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Use of Rhizobacteria to Increase Tolerance to Salinity Stress in Tomato Growing

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Research Article

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Abstract

Salinity stress is a major and increasingly critical factor that negatively impacts plant growth, productivity, and overall agricultural sustainability. Addressing this challenge has therefore become a central focus in modern agricultural research. This study aimed to investigate the potential of beneficial rhizobacteria to enhance salt stress tolerance in tomato plants. For this purpose, six tomato varieties were used, and ultimately, only the most tolerant and the most sensitive varieties were selected for the study. Among the 19 bacterial isolates selected from laboratory stocks, three of the most successful isolates, identified *in vitro* under salt stress conditions, were chosen for further testing under *in vivo* conditions. The *in vivo* pot experiments were repeated twice. In the *in vivo* salt stress experiment, the selected bacterial isolates were applied to the salt-tolerant and salt-sensitive tomato varieties using seed bacterization and root immersion methods. It was determined that bacterial applications increased the fresh and dry weight of both shoots and roots compared to untreated control plants. Based on molecular identification and sequence analysis, these bacteria were identified as *Pantoea ananatis*, *Acinetobacter calcoaceticus*, and *Pantoea vagans*. These halophytic bacteria, known for their potential to enhance plant growth in saline environments, were evaluated as promising in terms of salt stress tolerance, plant growth promotion, and yield improvement, city and regional planning, forestry, agriculture and aquaculture.

1. Introduction

Tomato (*Solanum lycopersicum*) is a perennial vegetable belonging to the Solanaceae family. It is a versatile, popular, and one of the most important vegetable crops worldwide. Due to its unique nutritional value and widespread global production, it is recognized as a significant food source. Tomatoes can be grown in a variety of climates, including relatively cold regions, as they can be grown both outdoors and under protected conditions in many countries.

Like many crops, tomato cultivation is frequently exposed to various stresses that hinder plant growth and productivity. These stresses may be biotic, such as bacteria, viruses, fungi, and nematodes, or abiotic, including drought, salinity, water stress, temperature fluctuations, and mineral deficiencies. In the twenty-first century, several global challenges affecting agricultural systems have intensified, including global warming, water scarcity, environmental pollution, and the increasing salinization of agricultural lands and water

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resources. Environmental stressors such as strong winds, extreme temperatures, soil salinity, drought, and flooding have significantly affected the productivity and management of agricultural lands, with soil salinity representing one of the most severe constraints.

The climate crisis and improper land and water management practices are accelerating soil salinization, which affects more than 10% of the Earth's land surface (Food and Agriculture Organization [FAO], 2024). According to the FAO, approximately 20% of the world's irrigated agricultural lands are impacted by salinity, and nearly 10 million hectares of arable land are lost annually due to soil salinization worldwide (FAO, 2024).

Soil salinity develops through both natural and anthropogenic processes. Natural causes include the weathering of salt-bearing rocks, volcanic activity, and the deposition of sea salts transported through the atmosphere by wind. Anthropogenic salinization is primarily associated with improper irrigation practices and inadequate drainage systems. Additional contributing factors include low precipitation, high evaporation rates, and intensive agricultural activities. Moreover, climate change, excessive groundwater extraction, the use of low-quality irrigation water, intensive irrigation in semi-arid and arid regions, and insufficient soil leaching further exacerbate the salinization process. According to the first global assessment conducted in fifty years, 10.7% of global land is affected by salinity, a phenomenon increasingly intensified by climate change and human activities (FAO, 2024).

The prevention and management of salt stress in agricultural soils can be achieved through a range of integrated strategies, including the assessment of soil electrical conductivity (EC) to quantify salinity levels, the cultivation of salt-tolerant perennial crops, the selection of salt-resistant plant varieties, the use of salt-tolerant cover crops, and regular monitoring of irrigation water salinity. In addition, appropriate irrigation management and effective drainage systems can facilitate the removal of excess salts from the soil profile, while periodic soil leaching helps reduce salt accumulation within the root zone.

In recent years, increasing attention has been directed toward the use of non-pathogenic rhizobacteria, commonly referred to as Rhizobacteria (RB) or Plant Growth-Promoting Rhizobacteria (PGPR), due to their potential roles in the biological control of plant diseases, stimulation of plant growth, and enhancement of plant tolerance to abiotic stresses such as drought and salinity.

A growing body of research highlights the effectiveness of PGPR in mitigating salt stress and enhancing crop productivity. Egamberdieva et al. (2017) demonstrated that soil salinity significantly influences plant growth while emphasizing the dual role of rhizobacteria in growth promotion and biological control. In tomato cultivation, Medeiros and Bettiol (2021) reported that *Bacillus* spp. alleviated salt stress and concurrently suppressed Fusarium wilt under saline conditions. Recent studies have further identified PGPR as key salt stress attenuators contributing to sustainable agricultural (Al-Turki et al., 2023). Similarly, de Souza Ribeiro et al. (2023) showed that PGPR application mitigated salt-induced morphological stress in strawberry plants, while Yahyaoui et al. (2024) demonstrated significant improvements in growth and productivity of maize and tomato under saline soils following PGPR treatment. Collectively, these findings underscore the considerable potential of PGPR as an effective and sustainable strategy for enhancing crop resilience to salinity stress.

The aim of this study is to evaluate the potential of RB to mitigate salt stress in tomato plants. Specifically, the study investigates the effects of RB application under saline conditions by assessing key plant growth parameters, including leaf number and the fresh and dry weights of roots and shoots. Through this approach, the research seeks to contribute to the development of sustainable, biology-based strategies for managing salinity stress and enhancing tomato growth and productivity.

2. Materials and Method

2.1. Materials

2.1.1. Bacterial isolates used in the study

From the extensive RB collection (including epiphytic and endophytic strains) available in the Bacteriology Laboratory of the Dept. of Plant Protection at Ege University, 19 bacterial isolates were selected. These isolates were chosen based on previous projects where they had successfully promoted plant growth, as well as considering various *in vitro* test results and species differences. Selection criteria included key plant growth parameters such as ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) activity, IAA (indole-3-acetic acid) production, phosphate solubilization activity, siderophore production, and other relevant traits.

2.1.2. Plant material used in this study

In this study, salt-tolerant and salt-sensitive tomato seeds were selected as test plants based on a preliminary trial conducted among widely cultivated commercial tomato varieties. For this purpose, seeds from six different tomato varieties (*Sakata F1 Jasmine 10, Sakata F1 Pink Pearl 13, Sakata Karasuta F-1 24, Sakata Beysin F-1 36, SC 2121,* and *Syngenta Torry 33*) were used.

2.1.3. Bacterial media and plant growth medium used in the study

In this study, King B (KB) and Tryptic Soy Agar (TSA) were used as culture media. KB was employed for culturing RB, while Nutrient Agar (NA) was used to evaluate growth kinetics to support production on a low-cost substrate (peat).

2.2. Method

2.2.1. *In vitro* tests

In this study, 19 bacterial isolates were evaluated for their tolerance to salinity. Bacterial cultures were obtained from 48-hour-old growth and streaked onto NA media supplemented with different concentrations of sodium chloride (NaCl) (0, 200, 400, 600, 850, 1000, 1500, and 2000 mM). The inoculated Petri dishes were incubated at 24 °C for 3–4 days, after which colony formation was assessed (Fischer et al., 2007; Ramadoss et al., 2013). Isolates capable of growth under high-salinity conditions were preserved at −80 °C in Nutrient Broth (NB) containing 10% NaCl and 30% glycerol for future use. Based on their growth performance, the most salt-tolerant isolates were selected for subsequent experiments.

In parallel, seeds of six tomato varieties were evaluated for germination percentage in accordance with International Seed Testing Association (ISTA) guidelines. Seeds were placed on filter papers moistened with NaCl solutions at concentrations of 0, 10, 20, 30, 40, 50, 100, 200, 1000, and 1500 mM. Prior to germination, seeds were surface-sterilized with 0.2% sodium hypochlorite (NaOCl), soaked in the respective salt solutions for 30 minutes, and then placed between double layers of filter paper moistened with the same solutions. Seeds placed on filter papers moistened with sterile distilled water served as the negative control. The experiment was arranged with 10 replicates per variety, each replicate consisting of 10 seeds. Seeds were incubated at 24 °C under high relative humidity (95%) for seven days, after which germination percentages were recorded.

Based on these results, one salt-sensitive and one salt-tolerant tomato variety were selected. The vigor index (VI) was determined under 0 and 40 mM NaCl treatments using the following formula:

VI = Germination rate (%) × (Radicle length + Hypocotyl length)

To evaluate the effect of salt-tolerant bacterial isolates on germination performance under salinity stress, the most salt-sensitive tomato variety was used. Based on preliminary screening, the seven most salt-tolerant bacterial isolates were selected. Forty-eight-hour-old bacterial cultures were suspended in a 0.1% carboxymethyl cellulose (CMC) solution to prepare inocula. Surface-sterilized tomato seeds (0.2% NaOCl) were coated with the bacterial suspensions at a ratio of 5 g seeds per 5 mL suspension, shaken at 121 rpm for 30 minutes, and air-dried between filter papers at 24 °C for one hour (Sarma & Saikia, 2013).

Bacteria-coated seeds were placed in 9-cm Petri dishes containing double-layered sterile filter papers moistened with sterile water supplemented with 40 mM NaCl and incubated at 24 °C for seven days. The experiment was conducted with three replicates per bacterial isolate, each replicate consisting of 10 seeds. Seeds without bacterial inoculation or salt treatment served as the negative control, while seeds treated only with 40 mM NaCl served as the positive control. At the end of the incubation period, germination percentage was recorded, and the VI was calculated using the formula described above. The bacterial isolates that produced the highest germination rates and VI values were selected for subsequent *in vivo* experiments.

2.2.2. *In vivo* tests

Following preliminary trials, *in vivo* experiments were conducted using seeds from two tomato varieties identified as the most salt-sensitive and salt-tolerant, together with three bacterial isolates exhibiting the highest salinity tolerance. Bacterial inoculum was prepared from cultures grown on Nutrient Agar (NA) for 24–48 hours and suspended in a 0.1% CMC solution. Tomato seeds (5 g seeds per 5 mL suspension), previously surface-sterilized with 0.2% NaOCl, were coated with the bacterial suspension by shaking at 121 rpm for 30 minutes. The treated seeds were then air-dried between filter papers for 1 hour at 24 °C (Sarma & Saikia, 2013).

The bacterized seeds were sown in trays containing sterile peat. The experiment was arranged in a randomized complete block design with five replicates per treatment, each replicate consisting of one plant, and was conducted in a controlled growth chamber maintained at 24 °C with a 16 h light/8 h dark photoperiod. When seedlings reached the two-leaf stage, they were transplanted into 10 cm diameter pots containing sterile peat. The potting medium was homogeneously amended with 40 mM NaCl (11.6 g NaCl per 100 g peat).

After transplanting, seedlings were irrigated with a bacterial suspension containing the selected root bacterial isolates at a concentration of 10^8 CFU mL^{-1} (50 mL per pot). For the negative control, seeds were coated only with 0.1% CMC, without bacterial inoculation or NaCl treatment. For the positive control, plants were grown in NaCl-amended peat (40 mM) without bacterial inoculation. Plants were monitored periodically for 6–8 weeks.

At the end of the experimental period, physiological and growth parameters, including plant height, number of compound leaves, and flowering status, were recorded. In addition, total plant biomass was determined by measuring fresh and dry weights for each replicate. Differences in biomass between positive and negative control treatments were quantified using the Abbott formula to calculate percentage effects. The *in vivo* experiments were repeated twice to ensure reproducibility and validation of results.

2.2.3. Molecular identification of salt-tolerant bacterial isolates

For molecular identification of the three bacterial isolates selected on the basis of *in vitro* and *in vivo* performance, including PGPR activity and salt stress tolerance, amplification of the 16S rRNA gene was performed using universal primer pairs 27F/1492R (Forward: 5′-AGA GTT TGA TCM TGG CTC AG-3′; Reverse: 5′-GGT TAC CTT GTT ACG ACT T-3′) (Hodkinson & Lutzoni, 2009).

The PCR reaction mixture was prepared as a master mix, aliquoted into PCR tubes (20 µL per reaction), and amplified using a thermal cycler under the following conditions: an initial denaturation at 95 °C for 5 minutes; 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 1 minute and a hold at 15 °C.

The amplified PCR products were resolved by electrophoresis on a 1.5% (w/v) agarose gel prepared in TAE buffer (0.5 M; Fermentas) and run at 80 V for 1 hour. DNA bands were visualized using a UV transilluminator, with amplicons observed at approximately 1460 bp. The PCR products were subsequently purified and submitted for sequencing, and the resulting sequences were compared with those available in the NCBI database to determine the taxonomic identity of the bacterial isolates.

2.2.4. Analysis of data

The experimental data were analyzed using one-way analysis of variance (one-way ANOVA). Differences among treatment means were assessed using Duncan's multiple range test at a 95% confidence level ($p \leq 0.05$).

3. Results

Within the scope of this study, bacterial isolates selected for salinity tolerance were initially evaluated under *in vitro* conditions and subsequently assessed under *in vivo* conditions.

3.1. *In vitro* results obtained from the determination of salt tolerance of bacterial isolates used in the study

In the study, a total of 19 bacterial isolates were cultured on NA medium to assess their salt tolerance at varying salt concentrations ranging from 0 to 2000 mM. After 72 hours of incubation at 24°C, the growth of the colonies was evaluated. Among the 19 bacterial isolates tested, 13 isolates exhibited good growth at a 1000 mM salt concentration. Among these, bacterial isolates 67, 121, 160, 213, 224, 300, and 302 demonstrated good growth even at a high salt concentration of 1500 mM (Figure 1).

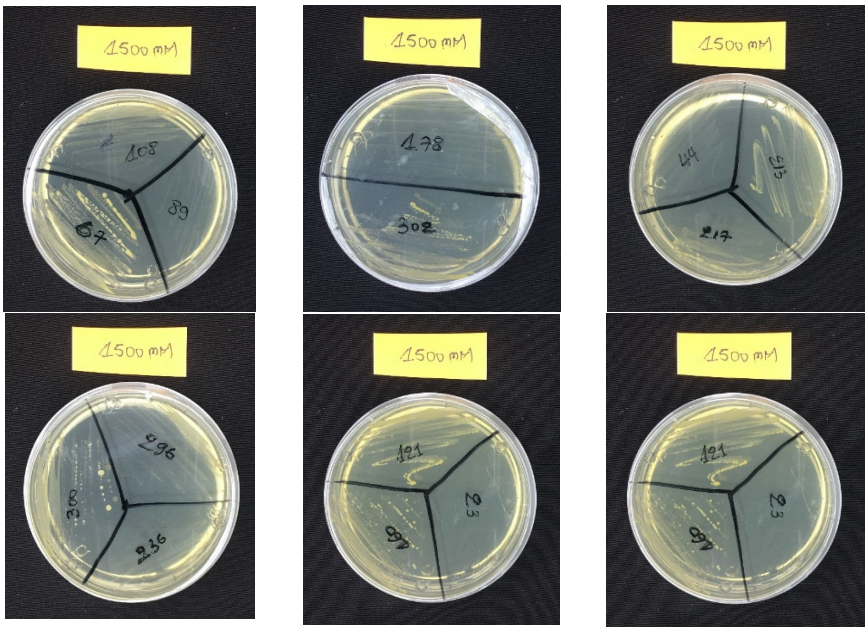

Figure 1. Growth of tested bacterial isolates on NA medium containing 1500 mM salt concentration

3.2. Results of the effects of different salt concentrations on germination rate (%) and VI of tomato varieties

The results obtained from testing the germination rate (%) of seeds from six different tomato varieties under various salt concentrations (0 mM, 10 mM, 20 mM, 30 mM, 40 mM) and later evaluating the VI using only the 0 mM and 40 mM doses are presented in Table 1.

Table 1. VI of tomato varieties at 40 mM salt concentration

Tomato cultivars	VI at different salt concentrations*	
	0 mM	40 mM
Syngenta 33	5143,33 bc**	9346,66 a**
Sakata 13	4224,66 bc	6223,33 b
Sakata 24	3464,66 bc	5564,33 bc
Sakata 36	3256,33 bcd	2333 d
Sakata 10	5688,60 bc	5456,66 bc
SC 21 21	2473,33 cd	2838 bcd

*Values represent the mean of four replicates, each consisting of ten seeds. Each experiment was conducted twice.
**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at P ≤ 0.05.

3.3. Results obtained from the effects of salt-tolerant bacterial isolates on germination rate (%) and VI in salt-sensitive tomato cultivars

Based on the results obtained in section 3.2, it was determined that the tomato variety most sensitive to salt was SC2121. Before proceeding to *in vivo* tests, seeds of the most sensitive tomato variety were treated with bacterial isolates numbered 67, 121, 160, 213, 224, 300, and 302, which were identified as tolerant to salt. The effects of these bacteria on germination rate (%) and VI under a salt concentration of 40 mM were tested. The results obtained are presented in Table 2. According to the data in Table 2, in terms of germination rate and VI compared to the untreated positive control, isolate 302 was found to be the least affected by salt stress (Figure 2), followed by isolates 67 (Figure 3) and 121, respectively. Moreover, under conditions without salt stress, all seven bacterial isolates tested succeeded in increasing the VI compared to the untreated negative control (Table 2). Thus, among the bacterial isolates with the highest salt tolerance, isolates numbered 67, 121, and 302 were selected for *in vivo* tests.

Table 2. The effect of salt-tolerant bacterial isolates on the germination rate (%) and VI of the salt-sensitive tomato variety

Isolate No	Mean germination rate at different salt concentrations (%)*		Mean VI at different salt concentration*	
	0mM	40mM	0mM	40mM
K-	66,6 ab**	50 b**	2238,30 abc**	1078,3 bc**
67	70 ab	70 ab	3585,00 abc	2355 abc
121	73,3 ab	63,3 ab	3635,60 abc	2228,3 abc
160	83,3 a	63,3 ab	4883,00 a	2148,3 abc
213	73,3 ab	53,3 ab	3314,00 abc	1445,6 bc
224	73,3 ab	60 ab	3040,00 abc	785,6 c
300	73,3 ab	53,3 ab	3025,00 abc	764,6 c
302	80 ab	83,3 a	3708,30 abc	3801,6 ab

*Values represent the mean of four replicates, each with ten seeds. Each trial was repeated twice.
**Acc. to Duncan's multiple range test, values sharing the same letter arenot significantly different at P ≤ 0.05.

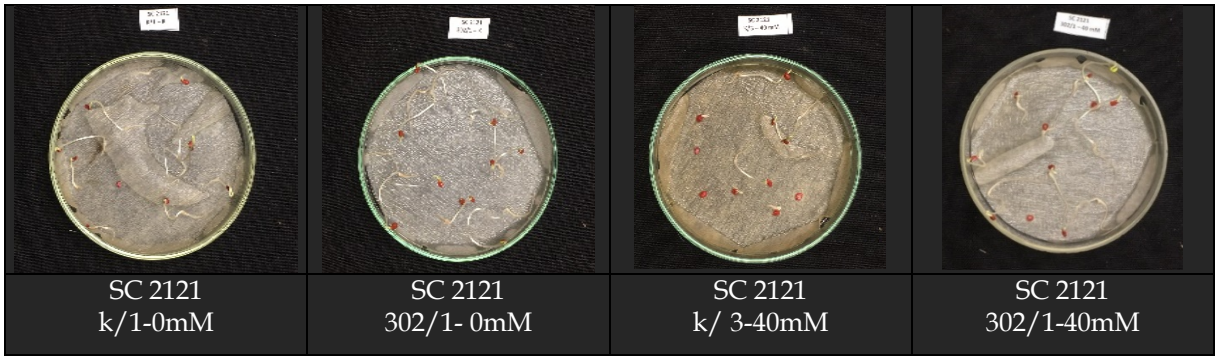

Figure 2. The effect of bacterial isolate number 302 on the germination of the SC2121 tomato variety in the absence and presence of salt stress (from left to right: Negative control, isolate 302 without salt stress, 40 mM salt stress (Positive Control), 40 mM salt stress + isolate 302)

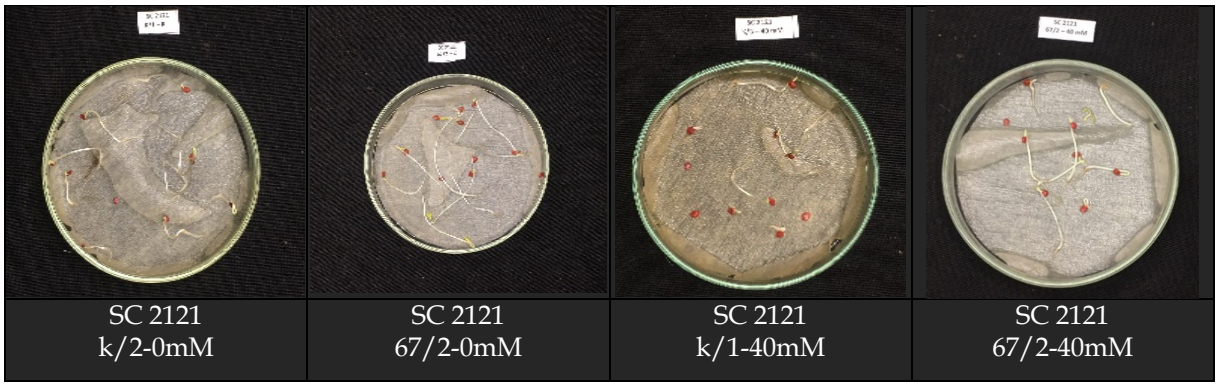

Figure 3. The effect of bacterial isolate number 67 on the germination of the SC2121 tomato variety in the absence and presence of salt stress (from left to right: Negative control, isolate 67 without salt stress, 40 mM salt stress (Positive Control), 40 mM salt stress + isolate 67)

3.4. *In vivo* test results

According to the results of the *in vitro* salt tolerance tests, *in vivo* tests were conducted in a plant growth chamber using two tomato varieties selected as the most sensitive and the most tolerant to salt, along with bacterial isolates numbered 67, 121, and 302, which were chosen from among the most salt-tolerant isolates. Tomato seeds treated via seed bacterization were transplanted into pots once they reached the two-leaf stage in seedling trays, in order to observe the effects of salt stress. The plants were monitored for growth under salt stress conditions for two months. The experiment was repeated twice, and the resulting shoot and root fresh and dry weight values are presented in Tables 3, 4, 5, and 6, respectively.

Table 3. The effect of bacterial treatments on shoot fresh weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial Mean shoot fresh weight (g/plant) *		II. Trial Mean shoot fresh weight (g/plant) *		Mean of two trials *	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	26,244 abcd**	24,092 abcd**	14,748 d**	30,608 abc**	20.496	27,350
	67	27,342 abcd	23,000 bcd	25,538 bc	32,815 ab	26,440	27,908
	121	28,444 abc	26,952 abcd	27,044 abc	33,784 a	27,744	30,368
	302	21,404 cd	26,712 abcd	26,114 bc	31,360 abc	23,759	29,036

SC 2121	K-	25,248 abcd	20,306 d	28,046 abc	30,282 abc	26,665	25,294
	67	29,146 ab	30,400 ab	25,026 c	32,364 ab	27,086	31,382
	121	27,034 abcd	30,442 a	28,328 abc	32,120 abc	27,681	31,281
	302	26,724 abcd	27,626 abcd	28,082 abc	28,382 abc	27,403	28,004

*Values represent the mean of five replicates, with one plant per replicate.
**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at P ≤ 0.05.

Based on the mean shoot fresh weight values from the two trials, all bacterial treatments increased shoot fresh weight in both tomato varieties under both saline and non-saline conditions compared with the untreated negative control (Table 3).

Table 4. The effect of bacterial treatments on root fresh weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial Mean root fresh weight (g/plant) *		II. Trial Mean root fresh weight (g/plant) *		Mean of two trials*	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	1,160 ab **	1,536 ab**	1,526 de**	2,800 ab**	1,343	2,168
	67	1,532 ab	0,904 b	1,290 e	1,976 bcde	1,411	1,440
	121	1,252 ab	1,978 a	1,680 de	2,640 abc	1,466	2,309
	302	1,374 ab	2,062 a	3,112 a	3,036 a	2,243	2,549
SC 2121	K-	1,276 ab	0,862 b	1,832 cde	2,210 abcde	1,554	1,536
	67	1,246 ab	1,792 ab	1,936 bcde	1,932 bcde	1,591	1,862
	121	1,358 ab	1,492 ab	1,644 de	1,642 de	1,501	1,567
	302	1,462 ab	0,876 b	2,774 ab	2,314 abcd	2,118	1,595

*Values represent the mean of five replicates, with one plant per replicate.
**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at P ≤ 0.05.

Based on the mean root fresh weight values obtained from the two trials, all bacterial treatments significantly increased root fresh weight in both tomato varieties under salt stress compared with the untreated negative control (Table 4). Under saline conditions, bacterial inoculation resulted in greater root biomass in the Syngenta 33 variety than in the SC2121 variety, with isolate 302 producing the most pronounced effect (Table 4).

Based on the mean shoot dry weight values from the two trials, bacterial treatments with isolates 121 and 302 increased shoot dry weight in the Syngenta 33 tomato variety under salt stress compared with the untreated negative control (Table 5). Overall, isolates 121 and 302 were the most effective bacterial treatments.

Table 5. The effect of bacterial treatments on shoot dry weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial Mean shoot dried weight (g/plant) *		II. Trial Mean shoot dried weight (g/plant) *		Mean of two trials*	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	2,922 abcd**	2,968 abcd**	1,846 d**	3,036 ab**	2,384	3,002
	67	3,402 abc	2,564 cde	2,206 cd	3,028 ab	2,804	2,796
	121	3,586 ab	2,934 abcd	2,556 abc	3,146 a	3,071	3,040
	302	2,426 de	3,730 a	2,590 abc	3,188 a	2,508	3,459
SC 2121	K-	2,746 bcde	2,014 e	2,392 bcd	2,376 bcd	2,569	2,195
	67	3,464 abc	3,404 abc	2,140 cd	2,622 abc	2,802	3,013
	121	3,242 abcd	3,356 abc	2,356 bcd	2,438 bcd	2,799	2,897
	302	3,078 abcd	2,766 bcde	1,992 cd	2,470 bcd	2,535	2,618

*Values represent the mean of five replicates, with one plant per replicate.
**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

Table 6. The effect of bacterial treatments on root dry weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial Mean root dried weight (g/plant) *		II. Trial Mean root dried weight (g/plant) *		Mean of two trials*	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	0,238 c**	0,250 c**	0,204 bc**	0,330 abc**	0,221	0,290
	67	0,304 c	0,202 c	0,148 c	0,272 abc	0,226	0,237
	121	0,346 bc	0,316 c	0,198 bc	0,328 abc	0,272	0,322
	302	0,224 c	1,060 a	0,376 ab	0,364 ab	0,300	0,712
SC 2121	K-	0,274 c	0,324 c	0,200 bc	0,250 bc	0,237	0,275
	67	0,376 bc	0,372 bc	0,198 bc	0,226 bc	0,287	0,299
	121	0,382 bc	0,366 bc	0,182 bc	0,196 bc	0,282	0,281
	302	0,294 c	0,670 b	0,290 abc	0,470 a	0,292	0,570

*Values represent the mean of five replicates, with one plant per replicate.
**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

Based on the mean root dry weight values from the two trials, all bacterial treatments increased root dry weight under salt stress in both tomato varieties compared with the untreated negative control. Among the treatments, bacterial inoculation with isolate 302 resulted in the greatest increase in root dry weight (Table 6).

Overall, bacterial treatments were more effective in increasing total plant dry weight (root + shoot) under salt stress in the salt-tolerant tomato variety Syngenta 33 than in the salt-sensitive variety SC2121.

The results obtained from the *in vivo* trials were also evaluated in terms of total plant biomass. Figure 4 presents the mean total fresh weight (root + shoot) of plants grown under salt stress, while Figure 5 shows the corresponding mean total dry weight (root + shoot).

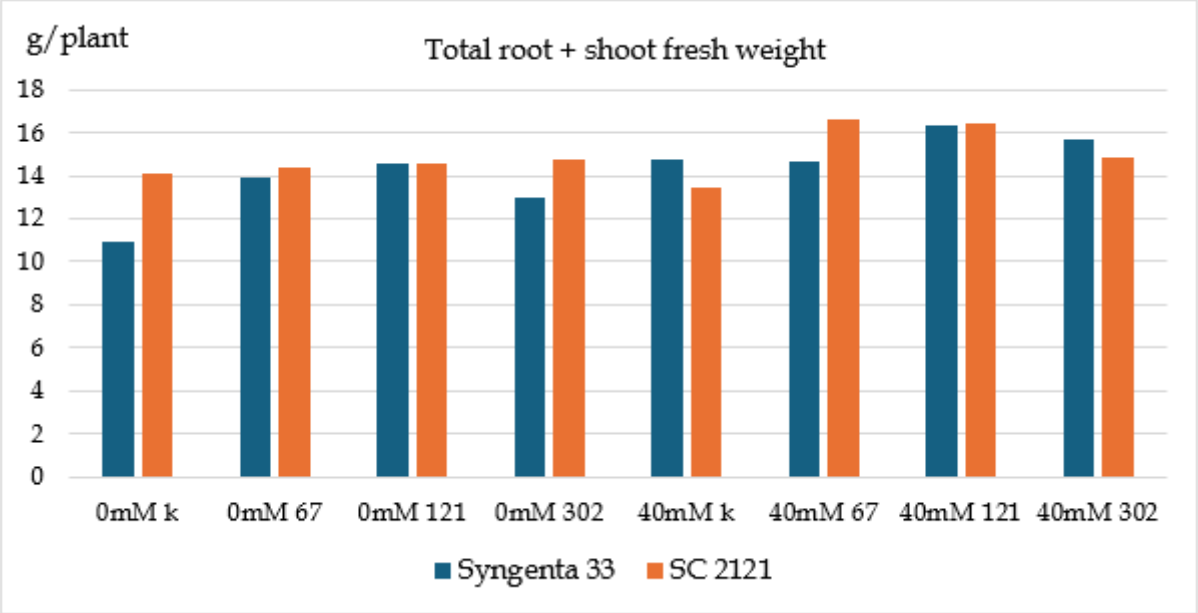

Figure 4. The effect of bacterial treatments on total plant fresh weight in the presence and absence of salt stress

As shown in Figure 4, bacterial applications exerted a more pronounced positive effect on total fresh plant weight (root + shoot) under salt stress than under non-saline conditions. In both tomato varieties exposed to salinity, all three bacterial treatments increased fresh plant weight.

Analysis of total dry plant weight under both saline and non-saline conditions (Figure 5) revealed that bacterial isolate 302 produced the greatest increase in dry biomass under 40 mM salt stress compared with the untreated control. This effect was followed by isolates 121 & 67.

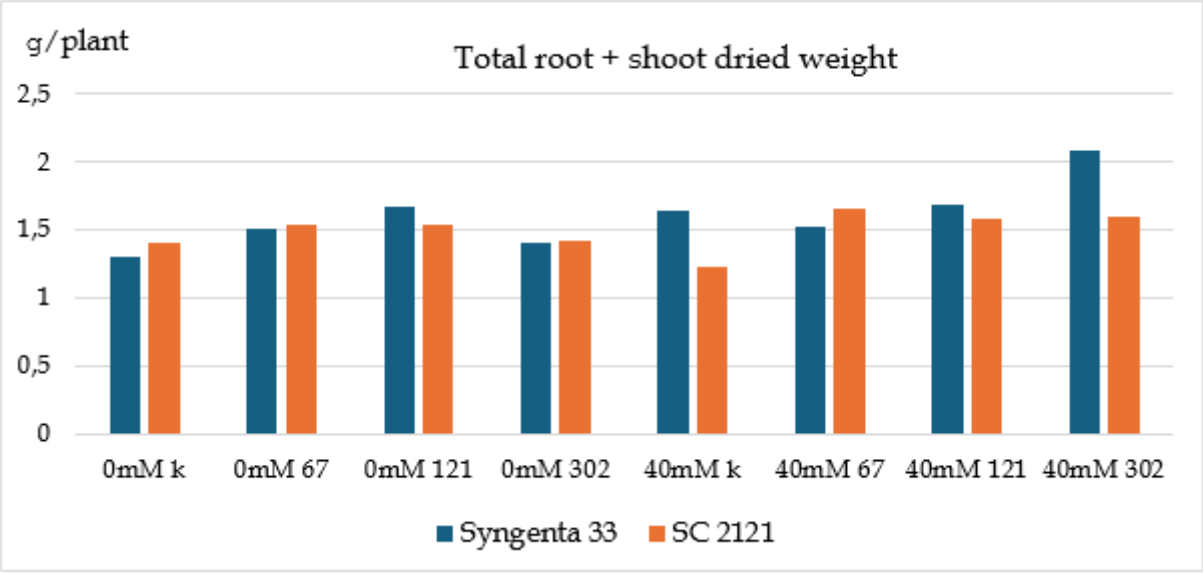

Figure 5. The effect of bacterial treatments on total plant dry weight in the presence and absence of salt stress

3.5. Molecular identification results of salt-tolerant bacterial isolates

In the molecular identification of the three bacterial isolates selected based on *in vitro* and *in vivo* trials (PGPR activity and salt stress), universal primer pairs designed for the 16S rRNA target region were used (Hodkinson and Lutzoni, 2009). The obtained PCR products were sent for sequencing, and the sequencing results were compared with information in the NCBI database to identify the bacterial isolates. As a result of the sequencing, isolate number 302 was found to be the most effective against salt stress in this study, followed by isolate number 121 and then isolate number 67 (Table 7).

Table 7. Sequence analysis of bacterial isolates effective against salt stress

Isolate code	Species / isolate no	NCBI accession number
FO67	*Pantoea ananatis* strain KD20/2	PQ009206
FO121	*Acinetobacter calcoaceticus* strain KD40/2	PQ009214
FO302	*Pantoea vagans* strain KD120/1	PQ009608

4. Discussion

Endophytic and rhizospheric bacteria play an increasingly important role in enhancing plant growth and resilience under adverse environmental conditions. Endophytes are non-pathogenic microorganisms that colonize internal plant tissues and often confer adaptive advantages to their hosts, particularly under biotic and abiotic stress (Vaishnav et al., 2018). Among abiotic stresses, soil salinity is one of the most severe constraints on plant growth and agricultural productivity worldwide. Consequently, sustainable, biology-based strategies—such as the use of PGPR—are gaining attention as effective alternatives to conventional breeding and genetic engineering approaches.

In the present study, salinity was selected as the primary abiotic stress factor, and bacterial isolates from laboratory stocks were evaluated for their capacity to mitigate salt stress in tomato plants under both *in vitro* and *in vivo* conditions. The isolates were initially screened based on key PGPR traits, including indole-3-acetic acid (IAA) production, siderophore secretion, phosphate solubilization, and 1-aminocyclopropane-1-carboxylate deaminase ($ACCD^+$) activity. These traits are widely recognized as reliable indicators of bacterial potential to enhance plant growth and stress tolerance. The results confirmed that preliminary *in vitro* screening based on these parameters is an effective approach for selecting salt-tolerant bacterial candidates.

A salinity level of 40 mM NaCl, previously reported to adversely affect tomato growth (Sahab et al., 2021), was used for *in vivo* evaluation. The selected bacterial isolates demonstrated a strong ability to tolerate high salt concentrations in *in vitro* assays and significantly improved plant growth under 40 mM NaCl in pot experiments. Increases in fresh and dry biomass indicated that bacterial inoculation effectively mitigated the inhibitory effects of salinity, confirming the suitability of this stress level for evaluating bacterial-mediated tolerance in tomato.

One of the most critical mechanisms underlying bacterial-induced salt tolerance is ACC deaminase activity. Under salt stress, plants produce excessive ethylene, which restricts root elongation, accelerates senescence, and triggers programmed cell death. $ACCD^+$ bacteria reduce stress-induced ethylene levels by degrading ACC, thereby promoting root growth and maintaining cellular viability (Choudhury et al., 2023). The bacterial isolates identified in this study—*Pantoea ananatis*, *Acinetobacter calcoaceticus*, and *Pantoea vagans*—exhibited strong $ACCD^+$ activity, which likely contributed to their consistent performance in improving tomato growth under saline conditions. These findings align with previous reports demonstrating

that ACCD$^+$ PGPR enhance biomass accumulation and delay stress-induced senescence through ethylene regulation.

In addition to ACC deaminase activity, IAA production and siderophore secretion were key traits associated with bacterial effectiveness in this study. High levels of IAA enhance root system development, increasing the plant's capacity for water and nutrient uptake under stress conditions. Siderophore production improves iron availability and may indirectly suppress pathogenic microorganisms, contributing to improved plant vigor. The strong expression of these traits among the selected isolates supports their role as reliable selection criteria for identifying effective PGPR under salinity stress.

Exopolysaccharide (EPS) production is another mechanism linked to bacterial-mediated salt tolerance. EPS can bind sodium ions, reduce ionic toxicity, enhance soil aggregation, and improve water retention in the rhizosphere. Previous studies have shown that EPS-producing bacteria promote plant survival and growth under saline conditions by acting as protective "stress molecules" (Tahmish & Naveen, 2023). The presence of effective *Pantoea* group isolates in the present study further supports the hypothesis that EPS production may have contributed to the observed improvements in plant biomass under salt stress.

The effectiveness of PGPR under saline conditions has been demonstrated across multiple crops. For example, *Bacillus velezensis* JB0319 enhanced lettuce growth, antioxidant enzyme activity, and osmotic balance under salt stress while also reshaping the rhizosphere microbial community (Bai et al., 2023). Similarly, PGPR applications alleviated NaCl-induced morphological and physiological damage in strawberry plants (Sahab et al., 2021). The positive effects observed in the present study using seed bacterization and root inoculation methods are consistent with these findings, highlighting the effectiveness of these application strategies for delivering beneficial bacteria into plant systems.

Increasing environmental challenges and global hunger have driven the development of stress-tolerant crops through genetic engineering and plant breeding. However, these approaches are time-consuming and difficult to implement on a large scale. As an alternative, sustainable agricultural practices involving beneficial bacteria have emerged as promising solutions. These bacteria enhance plant tolerance to salt stress by reducing salt uptake, notably through the regulation of specific ion transporters and by trapping ions within exopolysaccharide matrices (Bhat et al., 2020), which partly explains their observed effectiveness in this study.

Among the isolates identified, *Pantoea ananatis* emerged as a particularly promising halotolerant PGPR. Previous studies have shown that *P. ananatis* enhances plant growth, chlorophyll content, protein accumulation, and proline levels under salt stress (Lu et al., 2021). In agreement with these reports, the *P. ananatis* isolate identified in this study significantly promoted tomato growth under both saline and non-saline conditions, supported by strong ACCD$^+$ activity, IAA production, and siderophore secretion. Likewise, the effectiveness of the *Acinetobacter* isolate is consistent with earlier studies demonstrating the capacity of *Acinetobacter* spp. to enhance plant growth and seed germination under high salinity (Patel et al., 2022).

Overall, the findings of this study demonstrate that PGPR possessing ACC deaminase activity, IAA production, siderophore secretion, phosphate solubilization, and EPS production can effectively mitigate the adverse effects of salinity stress in tomato plants. Compared with conventional breeding and genetic engineering approaches—which often require long development periods—the use of beneficial bacteria offers a rapid, sustainable, and climate-smart strategy for improving crop resilience. Importantly, this study confirms that systematic *in vitro* screening based on key PGPR traits provides a reliable framework for selecting effective bacterial candidates for *in vivo* application in salt-affected agricultural systems.

5. Conclusion

Salinity is one of the most pervasive abiotic stresses threatening global food security and environmental sustainability, with projections indicating that up to 50% of arable land may be affected by salinization by 2050. Salt accumulation degrades soil fertility, limits crop productivity, and disrupts ecosystem functioning. In this context, PGPR offer a promising, environmentally sustainable strategy to enhance plant performance under saline conditions while contributing to the remediation of salt-affected soils.

In this study, several beneficial bacterial isolates demonstrated a strong capacity to mitigate salt stress in tomato plants. Among them, *Pantoea vagans* isolate 302 produced the most pronounced increases in fresh and dry plant biomass in both salt-sensitive and salt-tolerant tomato cultivars grown under 40 mM NaCl. Similarly, *Acinetobacter calcoaceticus* isolate 121 and *Pantoea ananatis* isolate 67 also significantly enhanced plant growth. Notably, bacterial inoculation improved plant biomass even relative to non-saline controls, highlighting the robust plant growth–promoting potential of these isolates.

Genotypic differences among tomato cultivars further influenced plant responses to salinity, emphasizing the importance of selecting salt-tolerant varieties as part of integrated stress management strategies. In addition to alleviating abiotic stress, the PGPR strains evaluated here exhibit multifunctional traits, including potential roles in biological control of plant pathogens and insect pests, supporting their application within integrated pest management frameworks.

Overall, the use of plant growth–promoting bacteria represents a sustainable and climate-smart approach to enhancing crop resilience. Future research should focus on field validation and the development of effective bioformulations to facilitate large-scale agricultural application.

Authorship Contribution Statement

FD carried out the growth chamber and colonization studies, and performed the statistical analysis, sequence alignment, and drafted the manuscript. HÖ designed and coordinated the study. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

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