SEED GERMINATION, EMERGENCE, BIOCHEMICAL CHANGES AND EARLY SEEDLING PERFORMANCE IN CLEOPATRA MANDARIN (CITRUS RETICULATA BLANCO) FOLLOWING CONTROLLED ENVIRONMENT STORAGE

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FAWUSI M. O. A. *Seed germination, emergence, biochemical changes ana early seedling performance in Cleopatra mandarin* (Citrus reticulata *Blanco*) *following controlled environment storage*. BIOTRONICS **18**, 29–35, 1989. Studies were carried out to investigate the effects of storage environment and duration of storage on viability and seedling performance in Cleopatra mandarin (*Citrus reticulata* Blanco) seeds. Storage in an environment where both temperature and relative humidity were controlled (16°C, 40% R. H.) proved to be the best for preserving germinability and seedling growth. Also, no significant changes were observed in seed moisture, protein, carbohydrate and fat contents under this environment. The ambient environment (22–32°C; 65–75% R. H.) kept the seeds viable for only two months. Water environment storage (22°C, 80% R. H.) proved unsatisfactory as seeds gained more moisture, despite the relatively water repellent nature of the plastic containers. In a deep freezer (−20°C) storage, all the seeds were dead within one month. While no significant changes were observed in the carbohydrate and fat contents, significant reductions were observed in the protein content, probably due to protein precipitation at the −20°C temperature of the deep freezer.

Key words: Cleopatra mandarin; *Citrus reticulata* (Blanco); seed viability; controlled environment; seedling vigour.

INTRODUCTION

Seeds offer a convenient way to store germplasm over time and to transport it over long distances even across international boundaries (*3*, *6*). Seeds are particularly valuable in citrus culture because of their importance in the establishment of nursery stocks and because seeds are known to be relatively free from the common citrus virus and fungal diseases (*7*, *9*). Moreover, citrus seeds are polyembryonic, developing nucellar seedlings that have the semblance of vegetatively propagated clones.

Preservation of viability in recalcitrant tropical tree crops seeds like citrus and cacao (*1*, 5) is dependent on the moisture content, storage temperature, relative humidity and carbon dioxide and oxygen pressures in the storage environment of such seeds (*10*, *11*).

Recalcitrant seeds, as opposed to orthodox seeds, cannot be dried below a comparatively high moisture content without immediate cell damage and consequent loss in germinability. They are short-lived under ambient tropical conditions. It is known that citrus seeds can withstand only slight drying. Barton (*2*), reported that grapefruit and sweet orange seeds were injured by drying to 52 and 25% moisture content, respectively. While grapefruit seeds with 78% moisture content, stored at 5°C gave more than 70% germination at the end of one year's storage. Cleopatra mandarin seeds shade dried to 32% moisture content and stored at 5°C retained high viability and germinability for a long storage time (5). The biochemical components of the causes of this observed rapid loss in viability is not well understood. Hence, this study was carried out to ascertain what chemical changes do occur during the storage of Cleopatra mandarin seeds and what relationships they might have with retention or loss of viability and seedling performance.

MATERIALS AND METHODS

Cleopatra mandarin (*Citrus reticulata* Blanco) seeds were extracted from ripe fruits harvested from the Crops Garden of the University of Ibadan. The seeds were throughly rinsed and shade dried to a moisture content of 25% (approximately 36 h). The seeds were treated with benlate fungicide at the rate of 2 g/kg seed. Two thousand seeds (approximately 200 g) were placed in water-resistant plastic bottles and wax sealed. The seeds in these bottles were then stored as follows.

(i) Storage in a controlled environment room maintained at 16°C and R.H. of 40%.

(ii) Storage in an ambient environment with day/night temperature fluctuating between 32°C and 26°C, and R.H. between 65–75%.

(iii) Storage in water containers: The sealed plastic bottles were placed in a bigger plastic bottle and sealed before being put in 20 liter water containers. There were three such containers to allow for monthly sampling. The containers were then placed in a cool room, maintained at 26°±2°C. The relative humidity in the room was 80%. The plastic bottles in water were weighed down by placing a stone on each bottle. Mean water temperature was 22°C.

(iv) Storage in a deep freezer maintained at −20°C.

Storage duration under each environment was three months. Seeds were sampled at monthly intervals and analysed for changes in seed moisture content, crude protein, total fats and carbohydrate changes with length of storage. Germination, emergence and seedling growth and dry matter yields were also observed. All data were subjected to statistical analysis and mean separation was by the use of least significant difference.

RESULTS

Data on the moisture content of the seeds stored under various environmental conditions and for varying lengths of time are presented in Table 1. After one month storage, there was no significant change in seed moisture content under the various storage environments. However, the moisture content of the seeds stored in the water environment increased significantly during the second and third months. There was a slight but non-significant dehydration of seeds stored in a deep freezer.

The worst storage environment, in this study, was in the deep freezer (−20°C) where no germination occurred only after one month (Fig. 1), while the best was the controlled environment storage (16°C, 40% R.H.) where germination dropped

Table 1. Moisture content of Cleopatra mandarin citrus seeds as influenced by storage environment and duration

Storage environment	Storage duration (months)				
	0	1	2	3	LSD 0.05
Controlled environment room (16°C, 40% R.H.)	25.3	25.7	24.9	25.0	2.9
Ambient environment (26–32°C, 65–75% R.H.)	25.3	25.1	25.0	25.6	2.8
Water environment (22°C, 80% R.H.)	25.3	26.3	33.7	37.6	7.1
Deep freezer (−20°C)	25.3	24.8	24.8	24.5	2.5
LSD 0.05	—	2.9	3.6	5.5	—

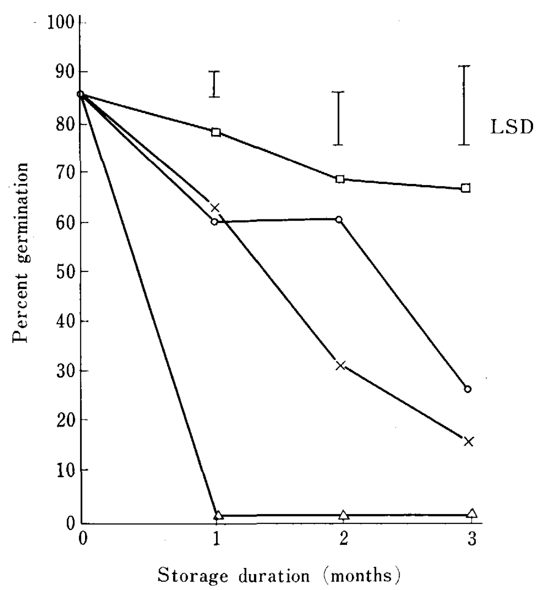

Fig. 1. Germination of Cleopatra mandarin citrus seeds as influenced by storage environment and duration. □, controlled; ○, ambient; ×, water; △, deep freezer environments.

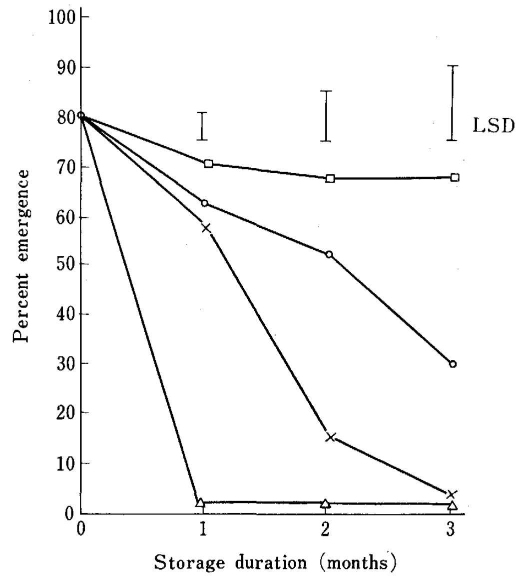

Fig. 2. Emergence of Cleopatra mandarin citrus seeds as influenced by storage environment and duration. □, controlled; ○, ambient; ×, water; △, deep freezer environments.

from the initial of 85% to 75% at the end of the three months storage. Germination dropped to 25% and 15% under ambient environment (26–32°C and 65–75% R.H.) and water environment (22°C) respectively, at the end of the storage period.

More than 70% emergence was still observed in seeds stored in controlled environment after three months storage (Fig. 2). Emergence drastically dropped to between zero and 30% within three months under other storage conditions. The ambient environment was even significantly better than both water environment and deep freezer storage.

Data showing the biochemical changes in the seeds, as storage time progressed, are presented in Table 2. Under controlled temperature and relative humidity, there were no significant changes in the protein, fats and carbohydrate content of the seeds over the three months storage period. Under the ambient environment, the seed protein content declined significantly by the third month. There was no significant change in the fat content, while the carbohydrate content declined rapidly as from the end of the first month and continued declining till the third month. Under water environment, a bigger and faster decline in both protein and carbohydrate contents were observed throughout the storage period. In the deep freezer storage, there was a big reduction in the protein content over the period of storage. There was no significant change in both the fat and carbohydrate contents.

Comparing the different environments, it was observed that there were significant reductions in protein content under the water and deep freezer storage compared with the controlled and ambient environments. There were no significant differences in the fat contents under all the storage environments. With regards to the carbohydrate content, the biggest reductions were observed under the ambient environment while the least change was observed under deep freezer storage.

Table 2. Crude protein, total fats and carbohydrate changes in Cleopatra mandarin citrus seeds as influenced by storage under various environmental conditions for one to three months

Storage environment	Storage duration (months)														
	0	1	2	3		0	1	2	3		0	1	2	3	
	Crude protein (%)					Total fats (%)					Soluble carbohydrate (%)				
					LSD					LSD					LSD
Controlled environment (16°C, 40% R. H.)	14.9	14.2	14.1	14.6	2.8	21.3	22.1	21.5	21.7	3.5	7.45	7.20	7.00	6.70	1.3
Ambient environment (26–32°C, 65–75% R.H.)	14.9	14.5	14.0	13.7	2.0	21.3	21.6	22.2	22.4	3.0	7.45	6.50	4.80	4.50	1.20
Water environment (22°C, 80% R.H.)	14.9	14.1	11.8	11.8	2.1	21.3	22.3	21.9	22.4	2.5	7.45	7.05	5.90	5.80	1.30
Deep freezer environment (−20°C)	14.9	11.7	11.8	11.6	1.7	21.3	22.6	21.1	22.6	2.7	7.45	7.38	7.35	7.37	0.30
LSD	—	2.2	1.9	1.6	—	—	0.6	0.5	0.9	—	—	0.60	1.20	1.4	—

Table 3. Seedling growth and dry matter yields in Cleopatra mandarin citrus seeds at 70 days after emergence following storage under various environmental conditions for one to three months

Storage environment	Storage duration (months)											
	1	2	3		1	2	3		1	2	3	
	Plant height (cm)				Stem diameter (mm)				Dry matter yields (g)			
				LSD				LSD				LSD
Controlled environment	6.8	7.0	6.1	1.02	2.0	1.8	1.7	0.18	0.24	0.22	0.22	0.03
Ambient environment	6.3	6.1	4.3	1.50	1.9	1.5	1.5	0.30	0.21	0.18	0.15	0.02
Water environment	6.2	4.5	3.8	1.30	1.9	1.2	0.8	0.35	0.18	0.13	0.09	0.04
LSD 0.05	0.25	0.8	1.2	—	0.15	0.5	1.0	—	0.02	0.03	0.05	—

Seedling growth and dry matter production data are shown in Table 3. There was no germination at all under deep freezer storage. Hence no seedling performance data could be reported. Under the controlled environment storage, there were no significant changes in all the plant parts measured throughout the duration of storage.

Both the ambient and water environments recorded significant reductions in plant height, stem diameter and dry matter yields over the period. While the controlled environment recorded the best seedling performance, the water environment was the worst.

DISCUSSION

Citrus seeds usually preserve better when stored with high moisture content (5). In this work, high moisture content combined with very low temperature storage virtually killed the seeds. Darrell *et al.* (*4*), working on freezing effects on the germination of sorghum seeds, reported that freezing brought about a dehydration of cells which caused protein precipitation. This resulted in loss of germination. They stated that the embryo seemed to be the first organ injured and that the injury increased with decreasing storage temperature particularly with seeds stored with high moisture content. Seeds in the water environment lost viability mainly because of the high relative humidity of that environment (80%). It is possible that water penetrated the plastic containers somehow thus keeping the humidity very high. Also it was observed that the seed moisture content increased under this storage environment. According to Toole(*12*), most crop seeds lose viability rapidly at a relative humidity approaching 80% and a temperature of between 21 and 31°C. Previous studies showed that citrus seeds store well with relatively high seed moisture content, in high relative humidity environment, but at cool temperature (5, *8*). It is suspected that the absorption of more moisture by citrus seeds during storage was particularly detrimental to seed longevity.

The ambient temperature was shown to sustain viability for two months. Changes in the chemical constituents of the seeds were greatest under this environment. Thus, rapid depletion of the carbohydrates and protein reserves in the seeds might be responsible for the loss in vigour. The best storage environment in this study was where both the temperature and relative humidity were controlled (16°C, 40% R.H.). Under this condition, changes in the seed protein and carbohydrate contents were minimal, indicating that these were the two chemical components easily influenced by storage environments in seeds. Since citrus seeds must be stored with relatively high moisture content (25–35%) and high relative humidity (40–65%) the temperature of the environment seems to be the critical limiting factor for the preservation of viability in these seeds.

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