Biological Forum
an International Journal (SI-AAAS: CS March 21&22, 2025)
17(5a): 107-110(2025)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Genotypic Variability in Floral Morphology, Flowering Phenology and Pollen Viability of Citrus Cultivars under Subtropical Conditions

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(Received: 31 March 2025; Accepted: 03 May 2025; Published online: 15 May 2025)
(Published by Research Trend)

ABSTRACT: Citrus is one of the most commercially important fruit crops globally, with reproductive efficiency and floral traits playing a crucial role in determining yield potential and suitability for hybridization. This study aimed to evaluate the floral morphology, flowering phenology and pollen viability of seven citrus cultivars: three mandarins ('Kinnow', 'Daisy', 'W. Murcott') and four sweet oranges ('Blood Red Malta', 'Jaffa', 'Mosambi', 'Valencia Late'), under subtropical conditions of Hamirpur, Himachal Pradesh. The experiment was conducted during 2019-2021 using four replications per cultivar in an Ultra High-Density Plantation system. Floral traits were recorded following IPGRI descriptors, while pollen viability was assessed using 2% acetocarmine stain. Significant genotypic variability was observed in flower size, filament and style length and pollen fertility. 'Mosambi' exhibited the largest flowers, whereas 'Kinnow' recorded the highest proportion of perfect flowers (93.50%) and pollen viability (92.50%). In contrast, 'Valencia Late' and 'Blood Red Malta' had higher percentages of staminate flowers, potentially limiting their pollination efficiency. The results highlight the genetic diversity among cultivars and underscore the importance of floral traits in breeding and orchard planning. Cultivars like 'Kinnow' and 'Mosambi' may serve as superior pollen parents in citrus breeding improvement programmes targeting enhanced fruit set and productivity.

Keywords: Citrus, Floral Morphology, Phenology, Pollen Viability, Acetocarmine Variability.

INTRODUCTION

Citrus is a globally significant fruit crop, with annual production surpassing 160 million tonnes (FAO, 2021). Its adaptability across diverse agro-climatic zones and high consumer preference have positioned it among the top fruit crops worldwide. India, as a part of the citrus centre of origin, harbours rich genetic diversity, especially in the North-Eastern and Western Himalayan regions (Swingle and Reece 1967; Singh and Chadha 1993). Beyond its economic role, citrus contributes substantially to human nutrition. Citrus fruits are rich in vitamin C, flavonoids, carotenoids, dietary fibre and various phytochemicals with antioxidant and health-promoting properties (Gil-Izquierdo *et al.*, 2001; Abeysinghe *et al.*, 2007).

Improving citrus productivity relies not only on agronomic practices but also on understanding reproductive traits such as floral morphology, phenology and pollen viability. These factors influence pollination efficiency, fertilization success and ultimately fruit set and yield (Davies and Albrigo 1994; Erickson *et al*., 2022). However, limited research exists on cultivar-specific floral biology, particularly under ultra-high-density planting (UHDP) systems in subtropical conditions. UHDP has gained momentum in citrus cultivation due to its potential to increase productivity per unit area. Although, successful implementation requires precise knowledge of flowering behaviour and reproductive compatibility among cultivars to ensure effective cross-pollination. Under such intensive systems, variation in floral traits and pollen fertility can critically affect yield outcomes, especially in partially self-incompatible species like citrus.

Floral traits such as flower size, filament and style length and the proportion of perfect and staminate flowers influence pollen transfer and fertilization efficiency. Pollen viability, another key parameter, directly affects the reproductive potential of a cultivar and its role as a male parent in breeding programmes. Therefore, understanding these parameters is essential for designing compatible orchard layouts, selecting suitable parents for hybridization and improving fruit set under specific regional conditions.

The present study was conducted to characterize the floral morphology, flowering dynamics and pollen viability of seven citrus cultivars: three mandarins (*Citrus reticulata*) and four sweet oranges (*Citrus sinensis*) grown under uniform agro-climatic conditions in an UHDP system in Hamirpur, Himachal Pradesh. This region, situated in the subtropical hills, experiences environmental fluctuations that can influence flowering and fertility. The investigation aimed to evaluate genotypic variability in reproductive traits to identify cultivars with superior floral characteristics and pollen performance. These findings can contribute to more informed selection of cultivars for breeding and orchard planning, especially in subtropical hill regions. By integrating floral and reproductive assessments, this research supports the development of improved citrus production strategies suited to UHDP systems and region-specific climatic conditions.

MATERIAL AND METHODS

Experimental site and planting material. The study was conducted during 2019-2021 at the Experimental Farm and Laboratory of the Department of Fruit Science, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh. The site lies in a subtropical zone at an altitude of 650 m above mean sea level, located at 31°41′47.6″ N latitude and 76°28′6.3″ E longitude.

The investigation involved seven citrus cultivars, including three mandarin cultivars (*Citrus reticulata* Blanco): 'Kinnow', 'Daisy' and 'W. Murcott' and four sweet orange cultivars (*Citrus sinensis* Osbeck): 'Blood Red Malta', 'Jaffa', 'Mosambi' and 'Valencia Late'. These trees were planted in 2016 under an Ultra High-Density Plantation (UHDP) system at a spacing of 2.5 × 2.0 m. Four healthy and uniformly growing trees per cultivar, free from insect-pest infestation and diseases, were selected for recording observations.

Qualitative floral traits. Morphological evaluation of qualitative floral traits was performed in accordance with the Citrus Descriptors of the International Plant Genetic Resources Institute (IPGRI, 1999), now renamed as Bioversity International, Rome, Italy. Ten randomly selected flowers per tree were assessed to document traits such as colour of open flowers, anther colour and number of petals per flower.

Flowering phenology. The starting stage of flowering was considered when approximately 5-10% of flower buds had opened. Full bloom was noted when 70-80% of the total floral buds were open, while the end date of flowering season was taken when nearly 90% of flowers had shed and 10% flower buds were still to open. Flowering observations were recorded separately for each cultivar over two consecutive flowering seasons.

Quantitative floral traits. Quantitative floral characteristics including flower length, flower diameter, staminate flower percentage, perfect flower percentage, filament length and style length were measured during the peak flowering stage. Ten fully open flowers per tree were randomly selected for morphometric measurements using a digital vernier calliper (Mitutoyo). The evaluation was carried out in accordance with the descriptors for citrus as outlined by the International Plant Genetic Resources Institute (IPGRI, 1999).

The experiment was laid out in a Randomised Block Design (RBD) with four replications per cultivar and one plant per replication for the quantitative floral traits, following the methodology suggested by Gomez and Gomez (2010). The data were statistically analysed to determine significant differences among cultivars. Critical difference (CD) at a 5% level of probability was calculated to assess the significance of variation in the floral traits using standard ANOVA procedures.

Pollen collection. Flower buds at the balloon stage were collected from each cultivar during morning hours (08:30 AM - 10:30 AM). Buds were taken to the laboratory in butter paper bags. Anthers were separated carefully from the filaments using sterilized dissecting needles and placed on clean, non-adhesive paper within sterile petri dishes. These were kept under shade at ambient room temperature (~25 ± 2°C) to allow natural anther dehiscence. Pollen grains, released as yellow powdery masses, were gently tapped out and collected in sterile, labelled glass vials for subsequent viability studies.

Pollen viability. Pollen viability was assessed using 2% acetocarmine stain, prepared by dissolving 2 g of carmine powder in 45 ml of glacial acetic acid and making the final volume up to 100 ml. The mixture was filtered through Whatman No. 1 filter paper to obtain a clear staining solution.

A small quantity of fresh pollen was dusted onto clean glass slides and one to two drops of acetocarmine solution were added. Slides were covered with a coverslip and allowed to rest for 5-10 minutes to facilitate adequate staining (Norton, 1966). Pollen grains that stained uniformly deep red were classified as viable and unstained or distorted grains as non-viable. The percentage of viable pollen was calculated using the following formula:

Pollen viability (%) = (Viable pollen grains / Total pollen grains counted) × 100

All observations were recorded following a Completely Randomised Design (CRD) to ensure statistical accuracy and experimental reliability. All slides were prepared fresh on the day of observation and each assessment was repeated three times per cultivar to ensure consistency and reproducibility of results.

RESULTS AND DISCUSSION

Floral morphology and flowering behaviour are key traits influencing reproductive efficiency and yield in citrus. Significant genotypic variation was observed among the seven studied cultivars with respect to flower structure, blooming period and pollen viability. The following results present a comparative analysis of these traits under uniform growing conditions in Hamirpur, Himachal Pradesh, highlighting their potential role in determining productivity and suitability for hybridization programmes.

Qualitative floral traits and flowering phenology. Floral morphological traits across the evaluated citrus cultivars showed a high degree of consistency, with only minor differences observed in certain parameters (Table 1). All cultivars exhibited five petals and the flower colour was consistently white. Similar results were previously recorded by Khan *et al.* (2008); Altaf and Khan (2009) in mandarins.

However, anther colour showed slight variation among the cultivars. While most genotypes, including 'Kinnow', 'Daisy', 'W. Murcott', 'Blood Red Malta' and 'Valencia Late', produced pale yellow anthers, a distinct yellow pigmentation was recorded in 'Jaffa' and 'Mosambi'. These differences may reflect underlying cultivar-specific differences in floral pigmentation, as noted by Singh *et al.* (2016) in various mandarin cultivars.

Flowering phenology varied considerably among cultivars and seasons (Table 1). The onset of flowering was first recorded in 'Blood Red Malta' (3rd March), followed by 'Jaffa' (4th March) and 'Mosambi' (5th March). In contrast, 'Kinnow' initiated flowering significantly later, around 12th March. The peak bloom period, when approximately 70-80% of flowers were open, occurred from mid to late March, varying by genotype. 'Daisy' attained full bloom between 16th-20th March, followed by 'W. Murcott' and 'Valencia Late' between 18th-26th March. 'Kinnow' exhibited the latest full bloom, from 24th-30th March.

Regarding the end of the flowering period, 'Blood Red Malta' and 'Mosambi' were the first to terminate their flowering by 24th March followed by 'Daisy' and 'Jaffa' (27th March) and 'Kinnow' was the last to complete flowering on 7th April. Kumatkar *et al*. (2016); Singh *et al*. (2016) also reported similar observations. Understanding the variation in flowering behaviour is critical for breeding and orchard management, especially in synchronising flowering periods for potential cross-compatibility and improving pollination efficiency.

Quantitative floral traits. A high degree of variability was noted among the studied citrus cultivars in terms of floral morphometric traits and pollen viability, highlighting their genotypic diversity (Table 2). The flower length ranged from 14.10 mm in 'W. Murcott' to 24.20 mm in 'Mosambi', while flower diameter was highest in 'Mosambi' (38.00 mm) and lowest in 'W. Murcott' (28.50 mm). These findings suggest a genotypic influence on floral dimensions, likely linked to species-specific growth habits and environmental adaptability. Larger flower dimensions, particularly in 'Mosambi' and 'Valencia Late', may improve the exposure and accessibility of reproductive structures, thereby facilitate more efficient pollen transfer and enhance fruit set efficiency, as suggested by Erickson *et al.* (2022). Similar genotypic differences in flower size have been reported in sweet oranges by Baswal *et al.* (2015), where 'Campbell Valencia' flowers were notably larger (41.20 mm diameter) than those of cultivars like 'Sanguinelli' (~25.7 mm).

Filament length ranged between 8.30 mm in 'Blood Red Malta' to 10.93 mm in 'Mosambi', while style length varied from 5.02 mm ('Kinnow') to 6.60 mm ('Valencia Late'). These structural differences influence the spatial arrangement of reproductive organs, affecting the efficiency of pollen deposition and stigma receptivity, which are critical for effective pollination and fertilization. Comparable variability in filament length among sweet orange cultivars was also reported by Gupta *et al.* (2020).

Variation was also evident in the percentage of staminate and perfect flowers. The highest proportion of perfect flowers was recorded in 'Kinnow' (93.50%), while 'Valencia Late' showed the lowest (90.30%). Conversely, the highest percentage of staminate (male) flowers was observed in 'Valencia Late' (9.70%) and 'Blood Red Malta' (9.00%). These proportions reflect the balance between functional reproductive structures within cultivars, which may influence yield potential. Cultivars like 'Kinnow' and 'Mosambi', with a greater proportion of perfect flowers, are likely to exhibit improved fruit set under open pollination conditions.

Similar trends in the balance of perfect and staminate flowers have been reported in sweet orange cultivars, where staminate flower percentage ranged from 6.62% in 'Mosambi' to 11.97% in 'Phule Mosambi', indicating a wide genotypic spectrum (Gupta *et al*., 2020). These findings support the hypothesis that a higher percentage of perfect flowers contributes positively to pollination success and overall productivity in citrus.

Pollen viability was highest in 'Kinnow' (92.50%), while the lowest value was observed in 'Valencia Late' (86.50%). High pollen viability is essential for successful fertilization and has a direct impact on fruit set and yield, particularly in cross-pollinated species like citrus. Similar findings were reported by Demir *et al*. (2015), who observed significant variation in pollen viability among seven lemon genotypes, with the 'Meyer' variety showing the highest viability (86.74%), followed by 'Kutdiken' (69.22%) and the lowest in 'BATEM Sarisi'.

Table 1: Floral Morphological and Phenological Characteristics of Citrus Cultivars.

Cultivars	Colour of open flower	Colour of anthers	Number of petals per flower	Flowering period		
				Start date of flowering	Full bloom date of flowering	End date of flowering
Kinnow	White	Pale Yellow	Five	12th March	24th – 30th March	7th April
Daisy	White	Pale Yellow	Five	7th March	16th – 20th March	27th March
W. Murcott	White	Pale Yellow	Five	11th March	21st – 26th March	29th March
Blood Red Malta	White	Pale Yellow	Five	3rd March	13th – 16th March	24th March
Jaffa	White	Yellow	Five	4th March	15th – 18th March	27th March
Mosambi	White	Yellow	Five	5th March	14th – 19th March	24th March
Valencia Late	White	Pale Yellow	Five	7th March	18th – 22nd March	29th March

Table 2: Quantitative Floral Characteristics and Pollen Viability of Citrus Cultivars.

Cultivars	Flower length (mm)	Flower diameter (mm)	Length of filament (mm)	Length of style (mm)	Staminate flower (%)	Perfect flower (%)	Pollen viability (%)
Kinnow	16.40	31.50	8.87	5.02	6.50	93.50	92.50
Daisy	15.06	29.50	8.50	5.20	8.75	91.25	91.50
W. Murcott	14.10	28.50	8.60	5.80	8.03	92.00	89.50
Blood Red Malta	19.10	30.41	8.30	5.08	9.00	91.00	88.50
Jaffa	22.40	32.50	9.90	6.14	8.50	91.50	87.70
Mosambi	24.20	38.00	10.93	6.49	7.00	93.00	87.00
Valencia Late	21.25	36.05	9.40	6.60	9.70	90.30	86.50
C.D. $_{(0.05)}$	1.44	1.51	0.08	0.05	1.43	1.78	0.18

CONCLUSIONS

This study highlighted genotypic variation in floral morphology, flowering phenology and pollen viability among seven citrus cultivars evaluated under subtropical conditions of Hamirpur, Himachal Pradesh. Differences in flower size, filament and style length reflected the cultivars' genetic diversity. Larger floral structures in cultivars like 'Mosambi' and 'Valencia Late' may enhance pollinator attraction and facilitate better fruit set. 'Kinnow', with the highest percentage of perfect flowers (93.50%) and pollen viability (92.50%), showed superior reproductive efficiency. Conversely, 'Valencia Late' and 'Blood Red Malta' had higher proportions of staminate flowers, which may reduce their effectiveness in natural pollination. These findings highlight the importance of floral characteristics and pollen fertility in determining fruit set, yield potential and breeding value of citrus cultivars.

FUTURE SCOPE

The findings from this study offer valuable insights for selecting parent cultivars in citrus breeding. 'Kinnow' and 'Mosambi', with superior floral traits and pollen viability, are promising parents for hybridization to improve fruit set and yield. Variations in flowering time and structure can guide orchard design for better pollination, especially in high-density systems. Future integration of floral trait data with molecular markers may accelerate selection through marker-assisted breeding. Given rising climate variability, further research on seasonal effects on flowering and pollen fertility is vital for developing adaptive strategies to sustain citrus productivity in subtropical regions.

Acknowledgement. The authors sincerely thank the Department of Fruit Science, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India, for providing the necessary resources, support and valuable guidance throughout the course of this investigation.

Conflict of Interest. None.

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How to cite this article: Shivali Chauhan, Vikas Kumar Sharma, Rupam Nehta, Lakesh Rahi, M.A. Kuchay and Sanjeev (2025). Genotypic Variability in Floral Morphology, Flowering Phenology and Pollen Viability of Citrus Cultivars under Subtropical Conditions. *Biological Forum, 17*(5a): 107-110.