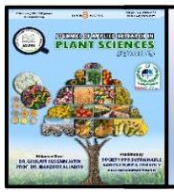
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Halotolerant PGPR Enhances Growth and Floral Performance of *Rosa chinensis* Under Saline Stress

Usama Bin Arif[1], Saqib Ayyub[2*], Shabana Ehsan[3], Tahir Saeed[1], Munawar Hussain Almas[4], Muhammad Faraz Anwar[3], Ahsan Akram[5], Muhammad Amjad Qureshi[6], Ifra Saleem[7]

[1]Horticultural Research Sub-Station Floriculture & Landscaping, AARI, Faisalabad, Punjab, Pakistan.
[2]Horticultural Research Institute, AARI, Faisalabad, Punjab, Pakistan.
[3]Soil & Water Testing Laboratory, AARI, Faisalabad. Punjab, Pakistan.
[4]Directorate of Floriculture (T&R), Punjab, Lahore, Pakistan.
[5]Institute of Horticultural Sciences, University of Agriculture, Faisalabad.
[6]Soil Bacteriology Section, Agricultural Biotechnology Research Institute, AARI, Faisalabad, Punjab, Pakistan.
[7]Soil Chemistry Section, AARI, Faisalabad, Punjab, Pakistan.
[*]**Corresponding author; saqibayyub218@gmail.com**

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ABSTRACT

Roses are susceptible to salinity stress, which has a substantial impact on their growth and the visual quality of their flowers. The growth and visual quality of ornamental plants are closely associated with their ability to tolerate abiotic stressors. In recent years, the use of plant growth-promoting rhizobacteria (PGPR) has emerged as a potential strategy for improving the growth and quality of ornamental plants in stressful environments. Therefore, the present study was conducted to identify commercially available PGPR species that could be used to develop salt stress tolerance in 'Surkha' roses for commercial production. We evaluated three salinity levels (3, 5 and 7 dS m^{-1} NaCl), applied alone or in combination with one of two PGPR genera. Compared with the corresponding non-inoculated treatments at the same salinity, inoculation with Bacillus spp. at 3 dS m^{-1} produced a significant increase in leaf area and numerical improvements in several other growth and floral traits; effects of Pseudomonas spp. and of both genera at higher salinity levels were generally small and non-significant. The non-saline control generally showed the highest values for most traits. In conclusion, under the conditions of this single-season pot study, halotolerant PGPR (particularly Bacillus spp.) partially alleviated salinity effects on Rosa chinensis 'Surkha', most clearly for leaf area at mild salinity.

KEYWORDS: Rosa chinensis, salinity stress, bacillus, pseudomonas, ornamental plants, floral quality

INTRODUCTION

Roses are the top-ranking cut flowers in the flower trade based on acreage, production and consumption. However, Pakistan's export of cut roses is negligible compared with that of other countries because of their short vase life and poor management practices (Almas *et al.,* 2023). Therefore, improved production management and postharvest practices are needed to reduce vase-life deterioration and expand the export potential of roses (Almas *et al.,* 2023). The rose is emblematic of love, grace, creativity, sensuality and religious belief, and is a symbol of visual pleasure for humanity. It is classified within the family Rosaceae and the genus Rosa, which includes about 200 species and over 18,000 cultivars (Zarif *et al.,* 2025). It has consistently been the most cherished bloom in the subcontinent. Pakistan, being an agrarian nation with varied agro-climatic conditions, has significant potential for cultivating rose blossoms (Ahmad *et al.,* 2010).

Roses are often sensitive to salinity above 3.0 dS m^{-1} electrical conductivity (EC) of soil, while some rose cultivars may endure up to an EC of 3.5 dS m^{-1} without compromising production and quality (Cabrera and Perdomo, 2003). The high ion concentration in saline soil is detrimental to plant growth, root formation, and blooming (Cai *et al.,* 2014). Soil salinity significantly hinders the growth of prevalent commercial crops in many dry and semi-arid areas globally, impacting plants via osmotic, specific-ion and oxidative stresses (Pitman and Läuchli, 2002). Salt stress may influence plant development via many mechanisms, including reduced water absorption, ion accumulation to harmful concentrations, and decreased nutrient accessibility (Tuncturk *et al.,* 2011). It is estimated that 20% of arable land and 33% of irrigated agricultural areas globally are affected by salinity. The Food and Agriculture Organization of the United Nations (FAO, 2018), along with various scientific publications, indicates that these percentages correspond to an estimated expanse exceeding 900 million hectares, of which roughly 77 million hectares may be classified as anthropogenic salt-affected soils

(Shahid *et al.,* 2018). Moreover, the area of salt-affected soil increases by about 10% annually owing to several factors, including climate change, inadequate irrigation methods, and other natural phenomena (Ayuso-Calles *et al.,* 2021).

Oceania and Asia are the continents most impacted, accounting for 39% and 34% of the world's salt-affected soils, respectively. In terms of anthropogenic salinization, Asia exhibits the highest percentages at over 70%, followed by Africa at 20% and Europe at 5% (Shahid *et al.,* 2018). The deterioration of cultivable land caused by salt adversely affects productivity and, therefore, the economy. The FAO (FAO, 2018) estimates that the yearly decline in agricultural output amounts to US$ 27–31 billion, with production losses ranging from 20% to 40%.

In light of this escalating issue, it is essential to explore strategies that may enhance agricultural productivity under saline conditions (Shahbani *et al*., 2023). The development of salt-tolerant crops through conventional breeding and genetic engineering is a costly and labour-intensive process that is not always straightforward to execute. Conversely, other viable and economical approaches exist, such as the use of beneficial microbes that enhance salt tolerance in plants (Ayuso-Calles *et al.,* 2021).

Recently, the use of PGPR has been shown to be an efficient method for improving plant growth and the nutritional quality of diverse crops such as lettuce (Lactuca sativa L.; Ayuso-Calles *et al*., 2020), maize (Zea mays L.; Bano and Fatima, 2009; Ehsan *et al*., 2023), wheat (Triticum aestivum L.; Upadhyay *et al*., 2012; Upadhyay *et al*., 2019), coriander (Coriandrum sativum L.; Jiménez-Gómez *et al*., 2020), soybean (Glycine max L.; Han and Lee, 2005) and peanut (Arachis hypogaea L.; Goswami *et al*., 2014), even under challenging environmental conditions such as soil salinity. These soil microorganisms may enhance crop water dynamics, modify ion equilibrium, or adjust phytohormone levels via direct and indirect processes that influence abiotic stress management (Ilangumaran and Smith, 2017). Therefore, the objective of this study was to investigate the growth and floral performance of roses under salt stress by using PGPR.

Considering the importance of the floriculture industry in Pakistan and the significance of roses as loose and cut flowers, a pot experiment was performed to evaluate the potential of commercially available halotolerant PGPR species in mitigating salt stress in Rosa chinensis ‘Surkha’ and to compare the relative efficacy of two bacterial genera across a salinity gradient (3, 5 and 7 dS m^{-1} NaCl), applied alone or in combination with inoculation, to improve growth and floral performance.

MATERIALS AND METHODS

Plant material and treatments: Rose plants were grown in the Floriculture Research Area, Horticultural Research Sub-Station Floriculture and Landscaping (31°24’16” N 73°2’54” E), Ayub Agricultural Research Institute, Faisalabad, Pakistan, from cuttings planted in January 2024. Uniform plants were transplanted into 12-inch plastic pots (height 10.5 inches) in the first week of November 2024. Each pot contained about 5 kg of a growing medium of silt, compost and garden soil (1:1:2). After establishment, the plants were placed in the nursery and irrigated fortnightly with saline water of known EC to induce the desired salinity levels. Subsequently, the plants were drenched with suspensions of the selected PGPR strains, which have previously been reported as halotolerant (Arora *et al.,* 2020). Data were collected at suitable intervals and analyzed. The experiment was laid out in a completely randomized design (CRD) with four replications and four plants per experimental unit. Standard methods were used to grow the plants, and all treatments received the same cultural practices throughout the study period. The ten treatments are listed in Table 1.

Table 1. Experimental treatments: Salinity levels, PGPR inoculation and combinations.

T_0 =	Control (1.9 EC)
T_1 =	3 dS m^{-1} NaCl
T_2 =	5 dS m^{-1} NaCl
T_3 =	7 dS m^{-1} NaCl
T_4 =	3 dS m^{-1} NaCl + *Bacillus* spp.
T_5 =	5 dS m^{-1} NaCl + *Bacillus* spp.
T_6 =	7 dS m^{-1} NaCl + *Bacillus* spp.
T_7 =	3 dS m^{-1} NaCl + *Pseudomonas* spp.
T_8 =	5 dS m^{-1} NaCl + *Pseudomonas* spp.
T_9 =	7 dS m^{-1} NaCl + *Pseudomonas* spp

Rose roots were inoculated before the onset of salt stress. During the transplanting phase, the root systems were immediately immersed in the respective PGPR suspensions for 30 min. Individual bacterial strains were cultured in a standard liquid medium, such as nutrient broth or Luria-Bertani broth, at 28–30°C for 24 to 48 h until they reached the exponential growth phase. The cells were then centrifuged, washed and resuspended in sterile distilled water or 0.85% physiological saline to get a standard optical density corresponding to a bacterial concentration. Further booster treatments were given by root drenching at 15-day intervals after transplanting to guarantee continuous colonization of rhizobacteria

throughout the trial period. Each rose plant was drenched with 50–100 mL of the bacterial suspension, poured around the base of the stem to evenly saturate the root zone, to help maintain the microbial population under the subsequent 3, 5, and 7 dS/m salinity regimes. Salinity stress treatments of 3, 5 and 7 dS/m were prepared by dissolving calculated amounts [30 mM (≈ 1.8 g/L) of salt was added to achieve the 3 dS/m threshold (mild stress for roses), 50 mM (≈ 3.0 g/L) for the 5 dS/m treatment (moderate stress) and 70 mM (≈ 4.1 g/L) for the 7 dS/m treatment] of analytical grade sodium chloride (NaCl) into a standard nutrient base such as a modified Hoagland's solution. The EC of each solution was checked using a calibrated, temperature-compensated handheld EC meter. These treatments were applied gradually over a period of 4 to 6 days.

Plant height, number of leaves, leaf area and stem diameter: The height of four healthy plants per treatment was measured from the soil surface to the top of the plant with a meter ruler and expressed in cm. The height of the plant was measured for all the four plants in each treatment (i.e. all the four replications) and the mean of the four plants was used as the treatment mean. These means were subjected to statistical analysis. The number of leaves per plant was counted manually on the same four plants used for height measurements. Leaf area was determined by measuring the maximum length and breadth from the center of a representative leaflet using a ruler, inserting these measurements into the formula [Leaf Area (cm2) = Length × Width × 0.68], and expressing the result in cm^2 (Arif and Ahmad, 2024). The stem diameter was measured with a vernier caliper and recorded in millimeters (mm).

Number of flowers, flower diameter, flower fresh weight, flower dry weight and flower visual quality: In each replicate, three plants were selected randomly, and the total count of flowers was noted. Flower diameter was measured across the center of the flower with a ruler and expressed in cm. Flower fresh and dry weights were determined with an electronic balance (Setra, BL-4100S, USA) and expressed in g. For dry weight, flowers were oven-dried at 65–70 °C for 72 h (or until constant weight) after an initial air-drying period. Pulverization was performed only for archival storage and was not required for the dry-weight determination. The visual quality of the flowers was evaluated by three assessors, who rated them on a scale from 1 to 9 where, 1 = poor quality flower; 5 = satisfactory flower quality; 9 = fresh and superior quality flower (Arif *et al.,* 2025).

Statistical assessment: Data were analyzed by Fisher's analysis of variance using Statistix 8.1 (Analytical Software, Tallahassee, Florida, USA). Treatment means were compared using the least significant difference (LSD) test ($P < 0.05$).

RESULTS AND DISCUSSION

Plant height, number of leaves, leaf area and stem diameter: Results indicated a significant difference among treatments in plant height (LSD0.05 = 15.27; Figure 1). Control (1.9 EC) plants had the maximum plant height, approximately 1.35-fold higher than the mean of the saline treatments. Among saline treatments, plant height was highest under 3 dS m^{-1} NaCl + Bacillus spp., but it did not differ significantly from 3 dS m^{-1} NaCl alone according to the LSD; the lowest values occurred at 7 dS m^{-1} NaCl. Inoculation effects at the same salinity level were generally small and non-significant for plant height.

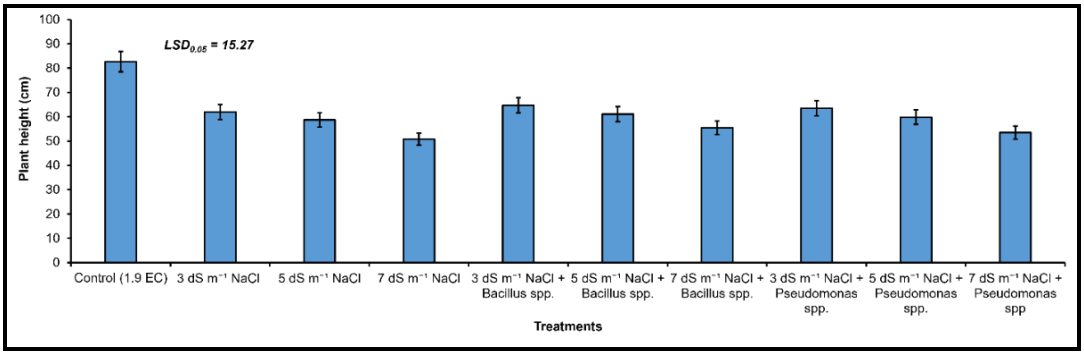


Figure 1. Effect of halotolerant PGPR species on plant height of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

Salinity stress considerably reduces the plant height, stem elongation, and total biomass of Rosa chinensis by inducing osmotic stress, ion toxicity, and chlorophyll content reduction (Islam *et al.,* 2021). Treatment with halotolerant PGPR mitigates the inhibiting impact of salt and results in a significant increase in plant height over non-inoculated plants in similar saline treatments (Palacio-Rodríguez *et al.,* 2023). Comparable findings of increased plant height were noted in *Carya illinoinensis* (Palacio-Rodríguez *et al.,* 2023) and eggplant (Fu *et al*., 2010) inoculated with PGPR under saline conditions.

The number of leaves varied numerically among treatments (LSD0.05 = NS; Figure 2). Control plants (1.9 EC) had approximately 105 leaves. Among saline treatments, 3 dS m^{-1} NaCl + Bacillus spp. produced

the highest count (approximately 120), which was numerically higher than the control, while 7 dS m^{-1} NaCl produced the fewest (approximately 79). Differences among treatments, including comparisons of inoculated versus non-inoculated plants at the same salinity, were not statistically significant.

PGPR treatment reduced the decline in leaf number under saline conditions. PGPR significantly increased the total number of leaves compared with uninoculated plants under salt stress (Arora *et al.,* 2020). Salinity often inhibits leaf production by inducing osmotic shock, ion toxicity and oxidative stress, leading to leaf chlorosis, premature senescence, and severe defoliation. Inoculation of Rosa chinensis with PGPR possibly activates multiple physiological and biochemical mechanisms that preserve existing foliage and promote new leaf development (Kumawat *et al.,* 2023). Similarly, the application of PGPR has augmented the leaf count and growth of crops such as wheat (Dudak *et al.,* 2026), okra (Habib *et al.,* 2016) and coriander (Rabiei *et al.,* 2020) under saline conditions.

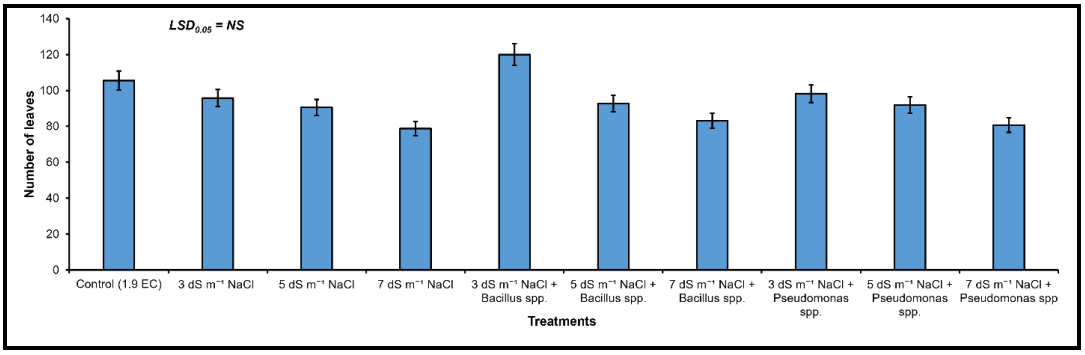


Figure 2. Effect of halotolerant PGPR species on number of leaves of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

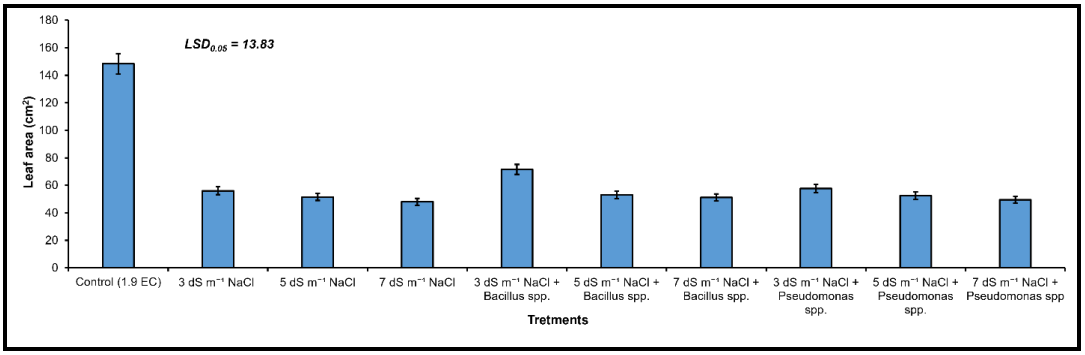


Figure 3. Effect of halotolerant PGPR species on leaf area of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

Treatments differed significantly in leaf area (Figure 3). Control (1.9 EC) plants had the largest leaf area. Among saline treatments, the largest leaf area was recorded under 3 dS m^{-1} NaCl + Bacillus spp., which was significantly higher than 3 dS m^{-1} NaCl alone (difference exceeding the LSD), while the smallest leaf area occurred at 7 dS m^{-1} NaCl. This was the clearest statistically supported benefit of Bacillus inoculation in the study.

PGPR inoculation markedly reduced the damaging consequences of salt stress on leaf area of *Rosa chinensis.* Salinity is a serious constraint to leaf growth owing to osmotic and ionic stress. Inoculation with PGPR strains can restore leaf growth and delay premature leaf senescence (Ramasamy and Mahawar, 2023). The leaf area is reported to be higher under salt stress in various crops such as potato (Batool *et al.,* 2020) and okra (Habib *et al.,* 2016) after inoculation with PGPR.

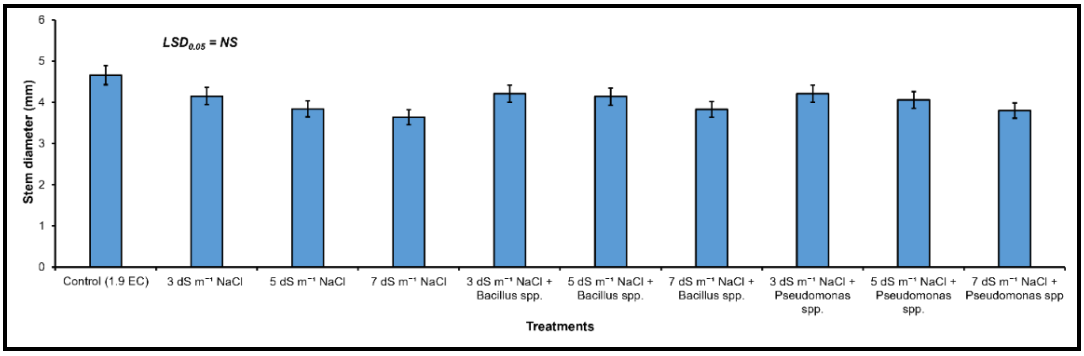


Figure 4. Effect of halotolerant PGPR species on stem diameter of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

Stem diameter differed numerically among treatments but the differences were not statistically significant (LSD0.05 = NS; Figure 4). Control plants had the largest stem diameter. Among saline treatments, the highest value was under 3 dS m^{-1} NaCl + Bacillus spp. and the lowest under 7 dS m^{-1} NaCl; inoculation effects at the same salinity were small and non-significant.

Inoculation with halotolerant PGPR greatly alleviates the detrimental effects of salt stress on stem diameter and total biomass of *Rosa chinensis*. Salt stress in plants causes osmotic pressure and ion toxicity, limiting cell division and resulting in thinner, stunted stems. It is possible that PGPR species promote stem diameter expansion by secreting phytohormones, trapping detrimental ions with exopolysaccharides and increasing nutrition and water intake (Ramasamy and Mahawar, 2023). Earlier research on oilseed crops under salt stress showed that combining PGPR such as *Bacillus* pumilus with biochar increased sunflower stem diameter by up to 45% (Tu *et al.,* 2025).

Number of flowers, flower diameter, flower fresh weight and flower dry weight: Number of flowers differed numerically among treatments but differences were not statistically significant (LSD0.05 = NS; Figure 5). Control plants showed the highest number of flowers. Among saline treatments, the highest count was under 3 dS m^{-1} NaCl + Bacillus spp. and the lowest under 7 dS m^{-1} NaCl; inoculation effects at the same salinity were small and non-significant.

Halotolerant PGPR may regulate phytohormone levels, limit oxidative damage and sustain cellular osmotic balance, which may enhance reproductive health and increase the number of blooms in Rosa chinensis under salt stress. Inoculation with salt-tolerant rhizobacteria mitigates osmotic and ionic stress. PGPR may produce phytohormones such as indole-3-acetic acid and gibberellins, which help the plant conserve metabolic energy and resume bud production, therefore maintaining flower numbers (Arora *et al.,* 2020). Under high salt stress, bacterial isolates like Azospirillum brasilense considerably increased the number of flower buds in sunflower (Ullah *et al.,* 2024). In okra, ACC deaminase-producing PGPR sustained biomass and flower output under saline conditions (Habib *et al.,* 2016).

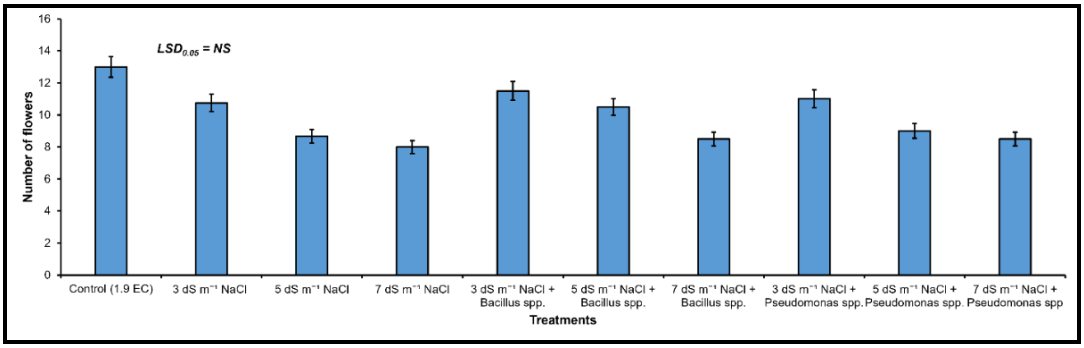


Figure 5. Effect of halotolerant PGPR species on number of flowers of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

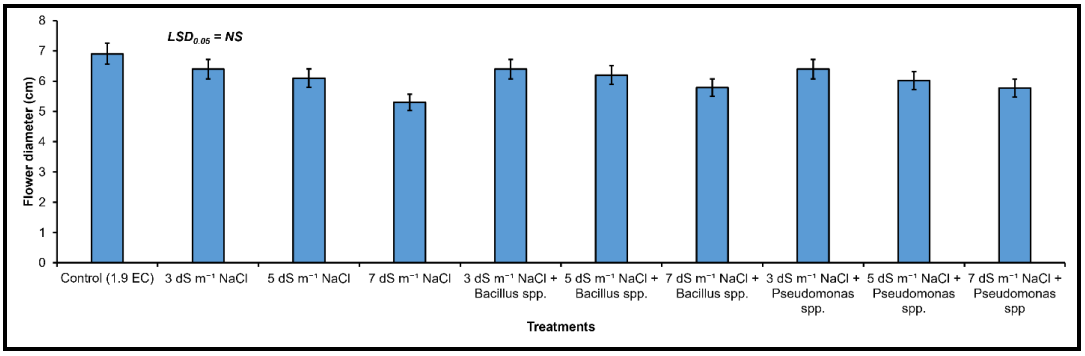


Figure 6. Effect of halotolerant PGPR species on flower diameter of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

Flower diameter showed only small, non-significant differences among treatments (LSD0.05 = NS; Figure 6). Control plants had the largest flower diameter. Values under 3 dS m^{-1} NaCl with and without Bacillus spp. were essentially identical; inoculation had no significant effect on flower diameter at any salinity level.

High salt concentrations often cause a considerable decrease in bloom diameter in uninoculated roses due to osmotic stress, ion toxicity, and limited nutrient absorption. Inoculation with several halotolerant PGPR (HT-PGPR) strains (e.g. *Pseudomonas* and *Bacillus* spp.) may help to preserve the vascular and metabolic health of the plant, so that cell growth can proceed normally, resulting in bigger flower diameters and healthier blooms (Islam *et al.,* 2021). Studies on the effect of *Azospirillum brasilense*, *Bacillus* megaterium and *Pseudomonas* fluorescens PGPR on sunflower cultivars "Suntastic" and "Hysun-33" and petunias have shown that PGPR inoculation greatly reduces the negative effects of salt, resulting in an increase or maintenance of flower diameter under saline conditions (Ullah *et al.,* 2024; South *et al.,* 2021).

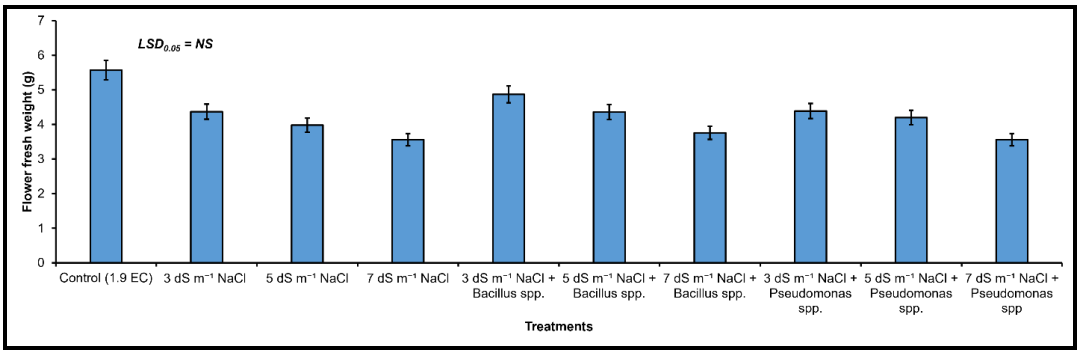


Figure 7. Effect of halotolerant PGPR species on flower fresh weight of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

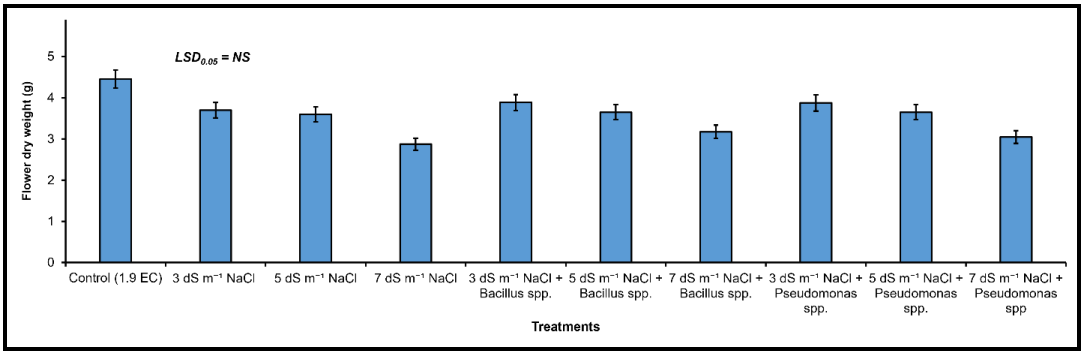


Figure 8. Effect of halotolerant PGPR species on flower dry weight of *Rosa chinensis* under saline stress. Vertical bars indicate ± S.E. n = 4 replicates

Flower fresh weight differed numerically among treatments (Figure 7); differences were not statistically significant (LSD0.05 = NS). Control plants showed the highest flower fresh weight. Among saline treatments, the highest value was under 3 dS m^{-1} NaCl + Bacillus spp. and the lowest under 7 dS m^{-1} NaCl; inoculation effects at the same salinity were small. Flower dry weight showed a similar numerical pattern (Figure 8). Note that the reported dry-matter percentages appear unusually high for rose

flowers and warrant verification of the drying protocol and data in future work.

Salinity stress usually leads to substantial decrease in flower output, chlorosis and loss of decorative value in Rosa chinensis. Inoculation with halotolerant PGPR strains (such as *Bacillus*, *Pseudomonas* or *Azospirillum* species) increased water retention and nutrient uptake, resulting in significant improvements in the fresh and dry weights of floral tissues compared with stressed, uninoculated plants (Li *et al.,* 2016; Kashyap *et al.,* 2019). Previously, PGPR such as *Bacillus* megaterium and strains such as KS-44 were reported to promote vigorous vegetative growth and increase flower weight under severe salt stress in sunflower (Ullah *et al.,* 2024). Tomatoes treated with salt-tolerant PGPR (e.g., *Azotobacter chroococcum* or *Pseudomonas* spp.) during moderate to severe salt stress significantly increased flower fresh weight (Egamberdieva *et al.,* 2019).

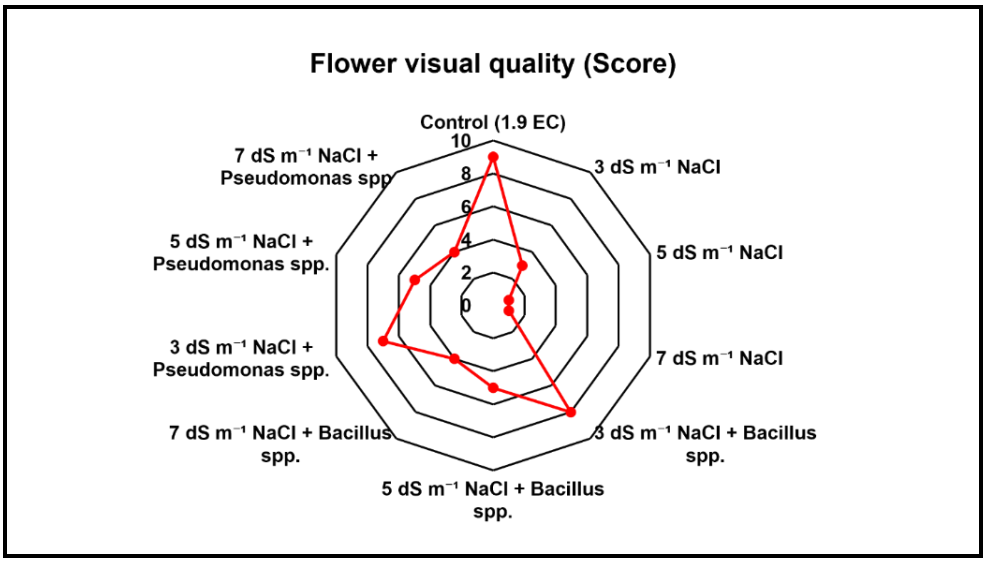


Figure 9. Effect of halotolerant PGPR species on flower visual quality of *Rosa chinensis* under saline stress. n = 4 replicates

Floral visual quality scores were highest for control plants and for 3 dS m⁻¹ NaCl + Bacillus spp., and lowest at the higher salinity levels (Figure 9). Because the figure lacks error bars and an LSD, formal significance cannot be assessed from the published plot; the pattern is consistent with a protective effect of mild-salinity Bacillus inoculation on visual quality. Future work should present these ordinal data with appropriate non-parametric analysis and a conventional bar chart.

CONCLUSION

It is concluded that, under the conditions of this single-season pot study with one cultivar, inoculation with halotolerant PGPR improved some growth and quality indices of Rosa chinensis 'Surkha' under mild salinity (3 dS m⁻¹ NaCl). The clearest statistically significant benefit was increased leaf area with Bacillus spp. at 3 dS m⁻¹; most other traits showed only numerical, non-significant improvements, and effects at 5 and 7 dS m⁻¹ were limited. Bacillus spp. generally performed better than Pseudomonas spp. Future research should validate findings at field scale, identify strains molecularly, optimise formulations, test a broader salinity range and additional cultivars, and integrate PGPR with other sustainable management techniques for commercial salt-tolerant ornamental production.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTION

UBA. Execution, data collection for the experiment and write-up, SA. Data Analysis, write up and references, SE. Paper write up and calculation of water salinity doses, TS. write up, MHA: technical guidance, MFA. Evaluation of soil and irrigation samples, AA. Provision of literature and technical guidance, MAQ. provision of PGPR species and IS. calculation and evaluation of salinity dose and data collection.

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