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Impact of pollination levels on quantitative and qualitative characteristics of custard apple (*Annona squamosa* L.) fruits genotypes

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Abstract
The present study was conducted at the Department of Horticulture, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India, during the year 2023-24, using a Completely Randomized Design with a three-factorial concept, with three repetitions and nine treatment combinations of the two factors. Genotype (G_1: Anand selection-1, G_2: Sindhan, G_3: Balanagar and Levels of pollen grain (P_1: Natural pollination, P_2: Hand pollination with 100% pollen grain and P_3: Hand pollination with 40% pollen grain + 60% corn starch). The treatments were repeated three times. Among different genotypes, Balanagar recorded significantly maximum number of fruit set (6.42), fruit length (7.81 cm), fruit volume (188.58 cc), pulp weight (86.00 g), lowest stone fruit (5.44 %), Lower seed weight (15.23 g) and number of seeds (46.10) was recorded with Anand selection-1 (G_1) in pooled data. In quality parameters, TSS (23.90 °Brix) was recorded with Balanagar during the pooled analysis. Over two seasons, the Balanagar cultivar consistently showed superior fruit set, yield traits, and quality. Pollination method mattered as hand pollination with 100% pollen outperformed other treatments across most metrics, while natural pollination gave the lowest results.

Keywords: Anand selection-1, Balanagar, custard apple, genotypes, hand pollination, TSS, pollination, pollen grain level

Introduction
Among the different fruits, custard apple (*Annona squamosa* L.) is a very important fruit. The fruit has an edible, soft, granular, juicy, and sugary pulp with a mild flavors and slight acidity. The creamy, granular edible portion offers an excellent balance of sweetness and acidity. The pulp is widely utilized in the production of custard powder, frozen pulp, and various beverages (Khodifad *et al.*, 2016) [16]. The fruit is reported to have moisture 70.5g, protein 1.6g, fat 0.4g, minerals 0.9g, fiber 3.1g, calcium 17.0mg, phosphorous 47.0mg, iron 1.5mg, thiamine 0.07mg, riboflavin 0.17mg, niacin 1.30mg, Vitamin C 37.0mg and energy 104Kcal (Gopalan *et al.*, 1987) [13]. Species of the genus *Annona* typically bear flowers on both current-season and previous-season shoots, with only occasional flowering on older woody sections. The flowers are characterized by three thick, white to greenish petals that form a partially enclosed floral chamber around numerous reproductive organs. They are hermaphroditic and strongly protogynous: stigmas become receptive well before anther dehiscence and can remain receptive for up to 24 hours, a phenology that promotes cross-pollination and limits self-fertilization (Gazit *et al.*, 1982) [10]. In custard apple (*A. squamosa*), flowering is protracted from March and April through July to August, peaking in April and May; however, effective fruit set is generally realized only with the onset of the rainy season, reflecting the interaction of floral biology with prevailing environmental conditions. Poor fruit set remains the key constraint to commercial production of annonaceous fruits. In custard apple, natural fruit set is typically only 1-8% despite abundant flowering (Hayes, 1957; George & Nissen, 1986; Ahmad, 1936; Venkataratnam, 1963; Thakur & Singh, 1965; Kumar *et al.*, 1977; George & Nissen, 1988) [14, 11, 1, 32, 30, 18, 12]. Protogynous dichogamy in custard apple that stigmas become receptive before anther dehiscence greatly restricts self-pollination (Campbell & Phillips, 1994) [6]. Consequently, despite numerous hermaphroditic, self-fertile flowers, only 1 to 2% set fruit under natural conditions, making economic yields unlikely without assisted pollination.

Hand pollination markedly improves fruit set and quality, with hand-pollinated fruits often fetching premium prices (Campbell & Phillips, 1994; Jalikop & Kumar, 2007; Campos *et al.*, 2004; Escobar *et al.*, 1986; Melo *et al.*, 2004; Matsuda & Higuchi, 2019; Motis, 2007) [6, 15, 4, 9, 21, 19, 23]. Hand (manual) pollination is a highly effective practice that increases fruit set and promotes larger, more uniform, and visually superior fruits.

Materials and Methods

The study was conducted during 2023-2024 at the Horticultural Research Farm, Department of Horticulture, B. A. College of Agriculture, Anand Agricultural University, Anand, using thirteen-year-old custard apple (*Annona squamosa* L.) trees of three genotypes: Anand Selection-1 (G1), Sindhan (G2), and Balanagar (G3). The experiment followed a Completely Randomized Design (factorial) with three replications and nine treatment combinations comprising two factors: (A) Genotype G1, G2, G3; and (B) Pollen grain level P1: natural pollination, P2: hand pollination with 100% pollen, and P3: hand pollination with 40% pollen + 60% corn starch. Observations were recorded during the experimental period, and data were analyzed by analysis of variance (ANOVA) appropriate for a factorial CRD, following Gomez and Gomez (1976).

Results and Discussion

The results revealed that among different genotypes Balanagar (G3), exhibit Significantly higher number of fruit set (6.27, 6.57 and 6.42) during the year 2023, 2024 and pooled analysis, More fruit length (7.77, 7.86 and 7.81 cm) during the year 2023, 2024 and pooled analysis, it might be due to Balanagar variety itself superior in fruit length as compared to other varieties of custard apple. Similar results were obtained by Bagul and Masu (2017) [3], higher fruit volume (188.19, 188.97 and 188.58 cc) during the year 2023, 2024 and pooled analysis, this might be due to heavier fruits usually exhibit greater volume. As balanagar showed higher fruit weight resulted in higher fruit volume A similar view was also shared by Bagul and Masu (2017) [3]. The higher pulp weight (84.92, 87.09 and 86.00 g) during the year 2023, 2024 and pooled analysis, this might be due to fruit weight of Balanagar was highest as compared to other genotypes. The results were in accordance with the findings of Rao and Subramanyam (2011) [26] and Bagul and Masu (2017) [3]. lowest stone fruit (5.43, 5.45 and 5.44 %) during the year 2023, 2024 and pooled analysis, Although the exact cause of stony fruit formation remains unclear, some researchers suggest that factors such as calcium content, moisture deficiency, and temperature fluctuations might be contribute to the condition. Significantly lower seed weight was recorded with Anand selection-1 (G1), *i. e.,* 14.92, 15.54 and 15.23 g during the year 2023, 2024 and pooled analysis. It might be due to lower size of fruit of Anand selection-1 as compared to Sindhan and Balanagar. A similar result were also shared by Bagul and Masu (2017) [3]. Minimum number of seeds was recorded with Anand selection (G1), *i. e.,* 45.62, 46.59 and 46.10 during the year 2023, 2024 and pooled analysis, respectively. The observed differences might be due to inherent genetic variation among the genotypes. A similar view was also shared by Bagul and Masu (2017) [3]. The higher non-reducing sugar (5.77, 5.79 and 5.79 %) during the year 2023, 2024 and pooled analysis, this might be due to non-availability of free ketone or aldehyde group. Similar observation was also recorded by Sanghani and Varu (2022) [27] and Meena *et al.* (2023) [20] and Usman *et al.* (2013) [31] in guava, Munir *et al.* (2020) [24] in date palm. The higher TSS (23.80, 23.99 and 23.90 °Brix) during the year 2023, 2024 and pooled analysis, respectively. It might be due to the hydrolytic degradation of complex carbohydrates, such as starch, cellulose, and pectin, which yields simpler sugars and thereby increases the content of soluble solids. The results were in accordance with the findings of Rao and Subramanyam (2011) [26] and Bagul and Masu (2017) [3]. The observed differences might be due to inherent genetic variation among the genotypes.

Among different pollen grain level number of fruit set per shoot. Highest was recorded in hand pollination with 100% pollen grain (P2), *i. e.,* 8.46, 8.66 and 8.56 during the year 2023, 2024 and pooled data, it might be due to Hand pollination with 100% pollen ensures full and uniform pollen deposition on receptive stigmas, leading to complete ovule fertilization and higher seed set. This triggers stronger hormonal responses, promoting better fruit initiation and resulting in a significantly higher number of fruits compared to natural pollination, which is often limited by poor pollen transfer and pollinator activity. The interaction effect of genotype and level of pollen grain on number of fruits set per shoot was found non-significant in the year of 2023, 2024 but significant in pooled analysis. maximum fruit length was recorded with hand pollination with 100% pollen grain (P2), *i. e.,* 7.85, 8.00 and 7.93 cm during the year 2023, 2024 and pooled analysis, respectively. Minimum fruit length was recorded in natural pollination (P1), *i. e.,* 6.99, 7.04 and 7.02 cm in the year of 2023, 2024 and pooled analysis, respectively. It could be due to a more uniform and efficient distribution of pollen on stigma of the female flowers, rather than simply an increased amount of pollen. The even pollen coverage enhances the chances of successful fertilization across the entire ovary, As a result, it promotes more active cell division and cell enlargement processes during fruit development, ultimately contributing to greater fruit length. Similar results were obtained by (Blanchet, Douault & Pouvreau, 1991) [4]. Sanghani and Varu (2022) [27] in custard apple, Melo *et al.* (2004) [21] in atemoya, Moreira *et al.* (2022) [22] and Boraiah *et al.* (2024) [5] in dragon fruit. Higher fruit volume was recorded with hand pollination with 100% pollen grain (P2), *i. e.,* 195.52, 196.85 and 196.16 cc during the year 2023, 2024 and pooled analysis, This might be due to efficient pollination, it resulted in uniform fertilization across all ovaries, resulting in balanced growth and maximized fruit volume. The results were in accordance with Munir *et al.* (2020) [24] in date palm. The pulp weight was recorded with hand pollination with 100% pollen grain (P_2),*i. e.*,84.92, 87.09 and 86.00 g during the year 2023, 2024 and pooled analysis, respectively, This might be due to the influence of pollen amount on ovarian tissue growth, mediated by hormones released from the developing endosperm and embryo, which diffuse into the ovary and promote fruit development resulted in high pulp weight. The results were in accordance with the findings of Shivkumar *et al.* (2018) [29], Pereira *et al.* (2019) [25], Sanghani and Varu (2022) [27] and Meena *et al.* (2023) [20] in custard apple Melo *et al.* (2004) [21] in atemoya and Munir *et al.* (2020) [24] in date palm, Usman *et al.* (2013) [31] in guava, Boraiah *et al.* (2024) [5] in dragon fruit. Minimum stone fruit percentage was recorded with

hand pollination with 100% pollen grain (P2), *i. e.*, 2.43, 2.59 and 2.51 during the year 2023, 2024 and pooled analysis, respectively. However, reason for stony fruits are still unknown but some researchers reported that Ca content, moisture deficient and temperature are possible reasons. Earlier similar kind of results has been found by Sanghani and Varu (2022) [27] and Meena *et al.* (2023) [20]. Maximum number of seeds was recorded with hand pollination with 100% pollen grain (P2), *i. e.*, 58.20, 59.46 and 58.83 during the year 2023, 2024 and pooled analysis, This might be due to proper ovule development because of successful fertilization initiates the formation of seeds. In aggregate fruits like custard apple multiple ovaries develop into individual areoles, each typically enclosing seed. Therefore, the number of pollen grains involved and the proper development of ovules are key factor influencing seed formation A similar view was also shared by Pritchard and Jalikop and Kumar (2007) [15], Sanghani and Varu (2022) [27] and Meena *et al.* (2023) [20] in custard apple, Schroeder (1941) [28] in cherimoya, Melo *et al.* (2004) [21] and Pereira *et al.* (2019) [25] in atemoya, Dag and Mizrahi (2005) [8] in dragon fruit, King and Ferguson (1991) [17] in kiwi.The lowest seed weight was recorded in natural pollination (P1), *i. e.*, 12.61, 13.02 and 12.81 g in the year of 2023, 2024 and pooled analysis, respectively. The variation might be due to more number of fertilized ovaries, as well as fruit shape, weight, and seed count. The results were in accordance with the findings of Pereira *et al.* (2019) [25], Sanghani and Varu (2022) [27] and Meena *et al.* (2023) [20], Munir *et al.* (2020) [24] in datepalm. The maximum percentage of non-reducing sugar was recorded with hand pollination with 100% pollen grain (P_2), *i. e.*, 5.86, 5.89 and 5.88 during the year 2023, 2024 and pooled analysis, respectively. This might be due to non-availability of free ketone or aldehyde group. Similar observation was also recorded by Sanghani and Varu (2022) [27] and Meena *et al.* (2023) [20] and Usman *et al.* (2013) [31] in guava, Munir *et al.* (2020) [24] in date palm. The Maximum TSS was recorded with hand pollination with 100% pollen grain (P_2), *i. e.*, 24.06, 24.43 and 24.25 °Brix during the year 2023, 2024 and pooled analysis, respectively. In larger fruits, a strong source-sink relationship and greater conversion of insoluble compounds into soluble solids may contribute to this phenomenon. Similar results were obtained by Melo *et al.* (2004) [21], Pereira *et al.* (2019) [25], Sanghani and Varu (2022) [27] and Meena *et al.* (2023) [20] and Usman *et al.* (2013) [31] in guava.

Table 1: Effect of genotype, level of pollen grain on number of fruit set per shoot, Fruit volume and Fruit length in custard apple

Code	Treatment Details	Number of fruit set per shoot			Fruit length (cm)			Fruit volume (cc)		
		2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled
Genotypes (G)										
G_1	Anand Selection-1	5.50	5.56	5.53	7.13	7.34	7.23	168.89	168.89	168.89
G_2	Sindhan	6.05	6.15	6.09	7.39	7.47	7.43	178.92	179.37	179.14
G_3	Balanagar	6.27	6.57	6.42	7.77	7.86	7.81	188.19	188.97	188.58
S. Em. ±		0.13	0.12	0.09	0.10	0.12	0.08	3.26	3.45	2.37
C. D. (P = 0.05)		0.38	0.36	0.25	0.30	0.35	0.23	9.70	10.24	6.81
Level of pollen grain (P)										
P_1	Natural pollination (Control)	3.72	3.77	3.75	6.99	7.04	7.02	159.81	159.26	159.54
P2	Hand pollination with 100% pollen grain	8.46	8.66	8.56	7.85	8.00	7.93	195.52	196.85	196.19
P_3	Hand pollination with 40% pollen grain + 60% corn starch	5.63	5.85	5.74	7.44	7.62	7.53	180.66	181.11	180.89
S. Em. ±		0.13	0.12	0.09	0.10	0.12	0.08	3.26	3.45	2.37
C. D. (P = 0.05)		0.38	0.36	0.25	0.30	0.35	0.23	9.70	10.24	6.81
Year		-	-	NS	-	-	NS	-	-	NS
Significant interaction		-	-	G×P	-	-	-	-	-	-
C.V. %		6.38	6.00	6.19	4.15	4.73	4.45	5.48	5.77	5.63

Table 2: Effect of genotype, level of pollen grain on Seed weight, Number of seeds and Stone fruit in custard apple

Code	Treatment Details	Seed weight (g)			Number of seeds			Stone fruit (%)		
		2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled
Genotypes (G)										
G_1	Anand Selection-1	14.92	15.54	15.23	45.62	46.59	46.10	6.91	7.06	6.99
G_2	Sindhan	16.54	16.46	16.50	48.44	48.90	48.67	6.06	6.04	6.05
G_3	Balanagar	17.51	18.33	17.92	51.67	52.77	52.22	5.43	5.45	5.44
S. Em. ±		0.26	0.29	0.19	0.88	0.82	0.60	0.13	0.18	0.11
C. D. (P = 0.05)		0.76	0.86	0.56	2.61	2.43	1.72	0.37	0.52	0.31
Level of pollen grain (P)										
P_1	Natural pollination (Control)	12.61	13.02	12.81	38.52	38.92	38.72	10.12	10.14	10.13
P2	Hand pollination with 100% pollen grain	19.35	19.72	19.54	58.20	59.46	58.83	2.43	2.59	2.51
P_3	Hand pollination with 40% pollen grain + 60% corn starch	17.01	17.58	17.30	49.02	49.88	49.45	5.86	5.83	5.84
S. Em. ±		0.26	0.29	0.19	0.88	0.82	0.60	0.13	0.18	0.11
C. D. (P = 0.05)		0.76	0.86	0.55	2.61	2.43	1.72	0.37	0.52	0.31
Year		-	-	NS	-	-	NS	-	-	NS
Significant interaction		-	-	-	-	-	-	-	-	-
C.V. %		4.70	5.20	4.96	5.42	4.96	5.19	6.10	8.53	7.42

Table 3: Effect of genotype, level of pollen grain on Non-reducing sugars and TSS in custard apple

Code	Treatment Details	Non-reducing sugars (%)			TSS (° Brix)		
		2023	2024	Pooled	2023	2024	Pooled
Genotypes (G)							
G_1	Anand Selection-1	5.32	5.35	5.34	21.55	21.86	21.70
G_2	Sindhan	5.50	5.52	5.52	22.98	23.23	23.11
G_3	Balanagar	5.77	5.79	5.79	23.80	23.99	23.90
S. Em. ±		0.09	0.08	0.06	0.34	0.36	0.25
C. D. (P = 0.05)		0.26	0.23	0.17	1.01	1.07	0.71
Level of pollen grain (P)							
P_1	Natural pollination (Control)	5.16	5.17	5.17	21.76	21.91	21.83
P2	Hand pollination with 100% pollen grain	5.86	5.89	5.88	24.06	24.43	24.25
P_3	Hand pollination with 40% pollen grain + 60% corn starch	5.57	5.59	5.59	22.51	22.75	22.63
S. Em. ±		0.09	0.08	0.06	0.34	0.36	0.25
C. D. (P = 0.05)		0.26	0.24	0.17	1.01	1.07	0.71
Year		-	-	NS	-	-	NS
Significant interaction		-	-	-	-	-	-
C.V. %		4.78	4.27	4.53	4.47	4.70	4.58

Conclusion

From the two-year field experiment, the Balanagar genotype consistently achieved the highest fruit set and outperformed others in both yield-contributing and quality attributes. Pollination method significantly influenced all measured traits, with hand pollination using 100% pollen delivering superior results across most parameters, whereas natural pollination produced the lowest values.

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