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Floral biology and pollen viability of different guava genotypes under the sub-tropics of Jammu and Kashmir

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ABSTRACT

Floral biology plays a crucial role in improving crop management, synchronizing flowering and increasing the efficiency of hybridization. Climatic conditions change with spatial variations, therefore, experiment was carried out in 2024 on different guava cultivars, including Lalit, Hisar Surkha, Hisar Safeda, Punjab Pink, L-49, Shweta, Lalima and Dhawal under sub-tropical conditions of Jammu in Jammu and Kashmir. Among different guava genotypes L-49 exhibited the earliest flowering initiation, starting from 7th August to 20th September 2024 revealing the shortest flowering period of 44 days whereas the longest flowering period was exhibited by Hisar Safeda (57 days). The largest flower diameter was recorded as 45.34 mm in cultivar L-49. However, the smallest flower size was noted in cultivar Lalit with measurements of 30.34 mm. All genotypes featured four green sepals, five to six white petals and an inferior ovary. Cultivar L-49 also had the highest counts for stamens (413.67), stamen length (19.76 mm) and pistil length (15.07 mm). The lowest count of stamens (202.00) and smallest pistil length (9.41 mm) was found in cultivar Dhawal, whereas, lowest stamen length was recorded in cultivar Lalit (11.37 mm). The highest pollen viability tested with 2 percent acetocarmine solution was recorded as 97.33 percent in cultivars L-49 and Lalit, whereas, minimum pollen viability was found in Punjab Pink and Lalima as 94.00 percent. All genotypes displayed a triangular pollen shape. Pollen germination was found to be higher with 10 percent sucrose + 50 ppm boric acid solution as compared to 5 percent sucrose + 50 ppm boric acid and 15 percent sucrose + 50 ppm boric acid solutions. Maximum pollen germination was recorded as 78.66 percent with 10 percent in cultivar Hisar Surkha. In contrast, the lowest pollen grain germination 45.33 percent was recorded in cultivar Dhawal.

Keywords: *Guava, Genotypes, Floral biology, Pollen viability, Pollen germination, Flowering phenology, Flowering intensity, Floral morphology measurements, Morphometric traits, Floral characteristics assessment, Genotypic variation, Pollen grain morphology.*

Introduction

Guava (*Psidium guajava* L.) is recognized as one of the most significant tropical and subtropical fruit crops within the family Myrtaceae. It is extensively cultivated due to its high nutritional value, especially abundance of vitamin C, minerals and bioactive compounds, which has earned it the nickname "poor man's apple" in various developing nations [1]. Guava ranks fourth most cultivated fruit crop in India, following mango, banana and citrus, with 363-thousand-hectares of area under cultivation and production volume of 5,446 thousand metric tons [2]. The primary states contributing to guava production in India include Uttar Pradesh, West Bengal, Bihar, Maharashtra, Chhattisgarh, Karnataka, Madhya Pradesh, Gujarat, Tamil Nadu and Andhra Pradesh. In Jammu and Kashmir, guava is cultivated in the subtropical zone of the Jammu region primarily in Kathua, Samba and Jammu districts.

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In Jammu and Kashmir, guava is cultivated on 2.831 thousand hectares with an annual production of 8.802 thousand tonnes [3] and still guava has a huge scope for cultivation in this region. However, increasing the production requires area expansion, climate-specific cultivars, improved cultivation practices and sustainable methods or pest control. For cultivar improvement and development of improved cultivation practices phenological stages and floral biology of the crop must be understood properly under specific climatic conditions. Floral traits such as anthesis time, the duration of flower opening and closing and floral morphology have a direct impact on pollination dynamics and reproductive success [4]. Similarly, research on pollen viability and germination offers crucial insights into male fertility, which is vital for hybridization and achieving successful fruit set in both natural and controlled pollination environments [5]. Given that guava displays significant variability in flowering behavior among different cultivars and seasons, comprehensive studies on floral biology are also beneficial for crop management, synchronization of flowering and enhancing hybridization efficiency [6]. Moreover, comprehending floral characteristics can assist in identifying appropriate pollinators and improve the effectiveness of breeding initiatives focused on creating high-yielding and

quality varieties. Consequently, this study on floral biology and pollen viability of guava was conducted with the aim of producing detailed information regarding flowering patterns, floral structure, dimensions of flower parts, pollen viability and germination across various guava genotypes, which would aid in enhancing breeding efficiency, pollination strategies and overall productivity in this significant fruit crop. In spite of its economic significance and broad adaptability, the fruit set and yield of guava are heavily reliant on the effectiveness of pollination and fertilization processes. Consequently, a comprehensive understanding of its floral biology is essential for effective crop management and breeding enhancement [7].

2. Materials and Methods

The study on floral biology and pollen viability of different guava cultivars namely Lalit, Hisar Surkha, Hisar Safeda, Punjab Pink, L-49, Shweta, Lalima and Dhawal planted at Advanced Center for Horticulture Research, Udheywala, SKUAST-Jammu, was carried out during 2024. Plants of 8-year age planted at 6.0×6.0 meter spacing were maintained uniformly during the course of experiment as per the package of practices for fruit crops published by Shere-e-Kashmir University of Agricultural Sciences and Technology of Jammu. Trial was laid during 2024 and observations were recorded for hastha bahar flowering season.

2.1 Observations recorded

2.1.1 Flowering phenology

The date of first flower opening (defined as when more than 5 % of the flowers had opened), the date marking the end of flowering (when over 95 % of the flowers had opened), and the duration of flowering (calculated as the number of days from the first flower opening to the end of flowering) were documented through visual observation for all the cultivars under study.

2.1.2 Floral characteristics assessment

The number of stamens, sepals petals along with sepal and petal color and the position of the ovary relative to the stamens, were assessed through visual observation.

The classification of the ovary as superior, semi-inferior or inferior is based on its position relative to the stamens.

The ovary positioned above the stamens is classified as superior, while one located below is deemed inferior, If it is aligned with the stamens, it is referred to as semi-inferior and were assessed through visual observation.

2.1.3 Floral morphology measurements

Measurements of flower size (diameter), stamen length and pistil length were recorded in millimetres by using a vernier calliper.

2.1.4 Pollen viability

Evaluation of pollen viability was performed by staining the pollen grains with a 2 percent acetocarmine solution. Fresh pollen grains were acquired by gently crushing and tapping the guava anthers, allowing them to fall onto a clean glass slide. A drop of 2 percent acetocarmine was poured gently on the pollen grains and a cover slip was placed over it. The pollen viability was subsequently assessed under a microscope by averaging and working out the percentage of viable and non-viable pollen grains in 5 microscopic fields. Pollen grains that absorbed the purple stain and appeared plump were identified as viable, whereas those the were shrivelled and unstained were considered non-viable.

2.1.5 Pollen shape

The shape of pollen was observed visually under a microscope.

2.1.6 Pollen germination

Pollen germination was assessed by germinating pollen grains in different sucrose concentrations using with hanging drop method. The freshly dehisced pollen grains were subjected to *in-vitro* pollen germination tests using the hanging drop technique. This experiment was performed with varying concentrations of Sucrose (5 %, 10 % and 15 %) and Boric acid (50 ppm) in combination. A drop of sucrose + boric acid solution was put in the cavity of the cavity slide. Fresh pollen grains were shed on the drop and gently mixed with the solution in the cavity slide without disturbing the shape of the drop. The cavity of the slide was sealed with a cover slip by applying vaseline on its sides. The cavity slides were promptly inverted, resulting in the formation of hanging drops, after which they were placed into a petri-dish containing moist filter paper to maintain consistent moisture conditions. Pollen germination was assessed after 12 hours, 24 hours and 72 hours. Pollen grains were considered germinated when the length of the pollen tube was at least double that of the pollen grain. The average percentage of germination was calculated in five microscopic fields.

2.2 Statistical analysis

Descriptive statistical parameters such as mean, minimum, maximum, standard deviation and standard error of the mean were calculated utilizing Microsoft Excel. A one-way analysis of variance (ANOVA) was executed following a randomized block design (RBD) via the OPSTAT statistical analysis system (opstat.pythonanywhere.com). Additional statistical analyses and data visualization were carried out using the SPSS software. [8].

3. Result and discussion

3.1 Flowering phenology

3.1.1 Flowering initiation

Data presented in Table 1 indicates that earliest flowering among different guava genotypes was recorded on 7th August 2024 in L-49 followed by Hisar Safeda and Lalit on 11 August 2024, followed by Hisar Surkha on 12 August 2024, Shweta on 14 August 2024, Punjab Pink on 16 August 2024, Lalima on 20 August 2024, and concluding with Dhawal on 21 August 2024. The present findings align with observations of Bakshi et al. [9], Sahoo et al. [10], Dolkar et al. [11] and Goswami [12], who reported that hastha bahar flowering appears in the month of August-September and also reported that earliest flowering has been recorded in L-49 genotype.

3.1.2 End of flowering

From Table 1, it is evident that L-49 exhibited the earliest end of flowering on 20th September 2024 followed by Lalit on 30th August 2024 and Punjab Pink on 2nd September 2024. In contrast, the latest flowering cessation was observed in the genotypes Hisar Safeda, Hisar Surkha, Shweta and Dhawal, all of which concluded on 5th October 2024. These findings align with the results reported by Sharma et al. [13], who indicated that L-49 had an early end of flowering, while Hisar Safeda exhibited the latest during winter season.

3.1.3 Flowering duration

From table 1, it is clear that the shortest flowering duration was observed in L-49, which took 44 days, followed by Lalima (45 days) and Dhawal (46 days), indicating relatively early and

compact flowering behavior. In contrast, the longest flowering duration was found in Hisar Safeda (57 days), followed by Hisar Surkha (55 days) and Shweta (53 days). Moderate flowering duration was noted in Lalit (51 days) and Punjab Pink (48 days). The variation in the flowering duration of different guava cultivars may be attributable to varietal precocity and the genetic makeup of the genotype. Similarly, Sharma et al. [13] also reported minimum flowering duration in L-49 (40 days) among different cultivars of guava. However, maximum flowering duration was observed as 45 days in Hisar Safeda during winter season.

3.2 Floral characteristics assessment

Data presented in table 2 reveal that all eight guava cultivars *viz*., Lalit, Hisar Surkha, Hisar Safeda, Punjab Pink, L-49, Shweta, Lalima and Dhawal were found to have four green sepals and an inferior ovary. All studied genotypes featured white petals. However, notable variation was found in the petal count per flower. While most genotypes, including Hisar Surkha, Hisar Safeda, Punjab Pink, Lalima and Dhawal, typically had five petals, cultivars such as Lalit, L-49, and Shweta were characterized by six petals. This difference in petal number may provide a valuable morphological characteristic for identifying genotypes and could influence factors such as pollination efficiency and the attractiveness of the flowers. While the consistency suggests a significant degree of floral structural stability among the cultivars, indicating that guava shows minimal variation in fundamental floral morphology despite genetic or environmental influences. The occurrence of four sepals in each cultivar corresponds with previous findings by Singh et al. [14] and Yadava et al. [15], who characterized guava flowers as regular, pentamerous to tetramerous, complete and bisexual, featuring 4–5 green sepals and white petals. The green coloration of the sepals plays a protective role for the developing floral buds and is a characteristic feature across the majority of *Psidium guajava* genotypes Kumar et al. [16].

3.3 Floral morphology measurements

The data displayed in Table 3 indicates that there is significant variation among guava cultivars regarding the dimensions of floral organs, specifically, the number of stamens, stamen length and pistil length, which varied considerably among different guava cultivars.

3.3.1 Flower size

Flower size of different cultivars presented in Table 3, revealed that L-49 exhibited the largest flower diameter at 45.34 mm, with Hisar Safeda followed by Hisar Surkha measuring 44.23 mm and 42.90 mm, respectively. Conversely, the smallest flower size was recorded in Lalit as 30.34 mm. This variation in flower size may be attributed to cultivar-specific higher allocation of assimilates to reproductive structures, a conclusion corroborated by Singh et al. [17] indicated that the largest flower size in L-49 measured 47.01 ± 0.26 mm, while the smallest was found in Lalit at 34.90±0.23 mm across different guava cultivars.

3.3.2 Number of stamens, length of the stamen and pistil length

The highest count of stamens (413.67) was noted in L-49, succeeded by Hisar Safeda (365.00), whereas the lowest was found in Dhawal (202.00). Kumar et al. [16] and Sharma et al. [18] have reported comparable variability in stamen counts among guava genotypes, highlighting those genotypic variations predominantly influence the proliferation of floral organs. The length of the stamen varied from 11.37 mm in Lalit to a peak of 19.76 mm in L-49, followed by Shweta measuring 17.07 mm. The pistil length reached its highest in L-49 at 15.07 mm, closely followed by Shweta at 15.06 mm, while the shortest pistil length was noted in Dhawal at 9.41 mm. Earlier research conducted by Singh et al. [14] and Kumar et al. [16] also emphasized that the length of the pistil in guava affects compatibility, which is vital for the success of hybridization.

3.4 Pollen viability

Results of pollen viability assessed with 2 % acetocarmine presented in Table 4 reveal that highest pollen viability was recorded in the L-49 and Lalit genotypes of guava, with a viability of 97.33 % followed by Shweta (97.00 %) and Hisar Safeda (96.00 %). The observations are depicted in PlateS 1 and 2. Singh et al. [19] found that among the tested guava genotypes, L-49 recorded the highest pollen viability (96.23 %). In contrast, the lowest viability was observed in the Punjab Pink and Dhawal genotypes, which had a viability of 94.00 %. This variation in pollen viability among different cultivars is due to their different genetic make-up.

3.5 Pollen shape

Among all the cultivars, the shape of guava pollen was determined to be triangular shown in Plate 1. Similarly, Goswami [12] also reported triangular pollen shape in different guava cultivars.

3.6 Pollen germination

The highest pollen germination (Table 5) across all guava genotypes was observed in a 10 % sucrose solution. Among different sucrose concentrations, the genotype Hisar Surkha exhibited the highest pollen germination percentage, specifically 5 % sucrose (72.00 %), 10 % sucrose (78.66 %) and 15 % sucrose (74.66 %) shown in Plates 3 and 4. These results align with the observations made by Sarkar et al. [20], indicating that the highest pollen germination rate was 81.52 % in Hissar Surkha when using a 10 % sucrose + 50 ppm boric acid solution. Lowest pollen germination was recorded in the Dhawal genotype, 45.33 % pollen germination in 5 %, 63.33 % pollen germination in 10 % sucrose and 15 % pollen germination in 51.33 % sucrose solution. This variation in pollen viability among different cultivars is due to their different genetic make-up.

Table 1: Time and duration of flowering in different guava cultivars

S. No.	Cultivars	Start of Flowering	End of Flowering	Flowering duration (days)
1	Lalit	11-08-2024	30-09-2024	51
2	Hisar Surkha	12-08-2024	05-10-2024	55
3	Hisar Safeda	11-08-2024	05-10-2024	57
4	Punjab Pink	16-08-2024	02-10-2024	48
5	L-49	07-08-2024	20-09-2024	44
6	Shweta	14-08-2024	05-10-2024	53
7	Lalima	20-08-2024	04-10-2024	45
8	Dhawal	21-08-2024	05-10-2024	46
	SE(m)	-	-	-
	$Cd_{0.05}$	-	-	-

Table 2: Sepal characters and ovary position in different guava cultivars

S. No.	Cultivars	Number of sepals	Sepal colour	Position of ovary: as compared to stamen	Number of petals	Petal colour
1	Lalit	Four	Green	Inferior	Six	White
2	Hisar Surkha	Four	Green	Inferior	Five	White
3	Hisar Safeda	Four	Green	Inferior	Five	White
4	Punjab Pink	Four	Green	Inferior	Five	White
5	L-49	Four	Green	Inferior	Six	White
6	Shweta	Four	Green	Inferior	Six	White
7	Lalima	Four	Green	Inferior	Five	White
8	Dhawal	Four	Green	Inferior	Five	White
	SE(m)	-	-	-	-	-
	$Cd_{0.05}$	-	-	-	-	-

Table 3: Flower size, number stamens, stamen and pistil length in different guava cultivars

S. No.	Cultivars	Flower size (mm)	Number of stamens	Stamen length (mm)	Pistil length (mm)
1	Lalit	30.34	286.33	11.37	10.60
2	Hisar Surkha	42.90	356.67	15.51	10.80
3	Hisar Safeda	44.23	365.00	15.81	10.37
4	Punjab Pink	40.64	268.00	13.50	11.73
5	L-49	45.34	413.67	19.76	15.07
6	Shweta	36.44	297.33	17.07	15.06
7	Lalima	36.20	311.33	13.52	10.12
8	Dhawal	33.68	202.00	12.58	9.41
	SE(m)	1.25	0.31	0.65	0.42
	$Cd_{0.05}$	3.78	0.93	1.97	1.27

Table 4: Pollen viability and pollen shape in of different guava cultivars

S. No.	Cultivars	Pollen viability (%)	Shape of pollen
1	Lalit	97.33 (80.70)	Triangular
2	Hisar Surkha	95.33 (77.551)	Triangular
3	Hisar Safeda	96.00 (78.68)	Triangular
4	Punjab Pink	94.00 (75.79)	Triangular
5	L-49	97.33 (80.70)	Triangular
6	Shweta	97.00 (78.68)	Triangular
7	Lalima	94.00 (73.83)	Triangular
8	Dhawal	94.67 (76.67)	Triangular
SE(m)		0.65	Triangular
$Cd_{0.05}$		1.96	Triangular

**Figures parenthesis are angular transformed values*

Table 5: Pollen germination percentage of different guava cultivars

S. No.	Cultivars	Pollen germination %		
		5 % sucrose + 50 ppm boric acid	10 % sucrose + 50 ppm boric acid	15 % sucrose + 50 ppm boric acid
1	Lalit	48.66 (44.21)	70.00 (56.77)	63.33 (52.71)
2	Hisar Surkha	72.00 (58.03)	78.66 (62.50)	74.66 (59.76)
3	Hisar Safeda	56.00 (48.43)	73.33 (58.93)	65.33 (53.92)
4	Punjab Pink	49.33 (44.60)	69.33 (56.36)	56.66 (48.82)
5	L-49	64.00 (53.11)	73.33 (58.90)	67.33 (55.13)
6	Shweta	60.00 (50.75)	74.66 (59.78)	68.66 (55.95)
7	Lalima	55.33 (48.04)	68.00 (55.53)	58.00 (49.58)
8	Dhawal	45.33 (42.30)	63.33 (52.71)	51.33 (45.74)
SE(m)		1.57	1.70	1.54
$Cd_{0.05}$		4.82	5.23	4.73

**Figures parenthesis are angular transformed values*

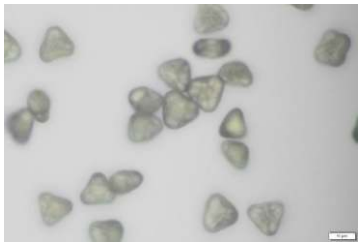

Plate 1: Unstained pollen grains

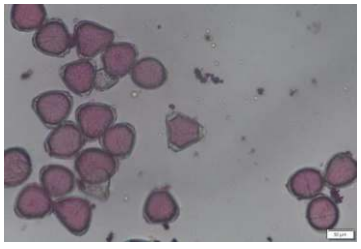

Plate 2: Pollen grains stained with 2 percent acetocarmine dye

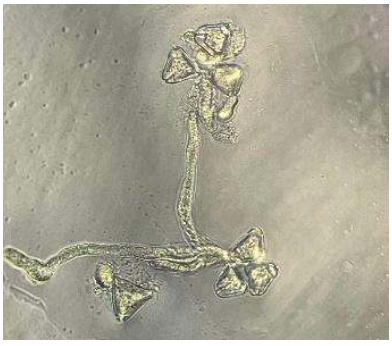

Plate 3: Pollen germination in 10 percent sucrose + 50ppm boric acid solution

Plate 4: Pollen germination in 10 percent sucrose + 50ppm boric acid solution

Conclusion

The investigation into the floral biology of various guava cultivars in the sub-tropical environment of Jammu demonstrated notable differences in flowering behavior and floral characteristics among the genotypes. The L-49 cultivar stood out as the most advantageous, exhibiting the earliest onset of flowering, the shortest flowering duration, the largest flower diameter, and the most significant dimensions of both stamen and pistil, alongside exceptional pollen viability. In contrast, Hisar Safeda was noted for having the longest flowering period, while Lalit and Dhawal displayed smaller floral structures and lower reproductive metrics. The optimal pollen germination was achieved using a solution of 10 % sucrose combined with 50 ppm boric acid, highlighting its potential for *in vitro* pollen research and hybridization efforts. Overall, the results indicate that L-49 and Hisar Surkha possess valuable floral and reproductive attributes that could be leveraged in future guava crop improvement and breeding initiatives.

Future scope

The current study on the floral biology of guava (*Psidium guajava* L.) genotypes has provided significant insights into the variations in flowering behavior, floral morphology and pollen characteristics across various cultivars. This knowledge would assist in selecting optimal parental combinations for hybridization initiatives designed to enhance fruit yield and quality. Moreover, investigations into pollination ecology, encompassing pollinator diversity and activity patterns, could play a crucial role in improving fruit set and productivity in field settings. The integration of floral biology findings with breeding and crop management strategies would promote better synchronization of flowering, effective hybridization, and overall advancements in guava cultivation.

Conflict of interest

The authors affirm that there are no conflicts of interest pertaining to the publication of this manuscript.

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