

Selection of conilon coffee clones tolerant to pests and diseases in Minas Gerais

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Abstract: *In the northern Minas region, the dry-warm climate predisposes coffee plants to the occurrence of leaf miners, mites, cercosporiosis, and leaf scald. Aiming for the development of a cultivar adapted to these conditions, Coffea canephora Pierre ex A. Froehner clones were selected through genetic parameters under an irrigated system, without agrochemicals. Eighteen agronomic traits were evaluated. The survival rate, number of nodes per plagiotropic branch, leaf miner infestation and cercosporiosis incidence were chosen as characteristics for selection of 'Vitória Incaper 8142', once they have shown superiority of the genetic parameters. The survival rate variable was used to rank the EMCAPA 8141 Robustão Capixaba clones. Clones V2, V4, V6, V13, RC7, and RC9 were selected as more tolerant to pests and diseases and can provide genetic improvements in conilon breeding program for region. The genetic dissimilarity identified between clones allowed suitable clone combinations to be proposed for use in future crosses.*

Keywords: *Coffea canephora, Vitória Incaper 8142, EMCAPA 8141 Robustão Capixaba, multivariate, mixed models.*


INTRODUCTION

Coffee is one of the commodities most exchanged in international markets, with an annual world production of 158 million 60kg bags of coffee beans. However, climatic conditions, such as drought and high temperature, adversely affects the occurrence of pests and diseases and plant development and production, causing economic damage to producers (OIC 2018).

Evidence suggests that recent shifts in climate have already affected the distribution and biology of disease and pest species of coffee (Ziska et al. 2018). Thus, in recent years, the use of pesticides in Brazil has increased, making the country the largest consumer of agrochemicals in the world (Pignati et al. 2017). The extensive use of pesticides in agricultural practices has been associated with human health problems and environmental contamination (Queiroz et al. 2018). In this context, an expressed demand exists for technologies that should be implemented to improve crop sustainability, especially regarding the availability of cultivars tolerant to diseases and pests. Plant screening and breeding are fundamental adaptation strategies to produce new cultivars with improved tolerance to biotic stresses and acceptable yields (DaMatta et al. 2018).

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The conilon coffee cultivation has been considered as an alternative to agriculture in the Northern Region of Minas Gerais, due to its characteristics of high productive potential, and large rusticity. It also has market potential, as a result of the growing world demand in blends with arabica coffee, soluble and espresso and other modern forms of consumption. This cultivation could be important for the generation of jobs and income and, mainly, to contribute to keeping the family farmer in the Northern Region of Minas Gerais bringing not only economic but also social benefits.

It is emphasized that land use by coffee cultivation is concentrated in the San Francisco River irrigated perimeter, and the temporal and spatial rainfall variability is overcome by the use of irrigation. However, the dry climate, with low relative air humidity when compared with other coffee producing regions, high insolation, and high temperatures, predisposes coffee plants to the occurrence of leaf miners, mites, cercosporiosis, and leaf scald (Venturin et al. 2013). Under these circumstances, the tolerant coffee can be grown using a lesser amount of pesticides are important as environmental protection and reliable food source for human health.

In the improvement of conilon coffee (*Coffea canephora* Pierre ex A. Froehner), the species are cross-fertilized and present self-incompatibility of the gametophytic type. Therefore, to produce fruit, the crops require clonal cultivars that produce a set of compatible clones or cultivars from seeds (Ferrão et al. 2017). Thus, to be obtained or selected as superior cultivars, clones must have favorable agronomic traits, environmental adaptations, and genetic compatibility (Moraes et al. 2018). The “EMCAPA 8141 Robustão Capixaba” and “Vitória Incaper 8142” cultivars present clones with these traits (Ferrão et al. 2017).

Expressive demands for production technologies of this species in these specific areas are highly needed, especially regarding the availability of cultivars adapted to the heat (Silva et al. 2017), as well as tolerance to diseases and pests. Aiming to the development of a cultivar adapted to these conditions, we selected conilon clones according to genetic parameters for tolerance to pests and diseases under irrigated systems and without the use of pesticides.

MATERIAL AND METHODS

This study was carried out since January 2012 at the Mocambinho Experimental Farm of the State Agricultural Research Agency (Empresa de Pesquisa Agropecuária do Estado de Minas Gerais-EPAMIG), which is located in a semiarid region in the extreme north of Minas Gerais, at lat 15° 05' S, long 44° 00' W, at alt 452 m asl. The climatic condition is characterized as semiarid the landscape is flat, and the soil is silty. The total annual precipitation is 750 mm, concentrated in November to March. Annual temperatures in the study area average 28 °C, whereas the sunlight averages 9.5 h day⁻¹, and the relative air humidity averages 48%.

Two different cultivars of conilon were evaluated under an irrigation system. Experiment E1 was composed of 13 clones of the cultivar Vitória Incaper 8142 (V1, V2, V3, V4, V5, V6, V7, V8, V9, V10, V11, V12, and V13). Ten clones of the cultivar EMCAPA 8141 Robustão Capixaba (RC1, RC2, RC3, RC4, RC5, RC6, RC7, RC8, RC9, and RC10) were used in the Experiment E2. Conilon seedlings were cultivated for six months in 500 mL polypropylene bags filled with subsoil and cattle manure in a 2:1 proportion, plus potassium chloride and superphosphate in the proportion of 1:10. After selection for uniformity in size and vigor, seedlings with four pairs of leaves were transferred to the cultivation area with a plant spacing of 3.0 × 1.0 m. The design was completely randomized and evaluated in a block with four replicates and eight plants per plot.

The drip irrigation system had a flow rate of 1.7 L hour⁻¹, and irrigation was applied every 50 cm. The water for the drip tubes was supplied by a 32-mm PVC pipe. Channel water was provided by the Jaíba Irrigation District. By providing a 1-m-wide wet strip (sandy soil), the drip system was able to apply 3.4 mm h⁻¹. Irrigation management was performed based on reference evapotranspiration (ET) data estimated by the Davis Vantage Pro Plus automatic weather station, which, after measuring temperature, relative air humidity, solar radiation, and wind, calculated hourly ET using the FAO Penman-Monteith equation (Allen et al. 1998). Regarding evapotranspiration values, variation from 3 to 9 mm day⁻¹ occurred, and the irrigation periods were approximately 1 to 2.5 hours. Considering the low water-storage capacity of the Jaíba soils, the design of the irrigation system made it possible to adopt a daily watering frequency to replenish crop evapotranspiration. For the initial phase, the Kc value was 0.70, and the application efficiency of the system was estimated at 90% (Ea = 0.90). The experimental planting and conditions were performed according to the technical recommendations for the culture of irrigated conilon. Pests and diseases were not controlled by the application of agrochemicals.

The survival rate (SUR) of the seedlings was determined in four evaluation periods (at 7, 24, 33, and 48 months following experiment installation). The vegetative characteristics were evaluated in three periods (at 7, 24, and 33 months of the beginning of the experiments) by means of the following variables: stem diameter (SD) (cm); height (H), measured from the base of the stem (ground surface) to the shoot apex (m); canopy diameter (CD), measured at superior crop canopy area; length of plagiotropic branch (LPB), which measured the reach of the largest branch in a transverse direction to the planting line; number of plagiotropic branches (NBP); and number of nodes per plagiotropic branch (NN), which were measured by counting those present in four alternating branches. The coffee leaf miner (LM) infestation and cercosporiosis (CER) incidence evaluations were carried out at 16, 18, 24, 29, 33, 42, and 45 months following experiment installation. The incidences of predatory mite (PM) infestation and leaf scalding (SCA) (cellular damage caused by full sun exposure) were analyzed at 24, 30, 34, 42, and 45 months after the start of the experiment. Pest and disease were monitored at 10 leaves collected per plant from both plant sides of the six central plants, from the 3rd or 4th leaf pairs, totaling 60 leaves per plot. The disease incidence and insect infestation were determined by calculating the percentage of leaves with lesions among the 60 leaves collected.

Measurements of agronomic traits were performed, including field productivity (PROD), processed coffee yield (YLD), and grain size, which was performed using sieve separation (% S10 and % S15-13). PROD was evaluated by determining the number of 60-kg bags of hulled coffee produced per hectare (bags ha⁻¹). The harvest was carried out in individual plots, and the volume of field coffee (coffee fruits of mixed maturity) per plot was considered the PROD. Subsequently, the volume of harvested coffee was converted to bags ha⁻¹ and considered the YLD. This determination was performed by collecting a representative sample of coffee produced from each plot. The grain samples were dried and processed, and the resulting coffee was graded using sieve techniques to determine grain quality. The analysis was carried out according to Normative Instruction No. 08 (MAPA 2003), and the grain grade was determined by calculating the percentages of grains retained in sieve 10 (% S10) and in sieves 15 and 13 combined (% S15-13). Pest and diseases assessment were evaluated according to Reis and Cunha (2010).

The growth variables were analyzed according to the mixed linear model in the matrix form (Resende 2016) with the following equation: $y = Xm + Zg + Wp + Ti + Qs + e$, in which y is the data vector, m is the vector of the effects of evaluation-replicate combinations (assumed to be fixed) added to the general mean, g is the vector of the genotypic effects (assumed to be random), p is the vector of the plot effects (random), i is the vector of the effects of genotype x evaluation interaction, s is the vector of the permanent environmental effects (random), and e is the error or residue vector (random). The uppercase letters represent the incident matrixes for the referenced effects.

Variables related to the incidence of disease, PM, SCA, and PROD and grain size were analyzed using the mixed linear model methodology (univariate additive) adapted by Resende (2016) with the following equation: $y = Xb + Za + Xc + e$, in which y is the data vector, b is the data vector of block effects (fixed), a is the vector of the genotypic effects (assumed to be random), c is the vector of the plot effects (random), and e is the error or residue vector (random). The uppercase letters represent the incident matrixes for the referenced effects.

Estimates of the genetic parameters were obtained by the REML/BLUP (restricted maximum likelihood/best linear unbiased prediction) procedure using SELEGEN software (Resende 2016). Likelihood ratio tests (LRT) ($p < 0.05$) were used to test the significance of random effects on the variance between clones (σ_g^2), whereby deviance analysis was performed for each characteristic (Resende 2016).

For the characteristics that were affected by the interactive effect of clones x crop year, analyses were carried out using the harmonic mean method of the relative performance of genetic values (HMRPGV). For the others, the Mulamba and Mock (1978)'s index was used to select the best clones in both varieties. In addition, a genetic divergence study was carried out using genotypic values predicted for each clone of the Vitória Incaper 8142 within each group, which were used to estimate Mahalanobis genetic distance matrixes. For the delimitation of the groups, the optimization technique proposed by Tocher and referred by Rao (1952) was used.

RESULTS

Genetic variability was observed among the clones of Vitória Incaper for the characteristics SUR, NN, CER, LM, and P10 (Table 1) and among the clones of EMCAPA 8141 Robustão Capixaba only for the SUR trait. The heritability of the

Table 1. Estimates of genetic parameters related to survival (SUR), stem diameter (SD), plant height (H), length of plagiotropic branches (LPB), canopy diameter (CD), number of plagiotropic branches (NPB), number of nodes per plagiotropic branch (NN), percentage of leaves with cercosporiosis (CER), percentage of leaves with coffee leaf miner (LM), percentage of leaves with predatory mites (PM), leaves with scalding (SCA), productivity (PROD), yield (YIELD), and sieve 10 (P10) and sieve 13 (P13) values of clones of Vitória Incaper 8142 (E1) and Robustão Capixaba (E2).

	SUR	SD	H	LPB	CD	NPB	NN	CER	LM	PM	SCA	PROD	YLD	% S13	% S10	
E1	σ_g^2	199.80*	3.86	8.61	0.41	9.75	11.26	2.20**	133.77*	32.38**	15.63	5.98	0.06	1.04	22.26	25.11**
	h_g^2	0.17*	0.02	0.02	0.00	0.01	0.06	0.19*	0.17**	0.10	0.08	0.00	0.04	0.14	0.56**	
	R_{gMED}	0.50	0.60	0.27	0.04	0.24	0.43	0.95	0.71	0.38	0.39	0.01	0.07	0.18	0.80	
	Mean	68.58	24.42	54.13	14.39	43.44	30.13	11.66	39.00	43.89	11.95	8.29	5.35	51.01	40.36	11.69
E2	σ_g^2	566.05**	0.60	0.43	0.21	16.92	0.51	0.01	24.80	9.22	0.11	25.35	28.57	13.36	0.10	4.61
	h_g^2	0.56**	0.00	0.00	0.00	0.02	0.00	0.00	0.12	0.04	0.00	0.21	0.54	0.00	0.29	
	R_{gMED}	0.91**	0.69	0.23	0.04	0.92	0.52	0.25	0.31	0.62	0.117	0.96	0.99	0.99	0.14	0.59
	Mean	29.51	19.95	52.98	13.64	39.25	24.09	7.87	35.35	41.15	15.84	9.01	6.83	48.44	15.03	13.72

σ_g^2 genotypic variance; h_g^2 heritability of individual plots in the broad sense, that is, of the total genotypic effects; R_{gMED} genotypic correlation through measurements; Overall mean of the experiment. *Significant at $p < 0.01$ and $p < 0.05$, respectively, by the likelihood ratio test.

parcels in the broad sense was of low magnitude for both cultivars. This indicates that the environmental conditions influenced these characteristics. In addition to the mentioned traits, CER, LM and P13 of the Vitória Incaper 8142 clones, also showed the same ranking in genotypic correlations throughout the evaluated periods.

For the SUR of the EMCAPA 8141 Robustão Capixaba clone, an interactive effect of evaluation periods and clones was demonstrated by the LRT test ($p < 0.05$), which indicates that the genetic and environmental effects are not independent. As shown by the high genotype correlation (0.91), the interaction is simple, indicating that the ranking of clones does not change throughout the periods evaluated.

The average temperatures during the years when the experiment were carried out were typical for the region, and the maximum temperatures were higher in 2014 and 2015, with averages above 32 °C, whereas the minimum temperatures were lower in 2012 (Figure 1). Relative air humidity was lower in 2015, and surface incident solar radiation was lower in 2012 (Figure 2). The high diurnal temperatures (often higher than 30 °C), low relative air humidity, and high insolation led to climatic conditions that were favorable to the occurrence of CER and LM; the general average occurrence of CER was 39.00% and LM was 43.89% for the Vitória Incaper 8142 clones. Similarly, in EMCAPA 8141 Robustão Capixaba, the average occurrence of CER was 35.35% and for LM, it was 41.15%.

The absence of any control measures for pests and diseases restricted the yield potential of these plants. The average productivity was 5.35 bags ha⁻¹ and 6.83 bags ha⁻¹ for Vitória Incaper 8142 and EMCAPA 8141 Robustão Capixaba,

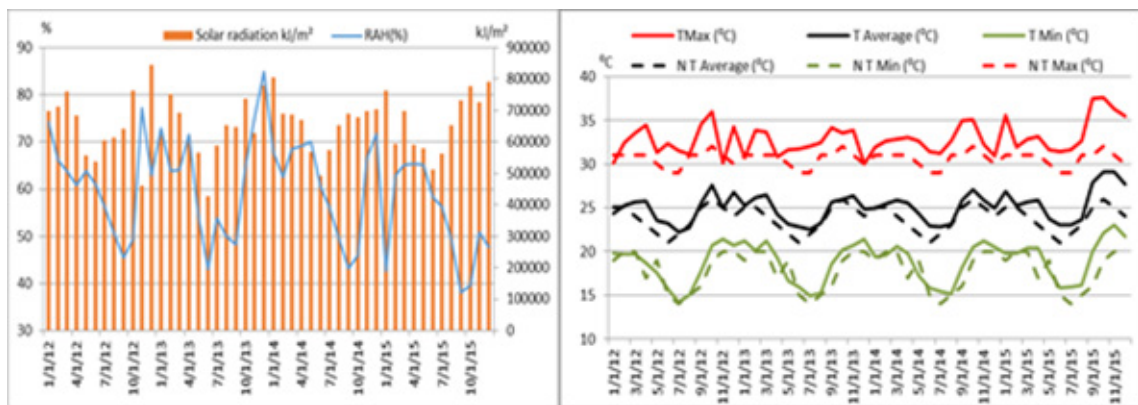


Figure 1. Meteorological variation (relative air humidity – RAH, incident surface insolation, average, maximum, minimum temperatures) and normal climate variation (average temperature) in the region of Mocaminho, MG, Brazil. Source: Adapted from INMET 2018 and Epamig 1982).

respectively. These values are below the yield of those cultivars in Espírito Santo state (Ferrão et al. 2017). These results may also have influenced the YLD of Vitória Incaper 8142 and EMCAPA 8141 Robustão Capixaba, which was approximately 30 and 50% lower, respectively, relative to the yield values described for the cultivars in other regions. The S10 values feature were satisfactory because they were already below the 21.40% described (Ferrão et al. 2017).

The SUR, NN, CER, and LM were chosen as major characteristics for the selection of clones of Vitória Incaper 8142, once these characteristics have shown genetic parameters superiority. The interactive effect of clones x crop year on the NN characteristic was analyzed, and the use of methodologies to identify the most well-adapted and stable clones throughout the coffee tree were applied with the method of harmonic mean relative performance of genetic values (HMRPGV), which allows analysis simultaneously by characteristic, stability, and adaptability (Resende 2016).

According to the Mulamba and Mock (1978)'s index, the sum of the "ranks" classified clones V2, V4, V6, and V13 as superior to the others (Table 2). In the selection of these improved clones, the predicted genotypic values favored genetic gains relative to the genotypic mean of the test, by a 5.23% increase in NN, 7.26% increase in CER, and 15.57% increase in SUR for the 13 clones of the Vitória Incaper 8142 variety. Relative to LM, the selection of these improved clones can increase the occurrence of the pests by 1.13%. However, the selection of clones with lower LM percentages implies lower gains for the other characteristics, including the SUR (Table 2).

The genetic divergence study, using by Tocher group analysis, showed that the Vitória Incaper 8142 clones were grouped into four classes (data not shown), with the highest clones concentration (V1, V4, V5, V6, V7, V10, and V12) allocated into the first group. The clones V2, V3, V8, and V13 were targeted to the second group. Clone V9 was one of the most divergent and was allocated into group 3, followed by clone V11 in group 4. The Tocher method has been efficient for distinct groupings in studies carried out with *C. canephora* progenies (Covre et al. 2016).

Based on the estimates of the variance components, the SUR variable was used to rank the EMCAPA 8141 Robustão Capixaba clones. The mean of the genotypic values predicted for the survival characteristic was 29.51% (Table 2), which represents a low survival rate of the clones in a drip-irrigated system in the absence of pest and disease control. Only clones RC7 and RC9 presented survival rates above 50%.

DISCUSSION

The average temperatures north of Minas Gerais are 25 to 26 °C, which was verified during the experiment, and these temperatures are considered suitable for the cultivation of conilons. Conilon cultivation originated in the tropical regions of Africa, where it is traditionally grown under the shade of native forests, with average temperatures ranging

Table 2. Estimates of stability and adaptability of genotypic values (HMRPGV) for the number of nodes per plagiotropic branch (NN), genotypic values ($\hat{\mu}+\hat{\alpha}$); Mock index (1978) (Ij), percentage of selection gain (SG%) for NN characters, percentage of leaves with cercosporiosis (CER), coffee leaf miner (LM), and percentage of survival (SUR¹) of thirteen clones of the Vitória Incaper 8142 cultivar. SUR² of ten clones of the EMCAPA 8141 Robustão Capixaba at the Experimental Farm of Mocambinho, municipality of Jaíba, MG

Clones	MHRPGV	NN ($\hat{\mu}+\hat{\alpha}$)	CER (%) ($\hat{\mu}+\hat{\alpha}$)	LM ($\hat{\mu}+\hat{\alpha}$)	SUR ¹ (%) ($\hat{\mu}+\hat{\alpha}$)	Ij	Clones	SUR ² (%) ($\hat{\mu}+\hat{\alpha}$)
V1	10.60	10.87	47,35	41.63	64.43	36	RC1	43.25
V2	12.92	12.70	45,69	40.58	80.32	18	RC2	32.54
V3	13.04	12.80	39,11	51.46	66.02	29	RC3	6.44
V4	12.88	12.59	38,62	42.77	67.08	25	RC4	7.42
V5	9.36	10.08	52,96	36.81	53,31	37	RC5	7.37
V6	13.97	13.33	26,49	49.47	82.97	15	RC6	11.88
V7	11.06	11.15	35,80	43.08	80.32	26	RC7	70.17
V8	11.46	11.49	38,00	54.86	78.73	31	RC8	25.42
V9	12.63	12.51	33,30	42.07	63.90	26	RC9	56.36
V10	9.80	10.35	39,21	39.75	64.96	30	RC10	34.23
V11	11.49	11.58	59,43	42.73	51.19	40		
V12	8.72	9.65	26,61	41.09	64.96	29		
V13	12.78	12.47	24,44	44.20	73.43	22		
SG%		5.23	7.26	1.13	15.57			114.36

from 24 to 26 °C (DaMatta et al. 2018). However, the maximum average temperature during the experiment was 33 °C and occurred in association with low relative air humidity and high surface incident solar radiation.

The climatic conditions mainly favored the occurrence of leaf miner and cercosporiosis at the studied clones. In fact, the incidence of leaf miner depends on meteorological factors since high levels of attack occur in months with higher temperatures and low relative air humidity (Silva et al. 2018) in irrigated coffee trees grown in warm climate regions (Custodio et al. 2009, Custodio et al. 2010).

The clones were evaluated without chemical control for pests and diseases because the objective was to select conilon coffee clones tolerant to pests and diseases for development of a cultivar that allows maximum agricultural efficiency. Therefore, we used mixed models to obtain estimates of genetic parameters and genetic values under imbalanced conditions, maximizing the genetic gains with selection (Rezende 2016). This method allows the correction for environmental effects and accurate and nonbiased predictions of genotypic values (Henderson 1984), thereby maximizing the genetic gain with selection.

The genetic gain is inversely proportional to the intensity of selection, which quantifies the number of selected individuals. Thus, one consideration was the importance of analyzing a larger number of individuals (four clones, 30% selection intensity) to ensure a minimum effective number that allows greater efficiency in the subsequent selection stages (Table 2).

Concerning the NN trait, the clones V6, V2, V4, and V13 showed adaptability and stability with a minimum of interactions in the evaluation periods (Table 2). This solid result could indicate reliable performance during successive evaluations. It was observed that LM infestations varied between 40.58 and 49.47% of leaves with necrosis and the percentage of leaves with CER lesion ranged from 24 to 45.69% in the selected clones. Even under these conditions, these clones presented a survival rate between 67 and 83%, indicating the ability of clones to withstand biotic pressure.

The lower incidence of LM (36% and 39.75%) was verified in the clones with lower SUR, suggesting that these clones may have been more sensitive to pest attack due to prior harm or previous damages. Many leaf-miner-infested leaves appeared yellow. The yellowing, due to the destruction of leaf chlorophyll induced by the presence of the LM, indicates high infestation of the pest in the field.

Due to their significance for genotypic effect and higher magnitudes for heritability and genotype correlation through measurements, the variables NN, SUR, CER and LM which were considered for the classification and selection of clones resistant to the coffee leaf miner and cercosporiosis. They are also tolerant under high semiarid temperatures and conditions.

The results regarding the stability and adaptability of the genotypic values (Table 2) indicate that the selected clones of Vitória Incaper 8142 show stability in unfavorable environments, are able to respond satisfactorily in favorable environments, and may provide base populations for the cultivation of this variety in the northern region of the state of Minas Gerais without pest control.

Advances in work were hampered by the low survival rate of some EMCAPA 8141 Robustão Capixaba clones; therefore, the SUR was adopted as a specific criterion for selection of clones of this variety (Table 2). The low survival rate can be attributed to the high incidence of CER and LM. In addition, drip irrigation favors the formation of a more superficial root system for plants propagated by cuttings, which leads to damping off of seedlings in sandy soil. The new mean predicted with the selection of two clones (RC9 and RC7), based on survival, was 63.26, a value that would allow a 114.36% increase in selection of these clones.

Given that coffee species have evolved from shaded habitats, the harmful consequences of climate change are being enhanced by high levels of irradiance resulting in leaf scald (DaMatta et al. 2018). However, in this study, the occurrence of leaf scald in the initial phase of development was low, showing that the coffee plant is more resilient than generally thought. Coffee trees can be assumed to have the necessary phenotypic plasticity to acclimate to changing light environments (Semedo et al. 2018) and high levels of irradiance, with the ability to trigger photo protection and antioxidant mechanisms and to reinforce the components of the photosynthetic machinery (Partelli et al. 2014).

Considering the four characteristics studied NN, CER, LM and SUR (Table 2), it was possible to identify the main

intra-population crosses to obtain greater genetic variability. Standing out among them is V2 x V4, V2 x V6, V13 x V4, and V6 x V13, thus increasing the probability of obtaining superior individuals in the progeny. According to Huang et al. (2015), genotypes of distinct groups may be crossed, to obtain greater genetic variability in the progeny or possible heterosis in traits that exhibit allele dominance.

The existence of genetic variability and selective potential among the conilon coffee genotypes was also demonstrated in other studies of genetic divergence through multivariate techniques using the mixed model method employed in populations of conilon (Silva et al. 2017, Oliveira et al. 2018).

Ferrão et al. (2017) recommend that “Conilon” clonal coffee cultivars be made up of a combination of at least eight different genotypes, although new clonal varieties have been developed by grouping nine to 14 genotypes. Therefore, studying new clones, including seed-propagated materials, in combination with the V2, V4, V6, V13, RC7, and RC9 clones is necessary to establish a population adapted to the cultural conditions in these areas. Finally, it is suggested that these new germplasm introductions may contribute substantially to future hybridization works for the improvement of conilon coffee for the Northern region of Minas Gerais.

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