Indian J. Pl. Genet. Resources 1(1&2) 103–107

# Seed Physiology in Relation to Genetic Conservation

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*The requirements for long and medium term seed storage are different. Even under most ideal conditions of storage, loss in seed viability can not be checked but it could be substantially reduced. The loss of seed viability leads to loss of genetic characteristics (genes). This requires frequent regeneration. The most ideal conditions which have been created for seed storage are energy dependent, capital intensive and expensive. Therefore, it has been suggested to conduct research in areas where the temperatures during most parts of the year are sub-zero, to study the suitability of the region for long-term storage of germplasm under natural conditions. It has also been suggested to study the suitability of various moisture vapour-proof containers for seed packaging under such conditions.*

The world's human population has reached 4.8 billion and is expected to increase. To feed the human population, the food production must increase. Most of the increase in food production would be expected to be achieved by evolving superior high yielding cultivars through plant breeding. To meet the growing needs of crop improvement programmes in the country, a large variability in genetic stocks is required. It is, therefore, necessary to explore genetic diversity, collect and conserve it adequately as seeds in gene banks and in case of crops that cannot be stored in the form of seeds, it may be conserved as *in vitro* cultures or in field gene banks. In this article only storage of seed in gene banks has been considered.

Plant species and their current forms can be broadly classified as (1) orthodox seeds : such seeds can be dried to a low moisture content (5% or less) and can be frozen without damage ; with reduction in seed moisture content the mean viability period of orthodox seeds increases e.g. seeds of cereals, oilseeds and pulses and (2) recalcitrant seeds or those seeds which cannot be dried below a relatively high moisture level or frozen without lowering its viability e.g. seeds of rubber, cacao and many species of tropical trees.

The storage requirements for these two categories of seeds are vastly different. Besides, a substantial variability is observed even in different genotypes of the same species in respect to their longevity (Agrawal, 1979 ; 1980). For example, seeds of green gram and okra are good storers, wheat and paddy are moderate storers while onion and soybean seeds are poor storers. Because of this variation in storage quality, viability and germination of stored seeds must be monitored regularly and conserved under proper conditions.

## SEED DETERIORATION

It is understood that even under the most ideal conditions of storage, loss in seed viability cannot be checked completely but it could be substantially reduced. Moreover, even much before the fall in germination, the process of seed deterioration is manifested by the loss of seed vigour (degree of aliveness), alteration in the metabolism and ultrastructure, increase in chromosomal aberrations, which bring genetic changes and affect the quality of the seed.

Even the genetically most homogeneous population is variable so far as its longevity is concerned. Each seed in a population is at a different level of vigour and viability whose storage potential is affected by its genotype, pre-storage environment (including conditions during ripening, harvesting, etc.) and storage environment. Thus, storability of a given seed sample is a function of seed factors and storage factors. Since the genotype and pre-storage environment would affect the life-span of the seeds from a lot to the same extent, the degree of deterioration would be a function of time, moisture content and temperature. Taking these factors into consideration, Roberts (1972) derived viability equations to predict the loss of viability of a given seed lot under a prescribed set of conditions.

### *Effect of moisture content and temperature on the survival of orthodox seeds*

Roberts (1972) observed that a linear relationship exists between storage temperature, moisture content and viability period of orthodox seeds. However, this linearity breaks down beyond a narrow limit and consequently the original viability equations would be inaccurate if extrapolated to extreme conditions such as sub-zero temperatures and excessively low moisture contents which is generally the requirement for long-term storage. Therefore, the viability equations were improved for estimating the slow rate of deterioration at sub-zero temperatures (Roberts and Ellis, 1977). In an attempt to quantify the relationship between temperature, moisture and viability period, Harrington (1963) suggested that the viability period of a seed lot is doubled by reducing its moisture content by 1% or the storage temperature by 5°C within a range of 1–14% and 0–50°C respectively. Excessive drying is reported to aggravate the seed deterioration during storage in peas, beans, papaya, etc. (Nakamura, 1975 ; Arumugam and Shanmungavelu, 1977). On the other hand, seeds with high moisture content cannot be stored at sub-zero temperatures because of the danger of ice crystal formation in the cells. Thus, the term 'safe' moisture content is relevant only when the temperature, period of storage and the required minimum percentage viability are also specified. If the seed is being stored in a sealed container, its moisture content should be lowered accordingly (for which proper drying facilities have to be created) before it is stored at sub-zero temperatures. The requirements would be different for storage under ambient conditions or even for storage at relatively higher temperatures.

## STORAGE REQUIREMENTS OF ORTHODOX SEEDS

For conservation of genetic resources, IBPGR has designated the following types.

*Base collections* : These are for long-term storage (20-50 years). Seed stored in base collections are not normally used for distribution.

*Active collections* : These are medium-term storage (5–8 years). Seeds stored in active collections are used for distribution, multiplication and characterisation.

*Working collections* : These are used for evaluation, testing, selection and hybridisation.

The storage requirements for the above-mentioned collections would be different. For making a decision on storage requirements for a particular situation, one has to consider the species to be stored, the genotypic variation in seed longevity within a species, period of storage, packaging material for seed storage, availability of drying facilities, etc.

The International Board for Plant Genetic Resources, has recommended 5%±1 seed moisture and—18°C, or less as storage temperature in air tight containers for long-term storage (IBPGR, 1976). Even under such conditions seed deterioration does occur but at a very slow rate. For medium-term storage a temperature of 2–4°C, and relative humidity of 40–45% is recommended. The seed moisture content should preferably not exceed 7% if they are stored, sealed in moisture proof containers. The National Bureau of Plant Genetic Resources, New Delhi, India has recently established long-term seed storage facility (gene bank) in which storage temperature of —18°C is maintained along with medium term storage at +4°C.

Long-term storage facilities all over the world are energy dependent and expensive, which necessitates alternate method for long-term seed storage under natural conditions of low temperature. More important regions such as Antarctic region, Dakshin Gangotri, where India has a base camp (latitude 70°5'S, longitude 12°E), may be considered for experimentation for long-term storage of seeds (base collection). The region is characterised by sub-zero temperatures during most part of the year. The minimum temperature ranges from —13.0°C (January) to —48.0°C (August), maximum from + 4.0°C (January) to —13.0°C (August), the average temperature being —4.0°C in January (warmest) and —30.0°C in August (coldest). The seeds here will be facing a changing environment nevertheless for most of the year, will be under subfreezing temperatures.

Research has to be conducted on packaging materials also. These materials should be moisture vapour proof, should be able to withstand changes in temperature (+4°C to —48°C), easy to seal, reseal (if necessary) and inexpensive. It should also be able to stand rapid changes in temperature which occurs during sealing. Laminated aluminium foil, pyrex glass ampules and polythene (800 gauge or more) bags may be experimented with for packaging. Plastic containers are not suggested as they may emit mutagenic gases.

## GENETIC STABILITY IN ORTHODOX SEEDS

To ensure genetic stability as mentioned earlier, seed under storage undergoes various metabolic changes albeit at a very slow rate. These include degradation of cellular membranes, shift in respiratory pathways (Kharlukhi and Agrawal, 1984), decrease in enzyme activities (Agrawal and Kharlukhi, 1987), and accumulation of chromosomal aberrations, etc. The latter process directly affects the genetic purity while the former ones result in the decrease in germination. Abdalla and Roberts (1969) observed that in peas and barley, any storage treatment which leads to a loss of viability of about 50 per cent induces recessive chlorophyll mutations in about 1-4 per cent of the surviving seeds. Considering that genes affecting chlorophyll synthesis are not outstandingly unstable than the others, actual extent of total mutation will be extremely high. Considerable accumulation of heritable changes can be obtained even when the loss in viability is small. In peas accelerated aging (40±2°C, 100% R.H.) resulted in mutation within the polygenic system controlling quantitative characters e.g. flowering and yield per plant (Purkar et al., 1982).

Villiers (1974) suggested that this situation can be recovered by storing the seeds under imbibed (hydrated) conditions. No chromosomal aberrations were observed in dormant lettuce seeds stored under full, imbibed conditions for 15 months while those stored in air-dry conditions showed an arrest of deterioration and reversal of cellular damage upon imbibition.

The damage in dry-stored seeds is considered to be due to peroxidation reactions causing deterioration of lipids and proteins of the cellular membranes. The process which is autocatalytic, can cause general damage including cross linkages within and between the nucleic acid molecules and its associated proteins in the chromatin. Thus, loss of viability is a process manifested in the damage of the membrane systems and enzymes as well as that of the gene.

## STORAGE OF RECALCITRANT SEEDS

One major problem concerning recalcitrant seeds is their correct classification. There are many specific examples of misinterpretation of orthodox seeds as recalcitrant. Coffee and citrus seeds are two such species. Coffee seeds in fact behave like orthodox seeds such as wheat and lettuce in a range of 10–20% and 30–40% moisture content respectively (Roberts et al., 1984). Similarly, citrus, which was earlier classified as recalcitrant, has been shown to be orthodox in nature where the germination is actually delayed due to dehydration.

However, the fact remains that many economically important species belong to the recalcitrant group. For such seeds, appropriate storage protocols need to be evolved by which viability can be maintained under hydrated conditions. There is no satisfactory method available at present for long-term storage of recalcitrant seeds. It has been reported (Grout, 1980) that treating tomato seeds with 15% dimethylsulphoxide (DMSO), a cryoprotectant, viability could be maintained in seeds with 33.4% m.c. at —196°C. Though tomato is an orthodox seed, similar

attempts may prove useful for recalcitrant seeds. National Bureau of Plant Genetic Resources has initiated researches both on *in vitro* conservation as well as cryopreservation of seeds, pollen and *in vitro* cultures.

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