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Foliar application of a Trichormus sp. lysate modifies leaf sugar, protein, and redox status in NaCl-treated tomato (Solanum lycopersicum cv. Micro-Tom)

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Research Article

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Posted Date: November 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11557179/v1

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Additional Declarations: No competing interests reported.

Foliar application of a *Trichormus* sp. lysate modifies leaf sugar, protein, and redox status in NaCl-treated tomato (*Solanum lycopersicum* cv. Micro-Tom)

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Abstract

Soil salinization affects approximately 1.4 billion hectares worldwide and limits the productivity of salt-sensitive crops such as tomato (*Solanum lycopersicum* L.). Cyanobacterial preparations have been proposed as biostimulants to improve salt tolerance, but the changes in the plant redox status they induce remain poorly understood. We evaluated the effects of foliar application of a cell lysate of *Trichormus* sp., a cyanobacterium from the Atacama Desert, on tomato cv. Micro-Tom grown with and without 200 mM NaCl, using four experimental groups: control (C), lysate (T), NaCl (S), and lysate plus NaCl (TS). Leaf pigments, sucrose, soluble protein, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), antioxidant enzyme activities, and growth and reproductive parameters were measured. NaCl increased H_2O_2 and MDA contents by 139% and 34%, respectively, and increased monodehydroascorbate reductase and glutathione reductase activities. Without NaCl, the lysate increased H_2O_2 content by 121% and guaiacol peroxidase activity by 76%, but lowered MDA content by 55%. Under NaCl, the lysate lowered MDA content by 28% relative to S, to control levels, without modifying the NaCl-induced changes in H_2O_2 content and enzyme activities, and increased sucrose content by 44% relative to S. Neither NaCl nor the lysate affected pigment content, growth, or reproductive parameters. These results show that the *Trichormus* sp. lysate modifies tomato leaf metabolism even when growth is not affected, supporting its further evaluation as a biostimulant for salt-affected crops.

Key index words: antioxidant enzyme activities; cyanobacteria; hydrogen peroxide; leaf pigments; lipid peroxidation; salinity.

Introduction

Soil salinization is one of the main constraints on agricultural productivity, particularly in arid and semi-arid regions, where high evaporation, low rainfall, and irrigation with brackish water promote the accumulation of soluble salts in the root zone (Hopmans et al. 2021; Shokri et al. 2024; FAO 2024). Tomato (*Solanum lycopersicum* L.), one of the most important horticultural crops worldwide (FAO 2025), is moderately sensitive to salinity: yield declines above a soil electrical conductivity of about 2.5 dS m^{-1} (~25 mM NaCl), at a rate of roughly 10% per additional dS m^{-1} (Saranga et al. 1991; Foolad 2004; Gálvez et al. 2012). Salinity reduces flowering, fruit set, and yield in tomato, and forces plants to redistribute resources among growth, reproduction, and defense (Guo et al. 2022; Roșca et al. 2023; Wang et al. 2023).

The negative effects of salinity result from the combined disruption of several homeostatic systems. The osmotic component lowers the water potential of the root zone, limiting water uptake and cell expansion; the accumulation of Na^+ and Cl^- then disrupts ionic homeostasis and the uptake of essential nutrients such as K^+ and Ca^{2+}; and the resulting metabolic imbalance increases the production of reactive oxygen species (ROS), which damage lipids, proteins, and nucleic acids (Munns and Tester 2008; dos Santos et al. 2022; Zhang et al. 2022). Plants counteract these effects

by accumulating compatible solutes such as trehalose and sucrose, by compartmentalizing Na^+, and by activating antioxidant systems, including superoxide dismutase, catalase, peroxidases, and the enzymes of the ascorbate–glutathione cycle (Munns and Tester 2008; Hasanuzzaman et al. 2021; Noctor 2025). ROS, particularly hydrogen peroxide (H_2O_2), also act as signals that trigger acclimation responses, and oxidative damage arises when their accumulation exceeds the plant's buffering capacity (Miller et al. 2010; Minkov et al. 2026). Markers of membrane damage such as malondialdehyde (MDA) are therefore needed, alongside ROS contents, to distinguish oxidative signaling from oxidative stress.

The Micro-Tom cultivar is a well-established model for salinity research owing to its small size, short life cycle, and capacity to activate adaptive responses across a wide range of NaCl concentrations (Bacha et al. 2017; Alves et al. 2021; Hoffmann et al. 2021). In Micro-Tom, increasing NaCl concentrations reduce plant height, chlorophyll content, and photosynthetic capacity (Leiva-Ampuero et al. 2020; Hoffmann et al. 2021). Hydroponic cultivation is well suited to such studies because it allows homogeneous and precise application of NaCl treatments while avoiding soil-derived variability (Pailles et al. 2020).

Among sustainable strategies to improve salinity tolerance, biostimulants have gained increasing attention (Rouphael and Colla 2020; Johnson et al. 2024). Plant biostimulants are defined by their effects rather than by their composition, as substances or microorganisms that, independently of their nutrient content, stimulate plant physiological processes to improve nutrient use efficiency, tolerance to abiotic stress, or crop quality (du Jardin, 2015; Regulation (EU) 2019/1009, 2019). This functional definition reflects a central limitation of the field: most biostimulants are complex mixtures of undefined composition, and their use is usually supported by agronomic outcomes, such as growth or yield, without knowledge of which components are active or which plant processes they modify (Yakhin et al. 2017; Backer et al. 2018). Consequently, the physiological basis of their effects remains largely unknown, which makes it difficult to predict their effects across crops and stress conditions and to standardize their production (Yakhin et al. 2017; Santini et al. 2021). Recent reviews propose that many biostimulants act by modulating the plant's own redox and hormonal signaling rather than by directly supplying nutrients or protective compounds (Abdel Latef et al. 2026; Minkov et al. 2026), but for most preparations this has not been tested experimentally. Addressing this knowledge gap requires evaluating, in a single experimental system, the effects of a biostimulant on plant performance together with the metabolic and redox processes that may underlie stress tolerance, such as compatible solutes accumulation, ROS content, membrane damage, and antioxidant enzyme activities.

Cyanobacteria a diverse group of photosynthetic organism existing in a broad range of habitats (Tiddia et al. 2026), are a promising source of biostimulants because they synthesize a wide range of bioactive molecules, including compatible solutes, amino acids, polysaccharides, phytohormones, and antioxidants (Toribio et al. 2020; Santini et al. 2021). Cell lysis improves the recovery of intracellular and cell wall-bound compounds, and foliar spraying is the most common application route (Ronga et al. 2019; Santini et al. 2021). Cyanobacterial preparations have mitigated salt stress in several crops. In tomato, a microalgae–cyanobacteria extract improved growth, K^+ uptake, and proline accumulation, and reduced lipid peroxidation under NaCl (Mutale-joan et al. 2021); similar effects have been reported in rice and wheat (Touzout et al. 2025; Paul et al. 2026). Nevertheless, the physiological mechanisms through which cyanobacterial lysates may improve salt tolerance remain poorly characterized, and in particular it is unclear whether they act as nutritional inputs, as direct sources of protective compounds, or as signals that modulate the plant's redox and metabolic responses (Sneha et al. 2021; Chabili et al. 2025).

Trichormus sp. is a filamentous cyanobacterium isolated from biological soil crusts of a semi-arid region at the southern limit of the Atacama Desert, Chile, an environment expected to select for stress-tolerance traits (Dahech et al. 2021; Marzban and Tesei 2025). Culture supernatants of this strain contain trans-zeatin and promote the growth of the grass *Polypogon australis* (Pontigo 2023). Its genome carries genes for the biosynthesis of proline, glycine betaine, trehalose, and γ-aminobutyric acid (GABA) (BioProject PRJNA1136538), and trehalose, GABA, and proline have been quantified in its cell lysate, trehalose being the most abundant of the three (Abello et al. 2024). Each of these compounds has been associated with salt tolerance: exogenous trehalose and GABA alleviated salt damage in tomato by modulating ROS metabolism, osmolyte synthesis, and ion homeostasis (Wu et al. 2020; Yang et al. 2022), and cyanobacteria-derived proline increased stress tolerance in *Arabidopsis thaliana* (Chua et al. 2020). However, the effects of *Trichormus* sp. cell lysates on crop plants, and in particular under salinity, have not been evaluated.

In this study, we evaluated the effects of foliar application of a *Trichormus* sp. cell lysate on tomato cv. Micro-Tom grown with or without 200 mM NaCl, and asked which of these effects depended on NaCl to determine if the cyanobacterial extract acts as an effective biostimulant to mitigate salt stress and promote sustainable plant growth by maintaining the redox status of the treated plants.

Materials and methods

Cyanobacterial strain and culture conditions

Trichormus sp. (Nostocales) was isolated from biological soil crusts of the Atacama Desert (Dahech et al. 2021) and deposited in the Chilean Collection of Microbial Genetic Resources under accession number CChRGM 3473. Its genome assembly (ASM5269260v1; BioProject PRJNA1136538) is available at NCBI under the name *Nodularia* sp. strain CChRGM 3473, and its formal taxonomic description is in progress. The strain was grown in BG11 medium (Sigma-Aldrich, Germany) adjusted to pH 7.0, inoculated at 10% (v/v) in 250 mL Erlenmeyer flasks closed with cotton and gauze plugs and aerated with a diaphragm pump (QL-348A, Quanlong) through 0.22 µm sterile filters. Flasks were incubated in an orbital shaker incubator (TOU-50, MRC; 25 mm orbit) at 120 rpm and 30 ± 1 °C, under a 16 h light/8 h dark photoperiod provided by white LEDs providing photosynthetic photon flux density at 60 µmol photons m^{-2} s^{-1}, following conditions adapted from Dahech et al. (2021). Growth was monitored as chlorophyll *a* (Chl *a*) concentration (Garg and Maldener 2021).

Preparation of the cyanobacterial lysate

Cultures were harvested in the late exponential phase (15 mg Chl *a* L^{-1}) by centrifugation at 5,000 *g* for 10 min (U-320R, BOECO; Xu et al. 2015). To remove residual culture medium, the cell pellet was washed twice with distilled water and centrifuged under the same conditions. The biomass was resuspended in 2 L of distilled water to 75 mg Chl *a* L^{-1} and immediately disrupted in a high-pressure homogenizer (PandaPLUS 2000, GEA Niro Soavi, Italy) at 100 MPa for one cycle. The lysate was frozen at −80 °C, lyophilized at −50 °C, and stored at room temperature in the dark. For application, the lyophilized lysate was resuspended in distilled water to 0.7 mg Chl *a* L^{-1}. Preliminary quantification of three compatible solutes in the lyophilized lysate yielded trehalose (309.75 ± 62.81 nmol mg^{-1}), GABA (127.53 ± 98.95 nmol mg^{-1}), and proline (112.97 ± 28.75 nmol mg^{-1} lyophilized lysate) (Abello et al. 2024).

Plant material and growth conditions

Seeds of *Solanum lycopersicum* L. cv. Micro-Tom (PanAmerican Seed, Netherlands) were surface-sterilized in 1% NaOCl for 2 min and rinsed twice with distilled water. Twelve seeds were sown per 9 cm Petri dish on autoclaved filter paper moistened with 7 mL of sterile distilled water (0 days after sowing, DAS); germination occurred at 4 DAS. At 8 DAS, two seedlings with fully

expanded cotyledons were transplanted into each pot (10 cm × 5 cm) filled with autoclaved perlite, and from 10 DAS onward, plants were sub-irrigated with 0.5× Hoagland solution (Hoagland and Arnon 1950). Plants were grown under a 15 h light/9 h dark photoperiod with LED lighting at 150 µmol photons m^{-2} s^{-1} at canopy level, 30 °C, and 70–75% relative humidity, conditions modified from Rothan et al. (2016).

Experimental design and experimental groups

The experimental setup was adapted from Campobenedetto et al. (2021). The experiment had a 2 × 2 factorial design with lysate application (lysate vs. mock spray) and salinity (0 vs. 200 mM NaCl) as factors, yielding four experimental groups: control plants (C), plants sprayed with cyanobacterial lysate (T), plants treated with 200 mM NaCl (S), and plants that received both treatments (TS). Because NaCl was supplied by sub-irrigation (bottom watering), each tray received a single treatment. Each experimental group comprised four trays holding 12 pots each, with two plants per pot. The tray was the experimental unit, and each biological replicate (n = 4) was obtained from a different tray. Trays were randomly positioned in the growth room and rotated weekly to minimize position effects; we therefore analyzed the experiment as a completely randomized design without a blocking factor.

Plants were sprayed with 8 mL per pot of lysate (0.7 mg Chl *a* L^{-1}) containing 0.01% (v/v) Tween 20; mock-treated plants received 0.01% (v/v) Tween 20. Sprays were applied within the first 3 h of the light period at 36, 43, 50, and 61 DAS. Salinity was imposed without gradual acclimation 24 h after the first lysate application (37 DAS, when plants had four to five leaf nodes) by sub-irrigating each tray three times per week with 1 L of 200 mM NaCl in 0.5× Hoagland solution (electrical conductivity, EC = 21 mS cm^{-1}); non-saline trays received 0.5× Hoagland solution (EC = 1.2 mS cm^{-1}). Throughout the experiment, trays were emptied and rinsed with tap water weekly to prevent salt accumulation. Salinity was maintained until 61 DAS (25 days), after which all trays were sub-irrigated with 0.5× Hoagland solution until the end of the experiment (88 DAS).

Growth and reproductive measurements

Plant height (from the stem base to the apical meristem) and the number of leaf nodes were recorded at 35 DAS (before the first lysate application) and at 75 DAS, 14 days after the end of the salinity period, and their increases were calculated as the difference between the two dates. Total flowers and fruits per plant were counted at 88 DAS, 27 days after the end of the salinity period.

Sampling and general assay conditions

At 62 DAS, 24 h after the last lysate application and 1 day after the last NaCl irrigation, the fifth and sixth true leaves (counted acropetally) of both plants in four pots per tray were collected and pooled into one composite sample per tray, frozen in liquid nitrogen, ground in liquid nitrogen, and stored at −80 °C. Aliquots of the same frozen powder were used for all analyses, and all extractions were performed on ice. Unless stated otherwise, assays were run in technical triplicate in 96-well UV-transparent microplates using a microplate reader (INNO, LTek, South Korea). Absorbance values were converted to a 1 cm path length using a correction factor of 1.8, determined empirically by comparing the absorbance of the same solutions in a 1 cm cuvette and in the microplate, before applying extinction coefficients.

Photosynthetic pigments

Chl *a*, chlorophyll *b* (Chl *b*), and carotenoids were extracted from 100 mg of frozen leaf powder with 80% (v/v) acetone. The extraction volume (20–40 mL) was adjusted so that absorbance remained within the linear range of the instrument, and the exact volume was recorded for each sample. A 1 mL aliquot of each extract was centrifuged at 14,000 *g* for 5 min at 4 °C, and the

absorbance of the supernatant was read in microplates at 663, 646, and 470 nm and corrected to a 1 cm path length. Pigment concentrations in the extract (µg mL⁻¹) were calculated according to Lichtenthaler (1987):

$$\text{Chl } a = 12.25\,A_{663} - 2.79\,A_{646}$$

$$\text{Chl } b = 21.5\,A_{646} - 5.1\,A_{663}$$

$$\text{Carotenoids} = \frac{[1000\,A_{470} - 1.82\,\text{Chl } a - 85.02\,\text{Chl } b]}{198}$$

where A_{663}, A_{646}, and A_{470} are the absorbances at 663, 646, and 470 nm, respectively. Pigment contents (mg g^{-1} FW) were then calculated as C × V / (1000 × W), where C is the pigment concentration in the extract (µg mL^{-1}), V is the extract volume (mL), W is the fresh weight of the sample (g), and 1000 converts µg to mg.

Soluble protein

Frozen tissue (150 mg) was homogenized in 1 mL of 50 mM phosphate buffer (pH 7.8) and centrifuged at 20,000 *g* for 25 min at 4 °C. The supernatant was mixed 1:1 with 50 mM sodium phosphate (pH 7.0), 2 mM EDTA, and 1% (w/v) polyvinylpyrrolidone (PVP). Protein was determined by the Bradford (1976) method using the A_{590}/A_{450} ratio to improve linearity (Zor and Selinger 1996). Protein was quantified against a bovine serum albumin standard curve and expressed as mg g^{-1} FW.

Sucrose and trehalose

Sucrose was quantified by a modified anthrone method (van Handel 1968). Soluble sugars were extracted from 100 mg of frozen tissue with 1 mL of 80% (v/v) ethanol and centrifuged at 10,000 *g* for 10 min, and the supernatant was dried at 40 °C for 24 h. The residue was resuspended in 100 µL of 30% (w/v) KOH and heated at 100 °C for 10 min to destroy reducing sugars. After cooling, 3 mL of anthrone reagent (150 mg anthrone in 100 mL of 72% H_2SO_4) was added, and samples were incubated at 40 °C for 15 min. Absorbance was read at 620 nm, and sucrose was quantified against a sucrose standard curve and expressed as µmol g^{-1} FW.

Trehalose was determined by the anthrone method of Li et al. (2014) with modifications. One gram of tissue was extracted with 5 mL of 80% (v/v) ethanol and centrifuged at 5,000 *g* for 20 min, and the supernatant was dried at 60 °C for 24 h and resuspended in 1 mL of deionized water. Sucrose and reducing sugars were removed by sequential hydrolysis with 100 µL of 0.2 N H_2SO_4 and oxidation with 75 µL of 0.6 N NaOH (100 °C, 10 min each). Then, 1 mL of anthrone reagent (0.05% w/v in 72% H_2SO_4) was added, samples were heated at 100 °C for 10 min, and absorbance was read at 630 nm. Trehalose was quantified against a trehalose standard curve and expressed as µmol g^{-1} FW.

Hydrogen peroxide

H_2O_2 was determined according to Junglee et al. (2014). Frozen tissue (150 mg) was homogenized in 1 mL of a solution containing 0.25 mL of 0.1% (w/v) trichloroacetic acid (TCA), 0.5 mL of 1 M KI, and 0.25 mL of 10 mM potassium phosphate buffer (pH 5.58) for 10 min at 4 °C, and centrifuged at 12,000 *g* for 15 min at 4 °C. Aliquots (200 µL) of supernatant were incubated for 20 min at room temperature, and absorbance was read at 350 nm. H_2O_2 was quantified against an H_2O_2 standard curve prepared in 0.1% TCA and expressed as µmol g^{-1} FW.

Lipid peroxidation

Lipid peroxidation was estimated as malondialdehyde (MDA) content. The supernatant obtained as in Section 2.8 (40 µL) was mixed with 160 µL of 20% (w/v) TCA containing 0.5% (w/v) thiobarbituric acid, incubated at 95 °C for 30 min, cooled on ice, and centrifuged at 10,000 *g* for 10 min. MDA was calculated from $A_{532} - A_{600}$ using ε = 155 mM^{-1} cm^{-1} (Heath and Packer 1968) and expressed as nmol g^{-1} FW.

Ascorbate–glutathione cycle enzymes

Ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) were extracted and assayed in microplates according to Murshed et al. (2008). The four enzymes were co-extracted by homogenizing frozen tissue (1 mL per 250 mg FW) in 50 mM MES/KOH (pH 6.0) containing 40 mM KCl, 2 mM $CaCl_2$, and 1 mM freshly prepared ascorbate (AsA). Homogenates were centrifuged at 14,000 *g* for 10 min at 4 °C, and supernatants were assayed immediately at 25 °C. Each assay consisted of 185 µL of reaction buffer, 10 µL of extract, and 5 µL of the reagent used to start the reaction (final volume, 200 µL).

APX (EC 1.11.1.11). The reaction buffer contained 50 mM potassium phosphate (pH 7.0) and 0.25 mM AsA. Non-specific AsA oxidation was recorded at 290 nm for 3 min before the reaction was started with 200 mM H_2O_2 (5 mM final). The decrease in A_{290} was recorded for 5 min (ε = 2.8 mM^{-1} cm^{-1}), correcting for non-specific AsA oxidation in the sample and for H_2O_2-dependent AsA oxidation in the absence of extract.

MDHAR (EC 1.6.5.4). The reaction buffer contained 50 mM HEPES (pH 7.6), 0.25 mM NADPH, and 2.5 mM AsA. After non-specific NADH oxidation was recorded, the reaction was started with 0.4 U of ascorbate oxidase per well to generate the monodehydroascorbate radical, and NADH oxidation was followed at 340 nm for 5 min (ε = 6.22 mM^{-1} cm^{-1}). The rate of non-specific NADH oxidation was subtracted.

DHAR (EC 1.8.5.1). The reaction buffer contained 50 mM HEPES (pH 7.0), 0.1 mM EDTA, and 2.5 mM reduced glutathione (GSH). The reaction was started with freshly prepared dehydroascorbate (DHA; 0.2 mM final), and the increase in A_{265} was recorded for 5 min (ε = 14 mM^{-1} cm^{-1}), correcting for non-enzymatic DHA reduction by GSH in the absence of extract.

GR (EC 1.8.1.7). The reaction buffer contained 50 mM HEPES (pH 8.0) and 0.2 mM NADPH. After non-specific NADPH oxidation was recorded, the reaction was started with 20 mM glutathione disulfide (GSSG; 0.5 mM final), and NADPH oxidation was followed at 340 nm for 5 min (ε = 6.22 mM^{-1} cm^{-1}). The rate of non-specific NADPH oxidation was subtracted.

Superoxide dismutase, catalase, and guaiacol peroxidase

Frozen tissue (1 g) was homogenized in 4 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1% (w/v) PVP and 2 mM EDTA and centrifuged at 10,000 *g* for 10 min at 4 °C, and the supernatant was stored at −80 °C until analysis (Ozden et al. 2009). Activities were determined following Taghipour et al. (2021), with the modifications to the SOD assay described below, adapted to 96-well microplates by scaling all volumes proportionally to a final volume of 200 µL per well, so that final reagent concentrations were unchanged.

SOD (EC 1.15.1.1). Activity was assayed as the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) in 50 mM potassium phosphate (pH 7.8), 13 mM L-methionine, 0.1 mM EDTA, 75 µM NBT, 2 µM riboflavin, and 10 µL of extract. Plates were incubated at 25 °C under white LED light of approximately 70–90 µmol photons m^{-2} s^{-1} (5,000 lx) for 15 min, following Xu et al. (2013). One unit (U) was defined as the amount of enzyme causing 50% inhibition of NBT reduction at 560 nm.

CAT (EC 1.11.1.6). Activity was measured as H_2O_2 decomposition at 240 nm for 1 min in 50 mM potassium phosphate (pH 7.0), 15 mM H_2O_2, and 10 µL enzyme extract, using ε = 36.6 M^{-1} cm^{-1}, and expressed as µmol H_2O_2 consumed min^{-1}.

POD (EC 1.11.1.7). Activity was measured as guaiacol oxidation at 470 nm for 1 min in 50 mM potassium phosphate (pH 7.0), 13 mM guaiacol, 5 mM H_2O_2, and 6.6 µL enzyme extract, using ε = 26.6 mM^{-1} cm^{-1}, and expressed as µmol guaiacol oxidized min^{-1}.

For all enzymes, soluble protein was quantified in a separate extract from the same frozen powder, and the activity of each replicate was normalized to its soluble protein content per gram of fresh weight. Activities are therefore expressed per mg protein.

Statistical analyses

The experiment was conducted once. Each biological replicate corresponded to one tray ($n = 4$ trays per experimental group). No a priori power analysis was performed; the number of replicates was determined by growth room capacity. For biochemical analyses, leaves from four pots (eight plants) per tray were pooled into one composite sample, and each sample was assayed in technical triplicate, whose mean was used as the value for that replicate. For growth and reproductive parameters, 14 plants (seven pots) per tray were measured and averaged per tray. Data were analyzed in R v. 4.3.0 (R Core Team 2023) using RStudio v. 2023.06.0. The four experimental groups were compared as a one-way layout. Normality of residuals was assessed visually with Q–Q and residual-versus-fitted plots (qqplotr and ggplot2 packages). Variables with approximately normal residuals (height increase per plant, number of fruits per plant, Chl *a*, Chl *b*, carotenoids, soluble protein, sucrose, trehalose, MDA, H_2O_2, POD, APX, MDHAR, and GR) were analyzed by Welch's ANOVA, which does not assume equal variances, followed by the Games–Howell post hoc test. Variables whose residuals deviated from normality (increase in leaf nodes per plant, number of flowers per plant, SOD, CAT, and DHAR) were analyzed with Monte Carlo permutation tests of independence (9,999 resamples; coin package), followed by pairwise permutation tests with Holm correction. All tests were two-sided, and the significance was set at $P \leq 0.05$. Effect sizes were estimated as η^2 for Welch's ANOVA, with the following formula:

$$\eta^2 = \frac{F \times df_1}{F \times df_1 + df_2}$$

where F is the Welch's ANOVA statistic, and df_1 and df_2 are its numerator and denominator degrees of freedom. And η^2 for permutation tests, with the following formula:

$$\eta^2 = \frac{\overline{Z^2}}{N}$$

where the numerator is the mean of the squared standardized permutation test statistics (Z^2), and N is the total number of observations, and classified as small ($0.01 \leq \eta^2 < 0.06$), medium ($0.06 \leq \eta^2 < 0.14$), or large ($\eta^2 \geq 0.14$) (Cohen 1988). Given the small sample size, effect sizes are reported for completeness but were not used to interpret non-significant differences. Letters indicating significant differences were assigned with the multcompView package. Figures show individual replicates and mean ± standard error (SE) and were produced with ggplot2 and ggpattern and assembled in Inkscape.

Results

Growth and reproductive parameters

Vegetative growth was assessed as the increase in plant height and in the number of leaf nodes between 35 and 75 DAS (Figure 1a, b). Height increase did not differ significantly among the experimental groups (Welch's ANOVA, $F_{3,6.30} = 1.15$, $P = 0.400$, $\eta^2 = 0.354$; Figure 1a). The increase in the number of leaf nodes was significant in the overall comparison among the four experimental groups (permutation test, $P = 0.018$, $\eta^2 = 0.342$; Figure 1b), but no pairwise comparison remained significant after Holm correction.

Reproductive parameters were assessed at 88 DAS. The number of flowers per plant differed significantly in the overall comparison among the four experimental groups (permutation test, $P = 0.033$, $\eta^2 = 0.360$; Figure 1c), but no pairwise difference remained significant after Holm correction. The number of fruits per plant did not differ among experimental groups (Welch's ANOVA, $F_{3,6.28} = 1.32$, $P = 0.350$, $\eta^2 = 0.386$; Figure 1d).

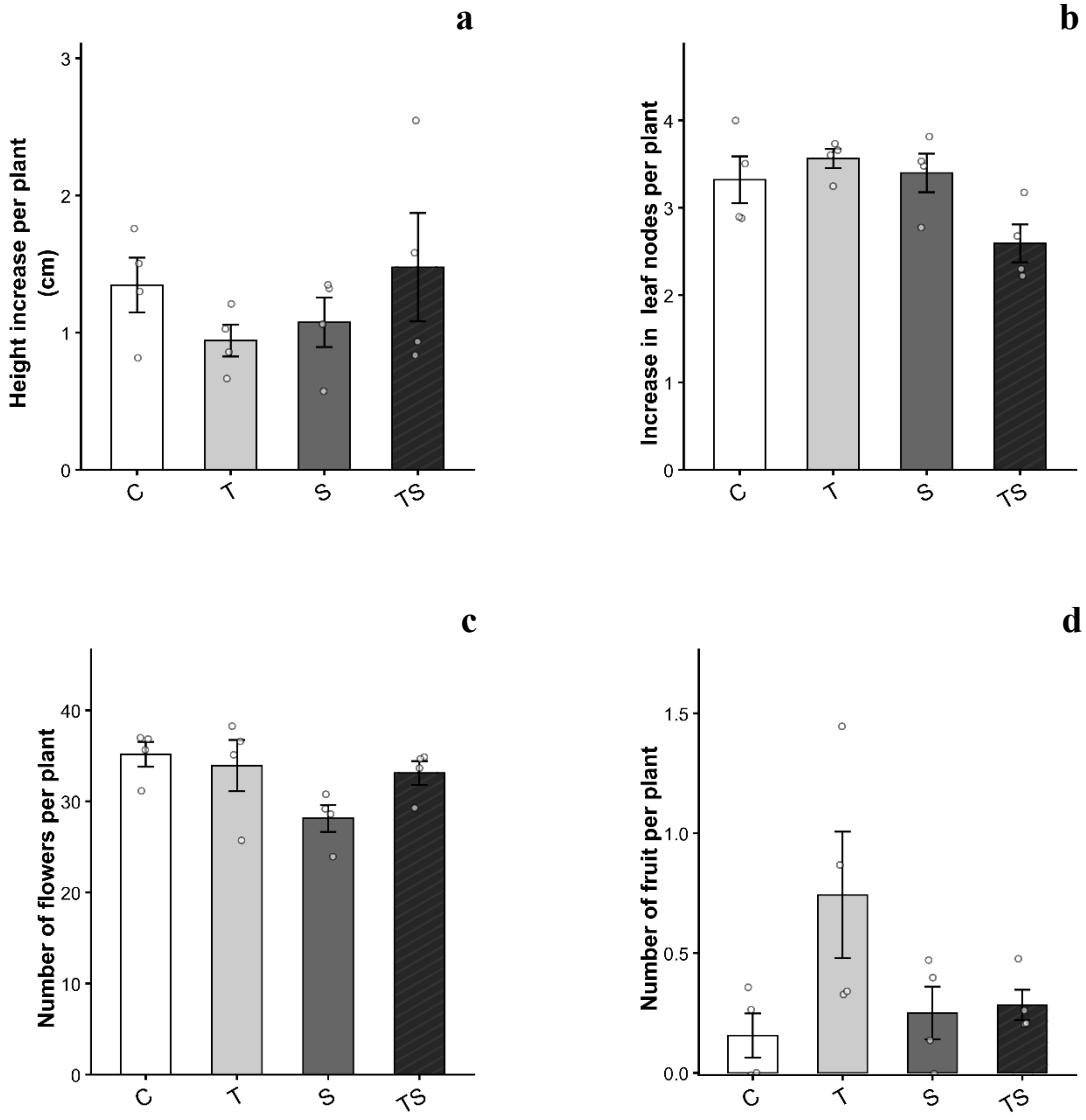


Fig. 1 Growth and reproductive parameters of *Solanum lycopersicum* cv. Micro-Tom plants with and without NaCl treatment and foliar application of a cyanobacterial lysate. (a) Increase in plant height and (b) increase in the number of leaf nodes between 35 and 75 days after sowing (DAS), and (c) number of flowers and (d) number of fruits per plant at 88 DAS. Data are presented as mean ± SE (n = 4 trays). Open circles represent individual biological replicates (tray means). Experimental groups: C (control), T (*Trichormus* sp. lysate), S (200 mM NaCl), and TS (*Trichormus* sp. lysate + 200 mM NaCl). Differences among groups were assessed by Welch's ANOVA (a, d) or by Monte Carlo permutation tests followed by pairwise permutation tests with Holm correction (b, c). In (b) and (c), the overall effect of experimental group was significant ($P < 0.05$), but no pairwise comparison was significant; in (a) and (d), no significant differences were detected ($P > 0.05$).

Photosynthetic pigments

Photosynthetic pigment content, chlorophylls (Chl) and carotenoids, did not differ among the four experimental groups. Neither the lysate nor the NaCl treatment changed the contents of Chl *a* in the treated plants (Welch's ANOVA, $F_{3,6.43} = 1.93$, $P = 0.220$, $\eta^2 = 0.474$), Chl *b* ($F_{3,6.44} = 1.95$, $P = 0.217$, $\eta^2 = 0.476$; Figure 2a), or carotenoids ($F_{3,6.38} = 2.96$, $P = 0.115$, $\eta^2 = 0.582$; Figure 2b).

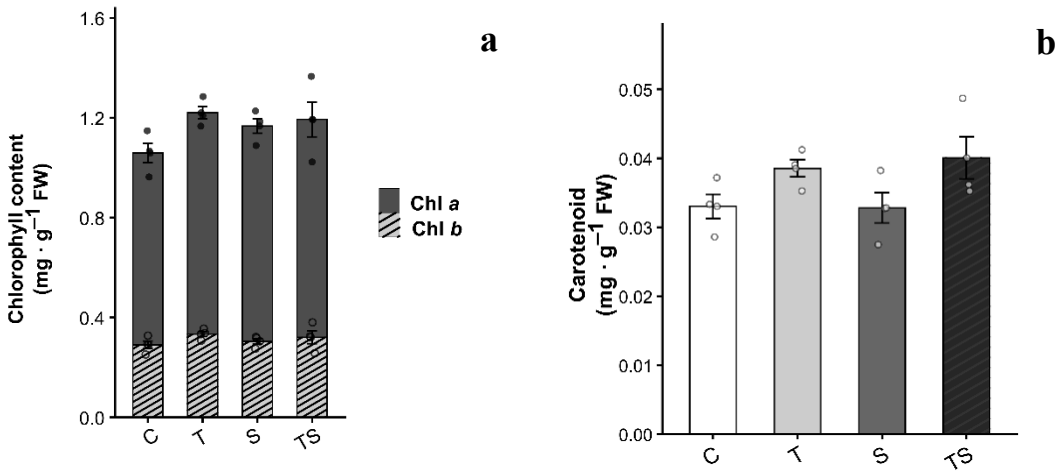


Fig. 2 Photosynthetic pigments in leaves of *Solanum lycopersicum* cv. Micro-Tom plants with and without NaCl treatment and foliar application of a cyanobacterial lysate, sampled at 62 days after sowing. (a) Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) contents, shown as stacked bars (Chl *a*, solid; Chl *b*, cross-hatched), and (b) carotenoid content. Experimental groups: control (C), *Trichormus* sp. lysate (T), 200 mM NaCl (S), and *Trichormus* sp. lysate + 200 mM NaCl (TS). Data are presented as mean ± SE (n = 4 trays) for each pigment. In (a), closed and open circles represent individual biological replicates for Chl *a* and Chl *b*, respectively; in (b), open circles represent individual biological replicates. Differences among groups were assessed separately for Chl *a*, Chl *b*, and carotenoids by Welch's ANOVA; no significant differences were detected (P > 0.05)

Soluble protein and sugars

Among plants treated with NaCl, those that received the lysate (TS) had more leaf soluble protein, although no experimental group differed from the control (Welch's ANOVA, $F_{3,6.48} = 31.01$, $P < 0.001$, $\eta^2 = 0.935$; Figure 3a). Protein content in the combined treatment (TS) was 48% higher than in plants treated with NaCl alone (S) ($P < 0.05$) and 30% lower than in plants treated with lysate alone (T) ($P < 0.05$). Sucrose content was 78% higher in the combined treatment (TS) than in control plants (C; $F_{3,5.64} = 21.00$, $P = 0.002$, $\eta^2 = 0.918$; Figure 3b). For trehalose content –a disaccharide that acts as an osmoprotectant and signaling molecule– the overall comparison among groups was significant ($F_{3,5.57} = 5.90$, $P = 0.036$, $\eta^2 = 0.760$), but the Games–Howell test did not detect significant differences between specific groups (Figure 3c).

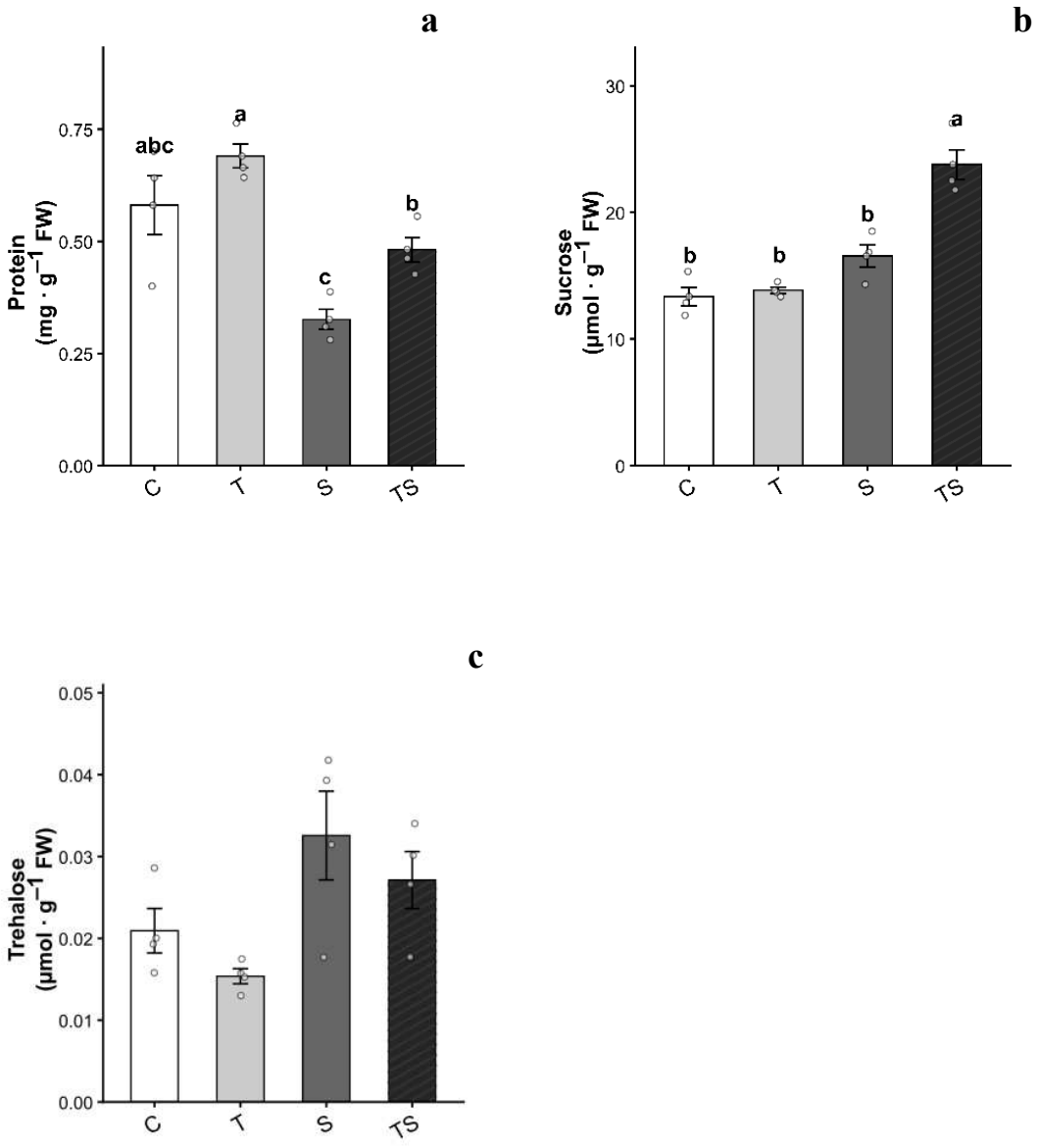
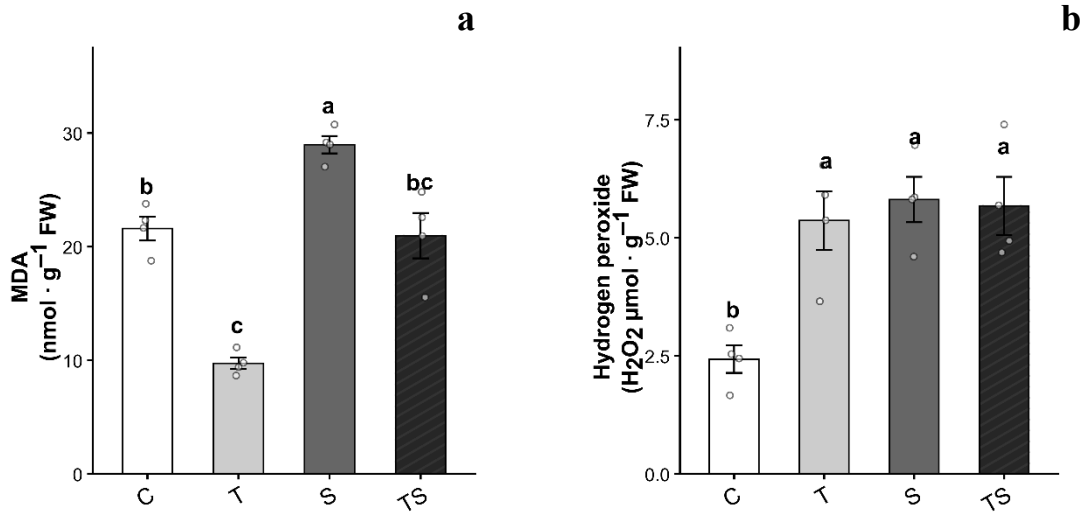


Fig 3 Soluble protein and sugar contents in leaves of *Solanum lycopersicum* cv. Micro-Tom plants with and without NaCl treatment and foliar application of a cyanobacterial lysate, sampled at 62 days after sowing. (a) Soluble protein, (b) sucrose, and (c) trehalose contents. Experimental groups: control (C), *Trichormus* sp. lysate (T), 200 mM NaCl (S), and *Trichormus* sp. lysate + 200 mM NaCl (TS). Data are presented as mean ± SE (n = 4 trays). Open circles represent individual biological replicates. Differences among groups were assessed by Welch's ANOVA followed by the Games–Howell post hoc test; means sharing a letter do not differ significantly ($P > 0.05$). In (c), the overall effect of experimental group was significant ($P < 0.05$), but no pairwise comparison was significant ($P > 0.05$)

Hydrogen peroxide and lipid peroxidation

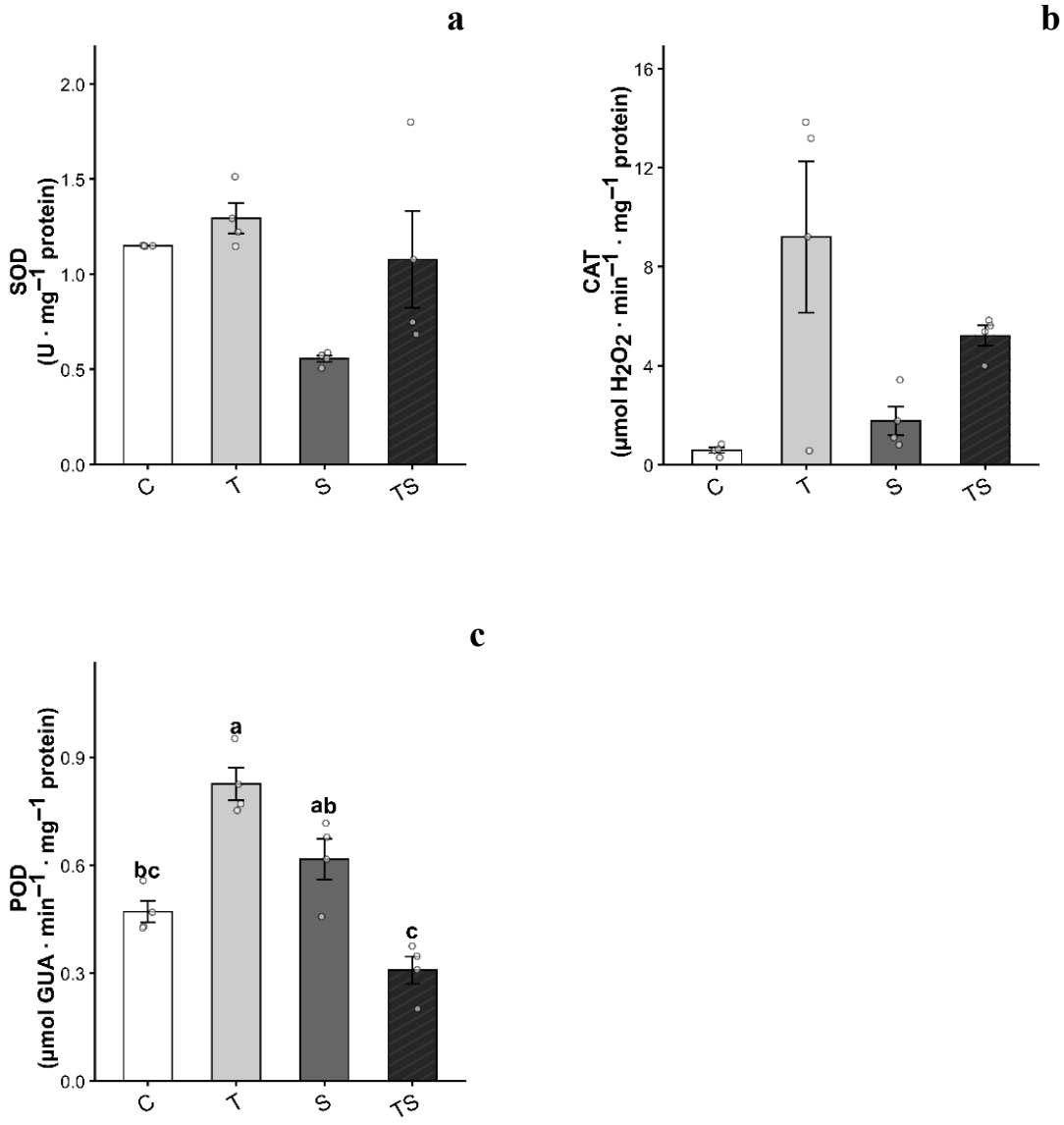
Regarding MDA content, plants treated with the cyanobacterial lysate alone (T) exhibited 55% lower MDA content compared to the control (C), whereas in plants submitted to the NaCl treatment (S), this content significantly increased by 34% (Welch's ANOVA, $F_{3,6.21} = 128.90$, $P < 0.001$, $\eta^2 = 0.984$; Figure 4a). Furthermore, the combined treatment of NaCl and lysate (TS) maintained MDA levels similar to those of the control (C) and the plants treated with the lysate alone (T), but was 28% lower than plants treated with NaCl alone (S). The content of H_2O_2 was 121% higher in plants treated with the lysate alone (T), 139% higher in plants treated with NaCl alone (S), and 134% higher in the combined treatment (TS) than in the control (C), with no differences among the NaCl and lysate treatment groups (Welch's ANOVA, $F_{3,6.31} = 15.22$, $P = 0.003$, $\eta^2 = 0.879$; Figure 4b).



386 **Fig 4** Lipid peroxidation and hydrogen peroxide content in leaves of *Solanum lycopersicum* cv.
387 Micro-Tom plants with and without NaCl treatment and foliar application of a cyanobacterial
388 lysate, sampled at 62 days after sowing. (a) Malondialdehyde (MDA) content, as an estimate of
389 lipid peroxidation, and (b) hydrogen peroxide (H_2O_2) content. Experimental groups: control (C),
390 *Trichormus* sp. lysate (T), 200 mM NaCl (S), and *Trichormus* sp. lysate + 200 mM NaCl (TS).
391 Data are presented as mean ± SE (n = 4 trays). Open circles represent individual biological
392 replicates. Differences among groups were assessed by Welch's ANOVA followed by the Games–
393 Howell post hoc test; means sharing a letter do not differ significantly ($P > 0.05$)
394

Superoxide dismutase, catalase and guaiacol peroxidase activities

SOD activity showed a significant comparison among the experimental groups (permutation test, $P = 0.002$, $\eta^2 = 0.575$), but no pairwise comparison remained significant after Holm correction (Figure 5a). The same happened for CAT activity (permutation test, $P = 0.039$, $\eta^2 = 0.428$; Figure 5b). The application of lysate alone increased POD activity compared to the control (C), whereas when was tested in NaCl treatment did not. POD activity was 76% higher in plants treated with lysate alone (T) than in the control (C), while NaCl alone (S) did not change it (Welch's ANOVA, $F_{3,6.52} = 22.91$, $P < 0.001$, $\eta^2 = 0.913$; Figure 5c). In the combined treatment (TS), POD activity was similar to that the control (C), but was 50% lower than in plants treated with NaCl alone (S) and 63% lower than in plants treated with the lysate alone (T).



406 **Fig 5** Antioxidant enzyme activities in leaves of *Solanum lycopersicum* cv. Micro-Tom plants with
407 and without NaCl treatment and foliar application of a cyanobacterial lysate, sampled at 62 days
408 after sowing. (a) Superoxide dismutase (SOD), (b) catalase (CAT), and (c) guaiacol peroxidase
409 (POD) activities, expressed per mg of soluble protein. Experimental groups: control (C),
410 *Trichormus* sp. lysate (T), 200 mM NaCl (S), and *Trichormus* sp. lysate + 200 mM NaCl (TS).
411 Data are presented as mean ± SE (n = 4 trays). Open circles represent individual biological
412 replicates. Differences among groups were assessed by Monte Carlo permutation tests followed
413 by pairwise permutation tests with Holm correction (a, b) or by Welch's ANOVA followed by the
414 Games–Howell post hoc test (c); means sharing a letter do not differ significantly ($P > 0.05$). In
415 (a) and (b), the overall effect of experimental group was significant ($P < 0.05$), but no pairwise
416 comparison was significant ($P > 0.05$)
417

Ascorbate–glutathione cycle enzymes

NaCl increased the activities of MDHAR and GR compared to the control (C), whereas the lysate did not change the activity of any enzyme of the cycle. APX activity did not differ from the control (C) in any experimental group, numerically higher in the S and TS groups than in T plants by 85% and 30%, respectively (Welch's ANOVA, $F_{3,6.15} = 12.33$, $P = 0.005$, $\eta^2 = 0.857$; Figure 6a). Compared to the control plants (C), the lysate alone (T) did not change MDHAR or GR activity, whereas NaCl alone (S) more than doubled MDHAR activity (0.013 vs. 0.005 μmol NADH min^{-1} mg^{-1}protein; Welch's ANOVA, $F_{3,5.00} = 17.41$, $P = 0.005$, $\eta^2 = 0.913$; Figure 6b) and approximately doubled GR activity (0.011 vs. 0.005 μmol NADH min^{-1} mg^{-1}protein; $F_{3,6.14} = 50.98$, $P < 0.001$, $\eta^2 = 0.961$; Figure 6d). In the combined treatment (TS), both activities were also higher than in the control (0.010 and 0.008 μmol NADH min^{-1} mg^{-1}protein, respectively) and did not differ from those of plants treated with NaCl alone (S). DHAR activity did not differ among the four experimental groups (permutation test, $P = 0.078$, $\eta^2 = 0.404$; Figure 6c).

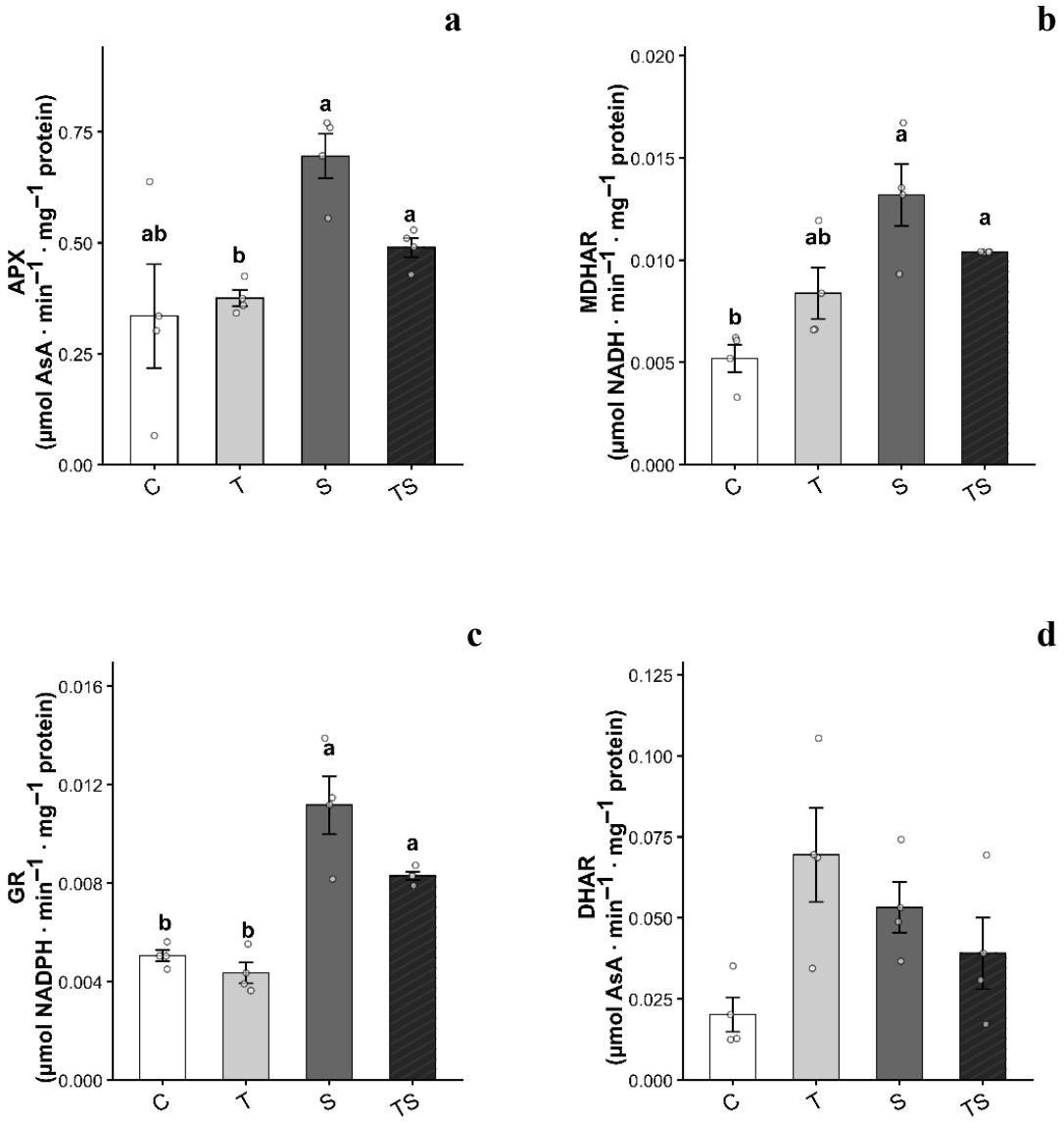


Fig 6 Ascorbate–glutathione cycle enzyme activities in leaves of *Solanum lycopersicum* cv. Micro-Tom plants with and without NaCl treatment and foliar application of a cyanobacterial lysate, sampled at 62 days after sowing. (a) Ascorbate peroxidase (APX), (b) monodehydroascorbate reductase (MDHAR), (c) dehydroascorbate reductase (DHAR), and (d) glutathione reductase (GR) activities, expressed per mg of soluble protein. Experimental groups: control (C), *Trichormus* sp. lysate (T), 200 mM NaCl (S), and *Trichormus* sp. lysate + 200 mM NaCl (TS). Data are presented as mean ± SE (n = 4 trays). Open circles represent individual biological replicates. Differences among groups were assessed by Welch's ANOVA followed by the Games–Howell post hoc test (a, b, d) or by a Monte Carlo permutation test (c); means sharing a letter do not differ significantly ($P > 0.05$). In (c), no significant differences were detected ($P > 0.05$)

Discussion

NaCl treatment induced oxidative stress without affecting growth of tomato cv. Micro-Tom

In plants, NaCl imposes osmotic and ionic stress (Munns and Tester 2008), which limits CO_2 fixation and increases the formation of reactive oxygen species (ROS), particularly in chloroplasts (Miller et al. 2010). Among these, H_2O_2 can give rise to hydroxyl radicals that attack the polyunsaturated fatty acids of membranes, leading to lipid peroxidation (Gill and Tuteja 2010; Sharma et al. 2012). Because H_2O_2 also acts as a signalling molecule (Miller et al. 2010), its accumulation alone does not indicate oxidative damage, whereas MDA content, an indicator of lipid peroxidation, does not show whether oxidants accumulated. A simultaneous increase in both is therefore consistent with oxidative stress, defined as an imbalance between oxidants and antioxidants in favour of the oxidants that leads to disrupted redox signalling and/or molecular damage (Sies 2015), and both variables are commonly used to assess it in salt-treated tomato (Li 2009; Mutale-joan et al. 2021; Ribera et al. 2026). In Micro-Tom, 200 mM NaCl applied from the three- to four-leaf stage until fruit harvest increased both H_2O_2 and MDA contents five- to six-fold (Habibi et al. 2023). In the present study, plants treated with 200 mM NaCl alone (S) from 37 DAS, when they had four to five leaf nodes, for 25 days had 139% higher H_2O_2 content and 34% higher MDA content in the fifth and sixth true leaves than control plants (C), which is consistent with oxidative stress. These increases were smaller than those reported by Habibi et al. (2023), in which NaCl treatment started at a similar developmental stage but lasted until fruit harvest. Compared with Micro-Tom plants treated with 50 mM NaCl from about 14 DAS, the increase in H_2O_2 content was similar, as H_2O_2 content doubled after two weeks, whereas the increase in MDA content was smaller than the three-fold increase reported in that study (Anee et al. 2025). In tomato, the effects of NaCl generally increase with concentration and duration of exposure, as observed for chlorophyll content and antioxidant enzyme activities in both salt-tolerant and salt-sensitive tomato lines (Zhang et al. 2026), and they are stronger when salt is applied early in development, as a short exposure at the seedling stage restricted growth of cv. Avalanche more than a prolonged exposure starting later (Dumbroff and Cooper 1974). The smaller increases observed here than in Habibi et al. (2023) may therefore reflect the shorter duration of NaCl exposure, and the smaller increase in MDA content than in Anee et al. (2025), despite a four-fold higher NaCl concentration, may reflect the later developmental stage at its onset, although differences in growth conditions and analytical methods among studies limit these comparisons.

Alongside this oxidative stress, NaCl treatment increased MDHAR activity more than two-fold and GR activity approximately two-fold compared with control plants, whereas the other five antioxidant enzymes measured (SOD, CAT, POD, APX, and DHAR) did not differ between S and control plants. This response differs from those reported in other studies with Micro-Tom, in which APX , CAT, and SOD activities increased by 40–120% in seedlings treated with 100 mM NaCl for 25 days (França et al. 2025), whereas in two-week-old plants treated with 50 mM NaCl, MDHAR activity decreased by 42–60%, DHAR, APX, and CAT activities decreased at one of the two sampling times, and GR activity did not change (Anee et al. 2025), which suggests that the antioxidant enzyme response to NaCl depends on the conditions of exposure. Both MDHAR and GR belong to the ascorbate–glutathione cycle, in which APX removes H_2O_2 using ascorbate, MDHAR and DHAR regenerate ascorbate from its oxidized forms, and GR regenerates the reduced glutathione required by DHAR (Hasanuzzaman et al. 2021; Foyer and Kunert 2024). MDHAR activity enhances the efficiency of the APX reaction in compartments where both enzymes are coupled, and GR maintains glutathione in its reduced state (Foyer and Kunert 2024). Higher MDHAR and GR activities may therefore help to maintain the ascorbate-dependent

removal of H_2O_2 without an increase in APX activity, as observed in maize seedlings treated with sea salt (12 dS m^{-1}) for 5 days, in which MDHAR activity increased mainly in tolerant genotypes and GR activity in all genotypes, whereas APX activity increased significantly in only one tolerant genotype, and tolerant genotypes showed smaller increases in H_2O_2 and MDA contents than susceptible ones (Methela et al. 2024). In tobacco, MDHAR overexpression increased ascorbate content and lowered H_2O_2 content under 0.3 M NaCl, which was attributed to the regeneration of ascorbate from MDHA before its conversion to DHA (Eltayeb et al. 2007). Such a route would be consistent with the unchanged DHAR activity in S plants, and in all but one of the maize genotypes studied by Methela et al. (2024). Because reduced glutathione is also oxidized in other reactions, such as those catalysed by glutaredoxins (Foyer and Kunert 2024), the higher GR activity may also reflect a broader demand for reduced glutathione. Even so, S plants had higher H_2O_2 and MDA contents than control plants, which indicates that the higher MDHAR and GR activities did not prevent H_2O_2 accumulation or lipid peroxidation under NaCl treatment. Ascorbate and glutathione pools and non-enzymatic antioxidants were not measured, so these interpretations remain to be tested.

Despite these changes in H_2O_2 and MDA contents and in MDHAR and GR activities, NaCl treatment did not affect the increases in plant height and leaf node number, flower and fruit number, pigment content, or sucrose and soluble protein contents. A similar dissociation between oxidative stress and growth has been reported in Micro-Tom, in which 50 mM NaCl tripled MDA content without affecting plant height (Anee et al. 2025), which indicates that lipid peroxidation in leaves can occur before, or without, a reduction in growth. In Micro-Tom, NaCl has been reported to reduce chlorophyll content by 45–53% (Palaniyandi et al. 2014; Bacha et al. 2017; Anee et al. 2025), plant height by 30–36% (Kiferle et al. 2022; Habibi et al. 2023), and leaf sucrose content by 44% (Habibi et al. 2025). In most of those studies, NaCl was applied from sowing or to young plants: at 180 mM from sowing for 60 days (Palaniyandi et al. 2014), at 150 mM from two weeks after germination for 10 weeks (Kiferle et al. 2022), and at 50 mM to two-week-old plants for one to two weeks (Anee et al. 2025). In the latter study, chlorophyll content decreased without changes in plant height, which suggests that pigment content responds to NaCl earlier than growth. In Habibi et al. (2023, 2025), 200 mM NaCl treatment started at a developmental stage similar to that in the present study but lasted until fruit harvest, whereas in Bacha et al. (2017), 150 mM NaCl applied from the six-leaf stage reduced chlorophyll content within 14 days. In tomato cv. Daniela, salt tolerance increased when NaCl was applied at later developmental stages (Del Amor et al. 2001), and in cv. Avalanche, salt stress imposed with concentrated nutrient solutions was most damaging during the seedling stage, from which plants also recovered more slowly (Dumbroff and Cooper 1974). The later onset of NaCl treatment relative to most of these studies, and its shorter duration relative to Habibi et al. (2023, 2025), may therefore be related to the absence of effects on growth. In addition, growth and reproductive parameters were recorded 14 and 27 days after the end of NaCl treatment, respectively, so recovery may have reduced earlier differences; this explanation does not apply to pigments, which were measured one day after the last NaCl application. Under the conditions of the present study, therefore, NaCl treatment induced oxidative stress in the leaves without measurable effects on growth or pigment content.

Effects of the cyanobacterial lysate

In the absence of NaCl, lysate application modified three of the variables evaluated: compared with control plants, T plants had 55% lower MDA content, 121% higher H_2O_2 content, and 76% higher POD activity. The increase in H_2O_2 content agrees with that reported in unstressed tomato plants 24 h after the application of *Arthrospira platensis* polysaccharides (Rachidi et al. 2021) and

in lettuce grown with the cyanobacterium *Oculatella lusitanica* (Brito et al. 2022), whereas foliar application of *A. platensis* extract every 5 days for 20 days did not change H_2O_2 content in wheat (Touzout et al. 2025). Because leaves were sampled 24 h after the last lysate application in the present study, this difference suggests that the increase in H_2O_2 content may be transient. In contrast, lysate-treated plants had lower MDA content, whereas MDA content did not change in lettuce or wheat treated with cyanobacteria in the absence of salt (Brito et al. 2022; Touzout et al. 2025). Thus, the lysate increased H_2O_2 content without causing lipid peroxidation, which is consistent with the role of H_2O_2 as a signalling molecule that causes oxidative damage only when its accumulation exceeds the capacity of antioxidant systems (Miller et al. 2010; Noctor 2025). The higher H_2O_2 content and POD activity suggest a response of the plant's own ROS metabolism, in agreement with the view that algal- and plant-derived biostimulants act mainly by modulating the plant's own ROS and hormone signaling rather than by directly supplying antioxidants (Minkov et al. 2026). However, there is no information on the effect of cyanobacterial preparations on lipid peroxidation in unstressed plants beyond the absence of changes in MDA content in lettuce and wheat (Brito et al. 2022; Touzout et al. 2025). An increase in H_2O_2 that induces defences without causing oxidative damage is also characteristic of a primed state (Conrath et al. 2015; Hilker and Schmülling 2019), but this could not be tested, because priming is defined by a faster or stronger response to a later stress, and leaves were sampled once, 24 h after the last lysate application, so the higher H_2O_2 content and POD activity of T plants may also reflect a short-term response to that application.

Despite these changes in ROS metabolism, the lysate alone did not affect growth, reproductive parameters, or pigment content, nor did it affect sucrose, trehalose and soluble protein contents. This contrasts with other cyanobacterial preparations, which increased tomato growth in the absence of stress. Foliar application of a *Leptolyngbya* sonicate (0.1 mg dry mass mL^{-1}) to cv. San Pedro seedlings with up to two true leaves increased stem length by 65% after three weeks (Toribio et al. 2021). Soil drenches of acid-hydrolysed *Aphanothece* sp., *Arthrospira maxima*, and *A. platensis* applied from 5 DAS increased shoot length by 16–48% and carotenoid content by 14–75% (Mutale-joan et al. 2020). Foliar application of an *A. platensis* protein hydrolysate (2.0 g L^{-1}, weekly) to cv. Itaipava plants transplanted at the four-true-leaf stage increased plant height by 28% during the vegetative stage, but not during the fruiting stage, and did not affect chlorophyll or carotenoid contents (Munaro et al. 2024). Because growth was promoted in that study in plants at a developmental stage similar to that in the present study, the absence of growth effects here is unlikely to be explained by developmental stage alone. It may instead reflect differences in organism, preparation, dose, or cultivar among studies; for instance, the hydrolysate used by Munaro et al. (2024) also supplied N, P, and K, which may have contributed to its effect on growth. Under the conditions of the present study, the lysate alone thus modified H_2O_2 and MDA contents and POD activity in the leaves without affecting growth.

The cyanobacterial lysate counteracts the NaCl-induced increase in MDA in tomato cv. Micro-Tom

Under NaCl, the lysate lowered MDA content to control levels. This agrees with the lower lipid peroxidation reported in salt-treated tomato after application of a microalgae–cyanobacteria extract (Mutale-joan et al. 2021) and in wheat after foliar application of *Arthrospira platensis* extract under 150 mM NaCl (Touzout et al. 2025). In those studies, however, lower lipid peroxidation was accompanied by lower H_2O_2 or higher antioxidant enzyme activities, whereas in the present study H_2O_2 content and the activities of most antioxidant enzymes measured did not differ between TS and S plants, and POD activity was even lower in TS than in S plants.

Conversely, in lettuce, the cyanobacterium *Oculatella lusitanica* lowered H_2O_2 content under salinity without affecting MDA content (Brito et al. 2022). The lower MDA content of TS plants therefore points to protective mechanisms other than H_2O_2 removal by the enzymes measured. Under NaCl, one candidate is the higher sucrose content of TS plants, because soluble sugars can act as ROS scavengers and protect membranes (Couée et al. 2006), although this would not explain the lower MDA content of T plants, in which sucrose content did not increase. Other candidates are non-enzymatic antioxidants that were not measured, such as phenolic compounds, whose synthesis can be stimulated by microalgal preparations (Rachidi et al. 2021).

Other effects of the lysate appeared only under NaCl treatment, the most marked being the 44% higher sucrose content of TS than of S plants. In Micro-Tom, NaCl alone reduced leaf sucrose content (Habibi et al. 2025), whereas in the present study it did not, and the lysate increased it, as reported for other biostimulants applied to salt-treated tomato (Naboulsi et al. 2022; Zuzunaga-Rosas et al. 2022). Soluble sugars contribute to osmotic adjustment and act as signalling molecules under stress (Couée et al. 2006; Shulaev et al. 2008), so this increase may contribute to the response of TS plants to NaCl. One possible link to the lysate is trehalose, the most abundant of the three compatible solutes quantified in a previous preliminary analysis of the lysate (Abello et al. 2024), because trehalose metabolism is connected to sucrose through trehalose-6-phosphate, which signals sucrose availability (Fichtner and Lunn 2021), and foliar trehalose alleviated salt stress in Micro-Tom (Yang et al. 2022). The higher soluble protein content of TS than of S plants, which did not differ from that of control plants, suggests that the lysate may have limited a decline in protein content under NaCl, as NaCl commonly reduces soluble protein content in tomato leaves (Debouba et al. 2006; Roșca et al. 2023; Habibi et al. 2025). Because both variables were measured at a single time point, however, these results do not show whether they reflect greater synthesis, lower use, or altered partitioning.

In our work, whereas the lysate alone increased POD activity, under NaCl treatment it lowered POD activity relative to S plants, to a level similar to that of control plants, although H_2O_2 content was similar in T, S, and TS plants. POD activity therefore did not follow H_2O_2 content, which might be related to the diverse functions of class III peroxidases. Guaiacol peroxidase activity measured *in vitro* includes many of these isoforms, which participate not only in H_2O_2 removal but also in cell wall modification and stress signalling (Mathé et al. 2010). Under NaCl, cell wall-bound peroxidases contribute to lignification, which stiffens cell walls and has been associated with growth restriction (Almagro et al. 2009; Neves et al. 2010; Mathé et al. 2010). The lower POD activity of TS plants might therefore reflect a lower involvement of peroxidases in cell wall modification under NaCl rather than lower H_2O_2 removal, although lignin content and peroxidase isoforms were not analysed in the present study. Overall, the lysate had an effect that occurred with and without NaCl (lower MDA content), effects that appeared only under NaCl treatment (higher sucrose and soluble protein contents), and an effect that depended on NaCl (POD activity), whereas it did not modify the response of the ascorbate–glutathione cycle enzymes to NaCl. These responses are compatible with the possibility that the lysate behaves as a plant biostimulant, defined as a substance or microorganism applied to plants to enhance nutrition efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of its nutrient content (du Jardin 2015). Under NaCl treatment, the lysate counteracted the increase in lipid peroxidation and increased sucrose content, a solute involved in osmotic adjustment and ROS buffering (Couée et al. 2006), as reported for other biostimulants applied to salt-treated tomato (Naboulsi et al. 2022; Zuzunaga-Rosas et al. 2022).

The components responsible for these effects cannot be identified from the present data, because the lysate composition was not analysed in this study. In a previous preliminary analysis, trehalose, GABA, and proline were quantified in the lysate (Abello et al. 2024), but cyanobacterial cell lysates also contain other compounds with documented bioactivity in plants, such as phycobiliproteins and polysaccharides (Santini et al. 2021; Righini et al. 2023), and culture supernatants of this strain contain trans-zeatin (Pontigo 2023). Several of these compounds have documented bioactivity in plants: exogenous GABA alleviated salt damage in tomato by modulating Na^+ uptake and ROS metabolism (Wu et al. 2020), cyanobacteria-derived proline increased stress tolerance in *Arabidopsis thaliana* (Chua et al. 2020), and phycobiliproteins from *Anabaena minutissima* promoted tomato seedling growth (Righini et al. 2023). *Trichormus* sp. was isolated from biological soil crusts at the southern limit of the Atacama Desert (Dahech et al. 2021), and its capacity to modify plant metabolism supports the exploration of cyanobacteria from extreme environments, given that the number of cyanobacterial species evaluated as biostimulants is still limited (Santini et al. 2021). Taken together, the lysate produced biochemical changes compatible with the functional definition of a biostimulant (du Jardin 2015; Caradonia et al. 2019), but because salinity did not affect growth under our conditions, these changes could not be linked to improved stress tolerance at the whole-plant level.

Conclusions

Foliar application of the *Trichormus* sp. lysate modified the leaf metabolism of tomato cv. Micro-Tom: it lowered lipid peroxidation both in the absence and in the presence of 200 mM NaCl, without lowering H_2O_2 content or increasing the activities of the antioxidant enzymes measured, and it increased sucrose content only under NaCl. However, these changes were measured at a single time point, 24 h after the last lysate application, and were not accompanied by effects on growth. Their persistence and their contribution to salt tolerance at the whole-plant level therefore remain to be established, and the potential of the lysate as a biostimulant for salt-affected crops warrants evaluation under conditions in which NaCl reduces growth.

Funding This work was supported by the Agencia Nacional de Investigación y Desarrollo (ANID), Chile, through FONDEF project ID23I10119 ("Polyfunctional biotechnological tool for adaptation to climate change"), the ANID National Doctoral Scholarship/2022-21221636, and by the European Union through the Erasmus+ programme project 2022-1-DE01-KA171-HED-000070710.

Availability of data and material The datasets generated and analysed during the present study are available from the corresponding author on reasonable request. The genome assembly of *Trichormus* sp. (*Nodularia* sp. strain CChRGM 3473) is available at NCBI under BioProject PRJNA1136538.

Code availability The R scripts used for statistical analyses and figures are available from the corresponding author on reasonable request.

Authors' contributions R.A.B. and C.O.C. conceived and designed the study, and acquired funding. R.A.B. performed the experiments, analysed the data, prepared figures 1–6, wrote the main manuscript text. C.O.C. supervised the work. H.H. and C.O.C. reviewed and edited the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

References

Abdel Latef AA, Hameed M, Khan Z, Khan S, Imran A, Fatima N (2026) Foliar stimulants: a sustainable strategy for enhancing plant resilience to abiotic and biotic stresses. Physiol Plant 178:e70741. https://doi.org/10.1111/ppl.70741

Abello R, Barros D, Vidal-Veuthey B, Ortiz C (2024) Metabolismo bioestimulante de la cianobacteria autóctona, *Trichormus* sp., para la mitigación del estrés salino en plantas [Biostimulant metabolism of the native cyanobacterium *Trichormus* sp. for the mitigation of salt stress in plants]. In: Reunión Anual Conjunta de Sociedades Botánica de Chile, Sociedad de Ecología y la Sociedad de Biología de Chile, La Serena, Chile, 5–8 November 2024. Abstract book, p 207 (in Spanish)

Almagro L, Gómez Ros LV, Belchi-Navarro S, Bru R, Ros Barceló A, Pedreño MA (2009) Class III peroxidases in plant defence reactions. J Exp Bot 60:377–390. https://doi.org/10.1093/jxb/ern277

Alves RD, Rossatto DR, da Silva JD, Checchio MV, de Oliveira KR, Oliveira FD, de Queiroz SF, da Cruz MC, Gratão PL (2021) Seed priming with ascorbic acid enhances salt tolerance in Micro-Tom tomato plants by modifying the antioxidant defense system components. Biocatal Agric Biotechnol 31:101927. https://doi.org/10.1016/j.bcab.2021.101927

Anee TI, Rachman RR, Ziqi Z, Suzuki N (2025) A combination of salt stress and waterlogging provides protection to tomato plants against the negative effects of waterlogging individually applied. Physiol Plant 177:e70116. https://doi.org/10.1111/ppl.70116

Bacha H, Tekaya M, Drine S, Guasmi F, Touil L, Enneb H, Triki T, Cheour F, Ferchichi A (2017) Impact of salt stress on morpho-physiological and biochemical parameters of *Solanum lycopersicum* cv. Microtom leaves. S Afr J Bot 108:364–369. https://doi.org/10.1016/j.sajb.2016.08.018

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473. https://doi.org/10.3389/fpls.2018.01473

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brito Â, Rocha M, Kaštovský J, Vieira J, Vieira CP, Ramos V, Correia M, Santos M, Mota R, Roque J, Pissarra J (2022) A new cyanobacterial species with a protective effect on lettuce grown under salinity stress: envisaging sustainable agriculture practices. J Appl Phycol 34:915–928. https://doi.org/10.1007/s10811-022-02692-4

Campobenedetto C, Mannino G, Beekwilder J, Contartese V, Karlova R, Bertea CM (2021) The application of a biostimulant based on tannins affects root architecture and improves tolerance to salinity in tomato plants. Sci Rep 11:354. https://doi.org/10.1038/s41598-020-79770-5

711 Caradonia F, Battaglia V, Righi L, Pascali G, La Torre A (2019) Plant biostimulant regulatory
712 framework: prospects in Europe and current situation at international level. J Plant Growth
713 Regul 38:438–448. https://doi.org/10.1007/s00344-018-9853-4

714 Chabili A, Hakkoum Z, Minaoui F, Douma M, Meddich A, Loudiki M (2025) Germination screen
715 of eco-extracts from soil cyanobacteria and microalgae for their biostimulant effects on
716 wheat seeds emergence and vigor. Algal Res 89:104087.
717 https://doi.org/10.1016/j.algal.2025.104087

718 Chua A, Sherwood OL, Fitzhenry L, Ng CK, McCabe PF, Daly CT (2020) Cyanobacteria-derived
719 proline increases stress tolerance in *Arabidopsis thaliana* root hairs by suppressing
720 programmed cell death. Front Plant Sci 11:490075.
721 https://doi.org/10.3389/fpls.2020.490075

722 Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. Lawrence Erlbaum
723 Associates, Hillsdale, NJ

724 Conrath U, Beckers GJM, Langenbach CJG, Jaskiewicz MR (2015) Priming for enhanced defense.
725 Annu Rev Phytopathol 53:97–119. https://doi.org/10.1146/annurev-phyto-080614-120132

726 Couée I, Sulmon C, Gouesbet G, El Amrani A (2006) Involvement of soluble sugars in reactive
727 oxygen species balance and responses to oxidative stress in plants. J Exp Bot 57:449–459.
728 https://doi.org/10.1093/jxb/erj027

729 Dahech P, Schlömann M, Ortiz C (2021) Light intensity stimulates the production of extracellular
730 polymeric substances (EPS) in a culture of the desert cyanobacterium *Trichormus* sp. J
731 Appl Phycol 33:2795–2804. https://doi.org/10.1007/s10811-021-02516-x

732 Debouba M, Gouia H, Suzuki A, Ghorbel MH (2006) NaCl stress effects on enzymes involved in
733 nitrogen assimilation pathway in tomato "*Lycopersicon esculentum*" seedlings. J Plant
734 Physiol 163:1247–1258. https://doi.org/10.1016/j.jplph.2005.09.012

735 Del Amor FM, Martinez V, Cerdá A (2001) Salt tolerance of tomato plants as affected by stage of
736 plant development. HortScience 36:1260–1263.
737 https://doi.org/10.21273/HORTSCI.36.7.1260

738 Dos Santos TB, Ribas AF, de Souza SG, Budzinski IG, Domingues DS (2022) Physiological
739 responses to drought, salinity, and heat stress in plants: a review. Stresses 2:113–135.
740 https://doi.org/10.3390/stresses2010009

741 du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. Sci
742 Hortic 196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021

743 Dumbroff EB, Cooper AW (1974) Effects of salt stress applied in balanced nutrient solutions at
744 several stages during growth of tomato. Bot Gaz 135:219–224.
745 https://doi.org/10.1086/336755

Eltayeb AE, Kawano N, Badawi GH, Kaminaka H, Sanekata T, Shibahara T, Inanaga S, Tanaka K (2007) Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. Planta 225:1255–1264. https://doi.org/10.1007/s00425-006-0417-7

FAO (2024) Global status of salt-affected soils – main report. FAO, Rome. https://doi.org/10.4060/cd3044en

FAO (2025) Agricultural production statistics 2010–2024. FAOSTAT Analytical Brief Series No. 121. FAO, Rome.

Fichtner F, Lunn JE (2021) The role of trehalose 6-phosphate (Tre6P) in plant metabolism and development. Annu Rev Plant Biol 72:737–760. https://doi.org/10.1146/annurev-arplant-050718-095929

Foolad MR (2004) Recent advances in genetics of salt tolerance in tomato. Plant Cell Tissue Organ Cult 76:101–119. https://doi.org/10.1023/B:TICU.0000007308.47608.88

Foyer CH, Kunert K (2024) The ascorbate–glutathione cycle coming of age. J Exp Bot 75:2682–2699. https://doi.org/10.1093/jxb/erae023

França VC, Campos WF, Cangussú LV, da Silva TP, Dobbss LB (2025) The inhibitory effects of salt stress on tomatoes are mitigated by humic acids independent of ethylene perception. Trop Plant Biol 18:5. https://doi.org/10.1007/s12042-024-09378-w

Gálvez FJ, Baghour M, Hao G, Cagnac O, Rodríguez-Rosales MP, Venema K (2012) Expression of LeNHX isoforms in response to salt stress in salt sensitive and salt tolerant tomato species. Plant Physiol Biochem 51:109–115. https://doi.org/10.1016/j.plaphy.2011.10.012

Garg R, Maldener I (2021) The dual role of the glycolipid envelope in different cell types of the multicellular cyanobacterium *Anabaena variabilis* ATCC 29413. Front Microbiol 12:645028. https://doi.org/10.3389/fmicb.2021.645028

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Guo M, Wang XS, Guo HD, Bai SY, Khan A, Wang XM, Gao YM, Li JS (2022) Tomato salt tolerance mechanisms and their potential applications for fighting salinity: a review. Front Plant Sci 13:949541. https://doi.org/10.3389/fpls.2022.949541

Habibi N, Aryan S, Amin MW, Sanada A, Terada N, Koshio K (2023) Potential benefits of seed priming under salt stress conditions on physiological, and biochemical attributes of Micro-Tom tomato plants. Plants 12:2187. https://doi.org/10.3390/plants12112187

Habibi N, Aryan S, Sediqui N, Terada N, Sanada A, Kamata A, Koshio K (2025) Enhancing salt tolerance in tomato plants through PEG6000 seed priming: inducing antioxidant activity and mitigating oxidative stress. Plants 14:1296. https://doi.org/10.3390/plants14091296

Hasanuzzaman M, Raihan MR, Masud AA, Rahman K, Nowroz F, Rahman M, Nahar K, Fujita M (2021) Regulation of reactive oxygen species and antioxidant defense in plants under salinity. Int J Mol Sci 22:9326. https://doi.org/10.3390/ijms22179326

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hilker M, Schmülling T (2019) Stress priming, memory, and signalling in plants. Plant Cell Environ 42:753–761. https://doi.org/10.1111/pce.13526

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Circular 347, California Agricultural Experiment Station, Berkeley

Hoffmann J, Berni R, Sutera FM, Gutsch A, Hausman JF, Saffie-Siebert S, Guerriero G (2021) The effects of salinity on the anatomy and gene expression patterns in leaflets of tomato cv. Micro-Tom. Genes 12:1165. https://doi.org/10.3390/genes12081165

Hopmans JW, Qureshi AS, Kisekka I, Munns R, Grattan SR, Rengasamy P, Ben-Gal A, Assouline S, Javaux M, Minhas PS, Raats PA (2021) Critical knowledge gaps and research priorities in global soil salinity. Adv Agron 169:1–191. https://doi.org/10.1016/bs.agron.2021.03.001

Johnson R, Joel JM, Puthur JT (2024) Biostimulants: the futuristic sustainable approach for alleviating crop productivity and abiotic stress tolerance. J Plant Growth Regul 43:659–674. https://doi.org/10.1007/s00344-023-11144-3

Junglee S, Urban L, Sallanon H, Lopez-Lauri F (2014) Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. Am J Anal Chem 5:730–736. https://doi.org/10.4236/ajac.2014.511081

Kiferle C, Gonzali S, Beltrami S, Martinelli M, Hora K, Holwerda HT, Perata P (2022) Improvement in fruit yield and tolerance to salinity of tomato plants fertigated with micronutrient amounts of iodine. Sci Rep 12:14655. https://doi.org/10.1038/s41598-022-18301-w

Leiva-Ampuero A, Agurto M, Matus JT, Hoppe G, Huidobro C, Inostroza-Blancheteau C, Reyes-Díaz M, Stange C, Canessa P, Vega A (2020) Salinity impairs photosynthetic capacity and enhances carotenoid-related gene expression and biosynthesis in tomato (*Solanum lycopersicum* L. cv. Micro-Tom). PeerJ 8:e9742. https://doi.org/10.7717/peerj.9742

Li Y (2009) Physiological responses of tomato seedlings (*Lycopersicon esculentum*) to salt stress. Mod Appl Sci 3:171–176. https://doi.org/10.5539/mas.v3n3p171

Li Z, Luo L-J, Zhu L-P (2014) Involvement of trehalose in hydrogen sulfide donor sodium hydrosulfide-induced the acquisition of heat tolerance in maize (*Zea mays* L.) seedlings. Bot Stud 55:20. https://doi.org/10.1186/1999-3110-55-20

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Marzban G, Tesei D (2025) The extremophiles: adaptation mechanisms and biotechnological applications. Biology (Basel) 14:412. https://doi.org/10.3390/biology14040412

Mathé C, Barre A, Jourda C, Dunand C (2010) Evolution and expression of class III peroxidases. Arch Biochem Biophys 500:58–65. https://doi.org/10.1016/j.abb.2010.04.007

Methela NJ, Islam MS, Das AK, Raihan HU, Rohman MM, Chowdhury AK, Mun BG (2024) Antioxidant mechanisms in salt-stressed maize (*Zea mays* L.) seedlings: comparative analysis of tolerant and susceptible genotypes. Appl Biol Chem 67:109. https://doi.org/10.1186/s13765-024-00963-x

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 33:453–467. https://doi.org/10.1111/j.1365-3040.2009.02041.x

Minkov P, Gechev TS, Kanojia A (2026) Terrestrial plant- and algal-derived biostimulants as modulators of ROS and hormone networks in crop abiotic stress resilience. Plants 15:992. https://doi.org/10.3390/plants15070992

Munaro D, Mazo CH, Bauer CM, da Silva Gomes L, Teodoro EB, Mazzarino L, da Rocha Veleirinho MB, e Silva SM, Maraschin M (2024) A novel biostimulant from chitosan nanoparticles and microalgae-based protein hydrolysate: improving crop performance in tomato. Sci Hortic 323:112491. https://doi.org/10.1016/j.scienta.2023.112491

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Murshed R, Lopez-Lauri F, Sallanon H (2008) Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. Anal Biochem 383:320–322. https://doi.org/10.1016/j.ab.2008.07.020

Mutale-Joan C, Rachidi F, Mohamed HA, Mernissi NE, Aasfar A, Barakate M, Mohammed D, Sbabou L, Arroussi HE (2021) Microalgae-cyanobacteria–based biostimulant effect on salinity tolerance mechanisms, nutrient uptake, and tomato plant growth under salt stress. J Appl Phycol 33:3779–3795. https://doi.org/10.1007/s10811-021-02559-0

Mutale-Joan C, Redouane B, Najib E, Yassine K, Lyamlouli K, Laila S, Zeroual Y, Hicham EA (2020) Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L. Sci Rep 10:2820. https://doi.org/10.1038/s41598-020-59840-4

Naboulsi I, Ben Mrid R, Ennoury A, Zouaoui Z, Nhiri M, Ben Bakrim W, Yasri A, Aboulmouhajir A (2022) *Crataegus oxyacantha* extract as a biostimulant to enhance tolerance to salinity in tomato plants. Plants 11:1283. https://doi.org/10.3390/plants11101283

855 Neves GY, Marchiosi R, Ferrarese ML, Siqueira-Soares RC, Ferrarese-Filho O (2010) Root
856 growth inhibition and lignification induced by salt stress in soybean. J Agron Crop Sci
857 196:467–473. https://doi.org/10.1111/j.1439-037X.2010.00432.x

858 Noctor G (2025) Hydrogen peroxide, ascorbate, and glutathione: building the Foyer–Halliwell–
859 Asada pathway. Planta 261:132. https://doi.org/10.1007/s00425-025-04702-4

860 Ozden M, Demirel U, Kahraman A (2009) Effects of proline on antioxidant system in leaves of
861 grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Sci Hortic 119:163–168.
862 https://doi.org/10.1016/j.scienta.2008.07.031

863 Pailles Y, Awlia M, Julkowska M, Passone L, Zemmouri K, Negrão S, Schmöckel SM, Tester M
864 (2020) Diverse traits contribute to salinity tolerance of wild tomato seedlings from the
865 Galapagos Islands. Plant Physiol 182:534–546. https://doi.org/10.1104/pp.19.00700

866 Palaniyandi SA, Damodharan K, Yang SH, Suh JW (2014) *Streptomyces* sp. strain PGPA39
867 alleviates salt stress and promotes growth of 'Micro Tom' tomato plants. J Appl Microbiol
868 117:766–773. https://doi.org/10.1111/jam.12563

869 Paul T, Jaiswal P, Govindasamy V, Pandey R, Kumar S, Abraham G (2026) Cyanobacterial seed
870 priming and inoculation mitigates salinity stress in transplanted rice through the regulation
871 of growth, photosynthesis and ion metabolism. J Appl Phycol.
872 https://doi.org/10.1007/s10811-026-03892-y

873 Pontigo D, Wiche O, Wilkens M, Schlömann M, Ortiz C (2023) Culture supernatants of the
874 cyanobacterium *Trichormus* sp. promote the growth of the grass *Polypogon australis*
875 Brong: Role of P and Mn. Algal Res 71:103032.
876 https://doi.org/10.1016/j.algal.2023.103032

877 R Core Team (2023) R: a language and environment for statistical computing. R Foundation for
878 Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 6 May 2023

879 Rachidi F, Benhima R, Kasmi Y, Sbabou L, Arroussi HE (2021) Evaluation of microalgae
880 polysaccharides as biostimulants of tomato plant defense using metabolomics and
881 biochemical approaches. Sci Rep 11:930. https://doi.org/10.1038/s41598-020-78820-2

882 Regulation (EU) 2019/1009 (2019) Regulation (EU) 2019/1009 of the European Parliament and
883 of the Council of 5 June 2019 laying down rules on the making available on the market of
884 EU fertilising products. Off J Eur Union L 170:1–114

885 Ribera LM, da Silveira Sousa Junior G, Meneses MD, Pimenta EP, Rolim GD, de Azevedo RA,
886 Gratão PL (2026) Machine learning model provides stress biomarkers for the classification
887 of abiotic stress in Micro-Tom. Sci Rep 16:7545. https://doi.org/10.1038/s41598-026-
888 39117-y

889 Righini H, Francioso O, Quintana AM, Pinchetti JL, Zuffi V, Cappelletti E, Roberti R (2023) New
890 insight on tomato seed priming with *Anabaena minutissima* phycobiliproteins in relation

to *Rhizoctonia solani* root rot resistance and seedling growth promotion. Phytoparasitica 51:763-781. https://doi.org/10.1007/s12600-023-01056-z

Ronga D, Biazzi E, Parati K, Carminati D, Carminati E, Tava A (2019) Microalgal biostimulants and biofertilisers in crop productions. Agronomy 9:192. https://doi.org/10.3390/agronomy9040192

Roșca M, Mihalache G, Stoleru V (2023) Tomato responses to salinity stress: from morphological traits to genetic changes. Front Plant Sci 14:1118383. https://doi.org/10.3389/fpls.2023.1118383

Rothan C, Just D, Fernandez L, Atienza I, Ballias P, Lemaire-Chamley M (2016) Culture of the tomato Micro-Tom cultivar in greenhouse. In: Botella JR, Botella MA (eds) Plant signal transduction. Springer, New York, pp 57–64. https://doi.org/10.1007/978-1-4939-3115-6_6

Rouphael Y, Colla G (2020) Editorial: biostimulants in agriculture. Front Plant Sci 11:40. https://doi.org/10.3389/fpls.2020.00040

Santini G, Biondi N, Rodolfi L, Tredici MR (2021) Plant biostimulants from cyanobacteria: an emerging strategy to improve yields and sustainability in agriculture. Plants 10:643. https://doi.org/10.3390/plants10040643

Saranga Y, Zamir D, Marani A, Rudich J (1991) Breeding tomatoes for salt tolerance: field evaluation of *Lycopersicon* germplasm for yield and dry-matter production. J Am Soc Hortic Sci 116:1067–1071. https://doi.org/10.21273/JASHS.116.6.1067

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012:217037. https://doi.org/10.1155/2012/217037

Shokri N, Hassani A, Sahimi M (2024) Multi-scale soil salinization dynamics from global to pore scale: a review. Rev Geophys 62:e2023RG000804. https://doi.org/10.1029/2023RG000804

Shulaev V, Cortes D, Miller G, Mittler R (2008) Metabolomics for plant stress response. Physiol Plant 132:199–208. https://doi.org/10.1111/j.1399-3054.2007.01025.x

Sies H (2015) Oxidative stress: a concept in redox biology and medicine. Redox Biol 4:180–183. https://doi.org/10.1016/j.redox.2015.01.002

Gr S, Yadav RK, Chatrath A, Gerard M, Tripathi K, Govindsamy V, Abraham G (2021) Perspectives on the potential application of cyanobacteria in the alleviation of drought and salinity stress in crop plants. J Appl Phycol 33:3761–3778. https://doi.org/10.1007/s10811-021-02570-5

Taghipour L, Rahemi M, Assar P, Mirdehghan SH, Ramezanian A (2021) Intermittent warming as an efficient postharvest treatment affects the enzymatic and non-enzymatic responses of

pomegranate during cold storage. J Food Meas Charact 15:12–22. https://doi.org/10.1007/s11694-020-00607-w

Tiddia F, Kodru S, Piano D, Farci D (2026) Ecological, functional, and phylogenetic determinants of cyanobacterial biomineralisation. Environ Microbiol Rep 18:e70281. https://doi.org/10.1111/1758-2229.70281

Toribio AJ, Jurado MM, Suárez-Estrella F, López-González JA, Martínez-Gallardo MR, López MJ (2021) Application of sonicated extracts of cyanobacteria and microalgae for the mitigation of bacterial canker in tomato seedlings. J Appl Phycol 33:3817–3829. https://doi.org/10.1007/s10811-021-02599-6

Toribio AJ, Suárez-Estrella F, Jurado MM, López MJ, López-González JA, Moreno J (2020) Prospection of cyanobacteria producing bioactive substances and their application as potential phytostimulating agents. Biotechnol Rep 26:e00449. https://doi.org/10.1016/j.btre.2020.e00449

Touzout N, Ainas M, Babaali M, Moussa H, Mihoub A, Ahmad I, Jamal A, Danish S, Ahmad R, Dewir YH, Székely Á. (2025) Potential effect of non-nitrogen fixing cyanobacteria *Spirulina platensis* on growth promotion of wheat (*Triticum aestivum* L.) under salt stress. Sci Rep 15:29029. https://doi.org/10.1038/s41598-025-14567-y

van Handel E (1968) Direct microdetermination of sucrose. Anal Biochem 22:280–283. https://doi.org/10.1016/0003-2697(68)90317-5

Wang X, Tian W, Zheng W, Shah S, Li J, Wang X, Zhang X (2023) Quantitative relationships between salty water irrigation and tomato yield, quality, and irrigation water use efficiency: a meta-analysis. Agric Water Manag 280:108213. https://doi.org/10.1016/j.agwat.2023.108213

Wu X, Jia Q, Ji S, Gong B, Li J, Lü G, Gao H (2020) Gamma-aminobutyric acid (GABA) alleviates salt damage in tomato by modulating Na^+ uptake, the GAD gene, amino acid synthesis and reactive oxygen species metabolism. BMC Plant Biol 20:465. https://doi.org/10.1186/s12870-020-02669-w

Xu J, Duan X, Yang J, Beeching JR, Zhang P (2013) Enhanced reactive oxygen species scavenging by overproduction of superoxide dismutase and catalase delays postharvest physiological deterioration of cassava storage roots. Plant Physiol 161:1517–1528. https://doi.org/10.1104/pp.112.212803

Xu Y, Milledge JJ, Abubakar A, Swamy R, Bailey D, Harvey P (2015) Effects of centrifugal stress on cell disruption and glycerol leakage from *Dunaliella salina*. Microalgae Biotechnol 1:20-27. https://doi.org/10.1515/micbi-2015-0003

Yakhin OI, Lubyanov AA, Yakhin IA, Brown PH (2017) Biostimulants in plant science: a global perspective. Front Plant Sci 7:2049. https://doi.org/10.3389/fpls.2016.02049

963 Yang Y, Yao Y, Li J, Zhang J, Zhang X, Hu L, Ding D, Bakpa EP, Xie J (2022) Trehalose
964 alleviated salt stress in tomato by regulating ROS metabolism, photosynthesis, osmolyte
965 synthesis, and trehalose metabolic pathways. Front Plant Sci 13:772948.
966 https://doi.org/10.3389/fpls.2022.772948

967 Zhang M, Wang Q, Liu Y, Liu H, Xu W, Yang X, Pang S (2026) Responses of processing tomato
968 genotypes under varying NaCl stress levels and durations. Plants 15:1450.
969 https://doi.org/10.3390/plants15101450

970 Zhang X, Yang H, Du T (2022) Osmotic adjustment of tomato under mild soil salinity can enhance
971 drought resistance. Environ Exp Bot 202:105004.
972 https://doi.org/10.1016/j.envexpbot.2022.105004

973 Zor T, Selinger Z (1996) Linearization of the Bradford protein assay increases its sensitivity:
974 theoretical and experimental studies. Anal Biochem 236:302–308.
975 https://doi.org/10.1006/abio.1996.0171

976 Zuzunaga-Rosas J, González-Orenga S, Tofei AM, Boscaiu M, Moreno-Ramón H, Ibáñez-
977 Asensio S, Vicente O (2022) Effect of a biostimulant based on polyphenols and glycine
978 betaine on tomato plants' responses to salt stress. Agronomy 12:2142.
979 https://doi.org/10.3390/agronomy12092142