Acta Scientiarum
http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v47i1.70207

Physiological and nutritional parameters of drought resistance in coffee seedlings genotypes

Elder Andreazi[1,3], Bruno Teixeira de Sousa[1,2], Halley Caixeta Oliveira[2], Inês Cristina de Batista Fonseca[1], Valdir Mariucci Junior[1,3], Luciana Harumi Shigueoka[3] and Gustavo Hiroshi Sera[3*]

[1]Departamento de Agronomia, Universidade Estadual de Londrina, PR-445, km 380, 86057-970, Londrina, Paraná, Brazil. [2]Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina, Londrina, Paraná, Brazil. [3]Departamento de Melhoramento Vegetal, Instituto de Desenvolvimento Rural do Paraná, Londrina, Paraná, Brazil. *Author for correspondence. E-mail: gustavosera@idr.pr.gov.br

ABSTRACT. Drought is an environmental condition that compromises the development of coffee plants. New coffee genotypes that are resistant to drought must be selected quickly and practically. The objectives of this study were to evaluate the resistance of five genotypes of *Coffea arabica*, including three new genotypes with introgression of genes from *Coffea racemosa* (H0113-40-26-1, H0113-40-26-19, and H0113-40-26-10), to water restriction and relate the intensity of plant wilting with physiological responses and nutrient accumulation. The experiment was conducted using 45 coffee seedlings obtained from seeds with six pairs of leaves cultivated in tubes. Some seedlings were subjected to two water restriction periods, whereas the remainder were kept under irrigation. The photosynthesis rate, transpiration rate, and wilting intensity were evaluated after each restriction period. Nutrient content was also evaluated after two periods of water restriction. The evaluation of wilting intensity corroborated the physiological parameters. There was a reduction in photosynthesis and transpiration rates under water restriction and nutrient accumulation in coffee seedlings H0113-40-26-1, H0113-40-26-19, and H0113-40-26-10 increased under these conditions. *C. arabica* genotypes carrying the genes of *C. racemosa* presented good drought resistance, with H0113-40-26-10 being the most resistant and showing the lowest wilt intensity.

Keywords: water deficiency; genetic breeding; *Coffea arabica*; *Coffea racemosa*.

Received on November 4, 2023.
Accepted on July 28, 2024.

Introduction

Brazilian coffee farming occupies a prominent position worldwide, placing Brazil as the largest producer and exporter of seeds (coffee beans), in addition to presenting a wide range of beverage qualities with particular characteristics of each producing region (Machado et al., 2020; Volsi et al., 2019). For the 2022 Brazilian harvest, approximately 35.7 million 60 kg bags of *Coffea arabica* L. green coffee are expected. However, prolonged dry periods were detrimental to the period defined for the productive potential of crops in all producing regions (Companhia Nacional de Abastecimento [CONAB], 2022). The fluctuation in coffee production worldwide is affected by temperature, precipitation, and vapor pressure deficit (VPD), which has recently been cited as a key indicator of global coffee productivity (Moat et al., 2019; Kath et al., 2019).

Drought is one of these limiting climatic factors that can compromise the development of coffee plants from the initial stages of young plant formation after planting until the grain filling of adult plants, although a period of water restriction is required for plants to flower (International Coffee Organization [ICO], 2022; Dias et al., 2007; Fialho et al., 2010; Carvalho et al., 2017; DaMatta et al., 2018). Stomatal closure is one of the first plant responses to drought to avoid water loss; however, it limits CO_2 uptake and biomass production (Buckley, 2005; McDowell et al., 2008). Water is fundamental for maintaining plant physiology and metabolism, and a reduction in water content leads to wilting (Hussain et al., 2018).

Moreover, water deficit may compromise the absorption of essential minerals nutrients, leading to deficiency symptoms and accentuating the growth reduction (Silva et al., 2011). Mineral nutrients are necessary for normal plant growth and development, as they act in the regulation of physiological processes such as enzymatic activation, photosynthesis, protein synthesis, and in the synthesis and transport of osmotic solutes (Ahanger et al., 2013). They are also useful in mitigating abiotic stresses (Waraich et al., 2011).

However, studying the effects of drought on the growth of coffee plants enables a projection of responses to climate change in the growing environment, in addition to aiding in the selection of cultivars and species that can survive this condition of abiotic stress (Fernandes-Brum et al., 2013; Melo et al., 2014; Tounekti et al., 2018).

Coffee plants can develop several drought resistance responses, such as alterations in the water content of leaves, reduction in the rates of CO_2 assimilation and transpiration, anatomical and morphological alterations, and alterations in the dynamics of nutrient absorption and accumulation (ICO, 2022; Fernandes-Brum et al., 2013; DaMatta, 2004; Maseda & Fernández, 2006). Therefore, genetic improvement studies on this species have been carried out in an exploratory manner. In addition, most studies evaluated a single event of water restriction throughout plant development, which differs from the actual potential for sequential events of water restriction (Menezes-Silva et al., 2017).

Evaluating leaf wilt in coffee seedlings has been cited as an important support methodology for genetic improvement, enabling the satisfactory identification of genotypes with greater resistance to drought (Carvalho et al., 2017). This evaluation is a quick and easy method, disregarding the use of equipment or the need to collect plant material for laboratory analyses. There is a strong correlation between the wilting intensity and grain production of coffee plants in the field (Mohammed et al., 2021). However, in addition to visual assessment, it is necessary to investigate the physiological mechanisms adopted by plants to present a more robust analysis of the behavior of resistant genotypes (Menezes-Silva et al., 2017).

Within the genus *Coffea*, the species *C. arabica* L. and *C. canephora* Pierre are the most widely cultivated, corresponding to approximately 55% and 45% of the world production of coffee beans, respectively (ICO, 2022); however, most cultivars of these two species are susceptible to drought. Other species of this genus, such as *C. racemosa* Lour., *C. canephora* Pierre ex A. Froehner, and *C. liberica* Hiern, have good drought resistance because of their origin in regions subjected to drought (Medina Filho et al., 1977; Pinheiro, 2005; Mazzafera & Carvalho 1987; Queiroz-Voltan et al., 2014). Thus, the insertion of genes from this species into *C. arabica* through genetic improvement could be a strategy for increasing drought resistance.

The objective of the present study was to evaluate the responses of physiological variables and the accumulation of nutrients in five genotypes of *C. arabica* under water restriction (among them, three carriers of genes of *C. racemosa*) and related them to the scale of visual scores of wilting intensity. Thus, it is expected that the results will validate wilting intensity as an effective method for determining drought resistance in coffee seedlings.

Material and methods

Plant material and growing conditions

Seedlings from seeds that were sown in cement-built sand germination boxes (1 × 1 × 20 m) in the seedling nursery of the *Instituto de Desenvolvimento Rural do Paraná* – IAPAR-EMATER (IDR-Paraná) in Londrina, Paraná State, Brazil, were used. Water irrigation was performed manually to keep the sand saturation at field capacity. Three F_4 progeny of *Coffea arabica* L. were introgressed with *C. racemosa* Lour. and *C. canephora* Pierre. The introgression of *C. racemosa* originated from the C1195-5-6-2 genotype, and the introgression of *C. canephora* originated in the cultivars IPR 104 and Tupi IAC 1669-33 (Andreazi et al., 2018; Carducci et al., 2019). Tupi IAC 1669-33 and IAPAR 59 were used as the susceptible and moderately drought-resistant controls, respectively (Carvalho et al., 2017) (Table 1).

Table 1. Genealogy and identification of coffee genotypes used for drought resistance testing.

Genotypes	Genealogy [1]
IAPAR H0113-40-26-1	IPR 104 x [Tupi x (IAPAR 81185 x Tupi)]
IAPAR H0113-40-26-9	IPR 104 x [Tupi x (IAPAR 81185 x Tupi)]
IAPAR H0113-40-26-10	IPR 104 x [Tupi x (IAPAR 81185 x Tupi)]
Tupi IAC 1669-33	Villa Sarchi x Timor Hybrid
IAPAR 59	Villa Sarchi x Timor Hybrid

[1]Villa Sarchi = Villa Sarchi CIFC 971/10; Timor Hybrid = Timor Hybrid CIFC 832/2; Tupi = Tupi IAC 1669-33; IPR 104 was originated from the crossing Villa Sarchi CIFC 971/10 x Timor Hybrid CIFC 832/2; IAPAR 81185 = F_2 plant of the F_1RC_2 genotype C1195-5-6-2 c.950 Ep209, originated from the crossing (*C. arabica* x *C. racemosa* C1195) x *C. arabica*] x *C. arabica*.

When the plants reached the stage of expanded cotyledonary leaves (8 cm height, approximately 45 days after sowing), they were transferred to 290 mL black tubes of non-toxic polypropylene (16 cm high, 16 cm

upper diameter, 5.9 cm lower diameter) filled with a commercial substrate based on autoclaved pine bark and vermiculite HF (MecPlant, Telêmaco Borba, Brazil), with the addition of the commercially formulated slow-release fertilizer N-P-K 15-09-12 (osmocote). Seedlings were grown in a glass greenhouse during the acclimatization process with daily irrigation until they reached six pairs of expanded leaves. The temperature of the culture environment was monitored constantly (Figure 1).

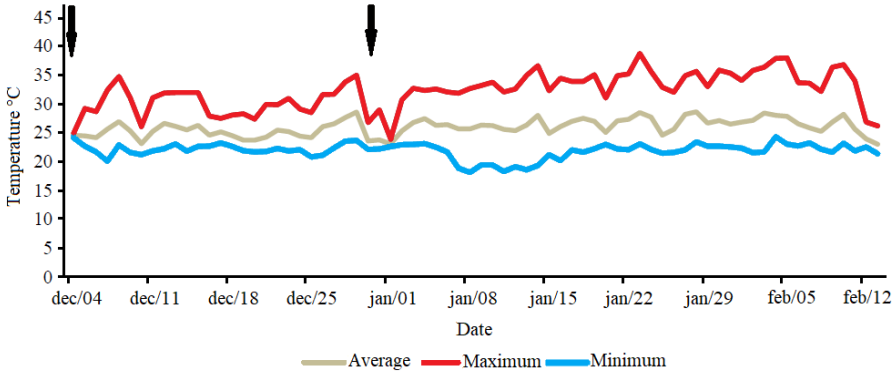

Figure 1. Maximum, average, and minimum temperatures that occurred during the experimental period in Londrina, Paraná State, Brazil. The arrows signal the beginning of water restrictions.

Before water restriction, the tubes with the seedlings were submerged in water (up to the stem base) for 15 minutes and then placed in a support to drain excess water. This process ensured that all seedlings reached field capacity before the water restriction period. The tubes were sealed with a plastic film in the upper and lower openings to prevent evaporation. The first irrigation restriction lasted 11 days, when 50% of the plants showed a wilting intensity of 3 (see topic *wilting intensity*). After this period, the seedlings were recovered and rehydrated. The plastic film was removed and the tubes with seedlings were submerged in water for 15 minutes. Water (100 mL) was added to each tube daily for 15 days. The tubes were submerged and sealed with plastic film (as described previously) and subjected to a second period of irrigation restriction, lasting 10 days (50% of plants showing a wilting intensity of 3).

Experimental design and treatments

The experiment was conducted using a completely randomized design and organized in a 5 × 2 factorial scheme (genotype × environment). The five genotypes are listed in Table 1. The environments were denoted as irrigated control (IC) and under stress (US) owing to water restriction. Five repetitions were used for evaluating physiological variables, and nine repetitions were used for evaluating macro- and micronutrient content and wilting intensity.

Wilting intensity

This assessment was carried out by assigning scores in two visual assessments, which ranged from 1 to 5, where 1 = turgid leaves without wilting symptoms; 2 = slightly drooping leaves; 3 = drooping leaves without discoloration and loss of gloss; 4 = completely drooping leaves with discoloration, loss of brightness, and partially dry; and 5 = completely drooping and dry leaves (Figure 2).

Figure 2. Visual scale for evaluation of wilting intensity in coffee seedlings.

Physiological parameters evaluated

Photosynthesis (*A*) and transpiration (*E*) parameters of the seedlings were evaluated using a portable infrared gas analyzer (IRGA) LC Pro SD (ADC BioScientific, Hoddesdon, United Kingdom). The equipment has a leaf chamber surface area of 6.25 cm^2. An artificial light of 1,000 µmol m^{-2} s^{-1} and a flow rate of 200 µmol mL^{-1} were used in the evaluations. The evaluations were conducted between 9:00 am and 11:00 am. The first measurement was performed 11 days after water restriction, and the second measurement was performed 10 days after water restriction (when 50% of the plants showed a wilting intensity of 3).

From the photosynthesis and transpiration data of the irrigated control, the percentage inhibition of physiological parameters (PP) was calculated using the following equation:

$$inhibition\ PP\ (\%) = \left[\frac{(PP\ irrigated\ control - PP\ under\ stress)}{PP\ irrigated\ control}\right] x\ 100 \quad (1)$$

The relative water content (RWC) was evaluated using leaf disks of 3.14 cm^2. They were weighed immediately after harvest to obtain the fresh mass (FM). The disks were stored in water-filled microtubes for 48 hours. After this period, the disks were weighed to obtain the turgid mass (TM). Then, they were dried for 72 hours at 70°C, and the dry mass (DM) was measured. RWC was calculated using the following equation:

$$RWC\ = \left[\frac{(FM-DM)}{(TM-DM)}\right] x\ 100 \quad (2)$$

Macro- and micronutrient content

The quantification of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), and boron (B) content was performed from the dry mass of the leaves of the coffee seedlings, according to the protocol established by Carmo et al. (2000). The extraction procedures were conducted based on the nutrient: nitric-perchloric (P, K, Ca, Mg, Cu, and Zn), sulfuric (N), and dry (B). The Kjeldahl (N), vanadate-yellow colorimetry (P), flame emission spectrometry (K), atomic absorption spectrometry (Ca, Mg, Cu, and Zn), and colorimetry with azomethine-H (B) methods were used for analytical determination.

Data transformations and statistical analysis

The PP variation data were transformed by arcsen$\sqrt{x}$. Micronutrient content data were transformed the $\left[\frac{1}{(x+1)}\right]$. Data from the variables photosynthesis, transpiration, and macro- and micronutrient content were tested for the normality of errors and homogeneity of variances and submitted to two-way analysis of variance (ANOVA) with $p \leqslant 0.05$, and a subsequent test of comparison of means by Tukey at $p \leqslant 0.1$. Variations in the physiological parameters and wilting intensity scores were also tested for normality of errors and homogeneity of variance. Subsequently, these data were submitted to one-way ANOVA with $p \leqslant 0.05$ and a subsequent test of comparison of means by Tukey at $p \leqslant 0.1$.

Principal component analyses (PCA) and heatmap visualization were also performed with physiological variables, macro- and micronutrient content, and wilting intensity scores. All statistical analyses were performed in RStudio (R Core Team, 2022).

Results

Wilting intensity

The mean scores attributed to the wilting intensity of coffee seedlings under water-restricted conditions are presented in Table 2. The results indicated that the H0113-40-26-10 genotype presented the lowest wilting effect imposed by the first water restriction and received the lowest score of all the genotypes (2.06), whereas the cultivar Tupi IAC 1669-33 (drought-susceptible control) received the highest scores (2.83). The other genotypes (H0113-40-26-1, H0113-40-26-9, and cultivar IAPAR 59) received intermediate scores.

After the second water restriction period, a general increase in the mean wilting intensity score was observed, but the difference between the evaluated genotypes was not significant. However, there was a tendency for the H0113-40-26-10 genotype to again present the lowest wilting intensity score.

Table 2. Wilting intensity in seedlings of five coffee genotypes after imposition of stress by water restriction in two periods.

	Wilting score [1]	
Genotype	1st Water restriction	2nd Water restriction
H0113-40-26-1	2.50 ab	3.53 a
H0113-40-26-9	2.61 ab	3.29 a
H0113-40-26-10	2.06 b	2.84 a
Tupi IAC 1669-33	2.83 a	3.74 a
IAPAR 59	2.17 ab	3.37 a

[1]The scores vary from 1 to 5; the closer to 1 the smaller the impacts caused by water restriction on the plants; the closer to 5, the greater the impacts caused by water restriction on the plants. Lowercase letters indicate the comparison between genotypes by Tukey test $p \leq 0.1$.

Physiological parameters

The imposition of stress by the first water restriction in coffee seedlings led to a reduction in photosynthesis (*A*) and transpiration (*E*), regardless of the genotype (Table 3). No significant interaction was observed between genotype and environment for either *A* or *E*. The inhibition of *A* under the stress condition (US) was between 74.7% and 91.1% in relation to the irrigated control environment (IC). In contrast, the inhibition of *E* in the US was between 85.3% and 95.7% in relation to IC. No significant differences were found between genotypes for the values of *A*, *E*, or inhibition of these variables in either environments.

Table 3. Physiological parameters of photosynthesis (*A*) and transpiration (*E*) after the imposition of seedlings of five coffee genotypes to the first water restriction.

	A (μmol $m^{-2} s^{-1}$)				*E* (mmol $m^{-2} s^{-1}$)			
	Environment				Environment			
Genotype	IC	US	Mean	Inhibition (%)	IC	US	Mean	Inhibition (%)
26-1	5.63 a A	1.42 a A	3.53 a	74.7 a	2.96 a A	0.297 a A	1.630 a	90.0 a
26-9	5.00 a A	1.01 a A	3.00 a	79.8 a	2.77 a A	0.229 a A	1.499 a	91.7 a
26-10	6.59 a A	1.15 a A	3.87 a	82.6 a	2.80 a A	0.412 a A	1.606 a	85.3 a
Tupi	5.95 a A	0.53 a A	3.24 a	91.1 a	3.09 a A	0.147 a A	1.618 a	95.2 a
IAPAR	5.68 a A	1.17 a A	3.42 a	79.4 a	3.21 a A	0.138 a A	1.675 a	95.7 a
Mean	5.77 A	1.06 B			2.97 A	0.245 B		

26-1: Genotype H0113-40-26-1; 26-9: Genotype H0113-40-26-9; 26-10: Genotype H0113-40-26-10; Tupi: Genotype Tupi IAC 1669-33; IAPAR: Genotype IAPAR 59; IC indicated irrigated control environment; US indicated under the stress environment. Capital letters indicate the comparison between cultivation environments at Tukey test $p \leq 0.1$. Lowercase letters indicate the comparison between genotypes at Tukey test $p \leq 0.1$. CO_2 reference average: 383.2 vpm. Leaf chamber temperature average: 31.2°C.

After the rehydration period and the imposition of stress by the second water restriction on coffee seedlings, there was a reduction in *A* and *E* for all evaluated genotypes (Table 4). No interaction was found between the genotypes and environment. The H0113-40-26-10 genotype had the highest mean *A* between the two environments (3.447 μmol $m^{-2} s^{-1}$) when compared to the other genotypes, and the Tupi IAC 1669-33 cultivar had the lowest mean (2.347 μmol $m^{-2} s^{-1}$). The inhibitions of *A* under US were between 68.7 and 90.2% in relation to IC, but did not differ between genotypes. *E* values did not differ between the genotypes in either evaluated environment, but there was a significant difference in their inhibition in the US. The H0113-40-26-10 genotype showed the lowest *E* inhibition under US (72.2%), whereas the Tupi IAC 1669-33 and IAPAR 59 cultivars showed the highest inhibitions (93.3 and 92.4%, respectively). Under these conditions, the H0113-40-26-10 genotype was the most resistant to drought, even when compared to the IAPAR 59 cultivar, which was used as a moderately resistant control.

Table 4. Physiological parameters of photosynthesis (*A*) and transpiration (*E*) after the imposition of seedlings of five coffee genotypes to the second water restriction.

	A (μmol $m^{-2} s^{-1}$)				*E* (mmol $m^{-2} s^{-1}$)			
	Environment				Environment			
Genotype	IC	US	Mean	Inhibition (%)	IC	US	Mean	Inhibition (%)
26-1	4.48 a A	0.871 a A	2.675 ab	80.6 a	2.49 a A	0.329 a A	1.408 a	86.8 ab
26-9	4.42 a A	0.828 a A	2.625 ab	81.3 a	2.55 a A	0.232 a A	1.389 a	90.9 ab
26-10	5.25 a A	1.642 a A	3.447 a	68.7 a	2.50 a A	0.695 a A	1.596 a	72.2 b
Tupi	4.32 a A	0.426 a A	2.374 b	90.2 a	2.38 a A	0.159 a A	1.268 a	93.3 a
IAPAR	4.31 a A	0.804 a A	2.557 ab	81.3 a	2.50 a A	0.191 a A	1.346 a	92.4 a
Mean	4.56 A	0.914 B			2.48 A	0.321 B		

26-1: Genotype H0113-40-26-1; 26-9: Genotype H0113-40-26-9; 26-10: Genotype H0113-40-26-10; Tupi: Genotype Tupi IAC 1669-33; IAPAR: Genotype IAPAR 59; IC indicated irrigated control environment; US indicated under the stress environment. Capital letters indicate the comparison between cultivation environments at Tukey test $p \leq 0.1$. Lowercase letters indicate the comparison between genotypes at Tukey test $p \leq 0.1$. CO_2 reference average: 388.1 vpm. Leaf chamber temperature average: 26.9°C.

The relative water content (RWC) after the first water restriction (Table 5) differed significantly only between environments (leaves from IC plants in the IC had a 7.7% higher RWC than those in the US). After the second water restriction, the difference between the environments increased, being 29% higher for the IC than for the US. In the second evaluation, Tupi IAC 1669-33 leaves showed the lowest RWC among the genotypes.

Table 5. Relative Water Content (RWC) in seedlings of five coffee genotypes after imposition of stress by water restriction in two periods.

	Relative Water Content					
	1st Water restriction			2nd Water restriction		
	Environment			Environment		
Genotype	IC	US	Mean	IC	US	Mean
H0113-40-26-1	89.51	72.90	81.21 a	88.08	61.60	74.84 a
H0113-40-26-9	86.66	75.11	80.89 a	89.75	57.04	73.40 a
H0113-40-26-10	82.61	77.53	80.07 a	88.88	67.17	78.03 a
Tupi IAC 1669-33	85.60	87.94	86.77 a	61.26	49.20	55.23 b
IAPAR 59	84.55	82.52	83.54 a	88.82	60.76	74.79 a
Mean	85.79 A	79.20 B		83.36 A	59.15 B	

IC indicated irrigated control environment; US indicated under the stress environment. Capital letters indicate the comparison between cultivation environments at Tukey test $p \leq 0.1$. Lowercase letters indicate the comparison between genotypes at Tukey test $p \leq 0.1$.

Macro and micronutrient content

The imposition of sequential periods of stress due to water restriction on coffee seedlings led to changes in macronutrient accumulation (Table 6). An interaction was found between genotype and environment for all evaluated macronutrients. The cultivar IAPAR 59 showed a higher N content in an environment US than in IC, whereas the other genotypes did not differ in N content with respect to environmental conditions. The IAPAR 59 cultivar also had the highest N content in the US compared to the other evaluated genotypes, and the Tupi IAC 1669-33 cultivar (susceptible to drought) had the lowest N content of all genotypes. The three genotypes with *C. racemosa* genes showed intermediate levels of N between the tolerant and susceptible cultivars.

Table 6. Content of macronutrients, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) extracted from seedlings of five coffee genotypes cultivated in a greenhouse under the irrigated control environment (IC) and under stress by water restriction (US).

	N (g kg^{-1})		P (g kg^{-1})		K (g kg^{-1})		Ca (g kg^{-1})		Mg (g kg^{-1})	
	Environment		Environment		Environment		Environment		Environment	
Genotype	IC	US	IC	US	IC	US	IC	US	IC	US
26-1	20.2 a A	20.0 ab A	2.7 bc B	3.8 a A	13.6 bc B	17.0 ab A	12.6 bc A	14.0 a A	5.3 b A	6.1 a A
26-9	20.5 a A	20.4 ab A	2.0 c B	3.1 a A	10.4 c B	14.9 ab A	9.2 d B	12.6 a A	4.0 b B	5.5 a A
26-10	21.5 a A	22.1 ab A	2.6 bc A	3.2 a A	13.8 bc B	17.2 ab A	12.0 cd A	13.4 a A	5.1 b A	5.8 a A
Tupi	21.5 a A	18.1b A	3.9 a A	3.3 a A	19.5 a A	14.7 b B	15.6 ab A	13.2 a A	7.4 a A	6.0 a B
IAPAR	17.9 a B	25.4 a A	3.3 ab A	3.9 a A	17.7 ab A	19.1 a A	16.1 a A	12.7 a B	7.5 a A	5.5 a B

26-1: Genotype H0113-40-26-1; 26-9: Genotype H0113-40-26-9; 26-10: Genotype H0113-40-26-10; Tupi: Genotype Tupi IAC 1669-33; IAPAR: Genotype IAPAR 59; IC indicated irrigated control environment; US indicated under the stress environment. Capital letters indicate the comparison between cultivation environments at Tukey test $p \leq 0.1$. Lowercase letters indicate the comparison between genotypes at Tukey test $p \leq 0.1$.

The H0113-40-26-1 and H0113-40-26-9 genotypes showed increased P content under US compared to that in the IC environment. In the IC environment, IAPAR 59 and Tupi IAC 1669-33 cultivars showed the highest P content compared to the three coffee genotypes with *C. racemosa* genes, whereas there was no difference in P content between genotypes in the US environment.

Potassium content increased for the three genotypes of coffee trees with *C. racemosa* genes in the US environment compared to that in the IC environment, whereas the cultivar Tupi IAC 1669-33 decreased. The cultivar IAPAR 59 maintained similar levels of K regardless of the environment. In the IC environment, the three genotypes of coffee plants with the *C. racemosa* genes showed lower levels of K in their leaves than the two cultivars, whereas in the US, the lowest K content of all genotypes was observed for the cultivar Tupi IAC 1669-33.

Calcium content increased in H0113-40-26-9 in the US environment compared to that in IC, whereas it was lower in the IC than US environment in cultivar IAPAR 59. In the IC environment, the Tupi IAC 1669-33 and IAPAR 59 cultivars had higher Ca content than the three genotypes with *C. racemosa* genes (H0113-40-26-1, H0113-40-26-9, and H0113-40-26-10). Under the US environment, the five genotypes did not differ in terms of Ca content. Magnesium content was reduced in Tupi IAC 1669-33 and IAPAR 59 cultivars under the US environment in relation to the IC environment, whereas the Mg content was higher in the US than in the

IC environment for the H0113-40-26-9 genotype. The IAPAR 59 and Tupi IAC 1669-33 cultivars showed higher Mg contents than the three genotypes with *C. racemosa* genes in the IC environment, whereas there was no difference in Mg levels among the five genotypes in the US environment.

Considering the accumulation of micronutrients, an interaction was also found that varied according to the growth environment of the coffee seedlings and between the evaluated genotypes (Table 7). No difference was observed in Cu content between the genotypes in the IC environment. However, the moderately resistant control IAPAR 59 showed lower Cu content in the US than in the IC environment, whereas the levels remained similar between the two environments for the other genotypes. The IAPAR 59 cultivar also showed the lowest Cu content of all genotypes in the US environment, whereas the highest levels were found in the genotypes H0113-40-26-1 and H0113-40-26-10.

Table 7. Content of micronutrients, copper (Cu), zinc (Zn), and boron (B) extracted from seedlings of five genotypes of coffee plants grown in a greenhouse under the irrigated control environment (IC) and under water restriction stress (US).

	Cu (mg kg^{-1})		Zn (mg kg^{-1})		B (mg kg^{-1})	
	Environment		Environment		Environment	
Genotype	IC	US	IC	US	IC	US
H0113-40-26-1	2.65 a A	3.31 a A	10.87 a A	14.83 a A	60.33 bc A	68.80 a A
H0113-40-26-9	2.03 a A	2.48 ab A	8.89 b B	10.53 a A	53.70 c B	68.38 a A
H0113-40-26-10	2.36 a A	3.35 a A	10.46 ab A	11.61 a A	60.34 bc A	70.32 a A
Tupi IAC 1669-33	2.46 a A	2.59 ab A	13.84 a A	10.81 a A	81.73 ab A	67.41 a A
IAPAR 59	3.06 a A	2.00 b B	14.46 a A	11.41 a A	84.86 a A	58.65 a B

IC indicated irrigated control environment; US indicated under the stress environment. Data transformed to 1/(x+1). Capital letters indicate the comparison between cultivation environments at Tukey test $p \leq 0.1$. Lowercase letters indicate the comparison between genotypes at Tukey test $p \leq 0.1$.

The Zn content was lower in the H0113-40-26-9 genotype in the IC environment than in the other genotypes. However, no significant differences were observed between the genotypes in the US environment. The H0113-40-26-9 genotype was the only genotype to differ in Zn content in relation to the environment, with the content under the US environment being higher than that in the IC environment. The three genotypes with *C. racemosa* genes showed the lower B contents in the IC environment than the IAPAR 59 and Tupi IAC 1669-33 cultivars. For the H0113-40-26-9 genotype, B content increased in the US environment relative to the IC environment. The B content in the cultivar IAPAR 59 (drought tolerant) was reduced under the US environment compared to the IC environment.

Principal component analysis

In the principal component analysis (PCA), results revealed the different groupings of the generated genotypes: the first with all genotypes under the US condition, the second with the two cultivars (IAPAR 59 and Tupi IAC 1669-33) under IC, and the third with genotypes with introgression of *C. racemosa* genes under IC (Figure 3A). The color scale indicated that the variables that contributed the most to the results were the rates of *A* and *E*, wilting scores (WS), macronutrients Ca and Mg, and micronutrient Zn. The N content of plants was the variable with the smallest contribution to the PCA results.

Under IC conditions, the Tupi IAC 1669-33 and IAPAR 59 cultivars showed a positive correlation with the levels of Ca and Mg, whereas the H0113-40-26-9 genotype showed a greater negative correlation with the levels of these macronutrients. The three genotypes with *C. racemosa* genes showed positive correlations with photosynthesis and transpiration in IC, indicating higher rates. Under US conditions, the five evaluated genotypes showed a positive correlation with the wilting scores, and a negative correlation with the physiological variables. The nutrient content was negatively correlated with the elements required in greater and lesser amounts (macro- and micro). Nutrients P and K had a stronger positive correlation with plants in the US condition.

The heatmap results (Figure 3B) separated the genotypes into two groups: the first group with all genotypes evaluated under the IC condition and the second with all genotypes under the US condition. The H0113-40-26-10 genotype led the group with the US condition genotypes, followed by the moderately resistant cultivar IAPAR 59. This result corroborates the physiological evaluations and macro- and micronutrient contents, demonstrating a greater balance in this genotype in these evaluations, as well as the lowest WS. When irrigated, the genotypes with *C. racemosa* showed a lower correlation with the macronutrients Ca, Mg, K, and P in relation to Tupi IAC 1669-33 and IAPAR 59; however, these genotypes presented a greater correlation with micronutrients, as also observed in the PCA.

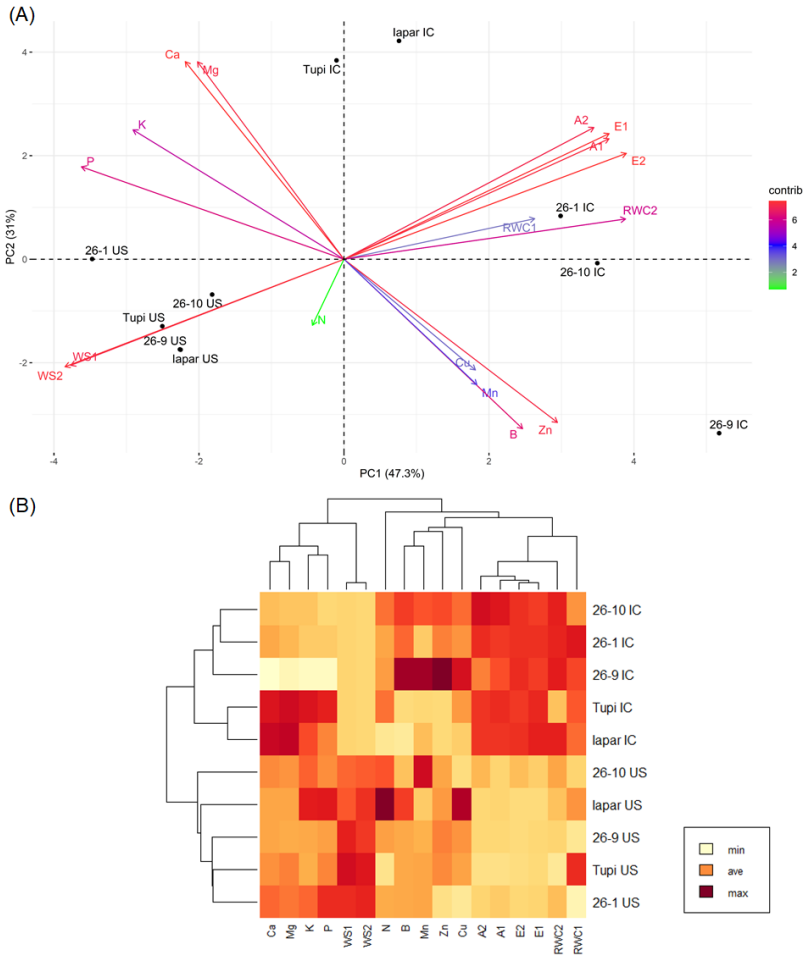

Figure 3. (A) Principal component analysis (PCA) and (B) heatmap visualization for the physiological variables photosynthesis and transpiration after the first water restriction (A1 and E1) and second water restriction (A2 and E2), wilting scores after the first water restriction (WS1) and second water restriction (WS2), relative water content after the first water restriction (RWC1) and second water restriction (RWC2), macronutrient content of nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg), and micronutrient content of zinc (Zn), manganese (Mn), copper (Cu), and boron (B). 26-1 IC/26-1 US: Genotype H0113-40-26-1 with irrigation/under stress; 26-9 IC/26-9 US: Genotype H0113-40-26-9 with irrigation/under stress; 26-10 IC/26-10 US: Genotype H0113-40-26-10 with irrigation/under stress; Tupi IC/Tupi US: Tupi IAC 1669-33 genotype with irrigation/under stress; Iapar IC/Iapar US: Genotype IAPAR 59 with irrigation/under stress. In B) the clusters were generated by applying the Canberra distance as a measure of dissimilarity and the average distance as a clustering method. The resulting cluster had a cophenetic correlation of 0.9242.

Discussion

Drought-resistant coffee plants regulate their survival through physiological, biochemical, and morphological mechanisms (Melo et al., 2014). One of the first physiological responses of coffee plants under water-deficient conditions is stomatal closure, which prevents excessive water loss through transpiration (Melo et al., 2014). However, stomatal closure also affects photosynthesis, which is a vital biological process in plants (Buckley, 2005). In the present study, a reduction in photosynthesis and transpiration rates was observed for all evaluated genotypes in both periods of water restriction (Tables 2 and 3).

The variation in transpiration rate may indicate a plant survival mechanism under conditions of water deficit; however, for prolonged periods, this can be detrimental to the growth and development of the plant, as it leads to carbohydrate depletion (Melo et al., 2014; Batista et al., 2010). Greater reductions in photosynthesis and transpiration indicate that plants undergo stomatal closure to promote greater use of water (Dias et al., 2007). Under conditions of water restriction, in plants of the Siriema cultivar, which is an Arabica coffee with introgression of *C. racemosa*, similar results were observed, with an increase in stomatal resistance and, consequently, a reduction in transpiration values (Melo et al., 2014).

Coffee plants exposed to multiple drought events can induce differential acclimatization to this condition, causing the expression of genes related to drought resistance and the reprogramming of metabolites and metabolic processes, allowing these plants to remain in a “state of alert” to deal with new drought events

(Menezes-Silva et al., 2017). The results of photosynthesis observed for the H0113-40-26-10 genotype under the second period of imposition of water restriction may be a sign of this "state of alert," which includes maintaining higher photosynthetic rates by plants after sequential drought events (Menezes-Silva et al., 2017).

In general, the *C. arabica* coffee genotypes with introgression of the *C. racemosa* genes presented lower accumulation of macronutrients under an irrigated environment; however, when these genotypes are subjected to dry conditions, their accumulation increases. Several metabolic processes in plants are regulated by N, such as the absorption of water and nutrients, protein metabolism, enzymatic activity, photosynthesis, and hormonal responses, due to its presence as a structural element of proteins, enzymes (including Rubisco), nucleic acids, and hormones (Ahmad et al., 2014; Shabbir et al., 2016). Under drought conditions, N absorption is reduced, and there is a decrease in the enzymatic activity of nitrate reductase and NO_3^- content in plants (Ahmad et al., 2014; Marur et al., 2000; Song et al., 2019). However, increased N availability can promote plant resistance to water deficits (Mohammed et al., 2021; Song et al., 2019).

According to our findings, IAPAR 59 (moderately resistant to drought) was the only cultivar that showed an increase in N content under the stressed environment, followed by the three genotypes (H0113-15 40-26-1, H0113-40-26-19, and H0113-40-26-10) with introgression of the genes of *C. racemosa*. It is possible that, in these genotypes, the inhibitory effect of drought on the synthesis and activity of proteins and enzymes (such as nitrate reductase) was lower than that in the Tupi IAC 1669-33 cultivar (susceptible to drought), or it may even indicate that there may be a mechanism that makes it possible to maintain the flow of N from the soil to plants even under low water availability.

The P content increased under the water-restricted environment for the H0113-40-26-1 and H0113-40-26-9 genotypes, indicating that its accumulation is beneficial for resistance to drought. Phosphate fertilization can improve drought resistance through physicochemical adjustments in plants, improving the ability to extract water from the soil and maintaining cell turgidity and stomatal conductivity (Waraich et al., 2011; Tarik et al., 2017). Furthermore, P is an important element in energy storage and transfer processes in biochemical activities such as photosynthesis, in addition to be a structural component of phospholipids, nucleic acids, nucleotides, coenzymes, and phosphoproteins (Waraich et al., 2011).

Potassium is known as a highly mobile, positively charged, efficient, and fast-acting nutrient element for the adjustment of the electrical balance and osmotic potential of cells and is necessary for the regulation of stomatal opening and closing (Nieves-Cordones et al., 2019; Zörb et al., 2014). In addition, increasing the amount of accumulated K is an "energetically cheap" alternative for maintaining cell turgor because it does not depend on the consumption of photoassimilates (Zörb et al., 2014). Together with N, these are important abiotic stress attenuators and are responsible for increasing plant productivity (Sedri et al., 2022). The three genotypes with *C. racemosa* genes showed an increase in K content in the environment under stress compared to the control environment, whereas the susceptible cultivar (Tupi IAC 1669-33) presented a reduced K content. These results corroborate the literature on the importance of this nutrient in drought resistance.

Calcium absorption has a synergistic effect with K absorption (Ramirez-Builes & Küsters, 2021). The Ca present in plant metabolism under drought conditions can help maintain the integrity of the cell structure, mediate enzyme activation, repair damage caused to cells, pump other nutrients into cells, control plant metabolism, and promote resistance to drought (Waraich et al., 2011). Magnesium is also involved in physiological and biochemical processes, contributing to plant growth and development; however, its presence in plants under drought conditions seems to be related to better root development and improved water and nutrient absorption (Waraich et al., 2011). Despite the importance of these macronutrients in increasing drought resistance, only the H0113-40-26-9 genotype had a greater accumulation of both nutrients (Ca and Mg) under stressed conditions, unlike the drought-tolerant IAPAR 59 cultivar.

Regarding the dynamics of macro- and micronutrient accumulation by coffee seedlings, it can be observed that, in general, genotypes of *C. arabica* with genes from *C. racemosa* present less accumulation under the irrigated environment and that this is increased under dry conditions. The role of micronutrients in aiding drought resistance is not well defined; however, together with macronutrients, micronutrients play a role in activating biochemical, physiological, and metabolic processes in plants (Waraich et al., 2011).

Zinc is strongly connected with maintaining the photosynthetic rate; Cu is related to lignin synthesis (which confers resistance to cells and prevents wilting), prevents the yellowing and death of leaves and branches, prevents stunted growth, and improves carbon and N metabolism; and B is related to reducing the effects of drought in plants, such as stunting (Waraich et al., 2011). For these nutrients, only the H0113-40-26-9 genotype showed an increase, whereas the IAPAR 59 cultivar presented a reduced Zn content.

In general, our results indicate that the main effect of drought is a reduction in photosynthesis and transpiration, as observed by the negative correlation of these variables with genotypes under water restriction. In addition, greater accumulation of mineral nutrients was observed, which was related to the control of plant metabolism for drought resistance. As a novelty, we demonstrated that the wilting intensity of the plants proved to be extremely positively correlated with stress due to water restriction, revealing its potential for use as a parameter for classifying resistance to drought in coffee seedlings.

With respect to evaluating genotypes, the physiological data showed that *C. arabica* coffee plants with introgression of *C. racemosa* genes performed well in coping with drought conditions, with the H0113-40-26-10 genotype representing a positive highlight of resistance to the drought simulated in the present study. This genotype was able to maintain a higher photosynthetic rate than the other genotypes under both cultivation conditions and obtained even lower visual scores of wilting intensity than the IAPAR 59 cultivar, which is already widespread in the market and is used as a moderately drought-resistant control. Genotypes H0113-40-26-1 and H0113-40-26-9 demonstrated similar behaviors to the cultivar IAPAR 59, showing good resistance to drought. However, the susceptibility of the Tupi IAC 1669-33 cultivar to drought was confirmed, with a more intense wilting intensity and the sharpest decrease in the rates of photosynthesis and transpiration among the genotypes.

Sources of drought resistance have already been identified in pure wild Arabica coffee trees from Ethiopia (Carvalho et al., 2017; Queiroz-Voltan et al., 2014) and in Arabica coffee trees with introgression of *C. racemosa* (Carvalho et al., 2017; Medina Filho et al., 1977), of *C. liberica*, such as BA-10 (Mazzafera & Carvalho, 1987; Queiroz-Voltan et al., 2014) and the cultivar IPR 100 (Carvalho et al., 2017), and of *C. canephora*, such as IAPAR 59 and IPR 103 (Carvalho et al., 2017). The resistance level of the H0113-40-26-10 genotype was higher than that of the moderately resistant control IAPAR 59, corroborating the results of Carvalho et al. (2017).

The progeny F4 H0113-40-26-10 and the other progenies with introgression of *C. racemosa* demonstrated great potential to become a new cultivar with a high level of resistance to drought, since progenies with similar parentages were identified in other studies and had high productivity (Andreazi et al., 2017), frost resistance (Mariucci Junior et al., 2022), moderate resistance to leaf miner (Andreazi et al., 2015), moderate resistance to red mite (Carducci et al., 2019), high resistance to coffee leaf rust (Andreazi et al., 2015), and high resistance to bacterial halo blight (Andreazi et al., 2018; Andreazi et al., 2015). These studies reported that resistance to coffee leaf rust originated from the Tupi IAC 1669-33 and IPR 104 cultivars (Sera et al., 2022), whereas resistance to other factors originated from the C1195-5-6-2 genotype, which was also used in the development of the progeny H0113-40-26-10. Seeds from this progeny can be used to develop a new cultivar with a high level of drought resistance and multiple resistances to several biotic and abiotic factors.

Conclusion

This study showed that physiological and nutritional analyses, as well as visual evaluation of wilting intensity, are suitable parameters for evaluating the drought resistance of coffee seedlings, which is very important in genetic improvement programs. Notably, physiological and nutritional parameters were positively related to the visual scores of wilting intensity, indicating that the latter could effectively determine drought resistance in coffee seedlings. Moreover, we observed that the *C. arabica* genotypes carrying *C. racemosa* genes had good resistance to drought, especially the H0113-40-26-10 genotype, which showed a higher level of resistance than the IAPAR 59 cultivar. Further studies must be conducted in the field to confirm the performance of these genotypes under different abiotic stress conditions (including evaluation of grain production).

Acknowledgements

This research was funded by *Consórcio Pesquisa Café* (grant number 02.13.02.067.00.02). E. Andreazi, B. T. Souzsa, and V. Mariucci Junior were supported by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) [Finance Code 001], and H. C. Oliveira was supported by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* – CNPq [311034/2020-9].

References

Ahanger, M. A., Tyagi, S. R., Wani, M. R., & Ahmad, P. (2013). Drought tolerance: Role of organic osmolytes, growth regulators, and mineral nutrients (1st ed.). In P. Ahmad, & M. Wani (Eds.), *Physiological mechanisms and adaptation strategies in plants under changing environment* (pp. 25-55). Springer.

Ahmad, R., Waraich, E. A., Ashraf, M. Y., Ahmad, S., & Aziz, T. (2014). Does nitrogen fertilization enhance drought tolerance in sunflower? A review. *Journal of Plant Nutrition, 37*(6), 942-963. https://doi.org/10.1080/01904167.2013.868480

Andreazi, E., Carducci, F. C., Sera, T., Pereira, C. T. M., Mariucci Junior, V., Carvalho, F. G., Shigueoka, L. H., Santos, W. G., Fonseca, I. C. B., & Sera, G. H. (2017). Early ripening cycle and yield in coffee genotypes derivatives from C1195-5-6-2. *Coffee Science, 12*(4), 575-582.

Andreazi, E., Sera, G. H., Faria, R. T., Sera, T., Shigueoka, L. H., Carvalho, F. G., Carducci, F. C., & Chamlet, D. (2015). Performance of F1 hybrids of arabica coffee with simultaneous resistance to leaf rust, bacterial blight and leaf miner. *Coffee Science*, *10*(3), 375-382.

Andreazi, E., Sera, G. H., Sera, T., Fonseca, I. C. B., Carducci, F. C., Shigueoka, L. H., Santos, W. G., & Pereira, C. T. M. (2018). Resistance to bacterial halo blight in Arabica coffee lines derivate from the genotype C1195-5-6-2 under natural infection conditions. *Crop Breeding and Applied Biotechnology, 18*(1), 110-115. https://doi.org/10.1590/1984-70332018v18n1n17

Batista, L. A., Guimarães, R. J., Pereira, F. J., Carvalho, G. R., & Castro, E. M. (2010). Leaf anatomy and water potential in the coffee cultivars tolerance to water stress. *Revista Ciência Agronômica*, *41*(3), 475-481. https://doi.org/10.1590/S1806-66902010000300022

Buckley, T. N. (2005). The control of stomata by water balance. *New Phytologist, 168*(2), 275-292. https://doi.org/10.1111/j.1469-8137.2005.01543.x

Carducci, F. C., Fonseca, I. C. B., Santos, W. G., Pereira, C. T. M., Mariucci Junior, V., Sera, T., & Sera, G. H. (2019). Resistance to red mite in *Coffea arabica* genotype introgressed with *Coffea racemosa* genes. *Australian Journal of Crop Science, 13*(5), 683-686. https://doi.org/10.21475/ajcs.19.13.05.p1254

Carmo, C. A. F. S., Araújo, W. S., Bernardi, A. C. C., & Saldanha, M. F. C. (2000). *Métodos de análise de tecidos vegetais utilizados na Embrapa Solos*. Embrapa Solos.

Carvalho, F. G., Sera, G. H., Andreazi, E., Sera, T., Fonseca, I. C. B., Carducci, F. C., Shigueoka, L. H., Holderbaum, M. M., & Costa, K. C. (2017). Drought tolerance in seedlings of coffee genotypes carrying genes of different species. *Coffee Science, 12*(2), 156-163.

Companhia Nacional de Abastecimento [CONAB]. (2022). *Acompanhamento da safra brasileira de café* (v. 9, n. 2, safra 2022, segundo levantamento). CONAB.

DaMatta, F. M. (2004). Exploring drought tolerance in coffee: a physiological approach with some insights for plant breeding. *Brazilian Journal of Plant Physiology, 16*(1), 1-6. https://doi.org/10.1590/S1677-04202004000100001

DaMatta, F. M., Avila, R. T., Cardoso, A. A., Martins, S. C. V., & Ramalho, J. C. (2018). Physiological and agronomic performance of the coffee crop in the context of climate change and global warming: A review. *Journal of Agricultural and Food Chemistry, 66*(21), 5264-5274. https://doi.org/10.1021/acs.jafc.7b04537

Dias, P. C., Araujo, W. L., Moraes, G. A. B. K., Barros, R. S., & DaMatta, F. M. (2007). Morphological and physiological responses of two coffee progenies to soil water availability. *Journal of Plant Physiology, 164*(12), 1639-1647. https://doi.org/10.1016/j.jplph.2006.12.004

Fernandes-Brum, C. N., Melo, E. F., Barquero, L. O. B., Alves, J. D., & Chalfun-Junior, A. (2013). Modifications in the metabolism of carbohydrates in (*Coffea arabica* L. cv. Siriema) seedlings under drought conditions. *Coffee Science*, *8*(2), 140-147.

Fialho, G. S., Silva, D. P., Reis, E. F., Fonseca, A. F. A., & Ferrão, M. A. G. (2010). Behavior of *Coffea arabica* L. plants exposed to water deficits in different phases of their initial development. *Idesia (Arica), 28*(3), 35-39. https://doi.org/10.4067/S0718-34292010000300005

Hussain, M., Farooq, S., Hasan, W., Ul-Allah, T. M., Tanveer, M., Farooq, M., & Nawaz, A. (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agricultural Water Management, 201*, 152-166. https://doi.org/10.1016/j.agwat.2018.01.028

International Coffee Organization [ICO]. (2022). *Coffee market report June 2022*. ICO. https://www.ico.org/documents/cy2021-22/cmr-0622-e.pdf

Kath, J., Craparo, A., Fong, Y., Byrareddy, V., Davis, A. P., King, R., Nguyen-Huy, T., van Asten, P. J. A., Marcussen, T., Mushtaq, S., Stone, R., & Power, S. (2022). Vapour pressure deficit determines critical

thresholds for global coffee production under climate change. *Nature Food, 3*, 871-880. https://doi.org/10.1038/s43016-022-00614-8

Machado, A. H. R., Puia, J. D., Menezes, K. C., & Machado, W. (2020). Coffee culture (*Coffea arabica*) in the agroforestry system. *Brazilian Journal of Animal and Environmental Research, 3*(3), 1357-1369. https://doi.org/10.34188/bjaerv3n3-053

Mariucci Junior, V., Shigueoka, L. H., Pereira, C. T. M., Carducci, F. C., Sera, T., & Sera, G. H. (2022). Resistance to frost in Arabica coffee lines introgressed with *Coffea racemosa* Lour. Genes. *Australian Journal of Crop Science, 16*(3), 338-342. https://doi.org/10.21475/ajcs.22.16.03.p2925

Marur, C. J., Mazzafera, P., & Magalhães, A. C. (2000). Nitrate reductase activity in cotton plants under water deficit and after turgescence recovery. *Scientia Agricola, 57*(2), 277-281. https://doi.org/10.1590/S0103-90162000000200013

Maseda, P. H., & Fernández, R. J. (2006). Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *Journal of Experimental Botany*, *57*(15), 3963-3977. https://doi.org/10.1093/jxb/erl127

Mazzafera, P., & Carvalho, A. (1987). Yield and drought tolerance evaluation of coffee plants (*Coffea arabica* L.). *Bragantia*, *46*(2), 403-415. https://doi.org/10.1590/S0006-87051987000200020

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G., & Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist, 178*(4), 719-739. https://doi.org/10.1111/j.1469-8137.2008.02436.x

Medina Filho, H. P., Carvalho, A., & Medina, D. M. (1977). Potential use of *Coffea racemosa* germplasm for breeding of Arabica coffee. *Bragantia*, *36*(1), 43-46. https://doi.org/10.1590/S0006-87051977000100040

Melo, E. F., Fernandes-Brum, C. N., Pereira, F. J., Castro, E. M., & Chalfun-Júnior, A. (2014). Anatomic and physiological modifications in seedlings of *Coffea arabica* cultivar Siriema under drought conditions. *Ciência e Agrotecnologia, 38*(1), 25-33. https://doi.org/10.1590/S1413-70542014000100003

Menezes-Silva, P. E., Sanglard, L. M. V. P., Ávila, R. T., Morais, L. E., Martins, S. C. V., Nobres, P., Patreze, C. M., Ferreira, M. A., Araújo, W. L., Fernie, A. L., & DaMatta, F. M. (2017). Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. *Journal of Experimental Botany, 68*(15), 4309-4322. https://doi.org/10.1093/jxb/erx211

Moat, J., Gole, T. W., & Davis, A. P. (2019). Least concern to endangered: applying climate change projections profoundly influences the extinction risk assessment for wild Arabica coffee. *Global Change Biology*, *25*(2), 390-403. https://doi.org/10.1111/gcb.14341

Mohammed, M., Shimbir, T., & Meskelu, E. (2021). Evaluation of coffee genotypes for drought tolerance in South Ethiopia. *Irrigation & Drainage Systems Engineering*, *10*(5), 1-5.

Nieves-Cordones, M., Ródenas, R., Lara, A., Martínez, V., & Rubio, F. (2019). The combination of K^+ deficiency with other environmental stresses: What is the outcome. *Physiologia Plantarum*, *165*(2), 264-276. https://doi.org/10.1111/ppl.12827

Pinheiro, H. A. (2005). Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. *Annals of Botany*, *96*(1), 101-108. https://doi.org/10.1093/aob/mci154

Queiroz-Voltan, R. B., Nardin, C. F., Fazuoli, L. C., & Braghini, M. T. (2014). Leaf anatomy characterization of *Coffea arabica* plants at different seasonal periods. *Biotemas*, *27*(4), 1-10. https://doi.org/10.5007/2175-7925.2014v27n4p1

R Core Team (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Ramirez-Builes, V. H., & Küsters, J. (2021). Calcium and potassium nutrition increases the water use efficiency in coffee: A promising strategy to adapt to climate change. *Hydrology, 8*(2), 1-11. https://doi.org/10.3390/hydrology8020075

Sedri, M. H., Roohi, E., Niazian, M., & Niedbala, G. (2022). Interactive effects of nitrogen and potassium fertilizers on quantitative-qualitative traits and drought tolerance indices of rainfed wheat cultivar. *Agronomy, 12*(1), 1-17. https://doi.org/10.3390/agronomy12010030

Sera, G. H., Carvalho, C. H. S., Abrahão, J. C. R., Pozza, E. A., Matiello, J. B., Almeida, S. R., Bartelega, L., & Botelho, D. M. S. (2022). Coffee leaf rust in Brazil: historical events, current situation and control measures. *Agronomy, 12*(2), 496. https://doi.org/10.3390/agronomy12020496

Shabbir, R. N., Waraich, E. A., Ali, H., Nawaz, F., Ashraf, M. Y., Ahmad, R., Awan, M. I., Ahmad, S., Irfan, M., Hussain, S., & Ahmad, Z. (2016). Supplemental exogenous NPK application alters biochemical processes to improve yield and drought tolerance in wheat (*Triticum aestivum* L.). *Environmental Science and Pollution Research*, *23*(3), 2651-2662. https://doi.org/10.1007/s11356-015-5452-0

Silva, E. C., Nogueira, R. J. M. C., Silva, M. A., & Albuquerque, M. B. (2011). Drought stress and plant nutrition. *Plant Stress*, *5*(Special Issue 1), 32-41.

Song, Y., Li, J., Liu, M., Meng, Z., Liu, K., & Sui, N. (2019). Nitrogen increases drought tolerance in maize seedlings. *Functional Plant Biology*, *46*(4), 350-359. https://doi.org/10.1071/FP18186

Tariq, A., Pan, K., Olatunji, O.A., Graciano, C., Li, Z., Sun, F., Sun, X., Song, D., Chen, W., Aiping, Z., Wu, X., Zhang, L., Mingrui, D., Xiong, Q., & Liu, C. (2017). Phosphorous application improves drought tolerance of *Phoebe zhennan*. *Frontiers in Plant Science*, *8*(1561), 1-12. https://doi.org/10.3389/fpls.2017.01561

Tounekti, T., Mahdhi, M., Al-Turki, T. A., & Khemira, H. (2018). Water relations and photo-protectionmechanisms during drought stress in four coffee (*Coffea arabica*) cultivars from southwestern Saudi Arabia. *South African Journal of Botany*, *117*, 17-25. https://doi.org/10.1016/j.sajb.2018.04.022

Volsi, B., Telles, T.S., Caldarelli, C.E., Camara, M.R.G. (2019). The dynamics of coffee production in Brazil. *PLoS ONE*, *14*(7), 1-15. https://doi.org/10.1371/journal.pone.0219742

Waraich, E. A., Ahmad, R., Saifullah, Ashraf, M. Y., & Ehsanullah. (2011). Role of mineral nutrition in alleviation of drought stress in plants. *Australian Journal of Crop Science, 5*(6), 764-777.

Zörb, C., Senbayran, M., & Peiter, E. (2014). Potassium in agriculture - Status and perspectives. *Journal of Plant Physiology*, *171*(9), 656-669. https://doi.org/10.1016/j.jplph.2013.08.008