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Lokesha R
Ph.D. Scholar, Department of Fruit Science, College of Horticulture, UHS, Bagalkot, Karnataka, India

Athani SI
Professor and Director of Extension, UHS, Bagalkot, Karnataka, India

Gollagi SG
Assistant Professor and Head, HREC, Tidagundi, UHS, Bagalkot, Karnataka, India

Sanjeevraddi G Reddy
Assistant Professor and Technical Assistant, Director of Research, UHS, Bagalkot, Karnataka, India

Nadaf AM
Assistant Professor and Head, Department of Entomology, KRCCH, UHS, Bagalkot, Karnataka, India

Prasanna SM
Assistant Professor and Head. Department of Soil Science and Agriculture Chemistry, COH, Bagalkot, UHS, Bagalkot, Karnataka, India

Patil DR
Professor, Department of Fruit Science, UHS, Bagalkot, Karnataka, India

Corresponding Author:
Lokesha R
Ph.D. Scholar, Department of Fruit Science, College of Horticulture, UHS, Bagalkot, Karnataka, India

Effect of different plant growth regulators on flowering and fruit set of custard apple (*Annona Squamosa* L.) cv. Balanagar under northern dry zone of Karnataka

Lokesha R, Athani SI, Gollagi SG, Sanjeevraddi G Reddy, Nadaf AM, Prasanna SM and Patil DR

Abstract
The experiment was carried out at Horticultural Research and Extension Centre, Vijayapura, Tidagundi on 12 years old, healthy and vigorous and uniformly grown custard apple trees plant to assess the Effect of different plant growth regulators on flowering and fruit set of custard apple (*Annona squamosa* L.) cv. Balanagar. The experiment was laid out in randomized block design with seven treatments and three replications. Different treatments were GA_3 at 50 and 100 ppm, NAA at 100 and 200 ppm and Brassinosteroid at 0.5 and 1 ppm, theses plant growth regulators were sprayed at 3 stages like before flowering, during flowering and after fruit set. The experiment was conducted in two season 2021 and 2022 in January to June. Among the different plant growth regulators sprayed treatment NAA 200 ppm recorded Minimum (22.33) days required for initiation of flowering, minimum (30.42 and 106.92) days required from flowering to fruit set and flowering to maturity, respectively. Maximum 26.17, 38.92 and 122.00 days required for initiation of flowering, days required from flowering to fruit set and flowering to maturity, respectively was recorded in control treatment. Similarly, the maximum number of flowers per shoot (63.14) and per plant (757.22) and pollen viability were recorded in NAA 200 ppm. More number of fruits per plant (83.75) and higher fruit set (13.03%) were recorded in NAA 200 ppm. Whereas, minimum flowers per shoot (43.72), per plant (523.81), pollen viability (50.65%), number of fruits per plant (47.25) and fruit set (9.18%) were recorded in control treatment (water spray).

Keywords: Custard apple, plant growth regulators, NAA, GA_3, brassinosteroid

1. Introduction
Annonas are very delicious fruits, consumed as a desert fruits. In different countries, the name custard apple is given to separate species of genus *Annona.* The custard apple botanically called as *Annona squamosa* L. belongs to the family Annonaceae and indigenous to tropical America. There are over 100 species of *Annona* several of these bear edible fruits (Mahadeem, 1990) [13], however, only six species produce fruits of commercial significance *viz*., *Annona squamosa, Annona cherimola, Annona reticulate, Annona muricata, Annona diversifolia and Annona muricata. Species Annona squamosa* is the most frequently grown fruit in India and probably in the world tropical and subtropical region. It is called by different names in different parts of the country like sugar apple, sweetsop and called by Sharifa in northern India and Sitaphal in southern India. It is produced on 41000 hectares in India, with an annual yield of 387.26 metric tonnes (Anon, 2021.) [3]. In India, it is grown commercially on smaller scale in Telangana, Bihar, Maharashtra, Madhya Pradesh, Odisha, Gujarat, Andhra Pradesh, Rajasthan, Uttar Pradesh, Assam, Karnataka and Tamil Nadu. Maharashtra stands first in production of custard apple with a total production of 120.88 metric tonnes. In Karnataka, it is grown in an area of 1800 hectare with the production of 13,400 tonnes and productivity of 7.4 tonnes per hectare (Anon, 2021) [3].
Annonas are mostly consumed as desert fruits, used in ice creams and other milk products and preserved as jam and jam jelly. The immature fruits, seeds, leaves and roots are known for their medicinal use in Ayurveda (Pareekh and Sharma, 1993) [15]. Sugar apples include a lot of vitamin C, dietary fibre, vitamin B6, magnesium, potassium, and a little amount of vitamin B2, as well as some complex carbohydrates.
Custard apple has short juvenile phase compare to other fruit crops starts bearing within three to four years of planting. Generally flowering starts February to March and continue up to June to July. Flowering period of custard apple coincides with the summer where temperature is very high and lesser relative humidity.

No fruit set occurs in summer even though flowers appears in spring and summer months, it occurs only in rainy season naturally and only one to eight percent of fruit set has been recorded under natural conditions (Thakur and Singh, 1965 and George and Nissen, 1988) [21, 7]. Poor pollination causes less fruit set in custard apple, which might be due to both external and internal factors such as low humidity at flowering, soil moisture stress and competition between vegetative and floral growth, hypogyny, dichogamy, poor pollen germination and a lack of insect pollinators.

The cultural practices includes close planting, regular irrigation in summer months, overhead spraying of water and mulching help in enhancing setting of fruits (Ahmed, 1936; Rao, 1974; George and Nissen, 1988) [1, 19, 7]. However, it has been asserted that hand pollination can boost fruit yield and quality, but it is time and money consuming. In an effort to replace hand pollination, growth regulators, namely GA_3, NAA, and Brassinosteroid to improve the flowering and fruit set during off season in custard apple cv. Balanagar.

2. Material and Methods

The field experiment was conducted on 12 years old, healthy and vigorous and uniformly grown custard apple trees of cv. Balanagar planted at a spacing of 4.5 x 4.5 m at Horticultural Research and Extension Centre, Vijayapura. Tidagundi. Karnataka located in a Northern dry zone of Karnataka at 16.59' North latitude, 75.45' East longitude and at an altitude of 513 m above mean sea level with an average annual rain fall of about 589 mm. Soil was alkaline with pH range from 7.5 to 8.5 having medium to black in colour. The experiment was laid out in Randomized Block Design consist of seven treatments and three replications and conducted in two season 2021 and 2022 in January to June. Different plant growth regulators were GA_3 50 and 100 ppm, NAA 100 and 200 ppm, Brassinosteroid at 0.5 and 1.00 ppm and water spray served as a control. These plant growth regulators were sprayed at three stages *i.e.*, before flowering, during flowering and at fruiting stage. Recommended dose of fertilizer is common for all the treatments (25 tonnes of FYM and 123 kg: 62 kg: 62 kg N: P: K per hectare (250:125:125 g of NPK/plant) and intercultural operations like pruning, defoliation (manual), weeding, irrigation and control of pest were performed during the right stages. All the observations were recorded by using the following methods.

Number of days required for initiation of flower (days) was recorded by using the date when the blossom first became apparent on each tree from start of the experiment was noted and recorded as same. The days taken from flowering to fruit set and flowering to maturity were recorded by recording the number of days required for the initiation of flowering on each tagged plants and on date of fruit set and maturity after flowering was recorded on the same tree and average number of days from flowering to fruit set and maturity was recorded.

To record the number of flowers per shoot, three shoots were tagged and counted the number of flowers manually and recorded the average number of flowers per shoot and number of flowers per shoot was multiplied with total number of shoots per plant to calculate the number of flowers per plant**.**

To assess the pollen viability aceto-carmine test was used (Jalikop and Kumar, 2007) [10]. The percentage of fruit set was calculated by dividing the total number of fruits set by total number of flowers per tree and multiplied by 100. The matured fruits were harvested and counted at each harvesting from each tagged plant for observation. The total number of fruits harvested during the entire harvesting period of each is counted as total number of fruits per plant.

3. Results

3.1 Reproductive parameters

The results obtained on reproductive parameters like number of days required for initiation of flowering (days), number of days required from flowering to fruit set (days), number of days from flowering to maturity (days), number of flowers per shoot, number of flowers per plant and pollen viability test (%) during the flowering period of both the years 2021 and 2022 in response to different plant growth regulators sprayed were compiled and presented in Table 1 and 2.

Days required for initiation of flowering, days required from flowering to fruit set and flowering to maturity in custard apple were found significantly influenced by the different plant growth regulators in both the season 2021 and 2022 and in pooled data.

In pooled data, the minimum number of days (22.33) required for the initiation of flowering was recorded in the treatment T_4 (NAA 200 ppm) and it was statistically on par with T_3 (NAA 100 ppm), T_1 (GA_3 50 ppm) and T_2 (GA_3 100 ppm) where they recorded 22.75 days, 23.33 days and 23.50 days, respectively. However, the maximum number of days for the initiation of flowering was recorded in control treatment (26.17 days). Treatment T_4 (NAA 200 ppm) recorded the minimum (30.42 days) number of days from flowering to fruit set and it was followed by T_3 (NAA 100 ppm) (32.17 days) and it was on par with T_1 (GA_3 50 ppm) and T_2 (GA_3 100 ppm) 33.33 days and 33.67 days, respectively. The maximum number of days from flowering to fruit set was recorded in control treatment (38.92 days).

The minimum number of days (106.92 days) required from flowering to maturity was recorded in T_4 (NAA 200 ppm) treatment and it was statistically on par with T_3 (NAA 100 ppm) (107.42 days) and it was followed by T_2 (GA_3 100 ppm) and T_1 (GA_3 50 ppm) they recorded 110.42 days and 113.25 days, respectively. The more number of days required from flowering to fruit set was recorded in T_7 (Control) treatment (122.00 days).

The data regarding effect of different plant growth regulators on number of flowers per shoot, per and pollen viability was found significant among the different plant growth regulators in both the season (2021 and 2022) in pooled data and presented in Table 2. In pooled data, it was found that maximum (63.14) number of flowers per shoot was recorded in T_4 (NAA 200 ppm) treatment and it was followed by T_3 (NAA 100 ppm) and T_2 (GA_3 100 ppm) they recorded (59.11 and 55.94 flowers/shoot, respectively.). However, minimum (43.72) number of flowers per shoot was recorded in control treatment.

Total number of flowers per plant was significantly influenced due to plant growth regulators. In pooled data, maximum (757.22) number of flowers per plant were found in the treatment T_4 (NAA 200 ppm) and it was followed by T_3 (NAA 100 ppm) it was recorded (709.56 flowers/plant) and was on par with T_2 (GA_3 100 ppm) (671.00 flowers/plant). While lowest values were recorded in control treatment (523.81 flowers/plant). The maximum pollen viability was recorded in the treatment T_4 (NAA 200 ppm) it recorded 65.79 (%) and followed by T_3 (NAA 100 ppm) it recorded 64.20 per cent and these were statistically on par with each other and minimum pollen viability was found in control treatment (50.65%).

Table 1: Days required for initiation of flowering, flowering to fruit set and flowering to maturity of custard apple cv. Balanagar as influenced by various plant growth regulators

Treatments	Days required for initiation of flowering			Days required from flowering to fruit set			Days required from flowering to maturity		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
T_1	24.50 ^{a}	22.17 ^{ab}	23.33 ^{abc}	34.00 ^{b}	32.67 ^{b}	33.33 ^{bc}	117.00 ^{b}	109.50 ^{bc}	113.25 ^{dc}
T_2	24.17 ^{a}	22.83 ^{b}	23.50 ^{abc}	35.17 ^{bc}	32.17 ^{b}	33.67^{b}	116.67 ^{b}	104.17 ^{a}	110.42 ^{cb}
T_3	23.83 ^{a}	21.67 ^{a}	22.75 ^{ab}	32.17 ^{a}	32.17 ^{b}	32.17 ^{b}	113.50 ^{a}	101.33 ^{a}	107.42 ^{ab}
T_4	23.33 ^{a}	21.33 ^{a}	22.33 ^{a}	31.83 ^{a}	29.00 ^{a}	30.42 ^{a}	113.33 ^{a}	100.50 ^{a}	106.92 ^{a}
T_5	23.83 ^{a}	23.50 ^{cd}	23.67 ^{bc}	36.00 ^{c}	32.50 ^{b}	34.25 ^{c}	116.00 ^{b}	111.50 ^{a}	113.75 ^{dc}
T_6	24.50 ^{a}	24.00 ^{d}	24.25 ^{c}	36.00 ^{b}	32.83 ^{b}	34.42 ^{c}	117.17 ^{b}	112.33 ^{c}	114.75 ^{d}
T_7	26.83 ^{b}	25.50 ^{e}	26.17 ^{d}	39.67 ^{d}	38.17 ^{c}	38.92 ^{d}	124.33 ^{c}	119.67 ^{d}	122.00 ^{e}
S.Em±	0.61	0.31	0.40	0.53	0.64	0.41	0.71	2.18	1.11
CD at 5%	1.87	0.96	1.22	1.63	1.97	1.26	2.19	6.72	3.43

Note: T_1 – GA $_3$ 50 ppm, T_2 - GA $_3$ 100 ppm, T_3 – NAA 100 ppm, T_4- NAA 200 ppm, T_5- Brassinosteroids 0.5 ppm, T_6 – Brassinosteroids 1.0 ppm, T_7 - Control (water spray). *PGR sprayed at three stages – 1. Before flowering 2. During flowering 3. After fruit set

Table 2: Number of flowers per shoot, flowers per plant and pollen viability custard of apple plants cv. Balanagar as influenced by various plant growth regulators

Treatments	Number of flowers/shoot			Number of flowers per plant			Pollen viability (%)		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
T_1	50.83 ^{bcd}	55.11 ^{c}	52.97 ^{cd}	609.72 ^{bcd}	651.28 ^{c}	630.50 ^{cd}	56.43 ^{b}	66.17 ^{bc}	61.30 ^{b}
T_2	51.83 ^{bc}	60.06 ^{b}	55.94 ^{c}	621.72 ^{abc}	720.28 ^{b}	671.00 ^{bc}	56.66 ^{b}	66.84 ^{abc}	61.75 ^{b}
T_3	54.61 ^{ab}	63.61 ^{b}	59.11 ^{b}	655.11 ^{ab}	764.00 ^{b}	709.56 ^{b}	60.54 ^{a}	67.87 ^{ab}	64.20 ^{a}
T_4	57.17 ^{a}	69.11 ^{a}	63.14 ^{a}	685.83 ^{a}	828.61 ^{a}	757.22 ^{a}	62.18 ^{a}	69.40 ^{a}	65.79 ^{a}
T_5	46.06 ^{de}	54.17 ^{c}	50.11 ^{de}	553.94 ^{de}	650.00 ^{c}	601.97 ^{d}	53.37 ^{c}	64.34 ^{cd}	58.86 ^{c}
T_6	47.50 ^{cd}	51.61 ^{c}	49.56 ^{e}	571.28 ^{cd}	649.22 ^{c}	610.25 ^{d}	51.05 ^{c}	62.36 ^{d}	56.71 ^{d}
T_7	41.39 ^{e}	46.06 ^{d}	43.72 ^{f}	494.56 ^{e}	553.06 ^{d}	523.81 ^{e}	46.70 ^{d}	54.59 ^{e}	50.65 ^{e}
S.Em±	1.72	1.27	1.04	20.88	16.13	14.58	0.97	0.85	0.56
CD at 5%	5.31	3.91	3.19	64.33	49.71	44.94	2.98	2.62	1.74

Note: T_1 – GA $_3$ 50 ppm, T_2 - GA $_3$ 100 ppm, T_3 – NAA 100 ppm, T_4- NAA 200 ppm, T_5- Brassinosteroids 0.5 ppm, T_6 – Brassinosteroids 1.0 ppm, T_7 - Control (water spray). *PGR sprayed at three stages – 1. Before flowering 2. During flowering 3. After fruit set

3.2 Number of fruits per plant and fruit set (%)

The effect of plant growth regulators on number of fruits per plant and fruit set (%) is presented in Table 3. The result clearly indicated that plant growth regulators significantly influenced the number of fruits per plant and fruits set (%) in both the season (2021 and 2022) and also in pooled data.

From the pooled data, more number of fruits per plant (83.75) was recorded under the treatment T_4 (NAA 200 ppm) and it was on par with T_3 (NAA 100 ppm) (79.00 fruits/plant). Next best treatment with respect to number of fruits per plant was T_2 (GA_3 100 ppm) it was recorded 73.83 fruits per plants and it was followed by T_1 (GA_3 50 ppm) and T_5 (Brassinosteroid 0.5 ppm) (67.83 and 65.83 fruits/plant, respectively.). However, the minimum number of fruits per plant was recorded in control treatment (47.25 fruits/plant). Maximum fruit set (13.03%) was recorded in the treatment T_4 (NAA 200 ppm) followed by T_3 (NAA 100 ppm) in this treatment 12.75 per cent fruit set was noticed and these are statistically on par with each other. The next best treatment with respect to fruit set was T_2 (GA_3 100 ppm) (11.67%) it was on par with T_1 (GA_3 50 ppm) and T_5 (Brassinosteroid 0.5 ppm) where they recorded 11.61 and 11.69 per cent, respectively. The lowest fruit set was recorded in control treatment (9.18%).

Table 3: Number of fruits per plant and fruit set (%) of custard apple cv. Balanagar as influenced by various plant growth regulators

Treatments	Number of fruits per plant			Fruit set (%)		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
T_1	72.17 ^{cd}	63.50 ^{c}	67.83 ^{c}	11.84 ^{abc}	11.37 ^{b}	11.61 ^{b}
T_2	76.83 ^{bc}	70.83 ^{b}	73.83 ^{b}	12.35 ^{abc}	11.00 ^{b}	11.67 ^{b}
T_3	82.83 ^{ab}	75.17 ^{ab}	79.00 ^{ab}	12.73 ^{a}	12.77 ^{a}	12.75 ^{a}
T_4	88.83 ^{a}	78.67 ^{a}	83.75 ^{a}	13.04 ^{a}	13.02 ^{a}	13.03 ^{a}
T_5	67.50 ^{de}	64.17 ^{c}	65.83 ^{cd}	12.43 ^{ab}	10.96 ^{b}	11.69 ^{b}
T_6	61.17 ^{ef}	62.67 ^{c}	61.92 ^{d}	10.81 ^{c}	10.61 ^{b}	10.71 ^{c}
T_7	53.67 ^{f}	40.83 ^{d}	47.25 ^{e}	10.88 ^{bc}	7.47 ^{c}	9.18 ^{d}
S.Em±	2.64	2.04	1.66	0.51	0.44	0.26
CD at 5%	8.15	6.28	5.12	1.56	1.36	0.80

Note: T_1 – GA $_3$ 50 ppm, T_2 - GA $_3$ 100 ppm, T_3 – NAA 100 ppm, T_4- NAA 200 ppm, T_5- Brassinosteroids 0.5 ppm, T_6 – Brassinosteroids 1.0 ppm, T_7 - Control (water spray). *PGR sprayed at three stages – 1. Before flowering 2. During flowering 3. After fruit set

4. Discussion

Flowering in custard apple takes place over a lengthy period of time, which commences form March-April and continues until July-August. Flowering is at its maximum in the months of April and May. Fruit set does not occur throughout the spring and summer months, and it only begins during the

rainy season. It is mainly due to increase in temperature and decrease in relative humidity during summer month (March-April) and there will be shortage of soil moisture for growth and development, hot shriveling winds has deleterious effect on flowers and reduces the fruit set and development. Productivity of custard apple in the field may be improved by cultural practices like overhead misting, windbreaks, efficient irrigation schedule and proper nutrient management. Hence, in the present study use of different plant growth regulators like GA3, NAA and Brassinosteroid were used to improve the flowering, fruit set of custard apple cv. Balanagar during off season.

4.1 Reproductive parameters

From the results of pooled data, it is indicated that minimum days took for the initiation of flowering was noticed in the treatment T_4 (NAA 200 ppm) and more number of days was recorded in control treatment. This might be due to the balanced C: N ratio, which helps in balanced management of vegetative and reproductive phases and thereby promote early flowering, these results are in conformity with the findings of Prajapati *et al.* (2016) [17] in custard apple cv. Local and also present study are in controversy with the results obtained by Goswami *et al.* (2013) [9] in pomegranate and this may be due to crop and variety.

Results on the days required from flowering to fruit set and days required from flowering to maturity was minimum in treatment T_4 (NAA 200 ppm) whereas, lowest values were recorded in control treatment. The early fruit set and fruit maturity under plant growth regulators than compared to control might be due to early initiation of flowering by NAA and application of NAA further boost up this process and plants remain physiologically more active to build up sufficient food stock for the developing flowers and eventually resulted in increased number of flowers. The application of GA_3 probably caused early floral bud initiation and flowering by decreasing the concentration of Abscisic acid (ABA). The findings are in conformity with the findings of Bhujbal *et al.* (2012) [4] in sapota and Manju and Rawat (2015) [14] in local malta.

The maximum number of flowers per shoot and per plant was recorded in T_4 (NAA 200 ppm) and minimum number of flowers per shoot and flowers per plant was recorded in control treatment. The increase in flowers per plant and shoot might be due to plants remain physiologically more active to build up sufficient food stock for the developing flowers and fruits production, ultimately resulted into flower set and auxins are also known to stimulate flower bud initiation. Hence, the increase in flowering may be due to enhanced photosynthesis which increased the potential of trees to develop more flower buds. These results are agreement with the findings of Chaudhari *et al.* (2016) [5] in custard apple. Akshay *et al.* (2020) [2] in sapota cv. Cricket ball and Phawa *et al.* (2017) [16] in pomegranate cv. Khandhari.

Pollen viability was highest in T_4 (NAA 200 ppm) and on par with T_3 (NAA 100 ppm) and lowest pollen viability was recorded in control treatment. The increase in pollen viability under NAA is mainly due to NAA is a compound of auxin it helps better pollen germination and viability and our findings are agreed with the findings of Kaur *et al.* (2018) [12] in sapota cv. Kallipatti.

In the present study, different growth regulators treatments varied significantly for the number of fruits per plant and fruit set (%) in custard apple. Among different plant growth regulators T_4 (NAA 200 ppm) increases the number of fruits per plant and fruit set and the lowest values were in control treatment. NAA reduces the flower drop and increases the fruit set and number of fruits per plant due to (NAA) auxins play significant role in fruit set due to their strong mobilization activity which helps to maintain ongoing physiological and biochemical process of inhibition of abscission. And this might be due to anti-abscission property of auxin. Abscisic acid causes dissolution of middle lamella and primary walls of the cell at the base of pedicel and peduncle which leads to detachment of plant organ. However auxin counter acts with the ABA and ethylene and enhanced the Auxin: ABA ratio that ultimately prevents fruit drop. Present study are in conformity with the findings of Sahu *et al.* (2018) [20] and Chavan *et al.* (2009) [6] in sapota. Ghosh *et al.* (2009) [8] in pomegranate cv. Ruby, Kaseem *et al.* (2011) [11] in ber and Pujari *et al.* (2021) [18] in custard apple.

5. Conclusion

Custard apple is dry land fruit crop its flowering commences from February to March and end up to June – July. Although it flowering commences in summer and spring no fruit set occurs naturally. Fruit set is very less in custard apple due to internal and external factors. Hence, to improve the flowering and fruit set spraying of plant growth regulators like GA_3 and NAA is one of the important strategy to boost the yield of custard apple. NAA 100 to 200 ppm helps in early flowering, reduce the days required from flowering to fruit set and flowering to maturity. Similarly, NAA 200 ppm improved the number of fruits per plant and fruit set. Therefore, by adopting all these practices we can get the fruits during off season and get higher prize in the Market.

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