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FLOWERING PHENOLOGY AND POLLEN BIOLOGY OF LOW-CHILLING APPLE CULTIVARS UNDER WARM HUMID CONDITIONS OF ASSAM INDIA

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ABSTRACT

The expansion of apple cultivation into humid subtropical regions has emerged as a promising strategy to address climate-induced chilling deficits in traditional temperate production zones. The present study evaluated the flowering phenology and pollen characteristics of three low-chilling apple cultivars, HRMN-99, Dorsett Golden, and Anna, under the sub-tropical climatic condition of Assam during 2024-2025. Flowering in all cultivars occurred from the third week of February to the last week of March, which advanced in 2025 by approximately five days compared to 2024. Among the cultivars, Anna exhibited the earliest bloom, followed by Dorsett Golden and HRMN-99. Bud development progressed sequentially through silver tip, green tip, and pink bud stages, culminating in king bloom, full bloom, and fruit set. Anthesis occurred between 6:00 am and 6:00 pm with peak flower opening during late morning hours, while anther dehiscence was highest around mid-day. Days to anthesis varied among cultivars, reflecting genotypic differences in floral development. Pollen studies revealed distinct morphological and functional variation, with Anna possessing triangular and comparatively larger pollen grains, whereas Dorsett Golden and HRMN-99 exhibited spheroidal pollen. The highest pollen viability was recorded in Dorsett Golden (90.12%), followed by Anna (88.68%) and HRMN-99 (82.76%). Overall, the results demonstrate the adaptability, synchronized flowering behaviour, and satisfactory reproductive potential of low-chilling apple cultivars under warm humid conditions, highlighting their suitability for orchard diversification, improved pollination management, and climate-resilient apple production in subtropical regions.

Keywords **:** Low-chilling apple, phenology, flowering behaviour, anthesis, pollen viability, Assam.

Introduction

Apple (*Malus domestica* Borkh.) is among the most widely consumed fruit crops globally, valued for its nutritional value, desirable sensory characteristics, visual appeal, and high market return**.** Sustained consumer demand for apples, both as fresh fruit and processed products, continues to drive expansion of global production systems. The global demand for this fruit is poised to increase by 3.42 million tons during 2020-2024 at a CAGR of over 1% (Technavio, 2020).

In India, apple cultivation occupies approximately 2.77 lakh hectares with an annual production of about 2.3 million metric tons, with nearly 90% of commercial orchards concentrated in the temperate high-altitude regions of Jammu & Kashmir, Himachal Pradesh, and Uttarakhand. Smaller but emerging cultivation zones are also observed in northeastern states like Arunachal Pradesh, Sikkim, Nagaland, Meghalaya and Manipur (Anonymous, 2018; Atkinson *et al.*, 2013).

Traditionally, apple cultivation has been strongly governed by winter chilling accumulation, which is essential for breaking bud dormancy and ensuring uniform flowering. Generally, commercial apple

cultivars require between 800 and 1,200 chilling hours**,** depending on genotype and climatic adaptation (Campoy *et al.,* 2011). However, recent studies indicate that climate warming and altered temperature regimes are disrupting chill accumulation patterns**,** particularly in northwest Himalayan regions, leading to delayed, prolonged, or irregular bud break and flowering (Luedeling & Brown, 2011; Atkinson *et al.*, 2013; Kumar *et al*., 2017). Such climatic shifts pose serious challenges to orchard productivity and stability.

To mitigate these constraints and sustain yield performance, increasing emphasis has been placed on the development and adoption of low-chilling apple cultivars, which typically require less than 300 chilling hours. These cultivars offer promising opportunities for expanding apple cultivation into lower elevation zones characterized by mild winters and warmer climates**,** thereby supporting diversification and climate resilience in apple production systems.

The blooming behaviour in apple represents a critical phenological event that strongly influences pollination success, fruit set, yield stability, and fruit quality**.** Apple exhibits a gametophytic self and cross incompatibility system, which necessitates the requirement of at least two genetically distinct cultivars for a stable fruit production (de Nettancourt, 2001; Ramírez & Davenport, 2013). Consequently, effective pollination management is fundamental for achieving consistent cropping in commercial orchards (Chauhan *et al*., 2008). In addition, reproductive efficiency in apple is closely associated with pollen viability, germination capacity, and pollen tube growth, traits that are of considerable importance to breeders, researchers, and orchard managers (Sanzol & Herrero, 2001; Pandit *et al*., 2017).

In regions characterized by mild winters, the flowering dynamics of low-chilling apple cultivars become particularly important for successful cultivation. The adaptation of these cultivars depends on their chilling requirements, bloom timing, and phenological responses to temperature variability**,** which collectively determine flowering synchronization and subsequent fruit development (Campoy *et al.*, 2011). A comprehensive understanding of bloom patterns and dormancy release behaviour is therefore essential for site selection, cultivar choice, and optimization of orchard management practices under changing climatic conditions.

Scientific information on the phenological adaptation and reproductive biology of low-chilling apple cultivars under the humid subtropical conditions of Northeast India remains extremely limited. Despite increasing interest in expanding apple cultivation into non-traditional production regions, systematic evaluations of flowering behaviour and pollen biology under warm humid environments are largely lacking. Therefore, the present study provides novel insights into the flowering phenology and pollen characteristics of low-chilling apple cultivars under the agro-climatic conditions of Assam.

Materials and Methods

Plant material and study area

Three low-chilling apple cultivars *viz.*, HRMN-99, Dorsett Golden, and Anna**,** were selected for the study. The cultivars were planted in raised beds at a spacing of 3 m x 3 m**.** The investigation was conducted at the Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat, Assam, during the years 2024 and 2025**.** The experimental site is geographically located at 26°47′ N latitude and 94°12′ E longitude, with an elevation of 86.9 m above mean sea level. The region is characterized by a subtropical humid climate, marked by hot summers and relatively cool winters. Rainfall is predominantly received during the monsoon season, typically commencing in the first week of June and extending through October. Pre-monsoon showers are common during March and April, whereas limited precipitation occurs from November to March. Relative humidity remains consistently high throughout the year, generally exceeding 80%. The recorded maximum temperature ranges between 34-35 °C, while the minimum temperature varies from 8-11 °C during the study period**.**

The soil texture at the experimental site was classified as sandy loam, with a pH of 5.5**,** rich in organic matter and exhibited adequate levels of nitrogen, phosphorus, and potassium.

Flowering Phenological

Phenological stages were observed through systematic visual assessment of the tagged trees. The parameters monitored included the season and duration of flowering, date of bud spur initiation, and date of the pink bud stage.

The date of initial bloom was noted when approximately 10% of flowers had opened on an individual tagged tree. The date of full bloom was recorded when nearly 75% of flowers were open, whereas the date of last bloom corresponded to the stage when complete flowering (100% open flowers) was observed. Further the time and duration of anthesis and the days to anthesis i.e., the time from visible bud swell to full flower opening was also studied. The

contraction of the floral whorl following full floral opening was considered the beginning of dehiscence and the time of dehiscence was recorded on an hourly basis.

The duration of flowering was calculated as the interval between the pink bud stage and the end of flowering. Additionally, the date of petal fall was documented to characterize the completion of the flowering phase.

Pollen Morphology and Viability Assessment

Fresh pollen grains collected from apple flowers were used to assess pollen size and viability. Pollen dimensions were measured under a microscope (ZEISS Primostar 3, FIX-K ÖHLER Versions, Germany) following acetocarmine staining and the mean size was calculated from measurements of 30 individual pollen grains per cultivar. Pollen viability was evaluated using the acetocarmine staining test (Popa, 2021), where deeply stained, morphologically intact grains were classified as viable, whereas poorly stained or shrivelled grains were recorded as nonviable.

The pollen viability was measured using the following formula and expressed in %.

$$\text{Pollen viability} = \frac{\text{Number of stained pollen grains}}{\text{Total number pollen grains observed}} \times 100$$

Results and Discussions

Flowering Phenological

Variations in flowering behaviour are strongly influenced by geographical factors such as latitude, longitude, and altitude, as well as climatic conditions, rootstock selection, and orchard management practices (Karacali, 2004).

The flowering duration of the three low-chilling apple cultivars extended from the third week of February to the last week of March. In the 2025 season, flowering commenced approximately five days earlier compared with the 2024 season. This shift in flowering phenology may be attributed to higher temperatures during late winter and early spring, which are known to accelerate bud development and influence dormancy release. Similar observations have been reported where elevated temperatures promoted earlier blooming and enhanced floral activity (Castro *et al.,* 2016). Studies conducted under North Indian conditions have likewise indicated that flowering and flower production in low-chill apple cultivars predominantly occur between February and March (Kumar *et al.,* 2023).

Under the agro-climatic conditions of Jorhat, the cultivar Anna exhibited earlier blooming relative to Dorsett Golden and HRMN-99. The precocious behaviour of Anna observed in the present investigation aligns with earlier reports describing Anna as an early maturing cultivar, recommended for harvest during the second week of July (Sumrah *et al.*, 2000; Sharma *et al.*, 2004).

Flower bud initiation was recorded in January for Anna and Dorsett Golden, whereas HRMN-99 exhibited bud initiation during February, indicating comparatively earlier floral development than reported for several other apple-growing regions. Previous studies have documented that flower bud initiation in low-chill apple cultivars generally occurs between February and March (Verma & Thakur, 2019; Kumar *et al.,* 2023). Among the cultivars evaluated, Anna and Dorsett Golden initiated flowering earlier, suggesting superior adaptation to the prevailing climatic conditions.

The floral bud development of the low-chilling apple cultivars progressed through four distinct stages. During the initial stage of bud development, the outer bud scales, which were initially compact, brown, and desiccated, gradually enlarged and transformed into a visibly swollen structure. Approximately five days after the commencement of observations, the buds reached the silver tip stage. By around the fifteenth day, the buds advanced to the green tip stage, characterized by the emergence of green tissue. From the twenty-first day onwards, the buds developed into the pink bud stage, distinguished by a clear pink pigmentation of the petals while the flowers remained unopened (Table 1 & Fig. 1).

Following the initiation of the pink bud stage, floral development proceeded sequentially through the king bloom stage, wherein the central flower of the cluster opened first. This was succeeded by the half bloom stage, marked by the opening of approximately 50% of the flowers within the cluster, and subsequently the full bloom stage, during which all flowers were fully opened. Thereafter, the petal fall stage was observed, ultimately culminating in the onset of fruit set (Fig. 2).

Anthesis in all three low-chilling apple cultivars was observed to commence at approximately 06:00 hours and continued until nearly 18:00 hours, with peak flower opening occurring between 10:00 hours and 12:00 hours. The timing and progression of anthesis varied depending on the seasonal and prevailing climatic conditions. An increase in ambient temperature was associated with an acceleration of floral bud opening.

Anther dehiscence occurred as a continuous process, reaching its maximum intensity during the midday period between 12:00 hours and 14:00 hours, and was generally completed by 16:00 h to 18:00 h. On the day of anthesis, nearly all anthers within an individual flower were observed to have dehisced. Comparable patterns of anthesis and anther dehiscence in apple have been previously documented (Sharma, 1970; Bhartiya, 1980).

The number of days required to reach anthesis differed among cultivars, being 23 days in Anna, 28 days in Dorsett Golden, and 26 days in HRMN-99. The duration of anthesis exhibited seasonal variation. During the February-March period, when temperatures were relatively lower, flower opening initiated around 06:30 hours and peaked at 12:00 hours, with a mean duration of approximately 5.5 hours. In the mid-flush period (March-April), anthesis began at nearly 06:00 hours and reached peak bloom by 12:00 hours, extending over roughly 6 hours. During the late flush, anthesis commenced around 06:30 hours, attaining peak bloom at approximately 12:15 hours, with an average duration of 5.75 hours.

Pollen Morphology and Viability Assessment

The pollen grains of cultivar 'Anna' exhibited a triangular shape while cultivar 'Dorsett Golden' and 'HRMN 99' were predominantly spheroidal in shape (Fig. 3). These observed differences in pollen shape may reflect underlying genetic distinctions among the cultivars and could potentially influence reproductive biology and compatibility within and between apple varieties. The cultivar 'Anna' exhibited relatively larger pollen grains, with an average size of 15.2-18.0 micrometers in diameter. In contrast, the 'Dorsett Golden' cultivar showed slightly smaller pollen grains, with an average size of 14.0-15.2 micrometers, while 'HRMN 99' possessed pollen size of 13.8-14.0 micrometers (Table 2). These differences in pollen size could be attributed to genetic variation (Mai *et al*., 2020).

In a comparative study of three low-chilling apple cultivars 'Anna', 'Dorsett Golden', and 'HRMN 99' significant differences in pollen viability were observed. Cultivars, 'Dorsett Golden' exhibited the highest pollen viability with 90.12%, followed by 'Anna' with 88.68% and in 'HRMN 99', with 82.76% viable pollen grains recorded under 40x (Table 2). These variations may be attributed to both genetic factors and prevailing environmental conditions, as suggested by earlier studies (Sharma *et al*., 2005; Popa *et al*., 2021).

Conclusion

The present investigation clearly indicates that all the three evaluated low chilling apple cultivars performed well under the warmer agroclimatic conditions of Assam. The observed variability in phenological behaviour among the cultivars highlights substantial genetic diversity, underlining the importance of their systematic evaluation, conservation and exchange for effective utilization in future breeding programmes aimed at developing improved, commercially viable apple cultivars of warm regions. Breeders and horticulturist must comprehend these differences among cultivars, particularly when choosing cultivars for hybridization programme or enhancing orchard pollination techniques.

Table 1: Time and duration of flowering of apple cultivars

Cultivar	Stages of bud development (days)				Days to anthesis	Days to last flowering	Duration of flowering (days)
	Stage I (Initiation of bud swell)	Stage II (Silver tip)	Stage II (Green tip)	Stage III (Pink bud)			
Anna	1	5	14	21	23	53	30
Dorsett Golden	1	5	16	23	28	55	27
HRMN 99	1	6	15	22	26	55	29

Table 2: Pollen size (µm) and viability (%) in apple cultivars

Cultivar	Pollen size (µm)		Mean	Total pollen grains		Pollen grains stained		Pollen viability (%)		Mean
	2024	2025		2024	2025	2024	2025	2024	2025	
Anna	16.6	16.7	16.65	186	185	163	166	87.63	89.72	88.68
Dorsett Golden	14.7	14.5	14.60	237	238	214	215	90.29	89.95	90.12
HRMN 99	13.9	13.9	13.90	215	220	179	181	83.25	82.27	82.76

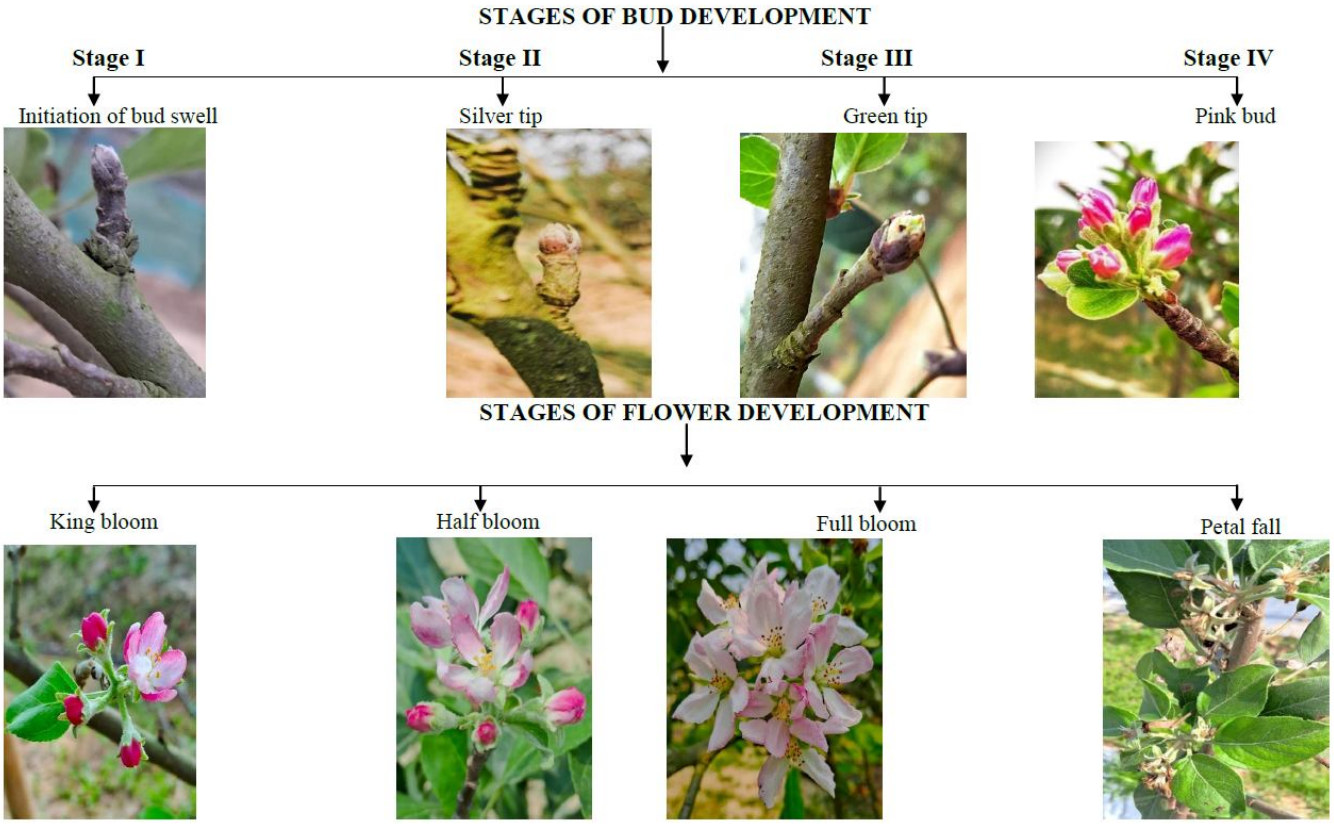

Fig. 1: Stages of apple bud and flower development

Fig. 2: Flowering of three apple cultivar

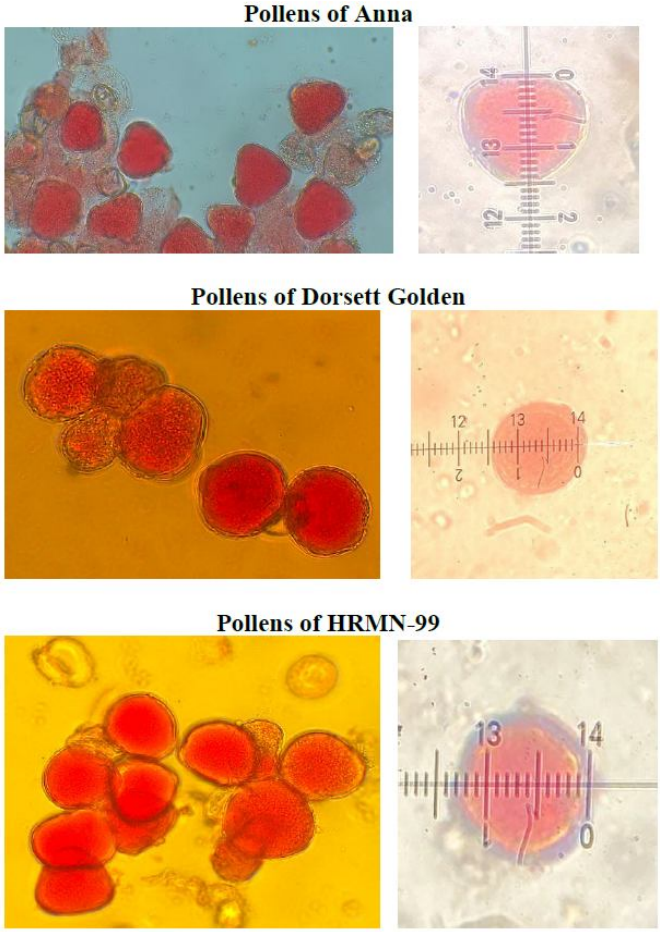

Fig. 3: Pollen study of three low chilling apple cultivars

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