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Original Research Paper

Floral biology and reproductive potential of Annona hybrid Arka Sahan

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ABSTRACT

Annona hybrid Arka Sahan has become a commercially important fruit in recent times and it needs an assisted pollination to get good size. Studies on floral biology of Annona hybrid Arka Sahan revealed that major bloom occurs during March-April. Most of the flowers start opening during the afternoon and continued till 7.00 pm, while, pollen dehiscence mainly occurred in early morning in the following day. The maximum stigma receptivity was recorded on the day of anthesis. Completely pendulous petal was more common in Arka Sahan flowers resulting improper pollination. The minimum number of beetles and bees were observed between 7.00 to 8.00 am. Pollen germination and viability were lower at anthesis (5.8% and 44%, respectively) and declined as the day progressed. The pendulous nature of petals eventually brings some un-pollinated stigmas potentially resulting in delayed selfing leading to misshaped fruits.

Keywords: Anther dehiscence, flowering, pollen germination, pollen viability, stigma receptivity

INTRODUCTION

In the world of tropical fruit cultivation, the Annona reigns supreme with its succulent flavors and captivating allure. However, its commercial cultivation faces a significant challenge i.e. the elusive secret of pollination. Neither self-pollination nor typical insect-assisted cross-pollination can fully unlock the potential of the Annona, hindered by its enigmatic protogyny and lack of attractive floral traits. Breeding efforts are entangled in the complexities of heterozygosity. Researchers, armed with curiosity and scientific acumen, embark on a quest to unravel the mysteries of Annona's floral morphology and pollen viability. They meticulously examine each flower, discovering hidden wonders within petals and pistils. They scrutinize pollen grains, uncovering their potential to fertilize and yield abundant fruits. Armed with this newfound knowledge, these researchers navigate the maze of Annona breeding with confidence. They select superior genotypes for hybridization, ensuring a future where fruitful abundance prevails.

The remarkable hybrid 'Arka Sahan', resulting from the crossbreeding of 'Island Gem' (*A. cherimola* x *A. squamosa*) with 'Mammoth' (*A. squamosa*), has been introduced for cultivation (Jalikop & Kumar, 2007). Despite its promising pedigree, 'Arka Sahan', like many other Annona species, displays suboptimal fruit yield characterized by variability in shape and size. This observation prompted an investigation into the reproductive phenology of Annona hybrid 'Arka Sahan', aiming to elucidate the ecological and evolutionary influences on its flowering patterns and their interaction with pollinators (Elzinga et al., 2007). The present study was designed to ascertain the flowering pattern, pollination dynamics, and evaluate pollen grain viability and germination kinetics at the moment of anther dehiscence, focusing specifically on hyrbid Arka Sahan under Bangalore region of Karnataka.

MATERIALS AND METHODS

The investigation was conducted at the Experimental Farm, ICAR-Indian Institute of Horticultural Research, Bengaluru, India, spanning from April to June of 2021 and 2022. The study focused on the floral biology of 'Arka Sahan'. Various floral characteristics were examined, including flowering time, flower bud diameter (mm), flower length (cm), duration of flowering, pedicel length, petal color, petal inner color, petal length, petal width, anthesis time, anther dehiscence, stigma diameter, angle of opening

during anther dehiscence, and the number of flowers per 10 cm shoot. Anthesis was defined as the point at which fully opened and reflexed petals at the flower base were observed.

For assessing stigma receptivity, hydrogen peroxidase activity (6%) was utilized as a determinant (Chen et al., 2013). Fruit set evaluation involved manual pollination of flowers daily, employing Balanagar pollen as the pollen source, conducted between 6:30 to 7:30 AM. The percentage of fruit set was recorded at harvest time (Kishore et al., 2010). Recording of flower visitors and visitation frequency was conducted throughout the research period, documenting all diurnal flower visitors. Pollen analysis was carried out using a ZEISS EVO 18 SEM, with pollen size ranging from 4 to 10 micrometers. For *in vitro* germination testing, pollen germination media containing 5% sucrose and 100 ppm boric acid was utilized (Subhash et al., 2019). After a 2-hour incubation period, germination rates were recorded using a stereo zoom microscope (Zeiss Axio Imager). Pollen viability was also assessed at hourly intervals from 6:00 AM to 11:00 AM using the Alexander staining method (Atlagic et al., 2012).

RESULTS AND DISCUSSION

In the context of Arka Sahan, the initiation of flower buds occurs approximately 40-45 days after pruning (Fig. 1A), with flowering commencing within 8-10 days following bud initiation. Notably, in the mild tropics of Karnataka, the major bloom periods traditionally fall in March and April. However, in March 2022, the first flower was observed during the first week, with flowering persisting for a duration of 45-55 days, peaking between March and April (Table 1a). The 10 cm stems bear 1-5 flower buds in axillary positions, occurring either singly, in pairs, or in multi-flowered fascicles on new branches. The distinctive floral chamber of Arka Sahan is formed by three pale-colored petals, measuring approximately 2.5 cm in length and 0.8 cm in width. These findings align with those of Bhutani et al. (2020). Despite their comparatively large size, these light green petals possess features that make them unattractive to pollinating insects, highlighting an intriguing dichotomy between visual appeal and functional aspects that warrants further investigation in botanical research.

The petals of the Arka Sahan, during the female phase, exhibit a unique opening behavior. Initially, they open slightly at an angle of about 10-15°. As time progresses and the anthers dehisce, the petals open wider to an angle of about 30-35°. This unique petal opening process plays a crucial role in the plant's reproductive phase. (Table 1a). Bisexual flowers exhibit protogynous dichogamy, with the receptivity

Table 1a : Floral characters of 'Arka Sahan' [Island gem (*Annona atemoya* Hort.) x Mammoth (*A. squomosa* L.)]

Floral character	Observation
Flowering period	March and April
Flower length	3.5 cm with pedicel
Peal outer colour	Yellow-green group, 144B
Petal inner colour	Yellow-green group, 150D
Angle of opining at peak female phase	10-15°
Angle of opining at anther dehiscence	30-35°
Pollination mechanism	Canthrophily/ Entomophily
Anthesis	Afternoon
Anther dehiscence	Early morning after anthesis
Pollen size (10µm focus) (tetrad)	27×39 µm (monad), 41×53 µm (dyad), 54×52 µm (triad), 64×49 µm
Stigma type	Wet
Stigma size	2.5 mm
Peak stigma receptivity	On the day of anthesis

of the 'wet' stigma typically commencing at the time of anthesis, often in the afternoon. Pollen dehiscence occurs on the next day after anthesis (Fig. 1E and 1F), characterized by longitudinal dehiscence of anthers in the early morning, releasing medium-sized pollen along the suture (Fig. 1B), consistent with findings by Bhutani et al. (2020). The transition from the female stage to the male stage occurs between 12:00 am and 4:00 am of the following day (2nd assessment day). During this interval, the vast majority of flowers reach the male stage, although a small portion may extend until 5:00 am of the same day (2nd assessment day). This transient phase of female to male flowers is characterized by full opening of flowers or drooping of petals and anther dehiscence. The morphological characteristics of the stigma change significantly during the 5-day period (2 days before and after anthesis along with the day of anthesis). Two days before anthesis, the stigma appears as light green with slightly wet papillae, accompanied by a small number of copious exudates with a diameter of 2.5 mm. On the day of anthesis, the stigma presents as light green with wet papillae and a high number of exudates. The Annona hybrid Arka Sahan exhibits a wet stigma similar to that previously reported in the closely related species *A. squamosa* (Vithanage, 1984). At 1 to 2 days after anthesis (DAA), the stigma dries, wilts from the base, and the papillae turn brown with a reduced stigma diameter (2 mm), indicating decreased support for pollen adhesion.

Stigma receptivity exhibited a notable temporal variation, beginning 2 days before anthesis and remaining receptive for 5 days. Peak receptivity to pollen occurred on the day of anthesis, evidenced by increased bubble formation around its peripheral areas (Fig. 2A), coinciding with the highest fruit set percentage (92%) (Table 1b). Up to 2 days before anthesis, the stigmatic surface accumulated numerous

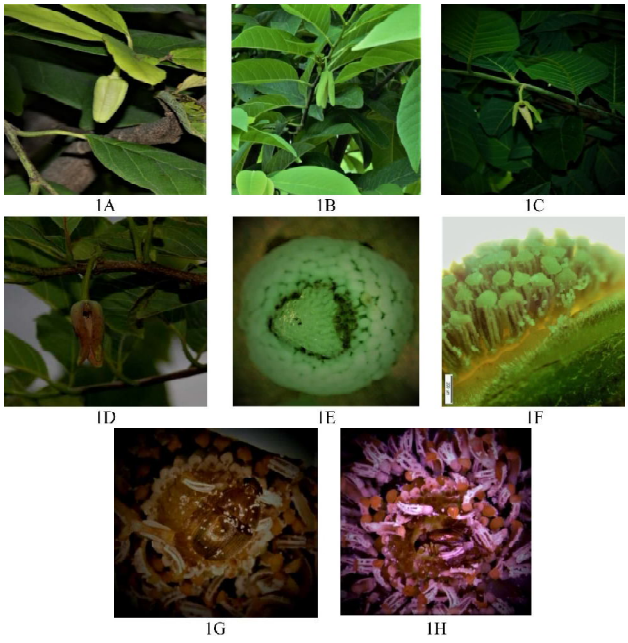


Fig. 1 : Illustration of the sequence of flowering stage of Annona hybrid Arka Sahan from closed flower bud to senescent. Closed flower bud (1A), anthesis (female stage) (1B), fully open flower (male stage) (1C), senescent flower (1D), receptive stigma at anthesis (1E), pollen dehiscence (1F), *Carpophilus domidiatus* (1G) and *Carpophilus hemipterous* (1H)

small and compact bubbles, resulting in lower fruit set percentages (32% and 86%) (Table 1a). As the stigma developed 1 to 2 days after anthesis, the density of bubbles notably decreased, accompanied by a very low fruit set percentage (30%) (Table 1b). This phenomenon has been previously observed in Balanagar (*A. squamosa*) (Nalawadi et al., 1975).

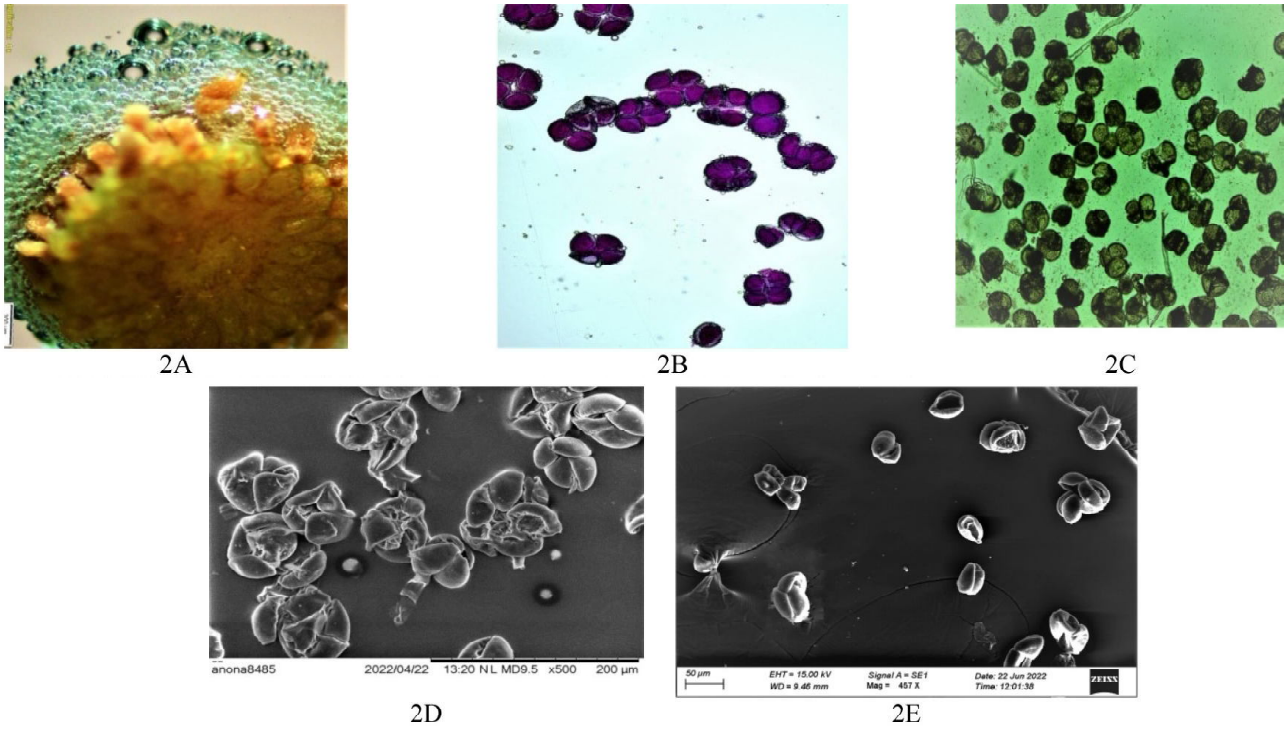
Flower visitation primarily involved two species of small nitidulid beetles and bees (Fig. 2B). The frequency of beetle visits was notably low, ranging from 1 to 2 per 10 minutes, occasionally even absent. Given that anther dehiscence occurs early in the morning, drooped petals restrict movement, and no

Table 1b : Stigma receptivity in terms of fruit set in Arka Sahan

Time of pollination	No. of flowers pollinated	No. of fruit set as observed after 3 weeks	Percentage of fruit set
Two days before the anthesis	50	16	32
One day before the anthesis	50	43	86
On the day of the anthesis	50	48	92
One day after the anthesis	50	15	30
Two days after the anthesis	50	nil	nil

pollen source is available during this time, aligning with findings by de Almeida-Junior et al. (2018). The collected beetles were sent for identification to ICAR-NBAIR, Bangaluru, where they were identified as nitidulid beetles; *Carpophilus domidiatus* and *C. hemipterous* (Fig. 1G and H). Insect visit counts were conducted hourly in the morning from 6 am to 9 am, as flowers exhibit prolonged opening during this period compared to daytime. Attempts to collect nectar were unsuccessful, indicating that Arka Sahan flowers do not produce nectar. Pollen serves as the sole reward for flower visitors. Arka Sahan exhibits aggregate pollens comprising monads, dyads, triads, and tetrads in varying percentages (1.4%, 6.55%, 6.09%, and 8.06%, respectively) (Fig. 2C) (Table 2). Tetrads are predominantly observed among aggregate pollen types (Fig. 2E). The hybrid Arka Sahan displays various types of tetrads, including rhomboidal, tetragonal, tetrahedral, and rarely decussate forms. Li and Xu (2019) also reported tetrahedral, tetragonal, rhomboidal, and decussate pollen in Annonaceae.

The pollen of Arka Sahan is characterized as inaperturate, polar, and radially symmetrical. In polar view, the pollen appears approximately circular, while, in equatorial view, it displays asymmetry with a rounded distal face and an angular proximal face. The



2A 2B 2C

2D 2E

Fig. 2: Stigma receptivity using peroxidase Test (2A), pollen viability (2B), pollen germination (2C), SEM image of pollen germination (2D) and SEM image of Pollen units with monad, dyad, triad and tetrads (2C)

Table 2 : Pollen units and pollen viability, germination pollen tube length (µm)

Treatment	Monads (%)	Dyads (%)	Triads (%)	Tetrads (%)	Pollen viability (%)	Pollen germination (%)	Pollen tube length (µm)
T1	1.32	6.83	5.48	75.9	44.25	5.85	125
T2	1.55	7.91	6.63	80.6	51.38	5.38	157
T3	1.70	5.43	5.75	72.9	68.54	6.19	138
T4	1.18	7.22	6.84	86.3	56.25	5.84	175
T5	1.31	5.36	5.75	89.6	42.55	6.62	138
Mean±SD	1.41±0.20	6.55±1.12	6.09±0.60	81.06±6.96	52.59±10.48	5.97± 0.46	146.6±19.55

longest axis of the pollen grain is observed within the tetrad. The formation of pollen in tetrads is a common trait in Annonaceae genera, as previously reported by Le Thomas (1980) and Le Thomas (1981).

In the present study, the highest pollen viability (52.59%) (Fig. 2B) and germination rate (5.97%) (Fig. 2C), along with pollen tube elongation (125-175 µm) (Table 2), were recorded for samples collected at 5:00 am. There was a noticeable decline in germination and viability over time, likely due to pollen desiccation leading to loss of viability. Samples collected in the early morning (5:00 am) exhibited the highest *in vitro* germination rates, with no germination observed after 10:00 am.

Maitha et al. (2022) similarly found that the highest percentage of pollen germination and tube length occurred at the anthesis stage for all cultivars. A greater pollen tube elongation value (175 µm/28°C) was recorded for pollen collected at 5:00 am, aligning with results reported by Subhash et al. (2019) in Custard apple.

CONCLUSION

Floral biology studies on Annona hybrid Arka Sahan revealed its unique reproductive traits, including protogynous dichogamy and temporal changes in stigma receptivity. Nitidulid beetles and bees are identified as potential pollinators, with pollen being the sole reward. Detailed analysis of pollen characteristics provides insights into reproductive success. This study lays ground work for further research, crucial for Arka Sahan's sustainable cultivation and conservation, with potential implications for improving crop productivity and quality.

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