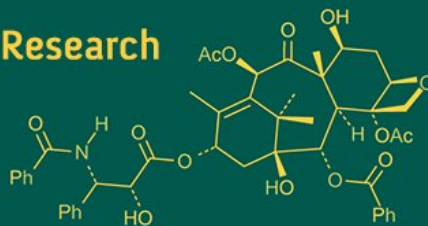


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Study of floral and pollen characteristics of selected custard apple genotypes under Akola conditions

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Abstract

The present investigation was conducted during 2025–26 at the Central Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, to study the floral biology and pollen behaviour of selected custard apple (*Annona squamosa* L.) genotypes. Five genotypes, namely Balanagar, CA-1, CA-2, RP-1 and RP-2, were evaluated for flower structure, pollen viability, pollen size, in-vitro pollen germination, pollen tube growth and stigma receptivity. The number of petals ranged from 3.0 to 3.2 per flower and did not differ significantly among the genotypes. Significant differences were observed for petal length and width. RP-2 recorded the longest petals (3.58 cm), whereas CA-2 had the widest petals (0.98 cm). Pollen viability ranged from 50.62 to 76.32%, with CA-2 recording the highest value (76.32%), statistically at par with RP-2 (76.26%). Pollen size was maximum in CA-2 (31.59 µm), while RP-2 recorded the highest in-vitro pollen germination (36.20%). The longest pollen tube was recorded in Balanagar (442.36 µm). Stigma receptivity was observed from approximately 24 hours before flower opening to about three hours after opening. Anthesis occurred between 5:30 and 7:30 a.m., whereas anther dehiscence occurred on the following day. The flowers therefore exhibited clear protogynous dichogamy, with the female phase preceding the male phase. The observed variation among genotypes for floral and pollen traits provides useful information for selecting parents and determining appropriate timing for controlled hybridization in custard apple.

Keywords: *Annona squamosa*, floral biology, pollen viability, pollen germination, pollen tube, stigma receptivity, protogyny

Introduction

Custard apple (*Annona squamosa* L.) is an important tropical and subtropical fruit crop belonging to the family Annonaceae. The crop is valued for its edible pulp, distinctive flavour and adaptation to relatively dry tropical environments. Despite its economic importance, fruit production can be affected by inefficient pollination, irregular fruit set and variation in fruit development. Understanding the reproductive biology of the crop is therefore important for both crop production and genetic improvement.

Flowering behaviour in *A. squamosa* is closely associated with the success of pollination. The species exhibits temporal separation between the female and male phases of the flower. Kishore *et al.* (2012) [4] reported protogynous dichogamy in *A. squamosa*, with stigma receptivity preceding anther dehiscence. This separation reduces the opportunity for direct self-pollination within a flower and has important implications for natural and controlled pollination. Floral stage is also associated with pollen performance. Araújo *et al.* (2021) [2] studied flower stages, pollen viability and germination in *A. squamosa* under tropical conditions and demonstrated that pollen performance varied with floral stage and storage conditions. These observations indicate that the timing of pollen collection is an important consideration in artificial pollination. Pollen viability alone does not provide a complete assessment of male reproductive performance. Germination and subsequent pollen-tube growth determine whether viable pollen can proceed towards fertilization. Mendes *et al.* (2012) [6] reported variation in pollen germination and fruit set in 'Brazilian seedless' sugar apple, emphasizing the importance of pollen performance in reproductive success. Similarly, Rodrigues *et al.* (2016) [5] demonstrated that pollen germination and pollen-tube growth in *A. squamosa* are influenced by temperature, indicating that environmental conditions can modify pollen behaviour. Time of pollen collection can also affect pollen performance.

Subhash *et al.* (2019) [8], working with sugar apple cv. Balanagar, reported the highest pollen germination at 6:00 a.m., followed by a rapid decline during the day. Such observations are particularly relevant to controlled hybridization because pollen collected at an unsuitable stage or time may show reduced germination even when pollen grains appear morphologically normal.

Floral morphology also varies among custard apple genotypes. Ghawade *et al.* (2018) [3], while characterizing custard apple genotypes, reported considerable variation in petal dimensions. Sushmitha *et al.*, (2024) [9] also reported the variation in floral biology within the hybrid Arka Sahan. Such variation provides useful information for distinguishing genotypes and understanding floral structure. Stigma characteristics are equally important in determining the timing of successful pollination. Vithanage (1984) [10] described the stigma of *A. squamosa* as a wet-type stigma with secretions associated with pollen adhesion and pollen-stigma interaction. The timing of stigma receptivity in relation to anthesis and anther dehiscence therefore needs to be considered when artificial pollination is undertaken.

Although several studies have described reproductive biology in *A. squamosa*, comparatively limited information is available on the combined floral and pollen characteristics of the genotypes maintained under the agro-climatic conditions of Akola. The present investigation was therefore undertaken to study and characterize selected custard apple genotypes with respect to floral structure, pollen viability, pollen size, in-vitro pollen germination, pollen-tube growth and stigma receptivity under Akola conditions.

2. Materials and Methods

2.1 Experimental site and plant material

The investigation was conducted during 2025–26 at the Central Research Station and Fruit Nursery, Department of Fruit Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra. The experimental plants were 17 years old and maintained at a spacing of 5 × 5 m. Five custard apple genotypes, namely Balanagar, CA-1, CA-2, RP-1 and RP-2, were used for the study.

2.2 Floral characters

The number of petals, petal length and petal width were recorded. The number of petals was counted from five opened flowers per plant per replication. Petal length was measured from randomly selected flowers, while petal width was measured at the widest portion of the petal by using vernier calliper instrument. Mean values were used for comparison among genotypes.

2.3 Pollen viability (%)

Pollen viability was assessed using 1% acetocarmine staining. Five slides were prepared for each genotype, and at least 100 pollen grains were examined per slide. Darkly stained pollen grains were considered viable.

$$\text{Pollen viability (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

2.4 Pollen size (µm)

Pollen size was measured using a compound microscope fitted with an (40x) and (10x) magnification for each genotype and size measured with the help of ocular

micrometer. The average size was worked out in micrometer (µm).

2.5 Pollen germination (%)

Pollen germination was assessed on a medium containing 300 mg l⁻¹ Ca(NO₃)₂·4H₂O, 200 mg l⁻¹ MgSO₄·7H₂O, 100 mg l⁻¹ KNO₃, 15% sucrose and 1% agar. Pollen grains were incubated for two hours at 28°C, after which at least 100 pollen grains were counted per slide.

$$\text{Pollen germination (\%)} = \frac{\text{Number of pollen grains germinated}}{\text{Number of pollen grains observed}} \times 100$$

2.6 Pollen tube length (µm)

Pollen tube length was measured using a compound microscope with (40x) and (10x) magnification for each genotype and size measured with the help of ocular micrometer. Average was worked out in micrometer (µm)

2.7 Stigma receptivity (hours)

Stigma receptivity was determined through visual observation using a hand lens. A stigma that appeared fresh and shiny and showed sticky secretion was considered receptive. Observations were continued during the period before and after flower opening to determine the duration of receptivity.

Anthesis and anther dehiscence were recorded by repeated observations of tagged flowers. The timing of flower opening and pollen release was used to characterize the reproductive phases of the flowers.

2.8 Statistical analysis

The observations on floral and pollen characters were subjected to statistical analysis as followed in the experimental study. The significance of differences among genotypes was assessed using the F-test, and the critical difference (CD) at 5% probability was used for comparison where the F-test was significant. The standard errors and critical differences recorded in the experiment are presented with the respective tables.

3. Results and Discussion

3.1 Floral structure

The five genotypes showed relatively little variation in the number of petals, whereas clear differences were observed in petal dimensions. The number of petals ranged from 3.0 to 3.2 per flower and the difference was non-significant. Balanagar, CA-2 and RP-2 recorded 3.0 petals, whereas CA-1 and RP-1 recorded 3.2 petals.

Table 1: Floral structure of selected custard apple genotypes

Sr. No	Genotypes	Number of petals	Length of petals(cm)	Width of petal(cm)
1	Balanagar	3.0	2.96	0.68
2	CA - 1	3.2	2.30	0.56
3	CA - 2	3.0	3.42	0.98
4	RP - 1	3.2	2.52	0.54
5	RP - 2	3.0	3.58	0.59
	F test	NS	Sig	Sig
	SE(m±)	0.16	0.09	0.01
	C.D. at 5%	-	0.27	0.04

NS = Non-significant; Sig = Significant.

Petal length differed significantly among the genotypes. RP-2 recorded the maximum petal length of 3.58 cm, followed by CA-2 (3.42 cm), Balanagar (2.96 cm) and RP-1 (2.52 cm). The minimum petal length was recorded in CA-1 (2.30 cm). Petal width also differed significantly. CA-2 recorded the maximum width (0.98 cm), followed by Balanagar (0.68 cm). RP-2 (0.59 cm) and CA-1 (0.56 cm), Whereas RP-1 had the minimum petal width (0.54 cm). Similar results have been reported by Ghawade *et al.* (2018) [3] in custard apple genotypes. The differences in petal dimensions indicate that the genotypes differed in floral morphology even though the basic petal number remained relatively stable. Similar variation in floral characteristics has been reported among custard apple genotypes. The observed differences may arise primarily from genotypic variation, although environmental conditions during floral development can also influence the final dimensions of floral organs.

Table 2: Pollen characteristics and stigma receptivity of selected custard apple genotypes

Sr. No	Genotypes	Pollen viability (%)	Pollen size (µm)	Pollen germination (%)	Pollen tube length (µm)	Stigma receptivity (hrs)	
						Before open of flower	After open of flower
1	Balanagar	50.62	29.13	35.44	442.36	24	3
2	CA- 1	68.02	23.72	30.89	432.58	24	3
3	CA- 2	76.32	31.59	35.26	422.81	24	3
4	RP- 1	51.51	20.81	31.74	412.29	24	3
5	RP- 2	76.26	22.48	36.20	434.72	24	3
	F test	Sig	Sig	Sig	Sig	NS	NS
	SE(m±)	1.51	0.09	1.09	2.41	-	-
	C.D. at 5%	5.00	0.30	3.56	8.00	-	-

NS = Non-significant; **Sig.** = Significant.

Variation in pollen viability among *A. squamosa* has also been reported under different growing conditions. Araújo *et al.* (2021) [2] and Rodrigues *et al.* (2016) [5] specifically demonstrated that pollen viability and germination vary with flower stage and post-collection conditions.

3.3 Pollen size

Significant differences were observed in pollen size. CA-2 recorded the maximum pollen size (31.59 µm), followed by Balanagar (29.13 µm). CA-1, RP-2 and RP-1 recorded 23.72, 22.48 and 20.81 µm, respectively.

The variation in pollen dimensions demonstrates differences among the genotypes. Pollen size can be influenced by genotype as well as pollen developmental stage and the conditions under which measurements are made. Therefore, comparisons are most reliable when pollen is collected at a comparable developmental stage and measured using the same microscopic procedure. Variation in pollen diameter among Annonaceae has also been documented by Azeez and Folorunso (2014) and Kishore *et al.* (2012) [1, 4].

3.4 In-vitro pollen germination

Pollen germination varied significantly among the genotypes and ranged from 30.89 to 36.20%. RP-2 recorded the highest germination (36.20%), followed by Balanagar (35.44%) and CA-2 (35.26%). RP-1 recorded 31.74%, while CA-1 showed the lowest value (30.89%).

The higher germination recorded in RP-2 indicates better in-vitro performance of its pollen under the medium and incubation conditions used in the experiment. The differences among genotypes may reflect variation in pollen physiological condition and genetic constitution. Pollen germination is also sensitive to pollen developmental stage,

3.2 Pollen viability

Significant variation was recorded in pollen viability among the five genotypes. Viability ranged from 50.62 to 76.32%. CA-2 recorded the highest pollen viability (76.32%), which was statistically at par with RP-2 (76.26%). CA-1 recorded 68.02%, while RP-1 and Balanagar recorded lower values of 51.51 and 50.62%, respectively.

The higher acetocarmine-stained pollen percentage in CA-2 and RP-2 indicates comparatively greater pollen viability under the conditions of the present study. However, stainability represents one aspect of pollen performance and should not be interpreted alone as proof of fertilization ability. Environmental conditions during pollen development and maturation can influence pollen physiological status.

collection time, temperature, humidity and the composition of the germination medium.

The result should therefore be interpreted specifically as in-vitro pollen germination under the experimental conditions, rather than as an absolute measure of field fertilization ability. Rodrigues *et al.* (2016) [5] demonstrated strong temperature dependence of pollen germination and pollen tube growth in sugar apple, with maximum germination observed at 25°C in their experiment.

3.5 Pollen tube length

Significant variation was observed for pollen tube length. Balanagar recorded the longest pollen tube (442.36 µm), followed by RP-2 (434.72 µm), CA-1 (432.58 µm) and CA-2 (422.81 µm). The shortest pollen tube was observed in RP-1 (412.29 µm).

The variation indicates differences in pollen-tube growth among the genotypes under the conditions of the experiment. Pollen tube growth is an important component of male reproductive performance because the pollen tube provides the pathway through which the male gametes reach the ovule. Its growth can be affected by pollen physiological status, genotype and environmental conditions.

An important observation was that pollen viability, pollen germination and pollen tube length did not follow the same ranking across all genotypes. CA-2 had the highest pollen viability, RP-2 had the highest pollen germination, whereas Balanagar produced the longest pollen tube. These findings are in close agreement with Rodrigues *et al.* (2016) [5] in *Annona squamosa* and Segundo *et al.* (2022) [8] in *Annona cherimoya* Mill. Thus, evaluation of more than one pollen parameter provides a better description of pollen performance than any single measurement.

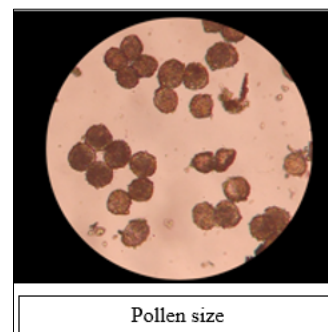
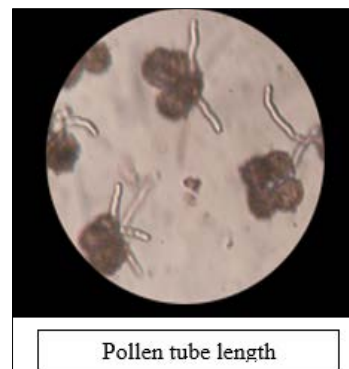
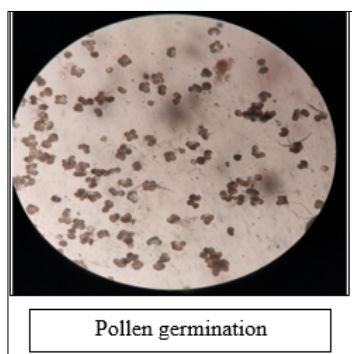
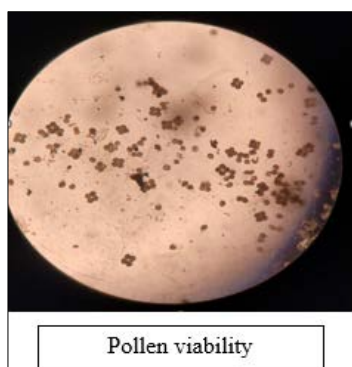
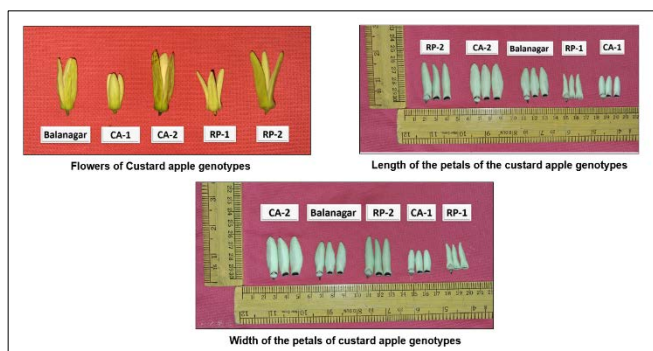
3.6 Stigma receptivity and reproductive phases

The stigma remained receptive for approximately 24 hours before flower opening and for about three hours after opening. The receptive period was associated with a fresh, shiny stigma and visible sticky secretion.

Anthesis occurred between 5:30 and 7:30 a.m., whereas anther dehiscence occurred between 12:00 noon and 2:00 p.m. on the following day. Thus, the receptive female phase preceded the effective pollen-release phase, demonstrating protogynous dichogamy in the flowers studied.

The present observation agrees with the general reproductive pattern reported for *A. squamosa*. Kishore *et al.* (2012)^[4] observed that the stigma becomes receptive before anther dehiscence and described the species as protogynous. Araújo *et al.* (2021)^[2] likewise demonstrated distinct female and male stages in sugar apple flowers, although the exact timing differed under their tropical environmental conditions.

The difference in clock time between studies emphasizes that the reproductive phases of custard apple should be determined under the local environmental conditions before undertaking controlled pollination. In the present investigation, the approximately one-day separation between stigma receptivity and subsequent anther dehiscence provides an important basis for scheduling pollen collection and pollination operations at Akola.



4. Conclusion

The study demonstrated clear genotypic variation in several floral and pollen characteristics of custard apple under the conditions of Akola. Petal number remained relatively stable, whereas petal length and width differed significantly among the genotypes. RP-2 recorded the longest petals, while CA-2 had the widest petals.

Marked differences were also observed in pollen behaviour. CA-2 recorded the highest pollen viability and pollen size, RP-2 recorded the highest in-vitro pollen germination, and Balanagar produced the longest pollen tubes. These differences show that pollen viability, germination and tube growth do not necessarily follow the same pattern among genotypes and should therefore be evaluated together when assessing pollen performance.

The stigma remained receptive from approximately 24 hours before flower opening until about three hours after opening. Anthesis occurred during the early morning, between 5:30 and 7:30 a.m., while anther dehiscence occurred on the following day between 12:00 noon and 2:00 p.m. The flowers therefore exhibited distinct protogynous dichogamy. The floral and pollen information generated in the present study provides a basis for identifying suitable flower stages, handling pollen and scheduling controlled pollination in future custard apple breeding work.

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