

Review Article

Flowering Behaviour and Synchronization in Avocado : A Comprehensive Review

Abstract

Understanding the flowering behaviour and synchronisation in avocado (*Persea americana*) is crucial for effective orchard management as it directly impacts fruit yield and quality. The flowering patterns of avocado trees are influenced by a variety of factors, including genetics, environment, and cultural practices. In this review, we will delve into the various factors that influence avocado flowering, with a particular focus on the physiological and environmental aspects. It is crucial to highlight the significance of maintaining optimal tree health and providing a well-balanced nutrition for successful avocado flowering. In this discussion, we will explore the role of chilling requirements, temperature, and photoperiod in flower initiation and development. An analysis is conducted on cultivation practices, specifically pruning and girdling, to assess their effects on flower synchronisation. While some practices, such as plant growth regulator applications, may offer potential avenues for manipulation, their efficacy in the context of avocado flowering remains a subject of ongoing research. This abstract highlights the importance of gaining a thorough understanding of avocado flowering dynamics in order to create sustainable orchard management strategies. By improving flower synchronisation, we can ultimately maximise fruit production in avocado cultivation.

1. Introduction

The avocado, scientifically known as *Persea americana* Mill., is a fruit that has a rich history spanning approximately 10,000 years. It is derived from tropical trees and is easily recognisable due to its distinctive pear shape and blackish-green colour. Not only does the avocado possess high nutritional value, but it also boasts a creamy texture and a one-of-a-kind taste [Birnbaum *et al.* (2003)]. The avocado is often referred to as the "Green Gold" due to its significant commercial value, as highlighted by Cervantes-Paz and Yahia (2021). Avocado cultivation is a noteworthy agricultural endeavour because of the widespread demand and economic significance of the fruit. Avocados are renowned for their nutritional value, boasting an abundance of healthy fats such as monounsaturated oleic acid, as well as essential vitamins and minerals like vitamin E, C, A, potassium, dietary fibres, and minerals [FAO (2000)]. Avocado cultivation has seen a significant global expansion in response to

growing consumer demand. Based on the data provided by **FAO (2022)**, the worldwide fruit production in 2021 reached approximately 8,685,672 metric tonnes. Additionally, the total area dedicated to avocado cultivation was 858,152 hectares, with Mexico, Peru, and Chile being the primary countries involved in avocado production. Unfortunately, the sources provided did not have the specific statistics on avocado production and area in India, likely due to a lack of information on commercial plantations. According to a study conducted by **Tripathi et al. (2014)**, it seems that the agro-climatic conditions in different regions of the country are suitable for expanding avocado cultivation. The cultivation practices can vary based on the specific regional climate and soil conditions. In the realm of agriculture, the cultivation of avocados holds immense significance, impacting both local and global economies.

Having a comprehensive understanding of the flowering behaviour of avocado is of utmost importance in order to maximise yield and ensure high-quality fruit. This is because the flowering behaviour directly influences pollination, which is a critical factor in determining fruit set [**Dymond et al. (2021)**]. The flowering pattern of avocados is quite distinctive, referred to as "synchronous dichogamy." This pattern plays a crucial role in determining how the flowers open and ultimately get pollinated. Having a clear understanding of orchard management is crucial for optimising the arrangement of various avocado varieties to ensure efficient cross-pollination, as highlighted by **Alcaraz and Hormaza (2014)**. The number of fruits that are set and the overall yield are directly influenced by effective pollination. Having a comprehensive understanding of flowering patterns is crucial for effective orchard layout planning and the management of pollinators, such as bees (**Estravis-Barcalaet et al., 2021**). The flowering behaviour is also affected by various environmental factors such as temperature and humidity. In addition, various varieties and cultivars demonstrate unique patterns of flowering. Gaining a comprehensive understanding of these relationships empowers growers to adjust cultivation practices according to specific local conditions, thereby guaranteeing a reliable and uniform flowering and fruiting process [**Davenport (1986)**].

This reviewpaper delves into the specifics of the synchronised dichogamy that is essential for efficient pollination as well as the distinctive blooming patterns of avocados. It studies how these characteristics impact fruit quality and production, investigates the genetic and environmental factors that influence these patterns, and explores different ways for synchronising blooming to increase cross-pollination and output. In addition, the study points

out where our present understanding is lacking and proposes avenues for future research to fill those gaps so that we can optimise avocado farming.

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2. Flowering Behaviour in Avocado

2.1. Synchronous dichogamy

According to **Mlcaraz and Hormaza (2021)**, it has been observed that an Avocado tree typically yields a significant number of flowers during the flowering period. However, a large portion of these flowers ultimately fail to bear fruit. Avocado flowers possess both female and male organs, making them bisexual. According to **Alcaraz *et al.* (2013)**, the flowers exhibit synchronous dichogamy, where each bisexual flower goes through two cycles of opening and closing. During the initial stage, the flower operates as a female, with its stigmas being receptive. However, on the following day, it undergoes a transformation and functions as a male, with the anthers dehiscing. This phenomenon was observed by **Alcaraz and Hormaza (2014)**. The stigma is typically white and becomes receptive to pollen prior to the anthers releasing the pollen grains from the pollen sacs. Only a small fraction, less than 1%, of the flowers that bloom is capable of producing fruits. The phenomenon is heavily influenced by environmental conditions. A study conducted in southern Spain also examined the practical stage of the pollination process to understand why some flowers detach prematurely while others stay attached to the tree [**Stern *et al.* (2021)**].

The female stage flower (Figure 1A) opens initially and then closes after a couple of hours, remaining closed for the remainder of the day and night. The flower opens again the following day, but the stigma is no longer receptive to pollen grains. In the male-stage flower depicted in Figure 1B, pollen is released and the flower subsequently closes [**McGregor (1976)**].

Avocado cultivars are categorised into two groups based on their flowering sequence: A type and B type cultivars. According to **X'etscherret *al.* (2000)**, type A cultivars have flowers that open in the morning as female, close at midday, and then reopen in the afternoon of the next day as male. In contrast, type B cultivars have flowers that open as females in the afternoon, close overnight, and then reopen the next morning as males.

The avocado presents intriguing possibilities for cross-pollination due to the synchronous dichogamous nature of its flowering and the presence of type A and B cultivars. Therefore, during the morning, it is possible to cross type A cultivars, which have functional female flowers, with type B cultivars, which have functional male flowers, in order to obtain pollen. Alternatively, during the afternoon, it is possible to cross B types that exhibit functional female characteristics with A types that display functional male flowers. If the

stigma is not working properly during the second opening, there is no chance of self-pollination occurring within the flowers or within the same cultivar [Gogolashvili (1980)].

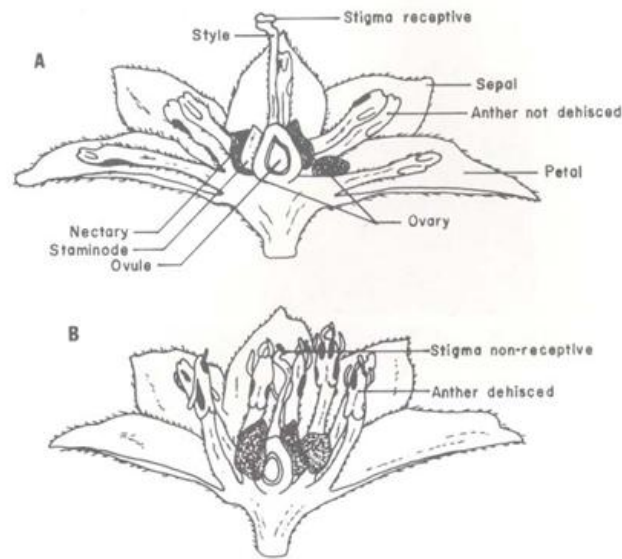


Fig 1. Schematic longitudinal section of avocado flower. **A**-Female stage, with stigma receptive, but stamens bent outward and anthers not dehiscent. **B**-Male stage, with stigma no longer receptive, but stamens upright and anthers dehiscent (from McGregor 1976).

2.2. Pollination

Avocado pollination occurs through three distinct modes. One type of pollination is cross-pollination, which typically takes place in warm weather conditions. According to Degani *et al.* (1989) and Markle and Bender (1992), pollen is transferred between male flowers of A-type and female flowers of B-type, and vice versa. The efficiency of the process is determined by the proximity between the pollen donor and the pollinated trees, as well as the duration of overlap between male and female-stage flowers. According to Davenport (2019), the second type of pollination occurs when neighbouring flowers on the same plant engage in close pollination. This happens during the overlap period of male and female stage flowers, when pollen from male flowers lands on the stigmas of female flowers. According to Stout (1923) and Anshman *et al.* (2004), self-pollination occurs when pollen grains reach the stigma within the same flower. This process takes place exclusively in a male-stage flower, where the stamen releases pollen that then falls onto the receptive stigma. The flowering behaviour of the Avocado is a complex process that serves to limit or even prevent successful self-pollination, nearby pollination, and cross-pollination. In order to enhance fruit sets and boost Avocado yields, it is crucial to gain a deeper comprehension of the pollination process. It is worth noting that a mere 1% of Avocado flowers actually result in fruit production, as

stated by **Davenport (1986)**. Effective pollination is a crucial process in the development of fruits. Flowers of type A exhibit a unique behaviour where they act as females in the morning and then switch to acting as males in the afternoon. On the other hand, type B flowers follow the opposite pattern, functioning as males in the morning and then transitioning to females in the afternoon. Therefore, the ability for self-pollination and close-pollination is limited, resulting in a change in the reciprocal cross-pollination between individuals from the two groups [**Anshman et al. (2004)**]. Several trials conducted by **Dymond et al. (2021)** yielded negative results when attempting artificial cross-pollination between types A and B. In contrast, other studies have shown that cross-pollination offers the greatest chance of fertilizing the ovum. In the event that this particular pollination process does not take place, the Avocado resorts to self-pollination as a result of various environmental factors. One issue arises when grains become windburned due to their connection with a sticky substance that coats Avocado pollen. This reliance on flying insects is crucial for the successful movement of pollen and the completion of the pollination process for Avocado flowers. In a study conducted by **Clark (1923)**, it was noted that bees tend to favour bee pastures over Avocado orchards. Bees play a crucial role in pollinating Avocado in California and Israel, while in Jamaica, Trinidad, and Florida, the primary pollinators are the Politest wasp and *Metabolybiasigulata*, as noted by **Papademetriou (1976)**. In certain regions and specific periods, bees play a vital role in pollinating Avocado trees. Additionally, various insects play a crucial role in the pollination process of Avocado. In order to enhance pollination, certain studies propose implementing various measures. To optimize honeybee pollination in the orchard, it is recommended to increase the number of hives as one hive is often not enough. Additionally, it is important to choose a sunny location for the hives and ensure that there are enough pollen-donor trees nearby. Lastly, it is beneficial to maintain an open canopy in the orchard [**Ish-Am (2005)**].

2.3.Factors affecting flower formation and fruit set

2.3.1. Environmental factors

According to **Davenport (1986)**, the dianthesis in avocado (*Persea americana*) flowers is highly influenced by environmental conditions. Having a clear understanding of how environmental factors impact avocado flowering is essential for ensuring successful avocado cultivation.

As per **Bergh (1986)**, the process of flower opening and closing exhibits a consistent pattern when the temperature remains above 70°F (21°C) during the night and reaches its

peak during the day. With the decrease in night minimum temperatures and day maximum temperatures, there is a noticeable delay in the opening of flowers. The opening of the female flower in certain cultivars may occur in the afternoon, while in others it may happen at night or the following morning. In cooler conditions, there may be a gap of 2-4 days between the opening of the female and male flower phases. At lower temperatures, the B flower cultivars may fail to open as female flowers. According to **Leslie and Bringham (1951)**, it was observed that the Fuerte (B flower) did not produce any female flowers when subjected to day temperatures ranging from 18 to 21 °C and night temperatures ranging from 7 to 12 °C. Lower temperatures can cause a delay in the opening of flowers. However, this delay can actually be advantageous in certain cases. It leads to an increase in the overlap between female and male flowers, which helps ensure pollination in a grove consisting of pure "A" type flowers like Hass. According to a study conducted by **Ish-Am and Eisikowitch (1989)**, it was observed that there is a period of overlap between 45 minutes to 90 minutes in Hass during the mid-day. Furthermore, lower temperatures (and overcast conditions) decrease the level of bee activity in the grove.

The flowering behaviour in the low-temperature regime underwent changes as previously explained, resulting in delayed flower openings. In the low-temperature regime, the germination of pollen tubes on 'Fuerte' stigmas seemed to proceed as expected. However, there was a noticeable limitation in their growth beyond the style, which significantly impacted the chances of successful fertilisation. A similar response was seen in plants that were moved to the low-temperature environment just 1 hour prior to pollination. It seems that colder temperatures directly impact the growth of pollen, rather than being influenced by the physiological condition of the flowers during pollination[**Sedgley and Grant (1983)**].

It is currently unclear whether changes in flowering behaviour are directly influenced by light intensity or if they are simply a result of the cooler temperatures typically associated with cloudy, overcast, and rainy weather. A recent study by **Sedgley (1985)** delved into the relationship between daylength and the regulation of flower opening. Through experimentation, it was discovered that the 'Hass' cultivar exhibited greater sensitivity to different daylengths, ranging from 1 to 12 hours, when compared to the 'Fuerte' cultivar. Observations were made on the changes in flower opening in both cultivars. According to **Sedgley (1985)**, the absence of light caused a delay in the male-stage opening and disrupted the floral cycle of both 'Hass' cultivars. This indicates that the day-night cycles play a crucial role in controlling the timing of floral anthesis. Some avocado varieties in California,

particularly 'Pinkerton', experience a noticeable decrease in blooming during the summer months. In a study conducted by **Buttrose and Alexander (1978)**, it was found that the 'Fuerte' variety of flowers bloomed successfully under both 15-hour and 9-hour day lengths.

2.3.2. Alternate bearing habit

A tree that exhibits alternate or biennial bearing does not consistently produce a crop every year. On the other hand, there is a pattern of alternating between high and low yields, as observed by [**Monselise and Goldschmidt (1982)**]. The phenomenon of avocado's alternating and irregular bearing can be observed in warm, humid subtropical regions as well as semi-arid Mediterranean production areas [**Scora et al. (2002)**]. Another possible cause could be a depletion of starch. According to scientific research, the theory of "starch depletion" suggests that when there is a heavy crop in one year, it can lead to reduced carbohydrate energy reserves in trees during the following year, affecting flowering and fruit set. This statement holds true for avocado trees, particularly when there is a delay in the harvesting process [**Whiley et al. (1996)**]. Reserve carbohydrate status serves as a valuable indicator of overall tree condition, although its reliability can vary across different time scales, environments, and management techniques, making routine predictive measurement unjustifiable. **Scholefield et al. (1985)** made a significant discovery regarding the connection between starch reserves and flowering in avocado.

2.3.3. Genetic factor

The transition from vegetative to reductive structure is a crucial developmental process in flowering plants. Based on molecular evidence, it has been observed that flowers of various species exhibit a wide range of diversity. However, it is interesting to note that there is a common set of genes that regulate the process of floral induction and flower initiation. These genes are influenced by external signals, such as changes in temperature, as well as internal factors like alterations in sugar or hormone levels. As an example, scientists have discovered proteins with a similar structure to the Arabidopsis FLOWERING LOCUS T (FT). These proteins act as signals that travel from the leaves to the shoot meristem, playing a role in the transition to flowering in different plant species [**Pin and Nilsson (2012)**]. FT is part of a small group of proteins that share structural similarities with mammalian phosphatidylethanolamine-binding protein (PEBP) [**Kardailskiy et al. (1999)**].

In the shoot meristem, FT collaborates with the b-ZIP transcription factor FD to facilitate floral transition by stimulating the expression of MADS box genes, such as APETALA1

(AP1), FRUITFULL (FUL), and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) [Baurle and Dean (2006)]. These MADS box transcription factors play a crucial role in the regulation of floral meristem identity genes, including Leafy (LFY). Leafy is a plant transcription factor that is essential for flower formation, as highlighted by Siriwardana and Lamb (2012). In avocado, despite the identification of several cDNA sequences with characteristics of flowering-related genes by the Floral Genome Project and the Ancient Ancestral Genome Project [Chanderbaliet al. (2009)], there is still a lack of information regarding their spatial and seasonal expression profiles during off and on years. It remains to be determined if an FT-like gene plays a role in the regulation of avocado inflorescence/flower induction, as suggested by Ziv et al. (2014).

2.3.4. Hormonal regulations

During the on-years, the abundance of fruit on the tree results in the production of plant growth regulators (PGRs) that hinder the initiation of flowers. It is widely believed that gibberellins (GA) produced by seeds play a crucial role in the suppression of flowering. According to the research conducted by Ebert and Bangerth (1981), Garcia-Luis et al. (1988), and Jonkers (1979), it has been observed that the presence of gibberellic acid in fruit seeds can hinder the process of flower induction. Research conducted by Kachru et al. (1971) has demonstrated that the use of gibberellins can lead to a decrease in flowering among subtropical and tropical evergreen trees. According to a study conducted by Scholefield et al. (1985), it was observed that flower initiation took place when vegetative growth stopped due to a decrease in carbohydrate levels. It is possible that the cessation of vegetative growth may be linked to a decrease in gibberellin production by the growing shoots, which in turn triggers flower initiation. In a study conducted by Salazar-Garcia & Lovatt (2000), the effects of GA₃ sprays on avocado flowering were examined. The researchers looked at the impact of these sprays on both individual branches and entire trees. Applying a 100mg/l GA₃ spray during early winter, prior to the onset of blooming, resulted in a decrease in the number of inflorescences, an increase in the number of vegetative shoots, and a 47% reduction in yield during blooming. These outcomes are considered favourable for potentially disrupting or altering an AB cycle. Further investigation is required regarding the concentration and timing of GA₃.

It is widely acknowledged that roots have a significant impact on the regulation of flowering and the promotion of bud development by providing growth regulators in a timely manner [Jackson (1993)]. At first, scientists didn't believe that cytokinins had any impact on

flower induction. However, recent evidence has shown that cytokinins can actually counteract the inhibitory effects of GA₄₊₇ on flowering in apples, as demonstrated by **Li *et al.* (2019)**. Roots play a crucial role in the production of cytokinins, which are important for stimulating the growth of flowers in the apical buds. In a study conducted by **Arpaia *et al.* (1996)**, it was observed that root growth in trees carrying a regular or light crop showed continuous growth throughout the year. However, during the winter months in on-years, root growth was found to be depressed and even stopped. Therefore, it is possible that inadequate root growth during a productive year, leading to a decrease in cytokinin availability, could play a role in the diminished flowering during the subsequent unproductive year [**Blanke and Lovatt (1996)**].

In a study conducted by **Bower *et al.* (1990)**, a model was put forth that highlights the importance of root physiology. This is because roots serve as the primary source of cytokinins and abscisic acid (ABA). They proposed that under conditions of low temperature and water-deficit stress, the roots produce more ABA. This hormone then halts the growth of vegetative shoots, allowing the transition from a vegetative apex to a floral apex. In this way, it regulates the development of floral shoots during periods of stress. According to **Gupta *et al.* (2022)**, the levels of ABA in avocado fruits increase as they mature. Based on the data, it can be inferred that during the period of flower induction, the levels of ABA would be higher in years when the phenomenon occurs compared to years when it does not. Hence, it is plausible that elevated levels of ABA during on-years may have inhibitory impacts on flower induction. Research has shown that as the fruit matures, it accumulates another inhibitor that may have inhibitory effects on flower initiation [**Gazit and Blumenfeld (2006)**]. Additional research is required to ascertain whether ABA, or another compound, acts as an inhibitor of flowering in avocado trees.

2.3.5. Phenology

Flower primordia can be observed in the terminal or subterminal buds of shoots that have experienced growth during the spring and summer periods. According to a study conducted by **Alcaraz *et al.* (2013b)**, it was discovered that the transition from vegetative buds to reproductive buds occurs towards the end of shoot expansion during the summer growth, typically in August in California. Eventually, these reproductive buds develop into complex, multi-branched panicles where the flowers emerge. According to **Hapuarachchiet *al.* (2022)**, it has been observed that a single tree can bear an impressive number of panicles, with the potential to produce up to a million flowers. The panicles typically exhibit a determinate nature, devoid of any leafy buds emerging from them. However, there are

instances where the panicles display an indeterminate characteristic, eventually giving rise to leafy buds. Typically, these clusters are located on the exterior of the tree, receiving the most sunlight. In a pruning programme, a challenge arises when it comes to hedgerow pruning or shaping later in the season. During this process, a significant number of shoots that are on the verge of forming panicles and flowers end up being removed [Bergh (1986)].



Fig 2. A. Indeterminate inflorescences; **B.** Determined inflorescences in *P. americana* cv. Hass. Sources: Alcaraz *et al.* (2013b).

The duration of the flowering season can vary depending on factors such as race, cultivar, and temperature, as observed in a study by Kinmonth-Schultz *et al.* (2019). Typically, Mexican cultivars tend to flower earlier, followed by West Indian cultivars, and pure Guatemalans flowering last. West Indian cultivars exhibit robust flowering patterns in tropical climates, yet their flowering capabilities tend to be subpar in the subtropical climate of Southern California [Sedgley and Annelis, (1981)]. In contrast, the flowering of Guatemalan and Guatemalan x Mexican hybrids is significantly hindered in tropical climates, while they exhibit abundant flowering in California. The absence of blooming in a tropical environment explains why 'Hass' (a hybrid of Guatemalan and Mexican varieties) is not cultivated as a commercially viable avocado cultivar in Florida and Hawaii [Pisani *et al.* (2017)].

2.4.Synchronization strategies

Effective synchronisation strategies play a vital role in maximising pollination and enhancing fruit set in avocado cultivation, especially in orchards that have a mix of Type A and Type B avocado trees.

2.4.1. Varietal selection of compatible avocado varieties

The alternating (complementary) synchronous dichogamy (protogyny) of avocado flowering appears to be an outcrossing mechanism, albeit with a fail-safe back-up [**Gonzalez et al. (2020)**]. Numerous studies have shown that environmental factors modify flower opening [**Acosta-Rangel et al. (2021)**]. Through careful selection and strategic planting of compatible varieties, growers can optimise the synchronisation of male and female flowering phases within the orchard. Choosing the right variety is crucial for establishing a harmonious and coordinated pollination ecosystem. Low temperatures disrupt group 'B' cultivars ('Fuerte', 'Sharwil', 'Ettinger') more than group 'A' cultivars ('Hass', 'Gwen', 'Pinkerton', 'Reed') [**Sedgley (1977); Whiley and Winston (1987)**], partly due to slower pollen tube growth [**Sedgley and Grant (1983)**]. **Whiley and Winston (1987)** were able to predict the performance of 'A' and 'B' group cultivars in different areas of Australia, based on mean temperatures during flowering. It is now accepted in the subtropics that group 'A' cultivars will yield better in cool climates, and that 'Fuerte', 'Sharwil' etc only achieve high yields in warm (spring) environments. **Lahav and Gazit (1994)** point out the significant preference for group 'A' cultivars in California, and that among the 12 new semi-commercial cultivars selected in Israel in the last decade, only two (both late-flowering) belong to the B group. The ability to delay flowering of group 'B' cultivars into a warmer time slot (perhaps through a rootstock effect), may significantly increase their yield potential. The necessity for interplanting cultivars of complementary groups and overlapping flowering times is still equivocal (**Davenport and Lahav, 1992**). The work of **Degani et al. (1989)** with genetic markers shows that 'Ettinger' (B) is a good cross-pollinator of 'Hass' (A), and that under environmental stress and vegetative growth competition it is the hybrid progeny rather than the self-pollinated fruitlets which develop the greater sink strength to survive the massive abscission. By implication and common observation, solid blocks of single cultivars (selfs) are more likely to yield adequately under low stress conditions at fruit set. **Lahav and Gazit (1994)** summarise succinctly: "Interplanting cultivars that belong to complementary flowering groups and bloom at the same time tends to increase pollination rates and promote cross-pollination and hence fruit set; yields are usually improved". Widespread adoption of interplanting is prejudiced by practical issues such as the need for closeness of pollinator and

pollinated, their often-incompatible management and requirements; and also, by insufficient research on the best pollinators.

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Table 1. Sequential opening of avocado flowers for both 'A' and 'B' types, focusing on significant cultivars[from **Pant *et al.* (2020)**]

Flower type	Day 1		Day 2		Cultivars
	Morning	Afternoon	Morning	Afternoon	
A	Female	-	-	Male	Reed, Wurtz, Rincon, Hass, Hazard, Lamb Anaheim, Gwen, Lamb Hass, Pinkerton, Reed, Simmonds, Perfecto, Pankay
B	-	Female	Male	-	Bacon, Ettinger, Fuerte, Nobel, Sharwill, Shepard, Zutano Bacon, Edranol, Nabal, Sir Prize, Walter Hole, Queen, Pollock, Panchoy

2.4.2. Pollination

Pollination is an essential process for plants to successfully reproduce by fertilising seeds. It plays a crucial role in ensuring the successful production of seeds. Most flowers do not receive pollen during their female stage. Inadequate pollination can result in a decreased yield, despite the fact that the required amount for fertilising the ovules is lower than the number of pollen grains that reach the stigma. Multiple studies have documented this phenomenon [**Burd (1994); Larson *et al.* (2000); Ashman *et al.* (2004)**]. Studies on Avocado production have shown that the main factor limiting fruit set is the insufficient transportation of pollen to the stigma of female flowers [**Alcaraz and Hormaza (2009)**].

Grower experience and research data have indicated the potential advantages of outcrossing in avocado groves. However, the question of whether placing bees and pollinizers in these groves actually promotes outcrossing has been a topic of debate for almost a century [**Kobayashi *et al.* (2000)**]. It has been suggested that the need for cross pollination between different cultivars may not be as straightforward as previously thought. Recent studies have shown that temperature fluctuations can lead to the male and female phases of plants overlapping within a canopy [**Sedgley and Grant (1983)**]. Close pollination can take place between flowers of the same tree or cultivar.

Insects are essential for the pollination of Avocado. Implementing various measures has been suggested by certain studies to enhance pollination. For optimal honeybee pollination in the orchard, it is advisable to consider increasing the number of hives, as a single hive may

not suffice. In order to optimise the conditions for the hives, it is crucial to select a suitable location that receives ample sunlight and has a sufficient number of nearby trees that can serve as sources of pollen. Finally, it is advantageous to keep an open canopy in the orchard, as suggested by **Ish-Am (2005)**.

2.4.3. Use of PGRs (Plant Growth Regulators)

In a study conducted by **Salazar-García and Lovatt (1998)**, they presented compelling evidence that GA₃ has the ability to cause predictable changes in the timing and structure of inflorescences. GA₃ was applied in November, prior to the initiation of a complete set of secondary inflorescence axes. This resulted in a decrease in flowering intensity as partially formed inflorescences were produced, each containing fewer flowers. However, it did promote the development of both inflorescences and vegetative shoots. The application had no impact on the inflorescence or flower count, but it did accelerate the growth of the vegetative shoot at the top of indeterminate inflorescences. As a result of the application of GA₃, there is a significant advancement in development. This leads to a change in the role of leaves, which now become sources of photo assimilates during fruit set instead of competing sinks [**Blanke and Lovatt (1998)**]. Both early and late foliar applications of GA₃ offer potential strategies for manipulating avocado flowering.

2.4.4. Cultural practices

The management and optimisation of flower synchronisation in avocado cultivation heavily rely on cultural practices. Cultural practices are primarily centred around creating optimal growing conditions, ensuring the well-being of trees, and effectively managing factors that impact the flowering process.

To further increase avocado yields, it is necessary to manipulate the trees in order to control their architecture and complexity. This will help increase fruitfulness, similar to the successful methods used with deciduous trees [**Cutting et al. (1994)**]. The pruning techniques used for temperate fruit trees can lead to excessive growth without fruit production when applied to evergreen avocado trees in subtropical or tropical regions. When it comes to pruning, it's often recommended to focus on redistributing growth. This involves limiting the primary extension and encouraging the growth of axillary buds, as suggested by **Thorp and Sedgley (1993)**. In their study, **Cutting et al. (1994)** experimented with two pruning techniques. One involved making a cut through the bud ring, while the other involved making a cut below the bud ring, specifically at the midpoint of the previous growth flush. The

pruning was performed on the wood from the current season, either in late summer or late autumn. There were noticeable variations in the responses based on the type of cut and, to a lesser degree, the time of cut. The incision made in the bud ring caused multiple buds to break, and the growth of the resulting shoots was regulated by competition. As a result, the initially strong response was somewhat diminished. In their study, **Snijder and Stassen (1995)** proposed a range of strategies to address the issues of an ageing and unproductive avocado orchard that is being encroached upon. When pruning, it is important to shape the plant into a pyramidal form. Additionally, the horizontal branches can be prompted to produce flowering units by gently tipping them. This results in the formation of additional shoots, leading to the development of an intricate network of branches. It is clear that the avocado tree can be effectively managed to maintain the optimal size and shape, which in turn promotes increased flowering in the orchards. In a study, **Miller (1960)** trimmed the robust growth of terminal twigs and branches that protruded beyond the overall shape of the tree. After a span of four years, the overall yield remained consistent between pruned and unpruned trees. It has been determined that performing a significant pruning before an expected abundant fruit production will result in a decrease in the current season's crop and an increase in the subsequent season's crop.

It is important to approach practices that involve girdling fruit trees, such as avocados, with caution, despite anecdotal evidence or localised practices suggesting their effectiveness in influencing flowering. In a study conducted by **Hodgson (1947)**, various horticultural practices were examined to determine their impact on the flower synchronisation pattern of avocado trees. One effective approach involved the timely harvesting of the fruit and implementing girdling. The positive outcomes were not replicated solely through early harvesting. In a study conducted by **Ticho (1970)**, it was discovered that ringing the same branch for two consecutive years yielded positive outcomes. However, the results in the second year were not as remarkable as those in the first year. According to his statement, the 'Hass' cultivar is not required to undergo girdling.

2.5. Possible avenues for future research and areas to enhance synchronisation in avocado

There are numerous potential avenues for future research and areas for improvement in synchronisation in avocado cultivation. These include enhancing pollination efficiency, optimising flowering patterns, and improving overall fruit production. There are several important areas that warrant further investigation.:

Genetic Studies:

Perform genetic research to analyse and gain insights into the molecular processes that govern the blooming patterns of different types of avocados. The findings of this study have the potential to pave the way for the creation of enhanced avocado varieties that exhibit better synchronisation and reduced tendencies for alternate bearing.

Climate Resilience:

Examine the effects of climate change on the timing of avocado blossoming. Having a clear understanding of how changes in temperature, precipitation, and other climatic factors impact the process of flowering can provide valuable insights for developing effective strategies to address potential challenges and ensure consistent synchronisation in the future.

Advanced Pollination Techniques:

Investigate and refine cutting-edge pollination methods, such as precise pollen delivery systems, to optimise the effectiveness of pollen transfer among avocado flowers. This may include the utilisation of drones, automated systems, or other cutting-edge technologies.

Integrated Pest Management (IPM):

Investigate the impact of pests and diseases on avocado flowering and examine strategies for integrated pest management to mitigate their effects. Effective pest control measures can have a positive impact on the health of the tree and help maintain synchronisation, ultimately leading to improved flowering.

Biochemical and Hormonal Regulation:

Examine the biochemical and hormonal processes involved in the regulation of avocado flowering, specifically focusing on the influence of plant hormones in the initiation and development of flowers. Gaining a comprehensive understanding of the physiological mechanisms can offer valuable insights into the effective manipulation of flowering for improved synchronisation.

Precision Agriculture Technologies:

Discover the practical implementation of precision agriculture technologies, including remote sensing and data analytics, for the purpose of monitoring and managing avocado orchards. These technologies have the potential to improve resource allocation, forecast flowering patterns, and optimise orchard management practices.

Microbial Ecology:

Explore the significance of the soil microbiome in the process of avocado flowering and pollination. Gaining insight into the ways in which beneficial microbes impact the health and reproductive processes of trees has the potential to pave the way for the creation of strategies that utilise microbes to improve synchronisation.

Rootstock-Scion Interactions:

Examine the relationships between rootstocks and scion varieties in grafted avocado trees. Gaining knowledge about the impact of various rootstocks on flowering patterns and tree vigour can greatly enhance orchard management strategies.

Modelling and Predictive Tools:

Create advanced tools for modelling and predicting flowering patterns by integrating environmental data, tree genetics, and management practices. These tools can help growers make well-informed decisions to optimise synchronisation.

These research directions have the potential to enhance our comprehension of avocado flowering and contribute to the creation of more effective and environmentally-friendly practices in avocado cultivation. Effective collaboration among researchers, growers, and industry stakeholders is crucial for tackling these challenges and enhancing synchronisation in avocado orchards.

2.6. Conclusion

After conducting a thorough analysis, it is evident that various factors play a crucial role in the flowering behaviour and synchronisation of avocado trees. The success of pollination and subsequent fruit set is greatly influenced by the complex interactions between genetic factors, environmental conditions, and management practices. Understanding the Type A and Type B flowering patterns, as well as the impact of genetic diversity, has become a crucial aspect of orchard management. Implementing synchronisation strategies is crucial for maximising fruit yield and minimising alternate bearing patterns. These strategies include optimal variety selection, effective pollinator management, and meticulous orchard practices. These strategies have far-reaching implications, not only for individual orchards, but also for the overall sustainability and profitability of the avocado industry.

Highlighting the significance of achieving flowering synchronisation is essential for avocado growers aiming to improve productivity and stability in their orchards. There are

several advantages that can be gained, such as enhanced fruit production, more consistent crop yields, and ultimately, higher overall output. Growers can achieve greater economic returns and operational efficiency by implementing effective synchronisation practices. In addition, implementing sustainable orchard management practices is crucial for ensuring the long-term success of avocado cultivation. These practices involve promoting biodiversity, making efficient use of resources, and minimising the environmental footprint.

With the rapid expansion of the avocado industry on a global scale, it is crucial to prioritise research and innovation in order to gain a deeper understanding of and enhance synchronisation in avocado flowering. Through the implementation of cutting-edge technologies, thorough investigation into the genetic factors influencing flowering, and the formulation of meticulous orchard management techniques, the industry can make significant strides towards enhancing its resilience and promoting sustainability. Having a deep understanding of how avocado flowering works and how to synchronise it is crucial for both individual growers and the overall success and sustainability of the avocado industry on a global scale.

References:

1. **Acosta-Rangel, A., Li, R., Mauk, P., Santiago, L. and Lovatt, C. J. (2021).** Effects of temperature, soil moisture and light intensity on the temporal pattern of floral gene expression and flowering of avocado buds (*Persea americana* cv. Hass); *Scientia Horticulturae*, **280**: 109940.
2. **Alcaraz, M. L. and Hormaza, J. I. (2009).** Avocado Pollination and Fruit Set—A Perspective from Spain. *Calif. Avocado Soc. Yearbook*, **92**, 113-135.
3. **Alcaraz, M. L. and Hormaza, J. I. (2014).** Optimization of controlled pollination in avocado (*Persea americana* Mill., Lauraceae); *Scientia Horticulturae*, pp180.
4. **Alcaraz, M. L. and Hormaza, J. I. (2019).** Reproductive biology of avocado (*Persea americana*); *Acta Hortic.* 1231, 23-28.
5. **Alcaraz, M. L., Hormaza, J. I. and Rodrigo, J. (2013).** Pistil Starch Reserves at Anthesis Correlate with Final Flower Fate in Avocado (*Persea americana*); *PLoS One*, **8**(10): e78467.
6. **Alcaraz, M. L., Thorpb, T. G. and Hormaza, J. I. (2013b).** Phenological growth stages of avocado (*Persea americana*) according to the BBCH scale; *Scientia Horticulturae*, **164**: 434-439.

7. **Arpaia, M. L., Robinson, P., Liu, X., Mickelbart, V. and Witney, G. (1996).** Development of a phenological model for California 'Hass' avocado. Proc. Avocado Research Symposium. California Avocado Society and University of California, Riverside, pp 7-11.
8. **Ashman, T. L., Knight, T. M., Streets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J. and Mitchell, R. J. (2004).** Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences, *Ecology*, **85** (9), 2408-2421.
9. **Baurle, I. and Dean, C. (2006).** The timing of developmental transitions in plants. *Cell*, **125**: 655–664.
10. **Bergh, B. O. (1986).** *Persea Americana*. Halevy, A.H. (ed.) Handbook of Flowering, Vol. 5. CRC Press, Boca Raton, Florida, pp. 253-268.
11. **Birnbaum, K., Desalle, R., Peters, C. M. and Benfey, P. N. (2003).** Integrating gene flow, crop biology, and farm management in on-farm conservation of avocado (*Persea americana*, Lauraceae). *Am J Bot.*, **90**, 1619-27.
12. **Blanke, M. and Lovatt, C. (1996).** Determinate versus indeterminate inflorescences in the 'Hass' avocados. Proc. Avocado Research Symposium. California Avocado Society and University of California, Riverside, pp 39-43.
13. **Bower, J., Cutting, J., Lovatt, C. and Blanke, M. (1990).** Interaction of plant growth regulators and carbohydrate in flowering and fruit set. *Acta Hort.* **275**:425-434.
14. **Burd, M. (1994).** Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot Rev.*, **60**, 83-139.
15. **Buttrose, M. S. and Alexander, D. (1978).** Promotion of Floral Initiation in 'Fuerte' Avocado by Low Temperature and Short-Day Length. *Scientia Horticulturae*. **8**: 213-217
16. **Cervantes-Paz, B. and Yahia, E. M. (2021).** Avocado oil: Production and market demand, bioactive components, implications in health, and tendencies and potential uses. *Compr Rev Food Sci Food Saf.*, **20**, 4120-4158.
17. **Chanderbali, A. S., Albert, V. A., Leebens-Mack, J., Altman, N. S. and Soltis, D. E. (2009).** Transcriptional signatures of ancient floral developmental genetics in avocado (*Persea americana*; Lauraceae). *Proc Natl Acad Sci.*, **106**: 8929–8934.
18. **Clark, O. I. (1923).** Avocado pollination and bees, California Avocado Association Annual Report; *Report*, **7**: 57-62.
19. **Cutting, J., Cocker, B. and Wolstenholme, B. (1994).** Time and type of pruning cut affect shoot growth in avocado. *J. Hort. Sci.* **69**:75-80.

20. **Davenport, T. L. (1986).** Avocado flowering. In: *J. Janick* (ed.) *Horticultural Reviews*; **8**: 257-289.
21. **Davenport, T. L. (2019).** ACross- vs. self-pollination in 'Hass' avocados growing in coastal and inland orchards of Southern California; *Scientia Horticulturae*, **246**: 307-316.
22. **Davenport, T. L. and Lahav, E. (1992).** "Is a pollinator required to maximise avocado production?" Proc. Second World Avocado Congr. II: 667-668.
23. **Degani, C., Goldring, A. and Gazit, S. (1989).** Pollen parent effect on outcrossing rate in 'Hass' and 'Fuerte' avocado plots during fruit development. *J. Amer. Soc. Hort. Sci.* **114**:106-111.
24. **Dymond, K., Celis-Diez, J. L., Potts, S. G., Howlett, B. G., Willcox, B. K. and Garratt, M. P. D. (2021).** The role of insect pollinators in avocado production: A global review; *Journal of applied entomology*, **145** (5): 369-383.
25. **Ebert, A., and Bangerth, F. (1981).** Relation between the concentration of diffusible and extractable gibberellin-like substances and the alternate bearing behaviour in apple as affected by chemical fruit thinning. *Sci. Hort.* **15**:45-52.
26. **Estravis-Barcala, M. C., Sáez, A., Graziani, M. M., Negri, P., Viel M. and Farina, W. M. (2021).** Evaluating honey bee foraging behaviour and their impact on pollination success in a mixed almond orchard; **52**: 860–872.
27. **FAO. (2000).** Avocado Production in Asia and the Pacific.
28. **FAO. (2022).** World Food and Agriculture – Statistical Yearbook 2022. In World Food and Agriculture – Statistical Yearbook 2022.
29. **Garcia-Luis, A., Fornes, F., Sanz, A. and Guardiola, J. (1988).** The regulation of flowering and fruit set in Citrus: Relationship with carbohydrate levels. *Israel J. Bot.* **37**:189-201.
30. **Gazit, S. and Blumenfeld, A. (2006).** Inhibitor and Auxin Activity in the Avocado Fruit; *Physiologia Plantarum*, **27**(1):77 – 82.
31. **Gogolashvili, L. A. (1980).** Biology of flowering and fruiting in avocado in Abkhazia. Tr. Prikl. Bot., *Genet. Sel.* **68**:53-59.
32. **Gonzalez, S., Gutierrez-Díez, A and Mayek-Perez, N. (2020).** Outcrossing Rate and Genetic Variability in Mexican Race Avocado; *J. Amer. Soc. Hort. Sci.*, **145**(1):53–59.
33. **Gupta, K., Wani, S. H., Razzaq, A., Skalicky, M., Samantara, K., Gupta, S., Pandita, D., Goel, S., Grewal, S., Hejnak, V., Shiv, A., El-Sabrou, A. M., Elansary, H. O., Alaklabi, A. and Brestic, M. (2022).** Abscisic Acid: Role in Fruit Development and Ripening; *Front Plant Sci.*; **13**: 817500.

34. Hapuarachchi, N. S., Kämper, W., Wallace, H. M., Bai, S. H., Ogbourne, S. M., Nichols, J. and Trueman, S. J. (2022). Boron Effects on Fruit Set, Yield, Quality and Paternity of Hass Avocado; *Agronomy*, *12*(6), 1479
35. Hodgson, R. (1947). Bearing habits of avocado. *Cal. Avoc. Soc. Yrbk.* *44*:35-39.
36. Ish-Am, G. (2005). Reproductive biology of the avocado - A review. New Zealand and Australia Avocado Growers' Conference, Tauranga, New Zealand.
37. Ish-Am, G. and Eisikowitch, D. (1989). Influence of Temperature on Flower Morphology and Flowering Phenology of the Avocado Cultvars 'Fuerte', 'Ettinger', and 'Hass'. *Alon Hanotea*, *33*: 747-766.
38. Jackson, M. (1993). Are plant hormones involved in root to shoot communication? *Adv. Bot. Res.* *19*:103-187.
39. Jonkers, H. (1979). Biennial bearing in apple and pear: a literature survey. *Sci. Hort.* *11*:303-317.
40. Kachru, R., Singh, R., and Chacko, E. (1971). Inhibition of flowering in mango (*Mangifera indica* L) by gibberellic acid. *HortScience*, *6*:140-141.
41. Kardailsky, I., Shukla, V. K., Ahn, J. H., Dagenais, N. and Christensen, S. K. (1999) Activation tagging of the floral inducer FT. *Science*, *286*: 1962–1965.
42. Kinmonth-Schultz, H. A., MacEwen, M. J. S., Seaton, D. D., Millar, A. J., Imaizumi, T. and Kim, S. (2019). An explanatory model of temperature influence on flowering through whole-plant accumulation of FLOWERING LOCUS T in *Arabidopsis thaliana*; *In Silico Plants*, *10*: 1093.
43. Kobayashi, M., Lin, J. Z., Davis, J., Francis, L. and Clegg, M. T. (2000). Quantitative analysis of avocado outcrossing and yield in California using RAPD markers; *Scientia Horticulturae* *86*: 135±149.
44. Lahav, E. and Gazit, S. (1994). World listing of avocado cultivars according to flowering type. *Fruits* *49*: 299-313.
45. Larson, B. M. H. and Barrett, S. C. H. (2000). A comparative analysis of pollen limitation in flowering plants, *Biol. J. Linn. Soc. Long.*, *69*, 503-520.
46. Lesley, J. W. and Bringhurst, R. S. (1951). Environmental Conditions Affecting Pollination of Avocados. California Avocado Society Yearbook. *35*: 169-173.
47. Li, Y., Zhang, D., An, N., Fan, S., Zuo, X., Zhang, X., Zhang, L., Gao, C., Han, M. and Xing, L. (2019). Transcriptomic analysis reveals the regulatory module of apple (*Malus × domestica*) floral transition in response to 6-BA; *BMC Plant Biol.*, *19*: 93.

48. **McGregor, S. E. (1976).** Insect pollination of cultivation crop plants. Agric. Handb. 496 U.S. Dept. Agric.
49. **Miller, M. (1960).** Avocado pruning to regulate crop production. Cal. Avoc. Soc. Yrbk. *44*:42-44.
50. **Mlcaraz, M. L. and Hormaza, J. I. (2021).** Fruit Set in Avocado: Pollen Limitation, Pollen Load Size, and Selective Fruit Abortion. *Agronomy*, *11*, 1603.
51. **Monselise, S. and Goldschmidt, E. (1982).** Alternate bearing in fruit trees. Hort. Rev. *4*:128-173.
52. **Pant, P., Singh, M. V. P., Panwar, R. and Jat, R. (2020).** Dichogamy and its Relevance in Fruit Crops: An Overview; *Int. J. Curr. Microbiol. App. Sci.*, *9*(8): 698-708.
53. **Papademetriou, M. K. (1976).** Some aspects of the flower behaviour, pollination and fruit set of avocado (*Persea americana* Mill.), in Trinidad. California Avocado Association Annual Report, *59*: 106-152.
54. **Pin, P. A. and Nilsson, O. (2012).** The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant Cell Environ*, *35*: 1742–1755.
55. **Pisani, C., Ritenour, M. A., Plotto, E. S. A., Alessandro, R., Kuhn, D. N. and Schnell, R. J. (2017).** Postharvest and Sensory Evaluation of Selected ‘Hass’ 3 ‘Bacon’ and ‘Bacon’ 3 ‘Hass’ Avocado Hybrids Grown in East-Central Florida; *HORTSCIENCE**52*(6):880–886.
56. **Salazar-Garcia, S. and Lovatt, C. J. (2000).** Use of GA3 to manipulate flowering and yield of 'Hass' avocado. *J. Amer. Soc. Hort. Sci.*, *125*: 25-30.
57. **Scholefield, P., Sedgley, M. and Alexander, D. (1985).** Carbohydrate cycling in relation to shoot growth, floral initiation and development and yield in the avocado. *Sci. Hort.* *25*:99-110.
58. **Scora, R. W., Wolstenholme, B. N. and Lavi, U. (2002).** Taxonomy and botany. In: AW Whiley, B Schaffer and BN Wolstenholme (eds): *The Avocado: Botany, Production and Uses*. CABI Publishing, Wallingford, U.K. Pp 15-38.
59. **Sedgley, M. (1977).** Flowering, pollination and fruit set of avocado. S. Afr. Avocado Growers' Assoc. *Yearb.* *10*: 42-43.
60. **Sedgley, M. (1985).** Some effects of day length and flower manipulation on the floral cycle of two cultivars of avocado (*Persea americana* Mill., Lauraceae), a species showing protogynous dichogamy. *J. Exp. Bot.* *36*:823-832.
61. **Sedgley, M. and Annells, C. M. (1981).** Flowering and Fruit Set Response to Temperature in the Avocado Cultivar ‘Hass’; *Scientia Horticulturae.* *14*: 27-33.

- 62. Sedgley, M. and Grant, W. J. R. (1983).** Effect of low temperatures during flowering on floral cycle and pollen tube growth in nine avocado cultivars. *Sci. Hort.* **18**:207-213.
- 63. Siriwardana, N. S. and Lamb, R. S. (2012).** The poetry of reproduction: the role of LEAFY in *Arabidopsis thaliana* flower formation. *Int J Dev Biol*, **56**: 207–221.
- 64. Snijder, B. and Stassen, P. (1995).** Strategies for renewal of unproductive older avocado orchards with severe encroachment problems. *S. Afr. Avoc. Grow. Assoc.* **18**:56-58.
- 65. Stern, R.A.; Rozen, A.; Eshed, R.; Zviran, T.; Sisai, I.; Sherman, A.; Irihimovitch, V.; Sapir, G. (2021).** Bumblebees (*Bombus terrestris*) Improve 'Hass' Avocado (*Persea americana*) Pollination. *Plants (Basel)*, **10**, 1372.
- 66. Stout, A. B. (1923).** A study in cross-pollination of avocado in southern California. *California Avocado Association Annual Report*, **8**: 29-45.
- 67. Thorp, G. and Sedgley, M. (1993).** Manipulation of shoot growth patterns in relation to early fruit set in 'Hass1 avocado (*Persea americana*, Mill). *Sci. Hort.* **56**:147-156.
- 68. Ticho, R. (1970).** Girdling as a means to increase avocado fruit production. *Cal. Avoc. Soc. Yrbk.*: 90-94
- 69. Tripathi, P. C., Karunakaran, G., Sakthivel, T., Sankar, V. and Senthilkumar, R. (2014).** Avocado cultivation in India. *Technical Bulletin2/2014*, ICAR-IIHR, Central Horticultural Experiment Station, Chettalli, Kodagu, Karnataka, Kodagu, Karnataka. PP18.
- 70. Whiley, A. W. and Winston, E. C. (1987).** Effect of temperature at flowering on varietal productivity in some avocado growing areas in Australia. *S. Afr. Avocado Growers' Assoc. Yearb.* **10**: 45-47.
- 71. Whiley, A. W., Rasmussen, T. S., Saranah, J. B. and Wolstenholme, B. N. (1996).** Delayed harvest effects on yield, fruit size and starch cycling in avocado (*Persea americana* Mill.) in two subtropical environments. 1. The early-maturing cv. Fuerte. *Scientia Hort.*, **66**: 23-34.
- 72. X'etscherr, A. E., Davenport, T., Shafit', S., Daga, A., Waser, N. and Arpaial, M. L. (2000).** A Review of Avocado Pollination and the Role of Pollinizers; *Subtropical Fruit News*, **8**: 1-2.
- 73. Ziv, D., Zviran, T., Zezak, O., Samach, A. and Irihimovitch, V. (2014).** Expression Profiling of *FLOWERING LOCUS T-Like* Gene in Alternate Bearing 'Hass' Avocado Trees Suggests a Role for *PaFT* in Avocado Flower Induction; *PLoS ONE*, **9**(10): e110613.

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