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Hermetic Seed Storage to Enhance Seed Longevity in Acid Lime (*Citrus aurantifolia* Swingle)

PALLAVI HM*[1], DHANANJAY B, BAPURAYAGOUDA PATIL[1], ANAND GN[1] AND SOWMYA KJ[2]

[1]University of Horticultural Sciences, Bagalkote, Karnataka, India
[2]Universtiy of Agricultural Sciences, Bengaluru, Karnataka, India
*pallavi.hm@uhsbagalkot.edu.in

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ABSTRACT: Storage capability of acid lime seeds are under question due to its intermediate to recalcitrant nature. Seed desiccation tolerance limits plays major role in storage of seeds with better viability for propagation in citrus. An experiment was conducted to explore the effect of desiccation limits on germination and influence of storage conditions on seed viability. Fresh seeds of kagzi acid lime were collected and studied for its germination and storability. Results showed that as the moisture content was reduced from 50.06% to 11.97%, the mean germination percentage was also reduced from 85.0% to 39.0% indicating sensitivity to desiccation tolerance and exhibited recalcitrant to intermediate storage behavior. The results of storage experiments showed that seeds packed in aluminum foil and stored at 10^0C could retain high germination (62%) and seedling growth parameters up to 3 weeks of storage.

Keywords: citrus, desiccation, germination, hermetic storage, longevity, recalcitrant

INTRODUCTION

Seeds of acid lime are considered non-orthodox but intermediate and lose viability at short period of time [1, 2]. Commercially acid lime is propagated both through seeds and vegetative means as grafts. Plants raised from seeds are known to grow as true to type due to nucellar embryony producing progeny similar to mother plant. Hence, seed propagation has economic importance in acid lime. However, seeds are desiccation sensitive and lose viability rapidly resulting in the loss of plant population [3]. Being intermediate/ recalcitrant seed, viability during storage varies as per the air dry environmental conditions. High moisture content inside recalcitrant seeds will keep embryo active throughout the period from maturity to shedding [4, 5], where delayed harvesting leads to vivipary. Lose of viability due to dehydration has lead to poor storage and death of seeds. For long term conservation of recalcitrant seeds, excision of explants for cryopreservation is also followed [6, 7].

Acid lime can tolerate desiccation more than true recalcitrant seeds. However, per cent survival is less than 35% after desiccation up to 11% seed moisture. Storage behavior of such seeds under conventional seed bank is difficult as moisture loss leads to loss of viability and freezing will lead to crystallization and cellular damage. The complications of desiccation tolerance limit in acid lime need to be clarified for storage under hermetic conditions. Loss of viability and poor germination is attributed to inhibitors in seed coat and seed size. Fresh seeds are poor storer and lose viability rapidly making storage difficult. Seed viability and longevity potentiality are main key factors for successful production of planting material of crops. Further, seeds are known to show dormancy and can be removed by seed treatment with hormones GA_3 to enhance seed germination by 20% more than the untreated control [8-12]. However, no treatment has resulted in complete removal of dormancy resulting in maximum germination. Implementation of seed treatment is influenced by seed behavior for moisture and seed longevity. Here, an attempt has been made to understand the behavior of recalcitrant seeds during storage conditions for conservation and commercial production of planting material.

MATERIAL AND METHODS

Fully matured fresh fruits of kagzi acid lime during March, were collected from *Bijapur*, Karnataka, India (altitude 16.827545 and the *longitude* is 75.725327 with gps coordinates of 16° 49' 39.1620'' N and 75° 43' 31.1772'' E). Completely matured fresh fruits were collected from

farmer's field and cut opened to extract seeds, washed in water before implementation of treatments and studies.

Desiccation tolerance limits

Fresh seeds were tested for desiccation tolerance [5] by recording seed moisture (%), germination (%), test weight (g) and seedling length (cm) for every six hours of air drying environment after extraction from fruits till moisture drops less than 11.0%. Seed moisture content was estimated on wet basis (%, w.b.) in hot air oven method and reported to the nearest 0.1% [13].

Seed germination test was conducted in between paper method at 27 °C and 95% RH, as described in Handbook of Seed Technology for Gene bank Volume I [13]. 400 seeds in 4 replications were tested in between paper method. Per cent germination was estimated on 24th day of test by counting normal seedlings. Because of poly-embryony only one seedling per seed was considered for calculating germination per cent.

Test weight was estimated by weighing 400 seeds in four replications and expressed in grams.

Seedling length was recorded on 24th day after germination test. Average of 10 randomly selected seedlings were measured from tip of the root to tip of the shoot and expressed in cm.

Seedling vigour index I is the product of germination (%) and Seedling length (cm) and expressed in whole number without any units [14].

Seedling vigour index II is the product of germination (%) and Seedling dry weight (mg) and expressed in whole number without any units [14].

Experiment was repeated for consecutive two years on fruits harvested during summer season and average was considered for analysis.

Seed longevity under hermetic storage

Freshly extracted seeds from fully matured fruits were used to study the seed storability under controlled storage conditions. CRD designed was applied with two hermetic storage containers (C_1: Polyethylene bag (200 gauge); C_2: Aluminum foil) and at two storage conditions (S_1: Room temperature (Average day temperature 36°C and night temperature 20°C with 52-55% RH) and S_2: 10°C & 40% RH) with 5 replications. Initially fresh seeds recording germination percentage of 86 and 35 per cent seed moisture seeds were stored as per the treatment details. Observations on seed germination and seedling vigour index were recorded at 7 days interval for 4 weeks of storage period.

Analysis of variance (ANOVA) and the means were statistically grouped by Tukey's test [15].

Plant material collection

The permission has been taken from farmers and university to collect seeds and conduct research at University of Horticultural Sciences, Bagalkot.

The study complies with local and national guidelines.

Table 1. Influence of desiccation on seed germination and vigour in Kazgi acid lime

Treat	Moisture content (%)			Test weight (g)			Germination (%)			Seedling length (cm)		
	I year	II year	Pooled analysis	I year	II year	Pooled analysis	I year	II year	Pooled analysis	I year	II year	Pooled analysis
D0	50.56	49.57	50.06	7.00	7.10	7.05	85	86	85.50	10.53	10.51	10.52
D1	45.78	45.92	45.85	6.10	6.52	6.31	84	85	84.50	10.18	10.47	10.32
D2	38.40	36.59	37.45	5.80	5.77	5.78	79	79	79.00	7.24	7.52	7.38
D3	28.58	28.93	28.75	4.60	5.43	5.01	62	62	62.00	6.12	6.18	6.15
D4	16.60	17.02	16.81	4.37	4.67	4.52	47	48	47.50	5.05	4.86	4.95
D5	11.73	12.22	11.97	4.31	4.3	4.3	39	39	39.00	4.09	4.21	4.15
Mean	31.94	31.61	31.77	5.36	5.63	5.49	65.92	66.29	66.1	7.2	7.29	7.24
S.Em +			0.727			0.095			0.7065			0.135
CD (p=0.01)			2.094			0.279			2.0375			0.405
C V %			7.615			3.208			4.715			5.8295

D0: before drying
D1: after 6 hrs of drying
D2: after 12 hrs of drying
D3: after 18 hrs of drying
D4: after 24 hrs of drying
D5: after 30 hrs of drying

RESULTS and DISCUSSION

Desiccation tolerance limits

Understanding desiccation tolerance of seeds is required for accessing the seed viability and longevity in acid lime for economic cultivation. Traditionally acid lime is seed propagated and demands study of desiccation tolerance limits for conservation and rapid multiplication or propagation. Studies on desiccation tolerance limit results revealed that, there is a statistically significant influence of desiccation on seed germination and seedling vigour. Present study conducted for two years pooled data showed significant results proving seed desiccation tolerance limit in relation to viability. Seed moisture immediately after extraction from ripened fruits reported to have 50.06% and later reduced to 11.97% after 30 hours of drying under room temperature. Initially drying was slower and rapid drying has occurred after 18 hours of air drying. Seed test weight has also followed the trend of moisture content initial test weight was 7.05 g and reduced to 4.15 g at the end of drying hours. Higher germination per cent was reported immediately after seed extraction (85.50%) and gradually decreased along with decrease in seed moisture content and reported 39.0 per cent at D5 hours. Similarly seedling length has also reported to reduce greatly from 10.52 cm to 4.15 cm from initial period to 30 hours of drying of seeds in acid lime. This confirmed seed viability in acid lime is highly sensitive to moisture desiccation. Higher moisture resulted in higher seed germination and better seedling performance. This shows acid lime seeds can tolerate intermediate desiccation compared to other recalcitrant seeds [16]. Similarly, seed germination also declined from 85 to 39 per cent. Seed drying rate is related to creating mechanical stress during desiccation leading to membrane damages in recalcitrant seeds [17, 18]. Reduced germination after drying is due to non tolerance to desiccation and exhibits intermediate type of storage behavior [19, 20]. Germination per cent and germination time is related to seed moisture content in non-orthodox seeds [21, 22].

Seed longevity under hermetic storage

Influence of seed storage containers and conditions on seed viability and vigour was studied on freshly harvested seeds and results revealed statistically significant differences among storage containers and conditions for seed moisture content, germination and seedling vigour (Table 2). Irrespective of storage container and storage conditions seed moisture has drastically reduced along with storage period in acid lime. At the end of IV weeks of storage seeds stored at 10°C has reported to have higher moisture (14.57%) compared to seed stored at room temperature (10.34%). Among containers seed packed in Aluminum foil pouch reported to have higher moisture (14.65%) compared to seeds packed in polythene bag (10.27%). In treatment interactions seeds packed in aluminum foil pouch and stored at 10^0C has recorded higher moisture (16.8%) among all other treatment combinations and least was recorded in seeds packed in polythene bag and stored at room temperature (8.07%). Initially higher seed moisture content seeds were stored under different conditions to study the impact on seed viability and longevity. Seeds survival is at highest during higher seed moisture content and critical moisture content level is necessary for minimum germination capacity [23, 24]. Seed moisture, rate of drying and drying period decides the percentage of damage seeds during storage in non-orthodox seeds [25].

With respect to seed germination studies, among storage conditions seeds stored at low temperature (S_1) of 10°C recorded significantly higher germination (58.0%) compared to room temperature (36.0%) after 3 weeks of storage period. At the end of 4 weeks of storage both S_2 (23.0%) and S_1 (11.0%) failed to maintain germination standards in acid lime seeds. Among containers, seeds stored in aluminum foil recorded higher germination (52.0%) after 3 weeks of storage compared to polyethylene bag (41.0%). However, both containers failed to maintain germination after 4 weeks of storage. Seeds packed in aluminum foil and stored at S_2 conditions recorded higher germination (62.0% and 25.0%) compared to all other treatments after 3 and 4 weeks of storage, respectively. Reduced germination after storage is due to non tolerance to desiccation and exhibits intermediate type of storage behavior [19, 20]. Germination per cent and germination time is related to seed moisture content in non-orthodox seeds [21, 22].

Seedling length at 24^{th} day of germination test has also showed significant differences among treatments. Seedling length has sequentially reduced from I week to IV week of storage among all the tested treatments. At the end of the storage period seeds stored at 10°C has higher seedling length (8.40 cm) followed by seeds stored at room temperature (8.18 cm). Seeds packed in aluminum foil pouch recorded higher seedling length (9.02 cm) and least in seeds packed in polythene bag

Table 2. Influence of storage container and storage conditions on seed moisture and seedling parameters of kagzi lime seeds (*Citrus aurantifolia* Swingle*)*

Treatments	Seed moisture content				Germination (%)				Seedling length (Cm)				Seedling Dry weight (mg)			
	I week	II week	III week	IV week	I week	II week	III week	IV week	I week	II week	III week	IV week	I week	II week	III week	IV week
S1	29.74	27.99	22.50	10.34	79	75	36	11	10.47	8.87	8.61	8.18	13.58	10.49	9.89	8.19
S2	31.71	30.56	26.51	14.57	85	83	58	23	11.43	9.38	8.66	8.40	14.47	12.26	11.21	9.40
Mean	30.72	32.56	24.50	12.45	82	79	47	17	10.95	9.12	8.63	8.29	14.02	11.37	10.55	8.79
S.Em±	0.25	0.27	0.26	0.26	0.66	0.47	2.05	0.45	0.19	0.07	0.10	0.09	0.32	0.14	0.150	0.14
CD @ 1%	1.01	1.12	1.07	1.09	2.73	1.94	8.49	1.89	0.80	0.29	0.41	0.37	1.36	0.61	0.62	0.59
C1	30.68	28.53	23.31	10.27	80	77	41	14	11.03	8.37	7.72	7.55	14.53	11.34	10.34	8.50
C2	30.78	30.03	25.70	14.65	84	80	52	19	10.87	9.88	9.55	9.02	13.51	11.41	10.76	9.17
Mean	30.73	29.28	24.50	12.46	82	78.50	46.50	16.50	10.91	9.08	8.63	8.28	14.01	11.37	10.55	8.83
S.Em±	0.25	0.27	0.26	0.26	0.66	0.47	2.05	0.45	0.19	0.07	0.10	0.09	0.32	0.14	0.150	0.14
CD @ 1%	1.01	1.12	1.07	1.09	2.73	1.94	8.49	1.89	0.80	0.29	0.41	0.37	1.36	0.61	0.62	0.59
S1C1	29.62	27.07	21.16	8.07	77	73	29	8	10.84	8.46	7.73	7.32	14.76	10.38	9.85	8.09
S1C2	29.86	28.92	23.84	12.62	81	76	42	13	10.10	9.28	9.48	9.03	12.40	10.59	9.93	8.3
S2C1	31.73	30.00	25.46	12.46	84	81	53	20	11.21	8.27	7.70	7.78	14.31	12.3	10.83	8.90
S2C2	31.70	31.13	27.55	16.68	86	85	62	25	11.65	10.48	9.61	9.04	14.62	12.22	11.6	10.04
Mean	0.35	0.38	0.37	0.37	82.00	78.75	46.50	16.50	10.95	9.12	8.63	8.29	14.02	11.37	10.55	8.83
S.Em±	1.43	1.58	1.52	1.54	0.93	0.66	2.90	0.64	0.27	0.09	0.14	0.12	0.46	0.21	0.21	0.20
CD @ 1%	29.74	27.99	22.50	10.34	3.87	2.75	12.01	2.67	1.13	0.41	0.59	0.53	1.92	0.87	0.88	0.84
CV %	2.52	2.92	3.35	6.68	2.56	1.90	13.92	8.76	5.63	2.44	3.70	3.48	7.43	4.17	4.52	5.17

S1: Room temperature S2:10 0C C1: Polythene bag C2: Aluminum foil pouches

Table 3. Influence of storage container and storage conditions on seedling vigour of kagzi lime seeds (*Citrus aurantifolia* Swingle*)*

Treatments	Seedling vigour index I				Seedling vigour Index II			
	I week	II week	III week	IV week	I week	II week	III week	IV week
S1	821	661	313	87	1063	781	353	85
S2	973	777	503	191	1231	1013	649	215
S.Em±	19.76	5.96	21.97	4.76	27.99	13.90	23.07	4.79
CD @ 1%	81.61	24.61	90.75	19.68	115.62	57.41	95.29	19.79
C1	884	643	317	105	1163	876	432	119
C2	910	795	499	173	1131	918	569	181
S.Em±	19.76	5.96	21.97	4.76	27.99	13.90	23.07	4.79
CD @ 1%	81.61	24.61	90.75	19.68	115.62	57.41	95.29	19.79
S1C1	830	618	223	57	1128	760	285	62
S1C2	813	703	403	118	998	802	420	109
S2C1	939	668	411	153	1199	993	579	176
S2C2	1006	886	596	229	1263	1033	719	254
Mean	897	718	408	139	1147	897	500	150
S.Em±	115.41	34.81	128.34	27.83	163.51	81.19	134.76	27.99
CD @ 1%	821.70	661.20	313.50	87.90	1063.44	781.30	353.00	85.70
CV %	6.69	2.62	14.56	10.07	7.71	4.91	14.26	10.07

S1: Room temperature S2:10 0C C1: Polythene bag C2: Aluminum foil pouches

(7.55 cm). Further, seeds packed in aluminum foil pouch at 10°C have recorded higher seedling length (9.04 cm) *on par* with seeds packed in aluminum pouch and stored at room temperature (9.03 cm). Lower seedling length was reported in seeds packed in polythene bag and stored at room temperature (7.32 cm).

Similar treatment effect has also been observed for seedling dry weight in the study. Seedling dry weight was higher in seeds stored at 10°C (9.40 mg) and lower in seeds stored at room temperature (8.19 mg). Among containers aluminum foil pouch packed seeds recorded higher seedling dry weight (9.17 mg) and lower in seeds packed in polythene nag (8.50 mg). Further, seeds packed in aluminum foil pouch and stored at 10°C have resulted in higher seedling dry weight (10.04 mg) and least in seeds packed in polythene bag and stored at room temperature (8.09 mg).

There was significant difference among treatments for seedling vigour index too (Table 3). Vigour index has gradually decreased along with storage period in both containers and storage conditions. Seedling vigour index I and seedling vigour index II was higher in seeds stored at 10°C (191 & 215, respectively) followed by seeds stored at room temperature (87 & 85, respectively). Among containers seeds packed in aluminum pouch have recorded 173 Seedling vigour I and 181 seedling vigour index II at the end of IV week storage. Among treatment interactions seeds packed in aluminum foil pouch and stored at 10°C has recorded higher seedling vigour index I and seedling vigour index II (229 & 254, respectively) by the end of IV week of storage. Seeds survival is at highest during higher seed moisture content and critical moisture content level is necessary for minimum germination capacity [24]. Seed moisture content, storage environment, rate of drying and drying period decides the seed germination and its vigour in non-orthodox seeds [25].

Study of seed moisture content and its effect on seed germination indicated that acid lime seeds can tolerate desiccation up to 30% seed moisture. Further decrease in moisture exhibited negative impact on germination and seedling vigour. Seed moisture, therefore should be maintained at 30% and above during storage for better germination (>70%) and seedling vigour in acid lime. Studies on storage behavior at different conditions revealed that seeds can be stored at low temperature (10°C) by packing in aluminum foil pouch for 3 weeks, beyond which seeds lose their viability rapidly. Seeds of acid lime may be used for seed propagation within three weeks of seed extraction for better germination and plant stand.

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