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Expression of self-incompatibility in *Coffea canephora* genotypes grown in the western Amazon

Abstract – The objective of this work was to characterize the expression of gametophytic self-incompatibility in a *Coffea canephora* breeding population, to assist in the management and development of new cultivars. For that purpose, 550 in vitro pollinations were carried out among 62 parent plants, of which 27 were from the conilon botanical variety and 35 from the robusta. Thirty-two genotypes compatible with all previously known testers were identified, suggesting the existence of new compatibility groups. From these results, hybridizations were carried out in a complete diallel design with reciprocal crosses to characterize new test plants. Based on the compatibility response with the test plants, the genotypes were clustered into the six following groups: group I, 11 (17.74%) genotypes; group II, 13 (20.97%); group III, 6 (9.68%); group IV, 9 (14.52%); group V, 8 (12.90%); and group VI, 15 (24.19%). The genotypes of the botanical variety robusta show a higher frequency of plants in compatibility group VI and a greater genetic variability, whereas those of the conilon variety have a higher frequency of plants in compatibility group II. The identification of new compatibility groups assists in new management practices that seek to increase the efficiency of pollination by favoring, through natural means, fully compatible crosses.

Index terms: coffee plant, conilon, gametophytic self-incompatibility, robusta.

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Expressão da autoincompatibilidade em genótipos de *Coffea canephora* cultivados na Amazônia Ocidental

Resumo – O objetivo deste trabalho foi caracterizar a expressão de autoincompatibilidade gametofítica em uma população de melhoramento do cafeeiro *Coffea canephora*, para fornecer subsídios ao manejo e ao desenvolvimento de novas cultivares. Para tanto, foram realizadas 550 polinizações in vitro entre 62 plantas matrizes, das quais 27 foram da variedade botânica conilon e 35 da robusta. Foram identificados 32 genótipos compatíveis com todos os testadores previamente conhecidos, o que indica a existência de novos grupos de compatibilidade. A partir desses resultados, foram realizadas hibridações em delineamento em dialelo completo com cruzamentos recíprocos para caracterizar novas plantas testadoras. Com base na resposta de compatibilidade com as plantas testadoras, os genótipos foram agrupados nos seguintes seis grupos: grupo I, 11 (17,74%) genótipos; grupo II, 13 (20,97%); grupo III, 6 (9,68%); grupo IV, 9 (14,52%); grupo V, 8 (12,90%); e grupo VI, 15 (24,19%). Os genótipos da variedade botânica robusta apresentam maior frequência de plantas no grupo de compatibilidade VI e maior variabilidade genética, enquanto os da variedade conilon mostram maior frequência de plantas no grupo de compatibilidade II. A identificação de novos grupos de compatibilidade fornece subsídios para novas práticas de manejo que buscam aumentar a eficiência da polinização pelo favorecimento natural de cruzamentos totalmente compatíveis.

Termos para indexação: cafeeiro, conilon, autoincompatibilidade gametofítica, robusta.

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Introduction

Self-incompatibility (SI) is a physiological mechanism present in the reproductive system of *Coffea canephora* Pierre Ex A. Froehner, which naturally favors the occurrence of cross pollinations (Asquini et al., 2011). In the nature, in the rate reductions of self-fertilization and hybridization between related plants favor allogamous populations, contributing to the reduction of the deleterious effects of inbreeding, as well as to the increase of genetic diversity (Vieira et al., 2021).

In the growing of clonal coffee plants, a reduced number of genotypes results in more limited genetic variability among plants, which represents a small sample of natural populations coming from their center of origin on the African continent (Moraes et al., 2018; Ferrão et al., 2021; Akpertey et al., 2022). The knowledge and management of self-incompatibility is important for growing clonal coffee plants, since the lack of knowledge can reduce the yield and quality coffee bean, resultant from lower pollination efficiency (Rocha et al., 2021).

In the species *C. canephora*, the expression of self-incompatibility is governed by only one multiallelic gene identified by the letter S (Berthaud, 1980). The gametophytic self-incompatibility reaction occurs between the pollen tube and the pollen grain, which may not share the same allele as the receptor plant (Nowak et al., 2011). The impediment to self-fertilization is caused by the action of ribonucleases that degrade the ribosomal RNA, impeding the growth of the pollen tube (Souza et al., 2021).

Although the incompatibility of *C. canephora* was reported already in the 1960s, few studies have been devoted to the characterization of the compatibility of this species. Some of the most notable studies are described as follows. Conagin & Mendes (1961) and Berthaud (1980) presented evidence that the self-incompatibility in *C. canephora* should be governed by the action of a single gene S, with three allelic forms (S_1, S_2, and S_3). Lashermes et al. (1996) obtained populations of doubled haploid plants that were homozygous for the S gene, and they showed that the development of the pollen tube can be observed using a fluorescence microscope. De Franceschi et al. (2012) described that the incompatibility is due to the interaction of specific glycoproteins in the stigma of the flower and in the pollen grains that combine to form dimers. Nowak et al. (2011) characterized the polymorphism of this gene among various species of the *Coffea* genus. Moraes et al. (2018) identified test plants of three different compatibility groups. And, more recently, Souza et al. (2021) presented a methodology for the evaluation of self-incompatibility using in vitro pollination associated with fluorescence microscopy, to reduce the rate of contamination and reduce the time necessary to diagnose the incompatibility.

Coffea canephora has two distinct botanical varieties that are grown in the western Amazon region: the conilon – characterized by plants of bush-type growth, drought tolerance, and greater susceptibility to diseases; and the robusta – characterized by upright growth, larger sized fruit and leaves, lower drought tolerance, and greater resistance to pests and diseases (Dubberstein et al., 2020; Rocha et al., 2021; Silva et al., 2022). Although evaluations at the center of origin suggest the existence of up to five allelic forms of the S gene (Omolaja & Fawole, 2005), evaluations outside the center of origin, in the germplasm cultivated in Brazil, suggest the occurrence of only three allelic forms in the expression of this trait (S_1, S_2, and S_3) (Conagin & Mendes, 1961; Moraes et al., 2018).

The objective of this work was to characterize the expression of gametophytic self-incompatibility in a *Coffea canephora* breeding population, to assist in the management and development of new cultivars.

Materials and Methods

The compatibility response of each genotype was evaluated in relation to tester plants. Genotypes 'BRS 1216' ('Conilon Emcapa03' × 'Robusta1675'), 'BRS 2299' (open pollination hybrid genotype), and BRS 3193 (open pollination hybrid genotype) were used as test plants of compatibility groups I, II, and III, respectively (Moraes et al., 2018; Teixeira et al., 2020). The first digit of these genotypes identify the compatibility groups.

The breeding population consisted of sixty-two plants with characteristics of the conilon and robusta botanical varieties, which were grown in a hybridization field set up in 2017, in an experimental field in the municipality of Porto Velho, in the state of Rondônia, Brazil. This breeding population was selected according to a greater genetic divergence estimated from the evaluation of agronomic and

morphological descriptors over three years (Oliveira et al., 2018). The genotypes of the conilon botanical variety were identified with the C prefix, and the parent plants of the robusta botanical variety were identified by the letter R.

The in vitro pollination consisted of the transfer of pollen grains from donor plants to stigmas of receptor plants. In the experimental field of Embrapa Rondônia in Porto Velho, the flowering was monitored weekly from January to December 2020. A small number of pollen grains and flowers necessary for the in vitro pollination allows of a large number of hybridizations to be performed over the year. In vitro pollination procedures were performed on three different occasions: 6/5/2020, 7/31/2020, and 9/9/2020, with 289, 108, and 153 hybridizations, respectively, totalizing 550 directed hybridizations. Prior to the in vitro pollination, germination tests of pollen grains were performed for each donor plant in 10% sucrose solution. To facilitate the pollen grain detachment, the anthers were immersed in 5.0 mL of 10% sucrose solution in Eppendorf type tubes. Using a pipette, the solution containing pollen grains was distributed homogeneously on a Petri dish. After 60 min, using a stereoscopic microscope (50X magnification), three replicates of 100 pollen grains were counted for each pollen donor. Pollen grains with tube length greater than the pollen grain diameter were considered germinated. Mean germination rates greater than 60% indicate good pollen grain viability (Souza et al., 2021) (Figure 1). In the day before anthesis, observed by the development of flowers in the field, inflorescences of the receptor and donor plants were collected, placed in paper bags, and taken to the laboratory of plant tissue culture of Embrapa Rondônia.

Stamens and petals of the receptor flowers were removed, and the inflorescences containing three, four, or five flowers were placed in containers with solid culture medium (water, 30% sucrose, 6% bacteriological agar, 1% amoxicillin), with the peduncle immersed in the culture medium (Souza et al., 2021). After the exposure to the environment, the antibiotic culture medium is viable for approximately four days, during a sufficient time for the development of the pollen tubes, which require approximately 36 hours. Inflorescences of the donor plants, with buds still closed, were placed in containers with the same culture medium, with the peduncle immersed in the medium. After that, all containers were sealed with plastic film, to prevent the contamination of pollen grains, and then they were kept in a plant growth room at 26±1°C and 16-hour photoperiod (50 μmoL m^{-2} s^{-1}). Each compatibility diagnosis was based on the evaluation of ten stigmas pollinated on one of the tips of the bifid stigma. On the day of the anthesis, pollen grains were collected from the donor flowers by scraping the dehiscent anthers with a scalpel (scraping should be carried out until pollen grains are visible to the naked eye on the scalpel). The scalpel blade was then delicately rubbed on one of the tips of the bifid stigma, to lead pollen grains to adhere to the stigma (Figure 1).

Compatibility diagnosis was based on the visualization of pollen tubes development in the pistil of compatible plants. Thirty-six hours after pollination, the stigmas were stored in FAA solution (10% formaldehyde, 10% glacial acetic acid, and 80% ethanol), by using penicillin vials (10 mL) with a rubber lid. The samples were stored in a refrigerator at 5°C until the preparation of slides, for which the pistils were removed from the FAA, washed with distilled water, and immersed in sodium hydroxide (1N NaOH) for 2 hours. After that period, the stigmas were washed with distilled water and stained for 12 hours, using 1% aniline blue stain prepared in a 0.1 mmol L^{-1} K_2PO_4 solution. After that, the stigmas were once more washed with distilled water. The sodium hydroxide

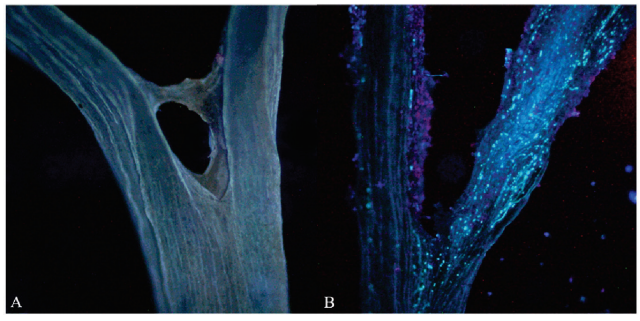

Figure 1. Visualization under a microscope at 50X magnification, showing: A, pollinated stigma without the development of pollen tubes; and B, pollination of only one of the sides of the bifid stigma, which favors the visualization under the microscope due to the contrast between the pollinated and nonpollinated styles – pollen tubes can be seen growing through the style tissue in the compatible hibridization.

solution causes alkaline hydrolysis of the ester type covalent bonds between the lignin and the structural carbohydrates of the cell wall, with solubilization of the hemicellulose and phenolic compounds.

For the visualization, ten pistils were placed on each slide, and a coverslip was delicately pressed on the pistils to flatten them on the slide. The material was visualized using a fluorescence microscope Leica DM2500 (Leica Microsystems, Wetzlar, Germany), equipped with a photodocumentation system. The pistils were visualized in 100X and 200X magnification, and the number of pistils with completely developed pollen tubes were counted. Ten stigmas were evaluated for each directed hybridization, and crosses with completely developed pollen tubes in the stigma style were considered compatible. Pollination efficiency (PE) was estimated from the ratio between the number of pollinated stigmas (NPS) and the total number of stigmas (TNS). In the diagnosis of compatibility, it is important to consider that each procedure has an error probability, due to the conditions of either the material handling or the environment. The in vitro pollination procedures allows of precise control of the transfer of pollen grains, to avoid false positive diagnoses that occur when two genotypes are incorrectly considered compatible, due to contamination. False negative type errors can also occur, when two genotypes are considered incompatible, due to some failure in the procedure. Thus, for the diagnosis of compatibility, the error rate at 5% was considered for each procedure.

For the quantification of genetic variability, the following characteristics were evaluated in the 2018/2019 and 2019/2020 crop years: plant height (PHEI); number of productive plagiotropic branches (NPLAG); distance between rosettes of the intermediate part of the plagiotropic branch (DROS); number of grains per rosette of the intermediate part of the plagiotropic branch (GROS); number of rosettes per plagiotropic branch (NROS); and plagiotropic branch length (PLAGL). Maturation time was determined using the criterion for plants bearing 70% of fruit in the cherry stage, recording the date of harvest (NDAYS). Genotypic values of production per plot (GV) were estimated based on the weight of processed coffee beans. In turn, coffee bean size was evaluated individually, using a set of 12 different sieves for samples of 250 g of processed coffee (PEN). Leaf length (LLEN) and width (LWID) were estimated in the evaluation of 10 leaves collected from the middle third of the plant which were measured using a digital caliper.

To quantify the genetic divergence, principal component analysis was used in association with reference points denominated centroids, obtained from the average behavior of each botanical variety (Rocha et al., 2005). Centroids were used to interpret the grouping of genotypes in relation to the characteristic botanical varieties.

Results and Discussion

The grouping of a genotype in a certain compatibility group is based on the diagnosis of incompatibility with one of the previously known testers (Moraes et al., 2018). Hybridizations were planned from the results observed in each experiment, considering individuals to be evaluated and the previously known test plants. The summarized results of 289 hybridizations show that 17 genotypes of the conilon botanical variety (27%) and 13 genotypes of the robusta botanical variety (21%) were grouped into compatibility groups I, II, and III (Table 1).

The transfer of pollen grains between donor anthers and receptor stigmas in an aseptic environment avoids false positive type errors caused by contamination in pollen grains coming from different plants (Souza et al., 2021). However, false negative type errors can occur when two genotypes are classified as incompatible due to a flaw in the procedure. Out of 289 hybridizations, 13 failed diagnoses were observed, which is equivalent to 4.5% error rate. The value of probability of the grouping of each genotype was estimated individually in accordance with the number of stigmas evaluated, considering 5% probability of error.

The pollination efficiency estimated from the comparison between the number of compatible diagnoses (233) and the total number of hybridizations (289) was 80%. In the evaluated population, a total of 62 genotypes, 10 of the conilon botanical variety (16%), and 22 of the robusta botanical variety (36%) were compatible with all the known testers, which indicates the existence of new compatibility groups.

In the center of origin of this coffee, plant in the African continent, there are reports of greater allelic variability of the S gene that governs expression of self-incompatibility (Omolaja & Fawole, 2005). Up

to the attaining of results of this study, evaluations of Brazilian populations indicated only three alleles and three compatibility groups. In the 1960s, Conagin & Mendes (1961) presented results showing the genotype grouping in three different groups and, more recently, similar results were observed by Moraes et al. (2018), showing grouped clones of new cultivars developed by Embrapa in only three compatibility groups (Teixeira et al., 2020).

The larger number of hybridizations in the present study, and the previous selection of parents of greater genetic divergence of the conilon and robusta botanical varieties appeared to be essential for the identification of new sources of variability (Oliveira et al., 2018). In the compatibility characterization of 10 cultivars from Embrapa, Moraes et al. (2018) observed a smaller diversity among genotypes with full-sib and half-sib degrees of kinship.

The identification of new compatibility groups provides assistance for new management practices to increase the efficiency of pollination, by natural means of favoring fully compatible crosses. In

Table 1. Hybridizations performed for 62 *Coffea canephora* genotypes with test plants of compatibility groups I, II, and III in the municipality of Porto Velho, in the state of Rondônia, Brazil[(1)].

n	Genotype	I	II	III	FN	FP	Group	p-value	n	Genotype	I	II	III	FN	FP	Group	p-value
1	C 167	(2)	1	1	0	0	I	<0.01	32	R 216	(2)	1	1	1	0	I	<0.01
2	C 799	(2)	1	1	0	0	I	<0.01	33	R 3	(2)	1	2	0	0	I	<0.01
3	C 986	(2)	1	1	0	0	I	<0.01	34	R 277	1	(2)	1	0	0	II	<0.01
4	C 154	(2)	1	1	0	0	I	<0.01	35	R 63	1	(2)	1	0	0	II	<0.01
5	C 452	(2)	1	1	0	0	I	<0.01	36	R 9	1	(2)	1	0	0	II	<0.01
6	C468	1	(2)	2	0	0	II	<0.01	37	R 99	3	(2)	2	0	0	II	<0.01
7	C 795	1	(2)	1	1	0	II	<0.01	38	R 164	2	2	(2)	0	0	III	<0.01
8	C 945	1	(2)	1	0	0	II	<0.01	39	R 32	3	1	(2)	0	0	III	<0.01
9	C 125	2	(1)	2	0	0	II	0.05	40	R 36	2	1	(3)	0	0	III	<0.01
10	C 184	1	(1)	1	0	0	II	0.05	41	R 20	3	2	3	0	0	New	-
11	C 59	1	(2)	1	0	0	II	<0.01	42	R 161	1	1	1	0	0	New	-
12	C 729	1	(1)	2	0	0	II	0.05	43	R 186	1	3	2	0	0	New	-
13	C 801	1	(2)	1	0	0	II	<0.01	44	R 222	2	1	1	0	0	New	-
14	C 46	1	(2)	2	0	0	II	<0.01	45	R 240	2	1	2	0	0	New	-
15	C 484	1	1	(2)	0	0	III	<0.01	46	R 302	1	1	2	1	0	New	-
16	C 968	1	1	(1)	0	0	III	0.05	47	R 212	1	2	1	0	0	New	-
17	C 694	2	1	(2)	0	0	III	<0.01	48	R 224	2	2	2	1	0	New	-
18	C 796	1	1	1	0	0	New	-	49	R 157	1	2	1	0	0	New	-
19	C 998	1	1	1	0	0	New	-	50	R 160	1	2	1	1	0	New	-
20	C 1048	2	3	3	1	0	New	-	51	R 220	1	3	1	0	0	New	-
21	C 1059	2	2	1	1	0	New	-	52	R 243	1	6	1	0	0	New	-
22	C 556	2	1	1	1	0	New	-	53	R 307	2	1	1	2	0	New	-
23	C 747	1	1	1	0	0	New	-	54	R 309	1	1	1	0	0	New	-
24	C 846	1	1	1	0	0	New	-	55	R 312	2	4	2	0	0	New	-
25	C 890	1	1	1	0	0	New	-	56	R 42	3	3	2	0	0	New	-
26	C 909	1	1	1	0	0	New	-	57	R 66	1	4	1	0	0	New	-
27	C 973	1	1	1	0	0	New	-	58	R 90	1	1	2	1	0	New	-
28	R 183	(2)	2	1	1	0	I	<0.01	59	R 1	1	2	1	0	0	New	-
29	R 30	(1)	4	1	0	0	I	0.05	60	R 131	2	4	2	0	0	New	-
30	R 5	(2)	1	1	0	0	I	<0.01	61	R 125	1	3	2	1	0	New	-
31	R 214	(2)	1	1	0	0	I	<0.01	62	R 155	1	2	1	0	0	New	-

[(1)]Non-compatible hybridizations are indicated by the number within parentheses, and compatible hybridizations are indicated by the numbers without formatting. Parameters: n, ordinal numbering; Genotype, identification of the receptor genotype; I, compatibility group I (one); II, compatibility group II (two); compatibility group III (three); New, a new compatibility group; FN, hybridizations with false negative type error; FP, hybridizations with false positive type error; Group, diagnosis of the compatibility group; and p-value, error probability of genotype classification in its respective compatibility group, considering a 5% error rate.

C. canephora, the percentage of peaberry coffee beans can be interpreted as a measure of the efficiency of pollination (Souza et al., 2017). Increases of up to 40% in the rate of fruit set were observed in studies of pollination efficiency. The importance of the pollination efficiency also increases in years of greater coffee yield, when a large number of flowers should be pollinated by different pollinating agents. Souza et al. (2017) observed increases of 40% up to 60% in the percentage of peaberry coffee beans between the first and second harvest of greater yield.

Hybridizations were made in a complete diallel design with reciprocal crosses aiming to cluster the genotypes that were compatible with all the previously known testers (Table 2). The genotypes were chosen at random within the group of plants classified in new compatibility groups. Branches from the six genotypes that had the highest germination rates of pollen grains at the time of hybridization were used as donors (germination rate ≥ 85%).

Essential for the study of quantitative characteristics inheritance, diallel designs are also useful for the compatibility characterization of a smaller number of genotypes with greater accuracy (Cruz, 2013). Reciprocal crosses are interpreted to revise the diagnoses of compatibility, confirming each one of the hybridizations made individually. The evaluated genotypes clustered in three new compatibility groups identified by the Roman numerals IV, V, and VI (Table 2). From this classification, plants C556 and C1059 of Group IV, plant R309 of Group V, and plants R161, R224, and C1048 of Group VI were chosen as test plants of their respective compatibility groups.

Unlike the normally accepted concept in plant breeding for the use of test plants to determine specific and general combining abilities, the use of test plants to determine compatibility groups is based on the evaluation of the compatibility response, in comparison with a plant of a genotype known for the S gene. The genotypes C556, R309, and R224 were used as test plants of compatibility groups IV, V, and VI, in the hybridizations made in September 2020. The summarized results of evaluation of 153 hybridizations indicate that among the 32 genotypes that were compatible with all the previously known testers, 9 clones were clustered in Group IV, eight clones in Group V, and fifteen clones in Group VI (Table 3). In the evaluation of these 153 hybridizations, 6 false negative errors were observed, which represents a rate of 3.9%.

Despite of their importance, much remains to be clarified in relation to the mechanisms of expression of compatibility in *C. canephora* coffee plant (Nowak et al., 2011). The characterization of the sequences of the allelic forms of the S gene is favored by the study of haploid or doubled haploid plants that are homozygous for this gene. Lashermes et al. (1993) reported the development of haploid plants from rare events of polyembryony in coffee that occur in less than 1% in seeds, and Lashermes et al. (1996), studying the linkage disequilibrium, observed RFLP and RAPD markers associated with this gene. The low spontaneous occurrence of polyembryonic seed and

Table 2. Hybridizations in a complete diallel design with self-fertilizations and reciprocal crosses of six *Coffea canephora* genotypes, with characteristics of the conilon and robusta botanical varieties that were compatible with all the previously known testers, showing: A, compatibility response of the directed hybridizations (NC, noncompatible; and C, compatible); and B, grouping of new compatibility groups designated as IV, V, and VI. Genotypes: C, plants of the conilon botanical variety; and R, plants of the robusta botanical variety.

A

Genotypes	C1059	C556	C1048	R309	R161	R224
C1059	NC[1]	NC	C	C	C	C
C556	NC[2]	NC[1]	C	C	C	C
C1048	C[2]	C[2]	NC[1]	C	NC	NC
R309	C[2]	C[2]	C[2]	NC[1]	C	C
R161	C[2]	C[2]	NC[2]	C[2]	NC[1]	NC
R224	C[2]	C[2]	NC[2]	C[2]	NC[2]	NC[1]

B

Groups	IV	V	VI
IV	C556	R309	R161
V	C1059		R224
VI			C1048

[(1)]Self-fertilizations. [(2)]Reciprocal crosses. IV, V, and VI, new compatibility groups identified.

the limited application of the linkage disequilibrium between molecular markers and the gene that governs the expression of this trait make it difficult the use of these results in the study of compatibility.

On the basis of the results of incompatibility with the tester genotypes, the 62 clones clustered in six groups identified as follows: 11 genotypes (17.74%) in group I; 13 genotypes (20.97%) in group II; 6 genotypes (9.68%) in group III; 9 genotypes (14.52%) in group IV; 8 genotypes (12.90%) in group V; and 15 genotypes (24.19%) in group VI (Table 4).

The characterization of the tester genotypes in two steps allowed of the compatibility genotypes of groups I, II, and III to be inferred; however, it limited the inference of the genotypes of groups IV, V, and VI, which may exhibit any combination of alleles of the previously known testers with the new allelic form of the new compatibility groups (Table 4). This limitation is due to the impossibility of differentiating the partially compatible hybridizations, which are those that occur between genotypes that share the same allele (for instance, $S_1S_2 \times S_1S_3$), from the fully compatible

Table 3. Hybridizations performed of 32 *Coffea canephora* genotypes with test plants of compatibility groups IV, V, and VI in the municipality of Porto Velho, in the state of Rondônia, Brazil[(1)].

n	Clone	IV	V	VI	FN	FP	Group	p-value
1	C 1048	4	3	(3)	0	0	VI	<0.01
2	C 1059	(3)	4	1	1	0	IV	<0.01
3	C 556	(3)	4	2	0	0	IV	<0.01
4	C 747	(2)	1	2	0	0	IV	<0.01
5	C 973	2	(1)	1	1	0	V	0.05
6	C 796	(1)	1	1	1	0	IV	0.05
7	C 846	4	3	(2)	0	0	VI	<0.01
8	C 890	2	(2)	1	0	0	V	<0.01
9	C 909	2	(2)	2	0	0	V	<0.01
10	C 998	2	(2)	2	0	0	V	<0.01
11	R 90	1	1	(1)	1	0	VI	0.05
12	R 1	(2)	1	2	0	0	IV	<0.01
13	R 125	1	1	(2)	0	0	VI	<0.01
14	R 131	1	1	(2)	0	0	VI	<0.01
15	R 155	2	1	(2)	1	0	VI	<0.01
16	R 157	1	1	(2)	0	0	VI	<0.01
17	R 160	1	2	(2)	0	0	VI	<0.01
18	R 161	1	1	(1)	0	0	VI	0.05
19	R 186	(2)	1	1	0	0	IV	<0.01
20	R 20	2	1	(2)	0	0	VI	<0.01
21	R 212	1	1	(2)	0	0	VI	<0.01
22	R 220	1	1	(2)	0	0	VI	<0.01
23	R 222	1	1	(1)	0	0	VI	0.05
24	R 224	1	1	(1)	0	0	VI	0.05
25	R 240	1	1	(1)	0	0	VI	0.05
26	R 243	(2)	2	1	0	0	IV	<0.01
27	R 302	2	(2)	1	1	0	V	<0.01
28	R 307	1	(1)	1	0	0	V	0.05
29	R 309	1	(2)	1	0	0	V	<0.01
30	R 312	1	(2)	1	0	0	V	<0.01
31	R 42	(1)	1	1	0	0	IV	0.05
32	R 66	(2)	2	1	0	0	IV	<0.01

[(1)]Non-compatible hybridizations are indicated by the number within parentheses, and the compatible hybridizations are indicated by the numbers without parentheses formatting. IV, V, and VI, compatibility groups; FN, hybridizations with false negative type error; FP, hybridizations with false positive type error; and p-value, error probability of the genotype classification in its respective compatibility group, considering 5% error rate.

hybridizations that occur between genotypes that do not share any allelic form in common (tha is, $S_1S_2 \times S_3S_4$).

The self-incompatibility is a pre-zygotic barrier that affects the germination of pollen grains, and it is not possible to differentiate the partially compatible and the fully compatible hybridizations either in the field or from the development of the pollen tubes. This result is associated with the development of the pollen tubes, since incompatible pollen grains which halt their development do not interfere with their development from compatible pollen grains. Working with plants able to carry out only partially compatible hybridizations, Moraes et al. (2018) also observed a high pollination efficiency in the field.

The six groups had different frequencies of plants of the conilon and robusta botanical varieties (Table 4). Group II showed the lower frequency of plants of the robusta botanical variety (30.8%), and Group VI showed the greatest frequency of plants of the robusta botanical variety (86.7%) (Table 4). Aiming to compare the compatibility groups with the diversity estimated from evaluations in the field, the dispersion of the first two principal components was interpreted in association with reference points (Figure 2).

The first two principal components, which represent 78% of the variability of the original data, show the separation between the two breeding populations, since the conilon genotypes were concentrated mainly in quadrant A, and the genotypes of the robusta botanical variety in quadrants B, C, and D (Figure 2). The greater dispersion of the genotypes of the robusta botanical variety is associated with its greater genetic variability observed among the accessions of the population.

Genotypes of this botanical variety are an important source of variability for the selection and development of new cultivars. Many studies have shown differences among the breeding populations of these two botanical varieties, which are exploited for the production of hybrid genotypes with the best characteristics of each one of these botanical varieties (Ferrão et al., 2019; Alkimim et al., 2020; Rocha et al., 2021; Partelli et al.,

Table 4. Grouping of 62 parent plants of *Coffea canephora* of the conilon and robusta botanical varieties, identified in their respective groups in accordance with the compatibility with test plants in the municipality of Porto Velho, in the state of Rondônia, Brazil[(1)].

n	I	II	III	IV	V	VI
	S_1S_2	S_1S_3	S_2S_3	$S_{(1,2,3)}S_4$	$S_{(1,2,3)}S_4$	$S_{(1,2,3)}S_4$
1	C 154	C 125	C 484	C 1059	C 890	C 1048
2	C 167	C 184	C 694	C 556	C 909	C 846
3	C 452	C 46	C 968	C 747	C 998	R 90
4	C 799	C468	R 164	C 796	C 973	R 160
5	C 986	C 59	R 32	R 243	R 309	R 222
6	R 183	C 795	R 36	R 186	R 312	R 20
7	R 214	C 801	-	R 1	R 302	R 161
8	R 216	C 945	-	R 42	R 307	R 131
9	R 3	C 729	-	R 66	-	R 125
10	R 30	R 63	-	-	-	R 155
11	R 5	R 9	-	-	-	R 157
12	-	R 99	-	-	-	R 224
13	-	R 277	-	-	-	R 212
14	-	-	-	-	-	R 220
15	-	-	-	-	-	R 240
Total	11	13	6	9	8	15
Conilon	5	9	3	4	4	2
Robusta	6	4	3	5	4	13
Robusta (%)	54.5	30.8	50.0	55.6	50.0	86.7

[(1)]Groups (Roman numbers) and their test plants: I, 'BRS1216', Clone 12; II, 'BRS2299', 'BRS2336'; III, 'BRS3210', 'BRS3193'; IV, C1059; V, R309, C890; VI, r160, C846, C1048. S_1, S_2, S_3, and S_4, different allelic forms of the S gene; and $S_{(1,2,3,)}$, unknown allelic form which, in accordance with the observed variability, may show variation among the alleles S_1, S_2, and S_3.

2022). Natural hybrids that show the smaller plant size and drought tolerance of conilon, with the larger sieve and resistance to pests and diseases of robusta have stood out in field evaluations (Oliveira et al., 2018; Mistro et al., 2019).

The scatter plot shows greater frequencies of genotypes of the robusta botanical varieties – 85%, 100%, and 87% –, respectively in the quadrants B, C, and D. These same quadrants exhibit a greater frequency of plants of the compatibility group VI – 39%, 48%, and 23%, respectively. In quadrant A, in which conilon genotypes are more frequent, the compatibility group II is the most frequent (31%).

The compatibility determination mechanism places the S gene under negative selection depending on the frequency, which means that the frequency of an allele is inversely proportional to its adaptive value (Moraes et al., 2018). The individuals that carry rarer allelic forms of this gene benefit from the greater frequency of potential pollen donor plants, making the fertility rate of an individual inversely proportional to the frequency of the allele in the population. These conditions have some important implications: none of the allelic forms can become fixed in the population, the rate of change is greater than in a neutral locus, and the individuals of the population will be highly heterozygous.

Although the self-incompatibility is an important trait for natural plant populations of *C. canephora*, the fruit production in a crop field depends on pollination between compatible plants. Under growing conditions, this trait should be managed to not reduce the efficiency of plant pollination, which affects yield in coffee fields.

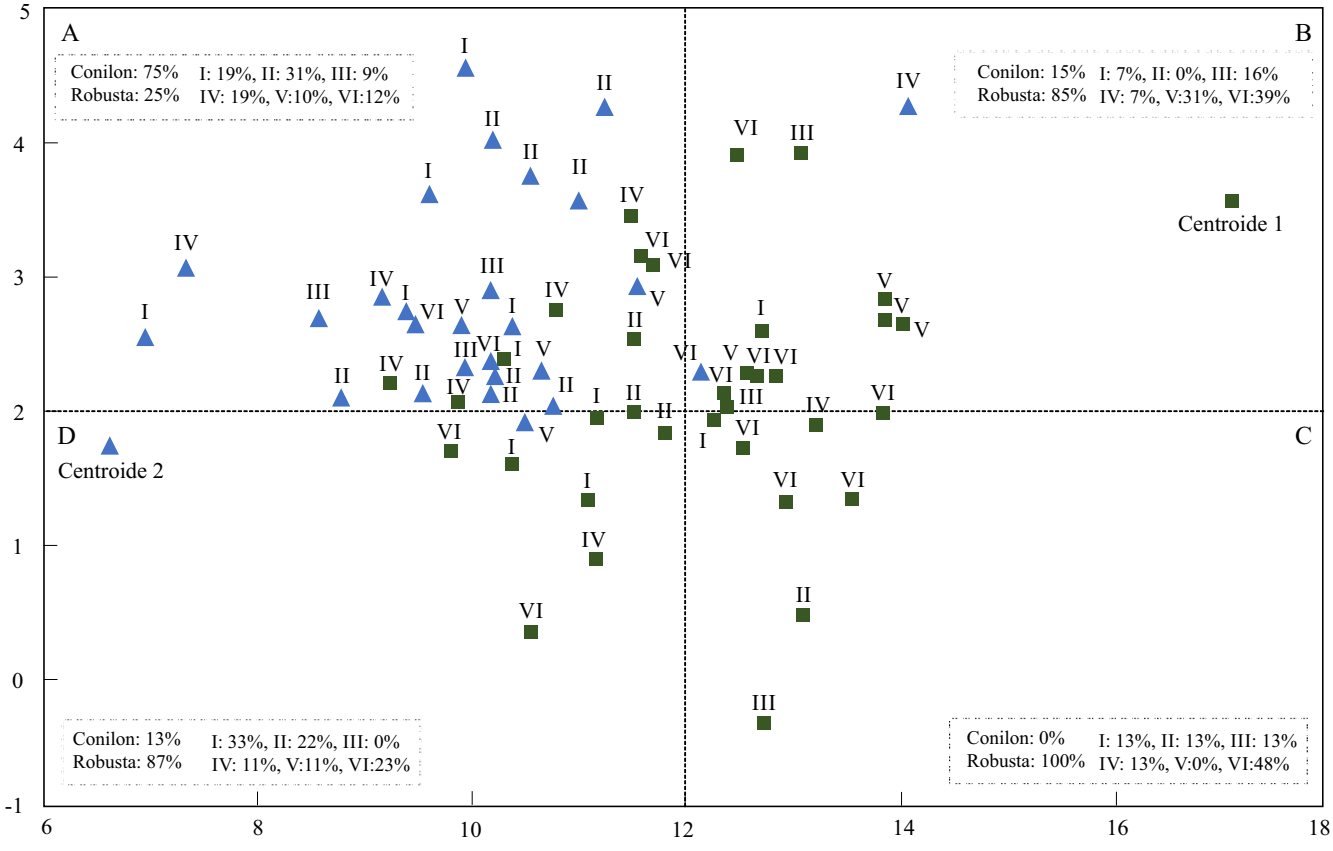

Figure 2. Scatter plot of the first two principal components of 62 *Coffea canephora* genotypes of the conilon and robusta botanical varieties, which are identified by geometric shapes (triangle, conilon; and square, robusta). The letters A, B, C, and D identify the quadrants of greatest similarity, and the centroids represent the plants with the typical characteristics of the conilon botanical variety (centroid 1) and robusta botanical variety (centroid 2). The Roman numerals represent the compatibility groups.

Conclusions

1. Through the use of previously known test plants, new compatibility groups not yet reported for *Coffea canephora* plants grown in Brazil are identified; the identification of new compatibility groups provides assistance for new management practices to increase the efficiency of pollination through natural means of favoring fully compatible crosses.

2. The comparison of the genotype grouping in their compatibility groups with the genetic diversity evaluated in the field indicates that group II exhibits the greatest frequency of plants of the conilon botanical variety (0.69), and group VI shows the greatest frequency of plants of the robusta botanical variety (0.87).

3. The larger number of hybridizations and the previous selection of the parents of greater genetic divergence of the conilon and robusta botanical varieties appears to be essential for the identification of new sources of variability.

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