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Optimization of pollen storage conditions for low chill peach cultivars

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ABSTRACT

The study was conducted to optimize conditions for long term pollen storage of low chill cultivars of peach. Pollen grains of three cultivars i.e. Florda Prince, Flordaglo and Prabhat were stored at variable temperatures (−80°C, −20°C, 3±1°C, 6±1°C and room temperature) for one year and were tested for their viability at 30 days interval by *in vitro* pollen germination and staining test using acetocarmine. The viability varied significantly according to the genotypes of peach, storage temperature and time. The maximum pollen germination percentage was found in pollen grains stored at −80°C after 30 days of storage followed by pollens stored at −20°C temperature. After 360 days of storage, only pollen grains stored at −80°C and −20°C showed germination in all cultivars. In staining test, pollen showed (94-100%) viability at different temperature after 30 days of storage and after 360 days of storage pollen viability ranged from 53.6% – 82.4%. The pollen viability and germination decreased with the increase in storage temperature and time in all the peach cultivars.

Key words: *Prunus persica*, pollen, storage, viability, germination.

INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is native to China and well adapted to temperate and sub-tropical regions. China is the leading peach producing country with about 54% share of the total world production followed by Italy, Spain and USA. Total world production and area under peaches and nectarines is 21.64 mt and 1.54 m ha, respectively and are produced commercially in 71 countries worldwide (FAO, 6). In India, peach occupies an area of 19 thousand ha and production is 95 thousand MT (NHB, 10). The area under peach is increasing rapidly in the sub-tropics of northern India due to its wider availability and higher returns per unit area.

Breeding of high quality peach cultivars is a major concern as the consumer preferences are changing rapidly. Peach fruit quality involves shape, size, skin colour, flesh colour, firmness, texture, freedom from loose fibre and non-browning of the flesh, beside the nutritive value. Improvement of all these traits is needed to produce the fruit of the high quality and can be improved by breeding techniques. For this peach needs large quantity and high quality of pollens for pollination and fertilization. In breeding programs, pollen availability during off time when the pollen of a variety is not there in the field is achieved by pollen storage and is a useful technique to establish a pollen bank which can be used by plant breeders for fertilization and hybridization. Another important application of pollen storage is to provides a convenient and simple means of pollen exchange amongst breeders within and between countries. In controlled hybridization, it is important to ensure that the stored pollen retains viability to produce fertile seeds. Therefore, pollen viability and germination capability are essential (Martinez-Gomez *et al*., 9) for successful peach breeding. Pollen staining and *in vitro* germination methods have long been used as indicators of viability. Pollen viability is determined by different methods viz. pollen culture on sucrose solution (2.5 to 20%), staining and, *in vitro* germination. Staining tests have advantages over pollen germination method as they are faster and easier than pollen germination.

Pollen germination tests are also very important as they determine the actual amount of viable pollen. Boavida and McCormick (5) indicated that the pollen grains have good growth and germination in the special environments. Imani *et al.* (8) evaluated the germination capacity of stored pollen of almond and peach and found highest pollen viability at lowest temperature (−80°C). Aslantus and Pirlak (4) evaluated the germination capacity of strawberry pollen and concluded that pollen germination percentage can be increased by storing them at low temperature. Sharafi *et al.* (12) reported that pollen germination and tube growth rate are the most important characteristics related to pollen quality and successful fertilization needs to high germination rates and fast tube growth because, low rates may lead to low fruit set caused by ovule degradation before the pollen tube reaches the ovary.

In peach breeding programs, the non-synchronous flowering among parents necessitates storage of pollen. When the pollen parent flowers earlier than

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the female parent it requires pollen storage just for few days or weeks. However, when the pollen parent flowers after the female parent then pollen is to be stored for one year. Further, in peach breeding programs crossing high chilling varieties from cold regions and low chilling varieties in sub-tropics is very useful strategy for the creation of diversity. The exchange of pollen among different breeding programs also necessitates information regarding the storage life of pollen. So, the objective of this experiment was to study the storage life of pollen grains of three peach cultivars at different temperatures and optimizing the best temperature for long term pollen storage.

MATERIALS AND METHODS

Pollen grains were collected from 5 year old plants of three low chill cultivars i.e. 'Florda Prince', 'Flordaglo' and 'Prabhat' planted at Fruit Research Farm, PAU Ludhiana Punjab during the flowering period in 2015. In these cultivars, flowering starts from the last week of January and among these, 'Florda Prince' flowers earlier than 'Prabhat' and 'Flordaglo'. Flower buds at balloon stage were collected and then their petals and sepals were separated and anthers isolated from flower bud and placed in petri dishes. Petri dishes were kept in desiccator at room temperature for 24 hours to release pollen from anthers. The pollen grains were stored in 10 ml plastic vials at −80°C, −20°C, 3±1°C, 6±1°C and room temperature. Pollen viability of these stored pollens grains were tested at 30 days interval upto one year of storage by acetocarmine staining and *in vitro* pollen germination. Two drops of acetocarmine solution (1%; Himedia Laboratories, India) was used to stain pollen grains. The deeply stained and normal pollen grains were considered to be viable, whereas shrivelled, lightly stained or colourless pollen grains were counted as non-viable. The pollen grains were uniformly dusted on thin layer of agar solidifying media plates for the *in vitro* pollen germination tests. The growing media consisted of sucrose (15%), $Ca(NO_3)_2$ (300 mg/L), $MgSO_4$ (200 mg/L), H_3BO_3 (100 mg/L), KNO_3 (100 mg/L) and Agar (10 mg/L) at pH 5.5. The number of germinated pollen grains were counted under an optical microscope (Magnus, MLM) at 40% magnification after incubation of 24 hours in dark at 26 ± 2°C temperature and at 30 days interval thereafter. Pollen tube growth were also measured after 24 hours of culture under stereoscope.

Experimental design was completely randomized design (CRD) with 5 treatments (5 different temperature conditions) and three replications (3 slides per cultivar for each storage temperature). Data were analyzed using SAS v9.0.0 software and means were compared using Least Significant Difference (Fisher's LSD) test at ≤0.05 level of significance.

RESULTS AND DISCUSSION

The data pertaining to the effect of different temperature conditions on the pollen germination of three peach cultivars is presented in Table 1 and Plate 1, 2 and 3. Pollen germination exhibited significant differences stored at different temperature conditions. Pollen grains stored at (−80 °C) showed best germination. In 'Florda Prince' cultivar, maximum germination percentage (94.13 %) was found in 30 days stored pollen at −80 °C and minimum germination (69.00 %) was recorded in pollens stored at room temperature. Non significant differences in pollen germination at −80 °C and −20 °C stored pollens was found initially after 30 days of storage, whereas the pollen stored at 3±1 °C, 6±1 °C and room temperature showed the significant differences. There is a considerable decrease in the pollen germination with increase in the storage period and germination stopped completely after 120 days of storage at room temperature, whereas in pollens stored at 3±1 °C and 6±1 °C germination failed after 210 days and

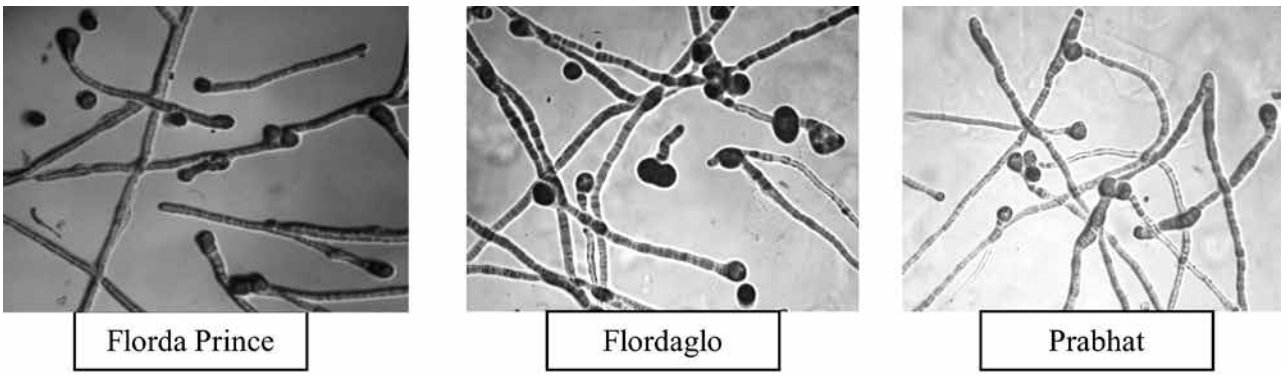

Plate 1. Pollen germination of three peach cultivars after 30 days of storage at -80°C temperature.

Table 1. *In-vitro* pollen germination (%) of three peach cultivars stored at different temperature conditions.

Florda Prince													
Storage time (Days) / Storage Temperature (°C)	30	60	90	120	150	180	210	240	270	300	330	360	Mean
−80	93.33	84.40	80.10	77.60	73.33	72.90	71.33	69.64	66.00	63.60	51.66	41.86	70.47[a]
−20	91.66	82.06	75.33	70.26	73.86	70.33	65.30	61.96	56.43	46.73	41.66	35.00	64.21[b]
3±1	81.93	76.93	61.13	44.00	32.33	23.66	14.53	11.73	0	0	0	0	28.85[c]
6±1	78.47	70.66	53.33	41.66	31.66	23.33	11.66	0	0	0	0	0	25.89[d]
Room temperature	69.00	65.66	42.33	30.00	20.00	0	0	0	0	0	0	0	18.91[e]
Mean	82.87[a]	75.94[b]	62.44[c]	52.70[d]	46.23[e]	38.04[e]	29.86[f]	26.32[fg]	24.48[fgh]	22.06[ghi]	18.66[hi]	15.37[i]	41.67
Flordaglo													
−80	95.00	87.33	87.66	83.60	79.00	76.43	75.26	74.63	69.63	62.60	53.56	46.90	74.3[a]
−20	91.66	86.66	86.53	81.66	76.50	74.23	68.86	65.60	56.66	49.33	44.33	40.20	68.51[b]
3±1	87.00	84.50	72.66	50.30	41.93	37.50	30.20	27.10	13.73	0	0	0	37.07[c]
6±1	86.50	77.66	66.66	42.93	34.00	32.33	23.33	13.40	0	0	0	0	31.40[d]
Room temperature	72.66	69.50	52.60	31.66	21.66	0	0	0	0	0	0	0	20.67[e]
Mean	86.56[a]	81.13[ab]	73.22[b]	58.03[c]	50.61[cd]	44.09[de]	39.53[ef]	36.14[fg]	28.00[gh]	22.38[hi]	19.57[hi]	17.42[i]	46.39
Prabhat													
−80	96.00	88.66	86.00	76.66	72.60	70.00	63.33	58.33	53.33	48.33	41.66	36.66	65.96[a]
−20	93.33	86.00	85.00	72.50	70.00	66.66	61.66	50.00	43.33	41.66	35.00	26.66	60.98[b]
3±1	88.33	83.33	71.66	50.00	40.86	34.50	28.33	13.33	0	0	0	0	34.19[c]
6±1	81.66	76.33	62.33	42.60	30.00	0	0	0	0	0	0	0	24.41[d]
Room temperature	70.00	66.66	49.33	30.00	0	0	0	0	0	0	0	0	17.99[e]
Mean	85.86[a]	80.19[a]	70.86[b]	54.35[c]	42.69[d]	34.23[e]	30.66[ef]	24.33[fg]	19.33[gh]	17.99[gh]	15.33[h]	12.66[h]	40.71
LSD 0.05		Florda Prince				Flordaglo				Prabhat			
Temperature		4.91				5.25				4.43			
Days		7.61				8.14				6.87			

Bars with the same letters are not significantly different according to Duncan's multiple range test at 5% level.

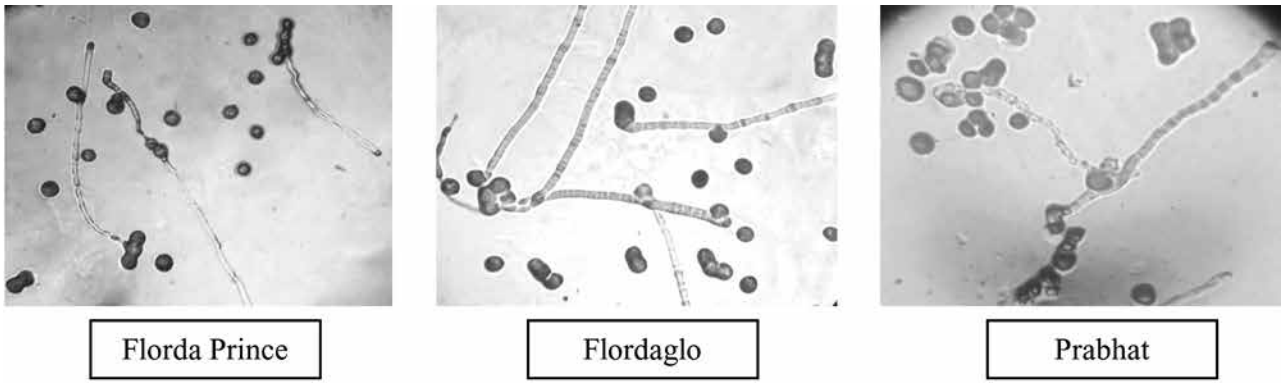

Plate 2. Pollen germination of three peach cultivars after 360 days of storage at -80°C temperature.

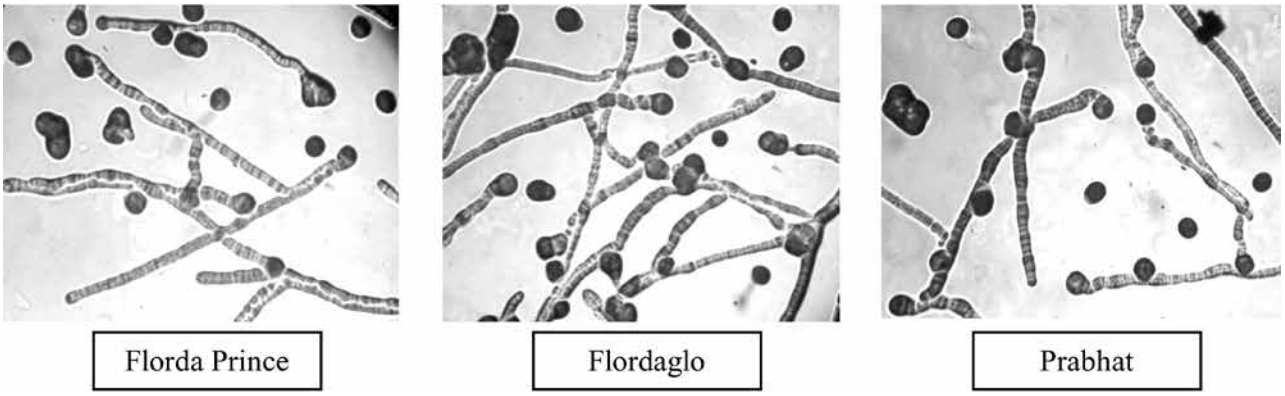

Plate 3. Pollen germination of three peach cultivars after 30 days of storage room temperature.

180 days of storage, respectively. The same trend of pollen germination was recorded in 'Flordaglo' and 'Prabhat' variety of peach with maximum germination at −80 °C after 30 days of storage and minimum at room temperature pollen grains and decreased with increase in storage period. Among the cultivars at −80 °C, maximum pollen germination (96.00 %) was found in 'Prabhat' variety after 30 days of storage followed by 'Flordaglo' variety (95.00 %) and minimum (94.13 %) in 'Florda Prince' variety. In 'Prabhat', pollen germination decreased very rapidly as compared to other two cultivars and showed less germination than 'Florda Prince' and 'Flordaglo' after 90 days and 60 days of storage at different temperature. After 360 days of storage, only those pollen grains showed germination which were stored at −80 °C and −20 °C temperature and 'Flordaglo' variety showed maximum pollen germination (46.9%) and minimum (36.66%) was observed in 'Prabhat' variety at −80 °C. Pollens stored at 3±1 °C and 6±1 °C germination was completely stopped after 240 days and 180 days of storage in 'Flordaglo' and 210 days and 150 days after storage in 'Prabhat'. At room temperature, 'Florda Prince' and 'Prabhat' variety didn't showed germination after 120 days of storage whereas in 'Flordaglo' germination failed after 150 days of storage. The results of the present study are in accordance with the findings of Imani *et al.* (8), who also found that temperature influenced the pollen viability in different way and lowering of temperature increased the level of viability. Anjum and Shaukat (2) also studied pollen germination of *M. pumila* L. for 48 weeks in the refrigerator (+4 °C), freezer (−20 and −30 °C) and freeze drier (−60 °C) in different concentration of sucrose and boric acid solution and reported that pollens which stored at low temperature had higher germination percentage as compared to pollens stored at +4 °C and in fresh pollen also, freeze dried pollen (−60 °C) showed the highest germination percentage.

Hedhly *et al*. (7) evaluated *in vitro* pollen germination of nine sweet cherry cultivars under two temperature regimes (15 and 30 °C) and found a highly significant effect of pollen genotype and temperature. Alburquerque *et al*. (1) also studied the influence of storage temperature on the viability of pollen in seven sweet cherry cultivars and reported that high percentage of pollen viability could be maintained after storage at −20 °C during one year for all studied cultivars. Temperature is a very basic factor in the control of the environmental conditions and influences pollen grain germination and longevity in stored pollens (Aparecida *et al*., 3).

The result of pollen viability using acetocarmine is shown in Table 2. The pollen viability of all cultivars was higher than the pollen germination in all storage temperature. Significant differences were recorded in pollen viability of all cultivars according to the storage conditions. The highest percentage of viable pollen grains was found in pollens stored at −80 °C and in −20 °C temperatures and lowest was found in pollens stored at room temperature. A non significant differences in pollen viability were observed in pollens stored at −80 °C, −20 °C and 3±1 °C temperature after 30 days of storage in three cultivars. Similarly pollen viability at 6±1 °C and room temperature was also found non significant after 30 days of storage. As the time passed, the percentage of pollen viability decreased gradually. In staining test, pollen grains showed less decrease in pollen viability as compared to *in vitro* pollen germination, and after 360 days of storage pollens showed (82.40 %) viability in 'Flordaglo', (82.13 %) in 'Florda Prince' and (75.13 %) in 'Prabhat' variety at −80 °C storage temperature followed by −20 °C. In both 'Florda Prince' and 'Flordaglo' cultivars pollen viability in −80 °C temperature was found at par with −20 °C stored pollen after 360 days of storage. Pollen stored at room temperature was also found viable after 360

Table 2. Pollen viability (%) of stored pollen of three peach cultivars using acetocarmine Bars with the same letters are not significantly different according to Duncan's multiple range test at 5% level.

Florda Prince													
Storage time (Days) / Storage Temperature (°C)	30	60	90	120	150	180	210	240	270	300	330	360	Mean
−80	100.00	100.00	98.13	96.06	94.38	94.06	92.18	90.01	86.44	85.28	84.20	82.13	91.90[a]
−20	100.00	100.00	94.16	93.33	92.93	92.20	91.03	89.60	85.60	82.12	80.26	80.00	90.10[a]
3±1	97.90	95.88	92.36	91.36	88.30	87.06	85.26	83.46	82.76	79.60	76.80	74.61	86.27[b]
6±1	94.80	96.66	92.10	90.40	87.60	86.06	84.46	80.21	77.14	74.76	73.40	71.10	84.05[c]
Room temperature	94.33	91.82	89.56	86.40	80.53	76.43	72.90	69.66	67.00	64.40	62.60	55.43	75.92[d]
Mean	97.40[a]	96.87[ab]	93.26[bc]	91.51[cd]	88.74[de]	87.16[ef]	85.16[fg]	82.58[gh]	79.78[hi]	77.23[ij]	75.45[jk]	72.65[k]	85.65
Flordaglo													
−80	100.00	100.00	98.33	96.74	94.70	93.02	92.26	90.34	87.53	85.50	84.26	82.40	92.09[a]
−20	100.00	100.00	97.60	95.54	93.79	92.33	90.26	89.32	86.33	82.79	80.37	80.06	90.69[a]
3±1	98.66	97.86	96.40	94.04	91.72	89.44	84.45	83.59	81.84	79.26	76.53	74.20	87.33[b]
6±1	97.93	96.33	94.24	90.98	88.78	85.93	84.00	80.84	78.46	74.83	73.37	71.73	84.78[c]
Room temperature	96.33	94.16	90.88	86.59	81.93	77.23	73.60	70.27	67.93	65.04	63.40	56.86	77.01[d]
Mean	98.58[a]	97.67[ab]	95.49[bc]	92.77[cd]	90.18[de]	87.59[ef]	84.91[fg]	82.87[gh]	80.41[h]	77.48[i]	75.58[ij]	73.05[j]	86.38
Prabhat													
−80	100.00	100.00	97.25	95.60	92.50	89.62	86.12	83.96	81.73	79.3	77.40	75.13	88.21[a]
−20	100.00	100.00	96.40	93.56	91.40	87.73	85.63	83.23	79.33	75.66	73.60	71.66	86.51[b]
3±1	99.33	98.86	94.06	88.96	86.16	83.54	81.23	78.73	76.87	72.16	70.86	68.66	83.28[c]
6±1	97.06	96.77	93.37	86.80	85.40	83.06	80.00	77.80	74.50	73.53	70.26	67.66	82.18[c]
Room temperature	96.23	95.20	89.40	83.63	79.68	75.13	69.70	66.93	63.66	61.66	58.46	53.60	74.44[d]
Mean	98.52[a]	98.16[a]	94.09[b]	89.71[c]	87.02[d]	83.81[e]	80.53[f]	78.13[f]	75.21[g]	72.46[h]	70.11[h]	67.34[i]	82.92
LSD 0.05		Florda Prince				Flordaglo				Prabhat			
Temperature		2.09				1.77				1.68			
Days		3.24				2.75				2.60			

days of storage with maximum viability (56.86 %) in 'Flordaglo' and minimum (53.60 %) in 'Prabhat'. These data are in accordance with the results of Parfitt and Ganeshan (11) in *Prunus* pollens. In present investigation, the pollens which didn't showed germination were found viable in staining test. In all the peach cultivars more than 50 % pollen viability was recorded even after one year of storage at room temperature, whereas germination was completely stopped after 120 and 150 days of storage under *in vitro* conditions. Parfitt and Ganeshan (11) examined that the pollen stain tests (acetocarmine, Alexander Stain's, TTC, MTT and NBT) are not reliable and are not positively correlated with *in vitro* germination tests. The stain test may be used to determine pollen viability in these species to provide only a rough estimate of viability. However, the exact amount of viable pollen may be determined *in vitro* by pollen germination. Temperature and humidity are the major influencing factors of pollen behaviour in different conditions. The most important factors for successful pollen conservation are storage temperature and moisture content of material; lowering of both tend to increase the period of viability.

Significant differences was recorded in pollen tube growth with respect to different storage temperature

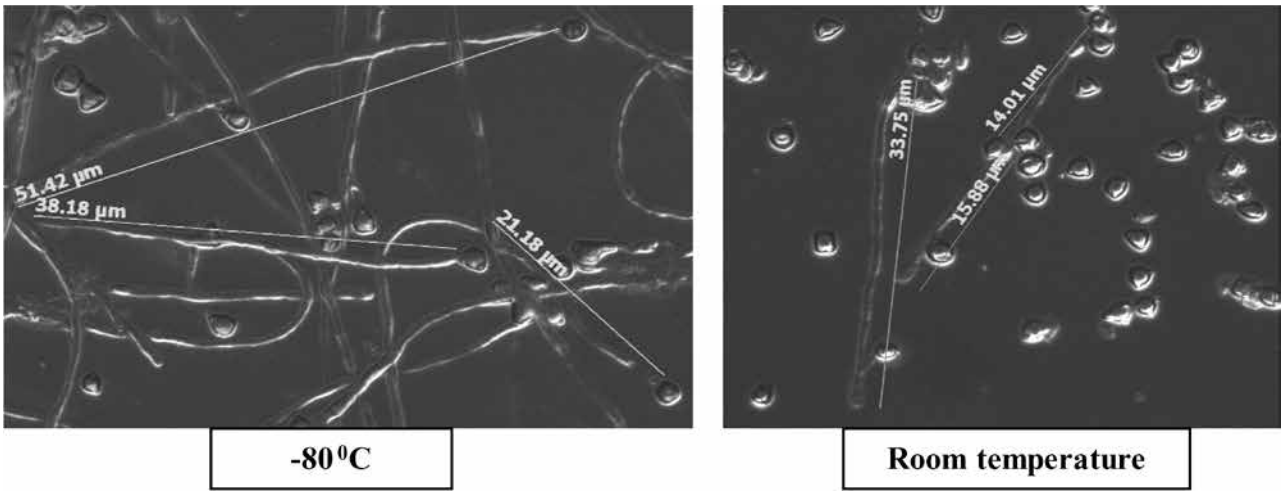

Plate 4. Pollen tube growth of stored pollen (-80°C and room temperature) after 24 hours of culture.

(Table 3 and Plate 4). Longest pollen tube (38.46 µm) was recorded when the pollen grains were stored at −80 °C which was non significant to the pollens stored at −20 °C. Smallest pollen tube (23.12 µm) was observed in pollen grains stored at room temperature. There was a non significant differences in pollen tube length of different varieties. It is clear from our data that pollen stored at low temperature has good germination capacity when cultured for 24 hours in dark at 26±2 °C temperature as compared to pollens stored at room temperature.

It is concluded that temperature and humidity are the major influencing factors of pollen viability in different conditions. Pollen stored at ultra low temperature freezer (−80 ºC) showed (36.66 - 46.90 %) of germination in different peach cultivars after 60 days of storage and (26.66 - 40.20 %) germination in pollens stored at −20 °C. In staining test also pollen showed maximum viability in −80 °C storage followed by −20 °C storage. Thus the most important factors for successful pollen conservation are storage temperature and moisture content of material; lowering both tend to increase the period of viability.

Table 3. Influence of different storage temperatures on pollen tube growth (µm) of peach cultivars.

Cultivars / Storage temperature	Florda Prince	Flordaglo	Prabhat	Mean
−80	40.73	38.19	37.01	38.46a
−20	40.70	37.60	36.53	38.27a
3±1	33.56	32.61	30.34	32.17ab
6±1	30.52	30.06	25.70	28.76bc
Room temperature	25.37	23.46	20.54	23.12c
Mean	34.17a	32.38a	30.02a	

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