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Scanning electron microscopy analysis of pollen grains of *Hibiscus rosa-sinensis* cultivars 'Playboy' and 'Gelia Castillo'

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Abstract

Pollen morphology of *Hibiscus rosa sinensis* cultivars 'Playboy' and 'Gelia Castillo' were studied under light and scanning electron microscope. The two cultivars exhibited spheroidal pollen with pantoporate aperture and spiny surfaces under a Scanning Electron Microscope (SEM). The pollen diameter of the cv 'Playboy' and cv 'Gelia Castillo' were 116.0 µm and 129.6 µm, respectively. The pollen apertures of both the cultivars were similar with round shape but in the cv. 'Gelia Castillo', the aperture was partially covered with an exine layer. The cv 'Gelia Castillo' exhibited a pollen fertility of 93.84%, whereas the cv 'Playboy' exhibited a pollen fertility of 88.57%. This study offers important information regarding the pollen traits of *H. rosa sinensis* cultivars 'Playboy' and 'Gelia Castillo'. Due to their attractive floral characters and high pollen fertility, both cultivars can be utilized as good male parents in crop improvement programs for breeding novel hibiscus varieties.

Key words: Hibiscus, SEM, pollen grains, pollen morphology, exine

Introduction

Hibiscus belongs to a large genus of flowering plants under the family Malvaceae which include approximately 275 species including several species like *H. rosa-sinensis, H. syriacus, H. mutabilis, H. moscheutos, H. sabdariffa, H. cannabinus* and *H. trionum*, mainly distributed in tropical and sub-tropical parts of the world. Among these, *H. rosa-sinensis* is widely cultivated worldwide due to its large, showy flowers in different forms and colours (Magdalita and Pascual, 2022). *H. rosa-sinensis*, also known as Chinese Hibiscus, China rose, Hawaiian Hibiscus and shoeblack plant, can be used both as an outdoor potted and a landscape plant (Vyas *et al.,* 2012). A variety of flower forms, flower colours, vigour, and growth forms exist in the genus *Hibiscus*. The beauty of the hibiscus makes it one of the most widely cultivated flowers in brilliant hues of red, orange, purplish-red, yellow, white, purple, pink and several other colour combinations that are either single or double petals (Pekamwar *et al.,* 2013).

Hibiscus flowers are typically bisexual, featuring the four main parts, calyx, corolla, androecium, and gynoecium and come in a variety of sizes. The male reproductive portion of the flower consists of slender filament-like structures, each topped with an anther that contains a significant amount of pollen, ranging from 250 to 500 grains (Valdoz *et al.*, 2017).

Pollen viability refers to the ability of the pollen grains to survive and form a pollen tube that grows towards a receptive stigma or pistil for fertilization and further development of fruit and seed (Edlund *et al.*, 2004). Fertile pollen is essential for successful plant reproduction (Razzaq *et al.*, 2019) and high viability of pollen grains leads to high chances for fruit setting (Naik *et al.*, 2024).

Pollen development in hibiscus is reported to be affected by the structure and shape of the flower and it offers a clear understanding of the seed development process. Understanding pollen characteristics is the first step in organized breeding initiatives. Pollen morphological studies help to distinguish between species and cultivars (Min *et al.*, 2008; Xu and Craene, 2013; Konzmann *et al.*, 2019; Sudha *et al.*, 2022). Pollen grains exhibit distinct exine architectural characteristics which are useful for comparative morphological studies. Scanning electron microscopy (SEM) was found effective in studying pollen morphology as it provides greater resolution than the light microscope (Ridgway and Skyvarla,1969; Aswath et al., 2022). SEM study of four genotypes of *H. rosa-sinensis* revealed the pollen grains as spheroidal, pantoporate, apolar and with radial symmetry. Variations were observed in pollen size, spine type, spine indexes and exine sculpture (Saensouk and Saensouk, 2021). In the present research, an attempt was made to study the pollen fertility and pollen morphology of two distinct cultivars of *H. rosa-sinensis viz.* 'Playboy' and 'Gelia Castillo'.

Materials and methods

Plant material: Two cultivars of *H. rosa-sinensis, viz.,* 'Playboy' and 'Gelia Castillo' were used for the study (Fig. 1). The plants were raised in pots and given uniform cultural practices. Observations on floral characters were recorded and pollen studies were conducted. Royal Horticultural Society (RHS) colour chart was used for recording the petal colour.

Pollen fertility assessment: For pollen fertility assessment of the cultivars, the pollen grains were collected and stained with 1:1 glycerin - acetocarmine stain (2%). Morphological observations of fertile pollen were made using a compound microscope with binocular lens of 40x eyepiece. The viability of pollen grains was assessed by separately counting fertile and sterile grains. Well-stained, plump, and normal-shaped grains were considered fertile,

Fig.1. The *H. rosa sinensis* cultivars used for the study: (a) Playboy (b) Gelia Castillo

while unstained, small, or shrivelled grains were deemed non-viable or sterile. This evaluation was performed in ten different microscopic fields, and the average percentage of viable pollen grains was calculated and expressed as pollen fertility percentage.

SEM analysis: The pollen microstructure observations were carried out in the Central Laboratory for Instrumentation and Facilitation (CLIF), University of Kerala, Karyavattom, Thiruvananthapuram, Kerala. For the Scanning Electron Microscope (SEM) study, flowers were collected from the field in a fully opened and complete anther dehiscence stage at approximately 9.00 am and packed separately in a labelled plastic container. In the same day itself, pollen from the flowers was prepared for scanning electron microscopy as per the method described by Erdtman (1953). Pollen grains were mounted on stubs and coated with gold using Quorum SC7620 and observed under a ZEISS EVO-18 Scanning Electron Microscope (SEM) at 15 kV. A representative field of vision was chosen to observe and capture photographs of the morphology of the pollen population as well as the spine height and width, inter-spine distance, aperture and pore diameter. Using the soft imaging system, pollen diameter measurements were taken from ten randomly selected pollen grains of both cultivars. Pollen pore diameter was measured from ten individual pollen pores from a single pollen grain and ten separate spines from the same pollen grain were measured to determine the spine height, width, and inter-spine distance. Spine index, the ratio between the height and the width of the pollen spine (Shaheen *et al.*, 2009) and D-spine index, the ratio between the radius of the pollen grain and average spine height (Andrade *et al.*, 2014) were calculated.

Table 1. Floral Characters of *H. rosa sinensis* cultivars 'Playboy' and 'Gelia Castillo'

Floral characters	'Playboy'	'Gelia Castillo'
Flower diameter(cm)	14-15.5	14-14.5
Flower longevity(days)	1	1
Petal length(cm)	7.4-7.8	7.5-8.1
Petal width(cm)	6.8-7.2	7-7.5
Staminal column length(cm)	4.1-4.3	4.2-4.3
Peduncle length(cm)	5.7-6.2	2.3-2.5
Epicalyx- number of lobes	8	6
Pistil length(cm)	7.9-8.1	6.8-7.1
Flower type	Single	Single
Bloom type	Ruffled	Cartwheel single
Flower main colour	Strong Reddish Orange (RHS 31A-226)	Brilliant Greenish Yellow (RHS 3B-248)
Undulation of petal margin	Medium	Absent or very weak
Flower eye zone	Present	Present
Colour of eye zone	Dark Red	Medium Purple Red
Stigma pad colour	Orange	Yellow

Results and discussion

Floral characteristics of the cultivars were recorded (Table 1). The cv 'Playboy' exhibited strong reddish orange (RHS 31A-226) coloured flowers with slight yellow highlights, dark red eye zone, overlapping ruffled type flower petals, large flowers and orange-coloured stigma pads. The cv 'Gelia Castillo' exhibited brilliant greenish yellow (RHS 3B-248) petals with deep maroon eye zone, and cartwheel single-type flowers with yellow stigma pads. Both the cultivars were upright bushy, well-branched and suited for pot cultivation.

Under light microscopy, the fertile pollens were stained and appeared spherical (Fig. 2). Pollen fertility is crucial for the success of any crop improvement programme, as it directly affects the fruiting percentage. Assessment of pollen viability of male parent is essential before the conduct of crop improvement programmes (Hu *et al.*, 2017). The cultivar 'Gelia Castillo' exhibited a pollen fertility rate of 93.84%, while cv 'Playboy' exhibited a pollen fertility of 88.57%. (Fig. 2). The pollen fertility of the two hibiscus cultivars exhibited only a slight difference. The high fertility exhibited by both the cultivars indicates that

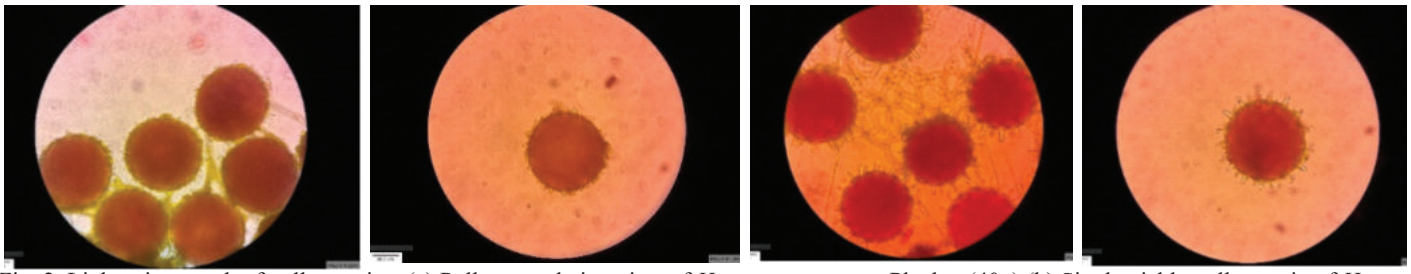

Fig. 2. Light micrograph of pollen grains: (a) Pollen population view of *H. rosa sinensis* cv. Playboy(40x) (b) Single viable pollen grain of *H. rosa sinensis* cv. Playboy(40x) (c) Pollen population view of *H. rosa sinensis* cv. Gelia Castillo(40x) (d) Single viable pollen grain of *H. rosa sinensis* cv. Gelia Castillo(40x)

Table 2. Pollen characters and fertility percentage of two *H. rosa-sinensis cultivars*

	Pollen diameter (µm)	Pollen shape	Pore diameter (µm)	Spine length (µm)	Spine width (µm)	Spine index	D spine Index	Inter-spine distance (µm)	Pollen fertility percentage
Playboy	129.6	Spheroidal	6.64	10.47	6.58	1.59	6.19	22.60	88.57
Gelia Castillo	116.0	Spheroidal	5.43	15.09	6.43	2.35	3.84	31.67	93.84

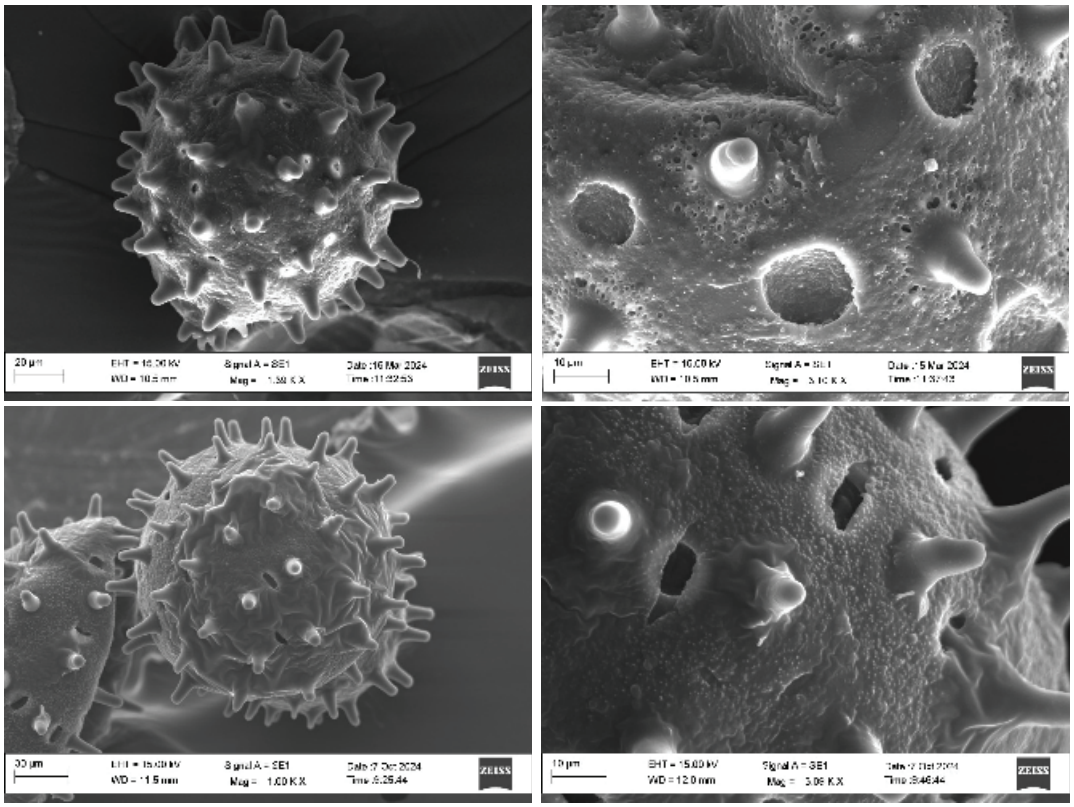

Fig. 3. SEM micrographs of the pollen grains: (a) Pollen grain of *H. rosa-sinensis* cv. Playboy (1390x) (b) Surface ornamentation, pollen pore and spine of *H. rosa-sinensis* cv. Playboy (1390x) (c) Pollen grain of *H. rosa-sinensis* cv. Gelia Castillo (1000x) (d) Surface ornamentation, pollen pore and spine of *H. rosa-sinensis* cv. Gelia Castillo (1000x)

both of them are good pollen parents in crop improvement programmes of hibiscus though the cv 'Gelia Castillo' is more likely to achieve successful pollination and fertilization compared to the cv 'Playboy'. This finding on pollen fertility of the present study aligns with the results of Naik *et al.* (2024), who conducted pollen fertility tests on various *H. rosa-sinensis* cultivars and reported viability percentages ranging from 62.85% to 95.12%.

Average pollen diameter of 129.6 µm was observed for the cv 'Playboy' whereas, 116.0 µm for the cv 'Gelia Castillo'. Bibi *et al.* (2008) reported a variation in pollen size in hibiscus among single-type and double-type flowers. Shaheen *et al.* (2009) reported that hibiscus pollen grains varied in diameter from 80 - 180 µm. Debut *et al.* (2013) documented an average pollen diameter of 81.5 µm for *H. rosa-sinensis*. The variability in pollen size reported by various authors indicates that pollen morphology is a varietal character in hibiscus.

The pollen aperture of both the cultivars was similar with round shape, but in the cv. 'Gelia Castillo' the aperture was partially covered with an exine layer. The cv. 'Playboy' had a larger aperture (6.64 µm) when compared to cv. 'Gelia Castillo' (5.43 µm). Both the cultivars featured blunt-shaped spine tips, where the cv 'Playboy' had short and wide spines with a spine length of 10.47 µm, while 'Gelia Castillo' had long and narrow spines with a spine length of 15.09 µm. The spines were reported to have very important roles in the mechanical structure of the pollen as well as its electrical properties which facilitate the pollination mechanism (Bowker and Crenshaw, 2007). The spines and mucilaginous substance present in the pollen were reported to help it attach to the stigma head and aid in pollination (Debut *et al.*, 2013). The distribution of spines equidistant from each other gives rise to repulsion forces due to electrical charges existing in the spines. In the pollination process, the negatively charged surface of pollen grains would be attracted by the positively charged surface in the legs of honey bees as opined by Katru *et al.* (2021).

Spine index (2.35) and inter-spine distance (31.67 µm) were found high for the cv. 'Gelia Castillo' compared to the cv 'Playboy' which recorded spine index and interspine distance values of 1.59 and 22.60 µm, respectively. Shaheen *et al.* (2009) obtained spine index values from 1.5 to 3.18 for four hibiscus cultivars and opined that the spine index defines the spine configuration and could be used as a taxonomic characteristic to delimit Malvaceous taxa. However according to Andrade *et al.* (2014), a defined ratio such as spine index could not be exact, as two similar spines would give the same spine index in two different stages of its growth, and it would not be reliable for the taxonomic identification of species within the same genera. They observed that morphological parameters like pollen diameter, pollen height and spine index were not uniform throughout the development of hibiscus. But D- spine index was reported to be more accurate for identifying species within the family Malvaceae. They obtained a D-spine index of 4.1 for mature pollen whereas, the values obtained in the present study were 3.84 and 6.19 respectively. The average measurements for spine length, spine width, pore diameter, and inter-spine distance observed in this study fall within the ranges specified by Bibi *et al.* (2008) for *H. rosa-sinensis* Linn. (Var. Double). The differences in the size, form, and surface distribution of the spines in pollen grains of Malvaceae are important at several taxonomic levels since they are observed not only across species within the same genus but also at the intergeneric level (El Naggar, 2003).

The observation and analysis of the morphology, surface characteristics, and exine ornamentation of pollen from two *H. rosa-sinensis* cultivars revealed that pollen from these species showed almost a similar micromorphology. The pollen of both cultivars was spheroidal in shape with pantoporate aperture and echinate surface ornamentation (Fig. 3), which was similar to the results of the study by Khalik *et al.* (2021). The relationship between pollen and its fertility is complicated because numerous factors, including pollen grain size, spine length, and spacing, as well as the pollinator species or their capacity to carry specific amounts of pollen, jointly influence the entire pollen developmental mechanism (Xiao *et al.*, 2023).

In this study, the pollen microstructure and pollen viability of two *H. rosa-sinensis* cultivars 'Playboy' and 'Gelia Castillo' showed that the pollen grains were single-grained and spheroidal and the pollen exine ornamentation was echinate. Hibiscus is an emerging ornamental plant in the potted plant industry. However, its heterozygous character, self and cross incompatibility, fruit shedding, uncommon seed set, pollen sterility, and endosperm failure lead to limitations in crop improvement. Since novelty is an important prerequisite for the ornamental flower industry, varieties suited to changing consumer demand are necessary. Newer varieties with good floricultural attributes can be developed by hybridization of potential parent plants. This study provides valuable evidence about the pollen characteristics of *H. rosa sinensis* cultivars 'Playboy' and 'Gelia Castillo' and both cultivars can be used as good male parents in crop improvement programmes for breeding novel varieties due to their high pollen fertility.

Conflict of interest: All authors read, approved the manuscript, and declared that they have no conflict of interest.

References

Andrade, K., S. Guerra and A. Debut, 2014. Fullerene-based symmetry in *Hibiscus rosa-sinensis* pollen. *PLoS One*, 9: 102123-102120.

Aswath, C.R., N.S. Bhandari and S. Gangola, 2022. SEM-based study for palynological and pollen germination of Lilium longiflorum cv. Pavia. Journal of Applied Horticulture, 24(3): 302-305. https://doi.org/10.37855/jah.2022.v24i03.53

Bibi, N., M. Hussain and N. Akhtar, 2008. Palynological study of some cultivated species of genus *Hibiscus* from North West Frontier Province (NWFP) Pakistan. *Pak. J. Bot.*, 40: 1561-1569.

Bowker, G.E. and H.C. Crenshaw, 2007. Electrostatic forces in wind-pollination–Part 1: Measurement of the electrostatic charge on pollen. *Atmos. Environ*., 8: 1587-1595.

Debut, A., S. Guerra and K. Andrade, 2013. Morphology of *Hibiscus rosa-sinensis* pollen grain from bud to senescence stage as criterion for taxonomy. *ESPE. Ciancia. Technol*., 4: 57-61.

Edlund, A.F., R. Swanson and D. Preuss, 2004. Pollen and stigma structure and function: The role of diversity in pollination. *Plant. Cell*., 16: 84-97.

El Naggar, S.M.I. 2003. Pollen morphology of Egyptian Malvaceae: An assessment of taxonomic value. *Turk. J. Bot.*, 28: 227-240.

Erdtman, G. 1953. *Pollen Morphology and Plant Taxonomy: Angiosperms*. First Edition. Almqvist and Wiksell, Stockholm, Sweden.

Hu, K.X., X.M. Zhang and Y.F. Zheng, 2017. Characteristics of pollen germination of *Primula obconica*. *J. Northwest. Univ*., 32: 170–173.

Katru, R., I. Johnson and S. Anbarasu, 2021. A nano analytical attempt on *Hibiscus rosa-sinensis* pollens using scanning electron microscopy (SEM) and UV-Vis spectroscopy. *Intl. J. Scientific Res. Sci. Technol*., 8: 525-529.

Khalik, K.A., A. S. Ruzayza, A. Assiri and A. Elkordy, 2021. Pollen morphology of Malvaceae genera from Saudi Arabia and its taxonomic significance. *Austral. J. Crop. Sci*., 5: 725-742.

Konzmann, S., S. Koethe and K. Lunau, 2019. Pollen grain morphology is not exclusively responsible for pollen collectability in bumble bees. *Scientific Rpt.*, 9: 4705-4713.

Magdalita, P.M. and S.A.O. Pascual, 2022. Floriculture and Ornamental Plants. p. 483-522. In: *Handbook of Crop Diversity: Conservation and Use of Plant Genetic Resources*: Hibiscus (*Hibiscus rosa-sinensis*): Importance and classification, S.K. Datta and Y.C. Gupta (eds.). Springer Nature.

Min, C.A.I., Z.H.U. Hua and W.A.N.G. Hong, 2008. Pollen morphology of the genus *Lasianthus* (Rubiaceae) and related taxa from Asia. *J. Syst. Evol.*, 46: 60-70.

Naik, K.T.P., J. Majumder, T. Koley and K. Adhikary, 2024. Effect of pollen morphology on hybridization and seed setting in Hibiscus (*Hibiscus rosa sinensis*). *Indian J. Hortic.*, 81: 135-141.

Pekamwar, S.S., T.M. Kalyankar and A.C. Jadhav, 2013. *Hibiscus rosa-sinensis*: A review on ornamental plant. *World. J. Pharm. Pharm. Sci*., 6: 4719-4727.

Razzaq, M.K., S. Rauf, M. Khurshid, S. Iqbal, J. A. Bhat, A. Farzand, A. Riaz, G. Xing and J. Gai, 2019. Pollen viability an index of abiotic stresses tolerance and methods for the improved pollen viability. *Pak. J. Agrl. Res.*, 32: 609-625.

Ridgway, J.E. and J.J. Skyvarla, 1969. Scanning electron microscopy as an aid to pollen taxonomy. *Ann. Missouri Bot. Gard*., 56: 121-124.

Saensouk, S. and P. Saensouk, 2021. Pollen morphology of subfamily Malvoideae (*Malvaceae sensu* lato) in Thailand. *Biodiversitas J. Biol. Diversity*, 22: 5633-5643.

Shaheen, N., M.A. Khan, M.Q. Hayat and G. Yasmin, 2009. Pollen morphology of 14 species of Abutilon and Hibiscus of the family Malvaceae (*Sensu stricto*). *J. Med. Plant. Res*., 3: 921-929.

Sudha, R., V. Niral, K. Samsudeen, V. Aparna, V. Selvamani and M. Neema, 2022. An insight into pollen morphology and evaluation of pollen viability, germination and mineral composition of some coconut (*Cocos nucifera* L.) genotypes. *S. Afr. J. Bot.*, 151: 485-494.

Valdoz, J.C., P.M. Magdalita, W.L. Absulio and R.C. Sotto, 2017. Morpho-anatomical characters and ethylene production in *Hibiscus rosa-sinensis* L. in relation to two-day floral retention. *Philipp. Agric. Sci*., 100: 169-177.

Vyas, A., S.S. Shukla, R. Pandey, V. Jain, V. Joshi and B. Gidwani, 2012. Chervil: A multifunctional miraculous nutritional herb. *Asian. J. Plant. Sci*., 11: 163-171.

Xiao, F., X. Wang, Y. Jiang, C. Chen, J. Chen, J. Zhang and Y. Wen, 2023. Combined morphological and palynological classification for *Hibiscus syriacus* L. (Malvaceae): Construction of the diagnostic classification framework and implications of pollen morphological variation on fruiting. *Agron*., 13: 828-846.

Xu, F. and L.P.R. Craene, 2013. Pollen morphology and ultrastructure of selected species from Annonaceae. *Plant. Syst. Evol*., 299: 11-24.

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