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Significance and Methods of Pollen Storage

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Systematic research on pollen storage started at the end of the 19th century. There is large number of crop species, including vegetables, fibre and fruit crops, forage and cereals, for which pollen storage strategies are desirable. Genetic conservation through pollen storage is desirable for a variety of horticultural plant species, since pollen is known to transmit important genetically heritable characters. Pollen is a product of genetic recombination and can provide a reliable source of nuclear genetic diversity at the haploid stage. Although genetic conservation through pollen storage does not accomplish the whole genome conservation, a plant breeder involved in genetic enhancement of a given horticultural crop could have access to a facility called 'Pollen Cryobank', from where he can draw pollen parents of his choice in the process of breeding a new cultivar

Keeping the viability and vigour intact the pollen grains can be suitably stored in appropriate containers like, glass or plastic vials for an extended period of time. Such containers are stored in desiccators with dehydrating agents to control humidity. Saturated solutions of different salts are used to obtain the required humidity. Lycopodium spores are used as diluents before storage to increase the bulk of pollen and prevent wastage of pollen sample during artificial pollination. This diluent has all the property of a good diluent, like non sticky and non-hydrating and in addition it keeps the viability rate quite high. It also provides its own growth factors, which leads to higher percentage of germination.

Comprehensive studies have been done to assess the different storage conditions that can prolong the viability of pollen grains. This storage can be conveniently grouped as short term and long-term storage methods.

Method of Pollen Storage:

I. Short Term Pollen Storage: It includes the effect of temperature and humidity, and pollen storage in organic solvent.

(i) Effects of Temperature and Humidity:

In general, low temperature and relative humidities are favourable for most taxa. However, pollen of a large number of taxa can be successfully stored for a limited period of time through the manipulation of storage temperature and humidity. Pollen grains can be suitably stored in glass or polythene vials or in appropriate containers.

In case of unsealed containers suitable dehydrating agents like silica gel, various concentrations of sulphuric acid or saturated solution of different salts are used to maintain required relative humidity.

Sticking together of the pollen grains can be prevented by using Lycopodium powder, talc, or corn, or wheat flour. Tricellular pollen of Gramineae requires sophisticated environmental conditions to preserve viability and fertility even for a short period storage.

(ii) Pollen Storage in Organic Solvent

Iwanami and Nakamura (1982) first demonstrated the use of different organic solvents in pollen storage. The organic solvents include benzene, petroleum, diethyl ether, acetone, chloroform, etc., whose efficiency varies greatly for different plant species. Pollen grains stored in non-polar organic solvents like benzene, diethyl ether and cyclohexane retained viability and showed very little leaching of phospholipids, sugars, and amino acids into the solvent. On the other hand, extensive leaching of substance and loss of viability was seen in polar organic solvents, thus establishing a correlation between the polarity of the solvents and its potency for pollen storage.

The Citrus pollen-maintained viability in different organic solvents for three months. Investigation in plants like, *Armenica vulgaris, Camellia japonica, Ginkgo biloba, Juglans regia, Malus pumila, Prunus triloba, Prunus percia, Salix babylonica,* and *Zea*

mays shows that the insect pollinated species stored in a suitable organic solvent at 4°C for 35-40 days exhibited the needed viability. *Chrysanthemum pacificum* pollen loses viability (12%) in dry conditions (25° C) within 60 minutes. When pollen grains stored in such dry conditions for 30 minutes are then transferred to diethyl ether, the viability remained at the level of 12% even after 20 days of storage.

Removable from the organic solvent thereafter, lead to complete loss of viability within 30 minutes. Literally while storage in organic solvent there was no loss of viability which has been referred as absolute dormancy. Inspite of the fact that the efficacy of individual organic solvents varies greatly for different plant solvent has proved to be better for the storage of any pollen than low temperature and humidity.

II. Long Term Pollen Storage

Storage at temperatures above 0°C slows down the metabolic activity of the pollen, resulting in gradual decrease and finally total loss of pollen viability. Thus for a long term preservation cryogenic technique seems to be more promising. Some of the methods of long term preservation are stated below.

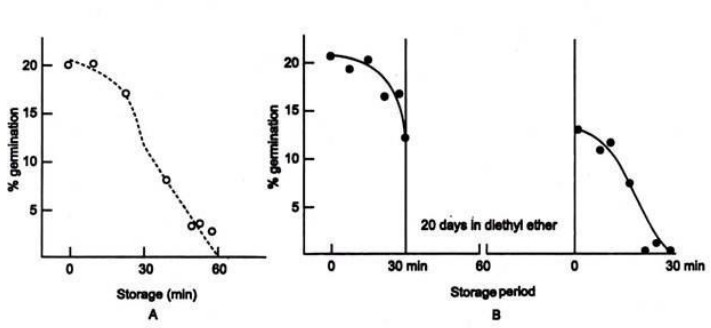

Fig 1: Induction of absolute dormancy in pollen of *Chrysanthemum pacificum* by diethyl ether

A:At 25^{0}C pollen vitality is lost in 60 minutes under dry conditions.

B:Storage in diethyl ether after 30 min. dry storage and tested after 20 days storage showed no loss of vitality during the period of storage in organic solvent

i. Storage at Sub-Zero Temperatures

Using a storage temperature of -10° C and – 34° C the longevity of bicellular pollen (desiccation tolerant) and pollen with original low content of moisture has been successfully extended between 1 and 3 years.

ii. Freeze or Vacuum Drying (lyophilization):

Pollen of different taxa especially the desiccation-tolerant pollen can be successfully preserved for a long period of time by freeze or vacuum dying method. Freeze-drying involves the rapid freezing of pollen to sub-zero temperature of -60° C or -80° C using inert gas helium or nitrogen, and then the gradual removable of water under vacuum sublimation.

In vacuum drying the pollens are directly exposed to a vacuum and simultaneous cooling. The moisture is later withdrawn by evaporative cooling. In number of taxa when freeze drying is combined with lyophilization then storage and viability of pollen has been found to be very effective.

iii. Cryopreservation by Deep-Freezing:

Long term preservation can also be done by ultra low temperature, ranging between -70° C and -196° C. Since the first half of 1950s, several studies have been conducted on the cryopreservation of pollen. A list of few important crop species whose pollen has been successfully stored at cryogenic temperature is presented below in Table1.

Table 1: Cryostorage of pollen from few crop species

Crops	Storage temperature (^{0}C)	Duration of storage
Beta vulgaris	-196	1 year
Brassica oleracea	-196	16 months
Capsicum annuum	-196	42 months
Carica papaya	-196	485 days
Glycine max	-192	21 days
Helianthus annuus	-76, -196	4 years
Lycopersicon esculentum	-196	1062 days
Prunus persica	-196	1 year
Pyrus communis	-196	7 months
Pyrus malus	-196	673 days
Solanum tuberosum	-196	24 months
Vicia faba	-196	1 month
Zea mays	-196	10 years

A reduction in the pollen water content below a threshold level before low temperature exposure seems to be important for achieving viability. Thus

partially dehydrated pollen possesses less freezable water and can survive deep freezing. However, further studies are needed to determine the critical moisture level of the pollen grains for a successful long-term cryo storage. Studies on the molecular structure of the membrane using Fourier transform infrared spectroscopy (FTIR) indicate that lipid transitions in membranes may cause major damage during freezing or warming after freeze-thaw has been completed.

The Graminaceous pollen contains nearly 35 to 60% water when shed, thus immediate freezing would cause irreversible structural damage as a result of ice formation. Thus, the water content of the pollen needs to be reduced before cryo storage, which is however, problematic, as there is rapid loss of viability with decreasing water content. Water loss without any detrimental effects in pollen viability ranges between 50 % and 80%, this however, again depends on the species and its genotype. Secale cereale and *Zea mays* can tolerate higher degrees of desiccation than the grains of Triticale. Triticum aestivum is however, intolerant to any degree of dehydration.

Some of the agronomically important Gramineae species shown in the Table 2 have been stored successfully at cryogenic temperatures or in deep freezer for long periods.

Table 2: Gramineae pollen in cryostorage

Species	Storage temperature (^{0}C)	Duration of storage
Avena sativa	-192	1 day
Saccharum spontaneous	-80	30 to 140 days
Secale cereale	-192	7 days
Triticum aestivum	1-192 to -196	1 day to 10 years
Zea mays	-196	10 years

Significance of Storage

I. The spatial and temporal isolation of parental species that enforce cross pollination barriers can be overcome.

II. In order to continue productivity supplementary pollinations like pollen sprays can be implemented.

III. In breeding programmes there is no need to grow the pollen parent continuously.

IV. Genetic property can be conserved and can be a source for germplasm in international exchange programmes.

V. In the study of pollen allergy and pollen biology it can serve as a continuous source of pollen.

VI. Exotic nuclear genetic diversity can be easily received and exchanged through pollen, thereby eliminating the need to go through a long juvenile phase, common in most fruit trees to produce pollen for hybridization at a desired location. Thus, stored pollen can be used to improve breeding efficiency.

VII. Fruit tree pollen is generally required to be stored for controlled crossings, either to achieve a desired breeding objective, or to overcome a constraint involved in commercial fruit production.

VIII. Pollen storage has come to the rescue, where stored pollen indexed as viable can be used in crossing with the desired female clone so as to accomplish the breeding objective.

References

Iwanami and Nakamura (1982). Efficacy of organic solvents for storing pollen grains of some leguminous taxa; Euphytics 31, 991-995

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