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Zinc and Phosphate Solubilizing by Rhizobacteria Promotes Lettuce (*Lactuca sativa* L.) Growth in Salty Conditions

Yusuf Çelik[1,a,*], Adem Özarslandan[2,b], Necibe Kayak[3,c]

[1]*Silifke Vocational School, Department of Plant an Animal Production, 33940, Silifke Mersin/Türkiye.*
[2]*Silifke School of Applied Technology and Business Administration, Department of Organic Agriculture Management, 33940, Silifke Mersin/Türkiye.*
[3]*Department of Horticulture, Faculty of Agriculture, Sakarya University of Applied Sciences, Sakarya/Türkiye*
[*]*Corresponding author*

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ABSTRACT

Due to climate change, the world is negatively affected by drought, temperature, salinity, and flood stress, leading to a significant decline in crop production. Lettuce is particularly significant when considering salt stress. To increase plant tolerance to salinity, various strategies are employed to support the development of agriculture. Rhizobacteria play a key role in regulating phosphorus (P) and zinc (Zn) homeostasis in plants. According to the study results, Rhizobium bacteria supported plant growth by improving the solubility of zinc and phosphate. These findings highlight the beneficial effects of plant growth-promoting rhizobacteria (PGPR) on the antioxidant system, which helps detoxify reactive oxygen species. The relationship between proline accumulation and antioxidant enzyme activities showed that PGPR inoculation enhanced the plant's defense mechanism against salt stress. In establishing this tolerance, increases in chlorophyll content, repair of membrane repair, and higher leaf relative humidity under salt stress were observed. PGPR also improved seedling height, diameter, and fresh and dry weight under stress by 70%, 51.4%, 55%, and 109%, respectively, due to the stress-mitigating effects of P and Zn. In conclusion, it is predicted that there will be a need to develop fertilization programs containing different rhizobacteria and Zn+P combinations. These programs would activate the antioxidant mechanism in saline soils, stabilize physiological processes, and positively impact plant growth.

[a] *ycelik33@mersin.edu.tr* *https://orcid.org/0000-0002-8590-6690*
[b] *mail* *https://orcid.org/0000-0001-7129-8543*
[c] *mail* *https://orcid.org/0000-0001-7104-8544*

Introduction

Lettuce (*Lactuca sativa* L.), a member of the *Asteraceae (=Compositae)* family, is cultivated worldwide and its leaves are commonly consumed as salad vegetables. Lettuce, which is an important food source for health, constitutes 3.41% of the total agricultural land in Türkiye and represents of 1.19% in vegetable production areas (TÜİK, 2019). According to TÜİK data, Türkiye produced 577,773 tons of lettuce in 2022. This highlights the significant contribution of lettuce to the overall vegetable production in the country.

Global warming, driven by climate change, represents a severe threat to the Earth's ecosystems (Borjas-Ventura et al., 2020). The main factor infulencing agricultural productivity is the climate (Adams, 1998). One of the major global issues negatively affecting agricultural productivity in arid and semi-arid regions is soil salinity (El hasini et al., 2019). According to Oster and Jayawardane (1998), soil salinization can impact surface water runoff, soil erosion, and seedling emergence. Salinity, for instance, can adversely effect on plant growth by hindering root penetration, reducing the plant's water-holding capacity, and disrupting the circulation of water and air in the soil. Soil osmotic stress initially increases as a result of salinity stress (Munns et al., 2008). A few minutes after salt accumulates in the root zone, the osmotic phase begins. In this phase, stomatal closure, an increase in leaf temperature, and restricted shoot elongation are the primary indicators in plants, due to the thick inner wall of the guard cells and the low soil water potential (Mukhopadhyay et al. 2021). Salt stress also induces oxidative stress by increasing reactive oxygen species (ROS), such as superoxide ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) (Alscher et al., 1997; Mittler, 2002; Neill et al., 2002).

Plants have developed various defense mechanisms against ROS, with the antioxidant defense system being crucial. This system includes enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and ascorbate peroxidase (APX) activities which are effective in mitigating oxidative stress (Farooq et al., 2008). An increase in the activity of antioxidant enzymes in response to salt stress has also been reported in various studies (Unlukara et al., 2008; Hela et al., 2011; Sardar et al., 2023)

To improve plant tolerance to salinity, various strategies are employed for sustainable agriculture. One promising approach is microbial technology, specifically the use of plant growth-promoting rhizobacteria (PGPR). Historically, the concept of the rhizosphere and the role of PGPR in mitigating salt stress and enhancing plant health were highlighted by Hilter in 1904 (Al-Barakah et al., 2019; Zhang et al., 2019). The most researched PGPR species are Bacillus and Pseudomonas, which are well-known for their ability to enhance plant health by solubilizing zinc and phosphate (Ku et al., 2019; Desoy et al., 2020). Lettuce, in particular, benefits from phosphate fertilizers, which significantly increase yield and improve quality (Johnstone et al., 2005; Hoque et al., 2010). The application of phosphate-solubilizing PGPR has been shown to increase soil phosphate availability and plant yield (Chabot et al., 1996).

Zinc is also crucial for various physiological functions, including photosynthesis, chlorophyll content, phytohormone synthesis, protein synthesis, and growth rate, antioxidant activity (Mousavi et al., 2013). Zinc deficiency can result in stunted development, chlorosis, and an increased vulnerability to diseases and other stressors (Tavallali et al., 2010). Ensuring adequate zinc supply can enhance growth, chlorophyll content, photosynthesis, and stress tolerance. The use os zinc-solubilizing PGPR has been proposed as a means of enhancing crop development and bioenrichment (Vaid et al., 2014; Mumtaz et al., 2017; Hussain et al., 2020).

Additionally, photosynthesis, the synthesis of phytohormones, growth rate, chlorophyll content, protein synthesis, and antioxidant activity are all dependent on zinc (Mousavi et al., 2013). Tropical areas are known for their high prevalence of zinc deficiency, which can lead to chlorosis, stunted growth, and heightened vulnerability to diseases and other stressors (Tavallali et al., 2010). Ensuring an adequate supply of zinc can improve growth, chlorophyll content, photosynthesis, and stress tolerance. According to several studies (Vaid et al., 2014; Mumtaz et al., 2017; Hussain et al., 2020), applying zinc-solubilizing PGPR can enhance crop development and bioenrichment.

Phosphorus (P) is one of the key plant nutrients whose absorption, transport, and distribution in plants are negatively impacted by salinity-stress (Dey et al., 2021). Crop productivity is limited by low a P availability mediated by salinity stress. It is often recommended to apply more P fertilizer to saline soils in order to control P deficits; however, in salt-affected soils, the low efficiency of available P fertilizer use limits P availability and P fertilizers can pose significant environmental risks (Dey et al., 2021). One of the most important PGPR in the salt-tolerant microbial communities is phosphate-solubilizing bacteria (PSB), which can produce a variety of metabolites that promote plant growth in saline conditions, such as phytohormone, siderophore, ACC deaminase, and anti-phytopathogens, as well as solubilize Phosphate, which aid in plant growth and include Nitrogen.

In Türkiye, salinity issues in agricultural soils are exacerbated by factors such as saline irrigation water, low rainfall, poor soil management, high evaporation rates, and excessive chemical use. In this context, PGPR offer a promising strategy to enhance plant resistance and productivity in saline environments. This study investigates the impact of PGPR on the growth and yield of lettuce under varying salinity conditions, emphasizing its potential to mitigate salinity stress and promote sustainable agriculture.

Materials and Methods

The Experiment's Variety and Characteristics of Lettuce

The experiment used the Lital lettuce variety from Hazar Seed Company's. It is a type of lettuce with a core and is resistant to heat and gets up late. The average head weight is 800 – 1100 gr. Harvest period is 55 – 70 days. The leaf color is light green, reminiscent of light yellow. The leaves are erect, brittle and broad. Leaf margins are wavy and slightly toothed.

Plants, Cultivation, and Treatments

This study was conducted in research greenhouses and as a pot experiment during the fall of 2022 at Mersin University Silifke Vocational School. Hybrid seeds of lettuce variety were used in the study. The trial planned as four replications according to the randomized blocks trial design; 7 applications including salt and nutrient solutions including control application and each application consisted of 40 plants. In the experiment, it was tried to determine the resistance of lettuce seedlings to drought stress, different salt concentrations and nutrient applications by using rhizobacteria, known to be effective in increasing stress tolerance in plants. Normal tap water was applied to the control plants. The seeds covered with bacteria in the laboratory were manually sown in seedling vials filled with the medium used in seedling production (peat + perlite + vermiculite) and then watered with appropriate methods. On the 21st day of the germination period, lettuce seedlings were transplanted into 750 ml pots in the same medium and were watered for one week for adaptation and necessary development. After this stage, salt and nutrient solutions were applied 5 times at intervals of 2 days and the experiment was terminated after 10 days. For measurements, 8 lettuce seedlings representing the average were selected from each plot and the average was taken. The greenhouse temperature during the experiment was conducted was 20-28 ^{0}C, with a humidity level of 65%. The fertilizers used in the experiment included a single dose of rhizobacteria, salt (NaCl) concentrations, 0 (control), 75 mM, 150 mM, Zinc ($ZnSO_4$); 5mg L^{-1} and phosphorus (P_2O_5); It was determined as 300 mg.kg^{-1} P and added to Hoagland nutrient solution for general nutrition of plants (Hothem et al., 2003).

Disinfection of Seeds to Be Used in the Study

Lettuce seeds were soaked in 70% ethanol for 5 minutes and then washed with distilled water (sdH_2O). Then, they were then treated with 5% NaOCl for 3 minutes and at the end of the time, the seeds were washed with sdH_2O. After the disinfection process was completed, the seeds were filtered and left to dry on blotting paper.

Isolation and Purification of Microorganisms

The culturable bacterial population was isolated from the rhizospheric soil using a serial dilution approach. One gram of soil was combined with nine milliliters of deionized water in a test tube and vigorously stirred with a vortex mixer. Following the method of Johnson and Curl (1972), dilutions were created up to 10^{-5}. On Nutrient Agar (HiMedia®) plates, 100 µl of each dilution was applied, and the plates were incubated for 24 hours at 30 °C. Then, using traits including color, texture, and shape, bacterial colonies were detected, enabling the computation of colony-forming units (CFU) and occurrence percentages. The CFU per gram of dry soil was determined using the formula: CFU g^{-1} dry soil = (Mean number of colonies / Dry weight of soil) × Dilution factor. For further purification, isolates were streaked on nutrient agar plates, and a single, well-isolated colony was selected and re-streaked to achieve a pure culture (Zhou, 1987).

Bacteria Used in The Study and Their Properties

YÖ41: Bacillus cereus GC subgroup A

- MIS similarity index (%): 78
- Nitrogen-fixing properties: Strong
- Phosphorus and zinc dissolving properties: Strongly positive
- Isolated from plant roots of Thymus vulgaris from rhizospheric soil in Türkiye

Phosphate Solubilization

The isolate's dissolution capacity was evaluated using the Tricalcium Phosphate (TCP) method as described by Wahyudi et al. (2011). In short, point inoculation of cultures was performed on Pikovskaya Agar (HiMedia®) plates, followed by incubation for 5 days at 30 °C. The appearance of a clear halo around the colonies indicated phosphate solubilization activity. Phosphate solubility was quantified as follows: fertile isolates were added to 100 ml of Pikovskaya solution in 250 ml Erlenmeyer flasks, along with 0.5 g of TCP as an insoluble phosphate source. The CFU per gram of dry soil was determined using the formula: CFU g^{-1} dry soil = (Mean number of colonies / Dry soil weight) × Dilution factor. The flasks were sterilized, and the medium was adjusted to an initial pH of 6.0. The isolates were then inoculated, and the flasks were incubated for 10 days at 27 °C. Each day, duplicate samples were taken, and the medium was filtered using Whatman 42 filter papers. The chlorostannous-reduced molybdophosphoric acid blue method was employed to measure the P_2O_5 content of the filtrates, with absorbance recorded at 700 nm using an Eppendorf BioSpectrometer. For zinc solubilization, the isolates were incubated for 48 hours in a modified PVK medium supplemented with 0.1% insoluble zinc compounds (ZnO, $ZnCO_3$, ZnS). Zinc dissolution was visually assessed by observing the clearance of the opaque medium, and the diameter of the clear zone was measured to calculate the Zinc Solubilization Index (ZSI) (Bapiri et al., 2012). Siderophore production was evaluated using Chrome Azurol S (CAS) agar (Schwyn et al., 1987). To assess the salinity tolerance (NaCl stress), selected isolates were grown in nutrient broth (NB) containing varying concentrations of NaCl (1.2% and 3% w/v) and their growth was monitored by measuring absorbance at 600 nm (Mahajan et al., 1920). Nitrogen fixation ability was determined by growing the isolate in nitrogen-free solid malate (Nfb) medium at 33 °C for 24 hours (Okon et al., 1977). Bacterial growth indicated nitrogen-fixing capacity. Quantitative assessment of nitrogen fixation by the YO41 isolate was performed using the acetylene reduction assay (ARA) (Hardy et al., 1968), a reliable method to evaluate the activity of nitrogenase, the enzyme responsible for nitrogen fixation. Nitrogenase reduces acetylene gas to ethylene, which is measured using flame ionization gas chromatography to determine nitrogen fixation rates.

Preparation of Bacterial Solutions and Vaccination Process

In the seed coating application, a bacterial suspension was first prepared. At this stage, bacteria preserved in 30% glycerol and liquid medium (Lauryl Broth) at -80 °C were seeded on Nutrient Agar solid medium. After the planted petri dishes were incubated for 48 hours in an incubator set at 27 ^{0}C, a loopful of each bacteria was taken and transferred to flasks containing 250 ml of Nutrient Broth. The broths contaminated with bacteria were incubated for 24 hours at 150 rpm in a shaker set at 27 °C for aerobic growth of bacteria. The prepared bacterial suspensions were diluted with sterile distilled water and the final concentration was adjusted to 107 cfu ml^{-1} by spectrophotometric measurement (Turan et al. 2014).

Covering Lettuce Seeds with Bacteria

Disinfected lettuce seeds were placed in bacterial suspensions with a concentration of 10^7 cfu/ml and left to incubate in a shaker at 140 rpm for 2 hours. At the end of the incubation period, the seeds were filtered and treated with sucrose to ensure the adhesion of the bacteria. The types of treatment to be applied in the trial is given in (Table 1)

Table 1. Trial subjects

1	Control (0 mM NaCl)
2	75 mM NaCl
3	150 mM NaCl
4	YÖ41
5	P +Zn+YÖ41
6	P+Zn+YO41+75 mM NaCl
7	P+Zn+YO41+150 mM NaCl

Membrane Permeability (% EC)

Sample plant seedlings with 5-6 true leaves representing the mean were harvested and three samples of 1 cm diameter were taken from the leaves of each in the laboratory. These samples were washed with distilled water and placed in brown glass bottles, and the analyzes were repeated 3 times by adding 10 ml of distilled water. The prepared bottles were kept in the shaker for 24 hours and after that time, the solutions in the bottles were poured into the tubes and the EC1 value was measured in the EC meter. Then, the solutions were poured back into the bottles and kept in an autoclave at 120 ºC for 20 minutes, and the EC value was calculated from the formula EC1 / EC2 x 100 by measuring the EC2 value at room temperature (Lutts et al., 1996).

Relative Water Content (% RWC)

The turgor weights of the leaf samples were determined after they were immersed in distilled water for four hours to measure their relative water content (RWC). Three 1 cm^2 leaf discs were obtained to check for membrane damage. The discs were then shaken in closed vials at 25 °C for 24 hours after adding 10 mL of water. The EC values were calculated right away. EC measurements were taken again after the same samples were autoclaved for 20 minutes at 120°C and allowed to cool to 25 °C (Lutts et al., 1996).

Chlorophyll Amount (SPAD)

The middle of the upper leaves of 4 randomly selected plants in each repetition for each application during the seedling period (5-6 leaves). With the SPAD device (SPAD-502 Chlorophyll) measuring devices; Konica Minolta, Tokyo, Japan) relative chlorophyll content was measured.

Antioxidant Enzyme Activity Assay

Fresh lettuce leaves were processed following the protocol outlined by Angelini and Federico (1989). The resulting samples were analyzed at 560 nm, as described by Agarwal and Pandey (2004), to determine SOD activity by measuring the enzyme content responsible for inhibition. CAT activity was assessed using the method of Havir and McHale (1987), with absorbance changes recorded at 240 nm. POD activity was measured at 470 nm, based on the procedure established by Chance (1955). APX activity was determined at 290 nm, based on procedure established by Chaoui (1997).

Proline Analysis (µmol/g fresh weight)

Proline levels were quantified using a spectrophotometric approach based on the acid-ninhydrin method, as described by Bates et al. (1973).

Dry Mass

After harvesting, the leaves from the sample plants were placed in an oven at 65 °C for 48 hours. When the last two weight measurements were equal, indicating that the leaves were completely dried, they were removed from the oven, and their dry weights were calculated (Kacar, 1972).

Statistical Analysis

The effects of varying irrigation levels and intervals on the yield and quality components were examined using variance analysis at two different probability levels (0.05 and 0.01), and the Duncan test was used to compare the averages. The statistical package IBM SPSS 23 (IBM Statistics for Windows, Version 23) was used to calculate all statistical values. Regression analysis was also used to determine the water-yield relationships.

Results

In the control group, the plant root length is 6.2 cm. In plants treated with 75 mM NaCl, the root length decreased to 5.7 cm. In plants treated with 150 mM NaCl, the root length decreased to 4.6 cm, indicating that root length decreases as salt concentration increases. The YO41 treatment increased root length to 7 cm. The P+Zn+YO41 treatment increased root length to 10.2 cm, achieving the highest value. The P+Zn+YO41 treatment with salt resulted in a root length of 9.7 cm with 75 mM NaCl and 7.4 cm with 150 mM NaCl. The highest root length was obtained with the P+Zn+YO41 treatment, demonstrating that this combination significantly enhances plant root development (Tables 2).

In the control group, the plant diameter is 4.2 cm. The plant diameter decreased to 3.7 cm with 75 mM NaCl and to 3.1 cm with 150 mM NaCl. The YO41 treatment increased the plant diameter to 5.6 cm. The P+Zn+YO41 treatment achieved the highest plant diameter of 6.2 cm. The P+Zn+YO41 treatment with salt resulted in a plant diameter of 5.6 cm with 75 mM NaCl and 4.7 cm with 150 mM NaCl. Overall, the P+Zn+YO41 treatment increased plant diameter, and this increase continued despite salt stress (Tables 2).

In the control group, the number of leaves is 7.3. In plants treated with 75 mM NaCl, the number of leaves decreased to 7, and to 5.5 with 150 mM NaCl. The YO41 treatment increased the number of leaves to 8.5. The P+Zn+YO41 treatment achieved the highest number of leaves, with 10 leaves. The P+Zn+YO41 treatment with salt resulted in 9 leaves with 75 mM NaCl and 8.9 leaves with 150 mM NaCl. The P+Zn+YO41 combination maintained the highest number of leaves, promoting plant growth (Tables 2).

In the control group, the leaf width is 3.5 cm. The leaf width decreased to 3.1 cm with 75 mM NaCl and to 2.9 cm with 150 mM NaCl. The YO41 treatment increased leaf width to 4.9 cm. The P+Zn+YO41 treatment increased leaf width to 4.7 cm. The P+Zn+YO41 treatment with salt resulted in a leaf width of 4.4 cm with 75 mM NaCl and 3.6 cm with 150 mM NaCl. The P+Zn+YO41 combination significantly increased leaf width (Tables 2).

In the control group, the root length is 7.7 cm. The root length decreased to 6.9 cm with 75 mM NaCl and to 4.9 cm with 150 mM NaCl. The YO41 treatment increased root length to 7.8 cm. The P+Zn+YO41 treatment increased root length to 8.4 cm, achieving the highest value. The P+Zn+YO41 treatment with salt resulted in a root length of 7.6 cm with 75 mM NaCl and 6.3 cm with 150 mM NaCl. The P+Zn+YO41 combination significantly increased root length and supported root development despite salt stress (Tables 2).

The P+Zn+YO41 combination significantly enhanced plant growth and protected the plant against salt stress. Notable increases were observed in root length, plant diameter, and number of leaves. As salt concentration increased, a general decrease in plant growth was observed, but the P+Zn+YO41 treatment largely compensated for this decrease. These data suggest that the P+Zn+YO41 treatment could be an effective method for improving plant growth and managing salt stress (Tables 2).

In the control group, the fresh and dry weights of the plants were 3.8 g and 0.7 g, respectively, and the fresh and dry weights of the roots were 1 g and 0.27 g, respectively. In plants treated with 75 mM NaCl, the fresh and dry weights of the plants decreased to 3.2 g and 0.6 g, respectively, and the fresh and dry weights of the roots decreased to 0.71 g and 0.21 g, respectively. This indicates that salt stress reduces both plant and root weights. In plants treated with 150 mM NaCl, the fresh and dry weights of the plants decreased to 2.7 g and 0.5 g, respectively, and the fresh and dry weights of the roots decreased to 0.62 g and 0.16 g, respectively. This shows that as salt concentration increases, plant and root weights decrease further (Table 3).

Table 2. The effects of P, Zn and PGPR (YO41) applications on lettuce seedlings exposed to salt stress on seedling growth parameters

Treatments	Plant Rootless Length (cm)	Plant Diameter (cm)	Number Of Leaves (Plant/Piece)	Leaf Width (cm)	Root Length (cm)
Control	6.2 ±0.2cd	4.2 ±0.1bc	7.3 ± 0.5c	3.5 ± 0.1b	7.7 ± 0.4ab
75 mM NaCl	5.7 ± 0.2de	3.7 ±0.1c	7 ± 0.4c	3.1 ±0.1b	6.9 ± 0.2bc
150 mMNaCl	4.6 ± 0.2e	3.1 ± 0.2d	5.5 ± 0.3d	2.9 ± 0.2b	4.9 ±0.2d
YO41	7 ± 0.3bc	5.6 ±0.2a	8.5 ± 0.3b	4.9 ± 0.1a	7.8 ±0.3ab
P+Zn+YO41	10.2 ± 0.7a	6.2 ± 0.2a	10 ± 0.4a	4.7 ±0.3a	8.4 ± 0.4a
P+Zn+YO41+75 mM NaCl	9.7 ± 0.5a	5.6 ± 0.2a	9 ± 0.4ab	4.4 ± 0.5a	7.6 ± 0.3ab
P+Zn+YO41+150 mM NaCl	7.4 ± 0.5b	4.7 ±0.3b	8.9 ±0.5b	3.6 ±0.2b	6.3 ±0.3c
Average	7.25	4.73	6.76	3.87	7.08

* When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

Table 3. The effects of P, Zn and PGPR (YO41) applications on lettuce seedlings exposed to salt stress on seedling growth parameters

Treatments	Plant Fresh Weight (g)	Plant Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Control	3.8 ± 0.2bcd	0.7 ± 0b	1 ± 0.1ab	0.27 ± 0d
75 mM NaCl	3.2 ± 0.2de	0.6 ± 0b	0.71 ±0.1bc	0.21 ± 0de
150 mM NaCl	2.7 ± 0.2e	0.5 ±0c	0.62 ±0c	0.16 ± 0e
YO41	4.2 ±0.2bc	0.9 ± 0.1a	0.8 4 ± 0.2bc	0.4 3 ±b
P+Zn+YO41	4.8 ± 0.3a	1 ± 0.1a	1.2 ± 0a	0.46 ± 0a
P+Zn+YO41+75 mM NaCl	4.4 ± 0.1ab	1 ±0.1a	1. 1 ± 0.1ab	0.44 ± 0b
P+Zn+YO41+150 mM NaCl	3.7 ± 0.2dc	0.7 ±0.1b	0.8 ±0.1bc	0.23 ±0c
Average	3.82	0.77	0.79	0.24

When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

The YO41 treatment increased the fresh and dry weights of the plants to 4.2 g and 0.9 g, respectively, and the fresh and dry weights of the roots to 0.84 g and 0.43 g, respectively. This demonstrates that YO41 supports plant growth. The P+Zn+YO41 treatment increased the fresh and dry weights of the plants to 4.8 g and 1 g, respectively, and the fresh and dry weights of the roots to 1.2 g and 0.46 g, respectively, achieving the highest values. This combination significantly enhances plant growth (Table 3).

In plants treated with P+Zn+YO41+75 mM NaCl, the fresh and dry weights of the plants were 4.4 g and 1 g, respectively, and the fresh and dry weights of the roots were 1.1 g and 0.44 g, respectively. This indicates that despite salt stress, this combination supports plant growth. In plants treated with P+Zn+YO41+150 mM NaCl, the fresh and dry weights of the plants were 3.7 g and 0.7 g, respectively, and the fresh and dry weights of the roots were 0.8 g and 0.23 g, respectively. This shows that even at high salt concentration, the combination helps maintain plant growth to some extent (Table 3).

Overall, the highest plant and root weights were obtained with the P+Zn+YO41 combination. Salt stress was observed to reduce plant and root weights, while YO41 and P+Zn+YO41 treatments mitigated these adverse effects (Table 3).

The table 4 presents the impact of P, Zn, and PGPR (YO41) applications on the antioxidant levels of lettuce seedlings under salt stress. SOD, CAT, POD), and APX activities were measured in enzyme units (EU) per gram of sample.

In the control group, SOD, CAT, POD, and APX activities were 116.4 EU g^{-1}, 324 EU $^{g-1}$, 57.5 EU $^{g-1}$, and 8.2 EU $^{g-1}$, respectively. Treatment with 75 mM NaCl decreased these activities to 100.2 EU g-1, 293.9 EU $^{g-1}$, 58.9 EU $^{g-1}$, and 6 EU $^{g-1}$, respectively. Under 150 mM NaCl, the activities further decreased to 97.8 EU $^{g-1}$, 237.6 EU $^{g-1}$, 55.7 EU $^{g-1}$, and 4.8 EU $^{g-1}$, respectively (Table 4).

YO41 treatment significantly increased all enzyme activities, with SOD at 231.8 EU $^{g-1}$, CAT at 517.8 EU $^{g-1}$, POD at 62.1 EU $^{g-1}$, and APX at 13 EU $^{g-1}$. P+Zn+YO41 treatment further increased these activities to 249.9 EU $^{g-1}$, 571.2 EU $^{g-1}$, 118.3 EU $^{g-1}$, and 15.3 EU $^{g-1}$, respectively (Table 4).

Combining YO41, P, and Zn with 75 mM NaCl resulted in intermediate activities, with SOD at 194.1 EU $^{g-1}$, CAT at 497 EU $^{g-1}$, POD at 102.9 EU $^{g-1}$, and APX at 10.4 EU $^{g-1}$. Similarly, with 150 mM NaCl, the activities were SOD 130.1 EU $^{g-1}$, CAT 315.7 EU $^{g-1}$, POD 101.7 EU $^{g-1}$, and APX 7.2 EU $^{g-}$1. Overall, application of YO41, P, and Zn, alone or combined, increased antioxidant enzyme activities in lettuce seedlings under salt stress, indicating a protective effect against oxidative stress (Table 4).

The table 5 presents the effect of P, Zn, and PGPR (YO41) applications on some physiological parameters of lettuce seedlings under salt stress. The parameters measured are membrane damage (%), chlorophyll ratio (%), relative humidity (%), and proline content (µg g^{-1}).

In the control group, the values for membrane damage, chlorophyll ratio, relative humidity, and proline content were 33.7%, 41.5%, 73%, and 170 µg g^{-1}, respectively. Treatment with 75 mM NaCl increased membrane damage to 64.1%, decreased chlorophyll ratio to 34.9%, decreased relative humidity to 58.7%, and decreased proline content to 110.6 µg g^{-1}. Under 150 mM NaCl, the values further increased to 71.4%, 33.1%, 54.5%, and 106 µg g^{-1}, respectively (Table 5).

Table 4. Effects of P, Zn and PGPR (YO41) applications on antioxidant levels of lettuce seedlings exposed to salt stress

Parameters	SOD (EU g^{-1})	CAT (EU g^{-1})	POD (EU g^{-1})	APX (EU g^{-1})
Control	116.4 ± 6.8cd	324 ±15.4c	57.5 ±1.5c	8.2 ± 0.5d
75 mM NaCl	100.2 ±7.3d	293.9 ± 5.2c	58.9 ±1.4c	6 ±0.3e
150 mM NaCl	97.8 ± 2.3d	237.6 ± 9.7d	55.7 ±1.6c	4.8 ±0.2f
YÖ41	231.8 ±-6.6a	517.8 ± 14.1b	62.1 ± 2c	13 ± 0.6b
P+Zn+YO41	249.9 ± 6.2a	571.2 ±4.1b	118.3 ±5.4a	15.3 ± 0.2a
YÖ41+P+Zn+75 mM NaCl	194.1 ± 9b	497 ± 14.6b	102.9 ±4.1b	10.4 ± 0.3c
YÖ41+P+Zn+150 mM NaCl	130.1 ±10.3c	315.7 ± 13.1c	101.7 ±5.6b	7.2 ±0.3d
Average	160.16	393.8	79.59	9.34

When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

Table 5. The effect of P, Zn and PGPR (YO41) applications on some physiological parameters of lettuce seedlings exposed to salt stress.

Parameter	Membrane Damage (%)	Chlorophyll Ratio (%)	Relative Humidity (%)	Prolin (µg g^{-1})
Control	33.7 ±1.1d	41.5 ±2.1bc	73 ±0.1b	170 ±6.6c
75 mM NaCl	64.1 ±3.1b	34.9 ±0.1d	58.7 ±1.5d	110.6 ±5.9d
150 mM NaCl	71.4 ±2.2a	33.1 ±0.1d	54.5 ±0.1e	106 ±3.4d
YÖ41	34.9 ±0.1d	43.6 ±1.3bc	71.8 ±2.2ab	315.5 ±9.8a
P+Zn+YO41	32.5 ±0.1d	53.2 ±1.9a	77.3 ±0.1a	344.7 ±9.3a
YÖ41+P+Zn+75 mM NaCl	56.6 ±1.5	44.9 ±0.1b	69.6 ±.6b	330.2 ±13.4b
YÖ41+P+Zn+150 mM NaCl	61.7 ±1.9	40.1 ±0.5d	64.6 ±0.1c	160 ±6.2c
Average	50.7	41.61	67.07	219.57

When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

YO41 treatment reduced membrane damage to 34.9%, increased chlorophyll ratio to 43.6%, slightly decreased relative humidity to 71.8%, and significantly increased proline content to 315.5 µg g^{-1}. P+Zn+YO41 treatment further reduced membrane damage to 32.5%, significantly increased chlorophyll ratio to 53.2%, slightly increased relative humidity to 77.3%, and significantly increased proline content to 344.7 µg g^{-1} (Table 5).

Combining YO41, P, and Zn with 75 mM NaCl resulted in moderate changes in the parameters, with membrane damage at 56.6%, chlorophyll ratio at 44.9%, relative humidity at 69.6%, and proline content at 330.2 µg g-1. Similarly, with 150 mM NaCl, the changes were membrane damage at 61.7%, chlorophyll ratio at 40.1%, relative humidity at 64.6%, and proline content at 160 µg g^{-1} (Table 5).

Overall, the application of P, Zn, and YO41, either alone or in combination, had varying effects on the physiological parameters of lettuce seedlings under salt stress, with significant improvements in most parameters observed with the combined treatment (Table 5).

Discussion

Salinity is a critical abiotic stress factor that significantly affects plant productivity and quality. Plants under salinity stress exhibit notable symptoms, including reduced turgor pressure, stunted leaf growth, and decreased photosynthesis rates. Salinity impairs growth, yield, and quality by disrupting essential metabolic processes in plants (Alp and Kabay, 2019; Kabay, 2019; Yılmaz et al., 2011). The increased severity of salinity also leads to mechanical, metabolic, and oxidative damage. In our study, we observed a marked decrease in lettuce growth as salinity stress intensified. Specifically, growth parameters such as root length, plant diameter, number of leaves, leaf width, plant fresh and dry weight, and root fresh and dry weight were significantly reduced under higher salt concentrations (Tables 2, 3). These findings are consistent with those of Ouhaddou et al. (2022), who reported that salinity adversely affected growth and physiological traits. However, their study also noted that compost application and mycorrhizal colonization under 100 mM NaCl positively influenced growth parameters.

Salinity negatively impacts physiological parameters, potentially reducing crop yields through three primary mechanisms: degradation of the thylakoid membrane, a decrease in photosynthetic efficiency, and a subsequent decline in plant growth (Baslam et al., 2020). Growth inhibition under salinity stress is commonly linked to increased osmotic pressure, nutrient deficiencies, and damage to physiological mechanisms (Alqarawi et al., 2014). Our results showed that PGPR inoculation improved root length in lettuce (Table 2), with plant growth enhancing upon application of PGPR isolates combined with P+Zn. However, bacterial activity decreased with higher NaCl concentrations, indicating a negative effect of salt on bacterial cell numbers. The observed growth promotion can be attributed to improved P-solubility and Zn uptake by the bacterial isolates, similar to findings by Qin et al. (2017).

During the seedling stage, plants are particularly vulnerable to salt stress, with parameters such as plant height, diameter, and fresh and dry weights responding to salt levels. Stress conditions lead to reduced root growth and stem elongation due to insufficient water uptake. Advanced salt applications further reduce stem dimensions and can lead to seedling mortality depending on severity. Significant reductions in dry matter and fresh weight under stress have been reported for various plants (Irshad et al., 2002; Ghoulam et al., 2002; Dasgan et al., 2002). Nevertheless, our study found that YO41, P, and Zn applications mitigated salt stress effects. Salt stress induces oxidative stress by altering water potential, causing

physiological drought, and imbalancing mineral elements, which triggers the release of reactive oxygen species (ROS) that inhibit growth and metabolism (Evelin et al., 2012). ROS accumulation also damages cell membranes. Plants counteract ROS with various defense systems involving both enzymatic (e.g., SOD and CAT) and non-enzymatic antioxidants (Navarro-León et al., 2020).

Using plant growth-promoting rhizobacteria (PGPR) offers an eco-friendly solution to combat abiotic stresses, including salinity (Glick and Bashan, 1997). The combined treatment with YÖ41, P, and Zn significantly enhanced enzyme activities even under NaCl stress, suggesting protective effects of these treatments. Similar findings were reported by Han and Lee (2005), where PGPRs like Serratia sp. and Rhizobium sp. reduced APX and GR activity under increasing salinity stress. Upadhyay et al. (2012) observed reduced antioxidant enzyme activities (APX, CAT, GR) in wheat leaves treated with PGPR strains under salinity stress. Kadmiri et al. (2018) noted increased antioxidant enzyme levels with phosphate-solubilizing and IAA-producing Pseudomonas fluorescens and Azospirillum brasilense in saline soil. Kallala et al. (2018) found that rhizobial inoculants alleviated stress in legumes by inducing antioxidant enzymes. Increased carbohydrate content in plants treated with R. radiobacter LB2 was also observed, attributed to enhanced photosynthesis (Kang et al., 2014). Our study supports these findings, demonstrating that PGPR positively impacts enzyme activities and stress resistance, aligning with existing literature.

Conclusion

Salinity poses a significant challenge to Turkish agricultural lands. The ability of plant growth-promoting rhizobacteria (PGPR) to thrive in saline soils offers a distinct advantage. The bacterial strain YO41, known for its role in enhancing plant defense mechanisms under stress, mitigates stress effects by improving the solubility of nutrients such as phosphorus (P) and zinc (Zn). During the development of salt tolerance, YO41 was found to increase chlorophyll content, repair membrane damage, enhance leaf relative humidity, proline content, and antioxidant capacity in response to salt stress. PGPR is believed to support plant growth through mechanisms such as auxin and siderophore production, with phosphorus solubilization balancing the antagonistic effects between soluble Zn and P.

As salinity levels increased, there was a noticeable decline in the fresh and dry weights of lettuce, as well as the fresh and dry weights of roots. Additionally, root height, diameter, leaf width, and root length were adversely affected. Plants subjected to 150 mM NaCl treatment ceased development after a certain period, whereas seedling growth was slower in treatments with YO41+P+Zn+150 mM NaCl. In contrast, control plants exhibited better growth compared to those treated with 150 mM NaCl. The combination of YO41+75 mM NaCl+P+Zn was found to alleviate the impact of salt stress. Notably, treatments with YO41+P+Zn+75 mM NaCl led to increased levels of SOD, CAT, APX, POD, and proline, whereas these components remained low in treatments with YO41+P+Zn+150 mM NaCl. Further research is needed to explore the effects of beneficial mineral applications in mitigating salt stress.

Declarations

Author Contribution

Y.Ç.: Investigation; conceptualization; funding acquisition; writing – original draft; methodology; validation; writing– review and editing.

A.Ö.: Investigation; writing – review and editing; methodology

N.K.: Conceptualization; methodol-ogy; writing – review and editing.

Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable reques.

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