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Sexual compatibility of two cacao genotypes (*Theobroma cacao L.*) in Santander, Colombia

Compatibilidad sexual de dos genotipos de cacao (*Theobroma cacao L.*) en Santander, Colombia

Verónica Coronado-Aleans [1]; Edinson Fernando Arguello Carreño[2]; Jaiver Enrique Morales Gómez [3]

[1.] *Instituto Universitario de la Paz, Colombia, veronica.coronado@unipaz.edu.co, https://orcid.org/0000-0002-0443-6640*
[2.] *Instituto Universitario de la Paz, Colombia, edinson.arguello@unipaz.edu.co, https://orcid.org/0009-0004-5988-5162*
[3.] *Instituto Universitario de la Paz, jaiver.morales@unipaz.edu.co, https://orcid.org/0009-0005-4840-8296*

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ABSTRACT

In the context of current research on sexual compatibility in cacao genotypes, previous studies have evaluated self-compatibility and intercompatibility in various materials, including genotypes FSV 1 and ICS 60. However, there remains a need to further characterize these reproductive interactions under specific local conditions, such as those found in the municipality of San Vicente de Chucurí (Santander, Colombia). The objective of this study was to evaluate the genetic compatibility of two cacao genotypes through assisted pollinations and to morphologically characterize the fruits resulting from directed pollinations involving the ICS 60 and FSV 1 genotypes. Field trials were conducted in San Vicente de Chucurí, Colombia, to assess variables associated with fertilization percentage, number of pollinated flowers, and number of unfertilized flowers. The results revealed significant differences in the sexual compatibility response of the FSV 1 and ICS 60 genotypes. FSV 1 showed 60% self-compatibility and 60% intercompatibility, both as a pollen donor and receptor. In contrast, ICS 60 exhibited a low self-compatibility percentage (11%) and 60% intercompatibility when acting as a pollen donor. In conclusion, these results indicate that both genotypes can be effectively used in polyclonal systems, as they perform well as pollen receptors in intercompatible crosses. This study provides valuable insights for the planning, management, and efficient establishment of these materials in cacao production and breeding programs.

Keywords: cacao; fertilization; flowers; genotypes; pollination; sexual compatibility

RESUMEN

En el contexto de las investigaciones actuales sobre compatibilidad sexual en genotipos de cacao, estudios previos han evaluado la autocompatibilidad y la intercompatibilidad en diversos materiales, incluidos los genotipos FSV 1 e ICS 60. Sin embargo, aún existe la necesidad de profundizar en la caracterización de estas interacciones reproductivas bajo condiciones locales específicas, como las presentes en el municipio de San Vicente de Chucurí (Santander, Colombia). El objetivo de este estudio fue evaluar la compatibilidad genética de dos genotipos de cacao mediante polinizaciones asistidas y caracterizar morfológicamente los frutos resultantes de polinizaciones dirigidas que involucraron a los genotipos ICS 60 y FSV 1. Los ensayos de campo se llevaron a cabo en San Vicente de Chucurí, Colombia, para evaluar variables asociadas con el porcentaje de fecundación, el número de flores polinizadas y el número de flores no fecundadas. Los resultados revelaron diferencias significativas en la respuesta de compatibilidad sexual de los genotipos FSV 1 e ICS 60. FSV 1 presentó un 60% de autocompatibilidad y un 60% de intercompatibilidad, tanto como donador como receptor de polen. En contraste, ICS 60 mostró un bajo porcentaje de autocompatibilidad (11%) y un 60% de intercompatibilidad cuando actuó como donador de polen. En conclusión, estos resultados indican que ambos genotipos pueden ser utilizados eficazmente en sistemas policlonales,

ya que presentan un buen desempeño como receptores de polen en cruzamientos intercompatibles. Este estudio aporta información valiosa para la planificación, el manejo y el establecimiento eficiente de estos materiales en programas de producción y mejoramiento de cacao.

Palabras clave: cacao; compatibilidad sexual; fecundación; flores; genotipos; polinización

INTRODUCTION

Cacao (Theobroma cacao L.) is a perennial species of the Malvaceae family, native to the Americas (Alverson et al., 1999; Motamayor et al., 2002; Arvelo Sánchez et al., 2017; Díaz-Valderrama et al., 2020). It holds significant economic importance in various countries worldwide, including Ivory Coast, Ghana, Cameroon, Indonesia, Venezuela, and Colombia. In 2019, global cacao production reached 4.7 million tons, with major contributions from Ivory Coast, Ghana, Indonesia, and Ecuador. African countries accounted for 77%, while the Americas and Asia/Oceania contributed 17% and 6%, respectively (International Cocoa Organization-ICCO, 2021).

In Colombia, cacao production in 2021 reached 69,040 tons, reflecting an 8.9% increase compared to 2020. Over the past 15 years, national production has surged 127%, rising from 30,357 to 69,040 t ha-1. In 2020, the preliminary planted area was 189 thousand hectares, with Santander (31.6%), Antioquia (8.7%), Nariño (8%), and Arauca (7.8%) being the primary contributors. The production in 2020 was approximately 63,000 tons, a 6% increase from 2019, concentrated in Santander with 27,000 tons (42%), followed by Antioquia with 5,600 tons (8.8%) and Arauca with 4,800 tons (7.6%) (FINAGRO, 2020).

Given the significant production of cacao in Colombia, particularly in Santander, where 34,000 hectares are dedicated to cacao cultivation, representing 30% of the country's total area, cacao farming is vital for the economy of 15,000 families, generating 2,600,000 labor days per year. San Vicente de Chucurí, known as the 'cacao Capital of Colombia,' stands out as a municipality with the highest cacao production in the department and is a leading national producer. Cacao constitutes 60% of the total agricultural production in the municipality, reaching 7,000 tons annually (Cámara de Comercio Barrancabermeja, 2017).

Currently, several factors limit the yield of cacao materials, attributed to genetic, ecological, physiological, pathological, and cultural aspects (Segovia, 2017). Among the genetic factors are barriers that hinder the normal fertilization process, commonly referred to as incompatibility. This issue is complex and reduces the crop's yield potential under field conditions (Quiroz Vera et al., 1992). Incompatibility refers to the physiological and morphological incapacity of plants, with normal pollen or ovules, to produce seeds after pollination (Poehlman, 1979).

Sexual reproduction in many flowering plants involves self-incompatibility, a mechanism which functional gametes are unable to achieve fertilization within the same plant. In contrast, cross-incompatibility occurs in different plants and acts as a genetic barrier that contributes to low production. In cacao, incompatibility can severely affect production. On a plantation, the number of healthy pods harvested is a critical factor. For a healthy pod to develop, cacao flowers must undergo successful pollination, and once fertilized (or set), they require favorable conditions to develop robustly and remain free from disease (Enríquez, 2004).

Pollen transfer in cacao cultivation is a delicate process, primarily due to the flower's physiological and genetic factors, which do not favor pollination. Natural

pollination has been identified as a limiting factor in many cases, and according to Soria (1971), it remains a subject of ongoing research in many cacao-producing countries, particularly in Tropical Continental America and Africa (Mindiola Véliz, 2017). An adult cacao plant, under normal conditions, can produce between 6,000 to 10,000 flowers per year, yet only about 0.01% of these develop into fruits (Hardy, 1961; Quiroz Vera et al., 1992; Suárez et al, 1994). In cacao, 80% of pollination occurs through entomophily, primarily facilitated by specific insect pollinators as the fly (Forcypomnia spp.), during the setting or fertilization period. Additionally, other genetic factors, such as the incompatibility of certain trees, contribute to low productivity in cacao plantations (Suárez et al., 2022).

Incompatibility remains a major constraint in cacao production. Understanding the compatibility relationship between genotypes is essential for ensuring efficient pollination, improved fruit set, and the achievement of maximum yield. This is particularly relevant in high-production areas such as San Vicente de Chucurí, where the selection of compatible genotypes can enhance both productivity and genetic diversity in plantations. Additional benefits of conducting compatibility studies alongside morphological characterization include obtaining valuable information that can support the development of improved planting materials through more efficiently breeding strategies (targeting disease resistance, quality improvement, and yield stability). Therefore, the objective of this study was to evaluate the genetic compatibility and the morphological characteristics of the fruit and seed in two cacao genotypes, ICS 60 and FSV 1, cultivated in the municipality of San Vicente de Chucurí, Colombia.

MATERIAL AND METHODS

Experimental Site

The study was conducted in the locality of La Pradera, on La Flor farm in the municipality of San Vicente de Chucurí. Situated in a Tropical Moist Forest (Bh-T) life zone, at a latitude of 6° 82'23" North and a longitude of 73° 43'118" West, at an altitude of 1025 meters above sea level. The average sampling temperature was 28°C, with an average relative humidity of 70% and annual precipitation ranging from 1,900 to 3,100mm (Instituto de Hidrología, Meteorología y Estudios Ambientales - IDEAM, 2022). The research was carried out between November and March 2021-2022, corresponding to the period of highest flowering activity for the studied materials.

Plant Material

Two cacao genotypes were used in the study, selected for their outstanding characteristics in terms of production, quality, and disease tolerance (Table 1).

Table 1. *Cacao plant material used in research*

Names	ICS 60	FSV 1
Origin	Trinidad	Federación Nacional de Cacaoteros de Colombia (FEDECACAO)
Type	Hybrid Trinitario × Criollo	Open Pollinated Indeterminate Generation
Production	1.076 kg/ha/year	1.600 kg/ha/year

Polanco-Díaz et al. (2020); Fedecacao (2015).

Experimental Design

As part of the experimental work, a completely randomized design (CRD) was implemented, consisting of six treatments. For each genotype, three trees were selected, and each material was replicated four times, resulting in a total of five experimental units (pollinated flowers) per replicate (20 pollinated flowers per treatment).

Statistical analyses were performed using the R program, version 3.5.2 (R-Core Team, 2021). Differences between treatments were assessed using a one-way ANOVA model, and mean comparisons were conducted by Tukey's honest significant difference test a at significance level of $p \leq 0.05$.

Experimental Sampling

Artificial Pollination in Cacao. Hybridizations were carried out through artificial pollination, following the methodology recommended by FEDECACAO (2015). Twenty pollinated flowers were used for each treatment (Table 2), resulting in 120 pollinated flowers for each genotype, for a total of 240 manually pollinated flowers. These pollinations were conducted between the second semester of 2022 and the first semester of 2023 to evaluate the self-compatibility and intercompatibility of the two cacao genotypes.

Table 2. *Treatments for the pollination process.*

N°	Description	Hybridization	
T1	Self-pollination same plant	ICS 60 F × ICS 60 M	FSV 1 F × FSV 1 M
T2	Self-pollination same plant	ICS 60 M × ICS 60 F	FSV 1 M × FSV 1 F
T3	Same genotype, different trees	ICS 60 M × ICS 60 F	FSV 1 M × FSV 1 F
T4	Same genotype, different trees	ICS 60 F × ICS 60 M	FSV 1 F × FSV 1 M
T5	Intercompatibility	ICS 60 F × FSV 1 M	FSV 1 F × ICS 60 M
T6	Intercompatibility	ICS 60 M × FSV 1 F	FSV 1 M × ICS 60 F

F: Female; M: Male

Pollinations were performed following the method described by Hardy (1961), by selecting closed and fully developed floral buds one day prior to pollination. These buds were encapsulated in transparent plastic tubes measuring 8 cm in length and 3 cm in diameter, which were attached to the tree and sealed with a ring of plasticine to prevent the entry of water and other external elements. To exclude pollinating insects and protect the flower, the distal end of each tube was covered with a layer of gauze and secured with a rubber band. Pollination was conducted the following morning, between 6:00 and 10:00 a.m. The plastic tube enveloping the flower was carefully removed, and the mother flower was emasculated, leaving the stigma exposed. The anthers from a male donor flower, previously detached from the tree, were rubbed onto the stigma of the emasculated female flower until the pollen grains adhered to the stigma, a process that took approximately 10 to 15 seconds. Subsequently, the pollinated flowers were again covered with the tubes and labeled to identify each cross. Fifteen days after pollination, the tubes were cautiously removed to avoid damaging fruit development. Pollination was considered successful when the presence of fruit was confirmed within this established period. Readings of fruit sets were then taken at three, nine, fifteen, and thirty days. The criterion established by Chavarro et al. (1982) was

considered, stating that "hybridizations are considered compatible when the fruit set percentage is equal to or higher than 30%; otherwise, they are determined as incompatible".

To determine compatibility and incompatibility, the criteria proposed by Chavarro et al. (1982) and Martínez et al. (2009) were employed. They assert that to define self-compatibility and inter-compatibility, the minimum threshold is 30%; in other words, a minimum of six flowers must be fertilized, as shown in the equation 1.

$$\%\ IR = \frac{Flowers\ successfully\ set\ in\ fruit}{Total\ number\ of\ flowers\ pollinated} \times 100 \qquad (1)$$

Morphological Characterization of Fruits Resulting from Directed Pollinations in genotypes ICS 60 and FSV 1. Morphological evaluations were conducted based on the descriptors developed by Restrepo & Urrego. (2018), which align with the guidelines established by the International Union for the Protection of New Varieties of Plants (UPOV, 2011) for DUS (Distinctness, Uniformity, and Stability) testing in Theobroma cacao L. Fourteen phenotypic variables were considered (Table 3), seven quantitative and seven qualitative, for both fruit and seed. For fruit characterization, five fruits were randomly collected from each genotype, and ten seeds were randomly taken from each fruit. This approach was similar to that reported by Guevara & Salazar (2015).

Table 3. *Morphological descriptors of cacao fruits and seeds.*

Variable	Description
Length of fruit (cm)	The linear distance between the ends of the fruit (from the apex to the base of the fruit) is measured.
Width of fruit (cm)	It is determined by the equatorial line of the pod.
Basal constriction	It is visually determined and classified according to the following scale: 0. Absent; 1. Slight; 2. Intermediate; 3. Strong
Shape of fruit apex	It is visually assessed; 1. Attenuated; 2. Toothed; 3. Acute; 4. Beaked; 5. Obtuse; 6. Rounded.
Color of ripe fruit	They must be fully physiologically mature and are classified according to the following scale: 1. Intense yellow; 2. Intense red; 3. Light yellow; 4. Intermediate yellow; 5. Intermediate red; 6. Orange-yellow; 7. Orange-red; 8. Light orange-yellow.
Color of unripe fruit	Fruits of 4 months of age are randomly selected. Colorations are related to the presence of anthocyanins in the fruits and are classified according to the following scale: 1. Intense green; 2. Light green; 3. Reddish green; 4. Intense violet; 5. Intermediate violet; 6. Light violet.
Fruit roughness	It is visually determined and classified according to the following scale: 0. Absent; 1. Slight; 2. Intermediate; 3. Strong.
Length of seed (mm)	It is measured from the embryo to the apex of the seed.

Variable	Description
Width of seed (cm)	It is measured on the flat part of the seed.
Shape in longitudinal section	A visual evaluation of the seed is performed and classified according to the following scale: 1. Oblong; 2. Elliptical; 3. Ovate; 4. Irregular.
Seed color	A visual evaluation of the seeds is performed and classified according to the following scale: 1. Creamy white; 2. Violet; 3. Purple.
Seed weight (g)	Using a scale, the mass of each pod is measured.

RESULTS

Genetic Compatibility of FSV 1 and ICS 60

Table 4 summarizes the significance of the mean squares of the treatments, mean values, standard deviation, coefficient of variation, and minimum and maximum values for the evaluated variables, which include fruit set days recorded at 3, 9, and 15 days after pollination for the FSV 1 and ICS 60 cacao genotypes. The ANOVA indicates the existence of significant differences ($p \leq 0.05$) or highly significant differences ($p \leq 0.01$) in the response of the FSV 1 and ICS-60 genotypes to the treatments, indicating variations in the fruit set days between each of the conducted crosses.

Table 4. *Mean squares, means, coefficients of variation, minimum and maximum values for fruit set days of FSV 1 and ICS 60 genotypes*

	FSV 1			ICS 60		
S. V	Three days	Nine days	Fifteen days	Three days	Nine days	Fifteen days
Treatment	84.3 ***	74.7***	57.6***	49.5**	21070**	279.9***
CV %	1.78	8.01	0.00	1.21	3.74	4.69
Error	3.06	2.50	2.00	2.05	2.30	2.31
Max	20.00	16.00	15.23	20.00	16.00	15.00
Mean	15.16	12.50	12.00	17.50	11.33	5.50
Min	11.00	8.00	8.00	13.00	5.00	1.00

S.V: source of variation; CV: coefficient of variation; * and **: significant for $p > 0.05$ and $p < 0.01$, respectively.

Table 5 presents the results of Tukey's test for the percentage of fertilized flowers at 3, 9, and 15 days after self- and inter-pollination of the two cacao genotypes. The treatments showed significant differences ($p \leq 0.05$).

The results at three days after pollination showed that both clones had high initial fruit set percentages. ICS 60 generally exhibited higher averages across treatments compared to FSV 1. For treatments 1, 2, and 3, ICS 60 consistently recorded high averages of 90.0%, while FSV 1 showed lower but still elevated values of 75.2%, 70.3%, and 55.0%, respectively. Notably, in treatments 4 and

5, FSV 1 exceeded ICS 60, reaching averages of 85.1% and 95.2%, respectively, compared to 65.1% and 95.1% in ICS 60. However, in treatment 6, ICS 60 had a lower fruit set (65.0%) than FSV 1 (70.3%), indicating greater early fruit set stability for FSV 1 in these treatments.

At 9 days after pollination, the fruit set percentages declined compared to 3 days in both genotypes, but FSV 1 consistently maintained higher averages than ICS 60 in most treatments. Specifically, in treatments 1 and 2, FSV 1 recorded 60.4% and 65.3%, outperforming ICS 60's 65.0% and 80.0%, where ICS 60 showed an unexpectedly higher average in T2. In treatments 3 and 4, FSV 1 exhibited markedly higher averages of 40.1% and 80.1%, compared to ICS 60's 25.2% and 25.2%, indicating better retention of fruit set. Similarly, for treatments 5 and 6, FSV 1 maintained averages of 75.3% and 55.1%, again exceeding ICS 60's 60.2% and 45.0%, reflecting FSV 1's greater capacity for sustained fruit set development up to 9 days.

Table 5. *Percentage of self-compatibility and genetic compatibility of two cacao genotypes*

Treatment	MATERIAL FSV 1 Average fruit set			Treatment	MATERIAL ICS 60 Average fruit set		
	3 days	9 days	15 days		3 days	9 days	15 days
T1	75.2 [b]	60.4	55.2 [b]	T1	90.0 [a]	65.0 [b]	5.0 [e]
T2	70.3 [b]	65.3 [b]	70.7 [a]	T2	90.0 [a]	80.0 [a]	25.4 [c]
T3	55.0 [c]	40.1 [c]	40.4 [c]	T3	90.0 [a]	25.2 [d]	10.2 [d]
T4	85.1 [a]	80.1 [a]	70.1 [a]	T4	90.3 [a]	65.1 [b]	5.0 [e]
T5	95.2 [a]	75.3 [a]	65.0 [a]	T5	95.1 [a]	60.2 [b]	50.2 [a]
T6	70.3 [b]	55.1 [b]	55.5 [b]	T6	65.0 [b]	45.0 [c]	45.0 [b]

(*) Equal letters in the same column indicate no statistically significant differences ($p \leq 0.05$).

At 15 days after pollination, fruit set was observed, with the FSV 1 genotype showing superior performance compared to the ICS 60 genotype under self-pollination conditions. FSV 1 achieved an average fruit set of 55.2% in treatment 1 and 70.7% in treatment 2, whereas ICS 60 showed significantly lower averages of 5.0% and 25.4% for the same treatments, respectively. In treatments 3 and 4, corresponding to pollinations between individuals of the same genotype (but from different trees), FSV 1 again outperformed ICS 60, with average fruit set values of 70.1% and 65.0%, respectively.

Regarding intercompatibility (treatments 5 and 6), FSV 1 also showed higher fruit set percentages, with means of 65.0% and 55.5%, while ICS 60 recorded lower values of 50.2% and 45.0%, respectively.

Table 6 presents the compatibility percentages observed among regional Theobroma cacao genotypes, evaluated in different crossing combinations, considering their behavior as female (pollen receptors) and male (pollen donors) parents. The results were classified according to the percentage of reproductive index (%RI), following the criteria proposed by Martínez et al. (2009).

Table 6. *Percentage of compatibility among regional cacao genotypes*

			Madre (♀)	
Padre (♂)	Genotypes		FSV	ICS
			1	60
	FSV	1	60%	60%
	ICS	60	60%	11%
	Intercompatible (≥ 30% and <70%)			
	self-compatibility (≥ 30%)			
	self-Incompatible (< 30%)			
	Intercompatible (<30%)			

Regarding self-pollination, the FSV 1 genotype, used as both female and male parent, exhibited a compatibility percentage of 60%, classifying it as self-compatible, i.e., capable of forming viable fruits from its own pollen (%RI ≥ 30). This behavior suggests that FSV 1 can efficiently reproduce without the need for external pollen, a desirable trait in conservation and breeding programs. In contrast, the ICS 60 genotype showed a low compatibility percentage when self-pollinated, with a value of 11%, classifying it as self-incompatible (%RI < 30). This result indicates a limited ability to reproduce via self-pollination, which could be a disadvantage in propagation systems where compatible genotypes are not guaranteed.

In interclonal crosses, the combination of FSV 1 as the female parent and ICS 60 as the male parent achieved 60% compatibility, classifying them as intercompatible. Similarly, when ICS 60 was used as the female parent and FSV 1 as the male parent, a 60% compatibility was also recorded. This reciprocity in compatibility between both genotypes suggests good reproductive affinity, which is advantageous for the generation of hybrids and the development of new genetic combinations in breeding programs. The fact that both crossing directions greatly exceed the 30% threshold reinforces the agronomic and reproductive viability of these combinations.

Overall, the results show that the FSV 1 material exhibits favorable reproductive behavior, displaying both self-compatibility and intercompatibility with the ICS 60 genotype. In contrast, the ICS 60 material exhibited clear self-incompatibility, although it maintained cross-compatibility with FSV 1. This information is relevant for planning genetic improvement strategies, as it allows the identification of combinations with a higher probability of success in obtaining vigorous and fertile progenies.

Description of Quantitative Variables

Table 7 summarizes the significance of the mean squares for genotype effects, along with the mean values, coefficients of variation (CV), and the minimum and maximum measurements for six agronomic variables evaluated in materials FSV-1 and ICS-60. The ANOVA revealed highly significant differences ($p \leq 0.01$) among genotypes for nearly all variables in both materials, highlighting the genetic variability present in these genotypes.

For FSV-1, significant differences were observed for fruit length (FL), fruit width (FW), almond length (LA), almond width (AW), and almond weight (ALW), indicating considerable variation in fruit and seed size within this material. The mean fruit length for FSV-1 was 20.16 cm, with a coefficient of variation of 7.87%, and values ranging from 0 to 24.5 cm. Fruit width averaged 9.7 cm with a CV of 7.63%. Almond weight showed particularly high variability (CV = 83.57%), ranging from 1.43 g to 30 g, reflecting substantial heterogeneity in seed development.

For ICS-60, significant differences were detected in fruit length (FL), fruit width (FW), almond width (AW), and almond weight (ALW), with fruit width showing especially strong differentiation among treatments ($F=125.60^{**}$). The average fruit length for ICS-60 was lower than for FSV-1, at 8.08 cm (CV=50.39%), with a range from 0 to 25.5 cm. Fruit width averaged 3.60 cm (CV = 41.98%), also indicating high variability. Almond thickness and weight in ICS-60 presented notable variation, with almond weight averaging 0.72 g (CV = 17.67%), but ranging from 0 to 1.78 g.

Overall, the high coefficients of variation observed in several variables, particularly in almond weight, reflect the influence of both genetic and pollination factors on the development of reproductive structures. The observed variability suggests opportunities for selecting superior individuals with desirable fruit and seed traits in breeding programs.

Fruit Characterization. The fruit length of material FSV 1 (Figure 1A) presented an average of 15.00 cm in the intercompatibility cross (FSV 1 Female × ICS 60 Male) and reached 23.44 cm in the self-compatibility treatment, indicating a clear reduction in fruit size when interclonal pollination occurred. Notably, the FSV 1 Male × ICS 60 Female cross did not yield a representative sample of fruits, limiting further evaluation in this combination. In contrast, material ICS 60 displayed a narrower range of fruit length, with averages of 10.5 cm for intercompatibility treatments (as pollen recipient) and 15.04 cm for self-compatibility (Figure 1B). These lengths are significantly lower than the values reported by Parco et al. (2021), who documented fruits reaching up to 30 cm in ICS 60 material cultivated in the Peruvian Amazon, suggesting a strong influence of genetic background and environmental conditions on fruit development.

Regarding fruit width, genotype FSV 1 again outperformed ICS 60, exhibiting average widths of 9.49 cm in self-pollinated fruits and 5.27 cm in interclonal crosses. Meanwhile, ICS 60 fruits showed average widths of 7.2 cm for self-compatibility and 5.2 cm for intercompatibility.

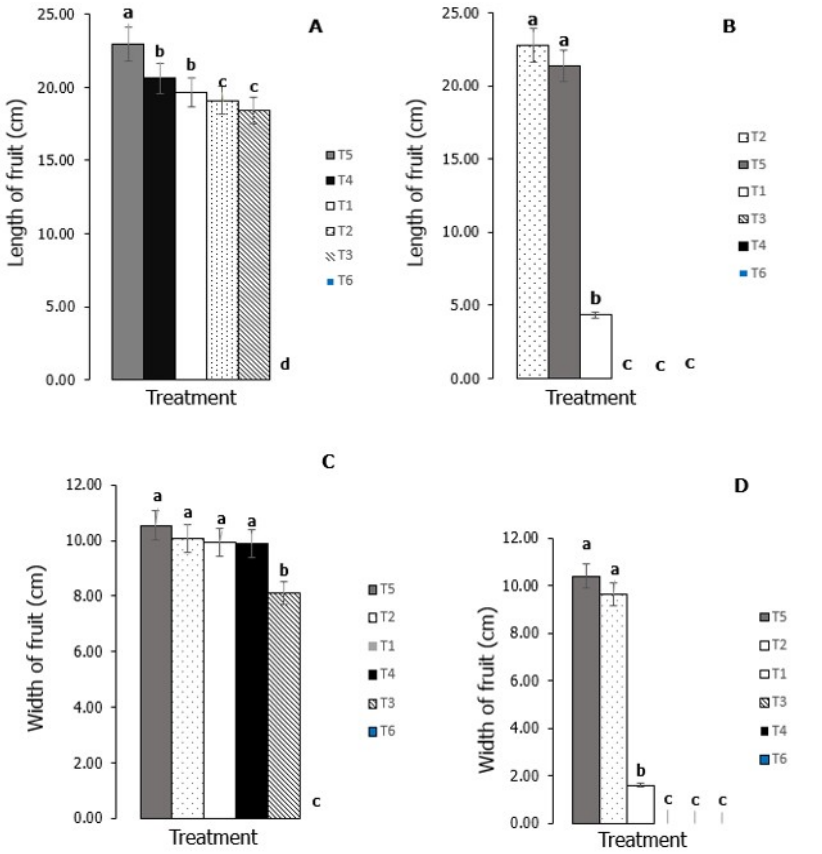


Figure 1. *Average of morphological descriptors of cacao fruit. A. Fruit length, FSV 1 genotype; B. Fruit length, ICS 60 genotype; C. Fruit width, FSV 1 clone; D. Fruit width, ICS 60 genotype.*

Table 7. *Mean squares, mean, standard deviation, coefficients of variation, and minimum and maximum values for six agronomic variables evaluated in two cacao materials in the Pradera locality of San Vicente de Chucurí, Santander, Colombia. Year 2024.*

	MATERIAL FSV-1						MATERIAL ICS-60				
S. V	LF (cm)	FW (cm)	LA (mm)	AW (mm)	AT (mm)	ALW (g)	LF (cm)	FW (cm)	LA (mm)	AW (mm)	ALW (mm)
Genotype	351.5**	81.88***	13.76***	3.98***	1.15**	78.97*	602.7**	125.60**	18.51**	6.503^{ns}	1.86**
Error	1.8	0.38	0.059	0.025	0.029	0.288	16.9	2.29	0.015	0.010	0.004
Mean	20.16	9.7	3,039	1.24	0.82	20.69	8.08	3.60	1.23	0.72	0.39
CV %	7.87	7.63	14.90	12.74	24.66	83.874	50.89	41.98	9.77	13.53	17.64
Min	0.00	0.00	1.00	0.00	0.00	1.43	0.00	0.00	0.00	0.00	0.00
Max	24.5	11.5	3.70	1.9	1.5	30.00	25.5	11.6	2.9	1.78	1.12

S. V: source of variation; CV: coefficient of variation; LF: fruit length; FW: fruit width; LA: almond length; AW: almond width; AT: almond thickness; PA: almond weight; ns: not significant; * and **: significant for $p > 0.05$ and $p < 0.01$, respectively.

Seed characterization. As shown in Table 6, the average almond length was 3.03 mm, with mean values of 2.89 mm for self-pollination treatments and 1.31 mm for interclonal pollinations. The ICS 60 genotype exhibited the narrowest variation, ranging from 1.60 mm to 1.30 mm (Figure 2B), indicating limited variability in this trait under the evaluated conditions.

For almond width, the FSV 1 material outperformed ICS 60, particularly under self-pollination, with an average value of 1.47 mm, compared to 1.20 mm in ICS 60 (Figure 2A). Interestingly, under interclonal crosses, ICS 60 showed a slightly higher average (1.30 mm) than FSV 1 (1.25 mm); however, this difference

was not statistically significant, suggesting that the observed variation may be due to environmental or experimental factors rather than genetic superiority.

Regarding almond thickness, the FSV 1 genotype also demonstrated superior performance, with an overall average of 0.82 mm, including 0.65 mm for self-pollination and 0.44 mm for interclonal treatments. In contrast, ICS 60 presented lower average values of 0.53 mm and 0.37 mm under the same conditions, respectively (Figure 2E and 2F). These results highlight the genotypic differences in seed traits and suggest that FSV 1 may have greater potential for producing larger and more vigorous seeds under both reproductive strategies.

The almond weight (ALW) showed a markedly higher average in the FSV-1 material (20.69 g) compared to the ICS-60 genotype (1.23 g). The coefficient of variation was also greater in FSV-1 (83.874%), reflecting higher dispersion in this material, while ICS-60 presented a lower variation (17.64%).

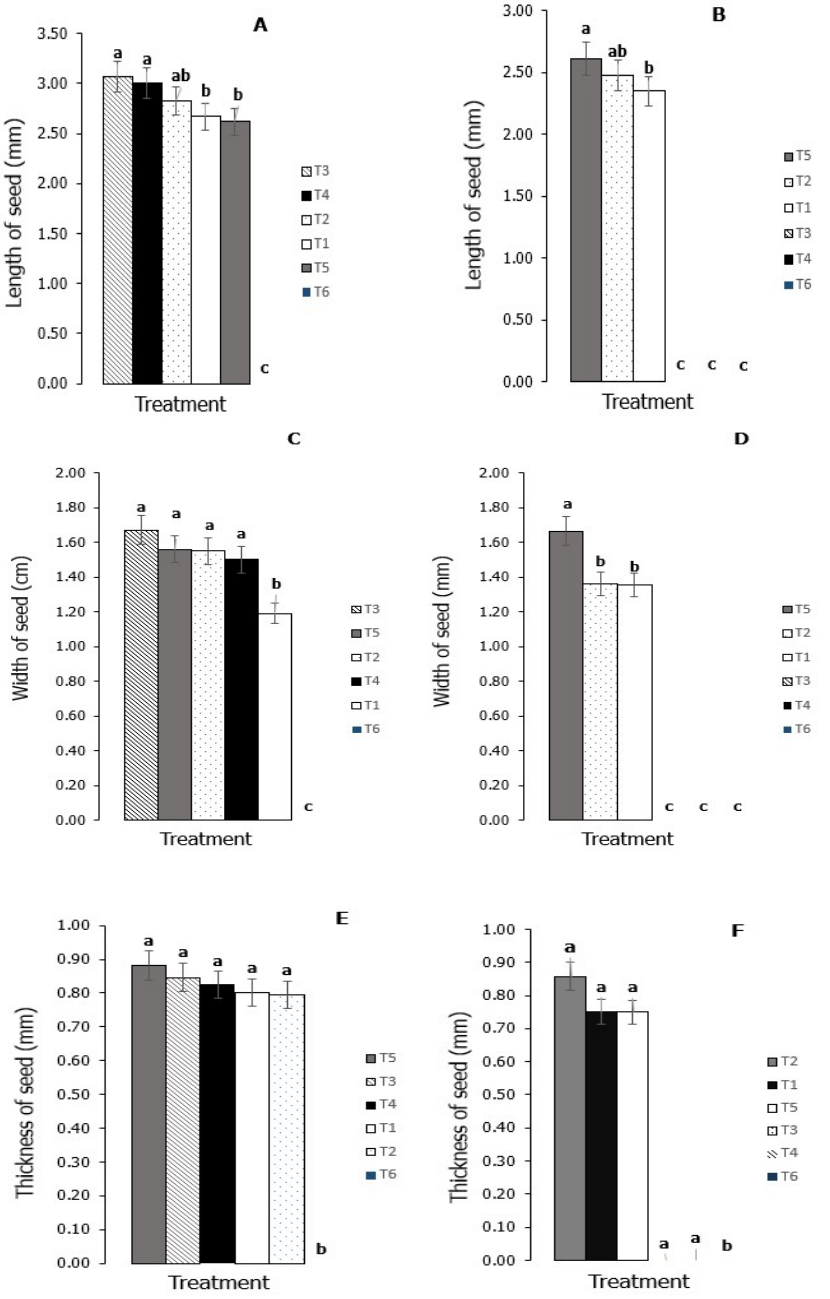


Figure 2. *Average of morphological descriptors of cacao fruit.* ***A****. Almond length, FSV 1 genotype;* ***B****. Almond length, ICS 60 genotype;* ***C****. Almond width, FSV 1 genotype;* ***D****. Almond width, ICS 60 genotype;* ***E****. Almond thickness, FSV 1 genotype;* ***F****. Almond thickness, ICS 60 genotype.*

Description of Qualitative Variables

Fruit Characterization. Regarding the self-compatibility treatments of the FSV 1 material, it predominantly exhibited an ovoid shape, intermediate roughness, intense red color (mature), and intense violet color (immature), with intermediate basal constriction and attenuated apex. In intercompatibility treatments, the fruit shape differed, with 60% being ovoid and 40% being oval; also, the apex shape always remained attenuated. ICS 60 showed uniformity in all treatments, with 70% of fruits being ovoid, intermediate basal constriction, attenuated apex, intense yellow color (mature), and intense green color (immature), with intermediate roughness. The results obtained are similar to those reported by Parco et al. (2021), who obtained immature fruit color as green and intermediate roughness.

Seed Characterization. In seeds, both genotypes exhibited high variability in shape, with ovate, oblong, and elliptical shapes being the most frequent. The cotyledon color was mainly purple or violet. FSV 1 was characterized by ovate seeds with an average of 70%, and they were predominantly purple or violet. In contrast, Parco et al. (2021) reported that ICS 60 had elliptical-shaped seeds, which differ from the findings in this study, where ovate (65%) or oblong (35%) seeds were obtained, but mostly with a purple color. Differences were found between genotypes for the evaluated variables. Fruit and seed analyses allowed for a comprehensive characterization of physical attributes and their variability.

DISCUSSION

Genetic Compatibility of FSV 1 and ICS 60

The results indicate that the FSV 1 genotype exhibits greater sexual compatibility than ICS 60 in both self- and cross-pollination scenarios. The higher fruit set in FSV 1, especially under self-pollination conditions, suggests a higher degree of self-compatibility, as also supported by Efombagn et al. (2009), who obtained pollination percentages of 38% to 43% in self-compatible cacao trees. This observation aligns with findings reported by Bekele et al. (2006), who noted that certain cacao genotypes possess partial or full self-fertilization capacity due to variations in the expression of self-incompatibility alleles.

Regarding intercompatibility, the moderate fruit set in ICS 60 (45.0%–50.2%) suggests partial compatibility with FSV 1, possibly influenced by allele diversity or physiological factors at the stigma–pollen interface (Dostert et al., 2011). In comparison, FSV 1 showed superior performance as both pollen donor and receptor, with consistent fruit set above 55%, supporting its potential use as a compatible genotype in controlled hybridizations or polyclonal systems (Guevara & Salazar, 2015). The results obtained in the present study regarding the genetic compatibility between genotypes FSV and ICS 60 are consistent with those reported by Suárez et al. (2022), who found flower set percentages of 70% for the cross ICS 60 × FSV and 65% for the cross FSV × ICS 60, values that indicate intercompatibility according to the classification proposed by Fedecacao (2015). This high level of fruit set in both directions of the cross suggests that there is no marked asymmetry of unilateral compatibility between the evaluated materials, which increases their potential for breeding programs aimed at generating hybrids with superior traits. On the other hand, the intercompatibility observed between FSV 1 and ICS 60, in both crossing directions, demonstrates reciprocal

sexual affinity, with values of 60% in both cases. This result is especially relevant for genetic improvement programs, as it indicates that these combinations have high potential to generate viable, fertile offspring with broad genetic diversity. As indicated by Aikpokpodion (2012), the success of controlled crosses in cacao largely depends on the sexual compatibility between parental genotypes, which ensures the efficiency of producing useful segregating progenies.

Díaz Tellez & Urbina Espino (2015) reported results similar to those obtained in our study, in which all crosses were intercompatible, with successful flower set ranging from 55% to 91%. However, N'Zi et al. (2017), in cross-pollinations, achieved a maximum of 24% successful flower set, which could be attributed to the use of materials with incompatibility genotypes different from those evaluated in our research, as well as to their distinct geographical origin and evolutionary stage. Mazeira Herrera (2013) also emphasized the importance of evaluating compatibility at different time intervals after pollination, highlighting that early assessments may overestimate fertilization rates due to delayed abscission of unfertilized flowers. This supports the decision to consider the 15-day post-pollination data as more reliable for determining true compatibility responses in both genotypes.

These findings are significant for breeding programs, as selecting genotypes with higher and more stable compatibility levels is crucial for maximizing fruit set and genetic gain. Furthermore, the variability observed underscores the importance of evaluating compatibility under local agroecological conditions, as environmental interactions can influence fertilization outcomes (Lanaud et al., 1995; Bekele et al., 2006).

Future research should incorporate molecular tools to characterize S-alleles and their expression in these genotypes, facilitating the prediction of compatibility patterns and improving cross-design efficiency in cacao breeding.

The results obtained reveal clear differences in the reproductive behavior of the evaluated cacao genotypes, particularly regarding sexual compatibility. The self-compatibility observed in the FSV 1 genotype, with a reproductive index of 60% under self-pollination, suggests the presence of favorable genetic mechanisms for the formation of viable fruits without the need for external pollen. This trait is especially valuable in production systems where natural pollination conditions may be limiting (Mazeira Herrera, 2013).

In contrast, genotype ICS 60 exhibited self-incompatible behavior, with only an 11% reproductive index. This response may be associated with gametophytic or sporophytic self-incompatibility mechanisms, particularly late-acting self-incompatibility (LSI), which are well-documented in Theobroma cacao L. These mechanisms regulate the failure of the pollen tube or gamete fusion when the pollen is genetically similar and are controlled by multiple loci (Ford & Wilkinson, 2012; Boza et al., 2014; Lanaud et al., 2017). According to Ruales Mora et al. (2011), the behavior of ICS 60 material was inconsistent, presenting lower yield results, affirming that this reaction is possibly linked to the self-incompatibility of the material. This finding is consistent with what was reported by Martínez et al. (2009), who noted that many criollo or improved genotypes exhibit high levels of self-incompatibility, which can negatively affect productivity in homogeneous monocultures if cross-pollination systems are not properly planned. Suárez et al. (2022) reported that the ICS 60 genotype exhibited 0% fruit set under self-pollination, classifying it as completely self-incompatible. In the present study, although a slightly higher reproductive index (11%) was obtained, this value remains well below the 30% threshold established to classify a genotype as self-

compatible (FEDECACAO, 2015).

Therefore, the classification of ICS 60 as self-incompatible is confirmed. The slight discrepancy between both studies could be attributed to variations in environmental conditions, differences in floral handling techniques during manual pollination, or intrinsic physiological factors of the evaluated material.

Additionally, the presence of intercompatible and self-compatible genotypes such as FSV 1 can be strategically leveraged in clonal propagation programs and the establishment of seed gardens. According to Mazeira Herrera (2013), in agro-industrial systems where genetic uniformity is desired, it is ideal to use clones that ensure high fertilization levels, either through self-pollination or planned crossing. Likewise, Guevara & Salazar (2015) emphasize that the selection of genotypes with good sexual compatibility and reproductive performance should be considered a priority when establishing germplasm banks and clonal gardens.

In this context, the results suggest that the FSV 1 genotypes represent a promising genetic resource, both for its capacity for autonomous reproduction and for its favorable response in crosses with other materials such as ICS 60. Conversely, although ICS 60 is self-incompatible, it can still be included in controlled crosses, as long as it is combined with intercompatible genotypes, thus optimizing its use in participatory breeding schemes and recurrent selection.

Moreover, environmental factors—particularly temperature, humidity, and rainfall patterns—can significantly influence pollination success and fruit set in cacao, thereby affecting the expression of compatibility responses. For instance, excessive rainfall can hinder pollinator activity and reduce effective pollen transfer, while high humidity may affect pollen viability or stigma receptivity (Daymond & Hadley, 2004; Bekele et al., 2006). Temperature fluctuations can also impact pollen tube growth and fertilization success, potentially masking or exaggerating the intrinsic compatibility of a given genotype. Therefore, the observed differences between FSV 1 and ICS 60 may not solely reflect genetic factors, but also the interaction with prevailing microclimatic conditions during the pollination period. This highlights the importance of evaluating compatibility responses under local agroecological conditions to ensure accurate selection of parent genotypes for breeding programs.

Description of Quantitative Variables

The observed differences in fruit morphology among treatments and genotypes are closely related to the reproductive behavior of the cacao genotypes. In self-compatible genotypes such as FSV 1, fruit set and development were more efficient under self-pollination, resulting in greater fruit length and width, as also reported by Guevara & Salazar (2015), who found that reproductive success in cacao is strongly influenced by compatibility type

Moreover, the notably smaller fruit sizes obtained in this study compared to those documented by Parco et al. (2021) for ICS 60 in the Peruvian Amazon point to the strong impact of environmental conditions, including temperature, rainfall, and soil fertility, on fruit development. Daymond & Hadley (2004) demonstrated that high humidity and optimal temperatures favor successful pollination and pod filling, while adverse conditions, such as drought or excessive rainfall, can reduce pod size and seed weight due to impaired floral biology or increased disease pressure.

In addition, the differences in fruit length and width between FSV 1 and ICS 60 genotypes highlight the influence of genetic variability and environmental modulation on pod morphology. The self-compatible FSV 1 consistently produced

larger fruits, suggesting superior reproductive efficiency and potential agronomic advantages. In contrast, the lower values observed in the ICS 60 genotypes under intercompatibility conditions may be related to incompatibility mechanisms or environmental stress factors affecting fruit development.

These findings align with those reported by Bidot Martínez et al. (2017), who documented substantial morphological diversity in traditional Theobroma cacao. plants in Cuba. Their study emphasized that traits such as fruit length, width, and shape are highly variable even within traditional varieties and are influenced by both genetic background and environmental conditions.

The absence of representative fruits in the FSV 1 Male × ICS 60 Female cross could result from pollen-pistil incompatibility or unsuccessful fertilization, a phenomenon observed in other cacao studies where specific interclonal crosses yielded low fruit set. This highlights the importance of selecting compatible parental genotypes to maximize productivity in breeding programs.

Collectively, these results underline the need for multi-environment testing of cacao clones to identify genotypes with stable and superior performance across diverse climatic conditions.

Almond weight constitutes a critical trait for cacao quality and industrial yield, as it directly determines the amount of dry cacao mass obtained per pod. In the present study, the FSV-1 genotype exhibited a mean almond weight of 20.69 g, markedly surpassing the ICS-60 genotype, which recorded an average of only 1.23 g. The pronounced differences observed in fruit and seed traits between genotypes FSV 1 and ICS 60 highlight the role of genetic control as well as the reproductive dynamics modulated by pollination mechanisms. Comparable observations were reported by Doaré et al. (2020), who demonstrated that in cacao, traits such as bean weight exhibit high heritability but are also significantly influenced by environmental factors, underscoring the importance of genotype × environment interactions in phenotypic expression.

CONCLUSIONS

In this study, significant differences in sexual compatibility between cacao genotypes FSV 1 and ICS 60 were recorded. It has been established that genotype FSV 1 is both self-compatible and intercompatible with a reproductive index of 60%, hence coming out as a potential production system and breeding program contributor. On the contrary, ICS 60 expressed self-incompatibility since it was only 11% compatible. However, it expressed intercompatibility with FSV 1, which indicated its strategic use in targeted hybridizations. Also, the shape study showed that FSV 1 did better than ICS 60 in most countable features like fruit length and width, seed length and thickness, and weight. For look features, FSV 1 gave round fruits with a strong red color when ripe, while ICS 60 gave round fruits with a strong yellow color. Therefore, it is recommended that future research work involve the study of sexual compatibility on a broader scale of genotypes, which includes genotypes with higher sources of resistance for diseases like Moniliophthora roreri (frosty pod rot of cacao). It also recommends carrying out compatibility analysis by identifying S-alleles to better understand the genetic mechanism and, hence, make parental selections more precise in the breeding programs of Theobroma cacao.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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