REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
**Vol**: 13.10
**DOI**: 10.66104/1b5x5b09
**Pages:** 1-25

# POLLINATION AND REPRODUCTIVE COMPATIBILITY TESTS IN CACAO (*THEOBROMA CACAO* L.) IN THE TRANSAMAZONIAN REGION

# POLINIZAÇÃO E TESTES DE COMPATIBILIDADE REPRODUTIVA EM CACAUEIRO (*THEOBROMA CACAO* L.) NA REGIÃO DA TRANSAMAZÔNICA

# POLINIZACIÓN Y PRUEBAS DE COMPATIBILIDAD REPRODUCTIVA EN CACAO (*THEOBROMA CACAO* L.) EN LA REGIÓN TRANSAMAZÓNICA

**Sidevaldo Santana de Jesus**
M.Sc. in Agronomy, Federal Rural University of the Amazon (UFRA), Brazil
E-mail: sidevaldoemater@yahoo.com.br

**Harleson Sidney Almeida Monteiro**
M.Sc. in Agronomy (Horticulture), Sao Paulo State University (UNESP), Brazil
E-mail: harleson.sa.monteiro@unesp.br

**Larissa Pacheco Nogueira**
Agricultural Engineer, Federal Rural University of the Amazon (UFRA), Brazil
E-mail: larissanogues@gmail.com

**Sinara de Nazaré Santana Brito**
M.Sc. in Agronomy (Horticulture), Sao Paulo State University (UNESP), Brazil
E-mail: sinara.santana@unesp.br

**Viviandra Manuelle Monteiro de Castro Trindade**
M.Sc. in Environmental Sciences, Sao Paulo State University (UNESP), Brazil
E-mail: viviandra.trindade@unesp.br

**Cinthia Mota Veiga**
Agricultural Engineer, Federal Rural University of the Amazon (UFRA), Brazil
E-mail: eng.agrocinthiaveiga@gmail.com

**Nayara Ferreira Barros da Silva**
Agricultural Engineer, Flor da Mata Environmental Consulting, Brazil
E-mail: nayarabarros101@gmail.com

**Deivid Almeida de Jesus**
M.Sc. of Science in Biological Sciences (Genetics), Federal University of Rio de Janeiro (UFRJ), Brazil
E-mail: dajesus.deivid.09@gmail.com

**Glenda Rafaele da Silva**
Agricultural Engineer, Federal Rural University of the Amazon (UFRA), Brazil

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
**Vol**: 13.10
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**Pages:** 1-25

E-mail: glendarafaelle@gmail.com

**Gleicilene Brasil de Almeida**
M.Sc. in Agronomy, Federal Rural University of the Amazon (UFRA), Brazil
E-mail: E-mail gleicilenebrasil@gmail.com

**Antonia Benedita da Silva Bronze**
Ph.D. in Agricultural Sciences, Federal Rural University of the Amazon (UFRA), Brazil
E-mail: antonia.silva@ufra.edu.br

## Abstract

Cacao cultivation *(Theobroma cacao L.)* has high socioeconomic, environmental, and productive importance for tropical regions, especially within family farming systems. However, crop productivity is strongly limited by the low efficiency of natural pollination and the occurrence of genetic self-incompatibility mechanisms, which significantly reduce fruit set. In this context, the present technical-descriptive review aimed to describe and discuss the main aspects related to floral biology, pollination, and genetic compatibility in cacao, with emphasis on the artificial pollination methodology used to determine sexual compatibility among genotypes. The analyzed literature demonstrates that cacao possesses highly specialized flowers, mainly dependent on insects of the family *Ceratopogonidae* for natural pollination. Nevertheless, environmental factors, ecological limitations, and late-acting self-incompatibility mechanisms compromise the reproductive efficiency of the species, resulting in low fruiting rates. The review also demonstrates that artificial pollination constitutes an essential tool for studies on genetic compatibility, selection of superior genotypes, and breeding programs. The procedure involves standardized stages of flower selection and isolation, pollen collection, manual pollination, and post-pollination monitoring, allowing the evaluation of fruit set under controlled conditions. Furthermore, self-compatible genotypes exhibit greater productive stability and lower dependence on natural pollinators, making them strategic for commercial plantations. It is concluded that knowledge regarding reproductive biology and genetic compatibility is essential for optimizing breeding programs, increasing reproductive efficiency, and contributing to the productive sustainability of cacao cultivation. Additionally, the reviewed studies indicate that understanding intercompatibility relationships among clones allows more efficient planning of polyclonal arrangements, favoring cross-fertilization, fruit formation, and productive stability in orchards. Thus, the standardization of artificial pollination protocols represents technical support for reproductive research and crop management strategies. These methodologies also contribute to the identification of compatible genetic materials with agronomic potential, supporting orchard establishment, reducing reproductive failures, and improving pollination efficiency under tropical environmental conditions characterized by climatic and variability.

**Keywords:** controlled pollination; self-incompatibility; genetic compatibility; fruit set.

## Resumo

A cultura do cacaueiro (*Theobroma cacao* L.) apresenta elevada importância socioeconômica, ambiental e produtiva para regiões tropicais, especialmente para sistemas de agricultura familiar. Entretanto, a produtividade da cultura é fortemente limitada pela baixa eficiência da polinização natural e pela ocorrência de mecanismos de autoincompatibilidade genética, os quais reduzem significativamente o pegamento de frutos. Nesse contexto, a presente revisão técnico-narrativa teve como objetivo descrever e discutir os principais aspectos relacionados à biologia floral, à polinização e à compatibilidade genética do cacaueiro, com ênfase na metodologia de polinização artificial utilizada para determinação da compatibilidade sexual entre genótipos. A literatura analisada demonstra que o cacaueiro apresenta flores altamente especializadas, dependentes principalmente de insetos da família *Ceratopogonidae* para a realização da polinização natural. Contudo, fatores

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ambientais, limitações ecológicas e mecanismos de autoincompatibilidade de ação tardia comprometem a eficiência reprodutiva da espécie, resultando em baixas taxas de frutificação. A revisão também evidencia que a polinização artificial constitui uma ferramenta fundamental para estudos de compatibilidade genética, seleção de genótipos superiores e programas de melhoramento genético. O procedimento envolve etapas padronizadas de seleção e isolamento floral, coleta de pólen, polinização manual e monitoramento pós-polinização, permitindo avaliar o pegamento de frutos sob condições controladas. Além disso, genótipos autocompatíveis apresentam maior estabilidade produtiva e menor dependência de polinizadores naturais, tornando-se estratégicos para plantios comerciais. Conclui-se que o conhecimento sobre biologia reprodutiva e compatibilidade genética é essencial para otimizar programas de melhoramento, aumentar a eficiência reprodutiva e contribuir para a sustentabilidade produtiva da cultura do cacaueiro. Adicionalmente, os estudos revisados indicam que a compreensão das relações de intercompatibilidade entre clones permite o planejamento eficiente de arranjos policlonais, favorecendo a fecundação cruzada, a formação de frutos e a estabilidade produtiva dos pomares. Dessa forma, a padronização de protocolos de polinização artificial representa suporte técnico para pesquisas reprodutivas e estratégias de manejo.

**Palavras-chave:** polinização controlada; autoincompatibilidade; compatibilidade genética; pegamento de fruto.

## Resumen

El cultivo del cacao (*Theobroma cacao* L.) posee elevada importancia socioeconómica, ambiental y productiva para las regiones tropicales, especialmente para los sistemas de agricultura familiar. Sin embargo, la productividad del cultivo está fuertemente limitada por la baja eficiencia de la polinización natural y por la ocurrencia de mecanismos de autoincompatibilidad genética, los cuales reducen significativamente el cuajado de frutos. En este contexto, la presente narrative review tuvo como objetivo describir y discutir los principales aspectos relacionados con la biología floral, la polinización y la compatibilidad genética del cacao, con énfasis en la metodología de polinización artificial utilizada para determinar la compatibilidad sexual entre genotipos. La literatura analizada demuestra que el cacao presenta flores altamente especializadas, dependientes principalmente de insectos de la familia Ceratopogonidae para la realización de la polinización natural. No obstante, factores ambientales, limitaciones ecológicas y mecanismos de autoincompatibilidad de acción tardía comprometen la eficiencia reproductiva de la especie, resultando en bajas tasas de fructificación. La revisión también evidencia que la polinización artificial constituye una herramienta fundamental para estudios de compatibilidad genética, selección de genotipos superiores y programas de mejoramiento genético. El procedimiento involucra etapas estandarizadas de selección y aislamiento floral, colecta de polen, polinización manual y monitoreo pospolinización, permitiendo evaluar el cuajado de frutos bajo condiciones controladas. Además, los genotipos autocompatibles presentan mayor estabilidad productiva y menor dependencia de polinizadores naturales, convirtiéndose en materiales estratégicos para plantaciones comerciales. Se concluye que el conocimiento sobre biología reproductiva y compatibilidad genética es esencial para optimizar programas de mejoramiento, aumentar la eficiencia reproductiva y contribuir a la sostenibilidad productiva del cultivo de cacao. Adicionalmente, los estudios revisados indican que comprender las relaciones de intercompatibilidad entre clones permite planificar arreglos policlonales eficientes, favoreciendo la fecundación cruzada, la formación de frutos y la estabilidad productiva de los huertos comerciales bajo condiciones ambientales tropicales.

**Palabras clave:** polinización controlada; autoincompatibilidad; compatibilidad genética; cuajado de fruto.

## 1. Introduction

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The cacao tree (*Theobroma cacao* L.) is a species native to Central and South America, including the Brazilian Amazon (Galvão et al., 2024). It belongs to the family Malvaceae and is characterized as a perennial woody species (Silva et al., 2022). Due to its high edaphoclimatic requirements, cacao exhibits optimal performance under warm climatic conditions, with temperatures above 21 °C and adequate annual water availability, as reported by Barroso et al. (2025). In addition, the species has successfully adapted to several tropical regions worldwide, including Africa, Asia, the Caribbean, and Latin America (Ceplac, 2020).

Cacao cultivation plays a major socioeconomic role in tropical regions, particularly in Brazil (Amaral et al., 2025). According to data from the Brazilian Institute of Geography and Statistics (IBGE), the state of Pará was the largest cocoa producer in Brazil in 2024, with emphasis on the Trans-Amazonian region, which currently concentrates a substantial portion of both state and national production. According to Brasil (2020), most cacao cultivation in the country is carried out by family farming systems, accounting for up to 60% of national production, thereby promoting employment opportunities and generating income for millions of Brazilians.

The Trans-Amazonian region of Pará State represents one of the main cacao-producing areas in Brazil, with production systems predominantly associated with family farming and agroforestry cultivation (Veloso et al., 2025). The environmental conditions of the region, characterized by high rainfall, elevated relative humidity, and heterogeneous shading systems, directly influence pollinator activity, floral biology, and fruit set dynamics in cacao plantations (Melo-Zipacon et al., 2025). Despite the economic importance of cacao production in the region, studies involving reproductive compatibility and controlled pollination under Trans-Amazonian conditions remain limited (Velosos et al., 2025).

Despite the economic importance of cacao, fruit production is directly associated with pollination efficiency, which is considered naturally limited under field conditions (Forbes, 2019). This process depends primarily on the activity of

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small insect pollinators, whose performance may be affected by environmental factors such as humidity, temperature, and crop management practices. Consequently, low fruit set rates are frequently observed, directly affecting crop productivity (Vansynghel et al., 2023). Furthermore, the occurrence of reproductive incompatibility mechanisms in certain plants may further restrict fruit formation, highlighting the need for strategies aimed at understanding and evaluating the reproductive behavior of different cacao genetic materials (Yamada et al., 2014).

In addition to the limitations associated with pollinators, the expression of genetic self-incompatibility mechanisms constitutes a determining factor for the reproductive success of cacao (Carletto and Soria, 1973). Self-incompatibility is a genetic system that prevents the fertilization of a flower by its own pollen, thereby promoting allogamy (cross-pollination) as an obligatory reproductive strategy in the species. This mechanism has direct implications for breeding programs and for the recommendation of clones for commercial plantations (Godoy et al., 2009). Therefore, when selecting a clone for cultivation, it is essential to understand its reproductive behavior. Self-incompatible clones, when planted in isolation, exhibit low or even absent fruit production, since they depend on pollen from compatible genotypes to achieve fertilization (Bekele and Phillips-Mora, 2019).

### 1.1 General Objective

The present technical-descriptive review aims to describe and discuss the artificial pollination procedure in cacao trees, with emphasis on the determination of plant sexual compatibility. Furthermore, it seeks to systematically present the stages involved in the process, thereby contributing to a broader understanding of this technique and its application in studies focused on genetic improvement and increased crop productivity.

## 2. MATERIALS AND METHODS OF THE REVIEW

This study was conducted as a technical-narrative review focused on the

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reproductive biology, artificial pollination, and compatibility assessment in cacao (*Theobroma cacao* L.). The manuscript was developed based on classical and contemporary scientific literature related to floral biology, pollination ecology, self-incompatibility, reproductive compatibility, and artificial pollination methodologies in cacao.

The bibliographic survey included scientific articles, technical bulletins, books, theses, and official publications indexed in databases such as Scopus, Web of Science, Google Scholar, ScienceDirect, and SciELO. The main descriptors used were: “Theobroma cacao”, “artificial pollination”, “self-incompatibility”, “reproductive compatibility”, “fruit set”, “pollination ecology”, and “cacao breeding”.

The selected literature was analyzed qualitatively, prioritizing studies with methodological relevance, applicability to cacao reproductive biology, and contributions to compatibility assessment and orchard management.

## 3. LITERATURE REVIEW

### 3.1 Floral biology and pollination of cacao

Cacao flowers exhibit a highly specialized and complex floral structure, evolutionarily adapted to pollination by small insects, particularly dipterans of the family Ceratopogonidae (biting midges) (Dani and Rokhmah, 2022; Jaramillo et al., 2024). According to Jaimez et al. (2022), cacao flowers are hermaphroditic, containing both male and female reproductive organs within the same floral structure.

Morphologically, each flower is composed of five sepals, five petals, five fertile stamens, five staminodes (sterile stamens), and a superior ovary containing five locules with numerous ovules, which may range from 40 to 70 depending on the genotype (Cuatrecasas, 1964; Bahia et al., 2013; Dani and Rokhmah, 2022). The floral structures involved in cacao reproduction are illustrated in Figure 1.

**Figure 1.** illustrates the main reproductive structures of the cacao flower.

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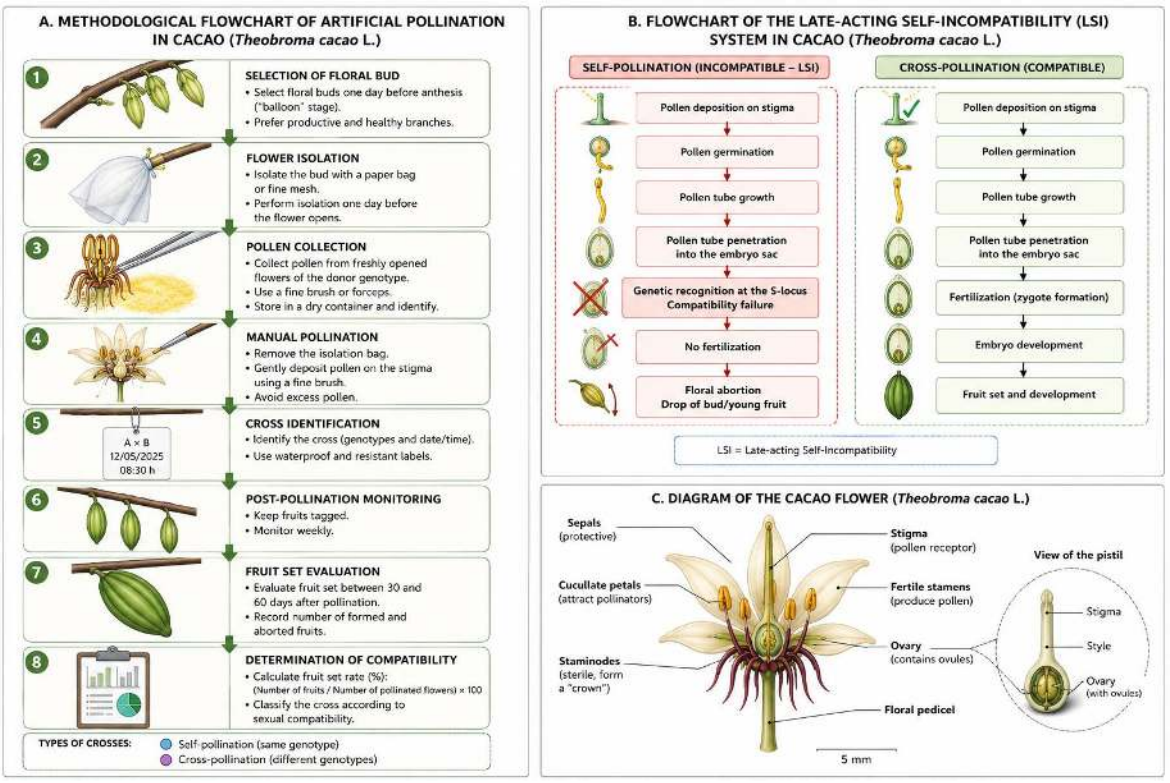


Source: Adapted from Dani and Rokhmah (2022), Jaramillo et al. (2024), Jaimez et al. (2022), Cuatrecasas (1964), and Bahia et al. (2013); prepared by the authors (2026).

In addition, the flowers are small, generally measuring between 1 and 2 cm in diameter, and are borne directly on the trunk and older branches, a reproductive characteristic known as cauliflory (Bridgemohan and Mohammed, 2019).

The floral morphology of cacao is directly associated with its pollination ecology. The petals exhibit a cucullate or hooded structure that partially encloses the stamens, while the sticky pollen grains and restricted floral opening make wind pollination inefficient or virtually absent (Jaramillo et al., 2024). Consequently, successful pollination depends almost exclusively on biological vectors capable of accessing the reproductive structures within the flower. Among these, biting midges (Ceratopogonidae) are traditionally considered the primary pollinators of cacao worldwide (Billes, 1941; Soria, 1971), although recent studies have demonstrated that other insect groups, including stingless bees, ants, and additional dipterans, may also contribute to pollen transfer under certain ecological conditions (Bridgemohan and Mohammed, 2019; Jaramillo et al., 2024).

The reproductive structures of the cacao flower also exhibit temporal and

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physiological adaptations associated with pollination success. The style is short and terminates in a five-lobed stigma that secretes a viscous exudate responsible for retaining pollen grains and facilitating pollen germination. Studies conducted with *T. speciosum* and *T. cacao* demonstrated that stigmatic receptivity is highest during the morning hours, coinciding with peak pollinator activity (Souza and Venturieri, 2010; Jaramillo et al., 2024). Floral anthesis generally begins during the night or early morning, with complete flower opening occurring at dawn, when anther dehiscence and stigma receptivity overlap (Figure 2), thereby maximizing the probability of successful fertilization (Souza and Venturieri, 2010; Bahia et al., 2013; Dani and Rokhmah, 2022).

**Figure 2.** Reproductive structures of the cacao flower, highlighting staminodes, stigma, and pollen deposition region.




Source: Authors (2026).

Under natural conditions, pollination occurs when insects visit flowers in search of nectar, pollen, or shelter. During this process, the insect's body first contacts the stigma and subsequently the anthers, promoting pollen transfer between flowers of different plants (Bridgemohan and Mohammed, 2019). This mechanism favors cross-pollination, which predominates in cacao due to the frequent occurrence of self-incompatibility systems. Despite the high flower production observed in cacao trees, which may reach thousands of flowers per

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flowering season, only a very small proportion successfully develops into mature fruits (Dani and Rokhmah, 2022). In most cases, fruit set rates range from 1 to 10 %, while many flowers abscise within 24 to 48 h after anthesis when pollination or fertilization fails (Dani and Rokhmah, 2022; Jaramillo et al., 2024).

This low reproductive efficiency is largely associated with pollen limitation and incompatibility mechanisms. In self-incompatible plants, pollen originating from the same genotype may germinate on the stigma; however, fertilization is subsequently interrupted through genetic recognition mechanisms that prevent zygote formation (Ford and Wilkinson, 2012). In cacao, this phenomenon is classified as late-acting self-incompatibility (LSI), since the incompatibility response occurs after pollen tube growth and sperm cell penetration into the embryo sac (Dani and Rokhmah, 2022). According to de Nettancourt (2013), the system is controlled by multiple loci, and its expression may vary substantially among genotypes.

As reported by Yamada et al. (2013), cacao clones exhibit distinct reproductive behaviors, ranging from complete self-incompatibility to high levels of self-compatibility (Figure 3). Some self-compatible clones may achieve fruit set rates between 50 and 100 % following self-pollination, whereas self-incompatible materials require compatible pollen donors to ensure fertilization and fruit production. This variability has major implications for breeding programs and commercial orchard planning, since isolated planting of self-incompatible clones may result in severe reductions in productivity. In contrast, highly self-compatible clones, such as CCN 51, are capable of maintaining satisfactory fruit production even under reduced pollinator availability or limited genetic diversity within plantations (Yamada et al., 2014).

**Figure 3.** Cacao floral bud at the pre-anthesis stage (swollen bud), representing the ideal phase for flower selection prior to artificial pollination.

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Source: Adapted from Jaramillo et al. (2024).

Recent studies have reinforced the importance of pollination efficiency as one of the major limiting factors for cacao productivity. Ríos-Moyano et al. (2025) reported that only a small fraction of flowers effectively receive viable pollen loads sufficient for fertilization, while Vansynghel et al. (2023), described this phenomenon as “pollination deficit.” This condition is considered one of the primary biological constraints affecting cacao yield worldwide, particularly in low-input production systems where pollinator abundance and habitat quality are reduced.

Moreover, environmental factors such as temperature, rainfall, humidity, canopy structure, and landscape connectivity strongly influence pollinator activity and floral visitation rates (Dani and Rokhmah, 2022; Ríos-Moyano et al., 2025). Consequently, understanding the floral biology, pollination ecology, and reproductive compatibility of cacao genotypes is fundamental for the development of breeding strategies, artificial pollination protocols, and management practices aimed at increasing fruit set and crop productivity.

### 3.2 Genetic compatibility of cacao

The reproductive biology of cacao is characterized by complex physiological and genetic barriers, with self-incompatibility representing one of the major limiting factors affecting overall crop productivity. Self-incompatibility is defined as the

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inability of plants, despite possessing morphologically normal pollen grains and ovules, to produce seeds following self-pollination (Coronado-Aleans et al., 2025). Physiologically, cacao exhibits a late-acting self-incompatibility (LSI) system, in which the pollen tube grows normally through the style and the male gametes are released into the embryo sac; however, a genetic failure subsequently prevents the final fusion of gametes and successful zygote formation (Lanaud et al., 2017). As a direct consequence of unsuccessful fertilization, floral abscission occurs, preventing fruit development and significantly reducing productive efficiency (López-Torres et al., 2021).

This incompatibility mechanism has profound implications for cacao reproductive success and orchard productivity. Although cacao trees are capable of producing thousands of flowers during a flowering cycle, only a small proportion successfully develop into mature fruits due to failures associated with pollination and fertilization (Dani and Rokhmah, 2022; Ríos-Moyano et al., 2025). In self-incompatible genotypes, reproductive failure occurs even when pollen deposition and pollen tube growth are apparently normal, demonstrating that the incompatibility response acts at advanced stages of the fertilization process (Dani and Rokhmah, 2022). According to Ford and Wilkinson (2012), this late-acting mechanism distinguishes cacao from many other species presenting conventional sporophytic or gametophytic self-incompatibility systems.

Conversely, self-compatibility occurs when favorable genetic mechanisms allow the plant to overcome these reproductive barriers and complete fertilization using its own pollen, frequently within the first 24 h after pollination (López-Torres et al., 2021). Genotypes considered self-compatible generally exhibit a fruit set index equal to or greater than 30 % under controlled pollination conditions, although many commercially important materials exceed 50 % fruit fixation efficiency (López-Torres et al., 2021; Coronado-Aleans et al., 2025). From an agronomic perspective, self-compatible genotypes provide considerable advantages because they promote greater production stability, facilitate the establishment of clonal gardens, and reduce crop dependence on pollinator abundance and compatible neighboring genotypes (Lanaud et al., 2017).

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The genetic control of self-incompatibility in cacao is highly complex and involves multiple loci associated with gamete recognition and fertilization processes. According to de Nettancourt (2013), the LSI system in cacao is regulated by different allelic interactions that determine the degree of compatibility between male and female gametes (Dani and Rokhmah, 2022). Depending on allelic combinations, self-pollination may result in partial or complete fertilization failure. Consequently, cacao genotypes may exhibit varying reproductive behaviors, ranging from complete self-incompatibility to full self-compatibility. This variability has become a major target in breeding programs aimed at developing highly productive and reproductively stable cultivars.

Among commercially important materials, clones such as CCN 51 are widely recognized for their high degree of self-compatibility and stable fruit production under commercial conditions (Lim et al., 2025). In contrast, several traditional and wild genotypes remain strongly dependent on compatible pollen donors and pollinating insects to achieve satisfactory fruit set. Therefore, understanding the compatibility profile of each genotype is essential for optimizing orchard design, maximizing pollination efficiency, and ensuring sustainable productivity.

In multiclonal commercial plantations, the productive interaction among trees is strongly influenced by intercompatibility and inter-incompatibility relationships (Coronado-Aleans et al., 2025). Intercompatibility, also referred to as cross-compatibility, corresponds to the reciprocal reproductive affinity that allows pollen from one genotype to successfully fertilize flowers of another genetically distinct plant. This compatibility is commonly evaluated through controlled artificial pollination combined with previous floral isolation to prevent unwanted pollen contamination (López-Torres et al., 2021). On the other hand, inter-incompatibility acts as a severe genetic barrier between different genotypes (Table 1), preventing successful cross-fertilization and negatively affecting orchard productivity when genetically incompatible materials are cultivated in close proximity.

**Table 1.** Operational definitions of reproductive compatibility categories in cacao.

| Category | Definition |
|---|---|

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| | |
|---|---|
| Self-compatibility | Ability to produce fruits after self-pollination |
| Self-incompatibility | Failure of fertilization after self-pollination |
| Partial self-compatibility | Intermediate fruit set after self-pollination |
| Cross-compatibility | Successful fertilization between distinct genotypes |
| Reciprocal compatibility | Compatibility observed in both crossing directions |
| Inter-incompatibility | Reproductive incompatibility between distinct genotypes |

Source: Adapted from Yamada et al. (2014); Lanaud et al. (2017); López-Torres et al. (2021); Coronado-Aleans et al. (2025); and Godoy et al. (2009); prepared by the authors (2026).

The degree of cross-compatibility not only determines fruit set efficiency but also directly influences important morphological and agronomic traits, including fruit vigor, seed size, seed weight, and overall bean quality (López-Torres et al., 2021; Coronado-Aleans et al., 2025). In addition, pollen source effects, commonly associated with xenia and metaxenia phenomena, may alter fruit and seed characteristics after fertilization (Dani and Rokhmah, 2022). Therefore, the establishment of polyclonal plantations should prioritize combinations of highly compatible genotypes in order to maximize fertilization success, stabilize production, and improve the qualitative and quantitative performance of cacao orchards.

### 3.3 Methodology of artificial pollination in cacao

The determination of sexual compatibility in cacao is primarily conducted through controlled artificial pollination assays, which enable the evaluation of reproductive behavior, self-compatibility, and cross-compatibility among genotypes under standardized conditions. This methodology constitutes one of the most important experimental approaches used in cacao reproductive biology and breeding programs, as it allows precise assessment of fruit set following self- or cross-pollination events. In addition, controlled pollination provides essential information for the selection of elite genotypes, establishment of compatible clonal combinations, and optimization of commercial orchard productivity.

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The procedure follows sequential stages involving floral selection, isolation of reproductive structures, manual pollen transfer, and post-pollination monitoring, as described in classical and contemporary studies on cacao reproductive biology (Pinto et al., 1998; Lanaud et al., 2017). Due to the high occurrence of late-acting self-incompatibility (LSI) in cacao, strict control of pollen origin and exclusion of natural pollinators are essential to ensure the reliability of compatibility assessments

### 3.3.1 Materials used in artificial pollination

Artificial pollination in cacao requires a set of simple yet technically indispensable materials to ensure floral isolation, prevent pollen contamination, and allow accurate manipulation of reproductive structures. The materials used during the procedure are illustrated in Figure 4.

**Figure 4.** Materials used during the artificial pollination procedure in cacao. Numbers indicate the different materials employed throughout the methodology.




Note: (01) Latex rubber bands (size 18): used to secure the isolation tube to the trunk or floral cushion region; (02) Materials used for tube assembly: foam, pieces of inner tire rubber, and transparent PVC tubing; (03) Crepe bandages: used to seal the upper extremity of the isolation tube, preventing the entry of insect pollinators and airborne contaminants; (04) Pins: used for flower identification and fixation of isolation tubes on thicker branches; (05) Forceps and scissors: used for floral manipulation, including removal of sepals, petals, and shortening of staminodes to facilitate access to the stigma

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and improve pollen deposition efficiency; (06) Container for storing identification pins; (07) Styrofoam container for temporary storage and protection of donor flowers carrying viable pollen; (08) Plastic storage box for transportation and organization of pollination materials during field activities. Source: Jesus, S. S. (2025).

The isolation tubes are generally prepared using transparent PVC tubing measuring approximately 7 cm in length and 1 inch in diameter. Transparent material is preferred because it allows visualization of floral development without disturbing the reproductive structures. Additionally, rectangular pieces of tire inner-tube rubber measuring approximately 7.0 × 6.0 cm or 10.0 × 5.0 cm are used to provide adequate fixation and sealing against the cacao trunk surface. The inner face of the rubber support is lined with foam to improve adherence and prevent the entry of pollinating insects, thereby ensuring complete reproductive isolation.

### 3.3.2 Selection and isolation of flowers

Floral selection is performed one day prior to pollination. Floral buds at the swollen pre-anthesis stage are selected because they are physiologically close to anthesis and are expected to open during the following morning. This developmental stage is characterized by enlargement of the floral bud and partial sepal separation, representing the ideal phase for controlled pollination procedures (Jaramillo et al., 2024).

Selected floral buds are isolated using transparent PVC tubes approximately 7 cm long, whose upper extremity is sealed with porous material to prevent the entry of natural pollinators while maintaining adequate ventilation (Figure 5). The tubes are fixed to the trunk or floral cushion using rubber bands, elastic ties, or pins, ensuring complete isolation of the reproductive structures throughout the pollination period.

**Figure 5.** Isolation system used for cacao flowers.

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ISSN 2178-6925

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Note: (A) Materials used for preparation of the isolation tube. (B) Transparent PVC tube installed on the cacao trunk for floral isolation. Source: Jesus, S. S. (2025).

Whenever possible, multiple floral buds from the same floral cushion are isolated simultaneously to increase the number of flowers available for pollination. On the following day, flowers that failed to reach anthesis are carefully removed using scissors, maintaining only fully opened flowers suitable for controlled pollination. This procedure minimizes mechanical disturbance to adjacent flowers and reduces the risk of accidental floral abscission.

Similarly, flowers intended as pollen donors must also be isolated one day before pollination to avoid contamination by external pollen sources and to ensure controlled pollen origin (Figure 6).

**Figure 6.** Isolation of cacao flowers prior to controlled pollination.



Source: Jesus, S. S. (2025).

The strict isolation of both donor and receptor flowers is particularly

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
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important in cacao due to the activity of numerous insect visitors capable of transferring pollen between flowers (Jaramillo et al., 2024).

### 3.3.3 Pollen collection and preparation of receptor flowers

Pollination procedures are generally conducted during the morning period, between 06:00 and 12:00 h, corresponding to the interval of maximum stigmatic receptivity and pollen viability in cacao flowers (Souza and Venturieri, 2010; Jaramillo et al., 2024). At this stage, previously isolated flowers that successfully reached anthesis are designated as receptor flowers.

Using fine forceps, the staminodes of receptor flowers are partially removed or shortened to facilitate access to the stigmatic surface and improve pollen deposition efficiency. This floral manipulation also facilitates subsequent visual identification of pollinated flowers during monitoring evaluations.

Simultaneously, freshly opened donor flowers exhibiting active anther dehiscence are collected and used as pollen sources (Figure 7). In cacao, anthers remain protected within hooded petal structures, and pollen release generally occurs during the early morning hours (Dani and Rokhmah, 2022). Therefore, freshly opened flowers provide the highest probability of obtaining viable pollen grains for successful fertilization.

**Figure 7.** Donor flower manually manipulated during artificial pollination.



Source: Jesus, S. S. (2025).

### 3.3.4 Controlled manual pollination

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
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Controlled artificial pollination is carried out through the direct transfer of pollen grains from a donor flower to the receptive stigma of a receptor flower under strictly controlled conditions. This methodology is widely employed in studies involving reproductive biology, compatibility assessment, and cacao breeding programs, as it enables the accurate determination of fruit set resulting from self- or cross-pollination events (Pinto et al., 1998; López-Torres et al., 2021).

The procedure consists of gently contacting pollen-bearing anthers with the stigmatic surface to ensure adequate deposition of viable and compatible pollen grains. In cacao, successful fertilization depends not only on pollen transfer itself but also on the quantity and viability of pollen deposited on the stigma, since insufficient pollen loads may compromise ovule fertilization and subsequent fruit development (Frimpong-Anin et al., 2014).

The execution of artificial pollination in cacao requires high technical precision due to the reduced floral size and the highly specialized morphology of the reproductive structures. Cocoa flowers possess hooded petals that partially enclose the anthers, sticky pollen grains, and narrow access to the reproductive organs, characteristics that make spontaneous self-pollination and wind-mediated pollination virtually ineffective (Ríos-Moyano et al., 2025).

Furthermore, the spatial arrangement between the staminodes and the style directly affects pollination efficiency. According to Frimpong-Anin et al. (2014), flowers with wider staminode-style distances tend to exhibit lower pollination success due to reduced contact between pollinating agents and the stigmatic surface. Consequently, manual pollination represents an indispensable approach for compatibility studies, particularly in genotypes exhibiting late-acting self-incompatibility mechanisms (Lanaud et al., 2017).

During the pollination process, donor flowers are carefully manipulated to expose freshly dehiscent anthers containing viable pollen grains. The pollen is subsequently transferred manually onto the receptive stigma of the receptor flower through gentle contact between the anthers and the stigmatic lobes. Floral manipulations are preferentially performed during the morning period, generally between 06:00 and 12:00 h, corresponding to the interval of maximum stigmatic

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
**Vol**: 13.10
**DOI**: 10.66104/1b5x5b09
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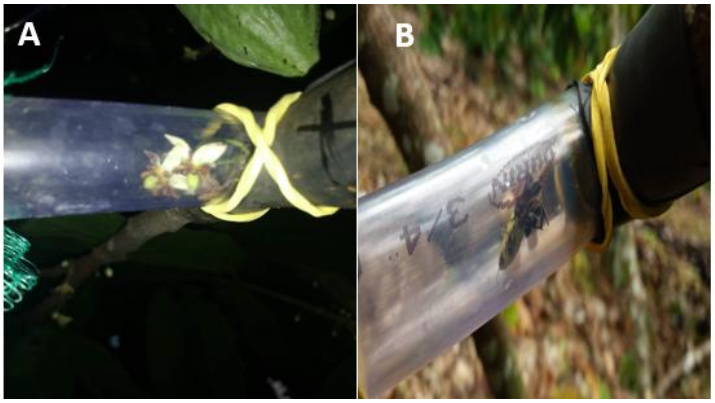
receptivity, pollen viability, and natural pollinator activity in cacao flowers (Souza and Venturieri, 2010; Ríos-Moyano et al., 2025). This synchronization between floral physiology and pollination timing significantly increases the probability of successful fertilization and fruit set.

Immediately after pollen transfer, receptor flowers are re-isolated using protective PVC tubes to prevent contamination by external pollen sources or natural pollinating insects. Floral isolation is particularly important in cacao because the species depends almost exclusively on insect-mediated pollen transfer under natural conditions, especially by dipterans belonging to the family Ceratopogonidae, such as *Forcipomyia* spp. and *Dasyhelea* spp. (Ríos-Moyano et al., 2025). Pollinated flowers are subsequently identified using pins or labels to facilitate post-pollination monitoring and compatibility evaluations.

### 3.3.5 Post-pollination monitoring

Post-pollination monitoring begins approximately three days after controlled pollination and represents a critical stage for evaluating reproductive success and compatibility responses in cacao. Sequential evaluations are commonly conducted on the 5th, 8th, and 15th days after pollination, enabling the identification of flowers that successfully initiated fruit development (Figure 8).

**Figure 8.** Flowers that successfully developed into fruits after artificial pollination.




Note: (A) Flowers at the 5th day after pollination. (B) Young developing fruits at the 8th day after pollination, suitable for removal of protective isolation tubes. Source: Jesus, S. S. (2025).

The persistence of pollinated flowers on the tree and the subsequent

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
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enlargement of the ovary are considered strong indicators of successful fertilization. According to Ríos-Moyano et al. (2025), flowers that remain attached to the plant for more than 24 h after anthesis are considered pollinated, whereas flowers persisting beyond 36 h generally indicate effective fertilization. Under successful reproductive conditions, the ovary progressively enlarges, the pedicel increases in thickness, and floral tissues gradually undergo senescence while remaining attached to the plant during the initial stages of fruit development.

Conversely, flowers that fail to achieve successful fertilization generally undergo rapid abscission within 24 to 72 h after anthesis, frequently without visible signs of senescence (Ríos-Moyano et al., 2025). In self-incompatible genotypes, reproductive failure may occur even after apparently normal pollen germination and pollen tube growth. In these materials, late-acting self-incompatibility mechanisms interrupt the final stages of fertilization after the pollen tube reaches the embryo sac, preventing gamete fusion and resulting in ovary abortion and floral drop (Lanaud et al., 2017; López-Torres et al., 2021). Therefore, the distinction between persistent and aborted flowers during the first days after pollination constitutes a reliable indicator of reproductive compatibility (Table 2) in cacao.

**Table 2.** Minimum methodological recommendations for reproductive compatibility tests in cacao.

| **Parameter** | **Recommendation** |
|---|---|
| Experimental unit | Pollinated flower |
| Minimum flowers per cross | 20–30 flowers |
| Number of plants | At least 3 plants per genotype |
| Evaluation period | 5, 8, 15 and 30 days after pollination |
| Pollen viability control | Recommended |
| Environmental monitoring | Temperature, humidity and rainfall |
| Replication | Temporal and biological replication |

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
**Vol**: 13.10
**DOI**: 10.66104/1b5x5b09
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| Parameter | Recommendation |
|---|---|
| Crossing types | Self- and cross-pollination |

Source: Adapted from Pinto et al. (1998), López-Torres et al. (2021), Yamada et al. (2014), Frimpong-Anin et al. (2014), and Ríos-Moyano et al. (2025); prepared by the authors (2026).

Environmental conditions also exert a strong influence on floral persistence and reproductive stability after pollination. Frimpong-Anin et al. (2014), demonstrated that pollinated flowers exhibit greater stability during humid periods, whereas flowers subjected to water deficit or unsuccessful pollination tend to abscise more rapidly under dry environmental conditions. According to these authors, seasonal factors such as reduced humidity and water stress can substantially decrease floral stability and compromise fruit set. Likewise, Ríos-Moyano et al. (2025), highlighted that environmental variables, including rainfall, temperature, and relative humidity, directly influence pollinator activity and pollination efficiency in cacao agroecosystems.

Recent studies have also demonstrated that the abundance of pollinating insects is positively associated with flower fertilization and fruit transition rates. Ríos-Moyano et al. (2025), reported strong positive correlations between the abundance of Ceratopogonidae pollinators and the percentage of fertilized flowers, reinforcing the fundamental role of insect-mediated pollination in cacao reproductive success. Therefore, monitoring flower persistence and early fruit development after controlled pollination not only allows compatibility determination but also provides important insights into the physiological and environmental factors regulating reproductive efficiency in cacao.

### 3.4 Determination of fruit set index

The efficiency of controlled artificial pollination is evaluated through the fruit set index (FSI), determined approximately 15 days after pollination. This index corresponds to the proportion of pollinated flowers that successfully developed into young fruits and is expressed as a percentage.

The fruit set index is calculated according to the following equation:

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
**Vol**: 13.10
**DOI**: 10.66104/1b5x5b09
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$$FSI = \left(\frac{SF}{PF}\right) \times 100$$

Where:

- FSI = Fruit set index (%);
- SF = Number of successful fruits (set fruits);
- PF = Total number of pollinated flowers.

The classification of cacao genotypes regarding self-compatibility is based on reference thresholds established in the literature. According to Lopes and Carletto (1995), plants are generally classified as self-compatible when they exhibit a minimum fruit set index of 10 % in a set of 26 controlled pollinations. When smaller experimental sets are used, such as 15 pollinations, the minimum threshold increases to 20 %.

These compatibility parameters are essential for cacao breeding programs because they enable the identification of genotypes with superior reproductive efficiency, greater fruiting stability, and enhanced agronomic potential. Furthermore, the characterization of compatibility relationships among clones supports the establishment of highly compatible polyclonal orchards, thereby improving pollination efficiency, reducing reproductive failure, and increasing overall crop productivity.

## 4. Final Considerations

This systematic review demonstrated that artificial pollination constitutes an essential tool for studies involving reproductive biology, sexual compatibility, and genetic improvement in cacao. The methodology enables the controlled evaluation of self-compatibility, cross-compatibility, and intercompatibility among genotypes, providing reliable information for the selection of superior plant materials and the establishment of more productive commercial orchards.

The literature analyzed also highlights that the reproductive success of cacao is strongly influenced by floral morphology, pollination efficiency, and late-acting self-incompatibility mechanisms, which frequently limit natural fruit set. In

REMUNOM
ISSN 2178-6925

**Received:** 13/03/2026 - **Accepted:** 18/05/2026
**Vol**: 13.10
**DOI**: 10.66104/1b5x5b09
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this context, controlled artificial pollination allows the identification of compatible genotypes capable of presenting greater reproductive stability and higher fruit production potential.

Furthermore, the standardization of procedures involving floral isolation, manual pollen transfer, and post-pollination monitoring contributes to increasing the precision and reproducibility of compatibility assessments. Therefore, the application of these methodologies represents an important strategy for supporting cacao breeding programs, optimizing orchard planning, and improving crop productivity under tropical production conditions.

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