

## Effects of Shell and Low Moisture Content on Peanut Seed Longevity

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### ABSTRACT

Peanut (*Arachis hypogaea* L.) germplasm accessions in ICRISAT genebank are conserved as pods under medium-term conditions (4 C and 30% RH). Pod storage requires far greater space than seed storage and is more likely to be expensive, especially in a controlled environment. With the objective to evolve cost effective strategies for conservation, the survival of in-shell and shelled seeds of two peanut cultivars, ICGS 76 (virginia bunch) and JL 24 (spanish), was studied under three different storage conditions—ambient (20-40 C and 30-80% RH), short term (23-25 C and 40-50% RH), and medium term (4 C and 30% RH). In-shell seeds had marginally greater longevity than shelled seed in all storage conditions. The differences in time for regeneration of in-shell and shelled seeds stored under medium term conditions were estimated to be less than 4 mo for both the cultivars. Because of the much reduced volume required for storage and the insignificant differences in regeneration interval, conservation of shelled seeds would be highly cost-effective under the controlled environmental conditions, as compared to in-shell seeds. Since storage at very low moisture contents was suggested as a simple and low cost option for conservation of seed lots required for short-term use, the longevity of peanut seeds (cv. ICGS 76) hermetically sealed with 3.6% moisture content was studied in comparison with seeds held at 5.8% moisture. The studies showed that peanut seeds hermetically stored at room temperature (23-25 C) with low moisture content (below 4%) could retain high germination (> 85%) for up to 8 yr.

Key Words: *Arachis hypogaea* L., germplasm, pod, storage.

Peanut (*Arachis hypogaea* L.) is an important crop grown in warm temperate and tropical regions of the world. The seeds are a rich source of edible oil and protein. The peanut germplasm collection assembled at the Int. Crops Res. Inst. for the Semi-Arid Tropics (ICRISAT) to support the crop improvement programs worldwide now consists of 15,342 accessions. The collection is conserved under medium term storage conditions (4 C and 30% RH) in the genebank. Since information available during establishment of the peanut program at ICRISAT showed that seeds are better preserved when stored in-shell than after shelling (Delouche *et al.*, 1973; Baskin, 1979; Navarro *et al.*, 1989), all germplasm accessions are conserved in the form of pods. In-shell seeds are protected from mechanical injuries during handling and from invasion of molds and storage fungi. However, pod storage requires far greater storage volume compared to shelled seeds; hence, it is likely to be more expensive, especially when the storage temperature and relative humidity are controlled by mechanical means. Although the quality of seeds inside the shell remains unknown, seeds often suffer considerable damage during shelling. Bass (1968) reported that hand-shelled peanut seed could be stored for 5 yr without loss of viability at 10 C. Shelled peanut seed held in sealed containers with no more than 6% moisture content at 2-5 C showed no appreciable loss in germination for at least 10 yr (Norden, 1981). Germplasm collections are usually conserved under optimal conditions that maximize seed longevity and minimize frequent regeneration, which risks genetic integrity. The international standards for germplasm conservation recommend that active collections of germplasm (available for immediate use) should be held under conditions which ensure that an accession's viability remains above at least 65% for 10-20 yr (FAO/IPGRI, 1994).

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If shelled seeds could meet these standards, active collections of peanut could be conserved in the form of seeds, thus reducing the conservation costs.

Ellis *et al.* (1990) found considerable benefit to seed longevity by reducing seed moisture content in hermetic storage to very low levels (2-4%), especially in oily seeds. For example, in peanut seeds stored at 50 C, decreasing moisture content from 5.0 to 2.0% was shown to increase longevity by a factor of 42. It was suggested that storage under extremely dry conditions (ultra-dry storage) would be worth pursuing for long-term seed storage under ambient conditions when funds do not allow refrigerated facilities. However, Vertucci and Roos (1993) reported that the water contents recommended by Ellis *et al.* (1990) are less than optimal for storage at cooler temperatures, which could result in more rapid loss of seed viability. Consequent to this, there has been considerable discussion on the use of ultra-dry technology for conservation of base collections of genetic resources (Ellis and Roberts, 1998; Walters *et al.*, 1998). Nevertheless, working collections of germplasm and breeder's seed are dynamic and do not require perpetual conservation. If longevity achievable at ambient temperatures is sufficiently long, low moisture content storage could provide a simple and low cost option for conservation of such material. In earlier studies, peanut seeds predried to 5.8% moisture content (mc) and packed in moisture barrier containers were safely stored for 3 yr or more at 20-30 C (Bass, 1968); while ultra-dry peanut seeds (2% mc) showed significantly greater survival compared to dry storage (5% mc) after 5 yr of storage at 20 C (Ellis *et al.*, 1996). Recently, Hong-yan *et al.* (1997) showed that peanut seeds stored at 3.3% mc could retain high vigor after 11 yr at ambient temperature (2-38 C). In this paper, we report the results of a study on survival of shelled and in-shell seeds under different conditions of storage. We also present the results evaluating storage of seeds with very low seed moisture contents as a cost-effective technique for conservation of working collections of germplasm and breeder's seed.

## Materials and Methods

**Experiment 1.** Two peanut cultivars, ICGS 76 (virginia bunch-type) and JL 24 (spanish-type), harvested from the 1993 postrainy season were used for the study. A portion of the pods was hand-shelled and the healthy seed were packed in airtight plastic bottles. In-shell seeds stored simultaneously in plastic bottles served as a control. The containers were kept under three storage conditions—ambient (20-40 C and 30-80% RH), short term (23-25 C and 40-50% RH), and medium term (4 C and 30% RH). The moisture content of the seeds was determined before storage by the low temperature oven method recommended by the Int. Seed Testing Assoc. (ISTA) (1993). The germination of the seeds was tested by the 'between paper' method using four replicates each of 25 seeds (Rao and Bramel, 2000). The pods were first hand-shelled and mature and healthy seeds, similar to those used for shelled storage, were selected for germination tests. The seeds were incubated at 20 C and the germination was expressed as the percentage of normal seedlings recorded after 10 d. Samples were drawn every 2 yr for 8 yr from all storage treatments and seed deterioration

was studied by conducting germination tests as described above.

**Experiment 2.** In the second experiment, seeds of cultivar ICGS 76 (virginia bunch) were dried to about 3.6% moisture content in a drying cabinet maintained at 20 C and 15-20% RH. The seeds were then sealed in aluminum foil envelopes and stored under the short- and medium-term conditions described above. These treatments also were applied to samples of the original seed lot that had a 5.8% moisture content. The moisture content and germination of the seeds before storage was tested following the same procedures described earlier. Subsequent tests for germination were made by drawing samples from the containers every 2 yr. After opening the seal of the aluminum packets and removing the sample, the packets were immediately resealed and restored to storage for a total of 8 yr. Prior to germination testing, seeds at the low moisture content were humidified by holding the seeds over water for 24 hr in a desiccator, to avoid imbibition injury.

## Results and Discussion

**Experiment 1.** The moisture content of shelled and in-shell seeds before storage ranged from 5.6 to 5.9% in both cultivars and no appreciable changes were detected after storage for 8 yr. After 2 yr of storage under ambient conditions, there was substantial loss in germination (Fig. 1). After 4 yr of storage, both shelled and in-shell seeds deteriorated completely under ambient conditions and there was substantial loss in germination in all seed lots stored under short-term conditions. However, the viability of the seeds

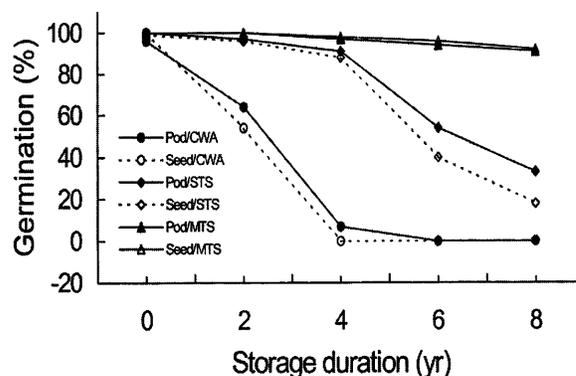


Fig. 1. Seed survival curves of in-shell (pod) and shelled (seed) peanuts stored under ambient (20-40 C/30-80% RH) (CWA), short- (23-25 C/40-50% RH) (STS), and medium-term (4 C/30% RH) (MTS) conditions for 8 yr.

stored under medium-term conditions remained high in all treatments. Under all storage conditions, in-shell seeds deteriorated more slowly than the shelled seeds. Thus, averaged over cultivars, storage environment, and storage duration, the mean viability of in-shell seeds was 68.3% compared to 65.4% in shelled seeds. There were genotypic differences in seed longevity; averaged over storage conditions and storage duration, the mean viability of cv. ICGS 76 was 64.2% compared to 69.5% of cv. JL 24.

Since continuous decline in viability was observed in all seed lots, the seed survival data were subjected to

probit analysis, in which a regression of transformed percentage germination was calculated against time in storage (Ellis and Roberts, 1980). Seed longevity was estimated as the half-viability period ( $P_{50}$ ), which is the number of days in storage for viability to decline to 50% (Table 1). The analysis of variance of the estimates of  $P_{50}$  showed significant effects of storage conditions on longevity of in-shell seeds ( $P < 0.001$ ). However, within each storage environment, differences in longevity between in-shell and shelled seeds were found to be statistically insignificant. The differences in longevity between the two genotypes were significant ( $P < 0.05$ ), but interaction with the storage method (i.e., in-shell or shelled storage) was insignificant.

The results presented here are consistent with those of Mathur *et al.* (1956) and Norden (1981). Mathur *et al.*

**Table 1. Estimates of half-viability period ( $P_{50}$ , in years) of in-shell and shelled peanut seeds stored under different conditions.<sup>a</sup>**

Storage condition	ICGS 76		JL 24	
	In-shell	Shelled	In-shell	Shelled
	-----yr -----		----- yr -----	
Ambient storage (20-40 C/30-80% RH)	1.94	1.84	2.88	2.28
Short-term storage (23-25 C/40-50% RH)	6.10	5.59	7.45	6.16
Medium-term storage (4 C/30% RH)	16.24	14.15	18.75	16.99

<sup>a</sup>LSD (0.05) for comparison of means = 3.04.

(1956) found only slight differences in performance of shelled and in-shell peanut seeds stored at room temperature (22-33 C) and no differences were observed when stored at low temperatures (0-13 C). Norden (1981) reported that hand-shelled peanut seeds at 5 to 6% moisture could be stored for at least 10 yr without appreciable loss in germination at 2 to 5 C, while germination declined to < 10% when held at 17 to 20 C. Norden (1981) observed that viability of spanish seed decreased at a slightly faster rate than virginia seed under similar storage conditions. However, the spanish cultivar (JL 24) used in this study had better storability than the virginia cultivar (ICGS 76), probably due to differences in chemical composition of seeds.

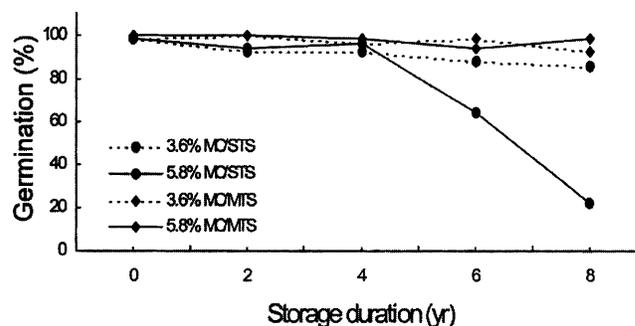
The present study showed no advantage, in terms of seed longevity, with peanut seeds stored in-shell. Consequently, the long-term costs of conservation of in-shell seeds under the controlled environment would be very high, considering the additional space required and the unknown quality of seeds as compared to shelled seeds. In-shell seeds occupy more than twice the volume required for storage of shelled seed and the mean viability of ungraded seed lots obtained after shelling also is expected to be low since they contain some low quality seeds. For example, in the present study, 1 kg of pods of ICGS 76 (2865 cc in volume) after manual shelling yielded 700 g (1040 cc) of healthy and sound seeds with

98% germination and 62 g of shriveled and discolored seeds with 69% germination. In JL 24, manual shelling of 1 kg of pods (3320 cc in volume) resulted in 771 g (1230 cc) of healthy seeds having 98% germination and 49 g of shriveled seeds with 80% germination.

In the ICRISAT genebank, peanut germplasm accessions are regenerated when the germination of the seeds falls below 75%. Based on the estimates of seed longevity derived by probit analysis, germination for in-shell seeds of ICGS 76 stored under medium-term conditions could be expected to decrease to 75% in about 11.7 yr compared to 11.4 yr in shelled seeds. Similarly in JL 24, the germination of in-shell seed is expected to decrease to 75% in 13.0 yr compared to 12.7 yr in shelled seeds. It is therefore evident that the differences in regeneration interval for in-shell and shelled storage under the medium-term storage are small—i.e., less than 4 mo.

In this study, viability of shelled seeds under medium-term conditions remained over 90% after 8 yr, suggesting that standards for conservation of active collections (FAO/IPGRI, 1994) could be met without requiring the more expensive in-shell storage. However, present studies included only two genotypes and further studies on a wide range of genotypes are warranted to recommend seed storage as general policy for management of ICRISAT's peanut collections.

**Experiment 2.** Seeds of cv. ICGS 76 hermetically stored with 5.8% moisture under short-term conditions retained high germination for about 4 yr, but substantial loss in viability occurred thereafter and the percentage germination decreased to 22% after 8 yr of storage (Fig. 2). However, seeds stored at 3.6% moisture still retained fairly high germination (86%) even after 8 yr under short-term conditions. On the other hand, storage under medium-term conditions showed no significant loss in viability at both moisture contents.



**Fig. 2. Effect of moisture content (MC) on germination of peanut seeds stored under short- (23-25 C/40-50% RH) (STS), and medium-term (4 C/30% RH) (MTS) conditions.**

The data on seed survival at the two moisture contents were subjected to probit analysis as described earlier. Based on the 75% regeneration standard, estimates of seed survival obtained by probit analysis indicated that the seeds stored under short-term conditions with 3.6% moisture content would be due for regeneration after 8.3 yr compared to 4.7 yr at 5.8% moisture content. By a similar comparison, under the medium-term conditions (although subject to considerable error because of insufficient deterioration), it would

become necessary to regenerate the seed lot after 47.7 yr when stored with 3.6% moisture content as compared to 19.3 yr for seeds stored with 5.8% moisture content. Thus, storage with low moisture content increased longevity by a factor of 1.8 at short-term conditions and by 2.5 under medium-term conditions compared to normal storage.

This study shows that seeds dried to low moisture contents can be stored successfully for reasonably long periods even at room temperatures (23-25 C). The findings have significant implications for conservation of peanut seed collections, especially by the resource poor national programs. Working collections of peanut germplasm, which are generally for short-term use, and breeder's seed, which require storage for only a few growing seasons, could be maintained in air-conditioned rooms for up to 8 yr in hermetically sealed containers following drying to moisture contents in equilibrium with 15-20% RH at 20 C.

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### Literature Cited

- Baskin, C.C. 1979. The effects of the shell on the viability and moisture content of peanut seeds in storage under alternating relative humidities. *Seed Res.* 7:1-4.
- Bass, L.N. 1968. Effects of temperature, relative humidity and protective packaging on longevity of peanut seeds. *Proc. Assoc. Off. Seed Anal.* 58:58-62.
- Delouche, J.C., R.K. Mathews, G.M. Dougherty, and A.H. Boyd. 1973. Storage of seed in tropical and sub-tropical regions. *Seed Sci. Technol.* 1:671-700.
- Ellis, R.H., T.D. Hong, D. Astley, A.E. Pinnegar, and H.L. Kraak. 1996. Survival of dry and ultra-dry seeds of carrot, groundnut, lettuce, oilseed rape, and onion during five years' hermetic storage at two temperatures. *Seed Sci. Technol.* 24:347-358.
- Ellis, R.H., T.D. Hong, E.H. Roberts, and K.-L. Tao. 1990. Low moisture content limits to relations between seed longevity and moisture. *Ann. Bot.* 65:493-504.
- Ellis, R.H., and E.H. Roberts. 1980. Improved equation for the prediction of seed longevity. *Ann. Bot.* 45:13-30.
- Ellis, R.H., and E.H. Roberts. 1998. How to store seeds to conserve biodiversity. *Nature* 395:758.
- FAO/IPGRI. 1994. *Genebank Standards*. Food and Agric. Org. U.N. and/Int. Plant Genetic Resources Inst., Rome.
- Hong-yan, C., Z. Guang-hua, J. Xin-ming, K. Tin-yun, T. Pei-song, T. Kar-Ling, Z. Ming-de, and D. Nai-xiong. 1997. Storage of peanut seeds with low moisture content for 11 years in ambient temperature. *Plant Genet. Resour. Newsl.* 110:35-40.
- Int. Seed Testing Assoc. (ISTA). 1993. International rules for seed testing. *Seed Sci. Technol.* 21(Suppl.):1-288.
- Mathur, P.B., M. Prasad, and K. Kripal Singh. 1956. Studies in cold storage of peanuts. *J. Sci. Food and Agric.* 7:354-360.
- Navarro, S., E. Donahaye, R. Kleinerman, and H. Haham. 1989. The influence of temperature and moisture content on the germination of peanut seeds. *Peanut Sci.* 16:6-9.
- Norden, A.J. 1981. Effect of preparation and storage environment on lifespan of shelled peanut seed. *Crop Sci.* 21:263-266.
- Rao, N.K., and P.J. Bramel. 2000. *Manual of Genebank Operations and Procedures*. Tech. Manual No. 6, ICRISAT, Patancheru, India.
- Vertucci, C.W., and E.E. Roos. 1993. Theoretical basis of protocols for seed storage II. The influence of temperature on optimal moisture levels. *Seed Sci. Res.* 3:201-203.
- Walters, C., E.E. Roos, D.H. Touchell, P.C. Stanwood, L. Towill, L. Wiesner, and S.A. Eberhart. 1998. Refrigeration can save seeds economically. *Nature* 395:758.