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Biometrical studies on characteristics of plagiotropic branches in *Coffea arabica* L. cultivated with high plant density

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Abstract

The great demand for improvements in productivity lead to the search of new information regarding the behaviour of new cultivars of arabica coffee. Improved genotypes need be studied in specific regions to evaluate their potential, such as the exploration on mountains, aiming to define variables that can be exploited for the study and selection of genotypes in those conditions. This study aimed to evaluate the genetic diversity of genotypes of *Coffea arabica* L. with the potential for cultivation in increased-density systems in the region of Caparaó (state of Espírito Santo, Brazil), based on the characteristics of plagiotropic branches in production, to evaluate the existence of correlations and to estimate the relative contributions of each of the aforementioned characteristics to the diversity. To this end, 25 characteristics were determined in 16 genotypes of arabica coffee cultivated under competition of density (evaluated in 2013, with the stabilisation of their reproductive phenological cycle). These genotypes cultivated with high plant density, IAPAR 59 and Catiguá MG2 presented a greater degree of dissimilarity. Of the 25 traits, the number of fruits per branch and the biomass contributed more to the variability among the genotypes (over 95% of accumulated contribution). A total of 111 significant correlations were observed, with values over |0.60| in 78% of cases. Strong positive and negative correlations are observed between the various characteristics, revealing the possibility of the simultaneous selection of correlated traits.

Keywords: coffee, correlation, genotypes, multivariate analysis, variability.

Abbreviations: LNG_Length of the plagiotropic branch; DIA_Diameter of the branch; NND_Number of nodes; VND_Number of vegetative nodes; RND_Number of reproductive nodes; LND_Length of the internodes; GFT_Number of green fruits; MFT_Number of mature fruits; NFT_Number of fruits; MAT_Percentage of maturation; WTF_Weight of 1,000 fruits; NLV_Number of leaves; TLA_Total leaf area; SLA_Specific leaf area; CLA_Chlorophyll a content; CLB_Chlorophyll b content; CLT_Total chlorophyll content; DMS_Dry matter of stems; DMF_Dry matter of fruits; DML_Dry matter of leaves; LAR_Leaf area ratio; SMR_Stem mass ratio; LMR_Leaf mass ratio; FMR_Fruit mass ratio.

Introduction

The cultivation of coffee is an agricultural activity of great importance for the social and economic development of Brazil, which is the largest producer and exporter of this product, with a total export of more than 26 million bags from May 2012 to April 2013 (ICO, 2013). Among the species of coffee, arabica coffee (Coffea arabica L.) is the most widely cultivated in the world. In the state of Espírito Santo, the production of arabica coffee is concentrated predominantly in the regions of Caparaó (37.4%), Serrana (36.7%) and South (15.3%) (Ferrão et al., 2008). The production of arabica coffee in Brazil for many decades occurred with the predominance of a small number of cultivars. However, breeding programs have developed and recommended new cultivars of arabica coffee, presenting productivities and qualities equal or superior to those grown traditionally, with the great advantage of associating new desirable agronomic traits, such as pest resistance and tolerance of adverse factors, such as environmental stresses (Oliveira and Pereira, 2008). The availability of new cultivars

has provided new options for famers, as with highly productive cultivars come facilities in cultivation and lower production costs. However, the specific characteristics of the soil and climate of each region create the need for studies that evaluate cultivar development under specific conditions of cultivation and of the regions. In addition to the development of new cultivars, one of the new technologies generated by coffee research is cultivation with high plant density, which consists in an increase in the number of plants that are cultivated per area, aiming to increase production and competitiveness in the agricultural sector (Braccini et al., 2005). In the state of Espírito Santo, where arabica coffee is mainly cultivated by farming families in mountainous region, the increased plant density becomes a practice of great importance to enhance the area utilisation and increase the crop yield (Matiello et al., 2005). For many decades, the main criteria for selecting coffee genotypes to create new cultivars was the yield. However, current breeding programs are investigating several other agronomic characteristics, aiming to increase the efficiency of the selection process (Severino et al., 2002). Studies of genetic diversity and the identification of variables that may be exploited for the characterisation of genotypes are very import to provide information regarding new criteria that may be employed in the selection of promising genotypes. The great demand for improvements in the cultivation techniques of arabica coffee and for information regarding the behaviour of new cultivars in the region of Caparaó creates the need for studies under field conditions of genotypes with the potential to be used in mountain plantations, as well as studies that may help to define variables that can be exploited in the study and selection of genotypes. The genetic variability among cultivars from different breeding programs may be exploited to identify those that are more suitable for cultivation with increased plant density and those that can develop better in the region of Caparaó-ES. Among the different characteristics of the coffee plant, the variables of the plagiotropic branches have special importance, as those are the branches that support the production of the fruits. There are many correlations between the variables of these branches and the genotypic potential for coffee production. Studies that help to define a group of variables of greater importance for the evaluation of genotypes can support the management and planning of future research and also generate valuable information for breeding programs. This study aimed to evaluate the genetic diversity of Coffea arabica L. genotypes with the potential for cultivation in increased-density systems in the region of Caparaó-ES based on the characteristics of plagiotropic branches in production, to evaluate the existence of correlations and to estimate the relative contributions of each of the aforementioned characteristics to the diversity.

Results

Dissimilarity between genotypes

The analysis of variance detected significant differences among the genotypes for all of the characteristics, with the exception of chlorophyll a content. The coefficients of variation (CV) were relatively low and were considered suitable for agricultural experimentation (Garcia, 1989), with the highest value of 19.07% derived from the study of the number of green fruits (Table 1). Table 2 shows the dissimilarity measures estimated by the Mahalanobis distance (D^2) between pairs of genotypes. A greater distance was observed between Catiguá MG2 and Oeiras MG 6851 $(D^2=367.34)$, while a smaller measure of dissimilarity was observed between Catuaí IAC 81 and Catuaí IAC 144 $(D^2=16.14)$, which are originated from similar genitors. Table 3 shows the cluster analysis of genotypes through the Tocher method. Using the characteristics of the plagiotropic branches, it was possible to identify seven distinct groups of genotypes. Groups VI and VII were formed by only one genotype each: IAPAR 59 and Catiguá MG2, respectively. Groups I, IV and, in particular, group II were formed by more genotypes, indicating that the study of divergence between the genotypes that compose them may be more complex. To study the genetic divergence, the technique of canonical variables requires that the first variables are able to accumulate at least 80% of the variation. Thus, it becomes necessary to supplement additional canonical variables of a higher order until this requirement is met. Following this criteria, the first four canonical variables were used to accumulate 82.87% of the available variation (Table 4) and, therefore, make the degree of distortion negligible.

Relative contribution

The relative contribution of each variable for the differentiation of genotypes is presented in Table 5. By analysing the relative contributions, it is possible to identify characteristics with greater or lesser importance in the evaluation of the genetic materials. This information allows for better planning of new trials in order to reduce the time and cost of experimental evaluations (Pereira, 1989; Cruz et al., 1990). The variables related to the number of fruits contributed the most to the variability among the studied genotypes, with a relative contribution of 45.20% for green fruits (GFT) and 39.66% for total fruits (NFT). This result, associated with the great importance of the number of fruits in the determination of the crop yield, reaffirms the need to explore this variable with the goal of differentiating between genotypes.

Correlations between characteristics

The correlation analysis between characteristics is presented in Table 6. Significant correlations were observed in 111 cases, with 84 correlations significant at 1% probability and 27 at 5% probability.

Discussion

The use different morpho-agronomic traits to study the variability between genotypes have been successfully used in many genetic studies for cultivated species (Ghosh et al., 2013; Roy et al., 2013). For species of coffee, it has been used to study diversity between progenies and improved genotypes in the breeding programs (Ivoglo et al., 2008; Rodrigues et al., 2012; Guedes et al., 2013). Resende et al. (2001) reported low genetic variability among the cultivars of arabica coffee when studying the characteristics of the plant as a whole (plant height, stem diameter and number of plagiotropic branches). However, Freitas et al. (2009) observed greater genetic variability for some characteristics, including the length of plagiotropic branches, in agreement with the present study. By analysing the results presented in Table 1, the estimate of genotypic variances $(\hat{\Phi}_g)$ showed elevated levels compared to the environmental variance ($\hat{\sigma}_{e}$) for most of the characteristics of the plagiotropic branches. Therefore, the greater proportion of phenotypic variance $(\hat{\sigma}_n)$ is associated with the effect of the differences between genotypes. For the chlorophyll a content, which did not differ between genotypes, the low variation observed may be related to the environmental effect. Characteristics that are greatly influenced by environmental conditions typically have low coefficients of genotypic determination (H^2) , usually less than 30%. In contrast, characteristics that are less influenced by environmental conditions have higher values (Ramalho et al., 2004). Even if it is reported relatively low values for estimate of genetic variance and heritability for many characteristics related to plant architecture and production; Freitas et al. (2009), studying different characteristics, reported high values of heritability for arabica coffee, indicating a considerable contribution of the genetic component to the expression of various characteristics. Among the characteristics of plagiotropic branches, disregarding the CLA, the estimates of the coefficients of genotypic determination were greater than 90% for many variables (GFT, NFT, MAT, TLA, DMB, DMF, DML, DMT, LAR, SMR, LMR and FMR), demonstrating that these characteristics are less influenced by the environment and; therefore, have advantages for use in the selection of

 Caparaó-ES, Brazil Parameters	$\frac{1}{1} (2013).$	DIA ²	NND ³	VND ⁴	RND ⁵
 MG	227.26**	1.07**	17.17.44	(70**	0.10**
MBg	237.30***	1.07***	17.10***	0.73***	9.19**
$\widehat{\sigma}_{p}$	59.34	0.26	4.29	1.68	2.29
$\widehat{\sigma}_{e}$	7.00	0.06	0.64	0.33	0.24
$\widehat{\Phi}_{g}$	52.33	0.20	3.64	1.34	2.05
$H^{2}(\%)$	88.19	76.53	84.90	80.08	89.38
CV (%)	8.35	7.87	7.85	11.45	9.51
CV _g (%)	11.42	7.10	9.31	11.48	13.79
$\mathrm{CV}_{\mathrm{g}}/\mathrm{CV}$	1.36	0.90	1.18	1.00	1.45
Mean	63.34	6.37	20.50	10.10	10.39
 Parameters	LND^{6}	VFT ⁷	MFT ⁸	NFT ⁹	MAT ¹⁰
 MSg	0.08*	3.384.16**	963.41**	3.662.49**	913.96**
$\widehat{\sigma}_{p}$	0.02	846.04	240.85	915.62	228.42
$\widehat{\sigma}_{e}$	0.01	26.36	32.43	51.73	8.80
$\widehat{\Phi}_{g}$	0.01	819.67	208.41	863.88	219.61
H ² (%)	53.63	96.88	86.53	94.34	96.14
CV (%)	6.40	19.07	17.50	12.09	10.48
CV _g (%)	3.44	53.17	22.18	24.71	26.16
CV _g /CV	0.53	2.78	1.26	2.04	2.49
Mean	3.09	53.84	65.06	118.90	56.64
 Parameters	WTF ¹¹	NFV ¹²	TLA ¹³	SLA ¹⁴	CLA ¹⁵
 MS_{g}	113.278.70**	45.40**	162.680.23**	5.54**	1.60 ^{ns}
$\widehat{\sigma}_{\mathrm{p}}$	28.319.67	11.35	40.670.05	1.38	0.40
$\widehat{\sigma}_{e}$	3.085.32	1.75	2.679.74	0.25	0.40
$\widehat{\Phi}_{g}$	25.234.34	9.59	37.990.31	1.13	0.00
$H^{2}(\%)$	89.10	84.53	93.41	81.61	-
CV (%)	9.76	15.47	15.77	17.04	3.09
CV _g (%)	13.96	18.08	29.69	17.95	-
CV _g /CV	1.42	1.16	1.88	1.05	-
Mean	1.137.49	17.12	656.39	5.92	41.86
 Parameters	CLB ¹⁶	CLT ¹⁷	DMS ¹⁸	DMF ¹⁹	DML^{20}
 MSg	36.28*	50.83*	15.54**	368.16**	22.15**
$\widehat{\sigma}_{\mathrm{p}}$	9.07	12.70	3.88	92.04	5.53

Table 1. Genotypic mean squares (MS_g), estimate of phenotypic ($\hat{\sigma}_p$), environmental ($\hat{\sigma}_e$) and genotypic ($\hat{\Phi}_g$) variances, coefficients of genotypic determination (H^2), coefficients of variation (CV), coefficients of genetic variation (CV_g), variation index (CV_g/CV) and means of 25 characteristics of plagiotropic branches of different genotypes of arabica coffee cultivated with increased plant density in Caparaó-ES, Brazil (2013).

$\widehat{\sigma}_{e}$	3.72	6.10	0.38	8.70	0.29
$\widehat{\varphi}_g$	5.34	6.60	3.50	83.33	5.24
H ² (%)	58.90	51.98	90.16	90.53	94.76
CV (%)	13.94	7.10	16.97	13.66	15.64
$\mathrm{CV}_{\mathrm{g}}(\%)$	8.34	3.69	25.69	21.13	33.26
CVg/CV	0.59	0.52	1.51	1.54	2.12
Mean	27.68	69.54	7.28	43.19	6.88
 Parameters	DMT ²¹	LAR ²²	SMR ²³	LMR ²⁴	FMR ²⁵
 MSg	525.74**	43.10**	0.01**	0.01**	0.01**
$\widehat{\sigma}_{p}$	131.43	10.77	0.01	0.01	0.01
$\widehat{\sigma}_{e}$	12.65	0.87	0.01	0.01	0.01
$\widehat{\Phi}_{g}$	118.77	9.89	0.01	0.01	0.01
H ² (%)	90.37	91.85	90.97	93.14	94.77
CV (%)	12.40	16.13	11.67	16.31	3.68
$\mathrm{CV}_{\mathrm{g}}(\%)$	18.99	27.09	18.53	30.06	7.85
CVg/CV	1.53	1.67	1.58	1.84	2.12
Mean	57.36	11.61	0.12	0.12	0.75

*Significant at 5%, **Significant at 1%, ^{ns}Non significant at 5% by the F test; ⁽¹⁾Length of the plagiotropic branch; ⁽²⁾Diameter of the branch; ⁽³⁾Number of nodes; ⁽⁴⁾Number of vegetative nodes; ⁽⁵⁾Number of reproductive nodes; ⁽⁶⁾Length of the internodes; ⁽⁷⁾Number of green fruits; ⁽⁸⁾Number of mature fruits; ⁽⁹⁾Number of fruits; ⁽¹⁰⁾Percentage of maturation; ⁽¹¹⁾Weight of 1,000 fruits; ⁽¹²⁾Number of leaves; ⁽¹³⁾Total leaf area; ⁽¹⁴⁾Specific leaf area; ⁽¹⁵⁾Chlorophyll *a* content; ⁽¹⁶⁾Chlorophyll *b* content; ⁽¹⁷⁾Total chlorophyll content; ⁽¹⁸⁾Dry matter of stems; ⁽¹⁹⁾Dry matter of fruits; ⁽²⁰⁾Dry matter of leaves; ⁽²¹⁾Total dry matter of branches; ⁽²²⁾Leaf area ratio; ⁽²³⁾Stem mass ratio; ⁽²⁴⁾Leaf mass ratio.

genotypes. For LNG, DIA, NND, VND, RND, MFT, WTF, NLV and SLA, the estimate showed a relatively high H^2 , between 76.53% and 89.38%. Only for LND, CLT and CLB was the estimative relatively low, with values between 51.98% and 58.90%. The estimate of coefficients of genetic variation (CVg) demonstrates the heterogeneity of the genotypes for most characteristics, which presents a favourable situation for the selection of superior genotypes. For the LNG, NND, VND, RND, GFT, MFT, NFT, MAT, WTF, NLV, TLA, SLA, DMB, DMF, DML, DMT, LAR, SMR, LMR and FMR characteristics, the estimated CV_g was superior to the CV, resulting in a variation index (CV_g/CV) greater than 1.00, indicating the predominance of genetic factors rather than environmental factors. Additionally, the estimate for this index for the DIA (0.90) still represents a value considered favourable for coffee breeding programs. Catiguá MG2 was one of the most dissimilar among the genotypes, participating in 7 of the 20 largest distances observed in the experiment (with IAPAR 59, Acauã, Catucaí Amarelo 24/137, Pau-Brasil MG1, Oeiras MG 6851, Catuaí IAC 81 and Catuaí IAC 144). In contrast, the genotype Catuaí IAC 44, although showing considerable distances with some genotypes, was more similar to the others, participating in 7 of 20 smaller distances (with Katipó, Acauã, Catuaí Amarelo 24/137, Pau-Brasil MG1, Catiguá MG3, Tupi and Catuaí IAC 81). We noticed that the genotype Catiguá MG2, which showed greater dissimilarity with most of the other genotypes, formed a group alone (Group VII), while Catuaí IAC 144, less dissimilar, was allocated in the largest group (Group II). Through the graphical dispersion of the first two

canonical variables (VC1 and VC2), it is already possible to observe the reliability of the Tocher grouping, showing the separation of the genotypes Catiguá MG2 and IAPAR 59 in relation to the other genotypes (Figure 1A). However, the addition of the canonical variables CV3 and CV4 (Figure 2B and 2C, respectively) was not sufficient to generate a perfect view of the dispersions of the other groupings. This same behaviour was observed by Fonseca et al. (2006) studying the genetic diversity of genotypes of C. canephora. The magnitude of the genetic correlations (|rg|) exceeded that of the phenotypic correlations $(|r_p|)$ in 81% of the cases, being lower in only 8%, indicating that genetic factors had a greater influence than did the environment in these cases. There are also differences between the signs of the genotypic and phenotypic correlations in 5% of cases, indicating that the genetic and environmental causes of variation can influence the characteristics of the plagiotropic branches by different physiological mechanisms (Falconer, 1981). Considering the significant correlations, values equal to or greater than |0.60|were observed in 78% of cases, indicating the presence of a strong correlation between these characteristics (Carvalho et al. 2004). Overall, the characteristics that showed the highest number of significant correlations with other variables were LNG (correlated with 14 features), TLA (14) and DMS (16). The strong positive correlations observed between the various characteristics indicate the possibility of obtaining gains through selection in more than one trait simultaneously. This fact is very desirable when planning new evaluations, allowing for the selection of a set of characteristics for simpler evaluation over more costly traits, still allowing a



Figure 1. Graphic dispersion of 16 genotypes of arabica coffee for the canonical variables VC1 and VC2 (A), VC1 and VC3 (B) and VC1 and VC3 (C) based on 25 characteristics of plagiotropic branches evaluated in Caparaó-ES, Brazil (2013).

good representation of the genotypes. The length and number of nodes of the plagiotropic branch were strongly correlated $(|\mathbf{r}_p|=0.93)$, showing that genotypes that present greater development in extension also tend to have a higher number of internodes in the branches. Mistro et al. (2007), evaluating four genotypes of arabica coffee, observed a low degree of correlation between these same characteristics and described the great influence of the environmental conditions on the interaction between features. This behaviour can be associated with differences in the experimental conditions, such as the region, number of genotypes studied and age of the plants during the evaluation. Freitas et al. (2009) also reported a strong positive and significant correlation between the length of the branch and the number of internodes, indicating that the length of the branch is a good indicator of the number of nodes that it can support, which also represents a major component of the potential production of the coffee plant. In addition, WeldeMichael et al. (2014) reported strong phenotypic and genotypic association between characteristics related to quality and chemistry of the fruits in accessions of Arabica coffee. As the chlorophyll content was similar between the genotypes, the variation observed in the total chlorophyll content was associated, to a greater degree, by the variation in the chlorophyll b content. Therefore, a strong correlation was observed between these variables ($|\mathbf{r}_p|=0.99$). Following the proportion of biomass accumulation, the dimensions of the stem (extension and thickness) were strongly correlated with the dry matter accumulated in the same ($|r_p| \ge 0.92$). Similarly, the dry matter accumulated in leaves was strongly correlated with the leaf area and number of leaves developed in each branch ($|\mathbf{r}_p| \ge 0.92$). This strong association between area and biomass can also be verified by the high degree of correlation between the leaf area ratio and leaf mass ratio ($|\mathbf{r}_p|=0.96$). A significant correlation between the total dry matter in the branch and the biomass accumulated in the stem indicates that branches with betterdeveloped and well-structured stems can sustain a higher proportion of leaves and fruits, resulting in a higher total accumulated biomass. However, due to the large proportion of biomass that the coffee plant allocates to the fruits, the dry matter accumulation of the plagiotropic branch as a whole is more strongly correlated with the dry matter accumulated in the fruits ($|r_p|=0.93$), compared to the accumulation in the other organs. Strong negative correlations were also observed between some characteristics, such as the percentage of maturation and the number of green fruits (|r_p|=0.87); as expected, the presence of green fruits directly influenced the percentage of mature fruits at the time of harvest. Overall, plagiotropic branches with higher growth, larger extension, more nodes and better leafiness also presented larger and thinner leaves with a lower specific leaf area. Therefore, there were strong negative correlations between the SLA and the variables LNG ($|r_p|=0.83$), NND ($|r_p|=0.84$), NLV ($|r_p|=0.96$), TLA ($|r_p|=0.86$), DMS ($|r_p|=0.81$) and DML ($|r_p|=0.91$). The dry matter accumulation in the fruits is negatively correlated with the development of vegetative buds and also with the biomass accumulation in other vegetal parts, indicating competition for assimilates among the different organs. There were strong negative correlations between the fruit mass ratio and the dry matter of leaves (|rp|=0.80), leaf area ratio $(|\mathbf{r}_p|=0.91)$, stem mass ratio $(|\mathbf{r}_p|=0.93)$, leaf mass ratio $(|\mathbf{r}_{p}|=0.97)$ and number of vegetative nodes $(|\mathbf{r}_{p}|=0.80)$.

Table 2. Dissimilarity measures between pairs of genotypes of arabica coffee obtained by Mahalanobis distance and estimated from the study of 25 characteristics of plagiotropic branches evaluated in Caparaó-ES, Brazil (2013). D² maximum: 367.34 (Catiguá MG2 and Oeiras MG 6851); D² minimum: 16.14 (Catuaí IAC 81 and Catuaí IAC 144). Genotypes: (1) IAPAR 59; (2) Katipó; (3) Acauã; (4) Paraíso MG H 419-1; (5) H419-3-3-7-16-4-1-1; (6) Araponga MG1; (7) Catucaí Amarelo 24/137; (8) Catiguá MG2; (9) Sacramento MG1; (10) Pau-Brasil MG1; (11) Catiguá MG3; (12) Oeiras MG 6851: (13) Tupi; (14) Catuaí IAC 44: (15) Catuaí IAC 81: (16) Catuaí IAC 144.

	Oenas M	0.0651,	(13) 1u	(14)		IC 44, (1	J) Catua	u IAC o	1,(10)	atual IA	IC 144.				
Genot.	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
(1)	109	129	262	277	211	148	292	187	149	179	89	102	132	99	55
(2)		64	85	127	80	54	156	144	37	33	102	23	18	40	55
(3)			168	208	57	29	245	228	56	112	73	63	53	56	56
(4)				132	140	89	151	164	95	42	301	104	79	85	120
(5)					108	167	34	186	159	65	312	119	100	193	202
(6)						67	159	224	84	74	168	75	55	87	102
(7)							201	184	26	72	127	63	47	39	57
(8)								157	208	81	367	121	122	237	230
(9)									157	117	281	108	150	160	159
(10)										64	113	61	52	40	75
(11)											211	34	29	67	86
(12)												124	146	116	103
(13)													26	58	56
(14)														52	59
(15)															16

 10^{-2} maximum: 367.34 (Catiguá MG2 and Oeiras MG 6851); 10^{-2} minimum: 16.14 (Catuaí IAC 81 and Catuaí IAC 144). Genotypes: (1) IAPAR 59; (2) Katipó; (3) Acauã; (4) Paraíso MG H 419-1; (5) H419-3-3-7-16-4-1-1; (6) Araponga MG1; (7) Catucaí Amarelo 24/137; (8) Catiguá MG2; (9) Sacramento MG1; (10) Pau-Brasil MG1; (11) Catiguá MG3; (12) Oeiras MG 6851; (13) Tupi; (14) Catuaí IAC 44; (15) Catuaí IAC 81; (16) Catuaí IAC 144.

Table 3. The grouping of 16 genotypes of a	arabica coffee by the Tocher meth	10d based on 25 characteristics of plagiotrop	pic
branches evaluated in Caparaó-ES, Brazil (2013).		

Group	Genotypes
Ι	Paraíso, Sacramento MG1, Catuaí Amarelo 24/137
II	H419-3-3-7-16-4-1-1, Pau-Brasil MG1, Catuaí Vermelho IAC 144, Araponga MG1
III	Oeiras MG 6851, Catuaí IAC 81
IV	Acauã, Catiguá MG3, Tupi
V	Katipó, Catuaí IAC 44
VI	IAPAR 59
VII	Catiguá MG2

Table 4. Estimate of the eigenvalues and cumulative variances of the canonical variables among 16 genotypes of arabica coffee
based on 25 characteristics of plagiotropic branches evaluated in Caparaó-ES, Brazil (2013).

Canonical variables	Eigenvalues	Cumulative variances (%)	Canonical variables	Eigenvalues	Cumulative variances (%)
VC1	25.24	42.83	VC14	0.07	99.96
VC2	11.12	61.70	VC15	0.02	100.00
VC3	8.42	75.99	VC16	0.00	100.00
VC4	4.06	82.87	VC17	0.00	100.00
VC5	3.11	88.16	VC18	0.00	100.00
VC6	2.24	91.96	VC19	0.00	100.00
VC7	1.81	95.04	VC20	0.00	100.00
VC8	1.11	96.92	VC21	0.00	100.00
VC9	0.66	98.04	VC22	0.00	100.00
VC10	0.37	98.67	VC23	0.00	100.00
VC11	0.32	99.21	VC24	0.00	100.00
VC12	0.21	99.56	VC25	0.00	100.00
VC13	0.16	99.84			

Materials and Methods

Experimental setup

The experiment was conducted in a competition field in a region where arabica coffee is typically cultivated in the district of Celina, municipality of Alegre, geographic coordinates 20°45'S and 41°33'W, in the region of Caparaó in the southern state of Espírito Santo. The area has in altitude of 690 m and presents an average annual temperature of 22 °C and rainfall between 1300-1800 mm per year, with the rainy season from October to April and the dry season from

May to September. The experiment followed a randomised block design with 16 genotypes, four replications and six plants per experimental plot. The plants were spaced 2.00×0.60 m with a total of 8,333 plants per hectare, configuring a high-density cultivation. The agricultural practices were established in accordance with those normally employed in the region according to their need and following the current

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Chara.	DIA ²	NND ³	VND^4	RND ⁵	LND^{6}	GFT ⁷	MFT ⁸	NFT ⁹	MAT ¹⁰	WTF ¹¹	NLV ¹²	TLA ¹³	SLA ¹⁴	CLA ¹⁵	CLB ¹⁶	CLT ¹⁷	DMS ¹⁸	DMF ¹⁹	DML ²⁰	DMT ²¹	LAR ²²	SMR ²³	LMR ²⁴	FMR ²⁵
LNG ¹	0.84^{**}	0.93**	0.62^{**}	0.73**	0.59^{*}	0.48	0.01	0.47	-0.33	-0.25	0.84^{**}	0.85**	-0.83**	0.16	0.33	0.31	0.95**	0.31	0.87^{**}	0.60^{*}	0.48	0.67^{**}	0.56^{*}	-0.63**
	0.92	0.97	0.65	0.76	0.71	0.49	-0.02	0.47	-0.36	-0.31	0.91	0.89	-0.91	0.71	0.40	0.40	0.99	0.29	0.90	0.60	0.52	0.71	0.61	-0.67
DIA ²	r _p	0.79^{**}	0.29	0.83**	0.46	0.56	0.22	0.65^{**}	-0.26	-0.18	0.66**	0.66**	-0.64**	-0.06	0.12	0.09	0.92**	0.53^{*}	0.69**	0.74^{**}	0.17	0.48	0.28	-0.38
	rg	0.89	0.31	0.93	0.60	0.60	0.20	0.69	-0.30	-0.27	0.78	0.74	-0.76	0.59	0.19	0.16	0.98	0.53	0.75	0.77	0.24	0.51	0.35	-0.43
NND ³		rp	0.68^{**}	0.78^{**}	0.26	0.38	-0.02	0.35	-0.23	-0.08	0.85**	0.84^{**}	-0.84**	0.04	0.33	0.29	0.89^{**}	0.28	0.86^{**}	0.57^{*}	0.48	0.62^{**}	0.57^{*}	-0.61*
		rg	0.66	0.79	0.52	0.40	-0.08	0.35	-0.27	-0.14	0.92	0.90	-0.92	0.66	0.39	0.35	0.93	0.26	0.91	0.57	0.54	0.66	0.62	-0.65
VND^4			rp	0.07	0.16	-0.14	-0.07	-0.17	0.11	0.02	0.76^{**}	0.74^{**}	-0.77**	0.48	0.63**	0.61^{*}	0.52^{*}	-0.21	0.78^{**}	0.07	0.78^{**}	0.66^{**}	0.83**	-0.80**
			rg	0.07	0.40	-0.16	-0.09	-0.21	0.11	-0.01	0.81	0.78	-0.83	0.82	0.75	0.78	0.55	-0.29	0.83	0.02	0.86	0.73	0.91	-0.86
RND ⁵				r _p	0.21	0.64**	0.03	0.63**	-0.41	-0.14	0.51*	0.51°	-0.49	-0.35	-0.07	-0.12	0.76**	0.58*	0.50*	0.72**	-0.01	0.27	0.06	-0.15
				r _g	0.36	0.68	-0.03	0.64	-0.46	-0.17	0.57	0.56	-0.55	0.21	-0.09	-0.16	0.80	0.59	0.54	0.74	0.01	0.29	0.08	-0.17
LND°					r _p	0.43	0.08	0.45	-0.36	-0.50	0.34	0.40	-0.33	0.33	0.13	0.17	0.55	0.18	0.41	0.33	0.21	0.42	0.24	-0.34
arm7					r _g	0.56	0.13	0.61	-0.47	-0.71	0.53	0.52	-0.51	0.85	0.29	0.39	0.75	0.21	0.53	0.42	0.30	0.59	0.35	-0.47
GFT'						rp	-0.18	0.86	-0.87	-0.63	0.37	0.39	-0.34	-0.22	-0.20	-0.21	0.56	0.58	0.32	0.64	-0.03	0.10	-0.04	-0.01
MET ⁸						rg	-0.19	0.87	-0.88	-0.67	0.40	0.40	-0.38	0.32	-0.26	-0.28	0.58	0.59	0.32	0.66	-0.03	0.10	-0.04	-0.01
MF1~							r _p	0.33	0.56	0.33	-0.13	-0.18	0.03	0.15	0.18	0.18	0.05	0.55	-0.13	0.44	-0.52	-0.39	-0.43	0.45
NIET ⁹							Ig	0.29	0.56	0.33	-0.16	-0.20	0.04	0.71	0.32	0.33	-0.01	0.50	-0.14	0.44	-0.34	0.43	-0.43	0.40
INF I								I _p	-0.34	-0.43	0.29	0.28	-0.51	-0.15	-0.10	-0.11	0.57	0.85	0.24	0.85	-0.30	-0.10	-0.27	0.21
MAT ¹⁰								Ig	-0.37	0.49	-0.31	-0.30	-0.33	0.40	-0.09	-0.11	-0.36	-0.19	-0.24	-0.27	-0.30	-0.22	-0.20	0.21
MAI									r r	0.79	-0.34	-0.30	0.29	0.65	0.10	0.10	-0.40	-0.19	-0.24	-0.27	-0.13	-0.22	-0.09	0.14
WTF ¹¹									Ig	r	-0.26	-0.10	0.19	-0.07	0.04	0.02	-0.26	0.09	-0.09	0.01	-0.12	-0.39	-0.12	0.22
										-p r_	-0.33	-0.12	0.24	0.34	0.16	0.16	-0.33	0.03	-0.11	-0.05	-0.09	-0.42	-0.10	0.22
NL V ¹²										19	r-	0.89**	-0.96**	0.23	0.42	0.40	0.81**	0.00	0.92**	0.42	0.67**	0.68**	0.76**	-0.75**
											r _a	0.92	-0.99	0.75	0.45	0.43	0.88	0.09	0.96	0.43	0.69	0.76	0.81	-0.80
TLA ¹³											×.	ľn	-0.86**	0.19	0.35	0.33	0.83**	0.18	0.97**	0.50^{*}	0.75**	0.63**	0.78**	-0.75**
												r _g	-0.91	0.75	0.39	0.38	0.87	0.18	0.98	0.50	0.76	0.66	0.79	-0.76
SLA ¹⁴												<u> </u>	r _n	-0.25	-0.40	-0.39	-0.81**	-0.19	-0.91**	-0.48	-0.59 [°]	-0.61 [°]	-0.70**	0.68^{**}
													rg	0.22	-0.35	-0.34	-0.89	-0.20	-0.95	-0.52	-0.62	-0.67	-0.73	0.72
CLA ¹⁵														r _p	0.84**	0.89^{**}	0.14	-0.16	0.29	-0.05	0.27	0.27	0.37	-0.35
														rg	0.97	0.97	0.71	0.54	0.76	0.62	0.78	0.72	0.73	0.17
CLB ¹⁶															rp	0.99**	0.31	-0.10	0.45	0.05	0.34	0.37	0.46	-0.44
															rg	0.99	0.39	-0.06	0.51	0.11	0.33	0.40	0.47	-0.47
CLT ¹⁷																rp	0.29	-0.12	0.43	0.03	0.34	0.37	0.46	-0.44
																rg	0.39	-0.08	0.51	0.10	0.34	0.41	0.49	-0.48
DMS ¹⁸																	rp	0.40	0.87	0.68	0.41	0.64	0.51	-0.59
D1 (7)																	r _g	0.38	0.90	0.68	0.46	0.64	0.57	-0.62
DMF																		rp	0.14	0.93	-0.47	-0.42	-0.44	0.45
D) (7 20																		r _g	0.14	0.93	-0.48	-0.45	-0.44	0.45
DML-*																			rp	0.47	0.73	0.69	0.81	-0.80
DMT ²¹																			Ig	0.46	0.74	0.72	0.82	-0.80
DMT																				I _p	-0.17	-0.10	-0.11	0.10
L A D ²²																				Ig	-0.10	-0.11	-0.09	0.10
LAIN																					r r	0.70	0.90	-0.91
SMR ²³																					1 g	0.01 r	0.97	-0.93**
5 MIX																						т _р	0.88	-0.95
LMR ²⁴																						1 g	г.	-0.97**
																							•p	0.08

Table 6. Phenotypic (r_p – top row) and genotypic (r_g – bottom line) correlations between 25 characteristics of plagiotropic branches of genotypes of arabica coffee cultivated with increased plant density in Caparaó-ES, Brazil (2013).

*Significant at 5%, **Significant at 1% by the t test; ⁽¹⁾Length of the plagiotropic branch; ⁽²⁾Diameter of the branch; ⁽³⁾Number of nodes; ⁽⁴⁾Number of vegetative nodes; ⁽⁵⁾Number of reproductive nodes; ⁽⁶⁾Length of the internodes; ⁽⁷⁾Number of green fruits; ⁽⁸⁾Number of mature fruits; ⁽⁹⁾Number of fruits; ⁽¹⁰⁾Percentage of maturation; ⁽¹¹⁾Weight of 1,000 fruits; ⁽¹²⁾Number of leaves; ⁽¹³⁾Total leaf area; ⁽¹⁴⁾Specific leaf area; ⁽¹⁵⁾Chlorophyll *a* content ⁽¹⁶⁾Chlorophyll *b* content ⁽¹⁷⁾Total chlorophyll content; ⁽¹⁸⁾Dry matter of stems; ⁽⁹⁾Dry matter of fruits; ⁽²⁰⁾Dry matter of branches; ⁽²²⁾Leaf area ratio; ⁽²³⁾Stem mass ratio; ⁽²⁵⁾Fruit mass ratio.

recommendations for the cultivation of arabica coffee in Brazil (Prezotti et al., 2007; Reis and Cunha, 2010).

Selection of genotypes

The 16 genotypes of *Coffea arabica* L. originated from the breeding programs of institutions that are references in the development of cultivars of arabica coffee, such as Instituto Agronômico de Campinas (IAC), Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Fundação Pro-Café (MAPA/Pro-café) and Instituto Agronômico do Paraná (IAPAR). The genotypes were selected according to their characteristics of high crop yield and quality, prioritising genotypes with a short canopy and rust resistance, agronomic characteristics that are highly important for cultivation with increased plant density.

Traits measured

The plants were cultivated until the stabilisation of their reproductive phenological cycle, being evaluated when in full production during their fourth year (2013). At the end of the fruit ripening stage, four plagiotropic branches located in the median region of the canopy, representative of the overall growth and fruit production of each plant, were identified and analysed. The length of the plagiotropic branch (LNG) in centimetres (cm) was determined using a graduated ruler, measuring from the insertion of the branch to its apex. The diameter of the branch (DIA) was measured in millimetres (mm) using a digital calliper on the middle portion of the third internode (from the insertion of the branch). The length of the internodes (LND) was determined by measuring the length in one basal, one median and one apical internode in the branch. The total number of nodes (NND) was determined by the direct counting of all gems along the branch. The separation between the nodes differentiated between vegetative structures (secondary branches and leaves), and rosettes were used to determine the number of vegetative nodes (VND) and the number of reproductive nodes (RND). The chlorophyll content was determined using a portable chlorophyllometer (ClorofiLOG®) in the fully expanded leaves of the third and fourth pairs (from the apex), measuring the contents of chlorophyll a (CLA), chlorophyll b (CLB) and total chlorophyll (CLT). After the measurements, the plagiotropic branches were collected and sent to the laboratory to determine the remaining variables. The primary branches were cut in order to separate each vegetal compartment between the stem, leaves and fruits. The fruits were counted to determine the total number of fruits (NFT) and weighed in a precision balance to determine the weight of 1,000 fruits (WTF). Later, the fruits were separated according to their stage of maturation to determine the number of mature fruits (MFT), the number of green fruits (GFT) and the percentage of maturation (MAT) according to the proportion of fully mature fruit present in the moment of the harvest. The leaves were counted to determine the number of leaves (NLV) and analysed on an area meter integrator (Area Meter, LI -COR® 3100) to determine the total leaf area (TLA). The specific leaf area (SLA) was obtained by the ratio between the leaf area and the dry matter of leaf discs of known area. Each vegetal compartment was separated and placed in paper bags and were oven-dried in a drying oven with forced air circulation at temperature of 65 °C until constant weight in order to determine the dry matter accumulation in the stems (DMS), leaves (DML) and fruits (DMF), as well as in the entire plant (DMT). The leaf area ratio (LAR) was calculated as the ratio between the leaf area

and the total dry matter of each plagiotropic branch. The stem mass ratio (SMR) was calculated as the ratio between the dry matter of the stem and the total matter. The leaf mass ratio (LMR) was calculated as the ratio between the dry matter of the leaves and the total dry matter. The fruit mass ratio (FMR) was calculated as the ratio between the dry matter of the fruits and the total dry matter of the plagiotropic branch.

Data analysis

The collected data were subjected to an analysis of variance using the F test at 1 and 5% probabilities in order to identify the characteristics that can be used to differentiate between groups of genotypes with different behaviours. For those characteristics, the genetic parameters were estimated based on the model $Y_{ij}=\mu+g_i+b_j+\varepsilon_{ij}$, considering the effect of genotypes as fixed. The genetic divergences between genotypes were estimated by multivariate analysis techniques for grouping. The Mahalanobis distance was used as dissimilarity measure, and the groups were delineated using an optimisation technique based on a proposal by Tocher (applied as described by Cruz and Carneiro, 2006). The analysis of the canonical variables was used to view the clusters in order to generate a graphical dispersion of scores based on the number of variables that allowed for an accumulation of variance of at least 80%. The correlations between the characteristics were calculated and their significance assessed by t test at 1 and 5% probabilities. The relative importance of each characteristic in the prediction of genetic divergence was also studies.

The analyses were performed using the statistical software GENES (Cruz, 2013).

Conclusions

The genotypes cultivated with increased plant density present variability for almost all of the characteristics evaluated, making it possible to identify different behaviours among them. For most of the characteristics of the plagiotropic branch, the genotypic effect show greater influence than did environmental factors, creating a desirable situation to identify superior genotypes for each agronomic trait. Among the studied genotypes, IAPAR 59 and Catiguá MG2 and 59 present greater dissimilarity. Between the 25 traits, the evaluation of the number of fruits per branch and the biomass are those traits with a greater relative contribution to the variability among the genotypes. Strong positive and negative correlations are observed between the various characteristics, revealing the possibility of simultaneous selection of correlated traits.

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