www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 2408-2413

www.thepharmajournal.com
Received: 16-06-2023
Accepted: 20-07-2023

Manju A
Ph.D. Scholar, Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Kerala Agricultural University, KAU Post, Thrissur, India

Sindhumole P
Assistant Professor (Plant Breeding and Genetics) and Head, AICRP on MAP & B, KAU-Thrissur, Kerala Agricultural University, Thrissur, India

Jiji Joseph
Professor and Head, Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, India

Pradheep K
Principal Scientist, ICAR-NBPGR Regional Station, Vellanikkara, Thrissur, Kerala, India.

Sangeeta Kutty M
Assistant Professor, Department of Vegetable Science, College of Agriculture, Vellanikkara, Thrissur, Kerala, India

Seeja Thomachan Panjikkaran
Assistant Professor and Head, Department of Community Science, College of Agriculture, Vellanikkara Kerala Agricultural University, Thrissur, Kerala, India

Corresponding Author:
Manju A
Ph.D. Scholar, Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Kerala Agricultural University, KAU Post, Thrissur, India

Reproductive biology of two *Moringa oleifera* L. varieties PKM1 and Jaffna under Kerala condition

Manju A, Sindhumole P, Jiji Joseph, Pradheep K, Sangeeta Kutty M and Seeja Thomachan Panjikkaran

Abstract
This study was conducted at College of Agriculture, Vellanikkara, Kerala Agricultural University, during 2022-23 to critically analyse the pollen morphology, viability, germination and stigma receptivity of *M. oleifera*, on each day of flower anthesis, specifically in PKM1 and Jaffna varieties for maximum reproductive success and genetic improvement. Flower anthesis in both varieties follows a forenoon pattern, with PKM1 from 7:00 am to 9:00 am and Jaffna from 6:30 am to 7:30 am. The impact of sucrose, sucrose + boric acid, potassium nitrate, calcium nitrate, and magnesium sulphate on freshly dehisced pollen germination resulted 96.67% in 10% sucrose + 150 ppm boric acid solution, with a mean pollen tube length of 1006 µm. Artificial *in-vivo* pollination on stigmas at each stage of flower opening using freshly dehisced pollen grains yielded the highest success rates on the 2nd and 3rd days (45%) in PKM1 and on the 2nd day (43%) in Jaffna suggested increased receptivity of the stigma during these specific days.

Keywords: Drumstick, viability, germination, stigma receptivity, anthesis

Introduction
Moringa oleifera, commonly known as drumstick or horseradish tree, originated in African countries and typically thrives in semi-dry, desert, or tropical soils. However, it has been successfully cultivated all around the world. This multi-purpose plant is highly valued for its medicinal and nutritional properties, with its leaves containing an impressive array of nutrients (Moyo *et al.*, 2011; Patil *et al.*, 2022; Shivangini *et al.*, 2022) [16, 19, 23]. The fresh leaves and dried leaf powder are abundant in vitamin A (beta carotene), calcium, iron, vitamin C, protein, potassium *etc*. (Fuglie, 1999; Prabhakar and Hebbar, 2008) [7, 21]. Due to its ability to thrive in dry periods and poor soils, this fast-growing species has high potential for cultivation worldwide (Morton, 1991) [15] and is regarded as one of the most important and beneficial trees (Anwar *et al.*, 2007) [1].

The pollination mechanism plays a crucial role in gene recombination in angiosperms. Successful pollination relies on key factors such as pollen viability, germination, and stigma receptivity, which must be thoroughly assessed on a species-specific basis. Since the drumstick plant is primarily valued for its tender fruits, quality fruit production in large scale is very important. Moreover, drumstick can propagate through both seeds and cuttings, there are reports that seed-derived progeny may yield inferior quality (Fotouo-M *et al.*, 2015) [26]. Therefore, gaining a detailed understanding of pollen viability, germination and stigma receptivity in *M. oleifera* is essential for producing genetically superior stocks.

This research examines the reproductive biology of drumstick with respect to the pollen behaviour and stigma receptivity on each day of flower opening specifically in PKM1 and Jaffna varieties. PKM1 and Jaffna are high yielding drumstick varieties suitable for Kerala condition. However, the yield potential is slightly lower here than the nearby dry regions. Therefore, in order to manage their plant breeding system a through comparision over these Informations on each day of flower opening is crucial for each cultivars, that will allow maximum reproductive success and genetic improvement of *Moringa oleifera*. Furthermore, such a comparision under Kerala condition among moringa cultivars for their reproductive success has barely been tested.

Materials and Methods

Drumstick flowers exhibit a prolonged blooming period, typically lasting four to five days. To document the flower opening process, we followed the methodology described by Mathur and Mohan Ram in 1986 [14]. Specifically, flower buds on the day prior to anthesis, as well as flowers on the first, second, third, and fourth day of anthesis, were collected from both PKM1 and Jaffna varieties in the field, typically between 8:00 and 8:30 am. These collected samples were allowed to air dry for 30 minutes to remove excess moisture. The study examined both the number of pollen grains per anther and pollen morphology in two flower varieties. Anthers were collected from freshly opened flowers on the first day. The quantification of pollen grains was done followed by the procedure of Mandal and Chanda (1981) [11]. Ten mature anthers from different flowers were placed on glass slides, crushed with 10% glycerine, and observed under a light microscope for pollen counting. The mean pollen grains per flower were calculated by averaging the counts from these anthers and multiplying by the total number of anthers. To study pollen morphology, freshly released pollen grains were collected on glass slides using a brush.

The study involved assessing the viability and germination of pollen grains obtained from fresh anthers of mature flower buds and flowers at various stages of anthesis. To determine pollen viability, collected pollen grains were stained with 1% acetocarmine due on a clean slide. After 10-minute incubation, a coverslip was applied, and the slides were examined under a Leica MZ75 microscope at 40x magnification. Pollen grains that exhibited full staining were considered fertile, while those that did not were considered sterile. This evaluation was conducted across five fields, using three different slides prepared from flowers at different days of opening. The percentage of viable pollen was calculated by observing 100 pollen grains from each field, following the method outlined by Manju and Raji (2018) [26]. To assess the impact of various chemical substances on pollen germination, freshly collected pollen from freshly opened flowers on the first day was exposed to sucrose, sucrose with boric acid (H_3BO_3), potassium nitrate (KNO_3), calcium nitrate $Ca(NO_3)_2$, and magnesium sulphate ($MgSO_4$). After storing the pollen at room temperature with natural light for 30 hours, slides were prepared with a drop of each medium containing pollen grains.

Pollen germination was evaluated by counting germinated pollen grains in five different fields for each treatment, and the germination percentage was determined. The medium with the highest germination rate was chosen for further investigation of pollen germination. Pollen was considered viable when the pollen tube length equal or exceeded the diameter of individual pollen grains, following the criteria established by Muhl *et al.* in 2013 [17]. Viability and germination of pollen grains from drumstick flowers at various stages of anthesis were observed using the most effective pollen germinating medium determined in the prior experiment. Additionally, the appearance of the stigma surface and its receptivity on each opening day was examined. To further investigate stigma receptivity, artificial pollination was conducted by applying freshly dehisced pollen grains to the stigmas of flowers at different stages of development. The results were collected and organized according to the methodology outlined in Shivanna and Rangaswamy (1993) [25].

Results and Discussion

In Kerala condition two flowering peaks are observed in drumstick trees and the flowers opened for four days (Fig.1A). *Moringa oleifera* was found to feature zygomorphic hermaphroditic flowers with a distinctive horizontal orientation, comprising five sepals, five petals, and five unequally sized stamens encircling a singular pistil. Remarkably, the posterior petal maintained an upright position, while the remaining petals and sepals reflexed, with the intermediate and front petals exhibiting an elegant forward curvature adorned with short trichomes on their ventral surfaces. The flowers follow a sequential pattern of opening, anther dehiscence, and stigma receptivity during anthesis. Notably, the anthers release pollen through longitudinal slits while the style maintains a parallel position with the anthers. Over time, the stigma gradually extended and, intriguingly, reached a position above the anther level on the same day (Fig.1C). These findings diverge slightly from the observations made by Jyothi *et al.* (1990) [9], who reported that the stigma was shorter than the stamens at anthesis, with the style elongating beyond the length of the anthers after 24 hours. Similar floral structure and phenological patterns were also documented by Decraene *et al.* (1998) [5] in *Moringa oleifera*. As the flower undergoes development, the color of the anthers transitions from dark yellow to light yellow and eventually to brown. The pistil exhibits a coronate structure with a smooth cell composition, serving as a seamless continuation of the style. The spatial separation of stamen and pistil shortly after anthesis implies a reduced chance of self-pollination.

Table 1: Floral characteristics of PKM1 and Jaffna varieties of *Moringa oleifera*

Floral characteristics	PKM1	Jaffna
Flowering Period	November – January April – June	October – January March - June
Flower Colour	Creamy white with greenish base	White with greenish base
Odour	Present	Present
Nectar	Present	Present
Anthesis time	7.00 am - 9.00 am	6.30 am - 7.30 am
Anther degiscence time	7. 30 am - 10.00 am	7.00 am - 9.00 am
Mean No. of anthers per flower	5	5
Range of anther filament length	3.00-6.2 mm	4.53-7.12 mm
Mean length of anther	2 mm	2.8 mm
Mean width of anther	1.12 mm	1.64 mm
Mean No. of pollens per anther	4586	6452
Mean No. of pollens per flowers	22930	32260
Mean No. of pollens per ovule	432	548
Pollen size Polar axis x equatorial axis (p x e)	38.67 x 34.50 µm	33.44 x 34.34 µm
p/e value	1.09	1.15
Pollen shape	Prolate spheroidal	Subprolate
Pollen type	Tricolporate	Tricolporate
Mean pore size	8.32 µ m	8.55 µ m
Mean exine thickness	2.02µ m	1.82 µ m
Stigma type	wet type Above anther level	wet type Above anther level
Mean length of stigma	8.32 mm	9.25 mm

The floral characteristics between the PKM1 and Jaffna varieties of drumstick exhibit notable differences, as detailed in Table 1. Specifically, the Jaffna variety stands out with larger floral parts and a higher quantity of pollen grains compared to PKM1 (Fig. 1B). Flower anthesis in both varieties follows a morning pattern, with PKM1 blooming from 7:00 am to 9:00 am, and Jaffna starting slightly earlier, from 6:30 am to 7:30 am. Subsequently, anther dehiscence and nectar secretion occur (Table 1). During this phase, the anthers promptly burst, tightly holding a bundle of pollen grains, which gradually desiccate before being released. Simultaneously, the nectar glands begin to secrete, appearing shiny. Similar observations regarding the anthesis of drumstick flowers were reported by Radice and Giordani (2018) [22].

Assessing individual flower potential within an inflorescence relies on counting total pollen grains per flower (Mandal & Ray, 1984) [12]. However, determining absolute pollen production remains challenging, as highlighted by prior research (Mandal & Chanda, 1981; Bhattacharya and Mandal, 1999) [11, 3]. Nevertheless, a correlation exists between pollen quantity per flower and per ovule, serving as a fertility indicator. The average pollen count falls between 22930 and 32260, with tricolporate pollen grains featuring three germinal furrows and germpores. These grains are typically globular with a psilate sculpturing, a common characteristic in dicotyledons. Pollen shape varies from prolate spheroidal (PKM1) to subprolate (Jaffna), as reported by Silva *et al.* (2011) [18], depending on the polar and equatorial axis ratio. PKM1 displays a prolate-spheroidal shape, while Jaffna exhibits a subprolate form (Fig. 1D).

The results of the *in vitro* pollen germination study, aimed at evaluating the impact of sucrose, sucrose + H_3BO_3, potassium nitrate, calcium nitrate, and magnesium sulphate, were presented in Table 2. A higher rate of active pollen tube growth was observed up to 9 hours after inoculation. In sucrose solution, maximum germinating pollen grains were observed at 83.67% in 10% solution, with a mean pollen tube length of 665 µm. The effect of sucrose indicated a direct correlation between pollen germination and sucrose concentrations up to 10%, beyond which the germination percentage declined. The optimal pollen germination (96.67%) and mean pollen tube length (1006 µm) occurred in 10% sucrose supplemented with 150 ppm boric acid solution.

Various concentrations (25-150 ppm) of potassium nitrate, calcium nitrate, and magnesium sulfate salts exhibited poor germination ability compared to the combined effect of sucrose and boric acid. The most favorable results were obtained at 75 ppm potassium nitrate with a germination rate of 59% and a mean pollen tube length of 321 µm, at 50 ppm calcium nitrate with a germination rate of 65% and a mean pollen tube length of 457 µm, and at 50 ppm magnesium sulfate with a germination rate of 41.67% and a mean pollen tube length of 478 µm.

Table 2: Effect of different concentrations of sucrose, 10% sucrose + boric acid, potassium nitrate, calcium nitrate and magnesium sulphate on germination of pollen-grains.

Effect of different concentrations of sucrose			Effect of 10% sucrose + Effect of different concentrations H_3BO_3			Effect of different concentrations of Potassium nitrate			Effect of different concentrations of Calcium nitrate			Effect of different concentrations of magnesium sulphate		
Conc. (%)	**Pollen germination (%)**	**Pollen tube length (µm)**	**Conc. (ppm)**	**Pollen germination (%)**	**Pollen tube length (µm)**	**Conc. (ppm)**	**Pollen germination (%)**	**Pollen tube length (µm)**	**Conc. (ppm)**	**Pollen germination (%)**	**Pollen tube length (µm)**	**Conc. (ppm)**	**Pollen germination (%)**	**Pollen tube length (µm)**
Distilled water	9	152	50	88.33	845	25	37	258	25	53	453	25	29.67	326
5%	79.67	520	100	92	911	50	53	300	50	65	457	50	41.67	478
10%	83.67	665	150	96.67	1006	75	59	321	75	46.33	424	75	34.33	453
15%	63	298	200	71	734	100	55	202	100	25	328	100	25	376
20%	43	216	250	53.33	356	150	13.33	98	150	15.67	254	150	9	103

In a study by Bhattacharya and Mandal (2004) [4], it was found that the optimal medium for pollen viability testing via *in vitro* pollen germination was 10% sucrose supplemented with 200 ppm H_3BO_3. Similar to the present study, their research highlighted the superior impact of sucrose or sucrose combined with boric acid compared to Ca $(NO3)_2$ and $MgSO_4$. Notably, they did not observe any pollen germination in mediums containing potassium nitrate.

The pollen viability, *invitro* pollen germination and success of artificial pollination on pre-anthesis to 4th day of flower opening was noted in the Figure 2. In the PKM1 and Jaffna varieties, the highest count of viable pollen grains was observed on the previous day and the first day of anthesis, ranging from 97% to 99% (Fig.1E). However, the highest germination rates were recorded exclusively among freshly dehisced pollen grains on the first day for both PKM1 (95%) and Jaffna (92%). Viability and germinability experience a gradual decline over time, ultimately dropping to below 10% by the fourth day following flower anthesis (Fig. 1F, 1G & 1J). This trend was similarly observed by Ponnuswami (2012) [20] in *Moringa oleifera*, where the highest pollen viability of 72% was noted at the time of anther dehiscence.

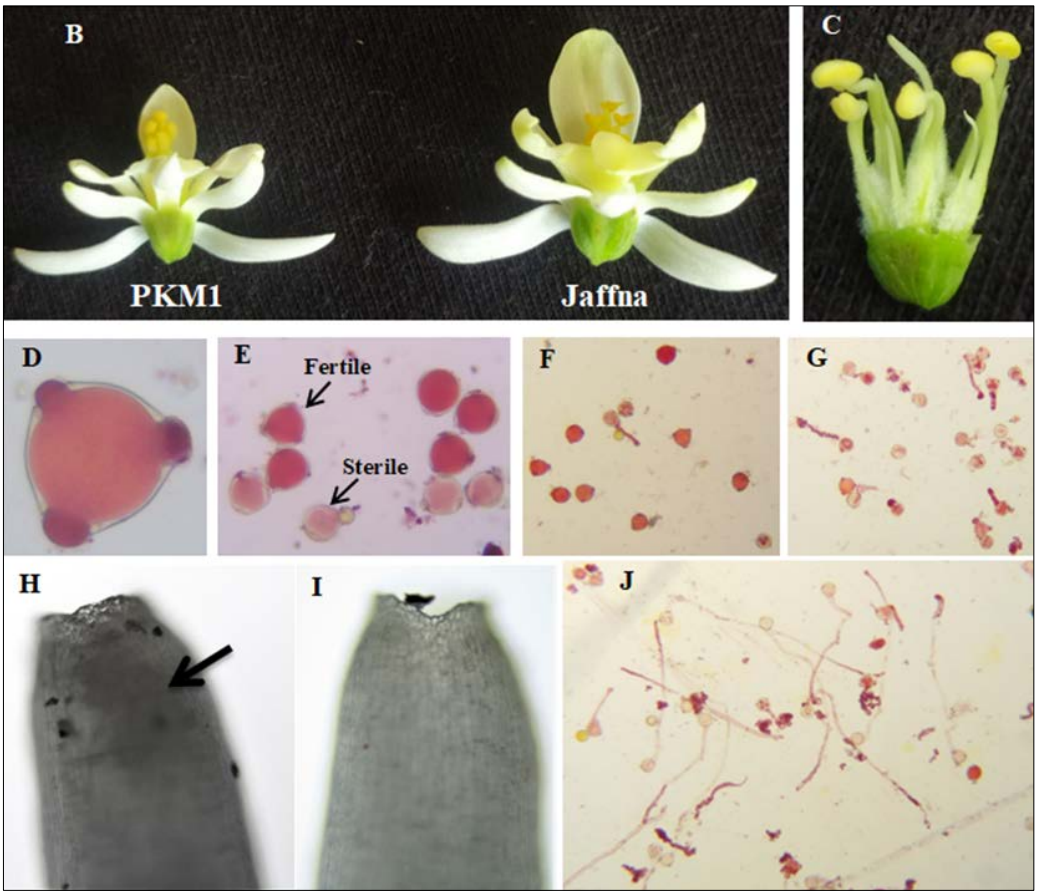
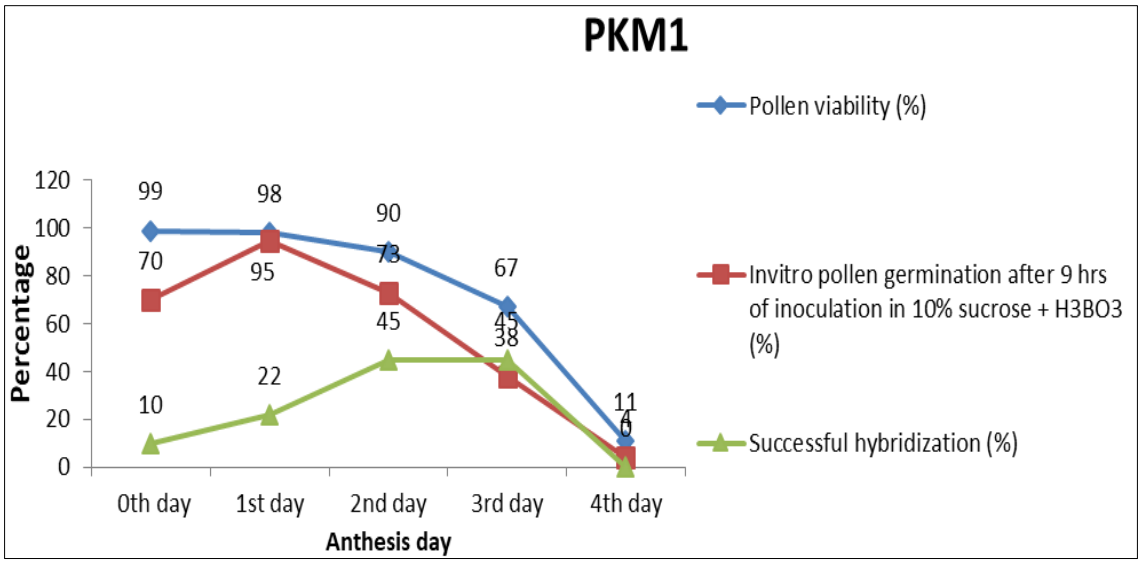


Fig 1: A) Flowers of *Moringa oleifera* on four consecutive days of anthesis B) Flower of PKM1 and Jaffna on first day of anthesis C) Stamens and style at the time of anthesis D) pollen morphology E) Viability of pollengrain F) *In-vitro* pollen germination on previous day of anthesis G) *In-vitro* pollen germination on 3rd day of anthesis H) Receptive stigma on 2nd day of anthesis, showing esterase accumulation just below the stigma head I) Non receptive stigma on 1st day of anthesis J) Pollen germination on 1st day of anthesis

Artificial *in-vivo* pollination was performed on stigmas at each stage of flower opening using freshly dehisced pollen grains known for their high viability and germinability. This approach yielded the highest success rates on the 2nd and 3rd days (45%) for PKM1, and on the 2nd day (43%) for Jaffna.

This trend suggests increased receptivity of the stigma during these specific days. A similar observation was made by Bhattacharya and Mandal (2004) [4] regarding delayed stigma receptivity in *Moringa oleifera*, specifically occurring on the third day of anthesis.



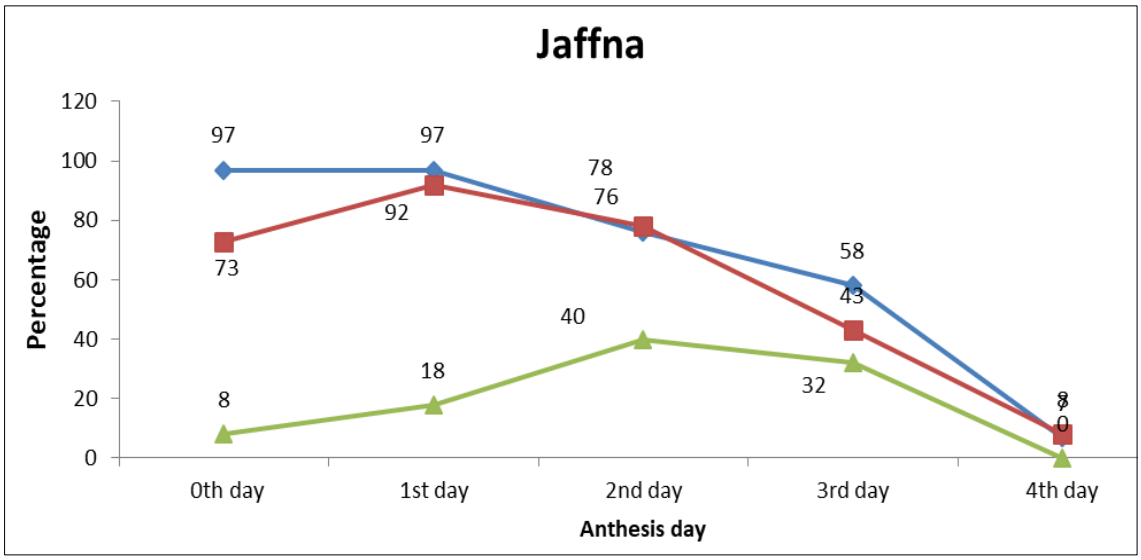


Fig 2: Pollen viability, germinability and successful hybridisation on different days of anthesis in PKM1 and Jaffna

In most plants, stigma receptivity coincides with anthesis, occurring simultaneously in flowers that bloom for a single day and extending throughout the entire day of anthesis in flowers that remain open for multiple days (Shivanna & Johri 1989; Manju, 2018) [24, 13]. However, the duration of stigma receptivity may vary between species (Joshirao & Saoji, 1989) [8]. Despite the peak of stigma receptivity being identified on the 2nd day in this study, it was extended to the 3rd day in PKM1 compared to Jaffna (Fig. 2). This variation indicates potential differences in the duration of stigma receptivity among species. As a sign of stigma receptivity, the deposition of a dense substance was observed near the stigma head during these anthesis days (Fig. 1H & 1I). This observation strongly aligns with the findings of Bhattacharya and Mandal (2017) [2], which noted the presence of dense esterase accumulation just beneath the stigma head and observed reaction products over the stigma surface in *Moringa oleifera*. Similar to this, Krieg *et al.*, (2017) [10] reported that when the stigma elongates over the stamens on second day of anthesis, it become receptive and the receptivity continued for next 48 hr. In contrast to these observations, Ponnuswami (2012) [20] reported that in *Moringa oleifera*, the stigma becomes receptive a day prior to anthesis and retains its receptivity on the day of anthesis. The differing receptive period of the stigma and the timing of anther dehiscence point towards the adaptation of the crop to cross-pollin

Conclusion

The critical analysis of pollen characteristics in PKM1 and Jaffna drumstick varieties under Kerala condition revealed slight variations in flower anthesis and morphology. Testing various substances, 10% sucrose + 150 ppm boric acid resulted in 96.67% pollen germination with a 1006 µm tube length. *In-vivo* pollination found the highest success rates on specific days (45% on 2nd and 3rd for PKM1, 43% on 2nd for Jaffna). Future research should aim to synchronize plant reproductive maturity through genetic, molecular, and physiological means, including genetic modification and practical field trials, considering sustainability and long-term impacts for crop yield and resilience in changing environments.

Conflict of Interest

The authors hereby declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors hereby acknowledge the financial assistance and laboratory facilities provided by Kerala agricultural University, Thrissur, Kerala, India for the smooth conduct of the experiment.

References

1. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytotherapy Research. 2007;21:17-25.
2. Bhattacharya A, Mandal S. Studies on *in vivo* pollen germination of some angiospermic plants. J Palynol. 2017;33:153-156.
3. Bhattacharya A, Mandal S. *In vitro* pollen germination in *Kleinhoevia hospita* Linn. – Science and Cult. 1999;65:327-328.
4. Bhattacharya A, Mandal S. Pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lamk. Grana. 2004;43(1):48-56.
5. Decraene LR, De Laet J, Smets EF. Floral development and anatomy of *Moringa oleifera* (Moringaceae): What is the evidence for a capparalean or sapindalean affinity? Ann. Bot. 1998;82:273-284.
6. Fotouo-MH, Du-Toit E S, Robbertse P J Germination and ultrastructural studies of seeds produced by a fast-growing, drought-resistant tree: Implications for its domestication and seed storage. Plants. 2015;7:1-5.
7. Fuglie LJ The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar; c1999. p. 68.
8. Joshirao JA, Saoji AA. Studies on *in vivo* germination of pollen of some alkaloid bearing plants, J Palynol. India.1989;25:45-50.
9. Jyothi PV, Atluri JB, Reddi CS. Pollination ecology of *Moringa oleifera* (Moringaceae). Proc. Plant Sci. 1990;100:33-42.
10. Krieg J, Goetze D, Porembski S, Arnold P, Linsenmair

KE, Stein K. Floral and reproductive biology of *Moringa oleifera* (Moringaceae) in Burkina Faso, West Africa. Acta Hortic. 2017;1158:63-69.
11. Mandal S, Chanda S. Aero-allergens of West Bengal in the context of environmental pollution and respiratory allergy. Biol. Mem. 1981;6:1-61.
12. Mandal S, Ray SK. Pollen production in weeds associated with some rice cultivars in Burdwan district, West Bengal, India. Geohydrology. 1984;14:4-81.
13. Manju A. Characterization and hybridization of *Nymphaea* spp. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur; c2018. p. 120.
14. Mathur G, Mohan RHY. Floral biology and pollination of *Lantana camara*. Phytomorphology 1986;36:79-100.
15. Morton JF. The horseradish tree, *Moringa pterygosperma* (*Moringaceae*) – a boon to arid lands? African journal of Biotechnololy. 1991;10:1295–1298.
16. Moyo B, Masika PJ, Hugo, A, Muchenje V. Nutritional characterization of Moringa (*Moringa oleifera*). African Journal of Biotechnology. 2011;10(60):12925-12933.
17. Muhl Q E, Elsa S, Du Toit, Joachim M, Steyn A. Bud development, flowering and fruit set of *Moringa oleifera* Lam. (Horseradish Tree) as affected by various irrigation levels. Journal of Agriculture and Rural Development in the Tropics and Subtropics. 2013;114(2):79-87.
18. Silva N, Mendes-Bonato AB, Sales JGC, Pagliarini MS. Meiosis and pollen viability in *Moringa oleifera*. 2011;10(3):1728-1732.
19. Patil SV, Mohite BV, Marathe KR, Salunkhe NS, Marathe V. Moringa tree, gift of nature: A review on nutritional and industrial potential. Current Pharmacology Reports. 2022;8(4):262-280.
20. Ponnuswami V. Advances in Production of Moringa. All India Co-ordinated Research Project-Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam; c2012. p. 604.
21. Prabhakar M, Hebbar SS. Underutilized and Underexploited Horticultural Crops. New India Publishing Agency, New Delhi; c2008. p. 430.
22. Radice S, Giordani E. Flower development and pollen vitality of *Moringa oleifera* Lam. grown in a humid temperate climatic condition. Advances in Horticultural Science. 2018;32(4):549.
23. Shivangini P, Mona K, Nisha P. Comprehensive Review: Miracle Tree Moringa oleifera Lam. Current Nutrition & Food Science. 2022;18(2):166-180.
24. Shivanna KR, Johri BM. The angiosperm pollen structure and function – Wiley Eastern Ltd., New Delhi; c1989. p. 340.
25. Shivanna KR, Rangaswamy NS. Pollen Biology – A laboratory manual – Narosa Publ. House, New Delhi; c1993. p. 278.
26. Manju A, Raji N. Pollen Morphology and Viability of Native *Nymphaea* Genotypes in Kerala. Research Journal of Agricultural Sciences. 2018;9(6):1190-1194.