

EFEITOS BIOATIVOS DO EXTRATO DE *Gracilaria birdiae* NO METABOLISMO DO TOMATE-CEREJA EM CONDIÇÕES DE DEFICIT HÍDRICO

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RESUMO: O crescimento populacional impacta diretamente a demanda agrícola, exigindo adaptações constantes nas práticas de produção de alimentos. Entre os principais fatores limitantes da produtividade das culturas estão os estresses abióticos, especialmente a escassez de água, que se intensificou nas últimas décadas devido às instabilidades climáticas. Nesse contexto, bioestimulantes derivados de macroalgas surgem como alternativas naturais promissoras para estimular o crescimento vegetal, melhorar a produtividade e aumentar a tolerância ao estresse abiótico. Com objetivo investigar as alterações metabólicas induzidas pelo extrato aquoso da alga vermelha *Gracilaria birdiae* como estratégia para mitigar os efeitos adversos do estresse hídrico moderado em plantas de tomate-cereja. As plantas foram cultivadas em mistura de areia, composto orgânico e vermiculita (1:1:1) e submetidas à restrição hídrica de 40% da capacidade de vaso durante 15 dias. O perfil metabólico dos tratamentos foi obtido por CG-EM. Os resultados demonstraram que as plantas de tomate submetidas à deficiência hídrica e tratadas com o extrato de *G. birdiae* na concentração de 1,0 g.L⁻¹ apresentaram mitigação significativa dos efeitos causados pelo déficit hídrico na raiz. Além disso, foi observado o acúmulo de metabólitos específicos no grupo tratado com 1,0 g. L⁻¹ que contribuíram para um padrão metabólico semelhante ao observado em plantas não estressadas, indicando o potencial do extrato em promover resiliência metabólica sob condições de déficit hídrico.

PALAVRAS-CHAVE: Bioinsumos, agricultura verde, biofertilizante, bioestimulante, metabólitos primários.

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BIOACTIVE EFFECTS OF *Gracilaria birdiae* EXTRACT ON CHERRY TOMATO METABOLISM UNDER WATER DEFICIT CONDITIONS

ABSTRACT: Population growth directly impacts agricultural demand, requiring constant adaptations in food production practices. Among the main limiting factors of agricultural productivity are abiotic stresses, especially water scarcity, which has intensified in recent decades due to climate instability. In this context, biostimulants derived from macroalgae emerge as promising natural alternatives to stimulate plant growth, improve productivity, and increase tolerance to abiotic stress. To investigate the metabolic changes induced by the aqueous extract of the red alga *Gracilaria birdiae* as a strategy to mitigate the adverse effects of moderate water stress in cherry tomato plants. The plants were cultivated in a mixture of sand, organic compost, and vermiculite (1:1:1) and subjected to a 40% water restriction of pot capacity for 15 days. The metabolic profile was analyzed by GC-MS. The results demonstrated that cherry tomato plants subjected to water deficit and treated with the *G. birdiae* extract at a concentration of 1.0 g.L⁻¹ showed significant mitigation of the effects caused by water deficit in the roots. Furthermore, the accumulation of specific metabolites in the group treated with 1.0 g.L⁻¹ contributed to a metabolic profile similar to that observed in non-stressed plants, indicating the potential of algal extract to promote metabolic resilience under water deficit conditions.

KEYWORDS: Bio-inputs, green agriculture, biofertilizer, biostimulant, primary metabolites.

INTRODUCTION

According to projections, the global population will grow exponentially from 7.8 to 9.7 billion by 2050 (United Nations, 2022), leading to an increasing demand for food amid the challenges posed by climate change and the need for environmental sustainability. However, current agricultural production may not be sufficient to meet the demands of this growing population (De Wrachien; Schultz & Goli, 2021). The use of mineral fertilizers, when compared to organic fertilization practices, can lead to reduced water retention and soil fertility (Maltas et al., 2018). Additionally, they often contain contaminants such as heavy metals derived from the extraction process (Rahman & Zhang, 2018). These concerns underscore the need to develop new fertilizers that enhance crop productivity while promoting environmental conservation (Kaur & Purewal, 2019). Consequently, there is a growing demand for strategies that strengthen domestic fertilizer production and introduce alternative inputs capable of

ensuring both food and environmental security. The application of microalgae-based biofertilizers to seeds has shown promising results, particularly in enhancing germination rates (Bumandalai & Tserennadmid, 2019). Besides, foliar spraying stimulates plant growth (Supraja; Behera & Balasubramanian, 2020). Beyond direct effects on plant physiology, seaweed extracts also play a broader role in the soil–water–plant continuum by serving as a substrate for microbial communities and enhancing soil organic matter. These changes improve the retention of water and nutrients in the soil, thereby supporting plant development and productivity (Górka et al., 2018). In addition to their preventive effects against stress conditions, macroalgae extracts can also enhance fruit Brix levels, an important quality indicator for the food industry, as observed with the foliar application of a mixture of two algae species (*Laminaria digitata* and *Ascophyllum nodosum*) on two tomato cultivars (Abidi; Snoussi & Bradea, 2021). However, further studies are needed to understand how these extracts alter plant metabolism and to optimize their application effectively. Once this gap in the literature was identified, the objective of this study is to investigate the use of *Gracilaria birdiae* extract as a metabolic modulator during the early growth of tomato plants under both irrigated and water deficit conditions.

MATERIALS AND METHODS

The germination of cherry tomato plants took place in a greenhouse, using seedling trays filled with vermiculite. The seeds were irrigated with desalinated water during the first seven days. On the eighth day after sowing, the plants were irrigated with half-strength Clark nutrient solution (Clark, 1975) for ten days. Subsequently, the plants were transferred to pots containing organic compost, sand, and vermiculite (1:1:1). For one week, they were maintained under irrigation equivalent to 80% of the field capacity (FC) for acclimatization. On the 28th day after sowing, the algal extract was prepared following literature and resuspended in deionized water (Pereira et al., 2025), at a concentration of 1.0 g/L, and 20 mL was applied to the soil. The plants were irrigated daily for another seven days, maintaining 80% of pot capacity (WW). On the 35th day after germination, irrigation was suspended for half of the plants until the soil reached 40% (WD), which was maintained until the 50th day, when samples were collected. Primary metabolite extraction was carried out using a methanol, chloroform, and ultrapure water solution (2:1:2, v/v/v), following a protocol adapted from Lisec et al. (2006). Samples were derivatized using N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA). The solution

components were separated and analyzed by gas chromatography coupled to mass spectrometry (GC-MS, QP-PLUS 2010, Shimadzu, Japan), using split mode (1:5). The experiment followed a 2×2 factorial design, testing two concentrations of the lyophilized extract: 0.0 g/L (negative control) and 1.0 g/L, combined with two water availability regimes: WW and WD. Each treatment included three replicates. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Tukey's test ($p \leq 0.05$) in R software. The data obtained in metabolomic were analyzed using Xcalibur™ 2.1 software; graphs were generated with MetaboAnalyst.

RESULTS AND DISCUSSION

The principal component analysis (PC1 and PC2) for leaves (Figure 1A) explained 55.4% of the total variation, showing a clear separation between irrigated and drought treatments. However, it was not possible to distinguish between algae extract-treated and untreated plants. In contrast, the analysis of root samples (Figure 1B) accounted for 53.5% of the variation, presenting an overlap among the WW treatments, and a spatial separation between WD treatments with and without algae extract. Notably, the proximity of the WD 1.0 g/L treatment to the WW cluster suggests that the algae extract induced significant metabolic changes in the roots, making the treated roots more similar to those that had not experienced water deficit. Among the metabolites that influenced this metabolic modulation, amino acids such as isoleucine, leucine, alanine, and serine stood out. It showed a similar pattern with WW 0.0 g/L group, highlighting their importance for stress resilience.

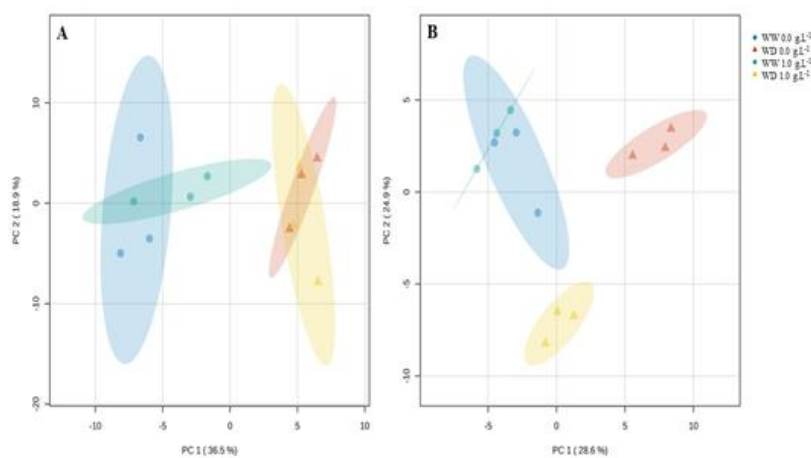


Figure 1. Principal component analysis (PCA) of metabolite profiles in (A) leaf and (B) root samples of plants subjected to different irrigation regimes and algae extract treatments (0.0 g.L^{-1} and 1.0 g.L^{-1}).

Following the PCA analysis, it became evident that the metabolic responses in the roots played a relevant role in the plant's adaptation to water deficit stress. Examination of the heatmap for the 25 most significant metabolites in the networks (Figure 2) revealed that water deficit conditions strongly shaped the metabolic profile. The proximity of the WD 1.0 g·L⁻¹ treatment to WD 0.0 g·L⁻¹ suggests that the extract application only partially mitigated the effects of water deficit, without fully restoring the metabolic profile to that of well-watered plants. Metabolites positively modulated under stress conditions included ribose, glucopyranoside, glycerol, and adipic acid, whereas well-watered conditions were associated with increased levels of ribose-5-phosphate, hexaric acid, quinic acid, galactonic acid, and xylose. The accumulation of free sugars and organic acids under low water availability may be related to osmoregulation, as well as to shifts in primary metabolism components (e.g., ribose-5-phosphate), reflecting a reallocation of metabolic priorities in energy pathways under stress. Application of the 1.0 g·L⁻¹ extract reduced the levels of carbohydrates and some organic acids compared with the untreated stress condition. This suggests a partial recovery from stress; however, the effect was insufficient to reestablish the metabolic state observed under control conditions.

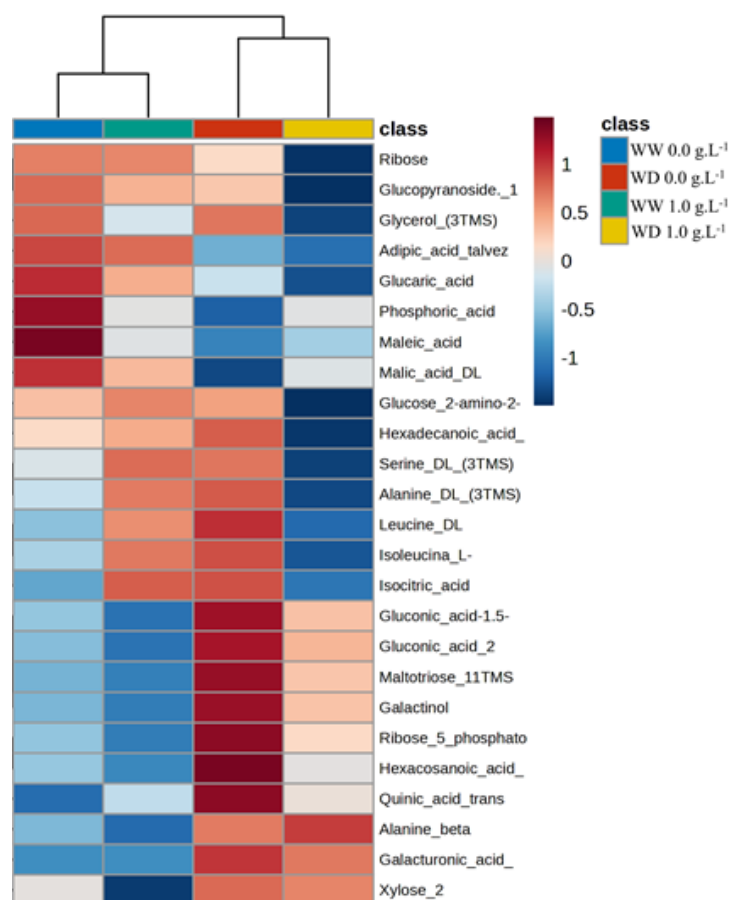


Figure 2. Heatmap of 25 metabolites with greater relevance using the T-test in roots samples of plants subjected to different irrigation regimes and algae extract treatments (0.0 g·L⁻¹ and 1.0 g·L⁻¹).

The potential benefits associated with the use of liquid seaweed extracts have been reported even at low dosages (SARKAR et al., 2018). The efficacy of such extracts lies in their ability to stimulate plant growth, increase the abundance of flowers, fruits, and roots, and enhance plant tolerance to salinity, drought, and high temperatures (BHATTACHARYYA et al., 2015). The results of this study indicate that the metabolic changes induced by the *G. birdiae* extract led to a distinct profile compared to the water deficit condition, corroborating previous reports that biochemical alterations are evident under such treatments (ANJUM et al., 2011). Moreover, morphological changes were also apparent in our experiment (Figure 3).

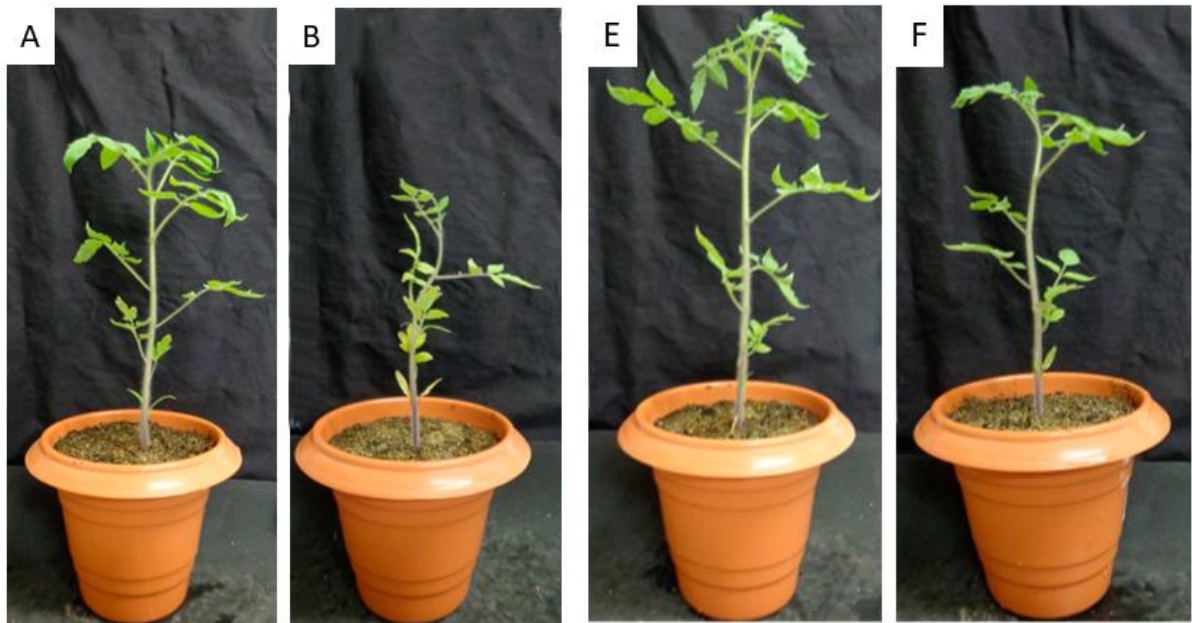


Figure 3. Cherry tomato plants 50 days after germination. A - WW 0.0 g/L; B - WD 0.0 g/L; E - WW 1.0 g/L; F - WD 1.0 g/L

CONCLUSIONS

The PCA and Heatmap shows that plants under water deficit have a very distinct metabolic profile compared to well-watered plants. Applying the $1.0 \text{ g}\cdot\text{L}^{-1}$ extract under stress slightly shifted the profile, mainly by lowering some free sugars and organic acids that were high during drought. However, this change was not enough to bring the plants back to the same metabolic state as the controls, suggesting that while the extract helps, its effect is only partial. The heatmap shows that plants under water deficit have a very distinct metabolic profile compared to well-watered plants. Applying the $1.0 \text{ g}\cdot\text{L}^{-1}$ extract under stress slightly shifted the profile, mainly by lowering some free sugars and organic acids that were high during drought. However, this change was not enough to bring the plants back to the same metabolic

state as the controls, suggesting that while the extract helps, its effect is only partial. In conclusion, the *G. birdiae* seaweed extract demonstrated the ability to alter the root metabolism of cherry tomato plants under stress conditions, allowing it to resemble control conditions. Although the same effect was not observed in the leaves, it is possible that with additional applications, full metabolic recovery could occur in these tissues. Overall, the extract also favored amino acid metabolism, highlighting the role of nitrogen metabolism in enhancing stress tolerance.

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