

# Adaptations to the drought season and impacts on the yield of ‘Híbrido de Timor’ coffee tree in the Minas Gerais State Cerrado (Brazilian Savanna)<sup>1</sup>

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## ABSTRACT

Climate change threatens the survival of commercial crops due to their narrow genetic base. One of the alternatives is the identification of plants with potential for abiotic stress tolerance. This study aimed to verify the physiological and anatomical adaptations to the drought period and the impacts on the yield of ‘Híbrido de Timor’ coffee tree accessions. The experimental design was randomized blocks, in a 7 x 2 factorial arrangement, being seven genotypes (UFV 377-21, UFV 377-21, UFV 442-42, BE 5 Wush-Wush x UFV 366-08, UFV 428-02, UFV 376-31 and UFV 427-55) and two seasonal periods (dry and rainy), with two replications. The stomatal conductance; predawn water potential; levels of hydrogen peroxide and malondialdehyde; activity of the enzymes catalase, superoxide dismutase and ascorbate oxidase; ascorbate content; cuticle thickness of the adaxial surface and leaf lamina; stomatal density; ratio between polar and equatorial diameter; phloem area; area, diameter and frequency of xylem vessels; relative hydraulic conductivity; vulnerability index; and yield were evaluated. The results showed acclimatization of the accessions to the dry period, with some of them maintaining higher water potential values in the predawn; induction of the antioxidant system with the increase in the activity of the superoxide dismutase enzyme and ascorbate levels; increase in the cuticle and leaf lamina thickness; and a higher ratio between polar and equatorial diameters. Most of the evaluated accessions showed a good productive performance, especially the ‘UFV 377-21’, ‘UFV 442-42’ and ‘UFV 376-31’, with adaptations to the dry period and yield potential.

KEYWORDS: *Coffea arabica* L., water deficit, leaf anatomy.

## RESUMO

Adaptações ao período seco e impactos na produtividade do cafeeiro Híbrido de Timor no Cerrado mineiro

As mudanças climáticas ameaçam a sobrevivência das culturas comerciais com sua base genética estreita. Uma das alternativas é a identificação de plantas com potencial para a tolerância a estresses abióticos. Objetivou-se verificar as adaptações fisiológicas e anatômicas ao período seco e os impactos sobre a produtividade de acessos de cafeeiro Híbrido de Timor. O delineamento experimental foi em blocos casualizados, em esquema fatorial 7 x 2, sendo sete genótipos (UFV 377-21, UFV 377-21, UFV 442-42, BE 5 Wush-Wush x UFV 366-08, UFV 428-02, UFV 376-31 e UFV 427-55) e dois períodos sazonais (seco e chuvoso), com duas repetições. Foram avaliados a condutância estomática; potencial hídrico na antemanhã; níveis de peróxido de hidrogênio e malondialdeído; atividade das enzimas catalase, superóxido dismutase e ascorbato peroxidase; conteúdo de ascorbato; espessura da cutícula da face adaxial e limbo foliar; densidade estomática; relação entre diâmetro polar e equatorial; área do floema; área, diâmetro e frequência de vasos do xilema; condutividade hidráulica relativa; índice de vulnerabilidade; e produtividade. Observaram-se aclimações dos acessos ao período seco, sendo que alguns mantiveram maiores valores de potencial hídrico na antemanhã; indução do sistema antioxidante com o aumento da atividade da enzima superóxido dismutase e de níveis de ascorbato; aumento da espessura da cutícula e do limbo foliar; e maior relação entre diâmetro polar e equatorial. A maioria dos acessos avaliados apresentou bom desempenho produtivo, com destaque para UFV 377-21, UFV 442-42 e UFV 376-31, com adaptações ao período seco e potencial produtivo.

PALAVRAS-CHAVE: *Coffea arabica* L., déficit hídrico, anatomia foliar.

## INTRODUCTION

Drought is the most important abiotic stress affecting crop yield (Canales et al. 2021). Climate

projections foresee for the coming years changes in the rainfall distribution, with periods of both rainfall excess and prolonged dry spells (Moat et al. 2017, Dubberstein et al. 2020).

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For coffee trees, initially the water restriction leads to stomatal closure as a way to avoid excessive transpiration. This behavior affects photosynthesis and, consequently, there is a reduction in the production of photoassimilates, reducing the crop growth and yield (Martins et al. 2019). In addition, water deficit associated with high temperatures causes leaf fall and scalding, and affects the reproductive phase and fruit set (Ruiz-Cárdenas 2015).

However, plants are able to acclimate to stress conditions, what allows them to survive (Hassan et al. 2021). For drought-tolerant plants, leaf anatomical adaptations such as increased leaf lamina thickness, cuticle thickness, changes in the stomata location and shape, as well as reduction in the diameter of xylem vessels, are observed (Castanheira et al. 2016). In addition, plants own enzymatic and non-enzymatic antioxidant molecules that are able to neutralize reactive oxygen species generated by water deficit (Nalina et al. 2021).

The germplasm bank of the Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig) has approximately 1,500 coffee tree accessions (Carvalho et al. 1991). Among them, there is a germplasm called 'Híbrido de Timor', a material used for the development of most cultivars resistant to coffee leaf rust (Carvalho et al. 1991, Guedes et al. 2013). In the 'Híbrido de Timor' population, there are genotypes with genetic variability for resistance to rust, cercospora, coffee fruit anthracnose, bacterial diseases and gall nematodes (Botelho et al. 2017, Peres et al. 2017); however, few studies have explored these accessions for tolerance to abiotic stresses.

The selection of genotypes based on physiological, anatomical and biochemical characteristics, and which, at the same time, meet the yield criteria, may assist in coffee genetic breeding programs aiming at the development of cultivars with multiple aptitudes. Thus, the present study aimed to verify the physiological and anatomical adaptations to the dry period and the impacts on the yield of 'Híbrido de Timor' accessions.

## MATERIAL AND METHODS

The experiment was carried out at the Epamig experimental field (18°59'26"S, 48°58'95"W and altitude of approximately 1,000 m), in Patrocínio, Minas Gerais State, Brazil. The spacing was 3.5 x 1.0 m between rows and between plants, respectively.

The soil is a 'Latossolo Vermelho-Amarelo' (Santos et al. 2013), corresponding to Oxisols (USDA 1999), and the topography is flat, with a slight slope (Santos et al. 2013). Cultivation followed the usual technical recommendations for coffee crops in the region.

The experimental design was randomized blocks, and the trial consisted of 14 treatments, in a 7 x 2 factorial arrangement, being seven promising 'Híbrido de Timor' accessions [UFV 377-21 (block 1); UFV 377-21 (selection of plants 1, 3 and 6 of block 2); UFV 442-42; BE 5 Wush-Wush x UFV 366-08; UFV 428-02; UFV 376-31; and UFV 427-55, aged seventeen years, belonging to the Epamig germplasm bank, selected based on yield, beverage quality and disease resistance parameters] and two seasonal periods (dry - Aug. 2019 and rainy - Jan. 2020), with two replications.

The climate in Patrocínio is classified as humid subtropical, with dry winter and rainy summer (Cwb), according to Köppen (Alvares et al. 2013). In 2019, the average maximum and minimum temperatures were 30.4 and 16.1 °C, respectively. The total annual rainfall was 1,299 mm (Figure 1A).

In August 2019, when the dry period assessment was carried out, there was absence of rainfall, as well as a maximum temperature of 29.6 °C and minimum of 14.5 °C (Figure 1A).

In 2020, the maximum and minimum temperatures averaged 29.4 °C and 15.9 °C, respectively, and the annual rainfall was 1,402 mm. In January 2020, the rainy season, there was rainfall of 282 mm and maximum and minimum temperatures of 30.3 °C and 18.7 °C, respectively (Figure 1B).

Three central plants from each experimental plot, which consisted of ten plants, were considered for the physiological, biochemical and leaf anatomical analyses, and were evaluated in two seasonal periods (dry - Aug. 2019 and rainy - Jan. 2020). Fully expanded leaves from the third or fourth pair of the plagiotropic branch, at the median part of the plant, were used. The whole experimental plot consisting of ten plants was evaluated for yield.

Stomatal conductance ( $gs - mmol m^{-2} s^{-1}$ ) was measured between 8 and 11 a.m., using a porometer (SC-1, Decagon Devices), on the abaxial side of the leaves. A Scholander-type pressure chamber (PMS Instruments Plant Moisture - Model 1000) was used to determine the leaf water potential, and the measurements were made before dawn (water potential in the morning).

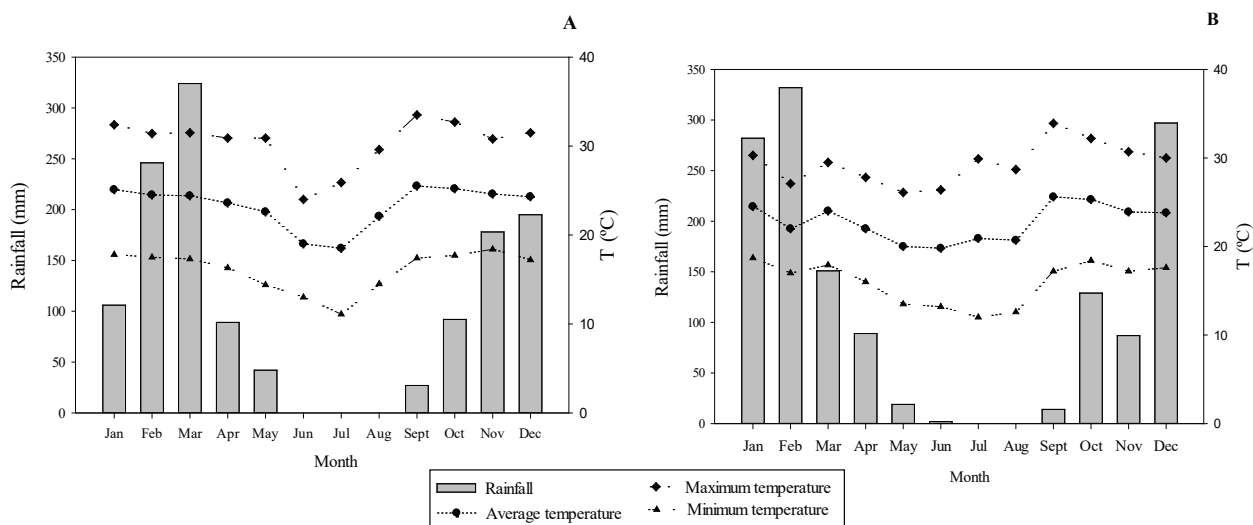


Figure 1. Maximum, average and minimum temperatures and rainfall recorded in 2019 (A) and 2020 (B). Source: Fazenda Boa Vista - DATERRA Atividades Agrícolas (Patrocínio, Minas Gerais State, Brazil).

For the biochemical analyses, the leaves were collected in the afternoon, between 12 and 1 p.m., placed in liquid nitrogen and stored in an ultra freezer (-80 °C).

For the quantification of hydrogen peroxide ( $H_2O_2$ ), 100 mg of the plant material were macerated in liquid nitrogen and polyvinylpyrrolidone (PVPP) and homogenized in 0.1 % trichloroacetic acid (TCA). The samples were centrifuged at 12,000 g for 15 min, at 4 °C. The concentration of  $H_2O_2$  was determined by the absorbance of the samples at 390 nm (Velikova et al. 2000). The determination of lipid peroxidation was performed by quantifying thiobarbituric acid reactive species (TBA) (Buege & Aust 1978).

To determine the antioxidant system enzymes activity, 100 mg of the plant material were macerated in liquid nitrogen and PVPP, then homogenized with 3.5 mL of the following extraction buffer: 100 mM of potassium phosphate (pH 7.8), 0.1 mM of EDTA and 10 mM of ascorbic acid. The extract was centrifuged at 13,000 g for 10 min, at 4 °C. The supernatants were collected and used for the analyses of the enzymes catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) (Biemelt et al. 1998).

The CAT activity was determined by the decrease in the absorbance of  $H_2O_2$  at 240 nm (Mengutay et al. 2013), the SOD activity by the ability of the enzyme to inhibit the photoreduction of nitrotetrazolium blue (NBT) (Giannopolis &

Ries 1977) and the APX activity by monitoring the ascorbate oxidation rate at 290 nm (Nakano & Asada 1981).

For the ascorbate (AsA) quantification, 50 mg of the plant material were used, macerated in liquid nitrogen and PVPP and homogenized with trichloroacetic acid (TCA 5 %) (m/v). Then, they were centrifuged at 10,000 g for 15 min, at 4 °C. The samples aliquots were added to the reaction medium composed of TCA (5 % - m/v), ethanol (99.8 % - v/v), ascorbic acid, phosphoric acid ( $H_3PO_4$ ) (0.4 % in ethanol - v/v), bathophenanthroline (0.5 % in ethanol - m/v) and iron (III) chloride ( $FeCl_3$ ) (0.03 % in ethanol - m/v). Then, this medium was homogenized and incubated at 30 °C, for 90 min. The samples were read at 534 nm (Arakawa et al. 1981).

Concerning the leaf anatomical evaluations, the leaf paradermal sections were obtained by printing the epidermis using the printing method with universal instant adhesive (cyanoacrylate ester) (Segatto et al. 2004). The stomatal density (ST - number of stomata  $mm^{-2}$ ) and the stomata polar and equatorial diameters were determined, from which the stomata polar diameter/equatorial diameter ratio (SPDED) was obtained.

For cross section analysis, the collected leaves were fixed in 70 % alcohol ( $v v^{-1}$ ) (Johansen 1940) and, after 72 hours, placed in a new 70 % alcohol solution ( $v v^{-1}$ ) to preserve the material, at room temperature, until the date of analysis.

The plant material was dehydrated in increasing ethyl series (80, 90 and 100 % - v v<sup>-1</sup>) and, after dehydration, went through the infiltration and polymerization processes in methacrylate-based historesin (Leica Microsystems, Wetzlar, Germany). Subsequently, it was sectioned about 8 µm thick, obtaining cross sections of the leaves, with the aid of a semi-automated rotary microtome model MRP 2015 of the brand Lupetec Tecnologia Aplicada (Lupe Indústria Tecnológica de Equipamentos para Laboratório, Brazil). The sections were stained with 1 % (m v<sup>-1</sup>) (O'Brien et al. 1964) and the slides prepared using vitreous varnish (Acrilex Tintas Especiais S. A.) as mounting medium.

The slides were observed and photographed in an optical microscope (model Red 200 from Kasvi/Motic) coupled to a digital camera (model Moticam 5MP from Motic). For each treatment replication, twelve photographs were taken, nine of slides containing transversal sections (three images of the main vein, three of the leaf lamina and three of the cuticle of the epidermis of the adaxial face) and three of slides with paradermal sections, always from different sections. Subsequently, the images were analyzed with the software UTHSCSA-Image Tool, version 3.0.

In cross sections, the following parameters were evaluated: adaxial face cuticle thickness (CUT - µm), leaf lamina thickness (LIM - µm), number of xylem vessels (NVX), metaxylem vessel diameter (XVD - µm), total area of the xylem region (XA - µm<sup>2</sup>), total area of the phloem region (PA - µm<sup>2</sup>), xylem vessel frequency (XVF - NVX/XA \* 1,000,000; mm<sup>2</sup>) and xylem vessel vulnerability index (VI = XVD/XVF) (Carlquist 1988). The relative hydraulic conductivity (RHC) was estimated using the Hagen-Poiseuille equation modified by Fahn et al. (1986):  $RHC = r^4 * XVF (\mu^4 m 10^6)$ , where r is the individual radius of the xylem vessels (Oliveira et al. 2018).

To determine the yield, harvests were made by total fruit stripping in 2019 and 2020, considering the ten plants of the experimental plot, and a 4-L sample was collected from each experimental plot and packed in woven polyethylene bags until drying reached approximately 11 % of moisture content. The samples were weighed before and after drying and after processing, to determine the yield and conversion of yield into bags ha<sup>-1</sup> of processed coffee, according to the yield of each experimental plot.

The data analyses were performed in the Sisvar software version 5.6 (Ferreira 2014) and the obtained means compared with each other using the Scott-Knott test, when significance was observed by the F test ( $p \leq 0.05$ ).

To verify the distinction between the genotypes in the dry and rainy periods, in addition to the correlations between the evaluated characteristics, the principal components analysis was used. The mean values were standardized to have zero mean and unit variance using the FactoMineR library and the R software version 4.1.1 (R Core Team 2020).

## RESULTS AND DISCUSSION

The predawn average values of stomatal conductance and water potential of *Coffea arabica* L. genotypes in two seasonal periods are shown in Figures 2A and 2B. No significant difference was found for the interaction between genotype and seasonal period. However, lower mean values of predawn water potential were found for the genotypes 5, 6 and 7 during the dry period.

Concerning the seasonal period, there were lower mean values of conductance and water potential in the predawn during the dry period (Table 1).

Stomatal closure is the first response of the plant to water restriction (Torre et al. 2021). Although there was no significant difference in water potential between the two evaluated seasonal periods, when considering the dry period, lower mean values of water potential in the predawn were observed for the genotypes 5, 6 and 7, when compared to the others, and there was a reduction in their stomatal conductance (Figures 2A and 2B). This reduction may affect photosynthesis, since it limits the CO<sub>2</sub> uptake (Martins et al. 2019, Torre et al. 2021).

There was no significant difference between the genotypes (G) and seasonal periods (P) for hydrogen peroxide, as well as for the interaction

Table 1. Mean values of stomatal conductance (gs - mmol m<sup>-2</sup> s<sup>-1</sup>) and water potential in the predawn (MPa) in genotypes of *Coffea arabica* L. evaluated in two seasonal periods.

Period	gs	MPa
Dry	130.88 b*	-1.11 b
Rainy	319.89 a	-0.32 a

\* Means followed by the same letter belong to the same group, according to the Scott-Knott test at 5 % of probability.

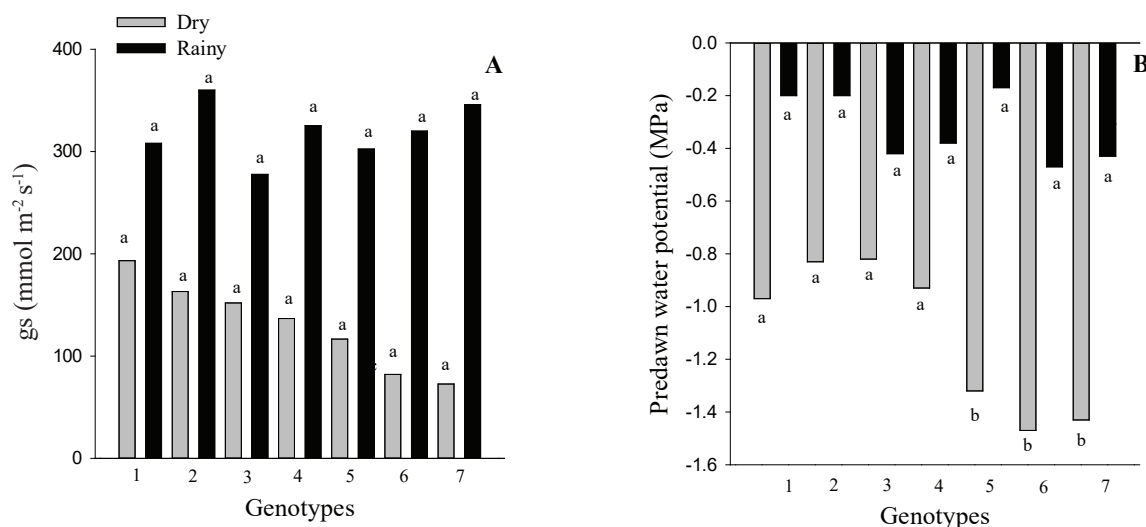


Figure 2. Mean values of stomatal conductance (A) and predawn water potential (B) of *Coffea arabica* L. genotypes belonging to the Epamig germplasm bank in two seasonal periods.

G x P. Nonetheless, concerning the seasonal period, there was a higher mean value of hydrogen peroxide in the dry period, when compared to the rainy period. For malondialdehyde levels, there was a difference only regarding the evaluated genotypes; however, all remained in the same group, according to the Scott-Knott test, for both the hydrogen peroxide and malondialdehyde content (Table 2).

The natural stress caused by changes in environmental conditions during the dry season (Figure 1) may lead to the overproduction of reactive oxygen species (ROS), such as the superoxide radical, hydrogen peroxide and hydroxyl radicals, which cause cell damage (Sachdev et al. 2021). However, plants own an antioxidant system which can keep ROS at non-harmful levels. Some studies

have identified a greater action of this system in plants with drought tolerance capacity (Hasanuzzaman et al. 2020, Sachdev et al. 2021, Salahvarzi et al. 2021). The SOD enzyme is the first line of defense of the antioxidant system and has the function of catalyzing the superoxide radical into H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> (Hassan et al. 2021). In this study, higher mean values of this enzyme activity, as well as higher levels of ascorbate (AsA), were observed in plants of the genotypes 1 and 2 in the dry period, when compared to the rainy period (Table 3). Ascorbic acid (AsA) is one of the main non-enzymatic antioxidants and acts as an electron donor in enzymatic and non-enzymatic reactions in the elimination of ROS (Hasanuzzaman et al. 2020).

Morpho-anatomical adaptations have been considered important features in plant survival under drought conditions. These anatomical adjustments may prevent dehydration, as well as optimize the transport of solutes (Alderotti et al. 2020, Hasanagic et al. 2020). During the dry period, the cuticle thickness of the adaxial surface (CUT) showed higher mean values for the genotypes 1, 2, 3, 4 and 6, when compared to the rainy period (Table 4).

However, the genotype 3 stood out due to the greater thickness of the cuticle in both seasonal periods (Table 4), an important mechanism of adaptation to dry periods. This layer is a barrier that can isolate internal tissues, increase radiation reflection and decrease the transpiration rate (Silva et al. 2004).

Table 2. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> - mmol H<sub>2</sub>O<sub>2</sub> g<sup>-1</sup> of fresh weight - FW) and malondialdehyde (MDA - mmol MDA g<sup>-1</sup> FW) levels in *Coffea arabica* L. genotypes evaluated in two seasonal periods.

Genotype	H <sub>2</sub> O <sub>2</sub>	MDA
1	3.17 a*	69.31 a
2	3.45 a	73.76 a
3	2.92 a	69.33 a
4	3.79 a	89.04 a
5	3.58 a	62.62 a
6	2.69 a	59.16 a
7	2.37 a	55.89 a

\* Means followed by the same letter belong to the same group, according to the Scott-Knott test at 5 % of probability.

Table 3. Activity of the enzymes catalase (CAT -  $\mu\text{M H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  of fresh weight - FW), superoxide dismutase (SOD -  $\text{SOD min}^{-1} \text{ mg}^{-1}$  FW), ascorbate peroxidase (APX -  $\mu\text{M AsA min}^{-1} \text{ mg}^{-1}$  FW) and ascorbate levels (AsA -  $\text{mg AsA g}^{-1}$  FW) in *Coffea arabica* L. genotypes evaluated in two seasonal periods.

Genotype	CAT		SOD		APX		AsA	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
1	0.004 aB*	0.031 aA	0.36 aA	0.24 bB	0.07 a	0.04 a	47.74 aA	43.39 aB
2	0.010 aB	0.026 aA	0.36 aA	0.22 bB	0.04 a	0.07 a	39.10 aA	34.45 bB
3	0.038 aA	0.028 aA	0.31 bA	0.31 aA	0.21 a	0.11 a	37.13 aB	40.81 aA
4	0.021 aA	0.025 aA	0.31 bA	0.30 aA	0.15 a	0.15 a	34.54 aA	33.99 bA
5	0.021 aA	0.017 aA	0.30 bA	0.29 aA	0.06 a	0.07 a	41.74 aA	29.22 bB
6	0.021 aA	0.022 aA	0.31 bA	0.30 aA	0.10 a	0.09 a	34.36 aA	32.67 bA
7	0.021 aA	0.019 aA	0.29 bA	0.32 aA	0.10 a	0.12 a	31.76 aA	32.93 bA

\* Means followed by the same lower case letter in the column and capital letter in the row belong to the same group, according to the Scott-Knott test at 5% of probability.

Table 4. Mean values of adaxial cuticle thickness (CUT -  $\mu\text{m}$ ) and leaf lamina thickness (LIM -  $\mu\text{m}$ ) evaluated in *Coffea arabica* L. genotypes in two seasonal periods.

Genotype	CUT		LIM	
	Dry	Rainy	Dry	Rainy
1	4.53 bA*	4.42 bB	320.19 aA	294.29 bB
2	4.57 bA	4.44 bB	267.43 cA	271.90 cA
3	4.91 aA	4.68 aB	232.73 cB	241.35 dA
4	4.52 bA	4.43 bB	276.37 cA	242.68 dB
5	4.41 cA	4.41 bA	307.58 bA	302.91 aA
6	5.51 bA	4.45 bB	252.46 cA	244.20 dB
7	4.36 dB	4.43 bA	255.86 cA	248.70 dB

\* Means followed by the same lower case letter in the column and capital letter in the row belong to the same group, according to the Scott-Knott test at 5% of probability.

Another way to avoid excessive transpiration is the leaf lamina thickening, mainly by the increment of the adaxial and abaxial epidermis, which work as a barrier against transpiration (Queiroz-Voltan et al. 2014). In this study, during the dry period, a greater thickness of this tissue was observed in the plants of the genotypes 1, 4, 6 and 7, when compared to the values seen in the rainy period (Table 4).

Maintaining the solute transport during the dry period is as important as avoiding transpiration (Queiroz-Voltan et al. 2014). These characteristics have been related by other studies to drought-tolerant plants (Queiroz-Voltan et al. 2014, Oliveira et al. 2018).

Concerning the conducting vessels, higher mean values were observed for the xylem area during the dry period for the genotypes 3, 4 and 7. Moreover, the genotypes 1, 2 and 3 remained with the highest mean values of xylem vessel diameter in the dry period associated with a higher RHC

(Table 5). These characteristics have been related to water transportation by Queiroz-Voltan et al. (2014) and may have benefited the maintenance of the water status of these genotypes during the dry period.

Nevertheless, the larger diameter of the vessels has been related to their vulnerability, leaving them more prone to cavitation events in the water column (Oliveira et al. 2018). Accordingly, the genotype 2, 3, and 4 would be subject to these events, as these showed higher mean xylem vessel vulnerability index values in the dry period, when compared to the rainy period (Table 5).

Concerning the seasonal periods, a greater phloem area and relative hydraulic conductivity were found during the dry period (Table 6).

The change in the shape, size and number of stomata are adaptations that can prevent plant dehydration. The higher ratio between SPDED also highlighted the plants of the genotype group 6 during the dry period, when compared to the rainy period (Table 7). This feature evidences the ellipsoidal shape of the stomata and favors the opening and closing dynamics, making the  $\text{CO}_2$  assimilation more efficient while avoiding excessive transpiration (Durand et al. 2019).

Although no significant difference was found for the stomatal density of the evaluated genotypes (Table 7), a lower stomatal density was observed during the dry period. This may be a mechanism to avoid excessive transpiration (Nóia Júnior et al. 2020) and has been related to drought-tolerant genotypes (Oliveira et al. 2018).

Figures 3A and 3B show the projections of the vectors and the dispersion of the genotypes in the dry and rainy periods, respectively. The projections of

Table 5. Mean values of the phloem and xylem area (PA, XA -  $\mu\text{m}^2$ ), diameter (XVD -  $\mu\text{m}$ ), frequency (XVF), vulnerability index (VI) and relative hydraulic conductivity (RHC -  $\mu\text{m}^4 10^6$ ) evaluated in *Coffea arabica* L. genotypes in two seasonal periods.

Genotype	PA	XA		XVF	
		Dry	Rainy	Dry	Rainy
1	72,100.13 a*	94,441.69 aA	92,941.11 aA	1,308.77 aA	1,398.31 aA
2	77,854.62 a	93,392.78 aA	91,941.56 aA	1,196.06 aB	1,329.04 aA
3	93,117.82 a	124,282.06 aA	104,350.98 aB	1,024.27 aB	1,221.59 aA
4	54,141.62 a	104,045.58 aA	85,082.96 aB	1,195.29 aB	1,442.67 aA
5	71,166.61 a	94,079.87 aA	93,886.93 aA	1,395.39 aA	1,371.35 aA
6	65,769.95 a	80,256.21 aA	83,345.14 aA	1,446.98 aA	1,399.09 aA
7	63,269.87 a	106,122.74 aA	91,744.76 aB	1,308.17 aA	1,411.07 aA

Genotype	XVD		VI		RHC
	Dry	Rainy	Dry	Rainy	
1	22.13 bA	21.40 aB	0.016 aA	0.015 bA	19.45 a
2	22.02 bA	20.73 bB	0.018 aA	0.016 bB	16.57 b
3	22.84 aA	21.46 aB	0.021 aA	0.018 aB	16.68 b
4	19.06 dA	17.64 dB	0.015 aA	0.012 cB	9.46 d
5	18.84 dA	18.79 cA	0.013 aA	0.013 cA	11.58 c
6	19.48 cA	18.75 cB	0.013 aA	0.013 cA	12.22 c
7	19.84 cA	18.59 cB	0.014 aA	0.013 cA	10.78 c

\* Means followed by the same lower case letter in the column and capital letter in the row belong to the same group, according to the Scott-Knott test at 5 % of probability.

Table 6. Mean values of phloem area (PA -  $\mu\text{m}^2$ ) and relative hydraulic conductivity (RHC -  $\mu\text{m}^4 10^6$ ) in *Coffea arabica* L. genotypes evaluated in two seasonal periods.

Period	PA	RHC
Dry	73,990.12 a*	14.48 a
Rainy	68,130.05 b	13.16 b

\* Means followed by the same lower case letter in the column and capital letter in the row belong to the same group, according to the Scott-Knott test at 5 % of probability.

Table 7. Mean values of stomatal density (SD - number of stomata  $\text{mm}^{-2}$ ) and stomata polar to equatorial diameter ratio (SPDED) evaluated in *Coffea arabica* L. genotypes in two seasonal periods.

Genotype	SD	SPDED	
		Dry	Rainy
1	149.57 a*	1.58 aA	1.64 aA
2	158.30 a	1.69 aA	1.61 aA
3	127.99 a	1.81 aA	1.84 aA
4	143.32 a	1.70 aA	1.63 aA
5	142.25 a	1.72 aA	1.73 aA
6	141.04 a	1.81 aA	1.65 aB
7	171.72 a	1.76 aA	1.68 aA

\* Means followed by the same lower case letter in the column and capital letter in the row belong to the same group, according to the Scott-Knott test at 5 % of probability.

variables related to the first two principal components account for 71.1 % of the data variation in the dry period and 69.6 % in the rainy period.

The principal components analysis revealed a predominance of cuticle thickness on the adaxial side, total area of the conducting vessels (xylem and phloem), vulnerability index and lower frequency of xylem vessels in the genotype 3, both in the dry and rainy periods (Figures 3A and 3B), which are probably intrinsic to the genotype. In the dry period, this genotype remained with higher mean values of water potential in the predawn period (Figure 2B), suggesting that the maintenance of the water status may be a function of these characteristics associated to other mechanisms of tolerance. The CAT and APX enzymes activity distinguished this genotype from the others during the dry period (Figure 3A), demonstrating biochemical responses to the water stress period (Hasanuzzaman et al. 2020, Sharma et al. 2012). These enzymes have the important role of neutralizing  $\text{H}_2\text{O}_2$  in plant cells (Sharma et al. 2012). The negative correlation among CAT, APX and  $\text{H}_2\text{O}_2$  was evidenced in the principal components analysis (Figure 3A).

During the dry period, the genotypes 5, 6 and 7 distinguished themselves from the others

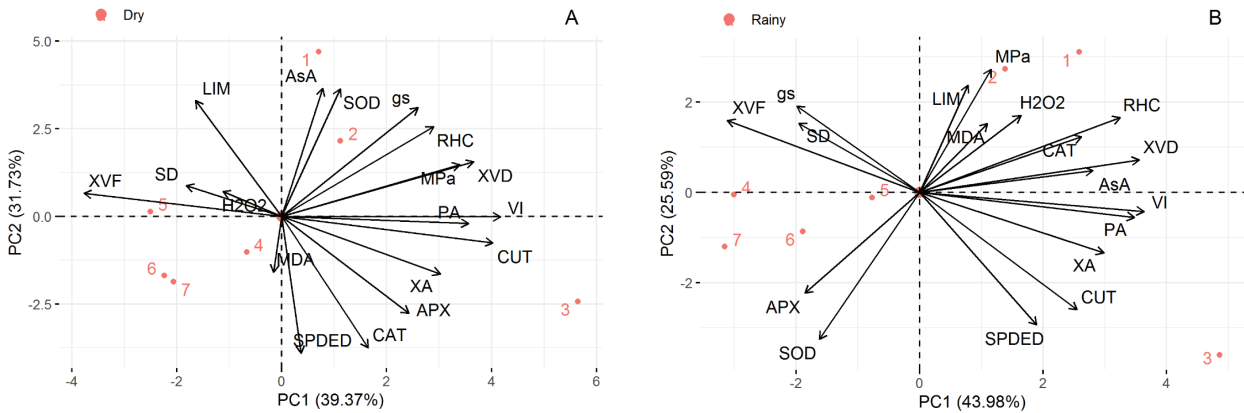


Figure 3. Projection of vectors and dispersion of *Coffea arabica* L. genotypes in the dry (A) and rainy (B) period, related to the first two principal components. Physiological variables: stomatal conductance (gs), water potential in the predawn (MPa); biochemical variables: hydrogen peroxide ( $H_2O_2$ ), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and ascorbate (AsA); leaf anatomical variables: leaf lamina thickness (LIM), adaxial cuticle thickness (CUT), stomatal density (SD), polar and equatorial diameter ratio (SPDED), phloem area (PA), xylem area (XA), xylem vessel diameter (XVD), xylem vessel frequency (XVF), vulnerability index (VI) and relative hydraulic conductivity (RHC).

by the lower scores of predawn water potential and stomatal conductance, as well as xylem vessel anatomical characteristics such as higher frequency of vessels, smaller diameter and xylem vessel vulnerability index (Figure 3A) and maintenance of relative hydraulic conductivity (Table 5), which may have benefited the solutes transportation during the dry period (Figure 3A). These anatomical characteristics were important in distinguishing these genotypes in both the dry and rainy periods, what may indicate the genetic preponderance of these characteristics.

On the other hand, the genotype 4 showed higher levels of malondialdehyde during the dry period, a reactive oxygen species that differentiated it from the others (Figure 3A).

For the genotypes 1 and 2, during the dry period, there was maintenance of stomatal conductance and water potential in the predawn period (Figure 3A). These materials stood out for their antioxidant response during the dry period, given by the higher scores of the SOD enzyme activity and the non-enzymatic antioxidant AsA (Figure 3A). Additionally, they presented a greater xylem and phloem area and xylem vessel diameter. The vascular system features may have favored the transportation of water, photoassimilates and maintenance of water status during the dry period, since a positive correlation was observed among XVD, RHC and MPa (Figure 3A).

In general, all the genotypes showed physiological, biochemical and anatomical adaptations during the dry season. However, to meet coffee tree genetic breeding programs, it is necessary that the genotypes meet agronomic aspects, particularly yield (Davis et al. 2021).

Regarding the evaluated genotypes yield, there were average values between 7.99 and 43.65 bags  $ha^{-1}$ , and the highest values were observed for the genotypes 1 and 2, followed by the genotypes 6, 7, 5 and 3. On the other hand, the lowest average yield values were seen in genotype 4 plants (Figure 4).

The ‘Híbrido de Timor’ genotypes have different adaptation strategies to the dry season in

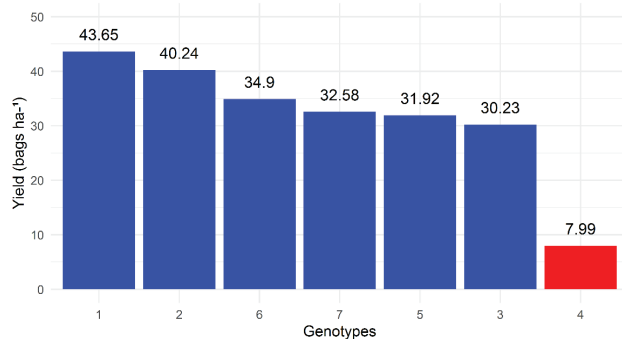


Figure 4. Average grain yield of *Coffea arabica* L. genotypes belonging to the Epamig germplasm bank. Columns with the same color represent the same group, according to the Scott-Knott test at 5% of probability.



the Brazilian Savanna. There are genotypes (1, 2 and 3) that showed a higher yield associated with the capacity of maintaining the water potential related to the anatomical characteristics and to the antioxidant system. The other group, composed of the genotypes 5, 6 and 7, maintained the yield even with the occurrence of the negative variation of the water potential correlated with the vascular system and stomata anatomical features seen for the genotype 6. On the other hand, the lower yield observed in the genotype 4 plants, as well as higher malondialdehyde levels (Figures 3A and 4) and low relative hydraulic conductivity (Table 5), are features indicating a low adaptability of this material to the climate conditions of this study (Figure 1).

This study combined physiological, biochemical and leaf anatomical approaches to understand the genotypes main adaptive mechanisms to water restriction periods. These results have great relevance in coffee tree genetic breeding, to contribute in the development of cultivars that meet the producers demands, with resistant characteristics to the main diseases of the crop, presenting a productive potential and, at the same time, being suitable for regions prone to drought.

### CONCLUSIONS

1. The 'Híbrido de Timor' accessions 'UFV 377-21', 'UFV 442-42' and 'UFV 376-31' stood out with adaptations to the dry period and yield potential;
2. The main adaptation strategies were the antioxidant system induction, water potential maintenance in the predawn, leaf anatomical features that benefit the solutes transportation during the dry period, and mechanisms with the function of preventing excessive transpiration;
3. The yield of the accession 'BE 5 Wash-Wash x Híbrido de Timor UFV 366-08' was affected, indicating a lower adaptation potential in the climatic conditions of the Minas Gerais State Cerrado.

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