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Studies on Pollen Morphology and Crossing Behavior of Guava (*Psidium guajava* L.) Cultivars in the Subtropics of North-West Himalayas

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ABSTRACT: The present investigation was conducted at the Fruit Research Farm located in Himachal Pradesh, to assess the pollen morphology, viability and hybridization potential of seven commercially important guava (*Psidium guajava* L.) cultivars, namely Arka Amulya, Allahabad Safeda, Shweta, Lucknow-49, Hisar Surkha, Lalit and Hisar Safeda. The study aimed to characterize the pollen attributes of genetically distinct genotypes and evaluate their cross-pollination potential in relation to fruit set. Pollen viability was assessed using a 2% acetocarmine staining method, varied between 84.83% and 96.27%, with 'Lalit' exhibiting the highest viability (96.27%) and 'Allahabad Safeda' the lowest (84.83%). These differences in viability are likely influenced by inherent genetic variation, cultivar-specific traits, and environmental conditions such as temperature and precipitation. Microscopic examination revealed consistently rounded triangular pollen morphology across all genotypes. Controlled hybridization trials involving ten distinct cross combinations indicated that the highest fruit set (68.33%) occurred in the 'Allahabad Safeda × Lalit' cross. Variability in fruit set percentages among the crosses may be attributed to differences in genotype compatibility, pollen viability, pollen tube growth dynamics and ambient environmental conditions during anthesis. The findings underscore the significance of pollen biology in identifying compatible parental combinations, thereby aiding guava breeding strategies aimed at improving fruit set and cultivar development.

Keywords: Guava, Pollen, Viability, Morphology, Acetocarmine, Hybridization, Cultivars.

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the significant fruit crops well adapted to both tropical and sub-tropical areas of the world. Guava is originated in tropical America extending from Mexico to Peru. In India Portuguese introduced guava during 17th century (Menzel, 1985). Guava is a highly popular fruit due to its rich nutritional content, affordable price, delightful aroma and flavor. It's enjoyed by everyone, regardless of their economic background, earning it the nickname "apple of the tropics." Guava is particularly notable for being an excellent source of Vitamin C and pectin. A typical guava fruit is composed of 82.5% water. It also contains 2.45% acids, 4.45% reducing sugars, and 5.23% non-reducing sugars, contributing to a total soluble solid (TSS) of 9.73%. Additionally, it has 0.48% ash and a remarkable 260 mg of Vitamin C per 100g of fruit. It's worth noting that these specific values can vary depending on the cultivar, its stage of maturity and the season (Dinesh and Vasugi 2010). Guava fruits are remarkably rich in essential nutrients, so much so that they're often called "Super-fruits." Consuming them, whether fresh or processed, offers a simple and effective way to help overcome dietary deficiencies (Chaplin Kramer *et al*., 2014; Sethi *et al.*, 2005). India is the largest producer of guava in the world with annual production of 5262.73 thousand MT with an area coverage of 357.64 thousand hectares (Anonymous, 2024). Guava is found growing from plains to higher hills owing to its wider adaptability. It is considered to be hardy fruit crop and also, is not very choosy for soil. The cultivation of guava in Himachal Pradesh covers 3,030 hectares, with an annual output of 5,090 metric tons and a productivity measure of 1.67 metric tons per hectare (Anonymous, 2024).

Pollen morphology in fruit species encompasses the structural attributes of pollen grains, which play a vital role in plant taxonomy, reproductive biology, and species differentiation. These characteristics include size, shape, surface ornamentation, and aperture type, all of which exhibit notable interspecific variability. For instance, pollen size varies widely, with peach producing some of the longest pollen grains, while walnut shows the greatest breadth (Evrenosoğlu & Misirli 2009). In terms of shape, common forms include prolate, suboblate and perprolate, with prolate spheroidal and subprolate types being predominant across many fruit-bearing species (Araújo *et al*., 2023). Surface ornamentation, another key diagnostic feature, may appear as striate, rugulate, or tectum imperforatum patterns, which assist in species-level identification. Distinct surface textures have been documented in species such as walnut and persimmon, contributing to their morphological differentiation (Evrenosoğlu & Misirli 2009). Additionally, pollen aperture types, such as inarperturate, colpate and colporate, vary across taxa, with a 3-colporate arrangement being the most frequently observed (Araújo *et al*., 2023). Despite its usefulness, pollen morphology may present challenges for identification due to overlapping features among closely related species within the same family (Yang *et al.,* 2012). These complexities underscore the importance of integrating palynological traits with other taxonomic tools for accurate classification and reproductive analysis.

Studies on the pollen morphology and crossing behavior of guava (*Psidium guajava* L.) provide valuable insights into its reproductive biology and have practical implications for cultivar improvement and orchard management. Research demonstrates that the selection of an appropriate pollen parent can significantly influence fruit quality and size through metaxenial effects. For example, the use of cv. Round as a pollen donor in crosses with cv. Pyriformed resulted in a 25.4% increase in fruit diameter and a 64 mg/100 g increase in ascorbic acid content in the pulp (Usman *et al.,* 2013). Such intervarietal crosses underscore the importance of pollen parent selection in breeding programs aimed at enhancing fruit traits. Regarding pollination dynamics, guava exhibits a positive response to cross-pollination, with hand cross-pollination increasing fruit set by 39.5% compared to self-pollination (Alves & Freitas 2007). The role of biotic pollinators is also critical, as open-pollinated flowers show superior fruit set compared to those under restricted pollination conditions. In terms of palynology, guava pollen grains display a range of sizes and shapes, typically measuring between 15.6 µm and 25.2 µm in diameter (Chezhiyan, 1989). Pollen viability is highly influenced by storage conditions, with low-temperature storage preserving viability for up to 210 days. Although cross-pollination generally promotes higher yields, certain guava cultivars exhibit self-compatibility, enabling fruit development in the absence of external pollinators; a trait advantageous for cultivation in isolated or pollinator-deficient environments. These findings collectively emphasize the importance of understanding pollen traits and pollination mechanisms for effective guava breeding and cultivation strategies.

MATERIALS AND METHODS

Place of Experiment: The experiment was conducted during the year 2018-19 at the experimental farm of the College of Horticulture and Forestry at Neri, Hamirpur with the coordinate of 31°41′47.6″N latitude and 76°28′6.3″ E longitude with an altitude of 650m above mean sea level in the sub-tropical zone of Himachal Pradesh for the evaluation of seven genetically diverse commercial guava (*Psidium guajava* L.) cultivars (Table 1) for pollen morphology and viability.

Pollen Collection: Flower buds at the half calyx break stage were collected separately for each variety. Using needles, anthers were carefully separated from the filaments and placed on non-sticky paper inside petri dishes. These dishes were kept under partial shade to promote anther dehiscence. Once dehiscence occurred, the released yellow powdery pollen mass was collected. The pollen grains were then stored in glass vials and small portions were taken out from these vials for viability testing and further analysis. For hybridization studies, freshly dehisced and small samples of pollen grains were drawn out from these vials for cross pollination studies.

Table 1: Guava genotypes used in current investigation.

Sr. No.	Cultivars/Hybrids	Parentage
1.	Lalit	Selection from half - sib population of Apple Colour
2.	Shweta	Selection from half - sib population of Apple Colour
3.	Lucknow-49	Open pollinated seedling selection from Allahabad Safeda
4.	Allahabad Safeda	Open pollinated seedling
5.	Arka Amulya	Allahabad Safeda × Seedless (Triploid)
6.	Hisar Safeda	Allahabad Safeda × Seedless
7.	Hisar Surkha	Apple Colour × Banarasi Surkha

Pollen viability (Acetocarmine solution 2% test): Freshly collected pollen grains of guava cultivars *viz*., Arka Amulya, Allahabad Safeda, Shweta, Lucknow-49, Hisar Surkha, Lalit and Hisar Safeda was used to determine the viability of pollen. The experimental design used was Completely Randomized Design.

Acetocarmine solution (2%): Carmine indicates that cytoplasm is present. Viable pollen stains pink to deep red with aceto-carmine, whereas sterile (mostly shrivelled) pollen does not take any stain and stays nearly white and transparent. The pollen nucleus is rich in chromatin material (Marutani *et al.*, 1993).

Procedure: Pollen viability was assessed using a freshly prepared 2% acetocarmine solution, following the protocol by Kumar and Kaur (2019). This solution was created by dissolving 2 grams of carmine powder in 45 ml of glacial acetic acid, then bringing the total volume to 100 ml with distilled water. After gentle boiling, the mixture was filtered through Whatman No. 1 filter paper. For examination, mature pollen grains were mounted in an equal parts glycerol-acetocarmine mixture. Pollen was carefully dusted onto a glass slide, subsequently treated with one to two drops of the staining solution, and then covered with a coverslip. To facilitate proper stain uptake, each slide remained undisturbed for 4 to 5 minutes. Microscopic analysis of approximately 500 to 700 pollen grains per slide was performed to determine both pollen viability and size. Across each cultivar, 10 to 20 fresh slides were prepared and thoroughly examined. Pollen grains exhibiting a full appearance and stained nuclei were classified as viable, whereas those that appeared shriveled and lacked stain were deemed non-viable.

Pollen morphology (size and shape): Pollen grain dimensions (length and breadth) were determined using a Digital Imaging System microscope. The resulting images were then analyzed for pollen morphology with Biovis Image Plus software. Pollen shape was additionally observed following staining with a 2% acetocarmine solution.

Hybridization studies: Hybridization studies with different cross combinations (10) were done in the Experimental farm at College of Horticulture and Forestry, Neri (Hamirpur) Himachal Pradesh. Based on their salient features (Table 1), 5 female parents (Arka Amulya, Allahabad Safeda, Shweta, Lucknow-49 and Hisar Safeda) and 2 male parents (Hisar Surkha and Lalit) were crossed with different cross combinations. All of the opened flowers and immature buds were removed from the chosen branches. To stop self-pollination, unopened blossoms at the calyx break stage were emasculated by removing the sepals, petals, and stamens. The emasculated flower clusters were appropriately tied, labelled and covered with muslin cloth bags. Fresh pollen (for cultivars whose flowering periods coincided) or stored pollen (for cultivars whose flowering periods did not coincide) were used to pollinate the emasculated buds on the day of flowering. After applying the pollen to the stigmas using a camel hair brush, the stigmas were bagged. The purpose of labelling each twig was to prevent contamination. Once the fruit had set, the bags were taken out. The ratio of the number of pollinated buds to the number of buds that set into fruits was used to determine the percentage of fruit set in pollinated flowers. Fruit set percentage was calculated as

$$\text{Per cent fruit set} = \frac{\text{Number of buds which set into fruit}}{\text{Number of buds pollinated}} \times 100$$

The data from this study were statistically analyzed using the Randomized Block Design with Three Replications (3 branches per replication) methods proposed by Gomez and Gomez (1984). The statistical analysis was performed using OPSTAT and MS-Excel software for the observed characters.

RESULTS AND DISCUSSION

Pollen viability: The guava germplasm under investigation showed considerable variation in pollen viability. The pollen viability percentage using a 2% acetocarmine solution ranged from 84.83 to 96.27 (Table 2). The maximum pollen viability percentage was recorded in cultivar Lalit (96.27%) which was closely followed by Lucknow-49 (95.68%) while, the minimum viability (89.19%) was recorded in Allahabad Safeda variety (Table 2). Variations in pollen viability among different guava cultivars are likely attributed to their inherent genetic differences and distinct varietal traits (Jha *et al.*, 2020). These variations may also be related to the traits of the fruits and seeds that the pollen donor genotypes produce (Silva *et al.*, 2017).

Furthermore, environmental factors particularly fluctuations in temperature and rainfall during the flowering period can significantly impact pollen viability.

Cultivars exhibiting higher pollen viability are well suited to be used as donor parents in hybridization programs. Pollen viability is affected by multiple factors such soil fertility, climatic conditions and genetic composition. Sarkar and Sarkar (2022) observed that the pollen viability ranged from 89.19 - 93.67% in different guava genotypes. Tandel *et al.* (2024) in their investigation found recorded pollen viability was in the range of 72.50–92.96%. The results observed in our case are also comparable with their studies.

Table 2: Pollen viability (%) and morphology of guava cultivars.

Cultivars	Pollen viability (%)	Pollen size (µm)	Pollen shape
Lalit	96.27(9.86)	26.94 × 22.49 - 22.00 × 18.19	Rounded triangular
Allahabad Safeda	84.83 (9.26)	24.16 × 19.61-19.00 × 16.73	Rounded triangular
Shweta	94.24 (9.76)	25.17 × 22.58 - 21.48 × 18.14	Rounded triangular
Lucknow- 49	95.68 (9.83)	26.32 × 23.45 - 22.10 × 20.86	Rounded triangular
Arka Amulya	92.81 (9.69)	24.99 × 21.53 - 20.29 × 17.68	Rounded triangular
Hisar Surkha	92.27 (9.66)	25.46 × 22.48 - 21.93 × 19.12	Rounded triangular
Hisar Safeda	93.15 (9.70)	25.56 × 23.00 - 22.38 × 19.88	Rounded triangular
$CD_{0.05}$	0.042		

***Figures in parenthesis are square root transformed values**

Pollen morphology (size and shape): The fresh pollen grain length in the guava genotypes under investigation varied from 19.00 µm in Allahabad Safeda to 26.94 µm in Lalit (Table 3). In Lucknow-49, the fresh pollen grains' width ranged from 23.45 µm to 16.73 µm in Allahabad Safeda. Under a microscope, the pollen grains of seven different guava varieties had a rounded, triangular shape (Fig. 1). Similarly, Tandel *et al.* (2024) noted similar variations. Our findings aligned with their study.

Hybridization studies: Hybridization is a conventional approach in plant breeding, specifically employed to amalgamate desirable traits present within disparate germplasm. Subsequent to this intercrossing, the resultant hybrid progeny undergoes rigorous screening for target characteristics, facilitating their selection in either the F1 generation or in later generations. This methodology can culminate in the development of a novel cultivar or establish a valuable breeding line for subsequent genetic improvement programs (Jalikop, 2010).

The percentage of fruit set is directly correlated with the quantity and presence of flowers on the plant. The environment and the germplasm's genetic composition directly affect flowering (Babu *et al.*, 2011). In present investigation, the highest fruit set per cent in 10 crosses was observed in cross Allahabad Safeda × Lalit (68.33 %) which was statistically at par with cross Shweta × Lalit (66.67 %), Lucknow-49 × Lalit (61.67 %), Hisar Safeda × Hisar Surkha (50.00 %), Shweta × Hisar Surkha (47.22%), Lucknow-49 × Hisar Surkha (47.22%) and Hisar Safeda × Lalit (41.67 %) , while no fruit set was observed Arka Amulya × Lalit which might be due to the incompatibility in this cross combination. Whereas, the minimum fruit set was recorded in cross of Arka Amulya × Hisar Surkha (11.11%) (Table 3).

Table 3: Fruit set (%) from hybridization in different cross combinations in guava cultivars.

Cross combinations	Percent fruit set (%)
Lucknow-49 × Lalit	61.67 (51.90)
Allahabad Safeda × Lalit	68.33 (56.12)
Shweta × Lalit	66.67 (59.99)
Hisar Safeda × Lalit	41.67 (39.98)
Lucknow-49 × Hisar Surkha	47.22 (43.23)
Allahabad Safeda × Hisar Surkha	33.33 (29.99)
Shweta × Hisar Surkha	47.22 (43.49)
ArkaAmulya × Hisar Surkha	11.11(11.75)
Hisar Safeda × Hisar Surkha	50.00 (44.98)
$CD_{0.05}$	29.61

***Figures in parenthesis are square root transformed values**

In the present investigation, results depicted that mean fruit set was higher when Allahabad Safeda is used as female parent in the cross combination, while mean fruit set was lower when Arka Amulya is used as female parent in cross combination. It could be due to variation of genetic makeup and differential response of the germplasm to climatic conditions of the locality. Similar results were also reported by Singh *et al.* (2017), who found that Allahabad Safeda × Lalit had the highest fruit set (61.22%), followed by Allahabad Safeda × Shweta (55.56%) and Allahabad Safeda × Purple Guava (54.16%), while Allahabad Safeda × Arka Kiran had the lowest fruit set (45.83%). According to Dhaliwal and Rachna (2003), during the winter months, the Selection 4/12 variety had the highest fruit set percentage of 92.42% and the Dharwad variety had the lowest at 68.09%. Sarkar and Sarkar (2022) also observed fruit set in different cross combinations in between 89.04 % - 40.71 % which are in line with our study. Pollen viability and germination percentages are likely influenced by variations in pollen tube growth rates. Additionally, temperature fluctuations during the anthesis period could significantly affect pollen performance. While genetic factors primarily determine the potential for fruit set, environmental parameters exert a substantial modulating influence on its ultimate expression.

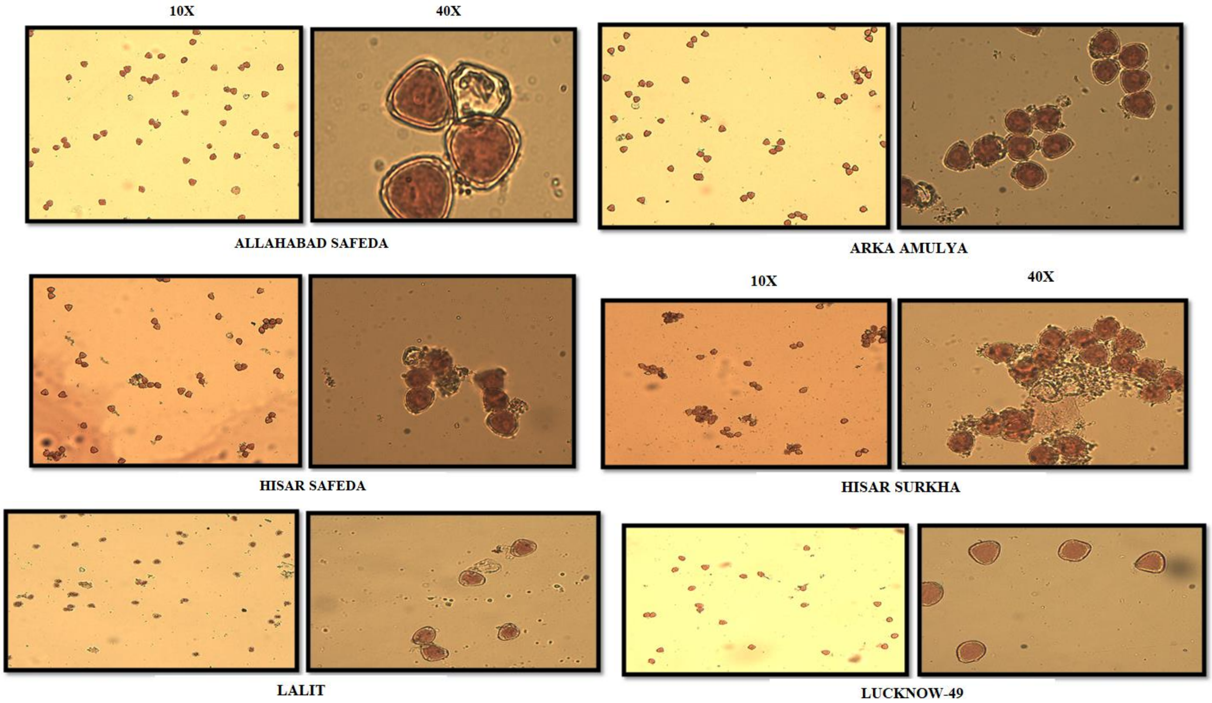

Fig. 1. Pollen Morphology of different guava cultivars.

CONCLUSIONS

The present investigation on pollen morphology, viability and hybridization potential of seven guava (*Psidium guajava* L.) cultivars provides meaningful insights into the reproductive biology of this economically important fruit crop. Significant variations were observed in pollen viability among cultivars, with 'Lalit' exhibiting the highest viability, making it a promising pollen donor for breeding programs. All cultivars demonstrated consistent rounded triangular pollen morphology, though pollen size varied notably across genotypes. These morphological and viability traits are crucial for understanding the compatibility and fertilization efficiency among different guava cultivars.

Hybridization studies revealed substantial differences in fruit set percentages across ten cross combinations. The cross 'Allahabad Safeda × Lalit' showed the highest fruit set, indicating a high degree of compatibility and hybrid vigor between these two cultivars. In contrast, combinations involving 'Arka Amulya' as the female parent exhibited poor fruit set, suggesting possible cross incompatibility or less favorable environmental-genetic interactions. Such variability underscores the importance of selecting compatible parent genotypes and optimizing environmental conditions for successful hybridization and fruit development.

Overall, this study highlights the relevance of palynological traits and controlled pollination strategies in guava improvement programs. Understanding pollen behavior, viability under storage, and cross compatibility can guide breeders in developing superior guava hybrids with enhanced fruit set, size, and nutritional quality. The findings not only support strategic parent selection in breeding programs but also provide a foundation for future research aimed at improving reproductive efficiency and yield potential in guava under diverse agro-climatic conditions.

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