

## Influence of Storage Temperature on Viability and *In Vitro* Germination Capacity of Pear (*Pyrus spp.*) Pollen

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### Abstract

Pollen viability and germination capability in three pear cultivars viz. Patharnakh, Punjab Beauty and Shinseiki were investigated up to 12 weeks (3 months) stored at different temperatures i.e. room temperature, refrigerator (40 °C), freezer (-20°C) and liquid N (-120°C). Viability was tested in 2 per cent acetocarmine solution whereas, for *in Vitro* pollen germination pollen grains were germinated in 10 percent sucrose solution at weekly intervals. The pollen viability and *in Vitro* germination percentages of the genotypes were significantly affected by storage temperatures. The pooled data for a period of two years revealed that average viability varied from 40.68% (Shinseiki) to 48.75% (Patharnakh) whereas, germination percentage varied from 34.93% (Shinseiki) to 42.81% (Patharnakh) among the cultivars under study. Pollen stored at low temperature (-120°C and -20°C) showed better viability and germination percentage as compared to pollen stored at room temperature and 4 °C. The cultivar Patharnakh had maximum viability and germination percentage when pollen were stored at -20 (67.40% and 59.62%, respectively) and -196°C (68.06% and 61.83%, respectively), followed by Punjab Beauty and Shinseiki. The results indicate that pollen collected and stored at sub-zero temperatures from early blooming pear varieties can be stored for very long period without any appreciable loss of viability and germination and can be used along the whole blooming season for hybridization programmes by fruit breeders even at distant places for the development of new strains so as to widen the genetic base and create variability in pear.

**Keywords:** cryopreservation, *in Vitro* pollen germination, viability, pollen grain, pear

### 1. Introduction

Pear is one of the most important temperate fruits of the world next to apple. Total world pear production reached 19.5 million metric tons in 2006 (FAOSTAT 2007) ranking second after apple, among global production of deciduous fruit tree species. In India the annual pear production is 1.76 lakh metric tons from an area of 38600 ha (Anonymous, 2006). In Punjab, it ranked 4<sup>th</sup> among fruit crops in terms of area after citrus, guava and mango and occupies an area of 2598 ha with an annual production of 58,643 mt (Aulakh & Gill, 2010). The area can be increased further and cultivation of this crop may prove to be a best alternative for diversification of agriculture.

Long-term storage of pollen grains is a useful means for conservation of gene pool and to overcome temporal and spatial isolation of the parent species in the breeding programmes. Cryopreservation is an effective method of long-term pollen storage. Under these conditions all metabolic processes in the biological systems, including pollen are virtually arrested, thus permitting maintenance of viability. Theoretically, cryogenic pollen storage permits maintenance of pollen viability for several decades but storage beyond 3-5 years has not been reported in many species.

Storage of pollen is necessary for controlled pollination either to achieve the desired breeding objectives or to solve some cultural constraints in fruit production. It is essential for breeding programmes, germplasm conservation and artificial pollination of dichogamous, self-incompatible, or male-sterile fruit species. In such cases, pollen grains have to be stored for extended periods of time without a significant loss of viability. Longevity of pollen varies greatly with plant species and storage conditions (Hanna & Towill, 1995; Dafni & Firmage, 2000). Pollen preservation is very similar to seed preservation. Some pollen are desiccation tolerant and others quite sensitive. Cryogenic storage of tolerant types is fairly easy. Considerable progress has been made in preservation methodology, handling, longevity and viability of pollen in pear (Alexander et al., 1996). Viability and germination capacity of stored pollen has been studied by several workers such as Pinny and Polito (1990) in olive pollen, Martinez et al. (2001) in almond, Aslantis and Pirlak (2002) in strawberry; Khan and Perveen (2006) in papaya. They reported better germination in pollen stored at low temperature than that at high temperature. Similarly, Perveen et al. (2008) examined pollen germination in *Malus pumila* stored at different temperatures and observed that pollen stored at low temperature (-196°C) showed better germination percentage as compared to pollen stored at 4°C.

Lack of overlap between blooming period in different pear cultivars leads to poor or no fruit set. In order to store the pollen of early flowering cultivars throughout the flowering season so as that they can be used for artificial pollination in late blooming cultivars and development of new strains. Pear pollen has a short viability and a high sensitivity to desiccation and, consequently, conservation is problematic. Since no previous reports are available on the conservation of pear pollen at subzero temperatures. Thus, the main objective of this work is to develop a method for short term storage of pear pollen (up to 3 months) comparing different sub-zero storage temperatures for viability and germination capacity of stored pollen.

## 2. Research Methods

Pollen from three pear cultivars viz. Pathernakh, Punjab Beauty and Shinseiki were used in this study. Flowers from male parents were collected at popcorn or balloon stage and were allowed to shed the pollen in shade for 3-4 hours under 100 watt lamp. Immediately after anther dehiscence, pollen were collected in vials and subjected to different storage conditions viz. room temperature (in anhydrous calcium chloride), 4°C, -20°C and cryogenic storage in liquid nitrogen (-196°C).

For sub zero temperature storage pollen was placed in 1.5 mL cryovials and stored at -20°C and -196°C in freezer and liquid nitrogen, respectively. Pollen was thawed at 1, 7, 15, 30, 60 and 90 days after storage by keeping the samples at room temperature for 5 min. After thawing pollen was hydrated in a covered tray with wet filter paper for 200 min at room temperature. To maintain pollen viability after removal from liquid nitrogen, the vials of pollen were immediately submerged in a 30°C water bath. This sudden rise in temperature is necessary to prevent moisture from crystalizing inside the pollen grain, which occurs between 0 and -20°C.

Pollen viability was tested in 2 per cent acetocarmine solution, which was prepared by dissolving 2 grams of carmine powder in 45 ml of glacial acetic acid and final volume was made to 100 ml by adding distilled water. Solution was boiled for 5 minutes and filtered through Whatman No.1 filter paper. The pollen grains were dusted on a glass slide and one to two drops of acetocarmine were put on these grains. After placing a cover slip over the stain it was left for five minutes for proper staining of pollen grains. Slides were observed under microscope. Deeply stained and normal looking pollen grains were considered to be viable whereas, shriveled, slightly stained or colorless pollen grains were counted as non viable. Three microscopic fields were observed and number of viable and non viable pollen grains were counted in each field.

For *in Vitro* pollen germination pollen grains were germinated in 15 percent sucrose solution at weekly intervals. For this 1-2 drops of sucrose solution were placed in the cavity of a cavity slide and pollen grains were dusted over it by camel brush. The slides were covered with cover slip and edges were smeared by molten wax and slide was inverted instantly so as to form a hanging drop on the cover slip. The cavity slides were then placed in petridishes containing moist filter paper to ensure uniform and high relative humidity. Pollen tube growth was assessed for each genotype under microscope after 24 hrs of incubation at 22±2°C. The pollen grains having pollen tube at least two times longer than pollen diameter were considered to be germinated. Pollen germination was observed under digital microscope. Data were collected on four Petri dishes with at least 200 pollen grains in each one.

Data were subjected to ANOVA analysis. Statistical analyses were performed with SPSS 12.0 statistical software (SPSS Inc. Chicago, USA).

### 3. Analysis of Results

Pollen viability was examined up to 12 weeks in different storage conditions viz. room temperature, 4 °C, -20 °C and -196 °C (Table 1 and Figure 1). The results obtained revealed that pollen viability was more than 65.00 per cent in all the three cultivars immediately after gathering in laboratory with maximum in Pathernakh (74.67 %) followed by Punjab Beauty (71.83 %) and minimum in Shinseiki (66.00 %). While comparing different storage temperatures the viability was lost immediately after storage in case of room temperature. However, maximum viability was observed at sub zero storage temperatures (-20°C, -196°C) during the whole period of storage duration with highest average value in cultivar Pathernakh (48.75 %) and lowest in Shinseiki (40.68 %). The viability showed a decreasing trend with increase in storage period and thus an inverse relation between viability and duration of storage was observed. Our results coincide with those obtained by Lora et al. (2006) for cherimoya, Weatherhead et al. (2006) for potato pollen, Gomes et al. (2003) for onion and Sharafi and Bahmani (2010) for loquat. In Pathernakh maximum viability was observed at -196°C (68.06%) and minimum at room temperature (6.11 %). Loss of viability was highest in pollen stored at room temperature where zero per cent viability was achieved after two weeks of storage while, 74.67 to 33.50, 59.17 and 60.17 per cent loss in viability was found at 4°C, -20°C and -196°C, respectively. After 12 weeks of storage the average viability declined from 74.67 to 48.75 per cent on an average. Similarly, in Punjab Beauty viability varied from 5.85 to 65.81 per cent at different storage temperatures with maximum value at -196°C (65.81 %). Lowest viability was observed at room temperature (5.37 %). A decreasing trend in viability was observed with increase in storage period at all the storage temperatures. However, minimum loss was observed at -196°C (71.83 to 55.67 %), followed by -20°C (71.83 to 49.67 %) and 4°C (71.83 to 25.33 %), respectively. Maximum loss in viability was observed at room temperature (71.83 to 0.00 %) after 12 weeks of storage. In cultivar Shinseiki, similar trend as that in Pathernakh and Punjab Beauty was observed. While comparing different storage temperatures, room temperature (5.37 %) had maximum while -196°C had minimum value for average loss of viability. The average viability lost from 66.00 to 40.68 per cent during a period of 12 weeks storage. Thus variability among different cultivars was observed for their viability potentials.

Table 1. Viability (%) studies of pollen stored at different temperature

Duration (days/ Weeks)	Patharnakh					Punjab Beauty					Shinseiki				
	Room temp.	4°C	-20°C	-196°C	Mean	Room temp.	4°C	-20°C	-196°C	Mean	Room temp.	4°C	-20°C	-196°C	Mean
1st day	74.67	---	---	---	74.67	71.83	----	-----	-----	71.83	66.00	---	---	----	66.00
1 <sup>st</sup> week	4.83	66.83	72.50	73.00	54.29	4.17	55.33	67.67	70.83	49.50	3.83	54.50	62.17	64.67	46.29
2	0	62.67	71.00	72.17	51.46	0	53.83	66.83	70.17	47.70	0	52.00	60.00	63.50	43.87
3	0	61.33	70.50	71.17	50.75	0	52.00	65.67	69.67	46.83	0	50.50	59.00	63.00	43.12
4	0	60.50	70.00	70.67	50.29	0	48.17	61.17	69.33	44.66	0	48.67	58.17	62.33	42.29
5	0	59.16	69.83	70.33	49.83	0	46.83	59.33	69.00	43.79	0	48.17	56.17	61.50	41.46
6	0	56.67	69.33	69.33	48.83	0	44.17	56.83	68.67	42.41	0	45.00	54.17	60.83	40.00
7	0	54.33	68.50	68.83	47.91	0	40.17	55.67	67.50	40.83	0	40.33	53.17	60.00	38.37
8	0	48.00	64.67	65.33	44.50	0	38.00	54.50	65.67	39.54	0	38.17	50.50	59.17	36.96
9	0	43.00	63.83	64.67	42.87	0	34.50	53.50	59.83	36.95	0	35.17	49.67	57.17	35.50
10	0	39.33	62.00	63.83	41.29	0	29.83	52.83	59.33	35.49	0	27.67	49.00	56.17	33.21
11	0	36.00	60.17	60.67	39.21	0	28.83	50.83	58.00	34.41	0	22.83	48.33	54.33	31.37
12	0	33.50	59.17	60.17	38.21	0	25.33	49.67	55.67	32.66	0	21.50	46.83	53.17	30.37
Mean	6.11	53.54	67.40	68.06	48.75	5.85	43.76	58.95	65.81	43.58	5.37	42.35	54.86	60.14	40.68
CD <sub>0.05</sub>	Duration:	6.93				Duration:	7.23				Duration:	8.35			
	Temperature:	7.56				Temperature:	5.11				Temperature:	6.89			
	Duration x temperature:	3.68				Duration x temperature:	4.21				Duration x temperature:	3.74			

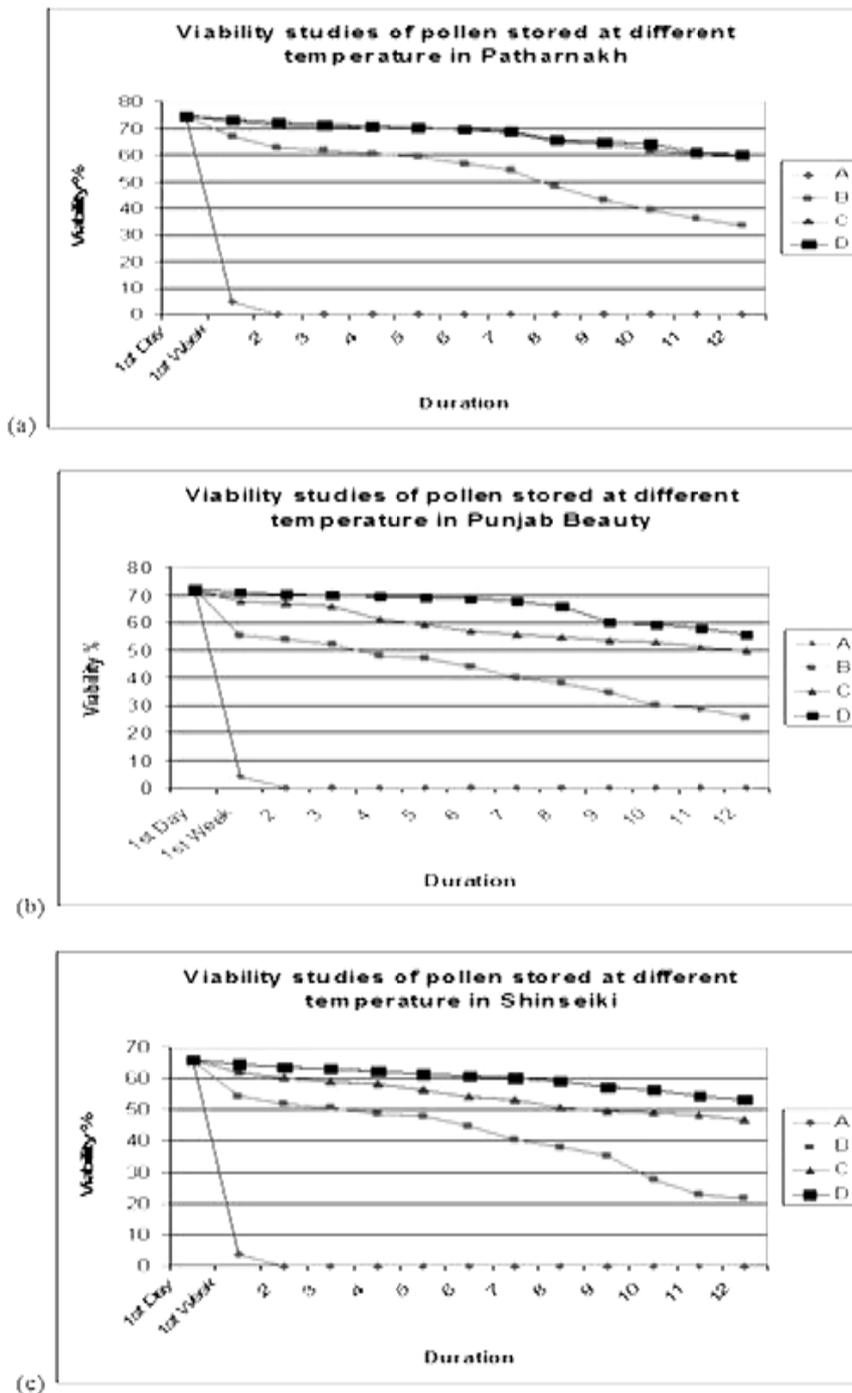


Figure 1. Effect of different storage temperatures on the rate of pollen viability in (a) Patharnakh (b) Punjab Beauty and (c) Shinseiki

Results of *in Vitro* pollen germination showed a significant higher germination of pollen stored at sub zero temperatures (-20°C and -196°C) with 61.83%, 57.73 % and 51.95 % germination in Patharnakh, Punjab Beauty and Shinseiki, respectively (Table 2 and Figure 2). Significant differences were observed among cultivars and maximum germination percentage (42.81 %) was observed in cultivar Patharnakh. Decline in pollen germination was observed with increase in storage duration. While comparing the different levels of storage temperatures, it was observed that germination was very poor at room temperature and excellent at ultra low temperatures.

Table 2. *In vitro* germination (%) of pollen stored at different temperature

Duration (days/ Weeks)	Patharnakh					Punjab Beauty					Shinseiki				
	Room temp.	4°C	-20°C	-196°C	Mean	Room temp.	4°C	-20°C	-196°C	Mean	Room temp.	4°C	-20°C	-196°C	Mean
1st day	66.12	--	--	---	66.12	65.50	--	--	--	65.5	61.17	---	---	---	61.17
1st week	1.50	57.67	66.00	66.07	48.96	2.50	53.83	62.17	63.33	45.45	1.67	48.83	55.67	60.17	41.58
2	0	56.33	65.67	66.00	47.45	0	51.83	60.50	62.67	43.75	0	46.17	53.67	58.83	39.66
3	0	55.17	64.00	66.00	46.71	0	50.17	59.17	62.33	42.91	0	45.17	51.67	57.67	38.62
4	0	52.33	63.67	66.00	45.66	0	48.17	54.83	61.00	41.00	0	42.50	48.67	57.17	37.08
5	0	52.17	61.50	65.67	44.83	0	45.83	53.83	60.00	39.91	0	41.33	47.00	53.33	35.41
6	0	50.00	59.50	62.50	43.00	0	40.83	49.83	58.67	37.33	0	37.67	45.83	50.50	33.50
7	0	47.00	58.17	60.33	41.37	0	38.83	48.00	57.83	36.16	0	35.50	44.50	49.17	32.29
8	0	40.50	57.50	59.17	39.29	0	35.67	46.17	57.33	34.79	0	32.00	43.00	47.83	30.70
9	0	33.00	55.33	57.83	36.54	0	31.67	44.67	54.67	32.75	0	29.67	41.50	47.17	29.58
10	0	29.33	54.00	54.67	34.5	0	29.17	40.83	51.50	30.37	0	21.00	39.33	45.67	26.50
11	0	26.00	53.33	54.17	33.37	0	23.50	39.17	48.83	27.87	0	17.83	37.33	43.67	24.70
12	0	11.50	50.00	53.67	28.79	0	20.83	37.83	46.83	26.37	0	14.50	35.50	43.00	23.25
Mean	5.20	44.28	59.62	61.83	42.81	5.23	41.22	50.96	57.73	38.78	4.83	36.41	46.53	51.95	34.93
	Duration:				11.44	Duration:				8.75	Duration:				10.03
CD <sub>0.05</sub>	Temperature:				9.24	Temperature:				9.87	Temperature:				8.57
	Duration x temperature:				7.32	Duration x temperature:				6.56	Duration x temperature:				5.96

Higher values for pollen germination at low storage temperature and decline at high storage temperature were observed by several workers (Hanna & Towill, 1995; Perveen et al., 2007; Perveen & Khan, 2008). In Patharnakh the mean value of pollen germination at different storage temperatures varied from 5.20 (room temperature) to 61.83 per cent (-196°C). A gradual decrease in germination percentage was observed from first day of storage till 12 weeks at all the storage temperatures. Minimum decrease was observed at -196°C (66.12 to 53.67 %) and was at par with -20°C (66.12 to 50.00 %). Maximum loss in germination percentage was observed at room temperature (66.12 to 0.00%). The average value ranged between 66.12 per cent at 1<sup>st</sup> day of storage to 28.79 per cent after 12 weeks of storage with a mean of 42.81 per cent. The average value at different storage temperature varied between 5.23 per cent at room temperature to 57.73 per cent at -196°C in Punjab Beauty. At room temperature germination percentage lost from 65.5 to 5.23 per cent whereas, the loss varied from 65.5 to 41.22 percent, 65.5 to 50.96 per cent and 65.5 to 57.73 per cent at 4°C, -20°C and -196°C, respectively after a period of 12 weeks of storage. Among different intervals of storage the germination percentage lost from 65.5 to 26.37 per cent with an average value of 38.78 per cent. Maximum germination at -196°C (51.95 %) and minimum at room temperature (4.83 %) was observed in Shinseiki. The loss in germination percentage was quite obvious at room temperature (61.17 to 0.00 %) than at 4°C (61.17 %), -20°C (61.17 to 35.50 %) and -196°C (61.17 to 43.00 %). The data further revealed that with increase in time of storage (1<sup>st</sup> day to 12<sup>th</sup> week) there was a gradual decline in germination percentage with average value ranging from 61.17 to 23.25 per cent and a mean value of 34.93 per cent.

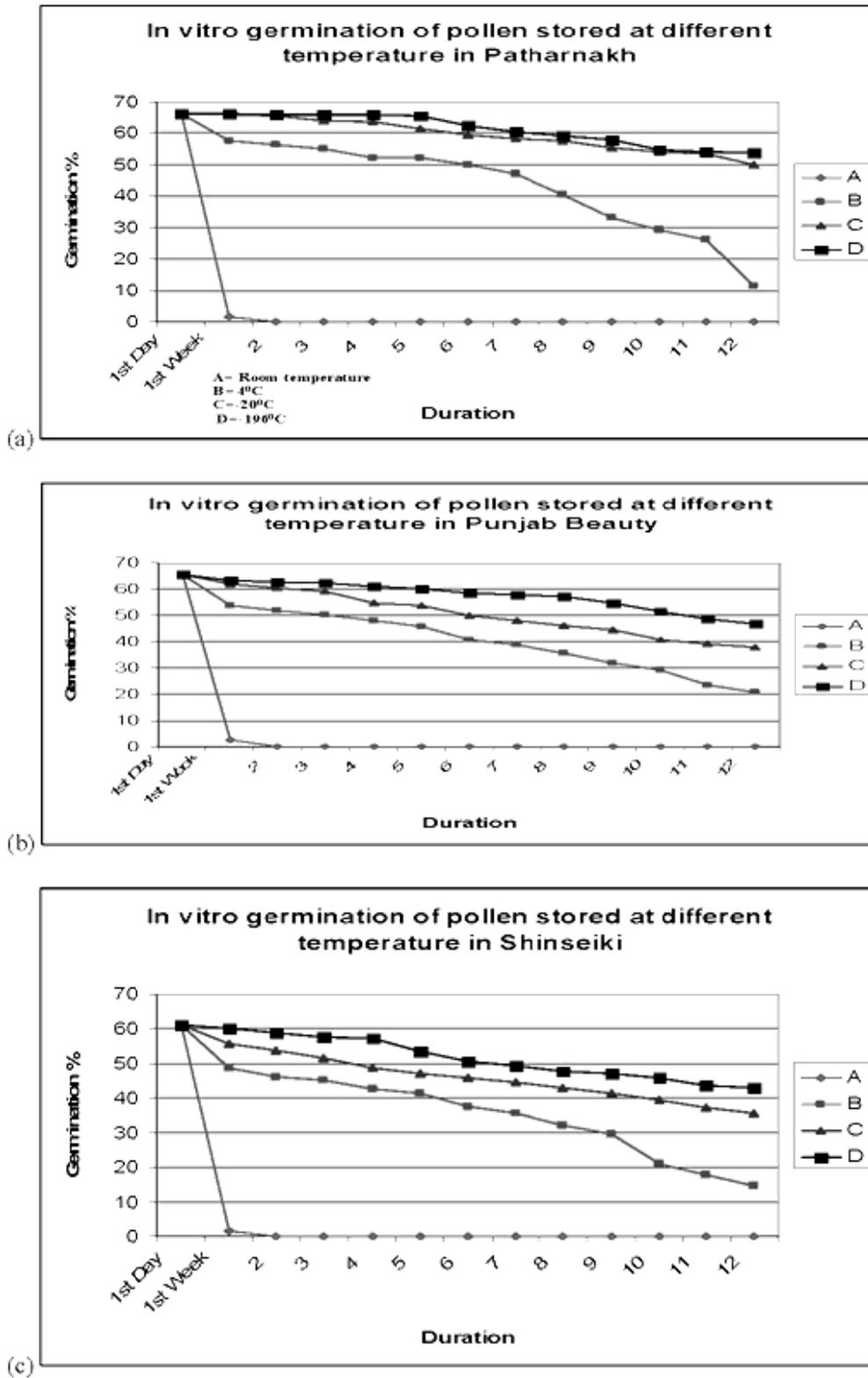


Figure 2: Effect of different storage temperatures on *in vitro* pollen germination in (a) Patharnakh (b) Punjab Beauty and (c) Shinseiki

**4. Discussion**

It is quite obvious that staining tests are not a reliable measure of pollen viability as compared to *in-vitro* pollen germination tests. Hence a combination of both viability and germination tests will provide a better

understanding about the pollen behaviour. In the present study wide variations in viability and germination of pollen grains was observed with different storage temperatures and durations among different pear cultivars. This variability may be due to pollen fertility, as a result of regular meiosis and activation of certain enzyme systems present in the pollen grain itself. Besides genotype and environmental interactions may also play an important role. This phenomenon indicates genetically differences among the genotypes which have been reported by many researchers in many of the fruit tree species and cultivars (Visser & Oost, 1981; Stosser et al., 1996; Pirlak & Bolat, 1999; Albuquerque et al., 2007; Sharafi et al., 2011). Anjum and Shaukat (2008) while studying pollen germination of *M. pumila* L., beyond 48 weeks in the refrigerator (4°C), freezer (-20°C, -30°C) and freeze drier (-60°C) reported higher germination percentage in pollen stored at low temperature as compared to pollens stored at 4°C; and in fresh pollen with highest germination percentage in freezer dried pollen (-60°C). The gradual loss of germination at low temperatures (-20°C and -196°C) observed in the present study may be attributed to frequent freezing and thawing of pollen grains. Furthermore low temperature might have lead to intracellular ice formation, cell death and thereby loss of germination. Our results clearly indicated that it is feasible to store pollen grains of pear at sub zero temperatures without any significant loss in their viability and germinability, and they may be used effectively throughout the flowering season for assisted pollination so as to broaden the pear genetic base.

### References

- Albuquerque, N., García, M. F., & Burgos, L. (2007). Influence of storage temperature on the viability of sweet cherry pollen. *Spanish J. Agricultural Res.*, 5, 86-90.
- Alexander, M. P. (1996). Different staining of aborted and non-aborted pollen. *Stain Technol.*, 44, 117-122.
- Anjum, P. and Shaukat, A. (2008). Maintenance of pollen germination capacity of *Malus Pumila* L., (Rosaceae). *Pakistan J Bot.*, 40(3), 963-966.
- Anonymous. (2006). Area and production of different fruit crops in India. Retrieved from: [www.faostat.org](http://www.faostat.org)
- Aslantis, R., & Pirlak, L. (2002). Storage and germination of Strawberry pollen. IV International Symposium on Strawberry, pollen. *Acta Hort.*, 2, 567.
- Aulakh, P. S., & Gill, M. S. (2010). Status and fruit cultivation in Punjab. *Proc National Seminar on Impact of Climate Change on Fruit Crops (ICCFC)*, pp 16- 21.
- Dafni, A., & Firmage, D. (2000). Pollen viability and longevity: practical, ecological and evolutionary implications. *Plant Systematic Aevol.*, 222, 113-132.
- FAO STAT. (2007). FAO Statistics data base on the world wide web. Retrieved from: [http // faostat.fao.org](http://faostat.fao.org)
- Gomes, P. R., Raseira, M. C. B., & Baudet, L. L. (2003). Onion (*Allium cepa* L.) Pollen Storage. *Revista Brasileira de Sementes*, 25, 14-17.
- Hanna, W. W., & Towill, L. E. (1995). Long-term pollen storage. *Plant Breeding Rev.*, 13, 179-207.
- Khan, S. A., & Perveen. A. (2006b). Germination capacity of stored pollen of *Solanum melongena* L. (Solanaceae) and their maintenance. *Pakistan J. Bot.*, 38(4), 921-923.
- Lora, J., Perez de Oteyza, M. A., Fuentetaja, P., & Hormaza, J. I. (2006). Low Temperature Storage and *In vitro* Germination of Cherimoya (*Annona cherimola* Mill.) Pollen. *Scientia Hort.*, 108, 91-94.
- Martinez, G., Dicenta, F., & Ortega, E. (2001). The germination of almond pollen. *J. Hort Sci.*, 58, 229-255.
- Perveen, A., & Khan, S. (2008). Maintenance of pollen germination capacity of *Malus pumila* L., (Rosaceae). *Pakistan J. Bot.*, 40, 963-966.
- Pinney, K., & Polito, V. S. (1990). Olive pollen storage and *In Vitro* germination. In: International Symposium on olive growing. *Acta Hort.*, 1, 286.
- Pirlak, L., & Bolat, I. (1999). An investigation on pollen viability, germination and tube growth in some stone fruits. *Turkey J. Agri.*, 23, 383-388.
- Sharafi, Y., & Bahmani, A. (2010). Study of pollen germination and tube growth in some Iranian Loquat cultivars and genotypes. *3th International Symposium on Loquat*, 22-25 May. Antakya. Turkey.
- Stosser, R., Hartman, W., & Anvari, S. F. (1996). General aspects of pollination and fertilization of pome and stone fruits. *Acta Hort.*, 423, 15-21.
- Visser, T., & Oost, E. H. (1981). Pollen and pollination experiments. III, the viability of apple and pear pollens affected by irradiation and storage. *Euphyt.*, 30, 65-70.

Weatherhead, M. A., Grout, B. W. W., & Henshaw, G. G. (2006). Advantages of Storage of Potato Pollen in Liquid Nitrogen. *Biomedical Life Sci.*, 21, 331-334.