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Early selection of drought-tolerant *Coffea arabica* genotypes at the seedling stage using functional divergence[1]

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ABSTRACT

The development of more drought-tolerant cultivars is essential for the maintenance of global agricultural production. This study aimed to perform an early selection of drought-tolerant *Coffea arabica* genotypes by evaluating their functional divergence using morphological, anatomical and physiological analyses. Seedlings of 14 genotypes were subjected to the drought stress imposed by irrigation for 18 days. Growth and anatomical parameters, leaf water potential and gas exchanges were measured. Under irrigated conditions and prolonged drought (18 days), the divergence among the genotypes was determined mainly by morphological traits, such as leaf area, stem diameter and, consequently, shoot dry mass. Under moderate drought (14 days), parameters such as water potential, cuticle thickness, stomatal density, number of xylem vessels and water-use efficiency were important for the divergence of the group with the highest ability to maintain its water status. The genotypes 1, 2, 4, 11 and 12 have characteristics that contributed to the maintenance of water status, such as greater cuticle thickness, stomatal density, smaller number of xylem vessels and phloem thickness, bigger root length and greater water-use efficiency. The functional divergence combining morphological, anatomical and physiological analyses in response to the moderate drought indicated the early selection of the genotypes 1, 2, 4, 11 and 12 as more drought tolerant during the seedling stage.

KEYWORDS: Coffee, water-use efficiency, leaf water potential.

RESUMO

Seleção precoce de genótipos de *Coffea arabica* tolerantes à seca em estágio de mudas utilizando-se divergência funcional

O desenvolvimento de cultivares mais tolerantes à seca é essencial para a manutenção da produção agrícola mundial. Objetivou-se realizar uma seleção precoce de genótipos de *Coffea arabica* tolerantes à seca, avaliando-se sua divergência funcional por meio de análises morfológicas, anatômicas e fisiológicas. Mudas de 14 genótipos foram submetidas ao estresse hídrico imposto pela irrigação por 18 dias. Foram medidos parâmetros de crescimento e anatômicos, potencial hídrico foliar e trocas gasosas. Em condições irrigadas e seca prolongada (18 dias), a divergência entre os genótipos foi determinada principalmente por características morfológicas, como área foliar, diâmetro do caule e, consequentemente, massa seca da parte aérea. Sob seca moderada (14 dias), parâmetros como potencial hídrico, espessura da cutícula, densidade estomática, número de vasos do xilema e eficiência do uso da água foram importantes para a divergência do grupo com maior capacidade de manter seu estado hídrico. Os genótipos 1, 2, 4, 11 e 12 possuem características que contribuíram para a manutenção do estado hídrico, como maior espessura de cutícula, densidade estomática, menor número de vasos xilemáticos e espessura do floema, maior comprimento de raiz e maior eficiência de uso de água. A divergência funcional combinando análises morfológicas, anatômicas e fisiológicas em resposta à seca moderada indicou a seleção precoce dos genótipos 1, 2, 4, 11 e 12 como mais tolerantes à seca durante a fase de muda.

PALAVRAS-CHAVE: Café, eficiência do uso de água, potencial hídrico foliar.

INTRODUCTION

The development of cultivars that are more tolerant to drought, as well as technologies that help plants to tolerate prolonged dry periods, are essential for maintaining the global agricultural production (Silva et al. 2018).

Given the challenge of developing technologies to overcome climate impacts on coffee, crop breeding programs are focused on developing drought-tolerant cultivars to maintain yields. To achieve this goal, segregating populations obtained by crosses between the Timor hybrid and commercial cultivars of the Catuaí group have been studied. These genotypes, developed by the Coffee Plant Breeding Program of Minas Gerais, Brazil, are of great interest, as they exhibit high yields, beverage quality and resistance to rust (*Hemileia vastatrix*) (Rezende et al. 2014),

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and may meet market demands to reduce the use of water resources, agricultural pesticides and production costs, as well as increase the income of coffee producers.

The combination of morphological and physiological traits through multivariate analysis to screen coffee progenies in response to drought during their initial development is recommended by Silva et al. (2013), since this tool allows for grouping and identifying variations in the degree of tolerance of genotypes at different levels and may, therefore, be useful when exploring a large number of genotypes in breeding programs.

Thus, the present study aimed to perform an early selection of drought-tolerant *Coffea arabica* genotypes at the seedling stage by evaluating their functional divergence in response to drought, combining morphological, anatomical and physiological analyses.

MATERIAL AND METHODS

This study was performed under greenhouse conditions at the Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig), in Lavras, Minas Gerais State, Brazil. Fourteen *Coffea arabica* genotypes were analysed: genotype 1 (H419-6-2-4-2-2 genealogy); genotype 2 (H419-6-2-7-1-1 genealogy); genotype 3 (Catuaí Vermelho IAC 144); genotype 4 (H516-2-1-1-7-1 genealogy); genotype 5 (H516-2-1-1-12-1 genealogy); genotype 6 (H516-2-1-1-14-3 genealogy); genotype 7 (H518-3-6-462 genealogy); genotype 8 (H419-3-3-7-16-2 genealogy); genotype 9 (H419-3-3-7-16-11 genealogy); genotype 10 (H419-3-4-4-13 genealogy); genotype 11 (H419-5-2-4-18 genealogy); genotype 12 (H419-5-4-5-6-1 genealogy); genotype 13 (Catuaí Vermelho IAC 99); and genotype 14 ("Siriema"). The genotypes 1, 2, 8, 9, 10, 11 and 12 originate from the Paraíso group; 4, 5 and 6 from the Araponga group; and 7 from the Pau-Brasil group. The "Siriema" has adaptive responses to water scarcity (Dias et al. 2007) and the cultivars from the Catuaí group are widely adopted by Brazilian producers.

From seeds obtained from these genotypes selected by the coffee breeding program conducted and coordinated by Epamig, the seedlings were formed in F_5 (7, 10 and 11) and F_6 generations (1, 2, 4, 5, 6, 8, 9 and 12), grown in polypropylene bags and then selected for uniformity, size and vigour. After reaching five pairs of leaves, they were transplanted into 26-L pots. A mixture of soil, sand and cattle manure (3:3:1; v/v/v) was used as substrate. The plants were kept in a greenhouse under a low-density polyethylene cover at 50 % of light and a maximum temperature of 28 ºC during the day and 19 ºC at night. After six months of growth, the seedlings were subjected to two water availability conditions, corresponding to daily watering maintaining 100 % of the field capacity (control) and drought conditions with the drought stress being imposed by the complete water withholding for 18 days. This period was defined by the occurrence of the first genotypes with values lower than -3 MPa. The experiment was carried out for a period of 15 months, ranging from sowing data to the evaluation of 18 days of water deficit (ending date).

A complete randomized blocks experimental design, with a 14 (genotypes) x 2 (irrigation suspension and soil at field capacity) factorial arrangement and five replicates, was used. Each experimental plot contained one plant. The analyses were performed at 0, 14 and 18 days after irrigation suspension (DAIS).

The morphological traits were evaluated one day before the onset of drought stress by assessing the stem diameter at the ground level (mm), plant height (cm) and leaf area, according to the leaf size method (Barros et al. 1973). In addition, the number of plagiotropic branches, the insertion angle with the orthotropic branch, as well as the length (cm) of the first plagiotropic branch, were determined. The insertion angle was obtained with a protractor of 0-180º, measuring the angle of insertion of the median plagiotropic branch with the orthotropic branch. At the end of the experiment, the tissues were placed in a forced-air oven at 70 ºC, for 96 h, until a constant weight was reached, to determine the shoot dry mass, root dry mass, root length and total dry mass. In addition, the root to shoot mass ratio and the root mass to leaf area ratio were estimated.

The leaf pre-dawn water potential (ψ_{pd}) was determined using a pressure pump chamber (Scholander et al. 1964), always in the morning (between 04:30 and 05:30 a.m.), at an average temperature of 17 ºC, to avoid the inhibitory effects of light and temperature on water potential. The gas exchange values were measured using a portable infrared gas analyser (IRGA-LI6400XT Portable Photosynthesis System, LI-COR, Lincoln, USA),

at 600 μmol of photons m^{-2} s^{-1}. The gas exchange analyses were performed on clear days between 08:00 and 11:00 a.m. (solar time). The following parameters were evaluated: net photosynthetic rate (A), substomatal CO_2 concentration (Ci), stomatal conductance (gs) and transpiration (E). The carboxylation efficiency (CE) and the instantaneous water-use efficiency (WUE) were obtained using the A/Ci and A/E ratios, respectively. Water potential and gas exchange traits were measured in fully expanded mature leaves on the third or fourth leaf pairs of plagiotropic branches in the middle third of plants, at 0, 14 and 18 days after the onset of water stress.

Anatomical analyses were performed before water stress was imposed on the fully expanded leaves of the plagiotropic branches in the middle third of the plants. The leaves were collected and stored in 70 % ethanol (v/v). The cross sections used for the anatomical analyses were obtained with an LPC table microtome. The sections were cleared with sodium hypochlorite (1.25 % active chlorine), triple washed with distilled water, stained with astra blue-safranin solution (0.1 % astra blue and 1 % safranin at a ratio of 7:3) and subsequently mounted on semipermanent slides with 50 % glycerol (v/v) (Kraus & Arduin 1997). The slides were observed and photographed under an Olympus BX 60 optical microscope coupled to a Canon A630 digital camera. The images were analyzed with the UTHSCSA ImageTool software.

The following traits were evaluated in the cross sections: thickness of the abaxial epidermis, adaxial epidermis, leaf blade, palisade parenchyma, spongy parenchyma, adaxial cuticle, xylem vessel and phloem, and number of xylem vessels. The stomatal density (number of stomata mm^{-2}) and polar to equatorial diameter ratio of the stomata were evaluated for the paradermal sections from the abaxial leaf surfaces.

For the univariate analysis, the data were first analysed by the Shapiro-Wilk normality ($p \geq 0.05$) and Bartlett's tests ($p \geq 0.05$) to assess the homoscedasticity of the variances. The data for which the assumptions of normality and homogeneity of variances were confirmed were subjected to analysis of variance (Anova) and the Scott-Knott test ($p \leq 0.05$), using the R statistical software (R Core Team 2013), specifically the ExpeDes, Lattice and ggplot packages. A multivariate analysis was performed using a canonical variate analysis (Can) in the R software with the Candisc package (Friendly & Sigal 2014). During the functional divergence analysis, the values for the canonical variables obtained for each genotype were used to estimate the Mahalanobis genetic distance matrices. For delimitation of the groups, the optimization technique proposed by Tocher was used (Rao 1952).

RESULTS AND DISCUSSION

Drought stress is multifunctional stress, so several characteristics must be considered in the study of parameters that define the capacity of a coffee tree to tolerate drought. Among the various scales of complexity, the morphological, anatomical and physiological parameters must be considered (Fathi & Tari 2016). During the analysis of biometric parameters (Table 1), the clustering of the groups differentiated the genotypes in terms of size, especially the genotype 7, which was classified into the superior group by its larger leaf area, plant hight, stem diameter, first plagiotropic branch and number of plagiotropic branches. Regarding the insertion angle of the plagiotropic branch with the orthotropic branch, two groups were formed, with the superior group consisting of the genotypes 1, 2, 3, 4, 5, 6, 8, 11 and 13.

No differences in root length were observed among the genotypes. However, there was a clustering of the groups with different biomass ranges (Table 1). For the root dry mass, three groups were formed, with the first consisting of the genotypes 4, 5, 7, 8 and 10. With respect to shoot dry mass, four groups were observed, with the genotype 7 standing out because it had the highest total dry mass. The root to shoot dry mass ratio was used to split the genotypes into two groups, in which the highest ratio consisted of the genotypes 1, 2, 3, 4, 5, 10, 11, 12 and 14. The genotypes 1, 2, 3, 11 and 14 also had a higher root dry mass to leaf area ratio.

Differences among the analysed genotypes (Table 1) were observed for adaxial cuticle thickness, stomatal density, number of xylem vessels and phloem thickness. The genotype 1 exhibited a thicker adaxial cuticle thickness than the others, while the group with the highest stomatal density was represented by the genotypes 1, 2, 3, 6, 8, 9, 11, 12, 13 and 14. Regarding the number of xylem vessels, the genotype 8, followed by the genotype 9, showed the highest number of vessels. The genotype 8

Table 1. Mean leaf area (LA), plant height (PH), stem diameter (SD), length and number of plagiotropic branches (LPL and NPL, respectively), insertion angle of the plagiotropic branch with the orthotropic branch (APL), root length (RL), root dry mass (RM), shoot dry mass (SM), total dry mass (TM), ratio between root dry mass and shoot dry mass (RM/SM), ratio between root dry mass and leaf area (RM/LA), mean thickness of the adaxial cuticle (AdC), adaxial epidermis (AdE), abaxial epidermis (AbE), palisade parenchyma (PP), spongy parenchyma (SP), mesophyll percentage represented by the palisade parenchyma (%PP), thickness of the leaf blade (LB), number of xylem vessels (XYL), phloem thickness (PHL), xylem diameter (DXYL), stomata polar diameter/equatorial diameter (PD/ED) and stomatal density (DEN) of the genotypes.

Genotype	LA	PH	SD	LPL	NPL	APL	RL	RM	SM	TM	RM/SM	RM/LA
	m^2	cm				º	cm	g			g g^{-1}	g m^{-2}
1	0.42 c*	46.6 b	11.3 b	12.9 c	10.5 b	72.6 a	51 a	39 b	89 d	129 c	0.45 a	95.1 a
2	0.40 c	45.8 b	10.9 c	14.3 c	10.5 b	72.7 a	49 a	38 b	83 d	121 c	0.45 a	93.6 a
3	0.38 c	51.5 b	10.1 c	13.3 c	10.6 b	73.9 a	51 a	35 c	82 d	115 c	0.43 a	93.1 a
4	0.51 b	46.6 b	11.3 b	11.6 c	11.4 b	71.9 a	48 a	44 a	96 c	140 b	0.46 a	86.6 b
5	0.54 b	50.9 b	11.6 b	13.5 c	10.6 b	72.2 a	50 a	46 a	101 c	146 b	0.45 a	85.0 b
6	0.50 b	47.4 b	10.8 c	12.1 c	11.1 b	77.8 a	46 a	39 b	97 c	136 b	0.39 b	78.8 b
7	0.66 a	56.5 a	12.7 a	18.0 a	12.8 a	63.1 b	51 a	47 a	128 a	175 a	0.37 b	72.5 b
8	0.56 b	48.4 b	12.0 b	15.9 b	11.3 b	71.9 a	49 a	44 a	110 b	154 b	0.39 b	78.6 b
9	0.49 b	48.3 b	11.5 b	15.4 b	10.8 b	68.3 b	47 a	38 b	100 c	138 b	0.38 b	78.0 b
10	0.43 c	52.0 b	11.7 b	15.0 b	11.2 b	66.3 b	50 a	43 a	94 c	138 b	0.46 a	99.2 a
11	0.37 c	44.3 b	10.7 c	12.4 c	11.3 b	75.6 a	51 a	36 c	81 d	118 c	0.45 a	98.0 a
12	0.49 b	47.5 b	11.3 b	12.5 c	11.3 b	62.2 b	50 a	41 b	87 d	128 c	0.46 a	83.6 b
13	0.42 c	45.7 b	10.4 c	13.8 c	11.1b	73.3 a	46 a	33 c	83 d	115 c	0.39 b	77.5 b
14	0.35 c	46.8 b	10.0 c	13.5 c	10.8 b	68.1 b	51 a	33 c	78 d	108 c	0.43 a	94.2 a
CV (%)	14.2	10.6	6.8	16.1	10.3	8.6	8.0	14.5	13.2	12.7	10.5	14.9

Genotype	AdC	AdE	AbE	SP	PP	%PP	LB	XYL	PHL	DXYL	PD/ED	DEN
	µm						µm		µm			n mm^{-2}
1	6.7 a	30.1 a	18.8 a	81.8 a	203.9 a	27.5 a	325.3 a	147.3 d	72.9 c	18.8 a	1.5 a	185.2 a
2	5.1 b	29.6 a	20.5 a	81.3 a	209.9 a	28.0 a	319.0 a	160.3 d	77.1 c	19.8 a	1.4 a	167.2 a
3	5.3 b	27.0 a	20.0 a	70.3 a	196.7 a	26.3 a	311.4 a	154.3 d	82.5 b	17.5 a	1.5 a	170.0 a
4	5.0 b	25.6 a	20.6 a	74.4 a	203.1 a	26.8 a	327.8 a	150.3 d	70.1 c	18.9 a	1.5 a	160.5 b
5	4.7 b	27.6 a	20.0 a	71.4 a	206.8 a	24.8 a	325.4 a	157.0 d	75.4 c	19.4 a	1.5 a	149.7 b
6	4.4 b	26.5 a	19.6 a	81.0 a	206.3 a	27.2 a	319.6 a	155.5 d	71.2 c	18.0 a	1.5 a	166.7 a
7	4.6 b	27.4 a	18.5 a	78.0 a	201.0 a	26.9 a	314.5 a	167.5 c	84.8 b	21.9 a	1.5 a	151.2 b
8	4.1 b	27.2 a	19.8 a	69.0 a	195.0 a	27.0 a	303.8 a	212.8 a	96.0 a	18.9 a	1.5 a	192.9 a
9	5.1 b	24.8 a	19.2 a	78.3 a	201.6 a	27.0 a	314.7 a	195.8 b	87.0 b	18.7 a	1.5 a	180.6 a
10	4.8 b	26.1 a	19.5 a	74.7 a	195.1 a	27.7 a	312.5 a	144.8 d	75.3 c	17.9 a	1.4 a	160.2 b
11	5.6 b	27.6 a	21.2 a	71.8 a	186.1 a	27.7 a	308.9 a	148.3 d	73.3 c	20.7 a	1.4 a	171.3 a
12	4.5 b	25.1 a	19.6 a	67.9 a	189.8 a	28.4 a	305.1 a	174.8 c	79.9 b	19.4 a	1.5 a	196.0 a
13	4.7 b	26.6 a	19.2 a	70.8 a	201.5 a	25.3 a	315.2 a	181.3 c	81.6 b	17.3 a	1.5 a	172.8 a
14	5.0 b	26.5 a	19.1 a	72.3 a	195.3 a	27.0 a	309.5 a	172.0 c	79.9 b	20.0 a	1.5 a	192.9 a
CV (%)	17.1	8.1	7.1	17.5	8.0	12.5	6.9	13.4	8.3	11.8	5.0	13.3

* Means followed by the same letter in each column do not differ from one another according to the Scott-Knott test.

also showed the thickest phloem, followed by the genotypes 3, 7, 9, 12, 13 and 14.

The ψ_{pd} of the irrigated plants remained close to -0.2 MPa over the entire experimental period, without differences among the genotypes (Table 2). In plants under drought stress, significant variations were observed, with a decrease in this physiological parameter in all the genotypes; however, these values were particularly pronounced in the genotype 7, starting at 14 days after irrigation suspension (DAIS), reaching values of -2.9 MPa. During this period, the genotypes 1, 2, 3, 11 and 14 had ψ_{pd} values greater than -1.0. The decreases in ψ_{pd} at 18 DAIS, as a consequence of drought stress, were higher in the clones 5, 7 and 8 (reductions of -3.5, -4.0 and -3.4, respectively).

During the water suspension period (14 and 18 DAIS), there was a reduction in the mean values of net photosynthetic rate, stomatal conductance and transpiration for all the genotypes (Figure 1A), as well as for most of them in carboxylation efficiency (Figure 2A). Regarding the carboxylation efficiency,

Table 2. Leaf water potential of irrigated plants (I) and plants under drought stress (D) for 0, 14 and 18 days.

Genotype	Condition	0 days	14 days	18 days
1	I	-0.19 ± 0.01 Aa*	-0.14 ±0.01 Aa	-0.14 ± 0.01 Aa
	D	-0.14 ± 0.02 Aa	-1.00 ± 0.21 Bb	-1.59 ± 0.19 Bb
2	I	-0.13 ± 0.02 Aa	-0.21 ± 0.05 Aa	-0.21 ± 0.05 Aa
	D	-0.13 ± 0.01 Aa	-0.73 ± 0.14 Aa	-1.83 ± 0.22 Cb
3	I	-0.14 ± 0.02 Aa	-0.15 ± 0.02 Aa	-0.15 ± 0.02 Aa
	D	-0.15 ± 0.03 Aa	-0.64 ± 0,10 Ab	-1.85 ± 0.25 Cb
4	I	-0.14 ± 0.02 Aa	-0.21 ± 0.02 Aa	-0.21 ± 0.02 Aa
	D	-0.14 ± 0.01 Aa	-1.33 ± 0.20 Cb	-3.08 ± 0.26 Db
5	I	-0.15 ± 0.02 Aa	-0.19 ± 0.01 Aa	-0.19 ± 0.01 Aa
	D	-0.16 ± 0.02 Aa	-1.56 ± 0.21 Cb	-3.51 ± 0.20 Eb
6	I	-0.16 ± 0.02 Aa	-0.16 ± 0.02 Aa	-0.16 ± 0.02 Aa
	D	-0.14 ± 0.02 Aa	-1.11 ± 0.06 Aa	-2.84 ± 0.29 Db
7	I	-0.15 ± 0.02 Aa	-0.25 ± 0.06 Aa	-0.28 ± 0.05 Aa
	D	-0.15 ± 0.02 Aa	-2.90 ± 0.24 Db	-4.00 ± 0.00 Fb
8	Control	-0.16 ± 0.02 Aa	-0.18 ± 0.01 Aa	-0.19 ± 0.01 Aa
	D	-0.15 ± 0.02 Aa	-1.49 ± 0.19 Cb	-3.44 ± 0.30 Eb
9	Control	-0.14 ± 0.02 Aa	-0.19 ± 0.04 Aa	-0.19 ± 0.04 Aa
	D	-0.14 ± 0.02 Aa	-1.38 ± 0.21 Cb	-3.19 ± 0.15 Db
10	Control	-0.16 ± 0.02 Aa	-0.14 ± 0.02 Aa	-0.14 ± 0.02 Aa
	D	-0.16 ± 0.02 Aa	-1.13 ± 0.15 Bb	-2.68 ± 0.37 Db
11	Control	-0.14 ± 0.02 Aa	-0.14 ± 0.02 Aa	-0.14 ± 0.02 Aa
	D	-0.14 ± 0.01 Aa	-0.73 ± 0.15 Ab	-2.00 ± 0.20 Cb
12	Control	-0.15 ± 0.02 Aa	-0.21 ± 0.05 Aa	-0.21 ± 0.05 Aa
	D	-0.15 ± 0.04 Aa	-1.11 ± 0.30 Bb	-2.25 ± 0.25 Cb
13	Control	-0.15 ± 0.02 Aa	-0.18 ± 0.03 Aa	-0.18 ± 0.03 Aa
	D	-0.15 ± 0.02 Aa	-1.18 ± 0.15 Bb	-3.14 ± 0.17 Db
14	Control	-0.16 ± 0.02 Aa	-0.18 ± 0.01 Aa	-0.18 ± 0.01 Aa
	D	-0.16 ± 0.02 Aa	-0.63 ± 0.12 Ab	-1.10 ± 0.11 Ab

* Uppercase letters indicate significant differences at 0.05 of probability among the genotypes within each evaluation time, and lowercase letters indicate a significant difference at 0.05 of probability between the control and drought conditions within each genotype.

the genotypes 4, 8 and 14 showed no difference from the control at 14 DAIS, and the same was observed for the genotype 14 at 18 DAIS.

According to the water-use efficiency (WUE) analysis (Figure 2B), there was an increase in plants under drought stress at 14 and 18 DAIS, which reached higher μmol CO_2/mmol H_2O values, relatively to the control, for the genotypes 1, 2, 3, 4, 5, 6, 8, 9, 11, 12 and 14, at 14 DAIS. In turn, at 18 DAIS, only the genotypes 2, 3, 5, 7, 8, 9, 10, 11, 13 and 14 showed an increased WUE, in relation to the irrigated plants.

Photosynthesis inhibition due to low soil water content occurs due to stomatal closure or non-stomatal limitation (Cavatte et al. 2012). The reduced values of net photosynthetic rate (A) under drought (Figure 1A) stress were accompanied by a significant decrease in the stomatal conductance (gs) (Figure 1B) and transpiration (E) (Figure 1C). In parallel, the decline in the photosynthetic rate to substomatal CO_2 concentration ratio (Figure 2) indicates that non-stomatal factors are acting and leading to a decrease in photosynthesis under stress conditions. However, the increase in the A/E ratio under drought stress occurs because stomatal conductance decreases more quickly than photosynthetic carbon assimilation, resulting in an increased WUE. These responses are characteristic of the coffee crop in response to drought, and may maximize the hydration maintenance under drought (Fernandes et al. 2021).

Univariate analyses provide more details for the interpretation of traits, but they do not cover the complexity of interactions between variables, while the multivariate techniques allow for the combination of all the data contained in the experimental unit, so it is possible to perform selections based on a large number of variables and identify desirable materials (Ferreira et al. 2003). In this context, given the large number of traits under consideration, the use of univariate analyses combined with multivariate

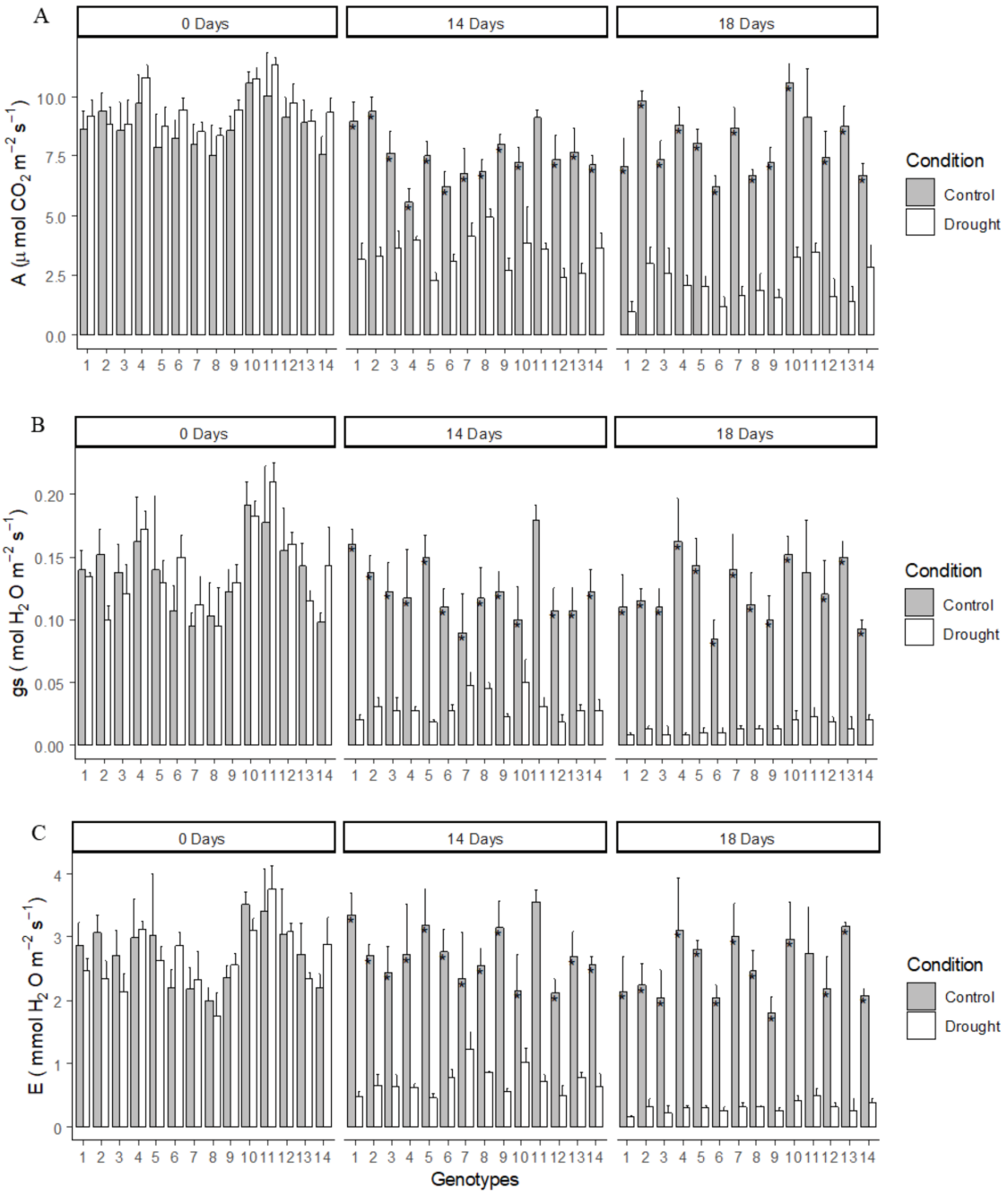

Figure 1. Photosynthesis (A), conductance (B) and transpiration (C) in the leaf tissues of irrigated plants and in plants under drought stress for 14 and 18 days after irrigation suspension. The bars represent the mean standard deviation of four replicates. * Significant difference at 0.05 of probability between the control and drought conditions within each genotype.

analysis is helpful in divergence analysis for the early selection of drought-tolerant genotypes (Ceolin et al. 2007, Sanwal et al. 2015).

In the functional divergence analysis of the irrigated controls at 0, 14 and 18 days, the first three canonical variables explained the total cumulative variances of 70.40, 84.97 and 82.36 %, respectively (Figures 3A, 4A and 5A). In general, the most important traits for discriminating among the irrigated genotypes in the canonical variables were leaf area, shoot dry mass and stem diameter in the first canonical variable (Table 3). For the irrigated controls, in the canonical variable 1, higher scores were obtained for the genotypes 7 and 8 (Figure 3A).

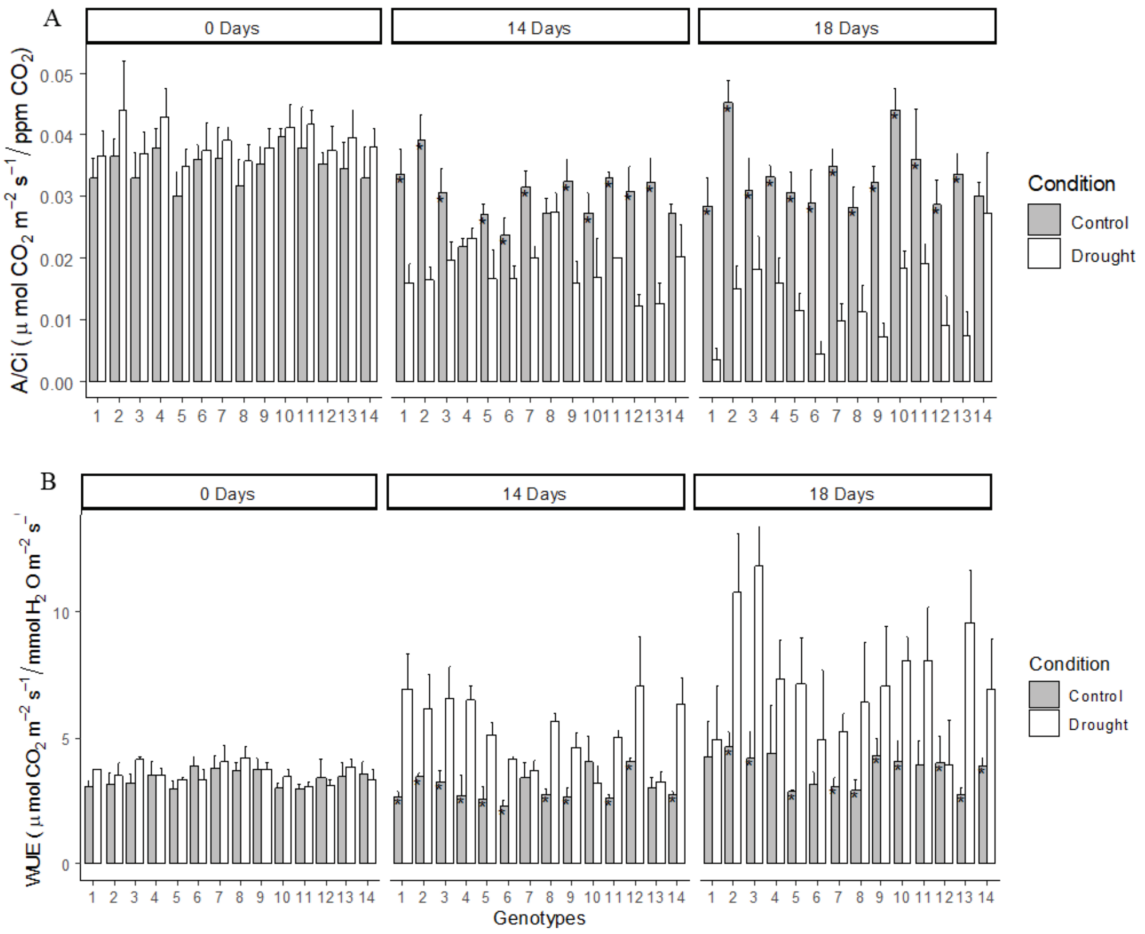

Figure 2. Carboxylation efficiency (CE) (A) and water-use efficiency (WUE) (B) in the leaf tissues of irrigated plants and plants under drought stress for 14 and 18 days after irrigation suspension. The bars represent the mean standard deviation of four replicates. * Significant difference at 0.05 of probability between the control and drought conditions within each genotype.

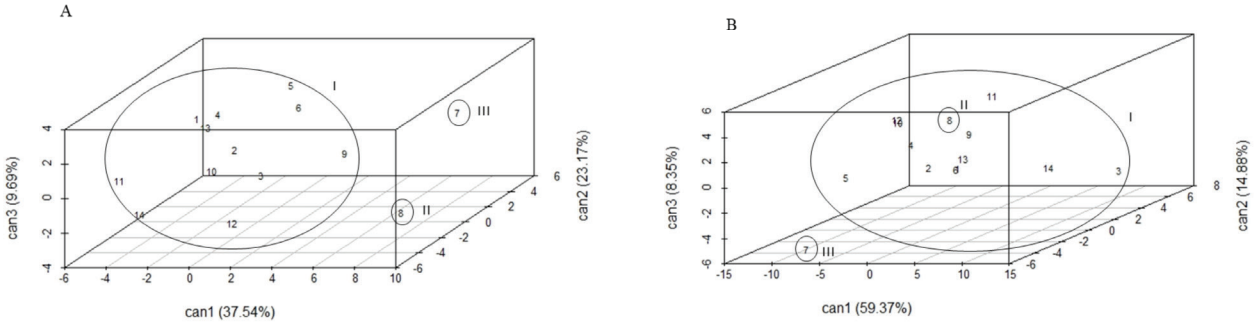

Figure 3. Dispersion of genotypes under irrigated (A) and drought (B) conditions, based on canonical variables, at 0 days after irrigation suspension, as established by a linear combination of standardized variables and cumulative variance (%) and weighting coefficients.

For the irrigated controls, the Tocher's clustering, based on the Mahalanobis distances according to the scores for the first three canonical variables, was used to separate the genotypes into three groups (Figure 3A). The group I was formed by the majority of the genotypes and the groups II and III consisted of the genotypes 8 and 7, respectively.

Considering the analysis of the variables at 14 days after the onset of drought stress, the evaluated characteristics were also represented by the first three

Table 3. Canonical variables at 0, 14 and 18 days after irrigation suspension (DAIS), as established by the linear combination of standard variables and cumulative variance of physiological, anatomical and growth variables.

	0 DAIS						14 DAIS						18 DAIS					
Variables*	Irrigated			Drought			Irrigated			Drought			Irrigated			Drought		
	Can1	Can2	Can3	Can1	Can2	Can3	Can1	Can2	Can3	Can1	Can2	Can3	Can1	Can2	Can3	Can1	Can2	Can3
Ψpd	-0.16	-0.01	0.16	-0.04	0.01	0.04	-0.25	-0.18	-0.16	0.71	-0.06	-0.11	-0.32	0.08	-0.07	0.65	0.42	0.38
A	0.02	0.23	-0.03	0.10	-0.09	0.53	-0.18	0.02	-0.30	-0.03	0.25	-0.01	0.01	-0.34	-0.29	0.16	0.07	0.17
gs	-0.26	-0.21	0.18	0.14	-0.18	0.57	-0.20	-0.17	-0.25	-0.24	0.17	0.25	0.09	-0.36	-0.02	0.04	0.00	0.24
E	-0.25	-0.24	0.17	0.14	-0.28	0.56	-0.13	-0.04	-0.20	-0.31	0.07	0.29	0.13	-0.34	-0.03	-0.02	-0.06	0.22
WUE	0.27	0.15	-0.08	-0.19	0.38	-0.31	0.13	-0.05	0.04	0.26	-0.06	-0.38	-0.17	-0.04	-0.13	0.23	0.16	-0.21
CE	-0.02	-0.08	0.05	0.11	-0.06	0.13	-0.07	0.20	-0.19	0.02	0.30	-0.07	-0.02	-0.15	-0.35	0.25	0.18	0.22
LA	0.78	0.07	0.29	-0.64	-0.03	-0.25	0.67	0.35	0.28	-0.61	0.30	0.01	0.82	-0.05	0.16	-0.61	-0.28	-0.05
PH	0.48	0.42	-0.09	-0.12	-0.14	-0.06	0.37	0.58	0.19	-0.23	-0.06	0.21	0.50	0.23	0.05	-0.11	-0.17	0.19
SD	0.15	-0.11	-0.40	-0.75	-0.12	0.05	0.69	0.05	0.06	-0.71	0.22	-0.23	0.72	-0.18	-0.03	-0.66	-0.35	0.16
NPL	0.23	-0.31	0.43	-0.35	-0.07	0.11	0.22	-0.21	0.16	-0.33	0.12	0.02	0.28	-0.48	0.32	-0.25	-0.41	-0.18
APL	-0.22	0.03	0.33	0.29	0.26	-0.12	-0.18	-0.18	-0.26	0.41	0.17	0.19	-0.19	-0.06	-0.16	0.24	0.23	-0.52
LPL	0.33	0.31	-0.03	-0.41	0.17	-0.31	0.35	0.47	-0.08	-0.38	0.30	0.15	0.37	0.23	-0.28	-0.52	0.19	0.19
RL	-0.10	-0.03	-0.09	0.05	-0.12	0.02	0.07	0.01	-0.35	-0.01	-0.17	-0.21	-0.02	-0.07	-0.39	0.08	-0.11	0.30
RM	0.42	-0.25	0.37	-0.65	-0.30	-0.27	0.56	-0.32	-0.05	-0.68	0.06	0.10	0.46	-0.35	-0.08	-0.50	-0.49	-0.07
SM	0.68	0.11	0.27	-0.66	-0.11	-0.50	0.74	0.13	0.02	-0.64	0.24	0.15	0.75	0.00	-0.14	-0.60	-0.28	-0.21
RM/SM	-0.35	-0.47	0.14	-0.08	-0.33	0.41	-0.20	-0.57	-0.14	-0.16	-0.26	-0.08	-0.36	-0.46	0.01	0.07	-0.36	0.23
RM/LA	-0.28	-0.39	0.03	0.28	-0.01	0.29	-0.11	-0.48	-0.33	0.24	-0.10	-0.02	-0.32	-0.26	-0.24	0.27	0.03	0.12
AdE	-0.04	0.19	0.11	0.09	-0.14	-0.13	-0.06	0.12	-0.34	0.07	-0.12	-0.11	-0.10	0.06	-0.34	0.12	0.04	0.03
AdC	-0.38	0.06	0.00	0.32	-0.12	0.08	-0.25	-0.04	-0.38	0.28	-0.20	-0.31	-0.33	0.00	-0.36	0.33	0.04	0.14
AbE	-0.09	-0.25	0.17	0.05	0.09	0.27	-0.08	-0.30	-0.05	0.09	0.02	-0.14	-0.12	-0.22	0.00	0.10	-0.08	-0.11
LB	0.04	0.38	0.44	-0.05	-0.24	-0.27	0.05	0.29	-0.38	-0.09	-0.10	0.17	0.06	0.09	-0.39	0.05	-0.19	-0.24
PP	-0.06	0.06	0.18	0.00	-0.12	-0.12	0.05	0.11	-0.24	-0.04	-0.08	0.03	0.02	0.07	-0.27	0.03	-0.04	-0.03
SP	0.00	0.52	0.39	0.00	-0.26	-0.38	-0.02	0.42	-0.33	-0.05	-0.12	0.30	0.04	0.15	-0.32	0.11	-0.19	-0.34
PD/ED	0.10	0.38	0.02	-0.01	-0.10	-0.34	-0.06	0.34	0.31	-0.03	0.01	0.09	0.12	0.10	0.33	-0.02	-0.10	-0.15
%PP	-0.05	-0.16	-0.06	-0.05	-0.01	0.15	0.05	-0.13	-0.05	-0.05	-0.02	-0.14	0.00	-0.07	-0.07	-0.06	0.04	0.17
DEN	-0.07	-0.20	-0.48	0.12	0.32	0.17	-0.17	-0.17	0.29	0.25	0.20	-0.24	-0.18	0.18	0.25	-0.04	0.42	0.24
XYL	0.44	0.04	-0.51	-0.18	0.57	-0.26	0.18	0.13	0.55	0.04	0.53	-0.02	0.27	0.44	0.33	-0.38	0.45	-0.12
PHL	0.13	0.07	-0.45	-0.06	0.56	0.03	-0.09	-0.04	0.48	0.18	0.50	-0.01	-0.04	0.34	0.36	-0.24	0.44	-0.13
DXYL	0.02	-0.18	0.19	-0.17	-0.22	-0.06	0.27	0.02	-0.19	-0.25	-0.18	-0.26	0.24	-0.17	-0.24	-0.10	-0.12	0.26

* A: net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}); gs: stomatal conductance (mol H_2O m^{-2} s^{-1}); E: transpiration (mmol H_2O m^{-2} s^{-1}); WUE: instantaneous water-use efficiency; CE: instantaneous carboxylation efficiency; LA: leaf area; PH: plant height; SD: stem diameter; NPL and LPL: number and length of plagiotropic branches, respectively; APL: insertion angle of the plagiotropic branch with the orthotropic branch; RL: root length; RM: root dry mass; SM: shoot dry mass; RM/SM: ratio between root dry mass and shoot dry mass; RM/LA: ratio between root dry mass and leaf area; AdE: adaxial epidermis thickness; AdC: adaxial cuticle thickness; AbE: abaxial epidermis thickness; LB: leaf blade thickness; PP: palisade parenchyma thickness; SP: spongy parenchyma thickness; PD/ED: stomata polar diameter/equatorial diameter; %PP: mesophyll percentage represented by the palisade parenchyma; DEN: stomatal density; XYL: number of xylem vessels; PHL: phloem thickness; DXYL: xylem diameter.

canonical variables, explaining a total cumulative variance of 79.96 % (Figure 4B). The most important traits for genotype discrimination in the first canonical variable were ψ_{leaf}, stem diameter, leaf area, root and shoot dry mass, and ψ_{leaf} was negatively correlated with these variables (Table 3). For the second canonical variable, the traits that made the highest contributions were phloem thickness and number of xylem vessels, which were positively correlated with photosynthesis, instantaneous carboxylation efficiency, leaf area and length of the first plagiotropic branch. For the third canonical variable, WUE was the largest contributing factor, being positively correlated with cuticle thickness, stomatal density and root length, and negatively correlated with stomatal conductance, transpiration and spongy parenchyma thickness. In the canonical variable 1, the highest positive scores were observed for the genotypes 3 and 14, and the highest negative scores were found for the genotype 7 (Figure 4B). In the canonical variable 2, the genotype 8 had the highest positive score, and the genotype 7 had the highest negative score. For the third canonical variable, the highest scores were observed for the genotype 6 (positive) and for the genotype 1 (negative).

At 14 days after the onset of drought stress, the Tocher's clustering separated the genotypes into five groups (Figure 4). The group I consisted

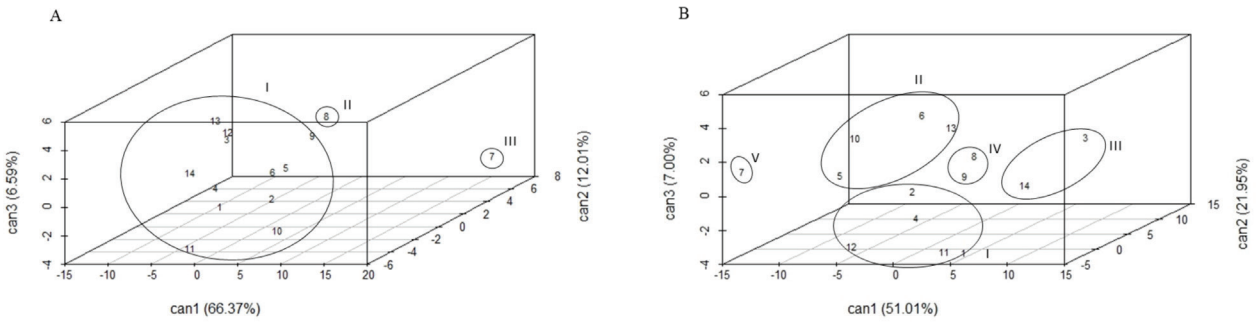

Figure 4. Dispersion of genotypes under irrigated (A) and drought (B) conditions based on canonical variables at 14 days after irrigation suspension, as established by a linear combination of standardized variables and cumulative variance (%) and weighting coefficients.

of five genotypes (1, 2, 4, 11 and 12), group II of four genotypes (5, 6, 10 and 13), group III of the genotypes 3 and 14, and group IV of the genotypes 8 and 9. Finally, the genotype 7 was the only genotype in group V. The group I was made up of genotypes with higher water potential values (-0.73 to -1.33 MPa). This group was defined primarily by traits that helped to maintain their water status, such as higher cuticle thickness, higher WUE, smaller number of xylem vessels and phloem thickness, greater stomatal density and the biggest root length. A higher cuticle thickness can maintain leaf hydration, since the lipidic nature of the cuticle can decrease transpiration and positively impact the WUE (Baliza et al. 2012). Since these genotypes also exhibited a smaller number of xylem vessels and phloem thickness, the conservation of leaf water potential could also have resulted from a greater hydraulic conductivity from the roots to the leaves, since there is a coordinated balance between the water supply capacity of the coffee stem/leaf system, which might be translated into higher gas exchange rates (Silva et al. 2013). The group II encompassed genotypes with water potentials ranging from -1.11 to -1.56 MPa and characteristics such as increased stomatal conductance, increased transpiration, greater spongy parenchyma thickness, and lower WUE and cuticle thickness. These characteristics together define genotypes with characteristics that promote transpiration and are also more vulnerable to cavitation and embolism under drought conditions (Wolfe et al. 2016).

In the functional divergence analysis at 18 days after the onset of drought stress, the evaluated characteristics were also represented by the first three canonical variables, explaining a total cumulative variance of 80.95 % (Figure 5), in which the traits that contributed the most to discrimination among the genotypes in the first canonical variable were leaf area, stem diameter and shoot dry mass with higher negative scores, and ψ_{leaf} and adaxial cuticle thickness with positive scores (Table 3). The second variable included the number of xylem vessels and phloem thickness with positive scores and root dry mass and number of plagiotropic branches with negative scores. The third canonical variable included

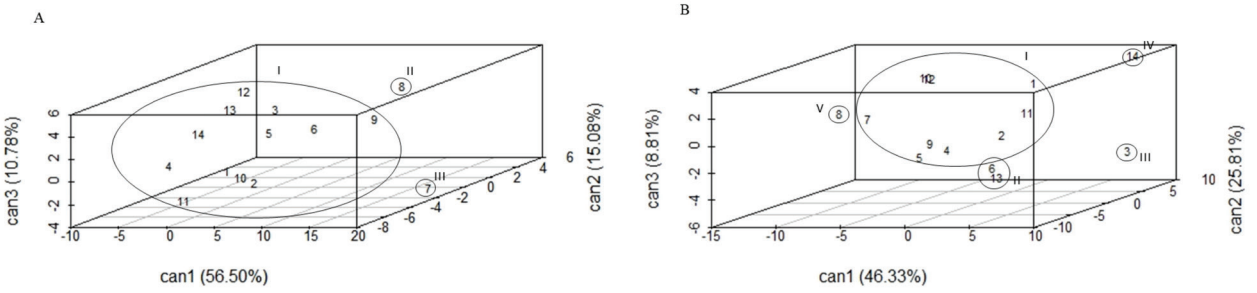

Figure 5. Dispersion of genotypes under irrigated (A) and drought (B) conditions, based on the canonical variables at 18 days after irrigation suspension, as established by the linear combination of the standardized variables and cumulative variance (%) and the weighting coefficients.

water deficiency (positive score) and the plagiotropic branches angle (negative score). For the canonical variable 1, the most negative score was observed for the genotype 8, and the highest positive scores were observed for the genotype 14 (Figure 5B). Considering the canonical variable 2, the genotype 8 had the highest positive score and the genotype 5 the highest negative score. For the third canonical variable, the highest scores were obtained by the genotype 13 (negative) and the genotypes 12 and 10 (positive). This result indicates that physiological parameters were more efficient at discriminating among the genotypes under moderate drought stress. In fact, at 18 days, the low water availability reduced the leaf photosynthesis rates in all the genotypes to very similar values.

At 18 DAIS, a functional divergence among the genotypes was determined primarily by morphological traits and architecture. The Tocher's clustering, based on the Mahalanobis distances, separated the genotypes into five groups (Figure 5). The group I consisted of 10 genotypes (1, 2, 3, 4, 7, 9, 10, 11 and 12), group II of two genotypes (6 and 13), and groups III, IV and V of the genotypes 5, 8 and 14, respectively. The group formed by the genotypes 6 and 13 presented the highest angle of plagiotropic branches correlated with low values of water potential (ranging from -2.84 to -3.14 Mpa). The greater angle of plagiotropic branches is characterized by genotypes with open crowns (lower boundary layer conductance), which are worse in postponing dehydration, if compared to cultivars with dwarf crowns, regardless of the given limits of their leaf areas (DaMatta 2004).

In this study, the univariate and multivariate analyses showed that, under irrigated conditions, the divergence among the genotypes was determined primarily by morphological characteristics such as leaf area and stem diameter, and, consequently, shoot dry mass. These characteristics allowed the discrimination of only the genotypes 7 and 8 from the other genotypes, and these genotypes showed a higher growth under adequate water availability. Under drought stress, the leaf area and stem diameter were also determinant parameters for the divergence of the genotypes 3 and 14 from the other genotypes. On the one hand, the genotypes 7 and 8 presented a higher leaf area and stem diameter, and these parameters had a negative correlation with the water potential, which reached low values of -2.9 Mpa and -4 Mpa at 14 and 18 DAIS, respectively. On the other hand, the genotypes 3 and 14 showed the lowest leaf area and stem diameter values, which were correlated with higher mean ψ_{pd} values of approximately -0.6 Mpa and -1.47 Mpa at 14 and 18 days, respectively. This finding is because genotypes with a greater leaf area have a greater total transpiration surface and, thus, a higher water uptake, more quickly reducing the water availability in the soil (Pizetta et al. 2016).

However, for the genotype 8, the leaf area and stem diameter were important variables for discriminating one genotype from the others under irrigated conditions and at 18 days after the onset of drought stress. The same effect was not observed at 14 days, indicating that other physiological or anatomical parameters influenced the response of this genotype to drought. In fact, at 14 days, in the genotypes 8 and 9, the ψ_{pd} values (-1.49 and -1.38 Mpa, respectively) were correlated with larger numbers of xylem vessels and higher phloem thickness, indicating a higher flow of water, minerals and carbohydrates in the leaves of these genotypes under moderate stress (Castro et al. 2009).

The functional divergence of the genotypes was evident under drought conditions, since the formation of five groups of genotypes was observed at 14 and 18 DAIS. This observation is consistent with those of Silva et al. (2013) and Castanheira et al. (2016), who emphasized that the screening and selection of water deficiency-tolerant coffee clones should be performed under drought conditions, because the performance of clones grown under drought conditions cannot be accurately predicted from the performance of clones grown under ample water conditions. However, a greater functional divergence was observed at 14 days than at 18 days after the onset of drought stress, what indicates that the drought period is a determinant of genotype responses and may affect the selection of genotypes more tolerant to drought. In addition, coffee is a tree species that goes through different development stages, each of which has particular characteristics. Each development stage may respond differently to environmental factors, depending on their intensity and duration. Taking this difference into account, it is important to verify if the drought tolerance responses of the genotypes in the seedling stage are repeated later when they are in the field under water restriction, in the adult plant stage. Therefore, it is important to associate data obtained in a greenhouse

with field data to select genotypes with higher productivity coupled with physiological phenotyping to obtain high-performance genotypes with multiple characteristics of interest.

CONCLUSION

The functional divergence combining morphological, anatomical and physiological analyses, in response to moderate drought, indicated the early selection of the genotypes 1, 2, 4, 11 and 12 as more drought tolerant during the seedling stage.

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