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**Short Communication**

# LONG TERM POLLEN PRESERVATION OF WILD TOMATO, BRINJAL SPECIES AND CULTIVARS IN LIQUID NITROGEN

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Preserving pollen of wild species and cultivated crops assume importance for safeguarding the pollen parents in any crop improvement program and dispense away with the need for growing the same frequently. It is well documented in literature (Roberts, 1975) that long term pollen preservation forms a part of an integrated gene resources conservation program. Pollen can be made available in abundant quantities and forms an ideal compact material for conservation of recombined genetic information present in pollen at the gametophytic stage. Although in nature pollen viability and longevity varies with different crop species, methods are available for preserving vegetable pollen for extended durations (Alexander and Ganeshan, 1989a) under cryogenic conditions. The present research paper relates to long term cryogenic preservation of wild species of tomato (*Lycopersicon pimpinellifolium* Mill.), *Solanum* species (*Solanum indicum* L.) and cultivars of *S.melongena* L. ('Arka Kusumakar' and 'Arka Shirish').

The procedures for pollen collection in tomato and brinjal were described earlier (Alexander and Ganeshan, 1989a). Pollen samples collected on a dry sunny morning at IIHR, Hessaraghatta, during 1983, 1988, 1989 and 1991 from *S.melongena* 'Arkà Kusumakar', 'Arka Shirish' *L.pimpinellifolium* and *Solanum indicum* were packed without desiccation in gelatin capsules, sealed in laminated aluminium pouches and stored in canisters of a liquid nitrogen Cryobiological System (Mach SM-33 of MVE Cryogenics, USA). Prior to storage, pollen samples were indexed for fresh pollen viability. After different durations of cryostorage (*S. melongena* CV 'Arka Kusumakar' 9 years, 'Arka Shirish' 3.5 years *L.pimpinellifolium* 2 years and *S.indicum* 9 months) pollen samples were thawed to ambient temperature and tested for *in vitro* viability (pollen germination) and fertility (field pollinations) in the year 1992, the results of which are presented below.

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**BRINJAl *Solanum melongena* CV. 'ARKA KUSUMAKAR'**

There was considerable viability decline in 'Arka Kusumakar' pollen sample cryostored for 9 years (Table 1). During this period of storage viability dropped from 51.71 to 12.74% (original mean value) as indexed by *in vitro* germination (Alexander and Ganeshan 1989b). Field pollinations on emasculated flowers of the same cultivar with the same duration cryostored pollen resulted in Ca. 90 percent fruit set, with seed number averaging to 569.5 seeds per fruit. This was comparable to fresh pollen (controls) used in field pollinations (Table 2), where 429.5 seeds could be recovered.

**Table 1. Pollen viability in brinjal and tomato after different durations of storage, indexed by percentage germination (*in vitro*)**

| | CROP/CULTIVAR/SPECIES | | | |
|---|---|---|---|---|
| | BAK (9YLN) | BAS (3.5Y LN) | SI(9M LN) | LP(2Y LN) |
| Fresh pollen viability | 45.96(51.71) | 48.32(55.72) | 50.51(59.55) | 45.55(51.00) |
| Stored pollen viability | 20.70(12.74) | 54.24(65.88) | 46.73(53.05) | 44.43(49.04) |
| SeM | 1.622 | 2.366 | 1.333 | 0.356 |
| LSD (p=0.05) | 6.369 | NS | NS | NS |
| CV% | 8.430 | 7.992 | 4.749 | 1.373 |

(FIGURES IN PARENTHESIS INDICATE ORIGINAL MEANS); BAK (9Y LN): Brinjal 'Arka Kusumakar' 9 year cryostored; BAS (3.5 Y LN): Brinjal 'Arka Shirish' 3.5 year cryostored; SI: (9 M LN) : *Solnum indicum* 9 months cryostored; LP: (2 Y LN); *Lycopersicon pimpinellifolium* 2 year cryostored

**BRINJAL *Solanum melongena* CV. 'ARKA SHIRISH'**

There was no significant reduction in viability *in vitro* as tested by modified cellophane procedure (Alexander and Ganeshan, 1989b) as shown in Table 1 after 3.5 years of cryostorage. Field pollinations (emasculated and selfed) with the same duration cryostored pollen resulted in Ca. 50 percent fruit set, with seed number averaging to 405 seeds per fruit. This was at par with that of fresh pollen (controls) used in field pollinations (Table 2), except that there was a reduced average seed number per fruit.

*Solanum indicum*

There was no significant reduction in viability tested by modified cellophane procedure (Alexander and ganeshan, 1989b) after 9 months of cryostorage (Table 1). Field pollinations (emasculated and selfed) with the same duration cryostored pollen resulted in 100 percent fruit set, but with a reduced average seed number per fruit (Ca. 54 percent of controls). The results are presented in Table 2.

**Table 2. Controlled pollinations with cryostored pollen**

| Crop/cultivar | Parents | | no. of flowers pollinated | No. of fruits set | no.seeds/ fruit recovered |
|---|---|---|---|---|---|
| | female | male | | | |
| 1. Brinjal | AK | AK (control) | 46 | 41 | 429.60 |
| (i) 'Arka Kusumakar' | AK | AK (9Y LN) | 37 | 34 | 569.50 |
| (ii) 'Arka Shirish' | AS | AS (control) | 20 | 13 | 237.75 |
| | AS | AS (3.5Y LN) | 17 | 10 | 405.00 |
| (iii) *S.indicum* | SI | SI (control) | 10 | 6 | 46.33 |
| | SI | SI (9M LN) | 7 | 7 | 25.33 |
| 2. Tomato | | | | | |
| *L.pimpinellifolium* | AS | LP (2Y LN) | 24 | 23 | 60.00 |

AK: 'Arka Kusumakar';AS: 'Arka Shirish'; SI: *Solanum indicum*; LP: *Lycopersicon pimpinellifolium*; Y LN: Years cryostored; M LN: months cryostored

*Lycopersicon pimpinellifolium*

There was no significant reduction in viability tested by the hanging drop method (Stanley and Linskens, 1974) after 2 years of cryostorage (Table 1). Pollinations with this pollen on emasculated tomato CV. 'Arka Saurabh' resulted in Ca. 95 percent fruit set, with an average seed number of 60 seeds per fruit (Table 2). Pollinations with fresh *L. pimpinellifolium* pollen could not be carried out, due to lack of synchrony in flowering.

The foregoing results clearly indicate the diverse behaviour of cryostored pollen in the different species and cultivars of tomato and brinjal, showing good resilience to cryostorage treatments imposed over durations ranging from 9 months to 9 years. The problem of asynchrony in flowering, if any, can well be circumvented between different species, if pollen can be collected, successfully stored and used in crosses with a desired female parent, as has been demonstrated with cryostored *L.pimpinellifolium* pollen. Although there was a significant reduction in viability for pollen cryostored for 9 years, field pollinations ultimately established the fertility state of this pollen, producing fruit and seed set comparable with controls. Such instances have been recorded in literature for onion (Ganeshan, 1986), grapes (Olmo, 1942) and walnut (Farmer and Barnett, 1974). Besides this, samples recording low germination profiles after storage could be due to one of the many reasons mentioned in the review of Stanley and Linskens (1974), Shivanna and Johri (1985) and Towill (1985). The protocols optimized for these economically important vegetable crops could therefore lead to establishment of pollen cryobanks, for conserving recombined genetic information present in the male gametophyte. Such a facility could help breeders and seed companies involved in developing

new cultivars for catering to the needs for human requirement, and improve the productivity of these crops.

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