

# SELECTION OF COFFEE PROGENIES WITH LARGE BEANS RESISTANT TO RUST AND CERCOSPORA LEAF SPOT

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**ABSTRACT:** Rust is the main disease of coffee. Recently, cercospora leaf spot has grown in importance, intensifying defoliation and decreasing grain yield in *Coffea arabica*. The Big Coffee VL is a variety of *C. arabica*, with large beans. There is still little information on this variety. Because of this, the aim of this study was to use a mixed model approach to select the best progenies of “Big Coffee VL” for resistance to rust and cercospora leaf spot. There were evaluated 12 progenies with high bean yields. Based on bean size, leaves and plant size, plants within each progeny were classified as “small” (P), “medium” (M) and “large” (G). The experimental design was in blocks completely randomized, with six replicates and one plant per plot. Six measurements were carried out every 15 days for each plant by selecting 20 fully exposed leaves with higher and lower sun exposures. The measurements summarized disease severity using two diagrammatic scales to obtain the area of the leaf under rust or Cercospora leaf spot. A mixed model approach was used to calculate genotypic value (GV) and heritability estimates. The Mulamba and Mock index was used to select the most resistant progenies, in which the sum of rankings was weighted by a heritability value. Five progenies were selected; three progenies were G (G17, G9, and G12), one progeny was M (M5) and another progeny P (P23). Among these, M5 and P23 progenies are the most productive and may have potential use in future studies. This work presents the potential in investigating the new *C. arabica* variety.

**Index terms:** *Coffea arabica*, *Hemileia vastatrix*, *Cercospora coffeicola*, simultaneous selection.

## SELEÇÃO DE PROGÊNIES DE CAFEIROS DE GRÃOS GRAÚDOS RESISTENTES A FERRUGEM E CERCOSPORIOSE

**RESUMO:** A ferrugem é a principal doença do cafeeiro. Recentemente a cercosporiose tem crescido em importância, intensificando a desfolha e diminuição na produtividade de grãos da espécie *Coffea arabica*. O Big Coffee VL é uma variedade de *C. arabica*, com característica de grão graúdo. Ainda existe pouca informação sobre essa variedade, diante disso, objetivou-se selecionar progênies de cafeeiro “Big Coffee VL”, para resistência à ferrugem e cercosporiose simultaneamente, com a abordagem de modelos mistos. Foram consideradas as 12 progênies mais produtivas e classificadas em “pequenas” (P), “médias” (M) e “grandes” (G), de acordo com o tamanho dos frutos, folhas e o porte da planta. O delineamento utilizado foi em blocos casualizado (DBC), com seis repetições e uma planta por parcela. Em cada planta foram realizadas seis avaliações, com intervalos de 15 dias, em 20 folhas do terço médio da planta da face da planta com maior e menor exposição solar. As avaliações foram realizadas com o auxílio de duas escalas diagramáticas a fim de obter a área abaixo da curva de progresso da doença, que resume a severidade da doença. Foi utilizada a abordagem de modelos mistos para o cálculo do valor genotípico (VG) e as estimativas de herdabilidade. Para selecionar as progênies mais resistentes foi considerado o índice de Mulamba e Mock, no qual a soma de postos foi ponderada pelo valor da herdabilidade. Na seleção de cinco progênies, três delas são do grupo G (G17, G9 e G12), uma M (M5) e uma P (P23). Dentre essas, as progênies M5 e P23 são as mais produtivas e tem potencial, em termos de resistência às doenças, para serem utilizadas em trabalhos futuros. Esse trabalho mostra o potencial em investigar a nova variedade de *C. arabica*.

**Termos para indexação:** *Coffea arabica*, *Hemileia vastatrix*, *Cercospora coffeicola*, seleção simultânea.

### 1 INTRODUCTION

Development of coffee cultivars that are resistant and/or tolerant to diseases is important for increasing grain yield and decreasing production costs. The lack of information on how new cultivars resist attacks from major diseases is one of the limiting factors in the selection of the

best cultivars (CARVALHO et al., 2010). Coffee rust caused by the biotrophic fungus *Hemileia vastatrix* Berk. et Br is the most economically important coffee disease in the world. It has been found in all coffee plantations in Brazil and has caused damages and losses to coffee cultivation (MAFFIA; HADDAD; MIZUBUTI, 2009, ZAMBOLIM, 2016). The disease can reduce

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yields by 30 to 50% depending on the level of resistance of the genotype, the inoculum potential, and the environment conditions (CAPUCHO et al., 2013).

Favorable conditions for fungal infection and development include temperatures between 21 °C to 25 °C, and a high relative humidity (BEDENDO, 2011). The symptoms appear on the upper surfaces of the leaves, showing translucent chlorotic spots 1–3 mm in diameter. As these spots gradually increase in diameter masses of orange urediniospores appear on the bottom of the leaves. The centers of the spots eventually dry and turn brown, while the margins of the lesions continue to expand and produce urediniospores. With leaf defoliation occurring before flowering, both flowers and fruits are affected, causing the formation of abnormal grains (MATIELLO et al., 2015).

In recent years, another important disease that has increased in importance is cercospora leaf spot. This increase is attributed to technologies used in managing coffee plantations, high-density planting of crops, and irrigation practices that have altered the microclimate, favoring an increase in the incidence of diseases (PAIVA et al., 2013). *Cercospora coffeicola* Berk & Cook (SOUZA et al., 2011) is the causal agent of Cercospora leaf spot. *C. coffeicola* is more prevalent with air temperatures between 17 and 22 °C and high relative humidity (ZAMBOLIM et al., 2005). The infection occurs on the bottom of the leaf, where there is penetration of the fungus through stomata, causing colonization into the parenchyma cells. The main symptoms appear as leaf and fruit lesions. The lesions are characterized by necrotic spots with light colors in the center surrounded by brown rings with yellowish halos. Early leaf defoliation caused by the necrotic spots may reduce bean yield and fruit attack can reduce bean quality (ZAMBOLIM et al., 2005). However, it is difficult to control this disease (PATRICIO et al., 2010).

Bernardo (2010) noted that information on statistical variability is fundamental to understanding population genetics. A recent prediction methodology to characterize populations of perennial species is the restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) (BALZARINI, 2001; PEREIRA et al., 2013). Data analysis in coffee experiments has benefited from using REML/BLUP because coffee is a perennial crop with

variable yields and unbalanced data in the field experiments. However, there remains a need for more accurate estimates (RODRIGUES et al., 2013; ANDRADE et al., 2001).

The objective of this study was to provide information on resistance to rust and cercospora leaf spot using the 12 most productive Big Coffee VL progenies.

## 2 MATERIAL AND METHODS

### Origin of the variety

The “Big Coffee VL”, known for its large beans, originated from an Acaia plantation in southeast Brazil, municipality of Capitólio-MG. The parent plants present vigorous canopies and large fruits and leaves. In 2011, seeds from the parent plants were collected and planted on the farm in Piumhi-MG. Based upon fruit, leaves and plant size, these Big Coffee VL seedlings were classified as “small” (P), “medium” (M) and “large” (G).

In February 2012, 100 of these Big Coffee VL seedlings were used in an experiment carried out at the Department of Agriculture of the Federal University of Lavras. This experiment was designed to expand the genetic base of Arabica coffee and to promote the research on genetic breeding of this variety.

### Experimental trials

The experiment was carried out at Department of Agriculture of the Federal University of Lavras, (21°14'43”S and 44°59'59”W, altitude of 919 m). All 100 progenies were distributed in a square lattice scheme. Twelve progenies of Big Coffee VL considered the most productive (SILVA, 2016) were selected (P5, P14, P32, M4, M4, M5, M11, M11, M20, G9, G12, G16, and G17) for resistance evaluation. A randomized complete block design was used with six replicates and one plant per plot. Each plant was evaluated every 15 days. Mean climatic data (precipitation, solar incidence, temperature and relative air humidity) were calculated for the 15 days prior to each measurement (Table 1).

During each evaluation, 40 leaves from each plant were selected from the middle-third of the plant. Twenty leaves were located on the side of the plant with the greatest sun exposure (North) and the other ones on the side with least sun exposure (South). For each leaf, the disease diagrammatic scales for coffee rust (CUNHA et al., 2001) and cercospora leaf spot (CUSTODIO et al., 2011) were used to calculate the severity of the disease.

**TABLE 1** - Mean precipitation, solar incidence, temperature and relativity humidity data\* 15 days prior to the day of plant evaluation.

Evaluations	Period	Precipitation (mm)	Solar incidence ** (hours)	Temperature (°C)	Relativity Humidity (%)
1	Feb 25 to Mar 10	4.3	5.0	22.9	78.1
2	Mar 11 to Mar 24	8.8	8.6	22.4	67.2
3	Mar 25 to Apr 07	0.2	8.1	21.4	68.3
4	Apr 08 to Apr 21	5.5	7.5	21.7	73.5
5	Apr 22 to May 05	2.2	5.6	19.8	76.6
6	May 06 to May 19	0.8	6.2	18.7	76.4

\*Data supplied by INMET <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>

\*\*Solar brightness length

For cercospora leaf spot, severity was calculated according to the formula:

$$x_i = \left[ \frac{(n0 \cdot 0) + (n1 \cdot 2.2) + (n2 \cdot 4.7) + (n3 \cdot 8.3) + (n4 \cdot 15.1) + (n5 \cdot 20.1) + (n6 \cdot 46.2)}{(nT \cdot 100)} \right] \cdot 100$$

where  $x_i$  is the severity at evaluation  $i$ , where  $i = 1 \dots 6$ ,  $n0$  is the number of leaves with a scale value = 0 up to  $n6$  (scale value = 6);  $nT$  is the total number of leaves evaluated.

For coffee rust, the severity of the disease was calculated according to the formula:

$$x_i = \left[ \frac{(n0 \cdot 0) + (n1 \cdot 1.5) + (n2 \cdot 4.5) + (n3 \cdot 9) + (n4 \cdot 18.5) + (n5 \cdot 37.5)}{(nT \cdot 100)} \right] \cdot 100$$

where  $x_i$  is the severity at evaluation  $i$ , where  $i = 1 \dots 5$ ,  $n0$  is the number of leaves with a scale value = 0 up to  $n5$  (scale value = 5);  $nT$  is the total number of leaves evaluated.

Using the disease severity results, a curve was plotted to assess the progression of disease severity based upon the estimate of the area under the disease progress curve (AUDPC) (SHANER; FINNEY, 1977).

$$AUDPC = \sum_i^{n-1} \left[ \frac{(x_i + x_{i+1})}{2} \right] \cdot t$$

where  $n$  is the number of evaluations,  $x$  is the disease severity,  $i$  is the number of evaluations and  $t$  is the interval between two consecutive evaluations.

For the mixed model approach, the following matrix model was followed:

$$Y = X_r + Z_g + \varepsilon \quad \text{and} \quad \begin{cases} y|r, V \sim N(Xr, V) \\ g \sim N(0, I\sigma_g^2) \\ \varepsilon \sim N(0, R) \end{cases}$$

where  $y$  is the data vector;  $r$  is the vector of the main effects for repetitions and  $g$  is the vector of progeny effects.  $X$  and  $Z$  are the design matrices related to fixed and random effects.

The significance of random effects was verified by the likelihood test ratio (LTR) using the lmerTest library (KUZNETSOVA; BROCKHOFF; CHRISTENSEN, 2016). Because it is a mixed model, the means of the progenies cannot be evaluated with a multiple comparison test. Therefore, parameter estimation and ordering of the progenies was performed by the predicted genotypic values obtained by R program (R CORE TEAM, 2014), using lme4 (BATES et al., 2004) and lattice libraries (SARKAR, 2008).

Broad-sense heritability was also estimated following Ramalho (2012):

$$h^2 = \frac{\sigma_g^2}{\sigma_f^2}$$

where  $\sigma_g^2$  is the variance of the genotypes; and

$\sigma_f^2$  is the phenotypic variance.

Progenies were classified in favorable order from the lowest to the highest disease severity value. To select promising progenies, the methodology of Mulamba and Mock (1978) as detailed by Cruz et al. (1997) was followed. After classification of each characteristic, the summation of the rankings was performed. Lower summation values indicated more disease-resistant progeny.

### 3 RESULTS AND DISCUSSION

The time required to fungus germination vary according to temperature, light regimes and exposure to light (SILVA et al., 2016). And the disease severity can be different according to the plant environment.

### Coffee rust

The rust disease severity was obtained for each evaluation (Figure 1).

The greatest severity was at evaluation 6 (beginning of May) for plants under the lowest. During the time interval of 15 days before evaluation, means of relative humidity, solar incidence time, and temperature were 76.4%, 5.6 h, and 19.8 °C, respectively (Table 1). Although the ideal temperature for infection of the fungus in plant is between 21 °C and 25 °C, the pathogen was able to colonize the leaf tissue. Montes, Paulo e Fischer (2012) reported similar results, where the incidence of the disease correlated negatively with temperature.

In evaluation 1, the plants under higher had the highest severity of rust. However, the level of severity was comparatively lower compared to plants under lower sun exposure. During the time interval of 15 days before evaluation, means for average relative humidity, solar incidence and temperature were 78.1%, 5 h, and 22.9 °C, respectively (Table 1). In evaluation 1, the combination of relative humidity and temperature favored disease development. Similar to Moller and Stukenbrock (2017), we observed that *H. vastatrix* has the ability to rapidly adapt to different environmental conditions. The increase in solar incidence and decrease in relative humidity in the subsequent evaluation intervals supported a reduction in disease severity, primarily in plants under higher sun exposure.

Estimates of progeny variance were 229.7 (lower solar incidence) and 59.2 (higher solar incidence). There was a significant difference in both cases (p-value:  $8,642 \times 10^{-7}$  and 0.009364, respectively) by the likelihood test ratio. Broad-sense heritability was 49.7% (lower solar incidence) and 36.2% (higher solar incidence).

According to Farias Neto et al. (2009), BLUP maximizes selective accuracy. In this work, the genotypic values are ordered from lower to higher values of rust severity (Table 2). The first position in both evaluations is associated with the G17 progeny. Considering the region of the plant with less solar incidence, two G progenies stand out (G17 and G12). Silvia (2016) observed that G progenies generally showed a lower average productivity, suggesting that resistance can incur a cost for grain yield as discussed by Bergelson and Purrington (1996). This is due to plants attempting to balance resource allocation between growth and defense, since a stress response can be costly and reduce growth and yield (TIAN et al., 2003).

Progenies P5 and P32 had the highest genotypic severity values in both evaluations. These are among the most productive progenies, confirming the negative correlation between grain yield and resistance. In general, the M progenies presented greatest mean values of severity as well as for yield (Silva et al., 2016).

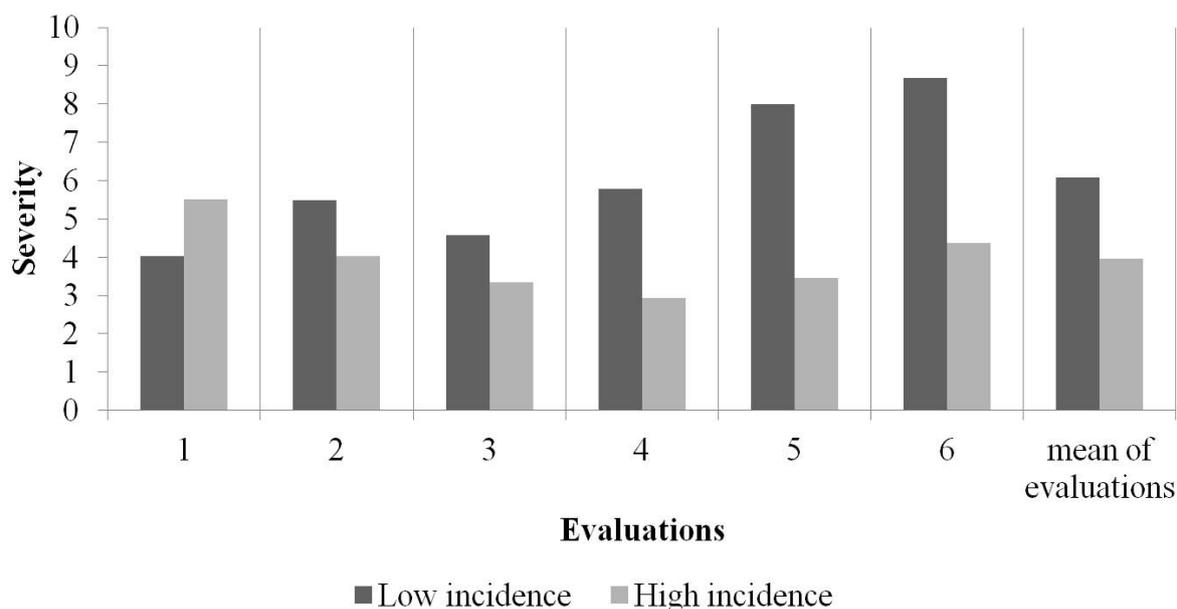


FIGURE 1 - Rust disease severity during the different evaluations in plants under low and high solar incidence.

**TABLE 2** – Genotypic values for mean rust severity for 12 progenies of Big Coffee VL, arranged by genotypic values and solar exposure.

Low		High	
Progenies	Genotypic values	Progenies	Genotypic values
G17	1061.94	G17	396.04
G12	1090.2	P23	398.76
M4	1103.56	G16	404.03
P23	1108.92	G9	412.43
M5	1148.98	M5	419.29
G9	1162.69	M11	431.03
G16	1173.6	P14	437.31
M11	1211.4	G12	444.62
P14	1212.52	M20	450.39
P32	1244.71	M4	451.99
M20	1247.35	P32	501.65
P5	1864.07	P5	563.29

### Cercospora leaf spot

The *Cercospora* leaf spot severity was obtained for each evaluation (Figure 2).

Few studies have investigated *C. coffeicola*; however, there are recent reports on new symptoms in coffee plantations caused by *Cercospora* leaf spot. Vale (2016) highlighted the importance of understanding the species identity and its relationship with a host. The likelihood test ratio was the only test that obtained a significant ( $p$ -value  $<0.05$ ) difference for severity in plants under the highest sun exposure. The broad-sense heritability was 33.4%, considered low estimate. Unlike what was observed for rust, two progenies (P32 and P23) stood out for potential resistance to *C. coffeicola* (Table 3). However, there appears to be no exceptional progeny that is resistant to attacks from rust and cercospora leaf spot. This raises the possibility that there may be different plant resistance mechanisms for each pathogen (NELSON et al., 2017).

### Selection of Disease Resistant Progenies

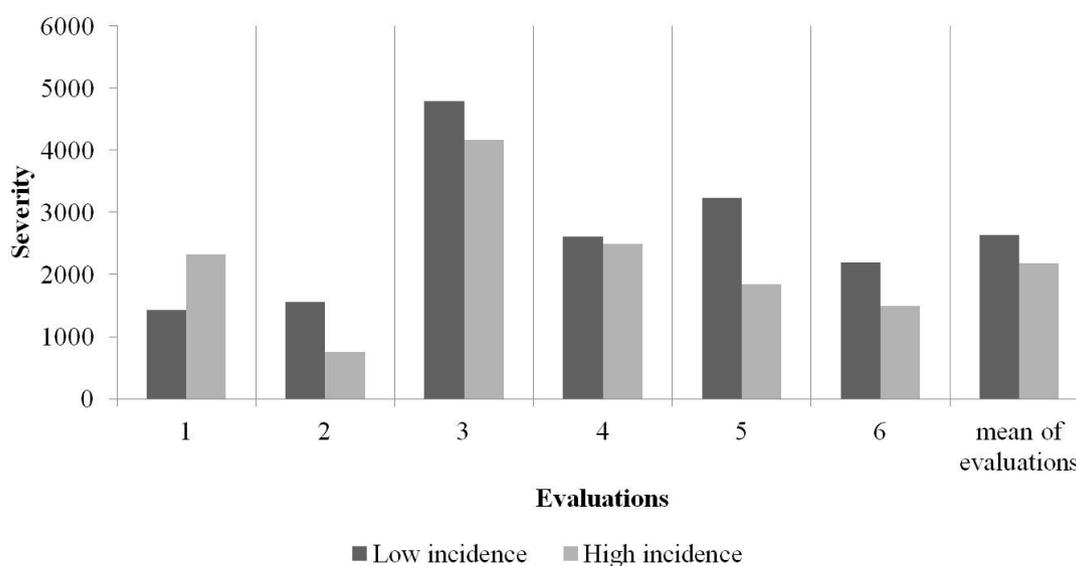
To select disease resistant coffee trees, it is important to consider the genetic characteristics of a population (CARVALHO et al., 2017; PAIVA et al., 2013). A mixed model approach was originally proposed for estimating heritability values in animal breeding. Later, perennial crops breeders tried this approach to remove problems related to selecting individual genotypes in unbalanced

experiments. Recently the genetic resistance of coffee plants to *C. coffeicola* (BOTELHO et al., 2017) and *H. vastatrix* (PETEK et al., 2008) has been studied using the mixed model approach. When associated with statistical procedures, the mix model approach allows greater reliability in breeding programs (OLIVEIRA et al., 2015).

For simultaneous selection (Table 4) for resistance to rust and cercospora leaf spot, the summation was weighted by heritability value in each case. Only genotypic values were considered due to the low solar incidence values for the rust and the high solar incidence values for the *Cercospora* leaf spots, at the time of the significance and value of the heritability, respectively.

Five progenies were selected; three of them are from group G (G17, G9 and G12), one from progeny M (M5) and one from progeny P (P23). There appears to be a positive correlation between vegetative development and fungus resistance, except for P progenies. The G progeny plants have large vegetative canopies, reducing possible photosynthetic losses due to reduction of the leaf area by fungus colonization (KUSHALAPPA; CHAVES, 1980).

A higher harvest index represents a greater investment in productive organs and consequently less investment in leaves and stem. The crops become less competitive and protected against losses of leaf area by pathogens (SADRAS; CALDERINI, 2015; ZAMBOLIM et al., 2005).



**FIGURE 2** - Cercospora leaf spot severity in the different evaluations in plants under low and high solar incidence.

**TABLE 3** - Genotypic values ordered for the Cercospora leaf spot severity in 12 progenies of Big Coffee VL.

High	
Progenies	Genotypic values
P32	236338
M5	248559
P23	254462
G9	262545
M20	271562
M4	280942
G17	285647
G12	287472
M11	298454
G16	299028
P14	302508
P5	353888

This is opposite to what we observed for the P23 and M5 progenies, which are among the most productive progenies (SILVA, 2016). Moreover, a lower disease severity may indicate horizontal resistance. Campbell and Madden (1990) found that under natural epidemic conditions, severity is the component that best discriminates between levels of horizontal resistance. The heritability estimates that were found below 50% indicate a possible presence of resistance genes that could be strongly influenced by the environment (BERNARDO,

2010). Partial and non-specific polygenic resistances have been found in *C. canephora*, in some *C. arabica* genotypes and in interspecific hybrids (SILVA et al., 2006). There is great genetic diversity within *H. vastatrix* (CABRAL et al., 2015), *C. coffeicola* (DELL'ACQUA et al., 2011) because of this durable long-term resistance in *C. arabica* has not been achieved so far. Therefore, breeding programs need to develop varieties with sustainable resistance for a highly fit and variable pathogen.

**TABLE 4** - Classification by the summation of rankings by Mulamba and Mock (1978) considering rust and cercospora leaf spot severity in Big Coffee progenies.

Progenies	Summation of rankings
G17	3.197
P23	3.714
M5	4.963
G9	5.766
G12	6.562
M4	7.115
G16	7.905
M11	9.154
P32	9.286
M20	10.395
P14	10.681
P5	14.316

#### 4 CONCLUSIONS

From the evaluated progenies it is possible to select superior plants more resistant to rust and Cercospora leaf spot simultaneously. The selection index associated with an advanced statistical approach related to genotype value prediction facilitates the selection of the genetically superior individuals, maximizing the success on selection.

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