the varied impact of rapid *versus* slow drying on the survival potential of recalcitrant seeds. In many studies, rapid drying has been reported to facilitate seed survival at lower water contents compared to slow drying (33,34,35) suggesting that if dehydration is slow, the seeds will have to spend extended times at "intermediate" water contents wherein aqueous based metabolism – linked damage accumulates resulting in loss of seed viability of *Ekebergia capensis* axes even at relatively high water contents (1.0 g g^{-1}). [24] was also of the opinion that when recalcitrant seeds were subjected to very slow-drying conditions, seed axes may be damaged by various deleterious processes ranging from the disruption of metabolic regulation to the failure of antioxidant systems. Further it was also hypothesized that uneven distribution of water in the seed tissues could improve the desiccation tolerance in the fast dried seed axes [35, 36]. [37] also found that loss of viability of *Mangifera persiciformis* seeds was faster during slow drying than that of rapid drying. Contrary to this hypotheses, the present study has unequivocally established that rapid drying was in fact more detrimental to seed viability of *L. perrottetei* compared to slow drying. In the lowest RH of 32.4% where highest rate of drying (17.62%) and electrical conductivity (2.67 dSm^{-1}) was recorded, the seed viability was found to be the lowest (50 per cent) even within first week of storage (Table 1). In 46.6 and 63.3% RH, where comparatively lower rate of drying as well as electrical conductivity was recorded, the seed viability observed was also higher to the level of 60 and 70 per cent, respectively during the same period. The data stands proof that the higher rate of drying had negatively influenced the membrane integrity of seeds of *L. perrotteiei* with concomitant reduction in seed viability as assessed through quick viability test. The results are supported by [24] who reported that electrolyte leakage began to increase and axis viability began to decrease even at high water contents. They put forth that under low RH the water potential of seed axes will change very rapidly. As a result, the uneven, rapid volumetric change would inevitably induce great damage within the well organized seed tissues (and also cells), unless the seed tissues are able to withstand such enormous mechanical stresses. Thus, the effect of drying rate on desiccation tolerance has not only governed the regulation of metabolisms (the physico-chemical aspects), but also with the physical process of dehydration itself (the mechanical aspects) [24]. [39] studied the relation between oxidative damage and viability loss of excised embryonic axes of *Antiaris toxicaria* subjected to rapid drying with silica gel at 15°C. They reported that viability loss of axes of *Antiarist toxicaria* under rapid drying with silica gel appeared to be more associated with mechanical or physical damage, rather than metabolic damage. It is therefore inferred that as suggested by [24], the rapid drying of *L. perrottettii*, could have caused mechanical damage of well organized seed tissues and cells, eventually leading to seed viability. Further, in the present experiment in which seeds of *L. perrotteiei* was stored in higher levels of silica gel ratio also it was repeatedly established that drying *pre se* was actually more critical to maintenance of seed tissue viability, irrespective of the rate of drying (Figure 2). The data proves that since the recalcitrant seeds are born with high seed moisture content, maintenance of seed volume by retaining high seed moisture content is quintessential to avoid mechanical damage to cell membrane as well as macromolecules, leaving no scope for prolonging the seed viability period by maintaining the 'lowest safe moisture content (LSMC)' as proposed by Tompset (1994). Such being the results, the concept of 'critical seed moisture content' loses it significance, as a determinant of seed viability period.

The embryos of recalcitrant seeds that shed from the mother tree, display high moisture content with correspondingly high respiration and metabolic rate making them prone to three categories of damage (i) mechanical damage, (ii) metabolism-induced damage, and (iii) macromolecular denaturation . The mechanisms have been reviewed in detail by [40]. Even as the seed

water is lost, aqueous-based metabolism continues but becomes unbalanced, leading to uncontrolled activity of free radicals as well as generation of reactive oxygen species (ROS) due to concurrent failure of the antioxidant system [14]. Cold conservation has been reported to remarkably prevent the oxidative damage which normally takes place during storage [41-42]. The fourth important finding of the present study is that management of storage temperature has a statistically significant effect on the seed viability period of *L. perrottetei.* Among the varied conditions of relative humidity, silica gel ratios and temperatures employed for study of seed storage, the seeds of *L. perrottetei* were found to possess a certain percentage of viable seeds even after four weeks of storage only at 10ºC (41% RH) (20 per cent), while none of the seeds showed viability in any other storage condition (Figure 1,2,3). Among the three temperature regimes *viz.,* ambient (Max 30.9°C, Min 18.2°C; 65% RH), 10°C (41% RH) and -3.3°C (27% RH), storage at 10°C (41% RH) alone was found to be the most optimum condition for storage since it was the only condition which could extend the seed viability potential of *L. perrotteiei* up to the maximum of four weeks (Figure 3). After four weeks of storage the seeds of *L. perrotteiei* was found to suffer higher seed viability loss both at ambient temperature (Max 30.9°C, Min 18.2°C; 47% RH), and -3.3°C (27% RH) however the loss of seed viability under ambient conditions was comparatively lower than under -3.3°C (27% RH) (Figure 3). Under ambient conditions, the higher RH level (47%) could have resulted in lower rate of drying, eventually leading to lower mechanical damage of tissues as measured by the electrical conductivity values, however, since the temperature was higher (Max 30.9°C, Min 18.2°C), it could have encouraged higher metabolic activity and metabolism-induced damage leading to loss of seed viability. On the contrary, under -3.3°C (27% RH), the low temperature (-3.3°C) could have reduced the metabolism-induced damage, but could not have prevented mechanical damage of cell membranes due to rapid drying at the lower RH of 27%, leading to quick loss of seed viability. Chilling is suspected to be detrimental to many recalcitrant seeds such as *Drybalanops aromatic* (Jensen, 1971) *Shorea curtesii, S.platycladus* [43] *Shorea ovalis* [44] and *Hopea odoratai* [45] suffer from chilling injury. [17, 34 & 3]also warned that low moisture content and sub-zero temperature storage will not reduce, but in most cases accelerate loss of viability of recalcitrant seeds. According to [17], freezing damage in moist seed is presumably associated with the formation of ice crystals. Besides, the deleterious effect of sub ambient temperature could also be due to protein denaturation [47] or change in membrane thickness and permeability [48].

However, under 10°C (41% RH), the optimum low temperature as well as higher relative humidity levels could have helped to lower both mechanical damage as well as metabolism-induced damage ultimately leading to extension of viability period of the recalcitrant seeds of *L. perrotteiei* upto four weeks. The results strongly support that viability period of recalcitrant seeds may a function of two factors *viz.,* optimum low temperature and sufficiently high relative humidity.

Taking a holistic view of the results obtained, it is concluded that, irrespective of the cause of drying *ie.,* low relative humidity or high seed: silica gel ratio, the embryonic axes of *L.perrottetii* seeds were more susceptible to dehydration damage compared to cotyledons. The study also established that the high rate of drying (witnessed in low RH and high ratio silica gel treatments) lead to faster deterioration of seed viability. The study disproves the hypothesis that 'fast drying' improves desiccation tolerance [38] due to lowered metabolic activity and uphold the findings of [24] that fast drying leads to great cell damage caused by enormous mechanical stress. The results of the present study unequivocally revealed that irrespective of the rate of drying, recalcitrant seeds of *L.perrottetii* demonstrated deterioration of tissue viability, leading to seed viability loss. Under such conditions, the concept of "Low Safe Moisture content" loses significance. Finally, among the storage methods explored, the seeds were found to store better at 10°C (41% RH) since the temperature level was optimum to reduce the 'metabolism induced damage' and the relative humidity level was higher to minimize the 'mechanical damage'. Therefore, it is recommended that, the fruits of *L.perrottetii* may be stored under refrigerated conditions (10°C; 41% RH) in order to extend the seed viability period at least for few weeks. However, future research may be conducted to study the seed viability potential of *L.perrottetii* seeds at a higher RH level and at a constant temperature of 10°C so as to achieve better control of 'metabolism induced damage' as well as 'mechanical damage' and increase the seed viability period.

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