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Preserving Viability and Fertility of Tomato and Egg Plant Pollen in Liquid Nitrogen

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Tomato (Lycopersicon esculentum *Mill cv. 'Arka Saurabh')* & *Egg plant* (Solanum melongena *L. cv. 'Arka Kusumakar')* *pollen cryopreserved over extended durations remained viable after retrieval from a cryopreserved state, inducing fruit and seed set equivalent to controls. Studies carried out at the experimental farm of IIHR showed that pollen stored under cryogenic conditions for a duration of 6 years could be successfully utilized by the breeder concerned, to produce seeds in the absence of fresh pollen normally collected during the current season. Varietal crosses involving frozen pollen did not deteriorate in their performance and capability to induce viable seed among the fruits set.* In vitro *pollen germination profiles though recorded, a considerable decline in egg plant does not appear to reflect the true performance potential of frozen pollen in the field. However, in tomato,* in vitro *pollen germination results appear to be in good fit with the results obtained using stored pollen in field pollinations. From this study it was concluded that it is advantageous to preserve pollen of these crops over extended durations in liquid nitrogen, since pollen retains its capability to fertilize and induce normal seed set. The study also establishes the fact that pollen preservation for genetic conservation in these crops could be accomplished, besides other conventional methods of germplasm storage involving seeds.*

Vegetable crop breeders require pollen at frequent intervals for affecting fertilization in their crop breeding programmes. Often, it has been felt that raising a crop exclusively for its pollen attributes and use as a pollen parent could be dispensed off, provided viable pollen could be cryopreserved for extended durations. The possibility of preserving valuable crop germplasm through pollen storage has been well recognised throughout the world. Pollen storage often enables crossing of different genotypes which are asynchronous in flowering. Protocols offering viable pollen after collection for several days to several years at different times and at different locations holds promise to accelerate the breeding programme efficiency since crossing could be accomplished almost round the year, irrespective of seasonal variation. Stored pollen could be effectively transported under refrigeration to several locations for breeding purposes, and thus enhances the output of seed production units. Besides, a genome could be preserved partially through pollen in a small cryovials. Conservation of a vast range of genetic diversity comprising wild species and landraces, sampled through pollen can be easily accomplished.

The 'pollen cryobanks' could form an integral part of the Gene bank, or repository.

Protocols for pollen preservation should be applicable to a wide range of germplasm. Extremely variable and cultivar dependent results were often encountered when attempts were made to preserve pollen for short durations, manipulating the two key factors that govern pollen viability; low temperature and relative humidity. Therefore, attempts were made to cryopreserve vegetable pollen world wide in crops like capsicum (Barnabas, 1984; Kristof and Barnabas, 1986), Onion (Ganeshan, 1986) tomato and eggplant (Alexander and Ganeshan, 1988). The present report establishes the viability state of cryopreserved tomato and egg plant pollen after a duration of 6 years, with fertility levels estimated under field conditions, in terms of fruit and seed set.

MATERIALS AND METHODS

(i) *Pollen collection and cryostorage*

Tomato (*Lycopersicon esculentum* Mill Cv. 'Arka Saurabh') and Egg plant (*Solanum melongena* L. Cv. 'Arka Kusumkar') cultivars released and popularised by IIHR, Bangalore were taken up for pollen cryostorage and viability assessment studies. Pollen extraction was accomplished by carefully clipping the anthers from healthy flowers at the time of dehiscence, and placing it in clean pertri-dishes in a desicator containing activated silicagel under ambient conditions. The anthers dehisce after a duration of 30-45 minutes, releasing pollen when tapped gently over a clean butter paper, pure pollen is thus collected and transferred into gelatin capsules which are in turn fastened using a high grade cellophane adhesive tape and enclosed in small laminated pouches with capsules are sealed airtight, using a heat sealer and then stacked at the bottom of the cannisters and lowered gradually into a liquid nitrogen cryoflask. Periodical refilling of cryoflask with liquid nitrogen ensures complete immersion of pollen samples. The cannisters are capped with perforated lids, to avoid samples from floating out into the cryoflask while refilling is done.

(ii) *Viability assessment*

Tomato pollen was germinated *in vitro* by the hanging drop technique (Stanley and Linskens, 1974). Egg plant pollen was germinated *in vitro* by the improved cellophane method developed in this laboratory. Egg plant pollen fails to germinate in hanging drops, since pollen sinks to the bottom of the drop. Hence, a different *in vitro* procedure (improved cellophane method) was developed. For both crops, the medium consisted of a carbohydrate source (15 per cent sucrose) supplemented with 300 ppm of Calcium nitrate, 200 ppm of Magnesium sulphate, 100 ppm of Potassium nitrate and 100 ppm of Boric acid. The preparations were incubated at $25° \pm 2°C$ for a duration of 4-6 hours, after which staining was accomplished using the versatile stain (Alexander, 1980). Three replicates each of fresh and cryostored pollen were made out when viability was assessed. Pollen

grains whose tube lengths were greater than the grain diameter were considered as germinated. More than 300 pollen grains per replicate were scored, using a binocular microscope and percentage germination was calculated.

In order to ensure viability of cryostored pollen, samples retrieved from a cryogenic state to ambient temperature were dusted on stigmas of excised pistils (Alexander, 1987) to study the pollen tube growth on styles. This was carried out prior to actual pollinations with cryostored pollen.

Pollinations with cryostored pollen (inter-varietal crosses) were carried out on healthy plants grown in pots. 'Arka Vikas' served as the female parent for tomato while 'Arka Shirish' served as female parent for egg plant. Flowers were emasculated a day prior to pollination and covered with butter paper bags, in order to avoid contamination. Cryostored pollen was applied on the receptive stigma the next day, and then immediately covered. For controls, stigma of emasculated flowers were applied with freshly collected pollen. Fruit and seed set was recorded with crosses made using fresh and cryostored pollen. Atleast 5 effective pollinations, using cryostored pollen were accomplished for each crop, from which fruit and seed set data was generated.

RESULTS AND DISCUSSION

In vitro pollen germination showed significant reduction after 6 years of cryostorage, both in tomato & egg plant (Fig. 1). More than 60 per cent germination

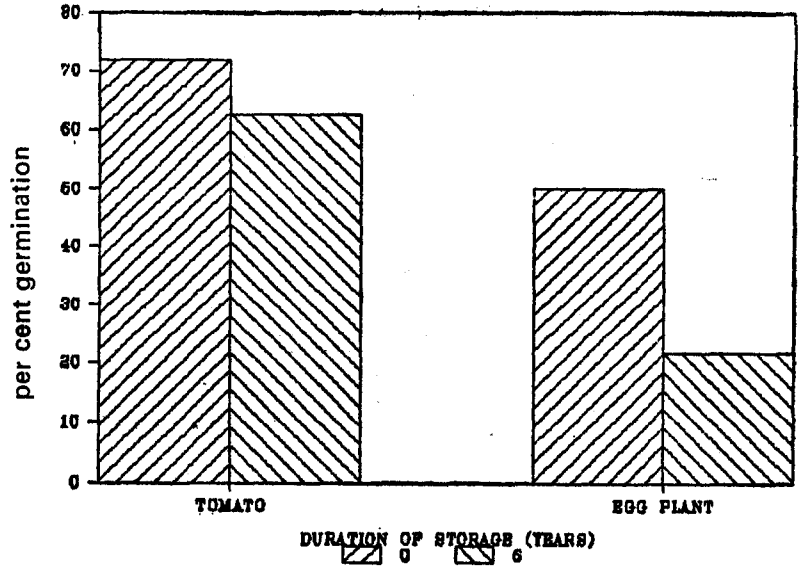

Fig. 1. Viability of Tomato and Egg Plant.

could be recovered in tomato (SE ± 2.36 & 0.64 for fresh and cryostored pollen). While only ca 35 per cent of egg plant pollen could germinate (SE ± 1.32 & 0.46 for fresh and cryostored pollen), in the media where fresh pollen germinated profusely. The trend in reduction was more drastic in egg plant than in tomato. However, this trend appears to have not much significance on observing the performance of cryostored pollen *in vivo* and controlled pollinations. Pollen tube growth was found to be normal on excised stylar material, in both the crops. In tomato controlled pollinations with cryostored pollen could induce ca. 50 per cent fruit and seed set, whereas cryostored egg plant pollen induced ca. 66 per cent seed set of control (Table 1). Seeds set through cryostored pollen germinated normally, with the vigour and vitality equivalent to seeds set through pollinations carried out with fresh pollen.

TABLE 1. CONTROLLED POLLINATIONS WITH CRYOSTORED POLLEN

Crop/Cultivar	Parents		Number of flowers pollinated	Number of fruits set	Number of Seeds/Fruit recovered
	Female	Pollen			
TOMATO :					
'Arka Saurabh'	'Arka Vikas'	× 'Arka Saurabh' (Control)	6	6	165
	'Arka Vikas	× 'Arka Saurabh' (6 years cryostored)	6	3	84
EGG PLANT :					
'Arka Kusumakar	'Arka Shirish'	× 'Arka Kusumakar' (Control)	6	6	840
	'Arka Shirish'	× 'Arka Kusumakar' (6 years cryostored)	6	4	738.25

The foregoing results indicate that it is possible to accomplish long-term cryogenic preservation of tomato and egg plant pollen retaining pollen fertility over extended durations. Although *in vitro* studies predict stored pollen viability, the capacity of cryostored pollen to fertilise and induce normal fruit and seed set could be established only by controlled pollinations. This is especially true in case of egg plant, where *in vitro* studies have indicated a poor viability of cryostored pollen, but when used in controlled pollinations under *in situ* conditions, fertility rates had not declined to a great extent, as shown by a good fruit and seed set. Poor germination rates were recorded for egg plant pollen cryostored for shorter durations. It is not uncommon, however, where a good fruit and seed set may occur even though the results of *in vitro* pollen germination assay indicate low viability (Stanley and Linskens, 1974). Maximum fruit and seed set was obtained with stored pollen viability as low as 6 per cent (Olmo, 1942) in grapes. In onion, with a germination of ca. 50 per cent for 1 year cryostored pollen, a high recovery of fruit and seed set was obtained by using the same in controlled pollinations (Ganeshan, 1986).

In conclusion, the present report emphasizes the role of cryogenic temperature for preserving fertility of tomato and egg plant pollen over prolonged durations. The technique is very simple, and could be effectively used in genebanks for preserving haploid male germplasm. Vegetable crop breeders can exploit such a facility for the requirement of specific pollen parents in their breeding programmes. Infact, breeders can preserve their pollen stocks in cryogenic containers and utilise cryostored pollen in hybridization schedules, as per their choice, and effectively manage their crop improvement strategies.

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