OTÁVIO VITOR SOUZA ANDRADE

MECANISMOS FISIOLÓGICOS INDUZIDOS PELO IODATO DE POTÁSSIO VIA SOLO NA MITIGAÇÃO DE ESTRESSE POR DÉFICIT HÍDRICO EM CAFEEIROS (*COFFEA ARABICA L.*)

LAVRAS – MG
2023

OTÁVIO VITOR SOUZA ANDRADE

MECANISMOS FISIOLÓGICOS INDUZIDOS PELO IODATO DE POTÁSSIO VIA SOLO NA MITIGAÇÃO DE ESTRESSE POR DÉFICIT HÍDRICO EM CAFEEIROS (*COFFEA ARABICA L.*)

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Agronomia / Fisiologia Vegetal, área de concentração em Fisiologia Vegetal para a obtenção do título de Mestre.

Paulo Eduardo Ribeiro Marchiori
Advisor

LAVRAS – MG
2023

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Andrade, Otavio Vitor Souza.
Mecanismos fisiológicos induzidos pelo iodato de potássio via solo na mitigação de estresse por déficit hídrico em cafeeiros *(Coffea arabica L.)* / Otavio Vitor Souza Andrade. - 2023.
41 p. : il.

Orientador(a): Paulo Eduardo Ribeiro Marchiori.

Dissertação (mestrado acadêmico) - Universidade Federal de Lavras, 2023.
Bibliografia.

1. Estresse abiótico. 2. Sistema antioxidante. 3. Iodo. I. Marchiori, Paulo Eduardo Ribeiro. II. Título.

OTÁVIO VITOR SOUZA ANDRADE

MECANISMOS FISIOLÓGICOS INDUZIDOS PELO IODATO DE POTÁSSIO VIA SOLO NA MITIGAÇÃO DE ESTRESSE POR DÉFICIT HÍDRICO EM CAFEEIROS (*COFFEA ARABICA L.*)

PHYSIOLOGICAL MECHANISMS INDUCED BY POTASSIUM IODATE VIA SOIL IN MITIGATING WATER DEFICIT STRESS IN COFFEE PLANTS (*COFFEA ARABICA L.*)

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Agronomia / Fisiologia Vegetal, área de concentração em Fisiologia Vegetal Plantas, para a obtenção do título de Mestre.

Aprovado em 15 Setembto de 2023.
Dr. Willian Macedo UFV
Dr. Fernando Zambrosi IAC

Paulo Eduardo Ribeiro Marchiori
Advisor

LAVRAS – MG
2023

ACKNOWLEDGMENTS

Firstly, to my family, my mother Madalena, my father Aguinaldo and my sister Cecília, for always supporting me at all times and for always fighting for me to achieve my dreams.

To my advisor Paulo Marchiori, for all his teachings, support and above all, for giving me the opportunity during periods of uncertainty.

To the members of the panel, for accepting my invitation and collaborating with my work.

To the friends I met during my master's degree and who have been with me throughout this journey, especially my great friend Jucelino Lima.

To the brothers of República Fim de Mundo, for all the friendship built up over the last eight years that I've been in Lavras.

To all my friends in Três Pontas, who, even from a distance, have always supported me.

The Federal University of Lavras, which has become my second home and has given me the opportunity to fulfill yet another dream.

This Study was finaced in part by the Coordernação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001

And finally, to all the people who have helped me directly or indirectly over the last few years.

RESUMO

O déficit hídrico impacta diretamente no crescimento e desenvolvimento das plantas, promovendo distúrbios fisiológicos que desencadeiam estresse oxidativo. Como alternativa a esse problema, a aplicação exógena de moléculas específicas pode minimizar esses danos e reduzir as perdas de produtividade. A aplicação de iodo (I) tem mostrado resultados consideráveis na melhoria da eficiência fotossintética e no estímulo ao sistema antioxidante em plantas estressadas. No entanto, há poucos resultados para mitigar o déficit hídrico em plantas de café. Assim, plantas de café foram cultivadas em vasos de 10 litros dispostos aleatoriamente. Quatro doses de iodato de potássio (KIO_3) foram testadas: 0,0, 2,5, 5,0 e 10,0 mg dm^{-3} de solo; em seguida, as plantas foram submetidas ao déficit hídrico e comparadas aos tratamentos sem KIO_3 e com déficit hídrico (Controle). Em nosso estudo, mostramos a influência do déficit hídrico na redução da biomassa e no crescimento relativo da planta de café. No entanto, a aplicação de 2,5 mg dm^{-3} de KIO_3 atenuou os sintomas do déficit hídrico, aumentando a eficiência fotossintética, o conteúdo relativo de água, o índice de tolerância ao déficit hídrico, o conteúdo de pigmentos fotossintéticos e os osmólitos compatíveis. Além disso, observamos o estímulo do sistema enzimático antioxidante, causando uma redução na degradação das membranas celulares. Doses de 5,0 e 10,0 mg dm^{-3} de KIO_3 induziram uma maior ativação do sistema antioxidante sem influenciar o desenvolvimento da planta, em comparação com plantas expostas ao déficit hídrico, implicando um possível efeito tóxico devido ao excesso de KIO_3. Portanto, observou-se que a aplicação de 2,5 mg dm^{-3} de KIO_3 via solo pode modular processos metabólicos e bioquímicos, permitindo uma melhoria no crescimento e desenvolvimento de plantas de café sujeitas ao déficit hídrico, sugerindo que isso poderia servir como uma estratégia viável para o manejo de plantas em condições de seca.

Palavras-chave: Estresse abiótico. Sistema antioxidante. *Coffea arabica* L. Iodo.

ABSTRACT

Water deficit directly impacts plant growth and development, promoting physiological disturbances that trigger oxidative stress. As an alternative to this problem, the exogenous application of a sort of molecule that can minimize these damages and reduce productivity losses. Applying iodine (I) has shown considerable results in improving photosynthetic efficiency and stimulating the antioxidant system in stressed plants. Nevertheless, there are few results for mitigating the water deficit in coffee plants. Thus, coffee plants were grown in 10-liter pots arranged wholly randomized. Four doses of potassium iodate (KIO_3) were tested: 0.0, 2.5, 5.0, and 10.0 mg dm^{-3} of soil, then the plants were subjected to water deficit and compared to treatments with no KIO_3 and water deficit (Control). In our stydy we show the influence of water deficit on biomass decline and relative growth of the coffee plant. However, the application of 2.5 mg dm^{-3} of KIO_3 attenuated the water deficit symptoms, increasing photosynthetic efficiency, relative water content, water deficit tolerance index, content of photosynthetic pigments, and compatible osmolytes. In addition, we observed the stimulation of the antioxidant enzymatic system, causing a decline in the degradation of cell membranes. Doses of 5.0 and 10.0 mg dm^{-3} of KIO_3 induced greater activation of the antioxidant system without influencing plant development, compared to plants exposed to water deficit, implying a possible toxicity effect due to excess KIO_3. Therefore, it was observed that the application of 2.5 mg dm^{-3} KIO_3 via the soil can modulate metabolic and biochemical processes, allowing an improvement in the growth and development of coffee plants subjected to water deficit, suggesting that it could serve as a viable strategy for managing plants under drought conditions.

Keywords: Abiotic stress. Antioxidant system. *Coffea arabica* L. Iodine. PhotosynthesisFotossíntese.

LIST OF FIGURES

SUMMARY

FIRST PART

GENERAL INTRODUCTION

Over the past few years, climate change has impacted food production in a scenario where the global population has an average of 800 million people suffering from hunger (FAO, 2023; UN, 2022). Among the prominent adversities that agriculture faces, the water deficit has become one of the main challenges that intensify the decrease in agricultural productivity (YANG et al., 2021). This scenario makes finding ways to deal with and guarantee global food security crucial, even with the increasingly common water scarcity scenario.

Water deficit is one of the leading causes of declining productivity, as it directly affects plant metabolism (YANG et al., 2021). Among the harmful processes, we have the reduction in photosynthesis, water status, nutrient transport, increased production of reactive oxygen species (ROS), and osmotic stress (ALI et al., 2023; NASIR; TOTH, 2022; WAHAB et al., 2022). Recently, strategies with the application of exogenous substances such as Silicon (Si), Selenium (Se), and, more recently, Iodine (I) have become a strategic alternative for mitigating the effects of the most varied abiotic stresses (AHSAN et al., 2023; CHERAGHI et al., 2023; ZAHEDI, 2020; LIMA et al., 2023).

Iodine is currently classified as a non-essential but beneficial element in plants and is related to antioxidant capacity and environmental adaptations (NASCIMENTO et al., 2022). This response occurs because the element activates several defense mechanisms, such as the production of enzymes of the antioxidant enzymatic system, the production of amino acids and sugars, in addition to adjusting the level of pigments, which together can provide an increase in photosynthetic activity, maintaining the development of plants even in unfavorable conditions (MACÍAS et al., 2016; NASCIMENTO et al., 2022; RIYAZUDDIN et al., 2023). The exogenous application of iodine has shown promising results for abiotic stresses, including salt stress, with studies on tomato (*Solanum licopersicum* L.) and strawberry (*Fragaria ananassa* L.) and more recently for water stress on soybean (*Glycine max* L.) (KIFERLE et al., 2022; MACÍAS et al., 2021; LIMA et al., 2023).

Given the above, it is possible to state that the exogenous application of iodine can be used as a strategy to mitigate the most varied abiotic stresses, and the present work aims to promote the application of potassium iodate (KIO_3) via soil to minimize the effects of water deficit in coffee plants (*Coffea arabica* L.).

REFERENCES

AHSAN, M. et al. Evaluation of Silicon Supplementation for Drought Stress under Water-Deficit Conditions: An Application of Sustainable Agriculture. **Agronomy**, v. 13, n. 2, p. 599, 2023. doi: https://doi.org/10.3390/agronomy13020599

ALI, J. et al. Biochemical response of okra (*Abelmoschus esculentus* L.) to Selenium (Se) under drought stress. **Sustainability**, v. 15, n. 7, p. 5694, 2023. doi: https://doi.org/10.3390/su15075694

CHERAGHI, M. et al. Silicon (Si): a regulator nutrient for optimum growth of wheat under salinity and drought stresses-a review. **Journal of Plant Growth Regulation**, v. 42, n.9 p. 5354–5378, 2023. doi: https://doi.org/10.1007/s00344-023-10959-4

FAO. Food and Agriculture Organization of the United Nations. **The State of Food Security and Nutrition in the World**. FAO; IFAD; UNICEF; WFP; WHO, 2023. Disponível em: <https://www.fao.org/publications/home/fao-flagship-publications/the-state-of-food-security-and-nutrition-in-the-world>. Acesso em 15 de Ago. 2023.

KIFERLE, C. et al. Improvement in fruit yield and tolerance to salinity of tomato plants fertigated with micronutrient amounts of iodine. **Scientific Reports**, v. 12, n. 1, p. 14655, 2022. doi: https://doi.org/10.1038/s41598-022-18301-w

LIMA, J. S. et al. Soybean Plants Exposed to Low Concentrations of Potassium Iodide Have Better Tolerance to Water Deficit through the Antioxidant Enzymatic System and Photosynthesis Modulation. **Plants**, v. 12, n. 13, p. 2555, 2023. doi: https://doi.org/10.3390/plants12132555

MACÍAS, J. M. et al. Use of iodine to biofortify and promote growth and stress tolerance in crops. **Frontiers in Plant Science**, v. 7, p. 1146, 2016. doi: https://doi.org/10.3389/fpls.2016.01146

NASCIMENTO, V. L. et al. On the role of iodine in plants: A commentary on benefits of this element. **Frontiers in Plant Science**, v. 13, p. 836835, 2022. doi: https://doi.org/10.3389/fpls.2022.836835

NASIR, M. W.; TOTH, Z. Effect of drought stress on potato production: A review. **Agronomy**, v. 12, n. 3, p. 635, 2022. doi: https://doi.org/10.3390/agronomy12030635

RIYAZUDDIN, R. et al. Iodine: an emerging biostimulant of growth and stress responses in plants. **Plant and Soil**, v. 486, n. 1-2, p. 119-133, 2023. doi: https://doi.org/10.1007/s11104-022-05750-5

UN. United Nations. **World population prospects**. Department of Economic and Social Afairs. Population division. UN DESA/POP/2021/N:3, 2022. Disponível em: <https://news.un.org>. Acesso em 15 de Ago. 2023.

WAHAB, A. et al. Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. **Plants**, v. 11, n. 13, p. 1620, 2022. doi: https://doi.org/10.3390/plants11131620

YANG, X. et al. Response Mechanism of Plants to Drought Stress. **Horticulturae**, v.7, n.3, p. 50, 2021. doi: https://doi.org/10.3390/horticulturae7030050

ZAHEDI, S. M. et al. Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. **Scientific reports**, v. 10, n. 1, p. 17672, 2020. doi: https://doi.org/10.1038/s41598-020-74273-9

SECOND PART – ARTICLE NBR 6022 (ABNT, 2018)

1 INTRODUCTION

Climate change has modified the direction of agriculture and directly affected the yield avarage in recent years (ABBASS et al., 2022). The current scenario indicates that 9.2% of the world's population is affected by hunger, representing about 800 million people in a circumstance where the population expectation for the year 2050 is 9.7 billion inhabitants (FAO, 2023; UN, 2022). These numbers show a need to increase food production under adverse weather conditions, including floods, high temperatures, and drought regimes, which are increasingly common. These phenomena directly affect plant metabolism and development, threating food security worldwide (WAHAB et al., 2022; YAHAYA; SHIMELIS, 2021).

Water deficit is among the principal abiotic stresses that cause yield declines since it impair the water status of plants, reducing growth and photosynthesis. As a consequence, it increases the production of reactive oxygen species (ROS) at cellular level, causing cellular damage (ALI et al., 2023; NASIR; TOTH, 2022; WAHAB et al., 2022). Plants can reduce this damage by adjusting the antioxidant enzymatic system and accumulating compatible osmolytes (ALI et al., 2023; LAXA et al., 2019; LIMA et al., 2023). Osmotic stress caused by water deficiency activates internal defense mechanisms, such as increased activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). Furthermore, the accumulation of organic molecules, such as proline (PRO), total free amino acids (TFAAs), and soluble carbohydrates, can improve the cellular water absorption, thus improving the water status (SILVA et al., 2021; BANDURSKA et al., 2022; GOWTHAM et al., 2022; NADARAJAH, 2020). Additionally, the adjustment in the levels of photosynthetic pigments (chlorophylls A and B) is an essential response in tolerance to water deficit, as it can enhance photosynthetic processes, providing maintenance of plant development even under adverse conditions (KHODABIN et al., 2020).

Among the phases of development of the coffee plant (*Coffea arabica* L.), the stage of formation and establishment of the coffee plantation, based on transplanting 6 month old plants from nursery, is greatly affected by the water deficit and, as it is a perennial crop, the initial development is a preponderant factor in obtaining crops with high productivity (MATIELLO et al., 2020). Coffee is the second most consumed beverage in the world, and according to the International Coffee Organization (ICO), consumption projections are accompanied by an upward

trend of around 2% per year in the last decade (ICO, 2021). Given this scenario, the grain production expectation for the year 2023 is 37.9 million bags of *Coffea arabica* L. This result represents an increase of approximately 16% compared with the 2022 harvest (CONAB, 2023). This increase is related to the favorable climatic conditions in the 22/23 season concerning the last two, where climatic adversities such as low rainfall, prolonged droughts, and above-normal temperatures harmed production (CONAB, 2023).

Hence, employing exogenous elements to mitigate the repercussions of water deficiency-induced stress represents a potential alternative. Their use has grown exponentially in crops of economic importance, such as rice (*Oryza sativa* L.), soybean (*Glycine max* L.), corn (*Zea mays* L.), and tomato (*Solanum lycopersicum* L.) (ALTAF et al., 2022; AZEEM et al., 2022; GHOURI et al., 2021; SOUZA JUNIOR, 2023). Beneficial elements such as silicon (Si), selenium (Se), and, more recently, iodine (I) have shown promising results for this purpose (AHSAN et al., 2023; CHERAGHI et al., 2023; ZAHEDI, 2020; LIMA et al., 2023).

Iodine for plants has been considered a beneficial element, with a positive effect, due to its direct relationship with its benefits for human health. It is commonly used in agronomic biofortification processes (NASCIMENTO et al., 2022; NEDIĆ, 2023). Several authors have already worked on the application of this element for biofortification. However, studies that correlate the application of iodine and the mitigation of abiotic stresses are still scarce (MACÍAS et al., 2016; MACÍAS et al., 2021; LIMA et al., 2023; RIYAZUDDIN et al., 2023). Iodine has the potential to mitigate stress because it can trigger some metabolic processes, such as regulation of the antioxidant enzymatic system, sugars, and photosynthetic pigments, in addition to enhancing photosynthesis, providing better plant development (BLASCO et al., 2011; LI et al., 2017; LIMA et al., 2023).

Based on this premise, our work hypothesizes that applying iodine via the soil, such as potassium iodate (KIO_3), can mitigate the stress from coffee plants' water deficit. This situation would be possible by regulating the water status of plants, increasing photosynthetic efficiency, stimulating the production of pigments and enzymes of the antioxidant system, and providing the accumulation of sugars and osmolytes, promoting the growth and development of coffee plants even in unfavorable conditions.

.

2 MATERIAL AND METHODS

2.1 Cultivation system and experimental design

Coffee plants were cultivated in a greenhouse locatedin Lavras city (latitude of 21° 14' 43 S, longitude of 44° 59' 59 E, and 919 m of altitude), Minas Gerais state, Brazil. 6-month-old coffee seedlings (5 pairs of leaves), cultivar Catuaí 99 (*C. arabica*), were used. The seedlings were transplanted into 10-liter pots, filled with 8.5 dm^{-3} of Red Latosol (Oxisol), duly corrected with limestone, to reach the pH of 5.8 and supplemented with the application of simple superphosphate (20 mg of P dm^{-3} soil). During the establishment of the plants, two applications of granulated fertilizers were made as topdressing. The first application, using the formulation 20-00-20, was done 30 days after planting and the second application, using ammonium sulfate (2 grams of nitrogen per application), was carried out 60 days after planting. A commercial Viça Café® mix (Café Brasil Fertilizantes LTDA) was used in two foliar applications at the same intervals as the topdressing applications (12.5 grams L^{-1}) to maintain micronutrient levels.

KIO_3 was applied via soil, also in coverage, on the 75th day after transplanting the seedlings. Later, the plants were kept in optimal irrigation conditions for 90 days allowing the roots to explore the entire dimension of the pot and KIO_3 could be absorbed. On the 90th day, the pots had their weights homogenized, and irrigation was restricted. Plants subjected to water deficit had the water content of their pots maintained for 20 days at 35% of the maximum water retention capacity (MWRC), while hydrated plants were kept at 80% of MWRC. To estimate the retention capacity, 200 g of soil was weighed, and 200 ml of water was measured. The 200 ml of water was added to 200 g of soil, quantifying how much water would be retained, this amount being considered 100% of the retention capacity. At the end of cultivation, the plants were collected for biomass evaluation. The evaluation of gas exchange, relative water, and chlorophyll content were evaluated during the maximum stress, atday 113. One day after, the plants were rehydrated and maitened at 80% of MWRC and at 123 th day, when the plants were totally harvested). Biochemical, photochemical, and biometric collections were conducted on days 113 and 120, encompassing both the stress period and the subsequent re-watering phase.

The pots were arranged in a completely randomized design with 4 replications in each treatment. Five treatments were evaluated: 4 doses of KIO_3 at concentrations of 0.0, 2.5, 5.0, and 10.0 mg dm^{-3} via soil, and under water deficit, tha were compared to an additional treatment (without application of KIO_3 and under adequate irrigation), used as a control, configuring a 2x4 factorial analysis scheme + 1 additional treatment. The experiment consisted of 20 experimental

units. The leaves samplings for antioxidant activity, oxidative damage, and osmolytes, were made under the deficit condition and after re-watering.).

2.2 Relative growth

Measurements to obtain relative growth were performed at the beginning of water restriction and after re-watering. For relative growth in height, a graduated ruler was used, and the measurement was carried out from the base of the stem to the cauline apex. The leaves were counted manually, and the stem diameter was measured using a digital caliper. Relative growth was calculated using the following equation:

$$\text{Relative Growth (\%)} = [(FH - IH) / (IH)] \times 100$$

Where: FH (final height) and IH (initial height)

2.3 Dry mass and water deficit tolerance index

To determine the dry matter of leaves, stems, and roots, after the destructive sampling the tissues were detached, separated in paper bags, and dried in an oven at 70 ºC until constant mass. Afterward, they were weighed, and after obtaining the data, it was possible to measure the RDM:SDM and WDTI ratios using the equations:

$$RDM:SDM = (RDM/SDM) \times 100$$

Where: RDM (root dry mass) and SDM (Shoot dry mass)

$$WDTI = (DMSP/DMIP) \times 100$$

Where: DMSP (dry mass of the stressed plant) and DMIP (dry mass of the irrigated plant)

2.4 Water relative content and chlorophyll content

The relative water content (RWC) was determined while the plants were subjected to water stress. Initially, a leaf was collected from each plant within each treatment. From this sampling, ten leaf discs of 5 mm in diameter were excised and weighed imediatly to obtain fresh weight (FW)

and left immersed for 24 hours in distilled water under low light. After 24 hours, the disks were weighed again, and the turgid weight (TW) was obtained. Subsequently, they were placed to dry in an oven at 70 ºC to acquire the dry weight (DW). The RWC was calculated as:

$$RWC = [(FW-DW)/(TW-DW)] \times 100\%$$

Where: FW (fresh weight), DW (dry weight), and TW (turgid weight).

Chlorophyll A and B contents were measured during stress in fully expanded leaves using a portable chlorophyll meter (Crorofilog CFL1030, Falker, Brazil). Subsequently, the total chlorophyll content and the chlorophyll A:B ratio were calculated.

2.5 Leaf Gas Exchange

Gas exchange analyses were performed during stress using an infrared gas exchange analyzer (LICOR 6400, Li-COR Biosciences, USA). The study was conducted between 8 am and 11 am when the values of the following variables were obtained: CO_2 assimilation rate *A* (μmol CO_2 m^{-2} s^{-1}), stomatal conductance g_s (mol H_2O m^{-2} s^{-1}), transpiration *E* (mmol H_2O m^{-2} s^{-1}) and internal carbon pressure C_i (Pa). From photosynthesis and internal carbon concentration results, it was possible to calculate the ratio [A/C_i (μmol CO_2 m^{-2} s^{-1} Pa^{-1})] and to determine estimates of water use efficiency [WUE (μmol CO_2 $mmol^{-1}$ H_2O)] through the results of photosynthesis and transpiration. Atmospheric CO_2 inside the leaf chamber was maintained at 400 μmols CO_2 mol air^{-1}, irradiance at 1500 μmols m^{-2} s^{-1}, and leaf temperature at 25 ºC. The pre-established minimum time for the stabilization of the readings was 120 seconds.

2.6 Oxidative damage and antioxidant activity

The fully expanded leaves were collected and macerated in liquid nitrogen with polyvinylpolypyrrolidone (PVPP) to reduce oxidation to evaluate the H_2O_2 and MDA content. After this process, a 0.2 g sample was weighed and homogenized in 1500 μL of trichloroacetic acid and centrifuged at 12.000 g for 15 minutes at 4 ºC. The supernatant was collected at the end of the process, and the analyses could be carried out.

For H_2O_2, an aliquot of 45 μL of the plant material extract was taken and added to a reaction medium composed of 10 mM potassium phosphate (pH 7.0) and 1M potassium iodide (Velikova et al. 2000). Then, the reading was performed in a spectrophotometer with absorbance at 390 nm.

The MDA quantification was performed according to Buege and Aust (1978). An aliquot of 125 μL from the supernatant was pipetted into a 1500 μL microtube containing 250 μL of the following reaction medium: 0.5% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA). Subsequently, the microtubes were placed in a water bath at 95° C for 30 minutes and then cooled on ice. Later, 350 μL of the reaction medium was collected and pipetted into microplates, where readings were performed in a spectrometer at 535 and 600 nm.

The MDA content was obtained according to the following equation:

$$MDA = (A535 – A600) / (\xi.b)$$

Where ξ: (molar extinction coefficient = 1.56 × 10^{-5} c^{-1}) and b: (optical length = 1). Lipid peroxidation was expressed in nmol (MDA) g^{-1} of fresh matter.

For the extraction and enzymatic evaluation, 0.2 g of fresh material macerated in liquid nitrogen and polyvinylpolypyrrolidone PVPP was collected with 1.5 mL of a buffered solution (0.1 mol L^{-1} of potassium phosphate pH (7.8), 0.1 mol L^{-1} EDTA (pH 7.0), 0.5 mol L^{-1}, DTT, 0.1 mol L^{-1} PMSF, 1.0 mmol L^{-1} ascorbic acid, and 22.0 mg PVPP). Then, the suspension was centrifuged at 13.000 g for 10 min at 4°C.

Subsequently, the supernatant was collected for analysis in a spectrophotometer (Epoch-BioTek-Elisa), according to the methodologies of Giannopolitis and Ries (1977), Havir and Mchale (1987), Nakano and Asada (1981) respectively for SOD, CAT and APX with specific adaptations to the method for coffee crop.

2.7 Osmolytes

For proline (PRO), 0.2 g of plant material was macerated in 3% sulfosalicylic acid (10 mL) and stirred for 60 minutes at room temperature. The material was filtered and added to tubes placed in a water bath at 100 °C for 60 minutes, as Bates et al. (1973) described. After this process, the tubes were cooled on ice, and the reading was performed in a spectrophotometer (Epoch-BioTek-Elisa) at an absorbance of 520 nm. Quantification was performed using a standard proline curve.

50 mg was collected from the macerated material (see 2.6), and ethanolic extraction was performed. First, 350 µl of 100% ethanol was added to a water bath at 70°C for 20 minutes, then centrifuged at 4°C and 14.000 RPM for 5 minutes. From there, the supernatant was collected, and once again, 350 µl of 80% ethanol was added to the pellet. The water bath and centrifugation process was repeated, and the supernatant was collected. Finally, 350 µl of 50% ethanol was added to the pellet and again taken to a water bath at 70°C for 20 minutes, undergoing centrifugation and subsequent collection of the supernatant. After these processes, the total soluble sugars were quantified by the anthrone method, described by Dische (1962), and the quantification of amino acids was performed by the ninhydrin method, as defined by Yemm and Cocking (1955). Readings were taken in a type of spectrophotometer (Epoch-BioTek-Elisa).

2.8 Statistical analysis

The data were submitted to the analysis of variance (ANOVA), and the basic assumptions were tested (normality of the residues by the Shapiro-Wilk test ($p > 0.05$) and homogeneity of variances by the Barlett test ($p > 0.05$). When the assumptions were met, the average of the treatments was compared by Tukey's test ($p < 0.05$), and the results were presented in the form of bar graphs. In case of non-compliance with the assumptions mentioned above, the data were submitted to a rank transformation (Conover and Iman, 1981; Holbert, 2022) and represented in a boxplot, allowing a better representation. Regarding the analysis carried out in a factorial scheme, the comparison between treatments was carried out according to the interaction or not of the studied factors, also using the Tukey test ($p < 0.05$). The R® software was used through the packages tidyverse (Wickham et al. 2019), multcomp (Hothorn et al. 2008), and rstatix (Kassambara, 2022) to perform the statistical analysis and make the graphs.

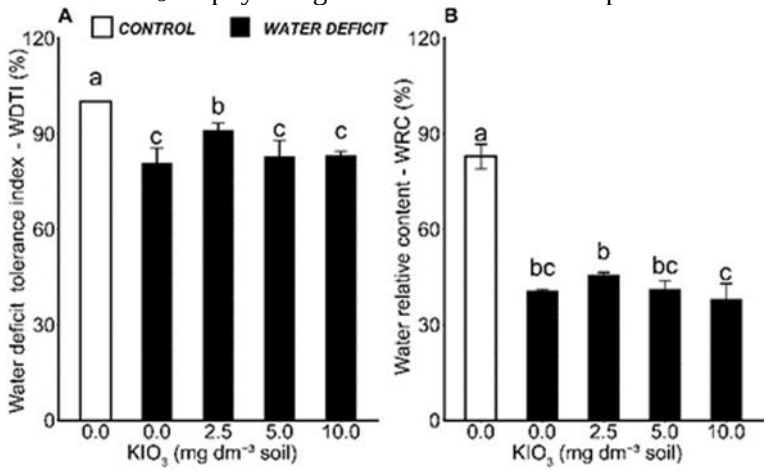
3 RESULTS

3.1 The water relations of coffee plants under water deficit

Significant difference ($p < 0.05$) was found for relative water content (WRC) and the water deficit tolerance index (WDTI) both when comparing irrigation conditions and for KIO_3 doses under water deficit (Figure 1). For (WDTI) it was possible to observe that the dose of 2.5 $mg.dm^{-3}$

KIO_3 promoted an average increment of 15% concerning the other treatments under water deficit (Figure 1A). The WRC was reduced by the water deficit, with a decrease of 51% for the plants without KIO_3 application, 54%, 49% e 45% for the doses of 10.0, 5.0 and 2.5 mg dm^{-3} of KIO_3, respectively. When considering only plants under deficit, 10.0 mg dm^{-3} of KIO_3 was 10% lower than the other treatments (Figure 1B).

Figure 1 – Effect of KIO_3 on physiological attributes of coffee plants under water stress.



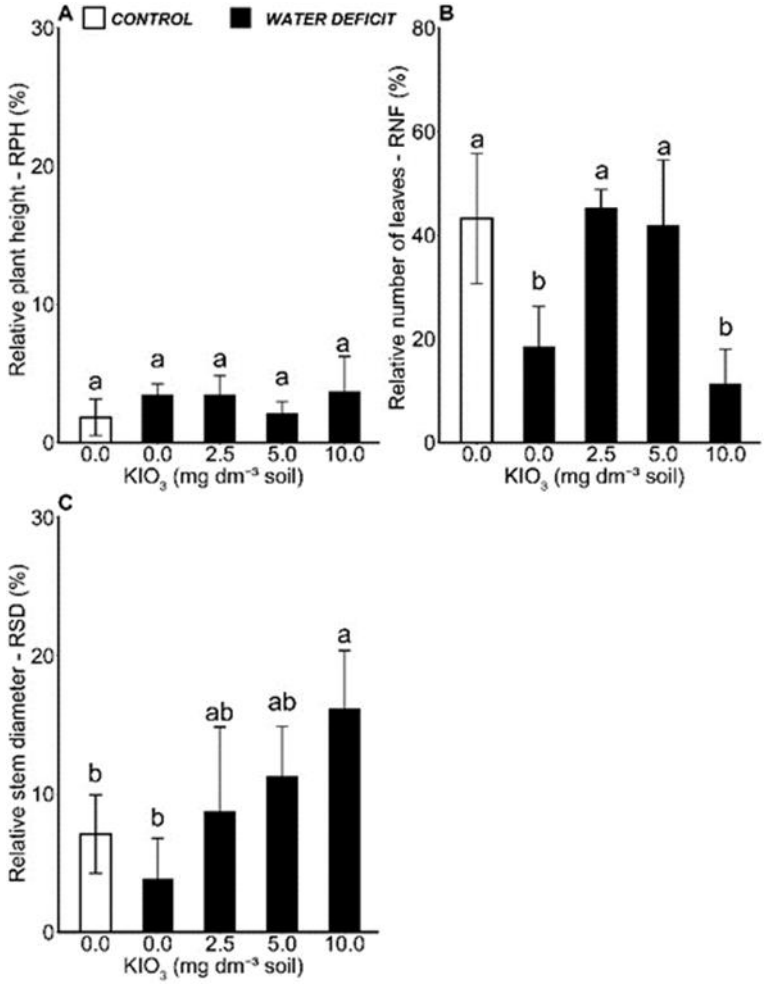
(A) water deficit tolerance index, and (B) water relative content. Treatment without application of KIO3 with adequate irrigation is represented with whitecolumns, while treatments with different concentration doses of KIO3 under water deficit are represented with black columns. Values are presented as average ± SD (n = 4). Same letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test.

Source: By the author, 2023.

3.2 Biomass production of the coffee plants to water deficit

The relative increment in number of leaves (RNL) and increase in stem diameter (RSD) were significantly affected ($p < 0.05$) by the tested treatments (Figure 2). For RNL, applying KIO_3 at doses of 2.5 and 5.0 mg dm^{-3} maintained the development of plants in water deficit conditions similar to plants grown without stress. Compared to plants without the application of KIO_3, in water deficit, the doses of 2.5 and 5.0 mg dm^{-3} KIO_3 increased by 27% and 23%, respectively. Still, for the variable RNL, a decrease of 34% was observed in the dose of 10.0 mg dm^{-3} of KIO_3 concerning the hydrated plants (Figure 2B). For RSD, mean increases of 10% and 13% were observed when the plants were submitted to a dose of 10.0 mg dm^{-3} of KIO_3 compared with plants without applying KIO_3, both under adequate and water deficit conditions (Figure 2C).

Figure 2 – Effect of KIO3 on relative growth attributes of coffee plants under water stress.



(A) Relative plant height - RPH, (B) Relative number of leaves - RNL, (C) Relative stem diameter - RSD. Treatment without application of KIO3 with adequate irrigation is represented with white bars, while treatments with different concentration doses of KIO3 under water deficit are represented with black bars. Values are presented as average ± SD (n = 4). * Equal letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test.

Source: By the author, 2023.

3.3 Plant dry mass

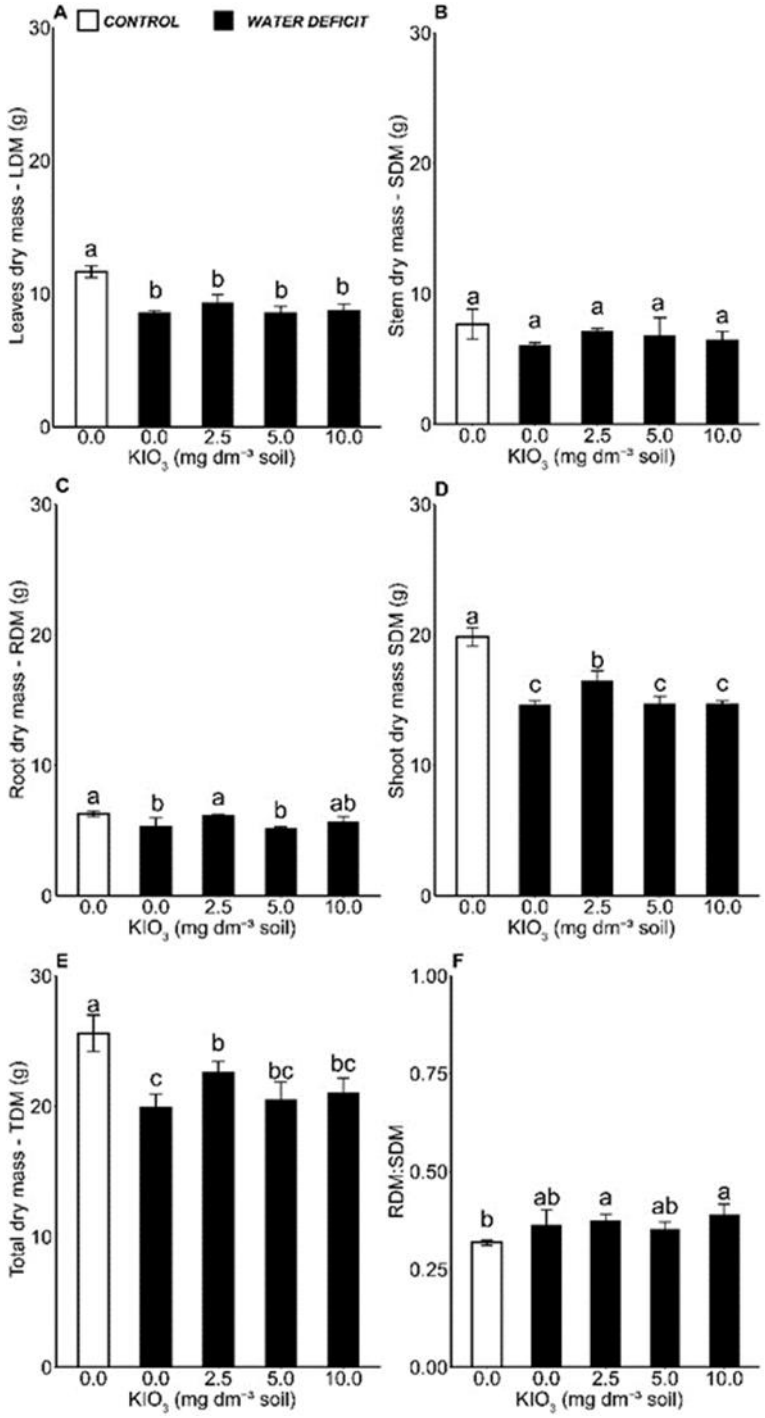
For root, shoot, and total dry mass, a significant difference ($p < 0.05$) was found between the plants submitted or not to water deficit and for the KIO_3 doses applied in the condition of water deficit (Figure 3). Regarding leaf dry mass (LDM), plants without water deficit had an average increase of 32% over plants under water deficit (Figure 3A). For stem dry mass, no differences were found for any of the treatments (Figure 3B).

In the root dry mass (RDM), the plants that were not exposed to KIO_3 and the dose of 5.0 mg dm^{-3} reduced by 21% the RDM concerning the plants without deficit. When analyzing only the plants submitted to water deficit, the dose of 2.5 mg dm^{-3} of KIO_3 maintained the development concerning the cultivation without deficit. It promoted an average increase of 20% concerning the plants without KIO_3, occurring the same concerning the plants subjected to a dose of 5.0 mg dm^{-3} of KIO_3.

For stem dry mass (SDM), there was an average increase of 34% for plants in adequate irrigation conditions compared with plants under deficit without KIO_3 and at doses of 5.0 and 10.0 mg dm^{-3} of KIO_3. However, this increase was only 20% compared with 2.5 mg dm^{-3} of KIO_3. Thus, the dry mass of aerial parts of plants under water deficit at the dose of 2.5 mg dm^{-3} of KIO_3 promoted an average increase of 12% concerning the other treatments that included water deficit (Figure 3D).

When observing the total dry mass (TDM), the plants under adequate irrigation increased the TDM by 29% concerning the plants under deficit and without KIO_3 application. The average increment was smaller for the other doses of KIO_3 (20%). Under deficit, the dose of 2.5 mg dm^{-3} of KIO_3 promoted an increment of 14% concerning those who did not receive the application of KIO_3 (Figure 3E). For the root: shoot dry mass ratio, the deficit increased by 16% regardless of the dose of KIO_3 applied (Figure 3F).

Figure 3 – Effect of KIO3 on biomass attributes of coffee plants under water stress.



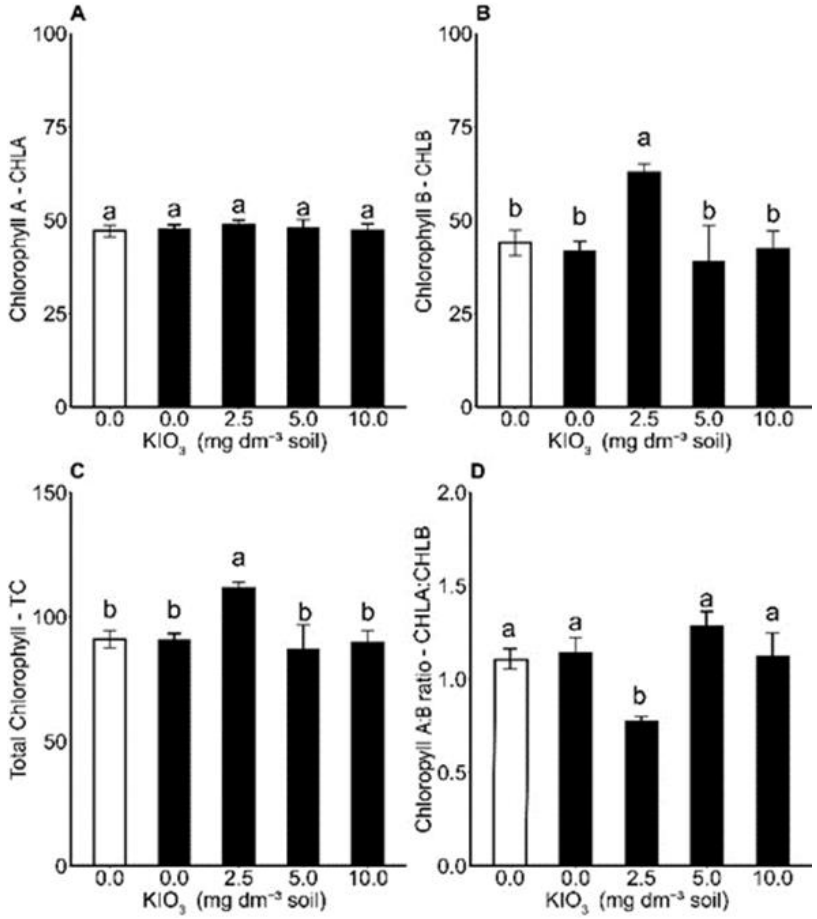
(A) Leaves dry mass - LDM, (B) Stem dry mass - STDM, (C) Root dry mass - RDM, (D) Shoot dry mass - SDM, (E) Total dry mass - TDM, and (F) Ratio between root dry mass and shoot dry mass - RDM:SDM. Treatment without application of KIO3 with adequate irrigation is represented with white bars, while treatments with different concentration doses of KIO3 under water deficit are represented with black bars. Values are presented as average ± SD (n = 4). * Equal letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test.

Source: By the author, 2023.

3.4 Physiogical responses of the coffee plants to water deficit

The dose of 2.5 mg dm^{-3} of KIO_3 promoted an average increment of 45% for chlorophyll B and 23% for total chlorophyll compared with the other treatments (Figure 4B and C). However, for the chlorophyll A:B ratio, a decrease of 43% was observed for the dose of 2.5 mg dm^{-3} of KIO_3 concerning the other treatments (Figure 4D).

Figure 4 – Effect of KIO3 on chlorophyll contents in coffee plants under water stress.



(A) Chlorophyll A - CHLA, (B) Chlorophyll B - CHLB, (C) Total chlorophyll - TC, and (D) Chlorophyll A:B ratio - CHLA: CHLB. Treatment without application of KIO3 with adequate irrigation is represented with white bars, while treatments with different concentration doses of KIO3 under water deficit are represented with black bars. Values are presented as average ± SD (n = 4). * Equal letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test.

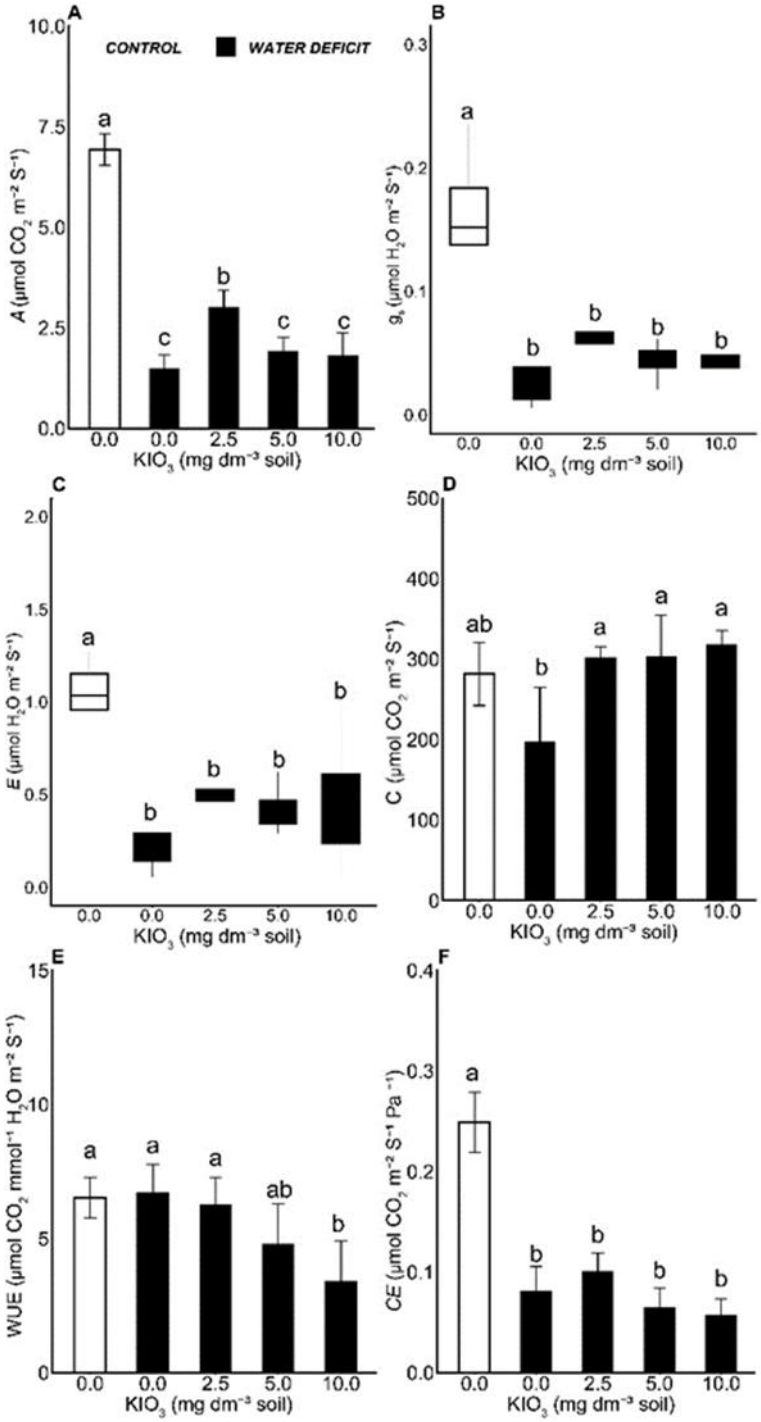
Source: By the author, 2023.

3.5 Leaf Gas Exchange

The gas exchange variables were significantly influenced ($p < 0.05$) by water deficit and KIO_3 application. A reduction in the CO_2 assimilation rate (*A*), stomatal conductance (g_s), transpiration (*E*), and carboxylation efficiency (*CE*) was found when plants were subjected to water deficit independent of exposure to KIO_3 (Figure 5). The water deficit promoted an average reduction of 74% *A* for plants without KIO_3, and for doses of 5.0 and 10.0 mg dm^{-3} KIO_3, this reduction was smaller for the dose of 2.5 mg dm^{-3} of KIO_3 (56%). When comparing only plants under water deficit, the application of 2.5 mg dm^{-3} of KIO_3 promoted an average increase of 74% concerning the other treatments (Figure 5A).

To stomatal conductance g_s and *E*, water deficit promoted an average reduction of 74% and 63%, respectively, regardless of the dose of KIO_3 applied (Figure 5B and C). Regarding C_i, the deficit promoted a decrease of 33% for plants without KIO_3 and maintained the levels for the other doses. When comparing only plants under water deficit, it is worth highlighting that the application of KIO_3, regardless of the dose, promoted an average increase of 64% compared with those that did not receive KIO_3 (Figure 5D). For WUE, an average reduction of 43% was observed only for the dose of 10.0 mg dm^{-3} KIO_3, regardless of the water condition of the plants (Figure 4E). It is also worth highlighting that the water deficit promoted an average decrease of 68% in *CE* concerning plants under adequate irrigation conditions (Figure 5E and F).

Figure 5 – Effect of KIO3 on gas exchange attributes in coffee plants under water stress.



(A) Assimilation rate - A, (B) Stomatal conductance - gs, (C) Transpiration - E, and (D) Internal CO2 concentration - Ci, (E) Water use efficiency - WUE, and (F) Carboxylation efficiency - CE. Treatment without application of KIO3 with adequate irrigation is represented with white bars, while treatments with different concentration doses of KIO3 under water deficit are represented with black bars. Values are presented as average ± SD (n = 4). * Equal letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test.

Source: By the author, 2023.

3.6 Biochemical responses of the coffee plants to wter deficit

Malondialdehyde (MDA) content and antioxidant enzymatic activity were significantly affected ($p < 0.05$) by both water deficit and KIO_3 application (Figure 6). Regarding the hydrogen peroxide (H_2O_2) content, a 40% increase was observed for treatments with water deficit (~11.39 U H_2O_2 min^{-1} mg FW^{-1}) when compared to hydrated plants (~8.19 U H_2O_2 min-1 mg FW^{-1}). Additionally, it was observed that water deficit promoted a 58% increase in H_2O_2 content when compared to re-watering (Figure 6A).

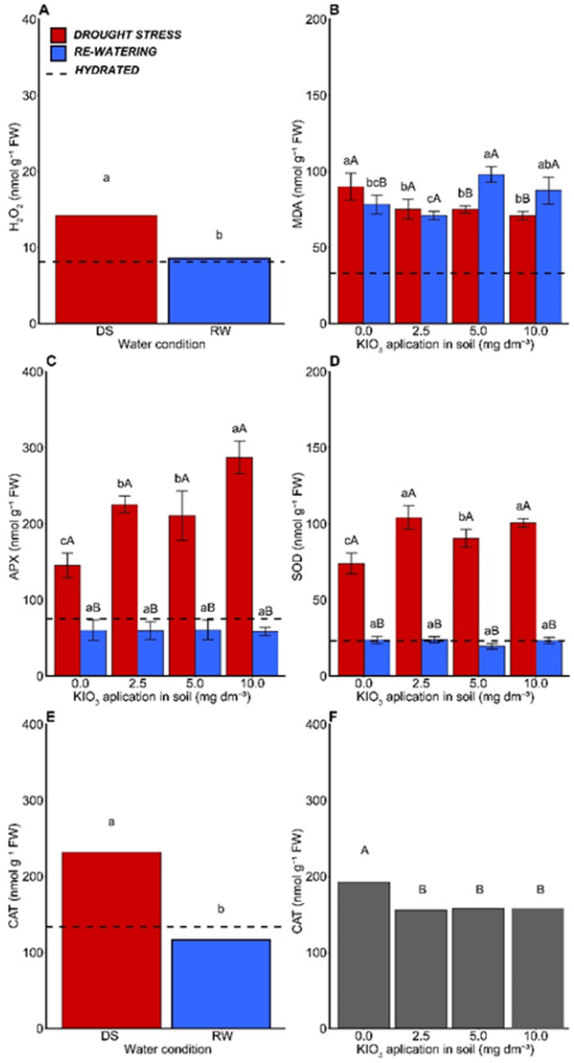
For the MDA content, there was an increase of 145% for plants with water deficit (~80.74 MDA min^{-1} mg FW^{-1}) compared to hydrated plants (~32.9 U MDA min^{-1} mg FW^{-1}). Furthermore, an average decrease of 18% was observed when plants were exposed to KIO_3 during stress. However, when rehydrated, it was found that the doses of 5.0 and 10.0 mg dm^{-3} of KIO_3 provided an average increase of 29% in the MDA content concerning the dose of 2.5 mg dm^{-3} of KIO_3. Still, for MDA, it was possible to notice that in the re-watering, there was an increase of 25% for the dose of 5.0 mg dm^{-3} of KIO_3 concerning those not exposed. When comparing water deficit and re-watering, there was no significant difference in the dose of 2.5 mg dm^{-3}. However, for plants without KIO_3, a decrease of 12% was observed after re-watering and inversely for doses of 5.0 and 10.0 mg dm^{-3} of KIO_3, which showed an increase of 29% and 22%, respectively (Figure 6B).

About APX, an average increase of 81% was observed for plants under water deficit (~138.1 AsA min^{-1} mg FW^{-1}) concerning hydrated plants (~75.9 min^{-1} mg FW^{-1}). Treatments with 2.5 and 5.0 mg dm^{-3} of KIO_3 provided an average increase of 55% during stress compared with plants that did not receive KIO_3. For the dose of 10.0 mg dm^{-3} of KIO_3, a rise of 97% was observed in the enzyme contents, also compared with the plants that did not receive KIO_3 and 27% concerning the doses of 2.5 and 5.0 mg dm^{-3} of KIO_3. However, after re-watering, no variations were found between treatments (Figure 6C).

Regarding SOD activity, it was possible to notice an increase of 145% in treatments with water deficit (~57.47 U SOD min^{-1} mg FW^{-1}) concerning hydrated plants (~23.00 U SOD min^{-1} mg FW^{-1}). Notably, the doses of 2.5 and 10.0 mg dm^{-3} of KIO_3 promoted an average increment of 38% compared with plants without KIO_3 and 13% concerning the dose of 5.0 mg dm^{-3} of KIO_3. Still, for SOD, the dose of 5.0 mg dm^{-3} of KIO_3 provided gains of 22% concerning the plants without application. No significant differences were found for SOD activity during re-watering (Figure 6D).

It is crucial to highlight that for the CAT activity, there were no interactions between the factors. Nevertheless, the average activity of the enzyme was increased by 32% in the plants without KIO_3 compared with the plants that received the application of KIO_3. However, there was an increase of 30% for plants under water deficit (~173.58 min^{-1} mg FW^{-1}) compared to hydrated plants (~133.45 U CAT min^{-1} mg FW^{-1}). Furthermore, it was observed that the deficit promoted a 64% increase concerning re-watering (Figure 6E and F).

Figure 6 – Effect of KIO3 on oxidative stress and antioxidant system in coffee plants under water stress.



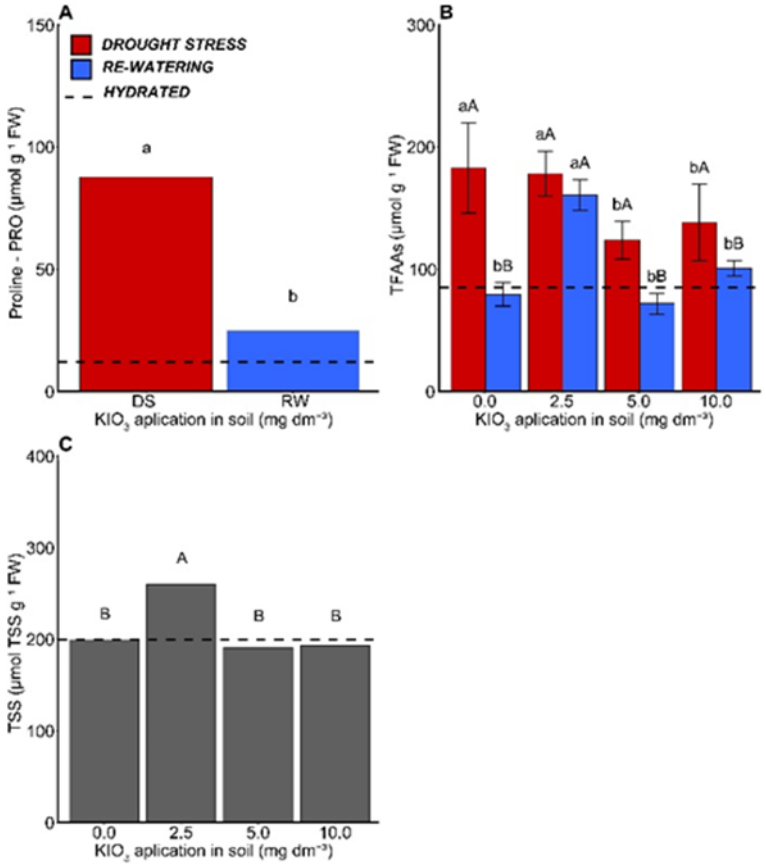
(A) Hydrogen peroxide - H2O2, (B) Malondialdehyde - MDA, (C) Ascorbate peroxidase - APX, (D) Superoxide dismutase - SOD, (E) and (F) Catalase - CAT. Water deficit is represented with red bars, re-watering is depicted with blue bars, and the gray bars represent the combination of data obtained during deficit and re-watering. Values are presented as average ± SD (n = 4). * Equal letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test.

Source: By the author, 2023.

Amino acid content was affected by both water deficit compared with plants under adequate irrigation and KIO_3 doses applied to plants under water deficit ($p < 0.05$) (Figure 7). For total soluble sugars (TSS), no significant interactions were observed between the factors. However, an average increase of 28% was observed for plants that were submitted to a dose of 2.5 mg dm^{-3} KIO_3 (~260.32 U TFF mg FW^{-1}) concerning the hydrated ones (~194.16 U TFF mg FW^{-1}) and 35% for the other KIO_3 concentrations tested (Figure 8C). Additionally, for proline, an increase of 370% was observed for treatments that were submitted to water deficit (~56.24 U PROL mg FW^{-1}) concerning hydrated plants (~11.95 U PROL mg FW^{-1}). It is also noteworthy that proline levels increased by 250% during the deficit when compared to re-watering (Figure 7A).

Regarding total free amino acids (TFAAs), an increase of 51% was observed for plants subjected to water deficit (~129.51 U TFAAs mg FW^{-1}) compared to hydrated plants (~85.05 U TFAAs mg FW^{-1}). Notably, during the deficit, there was an increase of 44% and 28% for the plants submitted to apply 2.5 mg dm^{-3} KIO_3 concerning the doses of 5.0 and 10.0 mg dm^{-3} of KIO_3, respectively. Even during the deficit in the 5.0 and 10.0 mg dm^{-3} doses, there was an average decrease of 28% concerning the plants that did not receive KIO_3. During re-watering, the dose of 2.5 mg dm^{-3} KIO^3 promoted an average increase of 90% compared with the other treatments. Furthermore, at the dose of 2.5 mg dm^{-3} KIO_3, the levels of TFAAs were maintained. At the same time, decreases of 56%, 42%, and 27% were observed at doses of 0, 5.0, and 10.0 mg dm^{-3}, respectively, compared to the deficit period and re-watering (Figure 7B).

Figure 7 – Effect of KIO3 on osmotic regulation in coffee plants under water stress.



(A) Proline - PRO, (B) Total soluble sugars - TSS, (C) Total free amino acids - TFAAs. Water deficit is represented with red bars, re-watering is depicted with blue bars, and the gray bars represent the combination of data obtained during deficit and re-watering. Values are presented as average ± SD (n = 4). * Equal letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test.

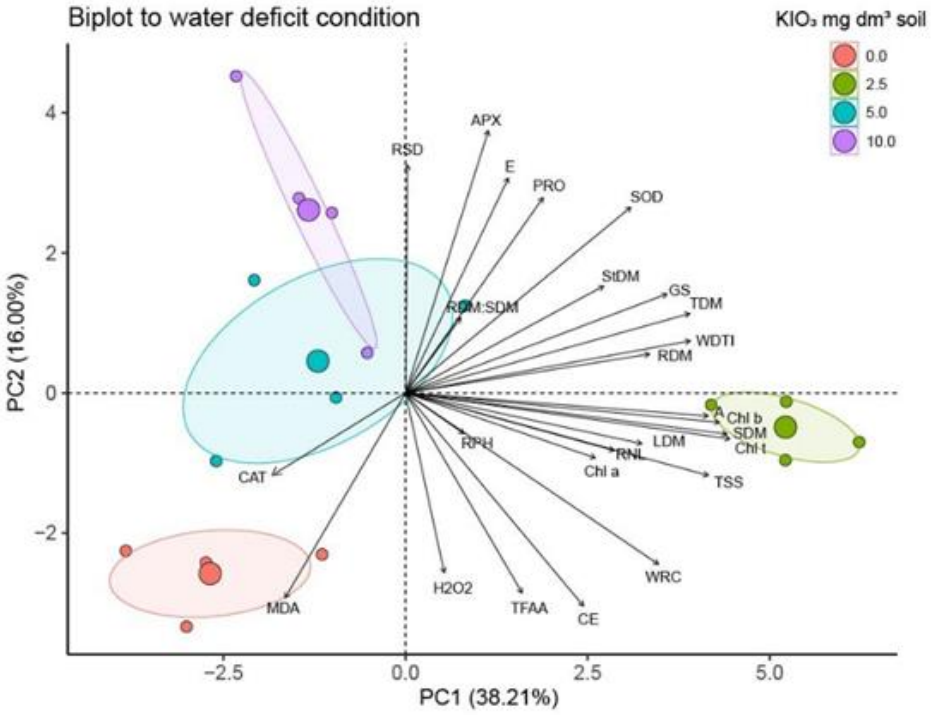
Source: By the author, 2023.

3.8 Multivariate data analysis

The principal component analysis (PCA) performed based on coffee plants' morphological, physiological, and biochemical characteristics under water stress (Figure 8) indicated that the first two components, PC1 and PC2, presented 51.5% of the total variance. The investigation was carried out only during the water deficit because the plants' primary metabolic and physiological responses were demonstrated during this period, as observed in the univariate analysis. The biplot for the deficit condition showed higher MDA in plants that did not receive KIO_3 fertilization, which negatively correlated with APX, SOD, and PRO. In contrast, the 2.5 mg dm^{-3} KIO_3 treatment tends to group *A*, TSS, CHLA, CHLB, TC, SDM, TDM, LDM, RDM, RNL, WRC, and WDTI that

correlate positively with each other and negatively with CAT which in turn was favored by the dose of 5.0 mg dm^{-3} KIO_3.

Figure 8 – Principal component analysis using morphological, physiological, and biochemical attributes in coffee plants in response to water deficit conditions under different concentration doses of KIO3 via soil.



Ascorbate peroxidase - APX; Superoxide dismutase - SOD; Catalase – CAT; Hydrogen peroxide - H2O2; Malondialdehyde – MDA; Proline – PRO; Total soluble sugars – TSS; Total free amino acids – TFAAs; Assimilation rate - A, Stomatal conductance - gs; Transpiration - E; Carboxylation efficiency – CE; Water Deficit Tolerance Index - WDTI; Water Relative Content - WRC; Chlorophyll A – CHLA; Chlorophyll B – CHLB; Total chlorophyll – TC; Leaves dry mass – LDM; Stem dry mass – STDM; Root dry mass – RDM; Shoot dry mass – SDM; Total dry mass – TDM; Relation root dry mass: Shoot dry mass - RDM:SDM; Relative plant height – RPH; Relative number of leaves – RNL; Relative stem diameter - RSD.

Source: By the author, 2023.

4 DISCUSSIONS

Water withholding drastically reduced growth and biomass production in coffee plants. However, our results clearly show that the KIO_3 fertilization at a dose of 2.5 mg dm^{-3} via soil attenuated these implications, minimizing the harmful effects of lack of water concerning plants that did not receive KIO_3 fertilization under water deficit. The damage caused by water restriction occurs due to the reduction in the relative water content, which causes photosynthesis limitation,

culminating in the increase in the overproduction of reactive oxygen species (ROS), promoting an increase in lipid peroxidation (RAZI; MUNEER, 2021; WANG et al., 2022; ZHANG et al., 2018; ZHANG et al., 2021).

Thus, the present work demonstrated the efficiency of KIO_3 application via soil in coffee plants under water deficit, which induced an increase in the chlorophyll content in the leaves, an improvement in the efficiency of the enzymatic antioxidant system and osmotic adjustment, which provided photosynthetic gains and an increase in the water deficit tolerance index (WDTI), in plants exposed to the lowest dose tested: 2.5 mg dm^{-3} KIO_3. Studies show that plants are sensitive to higher doses of Iodine. In soybean plants (*Glycine max* L.), the lowest dose, 10 µM of KI, was also the most adequate to mitigate the deleterious effects of lack of water, and the highest dose, 40 µM KI, considered phytotoxic (LIMA et al., 2023). Coffee plants fertilized with the highest doses of 5.0 and 10.0 mg dm^{-3} KIO_3 showed reductions in biomass concerning the dose of 2.5 mg dm^{-3} KIO_3. Applying elements in high amounts can cause a decrease in growth due to the accumulation of free radicals, which can influence metabolism, directly affecting the cellular integrity of plants (RAZA et al., 2022; SHARMA et al., 2020).

The increase in tolerance related to the dose of 2.5 mg dm^{-3} KIO_3 appears to be directly related to the increase in chlorophyll B, total chlorophyll, and photosynthetic efficiency in this treatment. Kiferle et al. (2021) demonstrated the participation of iodine in several proteins related to photosynthesis, where at least 31 iodinated proteins were linked to chlorophyll, thus indicating that iodine can participate in photosynthesis as a component of proteins that make up the chlorophyll molecule. Furthermore, another group of iodinated proteins can act in the electron transfer activity in the reaction center of photosystem II (PSII), in light capture, and in photosystem I (PSI) (KIFERLE et al., 2021). The photosynthesis of lettuce (*Lectuca sativa* L.) increased ~20% with the application of I via nutrient solution at adequate levels and similarly provided photosynthetic gains in soybean plants subjected to water deficit (BLASCO et al., 2011; LIMA et al., 2023).

On the other hand, applying 5.0 and 10.0 mg dm^{-3}, KIO_3 maintained the levels of photosynthesis and chlorophyll B and total content compared with plants without KIO_3. This result indicates that when iodine is made excessively available, it may have an inhibitory and toxic effect on plants (INCROCCI et al., 2019). In a study with basil (*Ocimum basilicum* L.), a significant decline in the total dry mass of the plants was observed when KI concentrations were greater than 50 mM (INCROCCI et al., 2019). Furthermore, due to the high concentration of iodine, there may be leaf chlorosis and burns, as observed in tomato plants (*Solanum licopersicum* L.) (LANDINI et

al., 2011). Thus, excess iodine can also influence photosynthesis, affecting chlorophyll synthesis through the accumulation of free radicals (KIFERLE et al., 2021; KIFERLE et al., 2022; ZHANG et al., 2022).

Additionally, we observed that when coffee plants were treated with 5.0 and 10.0 mg dm^{-3}, their lipid peroxidation increased after the re-watering period (Figure 6), which may indicate an intensification of oxidative stress in this condition. In general, when plants are subjected to water deficit followed by re-watering, ROS is rapidly released due to the resumption of metabolic activities. This factor may temporarily increase MDA content in response (CHEN et al., 2016; PATANÈ et al., 2022). Excess ROS in plant cells promotes oxidative stress, leading to lipid peroxidation, protein oxidation, enzyme inactivation, and even cell death in severe cases (LI et al., 2017; SAHU et al., 2022). Chen et al. (2016) showed that some maize genotypes had increased MDA content and reduced biomass after re-watering. A high MDA content indicates a high degree of lipid peroxidation of the plant membrane and a state of oxidative stress (REZAIZAD et al., 2019). Thus, excess iodine may have further intensified oxidative stress, increasing lipid peroxidation and, consequently, MDA content. Under high iodine concentration (5.0 mg L^{-1}), the concentration of MDA in pepper leaves (*Capsicum annuum* L.) almost doubled compared with the control group (LI et al., 2017).

In contrast, it was observed that the dose of 2.5 mg dm^{-3} KIO_3 promoted a balance for MDA content during deficit and re-watering. The application of KIO_3, regardless of the dose, increased the activity of antioxidant enzymes in coffee plants during water deficit, mainly SOD and APX (Figure 6). Previous researchers have already demonstrated that the exogenous application of iodine can promote the activation of several enzymes of the antioxidant system, such as APX, SOD, and CAT, for crops such as lettuce, tomato, and soybean under different cultivation forms (BLASCO et al., 2011; KIFERLE et al., 2022; LIMA et al., 2023). However, it was observed in the present study that only at the dose of 2.5 mg dm^{-3} KIO_3 there was more significant accumulation of TFAAs during re-watering (Figure 7). This result leads us to believe that only increases in enzymatic antioxidant activity by applying KIO_3 during the deficit were insufficient and that the accumulation of amino acids served as a crucial strategy in increasing the tolerance of coffee plants.

In addition to activating the enzymatic antioxidant system, the accumulation of osmolytes such as proline, free amino acids, and total soluble sugars are mechanisms of tolerance to abiotic stresses (OZTURK et al., 2021). The increase in the concentration of these substances can also confer resistance to water deficit as they act as osmoprotectors, osmotic regulators, and antioxidants

(IQBAL et al., 2023; YOU et al., 2019). Applying exogenous substances can induce the production of these compounds and increase plant tolerance to different types of stress (CHERAGHI et al., 2023; LEYVA et al., 2011; PU et al., 2021). The accumulation of amino acids during re-watering to the 2.5 mg dm^{-3} KIO_3 dose appears to have helped maintain the integrity of cell membranes and protected against excessive water loss and stress-related damage (OZTURK et al., 2020; TARKOWSKI et al., 2020).

Strzetelski et al. (2010) highlighted that KIO_3 fertilization provided increments in the TFAAs content in radish plants (*Raphanus sativus* L.). On the other hand, no increase in proline was observed with the application of KIO_3, which suggests that the production of simpler amino acids, with lower energy expenditure, was favored. Furthermore, the application of 2.5 mg dm^{-3} KIO_3 promoted an increase in total soluble sugars regardless of the studied condition. This result may be related to greater photosynthetic efficiency, where the stimulation of sugar production may have provided a more considerable amount of substrate for photosynthesis, enabling the maintenance of other metabolic activities. Studies that correlate the accumulation of sugars with the application of iodine under water deficit are scarce. However, some authors have already described gains in this regard with the application of the element to pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) in other conditions of cultivation (SMOLEŃ et al., 2015; LI et al., 2017).

The application of KIO_3, in general, has been shown to activate several mechanisms of tolerance to water deficit, and the dose of 2.5 mg dm^{-3} favored most of the variables observed during water deficit, mainly related to the accumulation of biomass, gas exchange, and content of photosynthetic pigments (Figure 8). Furthermore, the application of 2.5 mg dm^{-3} favored the accumulation of TFAAs during re-watering, which seems to have guaranteed higher protection to coffee plants against stress. Conversely, the highest concentrations (5.0 and 10.0 mg dm^{-3}), despite having favored high antioxidant enzymatic activity during the deficit, seem to have intensified oxidative stress after re-watering in plants, increasing the MDA content under these conditions.

5 CONCLUSION

Potassium iodate KIO_3 modulates several metabolic processes and defense mechanisms in coffee plantlets under water deficit, improving photosynthetic efficiency, redox homeostasis, and osmotic balance. The application of 2.5 mg dm^{-3} KIO_3 is the best alternative among those studied,

as it balances the metabolic processes, adjusting the antioxidant enzymatic system, accumulation of amino acids and sugars, promoting photosynthetic gains, and better plant development. The adoption of KIO_3 as a strategic management technique contributes to improving plant tolerance to adverse environmental conditions. Studies on applying iodine sources to mitigate abiotic stresses are still scarce, and many details still need to be elucidated. Thus, field studies must be carried out in other growth stages with variations of sources, forms of application, and interaction between nutrients.

REFERENCES

ABBASS, K. et al. A review of the global climate change impacts, adaptation, and sustainable mitigation measures. **Environmental Science and Pollution Research**, v. 29, n. 28, p. 42539-42559, 2022. doi: https://doi.org/10.1007/s11356-022-19718-6

AHSAN, M. et al. Evaluation of Silicon Supplementation for Drought Stress under Water-Deficit Conditions: An Application of Sustainable Agriculture. **Agronomy**, v. 13, n. 2, p. 599, 2023. doi: https://doi.org/10.3390/agronomy13020599

ALI, J. et al. Biochemical response of okra (*Abelmoschus esculentus* L.) to Selenium (Se) under drought stress. **Sustainability**, v. 15, n. 7, p. 5694, 2023. doi: https://doi.org/10.3390/su15075694

ALTAF, M. A. et al. Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. **Antioxidants**, v. 11, n. 2, p. 309, 2022. doi: https://doi.org/10.3390/antiox11020309

AZEEM, M. et al. Drought stress amelioration in maize (*Zea mays* L.) by inoculation of Bacillus spp. strains under sterile soil conditions. **Agriculture**, v. 12, n. 1, p. 50, 2022. doi: https://doi.org/10.3390/agriculture12010050

BANDURSKA, H. Drought stress responses: Coping strategy and resistance. **Plants**, v. 11, n. 7, p. 922, 2022. doi: https://doi.org/10.3390/plants11070922

BATES, L. S.; WALDREN, R. P a; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant and soil**, v. 39, p. 205-207, 1973. doi: https://doi.org/10.1007/BF00018060
BLASCO, B. et al. Photosynthesis and metabolism of sugars from lettuce plants (*Lactuca sativa* L. var. longifolia) subjected to biofortification with iodine. **Plant growth regulation**, v. 65, p. 137-143, 2011. doi: https://doi.org/10.1007/s10725-011-9583-0

BOGATI, K.; WALCZAK, M. The impact of drought stress on soil microbial community, enzyme activities and plants. **Agronomy**, v. 12, n. 1, p. 189, 2022. doi: https://doi.org/10.3390/agronomy12010189

BUEGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. In: FLEISCHER, S.; PACKER, L. (Eds.) **Methods in Enzymology.** v. 30, Academic Press, 1978. p. 302–310 doi: https://doi.org/10.1016/S0076-6879(78)52032-6

CHEN, D. et al. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. **Frontiers in Plant Science**, v. 6, p. 1241, 2016. doi: https://doi.org/10.3389/fpls.2015.01241

CHERAGHI, M. et al. Silicon (Si): a regulator nutrient for optimum growth of wheat under salinity and drought stresses-a review. **Journal of Plant Growth Regulation**, p. 1-25, 2023. doi: https://doi.org/10.1007/s00344-023-10959-4

CONAB. Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira de café. Levantamento Safra 2022/2023. Brasília, DF, v. 10, safra 2022/23, n.2, mai. 2023. Disponível em: <Conab - Página inicial>. Acesso em 15 de Ago. 2023.

CONOVER, W. J.; IMAN, R. L. Rank transformations as a bridge between parametric and nonparametric statistics. **The American Statistician**, v. 35, n. 3, p. 124-129, 1981. doi: https://doi.org/10.1080/00031305.1981.10479327

DISCHE, Zacharias. General color reactions. **Methods in carbohydrate chemistry**, v. 1, p. 478-512, 1962.

FAO. Food and Agriculture Organization of the United Nations. **The State of Food Security and Nutrition in the World**. FAO; IFAD; UNICEF; WFP; WHO, 2023. Disponível em: <https://www.fao.org/publications/home/fao-flagship-publications/the-state-of-food-security-and-nutrition-in-the-world>. Acesso em 15 de Ago. 2023.

GHOURI, F. et al.Effects of Silicon and Selenium in Alleviation of Drought Stress in Rice. Silicon v. 14, n. 10, p. 5453–5461, 2022. doi: https://doi.org/10.1007/s12633-021-01277-z

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: I. Occurrence in higher plants. **Plant physiology**, v. 59, n. 2, p. 309-314, 1977. doi: https://doi.org/10.1104/pp.59.2.309

GOWTHAM, H. G. et al. Insight into recent progress and perspectives in improvement of antioxidant machinery upon PGPR augmentation in plants under drought stress: a review. **Antioxidants**, v. 11, n. 9, p. 1763, 2022. doi: https://doi.org/10.3390/antiox11091763

HAVIR, E. A.; MCHALE, N. A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. **Plant physiology**, v. 84, n. 2, p. 450-455, 1987. doi: https://doi.org/10.1104/pp.84.2.450

HOLBERT, H., **Nonparametric Two-Way ANOVA.** 2022. Disponível em: <https://www.cfholbert.com/blog/nonparametric_two_way_anova/>. Acesso em 08 de Ago. 2023.

HOTHORN, T.; BRETZ, F.; WESTFALL, P. Simultaneous inference in general parametric models. **Biometrical Journal: Journal of Mathematical Methods in Biosciences**, v. 50, n. 3, p. 346-363, 2008. doi: https://doi.org/10.1002/bimj.200810425

INCROCCI, L. et al. Iodine accumulation and tolerance in sweet basil (*Ocimum basilicum* L.) with green or purple leaves grown in floating system technique. **Frontiers in Plant Science**, v. 10, p. 1494, 2019. doi: https://doi.org/10.3389/fpls.2019.01494

IQBAL, A. et al. Differential responses of contrasting low phosphorus tolerant cotton genotypes under low phosphorus and drought stress. **BMC Plant Biology**, v. 23, n. 1, p. 1-14, 2023. doi: https://doi.org/10.1186/s12870-023-04171-5

KASSAMBARA A. rstatix: **Pipe-Friendly Framework for Basic Statistical Tests**, 2023. Disponível em: <https://cran.rproject.org/web/packages/rstatix/index.html>. Acesso em 31 de Ago. 2023.

KHODABIN, G. et al. Effect of drought stress on certain morphological and physiological characteristics of a resistant and a sensitive canola cultivar. **Chemistry & biodiversity**, v. 17, n. 2, p. e1900399, 2020. doi: https://doi.org/10.1002/cbdv.201900399

KIFERLE, C. et al. Improvement in fruit yield and tolerance to salinity of tomato plants fertigated with micronutrient amounts of iodine. **Scientific Reports**, v. 12, n. 1, p. 14655, 2022. doi: https://doi.org/10.1038/s41598-022-18301-w

KIFERLE, C. et al. Evidences for a nutritional role of iodine in plants. **Frontiers in plant science**, v. 12, p. 616868, 2021. doi: https://doi.org/10.3389/fpls.2021.616868

LANDINI, M.; GONZALI, S.; PERATA, P. Iodine biofortification in tomato. **Journal of Plant Nutrition and Soil Science**. v. 174, p. 480-486, 2011. doi: https://doi.org/10.1002/jpln.201000395

LAXA, M. et al. The role of the plant antioxidant system in drought tolerance. **Antioxidants**, v. 8, n. 4, p. 94, 2019. doi: https://doi.org/10.3390/antiox8040094

LEYVA, R. et al. Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. **Plant Science**, v. 181, n. 2, p. 195-202, 2011. doi: https://doi.org/10.1016/j.plantsci.2011.05.007

LI, R. et al. Enhancing iodine content and fruit quality of pepper (*Capsicum annuum* L.) through biofortification. **Scientia Horticulturae**, v. 214, p. 165-173, 2017. doi: https://doi.org/10.1016/j.scienta.2016.11.030

LIMA, J. S. et al. Soybean Plants Exposed to Low Concentrations of Potassium Iodide Have Better Tolerance to Water Deficit through the Antioxidant Enzymatic System and Photosynthesis Modulation. **Plants**, v. 12, n. 13, p. 2555, 2023. doi: https://doi.org/10.3390/plants12132555

MACÍAS, J. M. et al. Enhancement to salt stress tolerance in strawberry plants by iodine products application. **Agronomy**, v. 11, n. 3, p. 602, 2021. doi: https://doi.org/10.3390/agronomy11030602

MACÍAS, J. M. et al. Use of iodine to biofortify and promote growth and stress tolerance in crops. **Frontiers in Plant Science**, v. 7, p. 1146, 2016. doi: https://doi.org/10.3389/fpls.2016.01146

MATIELLO J. B.; SANTINATO R.; ALMEIDA; G. **Cultura de café no Brasil.** Manual de recomendações para a cultura cafeeira. v. 6, 2020. Disponível em: <https://www.fundacaoprocafe.com.br/livros>. Acesso em 15 de Ago. 2023.

NADARAJAH, K. K. ROS homeostasis in abiotic stress tolerance in plants. **International journal of molecular sciences**, v. 21, n. 15, p. 5208, 2020. doi: https://doi.org/10.3390/ijms21155208

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. **Plant and cell physiology**, v. 22, n. 5, p. 867-880, 1981.

NASCIMENTO, V. L. et al. On the role of iodine in plants: A commentary on benefits of this element. **Frontiers in Plant Science**, v. 13, p. 836835, 2022. doi: https://doi.org/10.3389/fpls.2022.836835

NASIR, M. W.; TOTH, Z. Effect of drought stress on potato production: A review. **Agronomy**, v. 12, n. 3, p. 635, 2022. doi: https://doi.org/10.3390/agronomy12030635

NEDIĆ, O. Iodine: Physiological importance and food sources. **eFood**, v. 4, n. 1, p. e63, 2023. doi: https://doi.org/10.1002/efd2.63

OIC. Organização internacional do café. **Relatório sobre o mercado de café**. ago. 2021. Disponível em: <https://consorciopesquisacafe.com.br/arquivos/consorcio/publicacoes_tecnicas/relatorio_oic_agosto_2021.pdf>. Acesso em 15 de Ago. 2023.

OZTURK, M. et al. Osmoregulation and its actions during the drought stress in plants. **Physiologia plantarum**, v. 172, n. 2, p. 1321-1335, 2021. doi: https://doi.org/10.1111/ppl.13297

PATANÈ, C. et al. Relative water content, proline, and antioxidant enzymes in leaves of long shelf-life tomatoes under drought stress and rewatering. **Plants**, v. 11, n. 22, p. 3045, 2022. doi: https://doi.org/10.3390/plants11223045

PU, Y. J. et al. Coupling exogenous melatonin with Ca2+ alleviated chilling stress in *Dalbergia odorifera* T. Chen. **Trees**, v. 35, n. 5, p. 1541-1554, 2021. doi: https://doi.org/10.1007/s00468-021-02134-7

RAZA, A. et al. Plant hormones and neurotransmitter interactions mediate antioxidant defenses under induced oxidative stress in plants. **Frontiers in plant science**, v. 13, 2022.

RAZI, K.; MUNEER, S. Drought stress-induced physiological mechanisms, signaling pathways and molecular response of chloroplasts in common vegetable crops. **Critical Reviews in Biotechnology**, v. 41, n. 5, p. 669-691, 2021. doi: https://doi.org/10.1080/07388551.2021.1874280

REZAIZAD, M. et al. Photocatalytic effect of TiO2 nanoparticles on morphological and photochemical properties of stevia plant (Stevia rebaudiana Bertoni). **Sugar Tech**, v. 21, n. 6, p. 1024-1030, 2019. doi: https://doi.org/10.1007/s12355-019-00726-9

RIYAZUDDIN, R. et al. Iodine: an emerging biostimulant of growth and stress responses in plants. **Plant and Soil**, v. 486, n. 1-2, p. 119-133, 2023. doi: https://doi.org/10.1007/s11104-022-05750-5

SAHU, P. K. et al. ROS generated from biotic stress: Effects on plants and alleviation by endophytic microbes. **Frontiers in Plant Science**, v. 13, p. 1042936, 2022. doi: https://doi.org/10.3389/fpls.2022.1042936

SHARMA, A. et al. Nitric oxide-mediated regulation of oxidative stress in plants under metal stress: a review on molecular and biochemical aspects. **Physiologia plantarum**, v. 168, n. 2, p. 318-344, 2020. doi: https://doi.org/10.1111/ppl.13004

SMOLEŃ, S. et al. Iodine biofortification with additional application of salicylic acid affects yield and selected parameters of chemical composition of tomato fruits (*Solanum lycopersicum* L.). **Scientia Horticulturae**, v. 188, p. 89-96, 2015. doi: https://doi.org/10.1016/j.scienta.2015.03.023

STRZETELSKI, P. et al. The effect of diverse iodine fertilization on nitrate accumulation and content of selected compounds in radish plants (Raphanus sativus L.). **Acta Scientiarum Polonorum. Hortorum Cultus**, v. 9, n. 2, 2010.

TARKOWSKI, Ł. P.; SIGNORELLI, S.; HÖFTE, M. γ-Aminobutyric acid and related amino acids in plant immune responses: emerging mechanisms of action. **Plant, Cell & Environment**, v. 43, n. 5, p. 1103-1116, 2020. doi: https://doi.org/10.1111/pce.13734

UN. United Nations. **World population prospects**. Department of Economic and Social Afairs. Population division. UN DESA/POP/2021/N:3, 2022. Disponível em: <https://news.un.org>. Acesso em 15 de Ago. 2023.

VELIKOVA, V.; YORDANOV, I.; EDREVA, A. J. P. S. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. **Plant science**, v. 151, n. 1, p. 59-66, 2000. doi: https://doi.org/10.1016/S0168-9452(99)00197-1

WAHAB, A. et al. Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. **Plants**, v. 11, n. 13, p. 1620, 2022. doi: https://doi.org/10.3390/plants11131620

WANG, X. et al. Physiological response of soybean plants to water deficit. **Frontiers in Plant Science**, v. 12, p. 809692, 2022. doi: https://doi.org/10.3389/fpls.2021.809692

WICKHAM, H. et al. Welcome to the Tidyverse. **Journal of open source software**, v. 4, n. 43, p. 1686, 2019. doi: https://doi.org/10.21105/joss.01686

YAHAYA, M. A.; SHIMELIS, H. Drought stress in sorghum: Mitigation strategies, breeding methods and technologies—A review. **Journal of Agronomy and Crop Science**, v. 208, n. 2, p. 127-142, 2022. doi: https://doi.org/10.1111/jac.12573

YEMM, E. W.; COCKING, E. C.; RICKETTS, R. E. The determination of amino acids with ninhydrin. **Analyst**, v. 80, n. 948, p. 209-214, 1955. doi: https://doi.org/10.1039/AN9558000209

YOU, J. et al. Transcriptomic and metabolomic profiling of drought-tolerant and susceptible sesame genotypes in response to drought stress. **BMC plant biology**, v. 19, n. 1, p. 1-16, 2019. doi: https://doi.org/10.1186/s12870-019-1880-1

ZAHEDI, S. M. et al. Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. **Scientific reports**, v. 10, n. 1, p. 17672, 2020. doi: https://doi.org/10.1038/s41598-020-74273-9

ZENTGRAF, U.; ANDRADE-GALAN, A. G.; BIEKER, S. Specificity of H2O2 signaling in leaf senescence: is the ratio of H2O2 contents in different cellular compartments sensed in Arabidopsis plants?. **Cellular & Molecular Biology Letters**, v. 27, n. 1, p. 1-19, 2022. doi: https://doi.org/10.1186/s11658-021-00300-w

ZHANG, X. et al. Effects of drought stress and water recovery on physiological responses and gene expression in maize seedlings. **BMC plant biology**, v. 18, n. 1, p. 1-16, 2018. doi: https://doi.org/10.1186/s12870-018-1281-x

ZHANG, Y. et al. Superior iodine uptake capacity enabled by an open metal-sulfide framework composed of three types of active sites. **CCS Chemistry**, v. 5, n. 7, p. 1540-1548, 2023. doi: https://doi.org/10.31635/ccschem.022.202201966

ZHANG, Y. et al. Prediction and utilization of malondialdehyde in exotic pine under drought stress using near-infrared spectroscopy. **Frontiers in Plant Science**, v. 12, p. 735275, 2021. doi: https://doi.org/10.3389/fpls.2021.735275