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RELATION BETWEEN SEED LONGEVITY AND MOISTURE CONTENT IN HERMETIC AND OPEN STORAGE OF COTTON SEEDS

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Abstract

In seed storage, moisture content can be maintained by providing a stable relative humidity (*e.g*. over saturated salt solutions) or by hermetic storage, but two approaches provide different gaseous environments, which might affect longevity. Seeds of cotton were stored at 30°C, with different moistures contents maintained by hermetic storage in laminated aluminium foil bags, polythene bags, craftpaper bags or by desiccators above either saturated salt solutions or moistened silica gel. Seeds were withdrawn from storage at intervals of 1 to 15 d for up to 60 d and viability estimated. The values of germination percentage did not differ between storage in desiccators over either moistened silica gel or saturated salt solutions, whereas the germination percentage values were more in hermetic storage. This effect of storage method on seed longevity's sensitivity to moisture content implies that oxygen is relatively more deleterious to seeds at lower than at greater moisture contents and confirms that hermetic storage is preferable for long-term seed storage at low moisture contents.

Key words : Seed storage, bulk cotton seed, moisture content, seed survival.

Introduction

Seeds of high quality are those seeds which possess the highest genetic, physical, physiological characteristics of cultivar. Seed longevity may be affected by environmental and biological factors during seed formation and maturation and by handling conditions through harvesting, conditioning and storage (Justice and Bass, 1978). Storage environment influences seed longevity greatly. The principal environmental factors influencing seed deterioration and so seed survival are temperature, moisture content and oxygen partial pressure (Roberts, 1972). The single most important factor affecting seed quality in storage of both seed cotton and bulk cotton seed is moisture. In hermetic storage, at very high moisture contents (from greater than those used in air-dry storage up to fully imbibed), oxygen is greatly beneficial to seed survival, whereas at lower moisture contents (i.e. within the air-dry range) increase in oxygen partial pressure reduces seed survival periods (Roberts, 1972; Roberts & Ellis, 1989).

Cotton seed will come into equilibrium with the moisture content of the surrounding air and/or other material. In this way, relative humidity, green material, wet or damp lint etc. exert a great influence on the storability of seeds. Equilibrium moisture content of cotton seed is reached in eight to ten days and will range from less than 5 per cent at 10 per cent relative humidity to about 18 percent at 90 per cent relative humidity (Simpson and Miller, 1944).

In much of agriculture, open storage is used whereby the seed environment equilibrates (eventually) with ambient relative humidity and temperature while oxygen is freely available at atmospheric concentration. There has been a tendency in seed storage to ignore the role of oxygen and assume its effect on longevity in air-dry storage is modest, compared to those of temperature and moisture. There is indeed evidence that the seed viability equation and the parameter values derived from hermetic storage can be applied to predict the survival of agricultural crop seeds in open storage (*i.e*. with oxygen freely available) satisfactorily at a commercial scale (TeKrony *et al*., 1993).

Autoxidation is posited as a major cause of deterioration in air-dry seeds (Priestley, 1986). Smith (1992) has pointed out that controlling seed moisture content using hermetic containers or using desiccators and saturated salt solutions or desiccants provides different environments for research. In hermetic storage, volume

of air is small and seed (and associated micro-flora) respiration alter gaseous compositions (Roberts and Abdalla, 1968). In the second, desiccators with saturated salt solutions, seeds are in contact with a much larger volume of air. Moreover, if the lid is removed and then replaced (*e.g.* when taking a sample) then both gaseous composition and humidity will revert temporarily towards ambient. That is, the latter system can provide considerably more oxygen and humidity may fluctuate briefly. Seeds may also be exposed to more light in desiccators (*e.g.* Glass) than in hermetic containers (*e.g.* Metal can). Light can enhance the uptake of oxygen for oxidative processes (Vertucci and Leopold, 1987) and has been reported to be deleterious to seed longevity (Vertucci, Roos and Crane, 1994). Finally, saturated salt solutions may release gases which could be either deleterious or beneficial to seed survival (Vertucci and Roos, 1993).

The objective of this investigation was to determine whether or not the the relationship between seed storage longevity and moisture content in air-dry environments differed between sealed containers (hermetic storage) and desiccators providing controlled relative humidity environments.

Materials and Methods

Seeds of Cotton (*Gossypium hirsutum* L.) were selected for investigation, representing fibrous and oily seeds.

In the hermetic storage treatments, 200 seeds at the target moisture content were sealed in laminated aluminium foil bags, polythene bags and craft paper bags. The four target moisture contents (table 1) were provided by humidification in a desiccator over deionised water. In these treatments, each packet of 200 seeds represented a single treatment combination.

Two different treatments were provided by storage in desiccators:

1) four different relative humidities provided by saturated salt solutions *i.e.* NaCI, $NaNO_2$, NaBr, K_2CO_3.

2) four different relative humidities provided by silica gel adjusted with different amounts of deionised water.

Each 1000ml desiccators contained either 250ml of the appropriate saturated salt solution or 500g of silica gel. On a fine mesh sieve above the solution were placed 15g of seed. Prior to experimental storage at 30°C, seed moisture content were adjusted to close to the experimental values by exposure to the appropriate relative humidity in these desiccators for seven days at 20°C. In these treatments, desiccators were opened at intervals, a seed sample drawn at random and the remainder returned to the desiccators. This took no more than 30 seconds. To monitor relative humidity in these desiccators, temperature/ relative humidity sensor was inserted through the hole of the desiccator lid.

Seed moisture contents were determined twice, the first 1-2 weeks after the beginning of experimental storage and the second at the end of storage. Seed moisture contents were determined gravimetrically. Two samples of 3g of seeds were withdrawn from each treatment for each moisture content determination (wet basis, w.b.) at 103±2°C for 17 hours (ISTA, 2005). For the experimental storage treatments, the seeds whether in desiccators or bags were stored in one temperature, 30°C, for a experimental storage of 60 days. To determine the loss of viability during storage, seeds were withdrawn from storage at intervals of 15 days. They were then tested for the ability to germinate between germination papers moistened with water for 12 days (ISTA, 2005) with four replicates of 50 seeds. Each experiment was studied for the effect of container, storage time period and relative humidity (alone and as interaction) in 3 way ANOVA (Fact proa; CRD). Assumptions of normal distribution and homogenous variance were tested by the Kolmogrov Smirnov test and Cochran's C test, respectively. All statistical analysis were done following Chandel (2004).

Results and Discussion

Seed moisture content changed little during experimental storage i.e. between the first (beginning of storage) and final determination (end of storage): seeds tended to dry by 0.3% moisture content over saturated salt solutions, while those in hermetic storage remained within ± 0.2% of the original value. The storage moisture contents shown in table 1 are the mean of both determinations for all treatments. Note that in table 1 whereas the RH values reported for saturated salt solutions are provided from the literature, those for the moistened silica gel were determined directly. In the subsequent analysis, seed moisture status was quantified by seed moisture content since these values were determined for all treatments.

In present study, within each method of storage, there was a negative relation between longevity and moisture content. In hermetic storage, table 2 showed that effect of containers was significant on germination percentage. Polythene bags were the best storage containers (91%) among the three containers. Whereas seeds stored in craft paper bag turned out to be worst container (85%). Effect of storage time period was also significant on germination percentage. Maximum germination

percentage was observed after 15 days of storage and it decreased gradually as the storage period prolonged. Seed treated with 41% RH and 53% RH showed increase in germination percentage as compared to the seeds treated with 62% and 75% RH. Maximum germination percentage was observed at 41%.

Table 2 showed that effect of interaction of containers and storage time period was also significant. Maximum germination percentage (91.6%) was evident in seeds stored in polythene bags at 41% RH whereas minimum germination percentage (85.8%) was for seeds stored in craft paper bags when treated with 75% RH after 60 days of storage. The tables also showed that aluminium foil bags behaved at par with polythene bags. Table 2 also showed that effect of interaction of containers and relative humidity was also significant. These also showed that germination percentage decreased with increase in relative humidity in all three containers.

There was no difference between the values of germination percentage between storage in desiccators

Table 1 : Information on the seed storage environments.

Saturated salt solutions			Moistened silica gel		Hermetic storage
Salts	**RH* (%)**	**Moisture content (%,w.b.)**	**RH (%)**	**Moisture content (%, w.b.)**	**Moisture content (%, w.b.)**
Cotton					
NaCl	75	12.8	76.5	13.8	13.1
$NaNO_2$	62	10.9	66.3	12.3	10.9
NaBr	53	9.3	55.8	10.6	9.7
K_2CO_3	41	8.1	45.8	9.2	8.3

*Vertucci and Roos (1993)

Table 2 : Effect of treatment, storage time period and relative humidity on germination percentage in hermetic storage of cotton seeds.

Container	Time period	Control	Relative Humidity				Mean
			41%	53%	62%	75%	
Aluminium foil bag	15	99.70	98.60	98.10	97.30	97.00	97.75
	30	97.40	97.00	96.10	96.00	94.80	95.98
	45	95.00	94.10	93.90	92.80	91.40	93.05
	60	82.90	81.80	80.40	69.90	66.30	74.60
	Mean	93.75	92.88	92.13	89.00	87.38	90.34
Polythene bag	15	100.00	100.00	99.70	99.30	98.60	99.40
	30	98.30	97.90	96.50	96.70	95.30	96.60
	45	95.40	94.20	93.70	92.60	91.40	92.98
	60	83.70	82.50	82.10	70.50	68.70	75.95
	Mean	94.35	93.65	93.0	89.78	88.50	91.23
Craftpaper bag	15	98.70	98.40	98.20	98.00	97.90	98.13
	30	91.30	90.00	89.70	85.30	84.60	87.40
	45	83.70	82.30	81.20	80.10	80.00	80.90
	60	79.70	78.70	78.30	68.50	66.10	72.90
	Mean	88.90	87.93	87.08	83.18	82.15	85.08
Grand Mean	92.33	91.48	90.73	87.32	86.01	89.40	
CD-.05	C- 14.5	C*T-7.1	C*T*Tr-21.7			C	Container
	T-17.9	C*Tr-12.7				T	Time (Days)
	Tr-15.7	T*Tr-14.2				Tr	Treatment

Table 3 : Effect of treatment, storage time period and relative humidity on germination percentage in open storage (with saturated salt solutions) of cotton seeds.

Container	Time period	Control	Relative Humidity				Mean
			K_2CO_3 (41%)	NaBr (53%)	$NaNO_3$ (62%)	NaCl (75%)	
Desiccators Saturated Salt solutions	15	99.80	97.60	97.40	97.10	96.90	97.25
	30	96.30	96.10	95.90	95.80	95.30	95.78
	45	89.30	88.90	88.60	86.30	84.20	87.00
	60	82.40	82.00	81.70	73.20	71.50	77.10
	Mean	91.95	91.15	90.90	88.10	86.98	89.28
CD-0.05	C- 1.5	C*T-1.1	C*T*Tr-1.7			C	Container
	T-1.9	C*Tr-2.7				T	Time (Days)
	Tr-1.7	T*Tr-1.2				Tr	Treatment

over either moistened silica gel or saturated salt solutions for cotton seeds. Tables 3, 4 showed that the effect of storage time period was significant on germination percentage of cotton seeds. Maximum germination percentage was observed after 15 days of storage and it decreased gradually as the storage period prolonged, in both methods. After 60 days of storage, there was a decrease (20.15%) in saturated salt solutions and (20.09%) in moistened silica gel. Tables 3, 4 also showed that the effect of relative humidity was also significant on germination percentage in both methods of open storage. The values of germination percentage decreased in both methods of open storage with increase in relative humidity. Maximum germination percentage (97.6% in saturated salt solutions and 97.65% in moistened silica gel) was observed at 41% RH.

The results met the expectations (Ellis and Roberts, 1980) of a negative logarithmic relation between seed longevity and moisture content. There was, however, a substantial interaction with method of storage: improvement of longevity with reduction in seed storage moisture content was greater in hermetic storage (laminated aluminium foil bags, polythene bags, craftpaper bags) than in more open storage (desiccators in controlled relative humidities). This interaction resulted in similar longevities for the different storage methods at high moisture contents in equilibrium with about 75-80% relative humidity, but seven to ten fold greater longevity in hermetic storage compared to more open storage for drier seeds in equilibrium with about 35% relative humidity.

This interaction was not caused by toxicity problems of individual saturated salt solutions, because moistened silica gel provided the same relation (Tables 3, 4). Rather, it was due to differences in oxygen availability between the two methods of storage: very limited in hermetic storage (0.626 ml air per 0.384 g seed, *i.e.* 1 g of seed to 1.63 ml air) and considerable in the more open conditions provided by desiccators (1,250 ml air per 15 g seeds of cotton. Given that the lids of desiccators were regularly removed temporarily for sampling, the difference in oxygen availability is therefore much greater than the 19 to 51- fold given above. Evidence for such an interaction between the effects of oxygen and moisture content on seed survival in air-dry storage can be gleaned from earlier research. Roberts (1961) observed that seed longevity in rice (*Oryza sativa* L.) did not differ for hermetic storage with air, oxygen or nitrogen at 14.5% moisture content and 37°C, but a negative effect of oxygen partial pressure was detected at 37°C with 12.0% moisture content.

Priestley (1986) has emphasized a conundrum: damage to seeds by atmospheric autoxidation is potentially greatest at low moisture contents, but despite the consequent expectation for enhanced deterioration of this kind in very dry seeds the evidence for it has proved to be surprisingly elusive. The interaction between the methods of storage and the seed moisture content might be explained by enhanced autoxidation in the more open storage environment at progressively lower seed moisture contents.

The difference in seed longevity between hermetic storage and in desiccators with saturated salt solutions and moistened silica gel may also resolve contradictory reports for rates of loss of seed viability and vigour in lettuce (*Lactuca sativa* L.) and sunflower (*Helianthus annus* L.) at the same temperature (35°C) with low seed moisture contents. Vertucci and Roos (1990) using desiccators and saturated salt solutions reported much more rapid rates of deterioration [*e.g.* reduced viability

Table 4 : Effect of container, storage time period and relative humidity on germination percentage in open storage (with moistened silica gel) of cotton seeds.

Container	Time period	Control	Relative Humidity				Mean
			45.8%	55.8%	62%	76%	
Desiccators with moistened silica gel	15	99.80	97.65	97.50	97.10	96.90	97.29
	30	96.30	96.20	96.00	95.80	95.40	95.85
	45	89.50	89.10	88.70	86.30	84.90	87.25
	60	83.50	82.30	81.60	73.30	71.60	77.20
CD-0.05	Mean	92.28	91.31	90.95	88.13	87.20	89.40
	C-1.05	C*T-1.1	C*T*Tr-1.7			C	Container
	T-1.4	C*Tr-1.5				T	Time (Days)
	Tr-1.7	T*Tr-1.2				Tr	Treatment

within 4 months when stored below 19% RH (*i.e.* about 3.5 and 4.5% moisture content)] than Ellis *et al*. (1995) who used sealed laminated aluminium foil packets [no loss in viability within 16 weeks at 1.3 – 5% moisture content in sunflower, or 48 weeks at 1.3-5% moisture content in lettuce]. Presumably the greater rates of deterioration resulted from the greater availability of oxygen in the desiccators.

In practice, therefore, results from investigations in hermetic storage should only be used to estimate seed survival in hermetic environments and those from more open storage only in open environments, unless predictions are for comparatively moist air-dry environments, where the effect of variation in oxygen supply is negligible. Our results provide additional support to the long-standing practical recommendation that sealed containers be used to store seeds over the long-term for plant genetic resources conservation. The results emphasise the importance of providing hermetic conditions in genebanks, not merely to avoid the dry seeds taking up moisture but also to avoid ingress of oxygen during long-term storage.

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