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Research Article

Effect of plant growth-promoting rhizobacteria (PGPR) on growth and yield of shallots on saline soils

Deris Trian Rahmandhias, Anna Satyana Karyawati, Didik Hariyono, Mochammad Dawam Maghfoer*

Department of Agronomy, Faculty of Agriculture, Brawijaya University, Jl. Veteran, Malang 65145 East Java, Indonesia

*corresponding author: mdm-fp@ub.ac.id

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Abstract

Soil salinity is a limiting factor in agricultural productivity. One of the biological approaches to mitigate the impact of salt stress on plants is inoculating plant growth-promoting rhizobacteria (PGPR) to the plant roots. This study aimed to investigate the eff of PGPR dosage on the growth and yield of shallots at various salinity levels. This study was carried out in the experimental field of Poncokusumo, Malang. The treatments tested consisted of two factors. The first factor was soil salinity level, consisting of four levels: no salinity, NaCl 50 mM, NaCl 100 mM, and NaCl 150 mM. The second factor was PGPR concentration, consisting of four levels: no PGPR, PGPR 10 mL/L, PGPR 20 mL/L, and PGPR 30 mL/L. The sixteen treatment combinations were arranged in a randomized block design with three replications. The data obtained were subjected to the analysis of variance (ANOVA) at a significance level limit of 5%, followed by the Honestly Significant Difference (HSD) test at a 5% significance level for any significant differences. The results showed that the application of 30 mL/L of PGPR reduced EC of the soil and improved plant height, plant dry weight, leaf area, bulb diameter, bulb weight, and the number of bulbs per plant by 33%, 47.3%, 81%, 13%, 34.2%, 98.5%, and 31%, respectively, compared to the treatment without PGPR application under NaCl 150 mM salinity. The application of PGPR at 20 and 30 mL/L dosages significantly increased chlorophyll, flavonoid, and proline indices at NaCl at 100 mM and 150 mM salinity levels compared to the treatment without PGPR.

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Introduction

Soil salinity presents a significant challenge in agriculture as it restricts plant growth and productivity (Lipper et al., 2014). According to FAO (2022), 33% of saline land in the world, with 20% of saline land causing a decrease in agricultural productivity. Zhang (2014) reported that saline land in Indonesia reached 13 million hectares, which is estimated to continue to increase. The extent of saline land is caused by several factors, such as high levels of evaporation land, low precipitation levels, saline water irrigation, and seawater intrusion (Karolinoerita and Annisa, 2020). According to Shrivastava and Kumar (2015), saline soil with electrical conductivity (EC) above 4 dS/m can decrease agricultural land productivity and cause crop failure. Excessive salt content in the soil can cause sodic soil and reduce the cation exchange capacity of the soil, making it difficult for plants to absorb nutrients and water (Qadir et al., 2007).

Salinity causes an imbalance in osmotic pressure between the soil and the root surface, resulting in plant water deficiency and increasing the toxicity of Na^+ and Cl^- in plants (Balasubramaniam et al., 2023). Syamsiah et al. (2020) reported that agricultural land irrigated with water containing high salt can increase soil pH and accumulation of Na^+ in the soil, as well as proven to decrease growth, yield, and productivity of shallot

in a way significant if compared to irrigation land with no salinity. Soil treated with 150 mM sodium chloride (NaCl) also affected shallot productivity through decreased germination rate by 80%, root length by 82%, and tuber weight by 77% lower compared with non-saline soil (Çavuşoğlu, 2023). Hnilickova et al. (2021) also reported that the application of 300 mM NaCl to the soil caused a decline in land agriculture productivity with indication results of decreased photosynthesis, stomatal conductance, plant water potential, and carbon dioxide (CO_2) assimilation of *Portulaca oleracea* L.

Several efforts have been made to address the issue of saline land, which has a negative impact on agricultural land productivity. One of the biological approaches is the application of PGPR (plant growth-promoting rhizobacteria). It has been widely known that these microorganisms improve productivity in agricultural land by increasing nutrient uptake and water uptake under environmental stresses such as salinity (Gupta et al., 2021). PGPR uses direct and indirect mechanisms to improve agricultural land productivity through the enhancement of nutrient availability and osmoprotectant production, increase cation exchange capacity (CEC), especially K^+ and Na^+, as well as reduce the accumulation of Na^+ around rooting plants in saline conditions (Bhat et al., 2020). PGPR also produces secondary metabolites as Na^+ toxic ion chelator in saline soil so that plant roots will not absorb it.

The application of PGPR isolated from fertile soil has been proven to improve and increase crop yield in saline soil (Aini et al., 2023). Khan et al. (2016) reported that PGPR *Bacillus pumilus* inoculation in saline soil reduced Na^+ accumulation and increased *Oryza sativa* L. growth better compared with no PGPR application. Abd-Allah et al. (2018) reported that the application of *Bacillus subtilis* on saline soil alleviated salt stress on legumes through soil improvement and prevented accumulation of Na^+ in the soil around rooting, as well as modulation of the antioxidant system plant for suppress Reactive Oxygen Species (ROS).

Khan et al. (2023) proved that applying *Pseudomonas fluorescens* and *Azotobacter chroococcum* on saline soil could improve productivity, growth, yield, and antioxidant mustard better if compared with no PGPR application. According to Desoky et al. (2020), the application of PGPR *Pseudomonas aeruginosa, Serratia marcescens*, and *B. cereus* in saline soils can indirectly stimulate the production of proline plant as well as improve the yield of *Triticum aestivum* L. better if compared to without PGPR application. However, research on the effectiveness of PGPR application doses on saline soil is still limited.

This study aimed to evaluate the effect of PGPR effective concentration in improving soil quality and reducing the negative impact of salinity on the growth and yield of shallots.

Materials and Methods

The study was carried out from June to August 2023 in an experimental field located in Poncokusumo, a sub-district in Malang (8°02'34.7" S and 112°46'36.0" E - 8°02'34.7" S and 112°46' 6.0" E), at an elevation of 714 m above sea level and an average daily temperature of 27ºC. The soil of the study area has the following characteristics: EC = 0.88 dS/m, organic C = 0.72%, exchangeable Al = 0.11 $cmol_{(+)}$/kg, exchangeable H = 1.68 $cmol_{(+)}$/kg, total N = 0.14%, P_2O_5 = 202 ppm, exchangeable K = 0.27 $cmol_{(+)}$/kg, exchangeable Na = 1.10 $cmol_{(+)}$/kg, exchangeable Ca = 3.85 $cmol_{(+)}$/kg, exchangeable Mg = 0.03 $cmol_{(+)}$/kg, and CEC = 12.67 $cmol_{(+)}$/kg.

This study used a randomized block design with two treatment factors. The first factor was soil salinity levels, consisting of four levels: no salinity (S0), NaCl 50 mM (S1), NaCl 100 mM (S2), and NaCl 150 mM (S3). The second factor was PGPR concentration, consisting of four levels: no PGPR (P0), PGPR 10 mL/L (P1), PGPR 20 mL/L (P2), PGPR 30 mL/L (P3), resulting in sixteen treatment combinations. Three replications were carried out for each treatment, and each replication (plot) consisted of fifteen plants. The shallot seeds used were the "Tajuk" variety, which was sorted so they were of uniform seed size. Each planting hole was filled with one seed, and the polybag used had a diameter of 20 cm without holes under the polybag to avoid leaching.

The soil was from the same source and taken from the topsoil to a 0-30 cm depth. The weight of the media in the polybag was 5.34 kg soil and cow manure with a ratio of 1:4. Fertilizers given were Ponska (NPK 16:16:16) of 1.29 g and ZA of 0.64 g for each polybag. Fertilizers were applied twice as basal fertilizers and 30 days after planting (DAP). The salt used was industrial-grade NaCl with a purity of 90%. The NaCl solution preparation method was carried out by weighing the required NaCl divided by the relative mass of NaCl and the volume of water as a solvent using the following formula,

Molarity of NaCl = NaCl Mass / [Relative Molecular Mass NaCl × Solvent Volume (Water)] ……..(1)

NaCl solution was applied one day before planting with 500 mL volume in each polybag as an appropriate concentration treatment. The PGPR used in this study was isolates of a consortium of bacteria, including *Azospirillum* sp., *Azotobacter* sp., *Bacillus subtilis*, and *Pseudomonas fluorescens* with a density of $1x10^8$ CFU/mL. The PGPR isolates were obtained from the Plant Pathology Laboratory collection of the Department of Plant Pests and Diseases, Faculty of Agriculture, Brawijaya University. The PGPR was applied 7 days before planting and at 7, 14, 21, and 28 days after planting (DAP). Soil observations were carried out with composite samples in each replication by measuring soil EC after harvest. The plant growth was observed by measuring height, dry weight, and

leaf area. The plant yield observations at the harvesting time (8 weeks after planting) included the bulb diameter, bulb weight per plant, percentage of weight loss in bulbs, and the number of bulbs per plant. Meanwhile, the physiological observations were conducted by determining the shallot's chlorophyll index (SPAD), Relative Water Content (RWC), proline content, and total flavonoid content.

Relative Water Content (RWC) in plant

The RWC value was measured using the formula as follows:

RWC (%) = [(Fresh Weight – Dry Weight) / (Turgid Weight – Dry Weight)] × 100% ……..……..(2)

Proline content

The proline was measured based on the protocol suggested by Bates et al. (1973). In this study, 0.5 g of fresh leaves were rinsed with liquid nitrogen and ground into a fine powder using a mortar. The resulting homogenate was dissolved in 10 mL of 3% sulfosalicylic acid and filtered using Whatman No. 1 filter paper. Then, 2 mL of the filtrate was reacted with 2 mL of ninhydrin solution (1.25 g of ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid) and 2 mL of glacial acetic acid in a reaction tube at a temperature of 100°C for 1 hour. The mixture was then cooled in an ice bath. The solution was extracted with 4 mL of toluene and homogenized using a vortex for 30 seconds until two-phase layers were formed. The upper red layer, containing proline, was measured for absorbance using a spectrophotometer at a wavelength of 520 nm.

Flavonoid content

The flavonoid content was measured by grinding 1 g of dried leaf sample and extracting it with 10 mL of 70% ethanol for 1 hour. Then, the solution was filtered, and 0.5 mL of the filtrate was reacted with 1 mL of 10% $AlCl_3$ and 0.1 mL of 1 M CH_3COONa. The mixture was then added with 1.5 mL of 70% ethanol and 2.8 mL of distilled water. Afterward, the solution mixture was homogenized with a vortex and incubated for 30 minutes. A UV-Vis spectrophotometer was used to measure the absorbance of the solution with a wavelength of 430 nm.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) at a 5% significance level, followed by the Honest Significant Difference (HSD) test at a 5% significance level.

Results and Discussion

Soil electrical conductivity

The soil after harvest, especially those treated with salinity levels of 100 mM and 150 mM, had a soil EC above 4 dS/m; in other words, it is classified as saline soil (Zhovtonog et al., 2018). Treatment with a salinity level of 150 mM without PGPR (S3P0) application showed the highest EC, with a value of 9.67 dS/M. Meanwhile, the application of a PGPR concentration of 20 mL/L reduced EC values at all salinity levels. The salinity treatment of 150 mM and PGPR 30 mL/L (S3P3) showed a soil EC value of 7.33 dS/m. The application of PGPR 30 ml/L reduced EC values in all salinity treatments (Table 1).

Table 1. Soil electrical conductivity (ECs) values at shallot harvest time in various treatment combinations.

Treatments*		ECs (dS/m)
S0	P0	0.92
	P1	1.05
	P2	1.07
	P3	0.97
S1	P0	3.55
	P1	3.61
	P2	2.91
	P3	2.50
S2	P0	6.89
	P1	7.91
	P2	4.90
	P3	5.59
S3	P0	9.67
	P1	9.42
	P2	7.61
	P3	7.33

Notes: S1 = NaCl 50 mM; S2 = NaCl 100 mM; S3 = NaCl 150 mM; P0 = No PGPR; P1 = PGPR 10 mL/L; P2 = PGPR 20 mL/L; P3 = PGPR 30 mL/L. *Soil electrical conductivity (ECs) value before planting was 0.88 dS/m.

A decrease in soil EC values indicates a decrease in soil salinity levels, and in this context, it indicates a reduction of cations such as Na^+ (Zhovtonog et al., 2018). PGPR plays a role in reducing soil salinity or decreasing the accumulation of Na^+ in the soil, which can help plants absorb nutrients. PGPR has a strategy to overcome soil environmental salinity and Na^+ accumulation by producing biofilms such as exopolysaccharides.

Exopolysaccharides have functional groups such as carboxyl, carbonyl, hydroxyl, amino, and phosphate, which play a role in binding Na^+ so that it is not available in the soil so that it can indirectly balance osmotic conditions in the soil (Shrivastava and Kumar, 2015). PGPR improves soil quality through various means, such as reducing soil EC and adsorbing Na^+, to indirectly reduce the impact of salt stress on plants cultivated in saline soil (Ullah et al., 2022). According to Kasim et al. (2016), members of the PGPR genus *Bacillus*, *Pseudomonas*, and *Azotobacter,* which are applied to saline soil, have an excellent ability to reduce Na^+ accumulation in the soil by producing biofilms such as exopolysaccharides, which can indirectly improve the quality of stressed plant

growth by salinity. Improving qualities such as osmotic balance, reducing high soil EC values, and reducing exchangeable Na by PGPR in saline environments can indirectly influence the growth of shallot plants in absorbing nutrients in the soil (Arora et al., 2020).

Growth parameters

Based on the results of observations, leaf area, dry weight, and plant height, both with and without PGPR treatment on shallots, were greatly influenced by salt content. In the treatment without PGPR application, 150 mM salinity significantly reduced plant length, dry weight, and leaf area by 31.4%, 56.9%, and 65.1%, respectively, compared to without salinity (Table 2).

Soil with high salinity affects the accumulation of Na^+ and Cl^- in the soil, which impacts the continued plant growth of plants that absorb excess Na^+ in the soil. Saline soil reduces nutrient availability, thereby inhibiting plants in the growth process because it is difficult to absorb nutrients (Niste et al., 2014). Application of PGPR 30 mL/L can reduce this impact by improving plant growth. Based on Table 1, the decrease of EC values with the application of PGPR to the soil indicates a reduction in soil salinity and accumulation of Na^+ in the soil. PGPR indirectly influences plant growth by binding Na^+ to exopolysaccharides excreted in salt-stress environments. With this mechanism, PGPR can improve soil osmotic balance, reduce Na accumulation in the soil, and increase the availability of nutrient elements for plants such as N, P, and K (Ha-tran et al., 2021). The increase in parameters of leaf area and dry weight of shallot plants indicates that applying a PGPR concentration of 30 mL/L at various saline levels can improve plant nutrient absorption and reduce the availability of excess Na^+ in the soil. Tsegaye et al. (2022) stated that PGPR inoculation can increase growth through increasing nutrient absorption and increasing rhizosphere fertility.

Crop yield

The results of the observations on the shallot bulb parameters, such as bulb diameter, bulb weight per plant, weight loss of bulbs, and the number of bulbs per plant, indicated that the plant yield for both PGPR-treated and non-PGPR-treated was significantly affected by salinity (Table 3).

Table 2. The effect of PGPR on the growth variables of plant length, plant dry weight, and leaf area at various salinity levels.

PGPR	Salinity Levels			
	S0	S1	S2	S3
Plant Length (cm)				
P0	27.05 ^{cd}	22.60 ^{ef}	22.42 ^{ef}	18.55 ^{f}
P1	30.23 ^{abc}	25.03 ^{de}	21.35 ^{ef}	18.35^{f}
P2	31.78 ^{ab}	33.10 ^{ab}	29.62 ^{bc}	21.17 ^{ef}
P3	34.50 ^{a}	32.78 ^{ab}	28.92 ^{bcd}	24.73 ^{de}
Plant Dry Weight (g)				
P0	3.04 ^{def}	2.65 ^{efg}	1.88 ^{fgh}	1.31 ^{h}
P1	3.02 ^{def}	2.70 ^{efg}	2.46 ^{fgh}	1.51 ^{gh}
P2	4.09 ^{bcd}	4.99 ^{ab}	2.34 ^{fgh}	2.15 ^{fgh}
P3	6.17 ^{a}	4.64 ^{bc}	3.71 ^{cde}	1.93 ^{fgh}
Leaf Area (cm^2)				
P0	370.79 ^{efg}	271.55 ^{fghi}	265.79 ^{ghi}	129.21 ^{i}
P1	555.60 ^{cd}	316.27 ^{fgh}	224.50 ^{ghi}	124.35 ^{i}
P2	621.65 ^{bc}	764.14 ^{ab}	485.15 ^{cde}	210.16 ^{hi}
P3	852.89 ^{a}	746.37 ^{ab}	429.67 ^{def}	316.96 ^{fgh}

Notes: Numbers followed by different letters indicate significant differences in the HSD test at p = 0.05. Note S0 = no salinity-NaCl 0 mM; S1 = NaCl 50 mM; S2 = NaCl 100 mM; S3 = NaCl 150 mM; P0 = no PGPR; P1 = PGPR 10 mL/L; P2 = PGPR 20 mL/L; P3 = PGPR 30 mL/L.

Applying PGPR at a dose of 30 mL/L under various salinity levels produced better bulb diameter and weight than plants with no PGPR application (P0). The application of PGPR at 30 mL/L in salinity levels of 0 mM (S0), 50 mM, 100 mM, and 150 mM increased bulb diameter by 33%, 47%, 67%, and 34%, respectively compared to plants with no PGPR application (P0). Meanwhile, the application of PGPR at 30 mL/L in salinity levels of 100 mM and 150 mM increased the bulb weight per plant by 91% and 98.5%, respectively, compared to plants with no PGPR application (P0). The application of PGPR at 30 mL/L increased the number of bulbs produced at 50 mM and 100 mM by 47% and 33.4%, respectively, compared to plants with no PGPR inoculation. Further, the number of bulbs per plant with the application of PGPR at 20 mL/L and 30 mL/L increased by 41% and 59% under non-saline conditions (S0) and 58% and 47% at 50 mM of salinity. The application of 30 mL/L of PGPR under 100 mM salt stress increased the number of bulbs by 33% compared to plants with no PGPR application (Table 3). Regarding weight loss,

increasing salinity significantly reduced the weight loss of the bulbs. PGPR application at various doses did not significantly mitigate the impact of salinity. Salinity stress causes plants to absorb less water and accumulate more salt inside the cells (Yadav et al., 2019). Increasing the yield components of shallot bulbs (bulb diameter and bulb weight) is closely related to increasing plant photosynthesis. In general, a salinity level of 150 mM significantly reduced the yield components of shallot bulbs (Table 3). The low photosynthetic activity of shallot plants can cause this. El-Ramady et al. (2018) reported that saline soil would limit plants from absorbing essential nutrients such as N, K, and Mg as supporting components for photosynthesis. On the other hand, the accumulation of Na^+ absorbed by plants will inhibit the photosynthesis process and is toxic to plants (Balasubramaniam et al., 2023).

The application of PGPR 30 mL/L was proven to improve soil quality (Table 1) and increase the yield of shallot bulbs. According to Egamberdieva et al. (2019), applying PGPR to saline soil can improve soil quality by producing osmoprotectant compounds, such as proline, and secondary metabolites, such as exopolysaccharides. Apart from that, the application of PGPR in saline soil plays a role in stimulating plants to produce growth hormones such as IAA and activating antioxidants such as flavonoids (Bhat et al., 2020). Gau et al. (2021) reported that the application of *Bacillus subtilis* PGPR 108 CFU/mL improved nutrient uptake and significantly increased the weight and diameter of shallot bulbs. According to Widawati and Suliasih (2017), *Azotobacter* spp. PGPR can improve soil nutrient quality and mitigate salinity impact on plants, thereby increasing the growth and development of shallot bulbs.

Table 3. The effect of PGPR on yield variables bulb diameter, bulb weight per plant, bulb weight loss percentage, and number of bulbs per shallot plant at various salinity levels.

PGPR	Salinity Levels			
	S0	S1	S2	S3
Bulb Diameter (cm)				
P0	2.16 ^{cde}	1.86 ^{defg}	1.4 ^{ghi}	1.14 ^{ghi}
P1	2.20 ^{bcde}	2.06 ^{cdef}	1.57 ^{fghi}	1.23 ^{fghi}
P2	2.53 ^{abc}	2.73 ^{ab}	1.72 ^{efgh}	1.49 ^{efgh}
P3	2.89 ^{a}	2.75 ^{ab}	2.35 ^{abcd}	1.53 ^{abcd}
Bulbs Weight per Plant (g)				
P0	30.25 ^{defg}	24.68 ^{fgh}	19.56 ^{gh}	18.14 ^{h}
P1	33.87 ^{def}	26.96 ^{efgh}	22.95 ^{fgh}	24.47 ^{fgh}
P2	56.53 ^{ab}	40.26 ^{cd}	29.82 ^{defg}	28.65 ^{efgh}
P3	61.74 ^{a}	46.67 ^{bc}	37.36 ^{cde}	36.02 ^{cde}
Bulbs Weight Loss Percentage (%)				
P0	30.6 ^{a}	24.4 ^{b}	20.7 ^{cde}	18.4 ^{e}
P1	32.6 ^{a}	24.7 ^{b}	20.9 ^{cde}	18.6 ^{de}
P2	33.4 ^{a}	24.7 ^{b}	22.0 ^{bcd}	19.2 ^{cde}
P3	33.8 ^{a}	25.4 ^{b}	22.1^{bc}	20.2 ^{cde}
Number of Bulbs per Plant				
P0	6.50 ^{efg}	5.67 ^{efg}	5.50 ^{fg}	4.84 ^{g}
P1	6.67 ^{def}	6.84 ^{def}	5.84 ^{efg}	5.17 ^{fg}
P2	9.17 ^{ab}	9.00 ^{abc}	6.17 ^{efg}	6.00 ^{efg}
P3	10.34 ^{a}	8.34 ^{bcd}	7.34 ^{cde}	6.34 ^{efg}

Notes: Numbers followed by different letters indicate significant differences in the HSD test at p = 0.05. Note S0 = no salinity-NaCl 0 mM; S1 = NaCl 50 mM; S2 = NaCl 100 mM; S3 = NaCl 150 mM; P0 = no PGPR; P1 = PGPR 10 mL/L; P2 = PGPR 20 mL/L; P3 = PGPR 30 mL/L.

Physiological parameters

This study showed that proline levels increased when shallots were exposed to salt stress. The levels were elevated when the plants were inoculated with PGPR. (Figure 1a). The rise in proline levels due to PGPR simultaneously aids the plants in osmoregulation under saline environments. Vaishnav et al. (2015) found that when wheat experiences salinity stress, PGPR *Pseudomonas simiae* can induce the formation of proline as an osmolyte, reducing the accumulation of Na^+ salt around soybean roots induced by 100 mM of NaCl salinity stress. Abd-Allah et al. (2018) found that inoculation with *Bacillus subtillis* enhanced proline production and suppressed ROS levels under 200 mM NaCl salinity stress. Desoky et al. (2020) found that the inoculation of PGPR, *P. aeruginosa, Serratia marcescens*, and *B. cereus* under 150 mM of NaCl salinity increased proline while suppressing Na^+ and free radical (O_2^- and H_2O_2) accumulation. In high salt environments, some plant species sensitive to salinity can accumulate and increase proline concentration. It is accumulated in the cytosol to stabilize protein structures, maintain pH, and preserve cellular redox status (Hayat et al., 2012). It can be stimulated by rhizobacterial inoculation in plants.

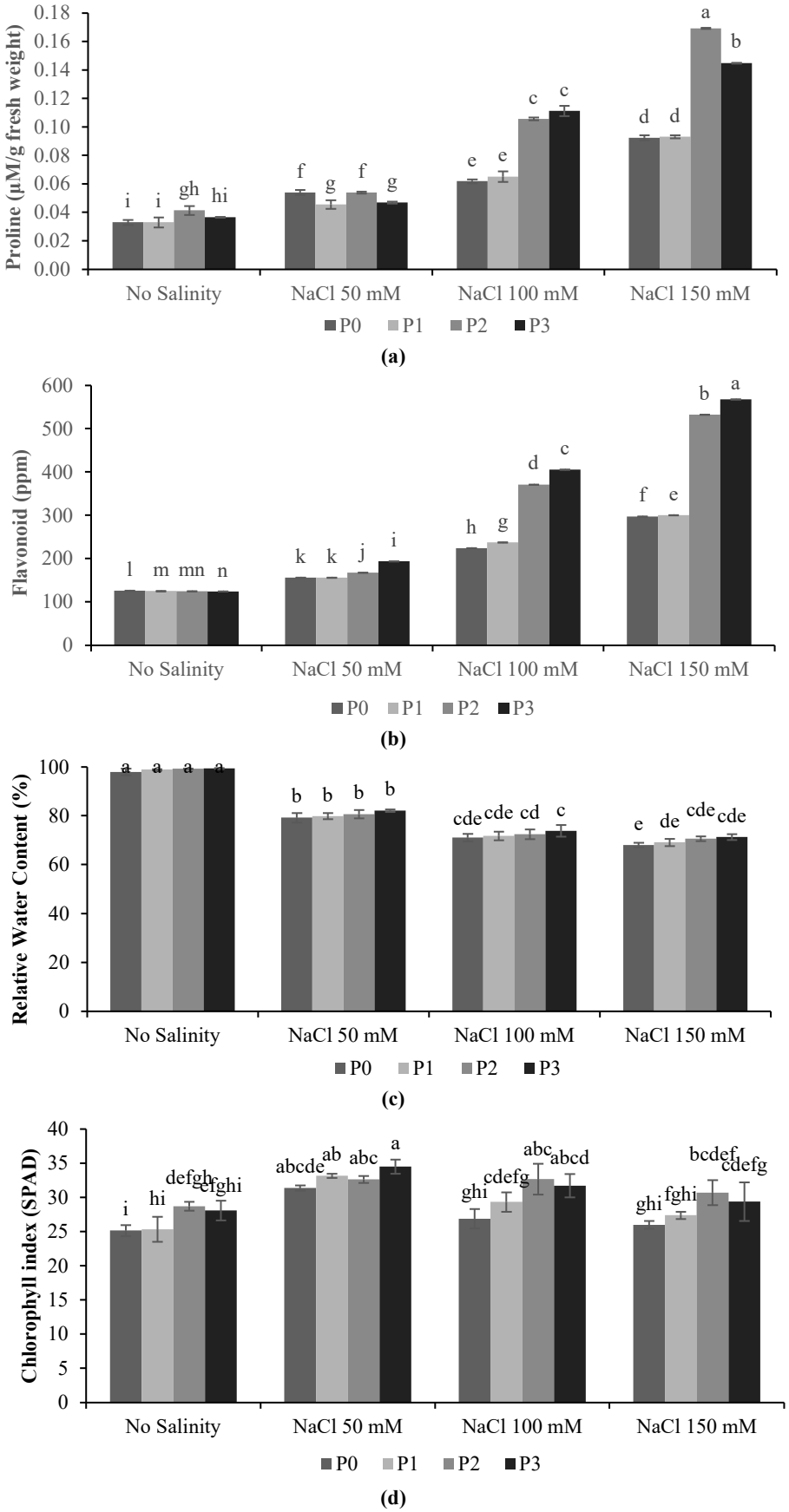

Figure 1. Effect of PGPR on (a) proline content, (b) flavonoid content, (c) RWC, and (d) SPAD chlorophyll index of shallots at various salinity levels. Notes P0 = no PGPR; P1 = PGPR 10 mL/L; P2 = PGPR 20 mL/L; P3 = PGPR 30 mL/L.

Further, it is synthesized more rapidly, and its concentration increases when plant roots are inoculated with PGPR, acting as an early protection mechanism against salt stress (Bharti et al., 2016).

The levels of flavonoids increased along higher salinity, although the levels relatively did not increase in the non-saline treatment (Figure 1b). The addition of 30 mL/L of PGPR at 150 mM of salinity (S3) led to the highest flavonoid levels of all other treatments. P3 treatment produced significantly higher flavonoid levels at 100 mM (S2) and 150 mM (S3), i.e., 80.8% and 91.3%, respectively, compared to plants with no PGPR application (P0). Higher synthesis and exudation of flavonoids can be stimulated by PGPR inoculation in plants. This study also found that PGPR inoculation could alleviate salt stress in shallots, as indicated by increased flavonoid levels, in comparison with plants with no PGPR application. This is similar to the finding of Khan et al. (2023) that the combined application of *Pseudomonas fluorescens* and *Azotobacter chroococcum* on *Brassica nigra* (mustard) under salinity stress can increase the plant flavonoid levels by 27.8%, in comparison with plants with no PGPR application. Ali et al. (2022) also reported that PGPR application, especially in saline environments, can enhance flavonoid production by 20-28% in maize compared to plants with no PGPR application.

Flavonoids play a role in mitigating salt stress through the homeostasis of the balance of Na^+ and K^+ ions in plant cells (Ismail et al., 2016). Studies conducted using *Azospirillum* sp., *Azotobacter* sp., *Bacillus subtilis*, and *Pseudomonas fluorescens* generally demonstrate the ability of the plant to alleviate salinity stress by improving growth and enhancing antioxidants (proline and flavonoids) when they are under salinity stress. Genera such as *Enterobacteria*, *Bacillus*, *Azotobacter*, *Ochrobactrum*, *Rhizobium*, *Stenotrophomonas*, *Serratia*, *Azospirillum*, and *Pseudomonas* are groups that have been widely reported as being able to suppress the effect of salinity on plants (Egamberdieva et al., 2019).

The RWC results indicated that an increase in salinity significantly reduced RWC. Salinity treatments with 50 mM, 100 mM, and 150 mM of NaCl significantly decreased RWC values by 23%, 37%, and 43%, respectively, compared to the non-saline treatment (S0). The application of PGPR could enhance RWC values at various salinity levels, although not yet statistically significant (Figure 1c). This research is in accordance with the report of Polash et al. (2018) that 100 mM salinity stress leads to a 21.7% reduction in RWC compared to non-saline conditions. Sanwal et al. (2022) demonstrated that potatoes cultivated with irrigation water containing 6 dS/m salinity resulted in an 11% decreased RWC compared to non-saline treatment.

The application of 20 mL/L and 30 mL/L of PGPR under 100 mM salinity (S2) improved chlorophyll index (SPAD) by 21.5% and 18%, respectively, compared to the non-PGPR treatment. Statistically, PGPR application at various doses has not significantly mitigated the impact of salinity on S0, S1, and S3, particularly in terms of chlorophyll index (Figure 1d). This confirms the study of Esan et al. (2020), reporting that the inoculation of *B. subtilis* PGPR enhances photosynthetic pigments, chlorophyll a, and chlorophyll b, particularly when tomatoes are subjected to salinity stress. Ansari et al. (2019) demonstrated that the inoculation of *P. fluorescens* increases the levels of total chlorophyll, chlorophyll a, and chlorophyll b in *Medicago sativa*, especially under salinity levels of up to 20 dS/m, when compared to non-inoculated conditions.

Conclusion

The application of PGPR at a concentration of 30 mL/L was more effective in reducing soil electrical conductivity values in saline soil. PGPR concentration of 30 mL/L was best in mitigating the negative impact of salinity on various parameters such as plant length, plant dry weight, leaf area, tuber diameter, tuber weight per plant, and number of tubers per plant, compared to without PGPR. The application of PGPR at 30 mL/L could increase proline levels, flavonoids, and chlorophyll index in shallot plants at 100 mM and 150 mM salinities compared to plants without PGPR application.

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