

Pollination characteristics and intervarietal hybridization of *Psidium guajava*

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ABSTRACT

The experiment was carried out on 11 guava genotypes, with various sorts of crosses used to decipher the reproductive biology, including intervarietal compatibility. Pollen grains of various types germinated in vitro, according to microscopic analysis. The 10 per cent sugar concentration resulted in the highest pollen germination in all the 11 guava genotypes. Pollen grain germination decreased as the sucrose concentration in the solution increased, except in the Lucknow-49 variety, where pollen grain germination increased to 1.79 per cent by increasing the sucrose percentage from 10 to 15% and then decreased as the sucrose concentration was increased further. The maximum fruit set (87.72%) was recorded under open pollination. Self pollination can also result in fruit set, however cross pollination results in a larger fruit set and yield. The intervarietal crosses revealed that the incompatibility between varieties is relatively low. The hybrid between Baruipur and Khaja resulted in enormous, high-quality fruits.

Keywords: Fruit set, guava, pollen viability, pollen germination and crossing

Guava (*Psidium guajava* L.) is a tropical and subtropical perennial fruit tree with significant economic potential (Sethi *et al.*, 2007; Pathak and Ojha, 1993). Guava fruits are abundant in high-profile nutrients and are frequently referred to as “Super-fruits.” If eaten as a fresh or processed fruit, it can help to solve nutritional problems (Chaplin Kramer *et al.*, 2014; Sethi *et al.*, 2007). The goal of this study was to gain a better understanding of guava reproductive biology and intervarietal hybridization. Guava breeding procedures are not well developed, so the majority of cultivated genotypes were discovered by studying solely fruit sizes and production characteristics.

Guava flowers appear alone or in a cyme of two or three buds that emerge from the young shoot’s leaf axils. Traditional breeding methods in guava are limited by its floral shape (epigynous flower with profuse inserted stamens of varying sizes), pollen viability, extended juvenile period, self-incompatibility and heterozygous nature (Coser *et al.*, 2012; Usman *et al.*, 2013). Honey bees (*Apis* spp.), solitary bees (*Lasioglossum* spp.), Bumble bees (*Bombus* spp.), Stingless bees (Heard, 1999) species of the genera *Trigona*, *Bombus mexicanus*, and *Xylocopa* sp. are the main pollinators (Hedstrom, 1988). Guava blooms can self-pollinate because pollen is commonly deposited on the stigma of individual flowers (Singh and Sehgal, 1968). Pollen does not display self-incompatibility, therefore fruit set through selfing is conceivable, while cross-incompatibility does occur in guava (Balasubramanyam, 1959). Guava pollination occurs in a variety of ways, although cross-pollination yields substantially more fruit than self-pollination and restricted pollination (Alves and Freitas,

2007). Guava blossoms are said to self-pollinate to the tune of 35-40%. There are additional reports of cleistogamy and the absence of self-incompatibility (Rachna-Singla and Dhaliwal, 2003; Zapori *et al.*, 2007). The mode of pollination in guava, on the other hand, is not well understood.

Guava is plagued by a variety of agronomic issues, including infections, primarily *Fusarium oxysporum* *pv. psidii*, high seed content, poor fruit growth, and short shelf life (Rai *et al.*, 2010), tree of medium height with coloured fruit pulp and wilt resistance (Chandra and Mishra, 2007). The breeding goals are to create a dwarf tree with a high yield, decent fruit size, and a long shelf life, as well as a dwarfing rootstock that is wilt resistant. Wide genetic basis, intervarietal hybridization, and interspecific hybridization to generate resistant rootstock and improved cultivars, dwarfing rootstock, and induced mutation and in-vitro change of somatic cells were also proposed by Negi and Rajan (2007).

In India, there are many cultivars of guava, but only a few commercially important types, such as ‘Allahabad Safeda’ and L-49 (Sardar), have been identified for commercial cultivation. The majority of guava cultivars in India have evolved through seedling selection and commercial multiplicities via vegetative propagation. However, not all members of the commercial population are faithful to type, and numerous traits differ from those of the parent population (Saxena *et al.*, 2007). The seedling population’s heterogeneity is owing to frequent natural pollination and heterozygosity, and new promising genotypes have emerged from the seedling population (Dinesh and Vasugi, 2010).

MATERIALS AND METHODS

Experimental site and germplasm used

The present experiment was undertaken at Horticultural Research Station (Fig. 1), Mondouri, Bidhan Chandra Krishi Viswavidyalaya, selecting 3 years old plantation of guava having 11 genotypes *viz.*, Arka Kiran, Kohir Safeda, Arka Amulya, Hissar Surkha, Safed Jam which were hybrids, while Khaja, Baruipur, Lalit, Allahabad Safeda, Lucknow-49, Sweta, Philipines, China, were selection. All genotypes were obtained from different university and nursery. Some of the genotypes were commercially cultivated in different locations for their high yielding and quality of fruits.

$$\text{Pollen germination (\%)} = \frac{\text{Total number of germinated pollen}}{\text{Total number of pollen in microscopic field}} \times 100$$

Pollen viability was assessed by collecting freshly opened flower from each genotype and brought to the laboratory. Pollens were shed in the petri dish from freshly dehiscence anthers and placed on the cavity slides. About 2 drops of freshly prepared 2 per cent acetocarmine solution were added to the cavity and covered gently with a cover slip. After staining, the pollen grains were examined by size, morphology and

$$\text{Pollen viability (\%)} = \frac{\text{Total number of viable pollen in microscopic field}}{\text{Total number of pollen in microscopic field}} \times 100$$

Pollination procedures and intervarietal crossing

To understand the natures of pollination, following treatments were used a) Self-pollination (by bagging): floral buds which would open the next day were bagged and protected from any possible visit of pollinator. b) Geitonogamy (manually): this treatment was performed before anthesis of flowers by emasculating at calyx breaking stage then covering the buds with brown paper bags. Following day, emasculated flowers were pollinated with the collected pollen from the freshly opened flower of the same tree and then covered with paper bags to restrict any contamination. c) Cross-pollination (manually): the emasculated flower was pollinated by pollen collected from the freshly opened flower of other variety, after pollination, flowers were covered to restrict contamination (Fig. 2). d) Open pollination: in order to know the extent of natural pollination of flowers by the action of pollinators in the orchard, just by tagging of flowers. 50 flowers were used in each treatment and treatments were replicated in 6 trees in a randomized design. After imposing the treatments, the developing fruits were counted at 15 days after anthesis. Fruit set was estimated by number of developing fruits divided by total number of flowers used in each treatment. Intervarietal crosses (Table 1)

In-vitro pollen germination and pollen viability

In-vitro germination was evaluated by utilizing freshly opened flowers by petri dish and agar method. The germination media were 10%, 15%, 20% and 25% sucrose along with 2% agar in each medium. The slides were placed in the moist chamber and incubated at 28° C. The slides were observed under the microscope frequently till a maximum number of pollen found germinated extended to 3 hrs. Germinating pollens in 3 random microscopic fields were counted. The pollen tube length was measured using a micrometre eyepiece (calibrated using a stage micrometre). The pollen germination percentage was calculated as

staining capacity. Pollen grains with more coloration, larger in size, and outline clear visible were considered viable, and pollen grains with lighter or no staining, smaller in size, and outline is irregular were classified as non-viable. Three slides were evaluated for each genotypes/ treatments.

were done by using female parent Allahabad Safeda and local most cultivated variety Baruipur Local which has long shelf life with medium fruit size.

Biochemical estimation

A digital hand refractometer was used to determine the total soluble solid (TSS) (^oBrix) content of fruit juice. With the 2,6-dichlorophenol indophenol dye, ascorbic acid (percent) was determined. By titrating a known volume of fruit juice against N/10 sodium hydroxide (NaOH) and using phenolphthalein as an indicator, titrable acidity (percent) was calculated. Using 10ml potassium ferricyanide + 5ml 20 percent N sodium hydroxide solution with methylene blue as an indicator, total sugar was calculated using the anthrone method. Pectin concentration was calculated using the gravimetric technique and expressed as percent calcium pectate /100 g pulp.

Statistical analysis

The study's findings were analysed using an RBD design, and the treatment means were compared using least significant difference (LSD) values at a significance level of $P \leq 0.05$ utilising Statistical Analysis System 9.3 methods.

RESULTS AND DISCUSSION

The result of pollen viability (Table 2) revealed that the viability ranged from 89.19 - 93.67% in different guava genotypes. The pollen grain had a maximum viability of 93.67% in China variety which was closely followed by 92.88% in Hissar Surkha variety, while the minimum viability (89.19%) was recorded in Sweta variety. Moreover, the varieties having more adaptations in a humid ecosystem like China (93.78%), Lucknow-49 (91.86%) showed more pollen viability than new introductions like Sweta and Lalit.

Regarding the data of pollen germination in Table 3 it varied significantly among the genotypes. For most genotypes, the maximum pollen grains were germinated in a 10% sucrose solution. However, in some varieties pollen germination was highest in 15% sucrose solution. The maximum pollen germination (81.52%) was observed in Hissar Surkha at 10% sucrose solution followed by Kohir Safeda (78.56%), where as minimum pollen germination (51.16%) was seen in Lalit at 25% sucrose solution. Except in Lucknow-49, where germination (76.65 per cent) increased in 15 per cent sucrose solution over 10 percent sucrose solution, pollen germination decreased as the proportion of sucrose concentration increased (72.86 per cent).

The data for nature of pollination (Table 4) showed significant variation on fruit set. More than 2000 flowers were pollinated to understand the nature of pollination. The result showed significant difference in nature of pollination on fruit set. The highest fruit set (89.03%, 86.40% and 87.72%) was recorded in open pollination; where as lowest fruit set (42.28%, 39.13% and 40.71%) was recorded in self-pollination during rainy, winter season and pooled respectively.

Among different crosses maximum fruit weight (Table 5, Fig. 3) was recorded in Baruipur × Khaja (132 gm) followed by Allahabad Safeda X Arka Kiran (130 gm) and Baruipur × Sweta (123.67 gm); however minimum fruit weight was observed in Allahabad Safeda × Sweta (107.67 gm) followed by Baruipur × Lucknow-49 (116 gm). The maximum fruit width was recorded in Baruipur × Khaja (6.17 cm) followed by Allahabad Safeda × Arka Kiran (6.00 cm); whereas minimum fruit width (5.00 cm) was observed in Baruipur × Lucknow-49 followed by Allahabad Safeda × Shweta (5.33 cm). Maximum fruit length was recorded in Allahabad Safeda × Arka Kiran (6.43 cm) followed by Allahabad Safeda × Sweta (6.23 cm); whereas minimum fruit length (5.17cm) was recorded in Allahabad Safeda × Lalit followed by Allahabad Safeda × Lucknow-49 (5.30 cm).

Table 1: Crosses combination of guava genotypes

Crossings			Number of crossings	Success of crossings	Success (%)
Allahabad Safeda	X	Sweta	57	23	40.35
Allahabad Safeda	X	Lalit	45	19	42.22
Allahabad Safeda	X	Arka Kiran	45	34	75.56
Allahabad Safeda	X	Khaja	54	26	48.15
Allahabad Safeda	X	L-49	50	31	62.00
Baruipur	X	Sweta	50	35	70.00
Baruipur	X	Lalit	46	36	78.26
Baruipur	X	Arka Kiran	50	41	82.00
Baruipur	X	Khaja	45	42	93.33
Baruipur	X	L-49	50	39	78.00

Table 2: Pollen viability percentage of different guava genotypes

Varieties/hybrids	Total no. of pollen grain studied	No. of pollen stained	Per cent of pollen viability
Lalit	641	576	89.85
Allahabad Safeda	594	534	89.89
Lucknow-49	590	542	91.86
Sweta	540	481	89.19
Phillipines	549	489	89.07
China	632	592	93.67
Kohir Safeda	576	529	91.89
Arka Amulya	612	552	90.19
Hissar Surkha	689	640	92.88
Safed Jam	548	498	90.87
Arka Kiran	563	512	90.94
SEm(±)		1.93	
LSD (0.05)		3.96	

Table 3: Effect of different sucrose concentrations on pollen germination (%) of guava genotypes

Varieties / Hybrids	10% sucrose + 2% agar	15% sucrose + 2% agar	20% sucrose + 2% agar	25% sucrose + 2% agar
Lalit	73.51±2.09	59.44±5.49	51.21±0.93	51.16±0.58
Allahabad Safeda	68.74±4.75	53.79±1.24	51.41±3.70	51.47±1.24
Lucknow-49	72.86±1.43	76.65±1.78	64.65±1.74	58.07±0.67
Sweta	73.40±5.07	65.73±1.91	63.52±1.92	54.66±3.41
Philippines	69.73±1.17	70.60±3.43	60.70±1.65	57.39±1.50
China	76.76±1.39	71.67±0.81	65.34±1.40	61.35±2.25
Kohir Safeda	78.56±0.57	70.02±3.71	66.90±1.51	63.64±2.77
Arka Amulya	75.19±1.91	68.52±3.12	63.67±1.26	64.29±4.95
Hissar Surkha	81.52±1.45	75.44±1.95	67.54±1.62	62.41±6.42
Safed Jam	76.53±2.53	70.66±1.84	66.36±0.99	66.57±3.30
Arka Kiran	75.25±1.00	66.25±1.00	64.25±1.00	63.25±1.00
LSD (0.05)	4.55	4.90	3.14	5.57

Table 4: Effect of different natures of pollination on fruit set (%)

Type of pollination	Rainy season	Winter season	Pooled
Self pollination	42.28	39.13	40.71
Geitonogamy (manually)	74.83	72.20	73.52
Cross-pollination (manually)	79.48	78.04	78.76
Open pollination	89.03	86.40	87.72
SEm (±)	1.80	0.92	0.83
LSD (0.05)	2.84	1.80	2.36

Table 5: Fruit and seed characteristics of different crossing combinations

Crossings	Fruit weight (gm)	Fruit width (cm)	Fruit Length (cm)	Outer flesh thickness (cm)	Total seed number fruit ⁻¹	Total seed weight fruit ⁻¹ (gm)
Allahabad Safeda × Shweta	107.67±4.93	5.33±0.21	6.23±0.15	1.07±0.06	240.67±4.51	2.16±0.05
Allahabad Safeda × Lalit	117.67±5.69	5.93±0.42	5.17±0.06	1.07±0.15	197.67±3.06	1.69±0.05
Allahabad Safeda × Arka Kiran	130.00±5.29	6.00±0.10	6.43±0.06	1.20±0.10	224.33±9.50	2.09±0.09
Allahabad Safeda × Khaja	119.67±4.04	5.50±0.10	6.13±0.06	1.27±0.06	176.33±8.08	1.87±0.02
Allahabad Safeda × Lucknow-49	118.00±7.55	5.70±0.10	5.30±0.26	1.43±0.06	177.33±9.71	1.51±0.05
Baruipur × Sweta	123.67±7.77	5.77±0.15	6.20±0.08	1.20±0.10	206.33±7.23	1.79±0.03
Baruipur × Lalit	121.67±9.29	5.63±0.12	6.04±0.05	1.33±0.06	166.33±8.08	1.22±0.06
Baruipur × Arka Kiran	120.00±4.00	5.43±0.15	5.93±0.10	1.10±0.17	177.33±8.02	1.44±0.02
Baruipur × Khaja	132.00±4.00	6.17±0.21	6.10±0.15	1.50±0.10	215.33±6.11	1.79±0.01
Baruipur × Lucknow-49	116.00±5.00	5.00±0.17	5.70±0.20	0.87±0.06	199.67±4.16	1.86±0.03
LSD (0.05)	10.84	0.35	0.24	0.17	12.92	0.08

Table 6: Biochemical analysis of different crossing combinations

Crossings	TSS (°Brix)	Acidity (%)	Ascorbic Acid (mg 100g ⁻¹ fruit)	Total Sugar (%)	Reducing sugar (%)	Non reducing sugar (%)	Pectin content (%)
Allahabad Safeda × Sweta	8.43±0.50	0.23±0.06	152.33±2.08	6.35±0.10	3.36±0.18	2.99±0.11	0.82±0.05
Allahabad Safeda × Lalit	10.17±0.06	0.21±0.04	150.67±1.53	6.32±0.17	3.54±0.10	2.78±0.13	0.70±0.01
Allahabad Safeda × Arka Kiran	10.63±0.45	0.17±0.03	175.00±1.73	7.18±0.06	4.08±0.12	3.10±0.10	0.86±0.03
Allahabad Safeda × Khaja	8.97±0.40	0.20±0.03	191.67±3.51	5.98±0.25	3.13±0.40	2.85±0.16	0.76±0.02
Allahabad Safeda × Lucknow-49	9.50±0.61	0.21±0.03	162.33±3.51	5.94±0.44	2.90±0.23	3.05±0.23	0.74±0.01
Baruipur × Sweta	8.97±0.21	0.19±0.01	165.00±2.89	6.81±0.57	3.85±0.35	2.96±0.23	0.78±0.01
Baruipur × Lalit	8.10±0.52	0.23±0.01	157.00±2.00	6.37±0.08	3.82±0.15	2.55±0.19	0.81±0.01
Baruipur × Arka Kiran	9.93±0.06	0.19±0.01	177.33±2.08	6.07±0.24	3.57±0.09	2.50±0.17	0.82±0.01
Baruipur × Khaja	9.50±0.52	0.22±0.02	186.33±1.00	6.01±0.13	2.82±0.21	3.19±0.34	0.80±0.01
Baruipur × Lucknow-49	9.07±0.23	0.20±0.01	162.33±0.58	6.28±0.16	3.54±0.08	2.74±0.23	0.80±0.02
LSD	0.73	0.1	10.35	0.46	0.71	0.37	0.04

Table 7: Morphological and seed character of F₁ fruit of different crossing combinations

Crossings	Fruit peel color	Flesh colour	Fruit surface	Seed size
Allahabad Safeda × Sweta	Yellow White	White	Smooth	Medium
Allahabad Safeda × Lalit	Light Green	White	Smooth	Medium
Allahabad Safeda × Arka Kiran	Yellow White	Creamish white	Smooth	Small
Allahabad Safeda × Khaja	Deep green	White	Rough	Bold
Allahabad Safeda × Lucknow-49	Light Green	White	Smooth	Medium
Baruipur × Sweta	Light Green	White	Smooth	Medium
Baruipur × Lalit	Light Green	White	Smooth	Small
Baruipur × Arka Kiran	Light Green	Creamish white	Smooth	Small
Baruipur × Khaja	Deep Green	White	Rough	Medium
Baruipur × Lucknow-49	Deep Green	Yellowish White	Smooth	Medium

The maximum outer flesh (1.50 cm) was recorded in Baruipur × Khaja which was closely followed by Allahabad Safeda × Lucknow-49 (1.43 cm); Allahabad Safeda × Sweta and Allahabad Safeda × Lalit crosses had no significant difference among them but were significantly minimum outer flesh thickness (1.07 cm). The data pertaining to total seed number/fruit (Fig. 4) was less recorded in Baruipur × Lalit (166.33) which was also minimum in total seed weight/fruit (1.22 gm).

Pollination of flowers in the field with the help of emasculation blooms prior to anthesis. In the experiment, flower buds were employed to obtain uncontaminated stigmas for pollen grain germination by mechanical pollination in all crossings. The maximum fruit set was in open pollination, which is important for understanding the nature of pollination

(87.72 percent). Alves and Freitas (2007) observed that the guava cv. Paluma has a number of pollination restrictions related to fruit set in their investigation of the pollination requirements of the guava cv. Paluma. As a result, when self-pollination was utilised, only 28% of the fruits were harvested, compared to 61.7 percent when open pollination was used. Pollen grains are already dispersible and have a high vitality (95.6 percent) at the time of anthesis, whilst stigmas are receptive (Siqueira *et al.*, 2012). Pollination stimulates auxin synthesis in the pistil by providing a stimulation to the ovary. The stimulus was not accessible in the case of self-pollination, resulting in more fruit drop. Cross-pollination helps to boost yield and adaptability. When compared to other cropping seasons, Dubey *et al.* (2004)

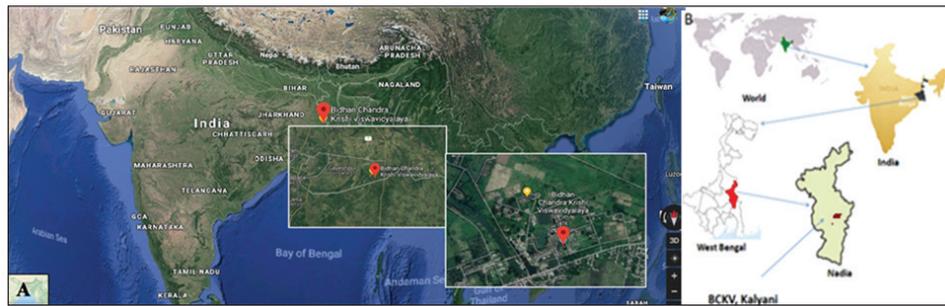


Fig. 1: Location map of experimental site (22.9452° N, 88.5336° E). A. Satellite view, B. Schematic view (not to scale).

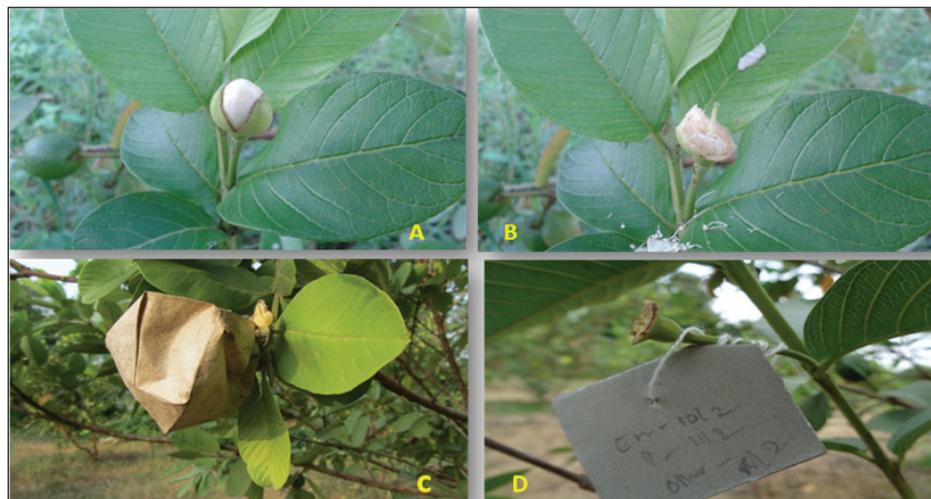


Fig. 2: A: Calyx breaking stage of flower; B: Emasculation of flower; C: bagged after emasculation of flower; D: Fruit set after crossing.



Fig. 3: F₁ fruits of different crossing combinations of guava



Fig. 4: Seeds of different crossing combinations

found that the winter season crop has the most fruit set and fruit retention.

The highest total soluble solids ($^{\circ}$ Brix) content was obtained in Allahabad Safeda X Arka Kiran (10.60 $^{\circ}$ Brix), which was significantly higher than other evaluated crosses (Table 6), whereas, the lowest titrable acidity was recorded also in Allahabad Safeda X Arka Kiran (0.17%) and the highest titrable acidity was recorded in Allahabad Safeda X Sweta and Baruipur X Lalit (0.23%) which were significantly higher than all other crosses. The highest ascorbic acid was recorded in Allahabad Safeda X Khaja (191.67 mg 100g $^{-1}$ fruit) which was closely followed by Baruipur X Khaja (186.33 mg 100g $^{-1}$ fruit), whereas the minimum ascorbic acid was recorded in Baruipur X Lalit (157 (186.33 mg 100g $^{-1}$ fruit). The highest total sugar, reducing and non-reducing sugar and pectin content was recorded in Allahabad Safeda X Arka Kiran (7.18, 4.08, 3.10 and 0.86 % respectively) cross, whereas the lowest pectin content (0.70%) was recorded in Allahabad Safeda X Lalit.

The fruit surface color of different crosses were, five crossings had light green color, three had deep green in color and two crossings had yellowish white (Table 7). Two crossings were showed creamish white color in Allahabad Safeda X Arka Kiran and Baruipur X Arka Kiran among all other crossings which may be due to Arka Kiran has pink flesh and has also small seed size with smooth fruit surface.

Intervarietal hybridization is a successful approach for crop improvement and variety development. Guava fruit quality is determined by the colour, surface, biochemical qualities, and seed content of the fruit. The major goals in guava breeding are to create new types with low seed content and good fruit quality. With the

goal of developing high yielding, superior quality, and low/soft seeded fruit, a total of 326 F1 hybrids were created using two female parents, Allahabad Safeda and Baruipur, and five male parents, Lalit, Shweta, Khaja, Arka Kiran, and Lucknow-49. Because guava is primarily a cross pollination crop, it has a high heterozygosity. Allahabad Safeda, a commercial guava variety, was crossed with other possible types with favourable agronomic characteristics such as low seed content and coloured flesh. The resulting hybrids have shown to be of good quality, with medium-sized fruits. Larger fruits with improved quality in terms of TSS, ascorbic acid, total sugar, and pectin content came from crosses between indigenous genotypes such as Baruipur and Khaja.

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