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Short communication

Low temperature storage of mango (*Mangifera indica* L.) pollen

S.K. Dutta [a], Manish Srivastav [a,*], Rekha Chaudhary [b], Krishan Lal [c], Pravin Patil [d], S.K. Singh [a], A.K. Singh [a]

[a] Division of Fruits & Horticultural Technology, Indian Agricultural Research Institute, New Delhi 110012, India
[b] Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources, New Delhi 110012, India
[c] Indian Agricultural Statistics Research Institute, New Delhi 110012, India
[d] National Research Centre for DNA Fingerprinting, National Bureau of Plant Genetic Resources, New Delhi 110012, India

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ABSTRACT

A study was conducted to investigate pollen viability of three polleniser mango cultivars, *viz.* 'Sensation', 'Tommy Atkins' and 'Janardan Pasand' up to 24 weeks under four storage conditions (room temperature, −4 °C, −20 °C and −196 °C). Three methods of pollen viability testing, *viz. in vitro* germination, fluorescein diacetate (FDA) and acetocarmine staining were used. Storage methods and interaction between storage methods and days of storage had highly significant effect on pollen viability ($p \leq 0.0001$). Room temperature storage of pollen in the three mango cultivars showed very low pollen viability after 4 weeks of storage, after which pollens were not viable. Irrespective of mango genotypes, cryo-stored (−196 °C) pollens showed significantly higher viability as compared to all the other storage conditions. The differential results obtained by using different pollen viability assay confirmed that *in vitro* germination test was more reliable compared to FDA or acetocarmine tests, where germination was often overestimated. From the present study, we suggest storage of pollen at −20 °C for pollination among cultivars having non-synchronized flowering in a season. However, for long term storage cryo method proved to be the best for pollen storage in mango.

1. Introduction

Mango (*Mangifera indica* L.) belonging to family Anacardiaceae is one of the commercially important fruit crop of India originated in the Indo-Myanmar region. Mango breeding programme is underway at several research institutes of India and several new releases have been reported over the last few decades. Indian Agricultural Research Institute, New Delhi is notable among them credited with the development of some commercially important mango hybrids with desirable traits for domestic as well as overseas markets. Existing diversity of mango with respect to important traits provides huge scope for breeding new cultivars with desirable characters (Ram and Rajan, 2003). Flowering period in mango in India range from November to April depending on agro-climatic zones and mango breeding programmes are on several occasions hampered by spatial and temporal isolation of the parents (Chaudhury et al., 2010).

Red peel colour is an important trait from export point of view and attentions have been paid to develop red peeled mango hybrids at I.A.R.I., New Delhi over the last 3–4 decades. In mango breeding programme, 'Amrapali' is employed as female parent due to its dwarfness, regularity and better fruit quality. For imparting red colour in the peel, mango cultivars such as 'Sensation', 'Tommy Atkins' and 'Janardan Pasand' are preferentially used as male donor parents due to their better combining ability with 'Amrapali'. Mango blooming season in north India starts in February and lasts through March, whereas the regular harvesting season extends from June to August. The problem of asynchronised flowering in certain mango cultivars restricts their use as parent in breeding programme and circumstances usually make it necessary to store the pollen from male donor parents for later pollination of the desired female parents. Thus, in order to optimize the pollination for both early and late blooming cultivars it would be necessary to conserve pollen from early flowering cultivars for pollination in late season in late blooming cultivars and *vice versa*. Mango pollen is characterized by short viability and high sensitivity to desiccation, consequently, conservation is problematic (Issarakraisila and Considine, 1994). In the present investigation, an attempt was made to determine the pollen viability of some polleniser mango cultivars under different storage conditions and to standardize the storage method(s) for short and long term pollens storage. The efficient storage of pollens from mango cultivars having asynchronised flowering would be highly useful in attempting strategic breeding for the development of desirable genotypes.

* Corresponding author. Tel.: +91 11 25843214; fax: +91 11 25843214.
E-mail address: mns_fht@rediffmail.com (M. Srivastav).

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2. Materials and methods

2.1. Plant material

Three important mango pollen parents, *viz*. 'Sensation', 'Tommy Atkins' and 'Janardan Pasand' were selected at the Main Orchards of Division of Fruits and Horticultural Technology, IARI, New Delhi. Trees of these pollen parents were fairly old (20–25 years), healthy and free from diseases and pests.

2.2. Pollen collection

Pollens were collected between 8.00 and 10.00 h in the morning. Panicles were selected and open flowers were removed and then bagged in the afternoon. In the next morning, fresh open male flowers with red or purple anthers were collected. These pollens were subjected to four storage conditions and six storage periods with three replications in each treatment. For each replication, a minimum of 50 flowers were placed in petri dish lined with moist filter paper disc and brought immediately to the laboratory. The flowers were then placed under sun to induce dehiscence and pollens were collected in small cryo vials and kept into desiccators for 3–4 h. After desiccation, the cryo vials were sealed and transferred to respective storage conditions.

2.3. Storage conditions

Pollens collected in cryo vials were subjected to four storage conditions (room temperature, −4 °C, −20 °C and −196 °C). For room temperature storage, the sealed vials were kept at clean and dry place free from any light exposure (which served as control treatment). For storage at −4 °C, refrigerator was used with constant temperature maintenance. Storage at −20 °C was also achieved through refrigeration. Storage of pollens at −196 °C (cryo-storage) was achieved through dipping the cryo-vials in sealed liquid nitrogen cylinders. Pollens collected in cryo-vials were stored for six different time intervals, *viz*. 4, 8, 12, 16, 20 and 24 weeks under different storage conditions. Pollen samples were taken out from the lot and tested for viability using *in vitro* germination (Stanley and Linskens, 1974), fluorescein diacetate (FDA) (Helpson-Harrison and Helpson-Harrison, 1970) and acetocarmine (Nassar et al., 2000) tests.

2.4. Pollen germination and viability tests

In vitro pollen germination was assessed by using hanging drop technique as suggested by Stanley and Linskens (1974). The liquid germination medium contained 150 g l^{-1} polyethylene glycol (PEG 4000), 4.88 g l^{-1} N-morpholinoethanosulfonic acid in potassium hydroxide buffer (MES-KOH, pH 6.4), 200 mg l^{-1} $MgSO_4$, 100 mg l^{-1} KNO_3, 100 mg l^{-1} H_3BO_3, 700 mg l^{-1} $Ca(NO_3)_2$ and 200 g l^{-1} sucrose at pH 5.5 (modified Vivian-Smith et al., 1992). A minimum of 100 pollen grains per slide were randomly counted under a light microscope (Leica DM 1000) at 20× magnification. Pollen was adjudged as having germinated when the length of the pollen tube was equal to, or exceeded the pollen diameter.

Fluorescein diacetate (Helpson-Harrison and Helpson-Harrison, 1970) and acetocarmine (Nassar et al., 2000) tests were used to assess the viability of fresh as well as stored pollen from different mango cultivars. In FDA test, preparations were observed under the fluorescent microscope (Leica DM 5000B) with fluorescence filters. Pollen grains showing bright fluorescence were taken as viable and scored. In acetocarmine test, pollen grains were observed through an optical microscope (light microscope 20× magnification). Pollens grains that presented visibly abnormal size, light colouring and reduced and/or absent protoplasm were considered non-viable, while those that presented intact exines and strongly coloured protoplasm with homogeneous distribution were classified as viable.

2.5. Statistical analysis

Data were analyzed by using Repeated Measure Technique. For the analysis of data, mixed procedure in SAS 9.2 has been used. Mixed procedure takes care of correlated structure of the error variance–covariance matrix. In mixed procedure, data was analyzed using the iteration procedure and ANOVA has been obtained.

3. Results and discussion

Germination of fresh pollens of three mango cultivars was examined using *in vitro* germination, FDA and acetocarmine tests. Regardless of cultivars, different viability tests showed differential results. Comparatively higher pollen viability was observed using acetocarmine test; however, *in vitro* germination test showed low pollen germinability. In the present study, the cultivars difference for pollen germinability and viability was found to be non-significant for fresh pollen ($p \geq 0.05$). Similarly, the interaction effect of cultivar and pollen viability testing method was also found to be non-significant ($p \geq 0.05$). Pollen viability tests also proved that fresh pollen in mango cultivars had higher viability. The maximum *in vitro* germination of fresh pollens was found in 'Sensation' followed by 'Tommy Atkins' and 'Janardan Pasand', which had non-significant differences. Fresh pollen *in vitro* germination and pollen viability when confirmed by acetocarmine test in all three mango cultivars was non-significant ($p \geq 0.05$). However, viability of fresh pollen observed using FDA test showed significant differences among mango cultivars ($p \leq 0.05$) and 'Sensation' had the highest pollen viability followed by 'Janardan Pasand' and 'Tommy Atkins' (Table 1). Earlier, Martinez-Gomez and Gradziel (2002) also reported higher germination of freshly collected pollen in almond.

The analysis of variance (ANOVA) clearly indicated that storage methods, days of storage and interaction between storage methods and days of storage had highly significant effect ($p \leq 0.0001$) on pollen viability as confirmed by *in vitro* germination, FDA and acetocarmine tests. Furthermore, the effect of mango cultivar and interaction between mango cultivar and storage method on *in vitro* pollen germination was significant at $p \leq 0.01$ level of significance (Table 2).

Pollen storage at room temperature showed only 2.11, 1.80 and 1.03% pollen germination in 'Sensation', 'Tommy Atkins' and 'Janardan Pasand', respectively up to 4 weeks of storage as confirmed by *in vitro* pollen germination test, after which the germination percentage declined to zero at later stages of storage (Fig. 1A). Similar trend was also observed when pollen viability was examined using FDA and acetocarmine assay methods (Fig. 1E and I). Irrespective of the three different tests and mango cultivars, pollen viability was found to be 0.0% at 8, 12, 16, 20 and 24 weeks after storage at room temperature (Fig. 1A, E and I).

Pollen storage at −4 °C showed non-significant differences for pollen viability among three mango cultivars on all observation dates as confirmed by FDA and acetocarmine tests. However, pollen viability determined by *in vitro* germination test showed significant variation among pollen parents ($p \leq 0.05$). In all mango cultivars, the pollen viability was found to be decreasing significantly ($p \leq 0.05$) with the increase in storage period as confirmed by all three pollen viability tests. In 'Sensation', the fresh pollen viability was found to be 50.14% (*in vitro* germination test), 88.27% (FDA test) and 87.71% (acetocarmine test), which reduced to 6.75, 14.77 and 21.01%, respectively after 24 weeks of storage at −4 °C. Similarly in 'Tommy Atkins', the fresh pollen viability was found to be 48.78% (*in vitro* germination), 83.42% (FDA test) and 88.28%

Table 1
In vitro germination and pollen viability of fresh pollens as confirmed by fluorescein diacetate (FDA) and acteocarmine tests in polleniser mango cultivars.

Cultivar	Pollen viability test		
	In vitro germination	FDA	Acetocarmine
'Sensation'	50.14 ± 4.06 (45.06)	88.27 ± 0.93 (69.95)	87.71 ± 4.01 (69.62)
'Tommy Atkins'	48.78 ± 3.64 (44.28)	83.42 ± 1.59 (65.96)	88.28 ± 4.47 (70.21)
'Janardan Pasand'	47.30 ± 7.75 (43.43)	85.48 ± 0.57 (67.58)	90.25 ± 3.58 (71.96)

CD at 5%	
Method of viability test	3.94
Cultivar	ns
Method of viability test × cultivar	ns

Values in the table show mean ± SD. values in parentheses are angular transformed values. ns, not significant ($p \leq 0.05$).

(acetocarmine test), which further reduced to 6.52, 12.00 and 20.32%, respectively after 24 weeks of storage at −4 °C. 'Janardan Pasand' showed almost similar trend and fresh pollen viability was reduced to 5.49% (*in vitro* germination), 15.73% (FDA test) and 16.43% (acetocarmine test) after 24 weeks of storage at −4 °C as compared to the viability of fresh pollen (47.30, 85.48 and 90.25%, respectively) (Fig. 1B, F and J).

Pollen storage at −20 °C showed almost similar trend as observed in case of −4 °C storage. The only difference noticed was the percentage viability as it was high in all three cultivars at all observation dates compared to pollen storage at −4 °C. In 'Sensation', pollen viability after 24 weeks of storage at −20 °C showed decrease of 79.23% *in vitro* germination (Fig. 1C), 79.04% by FDA test (Fig. 1G) and 62.15% by acetocarmine test (Fig. 1K) compared to fresh pollen viability. Similarly, in 'Tommy Atkins' and 'Janardan Pasand', the decrease in pollen viability after 24 weeks of storage at −20 °C was 74.94 and 76.57%, respectively as depicted by *in vitro* germination test (Fig. 1C). Fluorescein diacetate and acetocarmine tests also confirmed decrease in viability of pollen stored at −20 °C. Decrease in pollen viability of 'Tommy Atkins' was 75.90% as determined by FDA test (Fig. 1G) and 61.62% by acetocarmine test (Fig. 1K). In 'Janardan Pasand', the decrease was 78.07% (FDA test) and 60.46% (acetocarmine test) as compared to fresh pollen viability (Fig. 1G and K).

Storage of pollens at −196 °C showed significantly higher viability percentage as compared to all the other storage conditions as confirmed by *in vitro* germination, FDA and acetocarmine tests of pollen viability (Fig. 1D, H and L). Pollens of 'Sensation' showed a significantly higher *in vitro* germination percentage than 'Janardan Pasand' and 'Tommy Atkins' at all stages (Fig. 1D). Similarly, FDA pollen viability test also confirmed highest pollen viability (66.00%) in case of 'Sensation' after 24 weeks of storage (Fig. 1H). In contrast, results obtained from acetocarmine test showed highest pollen viability (78.15%) in 'Janardan Pasand' compared to pollen viability of other two cultivars stored at −196 °C on all observation dates (Fig. 1L).

Rate of reduction in pollen viability in all three mango cultivars was minimal in case of −196 °C storage compared to other methods which makes it most suitable technique for both short as well as long term pollen conservation in mango. Pollen viability under −196 °C storage condition as confirmed by *in vitro* germination test for the three mango cultivars showed non-significant differences between 4 and 24 weeks of storage. Similarly, pollen viability depicted by FDA and acetocarmine tests showed similar trend with few exceptions. In case of FDA test, the pollen viability of 'Sensation' cultivar decreased between 4 and 8 weeks and after 8 weeks non-significant decrease was noticed. Likewise, in case of 'Tommy Atkins', the pollen viability tested using acetocarmine showed significant decrease in pollen viability between 4 and 8 weeks; however, after 8 weeks pollen viability did not differ significantly up to 24 weeks. Results suggest that freshly collected pollens of all three cultivars had high pollen viability and upon storage at room temperature had viability up to 4 weeks. The decrease in pollen viability at room temperature during first 4 weeks might be attributed to high sensitivity of mango pollen to existing high temperatures and low relative humidity. The

Table 2
ANOVA showing effect of four storage methods and six storage periods on *in vitro* germination and pollen viability as confirmed by fluorescein diacetate (FDA) and acetocarmine tests in three polliniser mango cultivars.

Effect	Numerator degree of freedom	Denominator degree of freedom	*F*-Value	Sig.
In vitro germination				
Cultivar (C)	2	4	20.16	0.0081
Storage method (SM)	3	6	1348.22	<0.0001
Days (D)	5	10	63.95	<0.0001
C × SM	6	12	4.41	0.0139
C × D	10	20	0.43	0.9141
SM × D	15	30	20.98	<0.0001
FDA test				
Cultivar (C)	2	4	0.25	0.7872
Storage method (SM)	3	6	2640.09	<0.0001
Days (D)	5	10	181.54	<0.0001
C × SM	6	12	1.22	0.3602
C × D	10	20	0.99	0.4804
SM × D	15	30	48.75	<0.0001
Acetocarmine test				
Cultivar (C)	2	4	1.61	0.3069
Storage method (SM)	3	6	2416.84	<0.0001
Days (D)	5	10	100.10	<0.0001
C × SM	6	12	2.02	0.1413
C × D	10	20	0.64	0.7663
SM × D	15	30	26.85	<0.0001

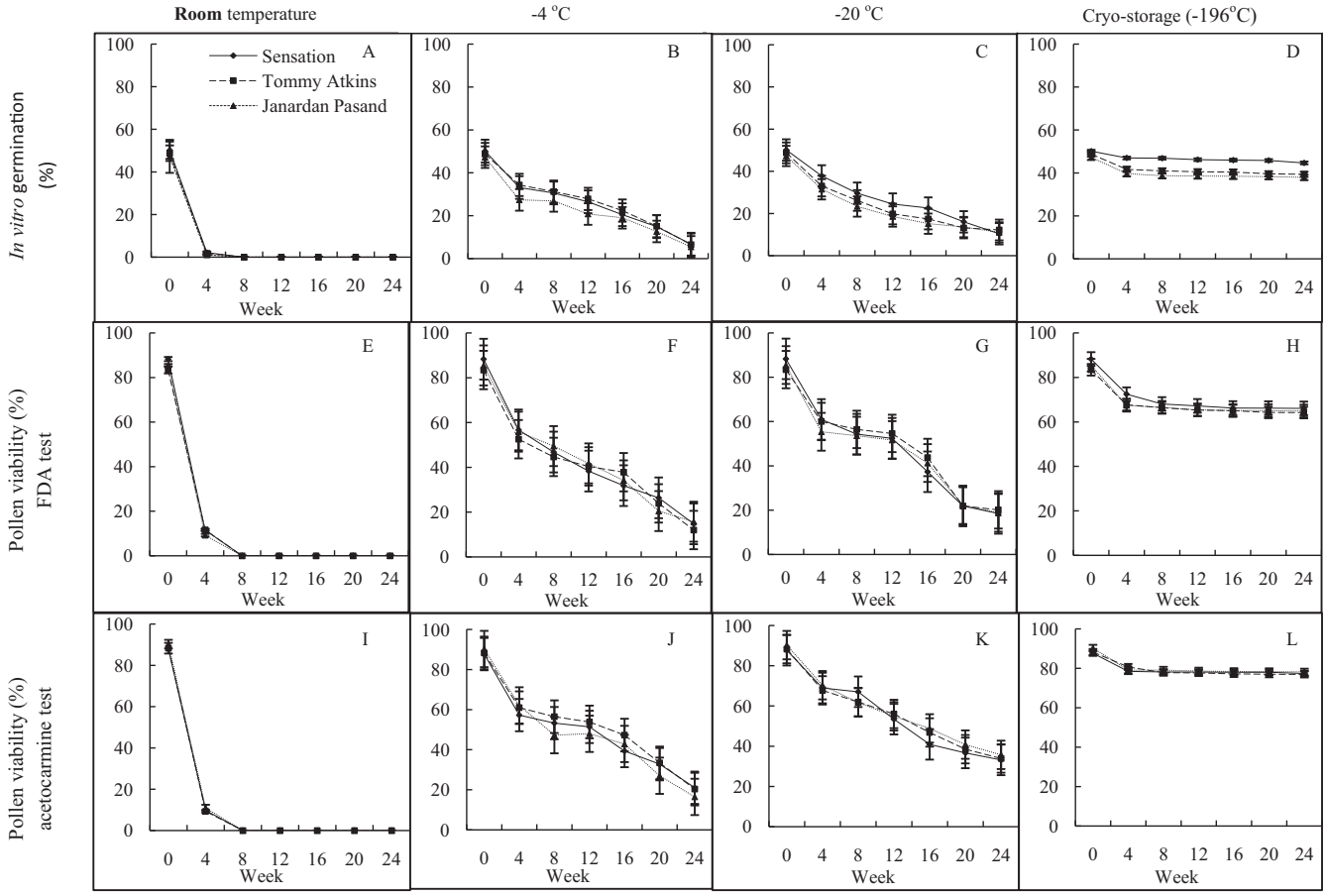

Fig. 1. (Panel A–L) Pollen viability of three polliniser mango cultivars stored at room temperature, −4 °C, −20 °C and −196 °C as evidenced by *in vitro* germination test (A–D), fluorescein diacetate test (E–H) and acetocarmine test (I–L). Vertical bars indicate ±standard error, $n = 5$.

differential rate of reduction in pollen viability stored at different temperature regimes may be because of rate of metabolic activities in pollen is temperature dependent. Mango pollen is known to be highly susceptible to desiccation and there is rapid moisture loss when maintained at a temperature above 25–27 °C. The metrological data observed during April month suggest that the average mean minimum temperature ranged between 17.6 and 22.3 °C while mean maximum temperature ranged between 37.7 and 42.2 °C further support the present findings. Earlier, Khan and Praveen (2009) while studying the germination capacity of three mango cultivars up to 48 weeks under different storage conditions, suggested that both freezer and freeze-dried conditions showed better results. In another comparative study between pollen viability and germination of 'Tommy Atkins', 'Kent' and 'Keitt' mango cultivars clearly indicated these parameters to be genotype dependent (Abourayya et al., 2011). The findings of Abourayya et al. (2011) were in agreement with those of previous findings of Singh (1954), Dahshan (1971), El-Kady (1973), Desai et al. (1986), El-Masry (2001) and Abd El-Hadi (2006) that pollen grain viability differed due to genotypes. Gehrke-Velez et al. (2011) reported that pollen showed acceptable viability (70–85%) and germination (14.5 and 1.75%) in hermaphrodite and male flowers. The results of present investigation are in agreement with the earlier reports of Chaudhury et al. (2010) on mango, and Vivian-Smith et al. (1992) in litchi and other species like cherimoya (Lora et al., 2006), *Zea mays* (Inagaki, 2000), and *Brassica campestris* (Mulcahy and Mulcahy, 1988).

The different pollen viability tests such as *in vitro* germination, FDA and acetocarmine revealed that significantly higher pollen viability was maintained due to storage at −196 °C followed by −20 °C and −4 °C irrespective of observation dates in all genotypes and the trend continued up to 24 weeks. Pollen longevity has been reported to be extended by using lower temperatures such as 5 °C, −20 °C, −80 °C and −196 °C and low moisture content (Towill and Walters, 2000). Pollen viabilities at 5 °C, −20 °C and −80 °C storage differed considerably among species and genotypes (Mishra and Shivanna, 1982). The pollen once cryo-stored at temperature below −160 °C would theoretically have infinite period of longevity (Stanwood, 1985). The differential results obtained by using different pollen viability assays confirmed that *in vitro* germination test was more reliable and accordingly the results obtained followed by FDA and acetocarmine tests could be overestimated. This might be attributed to the fact that these assays principally test the integrity of plasmalemma of the cell and subjected to various limitations. Helpson-Harrison et al. (1984) reported that a close correlation between FCR and germinability can only be expected when pollen is mature and medium is optimum. Similarly, they found that staining test is poorest guide to germinability. The tendency throughout is for an overestimation of the potential, false positive being given for immature, heat and DMSO treated pollens. Similar overestimation was observed with fresh mango pollen and after storage under various conditions (Chaudhury et al., 2010; Shivanna and Helpson-Harrison, 1981).

4. Conclusion

Present study revealed that at −196 °C cryo-storage of mango pollen could be best long term storage strategy for efficient conservation of genetic resources and for pollination both for

commercial fruit production and breeding. However, pollen can be efficiently stored for short periods at −20 and −4 °C temperatures for few weeks. In mango, blooming season start from February and lasts up to March under north Indian conditions, consequently, pollen collected and stored under different storage conditions in February and March can be used for pollinations throughout the blooming season. Low temperature storage of pollen of differentially blooming mango parents ensures availability of pollen during the same season and also in the succeeding seasons.

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References

Abd El-Hadi, S.M.K., 2006. Evaluation studies on some mango varieties. Fac. Agric., Al-Azhar Univ., Egypt, pp. 166 (M.Sc. Thesis).

Abourayya, M.S., Kassim, N.E., El-Sheikh, M.H., Rakha, A.M., 2011. Comparative study between inflorescence characteristics, pollen viability, germination and dimensions of Tommy Atkins, Kent and Keitt mango cultivars. Life Sci. J. 8 (1), 100–105.

Chaudhury, R., Malik, S.K., Rajan, S., 2010. An improved pollen collection and cryopreservation method for highly recalcitrant tropical fruit species of mango (*Mangifera indica* L.) and litchi (*Litchi chinensis* Sonn.). Cryo Lett. 31 (3), 268–278.

Dahshan, D.I.M., 1971. Studies concerning growth and fruiting in some mango varieties. Fac. Agric. Ain Shams Univ., Egypt, pp. 171 (M.Sc. Thesis).

Desai, A.G., Limaye, V.P., Gunjate, R.T., 1986. Floral biology of Alphanso, Goamankur and Kesar varieties of mango. J. Maharashtra Agric. Univ. 10 (2), 193–195.

El-Kady, M.I.E., 1973. Physiological studies on flowering and fruit set in some mango varieties especially in Taimour. Fac. Agric., Cairo Univ., Egypt, pp. 195 (M.Sc. Thesis).

El-Masry, S.M., 2001. Selection of some mango seedlings grown in Assiut. Fac. Agric., Assiut Univ., Egypt, pp. 195 (Ph.D. Thesis).

Gehrke-Velez, M.R., Castillo-Vera, A., Ruiz-Bello, C., Moreno-Martinez, J.L., 2011. Pollen viability and germination in mango (*Mangifera indica* L.) cv. Ataulfo. Interciencia 36 (5), 378–385.

Helpson-Harrison, J., Helpson-Harrison, Y., 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescien diacetate. Stain Technol. 45, 115–120.

Helpson-Harrison, J., Heslop-Harrison, Y., Shivanna, K.R., 1984. The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. Theor. Appl. Genet. 67, 367–375.

Inagaki, M., 2000. Use of stored pollen for wide crosses in wheat haploid production. In: Engelmann, F., Tagako, H. (Eds.), Cryopreservation of Tropical Plant Germplasm. Current Research Progress and Applications. International Plant Genetic Resources Institute, Rome, Italy, pp. 130–135.

Issarakraisila, M., Considine, J.A., 1994. Effects of temperature on microsporogenesis and pollen viability in mango cv. 'Kensington'. Ann. Bot. 72, 231–240.

Khan, S.A., Praveen, A., 2009. Pollen germination capacity of three mango cultivars (*Mangifera indica* L., Anacardiaceae) from Pakistan. Pak. J. Bot. 41 (3), 1009–1012.

Lora, J., Pe'Rez De Oteyza, M.A., Fuentetaja, P., Hormaza, J.I., 2006. Low temperature storage and *in vitro* germination of cherimoya (*Annona cherimola* Mill.) pollen. Sci. Hortic. 108, 91–94.

Martinez-Gomez, P., Gradziel, T.M., 2002. Short-term storage of almond pollen. HortScience 35 (6), 1151–1152.

Mishra, R., Shivanna, K.R., 1982. Efficacy of organic solvents for storing pollen grains of some leguminous taxa. Euphytica 31, 991–995.

Mulcahy, G.B., Mulcahy, D.L., 1988. The effect of supplemented media on the growth *in vitro* of bi- and trinucleate pollen. Plant Sci. 55, 213–216.

Nassar, N.M.A., Santos, E.D., David, S., 2000. The transference of apomixis genes from *Manihot neusana* Nassar to cassava, *M. eculenta* Crantz. Hereditas 132, 167–170.

Ram, S., Rajan, S., 2003. Status report on genetic resource of mango in Asia-pacific region. International Plant Genetic Resource Institute, Office for South Asia, Pusa Campus, New Delhi, India, pp. 196.

Shivanna, K.R., Helpson-Harrison, J., 1981. Membrane state and pollen viability. Ann. Bot. 47, 759–770.

Singh, R.N., 1954. Studies on floral biology and subsequent development of fruits in mango (*Mangifera indica* L.). Indian J. Hortic. 31, 16–22.

Stanley, R.G., Linskens, H.F., 1974. Pollen Biology, Biochemistry and Management. Springer-Verlag, Berlin, Germany.

Stanwood, P.C., 1985. Cryopreservation of seed germplasm for genetic conservation. In: Kartha, K.K. (Ed.), Cryopreservation of Plant Cells and Tissues. CRC Press, Boca Raton, FL, pp. 199–226.

Towill, L.E., Walters, C., 2000. Cryopreservation of pollen. In: Engelmann, F., Tagaki, H. (Eds.), Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Application. JIRCAS/IPGRI, Tsukuba, Japan, pp. 229, 115–129.

Vivian-Smith, A., Mcconchie, C.A., Batten, D.J., 1992. In vitro germination and growth of lychee (*Litchi chinensis* Sonn.). In: Control of fruit set and fruit retention in lychee. Rural Industries Research and Development Corporation Report, No. CS18A. CSIRO, Brisbane, Australia.