

2. SEED STORAGE, VIABILITY AND REJUVENATION

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Purpose of seed storage

Agricultural seeds often need to be retained for use for more than one season or year, and losses of viability occur if protection against heat, moisture, and pests is not provided. Even the most primitive agriculturist in remote history learned to protect his seeds from deterioration by drying them and by using closed containers to exclude moisture and pests, and any trade in seed is impossible without storage.

In the case of genetic resources, seed needs to be stored for a long period to be available to plant breeders in their quest for particular genes. There are two kinds of collections of genetic resources :

- i. Base collections for long-term storage as a precaution against loss.
- ii. Active collections under medium-term storage used for seed distribution, evaluation, and multiplication.

Principles of seed storage

Seed physiology is a rare scientific specialization and detailed comparative data on the subject are few and rather scattered; but the general principles are fortunately simple. Roberts (1972) and Justice and Bass (1978) have produced excellent handbooks. Barton (1961) is authoritative on the longevity of seeds.

Variation among species

Plant species can be divided into three seed storage groups.

1. Orthodox seeds are those which can be stored without any problem for long periods in dry and cool conditions, and they include chickpea, faba bean and lentil, most cereals, and many other agricultural crops. For example, barley seeds sealed in glass tubes and kept at room temperature had a germination of 12% after 123 years.
- ii. Recalcitrant seeds have a short life span and cannot be dried since they do not tolerate a loss of moisture. This group includes many tropical crops, such as cocoa, sugarcane, coconut, citrus, rubber, and tea. Their seed characteristics are much less well known than those of group i. Conservation *in vitro*, as meristem cultures, may be a solution to the storage problems of this group.

iii. Seeds with unknown, or little known, duration of viability under storage.

This group includes most wild species.

Natural selection, according to scientists at Fort Collins, is still much more important as a cause of variability than alterations occurring in storage; any mutant cells that are formed will mostly be swamped by normal cells as they are usually weaker or even lethal. The Leguminosae include hard-seeded species, the seeds of which may be long lived. For example, Trifolium seeds have been reported to survive for 100 years; Goodia for 105 years; Cassia for 158 years; and Albizia for 147 years. Faba beans, lentils, and chickpeas, that have been selected for edibility and cookability, do not have very hard seed coats and do not remain viable for so long.

Variation among cultivars

Variation among cultivars is often significant. For example, kabuli chickpeas survive shorter periods of storage than desi chickpeas, the latter having a thicker, harder seed coat. Inheritance studies of seed longevity of pulses have not been reported.

Condition of the seeds

Ideally, stored seeds should be fully-matured, of normal size, uninjured, without storage pests and micro-organisms and unaffected by extreme temperatures and moisture conditions during filling and ripening. Initial high viability confers better resistance than low viability to unfavourable storage conditions. The weather at harvest has an important influence on viability. Sometimes, therefore, plants sown for germplasm stocks are grown in off-seasons. For example, sorghum and millet in India produce better seeds in winter because they mature under drier conditions than in the main (rainy) cropping season.

Hand and mechanical threshing should be carried out with care because damaged seeds lose viability more quickly than undamaged seeds. Seed structure, the ease of removal of the seed from the pod, the moisture content of fruits, and the stage of maturity can influence seed damage.

Seed dormancy

Dormancy is found in several crop species. But not all seeds in one lot are necessarily dormant. Seed dormancy is often a reason for plant quarantine to reject samples as they are suspected to be dead. In wild plants dormancy is more common since cultivated plants have lost this attribute. Cicer montbretii, for instance, is known to survive in soil for three years prior to germination. This is probably because it has a hard seed coat, preventing the exchange of water and gas; but even scarified seeds show dormancy. Bracts, glumes, pericarp, and membranes are other seed structures inducing dormancy, whilst

embryo physiology and germination inhibitors can also be involved. For germplasm collection and maintenance purposes, seeds of wild Cicer and Cajanus are routinely scarified to reduce seed dormancy. Alternatively, seeds of at least 2-3 years old should be used. In cold storage dormancy will decrease more slowly than in ordinary temperatures. Genetic differences in dormancy have been found in lentils (Tosun et al., 1980).

Moisture content

This is one of the most important factors affecting seed longevity. In general, the drier the seed the greater its longevity.

Food legumes harvested in a dry climate usually have a moisture content of 8-11% in the seed. At these moisture levels there is no risk of freezing damage, if the seeds are placed in air-tight containers in a cold store. However, if the seed is dried to 5 or 6% moisture content then (assuming a reduction of about four percent in moisture content and a doubling of seed longevity for each one percent decrease in moisture content) an increase in longevity of 2 is obtained. Below 2% moisture content desiccation injury occurs at most temperatures.

High relative humidity causes the seed moisture content to be high. So, if containers are not vapour and air-proof a cooled store room needs to be dehumidified. The techniques used to reduce the moisture content of seeds for storage in gene banks are discussed by Witcombe (Chapter 3).

Studies relating to air and seed moisture content are available for many species. During the monsoon in India, chickpea seeds of kabuli cultivar L550 increased in moisture content from 7 to 12%, while seed of desi cultivars increased by only 1% (observation at ICRISAT).

Temperature

As a 'rule of thumb', between 0 and 50°C seed life is halved for each 5°C and 1% increase in moisture content (Harrington, 1970). If degrees Fahrenheit plus % RH total 100 or less, conditions are considered favourable for longevity (another rule of thumb suggested by James, see Roberts, 1972). Roberts (1972) developed viability nomographs or nomograms giving the time taken for viability to fall to any given level at any given temperature and moisture level.

If variability levels are plotted on a probability (log) scale, the resultant graphs are straight lines instead of normal distribution curves.

The IBPGR has established two standards relating to seed storage:

- i. Preferred standards: -18°C or less; seeds in airtight containers at a seed moisture content of 5 ±1% wet weight (a requirement for long-term storage).
- ii. Acceptable standards: +5°C or less in airtight containers at seed moisture

levels of 5-7%; or 5 C in unsealed containers, with a controlled relative humidity (RH) of 20%.

A level of 20% RH is not expensive to maintain in a well designed store (Witcombe, Chapter 3) and higher levels, of 30-40% RH, can easily be achieved.

The freezing of dry seeds considerably increases longevity, perhaps by centuries. In Fort Collins (USA) trials of storage in liquid nitrogen (-197 C) have so far resulted in no seed damage, although experiments have run for only a few years. Life processes are virtually stopped and also, presumably, the mutations that occur in storage. Repeated freezing and thawing, however, may damage seeds.

Vacuum and gas storage

Results of research on this subject are contradictory. At high moisture levels, oxygen shortens the viability of barley, pea, and other seeds. Living seeds' use up oxygen in sealed containers, so well-filled closed containers are recommended. For most situations the added expense of using storage atmospheres other than air appears unnecessary. Such atmospheres may be advantageous for very long-term storage, but samples cannot then be easily taken. The control of seed moisture and temperature are of much greater importance than that of the gas medium in seed storage.

Chemicals and pests

Chemicals applied to control fungi, bacteria, viruses, pests and rodents may affect germination and longevity. Dry seeds stored cool are little affected until germination, as the life processes of the parasites also cease. The use of naphthalene balls has proved effective and convenient (except for the odour and possible health damage to workers continually exposed to vapour) to avert insect damage, but in cool storage this is unnecessary. There is no established evidence that exposing seeds to naphthalene causes loss of viability.

Mercuric chloride, liquid organic mercurials, and hot water (used in disease control) all reduce seed longevity. Fungicidal dusts usually cause no injury, but there are many exceptions. At low temperatures injury from chemicals is reduced. Fumigants vary in their influence on seed germination. For example, organic mercury reduces the viability of eggplant seeds, but not of carrot, pea, pepper, and tomato. Methyl bromide reduces the vigour and viability of seedlings of several crops, especially when applied in high temperature or moisture regimes.

Fungi do not grow on seeds with a moisture content of less than 12%, and seeds containing less than 9% moisture are rarely attacked by insects.

Sample size

The number of seeds per sample should be at least 12000 for long-term storage. An accession of an inbred plant is sufficiently represented with 4000 seeds. In practice often the container size, or row length in the field, decides how many seeds are kept. The large-seeded grain legumes, in particular faba bean cultivars, require larger than average storage space. Opinions still differ on how many of such seeds should be kept.

Germination tests

After harvesting and drying, the seeds should be subjected to an initial germination test. Methods for germination tests are given in the International Rules for Seed Testing (ISTA, 1966), and they prescribe the optimum method and temperature for many species. Cultivar differences exist, and must be correlated to seed moisture content, determined by weight change on drying or electric moisture meters. Grain legumes are tested in sand or petri dishes, or between paper towels at favourable temperatures. Various equipment is used to facilitate the sampling, counting, and germinating of seeds (ISTA, 1966; Justice and Bass, 1978). A convenient method is to germinate the seeds on moist filter paper in 9-cm petri dishes at 20-30°C. The data should be recorded on standard forms and an example is shown in Appendix 4. Chemical tests (e.g., with tetrazolium) kill the seeds, but are very useful in research to pinpoint dead and living areas in the seeds.

In long-term cold storage 2-4 samples of 50-100 seeds should be taken every 5-10 years to measure their viability, although it may well be found that the viability period is very long. Sampling reduces diversity, both in respect of numbers as well as of populations if the sample is heterogeneous. Sampling should be at random. But it should be noted that even seeds in a homogeneous, homozygous sample will differ in viability.

To economize on seed, it is an excellent practical idea to keep a few samples of seed in large quantities in the cold store under the same conditions as the germplasm material. These samples can be then tested frequently, say yearly, using large samples.

Ellis et al. (1980) have proposed a method of germination testing, 'sequential testing', that uses fewer seeds than the ISTA method (Table 1).

An initial quantity of 40 seeds is tested. Depending on the results of this test the accession is either classified as requiring rejuvenation, or a further test is made. At this second test, and all subsequent tests in the sequence, there are three choices depending on the result of the test.

i. If the germination is below a certain level the accession requires

regeneration and no further testing is necessary.

- ii. If the germination' is at an intermediate level, the result is still unclear and a further test is required.
- iii. If the germination is high there is no need for further . testing and the accession is retained in the cold store.

Table 1. Sequential germination test plan for 85% regeneration standard, testing seeds in groups of 40 (Ellis et al., 1980)

No. of seeds tested	Regenerate if no. of seeds germinated is <=:	Retest accession if no. of seeds germinated between:	Keep in store if no. of seeds germinated is >=:
40	29	30-40	-
80	64	65-75	76
120	100	101-110	111
160	135	136-145	146
200	170	171-180	181
240	205	206-215	216
280	240	241-250	251
320	275	276-285	286
360	310	311-320	321
400	345	346-355	356
440	380	381-390	391
480	415	416-425	426
520	450	451-460	461
560	485	486-495	496
600	520	521-531	532

Storage of pollen

Pollen from a few families (Leguminosae, Primulaceae, Pipaceae) can remain viable for long periods if kept cool and desiccated. Gramineae pollen is short-lived. Ageing factors are mostly the same as for seeds: respiratory substrate exhaustion, enzyme inactivation, desiccation injury, blocking by secondary metabolic products. Pollen storage is complicated and collection of legume pollen is tedious, and is not yet a practical method of storage.

Rejuvenation

Theoretical considerations

Rejuvenation of germplasm is needed when either germination drops below a certain level (50%, 80%) or seed stock falls below an acceptable minimum, which varies with species, breeding system, and opinion. It is wise not to have less than 1000 seeds in stock, but in large-seeded legumes practice often dictates otherwise. Seed increase should preferably be carried out in the area of origin, or one similar to it, in the usual season, or if necessary in the off-season so that ripening takes place in dry weather.

Chickpea and lentil can be grown without isolation, because natural outcrossing is very low (<1%). Faba bean has a rate of outcrossing which varies between 20-40%; hence rejuvenation of faba germplasm must be done in isolation. The methods of isolation in faba bean are discussed in Witcombe (Chapter 12).

An outbreeding species ideally needs to be maintained as a population, however impractical this often is. Such populations contain considerable heterogeneity, which poses problems in characterization and evaluation. The number of accessions or populations could be reduced by merging similar accessions into gene pools. Timothy and Goodman (1979) discourage the formation of gene pools as tools for the maintenance of genetic resources unless they are carefully manipulated into separate usable units. The bulks would contain accessions brought together on the basis of similar characteristics, such as geographical background, or multivariate (cluster) analysis of evaluation data (see trait specific gene-pools in Witcombe, Chapter 12).

Management

In practice the plot size used for the maintenance of germplasm accessions is small. A plot of 2 x 2 m of chickpeas often gives enough seed to fill a bottle with 0.5-1.0 kg capacity. At ICRISAT chickpea is grown in double rows of 4 m, spaced 25 cm apart on the top of ridges spaced 60 or 75 cm apart, but rows or plots on the flat are equally suitable. The recommended row spacing for lentils is 25-30 cm to give a plant population of around 100 plants/m². This will ensure a high rate of seed multiplication. Grouping of material of similar maturity is useful, but this is often difficult to plan, especially if the accessions have not been previously evaluated.

Fertilizer application and irrigation are needed to ensure proper stands and satisfactory yields. The amount of irrigation required varies according to available soil moisture and rainfall. Since overdoses may lead to an excessive vegetative growth that reduces yield and makes harvesting cumbersome, or to

infestation with root diseases, starter dosage rates of 20 kg of N and 60 kg of P_2O_5 /ha are recommended.

Harvesting very small plots must necessarily be done by hand. Threshing can be done by small threshers, but care must be taken to empty the machine entirely between samples to avoid contamination. Drying is important. The drier the samples the less need there is for artificial drying. Seeds are first put into bags, preferably labelled both inside with a loose tag and outside with an attached label. In various cleaning operations the loose label can be shifted from tray to tray.

Germplasm rejuvenation (and evaluation) plots should be well protected against pests and diseases. Accessions not well adapted to the environment under which they are grown may otherwise be lost. Screening against pests and diseases should always be conducted separately. Great attention must be paid to avoid mistakes in labelling and the inadvertent mixing of accessions.

Observations

If germplasm has already been sufficiently evaluated, only a few notes such as flower colour, flowering date, and growth habit need to be taken to ensure the identity of the accession. Identification of off-types is necessary in both lines and populations. Off-types may be sufficiently interesting to be maintained separately.

Frequency of rejuvenation

The frequency of rejuvenation is often determined more by seed supply requirements than by considerations of the longevity of the seed. The storage of large amounts of seed is cheaper than frequent grow-outs, and reduces the chance of mistakes. Errors can be made at all levels, but must be reduced to the minimum.

Duplicate accessions

At ICARDA, ICRISAT, and other international institutes a number of samples is maintained that bear the same accession name ('administrative' duplicates) but differ in regard to seed and plant characteristics. Commonly, samples have been obtained from different sources under the same name. All such samples need to be maintained separately unless the description of the original line is known and staff can thus discard the wrongly labelled lines. Natural selection on a single variety of faba bean grown in different places will tend to result in

different populations being formed, because of the different selection pressures on the original heterogeneous variety and outcrossing.

The problem of maintaining real duplicates is more complex. If the size of a collection is manageable, it is better to be cautious and maintain duplicates, unless all descriptors match after several grow-outs. It is difficult to be sure that all genes of two genotypes are similar if the phenotypes are similar. An example is resistant sub-lines, containing genes or alleles for resistance not present in all seeds of an otherwise homogenous population sample. On the other hand, one should never attempt to split a collection into the most refined set of inbreds. For the purpose of germplasm maintenance, populations are preferable. Subsampling, except for useful genes, leads to an explosion of numbers and an unmanageable situation.

References

- Barton, L.V. 1961. Seed preservation and longevity. London: Leonard Hill.
- Ellis, R.H., Roberts, E.H. and Whitehead, J. 1980. A new, more economic and accurate approach to monitoring the viability of accessions during storage in seed banks. *Plant Genetic Resources Newsletter* 41: 3-18.
- Harrington, J.F. 1970. Seed and pollen storage for conservation of gene resources. Pages 501-521 in *Genetic Resources in Plants; Their Exploration and Conservation*, IBP Handbook no.11, eds. O.H. Frankel and E. Bennett, Oxford and Edinburgh: Blackwell.
- International Seed Testing Association. 1966. International rules for seed testing. *Proceedings of the International Seed Testing Association* 31: 1-152.
- Justice, O.L. and Bass, L.N. 1978. Principles and practices of seed storage. *USDA Agricultural Handbook* 506. Washington DC: Government Printer.
- Roberts, E.H. 1972. Viability of seeds. London: Chapman and Hall.
- Timothy, D.H. and Goodman, M.H. 1979. Germplasm preservation: the basis of future feast or famine. Genetic resources of maize: an example. Pages 171-200 in *The Plant Seed: Development, Preservation, and Germination*, ed. I. Rubenstein, New York and London: Academic Press.
- Tosun, O., Eser, D and Gecit, M.H. 1980. Dormancy in lentils. *LENS* 7: 42-46.

