

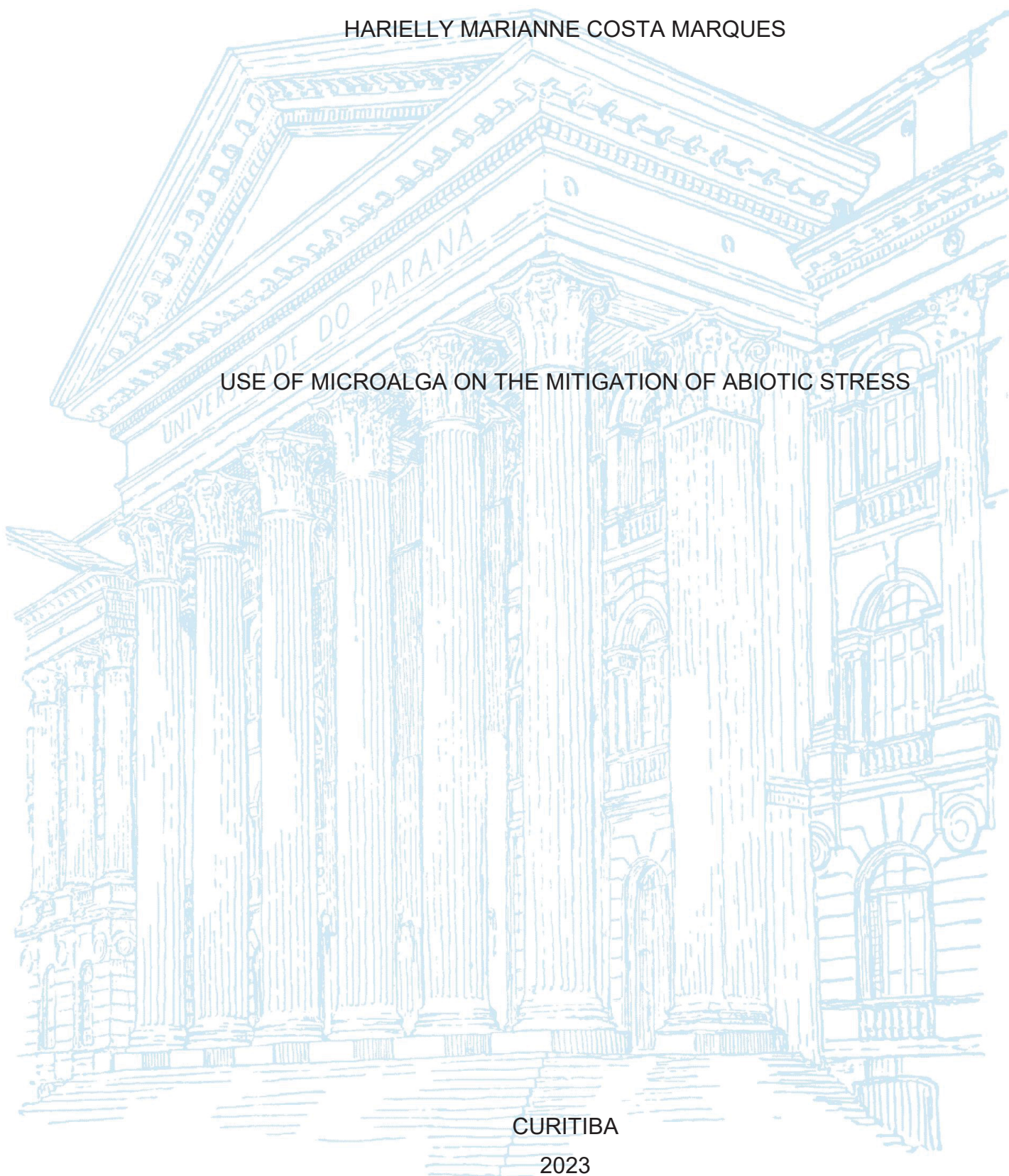
UNIVERSIDADE FEDERAL DO PARANÁ

HARIELLY MARIANNE COSTA MARQUES

USE OF MICROALGA ON THE MITIGATION OF ABIOTIC STRESS

CURITIBA

2023



HARIELLY MARIANNE COSTA MARQUES

USE OF MICROALGA ON THE MITIGATION OF ABIOTIC STRESS

Tese apresentada ao curso de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Departamento de Fitotecnia e Fitossanidade, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências.

Orientador: Prof. Dr. Bruno Francisco Sant' Anna dos Santos

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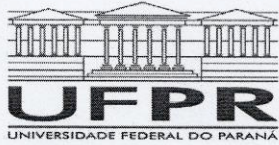
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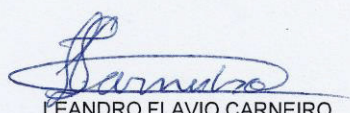
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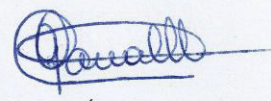
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A minha querida amiga Ely (in memoriam).

Dedico.

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RESUMO

As mudanças climáticas são uma das principais preocupações do mundo atual, com aumento na frequência de ocorrência dos estresses abióticos que causam danos no desenvolvimento e produtividade das plantas. Diante disso, torna-se necessário buscar alternativas sustentáveis que mitiguem os efeitos deletérios dos estresses abióticos nas plantas, como a aplicação de biofertilizantes. Assim, o objetivo deste trabalho foi estudar o potencial da microalga *Asterarcys quadricellulare* na mitigação do estresse salino em plantas de melão e no estresse hídrico em plantas de feijão. Os experimentos foram instalados na área de Olericultura da Universidade Federal do Paraná, no laboratório de biofertilizantes. A biomassa da microalga (código da cepa CCAP 294/1) foi obtida em sistema mixotrófico e fornecida pela empresa Alltech® Crop Sciences – Brasil. As etapas da pesquisa são apresentadas em três capítulos. No capítulo 1, plantas de melão das cultivares Dalí e Goldex foram cultivadas em vasos, com aplicação de *A. quadricellulare* nas concentrações de 0 ml L⁻¹, 0.5 ml L⁻¹, 1.0 ml L⁻¹, via foliar, por irrigação via gotejo e a combinação via foliar e gotejo, sendo irrigadas com solução salina com condutividade de 15 dS m⁻¹, além do controle sem salinidade. Foram realizadas avaliações bioquímicas, anatômicas e biométricas. Plantas tratadas com a microalga apresentaram acréscimos nos teores de clorofila, carotenóides, aminoácidos livres totais, proteínas, prolina, açúcares totais, compostos fenólicos, além do aumento da atividade de enzimas antioxidantes, com destaque para a aplicação da combinação das vias foliar e gotejo na concentração de 1.0 ml L⁻¹. No segundo capítulo as plantas de melão foram cultivadas em slabs e fertirrigadas com solução nutritiva e adição de cloreto de sódio em água para atingir condutividade elétrica de 8 dS m⁻¹, com aplicações semanais de *A. quadricellulare* na concentração de 1,0 ml L⁻¹, via foliar e a combinação da via foliar e gotejo, realizadas avaliações bioquímicas e biométricas. Plantas tratadas com a microalga apresentaram acréscimos nos teores de clorofila, carotenóides e açúcares. Houve ainda aumento na massa seca dos ramos produtivos, número de frutos, diâmetro, comprimento e peso dos frutos, acarretando no aumento de produtividade. No terceiro capítulo plantas de feijão das cultivares IAC 1850 e BRS ESTEIO foram cultivadas em vaso e submetidas a condições de estresse hídrico, com aplicação de *A. quadricellulare* nas concentrações de 0 ml L⁻¹, 0.5 ml L⁻¹, 1.0 ml L⁻¹ via foliar no estágio fenológico de V3. Foram realizadas avaliações bioquímicas, anatômicas e biométricas em três períodos: no terceiro dia de déficit hídrico, um e 10 dias após a reidratação. Houve aumento nos teores de clorofila, açúcares, proteína e enzimas antioxidantes nas plantas tratadas com a microalga. Aos dez dias após a reidratação, houve aumento na área foliar, massa fresca e na espessura da folha nas plantas tratadas. Nossos resultados demonstram alterações metabólicas, incluindo enzimas antioxidantes e melhorias de crescimento e produtividade, sendo assim, considera-se promissor o uso da microalga *A. quadricellulare* como biofertilizantes na mitigação de estresses abióticos.

Palavras-chave: *Asterarcys quadricellulare*; *Cucumis melo*; estresse salino; estresse hídrico; *Phaseolus vulgaris*

ABSTRACT

Climate changes are among the main concerns in the world today, with an increase in the frequency of occurrence of abiotic stresses that cause damage to the development and productivity of plants. Hence, the search for sustainable alternatives that mitigate the harmful effects of abiotic stresses on plants, such as the application of biofertilizers is paramount. Thus, the objective of this work was to investigate the potential of the microalgae *Asterarcys quadricellulare* in mitigating salt stress in melon plants and water stress in bean plants. The experiments were set in the Olericulture area of the Federal University of Paraná, in the biofertilizer laboratory. The microalgae biomass (strain code CCAP 294/1) was obtained in a mixotrophic system and supplied by Alltech® Crop Sciences – Brazil. The research steps are presented in three chapters. In Chapter 1, melon plants of the Dalí and Goldex cultivars were grown in pots, with the application of *A. quadricellulare* at the concentrations of 0 ml L⁻¹, 0.5 ml L⁻¹, 1.0 ml L⁻¹, via foliar irrigation, via irrigation drip and the combination via foliar and drip, irrigated with saline solution with conductivity of 15 dS m⁻¹, in addition to the control without salinity. Biochemical, anatomical, and biometric evaluations were performed. Microalgae-treated plants showed increases in the levels of chlorophyll, carotenoids, total free amino acids, proteins, proline, total sugars, and phenolic compounds, in addition to an increase in the activity of antioxidant enzymes, especially the application of the combination of foliar and drip routes in the concentration of 1.0 ml L⁻¹. In the second chapter, melon plants were grown in slabs and fertigated with nutrient solution and the addition of sodium chloride in water to achieve electrical conductivity of 8 dS m⁻¹, with weekly applications of *A. quadricellulare* at a concentration of 1.0 ml L⁻¹, via leaves and the combination of leaf and drip, biochemical and biometric assessments were carried out. Plants treated with microalgae showed increases in chlorophyll, carotenoids, and sugar levels. Na increase in the dry mass of the productive branches, number of fruits, diameter, length, and weight of the fruits were also observed, increasing productivity. In the third chapter, bean plants of the cultivars IAC 1850 and BRS ESTEIO were grown in pots and subjected to water stress conditions, with the application of *A. quadricellulare* at concentrations of 0 ml L⁻¹, 0.5 ml L⁻¹, 1.0 ml L⁻¹ leaf path at the V3 phenological stage. Biochemical, anatomical, and biometric evaluations were carried out in three periods: on the third day of water deficit, and one and 10 days after rehydration. The levels of chlorophyll, sugars, protein, and antioxidant enzymes increased in plants treated with microalgae. Ten days after rehydration, leaf area, fresh mass, and leaf thickness increased in the treated plants. Our results demonstrate metabolic changes, including antioxidant enzymes and improvements in growth and productivity, therefore, the use of *A. quadricellulare* microalgae as biofertilizers is considered promising in mitigating abiotic stresses.

Keywords: *Asterarcys quadricellulare*; salt stress; drought stress; *Cucumis melo*; *Phaseolus vulgaris*

LIST OF FIGURES

CHAPTER 1 - MICROALGAE MITIGATES SALT STRESS IN MELON PLANTS BY PROMOTING METABOLIC CHANGES

- Figure 1 **(a)** Chlorophyll *a* (mg g^{-1}), **(b)** Chlorophyll *b* (mg g^{-1}), **(c)** total chlorophyll (mg g^{-1}) and **(d)** carotenoid (mg g^{-1}) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).33
- Figure 2 **(a)** Total free amino acid ($\mu\text{g g}^{-1}$), **(b)** proteins ($\mu\text{g g}^{-1}$) and **(c)** proline ($\mu\text{g g}^{-1}$) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).34
- Figure 3 **(a)** Total sugars ($\mu\text{g g}^{-1}$), **(b)** reducing sugars ($\mu\text{g g}^{-1}$) and **(c)** non-reducing sugars ($\mu\text{g g}^{-1}$) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).36
- Figure 4 Phenolic compounds ($\mu\text{g g}^{-1}$) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).37
- Figure 5 **(a)** Activity of superoxide dismutase enzyme (SOD, U mg^{-1} protein), **(b)** peroxidase (POD, $\mu\text{mol de purpurogallin min}^{-1} \text{mg}^{-1}$ protein), **(c)** catalase (CAT, $\mu\text{Kat } \mu\text{g}^{-1}$ protein min^{-1}) and **(d)** malondialdehyde content (MDA, nmol g^{-1}) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).39
- Figure 6 **(a)** Leaf thickness, **(b)** palisade parenchyma, **(c)** lacunar parenchyma, **(d)** adaxial epidermis thickness **(e)** abaxial epidermis thickness and **(e)** cuticle (μm) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (0) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).40

Figure 7 (a) Fresh mass (g), (b) dry mass (g) and (c) leaf area (cm ²) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga <i>A. quadricellulare</i> (AQ).	42
Figure 8 (a) Stem fresh mass (g), (b) stem dry mass (g), (c) stem diameter (mm) and (d) stem length (cm) of melon plants of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga <i>A. quadricellulare</i> (AQ).	44
Figure 9 (a) Root diameter (mm), (b) root length (mm) and (c) root volume (mm ³) of melon plants of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga <i>A. quadricellulare</i> (AQ).	45
Figure 10 Number of flowers of melon plants of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga <i>A. quadricellulare</i> (AQ).	46

CHAPTER 2 – MIGROALGAE BIOMASS IMPROVES MELON CROP YIELD IN SALINE CONDITIONS

Figure 1. Chlorophyll a, (b) chlorophyll b and (c) total chlorophyll from cultivars 'Dalí' and 'Goldex' melon leaves with application of the biomass of the microalgae <i>A. quadricellulare</i> (AQ) in cultivation conditions with saline and non-saline water. Means followed by the same letter do not differ from each other using the test of Tukey. Lowercase letters compare treatments (T) - Control - nutrient solution EC; AQ.df - nutrient solution EC + AQ fertirigation and foliar application; NaCl - nutrient solution EC + 8 dS m ⁻¹ NaCl; NaCl+AQ.df - nutrient solution EC + 8 dS m ⁻¹ NaCl + AQ fertirigation foliar application; NaCl+AQ.f - nutrient solution EC + 8 dS m ⁻¹ NaCl + AQ foliar application. Capital letters compare cultivars (C) ANOVA: ns = not significant, * = significant at p≤0.01. Chl a: T = *; C = *; TXC = *. Chl b: T = *; C = *; TXC = *. Chl T: T = *; C = *; TXC = *	59
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Figure 2 Carotenoid content in 'Dali' and 'Goldex' cultivars melon leaves with the application of *A. quadricellulare* (AQ) microalgae biomass in cultivation solution using saline and non-saline water. Means followed by the same letter do not differ from each other using the test of Tukey. Lowercase letters compare treatments (T) - Control - nutrient solution EC; AQ.df - nutrient solution EC + AQ fertigation and foliar application; NaCl - nutrient solution EC + 8 dS m⁻¹ NaCl; NaCl+AQ.df - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ fertigation foliar application; NaCl+AQ.f - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar application. Capital letters compare cultivars (C) ANOVA: ns = not significant, * = significant at p≤0.01. ** = significant at p≤0.05. T = *; C = *; TXC = ns.....60

Figure 3 Relative chlorophyll index (N-Tester[®]) of melon cultivars 'Dali' and 'Goldex' leaves with the application of *A. quadricellulare* (AQ) microalgae biomass in cultivation conditions with saline and non-saline water. Means followed by the same letter do not differ from each other using the test of Tukey. Lowercase letters compare treatments (T) - Control - nutrient solution EC; AQ.df - nutrient solution EC + AQ fertigation and foliar application; NaCl - nutrient solution EC + 8 dS m⁻¹ NaCl; NaCl+AQ.df - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ fertigation and foliar application; NaCl+AQ.f - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar application. Capital letters compare cultivars (C). ANOVA: ns = not significant, * = significant at p≤0.01. T = *; C = ns; TXC = ns.....60

Figure 4(a) Total sugars, (b) reducing sugars and (c) non-reducing sugars of 'Dali' and 'Goldex' melon cultivars leaves with the application of *A. quadricellulare* (AQ) microalgae biomass in cultivation conditions with saline and non-saline water. Means followed by the same letter are not different from each other using the test of Tukey. Lower case letters compare treatments (T) - **Control** – nutrient solution EC; **AQ.df** - nutrient solution EC + AQ fertigation and foliar application; **NaCl** - nutrient solution EC + 8 dS m⁻¹ NaCl; **NaCl+AQ.df** - nutrient solution EC + 8 dS m⁻¹ de NaCl + AQ fertigation and foliar application; **NaCl+AQ.f** - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar

application. Upper case letters compare cultivars (C) ANOVA: ns = non-significant, * = significant at $p \leq 0.01$. ** = significant at $p \leq 0.05$.
 Total sugars: T = *; C = *; TXC = *. Reducing sugars: T = *; C = *; TXC = *. Non-reducing sugars: T = *; C = *; TXC = *62

CHAPTER 3 - USE OF MICROALGA *ASTERARCYS QUADRICELLULARIS* IN COMMON BEAN

Figure 1 Microalgae effects on biometric parameters of common bean plants under water deficit conditions, 1 day after rehydration (1AR). Fresh mass (a), dry mass (b), and fresh and dry mass ratio (g) (c). 16

Figure 2 Microalgae effects on biometric parameters of common bean plants under water deficit conditions, 10 days after rehydration (10AR). Leaf area (cm²) (a); fresh mass (b); dry mass (c) and ratio between fresh and dry mass (g) (d)..... 17

Figure 3 Microalgae effects on anatomical parameters of common bean plants under water deficit conditions, 10 days after rehydration (10AR). Leaf thickness (a), palisade mesophyll (b), spongy mesophyll (c), Upper epidermis thickness (d), lower epidermis thickness (e) (µm)..... 18

Figure 4 Visual aspect of common beans plants at 3RE. 19

LIST OF TABLE

CHAPTER 2 – MICROALGAE BIOMASS IMPROVES MELON CROP YIELD IN SALINE CONDITIONS

Table 1 Amount of fertilizers applied to melon plants grown in slabs through drip fertigation	56
Table 2. Treatments applied to melon plants with their abbreviations	56
Tabela 3. Productive and quality characteristics in melon (<i>Cucumis melo</i>) with applications of <i>Asterarcys quadricellulare</i> (AQ) in growing conditions with saline and non-saline water	63

CHAPTER 3 - USE OF MICROALGA *ASTERARCYS QUADRICELLULARIS* IN COMMON BEAN

Table 1. Chlorophyll <i>a</i> (mg g ⁻¹) of common bean leaves subjected to water deficit and foliar applications using <i>A. quadricellularis</i> in three periods	16
Table 2. Chlorophyll <i>b</i> (mg g ⁻¹) of common bean leaves subjected to water deficit and foliar applications using <i>A. quadricellularis</i> in three periods	16
Table 3. Total chlorophyll (mg g ⁻¹) of common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar application in three periods	17
Table 4. Carotenoids (mg g ⁻¹) in common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar spraying in three periods	17
Table 5. Total sugar (µg g ⁻¹) in common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar applications in three periods	18
Table 6. Reducing sugar (µg g ⁻¹) of common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar spraying in three periods	18
Table 7. Non-reducing sugars (µg g ⁻¹) in common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar applications in three periods	19
Table 8. Protein (µg g ⁻¹) in common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar spraying in three periods	19
Table 9. Total free amino acids (µg g ⁻¹) of common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar applications in three periods	18

Table 10. Proline ($\mu\text{g g}^{-1}$) in common bean leaves subjected to water deficit and leaf applications with <i>A. quadricellularis</i> foliar application in three periods	18
Table 11. Peroxidase (POD, $\mu\text{mol de purpurogallin min}^{-1} \text{mg}^{-1} \text{protein}$) of common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar application in three periods	19
Table 12. Activity of superoxide dismutase enzyme (SOD, $\text{U mg}^{-1} \text{protein}$) in common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar application in three periods.....	19
Table 13. Catalase (CAT, $\mu\text{Kat } \mu\text{g}^{-1} \text{protein min}^{-1}$) in common bean leaves subjected to water deficit and <i>A. quadricellularis</i> foliar application in three periods	20

LIST OF ABBREVIATIONS OR ACRONYMS

ANOVA - Analysis of Variance

BRIX - Refractometric Index

CAT - catalase

Cl⁻ - chlorine

CO₂ - carbon dioxide

DNA - deoxyribonucleic acid

EROS - Reactive Oxygen Species

Na⁺ - sodium

N^o - number

POD - peroxidase

SOD - superoxide dismutase

µm - micrometer

LIST OF SYMBOLS

® - registered trademark

° - degree

% - percentage

SUMMARY

1 GENERAL INTRODUCTION	16
2 LITERATURE REVIEW.....	16
2.1 ABIOTIC STRESS.....	16
2.1.1 Salt stress.....	16
2.1.2 Water stress	17
2.2 BIOFERTILIZERS	17
2.2.1 Microalgae-based fertilizers.....	18
2.2.2 Abiotic stress mitigation with the use of biofertilizers.....	19
2.3 MELON CROPS AND ECONOMICAL IMPORTANCE.....	21
2.4 SOYBEAN CROP AND ECONOMIC IMPORTANCE.....	22
CHAPTER 1- MICROALGA MITIGATES SALT STRESS IN MELON PLANTS BY PROMOTING METABOLIC CHANGES	24
1. INTRODUCTION.....	24
2. MATERIAL AND METHODS	26
2.1. EXPERIMENT	26
2.2 MICROALGA.....	27
2.3. EXPERIMENTAL DESIGN.....	27
2.4. TREATMENTS.....	27
2.5. BIOCHEMICAL ANALYSES.....	28
2.6. ANATOMICAL ANALYSES	29
2.7. BIOMETRIC ANALYSES.....	29
3. RESULTS AND DISCUSSION.....	30
3.1. BIOCHEMICAL ANALYSES.....	30
3.2 ANATOMICAL ANALYSES	40
3.3 BIOMETRIC ANALYSES.....	41
4. CONCLUSION	46
REFERENCES.....	47
CHAPTER 2 – MIGROALGAE BIOMASS IMPROVES MELON CROP YIELD IN SALINE CONDITIONS.....	53
1.INTRODUCTION.....	53
2. MATERIAL AND METHODS	54
2.1 FIELD EXPERIMENTS.....	55

2.2 TREATMENTS	56
2.3 BIOCHEMICAL ANALYSIS	57
2.4 RELATIVE INDEX OF CHLOROPHYLL, TOTAL SOLUBLE SOLIDS CONTENT (°BRIX) OF FRUITS, YIELD AND BIOMETRIC VARIABLES	57
2.5 EXPERIMENTAL DESIGN	58
3 RESULTS.....	58
4 DISCUSSION	64
5 CONCLUSION	66
CHAPTER 3 - USE OF MICROALGA <i>ASTERARCYS QUADRICELLULARIS</i> IN COMMON BEAN	70
1. INTRODUCTION	71
2. MATERIAL AND METHODS	72
2.1 PLANT MATERIAL AND GROWTH CONDITIONS	73
2.2 TREATMENTS	73
2.3 BIOCHEMICAL ANALYSIS	74
2.5 BIOMETRIC MEASUREMENTS.....	75
2.6 LEAF ANATOMY	76
2.7 EXPERIMENTAL DESIGN	76
3. RESULTS.....	77
3.1 BIOCHEMICAL ANALYSIS	77
3.2 BIOMETRIC ANALYSIS	17
3.3 LEAF ANATOMY	17
4. DISCUSSION	20
5. CONCLUSION	23
REFERENCES.....	23
FINAL CONSIDERATIONS	29
REFERENCES.....	30

1 GENERAL INTRODUCTION

Abiotic stresses (water deficit, excess of salt, high and low temperatures) have been classified as the factors that most harm the growth and productivity of crops around the world. It is estimated that abiotic factors affect plant development and global agricultural production by up to 70% (MANTRI et al., 2011). The losses are caused by inducing negative impacts on plants such as nutrient imbalance, reduction in photosynthesis, oxidative and/or osmotic stress, and damage to membranes, among others (MEDDICH, 2023).

In the scenario of an increase in the global population, the scarcity of agricultural areas, and climate change aggravating abiotic stresses, there is a great need to Search for sustainable alternatives that can mitigate the effect of these stresses. Biofertilizers are described in the literature as mitigating responses to abiotic stresses (BROWN and SAA, 2015).

Biofertilizers are defined as products that contain an active ingredient or organic agent, free from pesticides, capable of acting, directly or indirectly, on all or parts of cultivated plants, improving the performance of the production system, without considering their hormonal or stimulating values. The composition of a biofertilizer may contain amino acids, humic substances, algae extracts, and plant extracts (BRASIL, 2020).

The biofertilizing activity of algae extracts has been associated with the content of primary metabolites (carbohydrates and proteins, lipids), essential amino acids (arginine and tryptophan), vitamins, osmolytes (proline and glycine betaine), and polysaccharides (KHAN et al., 2009; BATTACHARYYA et al., 2015).

The use of microalgae biomass as biofertilizers has been the subject of recent studies (MÓGOR et al., 2017). Microalgae, are a highly diverse group of mainly photosynthetic microorganisms that include cyanobacteria (prokaryotic organisms) and green algae, euglenoids, and diatoms (eukaryotic organisms) (ALVAREZ et al., 2021).

To commercially exploit microalgae, the mixotrophic cultivation mode is important, being a unique cultivation strategy in which microalgae are able to simultaneously utilize inorganic (mainly CO₂) and organic carbon in the presence of light; thus, photoautotrophic and heterotrophic growth modes occur simultaneously

generating a sum of the biomass yields of the two cultivation modes (PATEL et al., 2020).

Microalgae can play an important role in the development of sustainable production systems (VUPPALADADIYAM et al., 2018). They have a promising place in the production of bioenergy, as they are a renewable source, have a high CO₂ mitigation rate and a high biomass yield compared to other bioenergy sources (BHARATHIRAJA et al., 2015), in addition to their role in wastewater treatment (GOYAL et al., 2023). They can also be used in the food and feed industry, the cosmetics industry and agriculture (SINGH et al., 2016).

In agriculture, microalgae are available as a new and promising source of nutrients, in addition to providing bioactive molecules (such as L-amino acids) acting to improve plant development as growth promoters, causing increases in productivity and mitigating the effects of abiotic and biotic stresses (BARONE et al., 2019). The main species of microalgae described in the literature with biofertilizing effects are *Chlorella* sp., *Acutodesmus dimorphus*, *Scenedesmus* sp., *Spirulina maxima*, and *Calothrix elenkinii* (CHIAIESE, 2018).

The chlorophyte microalgae *Asterarcys quadricellulare* was described for its high content of proteins, carbohydrates (GHOSH et al., 2017), and free amino acids (CORDEIRO et al., 2022a), in addition to its potential for the production of carotenoids (SINGH et al., 2019). Recent work shows growth promotion in potatoes (CORDEIRO et al., 2022a), onions (CORDEIRO et al., 2022b), tomatoes (LARA et al., 2022), and sugarcane (MÓGOR et al., 2022), increases in the levels of chlorophyll, amino acids and sugars in plants. Furthermore, less damage from salt stress was reported in soybean plants with applications of *A. quadricellulare* due to the increase in antioxidant enzymes and proline (PALMA et al., 2022).

Therefore, sustainable technologies that result in the reduction of the impacts of abiotic stresses on plants are more and more necessary. So, this work hypothesizes that the biomass of the microalgae *Asterarcys quadricellulare*, due to its high content of free amino acids, can mitigate saline stress in melon plants and water stress in bean plants, through antioxidant metabolism.

2 LITERATURE REVIEW

2.1 ABIOTIC STRESS

Global climate change alters environmental conditions that affect abiotic and biotic components, resulting in heat waves, rainfall intensity, CO₂ concentration, and temperature, limiting the growth and development of plants around the world (CHAUDHRY and SIDHU, 2021). Cramer et. al (2011) claim that around 96.5% of global rural lands are affected by stress conditions.

Drought and salt stress are the main abiotic factors affecting agricultural production worldwide, the presence or combination of them, as well as heat, are not uncommon and can lead to a drastic reduction in crop productivity (ABHINANDAN et al. al., 2018). It is estimated that up to 26% of arable land is subject to drought and more than 20% of irrigated land is affected by salt (RODZIEWICZ et al. 2014).

One of the effects caused by abiotic stresses is the uncontrolled increase in reactive oxygen species (ROS), which inhibit plant growth and reduce crop yields, as they generate lipid peroxidation, destabilization of cell membranes, and damage to DNA and proteins and, in turn, reduces photosynthesis efficiency and likely death of the plant (NAING and KIM, 2020).

2.1.1 Salt stress

According to Isayenkov (2012), almost 5.7 million hectares of agricultural land suffer from excess salinity, with estimates of rising to up to 17 million hectares by 2050.

One of the first effects of salt stress on plants is a reduction in the growth rate. Photosynthetic rates fall mainly due to the reduction in potential water consumption, in addition to the inhibition of the activity of enzymes involved in photosynthesis, such as the decrease in chlorophyll levels. Leaf growth and root growth are also reduced (PAHIRAR et al., 2014).

Salt stress also causes osmotic stress and nutritional imbalance (IMRAN et al., 2021). Osmotic stress causes water deficit by limiting water absorption, inducing responses similar to drought, such as stomatal closure, reduction of the CO₂ fixation, excessive reduction in the electron transport chain, and stimulation of photorespiration, leading to an increase in the ROS generation (SINGH and

THAKUR, 2018). Nutritional imbalance is generated due to excessive absorption of ions, particularly Na^+ and Cl^- (NAING and KIM, 2020).

2.1.2 Water stress

Drought (water stress) is one of the most important types of abiotic stress as it negatively influences the development and growth of plants through loss of turgor, disordered enzymatic activities, and reduction in the energy supply from photosynthesis (SALEHI-LISAR and BAKHSHAYESHAN-AGDAM, 2016). The size of the leaves and the extension of the stems and roots are reduced (ANJUM et al., 2011).

Water deficit leads to a decrease in photosynthesis, interrupting all the main components, such as the transport of electrons to the thylakoids, the stomatal closure, and, consequently, a decrease in CO_2 , a decrease in the activity of ribulose-1,5-bisphosphate carboxylase (Rubisco), in addition to reducing the activity of other photosynthetic enzymes. A drop in chlorophyll levels is also observed. This decrease is mainly due to damage to chloroplasts by reactive oxygen species (ROS) (KAUR and ASTHIR, 2017).

2.2 BIOFERTILIZERS

Following Normative Instruction No. 61 of July 8, 2020, of the Ministry of Agriculture, Livestock, and Supply

biofertilizers are products that contain an active ingredient or organic agent, free from pesticides, capable of acting, directly or indirectly, on all or part of the cultivated plants, increasing their productivity, without taking into account their hormonal or stimulating value. They can be classified into: (a) amino acid biofertilizer: product obtained from the fermentation or hydrolysis of natural organic materials; b) biofertilizer made from humic substances: product obtained from the decomposition and solubilization of organic materials and subsequent oxidation and polymerization, basically formed by humic acids, fulvic acids and humins; c) biofertilizer from algae extracts or processed algae: product obtained from the extraction and processing algae; d) biofertilizer from plant extracts: product obtained by extracting soluble organic compounds from the fermentation or processing of organic materials, free from biological contamination; e) compound biofertilizer: product obtained by mixing two or more biofertilizers from the amino acid

groups, humic substances, algae extracts, plant extracts and other approved organic principles or agents (BRASIL, 2020, no p.).

Also, according to Normative Instruction No. 61, the guarantee for biofertilizers is given by their total organic carbon content, and their bioactivity is verified through bioassays, in addition to research on plants evaluating biometric and biochemical variables to verify the action of these biologically active compounds. (BRAZIL, 2020).

A biofertilizer is any substance or microorganism applied to plants to increase nutritional efficiency, tolerance to abiotic stress, and/or quality characteristics of the crop, regardless of its nutrient content (DU JARDIM, 2015).

Biofertilizers are sustainable agronomic tools, with a growing market, with an annual growth rate of 12% and estimates of reaching 5.6 billion dollars in 2026. Several factors have influenced the increase in the biofertilizers market, such as changes in agricultural policies and environmental factors combined with climate change, driving the need for new alternatives to synthetic chemicals (LAU et al., 2022).

2.2.1 Microalgae-based fertilizers

Microalgae are microscopic, single-celled organisms capable of converting solar energy into chemical energy through photosynthesis. They are a broad group of organisms that include cyanobacteria and other eukaryotic organisms (green algae, euglenoids, diatoms, and others), which can grow in marine or freshwater systems (PRIYADARSHANI and RATH, 2012).

The production of microalgae has increased worldwide because of its promising economic potential and commercial viability, due to its relatively easy production, in addition to its role in environmental sustainability, as it can be grown and harvested using wastewater (GONZÁLEZ-PÉREZ et al., 2021).

Microalgae can be used for several applications such as pharmaceuticals, cosmeceuticals, biofuels, aquaculture, wastewater bioremediation, animal feed, and human food. Its use as a biofertilizer is recent and poorly investigated (JOHN et al., 2011; CHANDA et al. 2019).

According to Alvarez et al., (2021), microalgae biofertilizers are potentially versatile resources in agriculture as they improve soil aggregation and stabilization; influence soil microbial populations; produce phytohormones and other bioactive substances that influence plant growth, control pests and pathogens; can increase

the supply or availability of nutrients for plants; in addition to its role in mitigating abiotic stresses.

The microalgae *Asterarcys quadricellulare* is a green alga belonging to the phylum Chlorophyta, found in marine and fresh Waters. Its size ranges from 2 to 10 µm in diameter and is dependent on light for its autotrophic growth (HONG et al., 2012). Its composition contains high levels of carbohydrates and proteins (GHOSH et al., 2017), amino acids (CORDEIRO et al., 2022), and polysaccharides (VARSHNEY et al., 2018) in addition to high levels of carotenoids (SINGH et al., 2019).

According to Cordeiro et al., (2022), the main amino acids present in the biomass of the microalgae *Asterarcys quadricellulare* are aspartic acid, glutamic acid, serine, glycine, arginine, alanine, leucine, lysine, among others. These molecules, in the form of L-amino acids, can provide bioactivity, improving plant growth (MÓGOR et al., 2018) and resistance to abiotic stresses (BARONE et al., 2018).

The biomass of the microalgae *Asterarcys quadricellulare* is a potential biofertilizer, due to the promising results which had already been published, although in limited quantity. The first works using *A. quadricellulare* were carried out on onion, potato, sugarcane, tomato, and soybean crops (CORDEIRO et al., 2022a; CORDEIRO et al., 2022b; MÓGOR et al., 2022; LARA et al., 2022; PALMA et al., 2022). In potato, onion, and tomato crops, growth was promoted with applications of microalgae biomass, resulting in productivity gains. In addition to increases in leaf content of chlorophyll, sugars, free amino acids, and proteins. In sugarcane, additions of amino acids and non-reducing sugars were found in the initial stages of sprouting, indicating polyamines as the main compounds in nitrogen metabolism related to the sprouting and growth of these sprouts (MÓGOR et al., 2022).

2.2.2 Abiotic stress mitigation with the use of biofertilizers

Biofertilizers widely influence plant responses to stress at physiological, biochemical, and molecular levels (LANNA et al., 2021). The bioactive molecules contained in these compounds affect plant metabolism and activate metabolic pathways that are amplified and help plants adapt and/or overcome moments of stress. It is interesting to observe that among the metabolites increasing in plants treated with biofertilizers, there are often those with antioxidant properties. It is well known that these protective molecules have a central role in reducing the degenerative effects of ROS that accumulate in plant tissues under stressful

conditions (FRANZONI et al., 2022). Key antioxidant, ROS-scavenging enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and ascorbate peroxidase (APX), vital for plant tolerance to cellular oxidative stress (GILL and TUTEJA, 2010).

Another form of plant response against abiotic stresses with biofertilizer applications includes the accumulation of osmoprotective compounds such as glycine betaine, sugars, and amino acids such as proline (ZHANG et al., 2010). They act by reducing the osmotic potential of the cell, delaying the exit of water, and helping to maintain turgor. Through osmotic adjustment, organelles and cytoplasmic activities occur at a normal rate and help plants perform better in terms of growth, photosynthesis, and reproduction (FARROQ et al., 2009).

In addition, biofertilizers can change the morphology of the root system and aerial part of plants in stress conditions. Improvements in the absorption of water and nutrients are observed. These are caused by the improvement and change in the topology of the root system, increase in the root growth, and formation of lateral roots, and root hairs (LANNA et al., 2021).

In investigating the application of the microalgae *Chlorella vulgaris* in broccoli plants subjected to water stress, Kusvuran (2021) observed better growth performance when compared to plants under water stress that did not receive applications, in addition to an increase in photosynthetic pigments, flavonoids, phenolic compounds and of antioxidant enzymes (POD, SOD, and CAT) resulting in a reduction in the damage in the membrane, shown by the significant decrease in malondialdehyde levels in plants that received microalgae application. Applications of the microalgae *Nannochloris* sp. mitigate the effects of water stress on plant root development and reduce the negative influences on tomato plant height by almost 50% (OANCEA et al., 2013).

According to Cortés-Jiménez et al., (2013), the extract of the microalgae *Chlorella vulgaris* increased the length of the roots and the aerial part of tomato plants subjected to saline stress conditions. Arroussi et al., (2018), also studying tomato cultivation under conditions of saline stress, concluded that the application of the microalgae *Dunaliella salina* attenuated the decrease in the length of the shoot and root of the plant; improved concentrations of proline, phenolic compounds, and the activities of antioxidant enzymes that eliminate ROS (catalase, peroxidase, and superoxide dismutase).

The application of *Asterarcys quadricellulare* to soybean plants subjected to saline stress conditions showed that the microalgae acted to mitigate stress by maintaining plant growth, with increased levels of total free amino acids, proline, proteins, and antioxidant enzyme activity. In addition to an increase in the volume of root nodules (PALMA et al., 2022).

2.3 MELON CROPS AND ECONOMICAL IMPORTANCE

Cucumis melo L., popularly known as melon, is an angiosperm belonging to the Cucurbitaceae family (JEFFREY, 1990). The likely center of origin of the melon tree would be the African continent or Asia, with divergence among the authors (HARLAN et al., 1997; TRENTINI, 1998; SILBERSTEIN et al., 1999).

In Brazil, the genetic variability of *C. melo* was considerably increased by the introduction of accessions along the Brazilian coast, mainly in the Northeast Region, by African slaves and European immigrants during the 16th century. Since its introduction, the genetic material has undergone an intense process of selection and crossings that resulted in many local cultivars adapted to various types of soil and climatic conditions (ANDRADE et al., 2019).

In 2012, the melon was the eighth most-produced fruit in the world: the five largest producers are China, Iran, Turkey, Egypt, and India, accounting for more than 71% of the total produced that year. Brazil ranked ninth position, contributing with 1.8% of world production (FAO, 2016).

In Brazil, a large part of production is concentrated in the Northeast region, in the São Francisco Valley (Bahia and Pernambuco) and, mainly, in the Jaguaribe-Açu Complex (Ceará and Rio Grande do Norte), responsible for at least 97% of Brazilian exports in the last decade (CELIN et al., 2014). The main melon cultivars are from the Inodorus group, Yellow type; however, in the last years, there has been an increase in demand the marked for melons from the Cantalupensis group, which are aromatic, with salmon-colored flesh, with good flavor and high sugar content (°Brix). For melons of the Pele de Sapo, Gália, and Charentais types, the main opportunity for expanding the culture is the foreign market, especially the European one (SALVIANO, et al., 2017).

According to Agrianual (2019), in 2016, 23 thousand hectares of the crop were harvested with a total production of 596 thousand tons of fruit, of which almost 97%

of this production was in northeastern Brazil. In 2017, around 233 thousand tons were exported, particularly to the Netherlands, the United Kingdom, and Spain.

Meteorological elements such as temperature, solar radiation, and relative humidity are those that most affect fruit productivity and quality (PIVETTA, 2010). Suitable temperature ranges are different depending on the phenological stage of the crop. For germination, the range varies from 18 to 45°C, where the interval of 25 to 35°C is the ideal temperature range. For crop development, the optimum range is from 25 to 30°C (below 12°C, growth is ceased); and, for flowering, it is between 20 and 23°C. Shortening solar radiation harms plant growth with a reduction in leaf area. The successful development of the crop requires planting in regions that have sun exposure in the range of 2 to 3 thousand hours per year (SALVIANO et al., 2017). High rainfall levels and high relative humidity harms melon cultivation particularly because of the lack of leaf resistance to diseases and the deleterious effect of rain on fruit quality (MONTEIRO, 2007).

2.4 SOYBEAN CROP AND ECONOMIC IMPORTANCE

Phaseolus vulgaris L., the common bean, belongs to the Fabaceae family, with the legume-type fruit (or pod) being one of its main characteristics (VILHORDO, 1996). Genomic studies suggest that common beans may have originated in Central America and have two centers of domestication: Mesoamerican and Andean, with well-defined breeds within each gene pool. The Mesoamerican gene pool extends from Mexico through Central America Colombia and Venezuela, while the Andean gene pool is found in southern Peru, Chile, Bolivia, and Argentina (CHOUDHARY et al., 2018).

Beans are grown in different climates, such as the tropics, subtropics, and temperate zones and in many of these regions, they are considered a staple food crop. Furthermore, it is considered the most important legume for human nutrition worldwide, as it is a valuable source of high-quality proteins, carbohydrates, vitamins, minerals, fiber, phytonutrients (mainly phytosterols and flavonoids), and antioxidants (CATARCIONE et al., 2023).

The leading global producers of beans are Myanmar, India, Brazil, China, Mexico, Tanzania, the United States, Kenya, Uganda and Rwanda. The Leading

importers are India, China, Bangladesh, the United States, and Egypt (COÊLHO and XIMENES, 2020).

Common beans are grown in most Brazilian states, in different systems and technological levels. Sowing is carried out at three different times: the water crop, between August/October or November/December, with harvest between November and March. The second is carried out, particularly in the Northeast and Southeast regions, where sowing takes place between January and April and harvest is between April and July. In the Central-West and Southeast regions, irrigated winter beans are found (RICHETTI and ITO, 2015).

Bean crops depend on environmental factors. The average temperature for production is 21°C. However, it develops well between 15 and 29°C. The water demand of the crop is variable depending on its 24 phenological stages of development, increasing from a minimum value of germination (V0) to the maximum value in the flowering period (R5) and pod formation (R7), decreasing from the beginning of grain maturation (R9) (SILVA et al., 2020). Although the reproductive phase is the most sensitive to water stress, the deficit in the vegetative phase is also detrimental to productivity and crop development (STONE and MOREIRA, 2001).

In Brazil, according to Conab (2020), 2,927.3 thousand hectares of the crop were harvested with a total of 3,222.1 thousand tons of production in the 2019/2020 crop. Of this total, 858 thousand tons came from the Brazilian northeast, the region that leads production, despite the low productivity of 568 kg/hectare. In the southern region of the country, 490.9 thousand hectares were harvested, with a production of 760.2 thousand tons and a productivity of 1,549 kg/hectare. There are no large exportable surpluses for the crop, as is the case with other grains.

CHAPTER 1- MICROALGA MITIGATES SALT STRESS IN MELON PLANTS BY PROMOTING METABOLIC CHANGES

(Article formatted according to the journal *Ciência e Agrotecnologia*)

Abstract

High soil and water salinity are limiting factors for the growth and production of melon. Therefore, the search for sustainable alternatives to mitigate the deleterious effects of salt stress is highly relevant. The objective of this work was to evaluate the effects of concentration and form of application of the microalga *Asterarcys quadricellulare* on two cultivars of melon plants under saline conditions. The plants were grown in pots irrigated with and without NaCl addition, being the saline solution with the electric conductivity of 15 dS m⁻¹. They were submitted to treatments with application of *A. quadricellulare* at concentrations of 0 ml L⁻¹, 0.5 ml L⁻¹, and 1.0 ml L⁻¹ by foliar sprays, drip irrigation, and the combination of foliar and irrigation ways. Plants under saline conditions treated with the microalga showed an increase in chlorophyll, carotenoids, total free amino acids, proteins, proline, total sugars, phenolic compounds, and increased antioxidant enzyme activity. There was also an increase in fresh mass, diameter, and length of the stem, length, and volume of the roots, and an increase in the number of flowers in comparison to plants under salinity without microalga applications. Thus, *A. quadricellulare* stimulated metabolic changes mitigating the effects of salinity in both cultivars, with emphasis on the foliar + drip application at the concentration of 1.0 ml L⁻¹. Therefore, the application of this microalga is a sustainable alternative for mitigating the effects of salt stress in melon plants.

Keywords: Antioxidant enzymes; *Asterarcys quadricellulare*; biofertilizers; sustainability.

1. INTRODUCTION

Melon (*Cucumis melo* L.) belongs to the Cucurbitaceae family and has wide consumption worldwide, being considered an important horticultural crop ranking ninth in the world production ranking (Silva et al., 2020). Commonly grown in arid and semi-arid regions, its main producers are China, Iran, Turkey, Egypt, and India, with

Brazil occupying the eleventh position (FAO, 2018). In many production areas in these countries, there is a shortage of rainfall and low-quality water for irrigation (Tedeschi et al., 2011), which favors soil salinization. This is a problem that can limit land use and globally impact food production. It is estimated that approximately one-fifth of irrigated land in the world is affected by salinization which leads to losses of billions per year (Qadir et al., 2014).

Plants subjected to salinity present modifications in metabolism. Among these, there is a reduction in the assimilation of CO₂, which impairs vegetative development and yield (Ors et al., 2021). Additional problems in these environments are related to reduced nutrient uptake (Arif et al., 2020), in addition to increased ion toxicity, osmotic and oxidative stress (Nxele et al., 2017; Naveed et al., 2020). Therefore, finding sustainable solutions that minimize these effects of salinity is a demand of global interest.

Currently, to minimize the harmful effects of salt on crops tolerant cultivars and agronomic soil improvement practices are being developed, both of which are considered costly and time-consuming (Chourasia et al., 2022). An attractive and sustainable approach to alleviate salinity and other abiotic stresses involve the use of biostimulants and biofertilizers, which are products containing natural active ingredients or organic agents, free of pesticides, capable of acting on all or part of the plants, promoting the increase of osmoprotectant and antioxidant enzymes (Bulgari et al., 2015; Colla and Roupael, 2015; Van Oosten et al., 2017; BRASIL, 2020).

Among the possible biostimulants and biofertilizer sources, microalgae encompass a wide range of evolutionarily diverse phototrophic and unicellular organisms (Chiaiese et al., 2018; Mutale- Joan et al., 2021) and their effects are related to the high contents of free L-amino acids in their biomass (Mógor et al., 2018) and other bioactive compounds such as polysaccharides (Renuka et al., 2018), polyamines (Mógor et al., 2017; Amatussi et al., 2023), and hormones (Stirk et al., 2013).

The microalga *Asterarcys quadricellulare* is a species that presents high content of proteins and free amino acids, with results in growth promotion in potatoes (Cordeiro et al., 2022a), onions (Cordeiro et al., 2022b), tomatoes (Lara et al., 2022) and in sugarcane (Mógor et al., 2022), promoting increases in chlorophyll, amino acids and sugars contents of the plants. In addition, applications of this microalgae in

soybean plants mitigated the effect of salt stress by increasing antioxidant enzymes and proline (Palma et al., 2022).

To test the hypothesis that microalgae are able to mitigate the effects of salt stress, the aim of this work was to determine the best concentration and application form of *A. quadricellulare* (CCAP 294/1) microalga in melon grown under saline conditions.

2. MATERIAL AND METHODS

2.1. EXPERIMENT

The experiment was conducted in the Biofertilizers Laboratory of the Federal University of Paraná (UFPR), Brazil, under the coordinates of latitude 25°25' S, longitude 49° 08' and altitude 930 m, in the city of Pinhais, PR.

Both cultivars used were hybrid yellow melons, Goldex® (F1) from Agristar®, and Dalí® from Sakata®. These cultivars are adapted to the main producing regions of Brazil and the fruits are destined for export.

The melon seeds were sown in 3 L polyethylene pots containing substrate based on pine bark, peat, expanded vermiculite (Tropstrato®) mixed with organic compost (Provaso®), being the proportion 1:1, whose chemical analyses indicated: pH (CaCl₂) = 6.63, pH SMP = 7.03, Al⁺³ = 0, H⁺ + Al⁺³ = 2.31 cmol dm⁻³, Ca²⁺ = 12.34 cmol dm⁻³, Mg²⁺ = 3.52 cmol dm⁻³, K⁺ = 1.95 cmol dm⁻³, P = 193.81 mg dm⁻³, C = 55.21 g dm⁻³, V% = 88.5% e CTC = 20.12 cmol dm⁻³, Cu = 1.95 mg kg⁻¹, Mn = 31.80 mg kg⁻¹, Fe = 29.77 mg kg⁻¹, Zn = 2.30 mg kg⁻¹, B = 0.34 mg kg⁻¹, S = 141.12 mg kg⁻¹. Two seeds were sown per pot and placed in a greenhouse with suspended benches.

Irrigation control was done by gravimetric method (daily weighing of experimental units), considering the mass of substrate and water, with replacement of water lost by evapotranspiration. The water holding capacity was calculated according to the formula of Craven, Gulamhussein and Berlyn (2010). The pots irrigation had individual drippers and the water holding capacity maintained at 80%.

The saline treatments started to be applied when the plants had the first true leaf developed. The salinity of the water was corrected by increasing the electrical conductivity with the addition of sodium chloride P.A. (NaCl) until it reached a value of 15 dS m⁻¹, measured with a portable conductivity meter AKSO®. Salt was applied in all irrigations, except in the control plants, which were irrigated with clear water.

The plants were collected 60 days after sowing.

2.2 MICROALGA

Asterarcys quadricellulare (CCAP 294/1) (AQ) microalga biomass was supplied by Alltech® Crop Sciences - Brazil, obtained from mixotrophic culture and atomized by spray drying method producing a fine greenish powder. The sum of amino acids present in the biomass of *A. quadricellulare* is 30.31% (Aspartic acid = 3.32; Glutamic acid = 4.27; Serine = 1.66; Glycine = 1.54; Histidine = 0.71; Arginine = 2.17; Threonine = 1.45; Alanine = 2.41; Proline = 1.6; Tyrosine = 0.95; Valine = 1.81; Methionine = 0.51; Cysteine = 0.29; Isoleucine = 1.41; Leucine = 2.36; Phenylalanine = 1.37; Lysine = 2.11 and Tryptophan = 0.37) and 37.94% for crude protein (Cordeiro et al., 2022).

The AQ applications were started also with the first pair of truly developed leaves and performed weekly until the end of the experiment, performing seven applications. The AQ biomass was diluted in a suspension containing 0.25 g L⁻¹ concentration, from which aliquots of 0.5 ml L⁻¹ and 1.0 ml L⁻¹ were taken and diluted in distilled water for application. The foliar application was done using a Kawashima® electric knapsack sprayer with constant pressure (40 psi).

2.3. EXPERIMENTAL DESIGN

The experimental design was completely randomized with four replications (n=4) in a factorial scheme with 14 treatments x 2 cultivars. Each replication was composed of four pots containing two plants. The data obtained were tested by Bartlett's test and ANOVA and applied Scott-Knott's test (p<0.05), processed by the statistical program SISVAR (Ferreira, 2019).

2.4. TREATMENTS

Treatments were divided into plants grown in pots irrigated without salinity (clear water) and with salinity (NaCl addition): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ.

2.5. BIOCHEMICAL ANALYSES

Fully expanded leaves from the middle third of the plant were collected 60 days after planting between 09:00 and 10:00 am, subsequently, they were frozen and macerated in liquid nitrogen to determine biochemical variables.

Pigment analyses were performed according to Lichtenthaler (1987) by adding 0.3 g of plant material to 1.7 mL of 80% acetone with 0.1% CaCO₃ (w/v) and readings were performed in a UV-visible spectrophotometer at 663, 647 and 470 nm (Pompelli et al., 2013).

Total free amino acids were extracted according to Winters et al. (2002) and the colorimetric reaction was performed according to Magné and Larher (1992) with readings in a spectrophotometer at 570 nm.

The extraction of total sugars was performed according to Maldonado, Carvalho and Ferreira (2013). Samples were obtained by acid hydrolysis and subsequent reaction with DNS, with the extraction of reducing sugars and non-reducing sugars. The reading was performed in a UV-visible spectrophotometer at 540 nm.

Total soluble proteins were determined using the methodology described by Bradford (1976) using 50 µL of enzyme extract and the reading was performed in a UV-visible spectrophotometer at 595 nm.

Proline content was determined using the method of Bates, Waldern and Teare (1973), with 3% sulfosalicylic acid, 1% ninhydrin solution, and toluene. After a boiling water bath for 60 min, the reaction was read in a UV-visible spectrophotometer at 520 nm.

The determination of phenolic compounds followed the Prussian Blue method of Price and Butler (1977) with modifications. The readings were performed at 700 nm.

The malondialdehyde (MDA) content produced by lipid peroxidation was determined according to the technique described by Heath and Packer (1968), cited by Devi and Prasad (1998).

The activity of the superoxide dismutase enzyme (SOD) was determined according to Giannopolitis and Reis (1977) by adding 50 µL of diluted enzyme extract to a solution containing 13 nM of methionine, 75 µM of NBT, 100 nM of EDTA and 2 µM of riboflavin and 50 mM sodium phosphate buffer pH 7.8. The reaction was

performed in the presence of light for 10 minutes at room temperature (25°C) and read in a spectrophotometer at 560nm.

The peroxidase (POD) activity was determined according to Teisseire and Guy (2000) with the reaction system composed of 30 µL of diluted enzyme extract (1:10 in extraction buffer); potassium phosphate buffer 50 mmol L⁻¹ pH 6.5; pyrogallol (1,2,3-benzenetriol) 20 mmol L⁻¹ and hydrogen peroxide (H₂O₂) 5 mmol L⁻¹, measured in UV-visible spectrophotometer at 430 nm.

The enzyme catalase (CAT) was determined using diluted enzyme extract, sodium phosphate buffer, and H₂O₂ with readings in a UV-visible spectrophotometer at 240 nm (Peixoto et al., 1999).

2.6. ANATOMICAL ANALYSES

For the anatomical analyses, the most responsive treatments in biochemical and biometric variables were selected. Thus, applications 0.5fd and 1.0fd, which received salt (NaCl), were compared to the control treatments.

Leaf blade samples were collected 60 days after planting in the middle third of the plant. Leaf fragments of 0.5 cm² were taken from the central region of the leaves. The samples were fixed in FAA50 (Johansen, 1940) for 24 hours and, after dehydration in ethyl series, the samples were included in methacrylate (Historesin, Leica Instruments). Cross sections (7µm) were obtained using a rotary microtome and stained with toluidine blue, pH 7.2 (O'Brian and McCully, 1981). The slides were mounted in 500® vitreous varnish (Paiva et al., 2006) and analyzed and photographed under a light microscope (Zeiss Axiolab model) with a digital camera (Sony Cybershot 7.2 ned mb model) attached.

Data on leaf thickness and component tissues palisade parenchyma, lacunar parenchyma, adaxial epidermis, abaxial epidermis, and cuticle were obtained with the aid of the ANATI QUANTI software (Aguiar et al., 2007). Each of the five parameters was measured three times in each of the ten sections of each of the three slides for each repetition (n=3) totaling 1800 records per cultivar, as adapted by Sant'Anna-Santos and Azevedo (2010) and Rocha et al. (2014).

2.7. BIOMETRIC ANALYSES

The analyses were performed 60 days after planting. The leaves were collected for determination of the fresh mass (g) using a precision scale, after which

the samples were dried in a forced circulation oven at a temperature of approximately 65°C until a constant weight was obtained to obtain the dry mass (g). The leaf area (cm²) was obtained using the WinRhizo[®] computer program coupled to a LA1600 Scanner (Regent Instruments Inc., Canada).

Analyses of dry mass and fresh mass were performed on the stem of the plants as already described for the leaves. The diameter of the stem was measured with a digital pachymeter, standardized at 5 cm from the soil surface. The length of the stem was measured with a measuring tape in centimeters.

The diameter, length, and volume of the roots were measured using the WinRhizo[®] computer program, coupled with a LA1600 Scanner (Regent Instruments Inc., Canada).

The number of flowers present on the stem was counted manually, including open and closed flowers.

3. RESULTS AND DISCUSSION

Soil salinity is a major concern for agricultural production worldwide. The problems occur in plants due to ionic toxicity, altered enzyme activities, and oxidative injury, which compromises plant photosynthesis, growth, and development (Acosta-Motos et al., 2017; Ahanger; Agarwal, 2017).

In the present study melon plants subjected to saline growing conditions showed morphological, physiological, and biochemical modifications. For many of these damaging changes, it was observed that AQ applications helped to mitigate them.

3.1. BIOCHEMICAL ANALYSES

The use of AQ promoted an increase in chlorophyll *a* contents (Fig. 1a) in plants without salinity. In the Dalí cultivar there was a 2% increase in 0.5f and 14% in 1fd, in Goldex the increases were 24% in 0.5d, 18% in 1d and 14% in 0.5fd, when compared to the water control.

In plants subjected to salinity, there was a decrease in chlorophyll *a* levels in Goldex compared to the water control, as seen in other melon research (Sarabi; Ghashghaie, 2022). In addition, AQ applications provided improvements of chlorophyll *a* levels in plants under salinity. In Dalí there were increases of 22% at

0.5f, and in Goldex there were increases in all treatments that received AQ with remarkable increases up to 113% compared to the NaCl control.

Saline conditions resulted in increased chlorophyll *b* contents (Fig. 1b) for Dalí. In contrast, for Goldex there was a decrease in chlorophyll *b* contents in stressed plants compared to the water control. However, this effect was reversed when AQ was applied to the plants, leading to increased chlorophyll *b* levels in all salinity treatments.

The total chlorophyll contents (Fig. 1c) in Dalí showed increases in plants without salinity using AQ at 1df with increases of 13% compared to the water control. Cordeiro et al. (2022a) also found increased chlorophyll contents in potato plants with AQ applications. Plants subjected to salinity experienced increases in total chlorophyll compared to those without salinity in Dalí. This increase was also found by other authors (Sivritepe et al., 2005; Negrão; Schmöckel; Tester, 2017; Niu et al., 2017), and can be considered an important parameter for tolerance to the effects of salinity.

Unlike the Dalí cultivar, salt-stressed plants in the Goldex cultivar showed decreased total chlorophyll contents. Akrami and Arzani (2018) also found chlorophyll-related differences in different melon cultivars under saline cultivation. Thus, in Goldex, there was an increase in total chlorophyll contents in all treatments that received AQ application subjected to saline conditions, with increases of up to 194%. This effect is beneficial for the efficiency of the photosynthetic process, as reported by Palma et al. (2022) in salt-stressed soybean plants with applications of *A. quadricellulare*.

AQ also promoted increased carotenoid contents (Fig. 1d) for Dalí under salt conditions of 10% at 0.5d, 20% at 1d, and 15% at 0.5f and 1df. For Goldex there were carotenoid additions in all treatments that received AQ under saline conditions compared to the NaCl control. Carotenoids are described as antioxidants, capable of activating bioactive molecules, and inducing changes in gene expression leading to acclimation under stress conditions (Havaux, 2013; Wang et al., 2013; Kim et al., 2015).

There were increases in total free amino acid contents (Fig. 2a) in non-salinity plants with AQ applications, for Dalí the increases were 8% at 0.5d and 6% at 1f. In Goldex the increases were 7% at 1d and 0.5f; 5% at 1f, 8% at 0.5fd, and 4% at 1fd compared to the water control.

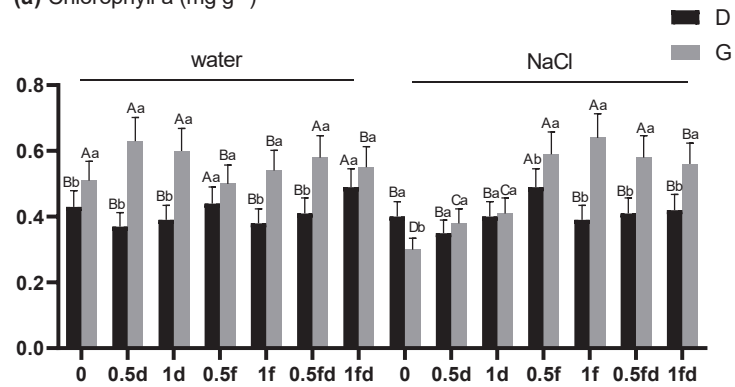
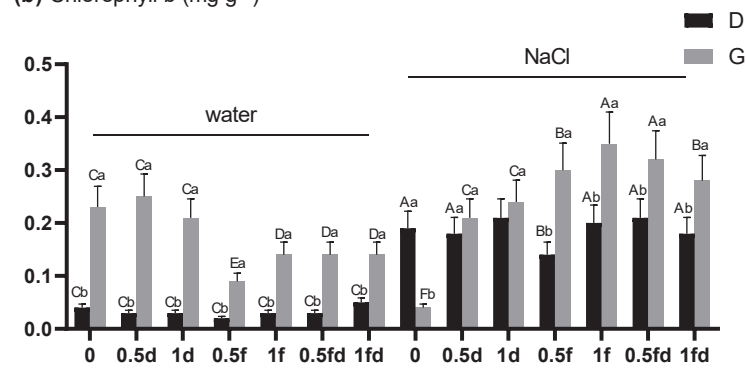
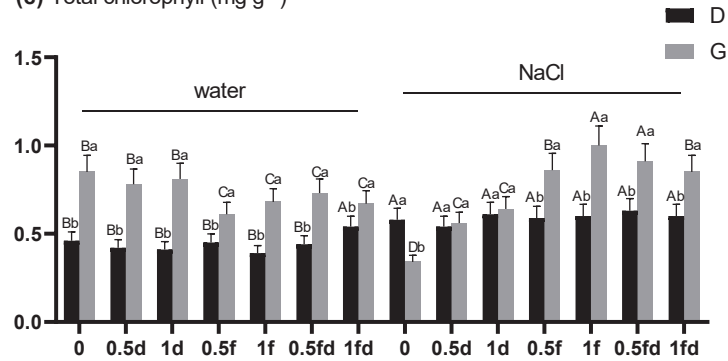
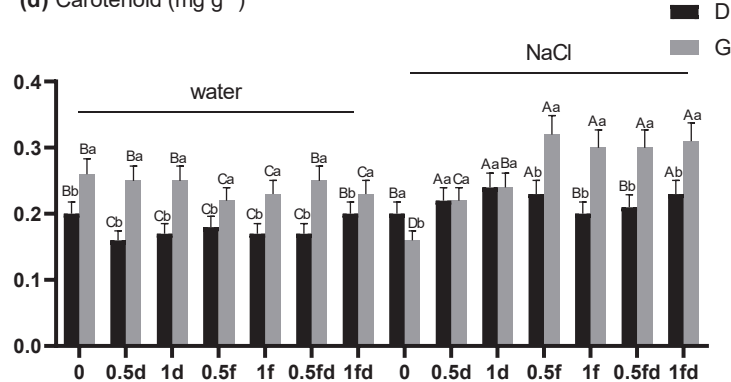
(a) Chlorophyll a (mg g^{-1})(b) Chlorophyll b (mg g^{-1})(c) Total chlorophyll (mg g^{-1})(d) Carotenoid (mg g^{-1})

Figure 1 **(a)** Chlorophyll *a* (mg g⁻¹), **(b)** Chlorophyll *b* (mg g⁻¹), **(c)** total chlorophyll (mg g⁻¹) and **(d)** carotenoid (mg g⁻¹) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = *; C = *; TXC = *; (b) T = *; C = *; TXC = *; (c) T = *; C = *; TXC = *; (d) T = *; C = *; TXC = *.

In accordance with Batista-Silva et al. (2018), the accumulation of free amino acids was observed in plants exposed to abiotic stresses. In this study, salinity led to an increase in total free amino acid contents in the leaves of melon plants in both cultivars. There was also an increase with the use of AQ in Dalí, the increases were 3% at 1d, 4% at 0.5f, 3% at 1f, 2% at 0.5fd, and 11% at 1fd. For Goldex the increase was 9% in 1fd when compared to the NaCl control.

In contrast to amino acids, salinity reduced the protein content in Dalí (Fig. 2b). This is explained by proteolysis which is essential to remove proteins that were damaged during stressful conditions. In addition, protein degradation is highly relevant for maintaining free amino acid contents in response to the dehydration caused by salt stress (Araújo et al., 2011; Hildebrandt, 2018).

As seen by Alvarez et al. (2021), regarding the increase of proteins in plants applied with microalgae, in this study there was an interaction between different cultivars and applications during salinity. Therefore, there were observed increased protein contents with AQ application in Dalí, the increase was 31% at 1f, 27% at 0.5fd, and 48% at 1fd, for Goldex the increases were 25% at 0.5d and 19% at 1fd compared to the NaCl control.

As well as total free amino acids, proline also showed an increase in salt-stressed plants (Fig. 2c) in both cultivars. The increase in proline is widely reported in the literature in salt-stressed plants in melon crops and other species (Kusvuran; Dasgan; Abak, 2013; Per et al., 2017; Hildebrandt, 2018). Furthermore, when applied with AQ there were increases in proline in salt-stressed plants. In Dalí the increases were 52% at 0.5d and 0.5fd and 92% at 1fd. In Goldex the increases were 68% at 1d and 5% at 1fd when compared to the NaCl control. Similar results were found in soybean under salt stress conditions, also with applications of *A. quadricellulare* (Palma et al., 2022).

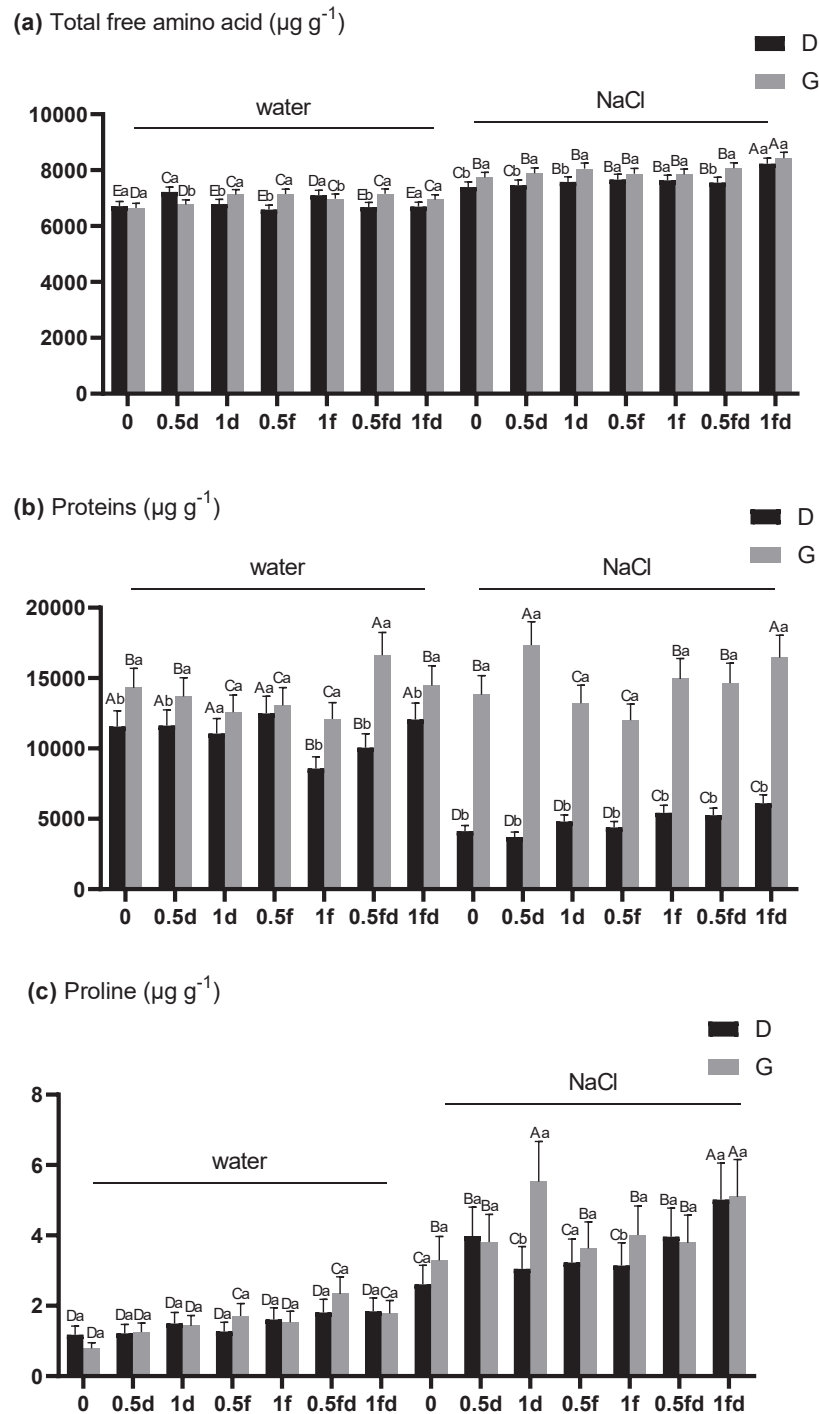


Figure 2 (a) Total free amino acid ($\mu\text{g g}^{-1}$), (b) proteins ($\mu\text{g g}^{-1}$) and (c) proline ($\mu\text{g g}^{-1}$) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L^{-1} AQ, (1.0d) drip application of 1.0 ml L^{-1} AQ, (0.5f) foliar application of 0.5 ml L^{-1} AQ, (1.0f) foliar application of 1.0 ml L^{-1} AQ, (0.5df) drip application of 0.5 ml L^{-1} AQ + foliar application of 0.5 ml L^{-1} AQ, (1.0df) drip application of 1.0 ml L^{-1} AQ + foliar application of 1.0 ml L^{-1} AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters

compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = *, C = *, TXC = *; (b) T = *, C = *, TXC = *; (c) T = *, C = *, TXC = *.

Total sugars (Fig. 3a) in melon leaves showed increases with AQ in plants without salinity, in Dalí there was an increase of 27% in 0.5f, 57% in 1f, 31% in 0.5fd and 48% in 1fd, for Goldex the increases were 20% in 1f and 34% in 0.5fd and 1fd when compared to the water control.

In reducing sugars (Fig. 3b) AQ promoted increases for both cultivars. In Dalí without salinity, the increase was 82% in 1f, 43% in 0.5fd, and 55% in 1df, while in Goldex the increases were 44% in 0.5fd and 49% in 1fd compared to the water control.

The non-reducing sugars contents (Fig. 3c), still in plants without salinity, for the Dalí cultivar, there was increases in all treatments that received AQ application up to 80%. For Goldex, the increases were 26% at 1d, 56% at 0.5f, 48% at 1f, 60% at 0.5fd, and 42% at 1.0fd compared to the water control.

When subjected to salt stress plants respond with increased sugars (Slewinski, 2011; Saddhe; Manuka; Penna, 2020). In the case of melon, plants subjected to salt stress showed an increase in total and reducing sugars compared to unstressed plants.

Additionally, there was an increase in total and reducing sugars in all treatments that received AQ application in the salinity-stressed plots of both cultivars. For Dalí cultivar, the highest increase in total sugars was at 1fd with an 82% compared to the NaCl control, in Goldex the increases were up to 41%. The reducing sugars contents in Dalí the biggest increase was in 1,0df with 85%, in Goldex the increases were up to 22% compared to the NaCl control.

In contrast to reducing sugars, there was a decrease in the levels of non-reducing sugars in salt-stressed plants in Dalí. In Goldex, there was no difference between the stressed and unstressed treatments. In addition, there was an increase in the levels of non-reducing sugars with the use of AQ, of 66% in 0.5f and 93% in 1fd in Dalí and in all the treatments that received AQ application in Goldex, with the largest increases in 0.5d with 118% and in 1fd with 90% compared to the NaCl control. This increase is considered beneficial because under abiotic stress conditions, non-reducing sugars, specifically sucrose, can function as a protective agent related to antioxidant activities such as ROS elimination (Bolouri-Moghaddam et al., 2010; Eggert et al., 2015).

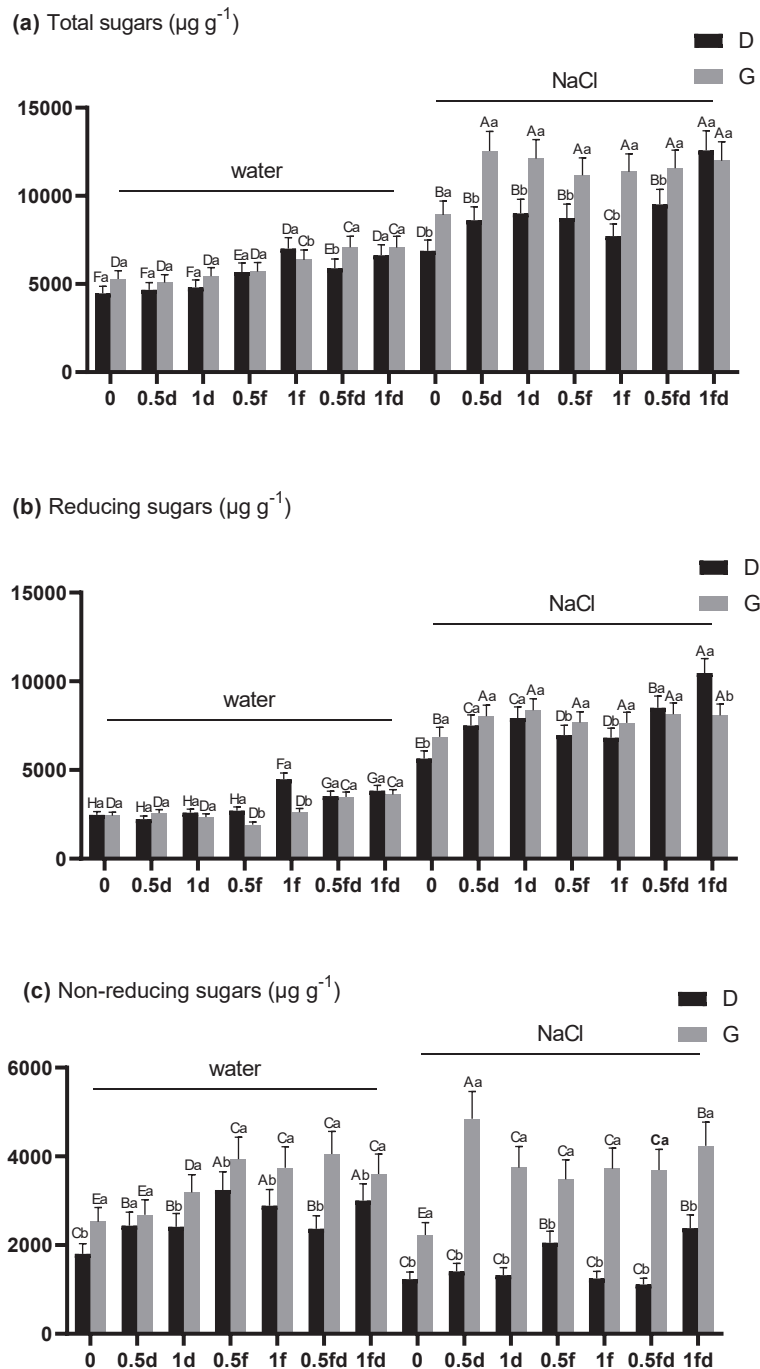


Figure 3 **(a)** Total sugars ($\mu\text{g g}^{-1}$), **(b)** reducing sugars ($\mu\text{g g}^{-1}$) and **(c)** non-reducing sugars ($\mu\text{g g}^{-1}$) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5fd) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0fd) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = *, C = *, TXC = *, (b) T = *, C = *, TXC = *, (c) T = *, C = *, TXC = *.

Thus, AQ showed efficiency to promote the accumulation of two major osmoprotectants compounds, the proline and sugars (Nahar; Hasanuzzaman; Fujita, 2016). The main role of osmoprotectant accumulation under abiotic stress conditions is to maintain osmotic balance in plants, maintaining cell turgor, protecting cellular components, in addition to their antioxidant role (Suprasanna; Nikalje; Rai, 2016).

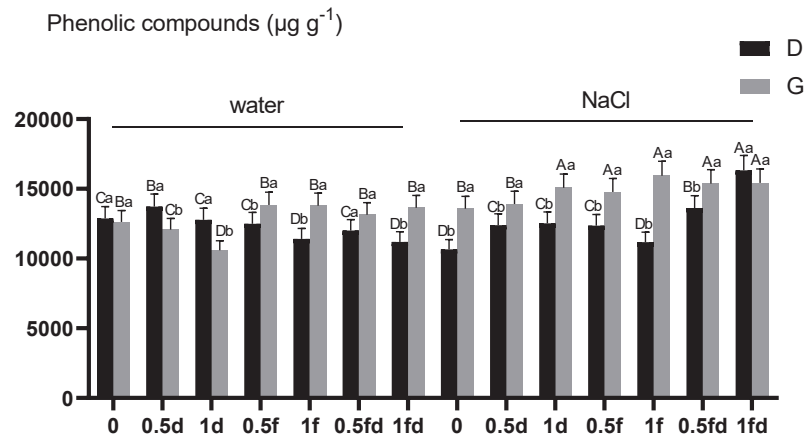


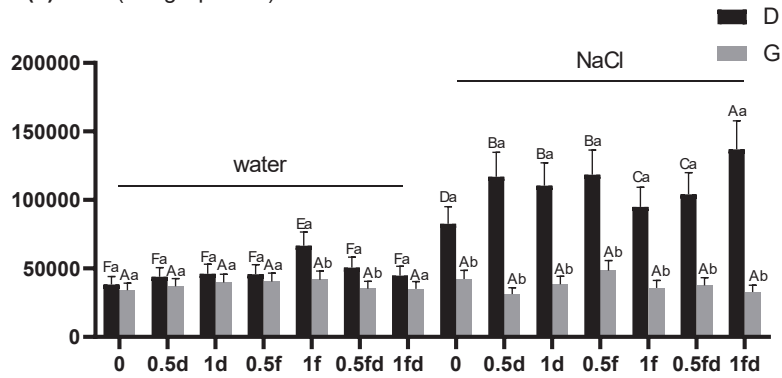
Figure 4 Phenolic compounds ($\mu\text{g g}^{-1}$) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5fd) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0fd) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. T = *; C = *; TXC = *.

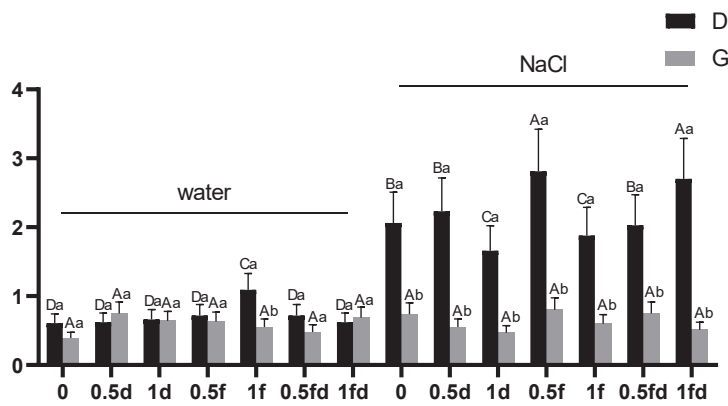
There was also an increase in phenolic compounds with AQ application (Fig. 4) in salt-stressed plants in both cultivars. These secondary metabolites confer plants a higher tolerance against stresses by eliminating ROS (Sharma et al., 2019). In Dalí the increases were 16% at 0.5d, 18% at 1d, 16% at 0.5f, 28% at 0.5fd, and 53% at 1fd compared to the NaCl control. In Goldex the increases were 11% at 1d, 9% at 0.5f, 17% at 1f, and 11% at 0.5fd and 1fd also compared to the NaCl control.

The enzymes SOD (Fig. 5a), POD (Fig. 5b), and CAT (Fig. 5c) were influenced by salinity with increases in the cultivar Dalí. Moreover, AQ still influenced the increase in the contents of these enzymes. There was an increase of SOD in all treatments that received AQ applications with the highest increase with 1fd of 66%

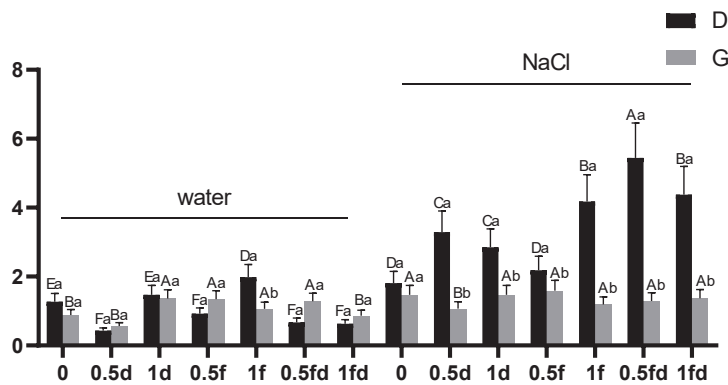
(a) SOD (U mg⁻¹ protein)



(b) POD ($\mu\text{mol de purpurogallin min}^{-1} \text{ mg}^{-1}$ protein)



(c) CAT, $\mu\text{Kat } \mu\text{g}^{-1} \text{ protein min}^{-1}$



(d) MDA (nmol g⁻¹)

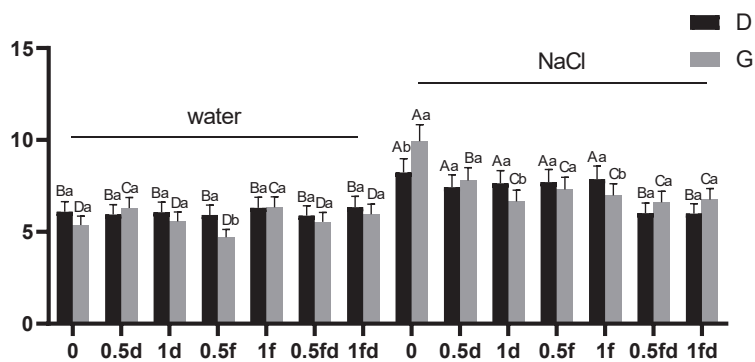


Figure 5 **(a)** Activity of superoxide dismutase enzyme (SOD, U mg⁻¹ protein), **(b)** peroxidase (POD, $\mu\text{mol de purpurogallin min}^{-1} \text{ mg}^{-1} \text{ protein}$), **(c)** catalase (CAT, $\mu\text{Kat } \mu\text{g}^{-1} \text{ protein min}^{-1}$) and **(d)** malondialdehyde content (MDA, nmol g⁻¹) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = *; C = ns; TXC = *; (b) T = *; C = *; TXC = *; (c) T = *; C = *; TXC = *; (d) T = *; C = *; TXC = *.

compared to the NaCl control. For the POD enzyme, the increases were 36% in 0.5f and 31% in 1f compared to the NaCl control. As for CAT, the increases were 82% in 0.5d, 57% in 1d, 131% in 1f, 200% in 0.5fd, and 142% in 1fd. These enzymes have the function of eliminating excess ROS, which causes oxidative damage in plants under stress conditions (Gill; Tuteja, 2010; Hasanuzzaman et al., 2020; Ali et al., 2022). As seen in melon, Palma et al. (2022) also found increases in the enzymes SOD, POD, and CAT with applications of the *A. quadricellulare* to salt-stressed soybean plants.

The malondialdehyde (MDA) content produced by lipid peroxidation is an indicator of membrane damage under ROS-induced oxidative stress (Farmer; Mueller, 2013; Rady et al., 2018). Thus, it is possible to note one of the effects of oxidative stress under salt-stressed melon plants by the increased MDA contents (Fig. 5d) compared with plants without this stress. Furthermore, in stressed plants that received AQ applications, there was a decrease in MDA levels showing that the microalga acted to mitigate the oxidative effects in both melon cultivars. In Dalí the drop was 27% at 0.5fd and 1fd compared to the NaCl control, in Goldex all treatments that received AQ applications showed drops of up to 22%.

The antioxidant effects of the microalga application were evidenced by the decrease in MDA levels due to the increase in antioxidant enzymes SOD, POD, and CAT in Dalí, in addition to the levels of amino acids such as proline, and phenolic compounds, and sugars in both cultivars. This effect of AQ in plants is new and can be related to its high content of free amino acids present in its biomass, such as glutamic acid (4.27%) and glycine-betaine (1.57%), besides polyamides (Mógor et al., 2022).

3.2 ANATOMICAL ANALYSES

Microalga application had no influence on the leaf thickness of melon (Fig. 6). Plants of the control treatment presented the abaxial face of the epidermis with 14% greater thickness (Fig. 6e) compared to plants exposed to salinity. Atabayeva et al. (2013) also found results of reduced abaxial epidermis thickness in barley leaves that were subjected to saline conditions.

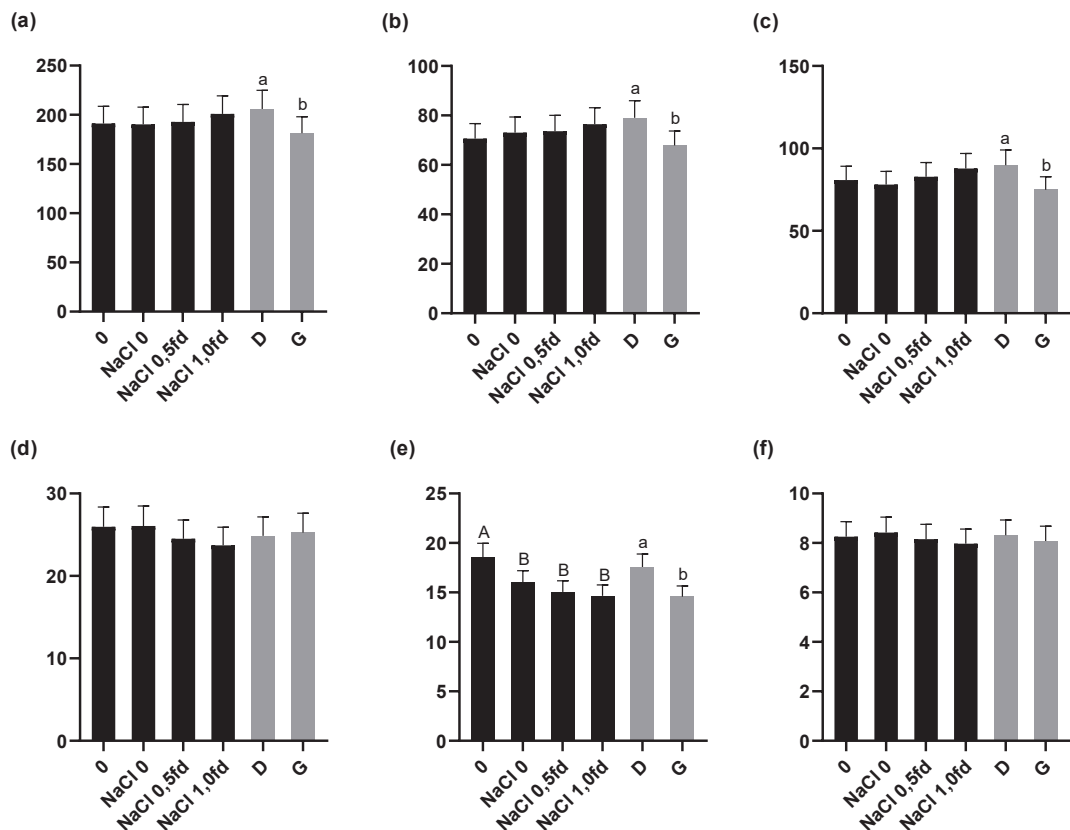


Figure 6 (a) Leaf thickness, (b) palisade parenchyma, (c) lacunar parenchyma, (d) adaxial epidermis thickness (e) abaxial epidermis thickness and (f) cuticle (μm) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (0) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = ns; C = *; TXC = ns*; (b) T = ns; C = *; TXC = ns; (c) T = ns; C = *; TXC = ns; (d) T = ns; C = ns; TXC = ns; (e) T = *; C = *; TXC = ns; (f) T = ns; C = ns; TXC = ns.

3.3 BIOMETRIC ANALYSES

Unstressed plants showed an increase in leaf, stem, and root biometric variables with AQ applications. In leaf fresh mass (Fig. 7a) in the cultivar Dalí with AQ applications, there was an increase of 18% at 0.5f and 14% at 1fd, in Goldex the increases were 28% at 0.5fd and % at 1fd. Furthermore, leaf dry mass (Fig. 7b) showed increases of 24% at 1fd. In the leaf area (Fig. 7c) increases were 19% at 1f, 28% at 0.5fd, and 21% at 1fd when compared to the control without NaCl. These results of biometric improvements are similar to those obtained in other crops like potatoes (Cordeiro et al., 2022a), onions (Cordeiro et al., 2022b), and tomatoes (Lara et al., 2022) with *A. quadricellulare* applications. This can be attributed to molecules present in microalgae with potential bioactive properties that act to increase cell division and expansion in plants (El-Naggar et al., 2020).

The fresh mass of the stem (Fig. 8a) also showed increases with AQ in plants without salinity in the cultivar Dalí of 17% in 0.5f. In Goldex the increases were 16% in 0.5f, 25% in 0.5fd, and 22% in 1fd when compared to the control without NaCl.

For the dry mass of the stem (Fig. 8b) in Dalí there were increases of 13% in 0.5f in plants without salinity, in Goldex there were increases of 32% in 1d, 16% in 0.5f, 20% in 1f, 42% in 0.5fd and 34% in 1fd when compared to the control without NaCl. Stem diameter (Fig. 8c) was also favored by AQ, in Dalí plants without salinity there was a 13% increase in 0.5f and 22% in 1fd. In Goldex there was an increase in all treatments that received AQ application when compared to the control without NaCl. There was an increase in stem length (Fig. 8d) in all Dalí treatments with AQ, when compared to the control without NaCl. Moreover, in Goldex, there were increases of 17% in 0.5f, 28% in 1f, 27% in 0.5fd, and 16% in 1fd.

The applications of AQ, in plants without salinity, promoted an increase in root diameter (Fig. 9a). In Dalí the increases were 17% in 1d, 23% in 0.5f, and 15% in 1fd. In Goldex there was an increase of 28% in 0.5d, 10% in 1d, 15% in 0.5fd, and 26% in 1fd when compared to the control without NaCl. In addition, root length (Fig. 9b) showed increases with the use of AQ with increases of 21% at 1f when compared to the control without NaCl.

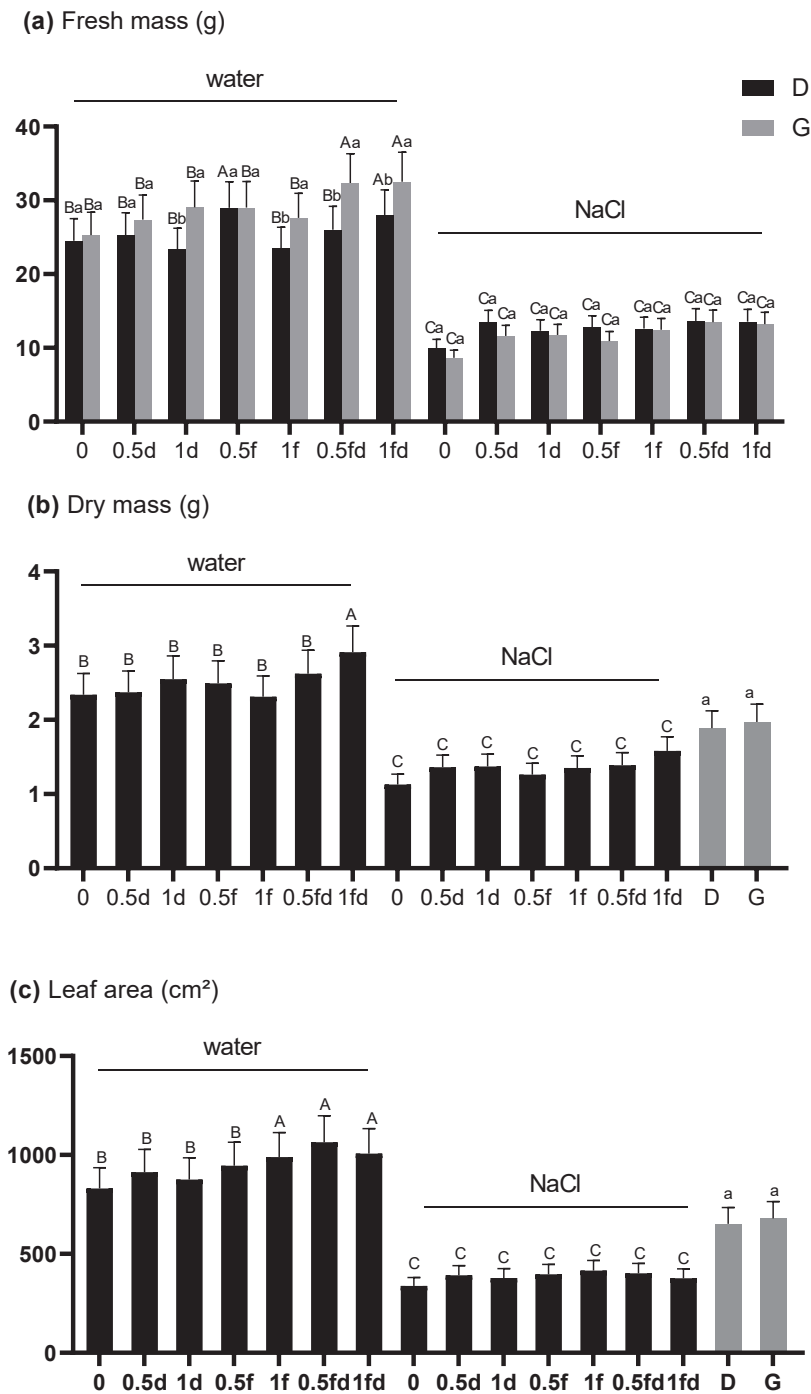
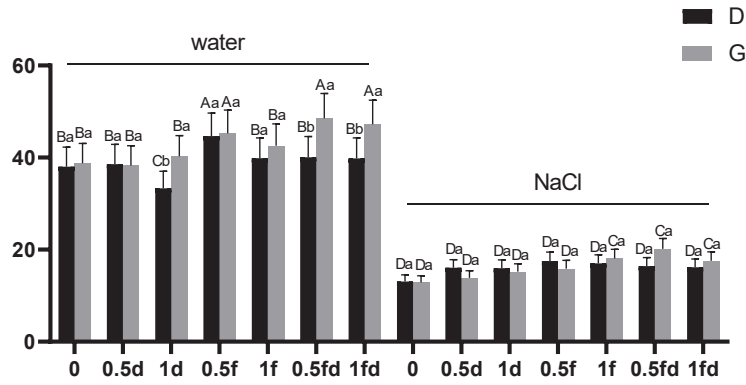


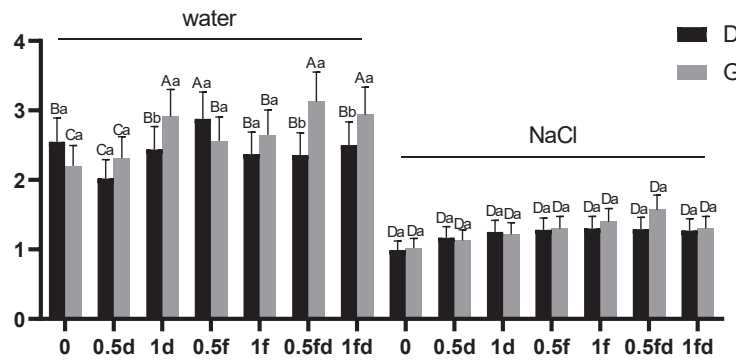
Figure 7 (a) Fresh mass (g), (b) dry mass (g) and (c) leaf area (cm²) of melon leaves of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = *; C = **; TxC = *; (b) T = *; C = ns; TxC = ns; (c) T = *; C = ns; TxC = ns.

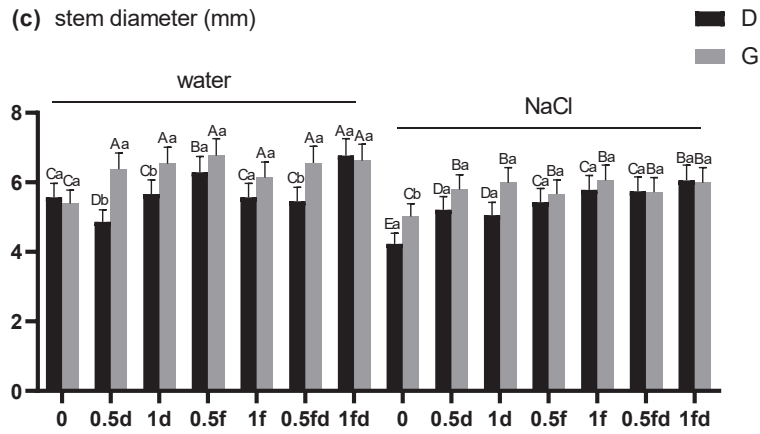
(a) Stem fresh mass (g)



(b) stem dry mass (g)



(c) stem diameter (mm)



(d) stem length (cm)

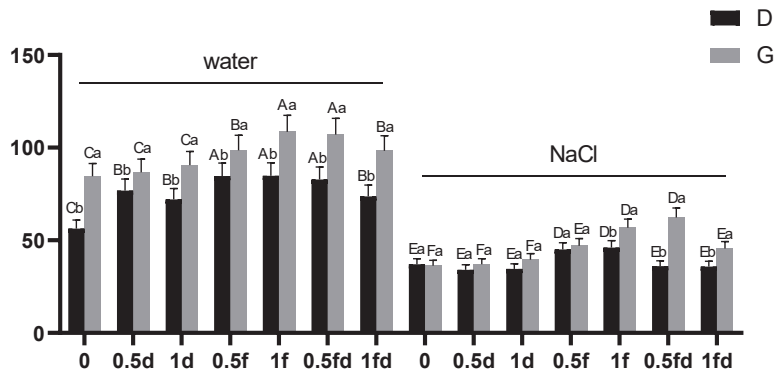


Figure 8 **(a)** Stem fresh mass (g), **(b)** stem dry mass (g), **(c)** stem diameter (mm) and **(d)** stem length (cm) of melon plants of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = *; C = *; TXC = **; (b) T = *; C = *; TXC = *; (c) T = *; C = *; TXC = *; (d) T = *; C = *; TXC = *.

In contrast, high salinity inhibits plant growth and development mainly due to osmotic stress and ion toxicity (Chen et al., 2021). Thus, as seen in the biometric data of leaves, stem, and roots, salt stress impaired biomass production and growth of melon plants. Similar negative impacts of salinity have been reported in melon and other crops (Jiang et al., 2017; Silva et al., 2020; Ali et al., 2021).

Under the analyzed conditions, it is added that there was a beneficial effect of the use of AQ on plants under saline conditions. In stem dry mass for Goldex, there were increases of 40% at 1f, 57% at 0.5fd, and 37% at 1fd compared to the NaCl control. In the diameter of the stem in Dalí there were increases in all treatments with AQ, with the largest diameter in 1df, with a 43% increase compared to the NaCl control. In Goldex there was an increase in all treatments with the use of AQ. The stem length of Dalí increased by 21% in 0.5f and 24% in 1f. In Goldex the increases were 30% in 0.5f, 57% in 1f, 72% in 0.5fd, and 26% in 1fd compared to the NaCl control.

Roots were also favored with AQ under saline cultivation conditions. For root length (Fig. 9b), the increases were 17% at 1d, 12% at 0.5f, 11% at 1f, 26% at 0.5fd, and 22% at 1fd compared to the NaCl control. Root volume (Fig. 9c) increased 108% at 1fd compared to the NaCl control. Roots are the main mechanism that plants use to absorb water and nutrients, thus their increase can positively affect salt tolerance by improving water and nutrient uptake for the plant (Chourasia et al., 2022; Gopalakrishnan et al., 2022).

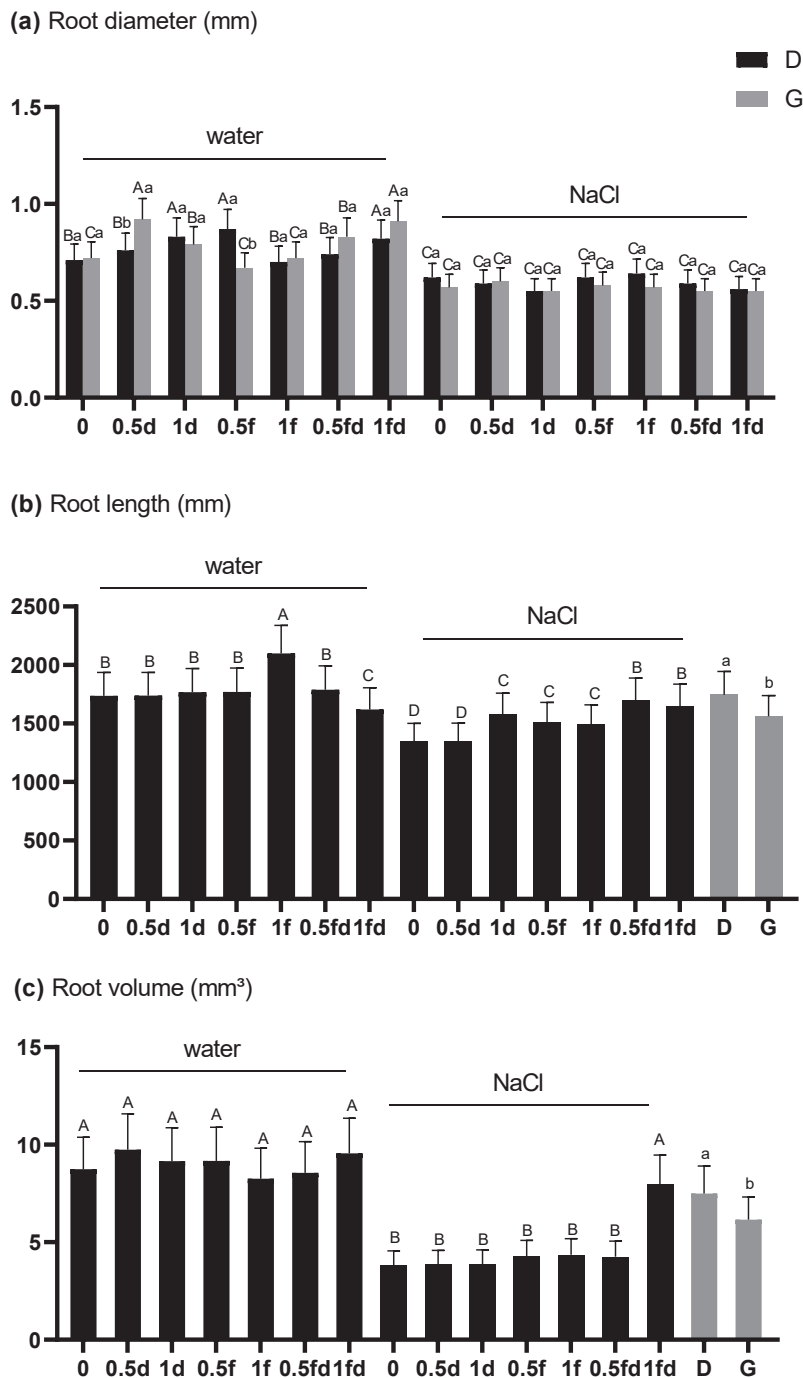


Figure 9 (a) Root diameter (mm), (b) root length (mm) and (c) root volume (mm³) of melon plants of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. (a) T = *; C = **; TXC = **; (b) T = *; C = *; TXC = ns; (c) T = *; C = **; TXC = ns.

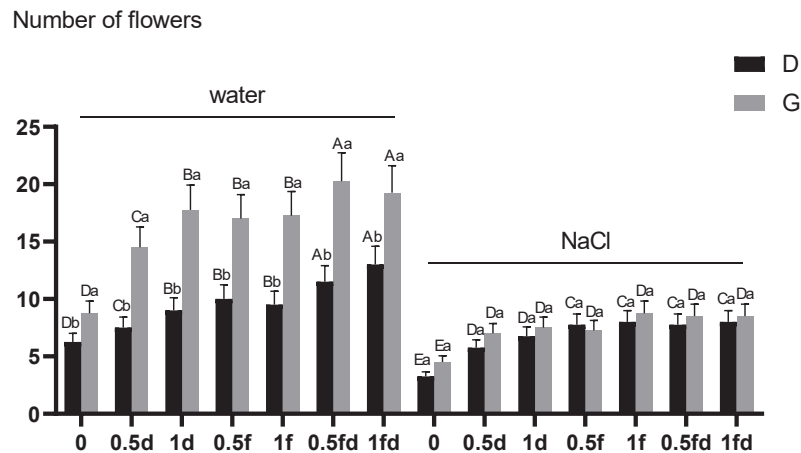


Figure 10 Number of flowers of melon plants of the cultivars (C) Dalí (D) e Goldex (G) without salinity (water) and with salinity (NaCl) and submitted to the application of the microalga *A. quadricellulare* (AQ).

Treatments (T): (0) control, no application of AQ, (0.5d) drip application of 0.5 ml L⁻¹ AQ, (1.0d) drip application of 1.0 ml L⁻¹ AQ, (0.5f) foliar application of 0.5 ml L⁻¹ AQ, (1.0f) foliar application of 1.0 ml L⁻¹ AQ, (0.5df) drip application of 0.5 ml L⁻¹ AQ + foliar application of 0.5 ml L⁻¹ AQ, (1.0df) drip application of 1.0 ml L⁻¹ AQ + foliar application of 1.0 ml L⁻¹ AQ. Means followed by the same letter do not differ by Scott-Knott's test ($p < 0.05$). Capital letters compare treatments. Lowercase letters compare cultivars. ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. T = *; C = *; TXC = *.

The number of flowers (Fig. 10) was also negatively affected by salinity. However, all treatments of both cultivars that received AQ applications showed increases in flower numbers. The increases were up to 113% in Dalí and up to 88% in Goldex in flower numbers suggesting that these plants experienced reduced stress compared to the NaCl control. The increase in flower production can also be seen as a potential improvement in the productive capacity of the plants.

4. CONCLUSION

The microalga *Asterarcys quadricellulare* (CCAP 294/1) efficiency to mitigate salt stress in melon cultivars was determined. The concentration of 1.0 ml L⁻¹ by foliar spray associated with drip irrigation proved to be more effective, especially by reducing lipid peroxidation. AQ acted by stimulating the antioxidant system and the accumulation of osmolytes in plant tissues. In view of the effects of AQ use, further work should be done to study under field conditions and also gene expression due to the difference in response form of the two melon cultivars, in addition to the signaling action of AQ, especially in transcriptomics.

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CHAPTER 2 – MICROALGAE BIOMASS IMPROVES MELON CROP YIELD IN SALINE CONDITIONS

Abstract

Biofertilizers are described as capable of promoting improvements in plant development in abiotic stress conditions. The use of microalgae biomass as biofertilizers has been the subject of recent studies. Therefore, the objective of the work is to evaluate the effect of the biomass of the microalgae *Asterarcys quadricellulare* on improving the growth and yield of melon crops under saline stress conditions. Dalí and Goldex cultivar melon plants were grown in slabs and fertigated with nutrient solution and addition of sodium chloride in water to achieve electrical conductivity of 8 dS m⁻¹ for salinized treatments. Weekly applications of *A. quadricellulare* were carried out at a concentration of 1.0 ml L⁻¹, sprays via and a combination of sprays and drip irrigation. Biochemical and biometric assessments were performed. Plants treated with microalgae showed an increase in chlorophyll, carotenoids and sugar levels. There was also an increase in the dry mass of the productive branches, number of fruits, diameter and length of fruits and weight, resulting in an increase in productivity. The results demonstrate that *A. quadricellulare* stimulated metabolic changes and increased productivity in melon plants grown, with emphasis on the application of microalgae biomass in the combination of sprays and drip irrigation.

Keywords: abiotic stresses, *Asterarcys quadricellulare*, *Cucumis melo* L., sustainability.

1. INTRODUCTION

Melon (*Cucumis melo* L.) is one of the main vegetables produced in the world, ranking ninth in the world in vegetable production, and being widely cultivated in arid and semi-arid regions (SILVA et al., 2020) that have soils generally affected by salinity (KAASHYAP et al., 2018).

Saline stress causes considerable losses in agricultural production worldwide, as it severely impacts plants (JHA et al., 2019). Salinity generates osmotic stress and

ionic stress in plants (SINGH and THAKUR, 2018), as well as an increase in reactive oxygen species (ROS) that can damage cell membranes through the oxidation of lipids, proteins and nucleic acids (FOYER and NOCTOR, 2000). In melon, salt stress reduces fruit yield and quality. However, fruit quality can be improved when the applied irrigation brackish water (7 dS m^{-1}) (BUSTAN et al., 2005).

Within this context, it is necessary to search for alternatives that enable melon production in saline conditions, especially for the use of biostimulants, which can improve plant tolerance to abiotic stresses (DEL BUONO et al., 2023).

Microalgae are being evaluated as new sources of plant biostimulants, as they produce bioactive substances such as phytohormones, vitamins and amino acids (ALVAREZ et al., 2021). The microalgae *Asterarcys quadricellulare*, which had been already described as a potential biofertilizer, is characterized by the high content of amino acids and proteins in its biomass (CORDEIRO et al., 2022a). The bioactivity of spraying microalgae biomass to plants has been attributed to changes in polyamine metabolism (MÓGOR et al., 2023).

Applications of this microalgae in bean plants under conditions of water stress have been described as having the potential to reduce damage to plants by increasing antioxidant enzymes and biochemical changes such as an increase in the content of carotenoids, proteins and amino acids (MARQUES et al., 2023). When applied to seed soybean under saline stress, it was effective in reducing damage to the plant growth with accumulation of osmolytes in plant tissues, such as chlorophyll pigments and sugars (PALMA et al., 2022).

Therefore, given the relevance of the melon production chain, the objective of this work was to evaluate the effect of foliar applications and the combination of foliar and drip routes of the microalgae *Asterarcys quadricellulare* (CCAP 294) /1) in improving the development and productivity of melon crops, grown under saline stress conditions in the field, based on biochemical and biometric variables.

2. MATERIAL AND METHODS

The biomass of the microalgae *Asterarcys quadricellulare* (CCAP 294/1) (AQ) was provided by the Alltech® Crop Sciences - Brazil, obtained from a mixotrophic culture and atomized using the spray drying method, producing a fine greenish powder. The sum of amino acids present in the biomass of *Asterarcys quadricellulare* is 9% (Aspartic acid = 3.32; Glutamic acid = 4.27; Serine = 1.66; Glycine = 1.54;

Histidine = 0.71; Arginine = 2.17; Threonine = 1.45; Alanine = 2.41; Proline = 1.6; Tyrosine = 0.95; Valine = 1.81; Methionine = 0.51; Cysteine = 0.29; Isoleucine = 1.41; Leucine = 2.36; Phenylalanine = 1.37; Lysine = 2.11 and Tryptophan = 0.37) and 37.94% for crude protein (CORDEIRO et al., 2022a).

2.1 FIELD EXPERIMENTS

The experiment was conducted at the Biofertilizer Laboratory of the Federal University of Paraná (UFPR), under the coordinates of latitude 25°25' S, longitude 49°08', and altitude 930 m. The climate according to Köppen's classification is temperate, humid mesothermal (Cfb), with annual precipitation between 1,400 and 1,800 mm, and well-distributed rainfall.

The cultivars used were hybrid yellow melons, Goldex (F1) from the company Agristar, and Dalí from the company Sakata. Both cultivars are adapted to the leading producing regions of Brazil and the fruits are intended for export. The Goldex cultivar has fruits with excellent flavor, excellent post-harvest characteristics, and a high Brix degree (AGRISTAR). The Dalí cultivar has an early cycle, fruits of ideal size and shape for packaging in boxes used for export, in addition to excellent flavor and post-harvest characteristics (SAKATA).

The cultivars were sown in polyethylene trays with 120 cells filled with composted pine bark substrate (Tropstrato®), in protected cultivation. Transplanting was carried out when the first true leaves appeared, 21 days after sowing. The experiment was conducted outdoors, in an area with soil covered with raffia (Allcrop®), with the melon seedlings placed in cultivation bags (slabs) measuring 50 cm x 1.20 cm, filled with substrate whose chemical analysis indicated (CaCl_2) = 6.43, pH SMP = 6.83, $\text{Al}^{+3} = 0$; $\text{H}^+ + \text{Al}^{+3} = 2.68 \text{ cmol dm}^{-3}$, $\text{Ca}^{2+} = 11.48 \text{ cmol dm}^{-3}$, $\text{Mg}^{2+} = 4.15 \text{ cmol dm}^{-3}$, $\text{K}^+ = 0.35 \text{ cmol dm}^{-3}$, P Mehlich = 22.04 mg dm^{-3} , V% = 85.7%, CEC = 18.66 cmol dm^{-3} . Throughout cultivation, the plants were fertigated with a nutrient solution, respecting the physiological stage of the culture as shown in Table 1 (TRANI et al., 2011).

Irrigation was carried out in a localized manner using drip tapes where drips were spaced 50 cm apart, and placed inside the slabs at the top. Irrigation was carried out every day of cultivation with the time calculated by determining the water retention capacity of the substrate according to the formula by Craven et al. (2010), with humidity maintained at 80% of the substrate's water retention capacity.

Table 1 Amount of fertilizers applied to melon plants grown in slabs through drip fertigation

Mellon growth phases (days after planting)	Amount of the fertilizer applied per day (kg ha ⁻¹)						
					Solution		Solution
	Uréia	K ₂ SO ₄	KNO ₃	MAP	EC (dS m ⁻¹)	Ca(NO ₃) ₂	EC (dS m ⁻¹)
1 to 5	-	-	-	-	-	1,25	0,93
6 to 10	5,00	-	-	-	0,22	2,08	1,3
11 to 20	7,08	5,42	-	4,58	5,4	4,58	3,01
21 to 27	5,83	4,58	6,67	1,25	6,92	11,25	6,19
28 to 37	4,58	6,67	4,58	9,17	8,92	7,50	4,52
38 to 48	1,25	-	-	-	0,22	2,08	1,3
49 to 62	-	13,33	-	-	8,71	0,83	0,63
63 to 75	-	15,83	-	-	9,91	2,08	1,3

Adapted from TRANI et al. (2011).

2.2 TREATMENTS

Treatments started after transplanting. It was used saline water with modification of electrical conductivity (EC) by adding sodium chloride (NaCl) and measured with an AKSO ® portable pH meter until the EC was 8 dS m⁻¹. The biomass of the microalgae *Asterarcys quadricellulare* was diluted in a suspension containing 0.25 g.L⁻¹ concentration, from which an aliquot of 1 mL L⁻¹ was taken, mixed in water and applied at intervals of seven days until the end of cultivation via fertigation and 1 mL L⁻¹ per foliar application using a Kawashima® electric portable sprayer at constant pressure (40 psi) with a rate of 400 L ha⁻¹. The plants were sorted into treatments according to Tab.2.

Table 2. Treatments applied to melon plants with their abbreviations

Control	Nutrient solution EC
AQ.df	Nutrient solution EC + AQ foliar and fertigation application
NaCl	Nutrient solution EC + 8 dS m ⁻¹ NaCl
NaCl+AQ.df	Nutrient solution EC + 8 dS m ⁻¹ NaCl + AQ foliar and fertigation application
NaCl+AQ.f	Nutrient solution EC + 8 dS m ⁻¹ NaCl + AQ foliar application

2.3 BIOCHEMICAL ANALYSIS

At the beginning of fruiting, leaves were collected from the median part of the main stem. The materials were frozen immediately after collection and subsequently macerated in liquid nitrogen until a fine powder was obtained. Chlorophyll and carotenoids were extracted with 80% acetone in distilled water and the addition of 0.1% CaCO₃ (w/v) and readings were taken on a UV-visible spectrophotometer at 663, 647, and 470 nm (LICHTENTHALER and BUSCHMANN 2001), and the values expressed in micrograms of total chlorophyll (Chl), chlorophyll a (Chl a) and chlorophyll b (Chl b) per gram of fresh plant material used.

Total sugars were quantified using a standard curve obtained with glucose at 1 mg mL⁻¹ (5.5 mM), with values ranging from 50 and 800 µg mL⁻¹. Readings were taken at 540 nm (MALDONADE et al. 2013) and values were expressed in micrograms of total sugars, reducing sugar, and non-reducing sugars per gram of fresh plant material.

2.4 RELATIVE INDEX OF CHLOROPHYLL, TOTAL SOLUBLE SOLIDS CONTENT (°BRIX) OF FRUITS, YIELD AND BIOMETRIC VARIABLES

At the beginning of fruiting, the relative chlorophyll content was quantified using the portable chlorophyll meter (N-Tester®) which presents average values from thirty random readings taken on leaves from the middle third of the plant (MÓGOR et al. 2013).

The fruits were harvested when they were detached from the plant at the abscission point. In addition to counting (NF = number of fruits), the fruits were also weighed (FW) on a digital scale. Production characteristics such as length (FL) and fruit diameter (FD), obtained by measurement using a digital caliper, were also evaluated.

Total soluble solids (TSS) were quantified by taking samples of the pulp and using a refractometer at room temperature and expressed in °Brix.

The dry mass of the productive branch (PBDM) was carried out after harvesting all the fruits. The samples were dried in a forced circulation oven, at a temperature of approximately 65°C until a constant weight was obtained on a precision scale.

2.5 EXPERIMENTAL DESIGN

The experimental design was completely randomized with four replications ($n = 4$) in a factorial scheme. Each repetition consisted of a slab containing three plants each. The data obtained were tested using the Bartlett test and ANOVA and the Tukey test was applied ($p < 0.05$), and processed through the statistical program SISVAR (FERREIRA, 2019).

3 RESULTS

Interactions were observed between cultivars and treatments in chlorophyll levels (Fig. 1). In the Dalí cultivar, the AQ.df treatment was 25% higher in *Chl b* levels compared to the control. In Goldex, the AQ.df treatment promoted an increase of 27% in *Chl b* compared to the control, and in NaCl+AQ.df, the increases were 300% compared to NaCl. Applications of AQ also promoted an increase of 42% in *Chl T* in the NaCl+AQ.df treatment for Goldex compared to NaCl.

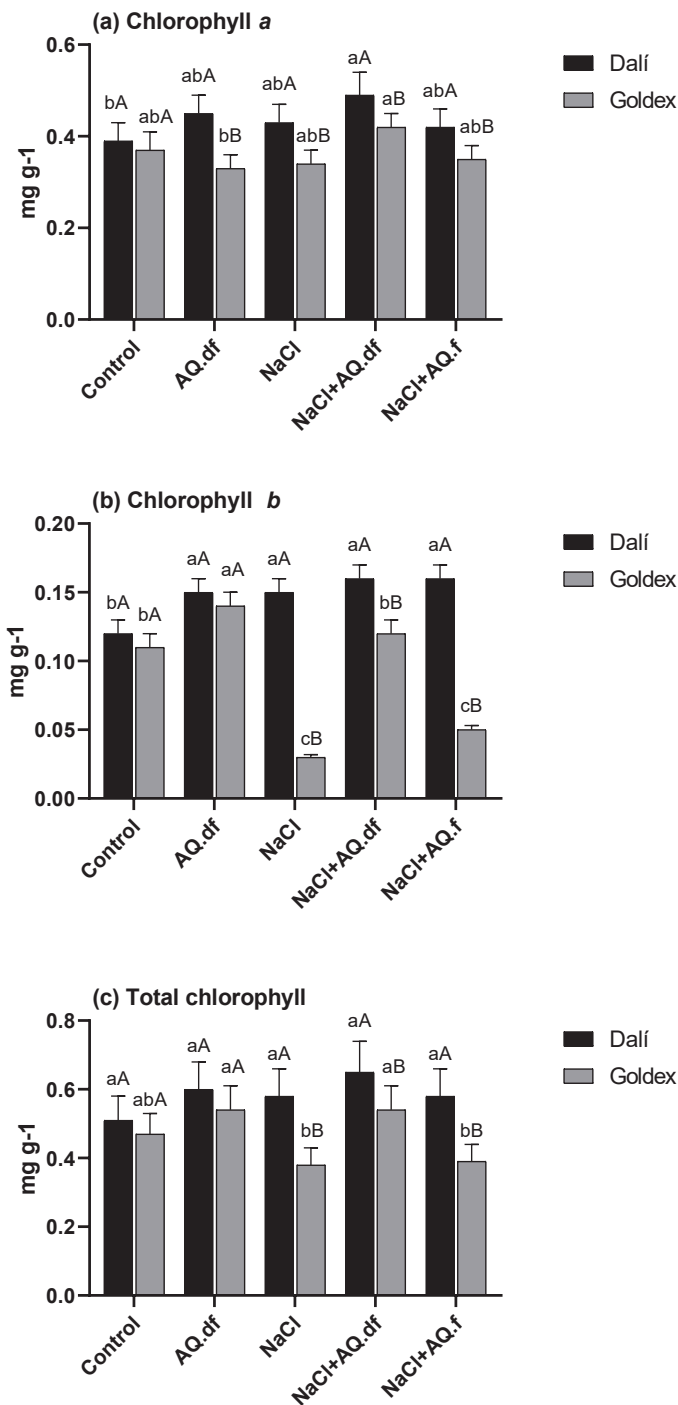


Figure 1. Chlorophyll a, (b) chlorophyll b and (c) total chlorophyll from cultivars 'Dalí' and 'Goldex' melon leaves with application of the biomass of the microalgae *A. quadricellulare* (AQ) in cultivation conditions with saline and non-saline water. Means followed by the same letter do not differ from each other using the test of Tukey. Lowercase letters compare treatments (T) - **Control** - nutrient solution EC; **AQ.df** - nutrient solution EC + AQ fertigation and foliar application; **NaCl** - nutrient solution EC + 8 dS m⁻¹ NaCl; **NaCl+AQ.df** - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ fertigation foliar application; **NaCl+AQ.f** - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar application. Capital letters compare cultivars (C) ANOVA: ns = not significant, * = significant at p≤0.01. Chl a: T = *, C = *, TXC = *. Chl b: T = *, C = *, TXC = *. Chl T: T = *, C = *, TXC = *

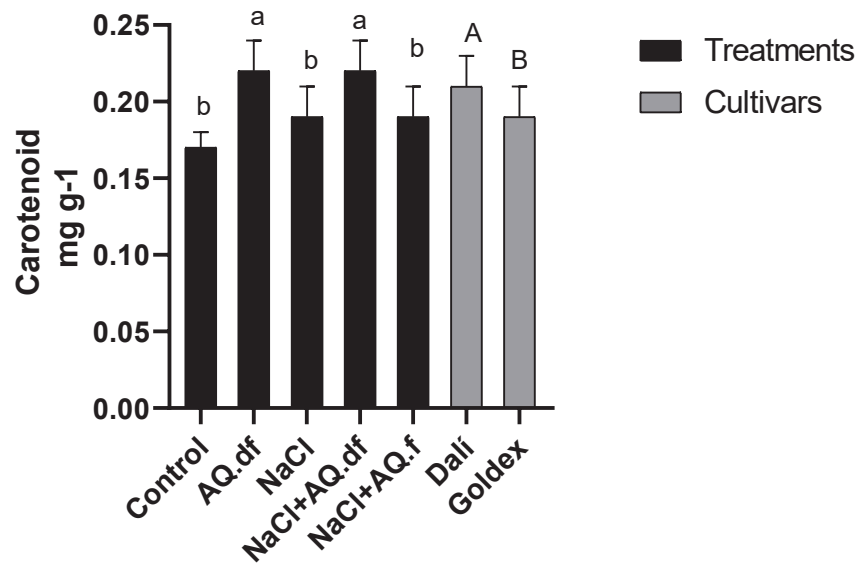


Figure 2 Carotenoid content in 'Dali' and 'Goldex' cultivars melon leaves with the application of *A. quadricellulare* (AQ) microalgae biomass in cultivation solution using saline and non-saline water. Means followed by the same letter do not differ from each other using the test of Tukey. Lowercase letters compare treatments (T) - Control - nutrient solution EC; AQ.df - nutrient solution EC + AQ fertigation and foliar application; NaCl - nutrient solution EC + 8 dS m⁻¹ NaCl; NaCl+AQ.df - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ fertigation foliar application; NaCl+AQ.f - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar application. Capital letters compare cultivars (C) ANOVA: ns = not significant, * = significant at p≤0.01. ** = significant at p≤0.05. T = *; C = *; TXC = ns.

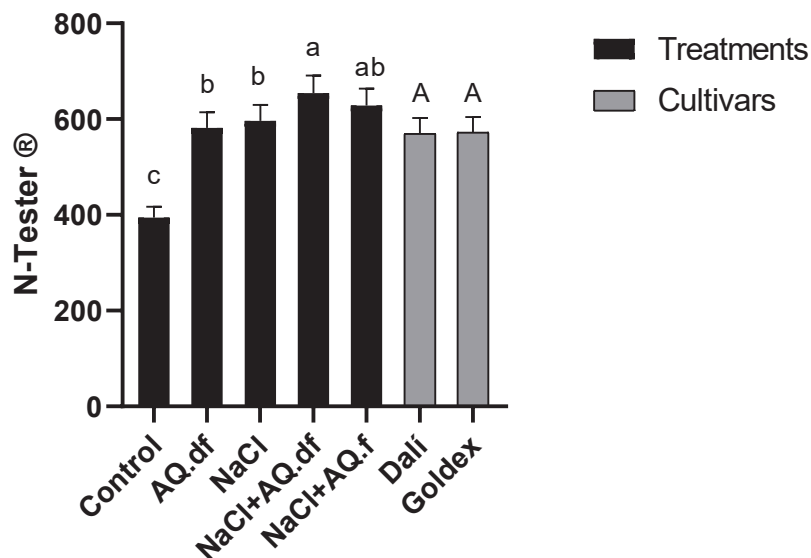


Figure 3 Relative chlorophyll index (N-Tester®) of melon cultivars 'Dali' and 'Goldex' leaves with the application of *A. quadricellulare* (AQ) microalgae biomass in cultivation conditions with saline and non-saline water. Means followed by the same letter do not differ from each other using the test of Tukey. Lowercase letters compare treatments (T) - Control - nutrient solution EC; AQ.df - nutrient solution EC

+ AQ fertigation and foliar application; NaCl - nutrient solution EC + 8 dS m⁻¹ NaCl; NaCl+AQ.df - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ fertigation and foliar application; NaCl+AQ.f - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar application. Capital letters compare cultivars (C). ANOVA: ns = not significant, * = significant at p≤0.01. T = *; C = ns; TXC = ns.

No interactions were found between treatments and cultivars in the levels of the carotenoid (Fig. 2). The AQ.df treatment promoted a 29% increase in carotenoid levels compared to the control; in NaCl+AQ.df, the increase was 16% compared to the NaCl treatment, with a higher value in Dalí compared among the cultivars.

For N-Tester, there were also no interactions between treatments and cultivars (Fig. 3). The increase was 47% in AQ.df compared to the control and 10% NaCl+AQ.df compared to the NaCl treatment.

Interactions were found between cultivars and treatments in terms of total sugars (Fig. 4a), with no interactions in terms of reducing sugars (Fig. 4b) and with interactions in non-reducing sugars (Fig. 4c). In the Dalí cultivar, the AQ.df treatment promoted an increase of 62% in total sugar content compared to the control; in NaCl+AQ.df, the increase was 14% compared to the NaCl treatment. In Goldex, AQ.df, the increase was 76% in total sugar content compared to the control, and in NaCl+AQ.df, the increase was 46% compared to NaCl besides the increase in total sugar content in NaCl compared to the control, in both cultivars. Also, AQ.df promoted an increase of 147% in reducing sugar levels compared to the control. In NaCl+AQdf, the increase was 38% compared to the NaCl treatment. In the Dalí cultivar, the NaCl+AQ.df treatment showed increases of 18% in non-reducing sugar levels compared to NaCl. In Goldex, in the AQ.df treatment, the increase was 34% in non-reducing sugars compared to the control, and in NaCl+AQ.df, this increase was 64% compared to NaCl.

No interactions were observed between cultivars and treatments for productive characteristics (Table 3). The AQ.df treatment promoted an increase of 54% in the dry mass of the productive branches. In NaCl+AQ.df and NaCl+AQ.f, the increase was 34% and 15%, respectively, compared to the NaCl treatment. The Goldex cultivar presented the highest dry mass value of the productive branches among the cultivars.

The comparison among treatments showed that AQ had an average increase of four fruits in AQ.df in comparison to control and 1.5 fruits in NaCl+AQ.df in

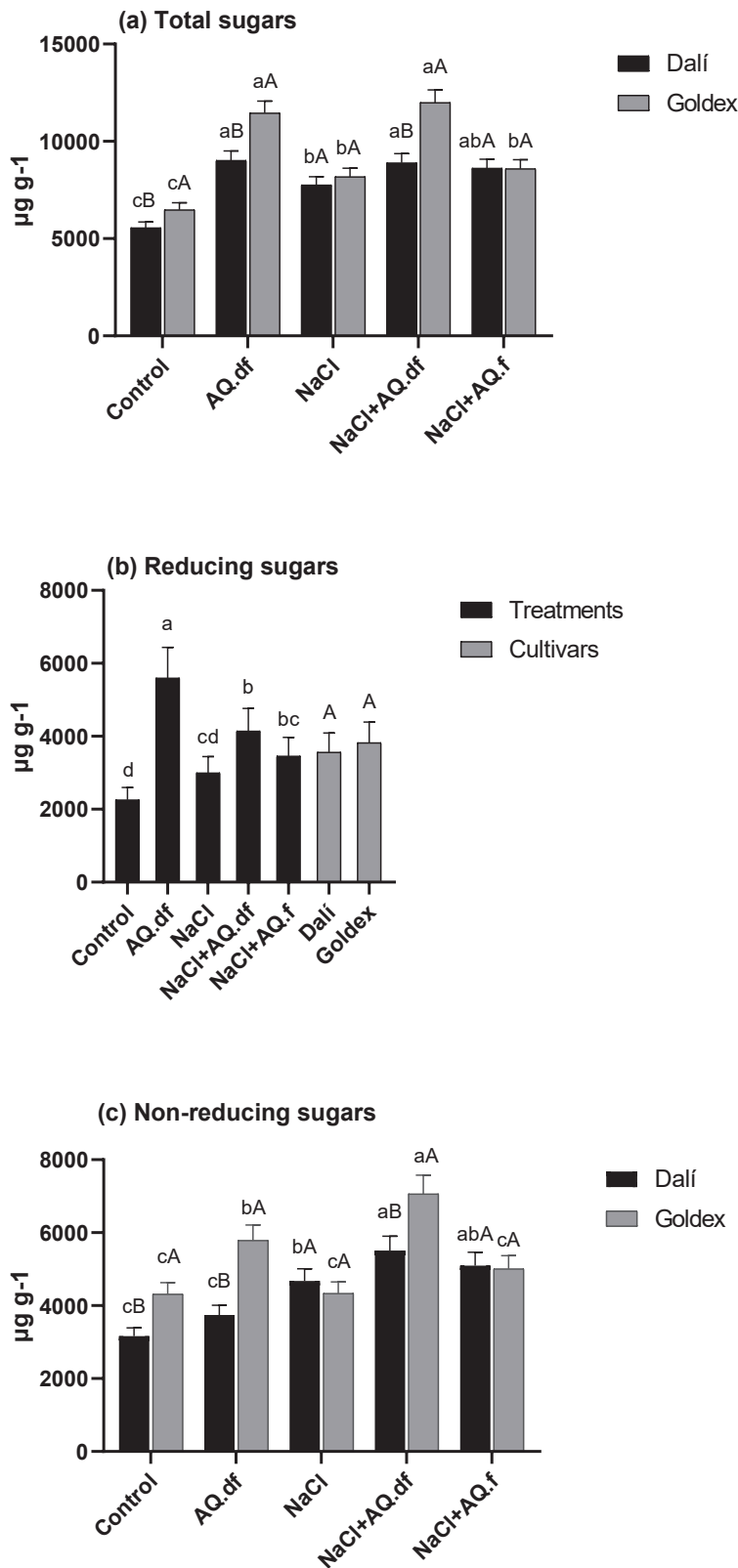


Figure 4(a) Total sugars, (b) reducing sugars and (c) non-reducing sugars of 'Dalí' and 'Goldex' melon cultivars leaves with the application of *A. quadricellulare* (AQ) microalgae biomass in cultivation conditions with saline and non-saline water. Means followed by the same letter are not different from each other using the test of Tukey. Lower case letters compare treatments (T) - **Control** - nutrient solution EC; **AQ.df** - nutrient solution EC + AQ fertigation and foliar application; **NaCl** - nutrient solution EC + 8 dS m⁻¹ NaCl; **NaCl+AQ.df** - nutrient solution EC + 8 dS m⁻¹ de NaCl + AQ fertigation and foliar application; **NaCl+AQ.f** - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar application. Upper case letters compare cultivars (C) ANOVA: ns = non-significant, * = significant at p≤0.01. ** = significant at p≤0.05. Total sugars: T = *; C = *; TxC = *. Reducing sugars: T = *; C = *; TxC = *. Non-reducing sugars: T = *; C = *; TxC = *

comparison to NaCl treatment. Goldex cultivar showed the greatest number of fruits among the cultivars.

The AQ.25df treatment promoted an increase of 169% in fruit weight compared to the control; in NaCl+AQ.df and NaCl+AQ.f, the increase was 59% and 44%, respectively compared to NaCl. Applications of AQ also promoted increases in fruit lengths and diameters in saline conditions. The NaCl+AQ.df treatment promoted an increase of 18% in fruit length and 12% in diameter; in NaCl+AQ.f, the increase was 14% in fruit diameter compared to the NaCl treatment.

Tabela 3. Productive and quality characteristics in melon (*Cucumis melo*) with applications of *Asterarcys quadricellulare* (AQ) in growing conditions with saline and non-saline water

	PBDM (g)	NF (per plot)	FW (g/per plot)	FL (mm)	FW (mm)	TSS (° Brix)
Control	12,45 c	3,37 c	1452,85 d	112,17 ab	79,55 b	6,75 c
AQ.df	19,16 a	7,00 ab	3911,93 bc	121,31 ab	89,52 ab	9,50 ab
NaCl	13,83 bc	6,37 b	3178,99 c	107,91 b	81,81 b	8,00 b
NaCl+AQ.df	18,59 a	7,87 a	5079,72 a	126,88 a	92,39 a	10,62 a
NaCl+AQ.f	15,93 ab	7,37 ab	4572,64 ab	122,62 ab	93,74 a	9,87 a
Dalí	14,8 B	6,00 B	3518,34 A	120,19 A	86,64 A	8,3 B
Goldex	17,70 A	6,80 A	3760,12 A	116,47 A	88,17 A	9,6 A
CV%	14,69	11,59	14,03	9,32	7,94	11,81

PBDM, productive branch dry mass, NF, number of fruits; FW, fruit weight; FL, fruit length; FW, fruit width; TSS, fruit total soluble solids. Means followed by the same letter do not differ from each other using the test of Tukey. Lowercase letters compare treatments (T) - Control - nutrient solution EC; AQ.df - nutrient solution EC + fertigation and foliar AQ application; NaCl - nutrient solution EC + 8 dS m⁻¹ NaCl; NaCl+AQ.df - nutrient solution EC + 8 dS m⁻¹ NaCl + fertigation and foliar AQ application; NaCl+AQ.f - nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar application. Capital letters compare cultivars (C). ANOVA: ns = not significant, * = significant at p≤0.01, ** = significant at p≤0.05. PBDM: T = *; C = *; TxC = ns. NF: T = *; C = **; TxC = ns. FW: T = *; C = ns; TxC = ns. CF: T = *; C = ns; TxC = ns. DF: T = *; C = ns; TxC = ns. SST: T = *; C = *; TxC = ns.

An increase was observed in levels of TSS in NaCl compared to the control. In AQ.df, there was an increase of 40% in TSS compared to the control. In the NaCl+AQ.df and NaCl+AQ.f treatments, the increase was 33% and 23%,

respectively compared to the NaCl treatment. The Goldex cultivar showed the highest TSS value among the cultivars.

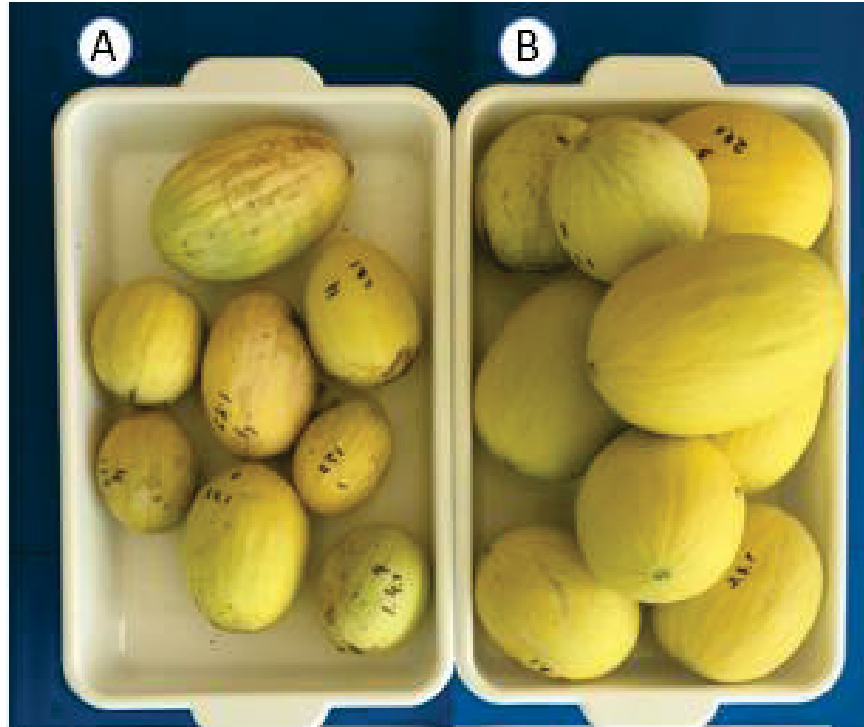


Figure 5. Melon fruits from the treatments (A) **NaCl** (nutrient solution EC + 8 dS m⁻¹ NaCl) and (B) **NaCl+AQ.df** (nutrient solution EC + 8 dS m⁻¹ NaCl + AQ foliar and fertigation application).

4 DISCUSSION

Salinity generates oxidative stress that affects the physiological processes of the plants such as photosynthesis, through the degradation of chlorophyll (AHANGER and AGARWAL, 2017), therefore explaining the reduction in the *Chl b* and *Chl T* index in the Goldex cultivar in saline cultivation conditions. The maintenance of chlorophyll levels in salt treatments with the drip and foliar application of AQ shows the effect of microalgae in protecting the photosynthetic apparatus of plants against oxidative damage caused by salt stress. This effect was also observed in bean plants subjected to water stress that received applications of *Asterarcys quadricellulare* (MARQUES et al., 2023).

In increase was also found in the levels of *Chl b* in plants that were not subjected to saline stress but that received AQ application. This may be attributed to

the composition of the microalgae with a predominance of amino acids, with L-glutamic acid in the highest concentration, in a total of 4.27%, this amino acid plays an important role in the synthesis and activation of chlorophyll (FORD and LEA, 2007; CORDEIRO et al., 2022a). The increases in chlorophyll with the application of AQ were also confirmed with increases in relative chlorophyll indices (N-Tester).

Carotenoid pigments in stress conditions are a type of antioxidant pigment of great importance in plant resistance due to their function in eliminating ROS and free radicals and maintaining redox balance (HAVAUX, 2013; HOU et al., 2016). Plants that received foliar and drip application of AQ showed increases in this pigment.

The accumulation of sugars also has antioxidant functions in stressed plants (ROSA et al., 2009). The AQ applications via foliar and drip also promoted an increase in the content of total, reducing, and non-reducing sugars. According to Mógor et al., (2022), in studies with sugar cane the free L-amino acid content of AQ (90.94 mg g⁻¹) may be a bioactive compound that triggers the metabolism of polyamines that improves remobilization from the source to the sink of non-reducing sugar. Similar results were found in soybean plants subjected to saline concentrations with increased non-reducing sugar levels with applications of the microalgae *Asterarcys quadricellulare* (PALMA et al., 2022).

The melon crop is reported to be moderately tolerant to salinity; however, this tolerance may be dependent on cultivars and the salinity level of the water applied (KUSVURAN et al., 2007; CORDÃO TERCEIRO NETO et al. 2013). The present work, the dose of salt used in irrigation (EC of 8 dS m⁻¹) did not cause the abortion of the flower, on the contrary, it increased the number of fruits compared to the control. Huang and collaborators (2012) also found no losses in the number of melon fruits in saline cultivation. Previous works have shown that between fruit development and harvest, the melon is salt tolerant while it is sensitive during the germination and seedling growth phases (NUKAYA et al., 1984; FRANCO et al., 1993).

Increases were found in the dry mass of the productive branches with the application of AQ, showing the effect of microalgae on plant growth. The promotion of growth after the application of microalgae is associated with greater nutrient absorption, and consequently an increase in photosynthetic products, greater biomass accumulation, and higher crop productivity (BARONE et al., 2018; KHOLSSI et al., 2019; GITAU et al., 2022). In line with our study, in addition to promoting growth, an increase was found in productivity in plants that received AQ application,

confirmed by a greater total fruit weight compared to the control and NaCl treatment that did not receive applications of microalgae. Productivity increases were also found in tomatoes and potatoes with foliar application of AQ (CORDEIRO et al., 2022a; LARA et al., 2022).

The number of fruits was also promoted with the application of AQ, as well as the length and diameter of the fruits in our work. The increase in length and diameter in fruits demonstrates the positive effect of microalgae on the size of these fruits, a result also found by Cordeiro et al. (2022b) in onion bulbs. This effect can be attributed to a greater redistribution of photoassimilates to the fruits, and to the molecules in microalgae with bioactive potential that act to increase cell division and expansion (EL-NAGGAR et al., 2020).

Overall, it is possible to associate the greater accumulation of mass in the productive branches of melon plants with the stimulation of the synthesis of pigments and sugars promoted by the foliar and drip application of AQ, producing a greater quantity of fruits, greater fruit mass and increment in sweetness in addition to better adaptation of plants subjected to saline concentrations.

5 CONCLUSION

The data indicated that the use of *A. quadricelulare* (CCAP 294/1) in foliar and drip application promoted the growth of melon plants, as well as increased productivity due to the increase in fruit numbers and yield, this effect may be in partly explained by the content of free amino acids present in the microalgae biomass.

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CHAPTER 3 - USE OF MICROALGA *Asterarcys quadricellularis* IN COMMON BEAN

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RESEARCH



Use of microalga *Asterarcys quadricellularis* in common bean

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Abstract

Common Bean (*Phaseolus vulgaris*) is considered one of the most affordable sources to tackle hunger in underdeveloped countries. However, its cultivation takes place in regions that suffer from water deficiency, which limits plant growth and crop yield. Therefore, it is necessary to seek sustainable alternatives that mitigate the deleterious effects of water shortage. The aim of this study was to evaluate the potential for mitigating the effects of water deficit with the application of the microalgae *Asterarcys quadricellularis* (CCAP 294/1) in two bean cultivars (IAC 1850 and BRS ESTEIO). The plants were grown in pots, with foliar application of microalgae at stage of third fully expanded trefoil at concentrations of 0.5 mL L⁻¹ and 1.0 mL L⁻¹. The water deficit lasted for 7 days, and, after that, the rehydration of the plants was performed. Evaluations were carried out in three periods: i. on the third day after the start of water restriction, ii. one day after rehydration, iii. 10 days after rehydration. The levels of chlorophyll, sugars, protein and antioxidant enzymes were increased in plants treated with microalgae. At ten days after rehydration, there was an increase in leaf area, fresh mass, and leaf thickness in the treated plants, characterizing the biostimulant effect of microalgae. Our results demonstrate the effects of *A. quadricellularis* (CCAP 294/1) in mitigating the deleterious effects of water deficit, accelerating the recovery of the two common bean cultivars, which

demonstrates its potential as a sustainable alternative to support food production under changing environment.

Keywords: Antioxidant activity, biostimulant, drought stress, *Phaseolus vulgaris*

1. INTRODUCTION

Climate change is one of the main concerns in the world today. These changes in the temperatures are caused by the increase in greenhouse gas emissions (Arora 2019). The rise in the temperatures can reduce the rates of rainfall, especially during summer, causing an increase in the dry periods (Staniak and Kocon 2015). Estimates indicate that different sources of abiotic stress (drought, salinity, extreme temperatures) can cause a reduction in crop yield of between 50 and 80% (Shinozaki et al. 2015), where drought is the most critical threat to food security in the world (Farooq et al. 2009).

Under water deficit conditions, plants suffer losses in growth and photosynthesis, accumulate reactive oxygen species (ROS) that inhibit protein synthesis, and oxidize photosynthetic pigments (Mahajan and Tuteja 2005; Farooq et al. 2012; Sharma et al. 2019). Thus, finding sustainable solutions that mitigate the effects of water deficit is a strategic theme for food security. The common bean (*Phaseolus vulgaris*) is one of the key legume sources for human diets in the world. It is the key source of protein in the diet in many developing countries (Broughton et al. 2003).

Within this context, biostimulant products that contain natural substances capable of acting on plant metabolism, increasing the tolerance of plants against different abiotic stresses stand out (Bulgari et al. 2015; Colla and Rouphael 2015; Van Ooesten et al. 2017; Ronga et al. 2019).

Microalgae include a wide range of evolutionarily diverse phototrophic and unicellular organisms (Chiaiese et al. 2018) with effects on plants related to the production of metabolites such as polysaccharides, amino acids, betaines, polyamines, vitamins, and hormones (Stirk et al. 2013; Mógor et al. 2017; Mógor et al. 2018; Renuka et al. 2018; Ronga et al. 2019).

The main species of microalgae described in the literature with biostimulant action are *Chlorella* sp., *Acutodesmus dimorphus*, *Scenedesmus* sp., *Spirulina* sp.,

and *Calothrix elenkinii* (Colla and Rouphael 2020). In addition, *Asterarcys quadricellularis*¹(¹The name of this alga is sometimes also spelled as *Asterarcys quadricellulare*, Guiry and Guiry (2023)) is a chlorophyta microalgae that have a high content of proteins and free amino acids, with promising results as a growth promoter in potato (Cordeiro et al. 2022a), onion (Cordeiro et al. 2022b), tomato (Lara et al. 2022) and sugarcane (Mógor et al. 2022). Among the metabolic effects of *A. quadricellulare* are the increment in the levels of chlorophyll, amino acids, and sugars in plants (Cordeiro et al. 2022a; Mógor et al. 2022). However, there are still no data in the literature on the effects of *A. quadricellulare* in mitigating water deficit deleterious effects.

Thus, the aim of this work was to investigate the effects of the use of *A. quadricellulare* biomass on *Phaseolus vulgaris* plants, aiming to answer the following questions: i) Does the application of microalgae induce greater tolerance of common beans plants to water restriction? ii) Does the application of microalgae help in the recovery of plants after rehydration? iii) Does the effect of the microalgae vary according to the dosage used (0.5 mL L⁻¹ and 1.0 mL L⁻¹)? iv) Does the effect of microalgae vary according to the common bean cultivars tested?

2. MATERIAL AND METHODS

The microalgae *Asterarcys quadricellularis* (CCAP 294/1) (AQ) biomass was provided by Alltech® Crop Sciences - Brazil, obtained from a mixotrophic system and atomized by spray drying method, producing a fine greenish powder. The microalga free amino acids content correspond to a concentration of 90.94 mg g⁻¹, which corresponds to 9% of the biomass, performed by colorimetric reaction (Magné and Larher 1992; Winters et al. 2002). The contents of the amino acid were determined using an SPC1000 amino acid analyzer adapted to the pre-column derivatization method with phenylisothiocyanate (PITC) and quantification by reverse-phase highperformance liquid chromatography (HPLC) using UV detection at 254 nm. The set consisted of a degasser, a quaternary pump module, a Rheodyne injection valve, an oven module, and a UV detection module, equipped with a LUNA C18 100 A 5µ column, 250 1 4.6 mm00G-4252-EQ. The amino acid score was calculated through the ratio between the values of essential amino acids in the samples (mg g⁻¹) and the standard values (FAO/WHO 1991).

The Percentage of free L-amino acids and proteins in the microalgae biomass are: Aspartic acid = 3.32; Glutamic acid = 4.27; Serine = 1.66; Glycine = 1.54; Histidine = 0.71; Arginine = 2.17; Threonine = 1.45; Alanine = 2.41; Proline = 1.6; Tyrosine = 0.95; Valine = 1.81; Methionine = 0.51; Cysteine = 0.29; Isoleucine = 1.41; Leucine = 2.36; Phenylalanine = 1.37; Lysine = 2.11 and Tryptophan = 0.37) and 37.94% for protein crude (Cordeiro et al. 2022a).

2.1 PLANT MATERIAL AND GROWTH CONDITIONS

The experiment was conducted at the Biofertilizers Laboratory of the Federal University of Paraná (UFPR), Brazil, under the geographic coordinates of latitude 25°25' S, longitude 49° 08' and altitude 930 m. The cultivation was carried out in a metal-arched greenhouse, coated with polyethylene, allowing the passage of light in it. The bean cultivars used were IAC 1850 (IAC) and BRS ESTEIO (BRS), with three plants in 3-L pots kept on benches containing substrate for plants based on pine bark, peat, expanded vermiculite, whose chemical analysis indicated: pH (CaCl₂) = 6.63, pH SMP = 7.03, Al³⁺ = 0; H⁺ +Al³⁺ = 2.31 cmol dm⁻³, Ca²⁺ = 12.34 cmol dm⁻³, Mg²⁺ = 3.52 cmol dm⁻³, K⁺ = 1.95 cmol dm⁻³, P = 193,81 mg dm⁻³, C = 55.21 g dm⁻³; V% = 88.5% and CEC = 20.12 cmol dm⁻³, Cu = 1.95 mg kg⁻¹, Mn = 31.80 mg kg⁻¹, Fe = 29.77 mg kg⁻¹, Zn = 2.30 mg kg⁻¹, B = 0.34 mg kg⁻¹, S = 141.12 mg kg⁻¹.

Also, 5 cm³ of expanded vermiculite was added to the upper part of the pots so that the foliar applications would not reach the roots, avoiding any interference with the absorption pathway. Irrigation control was performed by the gravimetric method (daily weighing of the experimental units), considering the substrate and water mass, and by replacing the water lost through evapotranspiration. The determination of the water holding capacity (WRC) was calculated according to the formula of Craven et al. (2010). The irrigation of the pots was conducted in a localized way with individual drippers per pot and maintained at 80% of the WRC until the V4 phenological stage (30 days after emergence).

2.2 TREATMENTS

The biomass was diluted in suspension containing 0.25 g L⁻¹ of concentration, from which aliquots of 0.5 mL L⁻¹ and 1.0 mL L⁻¹ were taken and mixed in water for

foliar application. These concentrations have been successfully used in previous works with AQ by Cordeiro et al. (2022) and Mógor et al. (2022).

There were established the following treatments: without water deficit (WO), with water deficit (WI), control without application of microalgae (AQ0), and foliar applications of microalgae at doses of 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0), that were divided into: WO+AQ0; WO+AQ0.5; WO+AQ1.0; WI+AQ0; WI+AQ0.5; WI+AQ1.0

Two applications of AQ were carried out, one when the common bean plants first trifoliolate was fully open and the second performed after 7 days. The WI was imposed one day after the first AQ application, when the plants were no longer irrigated, until WRC reaching up to 30%, establishing a water deficit condition, being maintained for seven days. After this period, the plants were rehydrated to 100% WRC.

Sampling was performed in three periods: on the third day after the start of water restriction (3RE), one day after rehydration (1AR), and 10 days after rehydration (10AR).

2.3 BIOCHEMICAL ANALYSIS

Samples were taken at 3RE, 1AR, and 10AR, using one plant per pot for each collection. The leaves were collected in the middle third of the plant, in the morning frozen and macerated in liquid nitrogen.

Leaves pigment analyses were performed according to Lichtenthaler (1987), adding 0.3 g of plant material to 1.7 mL of 80% acetone with 0.1% CaCO₃ (w/v) and readings were performed in a UV- visible at 663, 647, and 470 nm (Pompelli et al. 2013).

Total sugars were extracted according to Maldonado et al. (2013). The samples were obtained through acid hydrolysis and subsequent reaction with DNS, with the extraction of reducing and non-reducing sugars. The standard curve for total sugars was made with glucose at 1 mg mL⁻¹ (5.5 mM) with values between 50 and 800 µg mL⁻¹. The reading was performed in a UV-visible spectrophotometer at 540 nm.

Total soluble proteins were determined using the methodology described by Bradford (1976) using 50 µL of enzymatic extract and the reading was performed in a UV-visible spectrophotometer at 595 nm.

Total free amino acids were extracted according to Winters et al. (2002) and the colorimetric reaction was performed according to Magné and Larher (1992). The standard curve was made using glutamine and 2 mM asparagine with values between 28 and 140 $\mu\text{g mL}^{-1}$. The reading was performed in a UV-visible spectrophotometer at 570 nm.

The proline content was determined according to the method of Bates et al. (1973), using 3% sulfosalicylic acid, 1% ninhydrin solution, and toluene. After boiling in a water bath for 60 min, the reaction was read in a UV-visible spectrophotometer at 520 nm.

Peroxidase activity (POD) was determined according to Teisseire and Guy (2000) with the reaction system composed of 30 μL of diluted enzyme extract (1:10 in extraction buffer); 50 mmol L^{-1} potassium phosphate buffer pH 6.5; pyrogallol (1,2,3-benzenetriol) 20 mmol L^{-1} and hydrogen peroxide (H_2O_2) 5 mmol L^{-1} , at room temperature for 5 minutes. The purpurogallin formation was measured in a UV-visible spectrophotometer at 430 nm.

The enzyme superoxide dismutase (SOD) according to Giannopolitis and Reis (1977) was determined by adding 50 μL of an enzymatic extract diluted in a solution containing 13 nm of methionine, 75 μm of NBT, 100 nm of EDTA and 2 μm of riboflavin and 50 mm sodium phosphate buffer pH 7.8. The reaction was conducted in the presence of light for 10 minutes at room temperature (25°C). After the reaction, the blue compound formed (formazan) by the NBT photoreduction was determined by reading in a spectrophotometer at 560nm.

The catalase enzyme (CAT) was determined using diluted enzyme extract, sodium phosphate buffer, and H_2O_2 with UV-visible spectrophotometer readings at 240 nm (Peixoto et al. 1999).

2.5 BIOMETRIC MEASUREMENTS

At the 3RE, sampling was not performed for biometric analysis because the plants did not show any visual differences. The first sampling for biometric analysis was performed at 1AR. After determining the fresh mass (g) of the total aerial part using a precision scale, the samples were dried in a forced circulation oven at 65°C until achieving constant weight to obtain the dry mass (g) and fresh mass/dry mass ratio.

A second sampling was performed at 10AR on the plant stem that developed after the period of water deficit. For that, the last developed trifoliolate was marked on the day of rehydration and the section developed above was sampled for fresh and dry mass determination, and for the leaves area (cm²) measurement using the WinRhizo® software coupled to a Scanner LA1600 (Regent Instruments Inc., Canada).

2.6 LEAF ANATOMY

Leaf blade samples were collected at 10AR in the trifoliolate that developed after the period that the plant underwent water deficit. Leaf fragments measuring 0.5 cm² were removed from the midrib region of leaves in the middle third of the plant. The samples were fixed in FAA50 (Johansen 1940) for 24 hours and, after dehydration in an ethyl series, the samples were embedded in methacrylate (Historesin, Leica Instruments). Cross sections (7µm thick) were obtained using a rotating microtome and stained with toluidine blue, pH 7.2 (O'Brian and McCully 1981). The slides were mounted in 500® verniz vitral incolor (Paiva et al. 2006) and analyzed and photographed under a light microscope (Zeiss Axiolab model) with a digital camera (Sony Cybershot 7.2 ned mb model) attached to it.

Data on leaf thickness and tissue component palisade mesophyll, spongy mesophyll, upper epidermis, and lower epidermis were obtained with the aid of the ANATI QUANTI software (Aguiar et al. 2007). Each of the five parameters was measured three times in each of the ten cuts of each of the three slides for each repetition (n=3) totaling 1,350 records per cultivar, as adapted from Sant'Anna-Santos and Azevedo (2010); Rocha et al. (2014).

2.7 EXPERIMENTAL DESIGN

The experimental design was completely randomized with four replications (n = 4) in a factorial scheme 6 (treatments) x 2 (cultivars) for the biometric variables. In a triple factorial scheme with 6 (treatments) x 3 (periods) x 2 (cultivars) for the biochemical analyses and a factorial scheme with 3 (treatments) x 2 (cultivars) for the anatomical analyses. Each replicate consisted of two pots containing three plants each. The data obtained were tested by the Bartlett test and ANOVA and the Tukey test was applied (p <0.05) and processed by the SISVAR statistical program (Ferreira 2019).

3. RESULTS

3.1 BIOCHEMICAL ANALYSIS

The biochemical variables indicated that interactions were found among treatments, periods, and cultivars.

The IAC cultivar exhibit an increase in the chlorophyll content (Table 1) at 3RE in WO+AQ0.5 compared to the other treatments. At 10AR, in WO, the AQ0.5 and AQ1.0, promoted increase of 44% compared to WO control. In WI+AQ1.0 increased in 41% the chlorophyll a content compared to WI control. For WI+AQ1.0, comparing the chlorophyll a contents at 10AR with 1AR, an increase of 92% was found. The chlorophyll a content of BRS cultivar at 3RE was increased at WO+AQ0.5 compared to the other treatments.

For the IAC cultivar an increase of was found in the levels of chlorophyll b (Table 2) at 3RE and 1AR in WO+AQ0.5 compared to the other treatments. At 10AR, there was an increase of 250% and 166% in chlorophyll b levels in WO by AQ0.5 and AQ1.0, respectively, when compared to WO control. In WI, the AQ0.5 and AQ1.0, the increase was 100% and 141%, respectively, compared to WI control. For WI+AQ1.0, comparing the chlorophyll b contents at 10AR with 1AR, an increase of 45% was found.

For the BRS cultivar, no differences were found in chlorophyll b contents at 3RE. At 1AR, a 30% increase was observed in chlorophyll b levels for WO+AQ1.0, compared to WO control. At 10AR for WI+AQ0.5, there was a 120% increase in chlorophyll b content compared to WI control. For WI+AQ1.0 there was a 64% increase in chlorophyll b contents at 10AR compared to 1AR.

The IAC cultivar showed an increase in total chlorophyll levels (Table 3) at 3RE and 1AR for WO+AQ0.5 compared to the other treatments. At 10AR there was an increase of 79% and 61%, respectively, in the levels of total chlorophyll for WO+AQ0.5 and WO+AQ1.0 when compared to WO control. At WI, the foliar applications of AQ0.5 and AQ1.0 promoted increases of 33% and 74%, respectively, compared to WI control. Comparably, the BRS cultivar showed an increase in total chlorophyll contents at 3RE, in WO+AQ0.5.

The IAC cultivar displayed an increase in carotenoid contents (Table 4) at 3RE for WO+AQ0.5. When at 1AR there were no differences between treatments with and without microalgae. However, at 10AR, there was an increase in carotenoid levels of

50% and 62%, respectively for WO+AQ0.5 and WO+AQ1.0 when compared to WO control. In WI, the AQ0.5 and AQ1.0, showed increases of 21% and 60%, respectively, compared to WI control. For WI+AQ1.0, was found a 48% increase in carotenoid contents at 10AR compared to 1AR.

The BRS cultivar also had an increase in carotenoid levels at 3RE for WO+AQ0.5 compared to the other treatments.

The levels of total sugar (Tab.5) in IAC cultivar was increased to 18% at WI+ AQ0.5 at 3RE, when compared to WI control, and an increase of 24% at 10AR compared to 1AR.

For the BRS cultivar in WI, an increase of 25% was found in total sugar levels at 3RE by AQ0.5 application compared to WI control.

For the cultivar IAC, the levels of reducing sugars (Table 6) increases of 31% and 24% were found for WI by AQ0.5 and AQ1.0, respectively at 3RE, when compared to WI control. In comparison, the cultivar BRS had an increase of 37% in the levels of reducing sugars at 3RE in WI+AQ1.0, when compared to WI control.

The cultivar IAC, at 10AR showed increase in non-reducing sugars in WO+ AQ0.5 compared to the other treatments. In contrast, for the cultivar BRS, there was an increase in the levels of non-reducing sugars at 3RE for WO+AQ0.5 compared to the other treatments. Additionally, there was an increase of 41% in WI+AQ0.5 when compared to WI control.

The protein levels (Tab.8) of the IAC cultivar increased by 53% in WO+AQ1.0 at 3RE when compared to WO control. In WI, by the sprays with AQ0.5 and AQ1.0, the increases were of 25% and 36%, respectively, compared to WI control. For the BRS cultivar, there was an increase of 56% for WO+AQ1.0 in protein levels at 3RE when compared to WO control. At 1AR, there was an increase of 25% at WI+AQ0.5 when compared to WI control.

Considering the levels of total free amino acids (Table 9) of the cultivar IAC it was observed an increase in the at 3RE and 1AR in plants WI compared to those WO. Furthermore, at 3RE, there was an increase of 4% for WO+AQ1.0 in the total free amino acid content when compared to WO control. In WI, by AQ0.5 and AQ1.0, the increase was 2% when compared to WI control. At 1AR there was an increase in the total free amino acid content of 4% for WO+AQ0.5 when compared to WO control. As for WI+AQ0.5, the increase was 3% compared to WI control.

Table 1. Chlorophyll *a* (mg g⁻¹) of common bean leaves subjected to water deficit and foliar applications using *A. quadricellularis* in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	0.74 ± 0.07 Ba <u>a</u>	0.88 ± 0.09 Aa <u>a</u>	0.69 ± 0.07 BCa <u>a</u>	0.61 ± 0.06 Ca <u>a</u>	0.65 ± 0.06 BCa <u>a</u>	0.66 ± 0.06 BCb <u>a</u>
	BRS	0.58 ± 0.06 Bca <u>b</u>	0.72 ± 0.07 Aa <u>b</u>	0.66 ± 0.06 ABa <u>a</u>	0.53 ± 0.05 Ca <u>a</u>	0.57 ± 0.06 BCa <u>b</u>	0.56 ± 0.05 BCa <u>b</u>
1AR	IAC	0.67 ± 0.07 Aa <u>a</u>	0.76 ± 0.07 Ab <u>a</u>	0.75 ± 0.07 Aa <u>a</u>	0.54 ± 0.05 Ba <u>a</u>	0.51 ± 0.05 BCb <u>a</u>	0.41 ± 0.04 Cc <u>b</u>
	BRS	0.59 ± 0.06 ABa <u>a</u>	0.57 ± 0.06 ABb <u>b</u>	0.67 ± 0.07 Aa <u>b</u>	0.52 ± 0.05 Ba <u>a</u>	0.53 ± 0.05 Ba <u>a</u>	0.53 ± 0.05 Ba <u>a</u>
10AR	IAC	0.38 ± 0.04 Cb <u>b</u>	0.55 ± 0.05 Bc <u>a</u>	0.57 ± 0.06 Bb <u>a</u>	0.56 ± 0.05 Ba <u>a</u>	0.65 ± 0.06 Ba <u>a</u>	0.79 ± 0.08 Aa <u>a</u>
	BRS	0.62 ± 0.06 Aa <u>a</u>	0.5 ± 0.05 ABb <u>a</u>	0.47 ± 0.05 Bb <u>b</u>	0.49 ± 0.05 Ba <u>a</u>	0.58 ± 0.06 ABa <u>a</u>	0.55 ± 0.05 ABa <u>b</u>

Means followed by the same letter are not different from each other by the test of Tukey. Upper-case letters in the row compare treatments (T). Foliar spraying with *A. quadricellularis*: 0.5 ml L⁻¹ (AQ0.5) and 1.0 ml L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at ps0.01, ** = significant at ps0.05. C = *, T = *, P = *, CXT = *, CXP = **, TXP = *, CXTXP = *

Table 2. Chlorophyll *b* (mg g⁻¹) of common bean leaves subjected to water deficit and foliar applications using *A. quadricellularis* in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	0.34 ± 0.04 Ba <u>a</u>	0.41 ± 0.05 Aa <u>a</u>	0.32 ± 0.04 BCa <u>a</u>	0.28 ± 0.03 Ca <u>a</u>	0.31 ± 0.04 Bca <u>a</u>	0.27 ± 0.03 Ca <u>a</u>
	BRS	0.30 ± 0.03 Aa <u>b</u>	0.30 ± 0.03 Aa <u>b</u>	0.29 ± 0.03 ABa <u>a</u>	0.25 ± 0.03 ABa <u>a</u>	0.27 ± 0.03 ABa <u>b</u>	0.24 ± 0.03 Ba <u>a</u>
1AR	IAC	0.29 ± 0.03 BCb <u>a</u>	0.38 ± 0.04 Aa <u>a</u>	0.33 ± 0.04 ABa <u>a</u>	0.27 ± 0.03 Ca <u>a</u>	0.24 ± 0.03 CDb <u>a</u>	0.20 ± 0.02 Db <u>a</u>
	BRS	0.23 ± 0.03 Bb <u>b</u>	0.23 ± 0.03 Bb <u>b</u>	0.30 ± 0.03 Aa <u>a</u>	0.22 ± 0.03 Ba <u>b</u>	0.25 ± 0.03 ABa <u>a</u>	0.24 ± 0.03 ABa <u>a</u>
10AR	IAC	0.06 ± 0.01 Ec <u>a</u>	0.21 ± 0.02 BCb <u>a</u>	0.16 ± 0.02 CDb <u>a</u>	0.12 ± 0.01 Db <u>a</u>	0.24 ± 0.03 ABb <u>a</u>	0.29 ± 0.03 Aa <u>a</u>
	BRS	0.08 ± 0.01 ABc <u>a</u>	0.12 ± 0.01 Ac <u>b</u>	0.07 ± 0.01 ABb <u>b</u>	0.05 ± 0.01 Bb <u>b</u>	0.11 ± 0.01 Ab <u>b</u>	0.09 ± 0.01 ABb <u>b</u>

Means followed by the same letter are not different by the test of Tukey. Upper-case letters in the row compare treatments (T). *A. quadricellularis* foliar spraying: 0.5 ml L⁻¹ (AQ0.5) and 1.0 ml L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at ps0.01, ** = significant at ps0.05. C = *, T = *, P = *, CXT = *, CXP = *, TXP = *, CXTXP = *

Table 3. Total chlorophyll (mg g^{-1}) of common bean leaves subjected to water deficit and *A. quadricellularis* foliar application in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	1.10 ± 0.11 Ba \underline{a}	1.28 ± 0.12 Aa \underline{a}	1.00 ± 0.10 BCa \underline{a}	0.89 ± 0.09 Ca \underline{a}	1.00 ± 0.1 BCa \underline{a}	0.88 ± 0.08 Cb \underline{a}
	BRS	0.8 ± 0.08 BCab \underline{b}	1.02 ± 0.1 Aa \underline{b}	0.96 ± 0.09 ABa \underline{a}	0.78 ± 0.07 Ca \underline{a}	0.83 ± 0.08 BCa \underline{b}	0.8 ± 0.08 BCa \underline{a}
1AR	IAC	0.97 ± 0.09 BCb \underline{a}	1.14 ± 0.11 Ab \underline{a}	1.09 ± 0.10 ABa \underline{a}	0.83 ± 0.08 CDa \underline{a}	0.79 ± 0.08 Db \underline{a}	0.70 ± 0.07 Dc \underline{a}
	BRS	0.83 ± 0.08 ABa \underline{b}	0.77 ± 0.07 Bb \underline{b}	0.97 ± 0.09 Aa \underline{a}	0.73 ± 0.07 Ba \underline{a}	0.78 ± 0.07 Bab \underline{a}	0.80 ± 0.08 Ba \underline{a}
10AR	IAC	0.44 ± 0.04 Dc \underline{b}	0.79 ± 0.08 BCc \underline{a}	0.71 ± 0.07 Cb \underline{a}	0.66 ± 0.06 Cb \underline{a}	0.88 ± 0.08 Bab \underline{a}	1.15 ± 0.11 Aa \underline{a}
	BRS	0.70 ± 0.07 Ab \underline{a}	0.68 ± 0.07 ABb \underline{a}	0.53 ± 0.05 Bb \underline{b}	0.55 ± 0.05 ABb \underline{a}	0.69 ± 0.07 ABb \underline{b}	0.64 ± 0.06 ABb \underline{b}

Means followed by the same letter are not different by the test of Tukey. Upper-case letters in the row compare treatments (T). A. *quadricellularis* foliar spraying: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = *, T = *, P = *, CXT = *, CXP = ns; TXP = *, CXTXP = *

Table 4. Carotenoids (mg g^{-1}) in common bean leaves subjected to water deficit and *A. quadricellularis* foliar spraying in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	0.34 ± 0.03 Ba \underline{a}	0.39 ± 0.03 Aa \underline{a}	0.31 ± 0.02 Ba \underline{a}	0.31 ± 0.02 Ba \underline{a}	0.35 ± 0.03 ABa \underline{a}	0.34 ± 0.03 Bb \underline{a}
	BRS	0.25 ± 0.02 Ba \underline{b}	0.32 ± 0.03 Aa \underline{b}	0.28 ± 0.02 ABb \underline{b}	0.25 ± 0.02 Ba \underline{b}	0.28 ± 0.02 ABa \underline{b}	0.26 ± 0.02 Ba \underline{b}
1AR	IAC	0.31 ± 0.02 ABa \underline{a}	0.33 ± 0.03 ABb \underline{a}	0.34 ± 0.03 Aa \underline{a}	0.31 ± 0.02 Aba \underline{a}	0.30 ± 0.02 Bb \underline{a}	0.25 ± 0.02 Cc \underline{b}
	BRS	0.28 ± 0.02 ABa \underline{b}	0.26 ± 0.02 Bb \underline{b}	0.32 ± 0.03 Aa \underline{a}	0.27 ± 0.02 Ba \underline{b}	0.28 ± 0.02 ABa \underline{a}	0.29 ± 0.02 ABa \underline{a}
10AR	IAC	0.16 ± 0.01 Db \underline{b}	0.24 ± 0.02 BCc \underline{a}	0.26 ± 0.02 BCb \underline{a}	0.23 ± 0.02 Cb \underline{a}	0.28 ± 0.02 Bb \underline{a}	0.37 ± 0.03 Aa \underline{a}
	BRS	0.24 ± 0.02 Aa \underline{a}	0.23 ± 0.02 Ab \underline{a}	0.21 ± 0.02 Ac \underline{b}	0.20 ± 0.02 Ab \underline{a}	0.23 ± 0.02 Ab \underline{b}	0.22 ± 0.02 Ab \underline{b}

Means followed by the same letter are not different by the test of Tukey. Upper-case letters in the row compare treatments (T). A. *quadricellularis* foliar spraying: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = *, T = *, P = *, CXT = *, CXP = *, TXP = *, CXTXP = *

Table 5. Total sugar ($\mu\text{g g}^{-1}$) in common bean leaves subjected to water deficit and *A. quadricellularis* foliar applications in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	8683.14 ± 657.31 Ba <u>a</u>	9244.22 ± 699.79 Ba <u>a</u>	8841.63 ± 669.31 Ba <u>a</u>	9273.83 ± 702.03 Ba <u>a</u>	10985.35 ± 831.59 Aa	9792.90 ± 741.32 Ba <u>a</u>
	BRS	8982.38 ± 679.97 Ba <u>a</u>	9806.42 ± 742.35 Ba <u>a</u>	8756.72 ± 662.88 Ba <u>a</u>	9380.44 ± 710.10 Ba <u>a</u>	11771.5 ± 891.10 Aa <u>a</u>	9654.08 ± 730.81 Ba <u>a</u>
1AR	IAC	5481.10 ± 414.92 Ac <u>b</u>	6279.17 ± 475.33 Ac <u>b</u>	6077.90 ± 460.10 Ab <u>b</u>	5214.10 ± 394.71 Ab <u>b</u>	5256.41 ± 397.91 Ac <u>b</u>	6173.67 ± 467.35 Ab <u>b</u>
	BRS	7801.90 ± 590.60 Ab <u>a</u>	7703.72 ± 583.17 Ab <u>a</u>	7579.17 ± 573.74 Ab <u>a</u>	6304.75 ± 477.27 Bb <u>a</u>	6836.57 ± 517.53 ABb	7013.00 ± 530.88 ABb
10AR	IAC	6645.93 ± 503.10 ABb	7524.04 ± 569.57 Ab <u>a</u>	6645.74 ± 503.08 ABb	5834.89 ± 441.70 Bb <u>a</u>	6538.26 ± 494.95 ABb	5905.79 ± 447.07 Bb <u>a</u>
	BRS	6271.70 ± 474.77 ABc	6705.90 ± 507.64 Ac <u>b</u>	6967.69 ± 527.45 Ab <u>a</u>	6243.01 ± 472.60 ABb	5453.68 ± 412.84 Bc <u>b</u>	6308.66 ± 477.57 ABb

Means followed by the same letter are not different by the test of Tukey. Upper-case letters in the row compare treatments (T). A. *quadricellularis* foliar spraying: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = *, T = *, P = *, CXT = ns; CXP = **, TXP = *, CXTXP = **

Table 6. Reducing sugar ($\mu\text{g g}^{-1}$) of common bean leaves subjected to water deficit and *A. quadricellularis* foliar spraying in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	3419.65 ± 375.14 Ba <u>b</u>	3561.30 ± 390.67 Ba <u>a</u>	3612.53 ± 396.29 Ba <u>a</u>	3948.98 ± 433.20 Ba <u>a</u>	5198.09 ± 570.23 Aa <u>a</u>	4904.42 ± 538.01 Aa <u>b</u>
	BRS	4295.98 ± 471.27 Cb <u>a</u>	3871.52 ± 424.71 Cb <u>a</u>	4209.65 ± 461.80 Cb <u>a</u>	4571.98 ± 501.55 BCa	5422.36 ± 594.83 ABa	6300.04 ± 691.11 Aa <u>a</u>
1AR	IAC	3779.27 ± 414.59 Aa <u>b</u>	3996.81 ± 438.45 Aa <u>b</u>	4139.84 ± 454.14 Aa <u>b</u>	3290.88 ± 361.01 Aa <u>b</u>	3312.40 ± 363.37 Ab <u>b</u>	3327.66 ± 365.04 Ab <u>b</u>
	BRS	6380.38 ± 699.93 Aa <u>a</u>	5584.03 ± 612.57 ABa	5736.09 ± 629.25 ABa	4205.18 ± 461.31 Cab	4552.38 ± 499.4 Cb <u>a</u>	4975.23 ± 545.78 BCb
10AR	IAC	4032.21 ± 442.33 Aa <u>a</u>	3531.01 ± 387.35 Aa <u>a</u>	3924.39 ± 430.51 Aa <u>a</u>	3411.32 ± 374.22 Aa <u>a</u>	3568.52 ± 391.47 Ab <u>a</u>	3889.86 ± 426.72 Ab <u>a</u>
	BRS	3018.19 ± 331.10 Ac <u>b</u>	3503.24 ± 384.31 Ab <u>a</u>	3522.17 ± 386.38 Ab <u>a</u>	3463.60 ± 379.96 Ab <u>a</u>	2873.54 ± 315.23 Ac <u>b</u>	3118.55 ± 342.10 Ac <u>b</u>

Means followed by the same letter are not different by the test of Tukey. Upper-case letters in the row compare treatments (T). A. *quadricellularis* foliar spraying: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = *, T = *, P = *, CXT = ns; CXP = **, TXP = *, CXTXP = **

Table 7. Non-reducing sugars ($\mu\text{g g}^{-1}$) in common bean leaves subjected to water deficit and *A. quadricellularis* foliar applications in three periods

	WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE						
IAC	5263.49 ± 78320.73 Ba <u>a</u>	5682.93 ± 845.62 ABa <u>a</u>	5535.87 ± 823.74 ABa <u>a</u>	5324.86 ± 792.34 ABa <u>a</u>	6074.55 ± 903.89 Aa <u>a</u>	4888.47 ± 727.4 Ba <u>a</u>
BRS	4686.39 ± 697.33 Ba <u>a</u>	5934.89 ± 883.11 Aa <u>a</u>	4547.07 ± 676.6 Ba <u>b</u>	4640.69 ± 690.53 Ba <u>a</u>	6553.94 ± 975.23 Aa <u>a</u>	3498.72 ± 520.61 Ca <u>b</u>
1AR						
IAC	1702.69 ± 253.36 Ac <u>a</u>	2282.36 ± 339.62 Ac <u>a</u>	1938.06 ± 288.38 Ab <u>a</u>	1856.45 ± 276.24 Ab <u>a</u>	1944.01 ± 289.27 Ac <u>a</u>	2226.36 ± 331.28 Ab <u>a</u>
BRS	1421.53 ± 211.52 Ac <u>a</u>	2119.68 ± 315.41 Ac <u>a</u>	1843.08 ± 274.25 Ac <u>a</u>	2025.15 ± 301.34 Ab <u>a</u>	2395.33 ± 356.43 Ab <u>a</u>	2037.77 ± 303.22 Ab <u>a</u>
10AR						
IAC	2613.72 ± 388.92 BCb <u>a</u>	3993.03 ± 594.16 Ab <u>a</u>	2721.35 ± 404.94 BCb <u>b</u>	2423.58 ± 360.63 BCb <u>a</u>	3119.38 ± 464.16 ABb <u>a</u>	2015.94 ± 299.97 Cb <u>b</u>
BRS	3253.51 ± 484.12 Ab <u>a</u>	3202.67 ± 476.56 Ab <u>b</u>	3445.51 ± 512.69 Ab <u>a</u>	2590.76 ± 385.51 Ab <u>a</u>	2580.40 ± 383.92 Ab <u>a</u>	3190.11 ± 474.69 Aa <u>a</u>

Means followed by the same letter are not different by the test of Tukey. Upper-case letters in the row compare treatments (T). A. *quadricellularis* foliar spraying: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = ns; T = *; P = *; CXT = ns; CXP = *; TXP = *; CXTXP = *

Table 8. Protein ($\mu\text{g g}^{-1}$) in common bean leaves subjected to water deficit and *A. quadricellularis* foliar spraying in three periods

	WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE						
IAC	9384.58 ± 1325.10 Bb <u>a</u>	7967.91 ± 1125.07 Ba <u>a</u>	14374.53 ± 2029.68 Aa <u>a</u>	10233.11 ± 1444.92 Ba <u>a</u>	12888.88 ± 1819.91 Aa <u>a</u>	13951.18 ± 1969.91 Aa <u>a</u>
BRS	7549.40 ± 1065.98 Cb <u>b</u>	8621.73 ± 1217.39 BCa <u>a</u>	11790.61 ± 1664.83 Aa <u>b</u>	9984.73 ± 1409.84 ABCb <u>a</u>	9546.86 ± 1348.02 ABCb <u>b</u>	10136.18 ± 1431.23 ABb <u>b</u>
1AR						
IAC	12027.30 ± 1698.25 ABa <u>a</u>	5251.93 ± 741.57 Cb <u>b</u>	6475.17 ± 914.29 Cb <u>b</u>	10898.60 ± 1538.88 ABa <u>b</u>	9619.85 ± 1358.32 Bb <u>b</u>	13430.04 ± 1896.32 Aa <u>b</u>
BRS	9827.93 ± 1387.7 Ca <u>b</u>	9115.11 ± 1287.05 Ca <u>a</u>	11643.24 ± 1644.03 Ca <u>a</u>	14831.33 ± 2094.18 Ba <u>a</u>	18645.49 ± 2632.74 Aa <u>a</u>	16438.68 ± 2321.14 ABa <u>a</u>
10AR						
IAC	2928.59 ± 413.52 Ac <u>a</u>	3758.98 ± 530.77 Ab <u>a</u>	3814.92 ± 538.67 Ac <u>a</u>	5208.68 ± 735.47 Ab <u>a</u>	4295.07 ± 606.46 Ab <u>a</u>	5398.85 ± 762.32 Ab <u>a</u>
BRS	3963.88 ± 559.70 Ac <u>a</u>	3645.05 ± 514.68 Ab <u>a</u>	4046.77 ± 571.40 Ab <u>a</u>	4549.40 ± 642.38 Ac <u>a</u>	4982.33 ± 703.50 Ac <u>a</u>	4738.55 ± 669.08 Ac <u>a</u>

Means followed by the same letter are not different by the test of Tukey. Upper-case letters in the row compare treatments (T). A. *quadricellularis* foliar spraying: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lower-case letters in the column compare periods (P) among the same cultivar. Underlined lower-case letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = ns; T = *; P = *; CXT = ns; CXP = ns; TXP = *; CXTXP = *.

Similarly, for the cultivar BRS, there was an increase in the levels of total free amino acids at 3RE and 1AR in plants WI compared to those WO. At 3RE, increases by 3% were found in total free amino acids for WI by AQ0.5 and AQ1.0 when compared to WI control. At 1AR, there was an increase of 2% in WI+AQ1.0 when compared to WI control.

The concentration of proline in leaves (Table 10), as well as total free amino acids increased with water deficit compared to those without water shortage at 3RE in plant for cultivar IAC; at 1AR and 10AR, there was a decrease in proline levels for plants WI, similar to those WO, in addition to a decrease over the course from 10AR to 3RE. For cultivar BRS, there was an increase in proline levels at 3RE and 1AR in plants WI compared to those WO. At 10AR, there was a decrease in proline levels for plants with water deficit compared to 1AR for 3RE.

The POD enzyme levels in cultivar IAC showed an increase of 66% in WI by AQ0.5 and AQ1.0 (Tab.11) at 3RE when compared to WI control. Whereas, at 1AR, there was no difference between treatments with and without microalgae, at 10AR, there was an increase in POD content of 39% for WI+AQ0.5 when compared to WI control. In contrast, the BRS cultivar showed no differences in POD levels at 3RE and 1AR between treatments with and without microalgae. Nonetheless, at 10AR there was an increase in POD content of 44% for WI+AQ1.0 when compared to WI control.

The SOD enzyme levels in IAC cultivar (Tab.12) at 1AR, showed an increase of 100% in WO by AQ0.5 and AQ1.0. Moreover, at 10AR it was observed an increase in SOD content of 33% for WI+AQ0.5 when compared to WI control. Otherwise, for the BRS cultivar, no differences were found in SOD contents at 3RE, 1AR, and 10AR between treatments with and without microalgae.

The levels of CAT enzyme (Tab.13) in IAC cultivar displayed an increase at 3RE of 130% and 281% in WI by AQ0.5 and AQ1.0, respectively, when compared to WI control. At 1AR, CAT levels remained increased for WI by AQ0.5 and AQ1.0 with an increase of 53% and 118%, respectively, when compared to WI control.

For the BRS cultivar, an increase of 110% was found in the CAT enzyme content at 3RE in WI+AQ1.0 when compared to WI control. Also, at 1AR, there was an increase in CAT enzyme levels for WI by AQ0.5 and AQ1.0 with increases of 45% and 69%, respectively, when compared to WI control.

3.2 BIOMETRIC ANALYSIS

The IAC cultivar at 1AR, showed an increase in fresh mass (Fig. 1 a) in WO +AQ1.0 in comparison to the other treatments. Meanwhile, the cultivar BRS presented a decrease in plants WI compared to those WO, except for WI+AQ1.0, which was statistically similar to plants in WO.

The common bean plants dry mass (Fig. 1 b) and fresh mass/dry mass ratio (Fig. 1 c) showed difference only for plants WI and WO, that is, the microalgae did not promote changes for these variables. The comparison among cultivars showed that there was no significant difference.

At 10AR, an increase was found in the leaf area (Fig. 2 a), fresh mass/dry mass (Fig. 2 b) and fresh mass (Fig. 2 c) in WI by AQ1.0 compared to WI control of 42%, 45 % and 42%, respectively. Regarding dry mass (Fig. 2 d), there was a significant difference for plants among WO and WI and none influence of microalgae.

3.3 LEAF ANATOMY

An increase was found in leaf thickness (Fig. 3 a), palisade parenchyma (Fig. 3 b) and spongy parenchyma (Fig. 3 c) in WI by AQ1.0 when compared to WI control of 36%, 18% and 38%, respectively.

The upper epidermis and lower epidermis variables pointed to interactions between treatments and cultivars. For the IAC cultivar, an increase was observed in the thickness of the upper epidermis (Fig.3 d) and lower epidermis (Fig.3 e) in WI by AQ1.0 when compared to WI control of 44% and 46%, respectively. For cultivar BRS, an increase was found in lower epidermis thickness of 19% in WI by AQ1.0 when compared to WI control.

Table 9. Total free amino acids ($\mu\text{g g}^{-1}$) of common bean leaves subjected to water deficit and *A. quadricellularis* foliar applications in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	7545.10 \pm 72.43 Da <u>b</u>	7664.37 \pm 73.57 Da <u>b</u>	7861.96 \pm 75.47Ca <u>a</u>	8268.01 \pm 79.37 Ba <u>b</u>	8433.67 \pm 80.96 Aa <u>b</u>	8458.46 \pm 81.20 Aa <u>b</u>
	BRS	7834.98 \pm 75.21 CDa <u>a</u>	7910.99 \pm 75.94 Ca <u>a</u>	7743.30 \pm 74.33 Da <u>b</u>	8843.12 \pm 84.89 Ba <u>a</u>	9160.79 \pm 87.94 Aa <u>a</u>	9115.21 \pm 87.50 Aa <u>a</u>
1AR	IAC	7204.00 \pm 69.15 Eb <u>b</u>	7559.09 \pm 72.56 Da <u>a</u>	7225.67 \pm 69.36 Eb <u>b</u>	8109.21 \pm 77.84 Bb <u>b</u>	8372.14 \pm 80.37 Aa <u>a</u>	7910.81 \pm 75.94 Cb <u>b</u>
	BRS	7512.04 \pm 72.11 CDb <u>a</u>	7373.71 \pm 70.78 Db <u>b</u>	7641.90 \pm 73.36 Ca <u>a</u>	8284.80 \pm 79.53 Bb <u>a</u>	8238.59 \pm 79.09 Bb <u>b</u>	8444.21 \pm 81.06 Ab <u>a</u>
10AR	IAC	7199.31 \pm 69.11 BCb <u>b</u>	7285.63 \pm 69.94 Bb <u>b</u>	7200.23 \pm 69.12 BCb <u>a</u>	7447.45 \pm 71.49 Ac <u>a</u>	7508.22 \pm 72.07 Ab <u>a</u>	7054.99 \pm 67.72 Cc <u>b</u>
	BRS	7585.96 \pm 72.82 Ab <u>a</u>	7419.62 \pm 71.22 BCb <u>a</u>	7155.87 \pm 68.69 Db <u>a</u>	7454.89 \pm 69.80 ABc <u>a</u>	7271.45 \pm 69.80 CDc <u>b</u>	7442.74 \pm 71.45 ABc <u>a</u>

Means followed by the same letter do not differ by the test of Tukey. Upper case letters in the row compare treatments (T). *A. quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters in the column compare periods (P) between the same cultivar. Underlined lowercase letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = *; T = *; P = *; CXT = *; CXP = *; TXP = *; CXTXP = *

Table 10. Proline ($\mu\text{g g}^{-1}$) in common bean leaves subjected to water deficit and leaf applications with *A. quadricellularis* foliar application in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	12.02 \pm 1.22 Ba <u>a</u>	11.81 \pm 1.20 Ba <u>a</u>	12.12 \pm 1.23 Ba <u>a</u>	21.32 \pm 2.16 Aa <u>b</u>	23.30 \pm 2.36 Aa <u>a</u>	23.95 \pm 2.43 Aa <u>a</u>
	BRS	12.81 \pm 1.30 Ba <u>a</u>	11.43 \pm 1.16 Ba <u>a</u>	13.28 \pm 1.35 Ba <u>a</u>	23.37 \pm 2.37 Aa <u>a</u>	21.40 \pm 2.17 Aa <u>b</u>	20.86 \pm 2.11 Aa <u>b</u>
1AR	IAC	10.39 \pm 1.05 ABa <u>a</u>	8.56 \pm 0.87 Bb <u>a</u>	9.97 \pm 1.01 ABa <u>a</u>	11.63 \pm 1.18 Ab <u>b</u>	10.84 \pm 1.10 ABb <u>b</u>	11.05 \pm 1.12 ABb <u>b</u>
	BRS	11.51 \pm 1.17 BCab <u>a</u>	10.24 \pm 1.04 Cab <u>a</u>	10.87 \pm 1.10 Cb <u>a</u>	15.41 \pm 1.56 Ab <u>a</u>	13.92 \pm 1.41 ABb <u>a</u>	14.53 \pm 1.47 Ab <u>a</u>
10AR	IAC	11.79 \pm 1.19 Aa <u>a</u>	8.51 \pm 0.86 Bb <u>a</u>	10.09 \pm 1.02 ABa <u>a</u>	10.03 \pm 1.02 ABb <u>a</u>	10.15 \pm 1.03 ABb <u>a</u>	9.15 \pm 0.93 ABb <u>a</u>
	BRS	10.15 \pm 1.03 Ab <u>a</u>	9.01 \pm 0.91 Ab <u>a</u>	11.38 \pm 1.15 Aab <u>a</u>	10.74 \pm 1.09 Ac <u>a</u>	10.08 \pm 1.02 Ac <u>a</u>	10.56 \pm 1.07 Ac <u>a</u>

Means followed by the same letter do not differ by the test of Tukey. Upper case letters in the row compare treatments (T). *A. quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters in the column compare periods (P) between the same cultivar. Underlined lowercase letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0.01$, ** = significant at $p \leq 0.05$. C = *; T = *; P = *; CXT = ns; CXP = *; TXP = *; CXTXP = *

Table 11. Peroxidase (POD, $\mu\text{mol de purpurogallin min}^{-1} \text{mg}^{-1}$ protein) of common bean leaves subjected to water deficit and *A. quadricellularis* foliar application in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	2.51 ± 0.37 Bc a	2.12 ± 0.31 Bb a	2.23 ± 0.33 Bb a	2.51 ± 0.37 Bc b	4.18 ± 0.62 Ab a	4.17 ± 0.62 Ab a
	BRS	3.18 ± 0.47 ABCb a	2.25 ± 0.33 Cc a	3.01 ± 0.44 BCb a	3.98 ± 0.59 ABa a	3.42 ± 0.51 ABCb a	4.46 ± 0.66 Ab a
1AR	IAC	4.52 ± 0.67 BCb a	6.31 ± 0.93 Aa a	4.41 ± 0.65 Ca a	5.59 ± 0.83 ABCa a	6.25 ± 0.92 Aa a	5.83 ± 0.86 ABa a
	BRS	4.11 ± 0.61 Ab a	4.31 ± 0.64 Ab b	3.97 ± 0.59 Ab a	4.41 ± 0.65 Aa b	4.34 ± 0.64 Aab b	4.63 ± 0.68 Ab b
10AR	IAC	6.74 ± 1.00 Aa a	7.19 ± 1.06 Aa a	4.48 ± 0.66 BCa b	3.77 ± 0.56 Cb a	5.27 ± 0.78 Bab a	4.59 ± 0.68 BCb b
	BRS	6.08 ± 0.9 Aa a	5.52 ± 0.82 Aa b	5.81 ± 0.86 Aa a	4.03 ± 0.60 Ba a	4.77 ± 0.71 ABa a	5.81 ± 0.86 Aa a

Means followed by the same letter do not differ by the test of Tukey. Upper case letters in the row compare treatments (T). *A. quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters in the column compare periods (P) between the same cultivar. Underlined lowercase letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p < 0.01$, ** = significant at $p < 0.05$. C = **; T = *; P = *; CXT = *; CXP = *; TXP = *; CXTXP = **

Table 12. Activity of superoxide dismutase enzyme (SOD, U mg⁻¹ protein) in common bean leaves subjected to water deficit and *A. quadricellularis* foliar application in three periods

		WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC	52545.36 ± 8076.22 ABb a	61915.54 ± 9516.42 Ac a	34771.73 ± 5344.41 Bc a	47414.92 ± 7287.67 ABb a	53408.16 ± 8208.83 ABb a	38206.16 ± 5872.29 Bb a
	BRS	64592.6 ± 9927.88 Ab a	57193.41 ± 8790.63 ABb a	37594.2 ± 5778.23 Bb a	49099.08 ± 7546.53 ABb a	53226.53 ± 8180.92 ABb a	50285.24 ± 7728.84 ABb a
1AR	IAC	41200.72 ± 6332.55 Cb a	83173.04 ± 12783.7 Ab a	74620.34 ± 11469.15 ABb a	45037.19 ± 6922.22 Cb a	55459.7 ± 8524.16 BCb a	41815.92 ± 6427.11 Cb a
	BRS	50207.85 ± 7716.95 ABb a	51926.3 ± 7981.07 Ab b	39982.97 ± 6145.38 ABb b	32869.69 ± 5052.07 ABb a	27999.87 ± 4303.58 Bc b	32516.22 ± 4997.74 ABb a
10AR	IAC	153715.40 ± 23626.06 Aa b	131895.90 ± 20272.40 ABa a	121867.75 ± 18731.07 Ba a	87002.16 ± 13372.23 Ca a	115883.60 ± 17811.31 Ba a	92046.45 ± 14147.54 Ca b
	BRS	116544.70 ± 17912.92 ABa a	130074.50 ± 19992.45 Aa a	114736.33 ± 17634.97 Aba a	88682.92 ± 13630.56 Ca a	103016.40 ± 15833.62 BCa a	110532.7 ± 16988.88 ABCa a

Means followed by the same letter do not differ by the test of Tukey. Upper case letters in the row compare treatments (T). *A. quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters in the column compare periods (P) between the same cultivar. Underlined lowercase letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p < 0.01$, ** = significant at $p < 0.05$.

C = *; T = *; P = *; CXT = *; CXP = *; TXP = *; CXTXP = *

Table 13. Catalase (CAT, $\mu\text{Kat } \mu\text{g}^{-1}$ protein min^{-1}) in common bean leaves subjected to water deficit and *A. quadricellularis* foliar application in three periods

	WO+AQ0	WO+AQ0.5	WO+AQ1.0	WI+AQ0	WI+AQ0.5	WI+AQ1.0
3RE	IAC 0.057 ± 0.01 Cb <u>a</u>	0.079 ± 0.01 Ca <u>a</u>	0.078 ± 0.01 Ca <u>a</u>	0.091 ± 0.02 Ca <u>a</u>	0.210 ± 0.04 Ba <u>a</u>	0.347 ± 0.06 Aa <u>a</u>
	BRS 0.072 ± 0.01 BCa <u>a</u>	0.054 ± 0.01 Ca <u>a</u>	0.078 ± 0.01 BCa <u>a</u>	0.095 ± 0.02 Ba <u>a</u>	0.107 ± 0.02 Ba <u>b</u>	0.209 ± 0.04 Aa <u>b</u>
1AR	IAC 0.096 ± 0.02 Ca <u>a</u>	0.090 ± 0.02 Ca <u>a</u>	0.091 ± 0.01 Ca <u>a</u>	0.091 ± 0.02 Ca <u>a</u>	0.140 ± 0.02 Bb <u>a</u>	0.199 ± 0.04 Ab <u>a</u>
	BRS 0.058 ± 0.01 Ba <u>b</u>	0.068 ± 0.01 Ba <u>a</u>	0.072 ± 0.01 Ba <u>a</u>	0.093 ± 0.02 Ba <u>a</u>	0.135 ± 0.02 Aa <u>a</u>	0.158 ± 0.03 Ab <u>b</u>
10AR	IAC 0.098 ± 0.02 Aa <u>a</u>	0.061 ± 0.01 ABa <u>a</u>	0.093 ± 0.01 ABa <u>a</u>	0.065 ± 0.01 ABa <u>a</u>	0.056 ± 0.01 Bc <u>a</u>	0.066 ± 0.01 ABC <u>a</u>
	BRS 0.066 ± 0.01 Aa <u>b</u>	0.056 ± 0.01 Aa <u>a</u>	0.051 ± 0.01 Aa <u>b</u>	0.053 ± 0.01 Ab <u>a</u>	0.051 ± 0.01 Ab <u>a</u>	0.058 ± 0.01 Ac <u>a</u>

Means followed by the same letter do not differ by the test of Tukey. Upper case letters in the row compare treatments (T). *A. quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters in the column compare periods (P) between the same cultivar. Underlined lowercase letters in the column compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at p≤0.01, ** = significant at p≤0.05. C = *, T = *, P = *, CXT = *, CXP = *, TXP = *, CXTXP = *

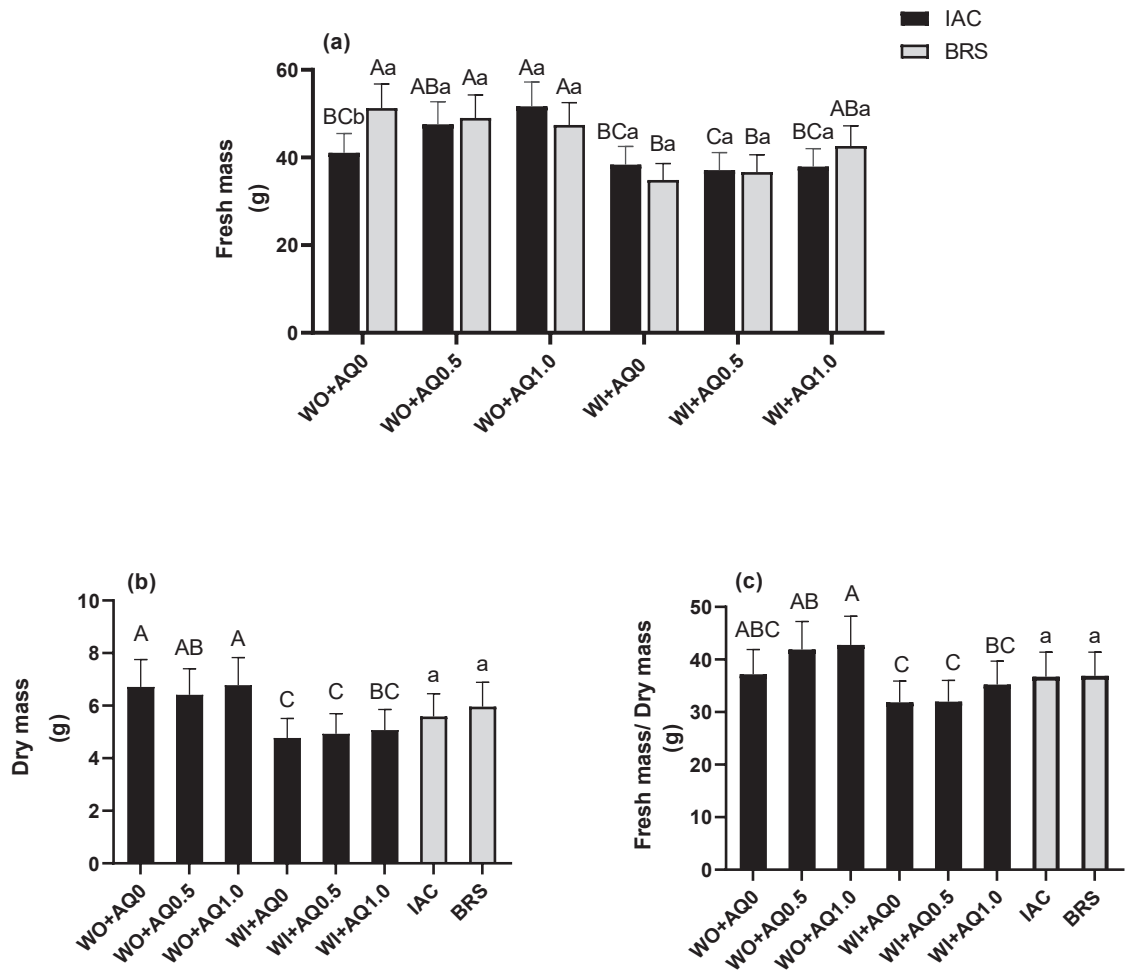


Figure 1 Microalgae effects on biometric parameters of common bean plants under water deficit conditions, 1 day after rehydration (1AR). Fresh mass (a), dry mass (b), and fresh and dry mass ratio (g) (c). Means followed by the same letter do not differ from each other by the test of Tukey. Capital letters compare treatments (T). A. *quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at p≤0.01, ** = significant at p≤0.05. Fresh mass: T = *; C = ns; TXC = **. Dry mass: T = *; C = ns; TXC = ns. Fresh mass/Dry mass: T = *; C = ns; TXC = ns

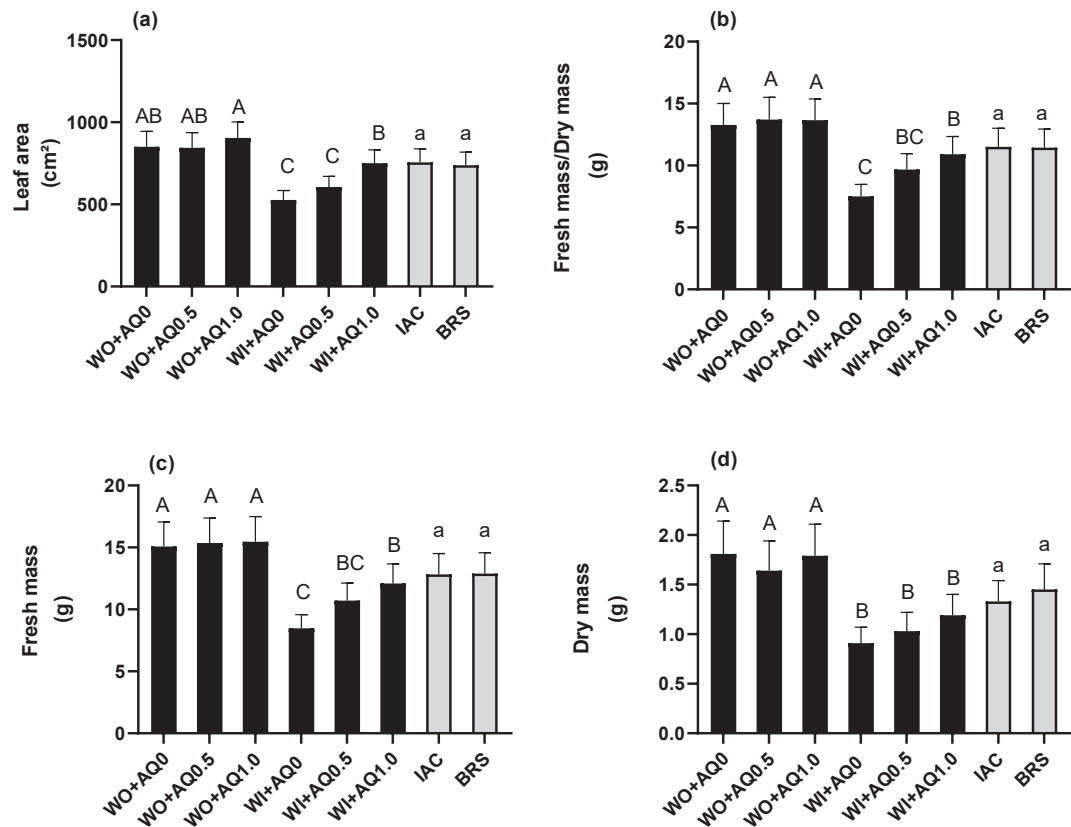


Figure 2 Microalgae effects on biometric parameters of common bean plants under water deficit conditions, 10 days after rehydration (10AR). Leaf area (cm²) (a); fresh mass (b); dry mass (c) and ratio between fresh and dry mass (g) (d). Means followed by the same letter do not differ by the test of Tukey. Capital letters compare treatments (T). *A. quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0,01$, ** = significant at $p \leq 0,05$. Leaf area: T = *; C = ns; TXC = ns. Fresh mass/dry mass: T = *; C = ns; TXC = ns. Fresh mass: T = *; C = ns; TXC = ns. Dry mass: T = *; C = ns; TXC = ns

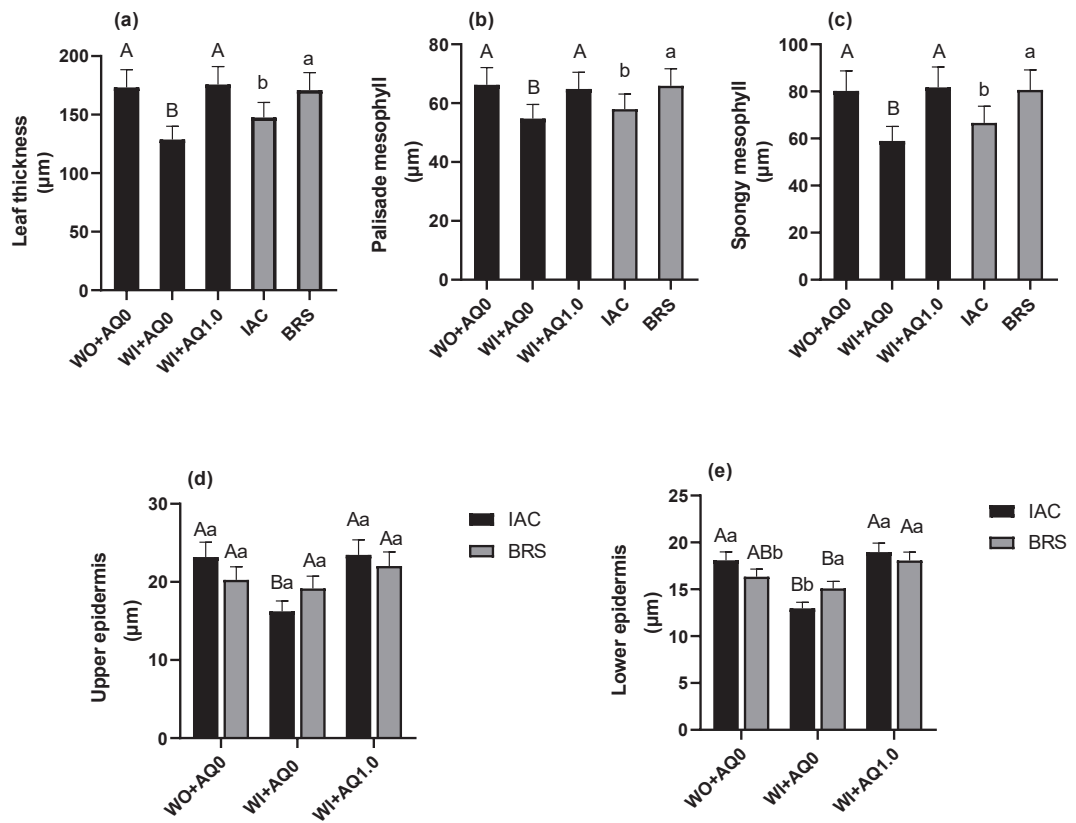


Figure 3 Microalgae effects on anatomical parameters of common bean plants under water deficit conditions, 10 days after rehydration (10AR). Leaf thickness (a), palisade mesophyll (b), spongy mesophyll (c), Upper epidermis thickness (d), lower epidermis thickness (e) (μm). Means followed by the same letter do not differ by the test of Tukey. Capital letters compare treatments (T). Leaf spray with *A. quadricellularis*: 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit. Lowercase letters compare cultivars (C). IAC 1850 (IAC), BRS ESTEIO (BRS). ANOVA: ns = not significant, * = significant at $p \leq 0,01$, ** = significant at $p \leq 0,05$. Leaf thickness: T = *; C = ns; TXC = ns. Palisade mesophyll: T = *; C = *; TXC = ns. Spongy mesophyll: T = *; C = *; TXC = ns. Upper epidermis: T = *; C = ns; TXC = *. Lower epidermis: T = *; C = *; TXC = *

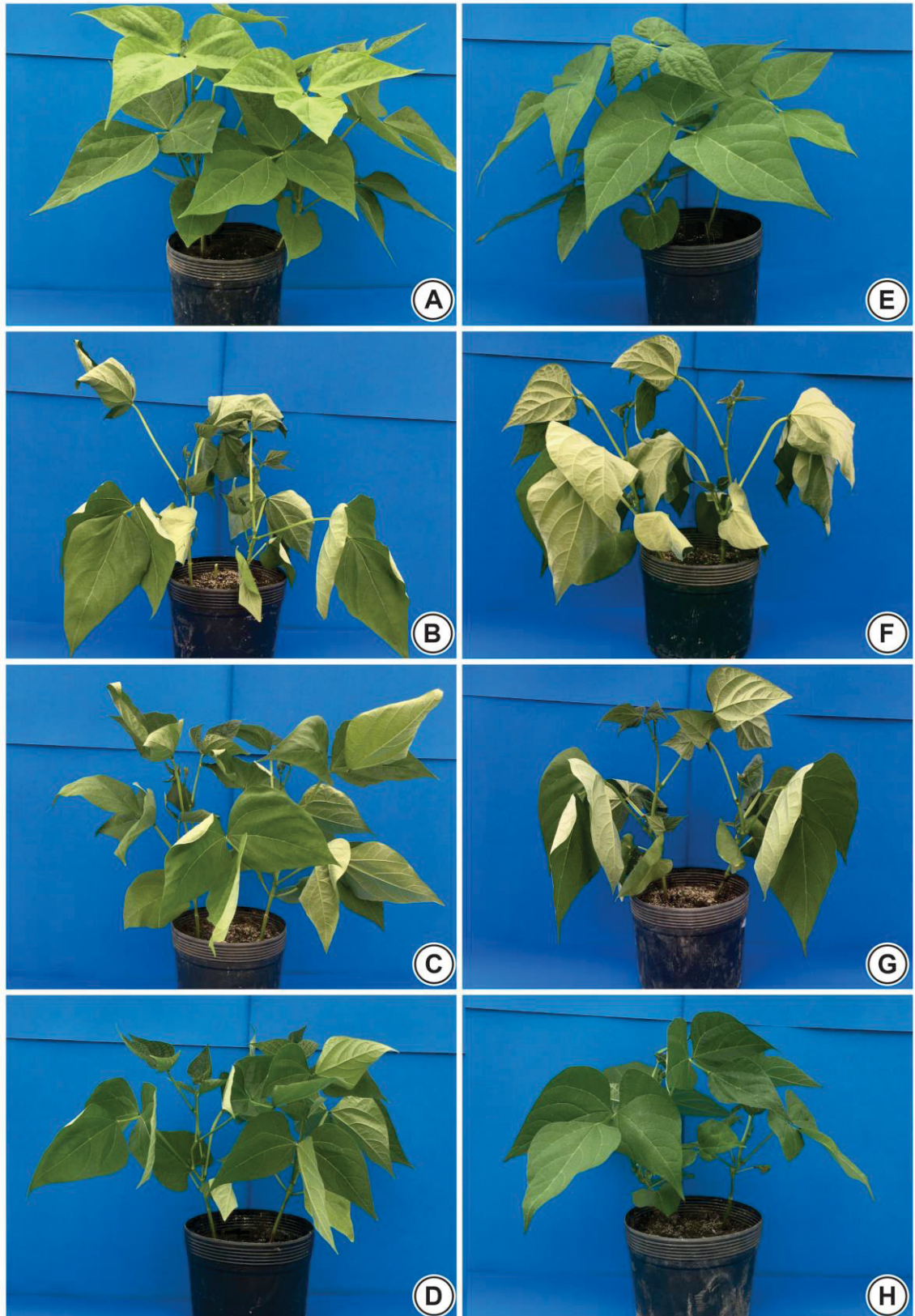


Figure 4 Visual aspect of common beans plants at 3RE. *A. quadricellularis* foliar spray: 0.5 mL L⁻¹ (AQ0.5) and 1.0 mL L⁻¹ (AQ1.0); WO: without water deficit, WI: with water deficit and cultivars IAC 1850 (IAC), BRS ESTEIO (BRS) (A) WO+AQ0, IAC (B) WI+AQ0, IAC (C) WI+AQ0.5, IAC (D) WI+AQ1.0, IAC (E) WO+AQ0, BRS (F) WI+AQ0, BRS (G) WI+AQ0.5, BRS (H) WI+AQ1.0, BRS

4. DISCUSSION

The photosynthetic efficiency of the plants depends on chlorophylls, which are the pigments responsible for absorbing the light energy used in the photochemical phase of photosynthesis (Rosa et al. 2020). The decrease in chlorophyll contents under water stress is mostly the result of the damage caused to chloroplasts by ROS (Smirnoff 1993). In our study, under water deficit conditions, common beans plants showed a drop in chlorophyll levels. However, at 10 days after rehydration (10AR), an increase was found in the chlorophylls in plants submitted to water shortage (WI) and to AQ (*Asterarcys quadricellularis* - CCAP 294/1) sprays at concentration of 1.0 mL L⁻¹ (WI+AQ1.0), demonstrating a beneficial effect of the microalgae, so that the damage caused to the photosynthetic apparatus was mitigated after rehydration. This beneficial effect of microalgae could be attributed, at least in part, to glycine betaine (Khan et al. 2009), and other amino acids in the AQ such as glutamic acid that participate of chlorophyll synthesis and adaptation to abiotic stresses (Marques et al. 2021). There was also an increase in chlorophyll levels in plants without water deficit by AQ application, demonstrating the effect of microalgae in the synthesis of chlorophyll, which was also observed in potato (Cordeiro et al. 2022a) and onion (Cordeiro et al. 2022b), effects also reported to be related to the content of free L-amino acids in the AQ biomass.

Carotenoids are substances involved in the plant's defense response under water stress conditions, through their role as antioxidants in the reduction and/or elimination of ROS (Zhang et al. 2020). Thus, the increase in carotenoid levels in plants of the cultivar IAC treated with the microalgae in treatments WI by AQ0.5 and AQ1.0 on the third day after the start of water restriction (3RE) demonstrates the potentiation of carotenoids synthesis triggered by AQ.

The effect on improving parameters related to foliar pigments was also observed with the use of other microalgae from the *Chlorophyta* division, such as *Chlamydomonas reinhardtii* and *Chlorella* sp. (Gitau et al. 2022).

The increment in the sugar levels at 3RE, in WI by AQ0.5 and AQ1.0, suggests the activation of one of the plant's defense mechanisms against water stress. Under stress, plants accumulate sugars as a form of defense, acting in the perception, signaling, and regulation of gene expression, ensuring osmotic

adjustment responses and maintaining cellular energy through carbon partition (Saddhe et al. 2020).

For osmotic adjustment, sugars act in the reduction of the osmotic potential of the plant cell, which attracts water into the cell and helps with the turgor maintenance. Through osmotic adjustment, organelles and cytoplasmic activities occur at a normal rate and aid plants perform better in terms of photosynthesis (Farroq et al. 2009). In this sense, Sperdouli and Moustakas (2012) found photosynthetic acclimatization by the increase of soluble sugars in *Arabidopsis thaliana* leaves for the onset of water stress. The increase in non-reducing sugar levels, specifically sucrose, in plants under stress may function as a protective agent related to antioxidant activities such as ROS elimination (Bolouri-Moghaddam 2010). Thus, the increase in the levels of non-reducing sugars in WI by AQ0.5 for the BRS cultivar are related to the increase in the antioxidant defenses of the plants stimulated by *Asterarcys quadricellularis*

The application of microalgae in plants increases the level of proteins (Alvarez et al. 2021). One of the defense mechanisms of plants under stress is related to the increase in the levels of specific proteins, which have EROS-sequestering functions with consequent protection of cell membranes (Sun et al. 2013). Our studies showed an increase in protein levels in WI by AQ0.5 and AQ1.0 at 3RE, indicating the activation of this plant defense mechanism for adaptation under water deficit. This finding is consistent with that found by Abd El-Baky et al. (2008) in which the application of the microalgae *Chlorella ellipoida* and *Spirulina maxima* in wheat plants stressed during the growth stages led to an increase in endogenous protein synthesis, as also found by Lara et al. (2022) using AQ to growth tomatoes, relating the improvements in plants proteins levels to the free L-amino acid contents in AQ biomass.

Over the water shortage period, the levels of amino acids increased, including proline, which contributed to the osmotic adjustment of cells, one of the plant's responses to water stress (Hayat et al. 2012). Beans are an accumulator of amino acids in response to water stress (Andrade et al. 2016) and proline, with several reports demonstrating increases in this amino acid (Rosales et al. 2012; Morosan et al. 2017; Arteaga et al. 2020). A rapid reduction of proline was observed after rehydration (1AR), which demonstrates the fast alteration of the plant's metabolism. Considering the total free amino acids, the decrease was slower, only being

observed at 10AR when comparing plants submitted to water deficit with those without water deficit. This increase in total free amino acids is even greater in plants treated with AQ in WI by AQ0.5 and AQ1.0, demonstrating a rise triggered by microalga in plants endogenous amino acid synthesis under water shortage condition.

The antioxidant enzymes POD, SOD, and CAT play an important role in the ROS elimination (Gill and Tuteja 2010), which demonstrates a clear activation of mechanisms that aim to reduce the oxidative stress caused by water restriction

In WI, by AQ sprays (AQ0.5 and AQ1.0), an increase was found in POD in the cultivar IAC and CAT in both cultivars at 3RE, while at 1AR the CAT additions remained, demonstrating that AQ enhances the activity of these complex of antioxidant enzymes, contributing to the ROS scavenging. This effect also was found with the application of the microalgae *Chlorella vulgaris* in broccoli plants subjected to water restriction also promoted an increase in the levels of antioxidant enzymes (Kusvuran 2021).

At 10AR for WI+AQ0.5 in the IAC cultivar, there was an increase in the levels of POD and SOD and for WI+AQ1.0 in the BRS cultivar, the levels of POD increased, demonstrating that the application of AQ also raises the action of antioxidant enzymes in the recovery of the plant after the period of water stress, eliminating the ROS that may still be causing damage to plants.

The data presented in this work indicated that the application of AQ through foliar sprays increases the tolerance levels of common bean plants under water deficit conditions. Although at 3RE it was already possible to see a significant decrease in dry mass and fresh mass of plants under water restriction, at 10AR, for WI+AQ1.0, an increase was found in fresh mass and leaf area, demonstrating that the microalga interfered reducing water losses from plant tissues, contributing to the mitigation of water stress.

Thus, plant growth and recovery were faster, probably due to protective biochemicals attributed to photosynthetic trimming and antioxidant activities attributed to free L- amino acids in microalgae (Mógor et al. 2022). The recovery of the plant after the period of water deficit is an important gain, considering that climate changes have been causing an increase in the periods without rainfall.

The AQ applications also improved the amount of water in the plant at 10AR for WI+AQ1.0 with increases in fresh/dry mass ratio. These results are similar to

those obtained by Faheed and Fattah (2008) who also observed an increase in water content with the application of the microalgae *Chlorella vulgaris* in lettuce plants. Water stress causes a reduction in water in cells and a consequent reduction in leaf thickness, and consequently a decrease in mesophyll thickness (Otto et al. 2017), similar to our study, where there was a reduction in the thickness of the leaf and its constituent tissues in plants not treated with AQ. In AQ-treated plants, there was no difference between WO control and WI+AQ1.0, indicating that the AQ was efficient to keep plant growth despite the water shortage, as shown in Fig. 4.

5. CONCLUSION

The microalgae *A. quadricellularis* CCAP 294/1 triggered biochemical changes for the IAC cultivar with the increase in the levels of chlorophyll, carotenoids, total and reducing sugars, proteins, amino acids, and the enzymatic activity of POD, SOD, and CAT. In the BRS cultivar, the levels of chlorophyll, total, reducing, and non-reducing sugars, proteins, amino acids, and the enzymatic activity of POD and CAT were increased. The recovery of plants after the deficit period, found from the biometric and anatomical parameters was observed in both cultivars, with more promising results for the 1.0 mL L⁻¹ dose. The results demonstrate that the microalga mitigate the deleterious effects of water deficit in common bean plants, effect that may be related, at least in part, to its free L-amino acid content, standing out as a potential source to reduce the damages of water shortage under changing environment.

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FINAL CONSIDERATIONS

The biofertilizer market is on the rise, due to the search for sustainable alternatives to the challenges encountered in agriculture, such as abiotic stresses. There are several studies in the literature demonstrating the use of microalgae in agriculture as a biofertilizer product, with benefits such as increased crop growth and productivity, as well as a role in mitigating abiotic stresses. However, studies on the microalga *Asterarcys quadricellulare* as a biofertilizer are recent, with its main results in promoting the growth of cultivated plants such as potatoes, onions and tomatoes. But its effect on mitigating abiotic stresses is unprecedented.

The use of *Asterarcys quadricellulare* biomass (CCAP 294/1) stimulated bean and melon plants under stress conditions through antioxidant defense systems such as the enzymes POD, SOD and CAT, non-reducing sugars, carotenoids, proteins and phenolic compounds, as well as the synthesis of protective substances such as proline. The results show that the microalgae attenuates the deleterious effects of water deficit in bean plants and salinity damage in melon plants, an effect that may be related, at least in part, to the content of free L-amino acids present in this microalgae.

In view of the beneficial effects of the use of microalgae, further work should be carried out with studies in field conditions, gene expression studies due to the difference in the form of response of the cultivars studied, in addition to the search for the signaling action of microalgae, through transcriptomics.

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