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Pollen sources and time of pollination for hand pollination affect biochemical and organoleptic attributes of sugar apple (*Annona squamosa* L.) cv. Arka Sahan

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Abstract

The present investigation aimed to know the effect of different pollen sources and time of pollination for hand pollination on biochemical and organoleptic properties in sugar apple cv. Arka Sahan. For the study, a total 130 flowers of cv. Arka Sahan were hand pollinated with three different pollen sources, such as Balanagar, Raydurg and APK-Ca 1 at three different times in the July. It was found that pollen source and timing significantly affected fruit quality attributes and organoleptic traits. Fruits of T_1 (first pollination with Balanagar) exhibited maximum pulp weight, fruit weight and seeds per fruit. The biochemical traits such as higher total soluble solids (32.33 ºBrix), lower titratable acidity (TA) (0.25%), higher total sugar (TS) (25.55%), reducing sugar (RS) (22.75%) and non-reducing sugar (NRS) (2.66 %) were also reported in T_1. Likewise, higher ascorbic acid (AA) (27.87 mg/100g) and minimum total phenols content (TPC) (66.76 µg GAE/100g) were also obtained in T_1 yielded fruits compared to the naturally pollinated (control). Evaluation of organoleptic revealed that pollen source and pollination time also affected the sweetness, sourness aroma, grittiness and overall acceptability (8.62) of pulp. Results of organoleptic evaluation revealed that pollen sources and pollination times also affected the sweetness, sourness, aroma, grittiness and overall acceptability (8.62) of the fruit pulp. Hence, Balanagar could be used as an effective pollen source for hand pollination in Arka Sahan during initial weeks of July to get higher quality and acceptability of the sugar apple fruits.

Key words: Pollen grain, fruit, ascorbic acid, total phenols.

Introduction

The sugar apple, also known as the custard apple (*Annona squamosa* L.), is an emerging fruit in the New World and belongs to the family Annonaceae. Many *Annona* members yield edible fruits like custard apple, sugar apple, cherimoya, sour sop, *Ramphal*, and atemoya (Pritchard and Edwards, 2006). Among these, the custard apple is the choicest and most superior fruit which is consumed and considered a 'super fruit' in the market due to its high nutraceutical properties. Though earlier, it was considered as an underutilized fruit but now it got a commercial status and revolutionized farmers.

Arka Sahan is the most important cultivar of sugar apple, having high pulp and low seeds content. In annonaceous fruits, proper pollination is a major constraint in achieving enough fruit set; thus, hand pollination is the only way to get the ideal yield (Jalikoop and Kumar, 2007). Earlier studies suggested that skewed sex ratio and dichogamy contribute to 8% of crop failure in pollination (Wurz *et al.,* 2021). In Arka Sahan, natural pollination is very difficult due to protogynous dichogamy (Shivakumar and Rao, 2018). In the case of the perfect flowers of this cultivar, maturation of carpels before pollen liberation on the anthers prevents self-pollination, resulting in almost no fruit set or sometimes one –two misshaped fruits set in the plant. It has been stated that even after producing hermaphrodite flowers, self-pollination in sugar apple varieties is almost impossible because its stigma becomes receptive or viable long before the pollen is released as a case of dichogamy (Meena *et al.,* 2017; Chander *et al.,* 2019). Again, timing and pollen source is also limiting factor owing to its long flowering period from April to August. Pollen sources and pollen viability from related species can affect fruit set, seed weight and quality of fruits (Chander *et al.,* 2019). Likewise, in guava Usman *et al.* (2011) found a positive impact of pollen parents on fruit quality. Again, the time of pollination is also crucial for fruit shape and internal quality. Sharifkhah *et al.* (2020) found that pistachio fruits had higher total phenolics, total flavonoid, and antioxidant activity when pollinated at lower florets opened and clusters were a green stage. Samnegård *et al.* (2019) reported that compatible pollen treatments affected fruit quality and storability.

Under South Indian conditions, hand pollination is carried out during April-May month, whereas under central and Northern parts, flowering starts in July month, thus, pollination needs to be carried out from the beginning of July to the first week of August. Highly pollinator-dependent crops such as sugar apples need assisted pollination due to defective organs and conditions (Jalikoop and Kumar, 2007; Awad and Al-Qurashi, 2012). However, only a few research works have been carried out on hand pollination in sugar apple with special attention to

physicochemical quality. Selection of the compatible pollen sources and time of pollination could help in getting quality produce. Hence, there is need to investigate the available superior genotypes for suitable pollination and further that could be used in breeding programmes to improve the crop traits.

With the facts mentioned above, we investigated the effects of different pollen sources and time of pollination on the fruit biochemical and organoleptic parameters of the Arka Sahan sugar apple.

Materials and methods

Location and details of treatments: The study was carried out during the year 2019-20 on the Arka Sahan cultivar planted at 5 × 5 m at the orchard of the Department of Fruit Science, College of Horticulture and Forestry, Jhalawar, Rajasthan (India) (Geolocation 24.5363297, 76.1496101). Three pollen sources *viz.* Balanagar, Raydurg and APK Ca-1 were selected along with three-time durations *viz.,* 1st pollination (8th-15th July), 2nd pollination (15th-22nd July) and 3rd pollination (22nd-29th July) for pollination. There were 10 treatment combinations used in this study which are as follows: T_0 = Control, T_1 = 1st pollination of Arka Sahan with Balanagar, T_2 = 1st pollination of Arka Sahan with Raydurg, T_3 = 1st pollination of Arka Sahan with APK-Ca 1, T_4 = 2nd pollination of Arka Sahan with Balanagar, T_5 = 2nd pollination of Arka Sahan with Raydurg, T_6 = 2nd pollination of Arka Sahan with APK-Ca 1, T_7 = 3rd pollination of Arka Sahan with Balanagar and T_8 = 3rd pollination of Arka Sahan with Raydurg, T_9 = 3rd pollination of Arka Sahan with APK-Ca 1. Control plants leftover for natural pollination.

Collection of pollen and pollination: Pollen from previously opened flowers was collected in a paper cup and most viable pollens were smeared on receptive flowers of Arka Sahan with the help of paint brush No. 2. The criteria for selection of flowers were already standardized and characterized by splitting of the female flower. The pollinated 130 flowers were tagged with different colour tagging materials with suitable notation. The hand pollination process was carried out in the early morning from 6.00 to 8.30 a.m., as previously suggested by (Jalikoop and Kumar, 2007).

Determination of fruit physical parameters: After the proper development of fruits, harvesting was done at the fully mature stage (light green colour and specific gravity 0.98). Fruits were procured to the Postharvest Lab of the Department of Postharvest Technology. Fruits were washed and cleaned with tap water. Fruits were divided into different lots based on treatments. Fruits were divided into different slots based on treatment. Fruit weight from each lot was measured by electronic balance and expressed in grams (g). Similarly, pulp weight was measured by scooping the pulp from the separated fruit and seeds. The pulp was weighed and expressed in grams. Pulp (%) was calculated by dividing pulp weight by total fruit weight and presented in percent. The number of seeds per fruit was calculated by separating seeds from the pulp and counting them on an individual fruit basis. Pulp: seed ratio was estimated by dividing pulp by seed weight.

Determination of fruit biochemical parameters: The total soluble solid (TSS) was measured by placing a drop of juice extracted from pulp on the prism of a digital hand refractometer (Model: Atago, Japan) (Meena and Asrey, 2018). Titratable acidity (TA) was determined by titration of a known volume of filtered custard apple juice with standard NaOH (0.1N) using phenolphthalein as an indicator and the average calculated value of TA was expressed in percent (Ranganna, 2001). Total sugars (TS), reducing sugars (RS) and non-reducing sugars (NRS) were estimated by titration method (Ranganna (2001). The results were recorded and expressed in per cent sugar content. Ascorbic acid was determined by titration method by using 2, 6-di-chlorophenol-indophenol dye (Ranganna, 2001). The TPC content of the fruit pulp was estimated by Folin Ciocalteu reagent (FCR) method (Singleton and Rossi, 1965).

Determination of organoleptic attributes: A hedonic scale score was used for organoleptic evaluation. A panel of ten trained experts was invited to take sensory parameters, *viz.* sweetness, acidity, flavour, texture and overall acceptability.

Statistical analysis: The data with respect to physicochemical and organoleptic attributes were subjected to analysis of variance (ANOVA) using CRD design. The mean value of three replicates were presented with LSD value ($P \leq 0.05$). All the statistical analysis was carried out by OPSTAT software package version 1.0 for agriculture.

Results and discussion

Fruit weight: Fruit weight was significantly affected by assisted pollination and pollination time (Table 1). In this study, treatment T_1 had the maximum fruit weight (326.32 g) while T_0 had the minimum (78.66 g) fruit weight. However, treatments T_3, T_6 and T_8 were statistically at par with each other in weight of the fruits but were significantly lower than the other treatments. Fruit weight is an important parameter in determining overall plant yield. Higher fruit weight might be attributed to a better distribution of pollen on all the female flowers' stigmas than to an additional contribution of pollen, thus increasing cell-to-cell arrangement and enlarging the fruit areoles (Blanchet *et al.,* 1991). It has been established that artificial pollination and distribution of pollination improve cell division and cell enlargement, leading to an increase in fruit size and weight (Mohammadi *et al.,* 2017)).

Pulp weight: Pulp weight was also significantly affected by assisted pollination and time. A maximum pulp weight (g) was recorded in T_1 (249.99 g), which was many folds higher compared to the control (49.67 g). Higher pulp content is a good quality trait from a consumer's point of view and marketing. Fruits produced as a result of Balanagar during 1st pollination contained higher pulp content, which might be due to the pollen source affecting the growth of ovarian tissues to hormones released by growing endosperm and embryo tissues, which diffuse into the ovarian tissue and exert a specific effect on the fruit growth. Earlier similar results have been found by El-Makhtoun and Abdel-Kader, (1993).

Pulp recovery: Likewise, maximum significant ($P \leq 0.05$) pulp recovery (76.94 %) was obtained in treatment T_1, while the minimum (63.14 %) was recorded in the T_0 (control). The larger fruit size and volume are attributed to the high pulp percent. Higher pulp weight results in a higher percentage of pulp. Hand pollination improved fruit quality parameters in kiwifruits, ascribed to a better pollen distribution on stigma than natural pollination (Blanchet *et al.,* 1991). Barhee date pollinated with Jarvis No.1 had maximum pulp content (Mohammadi *et al.,* 2017).

Table 1. Effect of pollen sources and time of pollination on fruit characters of Arka Sahan

Treatments	Pulp weight (g)	Pulp (%)	Pulp: seed ratio	Rind weight (g)	No. of areoles per fruit	No. of seeds/ fruit
T_0	49.67	63.14	12.76	25.30	80.00	10.00
T_1	249.99	76.94	24.24	65.33	63.00	28.00
T_2	233.66	76.60	24.18	60.00	66.49	27.67
T_3	142.32	72.12	19.85	47.67	70.67	20.67
T_4	195.99	73.77	21.12	49.67	72.00	27.00
T_5	173.99	73.20	20.86	54.67	74.68	22.67
T_6	150.99	73.64	19.53	46.00	78.82	21.00
T_7	194.32	75.70	24.08	53.67	73.80	25.26
T_8	149.99	72.81	18.63	47.67	76.26	24.00
T_9	144.49	72.85	19.08	46.33	78.00	22.00
LSD $P<0.05$	6.52	0.45	0.40	2.04	3.45	1.26

T_0 = Control, T_1 = 1st Pollination of Arka Sahan with Balanagar, T_2 = 1st Pollination of Arka Sahan with Raydurg, T_3 = 1st Pollination of Arka Sahan with APK-Ca 1, T_4 = 2nd Pollination of Arka Sahan with Balanagar, T_5 = 2nd Pollination of Arka Sahan with Raydurg, T_6 = 2nd Pollination of Arka Sahan with APK-Ca 1, T_7 = 3rd Pollination of Arka Sahan with Balanagar and T_8 = 3rd Pollination of Arka Sahan with Raydurg, T_9 = 3rd Pollination of Arka Sahan with APK-Ca 1. Control plants leftover for natural pollination.

Pulp: seed ratio: Changes in pulp/seed ratio due to pollen source and pollination time are presented in Table 1. The T_1 had significantly ($P\leq0.05$) higher pulp: seed ratio (24.24) than control (12.76). The maximum pulp: seed ratio in Balanagar pollinated plants could be due to proper fertilization and pollen tube germination, leading to a significantly improved Pulp: seed ratio compared to the control. Awad and Al-Qurashi (2012) found in date palms that the distribution of hormones like GA_3 after fertilization affects the pulp-seed ratio.

Rind content: Changes in the rind content as affected by assisted pollination were recorded (Table 1) Fruits produced with Balanagar pollination exhibited significantly ($P\leq0.05$) higher values during all three pollination times. pollination source and time could significantly affect the contribution of rind in fruit. Among all, T_1 had the highest rind weight (65.33 g). This is because of more cell division and increased cell density per unit volume (Bhat *et al.,* 2012). In addition, mineralogy, leaf shoot ratio and cell wall structuring compounds also play an equal role in rind development. A previous study by Shivkumar *et al. (*2018) supports our findings where they reported higher rind weight by hand pollination in sugar apple.

Areoles: Areoles are the major shape-forming phenomenon in the case of custard apple. The number of areoles also contributed to the seed in the fruits. Therefore, pollination is a crucial factor for areole formation. In the present study, data show that assisted pollination significantly ($P\leq0.05$) affected the number of areoles per fruit compared to the control. The minimum (63.00) number of areoles per fruit was recorded in T_1, on the other hand, the maximum areoles (80.00) were recorded in T_0 (control). Different genes determine areoles on the fruit surface. Arka Sahan has bit-flattened and fused areoles. Proper pollination imparts proper shape. The higher number of areoles in treatment T_1 might be due to proper pollination and the genetic character of the pollen source cultivar. In addition to that, individual ovaries might be attributed to areole development. The larger no of the ovary directly correlates to the number of areoles on the fruit surface.

Number of seeds: No. of seeds per fruit is the most important parameter in the case of custard apple. The characteristics and acceptability of fruit are significantly influenced by the seeds. Varieties with fewer seeds are more preferable for consumers. The total seeds in fruits depend on pollination and ovule formation, which is also influenced by the pollen source and the pollination time. Assisted pollination increased the No. of seeds/fruit significantly ($P\leq0.05$) over the natural pollination *i.e.,* T_0 (control). The number of seeds directly correlates with the size of fruits and the number of areoles. The No. of seeds/ fruit was recorded as a minimum (10.00) in treatment T_0 while a maximum (28.00) was recorded in the T_1. Ovule development as a fertilization process leads to final seed development. Being an aggregate fruit, multiple ovaries convert into areoles. Each areole almost contains seeds. The number of pollen grains and the development of ovules might be a possible reason for seed development. In a previous study, similar findings suggested that higher seed numbers in the fruits emerging from *A. squamosa* pollens could have triggered rapid fruit development. Numerous studies have documented the relationship between developing seeds and hormones, particularly auxin, indicating their significant role (Dag and Mizrahi, 2005). Higuchi *et al.* (1998) and Chander *et al.* (2019) also reported that warm temperatures and low pollen viability resulted in asymmetrical and small size fruits, which might produce small seeds.

Total soluble solids: Table 2 presents the impact of various pollination sources and time on the total soluble solids (TSS) value. TSS serves as a useful parameter for determining consumer preferences. In our study, there was no clear-cut trend for TSS reported. However, TSS was statistically at par in T_1, T_4 and T_7. The result indicates that the value of TSS was influenced only by the source of pollination but not by time. TSS at each pollination time was found statistically non-significant within the same treatment source but varied for different pollen sources.

After ripening, the TSS of fruit was found to be maximum (32.33 ^oBrix) in T_1 while the minimum value (28.33 ^oBrix) was reported in T_0 (control). Total soluble solids refer to soluble sugars, carbohydrates, proteins, or other minerals in ionic forms. In larger fruit, a strong source-sink ratio and higher depletion of insoluble compounds into soluble solids may be one of the reasons. Jalikoop and Kumar (2007) reported that hand pollination and pollen source significantly affected TSS value during a wet year, but moisture stress did not affect TSS. Therefore, it is assumed that along with the pollen source, climatic factors also contribute to TSS development in fruits. The results support the previous findings of Abdelal *et al.* (1983) and Samnegård *et al.* (2019), who reported a significant effect of pollen source on the TSS of the date palm fruit and apple dry matter, respectively.

Titratable acidity: Table 2 shows the changes in fruit titratable acidity subjected to various pollen sources and times of pollination. Likewise, TA exhibited a scattered trend and was found statistically at par in several treatments ($P<0.05$). Fruits of treatment T_1 had significantly lower acidity (0.28%) as compared to naturally pollinated fruits (0.37%) in T_0. However,

Table 2. Effect of pollen sources and time of pollination on TSS, TA, TS, RS, NRS, AA and TPC of sugar apple cv. Arka Sahan

Treatment	TSS (°Brix)	Titratable acidity (%)	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Ascorbic acid (mg/100g)	Total phenols (µg GAE/100g)
T_0	28.33	0.37	18.42	16.90	1.52	20.24	82.10
T_1	32.33	0.25	25.41	22.75	2.66	27.87	66.76
T_2	30.67	0.28	22.91	21.41	1.42	25.68	71.96
T_3	29.33	0.29	20.24	17.93	2.31	22.62	79.36
T_4	31.67	0.27	23.48	21.52	1.96	26.15	68.65
T_5	29.33	0.29	21.84	20.21	1.63	25.02	76.25
T_6	29.00	0.31	19.85	18.10	1.75	23.82	78.10
T_7	31.53	0.28	21.84	19.98	1.86	24.83	72.21
T_8	30.33	0.29	20.72	18.61	2.11	22.07	75.57
T_9	28.67	0.31	19.74	18.44	1.30	21.58	80.00
LSD ≤0.05	1.31	0.03	1.92	1.14	0.15	1.40	4.48

See note below Table 1 for treatment details

the TA of T_3, T_5 and T_8 was statistically at par and the mean value of T_6 and T_9 was also at par. Organic acids are the major substrate for furnishing fruit's physiological and other metabolic activities, especially in climacteric fruits like custard apples. Titratable acidity was affected by artificial pollination. In our study, acidity was influenced by the source of pollination and the time of pollination. The findings of Jalikoop and Kumar (2007) corroborate our research, demonstrating the effects of different pollen sources on total acidity (TA) content. In our study, the acidity levels varied with time, possibly influenced by environmental factors such as scattered rainfall observed in July. Jalikoop and Kumar (2007) also noted environmental sensitivity to changes in titratable acidity. Additionally, Mohammadi et al. (2017) discovered that the Barhee cultivar exhibited maximum total acidity when pollinated with Zahidi.

Total sugar, reducing and non-reducing sugars: Table 2 shows changes in total sugar (25.55%), reducing sugar (22.75%) and non-reducing sugar (2.66%), which were found maximum in T_1 while the minimum in T_0. Assisted pollination and its timing significantly affected the composition of total sugar, reducing sugar, and non-reducing sugars. The content of all three sugars was recorded to be highest in treatment T_1, where pollination was carried out with Balanagar pollens during 1st week of July. During the first week of July, there was the occurrence of little rainfall with a bit higher temperature. With limited fruit production on the tree, more photosynthates are directed towards developing fruits, resulting in a strong source-sink ratio. Additionally, higher temperatures on the sides of the tree and reduced competition for light may contribute to increased sugar synthesis. Studies suggest that supplying sufficient carbohydrates and essential nutrients to fruits can accelerate maturity, thereby enhancing fruit quality and overall sugar content. Similar observations were made by El-Kassas and Mahmoud (1984) in date palms. Shafique et al. (2011) also noted higher sugar levels in Dhakki date palms when pollinated with M-3 and M-2.

Ascorbic acid: The maximum (27.87 mg/100g) of ascorbic acid (AA) was recorded in T_1, whereas naturally pollinated fruits showed the least value for AA (Table 2). Ascorbic acid acts as an antioxidant because it scavenges the available free radicals in the fruits, thus preventing degradation during the oxidation process (Meena and Asrey, 2018). The variation in ascorbic acid contents of fruits pollinated by different pollen sources could be due to the metaxenial effect (Helail & El-Kholey 2000). Further, less ascorbic acid oxidase activity in T_1 fruits might be a reason for higher retention of ascorbic acid because ascorbic acid oxidase converts AA into dehydroascorbic acid and phenol oxidase. Sugars used during photosynthesis are responsible for ascorbic acid synthesis (Shafique et al., 2011) in date palms.

Total phenols: Phenols are the secondary metabolites present abundantly in sugar apple. It is evident from the data that the application of assisted pollination has significantly affected the total phenols. The minimum (66.76 µg GAE/100g) TPC content was recorded in fruits of T_1 whereas the maximum (82.10 µg GAE/100g) TPC content was recorded in the control. Phenolic compounds have strong antioxidant potential. Natural pollination accumulated more synthesis of TPC content as compared to other treatments. The difference in findings might be due to different mechanisms of hormones, temperature, pollination distribution and their reaction. The interaction between the tree and environmental factors might have triggered these differences. Total phenol content decreases due to increased activity of the polyphenol oxidase enzyme responsible for the oxidation of phenols. Sharifkhah *et al.* (2020) evaluated the influence of pollen time on TPC content and obtained significant results. A similar finding has been reported in date palms (Al-Turky *et al.,* 2010).

Sensory evaluation: Based on different sensory parameters, a score was given. A higher score was given to sweetness (8.0), sourness (8.5), aroma (9.0), grittiness (9.0) and overall acceptability (8.62) for T_1 fruit, which might be due to better TSS, acidity and blend taste of the fruit. Differences emerged among various biochemical and physical parameters based on pollen source and time. Treatment T1 received the highest rating for texture, primarily due to the presence of grittiness in the pulp. The enhanced development of sugars, texture, and

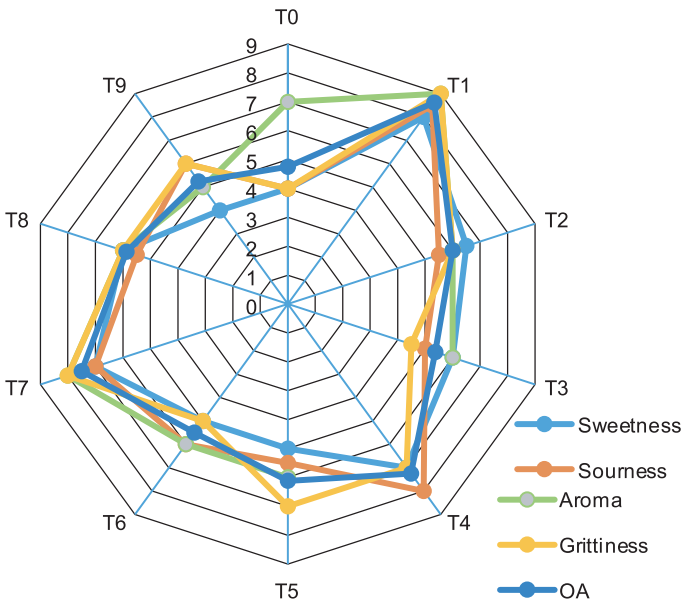

Fig. 1. Effect of pollen sources and time of pollination on organoleptic properties of sugar apple cv. Arka Sahan. See treatment details as footnote of Table 1.

aromatic compounds in T_1 fruits likely contributed to improved sensory properties and mouthfeel, as acknowledged by experts. Consequently, the overall acceptability of T_1 fruits might be attributed to the superior sensory traits identified by experts.

In conclusion, assisted pollination with Balanagar from 8th July to 15th July (T_1) proved to be best for assisted pollination in sugar apple. This treatment significantly retained higher physicochemical parameters such as fruit weight, pulp, seed ratio, TSS, TA, sugars and AA. Besides, T_1 treatment also obtained a higher sensorial score. Thus, Balanagar can be considered a good pollen source for hand pollination in the Arka Sahan sugar apple.

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