

Selection and genetic parameters for interpopulation hybrids between kouilou and robusta coffee

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ABSTRACT: Selection of hybrid coffee plants coming from crosses between divergent populations is particularly relevant for the success of breeding programs. This study aimed to outline the best selection strategy in a hybrid population of *Coffea canephora* var. kouilou and robusta by estimating intrapopulation genetic parameters. Twenty full-sib progenies obtained by North Caroline II were installed in a randomized complete blocks design, with one plant per elementary plot. The following traits were evaluated: vegetative vigor, reaction to rust, plant height, diameter of canopy projection, maturity time, and

bean yield. Significant individual genotypic variance and heritability estimates lead to an effective selection. The multi-trait selection index carried out between progenies and at individual level provided 5% and 40% gain, respectively. Thus, intrapopulation selection in a hybrid population is a viable strategy for the selection of superior individuals to compose new crosses and clones for cultivars in the breeding program of *C. canephora*, even with unbalanced data.

Key words: mixed models, multi-trait index, intrapopulation selection, *Coffea canephora*.

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INTRODUCTION

Coffee plant, originally from Africa, presents two species of economic importance: *Coffea arabica* L., known as arabica coffee, and *Coffea canephora* Pierre ex Froehner, known as robusta coffee. The latter represents about one-third of the coffee produced worldwide and has traits of great importance to the food and pharmaceutical industry, such as high caffeine content and high soluble solids content (Tran et al. 2016).

The species *C. canephora* is a diploid allogamous ($2n = 2x = 22$), with multistem shrubs and self-incompatible flowers (Carvalho 1946), being divided into two genetic groups. The Guinean group is phenotypically characterized by presenting long leaves, short size, short internodes, and for being tolerant to drought and susceptible to rust (caused by the fungus *Hemileia vastatrix* Berk. et. Br). The second group, referred to as Congolese, can be divided into five subgroups: SG1, SG2, B, C, and UW. Coffee plants of the SG1 subgroup are morphologically similar to those of the Guinean group. The individuals of the other subgroups present large, wide leaves; large grains; and are highly resistant to rust and highly susceptible to water stress (Fazuolli et al. 2009; Montagnon et al. 2012; Teixeira et al. 2017).

In the production chain of caffeinated coffee, those of the SG1 subgroup are known as *C. canephora* var. kouilou (in Brazil, they are known as conilon coffee), and the others are known as *C. canephora* var. robusta (or robusta coffee) (Montagnon et al. 2012). Studies carried out in Ivory Coast suggest that hybrids between these groups express heterosis due to the high genetic variability present in the species (Leroy et al. 1993; 2014). Hybridization between parents of these groups, found in Brazil, is of great interest since it gathers traits of both

groups in a single genotype and increases variability in the species' breeding. Thus, recurrent selection strategies can be used more efficiently.

This study aimed to estimate intrapopulation genetic parameters in *C. canephora*, to outline the best selection strategy in hybrid population between coffee plants of the kouilou and robusta groups by multi-trait selection index, and to select superior individuals to compose new crosses or clones for cultivars.

MATERIAL AND METHODS

Plant material and experimental design

Twenty full-sib progenies were obtained by North Carolina design II (NCII), consisting of five male parents of the kouilou group and five female parents of the robusta group, all of them belonging to the breeding program of *C. canephora* of EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais) (Table 1). The trial consisted of a randomized complete blocks design (RCBD), with a different number of individuals as representatives of each progeny, totaling 246 coffee plants. Elementary plots consisted of one coffee tree, spaced at 3.0×1.5 m apart. The experiment was installed in 2011 and evaluated in the years of 2013 and 2014, at the Experimental Field Oratórios, in the state of Minas Gerais (lat. $20^{\circ}25'51''$ S, long. $42^{\circ}48'21''$ W).

Evaluated traits

The evaluated agronomic traits were: vegetative vigor (VIG), evaluated by a scoring scale from 1 to 10, representing the worst plants and the best plants, respectively (Carvalho et al. 1979); fruit maturation cycle (MAT), classified by

Table 1. Progenies identification from the parents crosses between *Coffea canephora* var. robusta (female) and var. kouilou (male).

Robusta	Kouilou				
	513	3627-31	3628-2	3629-11	3629-25
3365-144	9 (15*)	**	13 (7*)	8 (16*)	17 (3*)
3366-139	2 (35*)	5 (32*)	12 (8*)	3 (35*)	**
3367-98	4 (35*)	**	11 (8*)	10 (10*)	16 (4*)
3373-36	18 (3*)	22 (1*)	15 (5*)	19 (2*)	20 (1*)
3374-28	14 (6*)	7 (19*)	**	21 (1*)	**

*number of plants per cross; **unsuccessful cross.

the scoring scale 1 = early, 2 = intermediate, and 3 = late; reaction to rust in the field (RUS), evaluated by the scoring scale 1 = immune, 2 = resistant, 3 = moderately resistant, 4 = moderately susceptible, and 5 = susceptible, modified from the scoring scale proposed by Eskes (1981); plant height (PH), measured in cm, from the soil to the last apical point of the coffee plant; diameter of canopy projection (DCP), measured in cm, perpendicular to the row, from the canopy center, with the greatest measure between both ends. Yield in 60 kg·ha⁻¹ bags of green coffee (Y) was evaluated by collecting all the fruits of the experimental plot. Afterward, the total volume in liters (Vol) was determined by the expression $Y = [(number\ of\ plants \cdot ha^{-1}) \cdot Vol] \cdot 360^{-1}$. For each bag of green coffee, 360 liters of freshly harvested coffee fruits were considered. Every trait was evaluated by year.

Statistical analyses

Genetic parameters were estimated according to the mixed model proposed by Resende (2007a). The model intended to estimate the variance components of individual genotypic effects, the genotype × harvest interaction, and the genetic parameters, given by (Eq. 1):

$$y = Xr + Zg + Wp + Ti + e \quad (1)$$

where y is the data vector; r is the vector of replication effects (assumed as fixed), added to the overall mean; g is the vector of individual genotypic effects (assumed as random); p is the vector of plot effects (assumed as random); i is the vector of genotype × harvest interaction (random); and e is the error vector (random). X , Z , W , and T are the incidence matrices for the vectors r , g , p , and i , respectively.

The estimated components of variance were: σ_g^2 = component of individual genotypic variance; σ_a^2 = component of additive variance, ignoring 1/4 of the variance due to dominance, and the fraction, due to epistasis; σ_p^2 = component of variance for plot effect; σ_i^2 = component of variance due to the genotype × harvest interaction; σ^2 = component of variance for residual error. The equations for genetic parameters, proposed by Resende (2002), are (Eqs. 2 to 5):

$$h_a^2 = \frac{\sigma_a^2}{\sigma_f^2} \quad (2)$$

narrow sense individual heritability.

$$h_{mp}^2 = (r_{aa})^2 \quad (3)$$

broad sense heritability at the progenies mean level.

$$r_{gcolh} = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_i^2} \quad (4)$$

genotypic correlation of the genetic material through harvests.

$$r_{aa} = \left[\frac{1 + (n-1)\frac{1}{2}}{n} \frac{\left[1 + (n-1)\frac{1}{2} \right] h_a^2}{1 + (n-1) \left[\frac{1}{2} h_a^2 + \frac{1}{4} (h_g^2 - h_a^2) \right]} \right]^{\frac{1}{2}} \quad (5)$$

selection accuracy of progenies means between replications and harvests

Selection was based on the multi-trait index proposed by Resende (2007b), obtained by the sum between the product of the predicted genotypic value and its respective economic weight for each trait, having Y as the main trait. The economic weight was established according to Viana and Resende (2014). Therefore, the individual additive genetic value was used for the selection of the recombinant population, and the individual genotypic values were used for the selection of clones to compose the index.

The genetic divergence between progenies was carried out using the Tocher's clustering method (Rao 1952) by the matrix of genetic distances of Mahalanobis, by Resende (2007a), obtained from the predicted values, using the variance and covariance matrix of these genetic values, as follows (Eq. 6):

$$D_{ii'}^2 = \delta' G \delta \quad (6)$$

where $D_{ii'}^2$ = Mahalanobis distance between genotypes i and i' ; G = matrix of genotypic variance and covariance; $\delta = [d_1, d_2, \dots, d_j]$, being $d_j = Y_{ij} - Y_{i'j}$; and Y_{ij} = mean of the i -th genotype in relation to the j -th variable.

The computational statistical package SELEGEN-REML/BLUP (Resende 2016) was used for the resolution of the genetic statistical analysis.

RESULTS AND DISCUSSION

Genetic parameters

Individual genotypic variance (σ_g^2) was significant (Table 2) for the traits RUS, PH, DCP ($p \leq 0.01$), and MAT ($p \leq 0.05$), allowing the exploitation of the variability of the hybrid progenies *per se*, the estimation of heritability, and

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Table 2. Estimates of the genetic parameters for the traits reaction to rust (RUS), plant height (PH), diameter of canopy projection (DCP), vegetative vigor (VIG), maturation time (MAT), and yield (Y) of a diallel cross in *Coffea canephora*, Oratórios – MG.

Parameters	RUS	PH	DCP	VIG	MAT	Y
σ_g^2	0.20**	154.85**	167.20**	0.12 ^{ns}	0.15*	64.63 ^{ns}
r_{aa}	0.72	0.75	0.67	0.66	0.75	0.59
r_{gcolh}	0.74	0.90	0.94	0.70	0.76	0.49
h_a^2	0.33	0.40	0.25	0.23	0.40	0.15
h_{mp}^2	0.52	0.56	0.45	0.44	0.56	0.45
Overall mean	1.64	163.40	175.40	6.03	1.95	33.40

σ_g^2 = individual genotypic variance, r_{aa} = accuracy, r_{gcolh} = genotypic correlation of harvest, h_a^2 = narrow-sense individual heritability, h_{mp}^2 = broad sense progeny mean heritability, ** p < 0.01, * p < 0.05 and ns = non-significant, at 1 g.l., by χ^2 test (1% = 6.63 e 5% = 3.84), by way of LRT test (Likelihood Ratio Test) of random effects.

the promotion of selection. Thus, narrow-sense individual heritability (h_a^2) was estimated, and mean values were calculated for all traits, ranging from 0.23 to 0.40, except for Y, which presented a low value of 0.15 (Table 2). These values are approximately the same as those estimated for *C. canephora* in Ivory Coast for Y heritability from crosses between Guinean and Congolese groups (Montagnon et al. 2003). These authors explain that estimates are influenced by a small progeny size and a few number of parents (around ten) between crosses. Yield is a quantitative trait largely influenced by the environment conditions. The low number of evaluations over the years can influence heritability estimations. The Y data evaluated over 14 years revealed higher heritability in Cameroon (Cilas et al. 2003). The literature shows low heritability for Y, being 0.0027 for arabica coffee from Brazil (Petek et al. 2008) and approximately 0.26 for arabica coffee from Cameroon (Cilas et al. 1998).

Broad-sense progenies mean heritability (h_{mp}^2) was estimated, and mean values for the traits ranged from 0.44 to 0.56. Carias et al. (2016) reported moderate magnitude h_{mp}^2 values in conilon coffee progenies, ranging from 0.22 to 0.53. The parameters estimator used in this work include unbalanced data and allow a better estimate, which is equivalent to that reported by Piepho and Möhring (2007).

According to Falconer (1987), population variability is essential to obtain selection gains, and heritability is the genetic parameter of greater importance for plant breeders since it determines the response to selection.

Thus, full-sib progenies have enough additive genetic variance for selection between and within progenies to be exploited in selection cycles, indicating intrapopulation recurrent selection (IRS) as a viable strategy to obtain superior hybrids and advance the selection cycles. Along

with recombinant population, superior individuals can be extracted to compose clonal tests.

High accuracy values (Table 2) are observed for RUS, PH, and MAT, ranging from 0.72 to 0.75, and intermediate values are detected for DCP, VIG, and Y, varying from 0.59 to 0.67. Resende (2007a) emphasizes that accuracy is a good measure to evaluate the quality of the experiment (r_{aa}), and the values observed in the present study are high ($r_{aa} \geq 0.70$) and intermediate ($0.40 \leq r_{aa} \leq 0.70$). High selective accuracy (r_{aa}) reveals that the predicted values are close to the real value, suggesting good accuracy of the selection method used, although the experiment was carried out under unbalanced data, with a single individual per elementary plot, and with a variable number of replications between treatments. Elementary plots containing a single individual and with more replications improve the statistical analysis in perennial species (Resende 2002).

Genotypic correlation between harvests (r_{gcolh}) was considered of high magnitude (above 0.70) for all traits, except for Y (Table 2). According to Resende (2007a), estimates equal to or greater than 0.70 indicate that the genotype \times harvest interaction is simple, whereas smaller values are complex. Simple interactions do not change the classification of genotypes in different harvests; conversely, complex interactions, such as crossover interaction, indicate difficulties in genotypes selection, changing their classification between measures and leading to selection based on the genotype \times environment interaction (Malosetti et al. 2013). Thus, for Y, selection can be more efficient if the trait is evaluated in multiple harvests. More evaluations over the years increase selection efficiency (Fonseca et al. 2004); however, for the other traits, selection efficiency is not influenced by the number of harvests.

Selection between and within progenies

The multi-trait index was used in the selection of the best progenies, based on the individual additive genetic value, aiming at the selection of individuals to compose the recombinant population. This method is beneficial when assigning weights to the traits since the index directly uses the predicted values and the weights related to the correlation with the main trait (Y). For the construction of the index, the economic weight for each trait was equal to RUS (0.1916), PH (0.1653), DCP (0.20), VIG (0.14), and Y (0.2972). MAT did not compose the index due to the interest in selecting individuals with different fruit maturation cycles, which consequently allowed obtaining populations with genotypes of early, intermediate, or late maturation.

The selection of the ten best progenies (5, 9, 22, 4, 12, 2, 10, 15, 11 e 16) provided 5% genetic gain by the index. Therefore, 28 plants were selected (the three best plants within each selected progeny). For progeny 22, only one plant was selected due to unbalance within progenies (Table 3). Selected plants are associated with the effective progeny size (Nef) of 15 progenies, which happens to be the equivalent number of unrelated individuals, resulting in a low coefficient of inbreeding of 0.033 ($F = 1/(2Nef)^{-1}$). To avoid selection of related individuals without reducing the selection intensity and genetic gain, the maximum number of selected genotypes should be restricted, as suggested by Resende and Barbosa (2005). Thus, for full-sib progenies, the authors describe Nef, which is given by (Eq. 7):

$$Nef = N(2n)(n + 1)^{-1} \quad (7)$$

where N is the number of progenies and n is the number of individuals per progeny.

The population selected (28 plants corresponding to the ten progenies) presented selection gains when the traits were analyzed separately (Table 3). MAT had an indirect selection in the negative direction (-31%), and thus the plants selected based on the other traits presented early maturation cycle. Gain for RUS was zero, which evidences, based on genetic values, the tolerance of plants to the disease causative agent. For PH and DCP, gains were 2% and 3%, respectively, leading to the selection of coffee plants slightly taller when compared with their general means and the selected genotypes.

VIG and Y had gains of 3% and 12%, respectively, which reflects in an increase (or selection gain) in the mean of the

selected population in approximately four bags of benefited coffee·ha⁻¹ more than that of the original population (Tables 2 and 3). This gain may be masked by environmental variance since these traits were not significant for individual genotypic variance. To obtain real increases in selection gains, the variability for these traits in the recombinant population must be increased. High values of genetic gain for yield hybrid progenies of *C. canephora* (65%) were reported by Leroy et al. (1997). Conversely, lower values were detected by Mistro et al. (2004), ranging from 15% to 8.15% when increasing the number of selected progenies of robusta coffee, based on the selection of only one variable.

Cluster analysis by the Tocher's method (Rao 1952) using the genetic distances of Mahalanobis as dissimilarity measure formed five progenies clusters, in which cluster I was formed by 13 progenies; cluster II was composed of three progenies; cluster III was formed by two progenies; and clusters IV and V were composed of one progeny each (Table 4). Clusters are different between and homogeneous within each other. The five clusters showed the most similar and divergent progenies, and those belonging to the distinct groups are more divergent when compared with those belonging to the same group, which simplifies the understanding of the population structure. Ivoglo et al. (2008) also identified four clusters between hybrid progenies from robust and conilon (koulou) coffee. The selected progenies (5, 9, 22, 4, 12, 2, 10, 15, 11, and 16) are of distinct clusters, containing representatives of clusters I, II, III, IV, and V. Therefore, the cross between progenies of different clusters increases the probability of favorable combinations in offspring to compose the crossing blocks. Resende et al. (2014) emphasize that if the goal is to create more variability or promote heterosis, the cross between genetically different clusters is preferable.

Clones Selection

The plants selected for cloning were originated from seven of the ten progenies obtained and evaluated in this work (5, 9, 22, 4, 12, 2 and 15). The best plants were selected based on the multi-trait index, using the calculated economic weights and the individual genotypic values, regardless of the selection of the best progenies. At 20% intensity selection, by selecting 48 coffee plants belonging to different progenies, 40% genetic gain was obtained. By evaluating Y of the selected genotypes, an increase in mean yield of 9.58 bags of benefited coffee per hectare was obtained when compared

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Table 3. Multi-trait selection index for the traits: reaction to rust (RUS), plant height (PH), diameter of canopy projection (DCP), vegetative vigor (VIG), maturation time (MAT), and yield (Y) with the selection of the three best individuals within the selected *Coffea canephora* progenies.

Order	Progenies	REP	RUS	PH	DCP	VIG	MAT	Y
1	5	4	2.36	169.18	191.95	6.37	1.34	45.21
2	5	1	2.56	168.18	189.86	6.35	1.34	46.24
3	5	29	2.44	172.41	192.06	6.39	1.34	44.87
4	9	8	1.17	171.45	175.48	6.09	1.34	41.52
5	9	14	1.21	174.15	176.45	6.08	1.33	42.30
6	9	6	1.19	174.32	176.01	6.08	1.34	40.37
7	22'	1	2.13	202.32	207.92	6.49	1.33	42.39
8	4	12	1.55	176.58	179.61	6.24	1.34	42.04
9	4	14	1.60	173.21	177.70	6.29	1.34	41.61
10	4	35	1.70	175.88	180.98	6.25	1.34	41.84
11	12	6	1.26	159.87	175.83	6.24	1.34	33.53
12	12	4	1.33	160.69	177.20	6.23	1.34	33.64
13	12	7	1.31	161.17	174.70	6.26	1.34	34.62
14	2	31	1.99	161.11	177.88	6.12	1.34	43.48
15	2	27	2.07	161.29	180.16	6.14	1.34	43.34
16	2	8	2.03	161.12	176.07	6.10	1.34	42.35
17	10	3	1.59	171.78	186.91	6.37	1.35	33.80
18	10	2	1.54	167.15	182.95	6.34	1.35	32.68
19	10	6	1.48	165.28	183.01	6.36	1.35	30.92
20	15	2	1.49	161.65	183.07	6.09	1.34	35.69
21	15	5	1.51	164.02	183.06	6.00	1.33	34.40
22	15	1	1.49	160.59	181.89	5.99	1.34	33.10
23	11	2	1.52	162.45	179.13	6.13	1.33	33.79
24	11	3	1.44	165.62	181.17	6.30	1.34	31.58
25	11	8	1.38	161.76	177.80	6.21	1.33	31.76
26	16	4	1.50	158.90	178.06	6.23	1.35	31.63
27	16	1	1.49	157.23	176.67	6.22	1.35	30.21
28	16	2	1.56	156.29	175.46	6.18	1.34	29.23
Mean			1.64	166.99	181.39	6.22	1.34	37.43
**SG			0.00	2.00	3.00	3.00	-31.00	12.00

REP = replications, *Progenies with only one representative individual; **SG = Percentage of selection gain.

Table 4. Clustering analysis by the Tocher's method between 20 *Coffea canephora* hybrid progenies for reaction to rust (RUS), plant height (PH), diameter of canopy projection (DCP), vegetative vigor (VIG), maturation time (MAT), and yield (Y).

Cluster	Genotypes													
1	16	20	7	3	15	14	13	18	12	11	17	10	19	
2	4	2	5											
3	21	22												
4	8													
5	9													

with the general mean of the population (Table 2), totaling a mean of 42.98 bags of benefited coffee per hectare (data not shown in tables).

This fact is fundamental for the *C. canephora* breeding program. The use of different genotypes for the locus controlling this trait should be considered in commercial varieties. Ferrão et al. (2007) suggest that a clonal variety is composed of at least eight different clones, which ensures good sustainability of the activity and prevents risks of genetic vulnerability.

Selection between and within progenies to compose the recombinant population should contain different crosses and ensure the genetic variability of the population since it will go through several IRS cycles in a breeding program. At the same time, individuals should be selected by the genotypic value and subsequently cloned, which will reduce the time to obtain a clonal cultivar, as proposed by Ferrão et al. (2007).

CONCLUSION

Genetic variability was observed by the estimates of intrapopulation genetic parameters, with selection gains successfully obtained from the multi-trait selection index, even with unbalanced data. Thus, the selection in hybrids

derived from crosses between the groups robusta and kouilou, combined with the robustness of mixed models methodology, proved to be a viable selection strategy for the *Coffea canephora* breeding program.

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