





A study on flower bud differentiation for off-season mango production using ultra high-density planting

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Abstract

The present study was conducted in Jain Irrigation System LTD from 2023 to 2024 to find the flower bud differentiation (FBD) process in ultra-high-density planting (UHDP) systems for off-season mango production using cv: Ratna and Bangalora and the associated morphological and biochemical changes under specific agro-climatic conditions. Observations were taken from August 10, 2023, to analyse FBD phases and their associated physiological changes. The process of fruit bud initiation began on August 20th in all cultivars. From August 20th to December 14th, the initiation and differentiation of fruit buds showed a progressive increase, with the highest number of fruit buds undergoing differentiation during this period. The differentiation process continued further, with the buds reaching an advanced stage of development by December 19th. Five distinct stages of FBD were recognised during the current study. The initiation of FBD was started in the second week of August, reaching its peak by the second week of December and concluding by the third week of December. During the progression of FBD, a significant increase in the total carbohydrate fraction (%) and C/N ratio was recorded, with the C/N ratio peaking at the maximum differentiation stage. Conversely, the total nitrogen fraction (%) exhibited a consistent decline, reaching its lowest at the peak FBD stage. The findings provide critical insights into optimizing UHDP practices for enhancing off-season mango flowering and production in the selected cultivars.

Keywords: C/N ratio; flower bud differentiation; mango; off-season; UHDP

Introduction

The mango (Mangifera indica L.) is widely regarded as one of the world's most delicious tropical fruits. It originates from Southeast Asia, specifically the Indo-Burma region in the Himalayan foothills (1). Mango trees are robust, able to thrive in a variety of soil types, require relatively little maintenance and are also known for their capacity to adapt to a wide range of climatic conditions. Mango ranks prominently among the world's top five most economically valuable fruit species (2). It is the second most consumed fruit globally, accounting for 28.2 % of total fruit consumption, following bananas at 29.4 %. Moreover, mango serves as the primary commercial fruit crop in India (3).

India contributes approximately 64 % of the world's total mango production, with other major producers including Mexico, Pakistan, Brazil, Philippines and Thailand. The country boasts an extensive repository of mango germplasm, encompassing over 1000 varieties cultivated nationwide (4). Despite this diversity, India's mango productivity remains

below its potential. This decline has been mostly caused by many issues, including poor soil quality, insufficient nutrient management and subpar water management techniques. Mango flowering marks a critical physiological event that signals the onset of fruit production (5). In tropical conditions, flowering is impacted by the age of the last vegetative flush in addition to climatic and internal factors. In mango, the flowering process involves several phases, including the initiation of shoots, differentiation of the apical bud into floral structures, emergence of panicles, elongation of inflorescences and eventual opening of flowers (6). This complex progression is governed by a combination of external environmental conditions and internal factors, such as the regulation of specific hormones and genes (7, 8). The timing of Flower Bud Differentiation (FBD) generally varies based on geographic location, ranging from mid-August to mid-November (9, 10). The interaction between environmental factors, carbohydrate levels and developmental phases, particularly under varying climatic conditions (11).

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Research indicates that an increase in total carbohydrate concentration, particularly starch, along with a high carbon-to-nitrogen (C/N) ratio, is commonly observed in the shoots of biennial mango varieties during FBD. The biochemical composition of mango shoots plays a pivotal role in triggering floral stimuli at the bud break stage. The maturity of terminal shoots and the accumulation of carbohydrates in the leaves and shoot apices are closely associated with the synthesis of floral stimuli in mango trees (12). Variations in the timing of FBD across different mango varieties and locations are attributed to prevailing temperature conditions, plant physiological and biochemical traits and cultural practices adopted before FBD initiation (13). Carbohydrate-nitrogen relations are so intimately associated with vegetative and reproductive performance that the various means that are effective in controlling carbohydrate-nitrogen relations become matters of practical interest. Understanding the factors influencing mango flowering, including physiological, biochemical and environmental determinants, is essential for optimizing productivity.

This research aims to explore the flower bud differentiation (FBD) in mango cultivars under an ultra-highdensity planting (UHDP) system that follows a distinct physiological and biochemical pattern, which can be optimized to enhance off-season flowering and yield. The study assumes that FBD initiation and progression are influenced by carbohydrate accumulation, C/N ratio dynamics and nitrogen levels, with peak differentiation aligning with the maximum C/ N ratio. It also postulates that UHDP alters microclimatic conditions and resource allocation, impacting FBD compared to conventional systems. Additionally, soil fertility, nutrient application and irrigation management are expected to play a crucial role in regulating FBD, while cultivar-specific responses may vary due to genetic and physiological differences. These insights aim to refine UHDP practices for improved mango productivity.

Material and Methods

An experiment was conducted on nine-year-old mango trees of cv. Ratna and Bangalora under the Ultra High-Density Planting (UHDP) system at Jain Irrigation Systems Limited Farms, Udumalpet, Tamil Nadu, India, for three consecutive years from 2022 to 2024. The study was carried out on uniform-sized trees spaced at 3 x 2 m. The experimental site, located at an altitude of 1208 feet above Meansea Level (MSL), experiences a mean annual rainfall of 501.40 mm and is surrounded by the Western Ghats on three sides. The average maximum temperature of the study area is 33.58 °C, the average minimum temperature is 21.08 °C and the average relative humidity is 70.83 %. The soil types in the region are black and red, with a pH of 6.56 and an electrical conductivity of 0.051 mS/cm.

In the experimental plot, trees and branches were earmarked for observation. Flower bud differentiation (FBD) was recorded from twenty tagged shoots distributed across all four quarters of the canopy and observed in thirty-six trees, with three replications for each variety. Before applying paclobutrazol for flower induction, various field maintenance tasks were performed. Initially, the shoot tips of branches were

pruned using secateurs to skip the regular flowering season and to promote lateral vegetative bud growth for off-season flowering under the UHDP system. Additional maintenance practices, including fertigation, pesticide application and irrigation through drip systems, were carried out as per the recommended package of practices by Jain irrigation Systems LTD. Meteorological data, such as daily maximum and minimum temperatures, sunshine hours, humidity and rainfall, were recorded at the Jain Irrigation Systems Limited meteorological observatory throughout the study period.

Observations were recorded three months after the application of paclobutrazol, tracking the progression from the initiation of flower buds to their differentiation. The number of shoots that emerged with flowering was estimated based on the emergence of flower panicles in 20 tagged shoots per tree. Panicle emergence observations were taken at five-day intervals from the first flower bud emergence. Additionally, the carbohydrate and nitrogen content in leaves was estimated at the same intervals. Fully mature middle leaves from the tagged shoots were sampled for biochemical and hormonal analyses. To study FBD, buds were categorized into five specific floral stages (0, I, II, III, IV). The proportion of floral differentiation at any given point was determined using 20 observations per tree. FBD was deemed to have begun when over 60 % of the observed buds advanced past Stage II. The flattening of meristematic tissue at the bud's apex marked the commencement of flower bud differentiation. Recorded observations included the timing of FBD, total carbohydrate content (%) and total nitrogen content (%) in flower buds undergoing differentiation. The analysis of carbon and nitrogen content was performed using the KEL PLUS Yellow Nitrogen Distillation Apparatus, ensuring precise measurement and accurate assessment of biochemical changes during the differentiation process. Randomized Block Design (RBD) was employed for the experimental layout to ensure precise treatment comparisons. The collected data were analyzed using R software (version 4.4.3), facilitating comprehensive statistical analyses and providing in-depth insights into the observed variations and trends.

Results and Discussion

The maturity of the terminal shoots and the accumulation of carbohydrates, nitrogen and the C/N ratio were closely associated with Flower Bud Differentiation (FBD) in mango. The relationship between Flower Bud Differentiation (FBD), carbohydrate accumulation, nitrogen content and the C/N ratio showed significant patterns. The initiation and differentiation of flower buds are significantly influenced by the accumulation of carbohydrates and nitrogen content in leaves. Starch serves as the primary carbohydrate reserve and is stored in the chloroplasts of photosynthetically active leaves. A study examining the interaction between carbohydrate levels and the expression of flowering-related genes (FT, LFY and AP1) in regular and alternate-bearing mango cultivars during the flowering stage emphasized the critical role of the carbon-to-nitrogen (C/N) ratio in fruit crops (14). An elevated C/N ratio is associated with improved fruit bud development and differentiation, leading to greater flowering and yield, whereas a lower ratio tends to promote vegetative growth instead (15, 16).

In Table 1, the carbohydrate content increased progressively until mid-September, coinciding with the initiation of FBD in both the Bangalora and Ratna cultivars. The maximum carbohydrate content was recorded on 14^{th} September in Ratna (14.5 %) and on 9^{th} September in Bangalora (14. 2 %), correlating with FBD percentages of 50 % and 35 %, respectively, indicating a close link between carbohydrate levels and floral induction. The significance of the C/N ratio is that a higher C/N ratio is essential for bud differentiation and is influenced by environmental and cultural practices as well (17).

Nitrogen content showed variability, with peaks observed earlier in the season. In Bangalora, nitrogen content reached a maximum of 1.44 % on 30th August, corresponding to 25 % FBD, while in Ratna, it peaked at 1.45 % on 25th August, aligning with 20 % FBD. This suggests that nitrogen content plays a critical role during the initial stages of shoot maturity before carbohydrate domination takes over for floral induction. The C/N ratio showed a progressive increase, reaching its highest values during the peak floral induction period. In Bangalora, the C/N ratio peaked at 11.7 on 9th September, while in Ratna, it reached 11.5 on 19th September. These higher C/N ratios support the hypothesis that elevated carbohydrate levels relative to nitrogen

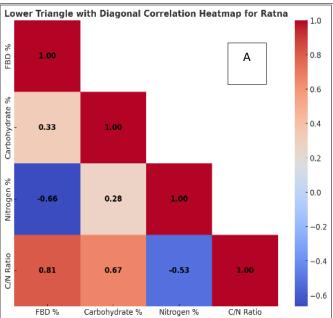
are essential for the synthesis of floral stimuli (18).

A decline in carbohydrate levels was observed after mid-September, likely due to their conversion into soluble carbohydrates for flower and fruit development. By 15^{th} December, the lowest carbohydrate levels were recorded in both cultivars, with Bangalora at 11.2~% and Ratna at 11.8~%, reflecting their utilization during the flowering process. These observations highlight the dynamic interplay of carbohydrates, nitrogen and the C/N ratio during floral induction, with variations influenced by genetic differences and environmental factors.

The percentage of FBD showed a strong positive correlation with carbohydrate content (r > 0.8) for both cultivars, suggesting that carbohydrate accumulation is essential during the process of flowering. In Fig. 1, a negative correlation between FBD percent and nitrogen content (r < -0.6) indicates a shift in metabolic priorities as flowering progresses, with nitrogen being reallocated to support flowering rather than vegetative growth. In Table 2, the Carbon/Nitrogen (C/N) ratio positively correlated with FBD percent, highlighting the importance of carbohydrate dominance in advanced flowering stages. FBD percent positively correlated with stage IV proportions (r > 0.7), emphasizing higher

Table 1. Total carbohydrate fraction, total nitrogen fraction and C/N ratio in twigs of Bangalora and Ratna

		Bangalora				Rat		
Date of Sampling	FBD %	Carbohydrate %	Nitrogen %	C/N Ratio	FBD %	Carbohydrate %	Nitrogen %	C/N Ratio
10-08-2023	0	10.7	1.24	8.6	0	10.2	1.24	8.2
15-08-2023	0	11.1	1.31	8.5	0	10.7	1.32	8.1
20-08-2023	15	12.3	1.37	9.0	10	11.6	1.38	8.4
25-08-2023	25	12.6	1.23	10.2	20	11.9	1.45	8.2
30-08-2023	25	13.5	1.44	9.4	20	12.4	1.42	8.7
04-09-2023	35	14.2	1.42	10.0	35	12.8	1.41	9.1
09-09-2023	35	14.2	1.21	11.7	40	13.5	1.37	9.9
14-09-2023	50	13.2	1.31	10.1	55	14.5	1.42	10.2
19-09-2023	50	13.8	1.35	10.2	55	14.6	1.27	11.5
24-09-2023	65	13.2	1.11	11.9	65	13.9	1.39	10.0
29-09-2023	70	12.9	1.33	9.7	70	13.2	1.17	11.3
04-10-2023	75	12.7	1.15	11.0	85	12.8	1.21	10.6
09-10-2023	90	11.9	1.14	10.4	90	12.4	1.18	10.5
14-10-2023	100	11.2	1.11	10.1	100	11.8	1.14	10.4
19-10-2023	100	11.8	1.17	10.1	100	11.2	1.11	10.1



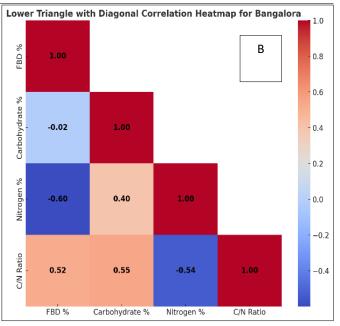


Fig. 1. Correlation analysis of the C/N ratio and its components with flower bud differentiation in mango cultivars (A: Ratna, B: Bangalora).

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Table 2. Flower bud differentiation stages and its development in Bangalora and Ratna

Date of sampling	Bangalora					Ratna				
	0	I	II	III	IV	0	I	II	III	IV
10-08-2023	20	-	-	-	-	20	-	-	-	-
15-08-2023	20	-	-	-	-	20	-	-	-	-
20-08-2023	17	3	-	-	-	18	2	-	-	-
25-08-2023	15	5	-	-	-	16	3	1	-	-
30-08-2023	15	2	3	-	-	15	3	2	-	-
04-09-2023	13	3	3	1	-	15	3	1	1	-
09-09-2023	13	1	2	4	-	13	4	2	1	-
14-09-2023	10	4	1	4	1	12	3	3	2	-
19-09-2023	10	2	3	3	2	9	5	3	2	1
24-09-2023	7	4	3	1	5	9	4	2	1	4
29-09-2023	6	5	2	2	5	6	6	2	2	4
04-10-2023	5	2	4	3	6	3	7	1	3	6
09-10-2023	2	4	3	4	7	2	5	4	2	7
14-10-2023	-	1	5	5	9	-	3	5	3	9
19-10-2023	-	-	4	6	10	-	1	3	4	12

FBD with advanced flowering stages. Carbohydrate content showed strong positive correlations with stage IV, whereas nitrogen content was more aligned with earlier stages. Both cultivars showed increased FBD percent over time, with Ratna reaching stage IV slightly earlier than Bangalora. Carbohydrate accumulation and stage IV proportions closely tracked FBD % progression, reflecting their critical roles in flowering. Ratna exhibited faster progression to advanced stages, likely due to its higher carbohydrate accumulation rate. Fig. 2 illustrates the relationship between Full Bloom Days (FBD %) and the progression to stage IV for both Bangalora and Ratna over time. The decline in nitrogen content and rise in the C/N ratio highlight resource reallocation during flowering, consistent with the findings of studies like (19, 20) which documented similar trends in mango flowering physiology.

Conclusion

This study emphasizes the crucial connection between carbohydrate accumulation, nitrogen levels and the C/N ratio in driving floral bud differentiation (FBD) in Bangalora and Ratna mango cultivars. The differences observed between these cultivars highlight the impact of genetic characteristics and environmental conditions on floral initiation. A higher carbohydrate reserve, along with an optimal C/N ratio, was found to encourage FBD, whereas excessive nitrogen led to increased vegetative growth, thereby inhibiting flowering.

These findings suggest that regulating nutrient inputs, particularly nitrogen and carbohydrates, is essential for maintaining a physiological balance that supports reproductive growth. Additionally, environmental variables such as temperature and light intensity interact with internal nutrient dynamics, further influencing flowering responses. Understanding these interactions can help in designing effective agronomic practices, including precise fertilizer applications, irrigation management, pruning techniques and foliar treatments, to maximize flowering and fruit set. Applying these insights in orchard management can improve the consistency and predictability of flowering, ultimately boosting fruit yield and quality in Bangalora and Ratna mango cultivars.

Authors' contributions

HT contributed to data curation, methodology, investigation, writing of the original draft and visualization. MI was responsible for conceptualization, supervision and methodology, as well as reviewing and editing the manuscript. PS provided resources and contributed to methodology and data curation. KM, BP, IRC and GD were involved in reviewing and editing the manuscript and validating the findings.

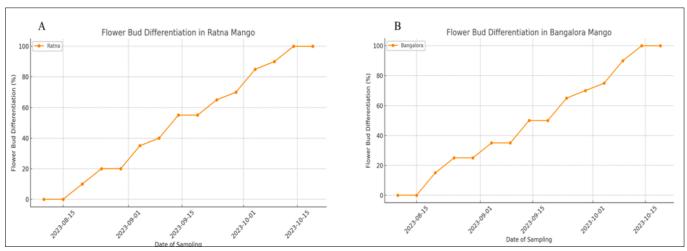


Fig. 2. Line plot illustrating flower bud differentiation over time in mango cultivars under UHDP during the off-season (A: Ratna, B: Bangalora).

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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