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Full Length Research Paper

Introgresion of the SH3 gene resistant to rust (*Hemileia vastatrix*) in improved lines of CASTILLO® variety (*Coffea arabica* L.)

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The genetic improvement at Cenicafé has developed varieties with resistance to Coffee Leaf Rust (CLR) (*Hemileia vastatrix*) derived from the Timor Hybrid. These varieties have durable rust resistance, especially against race II, the predominant race. In Colombia, these varieties have been cultivated for three decades and still show resistance. Recently, new rust races have appeared attacking these varieties. Consequently incorporation of different resistance genes into new varieties is being sought. The present study aims to determine the presence of the SH3 resistance gene using the SCAR marker BA-124-12K-f and to evaluate its effect in F1 and F2 progenies derived from crosses of 4 lines of the Castillo® variety (*C. arabica*) with the varieties S288/23 and BA-2 introgressed with the SH3 gene. Eight F1 populations were inoculated using the detached leaf method and evaluated by the incubation and the latency periods (IP and LP). Plants 9 months old from the F2 populations were inoculated in the greenhouse and compared for sporulation presence. All F1 and F2 populations whose progenitor contained the SH3 gene-marker were resistant. However, rust resistant plants that did not present the band in the F2 progenies derived from BA-2 progenitor also had the rust resistance SH2 gene and, some plants derived from S.288 progenitor, in addition to the SH3 gene, also had the SH1 gene. The resistant F2 plants were planted in the field to be evaluated for agronomic traits and to continue the breeding process. The best progenies will be the basis future varieties aimed for durable resistance to CLR.

Key words: *Coffea arabica*, *Hemileia vastatrix*, *Coffea liberica*, SH3 gene, marker assisted selection.

INTRODUCTION

According to the International Coffee Organization (ICO, 2015), Colombia is currently the third largest producer of coffee in the world. The coffee crop contributes to the economy of millions of families around the world and approximately 529,035 in Colombia, which is responsible for a production that represents between 16 and 17% of

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National Gross Domestic product (GDP), with an annual average of 11 million bags of 60 kg (Federación Nacional de Cafeteros de Colombia FNC, 2015).

Coffee leaf rust (CLR) (*Hemileia vastatrix*) affects productivity causing losses of up to 30% in the accumulated production of a four year cycle (Rivillas et al., 2011) in susceptible varieties such as Caturra. It is now known that there are more than 50 races of the fungus which have been identified mainly in the Research Center of the Coffee Rust (CIFC) in Oeiras, Portugal (Rodrigues et al., 1975; Talhinhas et al., 2017). In Colombia, it is believed that there are more than 10 races of the fungus, with race II being the predominant one (Rivillas et al., 2011).

In order to deal with the imminent outbreaks of rust in Colombia, the National Federation of Coffee Growers of Colombia launched different varieties. The Colombia variety was launched in the 1980s (Castillo and Moreno, 1987). Later in 2000, the Tabi variety (Moreno, 2002) and more recently in 2005, the Castillo® variety and its regional components (Alvarado et al., 2005), all of them with high resistance to the disease coming from different crosses between Caturra and the Timor Hybrid. This is the genetic resource most widely used as a donor of resistance to CLR, derived from a natural interspecific cross between *C. arabica* and *C. canephora* found on the island of Timor (Bettencourt, 1973). The Colombia variety has been cultivated in the field for more than 30 years in this country. However, the cultivation of these resistant varieties in extensive areas and for very long periods favors a high selection pressure that increases the probability of occurrence of new races of the fungus. As a consequence, in the next years the gradual appearance of compatible biotypes, capable of breaking the genetic resistance displayed in the field, is expected. This is a major challenge for the genetic improvement program of the FNC (Moreno and Alvarado, 2000).

In order to offer alternatives to this problem, in the last five years Cenicafé undertook a plan to introgress the CLR-resistant SH3 gene derived from *C. liberica* (Lashermes et al., 2010) in genotypes of the Castillo® variety. First, those accessions of the Colombian Coffee Collection (CCC) bearing the gene were identified and then crossed with a group of elite lines of Castillo® with excellent agronomic characteristics (González et al., 2009).

Nevertheless, the development of resistant lines takes approximately 25 years of continuous genealogical selection based on field evaluations, including resistance to rust. To assist the selection for CLR resistance there is a marker identified by Mahé et al. (2008), linked to the SH3 gene. This marker was used to select resistant progenies in this work, allowing an earlier and a more specific selection, to incorporate the SH3 gene in new elite varieties of coffee, maintaining a durability scheme of resistance, but without sacrificing the good agronomic and productive characteristics that distinguish the current varieties.

The present study was aimed to determining the presence of the SH3 gene in genotypes derived from crosses with advanced lines of Castillo® variety, and to establish its effect on genetic resistance to rust.

MATERIALS AND METHODS

Vegetal material

F1 populations

Eight F1 populations were developed using as male parents S288/23 and BA-2 (plants 1628 and 1621) which are *C. arabica* genotypes selected in India and introgressed with the SH3 gene. As female parents, four progenies of the Castillo variety, CX2848, CX2178, CU1843 and CU1852 selected for showing rust in the field were used. It should be annotated that these four progenitors possess genes SH6, SH7, SH8 and SH9 individually or in combination and the races compatible with those are already present in the field in Colombia but are resistant to race II.

F2 populations

When the F1 populations reached reproductive maturity, were collected seeds from each of the crosses to propagate 30 F2 plants per each one. The plants selected were:

(CX2848 x S288/23) plant 7 and (CX2848 x BA-2) plant 7
(CX2178 x S288/23) plant 2 and (CX2178 x BA-2) plant 1
(CU1843 x S288/23) plant 7 and (CU1843 x BA-2) plant 1
(CU1852 x S288/23) plant 9 and (CU1852 x BA-2) plant 5

As susceptible controls, Caturra variety and the female parents were used. As a resistant control, the differential for the SH3 rust gene HW35 was used.

Molecular marker

A sequence characterized amplified region (SCAR) derived from Bacterial artificial chromosome (BAC) sequences, known as the BA-124-12K-f marker and found linked to the SH3 gene (Mahé et al., 2008), and validated by González et al. (2009) was used to identify the plants that contain the resistance gene.

Inoculum of rust

The rust inoculum used for the assembly of the different detached leaf and nursery experiments was collected in each of the four female progenitors CX2848, CX2178, CU1843 and CU1852 growing in the field. These inoculums belong to new races emerging in the field in Colombia.

DNA extraction

DNA extraction from F1 plants was performed by the method of Bernatzky and Tanksley (1986).

For the DNA extraction of the genotypes of the F2 population, the Protocol Dnaeasy Plant Mini Kit® of Qiagen was used, since the plants were in seedling stage and hence little leaf tissue was available.

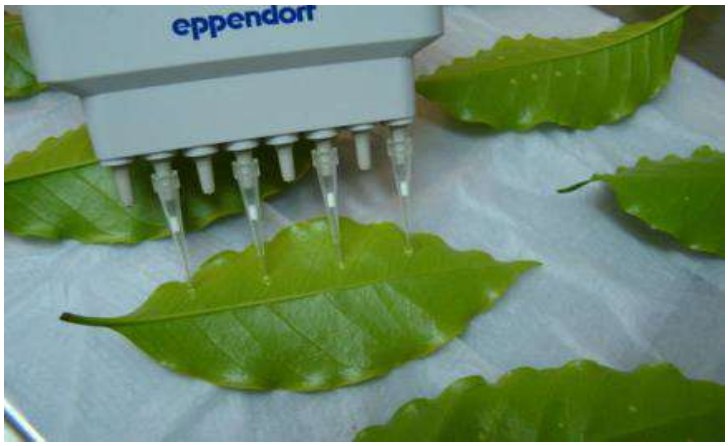

Figure 1. Inoculation of *Hemileia vastatrix.*

plants were in seedling stage and hence little leaf tissue was available.

DNA amplification

Amplification using the BA124-12K-f marker was done as described by Chen et al. (1997), with the following modifications in the PCR profile: initial denaturation was done at 94°C for 2 min, denaturalization at 94°C for 45 s, annealing at 60°C for 60 s, extension at 72°C for 75 s; then repeat the cycle from 2 to 4 for 35 cycles; next denaturation is done at 90°C for 45 s, annealing at 55°C for 60 s, extension at 72°C for 75 s; this second cycle is repeated for 30 times and terminated with an extension at 72°C for 8 min.

Marker band detection

All amplicons were evaluated on silver-stained polyacrylamide denaturing gels. Samples were prepared by mixing 10 μl of sequence loading buffer (95% formamide, 100-mM NaOH, 20-mM EDTA, 0.5 mg/mL of bromophenol blue, 0.5 mg/mL of xylene cyanol FF and deionized water) with 5 μL of amplicon, and the mix was denatured at 95°C for 5 min. For each sample, 6 μl was loaded per lane in a 4% polyacrylamide gel, and run at 2000 V, 75 W, 50 mA and 45^{o}C, maintained constant throughout the runs. Later the gels were stained with silver to visualize the bands. Gel images were scanned at high resolution and then scored by presence of the band linked to the SH3 gene.

Evaluation of rust resistance in the field

Field rust evaluations were carried out during the years 2013 to 2014, following the Eskes and Braghini scale (1981). They were done during a two years period in order to take into account the annual variation in the degree of rust attack. The evaluations were carried out in the following dates: October, 2nd, 2013, Feb.10th, July, 17th and December, 2014.

Detached leaf experiment (F1 plants):

It was carried out in the laboratory, at an average temperature of 25°C and a humidity of 80%. The light is alternated every 12 h. by means of a timer. A completely randomized experimental design was used. The experimental unit was the box, the sample unit the leaf and the number of replicates was three. From each of the plants, healthy young leaves from the second and third pair of each of the fourth upper branches exhibiting complete development were collected and immersed in a solution of DETEX® 2 ml/lt. which is a neutral liquid detergent, for 5 min and then were washed in distilled water. Eight leaves of the same genotype were placed in a pre-sterilized clear box, over a sterile paper napkin, moistened with 40 mL of sterile distilled water. The boxes were arranged randomly on a table under laboratory conditions which were measured with a thermo-hygrograph equipment. The temperature was 25°C ± 0.5 and relative humidity of 80% ±1.0. The inoculation solution with 50 mg of spores in 100 mL of sterile distilled water was applied to the underside of the leaves, depositing four drops with 10 μL of inoculum on each side of the central vein (Figure 1).

Evaluation of Incubation Period (IP) and Latency Period (LP) in each of the leaves was done according to Leguizamón (1983). Two evaluations were performed per week, starting 20 days after inoculation and for 45 days, totaling 13 evaluations.

Greenhouse experiment:

It was done for the F2 population, using 30 plants per population, 10 plants per repetition with three replicates and controls of 20 plants of Caturra and next to each crossing 6 plants belonging to the mother (the susceptible female progenitor). This method was used because the plants were only 9 months old and did not have enough mature leaves for the assembly of detached leaves; in addition the amount of genotypes to evaluate was very high to do it in the laboratory.

The inoculation was done by the spray method. The inoculum was prepared at a concentration of 50 mg of spores per 100 mL of sterile distilled water. A hose atomizer connected to a compressor was used at a distance of approximately 10 cm, to make the spray under 40 kg.m^{-2} of pressure. Eight leaves per plant were inoculated due to inoculum amount limitations in the field and the large number of plants to be assayed.

After inoculation, the plants were kept in a wet room, under dark for 48 h. A Bahnson humidifier was used to guarantee saturated atmosphere (100% relative humidity) necessary for rust infection. Subsequently, the plants were moved to the greenhouse, and organized under a randomized block design with tree repetitions. Each block or repetition was set with a row with 10 plants of each genotype, one row with 10 plants of the female parent and each

Figure 2. Distribution of blocks (lines) on the greenhouse experiment with F2 populations. Among the blocks there is a line of the Caturra variety susceptible to CLR (arrows).

block separated of the following one with a row of Caturra variety (Figure 2).

In order to identify the resistant and susceptible plants, they were evaluated twice a week until 90 days after the inoculation date, for the appearance of the rust symptoms (presence or absence of chlorosis and spores). Susceptible plants presented spores, otherwise there were resistant.

RESULTS

F1 populations

Verification of the SH3 gene-marker in the F1 populations

Molecular evaluations in three plants of each population allowed establishing the presence of the SH3 gene-marker in the F1 populations (Table 1). However, plant number 7 of the CX2178xBA-2 No.1621 cross did not present the marker, which was explained by the fact that there was no hybridization. All plants of the CU1843xBA-2 cross did not present the SH3 gene-marker. They had the BA-2 No. 1628 which was determined by molecular evaluation of the different plants from the BA-2 accession; this allowed us to conclude that the BA-2 No.1628 did not contain the SH3 gene-marker. Therefore, all of the crosses made using this plant did not present the SH3 gene-marker. On the other hand, the crosses made with the plant BA-2 No.1621 that was the progenitor that owns the gene SH3 did exhibit it. It was also observed that the susceptible controls, Caturra and females of the crosses, did not present the marker (3, a, f, g, h, i).

Detached leaves experiments

The F1 populations derived from the resistant progenitor S288/23 did not present incubation nor latency periods. They presented hypersensitivity reaction.

As expected, the control genotypes, Caturra variety and the susceptible female parents showed incubation and latency periods and the HW35 which is a differential for the SH3 gene did not present any symptoms.

The F1 populations derived from the resistant progenitor BA-2 1621 did not show incubation and latency periods with exception of one plant from the cross with CX2178. This result was explained before by the fact that there was no hybridization and it agrees with the results of the molecular analyses in which the SH3 gene-marker was not present in this plant (Figure 3j).

For the F1 population derived from the cross between CU1843 and BA-2 No. 1628 (Table 2) the plant “P1” presented incubation and latency periods; in contrast, the plants, “P2” and “P4” did not show them. As expected, none of these plants showed the SH3 gene-marker in the molecular analysis (Figure 3, c, d, e).

All of the F1 populations showing the band corresponding to the SH3 gene did not present IP and LP. For the CU1843xBA-2 No. 1628 cross, plant 1 showed susceptibility in detached leave tests and did not possess the SH3 gene; plants 2 and 4, belonging to the same cross did not have the SH3 gene band but were

Table 1. F1 Population, presence (+) and absence (-) of SH3 gene-marker.

F1 population	BA124-12K-f Gene- marker Present	BA124-12K-f SH3 gene-marker Absent
CX- 2848 x S-288/23	3	0
CU-1843 x S-288/23	3	0
CX-2178 x S-288/23	3	0
CU-1852 x S-288/23	3	0
CX-2848 x BA-2 (1621)	3	0
CU-1852 x BA-2 (1621)	3	0
CX-2178 x BA-2 (1628)	2	1
CU-1843 x BA-2 (1628)*	0	3
CX-2848 female progenitor	0	1
CU-1843 female progenitor	0	1
CX-2178 female progenitor	0	1
CU-1852 female progenitor	0	1
Caturra susceptible control	0	1
BA-2 1628* male progenitor	0	1
BA-2 1621 male progenitor	1	0
S288 male progenitor	1	0
HW35 differential of SH3 gene resistant control	1	0

*Highlighted and underlined are the crosses CX2178 and CU1843 by the BA-2 progenitor. They did not contain the SH3 gene-marker.

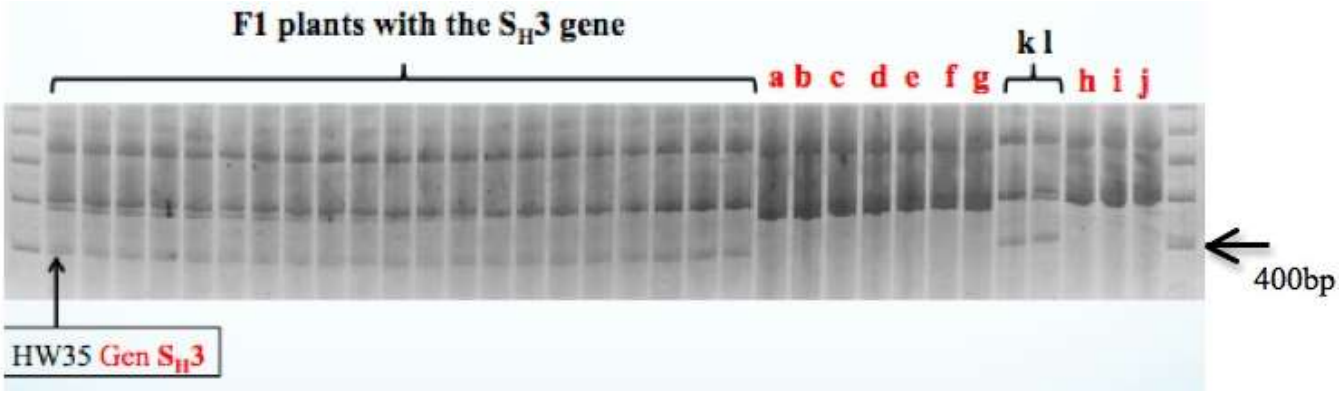

Figure 3. Image of the gel of DNA electrophoresis revealing the presence of the BA124-12K-f marker associated with the SH3 gene in F1 populations and negative controls for the SH3 gene in coffee genotypes. In order on the gel: HW35 differential that contains the band associated with the gene SH3, plants F1 with the marker; a: Caturra, b: CX2178xBA-2 P7, c: CU1843xBA-2 P1, d: CU1843xBA-2 P2, e: CU1843xBA-2 P4, f: CX2848, g: CX2178, h: CU1843, i: CU1852, j:BA-2 (1628) (Genotypes without the SH3 gene); K: BA-2 (1621) and l: S288 / 23 (progenitors with SH3 gene).

resistant. This result can be explained if the SH2 gene present in the BA-2 is in heterozygous form, and therefore, it produced resistant and susceptible plants (Castillo et al., 1976).

Plants 2 and 4 of crosses by BA-2 No. 1628 did not have the gene SH3, but they possessed the gene SH2, which also confers resistance to rust. The plant No. 1 of hybrid CU1843xBA-2 No. 1628 did not possess the SH3 gene, nor did the SH2 gene as explained above and showed IP and LP (Table 2).

The results of the field evaluations coincided with the detached leaves evaluations. Only plant 1, belonging to the cross of CU1843xBA-2 No. 1628 showed the disease, which is explained by the absence of the SH3 and SH2 genes. The rest of the plants so far are still unaffected by rust (*H. vastatrix*).

Table 2. Incubation and latency periods for populations of the CU1843 x BA-2 No. 1628 cross.

Genotype	Days to			SH3 gene-marker presence
	Box	IP	LP	
CU1843XBA-2 P1	1	30	41	-
CU1843XBA-2 P1	2	43	48	-
CU1843XBA-2 P1	3	42	43	-
CU1843XBA-2 P2	1	75*	75*	-
CU1843XBA-2 P2	2	75*	75*	-
CU1843XBA-2 P2	3	75*	75*	-
CU1843XBA-2 P4	1	75*	75*	-
CU1843XBA-2 P4	2	75*	75*	-
CU1843XBA-2 P4	3	75*	75*	-
HW35	1	75*	75*	+
HW35	2	75*	75*	+
HW35	3	75*	75*	+

*When the number of days for IP and LP is 75, it indicates that there was no incubation and latency periods. Presence (+), absence (-) of SH3 gene.

F2 populations

Greenhouse experiment

After DNA extraction, the SH3 gene was verified by the specific molecular marker BA124-12K-f. Thirty F2 plants of each of the 8 crosses of each of the female parents CX2848, CX2178, CU1843 and CU1852 by each of the male parents (BA-2 and S288 / 23) for a total of 240 plants, were evaluated for the presence or absence of the SH3-marker and for the presence or absence of rust symptoms in F2 populations (Table 3).

CX2848XS288/23 and CX2848xBA-2

For the 30 plants of the CX2848xS288/23 cross, 26 plants were resistant in the greenhouse tests and 15 of them showed the band corresponding to SH3 gene-marker.

In the case of the plants of the CX2848xBA-2 (1621) cross, 27 plants were resistant and 7 presented the band for the SH3 gene-marker.

CX2178XS288/23 and CX2178xBA-2

For the 30 plants of the CX2178xS288/23 cross, 23 plants did not present rust and from them 4 did not present the band associated to the SH3 gene. For the 27 plants of the CX2178 xBA-2 cross, 18 plants were resistant and 6 had the band linked to the SH3 gene.

CU1843xBA-2 and CU1843XS288 / 23

According to the results of Table 3, for the 30 plants of the cross CU1843x BA-2 none had the SH3 gene-marker. However there were 15 resistant plants. Looking at the 30 plants of the cross CU1843xS288/23, 23 plants showed resistance to CLR and 18 had the band associated to the SH3 gen.

CU1852xBA-2 and CU1852xS288 / 23

Of the 26 plants of CU1852xBA-2 cross, 25 presented resistance and 20 plants contained the band associated with the SH3 gene. For the 30 plants of the cross CU1852xS288/23 there were 29 resistant plants and 23 of them showed the band for the SH3 gene.

DISCUSSION

F1 populations

Evaluations of resistance to rust on detached leaf experiment

In the present study, 240 genotypes belonging to improved lines of Castillo® introgressed with *C. liberica* were evaluated molecularly using the BA-124-K-f marker associated to the SH3-gene that confers resistance to CLR, which was corroborated by detached leaves experiment and field evaluations (data not presented). CX2178xBA-2 No.1621 cross did not present the marker, which was explained by the fact that there was no hybridization. None of the plants from the cross CU1843 x BA-2 (1628) showed the SH3 gene-marker.

Molecular evaluations in three plants of each population allowed establishing the presence of SH3 gene in F1 populations. However, plant number 7 of the this could be

Table 3. Evaluation of the marker linked to the SH3 gene and its reaction to CLR in eight progenies F2.

F2 progeny	Marker linked to SH3 gene			
	Absence		Presence	
	Without rust	With rust	Without rust	With rust
CX2848xS288/23	11	4	15	0
CX2848xBA-2	20	3	7	0
CX2178xS288/23	4	7	19	0
CX2178xBA-2	12	9	6	0
CU1843xBA-2	15	15	0	0
CU1843xS288/23	5	7	18	0
CU1852xBA-2	5	1	20	0
CU1852xS288	6	1	23	0

explained by molecular evaluation of different plants of BA-2 accession, which allowed us to establish that the BA-2 No. 1628 did not contain the SH3 gene-marker. Therefore, all of the crosses made using this plant did not present the SH3 gene-marker. On the other hand, the crosses made with the plant BA-2 No.1621 that was the progenitor that owns the gene SH3 did exhibit it.

As mentioned, F1 populations were obtained from crosses with male parents carrying the SH3 resistance gene from crosses carried out by the breeding program of India. Specifically the male progenitor S288/23 was selected after a process of successive self-fertilization from the accession S.26 (*C. arabica* x *C. liberica*). The male progenitor BA-2, was derived from the F1 of S288/23 x Kent variety (Prakash et al., 2004). The molecular marker (BA-124-12K-f) was reported by Mahé et al. (2008) as a marker linked to the SH3 gene. Gonzalez et al. (2009) used it to identify the gene band in our F1 populations. The presence of the SH3-marker was confirmed by rust resistance observed in all plants of our F1 population.

The detached leaves evaluations coincided with the molecular evaluations, with the exception of crosses by CU1843xBA-2 (1628) plants 2 and 4 that did not possess the SH3 gene and yet did not develop a latency period, which is explained by the presence of the SH2 gene in the BA-2 parent (Castillo et al., 1976).

The remaining crosses that presented the band corresponding to the SH3 gene did not develop incubation period and latency period, indicating their resistance to rust. Similar results were obtained by Prakash et al. (2011) when he did MAS using the same marker in F2 progenies of crosses between *C. arabica* and the S. 795 donor of the SH3 gene.

The results of field evaluations were consistent with molecular and detached leaves evaluations. Those that presented the band linked to the SH3 gene and were resistant in the laboratory, also showed resistance in the field. This indicates that the races compatible with this resistance gene did not yet exist in the field.

In addition, the results for two plants of CU1843xBA-2 that did not possess the SH3 gene and yet did not develop a latency period was explained by the resistance conferred by the SH2 gene that is part of the genetic composition of the BA-2 (Castillo et al., 1976).

Therefore it is important to use the SH2 gene as a new source of resistance to rust, pyramided together with the SH3 gene, which may allow the achievement of more durable resistance.

F2 populations

Evaluations of resistance to rust on greenhouse experiment

In the evaluation, segregation of resistant and susceptible plants was observed, which is to be expected in this generation. In F2 populations the two alleles codifying for each trait are segregated during gamete production. This means that each gamete will contain a single allele for each gene allowing the maternal and paternal alleles to recombine to ensure variation in their offspring.

The fact that the susceptible control and the female progenitors showed CLR, guarantee that the inoculation was efficient and the inoculum used was not of race II. Because of that the results on the F2 plants is reliable. On the other hand the evaluation was done using very unequivocal variables such as presence or absence of chlorosis and spores twice a week for three months. Besides, the progenies in the greenhouse were organized on blocks with three repetitions. All of these factors allow the obtention of reliable results.

The plants that showed the SH3 gene-marker were resistant to rust (Table 3). This suggests that races that recognize it have not yet developed in Colombia. Besides, some plants that did not present the band associated to the SH3 gene showed resistance, which implies presence of other resistance genes.

In the case of crosses with the BA-2 progenitor, 52 resistant plants did not show the SH3 gene-marker, which is explained because, in addition to the SH3 gene,

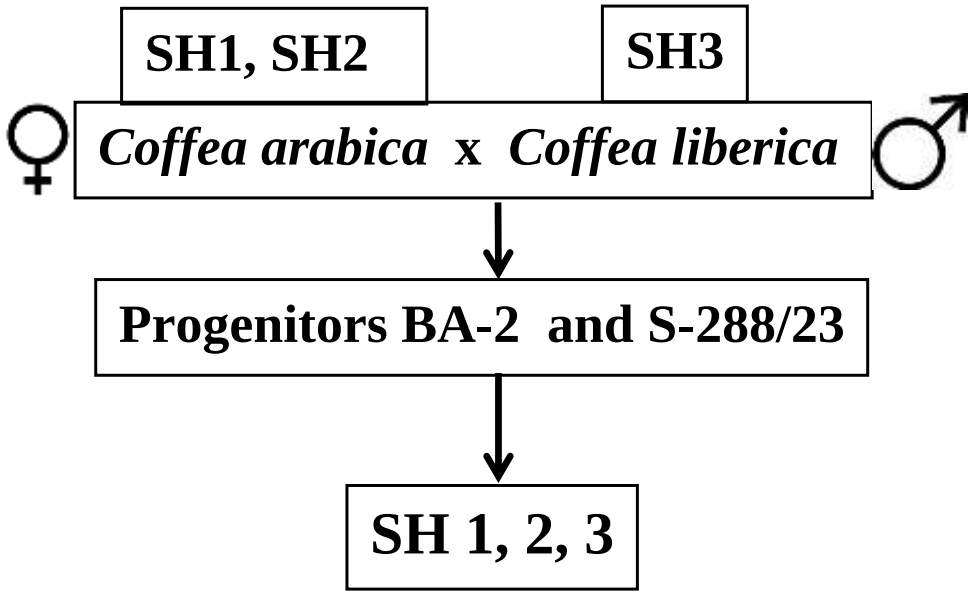

Figure 4. Genealogy of male progenitors BA-2 and S288/23 used to obtain F2 populations.

they also contain the SH2 gene (Castillo et al., 1976). Resistance in the 26 plants derived from crosses with the S.288 progenitor that did not have the presence of the SH3 gene, was probably due to the SH1 gene that according to Ramachandran and Srinivasan (1979) is also carried by this progenitor.

Comparing greenhouse and detached leaf evaluations, it can be said that both have advantages and disadvantages.

In the lab less time is required to obtain the results of inoculation, while in the greenhouse the experiment should be kept for longer periods of time to assure that there is enough time for the symptoms to appear. The inoculation is easier by aspersion in the greenhouse, although the amount of inoculum required is higher. From the point of view of plant breeding, the greenhouse evaluation is more convenient, given that during the breeding process, it is necessary to have the plants in greenhouse before planting them in the field. Furthermore, there is no limitation of infrastructure as there is in the detached leaf experiment.

So far, the resistance genes that have been used, have provided resistance with significant durability, but over time the rust races have evolved to the point of developing disease. For this reason, it is also important to use the plants that showed resistance in these evaluations, because they contained different rust resistance genes besides the SH3 (Figure 4). The genes SH1, 2, 3, 4 and 5 do not provide durable resistance because they have been used individually as mentioned by Prakash et al. (2005) in India. The use of those genes in mixture with the ones derive from Timor hybrid, in a multiline variety will allow durable resistance, as have been demonstrated with the Colombia variety, in which this strategy has been used and after 30 years is still resistant. In general, this strategy consist in utilize the higher number of resistance genes as possible as a diversity strategy (Browning and Frey, 1969). Van der Vossen (2005) also states that durable resistance is related to the effect of a combination of genes, either by stacking (pyramiding) of major genes, or by the accumulation of several minor genes. This implies that the CLR needs to have much more mutations for the variety to become susceptible.

In studies conducted by Hiroshi et al. (2007), SH1, SH2 and SH4 genes from *C. arabica* in combination have provided lasting resistance. Therefore, if the resistance of the genotypes belonging to the F2 population, which do not carry the SH3 gene, comes from some combination of SH1, and SH2, it increases the variability and durability of rust resistance (*Hemileia vastatrix*). Therefore, derived progenies containing all these resistance genes should be maintained in the successive selection cycles evaluating them for agronomic traits in order to select the best, and advance to the F3 generation.

According to Browning (1974), the equilibrium observed on natural ecosystems is based on combination of different mechanisms of resistance, which include general and specific resistance, tolerance and antagonisms between pathogens and non-pathogens. In Colombia, based on those principles, the breeding program has as strategy, namely genetic diversity, to develop resistant varieties.

That is to say that the varieties are composed of several lines with different combination of genes. This strategy have proved successful because after thirty year of the varieties liberation, they are still resistant. Indeed the combination of different sources of resistance (SH1,

SH2 and SH3) with resistance lines that have combinations of resistant genes from Timor Hybrid, will be of great value in the breeding program.

Unlike the field evaluations that have a response to the race or races prevalent in the field, the methodologies used for the evaluation of resistance allow to choose the inoculum from the race or races that need to be evaluated.

Conclusion

This work confirms that the SH3 gene from *C. liberica*, provides genetic resistance to rust to the genotypes derived from crosses with advanced lines of the Castillo® variety. The absence of rust in the progenies F1 and F2 evaluated, which contain the SH3 gene from *C. liberica*, indicates that the rust gene Vr3 compatible with this gene (SH3), is not yet recognized by the host plant, and consequently, at the moment there are no races that contain this gene in the field. The implementation of the BA-124-12K-f marker in selection of resistant plants that contain the SH3 gene allows doing earlier selection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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