Selected Beneficial Microbes Alleviate Salinity Stress in Hydroponic Lettuce and Pak Choi

Angela Karen Hirst[1], Sanzida Akhter Anee[1], Matthew Joseph Housley[1], Kuan Qin[1], and Rhuanito Soranz Ferrarezi[1]

KEYWORDS. biostimulants, *Brassica chinensis*, deep water culture system, *Lactuca sativa*, mycorrhizal inoculants, plant growth promoting microbes

ABSTRACT. Hydroponics is widely used in greenhouse and vertical farming production because these facilities can precisely control environmental conditions such as lighting, temperature, and vapor pressure deficit. However, the fertilizer solutions have a short life span, and they often do not have adequate microbial populations to enhance plant growth. Previous studies have shown the potential of beneficial microbes to promote plant production and alleviate abiotic and biotic stressors in the field, and studies on their use in controlled environments such as greenhouses and vertical farms are limited in the literature. In this study, we selected several plant growth promoting microbes (PGPMs) and tested their effects on alleviating salinity stress in 'Rex' lettuce (*Lactuca sativa*) and 'Red Pac' pak choi (*Brassica chinensis*) grown in deep water culture hydroponics. Our goal was to use one stressor, salinity, that induces profound symptoms in plant morphology. A three-cycle study was conducted using five PGPMs [*Bacillus*, *Glomus*, *Lactobacillus*, *Trichoderma*, and *Bacillus/Pseudomonas/Trichoderma* (B/P/T) mix] and two salinity levels [no salinity and salinity treatment, with 120 mM, 40 mM, and 80 mM sodium chloride (NaCl) solution used for the first, second, and third cycles, respectively]. We measured the effects of PGPMs and salinity on plant growth and quality and the solution pH and electrical conductivity (EC). Salinity stress decreased lettuce and pak choi leaf area and shoot fresh weight and increased plant leaf chlorophyll and anthocyanin contents with increased solution EC. Under high-salinity stress (120 mM NaCl), the addition of *Trichoderma* reduced pak choi leaf area and fresh weight but increased solution pH, whereas under low salinity stress (40 mM NaCl), *Trichoderma* increased pak choi leaf chlorophyll content. Under moderate-salinity stress (80 mM NaCl) condition, the addition of *Glomus* sp. increased lettuce fresh weight and leaf area, and B/P/T mix increased pak choi leaf area. In conclusion, using the selected PGPMs in low to moderate-salinity stress could increase lettuce and pak choi growth and quality parameters. These results have some practical applications in the future when more saline water is used for production.

Microbes play an important role in the health and lifecycle of plants by improving nutrient availability and uptake, suppressing pests and diseases, and stimulating/stabilizing plant hormone levels (Berg 2009). Having a diverse array of beneficial microbes present in the growing environment or within plant tissues or cells (such as rhizobacteria or mycorrhizal fungi) can help increase plant growth and enhance plant responses to environmental stress, which can also indirectly affect resistance to pathogens (Berg 2009). The strong evolutionary relationships between plants and microbes have been established by several researchers (Liu et al. 2020), and although beneficial microbes have been shown to assist in plant stress responses, the exact mechanisms behind plant/microbe interactions and their potential use in hydroponics systems are still not clearly understood and require further investigation. With the development of beneficial microbes as biostimulant products, such as fertilizers, supplements, and pest and disease control, research is needed to identify beneficial microbes to optimize their use in controlled environment agriculture.

Due to climate change/increased droughts, sea level rise, and anthropogenic activities, soil and water salinization has reduced plant growth in many production areas worldwide (Ullah et al. 2021). Salinity stress, reflected by an increased electrical conductivity (EC) of the soil or hydroponic solution, decreases plant photosynthesis, yield, and nutritional quality of plant tissues because of the accumulation of toxic ions such as sodium (Na^+) and chloride (Cl^-). In the past few years, salinity stress has caused as much as 20% reduction in global agricultural production (Schrader 2017; Shin et al. 2020; Svyantek et al. 2021). Even though water can be treated to remedy high salt concentrations, it is extremely expensive. Lack of proper soil management combined with other environmental concerns has resulted in issues like salinity negatively affecting populations of beneficial microbes in both conventional and organic agroecosystems (Lakhdar et al. 2023). Furthermore, the presence of strong microbial communities in symbiosis with plants can be enough to help each persist under stressful conditions. Many salt-tolerant microorganisms can help plants under high-salinity stress in several ways, such as inducing systemic resistance, activating antioxidant systems, or changing plant growth hormone levels (Gupta et al. 2021).

PGPMs in the genera *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Trichoderma*, and *Glomus* showed significant roles in promoting plant growth in either natural or hydroponic systems (Berg 2009). *Lactobacillus* play a crucial role in controlling the growth of pathogenic or harmful microorganisms by producing lactic acid, hydrogen peroxide, and other metabolites that suppress the growth of pathogens and contribute to nutrient cycling, enhancing plant nutrient availability/uptake. *Bacillus* and *Pseudomonas* are beneficial bacteria that help alleviate stress related to high salinity by facilitating nutrient cycling and promoting plant root growth by producing/synthesizing beneficial organic molecules such as abscisic acid or salicylic acid (Kim et al. 2017;

Received for publication 7 Feb 2024. Accepted for publication 28 Mar 2024.

Published online 13 May 2024.

[1]Department of Horticulture, University of Georgia, 1111 Miller Plant Sciences Building, Athens, GA 30602, USA

This research was funded by the University of Georgia and the College of Agricultural and Environmental Sciences (CAES) Undergraduate Research Grant. We thank Alan Huber, Christopher Nieters, Jonathan Cardenas, Thiago Gastaldo, Derek Bullard, George Hutchinson, Lan Nguyen, Husnain Rauf, Dr. T.C. Jayalath, Hannah Chaffee, and Samuel Poole for their technical support. We also thank Dr. Cari Peters and JR Peters for the fertilizer donation.

R.S.F. is the corresponding author. E-mail address: ferrarezi@uga.edu.

https://doi.org/10.21273/HORTTECH05403-24

Yasmin et al. 2020). *Bacillus* species have shown the ability to alleviate salt stress in cucumber (*Cucumis sativus*) and maize (*Zea mays*) seedlings (Kaloterakis et al. 2021; Song et al. 2023), *Bacillus* has also been shown to improve plant responses to drought stress in sugarcane (*Saccharum officinarum*) (Fonseca et al. 2022), and enhanced growth in hydroponic lettuce (*Lactuca sativa*) under normal conditions and high EC (Moncada et al. 2020). *Trichoderma* is a beneficial fungus with a symbiotic relationship with plants by enhancing resistance to root pathogens through the enzymatic breakdown of the pathogen's cell walls (Guzmán-Guzmán et al. 2023). In other studies, for example, *Trichoderma harzianum* was effective at improving tomato (*Solanum lycopersicum*) seedling growth and root development in nutrient-limiting soil and hydroponic solutions due to the function of mineral solubilization (via acidification, redox, chelation, and hydrolysis) (Li et al. 2015). *Trichoderma* has also been specifically studied and cited as a possible alternative to chemical fungicides that are currently being widely used around the world for agricultural production, contributing to soil and water contamination and raising concerns for human and animal health (Tyśkiewicz et al. 2022). A salt-tolerant strain of *Trichoderma* was also found to be effective in alleviating salt stress by decreasing the concentration of toxic ions in the soil (Liu et al. 2023). Lastly, *Glomus* is a type of mycorrhizal fungi cited as beneficial for plant root nutrient uptake (particularly phosphorus) and improves plant stress tolerance. *Glomus* could reduce the negative effects of salinity stress by regulating the redox state and ion homeostasis (Afrangan et al. 2023).

Using PGPMs in a hydroponic system can improve plant health, increase yield, and create a more resilient system (Liu et al. 2020). However, it is essential to maintain an optimal balance of microbial population, and testing the application of different PGPMs in hydroponic systems in which the environment is highly controlled becomes important. Furthermore, investigating the effects of PGPMs on different abiotic stresses in hydroponics will advance our understanding of plant-microbe interactions during stress conditions and explore the possibilities of integrating PGPMs into commercial hydroponic systems.

The objective of this study was to evaluate the effects of different types of PGPMs including rhizobacteria, mycorrhizal fungi, and products containing mixtures of other bacterial and fungal strains on salinity stress in lettuce and pak choi using deep water culture (DWC) hydroponics. The effects of the inoculants in a standard, nonstressed DWC system were also evaluated. The goal was to evaluate the microbes against various salinity stress levels, ranging from high, moderate, and low amounts of added salt.

Materials and methods

Study location and environmental conditions. This study was conducted in a greenhouse at the University of Georgia (College of Agricultural and Environmental Sciences, Department of Horticulture, Controlled Environment Agriculture Laboratory) in Athens, GA, USA (lat. 33°55′55.10″ N, long. 83°21′ 50.51″ W, altitude 198 m) for three cycles (5 Mar 2022 to 29 Mar 2022, 5 May 2022 to 1 Jun 2022, and 26 Oct 2022 to 22 Nov 2022). Environmental parameters inside the greenhouse were measured using a temperature and humidity sensor (HMP60; Vaisala, Vantaa, Finland) and a light extended photosynthetic active radiation (ePAR) sensor (SQ-610; Apogee Instruments, Logan, UT, USA) connected to a datalogger (CR1000X; Campbell Scientific, Logan, UT, USA). Daily light integral (DLI) and vapor pressure deficit (VPD) were calculated automatically using DLI = photosynthetic photon flux density × light hours per day × (3600/1,000,000), and VPD was calculated from the saturated and actual air vapor pressure using the air temperature and relative humidity data. Average temperature, relative humidity, VPD, and DLI for the first cycle plant growth period were 22.4 °C, 56.1%, 1.26 kPa, 22.31 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, respectively. Second cycle they were 23.8 °C, 77.5%, 0.68 kPa, 34.15 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, respectively. Third cycle they were 21.0 °C, 65.0%, 0.89 kPa, 12.74 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, respectively.

Plant material and cultivation system. Two leafy greens, 'Rex' lettuce (Johnny's Selected Seeds, Winslow, ME, USA) and 'Red Pac' pak choi (*Brassica chinensis*, Johnny's Selected Seeds) seeds were sowed on 1.0 × 1.0 × 1.5 $inch^3$ rockwool plugs (A0 36/40; Grodan, Roermond, The Netherlands) and placed inside a walk-in growth chamber (25 °C, 800 $mg{\cdot}L^{-1}$ carbon dioxide) watered daily by an automated ebb-and-flow subirrigation system. After 2 weeks of growth, seedlings were transplanted in 1.75 inches top diameter × 1.75 inches bottom diameter × 2.0 inches deep net cups (Orimerc Garden, Seattle, WA, USA) and placed in 10-gal (37.9 L) plastic containers (10 Gallon Stacker Tote; Sterilite, Townsend, MA, USA) simulating a DWC system with foam rafts on a 2 × 2 grid with four places to hold net pots and anchor the plants. The plastic containers were filled with a 15N–2.2P–12.4K nutrient solution (Jack's Professional LX 15–5–15 Ca–Mg; JR Peters, Allentown, PA, USA) with 120 $mg{\cdot}L^{-1}$ of nitrogen and aerated with one 2-inch air stone (Aquaneat, Madison, WI, USA) connected to 0.2-inch tubing and a 13.5 $m^3{\cdot}h^{-1}$, 48 kPa, 0.5-inch outlet aeration pump (EcoAir 7; Eco-Plus, Vancouver, WA, USA).

Microbes and salt treatments. We tested two factors, PGPMs and salt, in the form of sodium chloride (NaCl). For PGPMs, six treatments (including control with no added microbes) were mixed with the solution before transplanting the plants into the plastic containers using the recommended rate from the manufacturers: 1) *Bacillus* (Hydroguard; Botanicare, Vancouver, WA, USA) that contains *Bacillus amyloliquefaciens* (0.038% with at least 1 × 10^4 cfu/mL), application rate was 0.528 $g{\cdot}L^{-1}$; 2) *Glomus* (Micronized Endomycorrhizal Inoculant; BioOrganics, New Hope, PA, USA) that contains the mixture of *Glomus aggregatum*, *Glomus etunicatum*, *Glomus clarum*, *Glomus deserticola*, *Glomus intraradices*, *Glomus monosporus*, *Glomus mosseae*, *Gigaspora margarita*, and *Paraglomus brasilianum* (50 propagules/g), application rate was 0.472 $g{\cdot}L^{-1}$; 3) *Lactobacillus* (EM-1 Microbial Soil Amendment; Teraganix, Rusk, TX, USA) that contains *Lactobacillus casei* (1.0 × 10^6 cfu/mL), application rate was 7.488 $g{\cdot}L^{-1}$; 4) *Trichoderma* (RootShield Plus WP; BioWorks, Victor, NY, USA) that contains *T. harzianum* strain T-22 (1.15% with at least 1.0 × 10^7 cfu/g) and *Trichoderma virens* strain G-41 (0.61% with at least 5.3 × 10^6 cfu/g),

Table 1. Effects of plant growth promoting microbes (PGPMs) and sodium chloride (NaCl) treatments on lettuce growth performances (n = 3) during three cycles.

Cycle		120 mM NaCl (first cycle)				40 mM NaCl (second cycle)				80 mM NaCl (third cycle)			
		LA[i] (cm^2/plant)[ii]	SFW (g/plant)	Chl (CCI)	Ant (ACI)	LA (cm^2/plant)	SFW (g/plant)	Chl (CCI)	Ant (ACI)	LA (cm^2/plant)	SFW (g/plant)	Chl (CCI)	Ant (ACI)
PGPM	None	1,526	93.75	10.7	4.1	2,238	203.16	13.0	3.9	1,927 b[iii]	120.95 b	10.3 ab	3.8
	Bacillus	1,144	82.75	12.2	3.5	1,951	184.91	12.5	4.0	1,949 ab	123.71 b	12.8 a	3.6
	Glomus	1,103	73.25	10.9	3.9	2,261	223.57	14.9	4.0	3,070 a	208.65 a	6.9 b	2.8
	Lactobacillus	1,341	120.17	13.7	4.4	2,005	190.91	13.9	4.1	2,579 ab	162.21 ab	8.3 b	3.0
	Trichoderma	1,370	114.00	13.8	4.8	2,514	237.88	14.1	4.1	2,941 ab	192.04 ab	7.9 b	2.9
	B/P/T mix	1,008	98.00	8.5	3.2	1,841	158.72	14.8	3.9	2,740 ab	153.29 ab	8.2 b	3.2
NaCl	No NaCl	2,258 a	180.28 a	7.8 b	2.9 b	2,495 a	232.07 a	12.2 b	3.4 b	2,934 a	189.18 a	7.9 b	2.8 b
	NaCl	240 b	13.69 b	15.5 a	5.1 a	1,774 b	167.64 b	15.5 a	4.5 a	2,134 b	131.11 b	10.3 a	3.6 a
P value	PGPM	NS	NS	NS	NS	NS	NS	NS	NS	**	**	**	NS
	NaCl	***	***	***	***	***	***	***	***	***	***	**	***
	PGPM × NaCl	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[i] LA = leaf area; SFW = shoot fresh weight; Chl = leaf chlorophyll content; CCI = Chl with unit chlorophyll content index; Ant = leaf anthocyanin content; ACI = Ant with unit anthocyanin content index; B/P/T mix = *Bacillus/Pseudomonas/Trichoderma* mix.

[ii] 1 cm^2 = 0.1550 $inch^2$; 1 g = 0.0353 oz.

[iii] Different letters within the column from the same factor indicate significant differences at α = 0.05, according to Tukey's honestly significant difference test. NS, *, **, *** indicate nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

application rate was 0.30 $g \cdot L^{-1}$; 5) mixture of *Bacillus/Pseudomonas/Trichoderma* (B/P/T mix, Root Life; Key to Life, Broomfield, CO, USA) that contains *Bacillus firmus*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus azotoformans*, *Bacillus coagulans*, *Paenibacillus polymyxa*, *Paenibacillus durum* (1.0×10^8 cfu/g), and *Pseudomonas aureofaciens*, *Pseudomonas fluorescens*, *Streptomyces lydicus*, *Steptomyces griseus*, *Trichoderma reesei*, *T. harzianum* (2.0×10^7 cfu/g), application rate was 0.264 $g \cdot L^{-1}$.

For salt treatment, three salinity levels were tested in three cycles by adding NaCl (American Chemical Society reagent, ≥99.0%; Sigma-Aldrich, St. Louis, MO, USA) to the solution after transplanting. During the first cycle, 120 mM NaCl solution was prepared to represent high-salinity stress; for the second cycle, 40 mM NaCl was used to represent low salinity stress; and for the third cycle, 80 mM NaCl was created to represent moderate-salinity stress. No salt added as control was also included during each cycle.

MEASUREMENTS. After 4 weeks of growth, plants were harvested and shoot fresh weight was measured. Leaf area was determined using an area meter (LI-3100; LI-COR, Lincoln, NE, USA). Leaf chlorophyll content (CCM-200plus portable meter; Opti-Sciences, Hudson, NH, USA), leaf anthocyanin content (ACM-200plus portable meter; Opti-Sciences), and solution pH and EC (HI5522-01 bench meter; Hanna instruments, Smithfield, RI, USA) were measured accordingly.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS. For each cycle (salt added with 120 mM, 40 mM, and 80 mM NaCl for the first, second, and third cycles, respectively), a completely randomized design was used to arrange the full factorial design of six organisms as PGPM treatments (control, *Bacillus*, *Glomus*, *Lactobacillus*, *Trichoderma*, B/P/T mix) and two salt treatment levels (no salt and added salt). A total of 12 treatments (6 × 2) were randomly assigned to 36 plastic containers so that each treatment had three replications. Two crops (lettuce and pak choi) were randomly planted into the plastic containers with two plants per treatment replication. Results regarding each crop plant growth and

Table 2. Effects of plant growth promoting microbes (PGPMs) and sodium chloride (NaCl) treatments on pak choi growth performances (n = 3) during three cycles.

Cycle		120 mM NaCl (first cycle) LA[i] (cm^2/plant)[ii]	120 mM NaCl SFW (g/plant)	120 mM NaCl Chl (CCI)	120 mM NaCl Ant (ACI)	40 mM NaCl (second cycle) LA (cm^2/plant)	40 mM NaCl SFW (g/plant)	40 mM NaCl Chl (CCI)	40 mM NaCl Ant (ACI)	80 mM NaCl (third cycle) LA (cm^2/plant)	80 mM NaCl SFW (g/plant)	80 mM NaCl Chl (CCI)	80 mM NaCl Ant (ACI)
PGPM	None	642	72.83	16.7	12.7	1,641	232.31	52.1 ab[iii]	24.0	1,530 ab	166.84	36.9	41.3
	Bacillus	592	59.92	26.8	21.6	1,937	309.71	41.7 ab	25.9	1,039 b	119.18	37.1	35.4
	Glomus	634	74.17	32.2	25.2	1,464	229.87	50.4 ab	24.6	1,289 b	150.00	35.1	34.6
	Lactobacillus	696	67.33	31.8	19.4	1,552	207.32	41.2 ab	22.7	1,445 ab	150.96	32.1	37.5
	Trichoderma	380	37.50	26.2	20.3	1,751	250.44	58.7 a	27.8	1,281 b	122.63	32.4	33.3
	B/P/T mix	516	69.42	13.3	13.6	1,189	175.27	36.1 b	25.4	2,021 a	198.66	30.1	31.5
NaCl	No NaCl	1,143 a	125.94 a	30.1 a	23.5 a	1,959 a	270.37 a	44.6	23.2	1,845 a	193.82 a	30.7 b	33.7
	NaCl	10 b	1.11 b	18.9 b	14.1 b	1,219 b	197.94 b	48.8	26.9	1,023 b	108.93 b	37.2 a	37.5
P value	PGPM	NS	NS	NS	NS	NS	NS	**	NS	**	NS	NS	NS
	NaCl	***	***	*	**	***	***	NS	NS	***	***	**	NS
	PGPM × NaCl	*	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS

[i] LA = leaf area; SFW = shoot fresh weight; Chl = leaf chlorophyll content; CCI = Chl with unit chlorophyll content index; Ant = leaf anthocyanin content; ACI = Ant with unit anthocyanin content index; B/P/T mix = *Bacillus/Pseudomonas/Trichoderma* mix.

[ii] 1 cm^2 = 0.1550 $inch^2$; 1 g = 0.0353 oz.

[iii] Different letters within the column from the same factor indicate significant differences at $\alpha = 0.05$, according to Tukey's honestly significant difference test. NS, *, **, *** indicate nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

solution pH and EC were subjected to ANOVA using a statistical software (R version 4.2.2; R Foundation for Statistical Computing, Vienna, Austria) (R Core Team 2023) with package "agricolae," and the mean comparison was conducted using Tukey's honestly significant difference test at 5% probability ($P = 0.05$).

Results

Plant growth performance. The interaction effects between PGPM and additional salt treatments did not affect lettuce growth regardless of NaCl concentration ($P > 0.05$) (Table 1). Additional salt (40, 80, 120 mM NaCl) significantly decreased lettuce leaf area and shoot fresh weight and increased leaf chlorophyll and anthocyanin content (Table 1). The decrease in yield performances (leaf area and shoot fresh weight) and increase in pigmentation was more severe with high-salinity stress (120 mM NaCl), compared with low- (40 mM NaCl) and moderate-salinity stress (80 mM NaCl). In the trial with 80 mM NaCl, PGPM treatment significantly increased lettuce leaf area and shoot fresh weight, where *Glomus* product increased plant leaf area by 59.3% and shoot fresh weight by 72.5% compared with control ($P < 0.01$), *Bacillus*-treated plants had higher leaf chlorophyll content than other PGPM products ($P < 0.01$), regardless of the presence of salt stress or not.

Unlike lettuce, significant interaction effects were found between PGPM and additional salt treatments on pak choi growth when 120 mM NaCl was imposed (Table 2)—plants grown under *Trichoderma*-treated solution had lower leaf area than *Lactobacillus*-treated plants and lower shoot fresh weight than control, *Glomus*, and B/P/T mix-treated plants when no additional salt was added (Fig. 1A and B). Adding NaCl in the solution significantly reduced the pak choi leaf area and shoot fresh weight compared with control in all salinity stress treatments (Table 2). Although the additional 120 mM NaCl decreased leaf chlorophyll and anthocyanin contents, *Bacillus*, *Glomus*, *Lactobacillus*, and *Trichoderma* reversed this reduction (Fig. 1C). Leaf anthocyanin content did not change when adding 40 mM NaCl, and leaf chlorophyll content increased when moderate-salinity stress was implemented (80 mM NaCl). Under low salinity stress

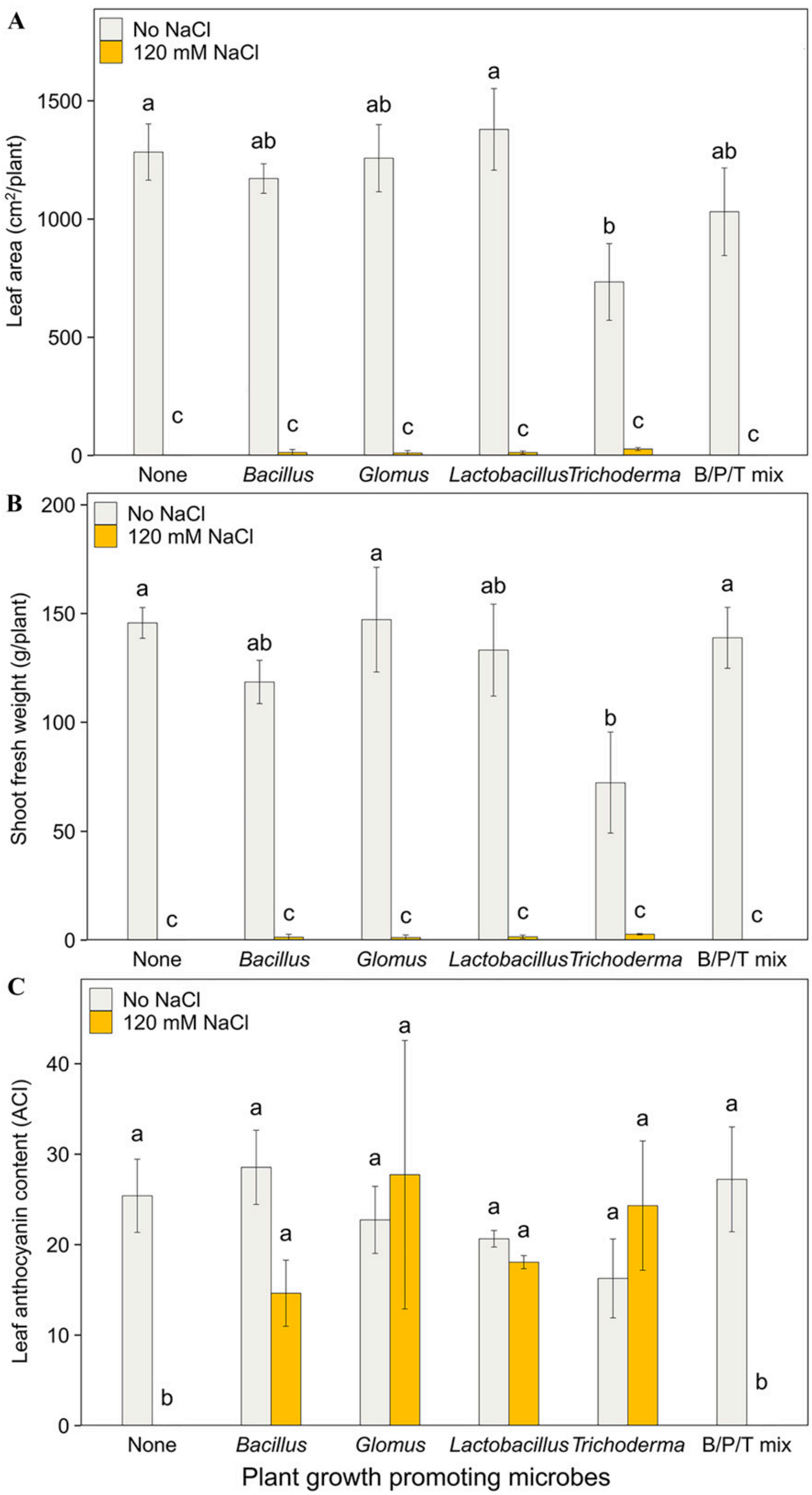

Fig. 1. Pak choi leaf area (A, per plant), shoot fresh weight (B, per plant), and leaf anthocyanin content (C, unit as anthocyanin content index, ACI) as affected by plant growth promoting microbes and sodium chloride (NaCl) treatments during the first cycle when high NaCl concentration (120 mM) was imposed. B/P/T mix = *Bacillus/Pseudomonas/Trichoderma* mix. Data present the mean ± *SE* of three replicates (n = 3), different letters above bars indicate significant differences ($P < 0.05$) among treatments by Tukey's honestly significant difference test. 1 cm^2 = 0.1550 $inch^2$, 1 g = 0.0353 oz.

(40 mM NaCl), *Trichoderma*-treated plants had higher chlorophyll content than other PGPM products regardless of salinity stress. In moderate-salinity stress condition (80 mM NaCl), addition of *Glomus* sp. increased lettuce fresh weight and leaf area (Table 1), and B/P/T-treated pak choi plants had the highest leaf area (Table 2).

Solution pH and EC changes. Solution treated with *Trichoderma* and B/P/T mix had significantly higher pH than high salt treatment (120 mM NaCl) with no salt (Table 3, Fig. 2). Also, low salt stress with 40 mM NaCl had no effect on the solution pH, while 80 mM NaCl significantly reduced the pH ($P < 0.001$). Adding NaCl increased solution EC, and the increased level was the highest when adding 120 mM NaCl, followed by 80 mM and 40 mM NaCl. PGPM treatment significantly affected solution environments during the third cycle, in which solutions mixed with *Glomus* and *Trichoderma* had higher pH than *Bacillus* regardless of salinity stress (Table 3).

Discussion

Additional salinity had the predominant effect on decreasing solution osmotic potential, leading to a higher solution EC, which led to significantly reduced lettuce and pak choi growth and biomass accumulation, even with a low salinity level (40 mM NaCl). Growth reduction was more severe in pak choi than in lettuce, especially under high-salinity stress (120 mM). Crop-specific tolerance is an important factor in overcoming the adverse effects of salinity stress, for example, hydroponic-grown lettuce can tolerate 20 mM NaCl, but when salinity reaches 60 mM NaCl, more than 50% reduction in biomass accumulation is observed (Breś et al. 2022). Pot-grown pak choi showed a significant yield reduction (40%) when salinity reached 50 mM NaCl (Keling and Zhujun 2010). Other leafy greens also have specific ranges of salinity tolerance: pot-grown spinach (*Spinacia oleracea*) had a similar growth when the salinity level reached 30 mM NaCl compared with no salinity, but more than 50% reduction in yield was reached when salinity was higher than 90 mM NaCl (El-Nakhel et al. 2022); hydroponic-grown sweet basil (*Ocimum basilicum*) had tolerance to 20 mM NaCl but not beyond 40 mM NaCl (Corrado et al. 2021); and pot-grown

Table 3. **Effects of plant growth promoting microbes (PGPMs) and sodium chloride (NaCl) treatments on solution pH and electrical conductivity (EC) changes (n = 3) during three cycles.**

	Cycle	120 mM NaCl (first cycle) pH	120 mM NaCl (first cycle) EC (mmho/cm)[i]	40 mM NaCl (second cycle) pH	40 mM NaCl (second cycle) EC (mmho/cm)	80 mM NaCl (third cycle) pH	80 mM NaCl (third cycle) EC (mmho/cm)
PGPM	None	6.0	8.90	7.3	4.60	5.5 ab[ii]	6.85
	Bacillus	5.8	9.07	7.3	4.83	4.8 b	6.74
	Glomus	6.0	9.00	7.3	4.66	6.5 a	6.45
	Lactobacillus	5.8	8.96	7.2	4.04	6.0 ab	6.66
	Trichoderma	6.1	9.38	7.7	4.66	6.3 a	6.69
	B/P/T mix[iii]	6.0	9.04	6.7	4.53	5.9 ab	6.78
NaCl	No NaCl	5.7 b	1.16 b	7.5	0.36 b	6.5 a	0.60 b
	NaCl	6.3 a	16.96 a	7.1	8.75 a	5.2 b	12.80 a
P value	PGPM	NS	NS	NS	NS	**	NS
	NaCl	***	***	NS	***	***	***
	PGPM × NaCl	*	NS	NS	NS	NS	NS

[i] 1 mmho·cm^{-1} = 1 dS·m^{-1}

[ii] Different letters within the column from the same factor indicate significant differences at α = 0.05, according to Tukey's honestly significant difference test. NS, *, **, *** indicate nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

[iii] B/P/T mix = *Bacillus/Pseudomonas/Trichoderma* mix.

perilla (*Perilla frutescens*) had reduced growth when salinity level reached 10 mM NaCl, and 40 mM NaCl could lead to 70% yield reduction (Rouphael et al. 2019).

Although salinity negatively affected plant biomass accumulation, moderate-salinity stress increased pigmentation in lettuce for chlorophyll and anthocyanin contents. Similar trends were also found in pak choi when mild salinity stress (80 mM NaCl) was imposed, whereas the high-salinity stress (120 mM NaCl) completely stopped pak choi growth and development so that pigments were not accumulated. Salinity stress could also increase plant biochemical compounds (e.g., ascorbic acid, flavonoids, phenols, carotenoids) and antioxidant capacities (Giordano et al. 2021). These results support the notion that low and moderate (nonharmful) salinity stress can increase pigmentation and stimulate the production of secondary metabolites in some leafy greens. That is important because consumers tend to prefer red cultivars because of the positive health benefits of anthocyanins.

Beneficial microbes form mutualistic, symbiotic, or commensal relationships with plants, the application of PGPM is often associated with improved plant growth, resistance to biotic and abiotic stress, and soil health due to their released bioactive compounds (e.g., vitamins, hormones, and enzymes), secreted biocontrol agents, enhanced resource availability (nutrients and water) and organic matter contents (Naik et al. 2019). Existing research on microbes in soil-based production systems supported that PGPMs alleviated salinity stress by improving plant responses (e.g., improved nutrient uptake, root development, and shoot biomass accumulation) rather than mitigating the salinity levels in the soil environment (Egamberdieva and Lugtenberg 2014). Mechanisms behind those improvements in plant responses can be summarized as follows: 1) increased nutrient availability and uptake due to extraradical hyphae; 2) induced plant systematic resistance to stress by promoting the production of phytohormones (e.g., auxin) and osmolytes (e.g., sugars, amino acids) to overcome osmotic stress; and 3) activating antioxidant systems to scavenge reactive oxygen species (ROS) (Egamberdieva and Lugtenberg 2014; Gupta et al. 2021; Lakhdar et al. 2023). Our results further extended the potential use of PGPMs, particularly *Glomus* mycorrhizal fungi, to improve lettuce growth and B/P/T mix to increase pak choi growth in hydroponics. However, unlike soil-based systems, additional PGPM in hydroponics also caused an alteration of the solution pH, mainly found under *Glomus* and *Trichoderma*-treated solution. As a genus of arbuscular mycorrhizal fungi, *Glomus* sp. affect water osmotic homeostasis due to the improved plant nutrient uptake (Lakhdar et al. 2023), and increased solution pH from our study could indicate *Glomus* promoted plants absorbing more anions than cations. *Trichoderma* sp. is often used as a biological control agent, and its use typically leads to reduced environmental pH due to the production of organic

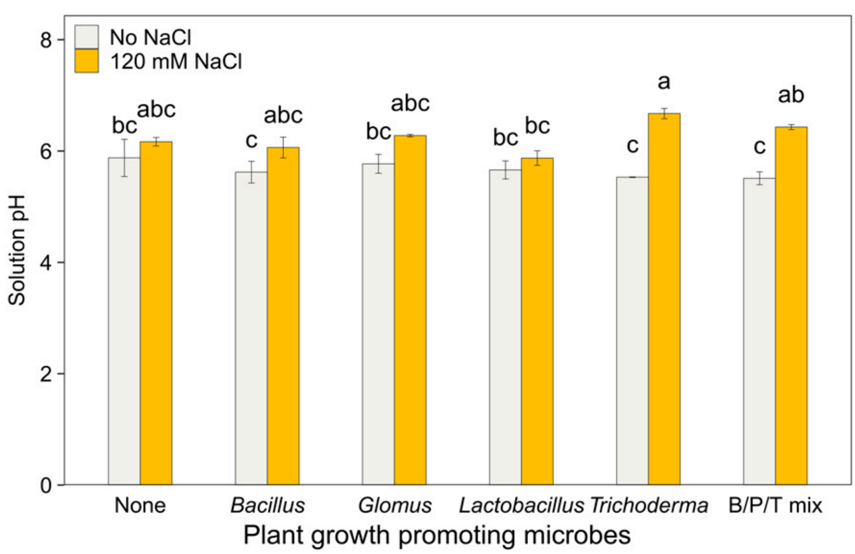

Fig. 2. Solution pH of deep water culture system affected by plant growth promoting microbes and sodium chloride (NaCl) treatments during the first cycle when high NaCl concentration (120 mM) was imposed. B/P/T mix = *Bacillus/ Pseudomonas/Trichoderma* mix. Data present the mean ± *SE* of three replicates (n = 3), different letters above bars indicate significant differences (*P* < 0.05) among treatments by Tukey's honestly significant difference test.

acids (e.g., gluconic and citric acids) (Tyśkiewicz et al. 2022). The opposite situation was observed in our study, in which *Trichoderma* application increased solution pH with negative effects on pak choi growth (leaf area and biomass). Although *Trichoderma* application was often associated with beneficial effects on plant growth under soil application, another study (Li et al. 2015) showed using *Trichoderma* resulted in negative outcomes due to potential competition for phosphorus (phytate) and zinc with plants by suppressing root development, releasing phytase and chelating minerals. The different effects between soil and hydroponic cultivation and the potential implementation for the use of *Trichoderma* needs further investigation, especially considering the interaction and competition of available nutrients and plant root growth due to *Trichoderma* application.

Bacillus used in this study was associated with improved lettuce chlorophyll and pak choi anthocyanin content without sacrificing biomass accumulation. Because anthocyanin can act as an antioxidant protecting plants from ROS, it can also increase the nutritional potential for the leafy greens' quality (Mattioli et al. 2020). These results provided new insights into improving plant quality using *Bacillus* under salt stress conditions in addition to the drought stress, and their application was reported to enhance gas exchange parameters (Fonseca et al. 2022); however, we did measure photosynthetic parameters (photosynthetic rates, stomatal conductance, transpiration) during the first cycle, but no differences were found among PGPMs and salt treatments (data not shown). Adjustment of the application of microbes in hydroponic systems relative to their soil application rates, as well as using techniques to identify a population of PGPMs in the solution, are important considerations when implementing the PGPMs into practical production. In addition, recent findings (Akhoundnejad and Baran 2023) indicated the synergistic use of PGPMs (e.g., arbuscular mycorrhizal fungi) and phytohormones (e.g., salicylic acid and jasmonic acid) could be a potential technique to alleviate abiotic stress. Because of the different responses of PGPM applications in soil-based and hydroponic systems, more research is needed on the practical implementation of PGPMs into hydroponic systems relative to the type of growth systems, crops, and the types of microbial inoculant (bacteria or fungi). Nutritional analysis of plant tissues also could be used to study the impacts on nutrient uptake in response to beneficial microbes or applied stress.

Conclusions

This study investigated beneficial microbes in a DWC system and showed that mycorrhizal inoculants *Glomus* and the mix of beneficial bacteria and fungi (B/P/T mix) were able to improve lettuce and pak choi leaf area and shoot fresh weight under low to moderate salt stress conditions (40–80 mM NaCl). Slight salt stress (40 mM NaCl) could potentially be used to increase plant secondary metabolites such as pigments, and there were potentials in using mycorrhizal inoculants (*Glomus*) to optimize the nutrient availability of hydroponic systems and using *Bacillus* to improve leaf chlorophyll content further.

References cited

Afrangan F, Kazemeini SA, Alinia M, Mastinu A. 2023. *Glomus versiforme* and *Micrococcus yunnanensis* reduce the negative effects of salinity stress by regulating the redox state and ion homeostasis in *Brassica napus* L. crops. Biologia. 78(11): 3049–3061. https://doi.org/10.1007/s11756-023-01479-3.

Akhoundnejad Y, Baran S. 2023. Boosting drought resistance in pepper (*capsicum annuum* L.) with the aid of arbuscular mycorrhizal fungi and key phytohormones. HortScience. 58(11):1358–1367. https://doi.org/10.21273/hortsci17370-23.

Berg G. 2009. Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol. 84(1):11–18. https://doi.org/10.1007/s00253-009-2092-7.

Breś W, Kleiber T, Markiewicz B, Mieloszyk E, Mieloch M. 2022. The effect of NaCl stress on the response of lettuce (*Lactuca sativa* L.). Agronomy. 12(2):244. https://doi.org/10.3390/agronomy12020244.

Corrado G, Vitaglione P, Chiaiese P, Rouphael Y. 2021. Unraveling the modulation of controlled salinity stress on morphometric traits, mineral profile, and bioactive metabolome equilibrium in hydroponic basil. Horticulturae. 7(9):273. https://doi.org/10.3390/horticulturae7090273.

Egamberdieva D, Lugtenberg B. 2014. Use of plant growth-promoting rhizobacteria to alleviate salinity stress in plants, p 73–96. In: Miransari M (ed). Use of microbes for the alleviation of soil stresses, volume 1. Springer, New York, NY, USA. https://doi.org/10.1007/978-1-4614-9466-9_4.

El-Nakhel C, Cozzolino E, Ottaiano L, Petropoulos SA, Nocerino S, Pelosi ME, Rouphael Y, Mori M, Di Mola I. 2022. Effect of biostimulant application on plant growth, chlorophylls and hydrophilic antioxidant activity of spinach (*spinacia oleracea* L.) grown under saline stress. Horticulturae. 8(10):971. https://doi.org/10.3390/horticulturae8100971.

Fonseca MCD, Bossolani JW, de Oliveira SL, Moretti LG, Portugal JR, Scudeletti D, de Oliveira EF, Crusciol CAC. 2022. *Bacillus subtilis* inoculation improves nutrient uptake and physiological activity in sugarcane under drought stress. Microorganisms. 10(4):809. https://doi.org/10.3390/microorganisms10040809.

Giordano M, Petropoulos SA, Rouphael Y. 2021. Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. Agriculture. 11(5):463. https://doi.org/10.3390/agriculture11050463.

Gupta S, Schillaci M, Walker R, Smith PM, Watt M, Roessner U. 2021. Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: Current knowledge, perspectives and future directions. Plant Soil. 461:219–244. https://doi.org/10.1007/s11104-020-04618-w.

Guzmán-Guzmán P, Kumar A, de los Santos-Villalobos S, Parra-Cota FI, Orozco-Mosqueda Md C, Fadiji AE, Hyder S, Babalola OO, Santoyo G. 2023. *Trichoderma* species: Our best fungal allies in the biocontrol of plant diseases–A review. Plants. 12(3):432. https://doi.org/10.3390/plants12030432.

Kaloterakis N, van Delden SH, Hartley S, De Deyn GB. 2021. Silicon application and plant growth promoting rhizobacteria consisting of six pure *Bacillus* species alleviate salinity stress in cucumber (*Cucumis sativus* L). Scientia Hortic. 288:110383. https://doi.org/10.1016/j.scienta.2021.110383.

Keling H, Zhujun Z. 2010. Effects of different concentrations of sodium chloride on plant growth and glucosinolate content and composition in pakchoi. Afr J Biotechnol. 9(28):4428–4433.

Kim M-J, Radhakrishnan R, Kang S-M, You Y-H, Jeong E-J, Kim J-G, Lee I-J.

2017. Plant growth promoting effect of *Bacillus amyloliquefaciens* H-2-5 on crop plants and influence on physiological changes in soybean under soil salinity. Physiol Mol Biol Plants. 23(3):571–580. https://doi.org/10.1007/s12298-017-0449-4.

Lakhdar A, Trigui M, Montemurro F. 2023. An overview of biostimulants' effects in saline soils. Agronomy. 13(8):2092. https://doi.org/10.3390/agronomy13082092.

Li RX, Cai F, Pang G, Shen QR, Li R, Chen W. 2015. Solubilisation of phosphate and micronutrients by *trichoderma harzianum* and its relationship with the promotion of tomato plant growth. PLoS One. 10(6):e0130081. https://doi.org/10.1371/journal.pone.0130081.

Liu H, Brettell LE, Qiu Z, Singh BK. 2020. Microbiome-mediated stress resistance in plants. Trends Plant Sci. 25(8):733–743. https://doi.org/10.1016/j.tplants.2020.03.014.

Liu Z, Xu N, Pang Q, Khan RAA, Xu Q, Wu C, Liu T. 2023. A salt-tolerant strain of *Trichoderma longibrachiatum* HL167 is effective in alleviating salt stress, promoting plant growth, and managing fusarium wilt disease in cowpeas. J Fungi. 9(3):304. https://doi.org/10.3390/jof9030304.

Mattioli R, Francioso A, Mosca L, Silva P. 2020. Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. Molecules. 25(17):3809. https://doi.org/10.3390/molecules25173809.

Moncada A, Vetrano F, Miceli A. 2020. Alleviation of salt stress by plant growth-promoting bacteria in hydroponic leaf lettuce. Agronomy. 10(10):1523. https://doi.org/10.3390/agronomy10101523.

Naik K, Mishra S, Srichandan H, Singh PK, Sarangi PK. 2019. Plant growth promoting microbes: Potential link to sustainable agriculture and environment. Biocatal Agric Biotechnol. 21:101326. https://doi.org/10.1016/j.bcab.2019.101326.

R Core Team. 2023. R-4.3.1 for Windows. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org. [accessed 10 Sep 2023].

Rouphael Y, Kyriacou MC, Carillo P, Pizzolongo F, Romano R, Sifola MI. 2019. Chemical eustress elicits tailored responses and enhances the functional quality of novel food *Perilla frutescens*. Molecules. 24(1):185. https://doi.org/10.3390/molecules24010185.

Schrader SE. 2017. Salinity tolerance of lettuce cultivars in controlled environment (Master's Thesis). University of Arizona, Tucson, AZ, USA.

Shin YK, Bhandari SR, Jo JS, Song JW, Cho MC, Yang EY, Lee JG. 2020. Response to salt stress in lettuce: Changes in chlorophyll fluorescence parameters, phytochemical contents, and antioxidant activities. Agronomy. 10(11):1627. https://doi.org/10.3390/agronomy10111627.

Song P, Zhao B, Sun X, Li L, Wang Z, Ma C, Zhang J. 2023. Effects of *Bacillus subtilis* HS5B5 on maize seed germination and seedling growth under NaCl stress conditions. Agronomy. 13(7):1874. https://doi.org/10.3390/agronomy13071874.

Svyantek AW, Wang Z, Rana B, Tatar I, Auwarter C, Hatterman-Valenti H. 2021. Performance of hydroponic pak choi (*Brassica rapa* subsp. *chinensis*) under elevated sodium conditions. Acta Hortic. 1321:133–140. https://doi.org/10.17660/ActaHortic.2021.1321.17.

Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J. 2022. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Int J Mol Sci. 23(4):2329. https://doi.org/10.3390/ijms23042329.

Ullah A, Bano A, Khan N. 2021. Climate change and salinity effects on crops and chemical communication between plants and plant growth-promoting microorganisms under stress. Front Sustain Food Syst. 5:618092. https://doi.org/10.3389/fsufs.2021.618092.

Yasmin H, Naeem S, Bakhtawar M, Jabeen Z, Nosheen A, Naz R, Keyani R, Mumtaz S, Hassan MN. 2020. Halotolerant rhizobacteria *Pseudomonas pseudoalcaligenes* and *Bacillus subtilis* mediate systemic tolerance in hydroponically grown soybean (*Glycine max* L.) against salinity stress. PLoS One. 15(4):e0231348. https://doi.org/10.1371/journal.pone.0231348.