

Original Research Article

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Effect of Different Varieties, Media and Storage Temperature on *in vitro* Pollen Germination in Mango (*Mangifera indica* L.)

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ABSTRACT

Keywords

Mango; Pollen storage, *In vitro* pollen germination, Storage media, Storage temperature

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A study was conducted to investigate *in vitro* pollen germination of four mango varieties, viz. Kesar, Alphonso, Mallika and Dudhpendo. Pollen grains were stored in three different media (n-hexane, paraffin oil and without media) under four different storage temperature (-20°C, -4°C, 4°C and room temperature). The experiment was laid out in Completely Randomized Design (CRD) with factorial concept. Germination media having 10% sucrose + 100 ppm H₃BO₃ + 300 ppm Ca(NO₃)₂ + 200 ppm MgSO₄ + 100 ppm KNO₃ used for *in vitro* pollen germination for all the varieties. Variation due to different varieties, media and temperature was found significant. Among four varieties, maximum germination was recorded in Kesar during 7, 14, 21, 28, 35, 42 and 56 days of storage, whereas, in Mallika at 49 days of storage. In storage media, n-hexane found best for retaining more germination ability during all storage days. Room temperature storage of pollen showed no germination from 21 days of storage. Maximum pollen germination was recorded at -20°C followed by -4°C. On the basis of interaction effect of results obtained, it could be concluded that mango pollen grains of Kesar and Mallika could be stored successfully up to 56 days in n-hexane at -20°C.

Introduction

Mango (*Mangifera indica* L.) belongs to the family Anacardiaceae. It is the most important crop among the tropical and subtropical fruit crops grown in more than 110 countries across the world. Mango (2n=40), is an allopolyploid, most probably amphidiploid and cross pollinated species.

Individual mango flower is small, ranging in size from five to ten mm in diameter. The three to nine (usually five) cream-coloured

petals typically change their colour before falling (Naik and Rao, 1943). Anthers are around 1.2 mm long, usually four-lobed, and dehiscence occurs longitudinally to reveal pollen grains (Scholefield, 1982). The ovary has one chamber that contains only one ovule (Free, 1993) and superior, sessile, free, yellow- coloured (Kosterman and Bompard, 1993; Galan-Sauco, 1999). Morphologically, pollen grains are 20–45 µm long, tricolpate and generally have three apertures along the longitudinal sides when they dry (Singh, 1961; Davenport, 2009).

Mango pollination is a complex physiological

phenomenon. It initiates with the contact of pollen with the stigmatic surface of the style. Pollen adhesion to stigmatic surface differs from cultivar to cultivar (Dutta *et al.*, 2013). Stigma receptivity commenced about 18 hrs prior to anthesis and continued for 72 h after anthesis and is most receptive during the first 6 hrs in mango (Pimentel *et al.*, 1984; Tyler and Schnell, 2009). After pollen deposition on the stigma, pollen tube germination and growth is the next step in the process of fruit setting. The minimum time required for pollen germination is 1.5 hrs in mango (Sen *et al.*, 1946; Singh, 1954; Spencer and Kennard, 1955). Depending upon ambient temperature, the rate of pollen germination differs among cultivars.

Pollen viability and its germination are cultivar dependent characters in mango (Singh, 1954; Abourayya *et al.*, 2011). Mango pollen requires some specific conditions of temperature and humidity as well as an artificial growth medium to achieve *in vitro* germination equaling that of pollen on stigmas *in vivo* (De Wet and Robbertse, 1986). To fulfil the above objective, an attempt was made to determine the *in vitro* pollen germination of different mango cultivars under different storage media and storage temperature for mango pollen storage.

Materials and Methods

Pollen collection and drying

Four mango varieties viz., Kesar, Alphonso, Dudhpendo and Mallika were chosen for present study available at Fruit Research Station, Sakkarbaug, Junagadh Agricultural University, Junagadh. The pollen used in this experiment was collected from freshly opened flowers of all four mango cultivars between 8:00 and 10:00 am ($\leq 23^{\circ}\text{C}$).

The harvested flowers were placed in the sun

to prompt anther dehiscence. Pollen grains visible on the anthers as a greyish powder, were collected in a petri dish using forceps and dried in a desiccator for two hours.

Pollen storage

Dried pollen grains were suspended in the different three storage media, viz. n-hexane, paraffin oil and without media and stored at room temperature in a controlled growth room (27°C) and at low temperatures in a cold-storage facility (4°C , -4°C , -20°C) at the Post Graduate Lab, College of Horticulture, Junagadh.

Sample preparation

The samples of pollen grains were taken from storage each week for observations. Pollen grains were separated from oil/organic solvent via a filtration process. Paraffin oil along with pollen grains was poured onto the filter paper directly from the petri dishes and allowed to filter through, leaving pollen grains on the paper. The pollen grains on the filter paper were then rinsed 4–5 times with n-hexane to remove residual oil, before they were dried for 10–15 minutes.

In vitro pollen germination

Germination media with different concentrations of sucrose, H_3BO_3 , $\text{Ca}(\text{NO}_3)_2$, MgSO_4 and KNO_3 was analyzed for *in vitro* pollen germination for all the varieties. Germination tests were incubated at $27 \pm 2^{\circ}\text{C}$ for 6 hours. Pollen grains were considered as germinated when pollen tube length surpassed their diameter. Germination media having 10% sucrose + 100 ppm H_3BO_3 + 300 ppm $\text{Ca}(\text{NO}_3)_2$ + 200 ppm MgSO_4 + 100 ppm KNO_3 was found best and was further used for *in vitro* pollen germination for all the varieties during the research program.

Statistical analysis

Statistical analysis of data of various characters was carried out as per Completely Randomized Design (Factorial). Analysis of variance was worked out using standard statistical procedures as described by Panse and Sukhatme (1985).

Results and Discussion

Effect of variety on *in vitro* pollen germination

Pollen germination is the most important parameter for long term pollen storage and its use for pollination. The variation due to varieties in pollen germination was found significant for all storage days. Significantly maximum pollen germination (53.95, 43.64, 31.62, 23.06, 16.04, 11.34 & 0.95%) was recorded in Kesar (V_1) followed by Mallika (V_3) during 7, 14, 21, 28, 35, 42 and 56 days. Whereas at 49 days of storage, maximum pollen germination (4.13%) was noted in Mallika (V_3) followed with Kesar (V_1). During all storage days, lowest pollen germination was observed in variety Alphonso (V_2) (Table 1).

Effect of media on *in vitro* pollen germination

The differences observed in pollen germination due to storage media was also found significant and maximum pollen germination (63.71, 52.63, 41.76, 32.64, 24.21, 15.99, 6.65 and 1.95%) was registered in pollen grains stored with media n-hexane (M_1) followed by paraffin oil (M_2) during all storage days. Similarly, lowest pollen germination (31.75, 21.11, 7.26 and 1.37%) were found in pollen grains stored without any media (M_3) at 7, 14, 21 and 28 days, respectively. From 35 days of storage, it was recorded to zero (Table 1).

Effect of storage temperature on *in vitro* pollen germination

In the present study, wide variation in germination of pollen grains was observed significant with different storage temperatures and durations among different mango cultivars. Maximum pollen germination (70.94, 60.30, 45.64, 33.24, 23.32, 16.99, 9.40 and 2.60%) was noted in pollen grains stored at temperature -20°C (S_1) followed by -4°C (S_2) during all storage days, respectively. However, minimum pollen germination was found in pollen grains stored at room temperature (S_4) for all storage days.

Interaction effect of variety and media on *in vitro* pollen germination

Variation due to interaction effect of variety and media on pollen germination was found significant at all days of storage. Maximum pollen germination (68.49, 59.98, 45.55, 37.22, 27.34, 20.47, 8.87 & 2.86%) was observed in treatment combination V_1M_1 at all days of storage followed by V_3M_1 at 7, 14, 21, 35, 42 and 49 as well as by V_4M_1 at 28 and 56 days. Lowest pollen germination was noted in V_2M_3 at 7 and 21 days of storage. At 14 and 28 days of storage, it was observed in V_3M_3 . Pollen germination exerted zero in no media (control) with all varieties i.e. treatment combination V_1M_3 , V_2M_3 , V_3M_3 and V_4M_3 after 35 days of storage (Table 2).

Interaction effect of media and storage temperature on *in vitro* pollen germination

Variation due to interaction effect of different media and storage temperatures on pollen germination was also found significant at all days of storage. Maximum pollen germination (85.48, 76.42, 63.43, 51.94, 42.05, 31.88, 21.43 & 7.81%) was noted in pollen grains stored with n-hexane at -20°C (M_1S_1) at all days of storage, respectively (Table 3).

Table.1 Effect of different mango variety, media and temperature on *in vitro* pollen germination

Treatments	<i>In vitro</i> pollen germination (%)							
	7	14	21	28	35	42	49	56
Factor A (Variety - 4)								
V ₁ : Kesar	53.95	43.64	31.62	23.06	16.04	11.34	3.97	0.95
V ₂ : Alphonso	46.66	36.79	25.15	16.41	10.38	5.88	0.89	0.00
V ₃ : Mallika	52.84	39.74	29.55	21.11	15.02	8.70	4.13	0.78
V ₄ : Dudhpendo	47.58	36.65	26.52	18.69	12.66	5.91	2.12	0.86
S.Em. ±	0.27	0.24	0.25	0.21	0.20	0.12	0.05	0.01
C.D. at 5%	0.75	0.67	0.71	0.60	0.56	0.34	0.15	0.03
Factor B (Media - 3)								
M ₁ : n-hexane	63.71	52.63	41.76	32.64	24.21	15.99	6.65	1.95
M ₂ : Paraffin oil	55.32	43.87	35.61	25.44	16.37	7.88	1.69	0.00
M ₃ : No media (Control)	31.75	21.11	7.26	1.37	0.00	0.00	0.00	0.00
S.Em. ±	0.23	0.21	0.22	0.19	0.17	0.11	0.05	0.01
C.D. at 5%	0.65	0.58	0.61	0.52	0.49	0.30	0.13	0.03
Factor C (Storage Temperature - 4)								
S ₁ : -20 °C	70.94	60.30	45.64	33.24	23.32	16.99	9.40	2.60
S ₂ : -4 °C	64.12	52.15	37.88	24.81	16.26	9.43	1.72	0.00
S ₃ : +4 °C	54.33	42.35	29.32	21.23	14.53	5.40	0.00	0.00
S ₄ : Room temperature	11.64	2.01	0.00	0.00	0.00	0.00	0.00	0.00
S.Em. ±	0.27	0.24	0.25	0.21	0.20	0.12	0.05	0.01
C.D. at 5%	0.75	0.67	0.71	0.60	0.56	0.34	0.15	0.03
Interactions								
V × M								
S.Em. ±	0.46	0.41	0.44	0.37	0.35	0.21	0.09	0.02
C.D. at 5%	1.30	1.15	1.22	1.04	0.97	0.59	0.26	0.05
V × S								
S.Em. ±	0.54	0.48	0.50	0.43	0.40	0.24	0.11	0.02
C.D. at 5%	1.50	1.33	1.41	1.20	1.12	0.69	0.30	0.06
M × S								
S.Em. ±	0.46	0.41	0.44	0.37	0.35	0.21	0.09	0.02
C.D. at 5%	1.30	1.15	1.22	1.04	0.97	0.59	0.26	0.05
V × M × S								
S.Em. ±	0.93	0.82	0.87	0.74	0.69	0.42	0.19	0.04
C.D. at 5%	2.61	2.31	2.44	2.08	1.95	1.19	0.52	0.11
C.V. %	3.20	3.64	5.34	6.49	8.88	9.20	11.59	10.10

Table.2 Interaction effect of variety and media on *in vitro* pollen germination

Treatments V × M	<i>In vitro</i> pollen germination (%)							
	7	14	21	28	35	42	49	56
V ₁ M ₁	68.49	59.98	45.55	37.22	27.34	20.47	8.87	2.86
V ₁ M ₂	60.14	46.59	38.60	28.90	20.78	13.55	3.05	0.00
V ₁ M ₃	33.23	24.34	10.72	3.08	0.00	0.00	0.00	0.00
V ₂ M ₁	60.32	49.31	39.68	28.24	19.35	11.95	2.67	0.00
V ₂ M ₂	51.42	41.28	31.61	21.00	11.79	5.68	0.00	0.00
V ₂ M ₃	28.23	19.77	4.16	0.00	0.00	0.00	0.00	0.00
V ₃ M ₁	66.01	52.68	41.99	32.52	<u>26.38</u>	16.70	<u>8.69</u>	2.35
V ₃ M ₂	57.37	47.06	38.39	28.38	18.69	9.40	3.71	0.00
V ₃ M ₃	35.15	19.47	8.26	2.41	0.00	0.00	0.00	0.00
V ₄ M ₁	60.02	48.54	39.82	32.59	23.75	14.82	6.37	2.59
V ₄ M ₂	52.34	40.54	33.85	23.49	14.22	2.90	0.00	0.00
V ₄ M ₃	30.39	20.86	5.88	0.00	0.00	0.00	0.00	0.00
S.Em. ±	0.46	0.41	0.44	0.37	0.35	0.21	0.09	0.02
C.D. at 5%	1.30	1.15	1.22	1.04	0.97	0.59	0.26	0.05
C.V. %	3.20	3.64	5.34	6.49	8.88	9.20	11.59	10.10

Table.3 Interaction effect of media and storage temperature on *in vitro* pollen germination

Treatments M × S	<i>In vitro</i> pollen germination (%)							
	7	14	21	28	35	42	49	56
M ₁ S ₁	85.48	76.42	63.43	51.94	42.05	31.88	21.43	7.81
M ₁ S ₂	77.99	66.91	55.00	42.46	28.65	18.53	5.17	0.00
M ₁ S ₃	69.88	61.17	48.62	36.16	26.13	13.54	0.00	0.00
M ₁ S ₄	21.49	6.02	0.00	0.00	0.00	0.00	0.00	0.00
M ₂ S ₁	74.49	65.04	54.86	42.29	27.90	19.10	6.76	0.00
M ₂ S ₂	69.03	59.03	48.25	31.96	20.13	9.75	0.00	0.00
M ₂ S ₃	64.32	51.40	39.34	27.52	17.46	2.66	0.00	0.00
M ₂ S ₄	13.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M ₃ S ₁	52.87	39.45	18.64	5.49	0.00	0.00	0.00	0.00
M ₃ S ₂	45.33	30.49	10.38	0.00	0.00	0.00	0.00	0.00
M ₃ S ₃	28.80	14.50	0.00	0.00	0.00	0.00	0.00	0.00
M ₃ S ₄	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.Em. ±	0.46	0.41	0.44	0.37	0.35	0.21	0.09	0.02
C.D. at 5%	1.30	1.15	1.22	1.04	0.97	0.59	0.26	0.05
C.V. %	3.20	3.64	5.34	6.49	8.88	9.20	11.59	10.10

Table.4 Interaction effect of variety and storage temperature on *in vitro* pollen germination

Treatments V × S	<i>In vitro</i> pollen germination (%)							
	7	14	21	28	35	42	49	56
V ₁ S ₁	71.31	61.52	48.72	35.59	<u>25.97</u>	21.42	12.30	3.82
V ₁ S ₂	65.94	57.42	42.18	30.47	17.87	14.01	3.60	0.00
V ₁ S ₃	63.50	51.27	35.60	26.20	20.32	9.93	0.00	0.00
V ₁ S ₄	15.06	4.34	0.00	0.00	0.00	0.00	0.00	0.00
V ₂ S ₁	69.44	59.16	43.45	29.57	18.74	12.06	3.56	0.00
V ₂ S ₂	60.03	48.90	31.92	19.08	12.02	7.63	0.00	0.00
V ₂ S ₃	50.71	39.10	25.23	16.99	10.77	3.82	0.00	0.00
V ₂ S ₄	6.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V ₃ S ₁	73.49	<u>61.31</u>	46.54	36.97	26.49	19.62	13.23	3.14
V ₃ S ₂	67.02	50.63	41.00	25.14	18.76	11.27	3.29	0.00
V ₃ S ₃	55.18	43.32	30.65	22.32	14.84	3.92	0.00	0.00
V ₃ S ₄	15.69	3.68	0.00	0.00	0.00	0.00	0.00	0.00
V ₄ S ₁	69.54	59.22	43.86	30.83	22.05	14.89	8.50	3.45
V ₄ S ₂	63.47	51.63	36.41	24.54	16.39	4.80	0.00	0.00
V ₄ S ₃	47.95	35.73	25.80	19.40	12.18	3.94	0.00	0.00
V ₄ S ₄	9.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.Em. ±	0.54	0.48	0.50	0.43	0.40	0.24	0.11	0.02
C.D. at 5%	1.50	1.33	1.41	1.20	1.12	0.69	0.30	0.06
C.V. %	3.20	3.64	5.34	6.49	8.88	9.20	11.59	10.10

Table.5 Combined interaction effect of variety, media and storage temperature on *in vitro* pollen germination

At 7 days of storage												
	V ₁			V ₂			V ₃			V ₄		
	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃
S ₁	<u>85.93</u>	73.50	54.50	84.21	74.96	49.15	87.20	76.79	56.46	84.58	72.70	51.35
S ₂	81.62	73.19	43.02	76.70	64.10	39.30	78.02	71.42	51.63	75.63	67.40	47.37
S ₃	80.44	74.67	35.38	66.54	61.10	24.48	69.23	63.80	32.50	63.30	57.72	22.83
S ₄	25.97	19.20	0.00	13.84	5.52	0.00	29.59	17.47	0.00	16.55	11.52	0.00
At 14 days of storage												
	V ₁			V ₂			V ₃			V ₄		
	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃
S ₁	79.61	63.71	41.25	76.13	63.28	38.07	75.27	69.79	38.86	74.67	63.38	39.61
S ₂	74.30	62.72	35.24	63.84	55.81	27.04	63.59	61.70	26.61	65.91	55.90	33.08
S ₃	72.99	59.94	20.88	57.28	46.04	13.98	60.81	56.74	12.40	53.59	42.87	10.74
S ₄	13.01	0.00	0.00	0.00	0.00	0.00	11.05	0.00	0.00	0.00	0.00	0.00
At 21 days of storage												
	V ₁			V ₂			V ₃			V ₄		
	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃	M ₁	M ₂	M ₃

S₁	63.30	55.76	27.09	61.74	51.96	16.66	<u>63.78</u>	57.12	18.72	64.88	54.61	12.09
S₂	59.10	51.67	15.77	52.69	43.08	0.00	56.94	51.74	14.32	51.27	46.50	11.45
S₃	59.82	46.98	0.00	44.29	31.40	0.00	47.24	44.71	0.00	43.12	34.27	0.00
S₄	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
At 28 days of storage												
	V₁			V₂			V₃			V₄		
	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃
S₁	50.08	44.40	12.30	49.46	39.25	0.00	54.79	46.45	9.66	<u>53.44</u>	39.04	0.00
S₂	52.90	38.50	0.00	33.40	23.83	0.00	41.45	33.97	0.00	42.09	31.54	0.00
S₃	45.90	32.69	0.00	30.08	20.90	0.00	33.86	33.11	0.00	34.81	23.38	0.00
S₄	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35 days of storage												
	V₁			V₂			V₃			V₄		
	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃
S₁	<u>46.66</u>	31.26	0.00	32.82	23.41	0.00	47.03	32.45	0.00	41.68	24.48	0.00
S₂	30.53	23.07	0.00	21.42	14.63	0.00	33.15	23.13	0.00	29.48	19.70	0.00
S₃	32.18	28.79	0.00	23.18	9.13	0.00	25.33	19.18	0.00	23.84	12.71	0.00
S₄	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
42 days of storage												
	V₁			V₂			V₃			V₄		
	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃
S₁	36.33	27.92	0.00	22.27	13.90	0.00	<u>35.83</u>	23.01	0.00	33.08	11.58	0.00
S₂	26.41	15.63	0.00	14.07	8.81	0.00	19.23	14.58	0.00	14.41	0.00	0.00
S₃	19.13	10.65	0.00	11.46	0.00	0.00	11.75	0.00	0.00	11.81	0.00	0.00
S₄	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
49 days of storage												
	V₁			V₂			V₃			V₄		
	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃
S₁	24.69	12.20	0.00	10.69	0.00	0.00	24.16	14.83	0.00	25.49	0.00	0.00
S₂	10.80	0.00	0.00	0.00	0.00	0.00	9.88	0.00	0.00	0.00	0.00	0.00
S₃	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S₄	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
56 days of storage												
	V₁			V₂			V₃			V₄		
	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃	M₁	M₂	M₃
S₁	11.45	0.00	0.00	0.00	0.00	0.00	9.42	0.00	0.00	10.35	0.00	0.00
S₂	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S₃	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S₄	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Days of storage	7	14	21	28	35	42	49	56
S.Em. ±	0.93	0.82	0.87	0.74	0.69	0.42	0.19	0.04
C.D. at 5%	2.61	2.31	2.44	2.08	1.95	1.19	0.52	0.11
C.V. %	3.20	3.64	5.34	6.49	8.88	9.20	11.59	10.10

Minimum pollen germination was observed in M₂S₄ at 7 days which turned to zero from 14 days of storage.

Interaction effect of variety and storage temperature on *in vitro* pollen germination

Similarly, variation due to the interaction effect of variety and storage temperature was also found significant and maximum pollen germination (73.49, 36.97, 26.49 & 13.23%) was noted in V₃S₁ during 7, 28, 35 and 49 days of storage. Elsewhere at 14, 21, 42 and 56 days of storage, maximum pollen germination (61.52, 48.72, 21.42 & 3.82%) were noted in V₁S₁. Similar trend was observed here also and Kesar and Mallika showed good pollen germination at -20°C might be due to low metabolic activities of pollen at sub-zero storage temperature. Minimum pollen germination was noted in variety Alphonso stored at room temperature (V₂S₄) at 7 days of storage but from 14 days of storage, it turns to zero germination in all varieties (Table 4).

Combined interaction effect of variety, media and storage temperature on *in vitro* pollen germination

Due to combined interaction effect of variety, media and storage temperature, the wide variation for pollen germination was also observed significant at all days of storage. Maximum pollen germination (87.20, 54.79 and 47.03%) was noted in V₃M₁S₁ at 7, 28 and 35 days of storage. Similarly at 14 and 42 days, it (79.61 and 36.33%) was noted in V₁M₁S₁ while at 21 and 49 days, it (64.88 and 25.49%) was noted in V₄M₁S₁. At 56 days of storage, all treatment combinations were recorded with zero per cent pollen germination except V₁M₁S₁ (11.45%) which was highest. Likewise, lowest pollen germination was noted in V₂M₂S₄, V₄M₃S₃, V₄M₃S₂ and V₁M₃S₁ at 7, 14, 21 and 28 days

of storage, respectively. Similarly, pollen grains stored without media at room temperature showed no germination for all varieties from 7 days (Table 5).

Effect of variety on *in vitro* pollen germination

The pollen germination differed depending on storage temperature, storage duration and cultivar. The differences in germination rates for the genotypes and cultivars might be due to genotype differences. The result was in confirming with those by Khan and Perveen (2009), Abourayya *et al.*, (2011) and Dutta *et al.*, (2013) in mango. Present finding were also in agreement with the results reported by Sahar and Spiegel (1984), Albuquerque *et al.*, (2007), Sharafi and Bahmani (2011), Bhat *et al.*, (2012), Gaaliche *et al.*, (2013), Marlon *et al.*, (2014), Khan and Perveen (2014) and Ahmed *et al.*, (2017) in avocado, sweet cherry, almond, pear, fig, custard apple and citrus, respectively.

Effect of media on *in vitro* pollen germination

The variation observed in pollen germination due to storage media was also found significant and might be due to the sensitivity of mango pollen to extended storage media exposure. However, decreased pollen germination from exposure to organic solvents may be the result of leaching of phospholipids, sugars, and amino acids from the stored pollen.

The loss of these compounds compromises pollen membrane integrity and consequently affects pollen germination (Jain and Shivanna, 1988). Results were also in close agreements with the finding of Kumar *et al.*, (2015) as well as with Jain and Shivanna (1988, 1990) in oil palm and crotalaria, respectively.

Effect of storage temperature on *in vitro* pollen germination

Wide differences in germination of pollen grains were observed significant with different storage temperatures and durations among different mango cultivars. Higher values for pollen germination at low storage temperature and decline at high storage temperature were observed by several workers (Khan and Perveen, 2008). The rate of reduction in pollen germination might be because of differential rate of metabolic activities in pollen at different temperature. From 21 days of storage, pollen grain stored at room temperature was completely lost its germination capacity. Our results clearly indicated that it is feasible to store pollen grains of mango at sub-zero temperatures (-20°C) without any significant loss in their germination capacity which was confirming with Bhat *et al.*, (2012) in pear. Present results were also in agreement with Salles *et al.*, (2007), Thaipong *et al.*, (2008) and Chander *et al.*, (2019) in citrus, grape and sugar apple, respectively.

Interaction effect of variety and media on *in vitro* pollen germination

Variation due to interaction effect of variety and media on pollen germination was found significant and might be due to the different genotype of the varieties and their interaction with storage media. Kesar had good pollen germination ability while stored in n-hexane could be due to very little leaching of sugars and amino acids into the solvent for good germination.

Interaction effect of media and storage temperature on *in vitro* pollen germination

Variation due to interaction effect of different media and storage temperatures on pollen germination was also found significant. As

mango pollen was more sensitive to desiccation and high temperature, zero pollen germination was noticed in pollen grains stored without media at room temperature (M₃S₄) from 7 days of storage. Results were also in agreement with Khan and Perveen (2008).

Interaction effect of variety and storage temperature on *in vitro* pollen germination

Similarly, variation due to the interaction effect of variety and storage temperature was also found significant. Kesar and Mallika showed good pollen germination at -20°C might be due to low metabolic activities of pollen at sub-zero storage temperature. Minimum pollen germination was noted in variety Alphonso stored at room temperature (V₂S₄) at 7 days of storage but from 14 days of storage, it turns to zero germination in all varieties. The decrease in pollen germination capacity at room temperature might be due to high sensitivity of mango pollen to existing high temperatures. However, results from this work were in agreement with those found by Albuquerque *et al.*, (2007), who found a highly significant effect of pollen genotype and temperature. Higher temperature reduced pollen germination. The result was also confirming with Imani *et al.*, (2011) and Bhat *et al.*, (2012) in apple and pear, respectively.

Combined interaction effect of variety, media and storage temperature on *in vitro* pollen germination

Due to combined interaction effect of variety, media and storage temperature, the wide variation for pollen germination was also observed significant at all days of storage. Results were observed due to different genotype of varieties, different nature of storage media and different metabolic activity rates of pollen at different storage temperature. Due to tolerability of Kesar

pollen against various storage conditions, less leaching of metabolites in n-hexane and very low metabolic activities at sub-zero (-20°C) storage temperature, its pollen could be stored more successfully up to 56 days compared to others.

On the basis of results obtained from the present investigation, it can be concluded that germination ability of mango pollen grains could be retained successfully in different storage media at low temperature up to 56 days. Among four varieties tested, Kesar and Mallika recorded as good in terms of pollen germination capacity. In storage media, n-hexane was found best in terms of retaining more germination ability in all four varieties but on the other hand success rate was found minimum in case of no media. Storage at -20°C gave better results in terms of *in vitro* pollen germination under study. Therefore, the best suitable temperature for long duration of pollen storage would be -20°C. At room temperature without media, pollen grains could not be stored more than a week due to rapid decrease in their germination capacity. Due to that, pollen grains stored without media at room temperature cannot be adopted. In case of interactions, pollen grains of Kesar, Mallika and Dudhpendo stored in n-hexane at -20°C were found best in terms of pollen germination up to 56 days.

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