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RESEARCH ARTICLE / *ARTIGO*

New coffee (*Coffea arabica*) genotypes derived from *Coffea canephora* exhibiting high levels of resistance to leaf rust and Ceratocystis canker

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ABSTRACT

The purpose of this study was to evaluate the resistance to coffee leaf rust (CLR) caused by *Hemileia vastatrix* and to Ceratocystis canker (Cc) in coffee genotypes derived from crosses of *Coffea arabica* var. Caturra with accessions of *C. canephora* backcrossed to Caturra. Twenty-three F_3BC_1 progenies including *C. arabica* var. Caturra and var. Colombia as controls were established in a field experiment. CLR evaluations were made during five years of natural infection, using an incidence rating scale. For Cc, artificial stem inoculations were made with an isolate of *Ceratocystis colombiana* and the results were assessed after one year. The selection process also included agronomic aspects such as plant height, canopy diameter, number of branch pairs, yield and grain characteristics. Twenty progenies showed >70% of rust resistance. Twelve progenies exhibited >80% of Cc resistance, while no resistance was observed in either of the controls. Only three progenies performed well for all criteria, including resistance to both pathogens and agronomic characteristics.
Key words: *Ceratocystis colombiana*, *Ceratocystis fimbriata*, *Hemileia vastatrix*, genetic resources, robusta coffee.

INTRODUCTION

Two major diseases that are currently responsible for significant yield reduction in Colombian coffee production are coffee leaf rust (CLR), caused by the obligate pathogen *Hemileia vastatrix* Berk. & Broome (Rivillas et al*.,* 2011) and Ceratocystis canker (Cc), caused by the soil-borne fungi *Ceratocystis papillata* Van Wyk & Wingf. and *C. colombiana* Van Wyk & Wingf. (Van Wyk et al., 2010). Of these, CLR is the best known and arguably the most damaging coffee disease in the world, resulting in crop losses of 20-40% where no control measures are used (Van der Vossen, 2005; Rivillas et al., 2011). Recently, reports from Central American countries suggest up to 80% defoliation caused by this disease (Cressey, 2013).

At least 49 rust races have been identified in coffee using a set of more than 40 coffee differentials, including the two commercial species cultivated in the world: *Coffea arabica* L. (tetraploid 2n=4x= 44) and *C. canephora* Pierre: A. Froehner (diploid 2n=2x=22) (Varzea & Marques, 2005; Gichuru et al., 2012). The pathogen *H. vastatrix* has (with various periods of delay), followed the spread of *C. arabica* around the world. This includes the Americas, where the traditional Typica and Borbón varieties, and their derivatives, are susceptible to race II (genotype v5 v5), the most predominant race in the world (Eskes et al., 1989; Van der Vossen, 2005). In Colombia, where only *C.* a*rabica* has been cultivated, CLR race II was reported for the first time in 1983 affecting var. Caturra (Leguizamón et. al., 1984). Subsequently, more than 10 different races have been identified (Castillo & Leguizamón, 1992; Gil & Ocampo, 1998; Alvarado, 2005; Cristancho et al., 2007). After the severe epidemics reported in Colombia during 2008-2011, with losses above 30% (Cristancho et al., 2012), molecular studies showed that race II and its derivatives prevail in this country (Rozo et al., 2012).

Several breeding programs have developed durable resistance against CLR, using the dominant major resistance genes (Sh) identified in some *Coffea* species (Van der Vossen, 2005). Nine dominant genes involved in resistance to *H. vastatrix* have been identified so far (Bettencourt & Rodriguez, 1988). Genes S_H1, S_H2, S_H4 are present in non-commercial varieties of *C. arabica*; S_H5 is present in commercial varieties of *C. arabica* such as Caturra, Typica and Borbón, among others (Bettencourt & Rodriguez, 1988). Gene S_H3 appears to come from *C. liberica* W.Bull.: Hiern. and genes S_H6 to S_H9 have been introgressed from *C. canephora* in some introductions of the Timor Hybrid (TH), which is a self-fertile spontaneous tetraploid (2n=44) originating from *C. arabica* and *C. canephora*. Among these TH introductions are CIFC-1343, CIFC- 832, CIFC-2570, and their resistant genes come from some accessions of *C. canephora* (Bettencourt & Rodrigues, 1988). Mahe et al. (2007) reported new sources of rust resistance in other

natural interspecific hybrids from New Caledonia, and more recently, Brito et al. (2010) identified one additional resistance gene to race II of *H. vastatrix* in TH-UFV-427-15. Fernandez et al. (2012) suggested potential novel rust resistance genes from genomic studies.

Most coffee breeding programs have used TH as the main source of CLR resistance (Van der Vossen, 2001). In Colombia, TH accession 1343 has been the source of major resistance genes transferred into the dwarf variety *C. arabica* var. Caturra (CLR susceptible), which resulted in the resistant varieties Colombia (Castillo & Moreno, 1988) and Castillo (Alvarado et al., 2005). TH 1343 is also the parent of the Tabi variety, arising from cross between Typica and Borbon (Moreno, 2002). These varieties are currently planted in ~ 58% of Colombian coffee growing areas (FEDERACAFE, unpublished).

Despite the success of CLR breeding programs, there is evidence that some improved commercial varieties derived from TH have lost their resistance due to the possible emergence of new virulent races of the pathogen (Várzea & Marques, 2005; Prakash et al., 2005; Alvarado, 2005, Gichuru, et al., 2012). Variability in virulence of the pathogen could be due to natural mutation processes, but it could also arise from other mechanisms such as cryptic sex, a hidden sexual reproduction of the pathogen (Carvalho et al., 2011). Therefore breeding programs have focused on efforts to broaden the genetic base of commercial coffee varieties. This especially has been through the exploration of alternative resistant resources, mainly from diploid species such as *C. canephora* or *C. liberica* and the introgression of genes into *C. arabica* (Varzea & Marques, 2005; Herrera et al., 2002a, 2002b). *Coffea arabica* is characterized by its autogamous nature and low genetic diversity, while diploid coffee species such as *C. canephora* are reported as allogamous with considerable variability (Berthaud & Charrier, 1988; Anthony et al., 2002). This includes resistance to other diseases, such as those caused by nematodes (Bertrand et al., 2001; Noir et al., 2003) and possibly to coffee berry disease (CBD) caused by *Colletotrichum kahawae* Waller & Bridges (Gichuru et al., 2006). Hence, the introgression of desired characters from diploid species into cultivars of *C. arabica* has been a priority in coffee breeding (Van der Vossen, 2001).

Ceratocystis canker (Cc), known in most Latin-American coffee producing countries as "llaga macana" or "trunk canker" is an important disease of coffee in Colombia (Castro et al., 2003). Fernández (1964) described the symptoms in coffee plants in some provinces of Colombia caused by the soilborne pathogen *Ceratocystis fimbriata* Ell.& Halst. Chlorosis, dieback and wilt are the external symptoms in plants that have been affected in their vascular tissues, primarily in the stem, where the dark lesions extend upwards or downwards, girdling the trunk and causing tree death. Currently the disease is found in all the Colombian coffee-growing areas (Marin et al., 2003) and all commercial coffee varieties (*C. arabica*) planted in this country are susceptible to the disease (Castro et al., 2003). Mechanical injuries (fresh wounds) are the main sources of entry for the pathogen in coffee plants. These wounds arise from farmers stabilizing their boots on the trunks of trees in order to support themselves on the steep slopes where coffee is cultivated, and also from pruning, leading to "llaga macana" (Castro et al., 2003).

Ceratocystis fimbriata sensu lato (s.l.) complex is associated with vascular diseases in a large number of plants in many parts of the world. Webster & Butler (1967) recognized that *C. fimbriata* probably represented more than one entity. Baker et al. (2003) hypothesize that local populations of *C. fimbriata* in some Latin American plants have become specialized on different hosts. Studies developed during the last twelve years with isolates from both soil and plants in affected Colombian coffee areas have revealed two phylogenetic lineages (Barnes et al., 2001; Marin et al. 2003). Based on morphological and DNA sequence comparison of isolates from different hosts in Colombia (coffee, cocoa, citrus and native forest), Van Wyk et al. (2010) named the two lineages *Ceratocystis colombiana* Van Wyk & Wingf. and *C. papillata* Van Wyk &Wingf. Both species are pathogenic, causing death of coffee plants artificially inoculated in their stems (Marin et al., 2003; Van Wyk et al., 2012).

Apparently all *C. arabica* commercial varieties are susceptible to Ceratocystis canker, however, an exceptional case of resistance has been reported in a line of *C. arabica* var. Borbón. Such resistance is characterized by the formation of lignified tissues surrounding infection sites and preventing the girdling of plant stems (Fernández, 1964). In the "immune" *C. canephora* and *C. liberica*, necrotic lesions do not develop due to rapid wound closure (Echandi & Fernández, 1961; Izquierdo, 1988). Recently such "immunity" was also recorded in the Colombian National Center of Coffee Research (Cenicafé, Colombia) in some accessions of *C. canephora* and *C. liberica* in artificial inoculations with *C. colombiana* and *C. papillata* (Bertha L. Castro, unpublished).

Selection and breeding for disease resistant/tolerant coffee cultivars remains an ongoing challenge and priority in Colombia. Besides developing rust resistant commercial coffee varieties, it has also been necessary to consider resistance to Ceratocystis canker in these. Using the resistant Borbón line, Castro & Cortina (2009) developed Caturra-like genotypes with resistance to "llaga macana," but these were susceptible to rust. Hence, development of varieties resistant to both rust and Cc is necessary. One approach would be to develop promising genotypes from gene introgression into *C. arabica* by the way of triploid hybrids, through crossing of *C. arabica* x *C. canephora* (Orozco, 1976; Alvarado & Cortina, 1997; Herrera et al., 2002a, 2002b). Following this strategy, researchers at Cenicafé crossed tetraploid *C. arabica* var. Caturra with accessions of diploid *C. canephora,* generating triploids backcrossed (BC) to Caturra. Based on rust resistant

tetraploid F_2BC_1 genotypes, the aim of this study was to select F_3BC_1 progenies simultaneously resistant to both CLR and Ceratocystis canker. Furthermore, the process of selection included desirable agronomic characteristics and bean attributes that will be useful for future commercial varieties.

MATERIAL AND METHODS

Genotypes and field experiment

Twenty-three F_3 progenies produced by crossing *C. arabica* var. Caturra with three accessions of *C. canephora* and backcrossed with var. Caturra (F_3BC_1) were included in the study. The different parental accessions were selected from Cenicafé's germplasm collection (Chinchiná, Colombia). These tetraploid interspecific hybrids, labeled as MEG 639, were generated by crossing the *C. arabica* var. Caturra as female parent (susceptible to both CLR and Cc) with accessions of *C. canephora* (BP.358-EA.93; BP.358-EA.239 and BP.46-EA.131) known to be CLR resistant, and backcrossed to Caturra. Two controls, *C. arabica* var. Colombia (CLR resistant, but Cc susceptible) and var. Caturra (susceptible to both pathogens) were included.

Field plots were established at Cenicafé Central Experiment Station (04°58' NL, 75°39' W, 1381 m) in 1998. The site has an annual average precipitation of 2556 mm, sunshine of 1816 hours/year, relative humidity of 78% and a mean temperature of 20.8°C (Cenicafé, 2008). Trees were planted in a square lattice (5 x 5) and two replicates were used per treatment in the experimental design. The experimental unit was a line of 10 plants with distance of 1.6 x 1.0 m between plants and 2.5 m between blocks. Standard agronomic management was applied.

Coffee leaf rust evaluation

Incidence of *H. vastatrix* infection was evaluated from January 2000 to August 2005, during periods of heavy rust outbreaks as defined by Sierra et al. (1991). The rating scale of Eskes & Braghini (1981) was used for plant evaluation. This scale grades the whole plant as a unit of observation on a visual scale (0 to 9), where 0 = absence of sporulating lesions; 1 = presence of one diseased branch; 2 to 8 = gradual increase in number of diseased branches with sporulating lesions; and 9 = maximum disease incidence. At each evaluation, the number of plants with rust was scored and grouped into three categories: uninfected plants (grade 0); plants with a low level, considered resistant (graded 1 to 4) and plants with high level of infection, as susceptible (graded 5 to 9). The maximum grade of each tree was recorded along with the frequency of plants of each grade, according to the criteria of Alvarado & Cortina (1997).

Ceratocystis canker evaluation

When the plants reached an age of seven years, they were inoculated with isolate CMW 34925 of *Ceratocystis colombiana* (GenBank accession number of ITS-rDNA sequence KF300545) (Van Wyk et al., 2010), previously identified as highly virulent by Castro & Cortina (2009). The inoculum was prepared as previously described by Marin et al. (2003). Drops of 70 µL containing approximately 3.0 x 10^4 ascospores/mL, were inoculated into inverted U-shaped wounds, approximately 2.0 cm in diameter, made on the stems at ~1.40 m above soil line. The spore suspension was inserted under the bark and sealed with parafilm.

Fifteen days after inoculation, the parafilm was removed and pathogen colonization was verified. Red paint was applied to the trunks below the site of inoculation to further identify plants and to aid in later assessments. One year after inoculation, the size of the lesions under the stem bark was determined. Three measurements were made for each plant, including stem circumference (SC), width of necrotic lesion (WNL) and lesion length (LL). The width of stem affected (WSA) by the necrotic lesion was expressed as percentage of the stem circumference (SC) girdled (WNL/SC x 100).

Evaluation of agronomic characteristics

All plants were evaluated for plant height (cm), canopy diameter (cm) and number of branch pairs at 24 months of age. Yield data per plant were also recorded from 2001 to 2005 in kg of fresh berries per tree. The percentage of bean defects such as empty beans in ripe coffee fruits and defects of dry parchment beans such as "peaberry" ("caracol") and "triangle" were evaluated in two peaks of yield in 2001 and 2002. For empty beans, the floating method was used. The size of dry beans (Supreme type) was determined as the percentage of husked beans (green coffee) retained by a 17/64 inches mesh. The assessment of these bean characteristics was made according to the methods of Castillo & Moreno (1988) and Moreno and Alvarado (2000), as well as the coffee quality standards (FEDERACAFE, 1988).

Data analysis and selection of promising genotypes

For CLR, a frequency distribution per progeny was developed for each assessment and the maximum rating was considered with these results expressed as percentage of plants affected. Data were grouped based on a severity scale from 0 (uninfected plants), grade 1 to 4, and grade 5 to 9. Progeny with ≥ 70% of plants graded 0-4 on the CLR scale were selected as resistant because at this level there is no effect on productivity (Eskes & Braghini, 1981; Alvarado & Cortina, 1997).

To analyze the Cc data, the mean values of the measurements for each coffee genotype were calculated and analysis of variance (ANOVA) was performed for the WSA and LL data at a significance level of P = 0.05. Tukey's test (P = 0.05) was used to compare the results for different progenies. All analyses were done using the SAS statistical program (SAS Statistical Software, 2010). Resistant genotypes were selected as those where >80% of plants had WSA values lower than 50% and with lesions

smaller than those of the susceptible controls, following the method previously used by Castro & Cortina (2009).

For agronomic characteristics, ANOVA was performed for plant height, canopy diameter and number of branch pairs as well as grain characteristics and annual yield over five years. The yield data were taken from harvests obtained over five years and the average (kg of fresh berries/tree/year) was transformed to kg of dry parchment coffee/plant/year, using the conversion factors of Montilla et al. (2008). Where statistical differences were found among the 23 progenies tested, Dunnett´s test (P = 0.05) was used to compare the results with those for var. Caturra. Average agronomic characteristics equal to or better than those of the controls were the criteria used to select promising plants for future use.

RESULTS AND DISCUSSION

Coffee leaf rust evaluation

On the basis of 12 evaluations from 2000 to 2005, clear differences among progenies, as well as between the progenies and the two control varieties, were found (Figure 1). The var. Caturra was the most susceptible of all genotypes tested, with all plants scoring between 5 and 9 on the rating scale. In contrast, progenies MEG639-410, MEG639-475, MEG639-705 and MEG639-708 were the most resistant, with 100% of plants graded 0 to 4 on the disease assessment scale. Eighteen progenies had greater than 70% of the plants graded 1 to 4, and these were also considered resistant.

Since 1983, when CLR (Race II) was detected in Colombia (Leguizamón et al., 1984), there has been a gradual increase in disease severity, depending on weather conditions (Sierra et al., 1991; Rivillas et al., 2011, Rozo et al., 2012). During the course of the current project, the highest CLR incidence (>60%) was found on var. Caturra in 2003 and 2005. In contrast, very low levels (<30%) of rust were observed in 2004. This behavior is related to climatic conditions as well plant vigor and size of the harvest, amongst other factors (Kushalappa & Eskes, 1989; Costa et al., 2006).

Overall, results of this study indicate that the selection of CLR resistant progeny made in earlier generations (F_2BC_1) assured an 87% level of resistance in the presently used progenies (F_3BC_1). The resistance found in var. Colombia indicates that, while the resistance genes in this variety come from the *C. canephora* through the TH/1343 (Castillo & Moreno, 1987), the rust resistant genes in the F_3 progenies studied are different from the ones present in the varieties Colombia, Tabi and Castillo, whose resistance introgressed from the unique TH. This is evident in our study, because at least seven progenies had some plants totally resistant, while all progeny of var. Colombia were infected, although at a low level. This possibility was also raised by Mahe et al. (2007), who studied the genetic diversity and rust resistance in natural interspecific hybrids between *C. arabica* x *C. canephora* from New Caledonia (HNC) and found resistance to all rust races in some progeny. Based on molecular data, they suggested a high level of genetic diversity of *C. canephora* progenitors at the origin of HNCs. For Colombian coffee, this new source of resistance genes may have important consequences. After the last severe rust outbreak of 2008-2011 (Cristancho et al., 2012), the susceptible var. Caturra has been replaced by commercial resistant multiline varieties derived from TH/1343. The increasing area planted with these lines may intensify the selection pressure on the pathogen, favoring the emergence of compatible races and thus threatening resistance durability. Therefore, the incorporation of new genotypes with different rust resistant genes, such as those selected in this study, into current breeding programs should increase the stability and durability of CLR resistance.

Ceratocystis canker evaluation

There was variability in the resistance reactions against Cc in the genotypes tested. Twelve progenies were statistically different to the susceptible control varieties in WSA, with <50% of the stem circumference affected by lesions (Figure 2). Eight of these progenies were also significantly different from the controls in LL (Figure 3). Progenies MEG639-708, MEG639-617, MEG639-841, MEG639-620, MEG639-601, MEG639-842, MEG639-704, MEG639-602, MEG639-609, MEG639-475, MEG639-771 and MEG639-705 were the most resistant to *C. colombiana* infection, compared with susceptible control varieties, which were killed by the pathogen. Progenies MEG 639-841, MEG 639-617 and MEG 639-708 exhibited the smallest lesions, suggesting a possible form of "immunity". This last resistant reaction was also noticed by Echandi & Fernández (1961) in inoculations with *C. fimbriata s.l* on *C. canephora* and *C. liberica* in Guatemala, and by Izquierdo (1988) in Cuba on *C. canephora*.

Among the progenies with resistance reactions, strong callus formation was observed surrounding the necrotic lesions (Figure 4A and 4B) with small amounts of discoloured tissue in the underlying wood (Figure 4C). In contrast, wood discoloration spread either upwards or downwards, and there was an absence of an obvious defense response in susceptible controls or susceptible progenies (Figure 4D). Our results showed clear evidence of resistance to *Ceratocystis* infection, probably conferred by the parental *C. canephora* through the hybridization, and the resistance persisted after backcrossing to the susceptible var. Caturra. This form of resistance is mentioned in other studies (Echandi & Fernández, 1961; Izquierdo, 1988); as well as in recent studies performed at Cenicafé (Castro unpublished) in inoculations with *C. colombiana* and *C. papillata* on the diploid coffee species *C. canephora* and *C. liberica*. The progenies selected in the present study may give rise to new resistant genes and may be valuable as efforts are made to reduce the damage caused by *Ceratocystis* spp. in Colombia.

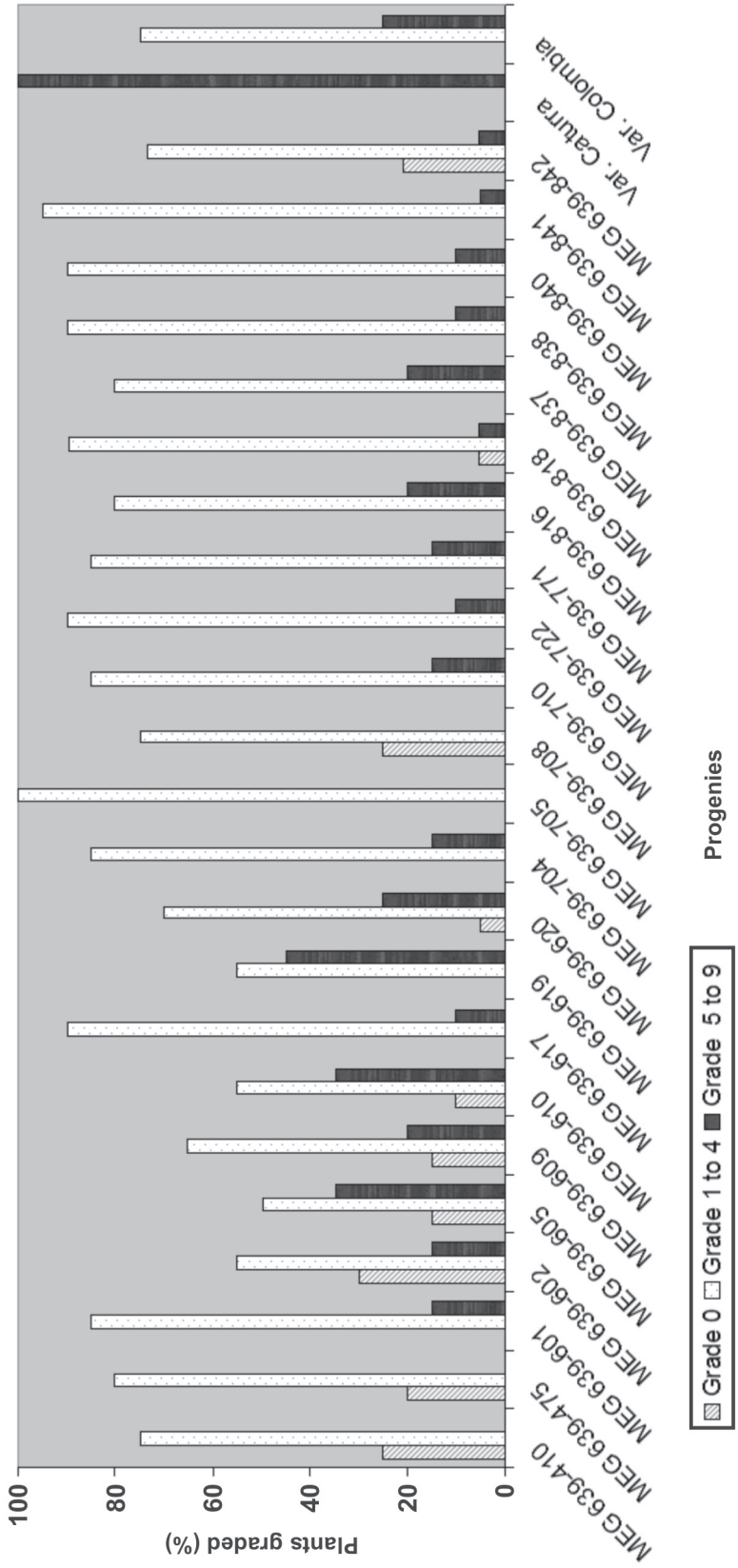

FIGURE 1 - Maximum percentage of plants infected by *Hemileia vastatrix* for each progeny set in 12 assessments, based on Eskes & Braghini scale (0 to 9).

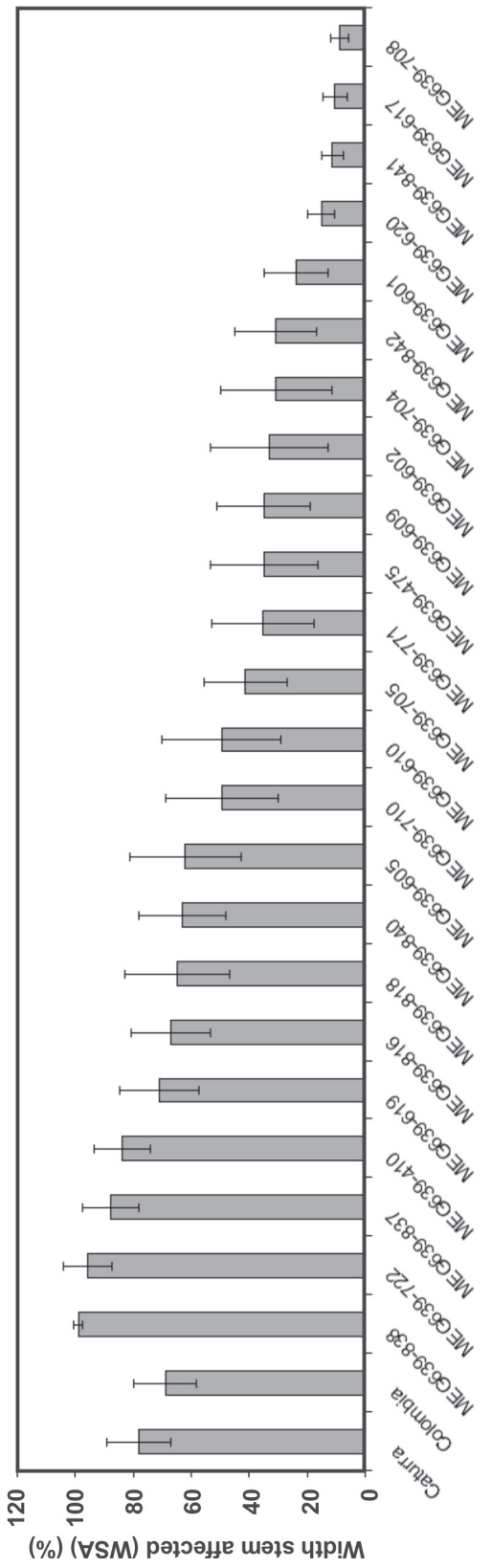

FIGURE 2 - Resistance reactions of coffee progenies to infection by *Ceratocystis colombiana*. Means (%) of width stem affected (WSA), one year after inoculation. Bars represent the standard error (F= 0.05%).

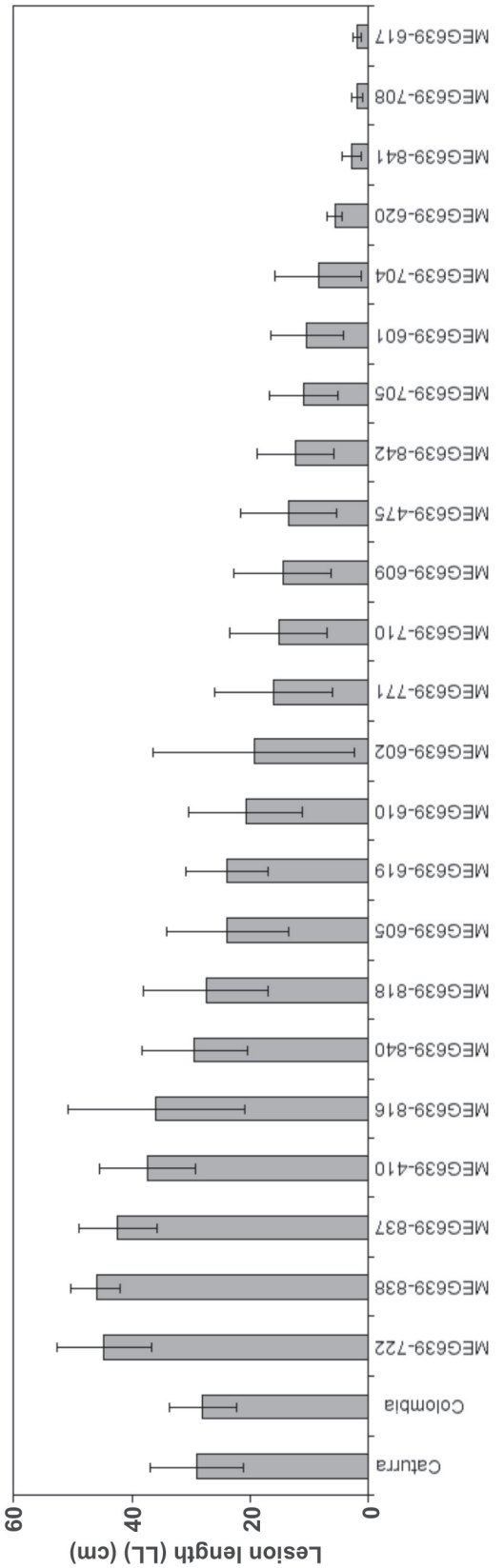

FIGURE 3 - Resistance reactions of coffee genotypes to infection by *Ceratocystis colombiana*. Means of lesion lengths (LL) in cm, assessed one year after inoculation. Bars represent the standard error (F=0.05%).

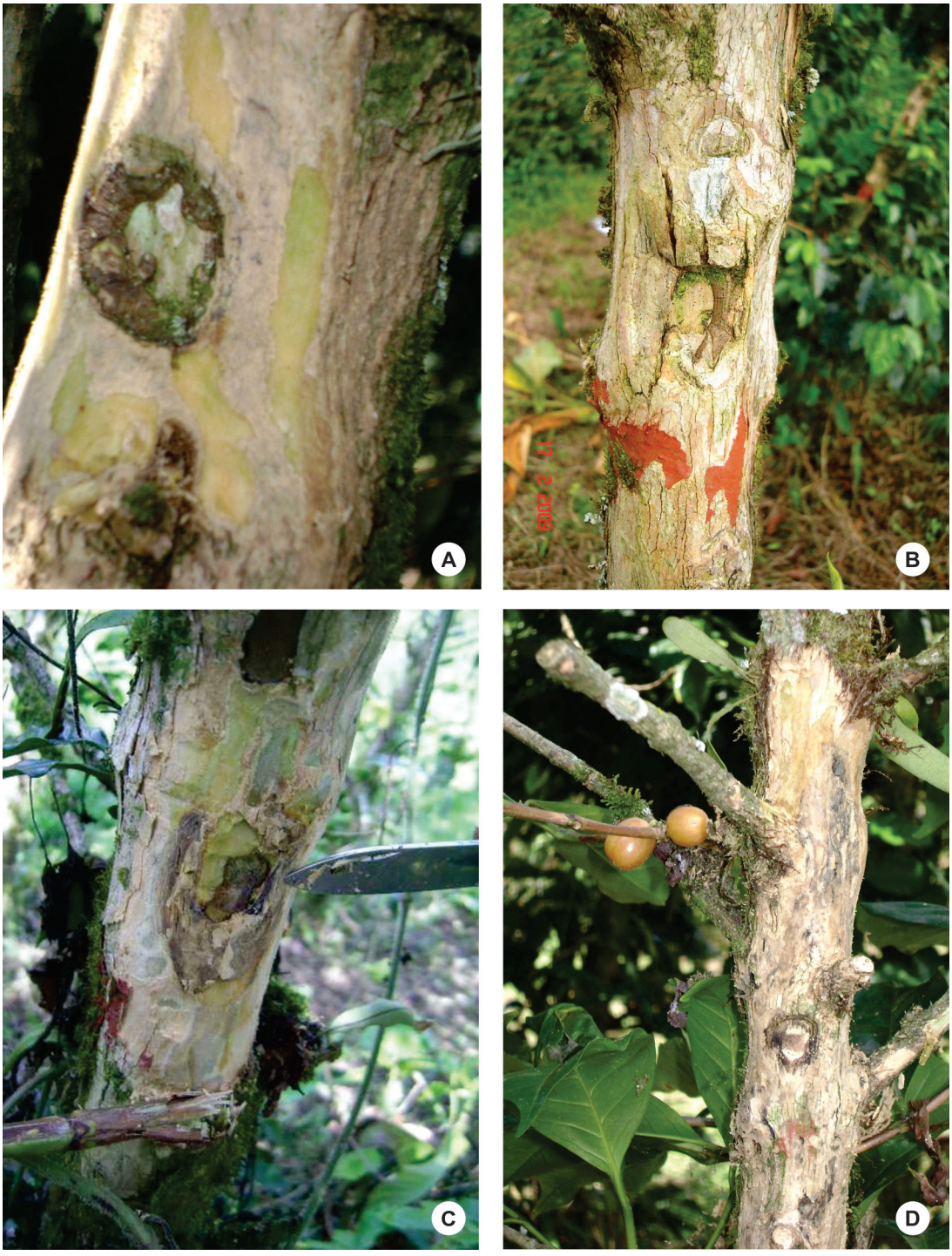

FIGURE 4 - Resistance reaction displayed by some progenies with strong callus formation (**A, B**) and reduced lesion area in the underlying wood (**C**), compared with wood discolouration (**D**) in controls and susceptible progenies F_3BC_1 derived from interspecific hybrids (*Coffea canephora x C. arabica*) one year after inoculation of *Ceratocystis colombiana.*

Evaluation of agronomic characteristics

The average plant height ranged from 131 to 161 cm in the progenies, while var. Caturra and var Colombia were an average height of 133 cm and 144 cm, respectively. Statistical differences ($P<0.0001$) were observed only for progenies MEG639-410 and MEG639-602, which were significantly taller than var. Caturra. No differences were noticed in the number of branches nor in canopy diameter for either the progenies or the controls. These phenotypic attributes are commercially accepted by Colombian coffee

growers and also mentioned by Castillo & Moreno (1988) and Alvarado & Cortina (1997).

Variable bean characteristics were observed in the progenies (Table 1). Dunnett´s test showed that ten of these had a higher percentage of empty beans than var. Caturra (3.9%) and var. Colombia (5.9%), while the rest of the progenies had acceptable ranges of bean production as defined by Castillo & Moreno (1988). Similarly, the progenies had higher peaberry values (average, 14.6%) than the two controls (average 8.2%). Dunnett´s test showed that 12 progenies had different values to those of var. Caturra (8.4%) and var. Colombia (9.6%). The frequency of triangle beans was low, with an average of 4%, which is within the acceptable range according to Castillo & Moreno (1987) and no differences were observed for this trait.

The average Supreme bean size (percent beans retained by a 17/64 inch screen) in the progenies was 61.0%, better than var. Caturra (41.0%), but lower than var. Colombia (66.0%). Dunnet's test showed that 14 progenies and var. Colombia had larger beans than var. Caturra. Progenies MEG639-771 (45.0 %) and MEG 639-818 had bean sizes that were much lower (37.0%) than expected (Table 1), according to the criteria established by Moreno & Alvarado (2000) for commercial varieties.

For average yield calculations (Table 1), differences ($P<0.0001$) and Dunnet's test showed that progenies MEG639-602, MEG639-705, MEG639-722, MEG639-771 and MEG639-841 were more productive than var. Caturra. There were no progenies statistically less productive than the controls. Thus progenies had variable yield and bean attributes, similar to what has been observed by other studies (Alvarado & Cortina, 1997). However, attributes such as bean size were close to the Colombian commercial varieties (Moreno & Alvarado, 2000).

Based on their resistance to rust and Ceratocystis canker, as well as on their most important agronomic characteristics (yield and bean size), three progenies (MEG 639- 601; MEG 639- 617 and MEG 639-704) were selected for future breeding development. This study has shown that it is possible to transfer desirable genes for resistance to the most important coffee pathogens to new genotypes. These genotypes will be valuable as new sources of resistance to these pathogens in the future.

TABLE 1 - Bean characteristics and yield (kg of dry parchment coffee/plant/year) for each progeny

Progeny nr.	Bean defects (%)			Bean size	Yield (Kg)
	Empty beans[a] (Mean ± SD)	Peaberry[b] (Mean ± SD)	Triangle[c] (Mean ± SD)	supreme[d] (%) (Mean ± SD)	(Mean ± SD)
MEG 639.410	5.1 ± 1.4	8.8 ± 2.0	5.8 ± 2.3	75.0 ± 3.8*	3.0 ± 0.9
MEG 639.475	7.8 ± 2.9	17.1 ± 4.1*	0.2 ± 0.2	53.5 ± 14.3	3.2 ± 1.2
MEG 639.601	6.6 ± 2.9	13.3 ± 4.0	3.2 ± 1.6	64.9 ± 4.3 *	3.2 ± 1.4
MEG 639.602	10.5 ± 4.1*	20.7 ± 8.4*	2.9 ± 2.1	54.6 ± 2.4	3.6 ± 1.2*
MEG 639.605	13.5 ± 5.8*	27.0 ± 13.3*	4.3 ± 2.3	58.7± 4.8*	3.0 ± 1.1
MEG 639.609	7.5 ± 3.0	12.6 ± 2.9	3.5 ± 1.3	50.5 ± 16.4	2.7 ± 1.1
MEG 639.610	6.4 ± 1.6	9.7 ± 2.4	6.7 ± 2.2	60.0 ± 8.8 *	3.1 ± 1.0
MEG 639.617	8.6 ± 3.2	10.7 ± 5.5	8.0 ± 1.8	70.3 ± 6.2*	3.1 ± 0.6
MEG 639.619	7.5 ± 3.8	11.0 ± 1.4	8.7 ± 2.4	55.6 ± 7.5	2.7 ± 0.9
MEG 639.620	11.1 ± 4.6*	18.7 ± 10.6*	1.9 ± 1.1	63.3 ±11.9*	3.1 ± 1.2
MEG 639.704	5.1 ± 1.7	12.4 ± 4.8	4.9 ± 2.6	66.6 ± 11.3*	3.1 ± 0.9
MEG 639.705	12.3 ± 7.7*	24.7 ± 9.2*	2.3 ± 0.8	64.5 ± 8.5*	4.0 ± 1.1*
MEG 639.708	7.2 ± 3.0	9.5 ± 1.9	5.9 ± 3.1	55.7 ± 9.2	3.1 ± 1.0
MEG 639.710	9.8 ± 4.0*	15.2 ± 7.9*	2.9 ± 1.3	61.8 ± 10.4*	2.6 ± 1.1
MEG 639.722	5.1 ± 1.3	8.1 ± 1.9	1.8 ± 0.6	70.6 ± 7.4*	4.2 ± 0.8*
MEG 639.771	9.9 ± 8.5*	15.7 ± 4.4*	7.0 ± 3.9	44.9 ± 19.2	3.5 ± 1.4*
MEG 639.816	9.5 ± 8.0*	18.2 ± 5.3*	0.9 ± 0.6	47.8 ± 16.9	3.2 ± 1.0
MEG 639.818	9.3 ± 4.5	20.8 ± 1.20*	0.5 ± 0.4	37.4 ± 19.6	3.3 ± 1.4
MEG 639.837	9.1 ± 3.3*	27.4 ± 11.4*	4.1 ± 2.3	62.2 ± 13.8*	3.1 ± 1.2
MEG 639.840	6.1 ± 2.8	14.1 ± 3.6	0.8 ± 0.6	74.9 ± 10.1*	3.1 ± 1.0
MEG 639.838	6.3 ± 3.6	16.3 ± 6.0*	4.1 ± 2.6	57.9 ± 13.6	2.9 ± 1.2
MEG 639.841	11.4 ± 10.2*	16.4 ± 3.5*	1.1 ± 0.7	70.3 ± 8.7*	3.5 ± 1.1*
MEG 639.842	4.7 ± 8.2*	12.3 ± 2.6	1.0 ± 0.6	73.8 ± 8.3*	3.1 ± 1.1
Var. Caturra	3.9 ± 1.0	8.4 ± 2.8	5.8 ± 1.2	41.0 ± 7.5	2.4 ± 0.8
Var. Colombia	5.9 ± 2.2	9.6 ± 2.6	2.3 ± 1.3	66.0 ±12.6*	3.2 ±1.0

[a] Empty beans: Average percent of 100 ripe coffee fruits floating in three samples of two harvest peaks
[b] Peaberry: Average percent in three samples of 400 dry parchment beans of two harvest peaks.
[c] Triangle: Average percent in three samples of 400 dry parchment beans of two harvest peaks
[d] Supreme: Average percent of three samples of 100 g of husked beans (green coffee) retained by a 17/64 inch screen.
*Statistical differences (0.05) according to Dunnett's test are indicated for each character.

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