



Germination Studies in Tomato Seeds Treated with Endophytic Bacteria Isolated from Curcuma longa

Shaju Reema Thankam and Suba G. A. Manuel

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675

E- ISSN: 2249-4538

Volume: 13

Issue: 05

Res. Jr. of Agril. Sci. (2022) 13: 1396–1404



Germination Studies in Tomato Seeds Treated with Endophytic Bacteria Isolated from *Curcuma longa*

Shaju Reema Thankam^{*1} and Suba G. A. Manuel²

Received: 12 Jun 2022 | Revised accepted: 22 Aug 2022 | Published online: 13 Sep 2022

© CARAS (Centre for Advanced Research in Agricultural Sciences) 2022

ABSTRACT

In the current scenario, the change in climatic conditions, excessive use of fertilizers, and lack of enough water in the soil have led to an increase in salt concentrations. This, in turn, has a major effect on seed germination and plant growth thus reducing plant productivity. The use of beneficial microorganisms that help enhance the stress resistance in plants can help in overcoming this situation and improve the growth of plants. Endophytes have the ability to enhance salt tolerance in host plants by enhancing their resistance to stress. Tomato seeds coated with endophytic bacteria were grown in different salt concentrations, and growth parameters which indicate the process of germination were observed. A pot study was also conducted to check the effect of different saline concentrations on the shoot length, root length, total height and total fresh weight of the plants. Seeds coated with endophytic bacteria had more tolerance to high salt concentrations than the control. They were able to withstand the high salinity stress and survive whereas, the seedlings in control wilted and dies within 30 days. There was a difference in weight, shoot length, percentage germinations, formation of lateral roots, true leaves between control and Test.

Key words: Percentage growth, Seed germination, Root length, Shoot length, Stress resistance, Endophytes

Endophytes are microbes that are present inside the plant tissue without causing any damage to the plant [1]. The plants and endophytes have a symbiotic relationship and understanding this relationship can help in studying their role as a biological control [2], in plant stress resistance [3] and environmental remediation [4]. One of the most commonly seen abiotic stress in plants are caused due to salinity in the soil and it possess a serious threat to agriculture [5-6]. Due to the lack of proper irrigation and uncontrolled fertilization, the arable land is turning to be more saline. Not all seeds can survive salinity in the soil and germinate to produce seedlings, thus reducing the productivity of the crop plants [7].

An increase in the population and the reduction in the arable land for the cultivation of crops are two main threats to agricultural sustainability [8]. The increase in soil salinity is one of the major problems that can lead to the reduction in arable land which can, in turn, reduce the quality and quantity of crop productivity [9]. Soil salinity being the major reason affecting most of the cultivable land, it has affected almost 7% of the earth's area [10] and 20% of the cropland [11]. In a study

conducted, almost 50% of the cultivated land will be affected by salinity by the end of 2050 [12]. Salinity in the soil increases the osmotic pressure of the plant which results in the reduction of the absorbance of water leading to late germination and affecting other metabolic and physiological processes [13-14], in some cases, it can also reduce or prevent the process of germination [15-16]. The crop plants are mostly more sensitive to salinity stress during the germination stage [17]. Tomato is one of the common crop plants grown all around the world. They are sensitive to the excess saline concentration in the soil. It has been observed that salinity can increase the time required for germination in tomato seeds and the germination of the seeds can be difficult when grown in soils with electric conductivity of 8 dS m⁻¹ and above [18], salt stress can affect root/shoot dry weight and Na⁺/K⁺ ratio in root and shoot [19].

The best way to grow plants in saline conditions is to grow breeds that are tolerant to these conditions [20]. Tolerance to salinity is an important criterion for any plant growth. Endophytic bacteria have the ability to survive high salt concentrations. The endophytic-plant association in increasing stress tolerance in plants caused due to the habitat is currently gaining more attention. These techniques are low-cost and eco-friendly and play an important role in protecting plants against stress and also increasing plant productivity in saline conditions [21].

In the present study, the effect of different salt concentrations (NaCl, 2%, 4%, 6%, 8%, 10%) on the germination of tomato seeds coated with endophytic bacterial isolates were observed and their impact on the rate and percentage

* **Shaju Reema Thankam**

✉ reemashaju8@gmail.com

¹ Department of Life Sciences, Bangalore University, Bangalore - 560 056, Karnataka, India

² Department of Life Science, Mount Carmel College, No. 58, Palace Road, Bangalore - 560 052, Karnataka, India

germination, shoot length, root length and an average weight of the plant were recorded.

MATERIALS AND METHODS

Isolation of endophytes from *Curcuma longa*

The six endophytic isolates from *Curcuma longa* showing plant growth-promoting activities were selected, identified, and inoculated into the seeds of *Solanum lycopersicum*.

Preparation of bacterial suspension

The 24-hour grown bacterial cultures were loop inoculated and grown in Luria broth (LB) treated with spectinomycin and incubated at $28 \pm 2^\circ\text{C}$ for two days at 180 rpm in a shaker incubator. The cells were separated from the broth aseptically by centrifugation at 2500 rpm and the pellet was re-suspended in phosphate buffer solution (PBS) (0.2 g/L KCl, 1.44 g/L Na_2HPO_4 , and 0.24 g/L KH_2PO_4 , in dH_2O , pH 7.4) using a vortex, with approximately 528 colony forming units (CFU)/ml. This bacterial suspension (1ml) was also plated onto nutrient agar and Tryptic soya agar (TSA) to check the successful transmission of the colonies into the PBS solution [22].

Inoculation of the seeds with endophytic bacteria

The seeds were surface sterilized using 70% ethanol (1 min), 5% sodium hypochlorite solution (3 min), 70% ethanol (30 seconds) and rinsed six times with sterilized distilled water (2 minutes each wash). The accuracy of the sterilization process was checked by plating aliquots of the sterile distilled water used in the final rinse onto tryptic soya agar plates supplemented with cycloheximide (100mg/L) and incubating the plates at 28°C for 2–15 days [23].

The surface-sterilized seeds were divided into two sets. One set was dipped in PBS solution (Test 1) and the other in PBS inoculated with bacterial cells (Test2) and was left for 5 hours. A control (seeds with no treatment) was also maintained [24].

Seed germination studies

The seeds (5 per plate) were transferred to Petri plates containing filter paper that was moistened with sterile distilled water. Five replicates were maintained for each concentration. The seeds were subjected to treatments of different salt (NaCl) concentrations (2%, 4%, 6%, 8%, and 10%) and PBS which was used as control. The salt solution was added daily for 20 days and the rate of germination, radicle length, and fresh weight were measured ones in every 5 days. The rate of germination was observed for 20 days. The seeds showing radicle protrusion through the seed coat were considered as germinated seeds [25].

The percentage of germination was calculated as:

Percentage germination = number of seeds germinated at a designated time period / Total number of seeds used for the test * 100

The Mean germination time (MGT), Germination Rate Index (GRI), Germination energy (GE), Uncertainty of Germination Process (U), and Vigour Index (VI) were also calculated as follows:

$$\text{MGT} = \sum (T_i \times N_i) / \sum N_i$$

where N_i is the number of newly germinated seeds at time T_i [26].

$$\text{GRI} = G1/1 + G2/2 + \dots + G_i/i$$

where G1 is the germination percentage on day 1, G2 is the germination percentage at day 2; and so on [27].

$\text{GE} = \text{Total number of seeds germinated at half of the observation time / the time period selected} * 100$

$\text{VI} = \text{Total root length} + \text{total shoot length} / \text{Percentage germination}$

Salinity stress in tomato seedlings

The salinity stress (2%, 4%, 6%, 8%, 10%) was induced to 3-week-old tomato seedlings continuously for a week [28]. The shoot length, root length, number of leaves, and biomass was measured every 5 days for 20 days.

Physical parameters of the soil

The physical parameters of the soil; pH and electrical conductivity were also measured to understand the change in pH and mineral content with respect to the introduction of salinity stress.

Measurement of pH of the soil

Soil samples (50g) were collected from the plots treated with different salt concentrations and 100mL of distilled water was added. The solution was mixed vigorously using a glass rod and was kept undisturbed for 30 minutes. The suspension was filtered and the filtrate was used to measure the pH (Elico pH meter) [29].

Measurement of electrical conductivity

Soil samples (50g) were mixed with 100mL of distilled water in a conical flask. The solution was filtered using Whatman filter paper (number 1). The filtration process was repeated several times until a clear solution was obtained. The filtrate was used for measuring electrical conductivity. Conductivity was recorded in milli Siemens/ centimetre [29].

Microscopic examination

The plants were uprooted after the 20th day and thin sections of the root samples were cut with smooth strokes using a sharp blade [30]. The sections were incubated in safranin for 1 min, washed with distilled water, and observed under a light microscope at a 10X objective lens [31].

RESULTS AND DISCUSSION

Isolation of endophytes from *Curcuma longa*

A total of 14 bacterial isolates were identified from *Curcuma longa*. Out of the 14 bacterial isolates from *Curcuma longa*, six endophytic bacteria that showed Plant Growth Promoting activities were selected and were inoculated into the selected crop plant. The six endophytes were identified using 16s rRNA sequencing and analysis and they showed similarities to the following bacterial strains (Table 1).

Table 1 Identification of endophytic bacterial isolates by 16 s rRNA sequencing and analysis

Colony Number	Identification	Accession Number
C5	<i>Kocuria rocea</i>	MT317205.1
C2	<i>Bacillus subtilis</i>	MT068199.1
C1	<i>Brevibacterium casei</i>	GQ365205.1
C8	<i>Actinobacterium JS14 strain</i>	AY372899.1
C7	<i>Bacillus amyloliquefaciens</i>	MT131178.1
C11	<i>Bacillus velezensis</i>	CP028204.1

Seed germination studies

The seed germination and post-germination growth were studied on tomato seeds grown in different salt concentrations that were uninoculated (only PBS solution) with and inoculated (PBS solution and bacterial isolates) with the bacterial endophytic isolates. The rate of the seed germination at

different salt concentrations was recorded by calculating their percentage growth, days required for germination, root length, shoot length, and biomass. A control with no endophytic inoculation or salt treatment was also maintained. The changes were observed at equal intervals of days: 1, 5, 10, 15, and 20 days (Table 2, Fig 1-2).

Table 2 Seed germination studies of tomato seeds treated with endophytic bacterial solution

Concentration	Sample	MGT	GRI	GE	U	VI
2%	Test 1	10.09	1.32	22.0	0.34	461.2
	Test 2	13.61	1.99	30.0	0.75	1837.7
4%	Test 1	9.54	0.63	16	0.44	194.56
	Test 2	13.57	1.06	26.0	0.61	526.2
6%	Test 1	7.90	0.45	8.0	0.14	38.16
	Test 2	11.48	0.85	20	0.50	160.58
8%	Test 1	6.00	0.13	6.00	0.15	16.83
	Test 2	11.14	0.97	16.0	0.40	133.66
10%	Test 1	3.0	0.44	2.00	0.05	4.18
	Test 2	10.65	1.22	18.00	0.35	110.80
0%	Control	10.41	1.61	26	0.61	677.94

*MGT- Mean germination time; GRI- germination rate index; GE: Germination energy; U- Uncertainty of germination process, VI- Vigour index



Fig 1 Tomato seedlings grown at 10% concentration after 20th day of germination. A) Test 2 B) Test 1



Fig 2 Tomato seedlings grown at 10% concentration after 20th day of germination. A) Test 1 B) Test 2

The rate of germination was slow in Test 1 when compared to Test 2 and control. The MGT, Vigour, and the germination rate index were more in seeds from Test 2 when compared to Test 1. The uncertainty of the germination process was also more in Test 1 when compared to Test 2 and control. The results for control were almost close to that of Test 2. Both Test 1 and Test 2 showed a decrease in the rate of germination when compared to control but the rate of germination was almost equal to control in seeds treated with endophytic bacteria. The mean germination time, germination rate index, germination energy, uncertainty of germination process, and vigour index were more in Test 2 followed by control when compared to Test 1. The results obtained were in accordance with the previous literature wherein a study where five endophytic bacterial isolates from four different desert plant species were introduced into *Arabidopsis thaliana* to check for their ability to enhance resistance against salt tolerance, it was observed that the inoculation of the *Arabidopsis* plant with the

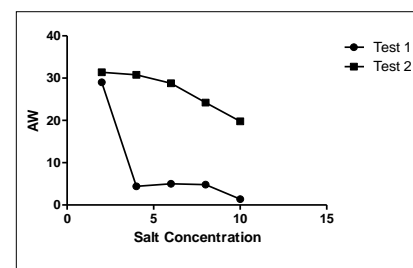
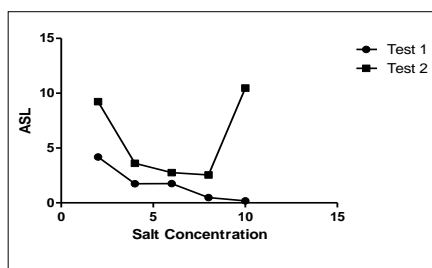
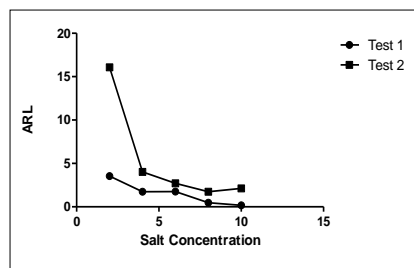
five isolates under salinity stress resulted in tissue-specific transcriptional changes of the ion transporters and thus reduced their Na^+/K^+ ratios in the root. This, in turn, helped the plant survive and grow under salt stress [32].

The average shoot length, root length and the average weight were also compared between Test 1, Test 2 and control. The results showed that the average shoot length, root length and weight decreased from salt concentration of 2% to 10% NaCl. The differences in the shoot length, root length and the weight were lesser in Test 2 when compared to Test 1. Graphs were also plotted for the same using GraphPad Prism software.

The average root growth decreased from 2% to 10% concentration; the growth was slow in Test 1 when compared to Test 2. There was a slight increase in the seeds grown at 8 salt concentration. But the overall root growth was lesser than Test 2. Test 2 showed the highest root length growth in seeds grown at 2% salt concentration. The least was seen in seeds grown at 8% NaCl concentration. There was a slight increase in

the root growth in the seeds grown at 10% NaCl concentration. The initiation of lateral root growth in Test 2 seeds started from day 6 of germination whereas the lateral roots were seen to grow only after 10 days in Test 1 (Fig 3). In a study conducted where endophytes isolated from different tissues of peanut plant were screened *in vitro* by seed germination bioassay to evaluate it

effects to alleviate salinity stress and to enhance yield in peanut plant, two cultivars were used in potted condition and were subjected to saline irrigation water. It was noted that nine endophytes that had the capability of producing IAA and ACC deaminase helped promote root growth and yield of the plant [33].



*ARL-Average root length

The rate of growth of the shoots was also noted for the respected time period

Fig 3 Average root growth in tomato seeds treated with endophytic bacterial solution

Fig 4 Average shoot growth in tomato seeds treated with endophytic bacterial solution

Fig 5 Average fresh weight of the seedlings emerged from the tomato seeds treated with endophytic bacterial solution

The average shoot length in both Test 1 and Test 2 decreased from 2% to 10% NaCl concentration. The maximum growth of the shoots was seen at 2% for Test 1. Whereas in Test 2 the maximum shoot growth was shown in seeds grown under 10% NaCl concentration. The seeds treated with endophytic inoculates were able to withstand and survive the high salinity and grow whereas the seeds from Test 1 showed slow rate of growth. There was a large difference in the height of the shoot grown in Test 1 when compared to Test 2 (Fig 4). The current study is in accordance with the previous studies where while studying the ability of endophytic bacteria isolated from medicinal plant *Thymus vulgaris* to enhance tomato plants resistance against salinity stress, it was noted that out of the three endophytic isolates selected, all three had the ability to promote plant growth under various salt concentrations (50–200 mM) compared to uninoculated control [34].

While comparing the average fresh weight of the seedlings after 20th day of germination it was noted that the seeds grown from Test 2 had a higher average fresh weight compared to that of Test 1. The seedlings from Test 2 showed a decrease in the average fresh weight from 2% to 10% NaCl concentration. The overall difference was significant but was not large. Whereas, the seedlings from Test 1 should have a larger difference in the average fresh weight from 2% to 10% NaCl concentration. The plants grown at 10% NaCl showed the least average fresh weight and most of them dried off by the end of the 20th day (Fig 5). While analyzing the effect of a natural halotolerant endophytic actinobacterium *Glutamicibacter halophytocola* KLBMP 5180 on enhancing the growth and salt tolerance in the tomato plant, it was observed that the inoculation of the bacterial strain into the seeds increased the seedling fresh weight, shoot length, root length, a number of fibrous roots, increased osmolyte content and regulation of ion homeostasis when compared to the non-inoculated seedlings [35]. In a study to analyze the ability of the plant growth-promoting endophytic bacteria (PGPEB) *Sphingomonas* sp. LK11 in alleviating the salinity stress in *Solanum pimpinellifolium* (currant tomato), it was observed that the combination of PGPEB and jasmonic acid can improve the growth of the crop plant and can help overcome the adverse effects of salinity stress [36].

Comparison using ANOVA

ANOVA test was conducted to understand the relationship between the effect of the different salinity concentrations (2%, 4%, 6%, 8%, 10%) on the average shoot length, root length, and the average mass of the tomato seedlings.

Null hypothesis: There is no effect of the treatment (Salinity stress) on the average shoot length, root length, and average mass of the tomato seedlings.

Alternate hypothesis: There is an effect of the Salinity stress on the average shoot length, root length, and the average mass of the tomato seedlings.

The F value obtained was 4.74 which was higher than the F crit value of 2.86 and the P value (0.007) was lesser than the value of alpha (0.05)

$$4.74 > 2.86 (F > F_{crit})$$

$$0.007 < 0.05 (P < \alpha)$$

Hence, the null hypothesis can be rejected and the alternate hypothesis was accepted that there is an effect of the salinity stress on the average shoot length, root length and the average mass of the tomato seedlings.

A histogram was plotted using the values obtained from ANOVA and it was noted that there was a decrease in the parameters from different salt concentrations (Fig 6).

A graph was also plotted to check the effect of salt concentrations on different parameters selected for Test 1 and Test 2. It was observed that when compared to Test 1, Test 2 showed increased values (Fig 7). The percentage germination was maximum in seeds from Test 2 when compared to Test 1. The highest rate of germination was observed in seeds grown at 2% NaCl concentration. The tomato seeds from Test 2 reached 100% germination after 20 days at 2% salt concentration. Whereas, the tomato seeds from Test 1 grown at 2% salt concentration showed only 44% germination.

There was an increase in the rate of germination in Test 2 seeds. The seeds treated with endophytic bacterial suspension were able to reach 50% germination by the 10th day in most of the salt concentrations. Whereas in Test 1, the seeds could not reach 50% germination even at the 20th day. The inoculation of the seeds with salinity tolerant endophytic bacteria helped in the survival and germination of the seeds at different salt

concentrations. The least percentage of germination was observed in seeds grown at 10% salt concentration. The seeds from Test 1 showed 4% germination on 20th day of germination and the seeds from Test 2 showed 48% and germination in tomato seeds on the 20th day of germination. The seeds grown in control showed a 50% germination rate after 11 days of seeds germination (Fig 8-9). These observations were in accordance with the previous studies where, in a study conducted to assess the role of endophytes in the growth and salt tolerance ability of a halophyte *Arthrocnemum macrostachyum*, it was noted that the seeds inoculated with endophytic bacteria showed increased kinetics of germination. The number of seeds germinated and

reaching 50% germination was more in inoculated seeds when compared to uninoculated seeds. It was observed that the inoculated seeds required 5 days to reach 50% inoculation whereas the non-inoculated seeds required a week [37]. In a study where non-rhizobial bacteria were isolated from Alfalfa (*Medicago sativa* L.) plant to understand their ability to improve growth under salinity stress, it was observed that the inoculation of the plant with the non-rhizobial bacteria resulted in an increase in growth compared to the non-inoculated plants thus indicating that the inoculation of the plant with the non-rhizobial bacteria provides promising ways to improve the effectiveness of growth in salt-affected soil [38].

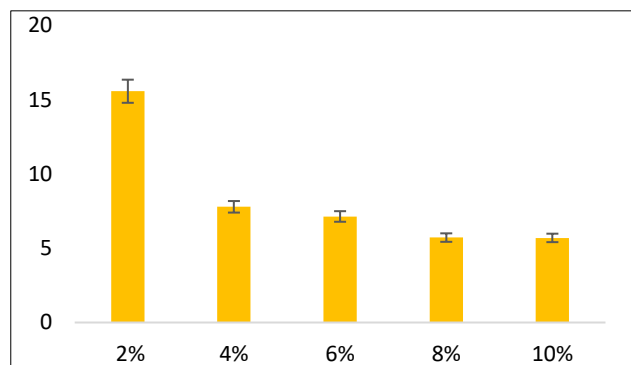


Fig 6 Comparison of the growth of the tomato seedlings at different salt concentrations

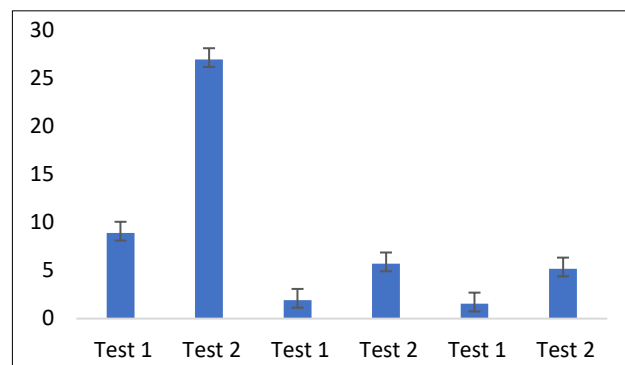


Fig 7 Comparison of the parameters for Test 1 and Test 2 at different salt concentrations

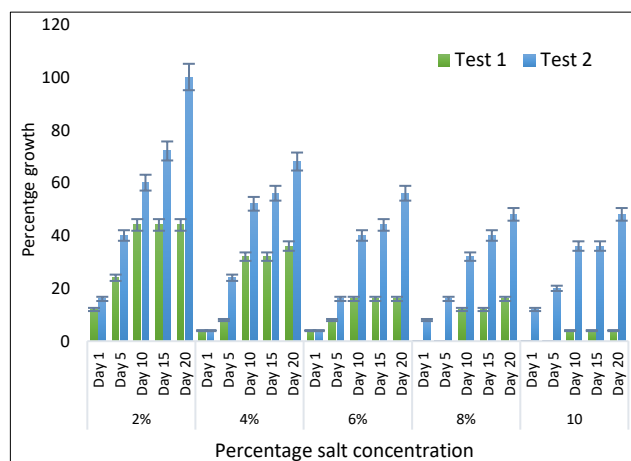


Fig 8 Percentage germination in tomato seeds treated with endophytic bacterial solution

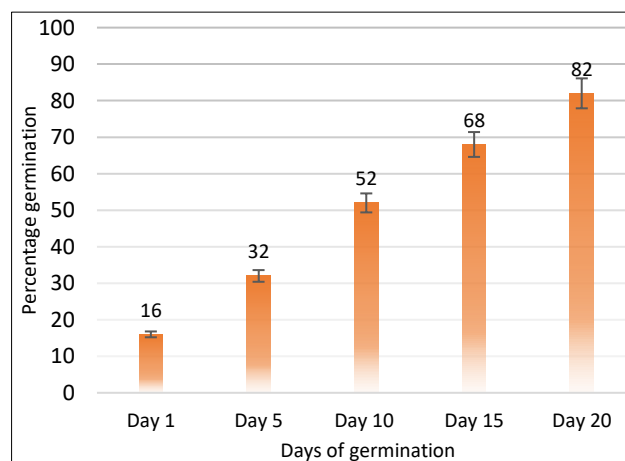


Fig 9 Percentage germination in tomato seeds with no treatment

Salinity stress in tomato seedlings

Young (8-week-old) plants were grown under salt stress by irrigating the plants with different concentrations of salt solution. The irrigation was done every alternate day for 20 days. One set of the plant (Test 2) was inoculated with the endophytic bacterial solution whereas, the second set (Test 1) was not inoculated with any bacterial endophytic solution. A control (without salt treatment or endophytic treatment) was also maintained.

Physical parameters of the soil

The change in pH and electrical conductivity (EC) of the soil can affect the biological activity and the chemical nature of the soil. Most of the microbial mediated processes can be affected with the slight change in pH and EC. Thus, the measurement of both pH and EC can provide a complete overview of the quality of soil [39].

The electrical conductivity (EC) and the pH of the soil were also analyzed (Table 3). It was noted that the electrical

conductivity of the soil increased with the increase in salt concentration. The EC of the soil treated with 2% salt solution was 8.36 ms/cm whereas, the EC for soil treated with 10% salt solution was 14.1ms/cm. The EC of soil which was not treated with any salt solution (Control) was 5.90ms/cm. There was a raise in the electrical conductivity of the soil from salt concentrations 2% to 10% NaCl. The pH of the soil collected from control was 7.5, whereas the pH of soil treated with 2% salt solution was 6.94 and for 10% salt solution was 6.33. There was a fall in the pH from the soil treated with 2% salt solution to 10% salt solution (Table 3, Fig 10). Similar results were also observed in previous papers wherein a study conducted to understand the effect of salinity on the nutrient status of soil, leaves, and kernels of corn it was observed that the soil samples with higher pH and EC had more salt content in them. The weight of the cob and the height of the plant decreased with an increase in salinity [40]. In a study conducted to understand the effect of secondary salinity on soil microbial mass, it was noted that there was a decrease in the organic carbon content and the

microbial biomass in soil with high salinity. The secondary salinity caused due to irrigation had a significant effect on the soil quality and the survival of soil microflora [41].

The effect of salinity stress on the tomato plants

The shoot length, the number of leaves, root length, and the mass of the plants were noted to understand the effect of salinity on the growth of the plants. The observation was made after every 5 days at 1, 5, 10, 15, and 20th days. The plants were grown under controlled conditions.

Table 3 Electrical conductivity and pH of the soil with different treatments

Physical parameter	Salt concentration					
	Control	2%	4%	6%	8%	10%
Electrical Conductivity (ms/cm)	5.90	8.36	9.62	6.52	11.6	14.1
pH	7.5	6.94	6.70	6.48	6.33	6.33

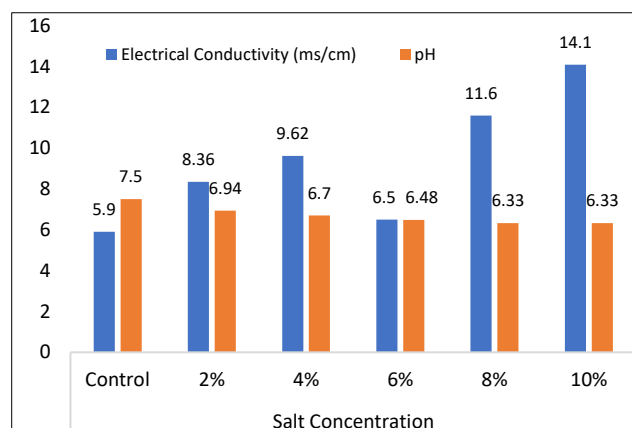


Fig 10 pH and electrical conductivity of the soil treated with different salt concentrations

It was observed that the uninoculated plants growing at higher concentrations of salt started to wilt after 5 days of observation. The leaves started to turn yellow and the stems started to bend and turn brown. The plants that were inoculated with endophytic bacteria had the ability to withstand the salt concentrations in the soil. The plants were able to survive even high concentrations of salt and grow. The number of leaves also increased from Day 1 to day 20.

The average root length of the tomato plants from Test 1 and Test 2 were carefully measured at Day 1, Day 5, Day 10, Day 15, Day 20. It was noted that the values of Test 1 almost remained the same throughout the observation period. There was a slight increase in the root length in Test 2 from Day 1 to Day 20 (Fig 11). In a study conducted to evaluate the effect of salt-tolerant plant growth-promoting bacteria on the growth and development of *Oryza sativa* under salinity stress, it was noted that the presence of two endophytic bacteria enhanced the growth of the plant and there was an increase in the root length when compared to control [42].

The shoot length of plants from Test 2 grown at different concentrations increased from Day 1 to Day 20. Even though the growth of the plants were slow at high concentrations when compared to control, the plants were able to survive to the high salt concentration. In plants from Test 1, the plants showed very slow growth. The plants started to wilt and die after 15 days of the observation (Fig 12). The present observations had similar results with the previous studies wherein a study conducted to understand the effect of salinity on the germination and development of *Sorghum* seeds, it was noted that the seeds grown under salinity stress had a reduced shoot length when compared to the ones grown in control [26].

The number of leaves started to decrease from day 1 to day 20 in plants from Test 1. The old as well as the young leaves wilted and dried off in plants from Test 1 after 5 days of the salt treatment. In Test 2 there was an increase in the number of leaves. The number of leaves of leaves remained almost the

same during Day 15 to Day 20. The number of leaves and Test 2 were almost similar. No wilting of leaves was observed in Test 2 and control (Fig 13).

The average fresh weight of the plants was also measured at equal intervals. The plants from Test 2 had a subsequent increase in the weight of the plants with the increase in days. The weight of plants in Test 1 remained the same from Day 5 to Day 20. The plants showed a decrease in its growth. The results for control and Test 2 were almost similar (Fig 14). The observations made were in par with the previous studies where, in a study conducted to assess the effect of salinity on the growth, sugar content and enzyme activity of rice, it was noted that the plants treated with 200 mM NaCl for 14 days showed decrease in fresh weight and dry weight by 95% and 75% respectively [43]. In a study conducted to understand the interactive effect of PGP bacterial endophytes and biochar in managing salinity stress in maize plants, it was observed that in the plants grown under greenhouse conditions, the combination of biochar and two bacterial strains *Burkholderia phytofirmans* (PsJN) and *Enterobacter* sp. (FD17) reduced the effect of salinity stress and decreased the concentration of Na⁺ in the xylem and helped maintain the nutrient balance within the plant. The results stated that the inoculation of the plants with the combination of endophytic bacteria and biochar helped the plant to sustain salinity stress and this method can be adopted for production of the crop in salt affected soil [44].

Microscopic examination

A cross-section was made to observe the changes in the vascular bundles of the roots of the plants. It was observed that the plants inoculated with endophytes had very little effect on the salt concentrations on the vascular bundle whereas, the vascular bundles of the plants uninoculated with the bacterial endophyte had ruptured vascular bundles with huge gaps in between. Even the cross section obtained from the plants taken from control did not show any rupture (Fig 20). The amount of rupture increased with the increase in the salt concentration. Maximum rupture was seen in the plants treated with 10% of salt concentration (Fig 15-19). The observations in the present study were in accordance with previous studies where the structural changes in the xylem present inside stems induced with salinity stress were studied in soybean, it was observed that the plants grown in plant stress had an increase in the thickness of the vascular cells and cuticle. The effect of salinity was dependent on the concentration of the saline solution [45]. The cells tend to become more lignified when the plants are under [46]. The lignification of the walls can inhibit the growth of the roots thus preventing proper absorption of water and minerals [47]. The endophytic bacteria other than enhancing the growth properties of the plant, phytohormone signaling, and enhancing plant metabolism also play a role in the resistance of the plant to abiotic and biotic stress. They are relatively protected from harsh conditions like abiotic and biotic stress thus helping in improved crop adaptations [47].

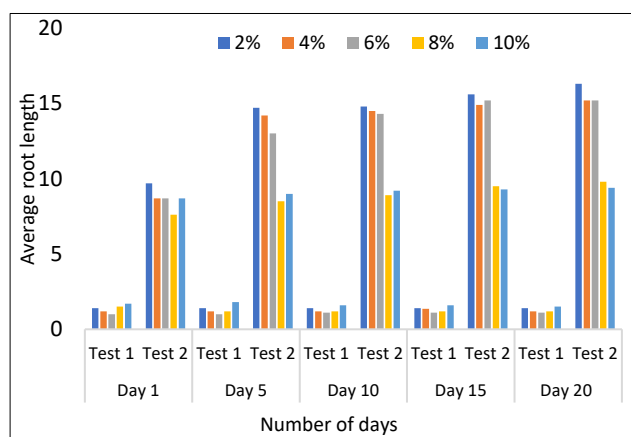


Fig 11 Comparison of average root length of tomato plants grown at different salt concentrations

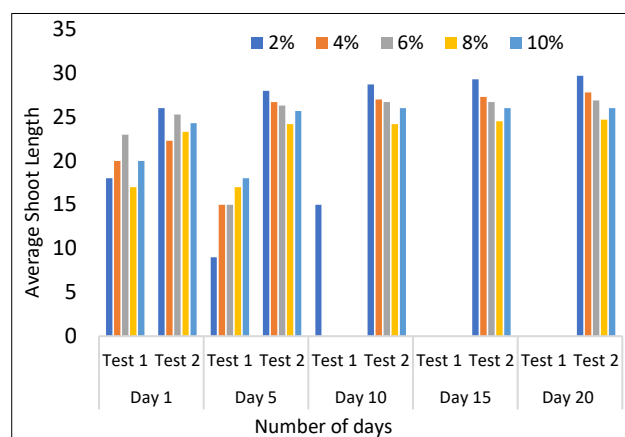


Fig 12 Comparison of average shoot length of tomato plants grown at different salt concentrations

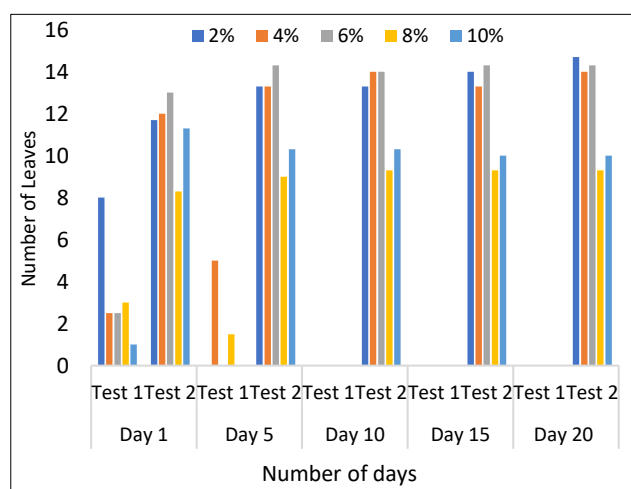


Fig 13 Comparison of number of leaves of tomato plants grown at different salt concentrations

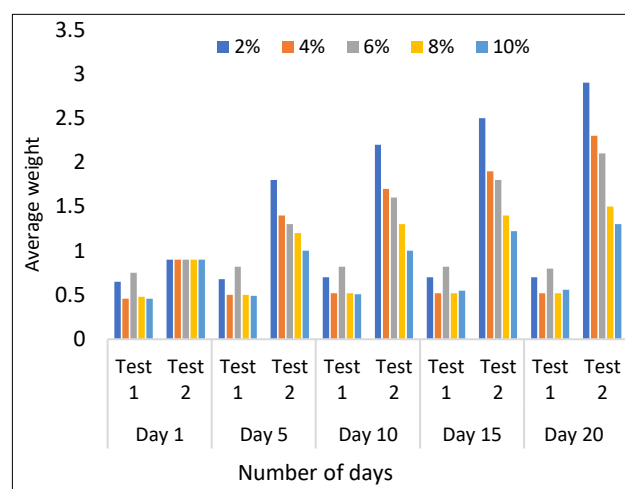


Fig 14 Comparison of average weight of tomato plants grown at different salt concentrations

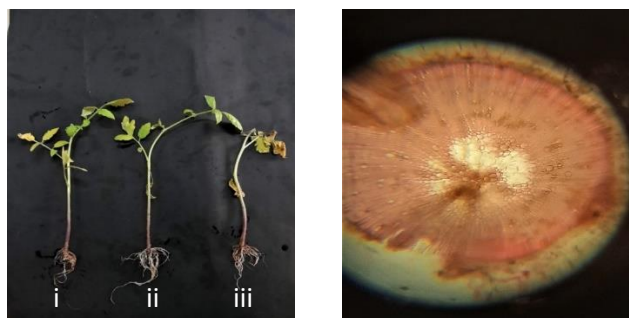


Fig 15 Tomato plants grown at 2% salt concentration (A) Comparison of the plants between i) Test 2, ii) Control, iii) Test 1 (B) Cross section of the root of the plant from Test 1



Fig 16 Tomato plants grown at 4% salt concentration (A) Comparison of the plants between i) Test 2, ii) Control, iii) Test 1 (B) Cross section of the root of the plant from Test 1



Fig 17 Tomato plants grown at 6% salt concentration (A) Comparison of the plants between i) Test 2, ii) Control, iii) Test 1 (B) Cross section of the root of the plant from Test 1



Fig 18 Tomato plants grown at 8% salt concentration (A) Comparison of the plants between i) Test 2, ii) Control, iii) Test 1 (B) Cross section of the root of the plant from Test 1



Fig 19 Tomato plants grown at 10% salt concentration (A) Comparison of the plants between i) Test 2, ii) Control, iii) Test 1 (B) Cross section of the root of the plant from Test 1

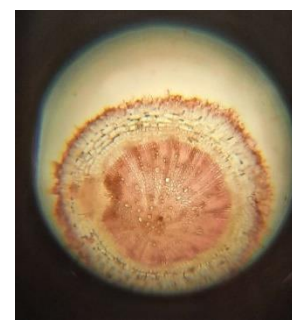


Fig 20 Cross section of the roots collected from (A) Control (B) Test 2

CONCLUSION

The interaction of the endophytes inside the plant tissues can not only help the plant in its growth but can also help the plant to withstand abiotic stress like the salinity stress. It was

recorded that the seeds and seedlings inoculated with endophytes had an ability to withstand high salinity stress when compared to the uninoculated seeds and seedlings. According to the results obtained, the set inoculated with endophytes showed similar growth like the seeds and seedlings which grew in normal conditions without any salinity stress.

LITERATURE CITED

1. Cocq KL, Gurrl SJ, Hirsch PR, Mauchline TH. 2017. Exploitation of endophytes for sustainable agricultural intensification. *Molecular Plant Pathology* 18(3): 469-473.
2. Senthilkumar M, Madhaiyan M, Sundaram SP, Kannaiyan S. 2009. Intercellular colonization and growth-promoting effects of *Methylobacterium* sp. with plant-growth regulators on rice (*Oryza sativa* L. Cv CO-43). *Microbiol. Res.* 164: 92-104.
3. Yaish MW, Antony I, Glick BR. 2015. Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their potential role in salinity tolerance. *Antonie Van Leeuwenhoek*. doi: 10.1007/s10482-015-0445-z.
4. Egamberdieva D, Mamedov NA. 2015. Potential use of licorice in phytoremediation of salt affected soils. In: (Eds) Öztürk M, Ashraf M, Aksoy A, Ahmad M, Hakeem K. Plants, Pollutants and Remediation. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-7194-8_13.
5. Hamwieh A, Tuyen DD, Cong H, Benitez ER, Takahashi R, Xu DH. 2011. Identification and validation of a major QTL for salt tolerance in soybean. *Euphytica* 179: 451-459.
6. Wang ZF, Wang JF, Bao YM, Wu YY, Zhang HS. 2011. Quantitative trait loci controlling rice seed germination under salt stress. *Euphytica* 178(3): 297-307.
7. Flowers TJ, Yeo AR. 1995. Breeding for salinity resistance in crop plants: where next? *Australian Journal of Plant Physiology* 22: 875-884.
8. Shahbaz M, Ashraf M. 2013. Improving salinity tolerance in cereals. *Critical Reviews in Plant Sciences* 32(4): 237-249.
9. Jägermeyr J, Frieler K. 2018. Spatial variations in crop growing seasons pivotal to reproduce global fluctuations in maize and wheat yields. *Sci. Adv.* 4: 4517.
10. Shrivastava, Kumar R. 2015. Soil salinity: A serious environmental issue and plant growth-promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences* 22(2): 123-131.
11. Yang Z, Wang BS. 2015. Present status of saline soil resources and countermeasures for improvement and utilization in China. *Shandong Agricultural Sciences* 47(4): 125-130.
12. Kumar A, Singh S, Gaurav AK, Srivastava S and Verma JP. 2020. Plant growth-promoting bacteria: Biological tools for the mitigation of salinity stress in plants. *Front. Microbiol.* 11: 1216. doi: 10.3389/fmicb.2020.01216
13. Kang HM, Saltveit ME. 2002. Chilling tolerance of maize, cucumber, and rice seedlings leaves and roots are differently affected by salicylic acid. *Physiol. Plant* 115: 571-576.
14. Ramin A. 2006. Effects of salinity and temperature on germination and seedling establishment of sweet basil (*Ocimum basilicum* L.). *Journal of Herbs, Spices and Medicinal Plants* 11(4): 81-90.
15. Keshavarzi MHB. 2012. The effect of different NaCl concentrations on germination and early seedling growth of *Artemisia annua* L. *International Journal of Agriculture: Research and Review* 2(3): 135-140.
16. Sharma S, Puri S, Jamwal A, Bhattacharya S, Dhindsa N, Thakur K. 2014. Effect of salt stress on seedling growth and survival of *Oenothera biennis* L. *International Research Journal of Environment Sciences* 3(9): 70-74.
17. Li W, Zhang H, Zeng Y. 2020. A salt tolerance evaluation method for sunflower (*Helianthus annuus* L.) at the seed germination stage. *Science Reporter* 10: 10626.
18. Jesús C, Fernández-Muñoz R. 1998. Tomato and salinity. *Scientia Horticulturae* 78(1/4): 83-125.
19. Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plant: a review. *Ecotoxicol Environ Safety* 60: 324-349.
20. Singh J, Sastry EVD, Singh V. 2012. Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) during the seed germination stage. *Physiol. Mol. Biol. Plants* 18: 45-50.
21. Vaishnav A, Shukla AK, Sharma A. 2019. Endophytic bacteria in plant salt stress tolerance: Current and future prospects. *Journal of Plant Growth Regulation* 38: 650-668.

22. Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, Berninger T, Naveed M, Sheibani-T R, von Maltzahn G, Sessitsch A. 2017. A New approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology* 8(11): DOI10.3389/fmicb.2017.00011.
23. Kuklinsky-Sobral J, Araújo R Mendes WL, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environ. Microbiology* 6: 1244-1251.
24. Navarro-Torre S, Mateos-Naranjo E, Caviedes MA, Pajuelo E, Rodriguez-Llorente ID. 2016. Isolation of plant-growth promoting and metal resistant cultivable bacteria from *Arthrocnemum macrostachyum* in the Odiel marshes with potential use in phytoremediation. *Marine Pollution Bulletin* 110: 133-142.
25. Redondo-Gomez S, Rubio-Casal AE, Castillo JM, Luque CJ, Alvarez AA, Luque T, Figueroa ME. 2004. Influences of salinity and light on germination of three *Sarcocornia* taxa with contrasted habitats. *Aquatic Botany* 78: 255-264.
26. Shultana R, Kee Zuan AT, Yusop MR, Saud HM. 2020. Characterization of salt-tolerant plant growth-promoting rhizobacteria and the effect on growth and yield of saline-affected rice. *PLoS One* 15(9): e0238537.
27. Dehnavi AR, Zahedi M, Ludwiczak A, Perez SC, Piernik A. 2020. Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy* 10: 859. doi:10.3390/agronomy10060859.
28. Al-Ansari F, Ksiksi T. 2016. A quantitative assessment of germination parameters: The case of *Crotalaria persica* and *Tephrosia apollinea*. *The Open Environmental Research Journal* 14: 13-21.
29. Mylavarapu R, Bergeron J, Wilkinson N. 1993. Soil pH and electrical conductivity: A County Extension Soil Laboratory Manual.
30. Ruzin SE. 1999. *Plant Microtechnique and Microscopy*. New York: Oxford University Press.
31. Frohlich WM. 1984. Freehand sectioning with parafilm. *Stain Technology* 59: 61-62.
32. Eida AA, Alzubaidy HS, de Zélicourt A, Synek L, Alsharif W, Lafi FF, Hirt H, Saad MM. 2019. Phylogenetically diverse endophytic bacteria from desert plants induce transcriptional changes of tissue-specific ion transporters and salinity stress in *Arabidopsis thaliana*. *Plant Science* 280: 228-240.
33. Pal KK, Dey R, Sherathia DN, Devidayal, Mangalassery S, Kumar A, Rupapara RB, Mandaliya M, Rawal P, Bhadania RA, Thomas M, Patel MB, Maida P, Nawade BD, Ahmad S, Dash P, Radhakrishnan T. 2021. Alleviation of salinity stress in peanut by application of endophytic bacteria. *Frontiers in Microbiology* 12: 791. 10.3389/fmicb.2021.650771.
34. Abdalla AMO, Jin-Biao M, Yong-Hong L, Daoyuan Z, Shao H, Shrikant B, Hedlund Brian P, Li Wen-Jun, Li L. 2020. Beneficial endophytic bacterial populations associated with medicinal plant *Thymus vulgaris* alleviate salt stress and confer resistance to *Fusarium oxysporum*. *Frontiers in Plant Science* 11: 47. DOI.10.3389/fpls.2020.00047
35. Xiong YW, Gong Y, Li XW. 2019. Enhancement of growth and salt tolerance of tomato seedlings by a natural halotolerant actinobacterium *Glutamicibacter halophytocola* KLBMP 5180 isolated from a coastal halophyte. *Plant Soil* 445: 307-322.
36. Khan AL, Waqas M, Asaf S, Kamran M, Shahzad R, Bilal S, Khan MA, Sang-Mo Kang, Yoon-Ha Kim, Byung-Wook Yun, Al-Rawahi A, Al-Harrasi A, In-Jung L. 2017. Plant growth-promoting endophyte *Sphingomonas* sp. LK11 alleviates salinity stress in *Solanum pimpinellifolium*. *Environmental and Experimental Botany* 133: 58-69.
37. Noori F, Etesami H, Zarini HN, Khoshkholgh-Sima NA, Salekdeh GH, Alishahi F. 2018. Mining alfalfa (*Medicago sativa* L.) nodules for salinity tolerant non-rhizobial bacteria to improve the growth of alfalfa under salinity stress. *Ecotoxicology and Environmental Safety* 162: 129-138.
38. Jeffrey LS, John WD. 1997. Measurement and use of pH and electrical conductivity for soil quality analysis. *Methods for Assessing Soil Quality* 49: <https://doi.org/10.2136/sssaspecpub49.c10>.
39. Rahman S, Vance GF, Munn LC. 1993. Salinity induced effects on the nutrient status of soil, corn leaves and kernels. *Communications in Soil Science and Plant Analysis* 24: 17-18.
40. Egamberdieva D, Renella G, Wirth S. 2010. Secondary salinity effects on soil microbial biomass. *Biology and Fertility of Soils* 46: 445-449.
41. Kumar K, Amaresan N, Madhuri M. 2017. Alleviation of the adverse effect of salinity stress by inoculation of plant growth-promoting rhizobacteria isolated from a hot humid tropical climate. *Ecological Engineering* 102: 361-366.
42. Amirjani MR. 2011. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. *International Journal of Botany* 7(1): 73-81.
43. Saleem AS, Neumann AM, Muhammad N, Ahmad ZZ, Fulai L. 2015. Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. *Functional Plant Biology* 42: 770-781.
44. Dolatabadian A, Modar R, Sanavy ES, Ghanati F. 2011. Effect of salinity on growth, xylem structure and anatomical characteristics of soybean. *Not. Sci. Biology* 3(1): 41-45.
45. Christensen JH, Bauw G, Welinder KG, Van Montagu M, Boerjan W. 1998. Purification and characterization of peroxidases correlated with lignification in poplar xylem. *Plant Physiology* 118: 125-135.
46. Cachorro P, Ortiz A, Barcelo AR, Cerda A. 1993. Lignin deposition in vascular tissues of *Phaseolus vulgaris* roots in response to salt stress. *Phyton-Ann. Rei. Botany* 33: 33-40.
47. Sturz AV, Christie BR, Nowak J. 2000. Bacterial endophytes: potential role in developing sustainable systems of crop production. *Critical Review of Plant Science* 19: 1-30.