Pollen Cryopreservation in Vegetable and Ornamental Species: Retrospects and Prospects

PE Rajasekharan and S Ganeshan
Pollen Storage Laboratory, Plant Genetic Resources Division, Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bangalore-560089, Karnataka

Key Words: Cryopreservation, Pollen, Nuclear Genetic Diversity

Conservation of nuclear genetic diversity (NGD) using pollen is desirable in horticultural species for a variety of reasons. Cryopreserved pollen can be a major access point for pre-breeding germplasm lines, hybrid seed production, biotechnological and other basic studies. In case of tree species, germplasm can be easily received and exchanged through pollen, eliminating a long juvenile phase. The objective of a useful pollen cryostorage protocol is to collect mature pollen from plant and treat it so as to retain its normal function, ultimately assessed by its ability to germinate *in vivo* and effect normal fertilization (Hanna and Towill, 1995). Hoekstra (1995) has assessed the merits and demerits of pollen as genetic resource. Ganeshan and Rajasekharan (1995) reviewed work on ornamental crop pollen storage. Recently Barnabas and Kovacs (1997) and Berthoud (1997) stressed the importance and need for pollen conservation. Response to cryopreservation experiments obtained with pollen conservation are presented in Table 1. In some of the recently cryostored pollen only feasibility tests were carried out. In most species, protocols are optimized for establishing pollen cryobanks. Besides the already existing role of pollen banks in breeding, there are many promising applications which has come to focus with the recent advances in allied bioscientific areas.

The protocols for pollen collection, viability assessment (pre- and post-storage), processing for cryopreservation, retrieval and fertility assessment followed at IIHR for vegetable (Rajasekharan and Ganeshan, 1994) and ornamental crops (Ganeshan and Rajasekharan, 1995) are described in detail.

Status of cryopreservation of pollen of various species is presented in Table 1. Extended availability of nuclear gene pool through application of cryogenic technology can help redesign crop breeding strategies, integrating conventional and biotechnological techniques for crop improvement. Exchange of dry pollen as germplasm pose relatively less quarantine problems and provides a 'snap shot' of nuclear genetic diversity of the species in question for a given ecosystem. A 'Pollen Cryobank' has been established at IIHR, maintaining more than 600 tropical pollen accessions collected during different seasons and years, kept under constant cryogenic conditions, through periodical replenishment of the cryogen (liquid nitrogen) over the past 18 years.

Crossing of desirable genotypes involves multiple and staggered plantings in order to synchronize flowering. This can be avoided when cryopreserved viable pollen is available, facilitating hybrids between genera, species and genotypes. This could effectively conserve field and greenhouse space. A pollen cryobank for a given crop can provide constant supply of viable and fertile pollen and can also allow supplementary pollination for improving seed set. The variability due to daily pollen collection can be nullified (Barnabas and Kovacs, 1997). Male sterile populations can be perpetuated by cryostored maintainer pollen, thus avoiding frequent planting of maintainer line. Large scale consolidation of potential pollen from male parents will ensure uninterrupted supply of the male gametophyte for hybrid seed production at a given location and pollen can be transported to different locations where seed parents are grown for crossing (Ganeshan and Rajasekharan, 1995). Cryogenic technology applied to pollen conservation facilitates integration of conventional breeding methods with modern biotechnological practices.

The international transfer of germplasm in the form of dry pollen is not generally restricted (Hoekstra, 1995). Moreover, this will eliminate the need of growing plant populations to produce pollen. Pollen is subjected to less stringent quarantine restrictions. So, it can be easily shipped and used. Through exchange of pollen, desired crosses can be made directly on the seed parent, allowing introgression of characters at a much faster rate. This would find favour especially in breeding of tree species with long juvenile phase. Conservation and management

Table 1. Response to pollen cryopreservation of different plant species

Species/Cultivars	Family	Pollen Longevity (Kozaki, 1975)	Longevity (CYP) (Year)	PSVFP # Accessions	Current status	Cryofeasibility
Gladiolus sp.	Iridaceae	Medium	11	V-7, F-7	20	
G. callianthus				Under study		
Allium cepa	Liliaceae	Medium	13	V-7, F-7	27	
A. fistulosum				Under study		
Rosa sp.	Punicaceae	Not reported	13	Under study	10	Good
R. indica	Rosaceae	Long	1-2	V-5, F-6		
R. multiflora				Under study	40	Good
Capsicum annuum				V-5, F-6		
C. baccatum	Solanaceae	Medium	6-9	V-6, F-6		
C. frutescens				Under study	63	Good
C. praertermissum				Under study		Good
Lycopersicon esculentum					Under study	Good
L. hisutum			9-10	V-9, F-9		
L. peruvianum				Under study	91	Good
L. pimpinellifolium				V-9, F-9		
Solanum erianthum					V-9, F-9	
S. indicum				Under study		Good
S. insanum				2 V-7, F-7		
S. integrifolium				2 V-7, F-7		
S. macrocarpum				2 V-7, F-7		
S.melongena				2 V-7, F-7		
S. sisymbrifolium			9-10	V-7, F-7	91	
S. torvum				2 V-7, F-7		
S. viarum				2 V-7, F-7		
S. xanthocarpum				2 V-7, F-7		
Viability and fertility scale				10-11	V-8, F-8	

1 very low, 2 very low to low 3 low 4 low to intermediate 5 intermediate 6 intermediate to high 7 high 8 high to very high 9 very high

PSVFP: Post Storage Viability and Fertility Profile, CYP: Cryopreservation

of NGD in plant species calls for constant cryobiological inputs in terms of material needed. These include cryobiological containers, constant supply of liquid nitrogen, its periodical replenishment, minimizing loss under ambience and above all, secure personnel safety. Stacking pollen samples in cryobiological systems needs to be done in sealed laminated poly-pouches, either in gelatin capsules or in bu"er paper packets. It is important to maintain a proper inventory of pollen samples under cryopreservation. Improper pouch sealing may lead to bursting of the sample packet, leading to loss of pollen. Unless and otherwise known periodical viability checks need to be carried out. It is preferable to cryopreserve sample with a high initial viability profile (Ganeshan, 1998).

The availability of pollen of quality in a pollen cryobank will provide constant supply of the same for extended durations. Pollen in such a state can be termed value added by virtue of its potential extended life, since it can be kept viable and fertile for extended duration to perform its natural function of fertilization, leading to formation of fruit and seed set *e.g.* pollen used in hybrid seed production. The process of genetic characterization further enhances the value of pollen, especially when it is established that it contains specific DNA sequences which are attributable to specific traits *e.g.* pollen with marker genes (Ganeshan and Rajasekharan, 1995).

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Conservation of Endangered Medicinal Plants: Challenges and Options

PE Rajasekharan, S Ganeshan and Sunitha Bhaskaran

Division of Plant Genetic Resources, Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bangalore-560089, Karnataka

Key Words: Endangered Species, *In Vitro*, Micropropagation, Hardening

India has about 8000 species of known medicinal plants. Using the current global rates of species extinction around 10 to 12% of the plants *i.e.*, around 800–1000 species are likely to be threatened. For developing conservation strategies it is essential to study their extent of occurrence (geographic range, endemism), assess their degree of threat based on International Union for the Conservation of Nature (IUCN) categories *viz.*- vulnerable, endangered, critically endangered *etc.*, harvesting practices, habitat specificity and growth forms (Mali and Ved, 1999). More of shrubs and less of herbs, which is expected from a random distribution among natural flora, appear to be among the rare/endangered (RE) group (Lokesha and Vasudeva, 1997). This suggests that shrubs have a higher risk of becoming endangered than herbs. Data on mode of dispersal suggest that a greater fraction of species that disperse their propagules through biotic agents than through wind, water or by passive means are likely to become RE. It may be important to use these syndromes as indications to identify the RE species and concentrate conservation efforts on these species. As a concept, rarity is a phenomenon in time as well as space (Harper, 1986).

Criteria used to assess the threat status of plant species as the class of rarity vary. Correct definition is important for formulating conservation policies especially for countries like India with 11% of world's floral diversity.

Plant tissue culture techniques are now being used globally for the multiplication of medicinally important plant species and monitoring of their secondary metabolites. The application of micropropagation techniques for medicinal plants gives many benefits to the breeders as it enables to increase the rate of rapid multiplication of plants which in a particular climate do not provide seeds or where seeds have low germination, the availability of plants throughout the year, producing uniform clones from highly heterozygous plants, production of plants with changed genotypes, conservation of genetic resources of species and threatened plant and improvement by regeneration technique (Srivastava and Pande, 1998). Successful *in vitro* regeneration of medicinal plants could be made possible with varied explants such as leaf and stem segments, shoot buds, hypocotyls, cotyledons, roots, and seedlings.

Micropropagation holds promise as a major component in medicinal plant breeding. Its benefits derive from *in vitro* culture and multiplication of axenic shoots excluding callus formation and association problems (Constabel, 1990). Axenic shoot (tip) cultures can be employed not only for multiplication, but also for storage.