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Self-Incompatibility and Pollination Efficiency in *Coffea canephora* Using Fluorescence Microscopy

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Abstract: In nature, the ability to avoid self-fertilization has evolved to prevent the deleterious effects of inbreeding. However, under cultivation conditions, self-incompatibility can reduce the pollination efficiency of *Coffea canephora*. The objective of this study was to characterize the self-incompatibility expression of the most cultivated genotypes in Western Amazonia, to improve the management of this coffee plant. In vitro pollinations were conducted among 45 genotypes, and the development of pollen tubes was evaluated using fluorescence microscopy. Pollination efficiency was evaluated considering the allelic variability within a breeding population from an ideal condition of maximum genetic variability. Based on the compatibility response, the genotypes were organized into six groups: group I (24.4%), group II (31.1%), group III (24.4%), group IV (2.2%), group V (2.2%), and group VI (15.6%). The lower frequencies of groups IV, V, and VI were associated with the lower frequency of the rarest allelic forms in this breeding population (p = 0.36, q = 0.26, r = 0.29, and s = 0.10). The correspondence between allelic and genotypic frequencies indicates that this population is in Hardy–Weinberg equilibrium (HWE) for this trait. Considering the cultivation of 2 to 10 clones, the population studied showed intermediate pollination efficiency between an ideal HWE population with p = q = r = s = 0.25 and a population with the rarest allelic forms (p = 0.48, q = 0.32, r = 0.19, s = 0.01). Efficiency estimates were stabilized from the cultivation of five clones, indicating that cultivating a minimum number of clones should be considered. Theoretically, maximum pollination efficiency is achieved by representing all alleles in equal proportions, whereas in practice, farmers should ensure the cultivation of plants from different compatibility groups, without significant imbalances.

Keywords: conilon; robusta; canephora coffee; compatibility

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1. Introduction

Coffee cultivation is a highly significant agricultural activity in tropical regions worldwide, providing employment and income for millions of people [1]. Of the 130 known coffee species [2], only two are commercially cultivated and represent approximately 99% of global production. The species *Coffea arabica* L. accounts for the majority of production (56%), while *Coffea canephora* L. Pierre ex A. Froehner makes up the remaining 44%; both species are vulnerable to climate change [3,4]. Coffee cultivation not only sustains local economies but also plays a crucial role in the exports of various countries. *C. canephora* stands out for its adaptation to tropical regions, significant genetic diversity, and high production potential [5–7].

The advancement in coffee production in Western Amazonia can be understood through the progress of this activity in its main production region. For the 2024 harvest in the state of Rondônia, 3.1 million bags are expected, with an average yield of 51 bags per hectare, produced in an area 80% smaller than in 2001 [8].

Coffee cultivation in this region is characterized by the growth of hybrid plants, featuring traits from the Conilon and Robusta botanical varieties [9]. The Conilon variety, originating from regions close to sea level on the African continent, is known for its smaller size, higher susceptibility to pests and diseases, and greater tolerance to water scarcity [10,11]. In contrast, the Robusta variety, also from equatorial forest regions in Africa, is characterized by its larger size, lower tolerance to water deficits, and higher resistance to pests and diseases. This coffee plantation originated in the 1980s, when seeds of the Conilon botanical variety were introduced by the farmers themselves, and seeds of the Robusta botanical variety were distributed by Embrapa [12].

From this genetic base, the farmers themselves initiated a selection process that led to the widely cultivated clones [13]. Although these genetic materials are intensively cultivated, many aspects, such as plant compatibility, remain unknown. Understood as a physiological mechanism that prevents self-pollination, *C. canephora* is characterized by gametophytic self-incompatibility, where the interaction between the pistil tissues and pollen grains occurs, and they must not share the same allele. In this species, self-incompatibility is determined by a single gene with multiple alleles (S gene) [11].

The incompatibility of *C. canephora* was originally reported in the 1960s, when Devreux et al. (1959) observed the cessation of pollen tube growth [14]. Subsequently, Conagin and Mendes (1961) and Berthaud (1980) found evidence that self-incompatibility is controlled by the action of a single multiallelic gene [15,16]. Lashermes et al. (1996) identified molecular markers associated with this trait through the development of double-haploid populations, demonstrating that pollen tube development can be observed using fluorescence microscopy [17]. Nowak et al. (2011) characterized the polymorphism of this gene in other *Coffea* species [18]. De Franceschi et al. (2012) showed that incompatibility is due to the interaction of specific glycoproteins in the stigma of the flower and pollen grains [19]. Asquini et al. (2011) and Vieira et al. (2021) observed that the cessation of pollen tube growth is mediated by the action of ribonucleases, which degrade ribosomal RNA [20,21]. More recently, Moraes et al. (2018) identified tester plants for three compatibility groups [22]. Souza et al. (2021) detailed in vitro pollination methods associated with fluorescence microscopy for compatibility diagnosis [23]. Depolo et al. (2022) expanded the known genetic variability by identifying tester plants for new compatibility groups [24].

Surveys in the field show that the genotypes GJ8 and GJ25 are present in 89% of the plantations, while the clone GJ3 is in approximately 80% of the properties in Western Amazonia. Following this, the clone P50 is cultivated in 64% of the properties, and the genotypes GJ5, SK80, and SK41 are present in 41%, 36%, and 29% of the properties, respectively [13]. More recently, the intermediate-cycle genotypes R22, LB10, and AS2, along with the early-cycle clone LB15, have been joining this group of genotypes selected by the coffee growers themselves and intensively cultivated. In addition to the genetic materials selected by farmers, there are cultivars adapted to the conditions of Western Amazonia, which differ due to their hybrid traits and the individualized characterization of each genotype [6,9]. These previously characterized cultivars were used as tester plants in compatibility diagnosis.

Higher occurrences of peaberry beans and lower yields are associated with lower pollination efficiency. In this species, the natural occurrence of peaberry beans happens due to the pollination of only one of the two ovary chambers, resulting in fertilized beans with a rounded shape [25,26]. In clonal coffee farming, planting incompatible genotypes can compromise both productivity and grain quality due to lower pollination efficiency and an increased incidence of peaberry beans [27].

The objective of this study was to characterize the self-incompatibility expression of the most cultivated genotypes in Western Amazonia using in vitro pollination methods and fluorescence microscopy, aiming towards the improved cultivation of this coffee plant.

2. Material and Methods

2.1. Tester Plants and Breeding Population

The breeding population consisted of forty-two genotypes cultivated in the public domain in the Western Amazon, along with three accessions from the Embrapa Germplasm Bank (Table 1). Genotypes BRS 1216, BRS 2299, and BRS 3193 were used as tester plants for compatibility groups I, II, and III, respectively, while genotypes R1, R309, and R160 served as tester plants for compatibility groups IV, V, and VI, respectively [24].

Table 1. List of studied genotypes in relation to their compatibility, sourced from the Embrapa Germplasm Bank and clones cultivated in the public domain in Western Amazonia, Brazil.

n	Genotype	Origin	n	Genotype	Origin
1	AR106	Aldinei Raasch [7]	24	LB10	Laerte Braun [5]
2	AS1	Ademar Schmidt [2]	25	LB12	Laerte Braun [5]
3	AS10	Ademar Schmidt [2]	26	LB15	Laerte Braun [5]
4	AS12	Ademar Schmidt [2]	27	LB160	Laerte Braun [5]
5	AS2	Ademar Schmidt [2]	28	LB20	Laerte Braun [5]
6	AS5	Ademar Schmidt [2]	29	LB33	Laerte Braun [5]
7	AS7	Ademar Schmidt [2]	30	LB68	Laerte Braun [5]
8	BAG24	Embrapa [1]	31	LB80	Laerte Braun [5]
9	BAG27	Embrapa [1]	32	LB88	Laerte Braun [5]
10	BAG28	Embrapa [1]	33	N13	Nivaldo Ferreira [6]
11	BG180	Adilson Berger [3]	34	N16	Nivaldo Ferreira [6]
12	CA1	Carlos Alves Silva [4]	35	N32	Nivaldo Ferreira [6]
13	GB1	Gilberto Boon [2]	36	N7	Nivaldo Ferreira [6]
14	GB4	Gilberto Boon [2]	37	N8[G8]	Nivaldo Ferreira [6]
15	GB7	Gilberto Boon [2]	38	P50	Valdecir Piske [2]
16	GJ2	Geraldo Jacomini [5]	39	R152	Ronaldo G Oliveira [2]
17	GJ20	Geraldo Jacomini [5]	40	R22	Ronaldo Vitoriano [2]
18	GJ25	Geraldo Jacomini [5]	41	SK244	Sergio Kalk [6]
19	GJ3	Geraldo Jacomini [5]	42	SK41	Sergio Kalk [6]
20	GJ30	Geraldo Jacomini [5]	43	SK80	Sergio Kalk [6]
21	GJ5	Geraldo Jacomini [5]	44	VP156	Valdecir Piske [2]
22	GJ8	Geraldo Jacomini [5]	45	WP6	Wanderley Peter [6]
23	L1	Alcides Rosa [3]			

[1] Ouro Preto do Oeste—RO, [2] Alta Floresta do Oeste—RO, [3] Rolim de Moura—RO, [4] Novo Horizonte do Oeste—RO, [5] Nova Brasilândia do Oeste—RO, [6] Cacoal—RO, [7] São Miguel do Guaporé—RO.

2.2. In Vitro Pollination

In vitro pollination involves the laboratory transfer of pollen grains from donor plants to the stigmata of recipient plants. At the Embrapa Rondônia experimental field in Porto Velho, RO, hybridization plots were monitored daily from January 2022 to December 2023. During this period, in vitro pollination procedures were conducted on six different occasions: 17 June 2022, 21 July 2022, and 25 August 2022, 12 June 2023, 28 July 2023, and 22 August 2023, totaling 335 diagnoses (Table 2).

Pollen grain viability tests were performed in a 10% sucrose solution by immersing the anthers in 5.0 mL of 10% sucrose solution in Eppendorf tubes. Pollen grain germination was evaluated after 60 min in a Petri dish using a stereoscopic microscope (50×), with the germination rate estimated from the average of three counts. Pollen grains with a germination rate higher than 60% were considered viable for pollination.

Table 2. Diagnosis of pollen tube development conducted among the 45 most cultivated clones in the Western Amazon, evaluated in the municipality of Porto Velho, Rondônia, Brazil. Each observation refers to the diagnosis of a slide with 10 stigmata, totaling 335 slides evaluated.

n	Genotype	C	NC	FN	Group	p(FN)	n	Genotype	C	NC	FN	Group	p(FN)
1	AR106	5	1	2	III	0.05	24	LB10	5	2	4	III	<0.01
2	AS1	5	2	1	VI	<0.01	25	LB12	5	1	3	II	0.05
3	AS10	5	1	4	VI	0.05	26	LB15	5	1	0	III	0.05
4	AS12	5	1	0	VI	0.05	27	LB160	5	1	4	I	0.05
5	AS2	5	1	0	III	0.05	28	LB20	5	1	2	II	0.05
6	AS5	5	1	1	II	0.05	29	LB33	5	1	1	III	0.05
7	AS7	5	1	1	II	0.05	30	LB68	5	1	2	II	0.05
8	BAG24	5	1	2	II	0.05	31	LB80	5	2	0	II	<0.01
9	BAG27	5	1	1	I	0.05	32	LB88	5	1	0	III	0.05
10	BAG28	5	1	0	III	0.05	33	N13	5	1	1	III	0.05
11	BG180	5	1	0	VI	0.05	34	N16	5	1	0	VI	0.05
12	CA1	5	3	1	VI	<0.01	35	N32	5	3	0	I	<0.01
13	GB1	5	1	1	I	0.05	36	N7	5	2	1	I	<0.01
14	GB4	5	1	2	II	0.05	37	N8[G8]	5	1	0	II	0.05
15	GB7	5	1	3	I	0.05	38	P50	5	1	1	II	0.05
16	GJ2	5	2	1	I	<0.01	39	R152	5	1	1	III	0.05
17	GJ20	5	1	0	III	0.05	40	R22	5	2	0	IV	<0.01
18	GJ25	5	1	0	II	0.05	41	SK244	5	1	0	I	0.05
19	GJ3	5	1	1	V	0.05	42	SK41	5	1	2	II	0.05
20	GJ30	5	2	0	I	<0.01	43	SK80	5	1	3	II	0.05
21	GJ5	5	1	1	II	0.05	44	VP156	5	1	1	VI	0.05
22	GJ8	5	1	2	I	0.05	45	WP6	5	2	2	I	<0.01
23	L1	5	1	1	III	0.05							

Note—n: ordinal numbering, C: compatible, NC: not compatible, FN: false negative, Group: compatibility groups identified by Roman numerals, I, II, III, IV, V, VI; p(FN): false negative probability considering a 5% error rate in each diagnosis.

Inflorescences were collected in gearbox-type containers the day before anthesis and transported to the Embrapa Rondônia Tissue Culture Laboratory. Anthers and petals were removed from receptive inflorescences, with their peduncle immersed in the culture medium (water, 30% sucrose, 6% bacteriological agar). Inflorescences from donor plants with closed buds had their peduncle immersed in the same culture medium. Finally, all containers were sealed with plastic film and kept in a growth chamber at 26 ± 1 °C with a photoperiod of 16 h (50 $\mu mol.m^{-2}.s^{-1}$).

On the day of anthesis, pollen grains were collected from donor flowers by scraping the dehiscent anthers with a scalpel. Approximately 18 anthers were scraped, and then the scalpel blade with pollen grains was gently rubbed onto one of the tips of the bifid stigma, allowing the pollen grains to adhere to the stigma.

2.3. Fluorescence Microscopy

The compatibility diagnosis was carried out by observing the pollen tubes in the pistils of compatible plants. Thirty-six h after pollination, the stigmata were stored at 5 °C in 10 mL penicillin vials containing FAA solution (10% formaldehyde, 10% glacial acetic acid, and 80% ethanol).

For slide preparation, the pistils were removed from the FAA, washed with distilled water, and immersed in 1N sodium hydroxide (NaOH) for 2 h. After this period, the stigmata were washed again with distilled water and stained for 12 h using 1% aniline blue dye, prepared in a 0.1 M K_2HPO_4 solution.

After the softening and staining steps, the coverslip was gently pressed onto ten pistils arranged on a slide. Visualization was performed using a Leica DM2500 microscope model (Wetzlar, Germany) equipped with a photodocumentation system. The visualization was

conducted at magnifications of 100 and 200 times, counting the number of pistils that showed developed pollen tubes (Figures 1 and 2).

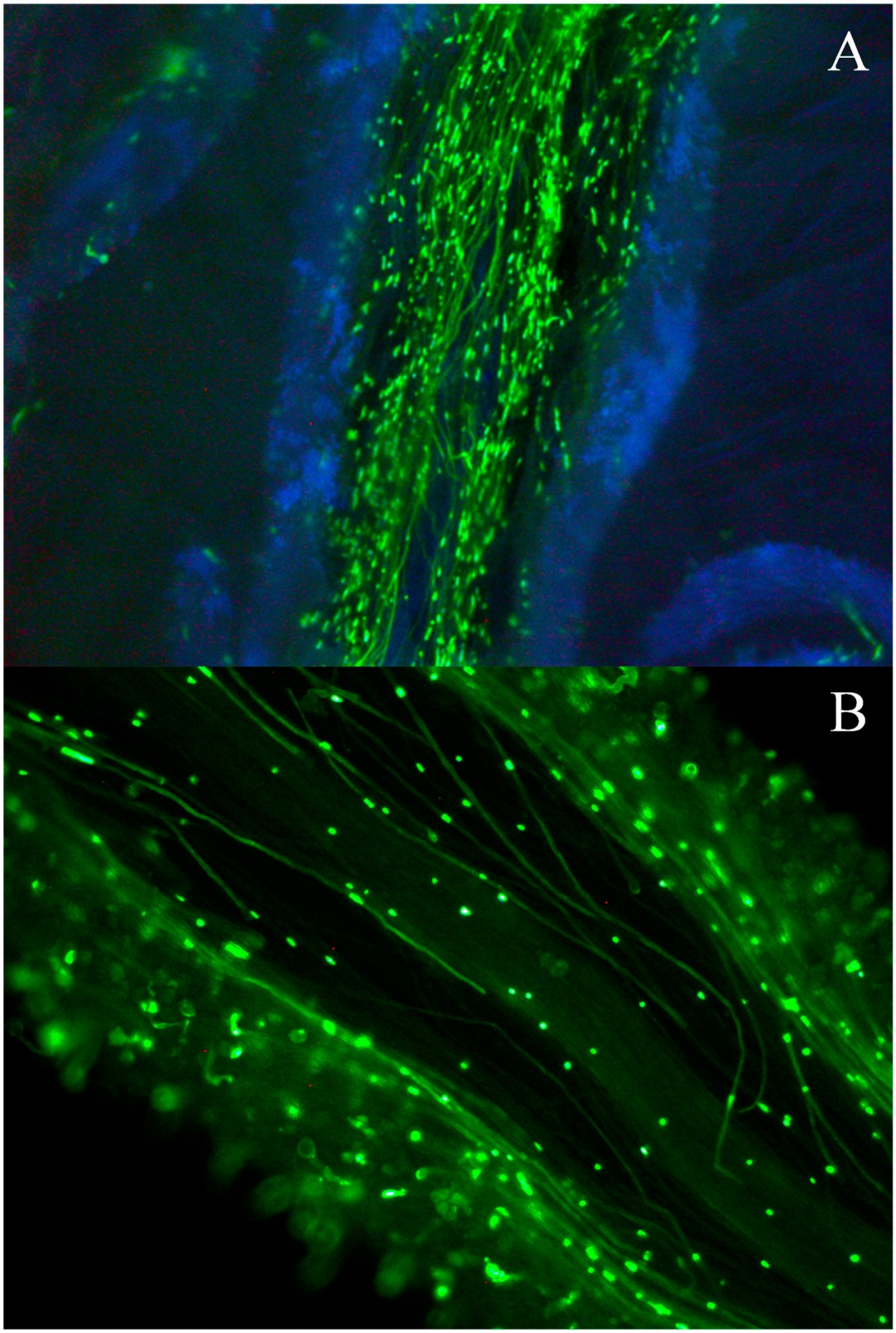

Figure 1. Pollen tubes developed inside a compatible stigma evaluated using fluorescence microscopy 36 h after in vitro pollination ((**A**): 200×, (**B**): 400×).

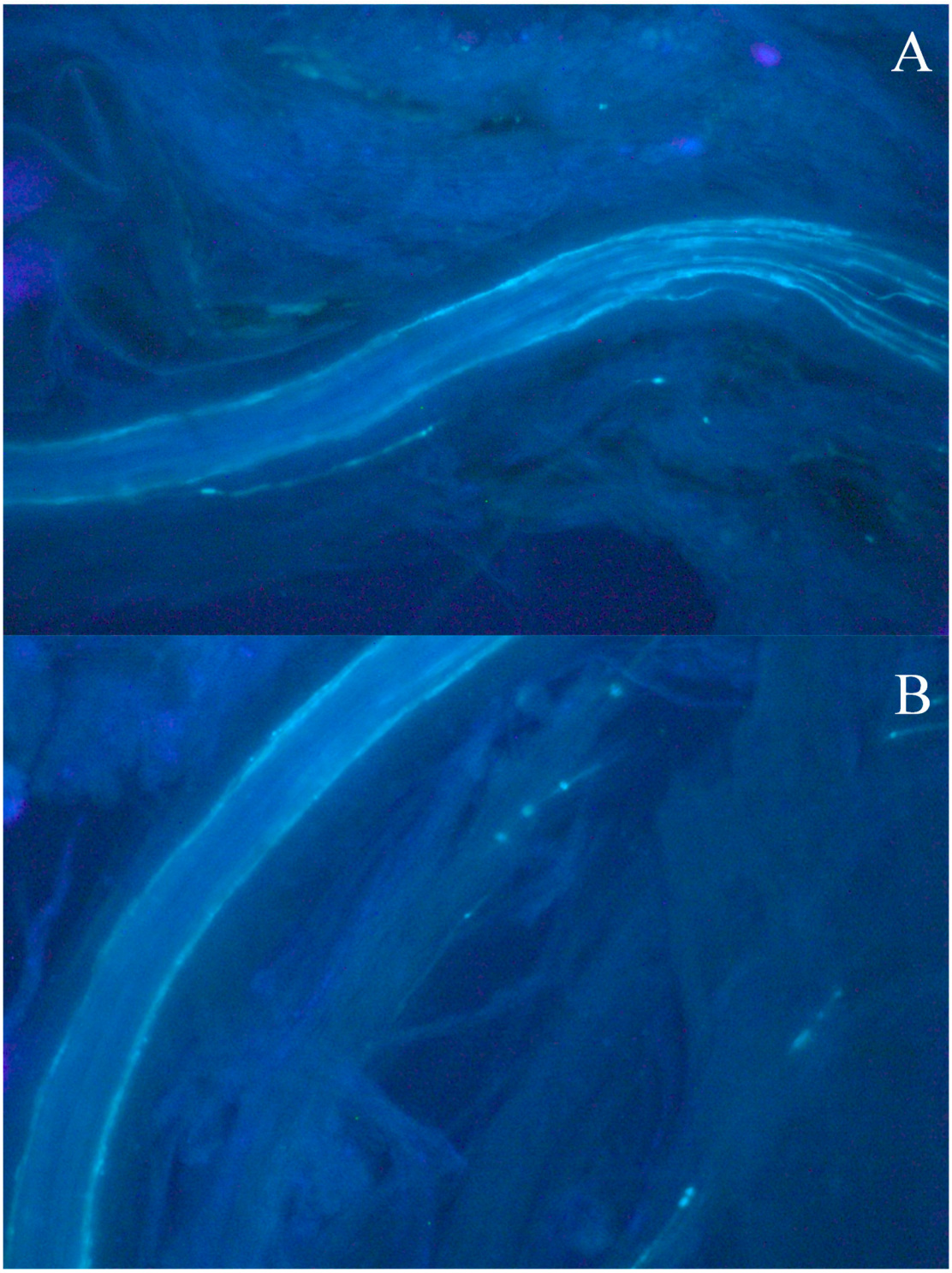

Figure 2. Styles and stigmata evaluated using fluorescence microscopy 36 h after in vitro pollination, showing no pollen tube development (200×). Images (**A**,**B**) show incompatible stigmas from different plants.

Ten pistils were visualized in each diagnosis, with cross-pollinations considered to be compatible when developed pollen tubes were observed in the style of the recipient flowers. The success rate was estimated based on the ratio of the number of observed slides to the number of slides with developed pollen tubes.

In vitro pollination enables precise control over pollen grain transfer, thereby avoiding false positive diagnoses, which arise from contamination and erroneously conclude compatibility between two genotypes. Conversely, false negative errors may occur when procedural mishaps prevent pollen tube development. Souza et al., in 2021 [23], observed error rates of 5% with the observation of ten stigmata per slide, as conducted in this evaluation.

2.4. Allelic and Genotypic Frequencies

Self-incompatibility can be understood as a pre-zygotic mechanism that influences the fertility of pollen grains, preventing any allelic form from becoming fixed in the population and ensuring that individuals are heterozygous for this gene [28]. To interpret the genotypic and allelic frequencies, the frequencies p, q, r, and s were considered in relation to the allelic forms S_1, S_2, S_3, and S_4, as follows:

P (S1S1) = p^2 = 0 (incompatible pollination);
P (S1S2) = 2pq;
P (S1S3) = 2pr;
P (S1S4) = 2ps;
P (S2S2) = q^2 = 0 (incompatible pollination);
P (S2S3) = 2qr;
P (S2S4) = 2qs;
P (S3S3) = r^2 = 0 (incompatible pollination);
P (S3S4) = 2rs;
P (S4S4) = s^2 = 0 (incompatible pollination).

The probabilities of occurrence of the genotypes were as follows:

$$P(S_1S_2) = \frac{2pq}{1-p^2-q^2-r^2};\ P(S_1S_3) = \frac{2pr}{1-p^2-q^2-r^2};\ P(S_2S_3) = \frac{2qr}{1-p^2-q^2-r^2}$$
$$P(S_1S_4) = \frac{2ps}{1-p^2-q^2-r^2};\ P(S_2S_4) = \frac{2qs}{1-p^2-q^2-r^2};\ P(S_3S_4) = \frac{2rs}{1-p^2-q^2-r^2}$$

2.5. Pollination Efficiency

The maximum pollination efficiency occurs when the four known alleles have the same frequency in the population (p = q = r = s = 0.25). Considering that, under this ideal condition, the variance of allelic frequencies is equal to zero (and that the further the deviation from this condition, the greater the variance between allelic frequencies), pollination efficiency was quantified as follows:

$$E = 1 - \sqrt{\frac{\sum\left(f_i - \overline{f}\right)^2}{n-1}}$$

where E: pollination efficiency, f_i: frequency of the i-th allele S_i, $\overline{f}$: average of allele frequencies, and n: number of alleles.

3. Results and Discussion

The summarized results of 335 hybridizations show that approximately 80% of the genotypes were grouped into compatibility groups I, II, and III, with frequencies of 24.4%, 31.1%, and 24.4%, respectively (Tables 2 and 3). A smaller percentage of genotypes (20%) were grouped into groups IV, V, and VI.

The characterization of self-incompatibility becomes more complex as the number of evaluated genotypes increases. Regarding the number of clones evaluated in this study, there are 990 possible ways to combine 45 genotypes in pairs. The use of previously characterized tester plants allows for the reduction in the number of hybridizations to the number of evaluated genotypes multiplied by the number of tester plants [23].

Table 3. Clustering of the 45 most cultivated clones in the Western Amazon according to the development of pollen tubes in compatibility groups identified by Roman numerals: I, II, III, IV, V, and VI.

n	I S_1S_2	II S_1S_3	III S_2S_3	IV $S_{(2,3)}S_4$	V $S_{(2,3)}S_4$	VI S_1S_4
1	BAG27	BAG24	AR106	R22	GJ3	AS1
2	GB1	AS5	AS2			AS10
3	GB7	AS7	BAG28			AS12
4	GJ2	GB4	GJ20			BG180
5	GJ8	GJ5	L1			CA1
6	GJ30	GJ25	LB10			N16
7	LB160	LB12	LB15			VP156
8	N7	LB20	LB33			
9	N32	LB68	LB88			
10	SK244	LB80	N13			
11	WP6	N8[G8]	R152			
12		P50				
13		SK41				
14		SK80				
Total	11	14	11	1	1	7
%	24.44	31.11	24.44	2.22	2.22	15.57

In the evaluation of new cultivars, Moraes et al. (2018) identified three compatibility groups (I, II, III) associated with three alleles (S_1, S_2, and S_3). This variability was expanded in a subsequent study, where the characterization of a germplasm bank with 80 accessions identified three new compatibility groups [24]. In this study, groups IV, V, and VI, associated with the S_4 allele, showed higher frequencies in coffee plants of the Robusta botanical variety. The lower frequency of these groups observed in this work seems to be associated with the genetic diversity of hybrid genotypes, with traits of the Conilon and Robusta botanical varieties.

The success rate estimated from the comparison between the number of compatible diagnoses (225) and the total number of hybridizations (335) was 67% (Table 2). Depolo et al. (2022) [24] and Souza et al. (2021) [23] observed success rate ranges from 41.7% to 80%, respectively. Several factors can limit the development of pollen tubes, such as the viability of pollen grains, the viability of floral structures maintained in vitro, climatic conditions, and the skill of the technician performing the directed pollination.

In vitro pollination in a sterile environment avoids false positive errors caused by pollen grain contamination. However, false negative errors, caused by procedural failures, can occur. The percentage of false negative errors (FN = 16%) observed in this study was higher than the percentages observed by Depolo et al. (2022) [24] and Souza et al. (2021) [23] (Table 2).

The principles of Mendelian genetics are fundamental for establishing an association between the diagnosis of pollen tube development and the classification into compatibility groups. Considering denominations previously presented [22], the genotype of group VI was designated as S_1S_4 based on the higher frequency of the S_1 allele (Table 4). The correspondence of allele and genotype frequencies indicates that the breeding population is in Hardy–Weinberg equilibrium, according to the chi-squared test at a 5% probability level. Comprising 45 genotypes from 16 origins, the population evaluated in this study represents the most cultivated materials in Western Amazonia (Table 1). This correspondence suggests that the selection practiced on these genotypes for better agronomic performance had a minimal effect on changing the allele frequencies of this trait (Table 4).

The ability to avoid self-fertilization has evolved as an important trait in natural populations, contributing to reducing the deleterious effects of inbreeding and increasing genetic variability. However, in cultivation conditions, self-incompatibility can limit pollination efficiency due to the low allelic diversity for this trait. The S gene is considered to be a neutral gene with reproductive success inversely proportional to its frequency [18]. In other words, individuals carrying less common allelic forms of this gene benefit from a greater

availability of pollen donor plants, while the fertility rate of individuals with more frequent alleles in the population is lower.

Table 4. Allelic and genotypic frequencies, observed and expected values, and chi-squared test results for assessing the null hypothesis that the breeding population is in Hardy–Weinberg equilibrium.

Alleles	Allelic Frequency	Groups	Genotypes	Genotypic Frequency	Expected Value
S_1	0.36	I	$P(S_1S_2)$	0.25	11.44
S_2	0.26	II	$P(S_1S_3)$	0.29	12.93
S_3	0.29	III	$P(S_2S_3)$	0.21	9.30
S_4	0.10	IV	$P(S_2S_4)$	0.07	3.22
		V	$P(S_3S_4)$	0.08	3.64
		VI	$P(S_1S_4)$	0.10	4.48
Groups	O	E	O-E	$(O-E)^2$	$(O-E)^2/E$
I	11	11.44	−0.44	0.19	0.02
II	14	12.93	1.07	1.14	0.09
III	11	9.30	1.70	2.91	0.31
IV	1	3.22	−2.22	4.92	1.53
V	1	3.64	−2.64	6.96	1.91
VI	7	4.48	2.52	6.37	1.42
χ^2					5.28 ^{NS}

O: observed value, E: expected value, χ^2: chi-squared test, ^{NS}: not significant.

Maximum pollination efficiency is achieved in a plantation when there are viable pollen grains for fertilization. This ideal condition is attained by the presence of all known allelic forms and in equal frequencies (p = q = r = s = 0.25). Although achieving this ideal condition in practice is challenging, plantations should be planned considering the representation of plants from different compatibility groups, in order to favor plant fertilization and fruit production.

The probability of cultivating non-compatible plants depends on both the allelic diversity of the S gene and the number of genotypes cultivated. Figure 3 compares a population in Hardy–Weinberg equilibrium (HWE) with equal allelic frequencies (p = q = r = s) with the breeding population evaluated in this study, where p = 0.36, q = 0.26, r = 0.29, and s = 0.10, considering the probability of randomly selecting non-compatible genotypes.

Lower probabilities of randomly cultivating non-compatible plants are observed with an increase in the number of cultivated plants. Cultivating a single clone does not produce fruits, due to the inability of this species to self-pollinate, and cultivating two or three genotypes presents significant probabilities of cultivating randomly selected non-compatible plants.

The breeding population evaluated in this study showed higher probabilities of cultivating non-compatible clones compared to the theoretical population with maximum allelic diversity. This probability decreases drastically with the cultivation of five or more genotypes, indicating that cultivating a small number of clones can decisively limit plants' pollination.

Large-scale cultivation of the clones GJ8, GJ25, GJ3, AS2, P50, and LB15 has been carried out for many years in Western Amazonia across thousands of hectares, without presenting problems of fruit set and filling. More recently, clones R22, LB10, GJ8, GJ25, LB15, and AS2 have been widely cultivated in the region.

The pollination efficiency values for all possible combinations in the cultivation of 2 to 10 clones is shown in Figure 4. While the cultivation of 2 clones is associated with 21 distinct combinations, the cultivation of 10 clones is associated with 3003 unique combinations (Figure 4). In this interpretation, three different scenarios were considered: (A) a population in Hardy–Weinberg equilibrium (HWE) with p = q = r = s = 0.25, (B) a population with a rarer allelic form (S_4 = 0.01), and (C) a population with the allelic frequencies observed in this study (Figure 5).

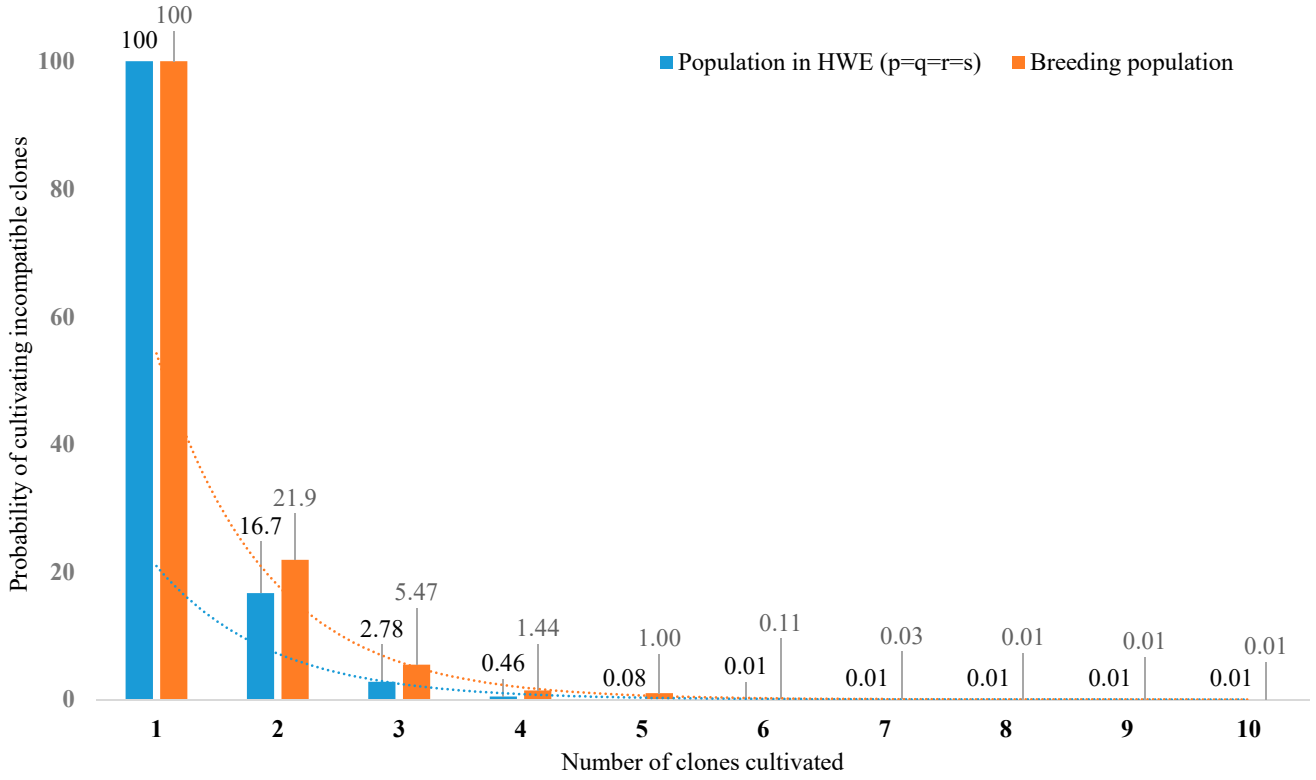

Figure 3. Probability of cultivating randomly chosen incompatible clones as a function of the number of cultivated plants, contrasting two populations: (i) a theoretical population in Hardy–Weinberg equilibrium with equal allelic frequencies (p = q = r = s), and (ii) a breeding population with p = 0.36, q = 0.26, r = 0.29, and s = 0.09.

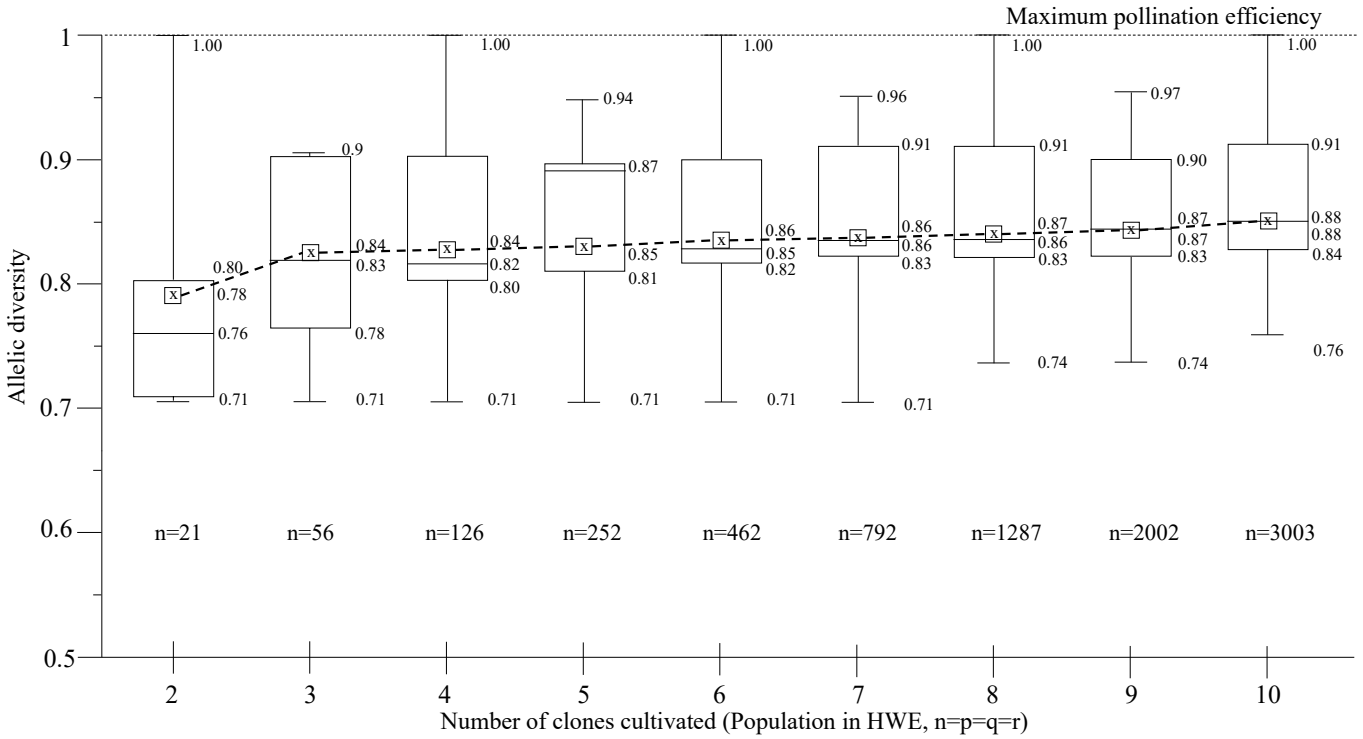

Figure 4. Pollination efficiency interpreted in a boxplot highlighting the mean, median, maximum, minimum, and the first and third quartiles considering a theoretical population in Hardy–Weinberg equilibrium with equal allelic frequencies (p = q =r = s).

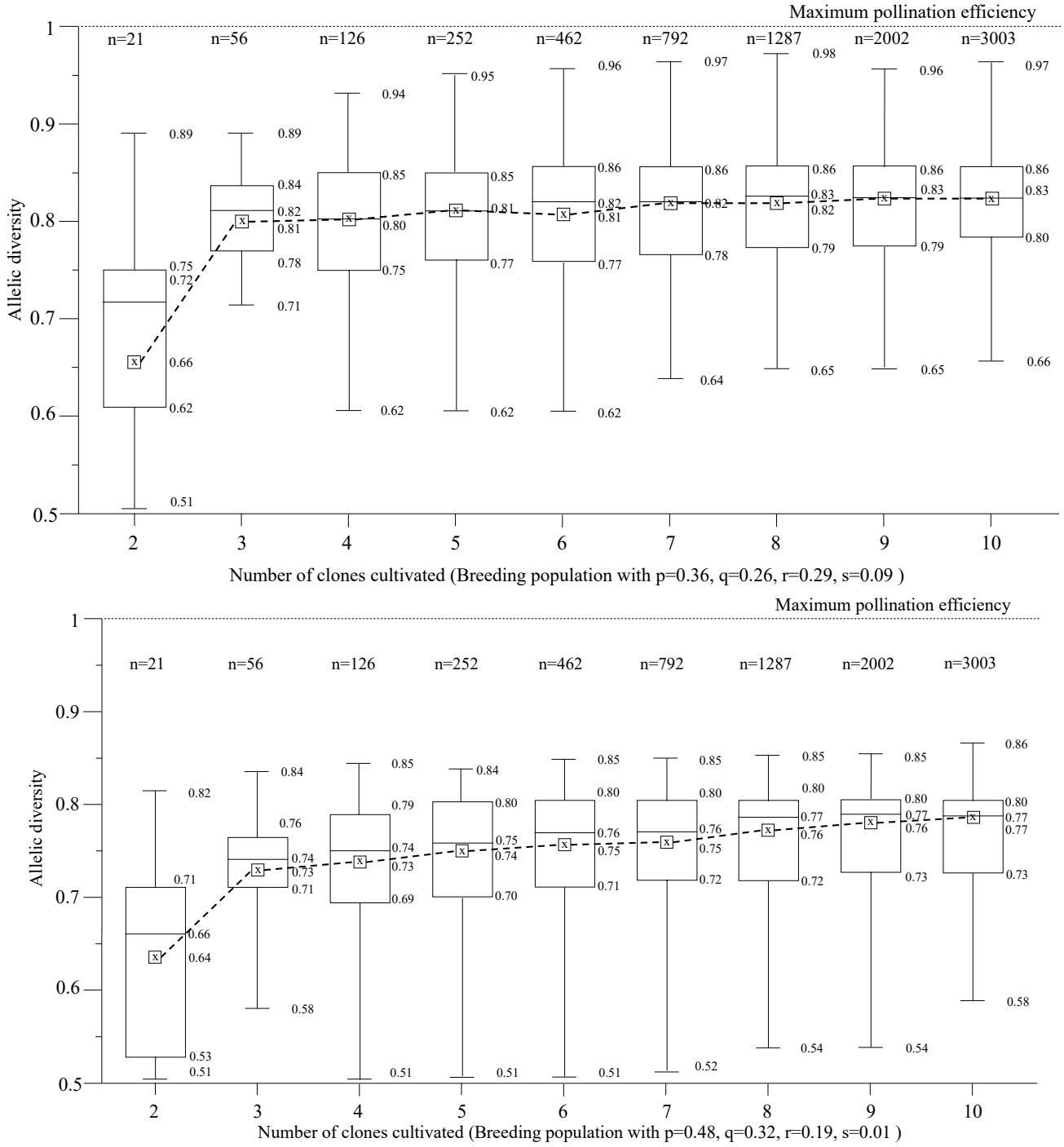

Figure 5. Pollination efficiency interpreted in a boxplot highlighting the mean, median, maximum, minimum, and the first and third quartiles considering (i) a breeding population with p = 0.48, q = 0.32, r = 0.19, and s = 0.01, and (ii) a breeding population with p = 0.36, q = 0.26, r = 0.29, and s = 0.09.

In this interpretation, pollination efficiency was quantified by the deviation of the breeding population from the ideal population with maximum genetic variability, which presents all known allelic forms in equal proportions. Pollination efficiency, defined by the unit subtracted from the standard deviation of allelic frequencies, is inversely proportional to the predominance of one allele over the others, with its upper limit equal to 1 and lower limit equal to 0.50, in a population where only one of the four allelic forms predominates.

Considering the cultivation of 2 to 10 clones in the plantation, the population in Hardy–Weinberg equilibrium (HWE) showed pollination efficiency averages ranging from 0.78 to 0.88, while the population with the rarer allelic form showed estimates ranging from 0.66 to 0.77. The population studied in this work showed intermediate behavior between these two populations, with estimates ranging from 0.66 to 0.83 (Figure 4). All three populations showed stability from the cultivation of five clones in the plantation.

Theoretically, maximum pollination efficiency is achieved by representing all alleles in equal proportions. In practice, farmers must ensure the cultivation of plants from different compatibility groups, without major imbalances. In this context, the minimum number of clones to be cultivated is one of the main considerations. The cultivation of 5 to 9 clones with the representation of at least three compatibility groups has shown good results in the field.

In this scenario of clones selected by farmers themselves and cultivated across thousands of hectares, self-incompatibility is an important issue for the safety of coffee farming. In the past, farmers took on this risk. Still in the 1980s, Ferrão et al. (2019) [27] reported productivity problems associated with the cultivation of a single clone or a few non-compatible clones.

From this initiative, however, arose an agricultural activity of great importance for Western Amazonia. While the clones GJ8, GJ25, GJ5, GJ3, and P50 were cultivated together on a large scale in the 2000s, more recently, the clones GJ8, GJ25, AS2, LB15, and R22 have been cultivated together. Based on the results of this study, we can infer that, in the 1980s, clones from compatibility groups I, II, and V predominated in the field at frequencies of 20%, 60%, and 20%, respectively, in comparison with the clones cultivated more recently, where compatibility groups I, II, III, and IV predominate at frequencies of 20%, 40%, 20%, and 20%, respectively. This indicates an increase in the genetic variability of this trait over the years.

In this context, the greatest risk for cultivation arises from relying on a small number of cultivated plants. Beyond the efficiency of pollination, challenges include unsynchronized flowering among few clones and a higher incidence of undesirable traits specific to certain clones within the plantation [23,27].

4. Conclusions

Based on the pollen tube development response, the genotypes were grouped into six groups: group I (24.4%), group II (31.1%), group III (24.4%), group IV (2.2%), group V (2.2%), and group VI (15.6%). The lower frequency of groups IV, V, and VI is associated with the lower frequency of the rarest allelic form in this population. The correspondence between allelic and genotypic frequencies indicates that selection practiced for better agronomic performance did not reduce variability for this trait. The breeding population exhibited intermediate behavior between a theoretical population of maximum genetic diversity and a population with a rare allelic form. Efficiency estimates stabilized with the cultivation of five clones, indicating that cultivating a minimum number of clones should be considered. The greatest risk for cultivation is associated with the cultivation of a small number of clones, and in practice, the farmer should ensure the cultivation of plants from different compatibility groups without significant imbalances.

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