JUCELINO DE SOUSA LIMA

IODINE APPLICATION AS A STRATEGY FOR MITIGATING WATER DEFICIT STRESS IN PLANTS

LAVRAS – MG
2023

JUCELINO DE SOUSA LIMA

IODINE APPLICATION AS A STRATEGY FOR MITIGATING WATER DEFICIT STRESS IN PLANTS

Thesis presented to the Federal University of Lavras, as part of the requirements of the Graduate Program in Plant Physiology area of concentration in Plant physiology to obtain the title of Doctor.

Professor Luiz Roberto Guimarães Guilherme,
Ph.D.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).

lima, Jucelino de Sousa.
Iodine application as a strategy for mitigating water deficit stress in plants / Jucelino de Sousa lima. - 2013.
78p.

Orientador(a): Luiz Roberto Guimarães Guilherme.
Coorientador(a): Vitor de Laia Nascimento, Paulo Eduardo Ribeiro Marchiori.
Tese (doutorado) - Universidade Federal de Lavras, 2013.
Bibliografia.

1. Plant Physiology. 2. Beneficial elements. 3. Drought stress. I. Guilherme, Luiz Roberto Guimarães. II. Nascimento, Vitor de Laia. III. Marchiori, Paulo Eduardo Ribeiro. IV. Título.

JUCELINO DE SOUSA LIMA

IODINE APPLICATION AS A STRATEGY FOR MITIGATING WATER DEFICIT STRESS IN PLANTS

APLICAÇÃO DE IODO COMO ESTRATÉGIA PARA MITIGAÇÃO DO ESTRESSE DO DÉFICIT HÍDRICO NAS PLANTAS

Thesis presented to the Federal University of Lavras, as part of the requirements of the Graduate Program in Plant Physiology in Plant physiology, to obtain the title of Doctor.

APPROVED on December 18, 2023.

D. Sc. Paulo Eduardo Ribeiro Marchiori	UFLA – MG
D. Sc. João Paulo Delfin Barbosa	UFLA – MG
D. Sc. Debora Domiciano	CTC – SP
D. Sc. José Lavres Junior	CENA/USP – SP

Professor Ph.D. Luiz Roberto Guimarães Guilherme Advisor
Professor D.Sc. Vitor de Laia Nascimento
Co-advisor
D. Sc. Paulo Eduardo Ribeiro Marchiori
Co-advisor

LAVRAS – MG
2023

To God, who has always blessed me. To my family and friends, who have consistently supported and helped me get to this point.
I dedicate!

ACKNOWLEDGMENTS

First and foremost, to God for allowing me to savor this experience with great discernment. To my entire family, who has always been by my side, offering support and encouragement. To my coursemates and lab colleagues who assisted me throughout this journey. To my advisor and co-advisors for their unwavering support. To the Plant Physiology Postgraduate Program at UFLA for the opportunity. To the Biology and Soil Science departments for providing laboratory facilities and structures for research development. To the University of Lavras for their hospitality and opportunities. To CAPES for funding the research and the scholarship. To CNPq and INCT Soil & Food Security for the research funding.

THANK YOU!

GENERAL ABSTRACT

As ongoing climate changes, marked by irregular precipitation patterns, pose a severe threat to food security, raising significant concerns about their impact on the food chain. Water deficit emerges as a critical factor for global agricultural stability and productivity. This deficit is a complex stressor affecting various morphophysiological characteristics throughout growth stages, resulting in considerable economic losses. Consequently, there is a growing exploration of new technologies and management practices to mitigate the damages caused by water deficit. The exogenous application of chemical elements has been considered a promising strategy to alleviate water deficit stress in plants, with special attention to elements classified as beneficial, such as selenium and silicon. However, certain elements in this category, like iodine, have been underexplored, despite demonstrating potential in mitigating other environmental stresses, such as saline stress and heavy metals. Thus, we conducted studies to assess the effectiveness of iodine applied in the form of potassium iodide (KI) via nutrient solution in mitigating water deficit in tomato and soybean plants. In the first experiment, we subjected tomato plants to water deficit and three concentrations of KI (0, 50, and 100 μM KI). We observed that exposing tomato plants to a concentration of 100 μM KI reduced oxidative damage caused by water deficit, increased photosynthetic efficiency, productivity, and improved fruit quality. In the second experiment, we applied increasing concentrations of KI (0, 10, 20, and 40 μM KI) to soybean plants grown under water deficit. It was possible to observe that KI at low concentrations (10 μM KI) promoted greater tolerance to water deficit in soybean plants, increasing photosynthetic efficiency, enzymatic antioxidant protection, and consequently biomass accumulation. However, at high concentrations (40 μM KI), phytotoxicity occurred, resulting in a reduction in the photosynthetic rate and biomass accumulation. We concluded from both studies that, when applied at the ideal concentration, iodine can increase plant tolerance to water deficit, inducing enzymatic antioxidant responses and promoting photosynthetic gains, which, in turn, lead to increased productivity. Additionally, this element also demonstrated the potential to improve fruit post-harvest quality. Our findings not only highlight the immediate benefits of iodine supplementation in plants grown under water deficiency but also elucidate some of the processes through which this element operates under these conditions. These studies represent a significant scientific advancement, providing a comprehensive understanding of iodine's multifaceted contributions to plant adaptation in water-scarce environments. Our work not only contributes to the scientific community but also offers transformative applications in agriculture, fostering a discourse on ensuring food security through innovative techniques.

KEYWORDS: Drought stress; Food security; Potassium iodide; Beneficial elements.

RESUMO GERAL

As mudanças climáticas em curso, marcadas por padrões irregulares de precipitação, representam uma séria ameaça à segurança alimentar, levantando preocupações significativas sobre seu impacto na cadeia alimentar. A deficiência de água surge como um fator crítico para a estabilidade e produtividade agrícola global. Essa deficiência é um estressor complexo que afeta várias características morfofisiológicas ao longo das fases de crescimento, resultando em consideráveis perdas econômicas. Consequentemente, há uma crescente exploração de novas tecnologias e práticas de manejo para mitigar os danos causados pela deficiência hídrica. A aplicação exógena de elementos químicos tem sido considerada uma estratégia promissora para aliviar o estresse de deficiência hídrica em plantas, com atenção especial a elementos classificados como benéficos, como selênio e silício. No entanto, certos elementos nesta categoria, como o iodo, têm sido pouco explorados, apesar de demonstrarem potencial para mitigar outros estresses ambientais, como estresse salino e metais pesados. Assim, conduzimos estudos para avaliar a eficácia do iodo aplicado na forma de iodeto de potássio (KI) via solução nutritiva na mitigação da deficiência hídrica em plantas de tomate e soja. No primeiro experimento, submetemos plantas de tomate à deficiência hídrica e três concentrações de KI (0, 50 e 100 μM KI). Observamos que expor plantas de tomate a uma concentração de 100 μM KI reduziu os danos oxidativos causados pela deficiência hídrica, aumentou a eficiência fotossintética, a produtividade e melhorou a qualidade dos frutos. No segundo experimento, aplicamos concentrações crescentes de KI (0, 10, 20 e 40 μM KI) a plantas de soja cultivadas sob deficiência hídrica. Foi possível observar que o KI em baixas concentrações (10 μM KI) promoveu maior tolerância à deficiência hídrica em plantas de soja, aumentando a eficiência fotossintética, a proteção antioxidante enzimática e, consequentemente, a acumulação de biomassa. No entanto, em concentrações elevadas (40 μM KI), ocorreu fitotoxicidade, resultando na redução da taxa fotossintética e da acumulação de biomassa. Concluímos a partir de ambos os estudos que, quando aplicado na concentração ideal, o iodo pode aumentar a tolerância das plantas à deficiência hídrica, induzindo respostas antioxidantes enzimáticas e promovendo ganhos fotossintéticos, o que, por sua vez, leva a uma maior produtividade. Além disso, esse elemento também demonstrou o potencial de melhorar a qualidade pós-colheita dos frutos. Nossas descobertas não apenas destacam os benefícios imediatos da suplementação de iodo em plantas cultivadas sob deficiência hídrica, mas também elucidam alguns dos processos pelos quais esse elemento opera nessas condições. Esses estudos representam um avanço científico significativo, fornecendo uma compreensão abrangente das contribuições multifacetadas do iodo para a adaptação das plantas em ambientes escassos de água. Nosso trabalho não apenas contribui para a comunidade científica, mas também oferece aplicações transformadoras na agricultura, promovendo um diálogo sobre a segurança alimentar por meio de técnicas inovadoras.

PALAVRAS-CHAVE: Deficit hídrico; Segurança alimentar; Iodeto de potássio; Elementos benéficos

SUMMARY

FIRST PART

1 GENERAL INTRODUCTION

As a result of climate change, water deficit has become one of the most common and detrimental abiotic stresses for plant growth, development, and productivity (RUIZ-LOZANO et al., 2016; SOURI et al., 2020). Forecasts suggest a heightened severity of this stress in forthcoming years, with anticipated surface temperature rises of up to 2.2°C over the next century. These increases are expected to diminish soil moisture levels, thereby exacerbating drought conditions for plants (WANG et al., 2017). In a scenario where 9.2% of the world's population is affected by hunger, representing about 800 million people (FAO, 2023), the need to enhance food production during adverse climatic conditions becomes increasingly critical.

The physiological disruptions associated with cell dehydration, excessive reactive oxygen species, lipid membrane peroxidation, and reduced photosynthetic rates (MANSOOR et al., 2022; XIE et al., 2018; XU et al., 2022) contribute significantly to decreased crop yields during water deficit. Photosynthesis, a vital process for plant growth, dry matter accumulation, and productivity, is notably impacted by water scarcity-induced stomatal closure, leading to decreased enzyme activities like Rubisco and PEPCase. This reduction in enzyme activity directly affects the plant's carbon dioxide assimilation capacity (XU et al., 2022). Consequently, the equilibrium between the light reaction and carbon dioxide fixation pathways is disrupted, resulting in excessive energy excitation in leaves and oxidative damage to photosystem II (PSII). This impairment further diminishes the photosynthetic capacity and inhibiting leaf metabolism (AHANGER et al., 2021; AHMAD et al., 2018; RAJA et al., 2022).

Plants possess mechanisms to adjust morphophysiological and biochemical functions, aiming to alleviate the deleterious impacts of water deficit stress. These adjustments involve intensified activity of antioxidant enzymes to remove reactive oxygen species, accumulation of compatible osmolytes for cellular protection and water potential maintenance, and modifications in gas exchange to enhance water use efficiency (AHANGER and AGARWAL, 2017; BEGUM et al., 2020; KAYA et al., 2020). However, the natural defense systems of plants against water deficit may not be fully effective. Consequently, the development of new technologies and management practices that aid these defenses becomes imperative.

The utilization of exogenous substances, particularly chemical elements, to alleviate the adverse effects caused by water deficit has emerged as a common and intriguing practice. Non-essential elements like silicon (Si), selenium (Se), and more recently, iodine (I), have displayed promising outcomes in mitigating various environmental stresses across diverse plant species

(ALI et al., 2018; BEZERRA et al., 2019; KIFERLE et al., 2022; RAVELLO et al., 2021; SOUSA et al., 2022; LIMA et al., 2023). Although these elements aren't traditionally classified as essential for plant nutrition, they are deemed crucial for human nutrition and are regarded as beneficial elements for plants. They have been observed to enhance crop productivity, especially under adverse conditions (BROWN et al., 2022; NASCIMENTO et al., 2020; RENGEL et al., 2022).

Iodine (I), despite being a relatively recent focus in plant studies, still lacks a comprehensive understanding of its application and functionalities. Kiferle et al. (2021) suggested that iodine might form part of the protein composition, contributing to the formation of iodinated proteins, particularly bound to chloroplasts, and involved in photosynthetic processes. Various investigations have indicated a significant increase in the activity of antioxidant enzymes with iodine supplementation (BLASCO et al., 2011; GUPTA et al., 2015; KIFERLE et al., 2022). Moreover, several studies have established a positive correlation between the amount of iodine and the antioxidant capacity of plants (BLASCO et al., 2013; INCROCCI et al., 2019; KIFERLE et al., 2019).

Nonetheless, investigations on iodine's effects concerning protection against water deficit have been limited (JAFARIAM et al., 2020). Our recent studies have unveiled the potential of iodine as a mitigator of water deficit stress in soybeans (LIMA et al., 2023a) and tomatoes (LIMA et al., 2023b). Under these growth conditions, iodine prompted heightened enzymatic antioxidant protection, diminishing oxidative damage, and increasing plant photosynthetic efficiency, thereby ensuring greater biomass accumulation and increased productivity. Remarkably, iodine supplementation improved tomato fruit quality by elevating antioxidant properties while reducing the ripening index. These studies underscore the potential of iodine in agricultural applications, offering enhanced tolerance to adverse conditions and elevated nutritional quality, either through direct biofortification or indirect post-harvest quality improvement pathways.

Recently, the Ministry of Agriculture, Livestock and Supply/Animal and Plant Health and Inspection Secretariat, through Normative Instruction No. 61 issued on July 8, 2020, classified Se, and Si as micronutrients, promoting their utilization in agriculture due to their significant agricultural and societal advantages. Considering iodine's demonstrated benefits across various crops and its essentiality for human health, its potential inclusion in this classification is vital. This step would encourage iodine's agricultural use, enhancing not only plant productivity under adverse conditions but also the production of high-quality food, ensuring societal food security. Brown et al. (2022) pointed out that exploring and integrating

new elements into agriculture will not only provide greater opportunities for the fertilizer industry in terms of product innovation and collaborative research but also enable farmers to comprehensively understand the integrated roles of plant nutrients in stress tolerance, resource utilization, crop quality, and overall system sustainability.

In summary, the challenges presented by water deficit in plants, exacerbated by climate change, demand innovative and sustainable agricultural practices. Our research contributes significantly by delving into the physiological and biochemical impacts of iodine under water deficit conditions in economically relevant plants, offering a promising strategy. Expanding our understanding of unconventional elements and integrating them sustainably into agricultural practices remains crucial. Our findings not only highlight the necessity for innovation in agricultural research but also underscore how such advancements are pivotal in ensuring food security and advancing global sustainability.

2 GENERAL OBJECTIVES

- Evaluation of the Efficacy of Iodine application in alleviating water deficiency in plants;
- Assessing the physiological and biochemical processes induced by iodine in plants under water deficit;
- Investigating the optimal threshold of iodine concentration for mitigating water deficit.
- Verify at which time the major metabolic responses induced by iodine occurred.

3 GENERAL HYPOTHESES

- When applied in ideal concentration, Iodine increases tolerance to water deficit in plants, through increased antioxidant defenses and photosynthetic gains;
- Iodine not only increases water deficit tolerance, but also improves fruit quality under these conditions.
- The increase in tolerance to water deficit induced by iodine occurs due to a priming effect before stress.

REFERENCES

AHANGER, M. A.; AGARWAL, R. M. Potassium up-regulates antioxidant metabolism and alleviates growth inhibition under water and osmotic stress in wheat (*Triticum aestivum* L). **Protoplasma**, v. 254, n. 4, p. 1471–1486, 1 jul. 2017.

AHANGER, M. A. et al. Improving growth and photosynthetic performance of drought stressed tomato by application of nano-organic fertilizer involves up-regulation of nitrogen, antioxidant and osmolyte metabolism. **Ecotoxicology and Environmental Safety**, v. 216, p. 112195, 15 jun. 2021.

AHMAD, P. et al. Silicon (Si) Supplementation Alleviates NaCl Toxicity in Mung Bean [*Vigna radiata* (L.) Wilczek] Through the Modifications of Physio-biochemical Attributes and Key Antioxidant Enzymes. **Journal of Plant Growth Regulation**, v. 38, n. 1, p. 70–82, 1 mar. 2019.

ALI, N. et al. Regulatory Role of Silicon in Mediating Differential Stress Tolerance Responses in Two Contrasting Tomato Genotypes Under Osmotic Stress. **Frontiers in Plant Science**, v. 9, 2018.

BEGUM, N.; AHANGER, M. A.; ZHANG, L. AMF inoculation and phosphorus supplementation alleviates drought induced growth and photosynthetic decline in Nicotiana tabacum by up-regulating antioxidant metabolism and osmolyte accumulation. **Environmental and Experimental Botany**, v. 176, p. 104088, 1 ago. 2020.

BEZERRA, B. K. L. et al. Physiological and biochemical impacts of silicon against water deficit in sugarcane. **Acta Physiologiae Plantarum**, v. 41, n. 12, p. 189, 16 nov. 2019.

BLASCO, B. et al. Does iodine biofortification affect oxidative metabolism in lettuce plants? **Biological Trace Element Research**, v. 142, n. 3, p. 831–842, 2011.

BROWN, P. H.; ZHAO, F.-J.; DOBERMANN, A. What is a plant nutrient? Changing definitions to advance science and innovation in plant nutrition. **Plant and Soil**, v. 476, n. 1, p. 11–23, 1 jul. 2022.

FAO - Food and Agriculture Organization of the United Nations. *The State of Food Security and Nutrition in the World.* Roma: 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 04 dez. 2023).

GUPTA, N. et al. Response of iodine on antioxidant levels of *Glycine max* L. grown under Cd^{2+} stress. **Advances in Biological Research**, v. 9, n. 1, p. 40–48, 2015.

HAYAT, S. et al. Role of proline under changing environments. **Plant Signaling & Behavior**, v. 7, n. 11, p. 1456–1466, 1 nov. 2012.

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, 2019.

JAFARIAN, S. et al. The Effect of Iodine On Increasing Drought Tolerance Of (*Carthamus* Tinctorius L.) In Seed Germination And Early Growth Stage. 6 abr. 2020.

KAYA, C. et al. Sulfur-enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (*Zea mays* L.). **Scientific Reports**, v. 10, n. 1, p. 6432, 14 abr. 2020.

KIFERLE, C. et al. Effect of Iodine treatments on *Ocimum basilicum* L.: Biofortification, phenolics production and essential oil composition. **PLoS One**, v. 14, n. 12, p. e0226559, 2019.

KIFERLE, C. et al. Evidences for a Nutritional Role of Iodine in Plants. **Frontiers in Plant Science**, v. 12, 2021.

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, 29 ago. 2022.

LIMA, J. DE 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, jan. 2023.

MANSOOR, S. et al. Reactive Oxygen Species in Plants: From Source to Sink. **Antioxidants**, v. 11, n. 2, p. 225, fev. 2022.

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, 22 mar. 2022.

RAJA, V. et al. Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses, and gene expression in Solanum lycopersicum. **3 Biotech**, v. 10, n. 5, p. 208, 23 abr. 2020.

RAVELLO, R. A. V. et al. Selenium application influenced selenium biofortification and physiological traits in water-deficit common bean plants. **Crop and Pasture Science**, 16 jul. 2021.

RENGEL, Z.; CAKMAK, I.; WHITE, P. J. **Marschner's Mineral Nutrition of Plants**. [s.l.] Academic Press, 2022.

RUIZ-LOZANO, J. M. et al. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. **Plant, Cell & Environment**, v. 39, n. 2, p. 441–452, 2016.

SOURI, Z. et al. Silicon and Plants: Current Knowledge and Future Prospects. **Journal of Plant Growth Regulation**, v. 40, n. 3, p. 906–925, 1 jun. 2021.

SOUSA, G. F. et al. Selenium enhances chilling stress tolerance in coffee species by modulating nutrient, carbohydrates, and amino acids content. **Frontiers in Plant Science**, v. 13, 2022.

WANG, J. et al. The implications of fossil fuel supply constraints on climate change projections: A supply-side analysis. **Futures**, v. 86, p. 58–72, 1 fev. 2017.

XIE, T. et al. Modulating the antioxidant system by exogenous 2-(3,4-dichlorophenoxy) triethylamine in maize seedlings exposed to polyethylene glycol-simulated drought stress. **PLOS ONE**, v. 13, n. 9, p. e0203626, 5 set. 2018.

XU, J.; GUO, L.; LIU, L. Exogenous silicon alleviates drought stress in maize by improving growth, photosynthetic and antioxidant metabolism. **Environmental and Experimental Botany**, v. 201, p. 104974, 1 set. 2022.

SECOND PART – ARTICLES

ARTICLE 1: Soybean Plants Exposed to Low Concentrations of Potassium Iodide Have Better Tolerance to Water Deficit through the Antioxidant Enzymatic System and Photosynthesis Modulation

Published in Plants

(https://doi.org/10.3390/plants1213255)

Jucelino de Sousa Lima[1], Otávio Vitor Souza Andrade[1], Leônidas Canuto dos Santos[2], Everton Geraldo de Morais[2], Gabryel Silva Martins[2], Yhan S. Mutz [2,3], Vitor L. Nascimento[1], Paulo Eduardo Ribeiro Marchiori[1], Guilherme Lopes[2], and Luiz Roberto Guimarães Guilherme[1*]

1 Department of Biology, Institute of Natural Sciences, Federal University of Lavras (UFLA).
2 Department of Soil Science, School of Agricultural Sciences, Federal University of Lavras (UFLA).
3 Department of Food Science, School of Agricultural Science, Federal University of Lavras (UFLA)

Abstract: Water deficit inhibits plant growth by affecting several physiological processes, which leads to the overproduction of reactive oxygen species (ROS) that may cause oxidative stress. In this regard, iodine (I) is already known to possibly enhance the antioxidant defense system of plants and promote photosynthetic improvements under adverse conditions. However, its direct effect on water deficit responses has not yet been demonstrated. To verify the efficiency of I concerning plant tolerance to water deficit, we exposed soybean plants to different concentrations of potassium iodide (KI) fed to pots with a nutrient solution and subsequently submitted them to water deficit. A decline in biomass accumulation was observed in plants under water deficit, while exposure to KI (10 and 20 µmol L^{-1}) increased plant biomass by an average of 40%. Furthermore, exposure to KI concentrations of up to 20 µM improved gas exchange (~71%) and reduced lipid peroxidation. This is related to the higher enzymatic antioxidant activities found at 10 and 20 µM KI concentrations. However, when soybean plants were properly irrigated, KI concentrations greater than 10 µM promoted negative changes in photosynthetic efficiency, as well as in biomass accumulation and partition. In sum, exposure of soybean plants to 10 µM KI improved tolerance to water deficit, and up to this concentration, there is no evidence of phytotoxicity in plants grown under adequate irrigation.

Keywords: iodine; abiotic stress tolerance; antioxidant defense.

Introduction

The rise in the world's population—which is expected to reach ~9.5 billion by 2050—requires continuous increases in crop production to ensure food security [1]. However, climate change is becoming one of the biggest threats to agricultural sustainability worldwide and, consequently, to the food supply chain, mainly due to increased temperatures that generate greater evapotranspiration and water scarcity [2]. Indeed, by 2050, water scarcity worldwide will create severe problems for plant growth and, consequently, yield, thus threatening global food security [3].

Under water deficit, one of the most typical consequences in leaves is the increase in reactive oxygen species (ROS) inducing lipid peroxidation [4,5]. Excessive accumulation of ROS causes the oxidation of nucleic acids, proteins, and lipids, which ultimately causes cellular dysfunction [6,7]. The excess production of ROS also causes disruption to the electron transport chain in the chloroplasts, as well as suppresses carbon fixation by inactivating the enzymes in the Calvin–Benson–Bassham cycle, resulting in a reduction in the photosynthetic rate and, consequently, in yield [6,8–10].

Among relevant crops cultivated globally, soybean (*Glycine max* (L.) Merrill) is one of the most economically important in the world, being the main cultivated oilseed and also an important protein source. Soybean is used by agroindustries in the production of vegetable oil as well as human and animal feed, in addition to being an alternative source for the manufacture of biofuels [11]. Brazil is the largest soybean producer in the world; data from the 2022/23 harvest indicate a planted area of 43,834.4 thousand ha^{-1}, a production of 154,810.7 thousand tons, and an average yield of 3.542 kg ha^{-1}, reaching historical records for planting area, production, and yield [12].

These high yields depend on several factors, such as water availability, which can be a crucial limitation to the expression of a crop's yield potential, especially in years with uneven distribution and a low volume of precipitation in non-irrigated crops [13]. Water deficit stress causes a decrease in the yield and quality of soybean, so strategies to mitigate this negative effect are needed [14]. The flowering stage is one of the most critical periods in terms of water supply, and a deficit at this time, depending on the intensity, can lead to premature flower drop, with a consequent decrease in the number of pods, one of the main productive components of this culture [13].

The exogenous application of beneficial elements has become an important strategy to partially mitigate the adverse effects of water deficit in plants. These elements are not

essential for survival, but they increase plant biomass and yield by stimulating various growth-promoting pathways, as well as helping to alleviate abiotic and biotic stresses [15–17]. Plant cells are generally protected by a complex antioxidant system, which may be enzymatic, including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and other peroxidases. SOD is the first line of defense against ROS, dismutating the radical superoxide (O_2^-) into peroxide (H_2O_2) and O_2, while the others prevent the formation of hydroxyl radicals (-OH), the most toxic and reactive radicals, which can react indiscriminately with all macromolecules [18]. Other works highlight the ubiquitous role of antioxidant enzymes in mitigating different abiotic stresses [19,20]. Recently, it was demonstrated that the exogenous application of iodine (I) reinforces the antioxidant capacity of lettuce, soybean, and tomato plants by stimulating the activity of the main ROS detoxifying enzymes, i.e., superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxide (POD) [18,21–23]. Higher antioxidant enzymatic activity in plants reduces oxidative damage promoted by ROS accumulation under water deficit [24,25]. Another point to highlight is the role of I in being covalently linked to at least 82 different proteins in the leaves and roots of Arabidopsis thaliana, and its presence in micromolar concentrations in a nutrient solution, which increased the accumulation of tomato plant biomass [26].

Iodine is currently classified as a non-essential element, but it is beneficial to plants, with the ability to increase tolerance to some types of stress, such as saline [16,26,27]. However, little is known about this beneficial effect on water deficit and on which mechanisms I can act to mitigate the resultant stress. Simultaneous evidence suggests that I can be used as a plant stimulant similar to silicon (Si), selenium (Se), and sodium (Na) [16]. Although there are indications of the beneficial effect of iodine on plants under conditions of abiotic stress (salinity and heavy metals, among others), there are no studies dealing with the effect of this element on plants under conditions of water deficit. Therefore, this study hypothesizes that exposure of soybean plants to I can improve plant tolerance to water deficit with stimulation of the enzymatic antioxidant system. Thus, the objective of the present study was to determine whether the application of I can improve the response to water deficit in soybean plants, increasing the enzymatic activity of the antioxidant system as well as the photosynthetic efficiency, thus improving the productive capacity of plants under water stress.

Results

Plant growth

The water deficit significantly affected ($p < 0.05$) the growth variables of soybean plants. However, the application of potassium iodide (KI) improved these variables under water deficit in a dose-dependent manner (Figure 1). Under good irrigation conditions, an average reduction of 56% in total dry mass (TDM) was observed when plants were supplemented with 40 µM of KI compared with the other treatments (Figure 1A). In addition, there was a 39% reduction in shoot dry mass (SDM) at the concentrations of 20 and 40 µM, compared with the other treatments (Figure 1A,B). However, under water deficit, mean increments of 43% in TDM and 37% in SDM were verified for plants treated with 10 and 20 µM KI.

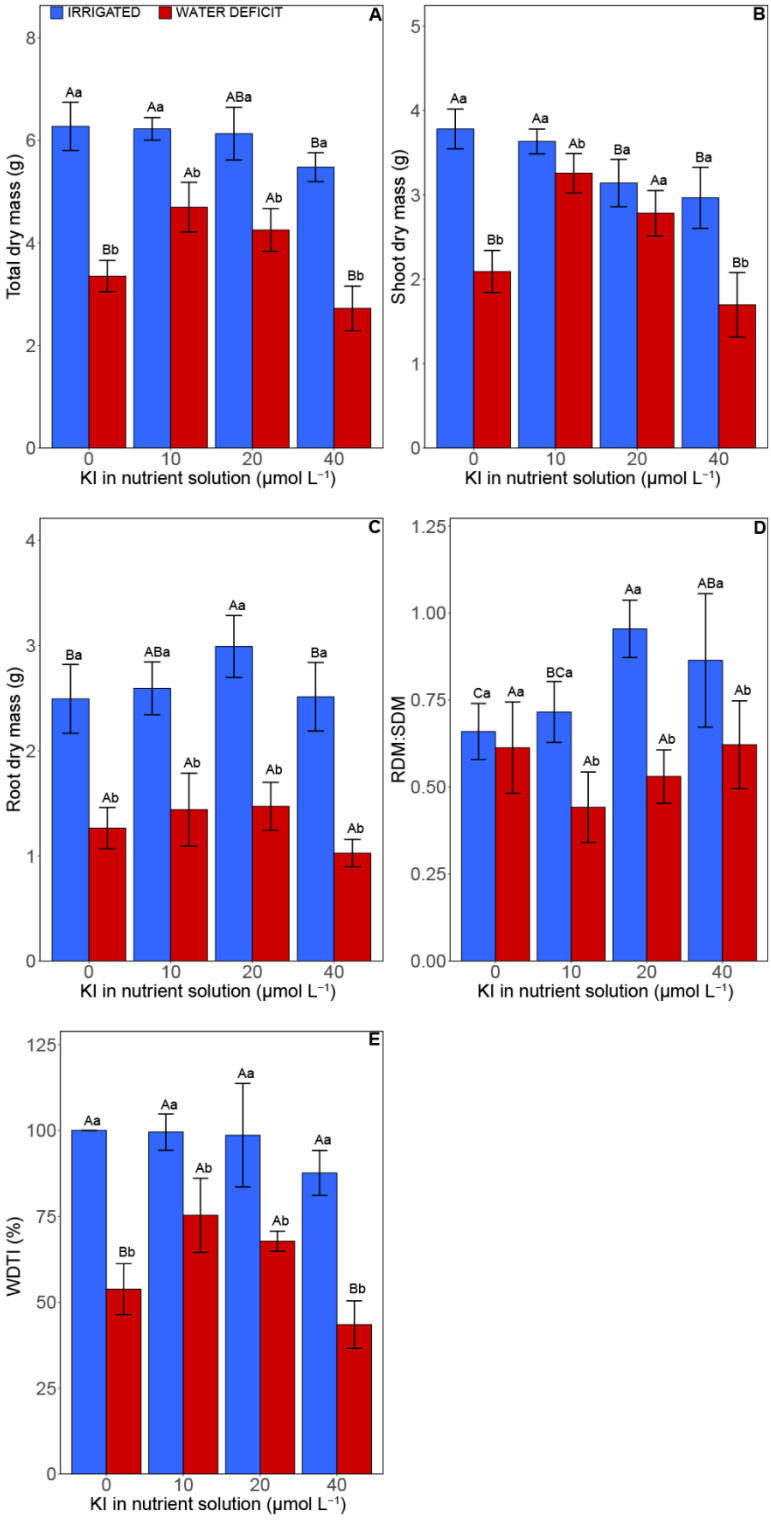
IRRIGATED
WATER DEFICIT
A
Total dry mass (g)
Aa
Bb
Aa
Ab
ABa
Ab
Ba
Bb
KI in nutrient solution (µmol L⁻¹)
B
Shoot dry mass (g)
Aa
Bb
Aa
Ab
Ba
Aa
Ba
Bb
KI in nutrient solution (µmol L⁻¹)
C
Root dry mass (g)
Ba
Ab
ABa
Ab
Aa
Ab
Ba
Ab
KI in nutrient solution (µmol L⁻¹)
D
RDM:SDM
Ca
Aa
BCa
Ab
Aa
Ab
ABa
Ab
KI in nutrient solution (µmol L⁻¹)
E
WDTI (%)
Aa
Bb
Aa
Ab
Aa
Ab
Aa
Bb
KI in nutrient solution (µmol L⁻¹)

Figure 1. Effect of KI on growth attributes of soybean plants under water deficit or not. (A) total dry mass, (B) Shoot dry mass, (C) root dry mass, (D) RDM:SDM—shoot root relationship, and (E) WDTI—water deficit tolerance index. Irrigated samples are represented with blue bars, on the left, while samples with water deficit with red bars, on the right. Values are presented as average ± SD (n = 5). Equal letters indicate no significant differences ($p > 0.05$) calculated using the Tukey test. Equal uppercase letters indicate no distinction among KI concentrations, while equal lowercase letters indicate no distinction between the irrigation conditions ($p > 0.05$).

When plants were treated with 20 µM of KI under conditions of full irrigation, the root dry mass (RDM) and root:shoot ratio (RDM:SDM) increased by 16% and 30%, respectively, compared with plants without KI (Figure 1C,D). It was also possible to observe that, under optimal irrigation, the root:shoot ratio improved by 23% in the treatment with 40 µM of KI compared with plants without KI, while under water suppression, no significant influence was observed between treatments with KI for RDM and RDM:SDM. For the water deficit tolerance index (WDTI), it was verified that KI concentrations did not significantly influence the plants with adequate irrigation (Figure 1E). However, under conditions of water suppression, the concentrations of 10 and 20 µM I increased the index by an average of 31% compared with the other treatments.

Leaf gas exchange

Leaf gas exchange variables were significantly influenced by water deficit and KI application ($p < 0.05$). Water deficit reduced the CO_2 assimilation rate (*A*), stomatal conductance (g_s), transpiration (*E*), and carboxylation efficiency (*CE*) irrespective of the applied KI concentration. On the other hand, under conditions of water deficit, an average increase of 86% and 71% in *A* was observed when plants were treated with 10 and 20 µM KI, respectively, whereas, in well-watered plants, a 70% reduction in A was observed with the application of 40 µM of KI (Figure 2A). When comparing all treatments under water deficit, the application of 10 µM KI promoted higher values of *A*, which reached 9.33 µmols CO_2 m^{-2} s^{-1}.

The g_s had an average increase of 47% in the concentration of 10 µM KI compared

with watered plants that received 10 and 40 µM KI (Figure 1B). Under water deficit, no significant difference was found for g_s between the treatments. For *E* under conditions of adequate irrigation, the highest value observed was in the treatment with 20 µM of KI (6.48 mmol H_2O m^{-2} s^{-1}) followed by the treatment with 10 µM (5.40 mmol H_2O m^{-2} s^{-1}) and without KI application (2.99 mmol H_2O m^{-2} s^{-1}), where all were significantly different from the KI 40 µmol treatment (1.69 mmol H2O m^{-2} s^{-1}) (Figure 2C). However, under water deficit, the concentration of 10 µM KI provided greater *E*, with an average increase of 73% when compared with the treatments of 0 and 40 µM KI. For the internal concentration of CO_2 (C_i), there was an increase of 16% for plants submitted to water deficit that did not receive the KI treatment when compared with the irrigated ones (Figure 1D). Under water deficit conditions, Ci was reduced by 14% with the application of 20 µM of I relative to plants without the use of KI. Regarding water use efficiency (WUE), there was an average reduction of 42% when soybean plants were supplemented with KI under adequate irrigation. On the other hand, under conditions of water deficit, concentrations of 10 and 20 µM KI increased WUE by 49% when compared with the other treatments, while for CE, an average reduction of 77% was observed when applying 40 µM KI under adequate irrigation conditions. When subjected to water deficit, increases of 86% and 73% in CE were observed for plants supplemented with 10 and 20 µM I, respectively, in comparison with the other treatments. It can also be highlighted that under water deficit, the plants treated with 10 µM KI had the highest *CE* (0.26 µmol CO_2 m^{-2} s^{-1} Pa^{-1}) among all the evaluated treatments.

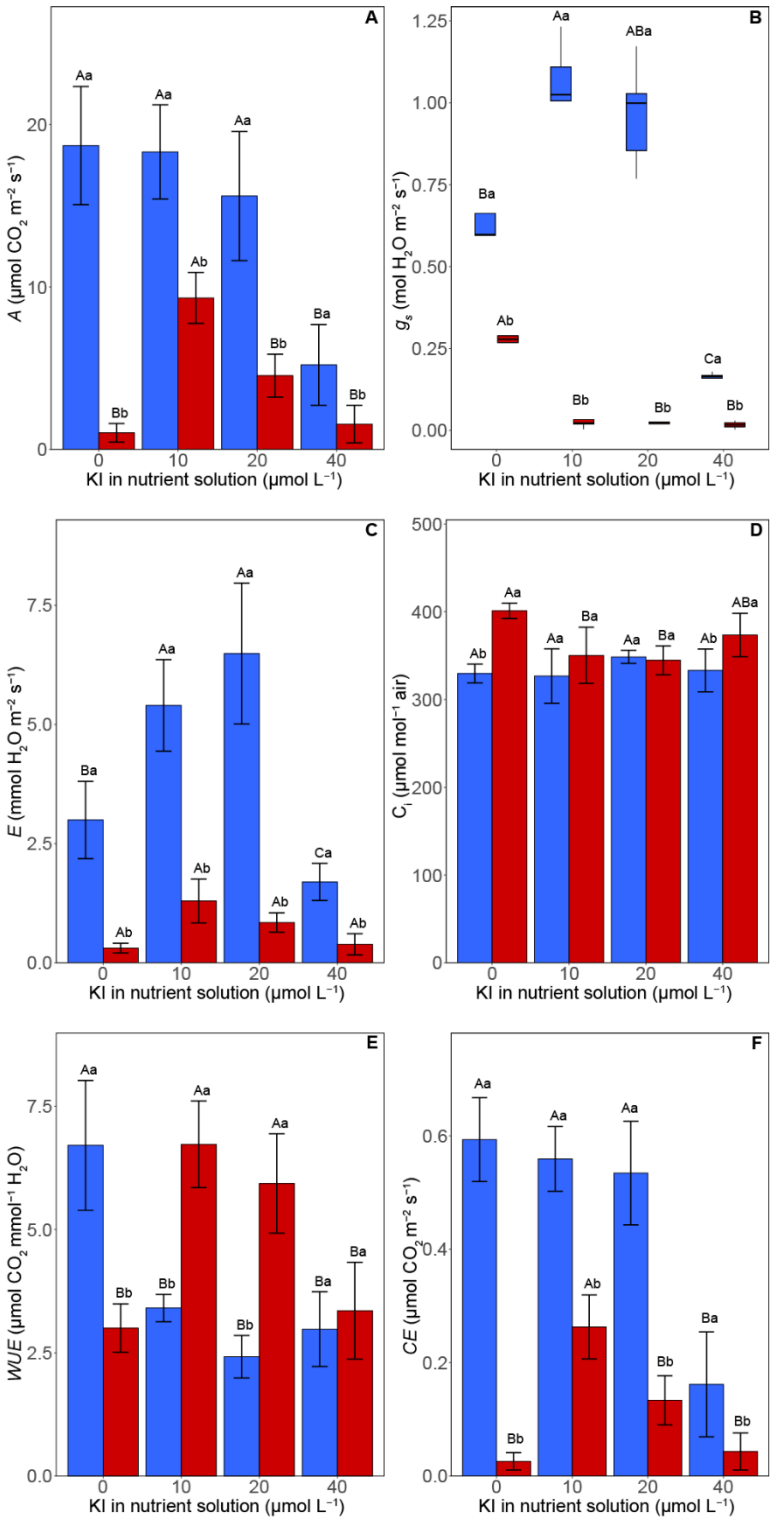

Figure 2. Effect of KI on gas exchange attributes of soybean plants under well-watered conditions (blue columns, on the left) and water deficit (red columns, on the right) one day after rehydration. (A) *A*—CO_2 assimilation rate, (B) g_s—stomatal conductance, (C) *E* - transpiration, (D) C_i—internal CO_2 concentration, (E) *WUE*—water use efficiency, and (F) *CE* — carboxylation efficiency. Values are presented as average ± SD (n = 5). Equal letters indicate no significant differences calculated using the Tukey test ($p > 0.05$). Different

uppercase letters indicate differences among KI concentrations, while distinct lowercase letters indicate differences between irrigation conditions. Graphs represented in the boxplot indicate that the data did not meet the assumptions of normality and homogeneity of variance, requiring a transformation by rank.

Oxidative damage

Malondialdehyde (MDA) content was significantly increased ($p < 0.05$) with water deficit irrespective of the applied KI dose (Figure 3A). However, under water deficit conditions, an average reduction of 18% in MDA was observed when plants were exposed to 10 and 20 µM I relative to plants grown without KI application.

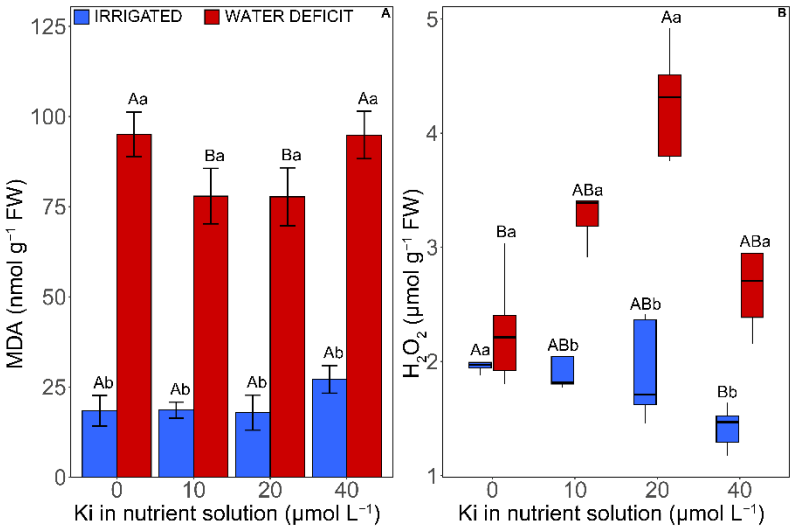

Figure 3. Effect of KI on oxidative damage attributes of soybean plants under well-watered conditions (blue columns, on the left) and water deficit (red columns, on the right) one day before rehydration. (A) MDA—malondialdehyde and (B) H2O2 —hydrogen peroxide. Values are presented as average ± SD (n = 5). Equal letters indicate no significant differences calculated using the Tukey ($p > 0.05$). Different uppercase letters represent statistical differences ($p < 0.05$) among KI concentrations, while lowercase letters indicate differences between irrigation conditions. Graphs represented in the boxplot indicate that the data did not meet the assumptions of normality and homogeneity of variance, requiring a transformation by rank.

Similarly, water deficit and KI concentrations significantly ($p < 0.05$) affected H_2O_2 content (Figure 3B). For adequate irrigation, treatment with 40 µM KI reduced the concentration of hydrogen peroxide (H_2O_2) by 28%, compared with plants without KI. However, under water deficit, a 46% increase was observed when the plants were exposed to

20 µM I compared with plants without KI under the same irrigation conditions. It is worth highlighting that when treated with KI (10, 20, and 40 µM) and subjected to water deficit, H_2O_2 increased by ~49% compared with irrigated plants cultivated with the same KI concentrations.

Antioxidant enzymatic activity

The antioxidant enzymatic activity was significantly influenced ($p < 0.05$) by both water deficit and KI concentrations (Figure 4). For adequate irrigation, there was an increase in SOD activity—corresponding to 53% and 70%—when plants received 10 and 20 µM KI, respectively, compared with the other treatments (Figure 4A). It can be highlighted that the highest SOD activity was found in the treatment with 20 µM (9.70 U mg^{-1} protein min^{-1}). Under water deficit, the activity of this enzyme increased by 69% when plants did not receive KI compared with their irrigated counterparts. However, when plants suffering water deficit were treated with 20 µM KI, an increase of 36% was observed compared with the plants without KI and with 10 µM, and 87% when compared with the treatment with 40 µM KI.

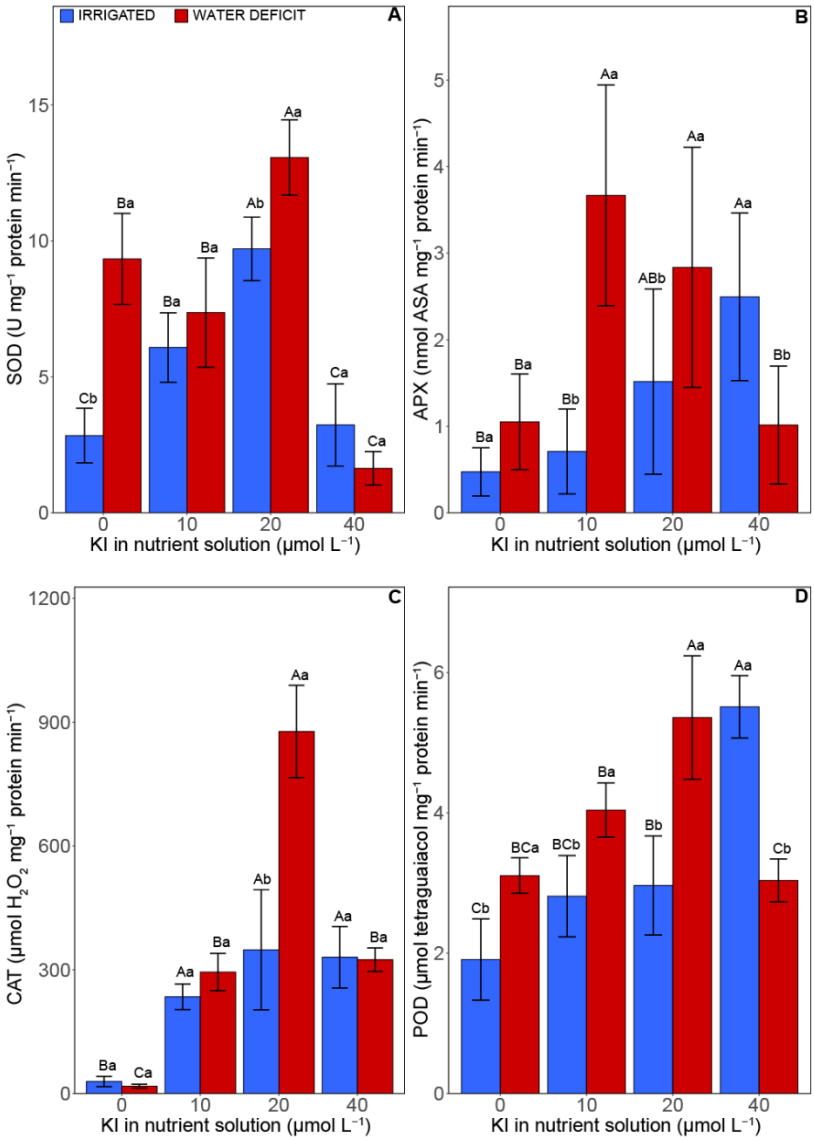

Figure 4. Effect of KI on antioxidant enzymatic activity attributes of soybean plants under well-watered conditions (blue columns, on the left) and water deficit (red columns, on the right) one day before rehydration. (A) (SOD—superoxide dismutase), (B) APX—ascorbate peroxidase, (C) CAT—catalase, and (D) POD—guaiacol peroxidase. Blue bars are irrigated samples and red bars are water deficit samples. Values are presented as average ± SD (n = 5). Equal letters indicate no significant differences calculated using the Tukey test ($p > 0.05$). Different uppercase letters represent statistical differences ($p < 0.05$) among KI concentrations, while lowercase letters indicate differences between irrigation conditions.

Regarding APX activity, plants supplemented with 40 µM KI and under optimal irrigation exhibited an average increase of 76% in relation to plants without and with 10 µM KI (Figure 4B). However, under water deficit, an average increase of 68% was observed for concentrations of 10 and 20 µM KI compared with the other treatments.

Under well-irrigated conditions, exposure to I increased the activity of CAT by an average of 90% compared with plants without KI. Under conditions of water deficit, there was an increase of 94% when plants received the treatment with 10 and 40 µM KI and 97% in the treatment with 20 µM when compared with the plants without KI. Notably, the highest CAT activity under water deficit conditions was observed in plants supplemented with 20 µM KI (877.29 µmols H_2O_2 mg^{-1} $protein^{-1}$) (Figure 4C).

POD activity increased between 35% and 65% when plants were treated with 20 and 40 µM KI and adequately irrigated, relative to plants without KI (Figure 4D). In the same irrigation condition, the treatment with 40 µM KI provided greater enzyme activity (5.51 µmols tetraguaiacol mg^{-1} protein min^{-1}). However, under water stress, the highest POD activity was related to the application of 20 µM KI (5.35 µmols tetraguaiacol mg^{-1} protein min^{-1}), which improved the activity of this enzyme by 42% compared with plants without KI and with 40 µM, in addition to 26% compared with treatment with 10 µM KI.

Proline

Soybean plants had significant changes ($p < 0.05$) in proline content in relation to the two studied factors (water deficit and KI application) (Figure 5). Under adequate irrigation, average increases of 55% were observed when plants were grown with 20 and 40 µM KI compared with plants without and with 10 µM KI. Proline contents increased under deficient irrigation compared with their adequate irrigation condition counterparts, regardless of the applied KI concentration. It is noteworthy that the highest concentration of proline in plants under water deficit was found in plants without KI (2.56 mg g^{-1} FW).

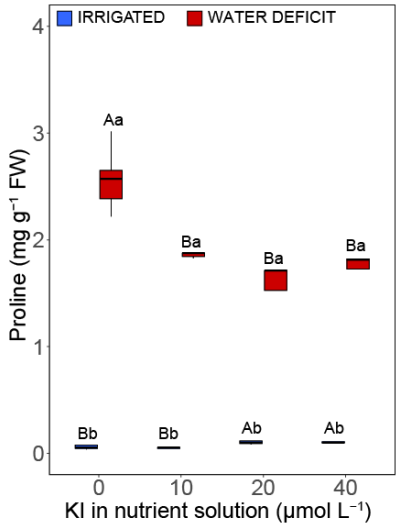

Figure 5. Effect of KI on content proline of soybean plants under well-watered conditions

(blue columns, on the left) and water deficit (red columns, on the right) one day before rehydration. Values are presented as average ± SD (n = 5). Equal letters indicate no significant differences calculated using the Tukey test ($p > 0.05$). Different uppercase letters represent statistical differences ($p < 0.05$) among KI concentrations, while lowercase letters indicate differences between irrigation conditions. Graphs represented in the boxplot indicate that the data did not meet the assumptions of normality and homogeneity of variance, requiring a transformation by rank.

Multivariate analyses

A principal component analysis (PCA) was performed using the morphological, physiological, and biochemical characteristics of soybean cultivated with adequate irrigation. The first two principal components (PC1 and PC2) presented 66.53% of the total data variance (Figure 6). The biplot for optimal irrigation conditions revealed a strong relationship between the treatment without KI and SDM and *WUE*. These two variables correlated positively and negatively with APX and POD activities, which were positively correlated with the highest KI dose (40 µMKI). On the other hand, the treatment with 10 µM I tended to favor TDM, SDM, *A*, *CE*, g_s, and H_2O_2, which had a positive correlation between themselves and a negative correlation with POD. Furthermore, TDM, SDM, *A*, and *CE* were negatively correlated with APX. Lastly, *A*, *CE*, and g_s also had a negative correlation with MDA. MDA tended to be favored by the 40 µM KI concentration. Further, MDA correlated negatively with SOD and *E*, which were positively correlated and tended to be favored by the treatment with 20 µM KI. In addition, RDM also tended to be favored by the 20 µM KI concentration and was positively correlated with SOD and *E*.

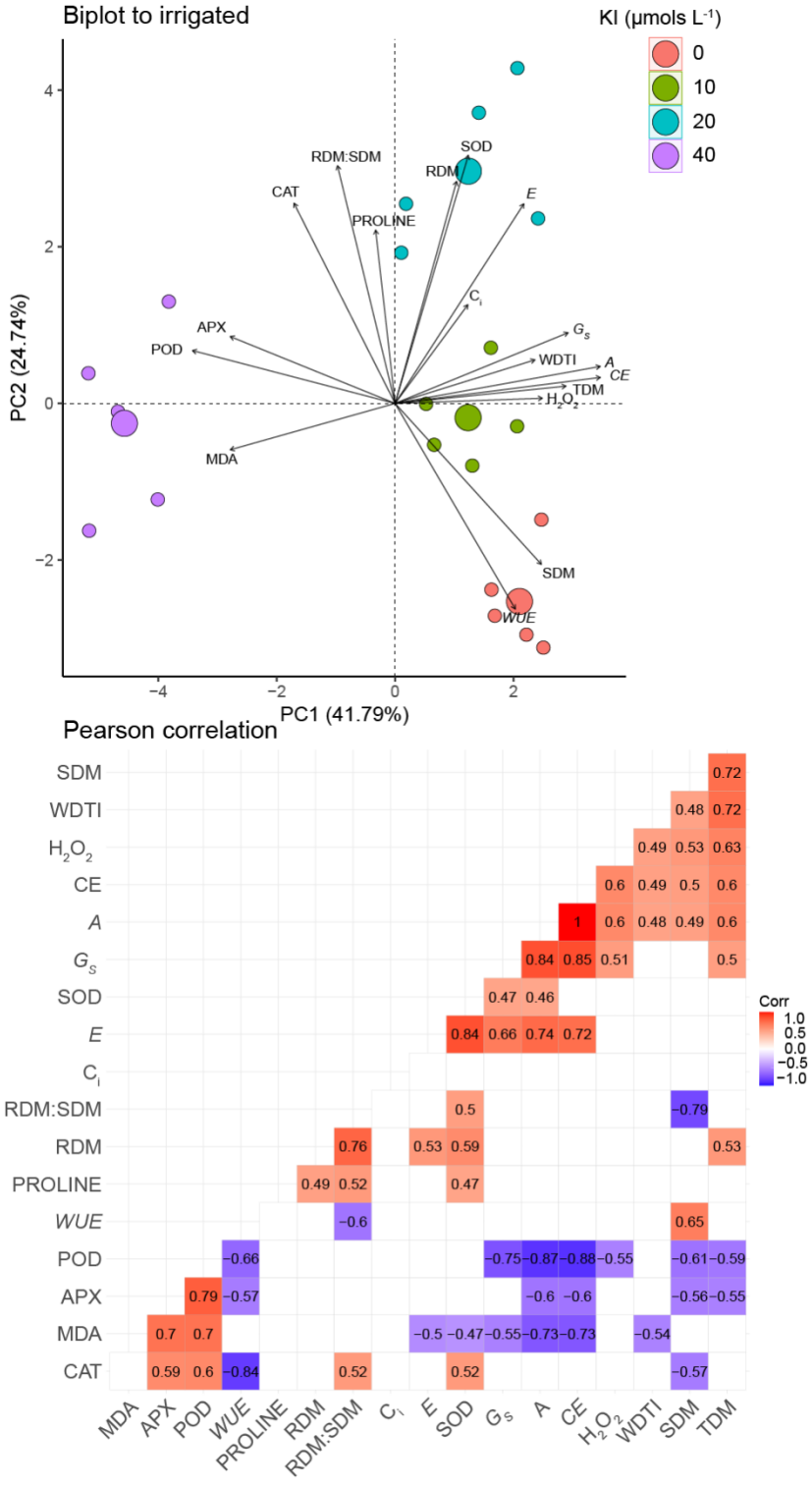

Figure 6. Principal component analysis and Pearson correlations using morphophysiological and biochemical data on soybean plants in response to well-watered conditions and KI application. Significant correlation coefficients ($q < 0.01$) are indicated with bold numbers, where positive and negative correlations are distinguished with red and blue, respectively. Non-significance is indicated with white boxes without numbers. TDM—total dry mass; SDM—shoot dry mass; RDM—root dry mass; WDTI—water index tolerance; H_2O_2—

hydrogen peroxide; MDA—malondialdehyde; *A*—CO_2 assimilation rate; g_s—stomatal conductance; C_i—internal concentration of CO_2; *E*—transpiration; *WUE*—water use efficiency; *CE*—carboxylation efficiency; POD—guaiacol peroxidase; APX—ascorbate peroxidase; CAT—catalase.

The cumulative variance in PC1 and PC2 under water deficit conditions was 68.43% (Figure 7). The concentration of 10 µM KI tended to be positively correlated with most of the analyzed variables, including WDTI, SMD, TDM, *A*, *E*, *CE*, and APX, which had positive correlations with each other. Furthermore, such concentration also indicated a favoring in RDM correlated positively with WDTI, TDM, and SDM. The treatment without KI, on the other hand, tended to correlate with proline, Ci, and gs, which correlated positively with each other and negatively with *WUE*. *WUE* presented a correlation with the treatment with 20 µM KI, which also favored POD and H_2O_2, with a positive correlation between all the mentioned variables. Furthermore, all these variables mentioned above for the 20 µM KI treatment had a negative correlation with MDA, which is favored by treatments without KI and with 40 µM KI. It is also noteworthy that MDA had a negative correlation with most variables that tended to be favored by the 10 µM KI concentration (WDTI, TDM, SDM, RDM, *A*, *E*, *CE*, and APX).

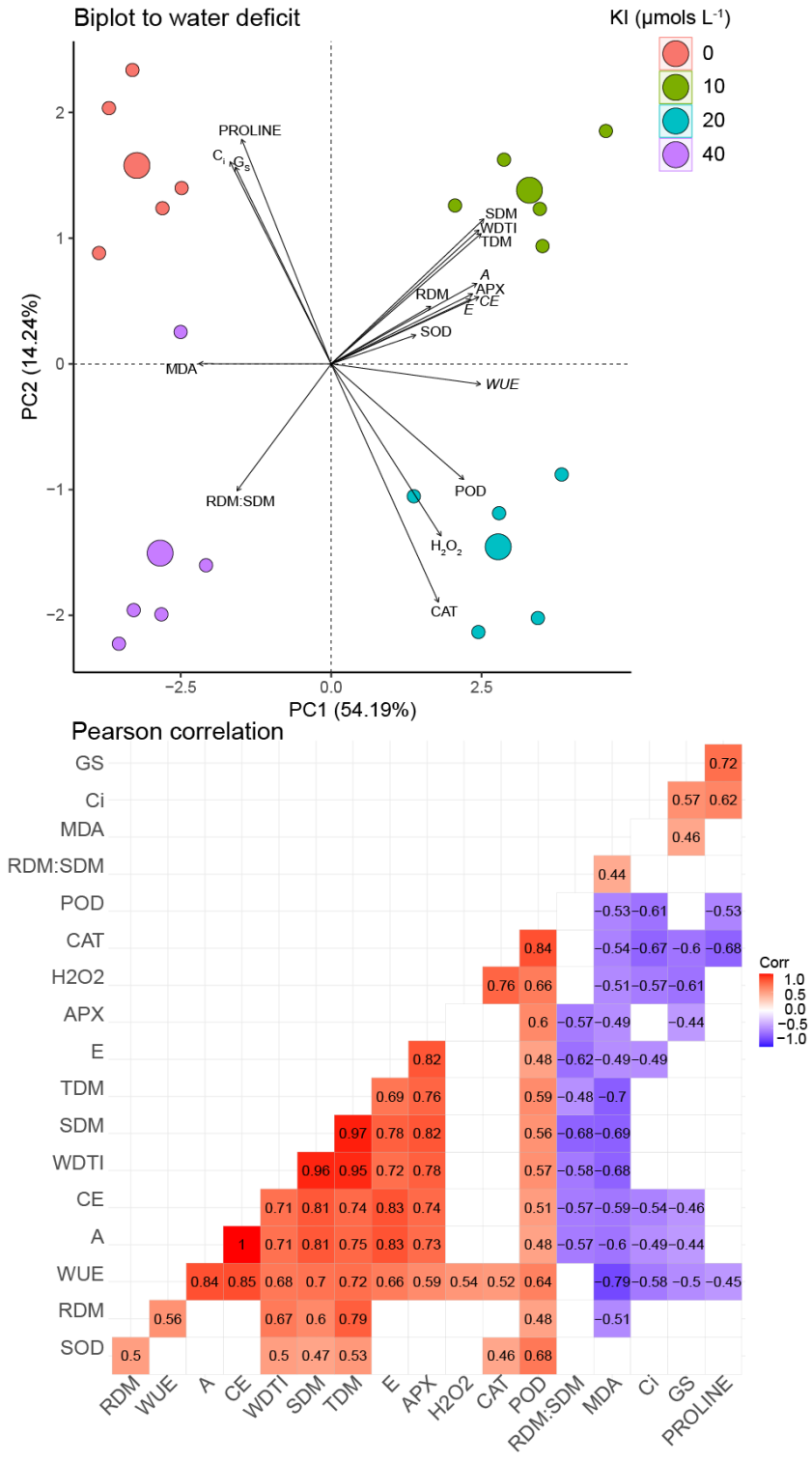

Figure 7. Principal component analysis and Pearson correlations using morphophysiological and biochemical data on soybean plants in response to water deficit and KI application. Significant correlation coefficients ($q < 0.01$) are indicated with bold numbers, where positive and negative correlations are distinguished with red and blue, respectively. Non-significance is indicated with white boxes without numbers. TDM—total dry mass; SDM—shoot dry mass; RDM—root dry mass; WDTI—water index tolerance; H_2O_2—hydrogen

peroxide; MDA—malondialdehyde; *A*—CO_2 assimilation rate; g_s—stomatal conductance; C_i—internal concentration of CO_2; *E*—transpiration; *WUE*—water use efficiency; *CE*—carboxylation efficiency; POD—guaiacol peroxidase; APX—ascorbate peroxidase; CAT—catalase.

Discussion

Stress in plants due to water deficit reduces their growth due to cell turgor reduction, followed by damages in the photosynthetic process, causing increases in oxidative damage [4,5,28]. However, these alterations promoted by stress may not necessarily harm plant development since supplementation of exogenous compounds or elements with beneficial properties can increase plant tolerance levels to specific stressful conditions [24,29,30]. In the case of water deficit stress, plants can circumvent damage by improving their antioxidant capacity and adjusting gas exchange [24,25].

In the present study, a reduction in growth due to water deficit was observed in the accumulation of biomass in soybean plants (Figure 1). A decrease in soybean biomass resulted mainly from a reduction in the efficiency of photosynthesis and an increase in lipid peroxidation due to oxidative stress, indirectly indicated by the accumulation of MDA [25]. Exposure of soybean plants to a low concentration of KI (10 and 20 µM) increased the antioxidant enzymatic activity, consequently reducing the MDA content, which improved photosynthetic efficiency, thus promoting greater tolerance to water suppression (WDTI). The effect of I related to water deficit is still little reported in the literature; however, reports point to its beneficial effect on the germination and growth of *Carthamus tinctorius* L. subjected to water deficit [31]. In addition, the effect of this element was also reported for other abiotic stresses, such as salinity [22,26,32] and heavy metals [21].

Our results indicated that exposure to iodine showed a capacity for photosynthetic improvement in plants under water deficit when concentrations of 10 and 20 µM KI were applied, which is reflected by the increase in biomass in these respective treatments. A study reported that iodinated proteins could improve photosynthesis and defense responses in plants [26]. The authors pointed out that most of the iodinated proteins in the shoot were connected with chloroplasts and were involved in photosynthetic processes. Corroborating our study findings, the photosynthetic rate of lettuce was shown to increase by ~20% due to the application of I, in the form of IO_3^{-1}, in concentrations of 20, 40, and 80 µM [18]. Furthermore, as already mentioned, the greater antioxidant protection at concentrations of 10

and 20 µM may also ensure greater photosynthetic performance in plants grown under water stress by effectively reducing the damage caused by ROS, thus balancing ROS synthesis and signaling to provide greater stress tolerance [33]. The excessive accumulation of ROS promoted with water deficit can cause oxidative damage to the electron transport chain, increase lipid peroxidation in chloroplasts and mitochondria, inactivate enzymatic activity, directly oxidize proteins and nucleic acids, and, finally, decrease photosynthesis and crop production [24,34].

Iodine is considered a potential antioxidant in plants since the antioxidant capacity of plants is positively correlated with the amount of I [35–38]. Furthermore, studies on soybeans and lettuce indicated that the application of I at concentrations of 20, 40, and 80 µM increases the enzymatic activities of SOD, APX, and CAT [18,21]. Evaluating different compounds containing I, a study reported decreases in POX (guaiacol peroxidase) activity for tomato plants exposed to increasing concentrations of I, with the exception of the highest doses (i.e., 50 mM I) [39]. The results cited above corroborate our findings. Furthermore, under water deficit, our results indicated greater APX, POD, and CAT activities, mainly when the plants were exposed to 10 and 20 µM KI. Exposure of soybean plants to 10 and 20 µM KI provided greater antioxidant enzymatic activity, which consequently may have guaranteed less oxidative stress, as observed by the lower MDA content. A high MDA content indicates a high degree of lipid peroxidation in the plant membrane and a state of oxidative stress [40]. This occurs when ROS levels are exceeded, causing lipid peroxidation, protein oxidation, and enzyme inactivation, which can even lead to cell death [41]. A high-performance antioxidant system contributes to lowering oxidative stress and helps plant growth. In *A. thaliana*, iodinated proteins were identified and confirmed as belonging to POD class III [26]. This may relate to ROS oxidizing I^-, which leads to the preferential iodization of close proteins, including enzymes from the antioxidant system [42].

An interesting finding from our study was the higher H_2O_2 content in plants grown under water deficit and exposed to 10 and 20 µM I, which were the treatments that provided the highest growth in this irrigation condition. H_2O_2 is a molecule capable of reacting with cellular components interfering with normal physiological and metabolic functions and unbalancing the cellular redox homeostasis [43,44]. However, H_2O_2 also plays important roles in plant development and physiological processes, including flowering [45], root system development [46–48], regulation of stomatal opening [49], and many others. Furthermore, the levels found in our study were below those generally considered harmful for some cellular compartments (basal H_2O_2 level of 5–15 µmols) [50,51]. The low levels

found in plants under water deficit and without KI may have been related to the H_2O_2 breakdown into other molecules as its dismutation into hydroxyl radicals (^{-}OH). These radicals, in turn, are more reactive than H_2O_2, leading to lipid peroxidation, and thus may explain the greater concentration of MDA in their respective treatment [52]. Thus, it was observed that in the plants treated with 10 and 20 µM KI, which had lower MDA content and higher H_2O_2 values, the dismutation products of H_2O_2 may have been H_2O and O^{2-} due to the greater antioxidant enzymatic activity in these treatments, enabling higher growth in conditions of water deficit [53,54]. This hypothesis represents a potential mechanism that suits this study's findings but needs more results to be confirmed.

Despite the beneficial effect of KI observed under the water stress condition, the treatment with the highest KI dose (40 µM) proved to be harmful to the plants since they had a higher MDA content and lower growth. These results indicate a possible toxicity of I at this concentration in plants subjected to water deficit, a fact related to the pro-oxidative effect of I at high concentrations [37,54]. The phytotoxic effect of I was also seen in the present study under conditions of adequate irrigation, as there was a change in the root:shoot ratio with the concentration of 20 µM I, and a reduction in the accumulation of biomass in plants treated with 40 µM I. In agreement with our results, Blasco et al. [18] observed that the photosynthetic rate of lettuce significantly decreased when the I concentration exceeded 40 µM, causing lower growth and biomass accumulation. In a study on basil, Incrocci et al. [37] observed a decline in plant height, leaf area, and biomass accumulation when KI concentrations were greater than 50 µM. Kato et al. [55] reported a 26% decrease in the shoot length of rice treated with 25 µM KI. Thus, it is observed that the beneficial effect of I depends on the concentration used, as in excess, I can potentially induce oxidative stress [38].

Although I accumulates to some extent in plants, it can reduce oxygen-containing free radicals and oxygen-containing molecules such as superoxide anions, H_2O_2, and ^{-}OH, [56]. Additionally, the formation of I proteins or the involvement of I as an inducing factor in protein synthesis may be the cause of I toxicity [54]. Thus, although plants under water deficit treated with 20 µM KI had higher activities of enzymes such as SOD, CAT, and POD and good accumulation of biomass, at this exposure concentration, I began to promote small disturbances in plants under adequate irrigation. Therefore, the concentration of 10 µM KI would be the most appropriate exposure treatment, as it promotes a beneficial effect under conditions of stress due to water deficit, without having a harmful effect under conditions of adequate irrigation. It is crucial to highlight that the morphophysiological and biochemical variables analyzed also pointed to 10 µM KI as the best treatment in a conjoint manner, as

depicted with the PCA evaluation.

Lastly, although plants under water deficit have increased proline content, this osmolyte was not responsible for soybean plants' increased tolerance induced using exposure to I. Thus, our findings suggest that the main process responsible for the increase in tolerance to water stress with the application of I was related to the increase in the production of antioxidant enzymes, which promoted better protection of the photosynthetic apparatus, thus allowing the accumulation of biomass in soybean plants under water deficiency.

Materials and Methods

Cultivation System, Experimental Design, and Treatments

Soybean plants were grown in pots with 1000 g washed sand, with one plant per pot. The dimensions of the pots were 15 × 9 × 9. The cultivation was carried out in a greenhouse located in the Experimental Area of the Plant Physiology Sector in the Biology Department at the Federal University of Lavras (UFLA) (21°14′45″ S, 44°59′59″ W; 920 m above sea level), southeastern Brazil. Plants were exposed to a mean temperature of 25 °C and an 11/13.5 h (winter/summer) photoperiod and were fertigated twice a week with 50 mL of Hoagland and Arnon's nutrient solution [57]. The treatments added to the pots were arranged in a completely randomized design, with five replications of each treatment. The experiment was carried out in a 4 × 2 factorial scheme, corresponding to four concentrations of KI added with a nutrient solution (0; 10; 20; 40 μmols L^{-1}) and two irrigation conditions (with and without water deficit). The experiment had a total of 40 experimental units. In total, there were 13 applications of 50 mL of the solution containing KI during the experiment, making a total of 650 mL. In this way, we can say that there was an application of approximately 0.0825, 0.165, and 0.33 g of I kg^{-1} of substrate in treatments of 10, 20, and 40 μM KI, respectively.

The treatments related to the application of I were carried out by adding KI to the nutrient solution starting 14 days after seed germination at stage V2 (two leaflet). Irrigation was suspended 60 days after germination (DAG) to subject the plants to water deficit, and when this was reached, immediate rehydration was performed. The water deficit was established by monitoring gas exchanges, i.e., when it reached a negative *A* value and an *E* value of less than 1 mmol H_2O m^{-2} s^{-1} (Figure 8). These values were achieved on the fourth day after the suspension of irrigation. The mean values of A and E for each treatment are

shown in Table 1.

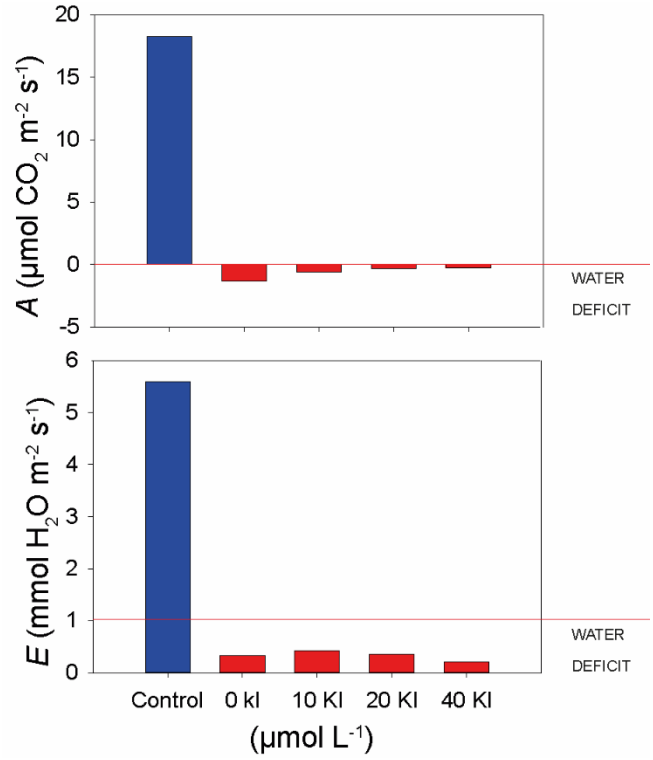

Figure 8. Establishment of water deficit by monitoring gas exchange in soybean plants subjected to different concentrations of KI. The graph shows the mean values of *A* and *E* on the fourth day after the suspension of irrigation.

TREATMENTS	*A* (µmol CO_2 m^{-2} s^{-1})	*E* (mmol H_2O m^{-2} s^{-1})
CONTROL	18.23	5.58
WATER DEFICIT + 0 µM KI	−1.26	0.32
WATER DEFICIT + 10 µM KI	−0.58	0.41
WATER DEFICIT + 20 µM KI	−0.28	0.35
WATER DEFICIT + 40 µM KI	−0.26	0.20

Table 1. Mean values of *A* and *E* for monitoring the water deficit of soybean plants exposed to different concentrations of KI.

The sample collection for the biochemical evaluations was carried out on the day the plants reached water deficit, and the analysis of gas exchanges was completed one day after rehydration. At 64 DAG, the experiment was completed, and the plants were collected for biomass evaluation, separating the plant into shoots and roots.

Biomass and WDTI

At the end of the experiment, the SDM, RDM, and TDM RDM:SDM were determined. To obtain dry mass, the tissues were dried at 70 °C in a forced-circulation oven until constant mass. In addition, the WDTI was calculated according to [58,59] using the following equation:

WDTI = (total dry mass of control / total dry mass of other treatments)×100

Leaf Gas Exchange

As previously mentioned, analyses were performed one day after the rehydration of the plants using an infrared gas exchange analyzer (IRGA, model LICOR 6400, Li-COR Biosciences, Lincoln, NE, USA). Data collection was performed between 8 am and 10 am, and the following variables were evaluated: CO_2 assimilation (*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 CO_2 concentration (C_i—μmol CO_2 mol air^{-1}). Based on the *A* a *E* results, water use efficiency estimates (*WUE* (μmol CO_2 $mmol^{-1}$ H_2O)) was calculated as *A*/*E*. The carboxylation efficiency (*EC*—μmol CO_2 m^{-2} s^{-1} Pa^{-1}) was estimated for the through the results of *A* and *Ci* (*A*/*Ci*). Atmospheric CO_2 inside the leaf chamber was maintained at 400 μmol CO_2 mol air^{-1}, irradiance at 1500 μmols m^{-2} s^{-1}, and leaf temperature at 25 °C. The pre-established minimum time for stabilization of the readings was 120 s.

H_2O_2 and MDA Content

To evaluate the H_2O_2 and MDA content, 0.2 g of fresh material was collected and macerated in a mortar with liquid nitrogen, homogenized in 1500 μL of trichloroacetic acid (TCA), and centrifuged at 12,000× *g* for 15 min at 4 °C. The H_2O_2 content was determined by collecting the supernatant and then reading its absorbance at 390 nm in a medium composed of 10 mM potassium phosphate (pH 7.0), 45 μL of plant material extract, and 1 M potassium iodide [60].

The MDA quantification was performed according to Buege and Aust [61]. For this, 125 μL of the extraction supernatant was collected and pipetted into a 1500 μL microtube containing 250 μL of the following reaction medium: 0.5% thiobarbituric acid (TBA) and 10% TCA. The microtubes then were placed in a water bath at 95 °C for 30 min, and after that, the

reaction was stopped using cooling on ice. Subsequently, 350 µL of the reaction medium was collected and pipetted into microplates and read using a spectrometer at 535 and 600 nm. The content of MDA was obtained according to the following equation:

$$MDA = (A535 - A600)/(\xi.b)$$

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

Antioxidant Enzymatic Activity

For the evaluation of SOD, APX, CAT, and POD enzymes, 0.2 g of fresh material was collected and then ground in liquid nitrogen with the subsequent addition of 1.5 mL of a buffer 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). Soon after, the suspension was centrifuged at 13,000× *g* for 10 min at 4 °C, and the supernatant was collected for analysis in a spectrophotometer (Epoch-BioTek, Miami, FL, USA).

The SOD activities in leaves were determined by quantifying the inhibition in photoreduction in nitrobluetetrazolium (NBT), following the protocol devised by Beauchamp and Fridovich [62]. The reaction solution was prepared by mixing: (i) 75 µL of NBT; (ii) 20 µL of riboflavin; (iii) 130 mL of L-methionine; and (iv) 100 µL of Na_2EDTA into a sodium phosphate buffer. Next, this solution (2.725 mL) was mixed with H_2O (0.25 mL) and 50 µL enzyme extract (supernatant) and placed in light conditions of 4.000 lux for 7 min. The absorbances of the samples were recorded at 560 nm using a spectrophotometer.

The activity of APX was determined using Nakano and Asada's [63] methodology. Briefly, the reaction solution contained 100 µL ascorbate solution (10 mM), 100 µL H_2O_2 (30%), and 100 µL enzyme extract (supernatant) in 2.7 mL of sodium phosphate buffer. After a gentle shake, the absorbance was read at 290 nm with on time scan (0–60s) using a spectrophotometer.

The reaction solution for POD contained 100 µL 30 mM H_2O_2, 100 µL guaiacol, and 100 µL enzyme extract (supernatant) in 2.7 mL sodium phosphate buffer. While for the estimation of CAT activity, the same reaction solution used for POD (except guaiacol) was used. The absorbance of POD and CAT samples was observed on time scan (0–60 s) at 470 and 240 nm, respectively, using a spectrophotometer [64].

To calculate the specific activity of antioxidant enzymes, the total soluble protein content was determined using the enzymatic extraction method. The microplates first received 294 µL of Bradford's solution [65] at a 1:5 dilution of the reagent. The readings were performed using an absorbance microplate reader (Epoch-BioTek) at a wavelength of 595 nm, and the results were obtained from a calibration curve with BSA.

Proline

Extraction and quantification were performed according to the methodology proposed by Bates et al. [66]. Briefly, 0.2 g of plant material was macerated in 3% sulfosalicylic acid (10 mL) followed by stirring for 60 min at room temperature, and then, the material was filtered and added to tubes and placed in a water bath at 100 °C for 60 min. The tubes were cooled on ice, and the reading was performed using a spectrophotometer at 520 nm. Quantification was performed using a standard proline curve.

Statistical Analysis

The data were submitted to Shapiro–Wilk normality tests and a Barlett's homogeneity of variance test, and when the assumptions were met, they were submitted to a two-way ANOVA with a post hoc Tukey test. Data were presented in bar graphs. When the data did not show normality or variance homogeneity, a rank transformation of the data was performed [67,68], and the data were represented in boxplots so that it was possible to better observe the data dispersion. A PCA was used to observe the multivariate correlation between all morphophysiological and biochemical variables and the treatment conditions. All statistical analyses and graphs were made with the R software environment using the tidyverse [69], multcomp [70], and rstatix [71] packages

Conclusions

Exposure to I (as KI) increased tolerance to water deficit in soybean plants through modulation of the antioxidant enzymatic system and increased photosynthetic efficiency, which consequently provided a greater accumulation of biomass. In plants without water stress, KI concentrations greater than 10 µM induced toxic effects of this element, thus altering

and increasing the root:shoot ratio as well as reducing the photosynthetic rate of soybean plants, whereas at higher concentrations (40 μM), it reduced the growth of soybean plants. Thus, based on the insights obtained for all the variables in conjoint using the PCA, the treatment with 10 μM KI provided the best performance since it did not change biomass accumulation under adequate irrigation conditions, in addition to promoting tolerance of plants subjected to water deficit. However, further studies must be carried out to better elucidate the molecular, biochemical, and physiological processes induced by I in other plants grown under water deficit. In addition, studies must be carried out with the application of different sources and forms of I application to identify the best management for mitigating water deficiency in commercial production conditions, as well as carrying out cultivations with I application in soils to evaluate its possible interactions with other soil elements/components.

Author Contributions

Conceptualization, J.d.S.L., O.V.S.A., E.G.d.M. and L.R.G.G.; methodology, J.d.S.L., O.V.S.A., L.C.d.S. and G.S.M.; software, J.d.S.L.; validation, L.R.G.G., V.L.N., P.E.R.M. and G.L.; formal analysis, J.d.S.L., O.V.S.A., L.C.d.S. and G.S.M.; resources, L.R.G.G. and P.E.R.M.; writing—original draft preparation, J.d.S.L., O.V.S.A., L.C.d.S., G.S.M. and Y.S.M.; writing—review and editing, J.d.S.L. and Y.S.M.; supervision, L.R.G.G., V.L.N., P.E.R.M., G.L. and E.G.d.M.; project administration, L.R.G.G.; funding acquisition, L.R.G.G., V.L.N., P.E.R.M., G.L. and E.G.d.M. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES) (grant code-001) and the National Institute of Science and Technology (INCT) in Plant Stress Physiology, CNPq grant 406455/2022-8. Partial funds were provided also by CNPq grant #406577/2022-6 (National Institute of Science and Technology on Soil and Food Security) and the São Paulo Research Foundation (FAPESP) grant 2023/00474-4.

Data Availability Statement

Not applicable.

Acknowledgments

We acknowledge The National Council for Scientific and Technological Development (CNPq), The Coordination for the Improvement of Higher Education Personnel (CAPES), and The Minas Gerais State Research Foundation (FAPEMIG) for financial support and scholarships provided to the authors. Special thanks to UFLA's Soil Science and Biology—Plant Molecular Physiology Lab—Departments. P.E.R.M. thanks CNPq for the fellowship grant #312663/2021-8.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of this manuscript; or in the decision to publish the results.

References

1. Ngumbi, E.; Kloepper, J. Bacterial-Mediated Drought Tolerance: Current and Future Prospects. *Appl. Soil Ecol.* **2016**, *105*, 109–125.
2. Kour, D.; Yadav, A.N. Bacterial Mitigation of Drought Stress in Plants: Current Perspectives and Future Challenges. *Curr. Microbiol.* **2022**, *79*, 248.
3. Barnawal, D.; Singh, R.; Singh, R.P. Chapter Six—Role of Plant Growth Promoting Rhizobacteria in Drought Tolerance: Regulating Growth Hormones and Osmolytes. In *PGPR Amelioration in Sustainable Agriculture*; Singh, A.K., Kumar, A., Singh, P.K., Eds.; Elsevier BV: Amsterdam, The Netherlands, 2019; pp. 107–128. ISBN 978-0-12-815879-1.
4. Abdelaal, K.A.A.; Rashed, S.H.; Hossain, A.; Sabagh, A.E. Yield and Quality of Two Sugar Beet (*Beta Vulgaris* L. ssp. Vulgaris Var. Altissima Döll) Cultivars Are Influenced by Foliar Application of Salicylic Acid, Irrigation Timing, and Planting Density. *Acta Agric. Slov.* **2020**, *115*, 273–282.
5. Canakci-Gulengul, S.; Yildiz, T.; Yilmaz, O.; Ozsahin, A. Effect of Cadmium on Some Biochemical Mechanism in the *Saccharomyces Cerevisiae*. *Fresenius Environ. Bull.* **2017**, *26*, 7334–7341.
6. Apel, K.; Hirt, H. REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction. *Annu. Rev. Plant Biol.* **2004**, *55*, 373–399.
7. Gill, S.S.; Tuteja, N. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. *Plant Physiol. Biochem*. **2010**, *48*, 909–930.
8. Allen, D.J.; Ort, D.R. Impacts of Chilling Temperatures on Photosynthesis in Warm-Climate Plants. *Trends Plant Sci.* **2001**, *6*, 36–42.

9. Ding, F.; Wang, M.; Zhang, S.; Ai, X. Changes in SBPase Activity Influence Photosynthetic Capacity, Growth, and Tolerance to Chilling Stress in Transgenic Tomato Plants. *Sci. Rep.* **2016**, *6*, 32741.
10. Ding, F.; Wang, M.; Zhang, S. Overexpression of a Calvin Cycle Enzyme SBPase Improves Tolerance to Chilling-Induced Oxidative Stress in Tomato Plants. *Sci. Hortic.* **2017**, *214*, 27–33.
11. Freitas, M. A Cultura Da Soja No Brasil: O Crescimento Da Produção Brasileira e o Surgimento de Uma Nova Fronteira Agrícola. *Enciclopédia Biosf.* **2011**, *7*, 1–12.
12. CONAB. Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira de Grãos, Brasília, DF, v. 10, safra 2022/23, n. 8 Oitavo Levantamento. 2023. Available online: https://www.conab.gov.br/info-agro/safras/graos/boletim-dasafra-de-graos (accessed on 1 May 2023).
13. Neumaier, N.; Farias, J.R.B.; Nepomuceno, A.L.; Mertz-Henning, L.M.; Foloni, J.S.S.; Moraes, L.A.C.; Goncalves, S.L. *Ecofisiologia Da Soja*; Embrapa: Londrinam, PR, Brazil, 2020.
14. Da Silva Cavalcante, W.S.; Da Silva, N.F.; Teixeira, M.B.; Cabral Filho, F.R.; Nascimento, P.E.R.; Corrêa, F.R. Eficiência Dos Bioestimulantes No Manejo Do Déficit Hídrico Na Cultura Da Soja. *Irriga* **2020**, *25*, 754–763.
15. Gómez-Merino, F.C.; Trejo-Téllez, L.I. The Role of Beneficial Elements in Triggering Adaptive Responses to Environmental Stressors and Improving Plant Performance. In *Biotic and Abiotic Stress Tolerance in Plants*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 137–172.
16. Nascimento, V.L.; Souza, B.C.; Lopes, G.; Guilherme, L.R. On the Role of Iodine in Plants: A Commentary on Benefits of This Element. *Front. Plant Sci.* **2022**, *13*, 836835.
17. Val-Torregrosa, B.; Bundó, M.; San Segundo, B. Crosstalk between Nutrient Signalling Pathways and Immune Responses in Rice. *Agriculture* **2021**, *11*, 747.
18. Blasco, B.; Ríos, J.J.; Leyva, R.; Cervilla, L.M.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Rosales, M.A.; Ruiz, J.M.; Romero, L. Does Iodine Biofortification Affect Oxidative Metabolism in Lettuce Plants? *Biol. Trace Elem. Res.* **2011**, *142*, 831–842.
19. Ravello, R.A.V.; de Oliveira, C.; Lessa, J.; Boas, L.V.V.; de Castro, E.M.; Guilherme, L.R.G.; Lopes, G.; Ravello, R.A.V.; de Oliveira, C.; Lessa, J.; et al. Selenium Application Influenced Selenium Biofortification and Physiological Traits in Water-Deficit Common Bean Plants. *Crop. Pasture Sci.* **2021**, *73*, 44–55.
20. Iwaniuk, P.; Borusiewicz, A.; Lozowicka, B. Fluazinam and Its Mixtures Induce Diversified Changes of Crucial Biochemical and Antioxidant Profile in Leafy Vegetable. *Sci. Hortic.* **2022**, *298*, 110988.
21. Gupta, N.; Bajpai, M.S.; Majumdar, R.S.; Mishra, P.K. Response of Iodine on Antioxidant Levels of *Glycine Max* L. Grown under Cd2+ Stress. *Adv. Biol. Res.* **2015**, *9*, 40–48.
22. Kiferle, C.; Gonzali, S.; Beltrami, S.; Martinelli, M.; Hora, K.; Holwerda, H.T.; Perata, P. Improvement in Fruit Yield and Tolerance to Salinity of Tomato Plants Fertigated with Micronutrient Amounts of Iodine. *Sci. Rep.* **2022**, *12*, 14655.
23. Pérez-Salas, S.; Medrano-Macías, J. Use of Iodine as a Tolerance Inducer in Tomato Seedlings Under Salinity Stress Uso Del Yodo Como Inductor a La Tolerancia En Plántulas de Tomate Bajo Condiciones de Estrés Por Salinidad. *Rev. Científica Univ. Autónoma Coahuila* **2021**, *13*, 14–22.
24. Altaf, M.A.; Shahid, R.; Ren, M.-X.; Naz, S.; Altaf, M.M.; Khan, L.U.; Tiwari, R.K.; Lal, M.K.; Shahid, M.A.; Kumar, R. Melatonin Improves Drought Stress Tolerance of

Tomato by Modulating Plant Growth, Root Architecture, Photosynthesis, and Antioxidant Defense System. *Antioxidants* **2022**, *11*, 309.
25. Imran, M.; Latif Khan, A.; Shahzad, R.; Aaqil Khan, M.; Bilal, S.; Khan, A.; Kang, S.-M.; Lee, I.-J. Exogenous Melatonin Induces Drought Stress Tolerance by Promoting Plant Growth and Antioxidant Defence System of Soybean Plants. *AoB Plants* **2021**, *13*, plab026.
26. Kiferle, C.; Martinelli, M.; Salzano, A.M.; Gonzali, S.; Beltrami, S.; Salvadori, P.A.; Hora, K.; Holwerda, H.T.; Scaloni, A.; Perata, P. Evidences for a Nutritional Role of Iodine in Plants. *Front. Plant Sci.* **2021**, *12*.
27. Medrano-Macías, J.; López Caltzontzit, M.G.; Rivas Martínez, E.N.; Narváez Ortiz, W.A.; Benavides Mendoza, A.; Martínez Lagunes, P. Enhancement to Salt Stress Tolerance in Strawberry Plants by Iodine Products Application. *Agronomy* **2021**, *11*, 602.
28. Gowtham, H.G.; Singh, S.B.; Shilpa, N.; Aiyaz, M.; Nataraj, K.; Udayashankar, A.C.; Amruthesh, K.N.; Murali, M.; Poczai, P.; Gafur, A.; et al. Insight into Recent Progress and Perspectives in Improvement of Antioxidant Machinery upon PGPR Augmentation in Plants under Drought Stress: A Review. *Antioxidants* **2022**, *11*, 1763.
29. Dobra, J.; Motyka, V.; Dobrev, P.; Malbeck, J.; Prasil, I.T.; Haisel, D.; Gaudinova, A.; Havlova, M.; Gubis, J.; Vankova, R. Comparison of Hormonal Responses to Heat, Drought and Combined Stress in Tobacco Plants with Elevated Proline Content. *J. Plant Physiol.* **2010**, *167*, 1360–1370.
30. Medrano-Macías, J.; Leija-Martínez, P.; González-Morales, S.; Juárez-Maldonado, A.; Benavides-Mendoza, A. Use of Iodine to Biofortify and Promote Growth and Stress Tolerance in Crops. *Front. Plant Sci.* **2016**, *7*, 1146.
31. Jafarian, S.; Sodaiezadeh, H.; Arani, A.; Hakimzadeh, M.a.; Sohrabizadeh, Z. The Effect of Iodine On Increasing Drought Tolerance Of (*Carthamus Tinctorius* L.) In Seed Germination and Early Growth Stage. *J. Environ. Sci. Stud.* **2020**, *5*, 2387–2393.
32. Fuentes, J.E.G.; Castellanos, B.F.H.; Martínez, E.N.R.; Ortiz, W.A.N.; Mendoza, A.B.; Macías, J.M. Outcomes of Foliar Iodine Application on Growth, Minerals and Antioxidants in Tomato Plants under Salt Stress. *Folia Hortic.* **2022**, *34*, 27–37.
33. Cao, L.; Jin, X.J.; Zhang, Y.X. Melatonin Confers Drought Stress Tolerance in Soybean (*Glycine Max* L.) by Modulating Photosynthesis, Osmolytes, and Reactive Oxygen Metabolism. *Photosynthetica* **2019**, *57*, 812–819.
34. Cui, G.; Zhao, X.; Liu, S.; Sun, F.; Zhang, C.; Xi, Y. Beneficial Effects of Melatonin in Overcoming Drought Stress in Wheat Seedlings. *Plant Physiol. Biochem.* **2017**, *118*, 138–149.
35. Blasco, B.; Leyva, R.; Romero, L.; Ruiz, J.M. Iodine Effects on Phenolic Metabolism in Lettuce Plants under Salt Stress. *J. Agric. Food Chem.* **2013**, *61*, 2591–2596.
36. Küpper, F.C.; Carpenter, L.J.; Leblanc, C.; Toyama, C.; Uchida, Y.; Maskrey, B.H.; Robinson, J.; Verhaeghe, E.F.; Malin, G.; Luther, G.W., III; et al. In Vivo Speciation Studies and Antioxidant Properties of Bromine in Laminaria Digitata Reinforce the Significance of Iodine Accumulation for Kelps. *J. Exp. Bot.* **2013**, *64*, 2653–2664.
37. Incrocci, L.; Carmassi, G.; Maggini, R.; Poli, C.; Saidov, D.; Tamburini, C.; Kiferle, C.; Perata, P.; Pardossi, A. Iodine Accumulation and Tolerance in Sweet Basil (*Ocimum Basilicum* L.) With Green or Purple Leaves Grown in Floating System Technique. *Front. Plant Sci.* **2019**, *10*, 1494.
38. Kiferle, C.; Ascrizzi, R.; Martinelli, M.; Gonzali, S.; Mariotti, L.; Pistelli, L.; Flamini, G.; Perata, P. Effect of Iodine Treatments on *Ocimum Basilicum* L.: Biofortification, Phenolics Production and Essential Oil Composition. *PLoS ONE* **2019**, *14*, e0226559.

39. Halka, M.; Smole´n, S.; Ledwozyw-Smole´n, I. Antioxidant Potential and Iodine Accumulation in Tomato (*Solanum Lycopersicum* L.) Seedlings as the Effect of the Application of Three Different Iodobenzoates. *Folia Hortic.* **2020**, *32*, 203–219.
40. Rezaizad, M.; Hashemi-Moghaddam, H.; Abbaspour, H.; Gerami, M.; Mueller, A. Photocatalytic Effect of TiO2 Nanoparticles on Morphological and Photochemical Properties of Stevia Plant (*Stevia Rebaudiana Bertoni*). *Sugar Tech* **2019**, *21*, 1024–1030.
41. Sahu, P.; Krishnappa, J.; Tilgam, J.; Gupta, A.; Nagaraju, Y.; Kumar, A.; Baba, S.; Singh, H.; Minkina, T.; Rajput, V.; et al. ROS Generated from Biotic Stress: Effects on Plants and Alleviation by Endophytic Microbes. *Front. Plant Sci.* **2022**, *13*, 1042936.
42. Takahashi, Y.; Satoh, K. Identification of the Photochemically Iodinated Amino-Acid Residue on Dl-Protein in the Photosystem II Core Complex by Peptide Mapping Analysis. *Biochim. Biophys. Acta (BBA) Bioenerg.* **1989**, *973*, 138–146.
43. Vranová, E.; Inzé, D.; Van Breusegem, F. Signal Transduction during Oxidative Stress. *J. Exp. Bot.* **2002**, *53*, 1227–1236.
44. Xie, X.; He, Z.; Chen, N.; Tang, Z.; Wang, Q.; Cai, Y. The Roles of Environmental Factors in Regulation of Oxidative Stress in Plant. *BioMed Res. Int.* **2019**, *2019*, e9732325.
45. Liu, J.; Macarisin, D.; Wisniewski, M.; Sui, Y.; Droby, S.; Norelli, J.; Hershkovitz, V. Production of Hydrogen Peroxide and Expression of ROS-Generating Genes in Peach Flower Petals in Response to Host and Non-Host Fungal Pathogens. *Plant Pathol.* **2013**, *62*, 820–828.
46. Liao, W.; Xiao, H.; Zhang, M. Role and Relationship of Nitric Oxide and Hydrogen Peroxide in Adventitious Root Development of Marigold. *Acta Physiol. Plant* **2009**, *31*, 1279–1289.
47. Ma, F.; Wang, L.; Li, J.; Samma, M.K.; Xie, Y.; Wang, R.; Wang, J.; Zhang, J.; Shen, W. Interaction between HY1 and H2O2 in Auxin-Induced Lateral Root Formation in Arabidopsis. *Plant Mol. Biol.* **2014**, *85*, 49–61.
48. Hernández-Barrera, A.; Velarde-Buendía, A.; Zepeda, I.; Sanchez, F.; Quinto, C.; Sánchez-Lopez, R.; Cheung, A.Y.; Wu, H.-M.; Cardenas, L. Hyper, a Hydrogen Peroxide Sensor, Indicates the Sensitivity of the Arabidopsis Root Elongation Zone to Aluminum Treatment. *Sensors* **2015**, *15*, 855–867.
49. Ge, X.-M.; Cai, H.-L.; Lei, X.; Zhou, X.; Yue, M.; He, J.-M. Heterotrimeric G Protein Mediates Ethylene-Induced Stomatal Closure via Hydrogen Peroxide Synthesis in Arabidopsis. *Plant J.* **2015**, *82*, 138–150.
50. Polle, A. Dissecting the Superoxide Dismutase-Ascorbate-Glutathione-Pathway in Chloroplasts by Metabolic Modeling. Computer Simulations as a Step towards Flux Analysis. *Plant Physiol.* **2001**, *126*, 445–462.
51. Mittler, R. Oxidative Stress, Antioxidants and Stress Tolerance. *Trends Plant Sci.* **2002**, *7*, 405–410.
52. García-Caparrós, P.; De Filippis, L.; Gul, A.; Hasanuzzaman, M.; Ozturk, M.; Altay, V.; Lao, M.T. Oxidative Stress and Antioxidant Metabolism under Adverse Environmental Conditions: A Review. *Bot. Rev.* **2021**, *87*, 421–466.
53. Rajput, V.D.; Harish; Singh, R.K.; Verma, K.K.; Sharma, L.; Quiroz-Figueroa, F.R.; Meena, M.; Gour, V.S.; Minkina, T.; Sushkova, S.; et al. Recent Developments in Enzymatic Antioxidant Defence Mechanism in Plants with Special Reference to Abiotic Stress. *Biology* **2021**, *10*, 267.
54. Zhang, Y.; Cao, H.; Wang, M.; Zou, Z.; Zhou, P.; Wang, X.; Jin, J. A Review of Iodine in Plants with Biofortification: Uptake, Accumulation, Transportation, Function, and Toxicity. *Sci. Total Environ.* **2023**, *878*, 163203.

55. Kato, S.; Wachi, T.; Yoshihira, K.; Nakagawa, T.; Ishikawa, A.; Takagi, D.; Tezuka, A.; Yoshida, H.; Yoshida, S.; Sekimoto, H. Rice (*Oryza Sativa* L.) Roots Have Iodate Reduction Activity in Response to Iodine. *Front. Plant Sci.* **2013**, *4*, 227.
56. Li, J.; Zhou, H.; Qian, K.; Xie, X.; Xue, X.; Yang, Y.; Wang, Y. Fluoride and Iodine Enrichment in Groundwater of North China Plain: Evidences from Speciation Analysis and Geochemical Modeling. *Sci. Total Environ.* **2017**, *598*, 239–248.
57. Hoagland, D.R.; Arnon, D.I. The Water-Culture Method for Growing Plants without Soil. *Circ. Calif. Agric. Exp. Stn.* **1950**, *347*, 13143–13151.
58. Fernandez George, C.J. Effective Selection Criteria for Assessing Stress Tolerance. In Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress, Taiwan, China, 13–18 August 1992; Kuo, C.G., Ed.; Asian vegetable research and Development Center: Shanhua, Tainan, Taiwan, 1992; pp. 257–270.
59. Sánchez-Reinoso, A.D.; Ligarreto-Moreno, G.A.; Restrepo-Díaz, H. Evaluation of Drought Indices to Identify Tolerant Genotypes in Common Bean Bush (*Phaseolus Vulgaris* L.). *J. Integr. Agric.* **2020**, *19*, 99–107.
60. Velikova, V.; Yordanov, I.; Edreva, A. Oxidative Stress and Some Antioxidant Systems in Acid Rain-Treated Bean Plants: Protective Role of Exogenous Polyamines. *Plant Sci.* **2000**, *151*, 59–66.
61. Buege, J.A.; Aust, S.D. Microsomal Lipid Peroxidation. In *Methods in Enzymology*; Fleischer, S., Packer, L., Eds.; Biomembranes—Part C: Biological Oxidations; Academic Press: Cambridge, MA, USA, 1978; Volume 52, pp. 302–310.
62. Beauchamp, C.; Fridovich, I. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Anal. Biochem.* **1971**, *44*, 276–287.
63. Nakano, Y.; Asada, K. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell. Physiol.* **1981**, *22*, 867–880.
64. Chance, B.; Maehly, A.C. *Assay of Catalases and Peroxidases: In Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1955; Volume 2, pp. 764–775.
65. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.
66. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid Determination of Free Proline for Water-Stress Studies. *Plant Soil.* **1973**, *39*, 205–207.
67. Conover, W.J.; Iman, R.L. Rank Transformations as a Bridge between Parametric and Nonparametric Statistics. *Am. Stat.* **1981**, *35*, 124–129.
68. Holbert, H. Nonparametric Two-Way ANOVA. 2022. Available online: https://www.cfholbert.com/blog/nonparametric_two_way_anova/ (accessed on 6 May 2023).
69. Wickham, H.; Averick, M.; Bryan, J.; Chang, W.; McGowan, L.; François, R.; Grolemund, G.; Hayes, A.; Henry, L.; Hester, J.; et al. Welcome to the Tidyverse. *J. Open Source Softw.* **2019**, *4*, 1686.
70. Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. *Biom J.* **2008**, *50*, 346–363.
71. Kassambara, A. _Rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.1. 2022. Available online: https://CRAN.R-project.org/package=rstatix (accessed on 7 May 2023).

ARTICLE 2: KI Increases Tomato Fruit Quality and Water Deficit Tolerance by Improving Antioxidant Enzyme Activity and Amino Acid Accumulation: A Priming Effect or Relief during Stress?

Published in Plants

(https://doi.org/10.3390/plants12234023)

Jucelino de Sousa Lima [1], Otávio Vitor Souza Andrade [1], Everton Geraldo de Morais [2], Gilson Gustavo Lucinda Machado [3], Leônidas Canuto dos Santos [2], Eduarda Santos de Andrade [1], Pedro Antônio Namorato Benevenute [2], Gabryel Silva Martins [2], Vitor L. Nascimento [1], Paulo Eduardo Ribeiro Marchiori [1], Guilherme Lopes [2], Eduardo Valério de Barros Vilas Boas [3] and Luiz Roberto Guimarães Guilherme [2,*]

1. Department of Biology, Institute of Natural Sciences, Federal University of Lavras (UFLA).
2. Department of Soil Science, School of Agricultural Sciences, Federal University of Lavras (UFLA).
3. Department of Food Science, School of Agricultural Science, Federal University of Lavras (UFLA)

Abstract: A water deficit can negatively impact fruit yield and quality, affecting critical physiological processes. Strategies to mitigate water deficits are crucial to global food security. Iodine (I) may increase the efficiency of the antioxidant system of plants, but its role against water deficits is poorly understood. This study aimed to evaluate the effectiveness of I in attenuating water deficits and improving fruit quality, investigating whether metabolic responses are derived from a "priming effect" or stress relief during water deficits. Tomato plants were exposed to different concentrations of potassium iodide (KI) via a nutrient solution and subjected to a water deficit. A water deficit in tomatoes without KI reduced their yield by 98%. However, a concentration of 100 µM of KI increased the yield under a water deficit by 28%. This condition is correlated with increased antioxidant activity, photosynthetic efficiency improvement, and malondialdehyde reduction. In addition, the concentration of 100 µM of KI promoted better fruit quality through antioxidant capacity and a decline in the maturation index. Therefore, KI can be an alternative for attenuating water deficits in tomatoes, inducing positive responses during the water deficit period while at the same time improving fruit quality.

Keywords: iodine; abiotic stress tolerance; drought; antioxidant defense; post-harvest.

Introduction

Drought can occur naturally, but climate change is contributing to the acceleration of this event, causing crop losses worldwide and posing a global threat to food security [1,2]. The world population is increasing and is expected to reach nine billion by 2050, consequently requiring a continuous increase in crop production [3]. However, drought is estimated to create serious plant growth problems with negative impacts on yield [4].

Water deficits are one of the principal stresses affecting plants' physiology and biochemistry. A consequence of water deficits in plants is the indiscriminate increase in reactive oxygen species (ROS), which may lead to oxidative stress in plants, promoting the oxidation of essential molecules for plant development associated with the lipid peroxidation of membranes [5]. To minimize such adverse effects, plants adopt mechanisms of tolerance by activating enzymes of the antioxidant system, such as superoxide dismutase (SOD) and catalase (CAT) [6]. The antioxidant system is an indispensable mechanism for neutralizing ROS and, consequently, reducing cell damage caused by these molecules [7]. Lima et al. [8] and Ravello et al. [9] highlighted the importance of antioxidant enzymes in mitigating different abiotic stresses. Another strategy to deal with water deficits is the biosynthesis of osmoprotectants: small, low-molecular-weight, and non-toxic molecules, such as free amino acids like proline and some carbohydrates. The accumulation of these metabolites promotes the protection of plants against oxidative damage and improves water absorption in a water deficit [10,11].

In addition, decreases in growth and yield under a water deficit may occur due to changes in plant photosynthesis. A series of molecular, metabolic, physiological, and morphological processes are triggered in plants in response to drought conditions [12]. There is a reduction in carbon assimilation by the leaves under a water deficit, causing changes in the partition of photoassimilates and hence declines in growth and crop yield [13]. In addition, cell toxicity can cause enzymatic dysfunction, reducing the photosynthetic rate and water use efficiency [14].

Another essential aspect of the production system is consumers' demand for quality food, highlighting its appearance, taste, nutritional value, and functional potential. However, adverse climatic conditions are often hard to control, and plants exposed to stressful environments usually lose fruit quality [15]. Quality attributes are affected by water deficits during the production of fruit-type vegetables [16]. A water deficit may increase the concentration of primary metabolites, such as organic acids and sugars, which affect flavor,

as well as secondary metabolites, such as flavonoids, anthocyanins, lycopene, β-carotene, and vitamin C [17], which affect coloration, the nutritional value, shelf life, and functional potential of the fruit. Therefore, in addition to reducing yield, a water deficit may impair fruit quality, accelerating deterioration [18].

The exogenous application of mineral elements is a vital strategy to alleviate the adverse effects of water deficits on plants. Recently, several studies have recognized that the application of iodine strengthens the antioxidant capacity of soybean (*Glycine max*), lettuce (*Lactuca sativa*), and tomato (*Solanum lycopersicum* L.) plants by stimulating the activity of the main ROS detoxifying enzymes, such as SOD, ascorbate peroxidase (APX), and CAT [7,8,19,20]. Another point to highlight is the nutritional role of iodine in plants, since this element can bind covalently to at least 82 different proteins in the leaves and roots of *Arabidopsis thaliana*; its presence in micromolar concentrations in the nutrient solution resulted in increased accumulation of plant biomass and timely flowering [21].

Tomato (*S. lycopersicum* L.) requires large amounts of water. Consequently, it is negatively affected by water deficits, especially during the reproductive phase, where photosynthesis is limited, with an intensification of floral abortion and, as a result, a reduction in yield [22,23]. This vegetable is the most cultivated in the world and is one of the most nutritionally and economically important crops [24]. Among horticultural crops, it is one of the essential model species, especially the Micro-Tom cultivar, which is helpful for studies on plant tolerance to environmental stresses due to its well-known genetic profile and convenient transformation techniques [25,26].

The exogenous application of iodine to tomato plants can be an alternative for mitigating a water deficit, as demonstrated for other species [8,27]. Also, iodine has shown positive effects on improving the post-harvest quality of fruits [28]. Thus, the current study aims to ascertain whether iodine can boost the response to a water deficit in the Micro-Tom tomato cultivar while at the same time leading to the production of high-quality fruits. This study holds considerable innovation potential, as it not only addresses novel approaches to alleviating water stress in plants but also emphasizes a concurrent impact on the quality of the ultimate agricultural produce. This, in turn, promises to enhance both yield and crop quality. Given iodine's limited exploration in agriculture, this research represents a pioneering step toward the incorporation of this element into plant nutrition programs, offering a comprehensive characterization of its role in plants and its beneficial effects for agricultural crops.

Results

Production

The water deficit affected ($p < 0.05$) the production and water deficit tolerance index (WDTI) of tomato plants (Figure 1). However, potassium iodide (KI) at a concentration of 100 µM increased the yield and WDTI when the plants were grown under a water deficit (Figure 1A,D). The yield was reduced by 91% in the water deficit plants at 0 and 50 µM KI concentrations compared with the control plants (optimal irrigation and 0 µM of KI) (Figure 1A). However, at the concentration of 100 µM of KI under the water deficit, the reduction in yield was 47% compared with the control. Furthermore, the concentration of 100 µM of KI increased the yield of the tomato plants by 23% compared with the other tested KI concentrations (0 and 50 µM KI) under the water deficit.

The water deficit provided a ~98% reduction in the WDTI in plants under the 0 and 50 µM KI treatments and 46% under the 100 µM KI treatment compared with the control treatment (Figure 1D). However, under the water deficit, the concentration of 100 µM of KI increased the WDTI of the plants by ~28% compared with the treatments without KI and with 50 µM of KI. There was no influence of the treatments on the number of fruits. However, the cultivation under the water deficit verified a reduction of ~71% in the dry mass per fruit compared with the control (Figure 1C).

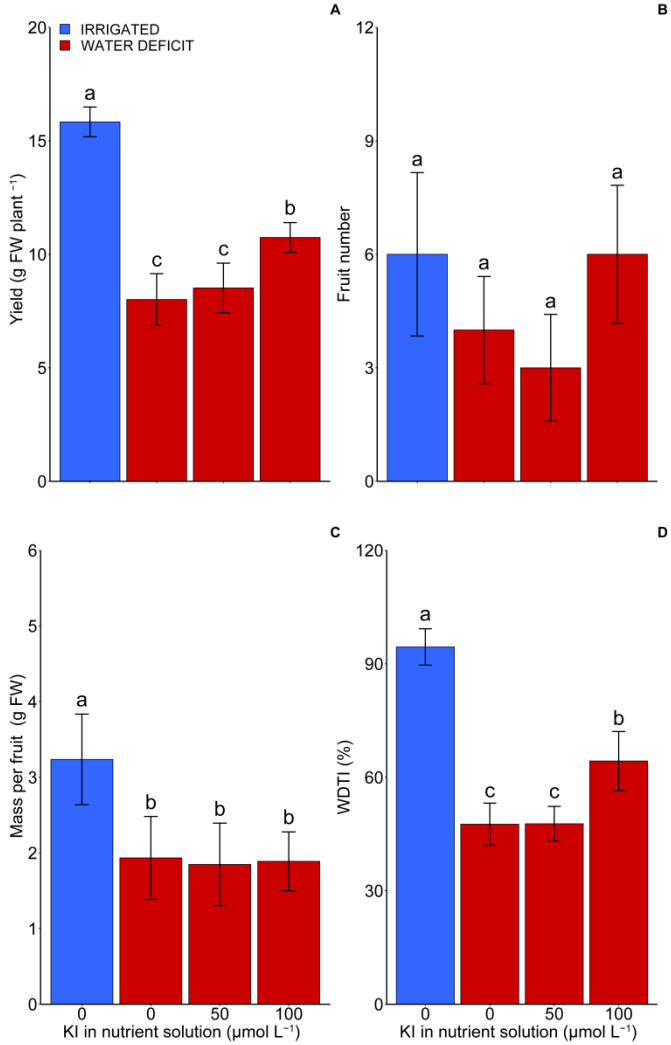

Figure 1. Effect of KI on yield (A), number of fruits (B), mass per fruit (C), and water deficit tolerance index—WDTI—(D) in tomato plants under water deficit or not. The control treatment (without water deficit) corresponds to the blue bars (first bar), while the second, third, and fourth bars, in red, represent the water deficit condition and the treatments with 0, 50, and 100 µmol L^{-1} of KI, respectively. Values are average ± SD (n = 4). The same letters indicate no significant difference ($p > 0.05$) in the Tukey test. The fruits were harvested 90 days after the seeds' germination, and all the clusters were collected.

Fruit Quality

The fruit quality changed depending on the water deficit and KI concentrations ($p < 0.05$). A ~3% reduction in the fruit pH occurred when the plants were subjected to a water deficit and supplemented with 50 and 100 µM of KI compared with plants without KI grown with and without a water deficit (Figure 2A). On the other hand, the titratable acidity was 65% higher in the fruits from the control treatment and those subjected to concentrations of 50 and

100 µM of KI under a water deficit when compared with the fruits under a water deficit and without KI application (Figure 2B).

Fruits from the plants treated with 50 µM of KI under a water deficit exhibited higher soluble solids content when compared with those from plants without KI treatment under a water deficit, showing an increase of 6.96%. No significant differences were observed concerning the treatments with irrigation or with 100 µM of KI under a water deficit (Figure 2C). The water deficit promoted an increase in the fruit maturation index (SS/AT), a fact mitigated by KI, in a dose-dependent manner (Figure 2D). The highest and lowest maturation index, 56.38 and 35.18, were observed in the fruits under a water deficit at 0 and 100 µM KI concentrations, respectively.

The water deficit increased the antioxidant activity of the fruits, measured through the ABTS method, by around 15% (Figure 2H). However, no differences occurred when evaluating it with the β-carotene/linoleic acid and phosphomolybdenum complex methods (Figure 2E,F). When assessing the effect of KI on a water deficit, it is noteworthy that fruits from the plants treated with 100 µM of KI had their antioxidant activity increased by around 25%, regardless of the determination method (Figure 2E,F,H). On the other hand, the dose of 50 µM of KI did not interfere with the antioxidant activity of the fruits produced by plants under a water deficit (Figure 2E,F).

As observed for antioxidant activity, the water deficit increased the total phenolic content, which was higher for the dose of 100 µM of KI (Figure 2G). Therefore, the highest concentration of total phenolics happened in the fruits harvested from the plants treated with 100 µM of KI under water deficit conditions (6233.65 mg GAE 100 g^{-1} FW) (Figure 2G). The tomato fruits produced under a water deficit had around 54% more total phenolics than the fruits not subjected to the water deficit, and the concentration of 100 µM of KI determined an increase of approximately 81% in this variable in the fruits under the water deficit.

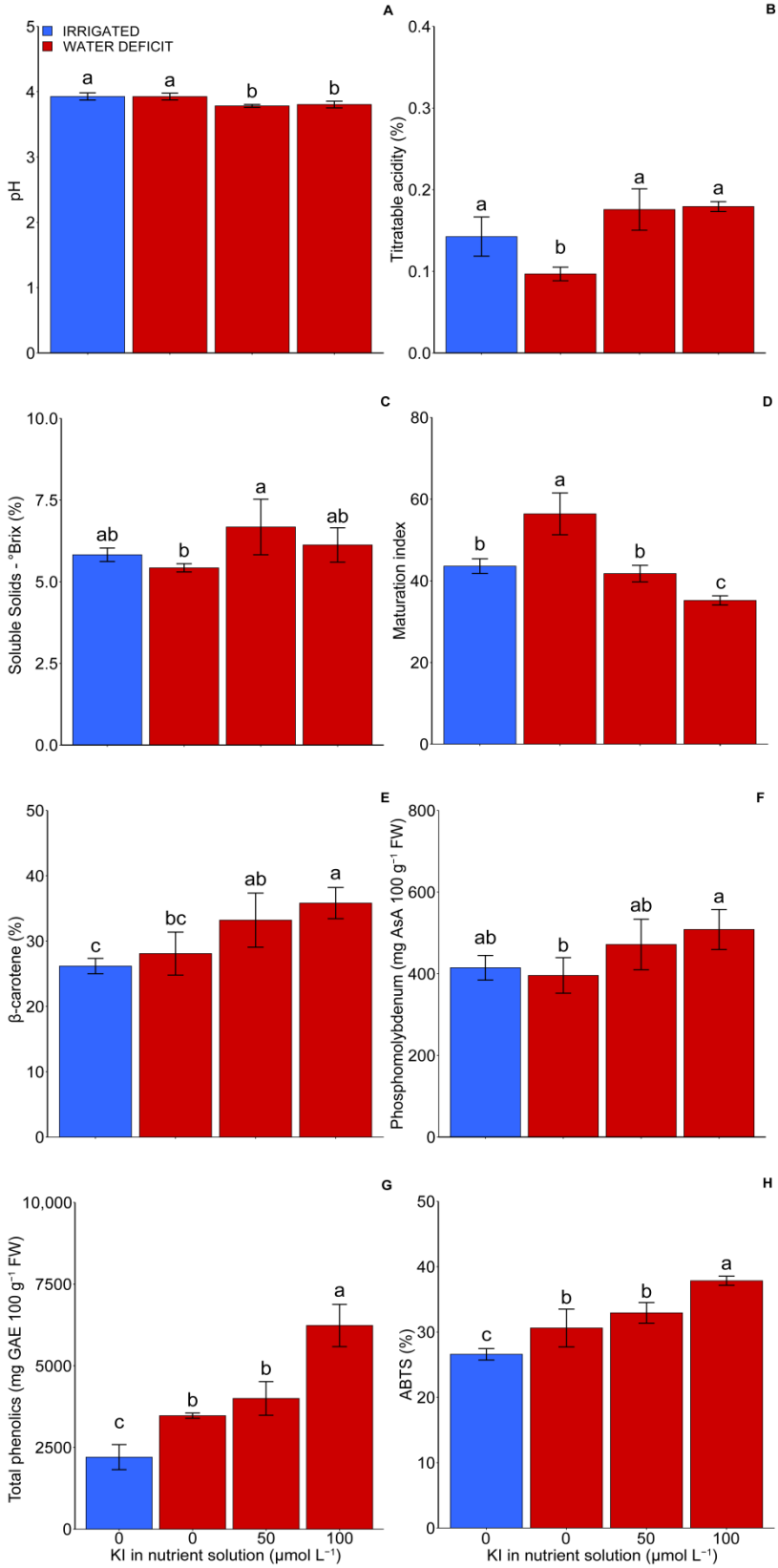

Figure 2. Effect of KI on pH (A), titratable acidity (B), soluble solids (C), maturation index (D), β-carotene, (E) phosphomolybdenum (F), total phenolics (G), and ABTS (H) in tomato fruits under water deficit or not. The control treatment (without water deficit) corresponds to the blue bars (first bar), while the second, third, and fourth bars, in red, represent the water

deficit condition and the treatments 0, 50, and 100 µmol L^{-1} of KI, respectively. Values are average ± SD (n = 4). The same letters indicate no significant difference ($p > 0.05$) in the Tukey test.

Photosynthesis

The water deficit drastically reduced ($p < 0.05$) the assessed photosynthetic variables, although the concentration of 100 µM of KI minimized this effect (Figure 3). An increase of ~224% in the net photosynthesis was observed when the plants were subjected to a water deficit and exposed to a 100 µM KI (1.07 µmol CO_2 m^{-2} s^{-1}) concentration compared with those without KI under the same irrigation conditions (0.33 µmol CO_2 m^{-2} s^{-1}) (Figure 3A). In the ETR, an increase of 51% was observed in the treatment with 100 µM of KI compared with that without KI, both under a water deficit (Figure 3B). Moreover, the highest values for the net photosynthesis and ETR were observed in the control treatment (11.06 µmols CO_2 m^{-2} s^{-1} and 83.55 µmols electrons m^{-2} s^{-1}, respectively).

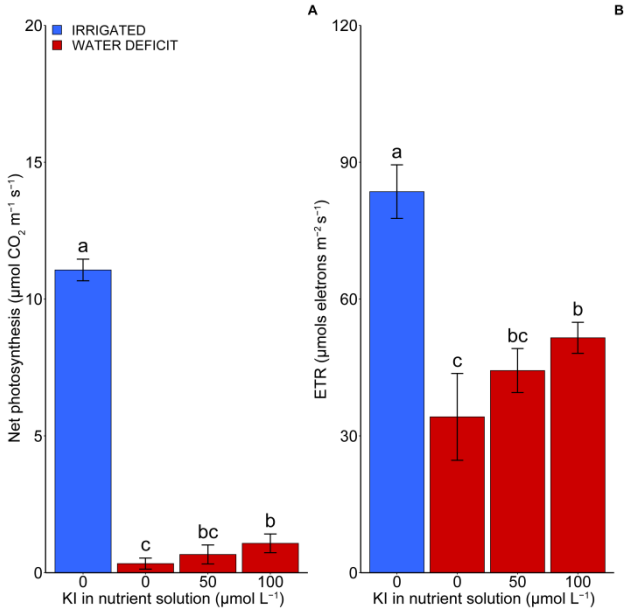

Figure 3. Effect of KI on net photosynthesis (A) and electron transport efficiency rate (B) in tomato plants under water deficit or not. The control treatment (without water deficit) corresponds to the blue bars (first bar), while the second, third, and fourth bars, in red, represent the water deficit condition and the treatments 0, 50, and 100 µmol $L^{-1}$of KI, respectively. Values are average ± SD (n = 4). The same letters indicate no significant difference ($p > 0.05$) in the Tukey test.

Oxidative Damage and Antioxidant Enzymes

The malondialdehyde (MDA) and H_2O_2 levels increased significantly ($p < 0.05$) during the water deficit (Figure 4A,B). However, the plants treated with 100 µM of KI did not change MDA content significantly before the water deficit period (Figure 4A). A ~56% reduction in MDA occurred in the plants with a treatment with 100 µM of KI (14.31 nmol MDA g^{-1} FW) compared with those without KI (31.80 nmol MDA g^{-1} FW) and 50 µM of KI (34.27 nmol MDA g^{-1} FW) during the water deficit. Regarding the concentration of H_2O_2, an increase of ~107% was found in the period of water deficit compared with the period before the water deficit (Figure 4B).

The antioxidant enzymatic activity also changed depending on the water deficit and KI concentrations ($p < 0.05$). For the SOD activity, an increase of ~120% was observed in the treatment without KI during the water deficit compared with the treatments before the deficit imposition (Figure 4C). Additionally, the 50 and 100 µM KI treatments increased the SOD activity during the water deficit by ~233% when compared with the same treatments before the water deficit and ~127% when compared with treatments without KI during the deficit. On the other hand, the CAT activity was only significantly influenced by the 100 µM KI treatment during the water deficit, with an increase of ~124% compared with the other treatments.

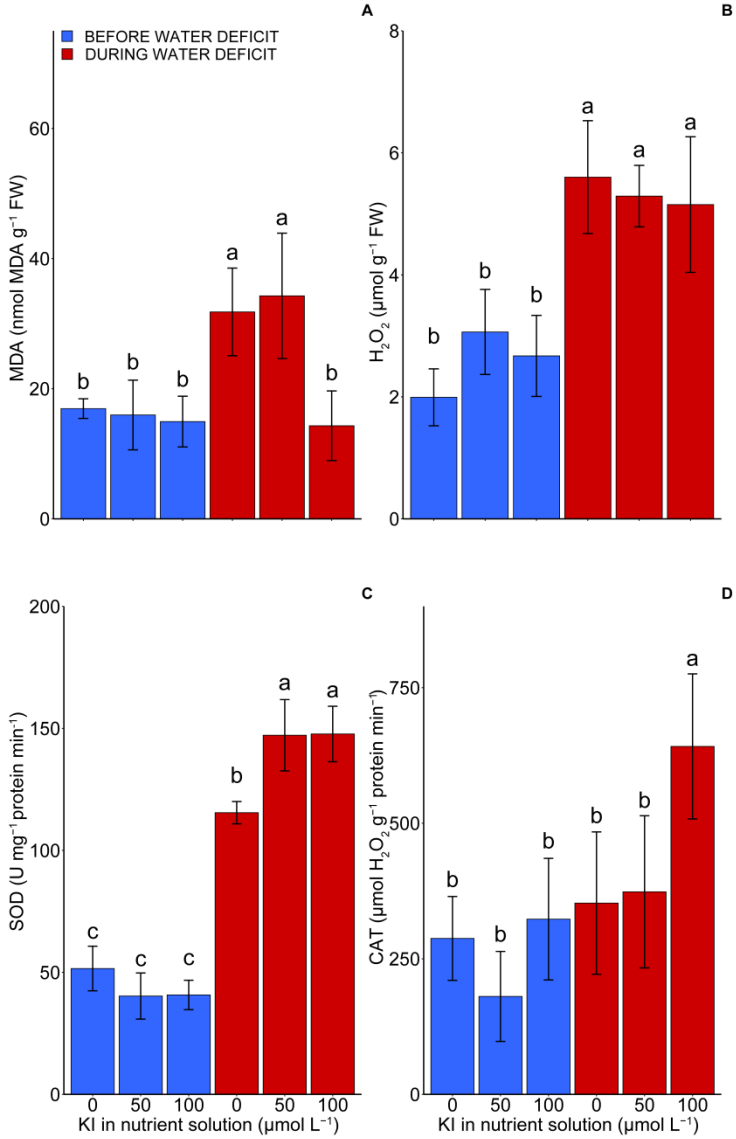

Figure 4. Effect of KI on malondialdehyde content (A), hydrogen peroxide (B), superoxide dismutase activity (C), and catalase activity (D) in tomato plants before and during water deficit. The first, second, and third blue bars represent the condition before the deficit and the treatments 0, 50, and 100 µmol L^{-1} of KI, respectively. In contrast, the first, second, and third red bars represent the condition during the deficit and the treatments 0, 50, and 100 µmol L^{-1} of KI, respectively. Values are average ± SD (n = 4). The same letters indicate no significant difference ($p > 0.05$) in the Tukey test.

Osmolytes

The compatible osmolyte levels changed as a function of the treatments applied ($p < 0.05$) (Figure 5). The water deficit resulted in a significant increase in the content of total soluble sugars in the leaves, with sucrose levels rising by approximately 96% and 46%,

respectively, in the treatments without and with 50 µM of KI. Notably, there was no statistically significant change in the treatment with 100 µM of KI (Figure 5A,B).

The total free amino acid levels were not affected by the water deficit, while the levels of the amino acid proline, specifically, increased as a function of stress (Figure 5C,D). KI, at concentrations of 50 and 100 µM, did not interfere with the levels of both variables under adequate water conditions. However, it caused a significant increase ($p < 0.05$) in their levels under water deficit conditions (Figure 5C,D). Potassium iodide determined an approximately 146 and 132% increase in the total free amino acids and proline, respectively, in the plants during the water deficit.

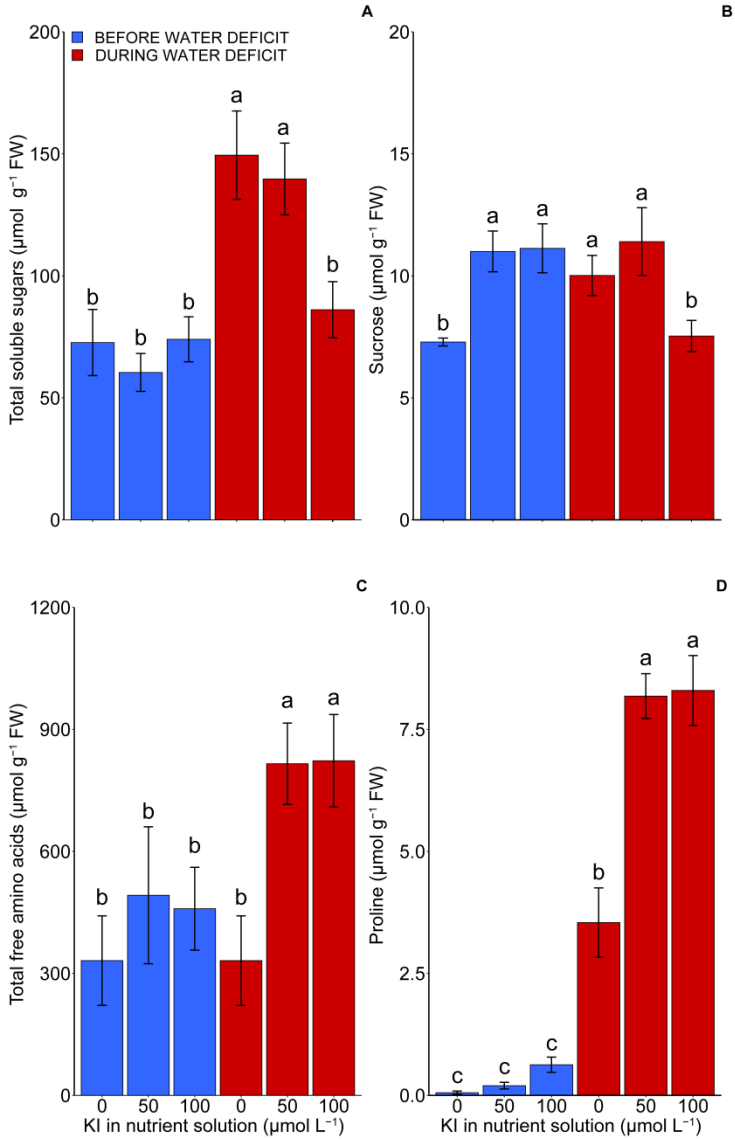

Figure 5. Effect of KI on total soluble sugars (A), sucrose (B), total free amino acids (C), and proline (D) in tomato plants before and during water deficit. The first, second, and third blue bars represent the condition before the deficit and the treatments 0, 50, and 100 µmol L^{-1} of KI, respectively. In contrast, the first, second, and third red bars represent the condition during

the deficit and the treatments 0, 50, and 100 µmol L^{-1} of KI, respectively. Values are average ± SD (n = 4). The same letters indicate no significant difference ($p > 0.05$), and different letters represent a significant difference ($p < 0.05$) in the Tukey test.

Multivariate analysis

Principal component analyses (PCA) and Pearson correlation were performed while considering the variables relating to the crop yield, fruit quality, and physiological and biochemical characteristics of the tomatoes grown under a water deficit and treated with different concentrations of KI. The first principal components (PC1 and PC2) presented 67.47% of the total data variance (Figure 6). The biplot revealed a strong relationship between the treatment without KI and the fruit ripening index and pH. These two variables correlated positively and negatively with SOD, proline, total free amino acids in leaves, titratable acidity, and ABTS in the fruits, with the respective variables being more favored by concentrations of 50 and 100 µM of KI.

The concentration of 100 µM of KI favored most of the variables analyzed, including the yield, water index tolerance, net photosynthesis, ETR, catalase, number of fruits, ABTS, phosphomolybdenum, and total phenols of the fruits. The yield, tolerance index, and net photosynthesis were positively correlated. Furthermore, the three variables mentioned above correlated positively with catalase activity. However, the yield and tolerance index correlated negatively with the content of malonaldehyde and sucrose in the leaves, which, in turn, were more favored by the concentration of 50 µM of KI. The concentration of 50 µM of KI also favored the accumulation of soluble sugars in the leaves, which is negatively correlated with the net photosynthesis. The fruit quality variables favored, in part, by the concentration of 50 µM of KI (titratable acidity and β-carotenes), in part by the concentration of 100 µM of KI (phosphomolybdenum, ABTS, and total phenols), except for β-carotene, correlated positively with net photosynthesis. However, the total phenolics, ABTS, and phosphomolybdenum correlated negatively with the total soluble sugar content in the leaves.

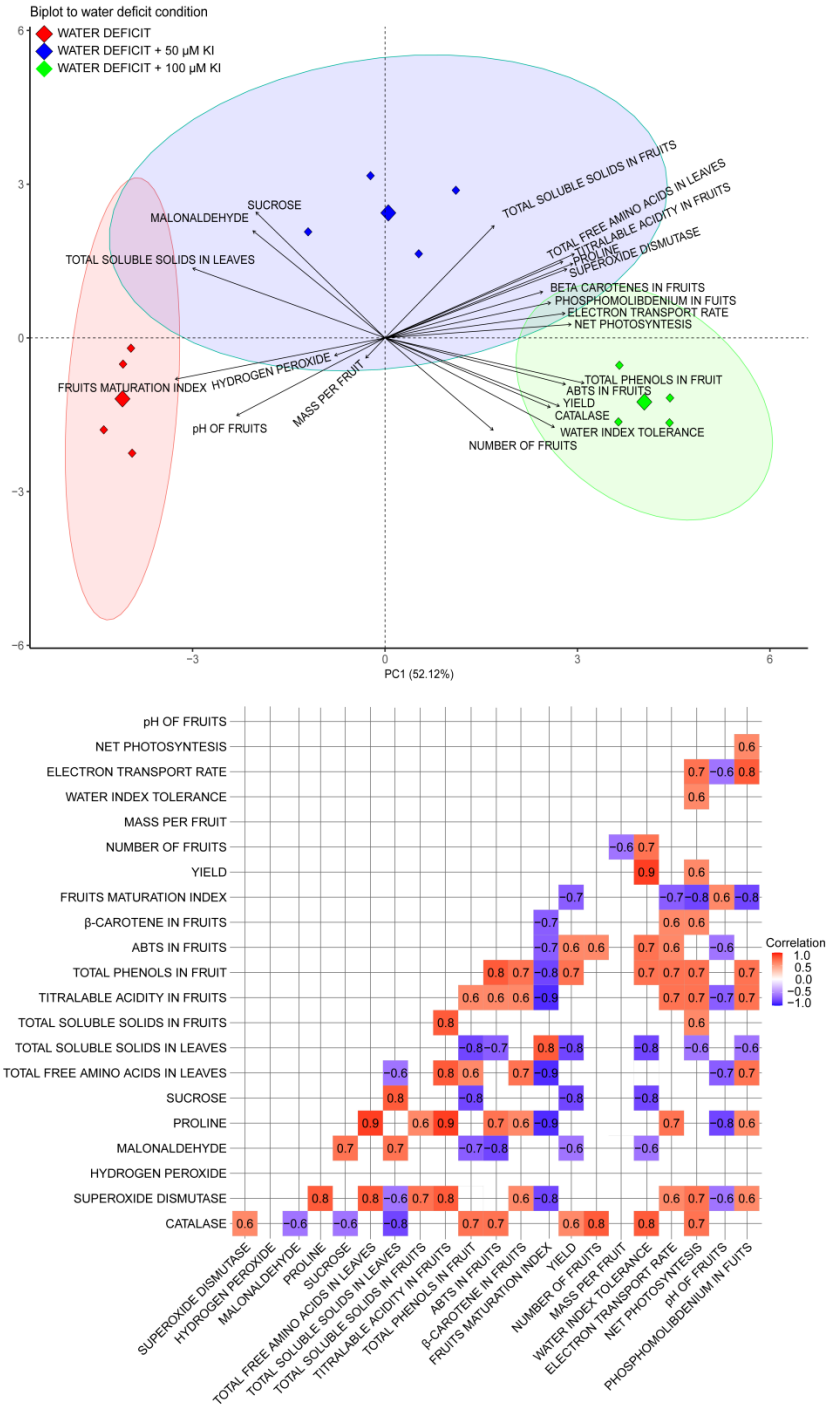

Figure 6. Principal component analysis and Pearson correlation using tomato plants' production, fruit quality, and physiological and biochemical characteristics data in response to water deficit conditions and KI application. Significant correlation coefficients ($p < 0.05$) are indicated by bold numbers, with positive and negative correlations distinguished by red and blue, respectively. White boxes indicate non-significance without numbers.

Discussion

Water is a vital environmental factor that affects plant growth, yield, and quality, mainly due to negative physiological and biochemical responses [29]. As in other crops, water scarcity causes a prominent inhibitory effect on tomatoes' physiological, biochemical, and yield responses [23]. Iodine is a strategic alternative for combating abiotic stresses [8,19,30,31]. However, the effects of this element on water deficit mitigation have been little explored [8].

Our findings showed that, under water deficiency, the tomato plants had a reduction in yield, which correlated with decreases in photosynthetic variables and an increase in cell damage (MDA) (Figures 1, 3, 4, 6, and 7). Water deficits can influence redox balance and cause secondary oxidative stress with an excessive generation of reactive oxygen species (ROS) in plant cells [32,33]. Consequently, oxidative damage to biological macromolecules, such as lipids and proteins, will interfere with cellular functions and reduce plant growth and yield [33]. In photosynthetic organisms, the main sites of ROS generation occur in photosystem I and II in the chloroplast, and oxidative stress is closely related to photoinhibition and a reduction in photosynthesis [32].

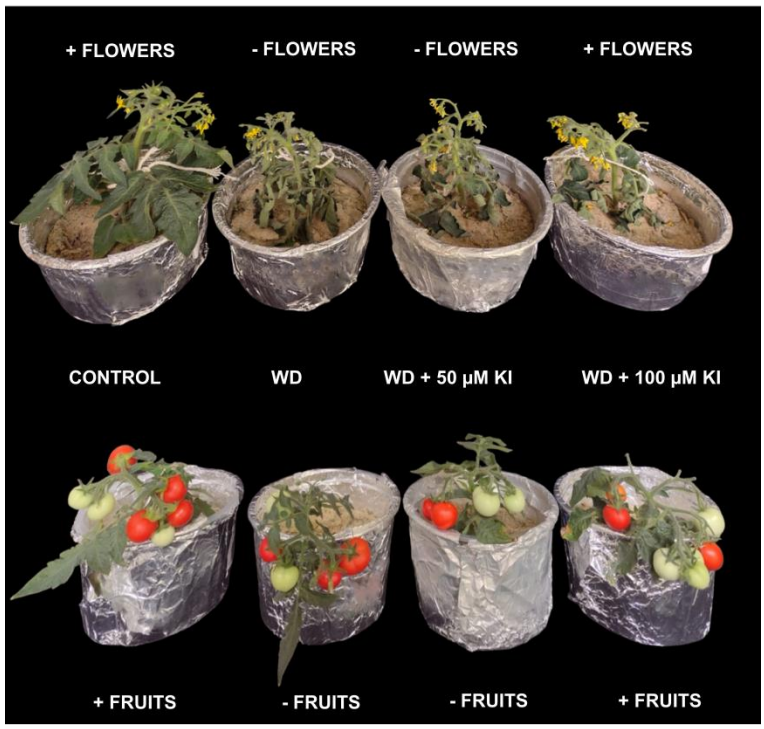

Figure 7. Representation of treatments in the flowering phase under water deficit and in the reproductive phase. Control: plants under optimal irrigation and without KI application; WD: plants subjected to water deficit; WD + 50 µM KI: plants subjected to water deficit and a

concentration of 50 µM of KI; and WD + 100 µM KI: plants subjected to water deficit and a concentration of 100 µM of KI.

Some studies have shown that the imposition of a water deficit on tomato seedlings increased the MDA content, which has been used as a biomarker of oxidative stress, also causing a decrease in ETR and net photosynthesis in tomato plants [29,34–36]. The results observed in the present study corroborate with those previous reports. However, applying KI at a concentration of 100 µM reduced the MDA content during the water deficit. Moreover, it improved the photosynthetic characteristics of the plants through increased antioxidant enzymatic activity, the accumulation of amino acids, and proline during the water deficit, thus providing greater yield in these plants (Figures 1 and 3–7). These results are similar to those observed by [8], studying soybean plants subjected to water deficits and exposed to different concentrations of KI. The authors found increases in the activity of antioxidant enzymes, which in turn provided a reduction in MDA and an increase in the photosynthetic rate of these plants. Additionally, other studies with soybeans and lettuce indicated that applying I at concentrations of 20, 40, and 80 µM increased the activities of SOD, APX, and CAT [7,30].

Regarding the accumulation of amino acids and proline promoted by KI during a water deficit (Figure 5C,D), our results contrast those found in the literature. Increasing KI concentrations did not affect proline accumulation in soybean plants grown under a water deficit [8]. Additionally, Kiferle et al. [19] observed that applying iodine reduced the accumulation of these osmolytes in tomatoes subjected to saline stress. Similarly, lettuce plants subjected to saline stress and treated with iodine (KI) had reduced proline accumulation [37]. This difference in responses may have occurred due to differences in species, intensities, and exposure time to stress since the accumulation of proline and other amino acids works as a tolerance mechanism for water deficits [38]. These molecules can accumulate during environmental stresses by plants to maintain water status through osmoregulation, which prevents damage to vital plant molecules, such as DNA, proteins, and lipids, caused by ROS [39,40].

In contrast, the plants that had the highest accumulation of sucrose in the leaves (0 and 50 µM of KI under a water deficit) were the ones that had the lowest yield (Figures 1A and 5B). A water deficit can inhibit plant growth, causing the accumulation of sucrose in the leaves and promoting a disturbance of the metabolic balance of sucrose [41]. Drought conditions generally increase sucrose phosphate synthase (SPS) activity and may increase sucrose accumulation [42–44]. Similarly to proline, the accumulation of soluble sugars (such as

sucrose) acts as a source of osmolites that maintain and protect plant macromolecules and structures from stress damage, eventually increasing plant tolerance to water deficits [45,46]. However, the control plants, i.e., those not submitted to the water deficit and those treated with 100 µM of KI, which had the deleterious effects promoted by the water deficit relieved by other mechanisms, possibly translocated carbohydrates to a more substantial sink, thereby reducing floral abortion and consequently increasing yield (Figures 1A and 7).

Our study indicated that the primary responses related to tolerance to water deficits promoted by KI in tomato plants were triggered during stress through relief during the deficit, dismissing a possible priming effect. During priming, a perception of stress is established, causing physiological, metabolic, and molecular changes, such as accumulating stress-responsive osmoregulatory metabolites or synthesizing protective proteins [47]. Some researchers propose that iodine can increase ROS, including H_2O_2, to stimulate enhanced activity of antioxidant enzymes in adverse conditions [48–50]. This was not observed in our study since KI did not influence the enzyme activities before the deficit, and the H_2O_2 content increased only when the plants were subjected to the water deficit, regardless of the KI concentrations (Figure 4).

Indeed, the water deficit promoted increased H_2O_2 levels (Figure 4B). Since H_2O_2 is toxic to cells, it must be eliminated, and CAT, which transforms H_2O_2 into H_2O and O_2, implements this. Potassium iodide at a concentration of 100 µM promoted the most significant increase in CAT (Figure 4D), which suggests its greater efficiency in controlling the deleterious effects of water deficits. Like CAT, SOD neutralizes ROS produced during oxidative processes, specifically the superoxide radical (O_2^-). The water deficit increased the SOD activity more pronouncedly in the fruits under the influence of KI, regardless of the dose used (Figure 4C). These results suggest, once again, the role of KI in controlling the harmful effects of water deficits, this time at the two doses tested. Malondialdehyde, an end product of lipid peroxidation, is commonly used to indicate oxidative stress. The production of free radicals increases lipid peroxidation and, consequently, MDA. The water deficit increased the production of MDA. However, this increase was significantly limited by KI at a concentration of 100 µM (Figure 4A), which demonstrates, once again, its potential to mitigate the adverse effects of this type of stress. Overall, the results point to the importance of KI in mitigating the harmful effects of free radicals and its potential as a mineral supplement in controlling or minimizing the impact of water deficits. These results are similar to those found in soybean plants treated with KI and grown under a water deficit [8].

A water deficit generally promotes the concentration of internal components (sugars,

organic acids, ascorbic acid, and carotenoids, among others) due to the decrease in water content in fruits [51–53]. However, the water deficit did not significantly change ($p < 0.05$) the SS content of the fruits, nor their pH, although it reduced their TA. The reduction in TA can be associated with the more active consumption of organic acids due to increased metabolism caused by stress. However, these variables should not be analyzed in isolation but together since SS gives an idea of sweetness, while pH and TA reflect tomato acidity. A balance between sweetness and acidity is vital in accepting the fruit. Thus, the maturation index, also known as the ratio SS/TA, provides more relevant information than the isolated assessment of each variable. When evaluating the maturation index, it was noted that the water deficit promoted its increase. A water deficit usually accelerates fruit maturation [54], which is generally associated with an increase in the ripening index. Regardless of the dose, but more intensely at 100 µM, KI reduced the increase in the ripening index caused by the water deficit, suggesting its stress-mitigating effect and increasing the post-harvest shelf life of tomatoes.

The increase in total phenolics caused by the water deficit (Figure 2G) can be associated with their fruit concentration. Since phenolics are potent reducing agents, this increase was reflected in an increase in the antioxidant activity, measured through the ABTS+ method, which did not agree with the data obtained with the β-carotene and phosphomolybdic complex methods (Figure 2). On the other hand, the supplementation of plants under a water deficit with 100 µM of KI induced a more significant accumulation of total phenolics and improved the antioxidant activities, based on the three methods used (Figure 2E–H). These results converge with those of MDA, H_2O_2, SOD, and CAT, highlighting the potential of KI in mitigating the harmful effects of free radicals produced from oxidative stress. The protective effect of KI on oxidative processes minimized the consumption of phenolics as reducing agents, resulting in fruits with enhanced antioxidant activity. Our results corroborate with the results of Mejía-Ramírez et al. [55], who observed an improvement in the quality of tomatoes when evaluating the priming effect of iodine in seeds, which increased, for example, the levels of phenolic compounds and β-carotenes. Maglione et al. [56] observed that treatment with $NaCl^+$ iodine improved the nutritional value and functional potential of lettuce plants in terms of bioactive compounds.

Therefore, the concentration of 100 µM of KI was the most efficient in promoting beneficial effects in conditions of stress due to a water deficit, highlighting that most of the production, quality, photosynthetic, and biochemical variables analyzed pointed to 100 µM of KI as the best treatment, as illustrated in the PCA (Figure 6). This respective concentration had the highest tolerance index to the water deficit among the plants grown under a water

deficit (Figure 1D). Our findings suggest that the primary process responsible for the enhancement in tolerance to the water deficit with the application of KI was related to the increase in the production of antioxidant enzymes, with an emphasis on CAT and SOD, which promoted better protection of the photosynthetic apparatus, thus allowing for a greater yield and better fruit quality under water deficiency. Furthermore, it was found that metabolic responses were induced mainly during the water deficit.

Materials and Methods

Cultivation System, Experimental Design, and Treatments

Tomato cultivation was carried out in a growth chamber with an average temperature of 22 °C and a photoperiod of 12/12 h, located in the Plant Physiology Sector of the Biology Department of the Federal University of Lavras (UFLA) (21°14′45″ S, 44°59′59″ W, 920 m above sea level), southeastern Brazil. Initially, the seeds were germinated in petri dishes and then transplanted into pots. The plants were cultivated in vases with 500 g of washed sand, with one plant per pot, and fertigated once a week with 20 mL of Hoagland and Arnon [57] nutrient solution for ten weeks. The KI concentrations applied weekly were 0, 50, and 100 µM. Therefore, the total amounts of I applied were approximately 1.27 and 2.53 mg of I kg^{-1} of substrate in the treatments of 50 and 100 µM of KI, respectively.

The treatments added to the pots were arranged in a completely randomized design with four replicates and two pots per experimental plot, so there was the possibility of collecting plants for evaluations in two moments (before and during water deficit) and evaluating yield. Therefore, the experiment had 32 experimental units, with whole plants collected before the water deficit (one day before), to assess possible priming effects on the antioxidant system, oxidative stress, and osmolyte content. In addition, one leaf per plant was collected during the deficit to evaluate the effects of the deficit (one day before rehydration). On both collection occasions, the samples were homogenized, forming a composite sample from the two pots of each treatment to minimize variations per plant. The experiment lasted 90 days until the species' reproductive cycle ended. The treatments used are shown in Table 1 below.

Table 1. Representation of treatments applied in the experiment.

Treatments	Description

Treatment 1 (Control)	Irrigation + 0 µM KI
Treatment 2	Water Deficit + 0 µM KI
Treatment 3	Water Deficit + 50 µM KI
Treatment 4	Water Deficit + 100 µM KI

Production and Water Deficit Tolerance Index

The fresh weight of the fruits on each plant was considered for the yield assessment. The number of fruits per plant and the average weight of a fruit were also evaluated. Furthermore, the WDTI was calculated according to [58] using the following equation:

$$\text{WDTI} = \left(\frac{\text{yield of reference plants}}{\text{yield of the other treatments}}\right) \times 100$$

Fruit Quality

The fruit pulp was crushed in water in a 1:3 ratio (m/v); the homogenate was filtered in an organza cloth; and the filtrate was used for the pH, titratable acidity (TA), and soluble solids (SS) determinations. The pH was determined using a Tecnal® pH meter, previously calibrated using buffer solutions (pH 4.0 and 7.0). The titratable acidity was determined via titration with a 0.01 N sodium hydroxide (NaOH) solution, using phenolphthalein as an indicator, according to the AOAC [59]. The results were expressed in mg of citric acid 100 g^{-1} of the sample. The soluble solids were determined in an ATA-GO PR-100 digital refractometer (Tokyo, Japan) with automatic temperature adjustment, and the results were expressed in %, as described by the AOAC [59]. The SS/TA ratio, the maturation index, was calculated.

Obtaining extracts for the quantification of total phenolic compounds and antioxidant activity was carried out according to the procedure of Rufino et al. [60]. Briefly, 0.5 g of a sample was homogenized, along with 4 mL of 50% methanol and 4 mL of 70% acetone, in a centrifuge tube for 30 min on a shaking table, protected from light. Then, the tubes were taken to an ultrasonic bath for 30 min, and the homogenate was filtered through filter paper (qualitative filter paper, 15 cm in diameter, Unifil®, Carvalhaes Produtos para Laboratorio LTDA, Rio Grande do Sul, Brazil). The filtrate was transferred to a 10 mL volumetric flask, and the volume was topped up with distilled water. The obtained extracts were placed in amber

bottles and stored in a freezer until the total phenolics and antioxidant activity were analyzed.

The total phenolic compounds were determined through the method described by Medina [61], using Fast Blue BB, with some adaptations. Fifty µL of the extract was mixed with 200 µL of distilled water, 25 µL of Fast Blue reagent (0.1%, *v*/*v*), and 25 µL of sodium hydroxide (5%, *w*/*v*), and the absorbance was measured at 420 nm after 1.5 h of incubation in the dark. All measurements were performed in triplicate using a microplate reader. The results were reported as gallic acid equivalents in mg 100 g^{-1} fresh sample mass (mg GAE 100 g^{-1} FM).

The antioxidant activity was determined with the β-carotene/linoleic acid, ABTS+, and phosphomolybdenum complex. The determination of the antioxidant activity through the β-carotene/linoleic acid method was based on β-carotene oxidation (discoloration) induced by the oxidative degradation products of linoleic acid ([60], with modifications). Solutions were prepared by mixing 270 µL of β-carotene/linoleic acid system solution and 30 µL of extract into each well of a 96-well, flat-bottomed microplate. The mixture was kept in a water bath at 40 °C, and the readings were performed at 470 nm after 2 h. The results were expressed as a percentage of oxidation inhibition.

Determining the antioxidant activity through the ABTS+ method was based on capturing the ABTS+ radical with an antioxidant. Briefly, the ABTS+ solution was prepared by reacting diammonium salt 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) at a concentration of 7 mmol L^{-1} with potassium persulfate 2.45 mmol/L at room temperature for 16 h. The obtained solution was then diluted with ethanol until an absorbance of 0.70 ± 0.05 at 734 nm was reached. Aliquots of 5 µL of sample extracts were pipetted into a 96-well, flat-bottomed microplate. An aliquot of 295 µL of solution (ABTS+) was added to each well. After 6 min of reaction time in the dark, the absorbance was measured at 734 nm. The results of antioxidant activity were expressed in % ABTS Reduction, according to the equation below [62].

$$\% \text{ ABTS Reduction} = (\text{ABS control} - \text{ABS sample})/(\text{ABS control}) \times 100$$

Antioxidant determination through the phosphomolybdenum complex, based on the reduction of Mo^{6+} to Mo^{5+}, was performed as described by Prieto et al. [63], with some adaptations. Fifty µL of the sample extract, 450 µL of distilled water, and 1.5 mL of the phosmolybdenum complex were pipetted into tubes with screw caps, which were closed, shaken, taken to a water bath at 95 °C for 90 min, and cooled in an ice bath. The absorbance

reading was performed using a spectrophotometer at 695 nm, and the results were expressed in mg ascorbic acid 100 g^{-1} fresh matter.

Net Photosynthesis and Electron Transport Rate (ETR)

The net photosynthesis and electron transport rate (ETR) analyses were performed on the last day of the water deficit. The net photosynthesis was measured using an infrared gas exchange analyzer (IRGA, model LICOR 6400, Li-COR Biosciences, Lincoln, NE, USA). Data collection was performed between 8 a.m. and 10 a.m. Atmospheric CO_2 inside the leaf chamber was maintained at 400 µmols CO_2 mol air^{-1}, irradiance at 1500 µmols photons m^{-2} s^{-1}, and leaf temperature at 25 °C. The pre-established minimum time for the stabilization of the readings was 120 s. ETR was measured using a MINI-PAM portable fluorescence meter.

H_2O_2 and MDA Content

A mass of 0.2 g of fresh material was collected and macerated in a mortar with liquid nitrogen, homogenized in 1500 µL of trichloroacetic acid (TCA) 0.1%, and centrifuged at 12,000× *g* for 15 min at 4 °C. The H_2O_2 content was determined through a reaction with potassium iodide (KI), according to Alexieva et al. [64]. Readings were performed in a spectrophotometer at 390 nm. The amount of H_2O_2 was expressed in µmol g^{-1} of fresh mass. The quantification of MDA was carried out through the reaction of TBA (2-thiobarbituric acid) with the final products of the lipid peroxidation process and the readings taken in a spectrophotometer at 535 and 600 nm, obtaining MDA values, which were calculated according to the equation described by Heath and Packer [65]. The amount of MDA was expressed in nmol g^{-1} of fresh mass.

Antioxidant Enzymatic Activity

For the evaluation of the enzymatic activity of SOD, and CAT, 0.2 g of fresh material was macerated in liquid nitrogen with the subsequent addition of 1.5 mL of a buffered solution (0.1 mol L^{-1} of potassium phosphate pH (7.8), 0.1 mol L^{-1} of EDTA (pH 7.0), 0.5 mol L^{-1} of DTT, 0.1 mol L^{-1} PMSF, 1 mmol L^{-1} of ascorbic acid, and 22 mg of PVPP). After the suspension was centrifuged at 13,000× *g* for 10 min at 4 °C, the supernatant was collected for analysis in a microplate reader.

The superoxide dismutase activity was determined by quantifying the inhibition of photoreduction in nitroblue tetrazolium (NBT), following the protocol developed by [66]. Sample absorbances were recorded at 560 nm. As for CAT activity, the reaction solution was made from a mixture of 30 mM of H_2O_2, an aliquot of the enzymatic extract (supernatant), and sodium phosphate buffer (100 mM and pH 6.0). The absorbance was observed in the time scan (0–60 s) at 240 nm [67].

The enzymatic extraction method determined the total soluble protein content to calculate the specific activity of antioxidant enzymes. The microplates initially received 294 µL of Bradford [68] solution in a 1:5 dilution of the reagent and aliquots of the enzymatic extract. Readings were performed at a wavelength of 595 nm, and the results were obtained from a calibration curve with BSA.

Compatible Osmolytes

The proline content was extracted according to the methodology proposed by Bates et al. [69]. Initially, 0.05 g of plant material was macerated in 10 mL of 3% (*w*/*v*) sulfosalicylic acid. Subsequently, quantification was carried out following the method described by Carillo et al. [70]. A reaction mixture was prepared, comprising 3 mL and consisting of 1 mL of the extract obtained after maceration, 1 mL of ninhydrin acid, and 1 mL of glacial acetic acid at 99.5% (*v*/*v*). The mixture was stirred and subsequently subjected to heating in equipment with water at 100 °C for 60 min. After this time, the samples were transferred to ice to stop the reaction for 10 min. The supernatant was collected, its absorbance was read at 520 nm, and the results were expressed in µmol proline g^{-1} of fresh mass.

The extraction total soluble sugars and total free amino acids was performed according to Zanandrea et al. [71], using 0.05 mg of dry weight and homogenized in 5 mL of potassium phosphate buffer 100 mM (pH 7,0) and then placed in a water bath for 30 min at 40° C. Homogenate was centrifuged at 5,000 g for 10 min and the supernatant was collected. The process was repeated twice, and supernatants were combined. Total soluble sugars and sucrose were quantified as described by Dische [72]. The determination of was based on the colorimetric method using ninhydrin, established by Yemm and Coccking [73].

Statistical Analysis

The data were subjected to Shapiro–Wilk normality tests and Barlett's homogeneity of

variance test. When the assumptions were met, they were subjected to a two-way ANOVA with the post-hoc Tukey test. The data were presented in bar graphs. When the data did not show normality or variance homogeneity, a rank transformation of the data was performed [74], and the data were represented in a boxplot so that it was possible to observe the data dispersion better. The PCA was used to analyze the multivariate correlation between all morphophysiological and biochemical variables and the treatment conditions. All statistical analyses and graphs were made under the R software environment using the tidyverse [75], multcomp [76], and rstatix [77] packages.

Conclusions

Exposure to iodine (as KI) increased tomato plants' water deficit tolerance through increased antioxidant enzymatic activity and the accumulation of amino acids. These responses were induced during a water deficit, providing greater photosynthetic efficiency and yield. In addition, iodine (I) promoted better fruit quality by increasing the antioxidant capacity of phenolic compounds and reducing the maturation index. Based on the insights gained for all variables in conjunction with the PCA, the treatment with 100 µM of KI offered the best performance. Based on the results, it was also evident that most responses induced by iodine occurred during periods of water deficit. This observation strongly suggests that iodine elicits relief responses rather than priming effects. Additional studies should be carried out with the application of different sources and forms of I to identify the best strategies to mitigate water deficiency under commercial production conditions, where the use of iodine, along with other products, would be evaluated to assess possible interactions of I with other elements/components in the soil. Also, an increasing sampling frequency should be considered for a better understanding of metabolic responses and the induction moments of the responses promoted by iodine under water deficits. Finally, the beneficial effects of I on the post-harvest quality of fruits, as well as on other relevant physical–chemical and nutritional characteristics, should be assessed in future investigations.

Author Contributions: Conceptualization, J.d.S.L., O.V.S.A., E.G.d.M. and L.R.G.G.; methodology, J.d.S.L., O.V.S.A., L.C.d.S., G.S.M., G.G.L.M., E.S.d.A. and P.A.N.B.; software, J.d.S.L.; validation, L.R.G.G., V.L.N., P.E.R.M., G.L. and E.V.d.B.V.B.; formal analysis, J.d.S.L., O.V.S.A., L.C.d.S., G.S.M., E.S.d.A. and P.A.N.B.; resources, L.R.G.G.; P.E.R.M.; writing—original draft preparation, J.d.S.L., O.V.S.A., L.C.d.S., G.S.M., E.S.d.A.

and P.A.N.B.; writing—review and editing, J.d.S.L. and P.A.N.B.; supervision, L.R.G.G., V.L.N., P.E.R.M., G.L., E.G.d.M. and E.V.d.B.V.B.; project administration, L.R.G.G.; funding acquisition, L.R.G.G., V.L.N., P.E.R.M., G.L., E.G.d.M. and E.V.d.B.V.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES) (Grant Code-001) and the National Institute of Science and Technology (INCT) on Plant Stress Physiology, CNPq Grant 406455/2022-8 as well as the National Institute of Science and Technology (INCT) on Soil and Food Security, CNPq grant #406577/2022-6.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq), and the Minas Gerais State Research Foundation (FAPEMIG) for financial support and scholarships provided to the authors. Special thanks to UFLA's Soil Science and Biology—Plant Molecular Physiology Lab—Departments. PERM thanks CNPq for the fellowship (#312663/2021-8).

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

1. Mukherjee, S.; Mishra, A.; Trenberth, K.E. Climate Change and Drought: A Perspective on Drought Indices. *Curr. Clim. Change Rep.* **2018**, *4*, 145–163. https://doi.org/10.1007/s40641-018-0098-x.
2. Zargar, S.M.; Gupta, N.; Nazir, M.; Mahajan, R.; Malik, F.A.; Sofi, N.R.; Shikari, A.B.; Salgotra, R.K. Impact of Drought on Photosynthesis: Molecular Perspective. *Plant Gene* **2017**, *11*, 154–159. https://doi.org/10.1016/j.plgene.2017.04.003.

3. Ngumbi, E.; Kloepper, J. Bacterial-Mediated Drought Tolerance: Current and Future Prospects. *Appl. Soil. Ecol.* **2016**, *105*, 109–125. https://doi.org/10.1016/j.apsoil.2016.04.009.
4. Barnawal, D.; Singh, R.; Singh, R.P. Chapter Six—Role of Plant Growth Promoting Rhizobacteria in Drought Tolerance: Regulating Growth Hormones and Osmolytes. In *PGPR Amelioration in Sustainable Agriculture*; Singh, A.K., Kumar, A., Singh, P.K., Eds.; Woodhead Publishing: Sawston, UK, 2019; pp. 107–128, ISBN 978-0-12-815879-1.
5. Abdelaal, K.A.A.; Rashed, S.H.; Hossain, A.; Sabagh, A.E. Yield and Quality of Two Sugar Beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima Döll*) Cultivars Are Influenced by Foliar Application of Salicylic Acid, Irrigation Timing, and Planting Density. *Acta Agric. Slov.* **2020**, *115*, 273–282. https://doi.org/10.14720/aas.2020.115.2.1159.
6. Zulfiqar, F.; Ashraf, M. Antioxidants as Modulators of Arsenic-Induced Oxidative Stress Tolerance in Plants: An Overview. *J. Hazard. Mater.* **2022**, *427*, 127891. https://doi.org/10.1016/j.jhazmat.2021.127891.
7. Blasco, B.; Ríos, J.J.; Leyva, R.; Cervilla, L.M.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Rosales, M.A.; Ruiz, J.M.; Romero, L. Does Iodine Biofortification Affect Oxidative Metabolism in Lettuce Plants? *Biol. Trace Elem. Res.* **2011**, *142*, 831–842. https://doi.org/10.1007/s12011-010-8816-9.
8. Lima, J.d.S.; Andrade, O.V.S.; Santos, L.C. dos; Morais, E.G. de; Martins, G.S.; Mutz, Y.S.; Nascimento, V.L.; Marchiori, P.E.R.; Lopes, G.; Guilherme, L.R.G. Soybean Plants Exposed to Low Concentrations of Potassium Iodide Have Better Tolerance to Water Deficit through the Antioxidant Enzymatic System and Photosynthesis Modulation. *Plants* **2023**, *12*, 2555. https://doi.org/10.3390/plants12132555.
9. Ravello, R.A.V.; de Oliveira, C.; Lessa, J.; Boas, L.V.V.; Castro, E.M. de; Guilherme, L.R.G.; Lopes, G.; Ravello, R.A.V.; Oliveira, C. de; Lessa, J.; et al. Selenium Application Influenced Selenium Biofortification and Physiological Traits in Water-Deficit Common Bean Plants. *Crop Pasture Sci.* **2021**, 73, 44–55. https://doi.org/10.1071/CP20519.
10. Jogawat, A.; Yadav, B.; Chhaya; Lakra, N.; Singh, A.K.; Narayan, O.P. Crosstalk between Phytohormones and Secondary Metabolites in the Drought Stress Tolerance of Crop Plants: A Review. *Physiol. Plant.* **2021**, *172*, 1106–1132. https://doi.org/10.1111/ppl.13328.
11. Singh, M.; Kumar, J.; Singh, S.; Singh, V.P.; Prasad, S.M. Roles of Osmoprotectants in Improving Salinity and Drought Tolerance in Plants: A Review. *Rev. Environ. Sci. Biotechnol.* **2015**, *14*, 407–426. https://doi.org/10.1007/s11157-015-9372-8.
12. Mahmood, T.; Khalid, S.; Abdullah, M.; Ahmed, Z.; Shah, M.K.N.; Ghafoor, A.; Du, X. Insights into Drought Stress Signaling in Plants and the Molecular Genetic Basis of Cotton Drought Tolerance. *Cells* **2019**, *9*, 105. https://doi.org/10.3390/cells9010105.
13. Kaur, H.; Kohli, S.K.; Khanna, K.; Bhardwaj, R. Scrutinizing the Impact of Water Deficit in Plants: Transcriptional Regulation, Signaling, Photosynthetic Efficacy, and Management. *Physiol. Plant.* **2021**, *172*, 935–962. https://doi.org/10.1111/ppl.13389.
14. Joshi, R.; Wani, S.H.; Singh, B.; Bohra, A.; Dar, Z.A.; Lone, A.A.; Pareek, A.; Singla-Pareek, S.L. Transcription Factors and Plants Response to Drought Stress: Current Understanding and Future Directions. *Front. Plant Sci.* **2016**, *7*, 1029. https://doi.org/10.3389/fpls.2016.01029.
15. Bisbis, M.B.; Gruda, N.; Blanke, M. Potential Impacts of Climate Change on Vegetable Production and Product Quality—A Review. *J. Clean. Prod.* **2018**, *170*, 1602–1620. https://doi.org/10.1016/j.jclepro.2017.09.224.
16. Hou, X.; Zhang, W.; Du, T.; Kang, S.; Davies, W.J. Responses of Water Accumulation and Solute Metabolism in Tomato Fruit to Water Scarcity and Implications for Main Fruit Quality Variables. *J. Exp. Bot.* **2020**, *71*, 1249–1264. https://doi.org/10.1093/jxb/erz526.

17. Cirillo, V.; D'Amelia, V.; Esposito, M.; Amitrano, C.; Carillo, P.; Carputo, D.; Maggio, A. Anthocyanins Are Key Regulators of Drought Stress Tolerance in Tobacco. *Biology* **2021**, *10*, 139. https://doi.org/10.3390/biology10020139.
18. Chen, J.; Kang, S.; Du, T.; Guo, P.; Qiu, R.; Chen, R.; Gu, F. Modeling Relations of Tomato Yield and Fruit Quality with Water Deficit at Different Growth Stages under Greenhouse Condition. *Agric. Water Manag.* **2014**, *146*, 131–148. https://doi.org/10.1016/j.agwat.2014.07.026.
19. Kiferle, C.; Gonzali, S.; Beltrami, S.; Martinelli, M.; Hora, K.; Holwerda, H.T.; Perata, P. Improvement in Fruit Yield and Tolerance to Salinity of Tomato Plants Fertigated with Micronutrient Amounts of Iodine. *Sci. Rep.* **2022**, *12*, 14655. https://doi.org/10.1038/s41598-022-18301-w.
20. Pérez-Salas, S.; Medrano-Macías, J. Use of Iodine as a Tolerance Inducer in Tomato Seedlings Under Salinity Stress Uso Del Yodo Como Inductor a La Tolerancia En Plántulas de Tomate Bajo Condiciones de Estrés Por Salinidad *Rev. Científica Univ. Autónoma Coahuila.* **2021**, *13, 14–22.*
21. Kiferle, C.; Martinelli, M.; Salzano, A.M.; Gonzali, S.; Beltrami, S.; Salvadori, P.A.; Hora, K.; Holwerda, H.T.; Scaloni, A.; Perata, P. Evidences for a Nutritional Role of Iodine in Plants. *Front. Plant Sci.* **2021**, *12*, 616868.
22. Barsanti, L.; Coltelli, P.; Gualtieri, P. Paramylon Treatment Improves Quality Profile and Drought Resistance in *Solanum lycopersicum* L. Cv. Micro-Tom. *Agronomy* **2019**, *9*, 394. https://doi.org/10.3390/agronomy9070394.
23. Murshed, R.; Lopez-Lauri, F.; Sallanon, H. Effect of Water Stress on Antioxidant Systems and Oxidative Parameters in Fruits of Tomato (*Solanum lycopersicon* L., Cv. Micro-Tom). *Physiol. Mol. Biol. Plants* **2013**, *19*, 363–378. https://doi.org/10.1007/s12298-013-0173-7.
24. Morison, J.I.L.; Baker, N.R.; Mullineaux, P.M.; Davies, W.J. Improving Water Use in Crop Production. *Philos. Trans. R. Soc. B Biol. Sci.* **2008**, *363*, 639–658. https://doi.org/10.1098/rstb.2007.2175.
25. Cuartero, J.; Bolarín, M.C.; Asíns, M.J.; Moreno, V. Increasing Salt Tolerance in the Tomato. *J. Exp. Bot.* **2006**, *57*, 1045–1058. https://doi.org/10.1093/jxb/erj102.
26. Yin, W.; Hu, Z.; Hu, J.; Zhu, Z.; Yu, X.; Cui, B.; Chen, G. Tomato (*Solanum lycopersicum*) MADS-Box Transcription Factor SlMBP8 Regulates Drought, Salt Tolerance and Stress-Related Genes. *Plant Growth Regul.* **2017**, *83*, 55–68. https://doi.org/10.1007/s10725-017-0283-2.
27. Jafarian, S.; Sodaiezadeh, H.; Arani, A.; Hakimzadeh, M.A.; Sohrabizadeh, Z. The Effect of Iodine on Increasing Drought Tolerance Of (*Carthamus tinctorius* L.) In Seed Germination and Early Growth Stage. *J. Environ. Sci. Stud.* **2020**, *5*, 2387–2393.
28. Hora, K.; Holwerda, H.T. Loss of Yield or Lower Fruit Quality Due to Iodine Deficiency in Tomato and Sweet Pepper Crops under Heat Stress Can Be Prevented by Application of Potassium Nitrate with Iodine. *Acta Hortic.* **2023**, 1372, 251–258. https://doi.org/10.17660/ActaHortic.2023.1372.33.
29. Altaf, M.A.; Shahid, R.; Ren, M.-X.; Naz, S.; Altaf, M.M.; Khan, L.U.; Tiwari, R.K.; Lal, M.K.; Shahid, M.A.; Kumar, R.; et al. Melatonin Improves Drought Stress Tolerance of Tomato by Modulating Plant Growth, Root Architecture, Photosynthesis, and Antioxidant Defense System. *Antioxidants* **2022**, *11*, 309. https://doi.org/10.3390/antiox11020309.
30. Gupta, N.; Bajpai, M.S.; Majumdar, R.S.; Mishra, P.K. Response of Iodine on Antioxidant Levels of *Glycine max* L. Grown under Cd^{2+} Stress. *Adv. Biol. Res.* **2015**, *9*, 40–48. https://doi.org/10.5829/idosi.abr.2015.9.1.9183.
31. Fuentes, J.E.G.; Castellanos, B.F.H.; Martínez, E.N.R.; Ortiz, W.A.N.; Mendoza, A.B.;

Macías, J.M. Outcomes of Foliar Iodine Application on Growth, Minerals and Antioxidants in Tomato Plants under Salt Stress. *Folia Hortic.* **2022**, *34*, 27–37. https://doi.org/10.2478/fhort-2022-0003.
32. Gill, S.S.; Tuteja, N. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. *Plant Physiol. Biochem*. **2010**, *48*, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016.
33. He, W.; Yan, K.; Zhang, Y.; Bian, L.; Mei, H.; Han, G. Contrasting Photosynthesis, Photoinhibition and Oxidative Damage in Honeysuckle (*Lonicera japonica* Thunb.) under Iso-Osmotic Salt and Drought Stresses. *Environ. Exp. Bot.* **2021**, *182*, 104313. https://doi.org/10.1016/j.envexpbot.2020.104313.
34. Fracasso, A.; Telò, L.; Lanfranco, L.; Bonfante, P.; Amaducci, S. Physiological Beneficial Effect of Rhizophagus Intraradices Inoculation on Tomato Plant Yield under Water Deficit Conditions. *Agronomy* **2020**, *10*, 71. https://doi.org/10.3390/agronomy10010071.
35. Fan, S.; Wu, H.; Gong, H.; Guo, J. The Salicylic Acid Mediates Selenium-Induced Tolerance to Drought Stress in Tomato Plants. *Sci. Hortic.* **2022**, *300*, 111092. https://doi.org/10.1016/j.scienta.2022.111092.
36. Zhang, Z.; Cao, B.; Gao, S.; Xu, K. Grafting Improves Tomato Drought Tolerance through Enhancing Photosynthetic Capacity and Reducing ROS Accumulation. *Protoplasma* **2019**, *256*, 1013–1024. https://doi.org/10.1007/s00709-019-01357-3.
37. Leyva, R.; Sánchez-Rodríguez, E.; Ríos, J.J.; Rubio-Wilhelmi, M.M.; Romero, L.; Ruiz, J.M.; Blasco, B. Beneficial Effects of Exogenous Iodine in Lettuce Plants Subjected to Salinity Stress. *Plant Sci.* **2011**, *181*, 195–202. https://doi.org/10.1016/j.plantsci.2011.05.007.
38. Moreno-Galván, A.E.; Cortés-Patiño, S.; Romero-Perdomo, F.; Uribe-Vélez, D.; Bashan, Y.; Bonilla, R.R. Proline Accumulation and Glutathione Reductase Activity Induced by Drought-Tolerant Rhizobacteria as Potential Mechanisms to Alleviate Drought Stress in Guinea Grass. *Appl. Soil. Ecol.* **2020**, *147*, 103367. https://doi.org/10.1016/j.apsoil.2019.103367.
39. Daffonchio, D.; Hirt, H.; Berg, G. Plant-Microbe Interactions and Water Management in Arid and Saline Soils. In *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*; Lugtenberg, B., Ed.; Springer International Publishing: Cham, Switzerland, 2015; pp. 265–276, ISBN 978-3-319-08575-3.
40. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A. Plant Drought Stress: Effects, Mechanisms and Management. In *Sustainable Agriculture*; Lichtfouse, E., Navarrete, M., Debaeke, P., Véronique, S., Alberola, C., Eds.; Springer Netherlands: Dordrecht, The Netherlands, 2009; pp. 153–188, ISBN 978-90-481-2666-8.
41. Du, Y.; Zhao, Q.; Chen, L.; Yao, X.; Zhang, W.; Zhang, B.; Xie, F. Effect of Drought Stress on Sugar Metabolism in Leaves and Roots of Soybean Seedlings. *Plant Physiol. Biochem*. **2020**, *146*, 1–12. https://doi.org/10.1016/j.plaphy.2019.11.003.
42. Yang, J.; Zhang, J.; Wang, Z.; Zhu, Q. Activities of Starch Hydrolytic Enzymes and Sucrose-phosphate Synthase in the Stems of Rice Subjected to Water Stress during Grain Filling. *J. Exp. Bot.* **2001**, *52*, 2169–2179. https://doi.org/10.1093/jexbot/52.364.2169.
43. Geigenberger, P.; Reimholz, R.; Geiger, M.; Merlo, L.; Canale, V.; Stitt, M. Regulation of Sucrose and Starch Metabolism in Potato Tubers in Response to Short-Term Water Deficit. *Planta* **1997**, *201*, 502–518. https://doi.org/10.1007/s004250050095.
44. Xu, W.; Cui, K.; Xu, A.; Nie, L.; Huang, J.; Peng, S. Drought Stress Condition Increases Root to Shoot Ratio via Alteration of Carbohydrate Partitioning and Enzymatic Activity in Rice Seedlings. *Acta Physiol. Plant* **2015**, *37*, 9. https://doi.org/10.1007/s11738-014-1760-0.

45. Khaleghi, A.; Naderi, R.; Brunetti, C.; Maserti, B.E.; Salami, S.A.; Babalar, M. Morphological, Physiochemical and Antioxidant Responses of Maclura Pomifera to Drought Stress. *Sci. Rep.* **2019**, *9*, 19250. https://doi.org/10.1038/s41598-019-55889-y.
46. Wu, H.-H.; Zou, Y.-N.; Rahman, M.M.; Ni, Q.-D.; Wu, Q.-S. Mycorrhizas Alter Sucrose and Proline Metabolism in Trifoliate Orange Exposed to Drought Stress. *Sci. Rep.* **2017**, *7*, 42389. https://doi.org/10.1038/srep42389.
47. Hilker, M.; Schmülling, T. Stress Priming, Memory, and Signalling in Plants. *Plant Cell Environ.* **2019**, *42*, 753–761. https://doi.org/10.1111/pce.13526.
48. Kuepper, F.C.; Carpenter, L.J.; McFiggans, G.B.; Palmer, C.J.; Waite, T.J.; Boneberg, E.-M.; Woitsch, S.; Weiller, M.; Abela, R.; Grolimund, D.; et al. Iodide Accumulation Provides Kelp with an Inorganic Antioxidant Impacting Atmospheric Chemistry. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6954–6958. https://doi.org/10.1073/pnas.0709959105.
49. Luther, G.W.I.; Wu, J.; Cullen, J.B. Redox Chemistry of Iodine in Seawater. In *Aquatic Chemistry*; Advances in Chemistry; American Chemical Society: New York, NY, USA, 1995; Volume 244, pp. 135–155, ISBN 978-0-8412-2921-1.
50. Medrano-Macías, J.; Mendoza-Villarreal, R.; Robledo-Torres, V.; Fuentes-Lara, L.O.; Ramírez-Godina, F.; Pérez-Rodríguez, M.Á.; Benavides-Mendoza, A.; Medrano-Macías, J.; Mendoza-Villarreal, R.; Robledo-Torres, V.; et al. The Use of Iodine, Selenium, and Silicon in Plant Nutrition for the Increase of Antioxidants in Fruits and Vegetables. In *Antioxidants in Foods and Its Applications*; IntechOpen: London, UK, 2018; ISBN 978-1-78923-379-7.
51. Barickman, T.C.; Kopsell, D.A.; Sams, C.E. Abscisic Acid Improves Tomato Fruit Quality by Increasing Soluble Sugar Concentrations. *J. Plant Nutr.* **2017**, *40*, 964–973. https://doi.org/10.1080/01904167.2016.1231812.
52. Ibrahim, M.F.M.; Elbar, O.H.A.; Farag, R.; Hikal, M.; El-Kelish, A.; El-Yazied, A.A.; Alkahtani, J.; El-Gawad, H.G.A. Melatonin Counteracts Drought Induced Oxidative Damage and Stimulates Growth, Productivity and Fruit Quality Properties of Tomato Plants. *Plants* **2020**, *9*, 1276. https://doi.org/10.3390/plants9101276.
53. Pék, Z.; Szuvandzsiev, P.; Daood, H.; Neményi, A.; Helyes, L. Effect of Irrigation on Yield Parameters and Antioxidant Profiles of Processing Cherry Tomato. *Open Life Sci.* **2014**, *9*, 383–395. https://doi.org/10.2478/s11535-013-0279-5.
54. Bartolini, S.; Leccese, A.; Andreini, L. Influence of Canopy Fruit Location on Morphological, Histochemical and Biochemical Changes in Two Oil Olive Cultivars. *Plant Biosyst.* **2014**, *148*, 1221–1230. https://doi.org/10.1080/11263504.2014.980360.
55. Mejía-Ramírez, F.; Benavides-Mendoza, A.; González-Morales, S.; Juárez-Maldonado, A.; Lara-Viveros, F.M.; Morales-Díaz, A.B.; Morelos-Moreno, Á. Seed Priming Based on Iodine and Selenium Influences the Nutraceutical Compounds in Tomato (*Solanum lycopersicum* L.) Crop. *Antioxidants* **2023**, *12*, 1265. https://doi.org/10.3390/antiox12061265.
56. Maglione, G.; Vitale, E.; Costanzo, G.; Polimeno, F.; Arena, C.; Vitale, L. Iodine Enhances the Nutritional Value but Not the Tolerance of Lettuce to NaCl. *Horticulturae* **2022**, *8*, 662. https://doi.org/10.3390/horticulturae8070662.
57. Hoagland, D.R.; Arnon, D.I. The Water-Culture Method for Growing Plants without Soil. *Circular. Calif. Agric. Exp. Stn.* **1950**, *347*, 32.
58. Sánchez-Reinoso, A.D.; Ligarreto-Moreno, G.A.; Restrepo-Díaz, H. Evaluation of Drought Indices to Identify Tolerant Genotypes in Common Bean Bush (*Phaseolus vulgaris* L.). *J. Integr. Agric.* **2020**, *19*, 99–107. https://doi.org/10.1016/S2095-3119(19)62620-1.
59. Association of Official Analytical Chemistry. *Official Methods of Analysis*, 21st ed.; AOAC: Gaithersburg, MD, USA, 2019.
60. Rufino, M.D.S.M.; Alves, R.E.; de Brito, E.S.; Pérez-Jiménez, J.; Saura-Calixto, F.;

Mancini-Filho, J. Bioactive Compounds and Antioxidant Capacities of 18 Non-Traditional Tropical Fruits from Brazil. *Food Chem.* **2010**, *121*, 996–1002. https://doi.org/10.1016/j.foodchem.2010.01.037.
61. Medina, M.B. Determination of the Total Phenolics in Juices and Superfruits by a Novel Chemical Method. *J. Funct. Foods* **2011**, *3*, 79–87. https://doi.org/10.1016/j.jff.2011.02.007.
62. Auzanneau, N.; Weber, P.; Kosińska-Cagnazzo, A.; Andlauer, W. Bioactive Compounds and Antioxidant Capacity of Lonicera Caerulea Berries: Comparison of Seven Cultivars over Three Harvesting Years. *J. Food Compos. Anal.* **2018**, *66*, 81–89. https://doi.org/10.1016/j.jfca.2017.12.006.
63. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341. https://doi.org/10.1006/abio.1999.4019.
64. Alexieva, V.; Sergiev, I.; Mapelli, S.; Karanov, E. The Effect of Drought and Ultraviolet Radiation on Growth and Stress Markers in Pea and Wheat. *Plant Cell Environ.* **2001**, *24*, 1337–1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x.
65. Heath, R.L.; Packer, L. Photoperoxidation in Isolated Chloroplasts. I. Kinetics and Stoichiometry of Fatty Acid Peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.
66. Beauchamp, C.; Fridovich, I. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Anal. Biochem.* **1971**, *44*, 276–287. https://doi.org/10.1016/0003-2697(71)90370-8.
67. Chance, B.; Maehly, A.C. [136] Assay of Catalases and Peroxidases. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1955; Volume 2, pp. 764–775.
68. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.
69. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid Determination of Free Proline for Water-Stress Studies. *Plant Soil.* **1973**, *39*, 205–207. https://doi.org/10.1007/BF00018060.
70. Carillo, P.; Kyriacou, M.C.; El-Nakhel, C.; Pannico, A.; dell'Aversana, E.; D'Amelia, L.; Colla, G.; Caruso, G.; De Pascale, S.; Rouphael, Y. Sensory and Functional Quality Characterization of Protected Designation of Origin 'Piennolo Del Vesuvio' Cherry Tomato Landraces from Campania-Italy. *Food Chem.* **2019**, *292*, 166–175. https://doi.org/10.1016/j.foodchem.2019.04.056.
71. Zanandrea, I.; Alves, J.D.; Deuner, S.; Goulart, P. de F.P.; Henrique, P. de C.; Silveira, N.M. Tolerance of Sesbania Virgata Plants to Flooding. Aust. J. Bot. 2010, 57, 661–669, https://doi.org/10.1071/BT09144.
72. Dische, Z. Methods in carbohydrates chemistry. In: Whistler R, Kearsley M.W., Eds.; Methods in carbohydrates chemistry, 2nd ed. Academic Press, New York, **1962**; pp. 478–512.
73. Yemm, E.W.; Cocking, E.C.; Ricketts, R.E. The Determination of Amino Acids with Ninhydrin. Analyst. **1955**, *80*, 209-214. https://doi.org/10.1039/AN9558000209.
74. Conover, W.J.; Iman, R.L. Rank Transformations as a Bridge between Parametric and Nonparametric Statistics. Am. Stat. 1981, 35, 124–129. https://doi.org/10.1080/00031305.1981.10479327.
75. Wickham, H.; Averick, M.; Bryan, J.; Chang, W.; McGowan, L.; François, R.; Grolemund, G.; Hayes, A.; Henry, L.; Hester, J.; et al. Welcome to the Tidyverse. *J. Open Source Softw.* **2019**, *4*, 1686. https://doi.org/10.21105/joss.01686.
76. Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric

models. *Biom. J.* **2008**, *50*, 346–363. https://doi.org/10.1002/bimj.200810425.
77. Kassambara, A. _rstatix: Pipe-Friendly Framework for Basic Statistical Tests_. R Package Version 0.7.1. 2022. Available online: https://CRAN.R-project.org/package=rstatix (accessed on 25 June 2023).

CONCLUDING REMARKS

Iodine has demonstrated its beneficial role in alleviating water deficit, both in tomatoes and soybeans. When applied at ideal concentrations, this element promoted greater photosynthetic efficiency and enzymatic antioxidant protection, consequently reducing lipid peroxidation and enhancing biomass accumulation and productivity in crops. Moreover, a concentration of 100 µM resulted in improved tomato fruit quality, suggesting that iodine also has the potential to ensure better post-harvest quality. In soybean plants, concentrations exceeding 20 µM were noted to induce a toxic effect, diminishing biomass accumulation. The concentration of 10 µM was identified as optimal, both in irrigated conditions and under stress. Thus, at ideal concentrations, iodine increases tolerance to water deficit, leading to additional positive responses such as enhancements in fruit quality. It is essential to underscore the need for further studies addressing the timing and best source for the application of this element, in addition to tests under field conditions and on more species of socioeconomic interest. Additionally, the indication of a dose and form of application that meets both biofortification and the beneficial physiological effect of this element is also a challenge.