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Effect of storage temperature on pollen viability of moringa hybrids

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Abstract
Moringa oleifera Lam, belong to a monotypic genius of the family Moringaceae, consider as one of the most beneficial trees currently cultivating all over the tropics of the world due to the nutritional and medicinal value. The pollination behaviour of moringa is found to be highly cross pollinated and pollen is the basic source for gene transfer and recombination in the intraspecific hybrids of moringa. Besides, pollen viability is considered as an important factor for pollen germination and pollen tube penetration in to the sigma. The objective of the present experiment was formulated to compare the effect of different storage temperatures on the pollen viability of intraspecific moringa hybrids. This experiment was carried out with five intraspecific F_1: hybrids *viz*., H_1: (CBEMO-1 X CBEMO-15) (Annual X Annual), H_2: (CBEMO-1 X CBEMO-29), H_3: (CBEMO-4 X CBEMO-29), H_4: (CBEMO-2 X CBEMO-32), H_5: (CBEMO-4 X CBEMO-29) (Annual X Perennial). The Pollen were collected from the field at the time of anthesis and stored at different temperatures *viz*., 25 °C, 4 °C, -20 °C and -80 °C to assess the effect of temperature on pollen viability. The storage temperature significantly affects the pollen viability of the intraspecific hybrids. All the F_1 hybrids where, the pollen stored at the low temperature -80 °C at various times showed maximum pollen viability ranged from 83.60 to 94.80. Whereas, the minimum pollen viability at various times was recorded at room temperature 24 °C ranged from 54.20 to 86.20. While, the hybrid H_5: (CBE MO-14 X CBE MO-29) recorded maximum viability 94.80 at various time and the least viability was observed in (CBE MO-2X CBE MO-54.2). The result showed that pollen viability of intraspecific moringa hybrids increases with decreasing temperature with minimum time.

Keywords: Pollen viability, intraspecific hybrids, moisture content, enzymes, de-esterification, humidity

Introduction
Moringa oleifera Lam. is a multipurpose and exceptionally nutritious vegetable tree with a variety of potential uses. It was believed to be originated from the Sub-Himalayan tracts of India, many of the moringa species are known by several regional names (Fahey, 2005) [6]. The majority of Asia, nearly all parts of Africa, South America, Southern part of North America, and certain parts of Europe had been occupied by the moringa tree. In many nations, particularly in India, Pakistan, Philippines, Hawaii, and many regions of Africa, the leaves, fruit, blossoms, and immature pods of these tree are consumed as a highly nutritious vegetable. In moringa, anthesis time is influenced by the external environment, where the temperature of 27-29 °C with relative humidity 68-78 per cent is favourable for anthesis. The panicle consists of protandrous and herkogamous flowers, where the anthesis takes place at early morning in the range of 7.00h-13.00h and the anthers dehisce through the longitudinal slits opening which releases the spheroidal pollen grains (~35 µm). Each flower produces 23525 pollen grains in an average and per ovule 523 pollen grains are present. The style of the flower remains shorter than the anthers, after a period of 24 hrs the style of the flowers elongates beyond the length of the anthers and stigma becomes receptive takes place and receptivity continued for next 48 hrs. (Bhattacharya and Mandal, 2004) [2].

Pollination is considered as a basic source for gene transfer and recombination in the intraspecific moringa hybrids (Ashoke *et al*., 2017) [19]. Which is mainly depends on the pollen viability, only the mature viable pollen alone can germinate and penetrate in to the sigmatic surface, which allows the pollen tube to grow and fertilize the ovule and developed in to a seed. Hence the pollen viability is considered as an essential criteria for fertilization and seed development (Singh *et al*., 2013) [5]. The preservation of pollen is difficult since, the viability of the pollen is mainly depending on the moisture content of the pollen and it is mainly influenced by temperature and relative humidity. The percentage of pollen viability may also varies between species to species and within the cultivars.

Hence the present study was to compare the different storage temperature on the pollen viability of moringa and to standardised the best storage temperature of the pollen to assist the plant breeders to have more flexible crossing during off season moringa flowering to take up the crossing work.

Materials and Methods

The present experiment was conducted in Horticulture College and Research Institute, Orchard, Tamil Nadu Agricultural University, Coimbatore during 2021-20122. The experimental site consists of warm tropical climate with deep sand loamy soil located at a latitude 432.37 above MSL. A total of fifteen intraspecific F_1 hybrids were layout in an Augmented Block Design. Among those for this study only five intraspecific F_1 hybrids belong to Annual X Annual, Annual X Perennial were selected from the field based on their fruit set per cent.

Table 1: The details of the intraspecific F_1 hybrids selected for this study includes the following

H_1	CBEMO-1 X CBEMO-15	(Annual X Annual)
H_2	CBEMO-1 X CBEMO-29	(Annual X Perennial)
H_3	CBEMO-4 X CBEMO-29	(Annual X Perennial)
H_4	CBEMO-2 X CBEMO-32	(Annual X Perennial)
H_5	CBEMO-14 X CBEMO-29	(Annual X Perennial).

Pollen collection and storage

From the five intraspecific F_1 hybrids, the flowers were collected from the field at the time of anthesis and flowers were allowed to air dry for 30 minutes for the removal of moisture content in the flower. The pollens were gently collected from the flowers and the collected pollens were stored at different temperature *viz*., 25 °C, 4 °C, -20 °C and -80 °C against the various time interval such as, 24 hrs, 48 hrs, 72 hrs and 1 week.

Pollen viability test

At frequent time interval the pollen viability test was carried out with aceto-caramine dye with the following standard procedure given by McKellar and Quesenberry, 1992; Marutani, *et al.* 1993 [20, 9]. The number of viable pollen grain to the non-viable pollen grain are counted visually with the help of electron microscope and expressed as per centage of pollen viability. The data on pollen viability per centage were statistically analysed.

Acetocarmine stain preparation: 1 g of caramine powder was added to the 45% of glacial acetic acid, then the solution was cooled rapidly. After cooling, the solution was filtered and stored in a dark glass jar.

Procedure

The stored pollen samples at frequent interval, were taken on the grooved slide and stained with the aceto-caramine dye, allowed to rest for 5 min and observed under the light microscope to check the pollen viability. The viable pollen grain is stained with pink to deep crimson with help of aceto-carmine dye, while the non-viable pollen grain/sterile (mainly shrivelled) pollen gain remains unstained which appeared as white and transparent.

The percentage of the pollen viability was calculated using the formula given by (McKellar and Quesenberry, 1992; Marutani, *et al.*, 1993) [20, 9].

$$\text{Pollen viability (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains on slide}} \text{ X } 100$$

Results and Discussion

According to the ANOVA all the genotypes in relation to temperature treatment and time interval showed positive significant difference among themselves at $p<0.01$ level of significance. Where given in Table 2 and Fig 1.

The present study showed that pollen viability of intraspecific hybrids of moringa was significantly affected by temperature at various time interval and also significant difference existed in hybrids at all the temperature treatments including time intervals. It also showed that maximum viability was observed in minimum time interval with maximum temperature treatment. Many studies showed that pollen viability declined rapidly over time due to disarrangement of intercellular integrity, decreased activity of enzymes, accumulation of free radicals, de-esterification and lipid peroxidation leading to increases leakage of cellular component by Taylor and Hepler (1997) [15]. The pollen viability in apple cultivar, when stored at room temperature decreases over time than that stored at lower temperature and the present findings are in agreement with the results of Dusica Calic *et al.* 2021 [13]. Where, the time interval of 24 hrs all the interspecific hybrids showed maximum pollen viability of 81.20% to 95.10%, while the pollen stored for 1 week had the pollen viability of 54.20% to 83.60% irrespective of various temperature treatment. Among all the hybrids under this study the hybrid H_5: (CBEMO-14 X CBEMO-29) recorded maximum pollen viability of 59.20% to 95.10%. These may be due to the innate ability of the hybrid and also the nutritional status of the flowers, where the presence of components like proteins and starches may be related to the increased in the pollen viability of the hybrid. whereas, the hybrid H_4: (CBEMO-2 X CBEMO-32) observed minimum pollen viability of 54.20% to 90.30% irrespective of various temperature treatment and time interval. At a time interval of 24 hrs the hybrid H_5: (CBEMO-14 X CBEMO-29) recorded maximum pollen viability of 95.10% at -80 °C temperature followed by 94.20% at a temperature of -20 °C. Whereas, the hybrid H_4: (CBEMO-2 X CBEMO-32) observed minimum pollen viability of 81.20% at 24 °C temperature. This may be due to the high temperature and humidity, strong activity of respiration and metabolism, severity of water loss and rapid decline of vitality all these lead to poor pollen viability. This result is in accordance with the findings of Du *et al*., (2018) [22]. While, the time interval of 48 hrs the hybrid H_5: (CBEMO-14 X CBEMO-29) showed maximum pollen viability of 94.20% at -80 °C temperature followed by 92.70% at a temperature of -20 °C. Whereas, the hybrid H4- (CBEMO-2 X CBEMO-32) observed minimum pollen viability of 74.00% at 24 °C temperature. During 72 hrs time interval the hybrid H5- (CBEMO-14 X CBEMO-29) showed maximum pollen viability of 92.20% at -80 °C temperature followed by 91.00% at a temperature of -20 °C. While, the hybrid H_4: (CBEMO-2 X CBEMO-32) recorded minimum pollen viability of 67.20% at 24 °C temperature. The hybrid H_5: (CBEMO-14 X CBEMO-29) exhibited maximum pollen viability of 89.10% at -80 °C temperature followed by 87.30% at a temperature of -20 °C. Whereas, the hybrid H_4: (CBEMO-2 X CBEMO-32) recorded minimum pollen viability of 54.20% at 24 °C temperature treatment during 1 week time in.

Table 2: Effect of Temperature treatment and Time interval on the pollen viability of intraspecific F_1 hybrids of moringa

Time Interval (Hrs.)	Hybrids	Temperature treatments (°C)			
		24 °C	4 °C	-20 °C	-80 °C
24 Hrs.	H1	83.80	89.00	90.50	90.80
	H2	85.80	92.40	92.90	94.20
	H3	84.20	89.40	91.60	91.70
	H4	81.20	87.80	89.20	90.30
	H5	86.20	93.20	94.20	95.10
48 Hrs.	H1	75.20	86.70	88.20	90.15
	H2	77.40	88.50	91.20	93.80
	H3	76.30	87.30	88.60	90.70
	H4	74.01	84.20	88.01	89.80
	H5	78.60	89.40	92.70	94.20
72 Hrs.	H1	71.80	79.30	86.73	87.70
	H2	73.80	81.66	90.81	90.30
	H3	72.60	80.42	87.82	89.90
	H4	67.20	78.20	83.40	87.30
	H5	75.20	82.91	91.02	92.20
1 week	H1	55.60	74.20	79.20	84.80
	H2	58.90	75.60	85.70	88.80
	H3	56.80	74.90	81.90	86.60
	H4	54.20	73.50	76.90	83.60
	H5	59.20	79.20	87.30	89.10
	S.Ed	CD (0.01)			
Hybrids	0.086	0.23**			
Temperature	0.078	0.20**			
Time interval	0.079	0.21**			

Significant 0.01**

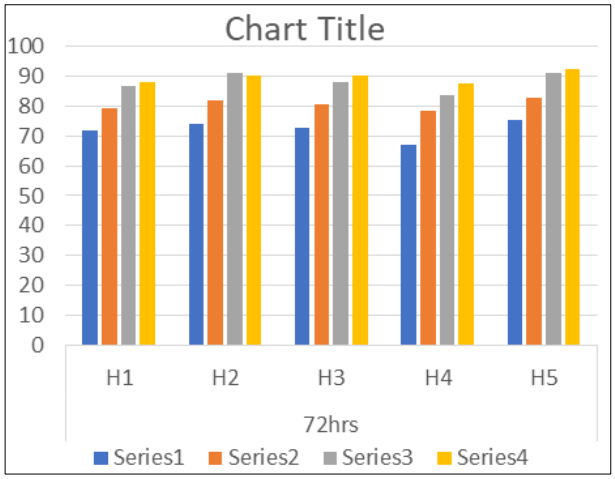


Fig A: Pollen viability of different hybrids at 24 Hrs.

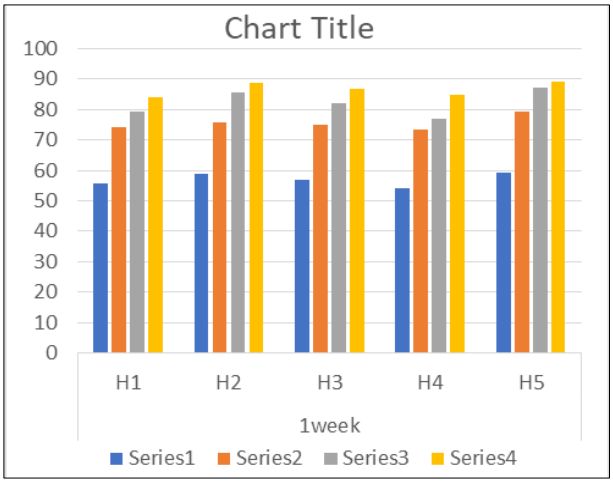
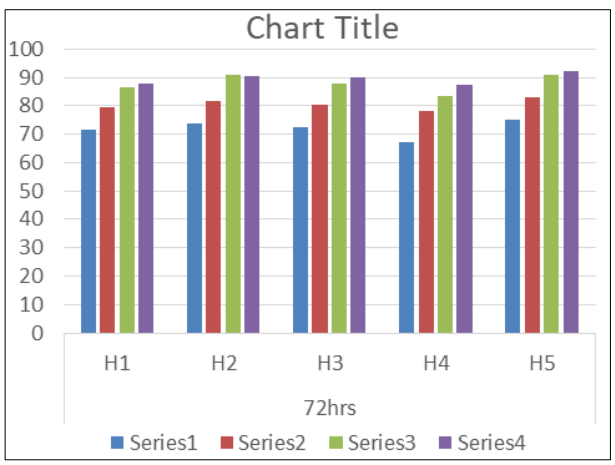


Fig B: Pollen viability of different hybrids at 48 Hrs.



C: Pollen viability of different hybrids at 72 hours

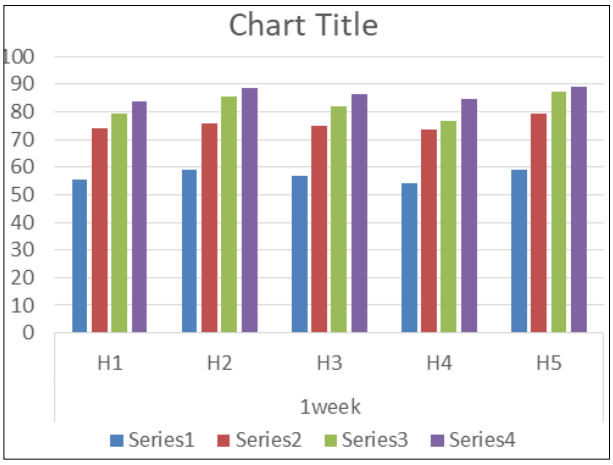


Fig D: Pollen viability of different hybrids at 1 week interval

Fig 1: Effect of Storage Temperature on Pollen Viability of Moringa Hybrids

Images showing pollen viability of H5-(CBEMO 14X CBEMO 29) at 1 week interval

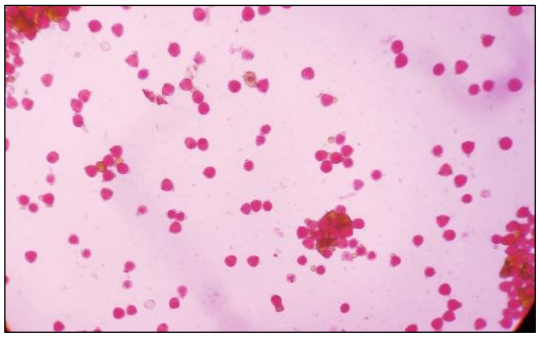


Fig A: 80 °C (89.10%)

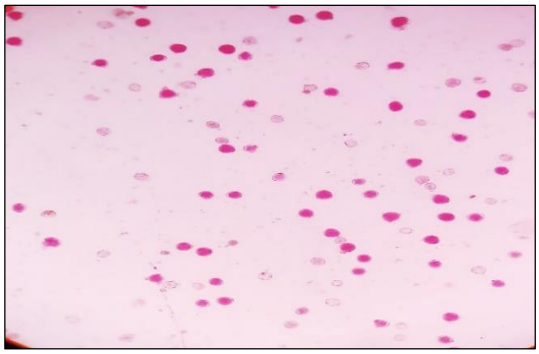


Fig B: Room Temp (59.20%)

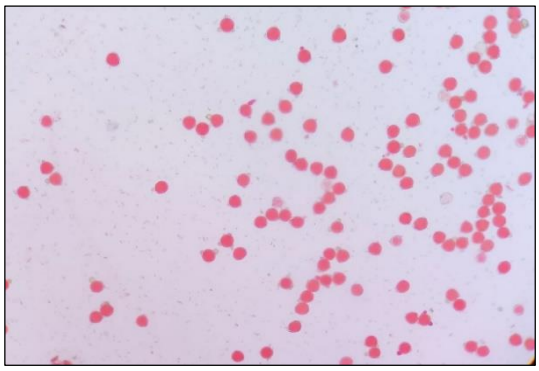


Fig C: 20 °C (87.30%)

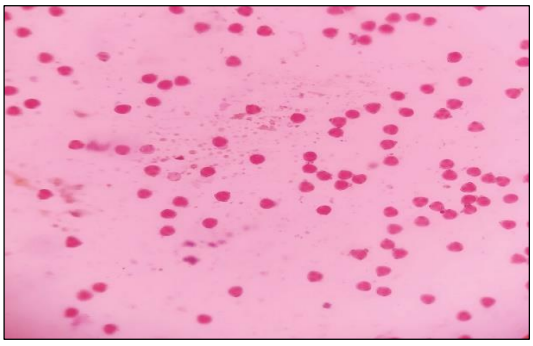


Fig D: 4 °C (79.20%)

Conclusion

The present study revealed the relation between the pollen viability of intraspecific moringa hybrids in relation to various temperature treatment under different time interval. Pollen viability was significantly affected by temperature with respect to time interval and also significant difference was existed in all the hybrids at various temperature treatments with respect to time intervals. Of which the maximum viability was observed in minimum time interval with minimum temperature treatment and pollen viability stored at room temperature decreases over time then that stored at lower temperature. Among all the hybrids under this study hybrid H_5 i.e. (CBEMO-14 X CBEMO-29) exhibited maximum pollen viability 95.10% at -80 °C temperature followed by 94.20% at -20 °C. Whereas, the hybrid H4-(CBEMO-2 X CBEMO-32) recorded minimum pollen viability of 54.20% at 24 °C temperature treatment during 1 week time interval.

References

1. Bhat ZA, Dhillon WS, Shafi RHS, Rather JA, Mir AH, Shafi W, *et al*. Influence of storage temperature on viability and *in vitro* germination capacity of pear (*Pyrus* spp.) pollen. Journal of Agricultural Science. 2012;4(11):128.
2. Bhattacharya A, Mandal S. Pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lamk. Grana. 2004;43(1):48-56.
3. Ćalić D, Devrnja N, Kostić I, Kostić M. Pollen morphology, viability and germination of *Prunus domestica* cv. Požegača. Scientia Horticulturae. 2013;155:118-122.
4. Du G, Xu J, Gao C, Lu J, Li Q, Du J, *et al*. Effect of low storage temperature on pollen viability of fifteen herbaceous peonies. Biotechnology Reports, 21, 2019.
5. Dutta SK, Srivastav M, Chaudhary R, Lal K, Patil P, Singh SK, *et al*. Low temperature storage of mango (*Mangifera indica* L.) pollen. Scientia Horticulturae. 2013;161:193-197.
6. Fahey JW. Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1. Trees for life Journal. 2005;1(5):1-15.
7. Khosh-Khui M, Bassiri A, Niknejad M. Effects of temperature and humidity on pollen viability of six rose species. Canadian Journal of Plant Science. 1976;56(3):517-523.
8. Kumari M, Prasad A, Ur Rahman L, Mathur AK, Mathur A. *In vitro* germination, storage and microscopic studies of pollen grains of four *Ocimum species*. Industrial Crops and Products. 2022;177:114-445.
9. Marutani M, Sheffer RD, Kamemoto H. Cytological analysis of Anthurium andraeanum (Araceae), its related taxa and their hybrids. American Journal of Botany. 1993;80(1):93-103.
10. Martinez-Gomez P, Gradziel TM, Ortega E, Dicenta F. Low temperature storage of almond pollen. Hort. Science. 2002;37(4):691-692.
11. Mesnoua M, Roumani M, Salem A. The effect of pollen storage temperatures on pollen viability, fruit set and fruit quality of six date palm cultivars. Scientia Horticulturae. 2018;236:279-283.
12. Rathod V, Behera TK, Munshi AD, Durgesh K, Jat GS, Krishnan BG, *et al*. Pollen viability and *in vitro* pollen germination studies in *Momordica species* and their intra and intraspecific hybrids. International journal of chemical studies. 2018;6(6):32-40.
13. Rhee HK, Lim JH, Cho HR, Van Tuyl JM. The relationship of storage and viability of lily pollen. Acta

Horticulturae. 2003;620:319-324.
14. Silva N, Mendes-Bonato AB, Sales JGC, Pagliarini MS. Meiotic behavior and pollen viability in *Moringa oleifera* (Moringaceae) cultivated in southern Brazil. Genetics and Molecular Research. 2011;10(3):1728-1732.
15. Taylor LP, Hepler PK. Pollen germination and tube growth. Annual review of plant biology. 1997;48(1):461-491.
16. Thiberge C. Flower structure, Ontogeny and Pollen viability of *Moringa oleifera* Lam (Doctoral dissertation, Università Degli Studi Di Firenze); c2014.
17. Vishwakarma PK, Vincent L, Vasugi C, Rajasekharan PE. Effect of cryopreservation on pollen viability, fertility and morphology of different *Psidium species*. Cryobiology. 2021;98:112-118.
18. Zlesak DC, Zuzek K, Hokanson SC. Rose pollen viability over time at varying storage temperatures. In IV International Symposium on Rose Research and Cultivation. 2005 Sep;751:337-343.
19. Ashoke B. Effect of flower density on pollen viability and fruit set of Calceolaria tripartita Ruiz & Pav. (Scrophulariaceae) in Darjeeling Himalaya. Trends in Biosciences. 2017;10(31):6572-7.
20. McKellar MA, Quesenberry KH. Chromosome-Pairing and Pollen Viability in *Desmodium ovalifolium* Wall× *Desmodium heterocarpon* (L) DC. Hybrids. Australian journal of botany. 1992;40(2):243-7.
21. Ćalić D, Milojević J, Belić M, Miletić R, Zdravković-Korać S. Impact of Storage Temperature on Pollen Viability and Germinability of Four Serbian Autochthon Apple Cultivars. Front. Plant Sci. 2021;12:709231. Doi: 10.3389/fpls.2021.709231
22. Du D, Wang-Kan X, Neuberger A, Van Veen HW, Pos KM, Piddock LJ, *et al*. Multidrug efflux pumps: structure, function and regulation. Nature Reviews Microbiology. 2018 Sep;16(9):523-39.