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Performance of intraspecific hybrids (Kouillou x Robusta) of *Coffea canephora* Pierre

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The experiment was carried out in 2005 in the municipality of Ouro Preto do Oeste at the experimental station of Embrapa Rondônia. 256 genotypes were evaluated, from nine hybridizations among parents of Kouillou (Cpafro 121, Cpafro 194, Cpafro 199 and Emcapa 03) and Robusta (Robusta 1675, Robusta 2258 and Robusta 640). The experimental design was a randomized complete block design, where each cross was represented by a maximum of 32 plants, distributed in four replications. The spacing used was 3.5 x 1.5 m. The average of six harvests and the yield of hulled coffee was 74.90 bags ha⁻¹. Highlight for progeny is Emcapa 03 x Cpafro 194 with average yield of 97.71 bags ha⁻¹. The estimated heritability observed for yield of coffee benefited, was 78.96%. The evaluation of six production seasons was allowed to verify the reliability of the genetic parameters in each set of crops. It was possible to observe that only the evaluation of the 1st and 2nd crops would be enough to estimate the genetic parameters with efficiency, with a coefficient of determination (R^2) of 79.10%. With the inclusion of the 3rd crop, R^2 estimates rose to 81.10%. The individual evaluations (per plant) carried out in 2006/2007, 2007/2008 and 2008/2009 crops showed the superiority of the plants from crosses 7, 8 and 9. In relation to resistance of orange rust, most of the genotypes were resistant to disease, and 75% of the genotypes did not present any type of infection symptoms. Among the susceptible genotypes, there was a variation between 1 and 15% in the degree of lesion intensity.

Key words: Coffee breeding, amazon, quantitative genetic.

INTRODUCTION

Brazil is currently the largest producer and exporter of coffee beans, achieving a record harvest of 56.1 million

bags in 2016. Of this amount, 81% of the beans are represented by the species *Coffea arabica*, and 19% by

the species *Coffea canephora* (Porto et al., 2015). The International Coffee Organization (ICO) estimates that coffee consumption in the world in 2020 will be 166.10 million bags, which represent an increase of 17% compared to production of 142 million bags in 2012.

The state of Rondônia is the sixth largest producer of coffee in Brazil with 1.63 million bags, and it is the second largest producer of the species *C. canephora*. Mean yield in the state of Espírito Santo is 25 bags ha⁻¹, in Rondônia the yield is not more than 20 bags ha⁻¹ (Abic, 2015; Porto et al., 2015).

Commercial crops of *C. canephora* in the state of Rondônia began from introduction made by farmers themselves, without defined genetic origin, resulting from natural recombination and phenotypic selection undertaken by them in coffee fields of Espírito Santo and São Paulo (Ferrão et al., 2007; Souza and Santos, 2009).

The species *C. canephora* is more recommended for low latitude regions with high mean annual temperatures (22 to 26°C) and, within certain limits in regions with a longer dry period because, in general, canephora coffee clones show greater tolerance to water deficit than genotypes of arabica coffee (Camargo, 2010).

Phenotypic, biochemical, and molecular evaluations have been used to study the genetic diversity and population structure of *C. canephora* in natural populations and in germplasm collections (Berthaud, 1986; Dussert et al., 1999; Cubry et al., 2008). These studies converge toward the existence of two groups: Guinea, which is composed of genotypes from west Africa (Guinea and Ivory Coast), with smaller leaves, less vigor, smaller plant size, small fruit, lower quality beverage, drought tolerant, and susceptible to coffee leaf rust (*Hemileia vastatrix* Berk. et Br.); and Congolese, composed of genotypes from the central region of Africa divided into four subgroups: SG1, SG2, B, and C.

Recently, a new subgroup composed of wild accessions from Uganda was proposed (Musoli et al., 2009; Montagnon et al., 2012). The SG1 group gathers genotypes Kouillou (Conilon) which are present from Benin to Gabon and have similar adaptive characteristics from the Guinea group, such as drought tolerance and susceptibility to coffee leaf rust. Subgroups SG2, B, and C are composed of the Robusta genotypes, which consist of taller, more vigorous plants, with larger leaves and fruit, better beverage quality, greater rust resistance, and greater sensitivity to drought (Montagnon et al., 2012).

Within this context, the aim of this study was to select intrapopulational hybrids of *C. Canephora* (Conilon x

Robusta) of high yield that show resistance to rust and drought tolerance.

MATERIALS AND METHODS

The experiment was set up in September, 2004 in the Embrapa experimental field, in municipality of Ouro Preto do Oeste, RO, Brazil, located at the geographic coordinates 10°44'53"S and 62°12'57"W. Climate in the region is classified as Tropical Rainy, Aw (Köppen), with mean annual temperatures of 25.8°C and mean rainfall of 2000 mm/year. Mean altitude of the region is 240 m, with relative humidity close to 82%, most of the year.

256 genotypes were evaluated from nine hybridizations among parents of the 'Conilon' (Cpafro 121, Cpafro 194, Cpafro 199, and Emcapa 03) and 'Robusta' cultivar (Robusta 1675, 2258, and 640). The experimental design used was randomized blocks in which each cross was represented by at most 32 plants, distributed in four replications. The spacing used was 3.5 x 1.5 m. The experiments were conducted according to fertilization recommendations for the coffee crop. The normal management practices used for the crop were adopted.

In 1970s and 1980s, coffee breeding activities at Embrapa Rondônia were limited to evaluations of clones developed in others Brazilian coffee research institutions, such as the Agronomic Institute of Campinas (IAC), the Federal University of Viçosa (UFV), the Agronomic Institute of Paraná (IAPAR) and the Capixaba Institute for Research, Technical Assistance and Rural Extension (INCAPER). During this period, the main objectives were the adaptation of cultivars and management techniques. Among those activities, there was an introduction of *C. canephora* progenies from the Agronomic Institute of Campinas - IAC, in São Paulo (Souza et al., 2013).

More recently, in order to implement new strategies to generate genetic variability, breeding populations were structured using superior genotypes from 'Conilon' and 'Robusta' botanical varieties. The hybrid vigor which is defined as the superior performance of progenies from divergent crosses is a characteristic of this species. Combinations among divergent parents express a greater heterotrophic effect, and the selection of parents should consider both genetic divergence and superior agricultural merit (Resende, 2002). Berthaud (1980) and Montagnon et al. (2008) observed that crosses between genotypes of high genetic divergence produced progenies with yields of 20 to 50% above the average of the control clones in conilon coffee. Ferrão et al. (2008) and Leroy et al. (1997) also report the heterotic potential of divergent combinations.

The yield characteristic was evaluated in 60 kg bags of hulled coffee per hectare (bags ha⁻¹). To estimate the coffee bean yield in terms of bags of hulled coffee per hectare (bags ha⁻¹), each plot was harvested and then weighed on an analytic balance. Conversion of total coffee fruit from the plots in different stages of maturation to hulled coffee (60 kg bags) was performed based on plant spacing and harvest production. In 2006/2007, 2007/2008, 2008/2009, and 2011/2012 crop seasons, coffee was harvested from each plant individually.

In the following crop seasons, harvest was carried out per plot, each plot being represented by eight plants. The size of the coffee

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beans (average size from sieve classification – SC) was measured using sieves from number 8 to 19. To do so, 100 g samples of dry hulled coffee were separated. Classification was made by means of a series of sieves. The percentage of “flat” type beans was considered resulting from the sum of the round mesh sieves from number 12 to 19. The percentage of “mocha” type beans (%) was obtained by the sum of all the sieves of oblong mesh from 8 to 13 (Brasil, 2003).

Evaluations in regard to resistance to coffee leaf rust were performed in a laboratory using leaf disks according to the methodology described by Eskes (1982). Urediniospores of *H. vastatrix* were collected with the aid of capsules from diseased leaves of coffee plants. The capsules were kept within a container with a desiccant under refrigeration at 4°C until use. Before inoculation on leaf disks, the viability of the inoculum was evaluated by germination of urediniospores in 2% agar-water using the method described by Zambolim and Chaves (1974). Eight leaves were collected from each genotype, from which a leaf disk was removed, such that each genotype was represented by eight leaf disks. The disks were placed in gerbox (clear polystyrene) boxes and inoculated with a concentration of 2.37×10^5 of rust urediniospores. After 30 days, the leaf disks were evaluated.

The data obtained in regard to hulled coffee yield were subjected to analysis of variance, with the significance of the effects verified by the F test at 5% probability. Selective accuracy (\hat{f}_{gg}), is determined by means of the expression $\hat{f}_{gg} = (F-1/F)^{1/2}$, in which F is the value of the Snedecor F test for the genotype effect (Resende and Duarte, 2007). This was estimated to check experimental precision. The analyses of variance and specific combining ability (SCA) were carried out using the Genes computational software (Cruz, 2013).

RESULTS

Through combined analysis of all the crop seasons, a significant difference was observed among progenies for the characteristic of hulled coffee yield, showing that there is a wide genetic variability among the genotypes evaluated (Table 1). The same significance was observed among crops seasons, indicating environmental variation throughout the years of evaluation. The progeny x crop season interaction did not exhibit significant difference, showing that the response of the progenies coincided in all the crop seasons (Table 1).

All the crop seasons exhibited selective accuracy estimates of high magnitude ($52.93\% < \hat{f}_{gg} < 82.41\%$), indicating good experimental precision. The lowest mean yield of hulled coffee was observed in 2006/07 crop season (17.68 bags ha^{-1}). The reason for low yield was that, it was the first crop season for production. The 2007/08 and 2008/09 crop seasons obtained satisfactory production, with mean yields of 58.50 and 64.31 bags ha^{-1} , respectively. The 2011/2012, 2012/2013, and 2013/2014 crop seasons, for their part, obtained expressive harvests, achieving yields above 93 bags ha^{-1} (Table 2). In the mean of six harvests, the hulled coffee yield was 74.90 bags ha^{-1} . The progeny Emscapa 03 x Cpafró 194 stood out, with mean yield of 97.71 bags ha^{-1}

(Table 2). The heritability estimate observed for hulled coffee yield was 78.96% (Table 1).

The evaluation of six production crop seasons made it possible to verify the reliability of the genetic parameters in each set of crop seasons (Table 3). It could be observed that evaluation of the 1st and 2nd crop season alone would already be enough to estimate the genetic parameters efficiently, with a coefficient of determination (R^2) of 79.10%. With inclusion of the 3rd crop season, the R^2 estimates rise to 81.10%. In breeding programs in which there were needed to advance generations, the earlier selection can be carried out; the more dynamic the process of obtaining lines is. In this specific case, selection of the best progenies could be carried out in the second harvest, ensuring efficiency in the selection process.

The individual evaluations (per plant) carried out in 2006/2007, 2007/2008, and 2008/2009 crop seasons showed the superiority of the plants derived from crosses 7, 8, and 9. Figure 1 shows hulled coffee production (kg/plant) of the 12 best plants evaluated and the respective families from which they came.

In relation to resistance to coffee leaf rust, most of the genotypes proved to be resistant to the disease; 75% of the genotypes did not show any type of symptom of infection. However, among the susceptible genotypes, there was a variation from 1 to 15% in the degree of intensity of the lesions. Figure 2 shows the difference between susceptible and resistant genotypes in regard to the presence of lesions on the leaf disks.

DISCUSSION

All the crop seasons showed high selective accuracy. This indicates that the trials were well conducted throughout all the crop seasons, providing reliability to the genotypic values obtained for hulled coffee yield.

The use of accuracy as a measure of experimental precision, suggested by Resende and Duarte (2007), has the advantage of not depending on the mean, which provides a higher degree of reliability in the use of phenotypic expression as an indicator of genotypic variation. Accuracy values above 70% indicate high experimental precision.

The estimate of heritability near 79% ensures success in selection of superior genotypes and indicates the proportion of genetic variance in total phenotypic variance, that is, the heritable proportion of total variability (Mohsin et al., 2009).

Conclusion

The mean yields of the progenies with best performance

Table 1. Summary of combined analysis of variance and estimates of heritability (h^2) and selective accuracy (\hat{r}_{gg}) for hulled coffee yield (bags ha⁻¹) in reference to the years 2007/08, 2008/09, 2009/10, 2011/2012, 2012/2013, and 2013/2014 crop seasons.

SV	DF	Mean Square
		Hulled coffee yield (bags ha ⁻¹)
Blocks	3	1121.37
Progenies	8	3344.88 ^{**}
Crop seasons	5	44676.34 ^{**}
Progenies x Crop Seasons	29	485.98 ^{ns}
Residue	96	703.52
Overall Mean	74.90	
Heritability (h^2)	78.96	
Selective Accuracy (\hat{r}_{gg})	88.87	

^{**}: Significant at 1% and 5% by the F test, respectively.

Table 2. Performance of the nine (9) progenies in regard to hulled coffee yield (bags ha⁻¹), in reference to the 2007/08, 2008/09, 2011/12, 2012/13, and 2013/14 crop seasons. Ouro Preto do Oeste, RO, Brazil, 2014.

Cross	Hulled coffee yield (bags ha ⁻¹)							Average sieve classification (SC)	Mocha beans (%)
	2006/07	2007/08	2008/09	2011/12	2012/13	2013/14	Overall Mean		
Robusta 640 x Cpafró 199	14.05	66.22	52.93	101.32	99.22	79.28	68.84	15.8	62.3
Robusta 640 x Cpafró 194	16.08	49.78	58.97	88.81	91.57	96.05	66.88	15.1	59.2
Cpafró 199 x Cpafró 121	15.47	60.67	71.59	118.20	105.26	93.87	77.51	15.5	78.3
Emcapa 03 x Robusta 640	25.65	57.27	63.14	115.30	92.90	88.09	73.72	14.6	73.5
Emcapa 03 x Cpafró 121	14.61	55.63	60.61	146.30	117.82	99.34	82.38	15.0	81.5
Emcapa 03 x Cpafró 194	11.38	39.27	59.11	90.49	75.94	76.00	58.70	14.2	63.5
Emcapa 03 x Robusta 1675	27.81	79.33	91.39	143.98	123.08	120.68	97.71	15.4	55.8
Emcapa 03 x Robusta 2258	17.05	58.35	43.38	92.95	101.19	77.41	65.05	15.7	70.0
Robusta 1675 x Cpafró 194	17.07	60.07	77.68	121.13	110.33	113.30	83.26	16.2	35.8
Overall Mean	17.69	58.51	64.31	113.16	101.92	93.78	74.89	15.28	64.43

Table 3. Estimate of the coefficient of determination (R^2) in relation to the number of harvests and time of harvest. Values in regard to sets of two, three, four, five and six crop seasons. Ouro Preto do Oeste, RO, Brazil, 2014.

No. of harvests	Crop seasons	Component	R^2
2	1st and 2nd	0.65	79.10
3	1st, 2nd, and 3rd	0.58	81.10
4	1st, 2nd, 3rd, and 4th	0.58	84.87
5	1st, 2nd, 3rd, 4th, and 5th	0.61	88.92
6	All the crop seasons	0.64	91.60

were superior to observations of trials of the cultivar BRS Ouro Preto presented by Ramalho et al. (2015), in the climatic conditions of the state of Rondônia. Thus, the results obtained show the high potential of some clones

for purposes of recommendation of new cultivars for Rondônia.

The high productive potential observed in the descendants of families 7, 8, and 9 suggest high

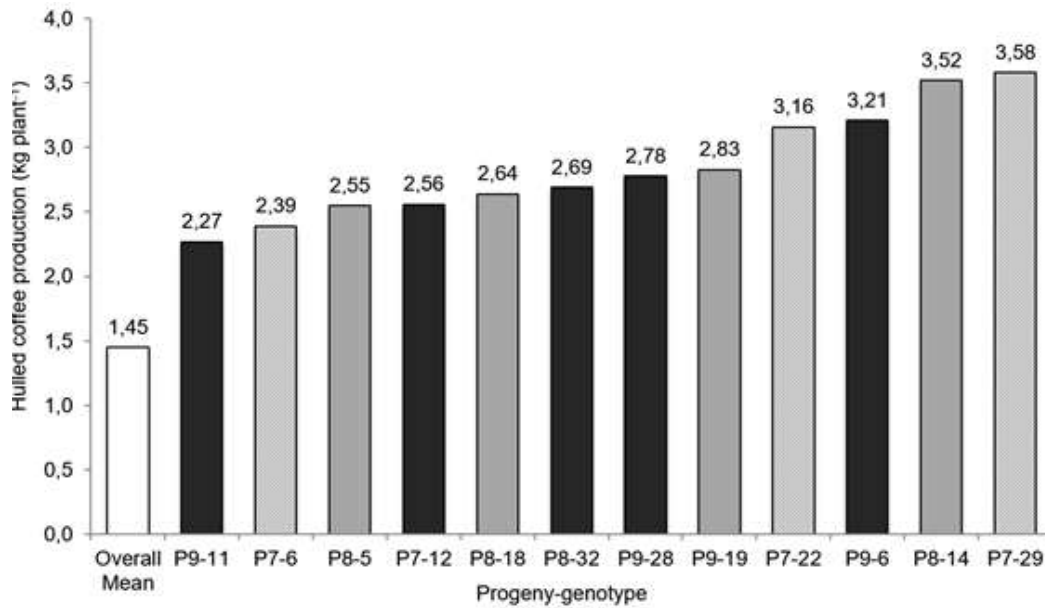


Figure 1. Production of hulled coffee (kg/plant) of the 12 plants that showed highest production in their respective families and the experimental mean. Mean of the years 2007, 2008, 2009, and 2012, Ouro Preto do Oeste, RO, Brazil.

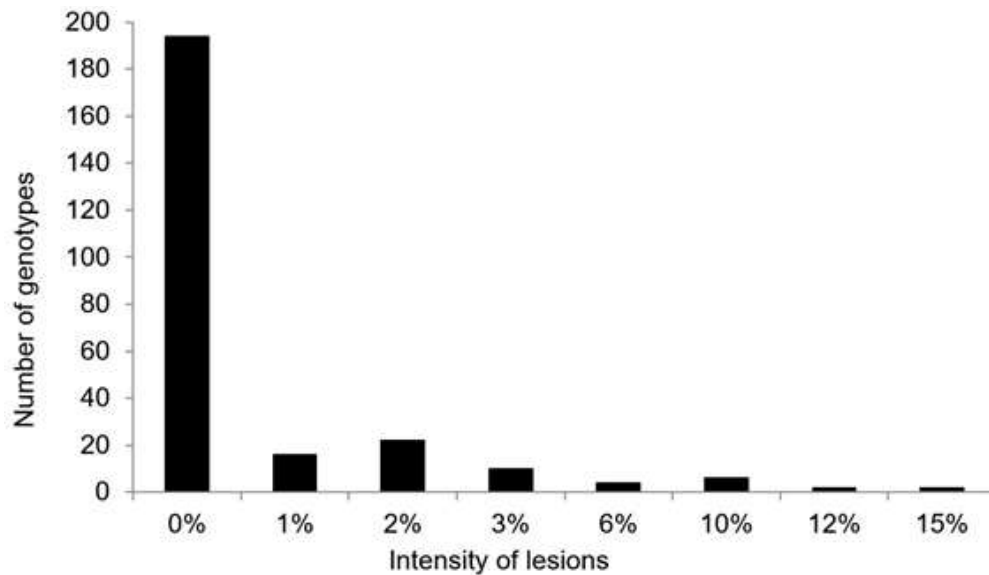


Figure 2. Frequency distribution in regard to intensity of lesions in 256 genotypes evaluated. Porto Velho, RO, Brazil, 2012.

expression of heterosis in crosses between divergent or unrelated parents. These results reinforce the predominance of heterosis in hybrids of *C. canephora*. This fact has guided breeding programs in the search for

high yielding hybrids that aggregate desirable characteristics of both 'Conilon' and 'Robusta' varieties. It is up to the breeder to choose the most promising parents and carry out the greatest possible number of

crosses, so as to exploit all the variability within the species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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