Impact Of Individual And Synergistic Application Of Seaweed Extract And PGPR On Growth And Physiology Of Tomato (*Solanum Lycopersicum L.*) Under Salinity Stress

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Abstract
In this study, we investigated the individual and combined effects of a liquid extract from the brown seaweed *Cystoseira compressa* and a plant growth-promoting rhizobacterial strain *Bacillus cereus MR64* on the growth and physiological responses of tomato plants (*Solanum lycopersicum L.*) under salt stress. The bacterial strain *Bacillus cereus* MR64 was selected for its remarkable NaCl tolerance and plant growth-promoting traits. A greenhouse pot trial was conducted, wherein tomato plants were treated with either a bacterial suspension, three concentrations (5%, 10%, and 15%) of the seaweed extract, or their combinations applied to the soil. The plants were subjected to 150 mM NaCl. Growth parameters, photosynthetic pigments, leaf relative water content, and leaf electrolyte leakage were measured. Results indicated that the extract was more effective than the bacterial strain alone in enhancing shoot growth, photosynthetic pigments, and membrane integrity. When applied alone, the bacterial strain notably increased root length and biomass compared to the extract and the control. Significant improvements in growth parameters, chlorophyll content, and electrolyte leakage were observed when the bacterial strain was combined with the extract at concentrations of 10% and 15%, showing notable synergistic effects.

Keywords: Seaweed, PGPR, Salt stress, Growth, Physiology, Tomato

1. Introduction

Modern agriculture confronts global challenges arising from population growth, climate change, and environmental pollution, all of which have detrimental impacts on worldwide food production (Peters et al., 2009). A significant contributor to agricultural losses is salt stress, which affects approximately 20% of irrigated land globally, especially in Mediterranean regions (Tomaz et al., 2020). The consequences of salt stress include diminished water potential, ion toxicity from sodium and chloride accumulation, and oxidative damage from reactive oxygen species (Gill & Tuteja, 2010; H.A. et al., 2019). Consequently, this leads to reduced water and nutrient uptake and adversely affects essential physiological processes such as germination, growth, and photosynthesis (Hassen et al., 2016). A prominent proposal in this regard focuses on novel sustainable solutions, particularly the use of biostimulants (W. Khan et al., 2009; Santini et al., 2021). Biostimulants are substances or microorganisms that stimulate natural processes and enhance nutrient uptake, efficiency, abiotic stress tolerance, and crop quality when applied to plants or the rhizosphere (du Jardin, 2015). They encompass various categories, such as seaweed extracts, protein hydrolysates, chitosan, silicon, humic and fulvic acids, plant growth-promoting rhizobacteria, and arbuscular mycorrhizal fungi (Van Oosten et al., 2017). Plant Growth-Promoting Rhizobacteria (PGPR) are a group of bacteria found in the plant rhizosphere, first recognized in 1978, that have a positive impact on plant growth and productivity (Pérez-Montaño et al., 2014). Microorganisms like *Bacillus*, *Pseudomonas*, *Azotobacter*, and *Azospirillum* function as PGPRs, documented for promoting plant growth and mitigating abiotic stresses such as salinity and drought in various crops (Batool et al., 2020; Cordero et al., 2018a; Kiran et al., 2022; Ruíz-Sánchez et al., 2011). Alongside PGPRs, seaweeds are key types of biostimulants, classified as macroalgae, are crucial components of marine ecosystems, serving as abundant reservoirs for valuable compounds (Leandro et al., 2020; Satpati et al., 2022). Brown seaweeds are widely used in agriculture due to their abundance and play a pivotal role in improving plant growth and resilience, particularly against salt stress (Battacharyya et al., 2015; Carillo et al., 2020). Microbial-based bioformulations, especially when combined with other biostimulants, are vital for ecological sustainability and boosting crop productivity (Sani & Yong, 2021; Santana et al., 2022). The objective of this study was

investigating the effects of the individual and combined application of a selected PGPR *Bacillus cereus MR64*, and the aqueous extract of *Cystoseira compressa* on growth parameters, photosynthetic pigments, leaf relative water content, and leaf electrolyte leakage of tomato (*Solanum lycopersicum L.*) against salinity stress (150 mM NaCl).

2. Materials and methods

2.1. Seaweed collection and preparation of the extract

The brown seaweed *Cystoseira compressa* was harvested from the coast of Tipaza, Algeria (36°37'03.8″ N, 2°38'21.2″ E). The collected samples underwent washing with seawater and tap water to eliminate residues, air-dried, powdered, and stored in sterile glass jars. A quantity of 100 g of seaweed powder was heated at 60°C for 45 minutes in 1 liter of sterile distilled water. The resulting extracts were filtered and stored. Various concentrations of seaweed liquid extract, including 5%, 10%, and 15%, were prepared by diluting the filtrate with distilled water and then stored at 4°C, with the original filtrate considered as 100% concentration (Hamouda et al., 2022).

2.2. Selection of the bacterial strain

Pure cultures rhizobacterial strains MR62, MR63, and MR64 were obtained from the Laboratory of Genetics, Biochemistry and Plant Biotechnology, University of Constantine, Algeria isolated previously from wheat field. The strains were grown in nutrient agar medium, kept at 30±2°C for 48 h, and then suspended in sterile flasks with 250 ml of Lauria Bertani medium. The flasks were shaken continuously for 48 h at 30°C as described by Barra et al. (2016). Rhizobacterial strains were screened for halotolerance by observing their growth on Luria Bertani medium (Bertani, 1951) at 37°C supplemented with different concentrations of NaCl ranging from 2% to 8%. Bacterial growth was determined by measuring OD_{600nm} after 3 days.

Strains were qualitatively screened for plant growth-promoting traits. IAA production was evaluated following the method of Bric et al. (1991), while phosphate solubilization followed Katznelson & Bose, (1959) procedure. HCN production was tested as per Lorck, (1948) method. Each assay was conducted in triplicate. The identification of the selected bacterial strain involved analyzing the 16S rDNA gene sequence. DNA extraction utilized a thermal shock procedure on an isolated colony from a young bacterial culture, involving cycles of freezing and heating. Universal primers were used to amplify 16S rDNA. amplified 16S rDNA, and PCR products underwent gel electrophoresis. Sequences were extracted using Mega (version11), followed by neighbor-joining (NJ) algorithms, and Blastn (2.8.0+) analysis identified related sequences in the NCBI GenBank database. A phylogenetic tree was constructed with MEGA using NCBI GenBank accession numbers (Weisburg et al., 1991).

2.3. Bioassay

The pot trial was conducted in a greenhouse at the Department of Biotechnology, Faculty of Nature and Life Sciences, Blida 1 University, from January to April, 2023. The soil used was sterilized in an oven for 1 hour at 120 °C. The soil has the following characteristics: soil texture: clay loam, electrical conductivity: 0.29 dS/m, pH: 6.43, total nitrogen N%: 2.24, total $CaCO_3$ %: 13,13, assimilable P_2O_5 ppm: 65.65, assimilable K_2O ppm: 21,46, C%: 0.72, organic matter MO%: 1.24, Ca^{2+} (meq/100g): 0.73, Na^+ (meq/100g): 0.44, Cl^- (meq/100g): 0.50, Co3- (meq/100g): 0.75. The tomato (*Solanum lycopersicum L.*) variety used in this study was the Saint Pierre variety, this variety is genetically fixed, approved, and certified. Seeds were obtained from the Technical Institute of Market Gardening and Industrial Crops, Alger, Algeria. Tomato seeds were surface-sterilized as described by (Götz et al., 2006). Seeds were germinated in Petri dishes at 25 °C. After germination, seedlings were transferred to pots of 11 cm in height and 12 cm in diameter and containing 6 kg of sterilized soil, they were arranged on a metal bench with a daily maximum and minimum temperature of 25 ± 3 °C and 18 ± 2 °C, respectively and relative humidity of 60%. The experiment was carried out using a randomized block design with six repetitions for 48 pots, starting at the 3-leaf stage, 15 days after transplanting. A bacterial suspension of the selected strain was prepared from young culture of *Bacillus cereus* MR64 using distilled water. Spectrophotometric measurements were used to adjust the bacterial suspension concentration to approximately 10^8 cfu/ml (OD_{600nm} = 0.8). Tomato plants were equally irrigated with a saline solution of 150 mM NaCl twice weekly and treated individually according to eight experiments: experiment 1: control (irrigated with saline solution only), experiment 2: 10 ml of *B. Cereus* MR64 bacterial suspension, experiments 3,4,5: 10 ml of 5, 10, and 15% of *C. compressa* aqueous extract respectively, experiments 6,7,8: 10 ml of *B. cereus* MR64 bacterial suspension + 10 ml of 5%, 10%, and 15% of *C. compressa* aqueous extract, respectively. Three applications of the treatments were applied once every 10 days. Parameters were measured 10 days after the third application.

2.4. Growth parameters

Shoot length was measured using a scale from the root-shoot junction to the tip of the longest leaf. Root length was measured from the root-shoot junction to the tip of the longest root. The number of leaves on each plant was counted. The fresh and dry weights of shoots and roots were determined by cutting off the plant parts at the junction and measuring their weights immediately and then placed in an oven at 80 °C until obtaining a stable weight to obtain the dry weight. Total fresh weight and total dry weight were estimated from shoot and root fresh and dry weights.

2.5. Photosynthetic pigments

The chlorophyll content was estimated using the method described by Arnon, (1949). Fresh leaves 100 mg were ground with 10 ml of ice cold 80% acetone. The extract was filtered through Whatman filter paper, and 1 ml of the filtered extract was used for spectrophotometric determination at 645 and 663 nm. Chlorophyll a, b and total chlorophyll contents were calculated using the following formulas:

Chlorophyll a = $11.23A_{663}–2.04A_{645}$
Chlorophyll b = $20.13A_{645}–4.19A_{663}$
Total Chlorophyll = $7.05A_{663} + 18.09A_{645}$
Where A is the absorbance at specific wavelength

2.6. Leaf relative water content

Leaf relative water content (LRWC) was determined according to Turner, (1981). The fresh weight of leaves was measured immediately after harvest. The samples were then immersed in distilled water for a 24-hour period to achieve full turgidity. After the excess water was removed, their turgid weight was recorded. Subsequently, the samples were placed in an 80 °C oven until a constant weight was achieved to determine the dry weight. The LRWC was calculated using the following formula:

$$\mathbf{LRWC}\ (\%) = [(\mathbf{FW} - \mathbf{DW}) / (\mathbf{TW} - \mathbf{DW})]\ \mathbf{x\ 100}$$

Where FW is the fresh weight (g), DW is the dry weight (g) and TW is the turgid weight (g).

2.7. Electrolytes leakage

Electrolytes leakage was evaluated according to the method described by Sun et al. (2006). Tomato leaves 200 mg were washed with distilled water, cut into small pieces, and placed in test tubes containing 20 ml of distilled water. The electrical conductivity C1 of the solution was measured using an electrical conductivity meter after incubating it at 25 °C for 30 min. Samples were then incubated at 100 °C for 30 min and cooled to room temperature. The second conductivity C2 was measured after stabilization at 25 °C. EL % was determined using the following equation:

$$\mathbf{EL}\ \% = [\mathbf{C1/C2}] \times \mathbf{100}.$$

Where C1 is the electrical conductivities of the solution before incubating at 100 °C, C2 is the electrical conductivity after incubating at 100 °C.

2.8. Statistical analysis

The significant differences between the mean values of the control and treated plant samples were determined by two-way ANOVA and Tukey's test using the 18th edition of the Genstat software. Tukey's test was used to separate the significant differences in treatment means with a significance threshold ($P < 0.05$). Data are expressed as mean ± standard deviation.

3. Results

3.1. Bacterial characterization

The plant growth-promoting traits and halotolerance of the rhizobacterial isolates are presented in Figure 1 and Table 1, respectively. Specifically, MR62 exhibited the capacity to tolerate up to 4% NaCl. However, growth was inhibited in the presence of high NaCl concentrations. In contrast, both MR63 and MR64 exhibited improved tolerance when exposed to concentrations exceeding 4% NaCl. Notably, MR64 and MR62 exhibited the ability to produce indole-3-acetic acid (IAA). BS64N also tested positive for hydrogen cyanide (HCN) production. Conversely, MR63 did not exhibit any PGP traits, and all three isolates yielded negative results in phosphate solubilization screening tests. MR64 was selected based on its NaCl tolerance and PGP traits.

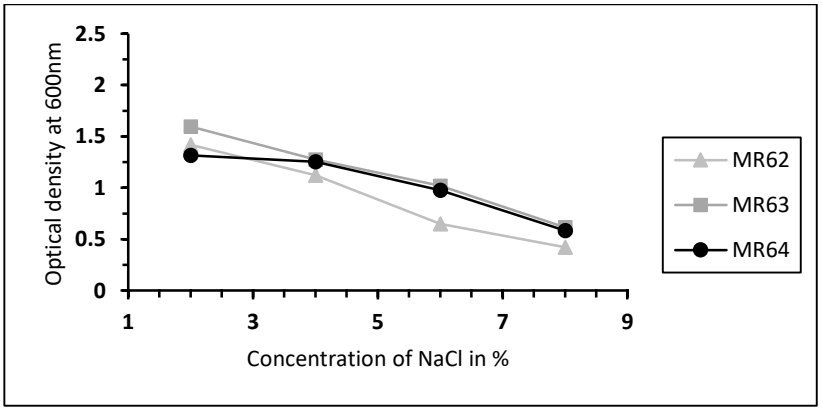


Figure 1: Effects of NaCl concentrations on OD_{600nm} of the strains MR62, MR63 and MR64

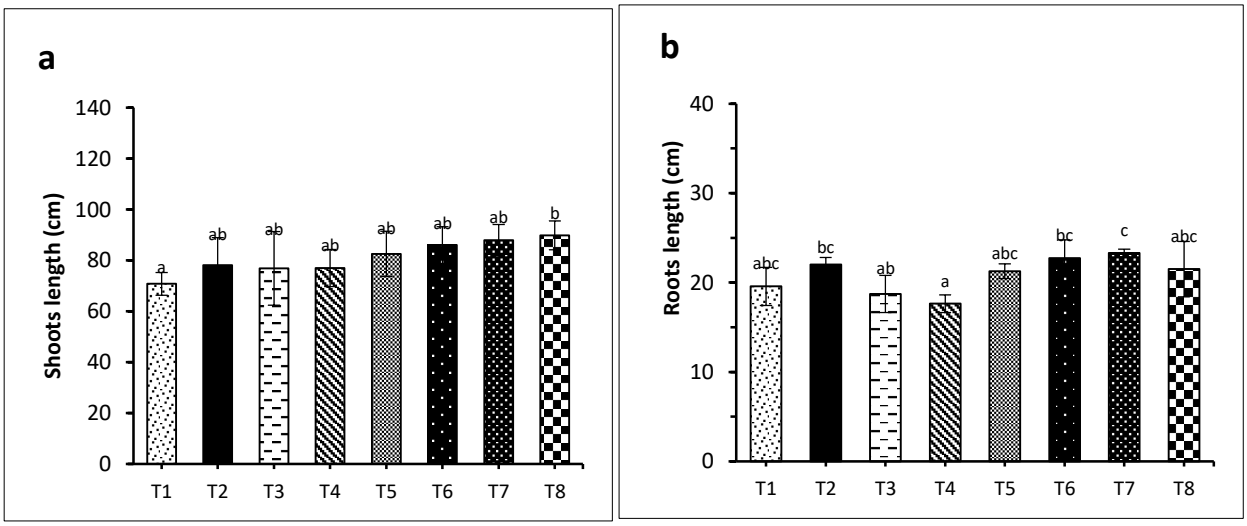
The Blastn results analysis revealed significant alignments of the strain MR64 with the *Bacillus cereus* strains group. The majority of sequences within the *B. cereus* group displayed identities surpassing 97%. Specifically, the sequence of MR64 exhibited a similarity of 97.75% with *Bacillus cereus* strain VMI2 (GenBank: ON680844.1) and a similarity score of 97.60% with *Bacillus cereus* strain S1-C (GenBank: MK185697.1).

Table 1: The PGP traits screening tests for the strains MR62, MR63, and MR64

Test	MR62	MR63	MR64
IAA Production	+	-	+
Phosphate solubilization	-	-	-
HCN production	-	-	+

3.2. Tomato growth and physiological responses

The morphological and physiological responses of tomato plants to treatments of different concentrations (5%, 10%, and 15%) of *Cystoseira compressa* aqueous extract, the PGPR *Bacillus cereus* MR64, and their synergistic application under salt stress (150 mM NaCl) are presented in Figure 2, and Figure 3. The co-application of both biostimulants, had a significant effect on the growth parameters of tomato plants, including shoot and root lengths, fresh and dry weights, as well as resulting remarkable enhancement in the physiological responses, such as an increase in total chlorophyll and chlorophyll A, and a decrease in electrolyte leakage ($p > 0.05$). However, it is essential to note that there were no significant effects on leaf number or leaf relative water content ($p < 0.05$). The combined treatments significantly improved various morphological parameters. Specifically, T8 (PGPR + 15% extract) and T7 (PGPR + 10% extract) showed enhanced shoot lengths (89.8 ± 5.64 and 23.3 ± 0.43 cm, respectively) compared to control plants (70.8 ± 4.49 and 19.57 ± 2.12 cm, respectively), higher shoot fresh and dry weights (93.3 ± 10.1 and 26.23 ± 1.81 g, respectively) than the control (76 ± 7.81 and 19.29 ± 2.81 g, respectively). In addition to root fresh and dry weights (26.11 ± 5.62 and 10.88 ± 0.08 g, respectively), in contrast to the control (17.48 ± 1.62 and 8.85 ± 0.48 g). Seaweed extract individually under T5 (15% extract) resulted in increased shoot length (82.6 ± 8.81 cm), shoot fresh and dry weights (83.7 ± 9.60 and 22.69 ± 1.50 g, respectively), and root dry weight (9.61 ± 0.38 g) at with T7 (10% extract) compared to PGPR alone. Conversely, PGPR individually (T2) enhanced root length and fresh weight (22 ± 0.8 cm and 21.71 ± 1.75 g, respectively) compared to seaweed extract alone at 10% and 15% extract. Regarding physiological responses, the combined application of the PGPR strain with the seaweed extract exhibited notable improvements, with the highest means (1.04 ± 0.16, 1.93 ± 0.15 mg/g FW, respectively) observed in chlorophyll A and total chlorophyll, respectively, recorded with T8 compared to the control (0.55 ± 0.03, 1.21 ± 0.13 mg/g FW, respectively). Additionally, the lowest mean in electrolyte leakage (69.29 ± 5.95 %) was observed under treatment T6 (PGPR + 5% extract), compared with the control (80.95 ± 2.07 %). Furthermore, seaweed extract alone proved more effective than PGPR alone. Specifically, all seaweed extract treatments T3 (5% extract), T4 (10% extract), and T5 (15% extract) recorded higher means for chlorophyll A (0.93 ± 0.06, 0.87 ± 0.11, 0.97 ± 0.17 mg/g FW), respectively and (1.53 ± 0.09, 1.70 ± 1.64, 1.66 ± 0.16 mg/g FW) respectively for total chlorophyll compared to the same variables observed with T2 (0.65 ± 0.25 mg/g FW) for chlorophyll A and (1.44 ± 0.27 mg/g FW) for total chlorophyll.



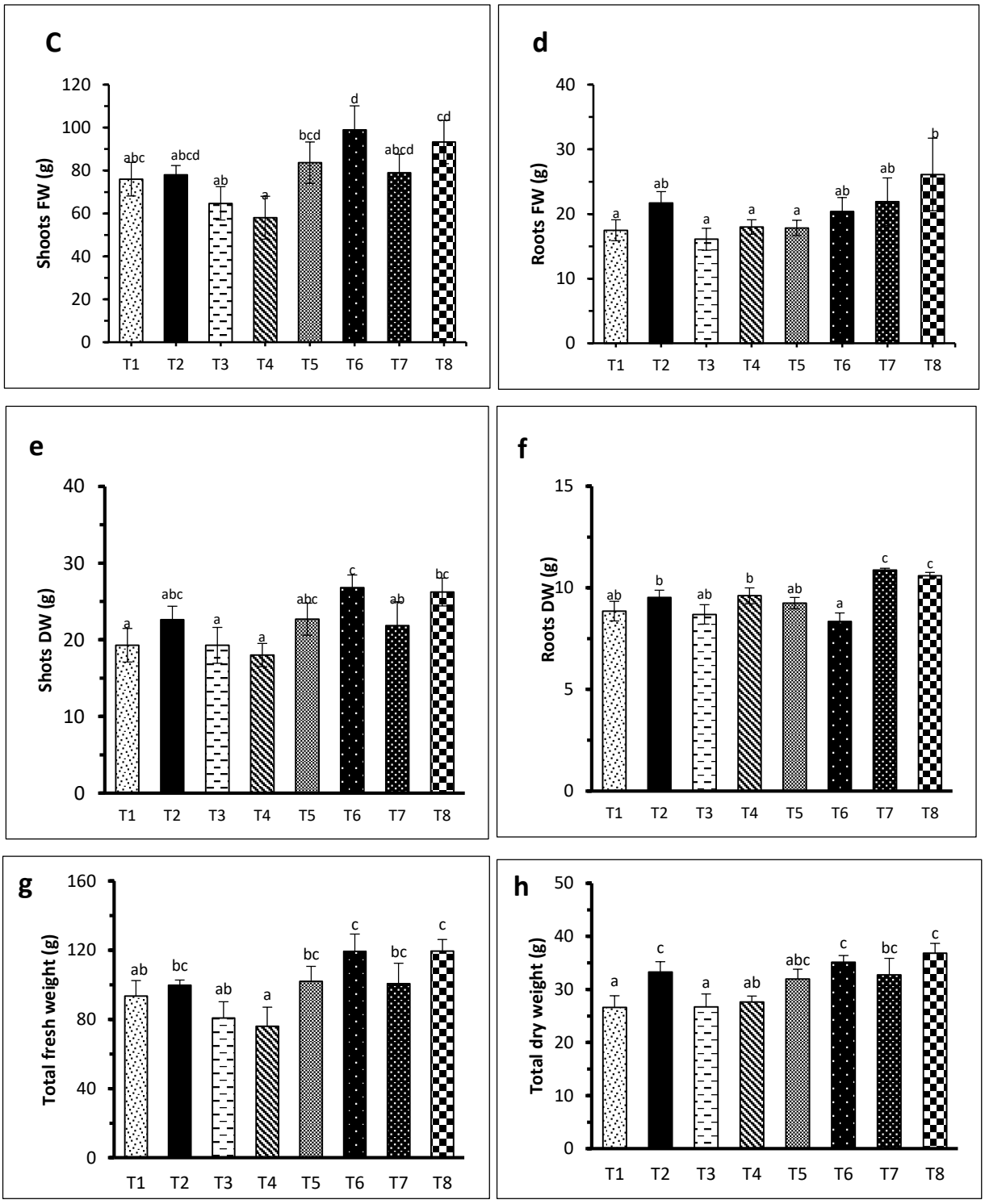


Figure 2: Effects of single and combined use of PGPR *Bacillus cereus* MR64 and three doses (5%, 10%, and 15%) of aqueous *C. compressa* extract under salt stress (150 mM NaCl) on tomato plants (**a, b**) shoot and root length, (**c, d**) fresh weight of shoot and root, (**e, f**) dry weight of shoot and root, (**g, h**) total fresh and dry weight, **T1**: control (150 mM NaCl), **T2**: *Bacillus cereus* MR64 + 150 mM NaCl, **T3, T4, and T5**: 5%, 10%, and 15% of *C. compressa* aqueous extract + 150 mM NaCl, respectively, **T6, T7, and T8**: 5%, 10%, and 15% of *C. compressa* aqueous extract + *Bacillus cereus* MR64 + 150 mM NaCl, respectively. Results are represented as means ± SD. Two-way ANOVA was applied at the 5% significance level. Values followed by the same letter are not significantly different according to Tukey's test ($P < 0.05$).

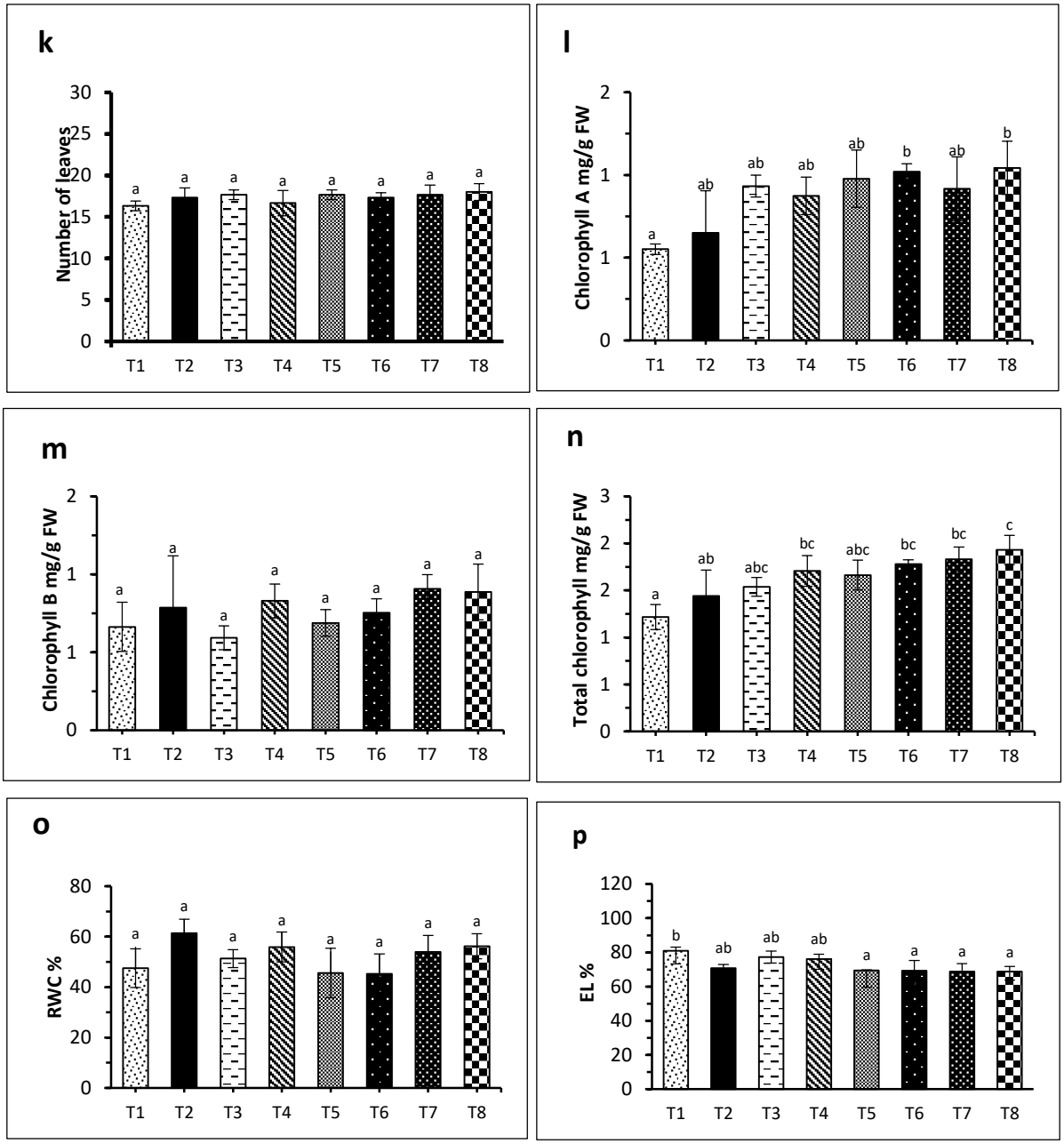


Figure 3: Effects of single and combined use of PGPR *Bacillus cereus MR64* and three doses (5%, 10%, and 15%) of aqueous *C. compressa* extract under salt stress (150 mM NaCl) on tomato plants. (**k**) leaves number, (**l, m, n**) chlorophyll A, B, Total, (**o**) leaf relative water content %, (**p**) leaf Electrolytes leakage %, **T1**: control (150 mM NaCl), **T2**: *Bacillus cereus MR64* + 150 mM NaCl, **T3, T4, and T5**: 5%, 10%, and 15% of *C. compressa* aqueous extract + 150 mM NaCl, respectively, **T6, T7, and T8**: 5%, 10%, and 15% of *C. compressa* aqueous extract + *Bacillus cereus MR64* + 150 mM NaCl, respectively. Results are represented as means ± SD. Two-way ANOVA was applied at the 5% significance level. Values followed by the same letter are not significantly different according to Tukey's test ($P < 0.05$).

4. Discussion

Salinity induces osmotic stress, ion toxicity, nutrient imbalances, and excessive ROS production. These effects have substantial effects on cellular components and biological membranes, resulting in decreased growth and biomass (Alzahrani et al., 2019; Loudari et al., 2020). The impact extends to reduced leaf relative water content and diminished levels of photosynthetic pigments due to lowered water potential. Furthermore, NaCl impedes the uptake of crucial elements like nitrogen (N) and magnesium (Mg), pivotal for chlorophyll structure (Kaya et al., 2009). The accumulation of Na+ ions from NaCl salinity disrupts ion balance within plant cells, potentially damaging cell membranes and increasing permeability to ions, particularly sodium and chloride ions (Cl-). Consequently, this leads to heightened electrolyte leakage and reduced K+ content (Shelke et al., 2019). when compared to using only the PGPR strain and control treatments, the exclusive use of *C. compressa* extract showed greater effectiveness in enhancing shoot parameters, increasing chlorophyll levels, and improving membrane integrity by reducing electrolyte leakage percentages, particularly

at concentrations of 10% and 15%. Conversely, the PGPR treatment had a more positive influence on root parameters, such as length and biomass, in comparison to both extract-treated and control plants. Seaweed extracts exhibit stimulant characteristics known to enhance plant growth and productivity in saline environments (Ali et al., 2021a; Deolu-Ajayi et al., 2022). These extracts comprise various bioactive components, including phytohormones, polysaccharides, polyphenols, vitamins, amino acids, peptides, and proteins (W. Khan et al., 2009). The possible presence of these compounds altogether influencing plant cellular metabolism, ultimately resulted in a significant boost in overall growth (W. Khan et al., 2009). Furthermore, it's likely that the presence of osmoprotectants such as free amino acids, polysaccharides within seaweed extract of *C. compressa* can play a vital role in supporting and modulating the physiology of the tomato plants facing salinity conditions (Y. Wang et al., 2017). According to Patel et al. (2018), applying *Kappaphycus alvarezzi* extract to various wheat varieties facing salinity and drought stress resulted in several positive effects. These included increased root length, higher levels of chlorophyll and decreased electrolytes leakage. In addition, it reduced the Na+/K+ ratio, and boosted calcium content, thus alleviating ionic imbalances in the plants. In a separate study by Hussein et al. (2021), seed priming with seaweed liquid extracts, such as *Cystoseira compressa*, enhanced the growth of *V. sinensis* and *Z. mays* under salinity stress. Godlewska et al. (2016) proposes that seaweed's growth-promoting ability may vary depending on concentration and extraction methods. These findings are consistent with those of this study, where the application of *C. compressa* liquid extract at concentrations of 10% and 15% improved many growth and physiological attributes of tomato plants despite salt stress, aligning with previous research (Bensidhoum & Nabti, 2021; Chanthini et al., 2022; Latique et al., 2017). The rhizobacterial strain utilized in this study demonstrated salt tolerance characteristics and produced essential plant growth-promoting traits, including acid indole acetic acid production, likely contributing to observed improvements in tomato plants, consistent with prior research (Cordero et al., 2018b; Kaloterakis et al., 2021; Zameer et al., 2016). Application of plant growth-promoting rhizobacteria (PGPRs) offers various benefits through hormone synthesis, phosphate solubilization, and nitrogen fixation (Gamalero & Glick, 2011). The observed plant parameter enhancements align with known responses to *Bacillus* strain inoculation (Julia et al., 2020). Previous studies, such as that by Zhou et al. (2022), examined the effectiveness of halotolerant *Bacillus cereus* (4% NaCl) producing indole-3-acetic acid (IAA) in promoting cucumber seedling growth and mitigating salt stress, resulting in increased plant height, stem diameter, fresh weight, and dry weight, as well as root length and biomass. Shultana et al. (2020) reported that application of *B. tequilensis* and *Bacillus aryabhattai* improved rice crop productivity under salt stress conditions. In a study by M. A. Khan et al. (2019), halotolerant *Bacillus* strains substantially increased soybean plant chlorophyll content compared to salt-treated plants (200 mM NaCl). Additionally, electrolyte leakage, indicative of plasma membrane damage under various stresses, exhibited reduced levels in salt-stressed radish plants inoculated with PGPR compared to non-inoculated stressed plants (Yildirim et al., 2008). The combined use of both biostimulants resulted in the highest enhancements in various parameters, demonstrating the efficacy of this combination in promoting plant growth and alleviating salt stress. No statistically significant changes were observed in leaf number, leaf relative water content, and chlorophyll B levels across our treatments, possibly due to other contributing factors. Leaf relative water content percentage is commonly used as an indicator of plant water status, with variations potentially linked to soil properties and environmental factors (Anderson & McNaughton, 1973; Bowman, 1989; Z. Q. Wang et al., 2009). Similarly, leaf number can be influenced by factors such as growth stage, environmental conditions, and genetic characteristics (Dieleman & Heuvelink, 1992). Noteworthy improvements were observed in most morphological and physiological aspects of tomato plants treated with T7 (MR64+ 10% extract) and T8 (MR64 + 15% extract). These effects may be attributed to the synergistic interaction between *C. compressa* extract and the PGPR *Bacillus cereus MR64* (Rouphael & Colla, 2018). Ali et al. (2021b) suggested that seaweed extracts could positively impact the soil microbiome, potentially enhancing plant growth promotion (PGP) characteristics of rhizospheric microbes. Previous studies have demonstrated the effectiveness of combining various plant biostimulants with PGPR, showcasing their ability to improve plant growth and productivity through additive and synergistic mechanisms. For instance, the combination of the microbial biostimulant *R. intraradices BEG72* and *T. atroviride MUCL45632* with a foliar application of plant-derived protein hydrolysate synergistically enhanced various growth parameters (Rouphael et al., 2017). Similarly, the combined application of *Azospirillum brasilense Az39* bacteria and *Macrocystis pyrifera* algae extracts positively impacted lettuce plant growth under drought stress compared to the control group (Julia et al., 2020). Additionally, combining PGPR *Pseudomonas fluorescens* (ATCC 13525) with *Kelpak*, a commercial seaweed extract, resulted in noticeable improvements in onion growth parameters (Gupta et al., 2021). These findings highlight the potential of synergistic interactions between microbial and non-microbial biostimulants (González-González et al., 2020). Recognizing that biostimulants are derived from living organisms or natural sources, understanding their properties may require examining the combined traits of all components rather than individual traits or specific combinations (Yakhin et al., 2017). Consequently, the synergistic application of PGPR and seaweed extracts holds significant promise as an environmentally sustainable approach to enhance overall plant growth and vitality (Ngoroyemoto et al., 2020).

5. Conclusion

In conclusion, the application of *C. compressa* liquid extract and/or the PGPR *Bacillus cereus MR64* has yielded valuable insights into their potential roles in enhancing plant growth, under salinity stress conditions. The bacterial characterization demonstrated that the strain Bacillus cereus exhibited tolerance to elevated salt concentrations and displayed plant growth-promoting traits, including indole acetic acid (IAA) production. The interactive application of *Bacillus cereus MR64* and the *C. compressa* liquid extract to tomato plants subjected to salinity stress demonstrated a more pronounced positive

impact on certain parameters at specific concentration combinations, suggesting a synergistic relationship between the microbial and non-microbial biostimulants and showcasing the ability for growth promotion and mitigating the detrimental effects of salinity stress by modulating growth and physiological responses. These findings serve as a potential basis for the formulation of biostimulants adapted for optimal plant growth and resilience under salinity stress conditions. Although the effects of these biostimulants need to be investigated further, they significantly contribute to the ongoing discourse on effective biostimulants for sustainable agricultural practices.

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Conflicts of Interest
The authors declare no conflicts of interest regarding the publication of this paper.

References
1. Ali, O., Ramsubhag, A., & Jayaraman, J. (2021a). Biostimulant Properties of Seaweed Extracts in Plants: Implications towards Sustainable Crop Production. Plants, 10(3), 531. https://doi.org/10.3390/plants10030531
2. Ali, O., Ramsubhag, A., & Jayaraman, J. (2021b). Biostimulant Properties of Seaweed Extracts in Plants: Implications towards Sustainable Crop Production. Plants, 10(3), 531. https://doi.org/10.3390/plants10030531
3. Alzahrani, S. M., Alaraidh, I. A., Migdadi, H., Alghamdi, S., Altaf Khan, M., & Ahmad, P. (2019). Physiological, biochemical, and antioxidant properties of two genotypes of Vicia faba grown under salinity stress. Pakistan Journal of Botany, 51(3). https://doi.org/10.30848/PJB2019-3(3)
4. Anderson, J. E., & McNaughton, S. J. (1973). Effects of Low Soil Temperature on Transpiration, Photosynthesis, Leaf Relative Water Content, and Growth Among Elevationally Diverse Plant Populations. Ecology, 54(6), 1220–1233. https://doi.org/10.2307/1934185
5. Arnon, D. I. (1949). COPPER ENZYMES IN ISOLATED CHLOROPLASTS. POLYPHENOLOXIDASE IN BETA VULGARIS. Plant Physiology, 24(1), 1–15. https://doi.org/10.1104/pp.24.1.1
6. Barra, P. J., Inostroza, N. G., Acuña, J. J., Mora, M. L., Crowley, D. E., & Jorquera, M. A. (2016). Formulation of bacterial consortia from avocado (Persea americana Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. Applied Soil Ecology, 102, 80–91. https://doi.org/10.1016/j.apsoil.2016.02.014
7. Batool, T., Ali, S., Seleiman, M. F., Naveed, N. H., Ali, A., Ahmed, K., Abid, M., Rizwan, M., Shahid, M. R., Alotaibi, M., Al-Ashkar, I., & Mubushar, M. (2020). Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. Scientific Reports, 10(1), 16975. https://doi.org/10.1038/s41598-020-73489-z
8. Battacharyya, D., Babgohari, M. Z., Rathor, P., & Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. Scientia Horticulturae, 196, 39–48. https://doi.org/10.1016/J.SCIENTA.2015.09.012
9. Bensidhoum, L., & Nabti, E. hafid. (2021). Role of Cystoseira mediterranea extracts (Sauv.) in the Alleviation of salt stress adverse effect and enhancement of some Hordeum vulgare L. (barley) growth parameters. SN Applied Sciences, 3(1). https://doi.org/10.1007/s42452-020-03992-5
10. Bertani, G. (1951). STUDIES ON LYSOGENESIS I. Journal of Bacteriology, 62(3), 293–300. https://doi.org/10.1128/jb.62.3.293-300.1951
11. Bowman, W. D. (1989). The relationship between leaf water status, gas exchange, and spectral reflectance in cotton leaves. Remote Sensing of Environment, 30(3), 249–255. https://doi.org/10.1016/0034-4257(89)90066-7
12. Bric, J. M., Bostock, R. M., & Silverstone, S. E. (1991). Rapid In Situ Assay for Indoleacetic Acid Production by Bacteria Immobilized on a Nitrocellulose Membrane. Applied and Environmental Microbiology, 57(2), 535–538. https://doi.org/10.1128/aem.57.2.535-538.1991
13. Carillo, P., Ciarmiello, L. F., Woodrow, P., Corrado, G., Chiaiese, P., & Rouphael, Y. (2020). Enhancing Sustainability by Improving Plant Salt Tolerance through Macro- and Micro-Algal Biostimulants. Biology 2020, Vol. 9, Page 253, 9(9), 253. https://doi.org/10.3390/BIOLOGY9090253
14. Chanthini, K. M.-P., Senthil-Nathan, S., Pavithra, G.-S., Malarvizhi, P., Murugan, P., Deva-Andrews, A., Janaki, M., Sivanesh, H., Ramasubramanian, R., Stanley-Raja, V., Ghaith, A., Abdel-Megeed, A., & Krutmuang, P. (2022). Aqueous Seaweed Extract Alleviates Salinity-Induced Toxicities in Rice Plants (Oryza sativa L.) by Modulating Their Physiology and Biochemistry. Agriculture, 12(12), 2049. https://doi.org/10.3390/agriculture12122049
15. Cordero, I., Balaguer, L., Rincón, A., & Pueyo, J. J. (2018a). Inoculation of tomato plants with selected PGPR represents a feasible alternative to chemical fertilization under salt stress. Journal of Plant Nutrition and Soil Science, 181(5), 694–703. https://doi.org/10.1002/jpln.201700480
16. Cordero, I., Balaguer, L., Rincón, A., & Pueyo, J. J. (2018b). Inoculation of tomato plants with selected PGPR represents a feasible alternative to chemical fertilization under salt stress. Journal of Plant Nutrition and Soil Science, 181(5), 694–703. https://doi.org/10.1002/jpln.201700480

17. Deolu-Ajayi, A. O., van der Meer, I. M., van der Werf, A., & Karlova, R. (2022). The power of seaweeds as plant biostimulants to boost crop production under abiotic stress. Plant, Cell & Environment, 45(9), 2537–2553. https://doi.org/10.1111/pce.14391
18. Dieleman, J. A., & Heuvelink, E. (1992). Factors affecting the number of leaves preceding the first inflorescence in the tomato. Journal of Horticultural Science, 67(1), 1–10. https://doi.org/10.1080/00221589.1992.11516214
19. du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae, 196, 3–14. https://doi.org/10.1016/j.scienta.2015.09.021
20. Gamalero, E., & Glick, B. R. (2011). Mechanisms Used by Plant Growth-Promoting Bacteria. In Bacteria in Agrobiology: Plant Nutrient Management (pp. 17–46). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-21061-7_2
21. Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48(12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016
22. Godlewska, K., Michalak, I., Tuhy, Ł., & Chojnacka, K. (2016). Plant Growth Biostimulants Based on Different Methods of Seaweed Extraction with Water. BioMed Research International, 2016, 1–11. https://doi.org/10.1155/2016/5973760
23. González-González, M. F., Ocampo-Alvarez, H., Santacruz-Ruvalcaba, F., Sánchez-Hernández, C. V., Casarrubias-Castillo, K., Becerril-Espinosa, A., Castañeda-Nava, J. J., & Hernández-Herrera, R. M. (2020). Physiological, Ecological, and Biochemical Implications in Tomato Plants of Two Plant Biostimulants: Arbuscular Mycorrhizal Fungi and Seaweed Extract. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.00999
24. Gupta, S., Stirk, W. A., Plačková, L., Kulkarni, M. G., Doležal, K., & Van Staden, J. (2021). Interactive effects of plant growth-promoting rhizobacteria and a seaweed extract on the growth and physiology of Allium cepa L. (onion). Journal of Plant Physiology, 262, 153437. https://doi.org/10.1016/j.jplph.2021.153437
25. H.A., H., H.A., M., S.A., E.-K., & R.A., H. (2019). The Potentiality of Marine Macro-Algae as Bio-Fertilizers to Improve the Productivity and Salt Stress Tolerance of Canola (Brassica napus L.) Plants. Agronomy, 9(3), 146. https://doi.org/10.3390/agronomy9030146
26. Hamouda, M. M., Saad-Allah, K. M., & Gad, D. (2022). Potential of Seaweed Extract on Growth, Physiological, Cytological and Biochemical Parameters of Wheat (Triticum aestivum L.) Seedlings. Journal of Soil Science and Plant Nutrition, 22(2), 1818–1831. https://doi.org/10.1007/s42729-022-00774-3
27. Hassen, A. I., Bopape, F. L., & Sanger, L. K. (2016). Microbial Inoculants as Agents of Growth Promotion and Abiotic Stress Tolerance in Plants. In Microbial Inoculants in Sustainable Agricultural Productivity (pp. 23–36). Springer India. https://doi.org/10.1007/978-81-322-2647-5_2
28. Hussein, M. H., Eltanahy, E., Al Bakry, A. F., Elsafty, N., & Elshamy, M. M. (2021). Seaweed extracts as prospective plant growth bio-stimulant and salinity stress alleviator for Vigna sinensis and Zea mays. Journal of Applied Phycology, 33(2), 1273–1291. https://doi.org/10.1007/S10811-020-02330-X/METRICS
29. Julia, I., Oscar, M., Analía, L., Zocolo Guilherme, J., & Virginia, L. (2020). Biofertilization with Macrocystis pyrifera algae extracts combined with PGPR-enhanced growth in Lactuca sativa seedlings. Journal of Applied Phycology, 32(6), 4361–4371. https://doi.org/10.1007/s10811-020-02202-4
30. Kaloterakis, N., van Delden, S. H., Hartley, S., & De Deyn, G. B. (2021). Silicon application and plant growth promoting rhizobacteria consisting of six pure Bacillus species alleviate salinity stress in cucumber (Cucumis sativus L). Scientia Horticulturae, 288, 110383. https://doi.org/10.1016/j.scienta.2021.110383
31. Katznelson, H., & Bose, B. (1959). METABOLIC ACTIVITY AND PHOSPHATE-DISSOLVING CAPABILITY OF BACTERIAL ISOLATES FROM WHEAT ROOTS, RHIZOSPHERE, AND NON-RHIZOSPHERE SOIL. Canadian Journal of Microbiology, 5(1), 79–85. https://doi.org/10.1139/m59-010
32. Kaya, C., Ashraf, M., Sonmez, O., Aydemir, S., Tuna, A. L., & Cullu, M. A. (2009). The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. Scientia Horticulturae, 121(1), 1–6. https://doi.org/10.1016/j.scienta.2009.01.001
33. Khan, M. A., Asaf, S., Khan, A. L., Adhikari, A., Jan, R., Ali, S., Imran, M., Kim, K.-M., & Lee, I.-J. (2019). Halotolerant Rhizobacterial Strains Mitigate the Adverse Effects of NaCl Stress in Soybean Seedlings. BioMed Research International, 2019, 1–15. https://doi.org/10.1155/2019/9530963
34. Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., Critchley, A. T., Craigie, J. S., Norrie, J., & Prithiviraj, B. (2009). Seaweed Extracts as Biostimulants of Plant Growth and Development. Journal of Plant Growth Regulation, 28(4), 386–399. https://doi.org/10.1007/s00344-009-9103-x
35. Kiran, S., Furtana, G. B., & Ellialtioglu, Ş. Ş. (2022). Physiological and biochemical assay of drought stress responses in eggplant (Solanum melongena L.) inoculated with commercial inoculant of Azotobacter chroococum and Azotobacter vinelandii. Scientia Horticulturae, 305, 111394. https://doi.org/10.1016/j.scienta.2022.111394
36. Latique, S., Mohamed Aymen, E., Halima, C., Chérif, H., & Mimoun, E. K. (2017). Alleviation of Salt Stress in Durum Wheat (Triticum durum L.) Seedlings Through the Application of Liquid Seaweed Extracts of Fucus spiralis. Communications in Soil Science and Plant Analysis, 48(21), 2582–2593. https://doi.org/10.1080/00103624.2017.1416136
37. Leandro, A., Pacheco, D., Cotas, J., Marques, J. C., Pereira, L., & Gonçalves, A. M. M. (2020). Seaweed's Bioactive Candidate Compounds to Food Industry and Global Food Security. Life, 10(8), 140. https://doi.org/10.3390/life10080140

38. Lorck, H. (1948). Production of Hydrocyanic Acid by Bacteria. Physiologia Plantarum, 1(2), 142–146. https://doi.org/10.1111/j.1399-3054.1948.tb07118.x
39. Loudari, A., Benadis, C., Naciri, R., Soulaimani, A., Zeroual, Y., Gharous, M. El, Kalaji, H. M., & Oukarroum, A. (2020). Salt stress affects mineral nutrition in shoots and roots and chlorophyll a fluorescence of tomato plants grown in hydroponic culture. Journal of Plant Interactions, 15(1), 398–405. https://doi.org/10.1080/17429145.2020.1841842
40. Ngoroyemoto, N., Kulkarni, M. G., Stirk, W. A., Gupta, S., Finnie, J. F., & van Staden, J. (2020). Interactions Between Microorganisms and a Seaweed-Derived Biostimulant on the Growth and Biochemical Composition of Amaranthus hybridus L. Natural Product Communications, 15(7), 1934578X2093422. https://doi.org/10.1177/1934578X20934228
41. Patel, K., Agarwal, P., & Agarwal, P. K. (2018). Kappaphycus alvarezii sap mitigates abiotic-induced stress in Triticum durum by modulating metabolic coordination and improves growth and yield. Journal of Applied Phycology, 30(4), 2659–2673. https://doi.org/10.1007/s10811-018-1423-4
42. Pérez-Montaño, F., Alías-Villegas, C., Bellogín, R. A., del Cerro, P., Espuny, M. R., Jiménez-Guerrero, I., López-Baena, F. J., Ollero, F. J., & Cubo, T. (2014). Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. Microbiological Research, 169(5–6), 325–336. https://doi.org/10.1016/j.micres.2013.09.011
43. Peters, C. J., Bills, N. L., Wilkins, J. L., & Fick, G. W. (2009). Foodshed analysis and its relevance to sustainability. Renewable Agriculture and Food Systems, 24(1), 1–7. https://doi.org/10.1017/S1742170508002433
44. Rouphael, Y., Cardarelli, M., Bonini, P., & Colla, G. (2017). Synergistic action of a microbial-based biostimulant and a plant derived-protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. Frontiers in Plant Science, 8(FEBRUARY). https://doi.org/10.3389/FPLS.2017.00131/PDF
45. Rouphael, Y., & Colla, G. (2018). Synergistic Biostimulatory Action: Designing the Next Generation of Plant Biostimulants for Sustainable Agriculture. Frontiers in Plant Science, 9. https://doi.org/10.3389/fpls.2018.01655
46. Ruíz-Sánchez, M., Armada, E., Muñoz, Y., García de Salamone, I. E., Aroca, R., Ruíz-Lozano, J. M., & Azcón, R. (2011). Azospirillum and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. Journal of Plant Physiology, 168(10), 1031–1037. https://doi.org/10.1016/j.jplph.2010.12.019
47. Sani, Md. N. H., & Yong, J. W. H. (2021). Harnessing Synergistic Biostimulatory Processes: A Plausible Approach for Enhanced Crop Growth and Resilience in Organic Farming. Biology, 11(1), 41. https://doi.org/10.3390/biology11010041
48. Santana, M. M., Rosa, A. P., Zamarreño, A. M., García-Mina, J. M., Rai, A., & Cruz, C. (2022). Achromobacter xylosoxidans and Enteromorpha intestinalis Extract Improve Tomato Growth under Salt Stress. Agronomy, 12(4), 934. https://doi.org/10.3390/agronomy12040934
49. Santini, G., Biondi, N., Rodolfi, L., & Tredici, M. R. (2021). Plant Biostimulants from Cyanobacteria: An Emerging Strategy to Improve Yields and Sustainability in Agriculture. Plants, 10(4), 643. https://doi.org/10.3390/plants10040643
50. Satpati, G. G., Sengupta, S., & Pal, R. (2022). Seaweeds: The ecological roles, the economic benefits and the threats for changing the carbon cycle. Sustainable Global Resources Of Seaweeds Volume 1: Bioresources, Cultivation, Trade and Multifarious Applications, 295–311. https://doi.org/10.1007/978-3-030-91955-9_16/COVER
51. Shelke, D., Nikalje, G., Nikam, T., Maheshwari, P., Punita, D., Rao, K., Kavi Kishor, P., & Suprasanna, P. (2019). Chloride (Cl^-) uptake, transport, and regulation in plant salt tolerance. In Molecular Plant Abiotic Stress (pp. 241–268). Wiley. https://doi.org/10.1002/9781119463665.ch13
52. Shultana, R., Kee Zuan, A. T., Yusop, M. R., & Saud, H. M. (2020). Characterization of salt-tolerant plant growth-promoting rhizobacteria and the effect on growth and yield of saline-affected rice. PLOS ONE, 15(9), e0238537. https://doi.org/10.1371/journal.pone.0238537
53. Sun, X.-C., Hu, C.-X., & Tan, Q.-L. (2006). Effects of molybdenum on antioxidative defense system and membrane lipid peroxidation in winter wheat under low temperature stress. Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao = Journal of Plant Physiology and Molecular Biology, 32(2), 175–182. http://europepmc.org/abstract/MED/16622316
54. Tomaz, A., Palma, P., Fialho, S., Lima, A., Alvarenga, P., Potes, M., Costa, M. J., & Salgado, R. (2020). Risk Assessment of Irrigation-Related Soil Salinization and Sodification in Mediterranean Areas. Water, 12(12), 3569. https://doi.org/10.3390/w12123569
55. Turner, N. C. (1981). Techniques and experimental approaches for the measurement of plant water status. Plant and Soil, 58(1–3), 339–366. https://doi.org/10.1007/BF02180062
56. Van Oosten, M. J., Pepe, O., De Pascale, S., Silletti, S., & Maggio, A. (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chemical and Biological Technologies in Agriculture, 4(1), 5. https://doi.org/10.1186/s40538-017-0089-5
57. Wang, Y., Gu, W., Meng, Y., Xie, T., Li, L., Li, J., & Wei, S. (2017). γ-Aminobutyric Acid Imparts Partial Protection from Salt Stress Injury to Maize Seedlings by Improving Photosynthesis and Upregulating Osmoprotectants and Antioxidants. Scientific Reports 2017 7:1, 7(1), 1–13. https://doi.org/10.1038/srep43609
58. Wang, Z. Q., Wu, L. H., & Animesh, S. (2009). Nutrient shift in Parthenocissus tricuspidata seedling in response to soil water stress. Scientia Horticulturae, 123(2), 253–257. https://doi.org/10.1016/j.scienta.2009.08.006
59. Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

60. Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A., & Brown, P. H. (2017). Biostimulants in Plant Science: A Global Perspective. Frontiers in Plant Science, 7. https://doi.org/10.3389/fpls.2016.02049
61. Yildirim, E., Turan, M., & Donmez, M. F. (2008). Mitigation of salt stress in radish (Raphanus sativus L.) by plant growth promoting rhizobacteria. Roumanian Biotechnol Lett, 13, 3933–3943.
62. Zameer, M., Zahid, H., Tabassum, B., Ali, Q., Nasir, I. A., Saleem, M., & Butt, S. J. (2016). PGPR Potentially Improve Growth of Tomato Plants in Salt-Stressed Environment. Turkish Journal of Agriculture - Food Science and Technology, 4(6), 455. https://doi.org/10.24925/turjaf.v4i6.455-463.614
63. Zhou, Y., Sang, T., Tian, M., Jahan, M. S., Wang, J., Li, X., Guo, S., Liu, H., Wang, Y., & Shu, S. (2022). Effects of Bacillus cereus on Photosynthesis and Antioxidant Metabolism of Cucumber Seedlings under Salt Stress. Horticulturae, 8(5), 463. https://doi.org/10.3390/horticulturae 8050463