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PSEUDOMONAS CHLORORAPHIS HAST17 BACTERIA OBTAINED FROM *HALOCNEMUM STROBILACEUM'S* ROLE AS GROWTH STIMULANT IN COTTON UNDER SALINITY STRESS CONDITIONS

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SUMMARY

Plants use several biological defense mechanisms associated with microorganisms in their microbiota to mitigate the effects of abiotic stress factors such as salinity and drought. This situation enhanced this research interest aimed at isolating beneficial bacteria from plant microbiota and using them to combat salinity stress conditions. This study evaluated the effect of five bacterial strains, viz., *Priestia megaterium* HAST2, *Priestia aryabhattai* HAST7, *Pseudomonas plecoglossicida* HAST9, *Pseudomonas putida* HAST10, and *Pseudomonas chlororaphis* HAST17, on the growth and development parameters of cotton under a saline environment. The strains came from the endomicrobiota of the vegetative organs of glasswort (*Halocnemum strobilaceum*). The results showed the bacterial strain *P. chlororaphis* HAST17 considerably stimulates cotton germination, shoot and root growth and development, and seed cotton yield traits under salinity conditions. The findings revealed such types of bacterial strains can increase the diversity of eco-products that stimulate crop growth and production under salinity conditions.

Keywords: Bacterial strains, cotton, endophyte, glasswort (*H. strobilaceum*), salinity stress, seed germination, seed treatment, yield

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Key findings: The study evaluated the potential of endophytic bacteria isolated from glasswort (*H. strobilaceum*) as a promising stimulator for sustainable cotton cultivation under salinity conditions, selecting the bacterial strain *P. chlororaphis* HAST17 from among them.

INTRODUCTION

Food security is a fundamental need of any society all over the world. Experts predicted the world population will reach approximately 10 billion people in the next 50 years. Therefore, to meet the additional food demand of humanity, it will be necessary to vertically enhance the productivity of staple food crops by at least 50 percent (Godfray *et al*., 2010). Shrivastava and Kumar (2015) reported that in today's rapidly growing world population, the arable lands in arid and semi-arid regions worldwide are also shrinking by 1%–2% annually due to soil salinization. In these regions, low rainfall and high temperatures hasten salinization.

Soil salinization is becoming a crucial limiting factor for the growth and development and production of intolerant crop plants and even some halophytes (Abduganiyeva *et al*., 2025). The ability to adapt to sudden changes in stressful environmental factors is present up to some extent in the living organisms such as fungi, bacteria, and archaea. Among all those microorganisms with these features, halotolerant bacteria proved of particular importance, as they can grow under non-saline conditions and in environments with maximum levels of salinity (Khan *et al*., 2016; Alikulov *et al*., 2022).

With these features, halotolerant bacteria emerged as suitable for inoculation into crops grown in areas affected by salinity stress conditions. The biological properties of bacteria isolated from plant microbiota grown under stressful environments gained preservation in other crops under stressful conditions (Akramov *et al*., 2023). Reports of similar findings have also come from Zhu *et al*. (2011) in experiments with the halophilic (salt-loving) bacteria strain *Kushneria* sp. YCWA18, isolated from saline soil and plants grown in China. The bacterial strain *Kushneria* sp. YCWA18 appeared to have the potential to retain the ability to degrade insoluble phosphate even under the higher salinity levels.

Studies of several salt-loving plant species have been progressing, such as the species *Sesuvium portulacastrum* (Anburaj *et al*., 2012); *Salicornia bigelovii* (Rueda-Puente *et al*., 2010); glasswort or saltwort (*Halocnemum strobilaceum*) (Al-Mailem *et al*., 2010); *Salicornia brachiata* (Jha *et al*., 2012); and *Acacia* spp.—widely distributed in saline regions (Boukhatem *et al*., 2012). Their selection for cultivation was due to bacterial diversity in the microbiota of vegetative organs of these plants and the optimal conditions for them to flourish. Specific recommendations came about for the practical use of the isolated bacteria. Shi *et al*. (2012) emphasized in their research that microorganisms from the plant endomicrobiota, when inoculated into another plant, have almost no difficulty in adapting to the new host organism.

One of the effective ways to boost crop yield by improving plant tolerance to salinity is to use salinity-tolerant microorganisms that accelerate crop growth and development (Jurakulov *et al*., 2023; Ergashev *et al*., 2025). The promising study aimed to select a bacterial strain that considerably stimulates the growth and development and enhances productivity of cotton crops under salinity conditions among endophytic bacterial strains isolated from *H. strobilaceum*.

MATERIALS AND METHODS

Research objects

The following five bacterial strains, obtained from the endomicrobiota of the glasswort (*H. strobilaceum*), were *Priestia megaterium* HAST2 (GenBank account number: OK594050), *Priestia aryabhattai* HAST7 (OK594051), *Pseudomonas plecoglossicida* HAST9 (OK594052), *Pseudomonas putida* HAST10 (OK594053), and *Pseudomonas chlororaphis* HAST17 (OK594054), selected as objects of the presented research (Alikulov *et al*., 2023). They are widespread in saline areas of deserts of Uzbekistan's southwestern regions (39°-31′45.5″ N, 65°-16′41.6″ E; 41°-04′14.4″ N, 64°-32′29.0″ E; 41°-04′14.4″ N, 64°-32′29.0″ E).

Bacterial cultivation

Bacterial strains underwent plating on solid LB (Luria Bertani: tryptone-10, yeast extract-5, NaCl-10, agar-20) medium and incubation at 30 °C for 72 h. Using the five grown colonies in studies served as bacterial strains (Mukhtorova *et al*., 2024).

Determining seed germination indicators

The cultured bacterial strains received inoculation into liquid LB medium and incubation at 30 °C for four days. The number of cells per ml of bacterial culture sustained an increase to 10^8 by serial dilution. Cotton seeds attained surface sterilization by soaking in 2.5% sodium hypochlorite for three minutes. The washing of cotton seeds used clean water, with sterile seeds treated by keeping them in a 20% suspension of bacterial cultures for six hours. Filter papers entailed soaking in 0.1%, 0.25%, 0.5%, 1.0%, 1.5%, and 3.0% NaCl solutions before placing them in Petri dishes. Ten cotton seeds preceded their transfer to sterile Petri dishes with wet filter paper. Cotton seeds treated with water served as the control. The conducted experiments were in triplicate (n=3) for each variant. Petri dishes remained in a thermostat at a temperature of 25 °C. Calculating the seed germination rate used the following formula (Khojakulov *et al*., 2024; Sultonova *et al*., 2025).

$$\textbf{\textit{Germination rate}}\ (\%) = \frac{\textit{Number of seeds germinated on day 7}}{\textit{Total number of seeds}} * 100$$

Vegetative experiments

The cultivated bacterial strains underwent inoculation into a liquid LB medium before incubation at 30 °C for four days. Increasing to 10^8 the number of cells per ml of bacterial culture continued by serial dilution. Cotton seeds' surface sterilization by soaking in 2.5% sodium hypochlorite took three minutes, with the seeds washed in clean water thereafter (Ergasheva *et al*., 2024). The cotton seeds underwent drying before leaving in a 20% working suspension prepared by mixing bacterial strain cultures with water for six hours. Then removing the seeds from the mixture, drying them followed at room temperature. The 0.5 L containers filled with 400 g of sandy soil underwent sowing with three cotton seeds in each container. The values obtained in experiments with water-treated seeds served as a control. Vegetative experiments transpired in the greenhouse of the Institute of Biochemistry of Sharof Rashidov Samarkand State University.

On the 14th day of the experiment, measuring the length of stems and roots of the seedlings grown in pots ensued (Norboyev *et al*., 2024). The prepared special saline soil medium with 0%, 0.45%, 0.9%, and 1.8% chloride salinity irrigated the sandy soil with solutions of different concentrations of NaCl in a ratio of 3:10. The remaining experiments continued in the same order as described above. Water-soaked dry seeds served as the control. The experiments commenced at room temperature, with plants irrigated with solutions of 0%, 0.45%, 0.9%, and 1.8% chloride salinity.

Field experiments

The study left cotton seeds in a 20% working suspension of bacterial cultures for six hours. Then, removing the seeds from the mixture proceeded to being dried at room temperature. The seeds entailed sowing and caring according to generally accepted agrotechnology processes. Small-scale experiments proceeded on plots of 140 m^2 each, based on recommended methods. In small-scale experiments, researchers determined the number of sprouted plants (before germination), stem length, root mass in the 0–60 cm soil layer, 1000-seed weight, and seed cotton yield using typically accepted methods (Eschenko *et al*., 2009). Using dry and water-treated seeds under farm conditions served as the control.

Soil composition

In field experiments, the determination of the soil's chemical composition and nutrient content used modern methods (Bazarov *et al*., 2024). By calculating the pH value of the soil, the H^+ or OH^- ions were also evident. The electrical conductivity of the soil, as detected, employed the conductometric method. In this case, comparing the amount of dissolved salts in the aqueous extract of the soil succeeded. In the soil, the total alkalinity estimation depended on the amount of simple carbonates and bicarbonates. Several indicators, including dry matter, the proportion of soluble salts in the soil (%), the chlorine ions, and the humus content, succeeded in their detection by the titrimetric method. The estimation of the amount of Na_2O and K_2O in the soil (%) engaged the photometric method. The amount of nutrients in the soil entailed calculation spectrophotometrically according to the ions method corresponding to mg/kg.

Data analysis

All the obtained data based on various traits in different experiments reached analysis according to the methodology of Lakin (1990). The processed values entailed graphic representation using the OriginPro (2024) program. The arithmetic means of the data obtained in 3–10 repeated experiments succeeded in their evaluation at the significance level of $p \leq 0.05$ (Rayimova *et al*., 2024; Ulugbek *et al*., 2025).

RESULTS

Bacterial strains' effect on seed germination

The stimulation of plant growth had cotton seeds treated with suspensions of various bacterial strains before sowing. These bacterial strains have biostimulatory effects, such as early establishment of soil-plant relationships, enhanced nutrient uptake, and increased resistance to abiotic stress conditions. The ability of endophytic microorganisms, including bacteria, to live in host plant tissues without adversely affecting them and have a certain positive effect on plant growth and development suggests these bacteria are potentially useful in different crop plants. A lot of evidence shows plant survival in arid and saline ecosystems depends on specific bacterial communities that facilitate plant adaptation, improve plant functions, and protect plants from abiotic environmental stress conditions. Therefore, the effect of inoculation with a suspension of different bacterial strains on the cotton seed germination under saline environments underwent initial determination in laboratory conditions (Figure 1).

The results revealed that seed treatments with different endophytic bacterial suspensions improve the germination of cotton seeds under salinity conditions (Figure 1). Although the seed germination rate decreased with increasing salinity in the environment, the germination values in all variants were more positive than those in the control variant. The seed germination rates' comparison at different levels of salinity among bacterial strains indicated that the variant treated with the bacterial suspension of the *P. chlororaphis* HAST17 strain had the highest seed germination rate among the studied bacterial strains. Under conditions of 3% salinity, where the stress factor was the maximum, the germination rate of cotton seeds treated with this strain was 60.1% ± 2.0%. This indicator appeared to be 24% higher than the values in the control variant and 3.8%–13.2% higher than the values in variants treated with other bacterial strains. These results support the idea that treatment with a suspension of endophytic bacteria isolated from *H. strobilaceum* stimulates seed germination in cotton crops under a saline environment.

Bacterial suspension effect on plant growth

The conducted vegetative experiments evaluated the effect of bacterial strains on cotton growth and development. The results revealed that two controlled variants (control 1: farm conditions, i.e., untreated; control 2: seeds treated with water for six hours) showed

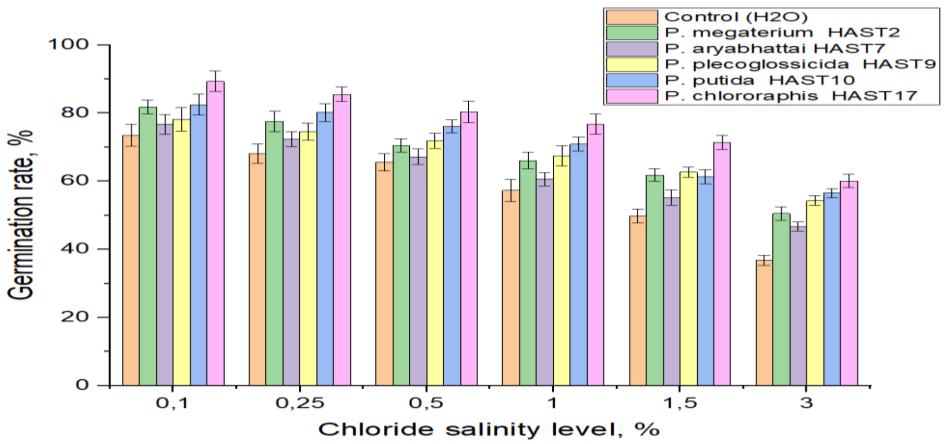

Figure 1. Effect of treatment with *H. strobilaceum* endophytic bacterial strains on cotton seed germination under salinity conditions (n=3, treatment time: 6 h, 24 °C).

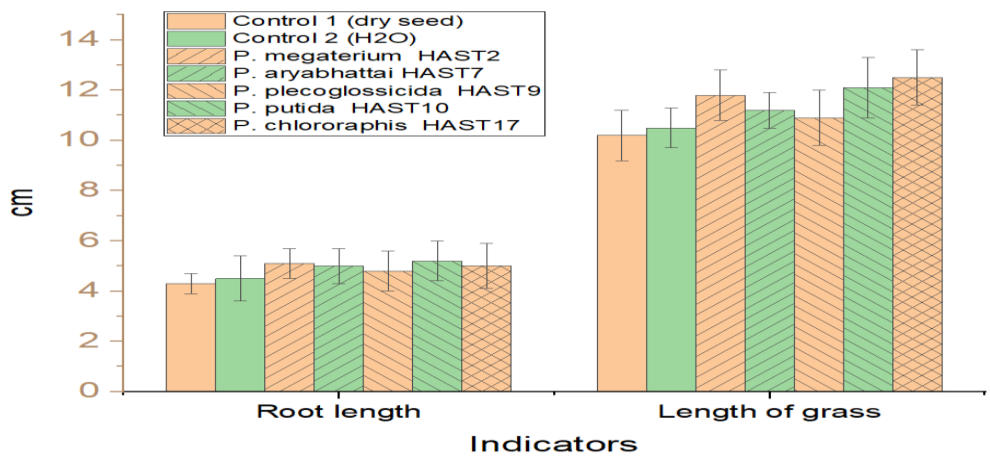

Figure 2. Effect of treatment of cotton seeds with *H. strobilaceum* endophytic bacterial strain on plant growth and development under normal soil environment (vegetative experiments), n=3.

seed treatment with bacterial suspensions of endophytic bacterial strains affected the growth and development of cotton shoots and roots (Figure 2).

According to results about the effect of endophytic bacteria *H. strobilaceum* treatment on cotton plants, treating with the *P. megaterium* HAST2 strain gave a root length of 5.1 ± 0.6 cm, and the blade length was 11.8 ± 1.0 cm. By seed treatment with bacterial strain *P. aryabhattai* HAST7, these indicators were 5.0 ± 0.7 cm and 11.2 ± 0.7 cm, respectively. In cotton seeds treated with other bacterial strains, *P. plecoglossicida* HAST9, *P. putida* HAST10, and *P. chlororaphis* HAST-17, the root length had ranges from 4.8 ± 0.8 to 5.2 ± 0.8 cm, and the blade length range was 10.9 ± 1.1–12.1 ± 0.9 cm. A comparative analysis of the length of roots (4.3 ± 0.4 cm, 4.5 ± 0.9 cm) and shoots (10.2 ± 1.0 cm, 10.5 ± 0.8 cm) in the untreated and water-treated control variants succeeded. Comparing variants treated with endophytic bacterial strains showed plants treated with bacterial strains *P. putida* HAST10 and *P. chlororaphis* HAST-17 had the best root and shoot development versus the control variants. In these variants, the cotton root length was 0.9 cm longer than in the control variant 1; it was 0.7 cm longer than in the control variant 2, and the length of the shoots was 1.9 cm and 1.6 cm longer than both controls, respectively.

Crop growth promoters should retain their plant growth-stimulating properties and potential to some extent when used under normal conditions and in areas exposed to stress factors that negatively affect crop growth and development. Such types of studies require testing of promising microorganisms for their beneficial properties in stress conditions. Therefore, in the course of this research, we conducted experiments to determine the effect of seed treatment with bacterial suspensions of promising endophytic bacteria strains on the development of shoots and roots in cotton plants under saline conditions (Table 1).

Determining effects of endophytic bacteria isolated from *H. strobilaceum* on cotton development, the variant treated with the strain *P. megaterium* HAST2 grown under 0.45%, 0.9%, and 1.8% chloride conditions gave longer roots (0.6, 0.4, and 0.4 cm, respectively) than the water-treated control variant. The shoots were 1.5, 0.3, and 0.7 cm longer, respectively. In the cottonseed treated with the strain *P. aryabhattai* HAST7, the root length was 4.7 ± 0.5 cm under 0.45% chloride salinity conditions and 3.9 ± 0.6 and 3.4 ± 0.6 cm under 0.9% and 1.8% chloride salinity conditions, respectively. In cottonseed treated with this bacterial strain and grown under 0.45%, 0.9%, and 1.8% chloride conditions, the leaf blade length decreased in the sequence of 10.0 ± 0.8, 8.2 ± 0.6, and 6.6 ± 0.6 cm as salinity increased.

In variants treated with the bacterial suspension of the *P. plecoglossicida* HAST9 strain, the root length reached 3.4 ± 0.7 cm, and the leaf blade length reached 6.7 ± 0.9 cm under the highest saline environment (1.8% chloride). Meanwhile, the root length improved by 21.4% and the leaf blade length by 8.0% compared with the control. In cotton treated with the bacterial strain *P. putida* HAST10, the root length decreased from 5.2 ± 0.6 to 3.4 ± 0.5 cm, and shoot length decreased from 12.1 ± 0.6 to 7.0 ± 0.5 cm at different salinity levels. By treating cotton seeds with *P. chlororaphis* HAST17, the root and shoot lengths were 4.6 ± 0.7 and 10.7 ± 0.9 cm at 0.45% chloride, while these values were 4.0 ± 0.8 and 9.2 ± 1.1 cm in 0.9% chloride solution, respectively. The results further enunciated that cotton seeds treated with the bacterial strain *P. chlororaphis* HAST17 at 1.8% chloride, the root and shoot lengths were 0.8 and 1.2 cm longer than the control, respectively. The results disclosed that bacterial strain *P. chlororaphis* HAST17 has the potential to stimulate cotton growth and development to a greater extent than other bacterial strains. Therefore, further studies progressed with this strain.

Pseudomonas chlororaphis as a growth stimulant

It is important to study the possibility of promising bacterial strains to maintain their positive effects on crop growth, development, and yield under field conditions. In this research, the conducted experiments sought to determine the effect of the bacterial suspension of the *P. chlororaphis* HAST17 strain culture on some growth and yield-related traits of cotton under field conditions. In experiments, the chemical composition of the soil and the amount of nutrients also incurred detection in the fields belonging to the "Rakhimjon Bekmurov" farm in the District of Narpay, Samarkand Region, Uzbekistan (39°58′34.2″N, 66°02′21.1″E), where field experiments transpired (Table 2).

As seen from the data in Table 2, the chlorine ions in the soil in the sampled fields exceeded the normative values by up to two times. Comparative analysis of chlorine ions in the cross-section of the layers showed the soil sustained secondary salinization. One can also note that the amount of nutrients in the soil of these fields was slightly lower than the required normative values. In the experiments, it was noticeable that treatment with a bacterial suspension of the *P. chlororaphis* HAST17 strain had a positive effect on the growth, development, and seed cotton yield of cotton plants (Table 3).

In field experiments, the cotton seeds' treatment with suspension of the bacterial strain *P. chlororaphis* HAST17 increased the stem length by 11.6%, stem diameter by

Table 1. Effect of cotton seed treatment with *H. strobilaceum* endophyte bacterial strain on plant growth and development under saline soil environments (vegetative experiments), n=3.

Type of seed treatment	Salinity level (%)	Root length (cm)		Grass length (cm)	
		Experience Estimates	Difference (C)	Estimates	Difference (C)
H_2O (Control)	0	4.5±0.5	0	10.5±1.0	0
	0.45	4.1±0.8	0	9.0±0.9	0
	0.9	3.5±0.5	0	7.9±0.8	0
	1.8	2.8±0.3	0	6.2±0.9	0
P. megaterium HAST2	0	5.1±0.6	+0.6	11.8±1.0	+1.3
	0.45	4.7±0.8*	+0.6	10.5±0.8	+1.5
	0.9	3.9±0.5	+0.4	8.2±0.8*	+0.3
	1.8	3.2±0.5	+0.4	6.9±1.1	+0.7
P. aryabhattai HAST7	0	5.0±0.9	+0.5	11.3±1.2	+0.7
	0.45	4.7±0.5	+0.6	10.0±0.8	+1.0
	0.9	3.9±0.6	+0.4	8.2±0.6	+0.3
	1.8	3.4±0.6	+0.6	6.6±0.6**	+0.4
P. plecoglossicida HAST9	0	4.8±0.4	+0.3	10.9±1.0	+0.4
	0.45	4.5±0.8	+0.4	10.1±1.0	+1.1
	0.9	4.0±0.5**	+0.5	8.5±0.8	+0.6
	1.8	3.4±0.7	+0.6	6.7±0.9	+0.5
P. putida HAST10	0	5.2±0.6	+0.7	12.1±0.6	+1.6
	0.45	4.5±0,6	+0.4	10.8±0.9	+1.8
	0.9	3.9±0.7	+0.4	9.1±0.9	+1.2
	1.8	3.4±0.5	+0.6	7.0±0.5**	+0.8
P. chlororaphis HAST17	0	5.2±0.8*	+0.7	12.0±0.9	+1.5
	0.45	4.6±0.7	+0.5	10.7±0.9	+1.7
	0.9	4.0±0.8	+0.5	9.2±1.1*	+1.3
	1.8	3.6±0.5	+0.8	7.4±0.9	+1.2

Note: *Statistically significant at $p < 0.05$, ** at $p < 0.01$.

Table 2. Soil composition of the experimental field.

Indicators	Standard (control)	Layers (cm)		
		0-30	30-70	70-100
Chemical composition of soil				
pH	6.5-7.5	6.94	7.27	7.15
Conductometer (μS/cm)	<500	1080	1110	1120
Dry residue (%)	< 0.3	1.21	1.34	1.27
Total alkalinity (%)	< 0.061	0.039	0.044	0.040
Chlorine ion (%)	< 0,01	0.021	0.018	0.016
Na_2O (%)	0.023	0.010	0.009	0.009
K_2O (%)	0.01	0.0054	0.0051	0.0051
$CaCO_3$ (%)	5-15	5.2	4.7	4.6
The amount of nutrients in the soil				
$N\text{-}NH_4$ (mg/kg)	31-45	24.2	26.1	25.0
P_2O_5 (mg/kg)	31-45	10.8	11.4	10.1
K_2O (mg/kg)	201-300	192	187	190
Hymus (%)	0.8-1.2	0.84	0.69	0.51

Table 3. Effect of seed treatment with *P. chlororaphis* bacterial strain on cotton performance and seed cotton yield.

Indicators	Control 1: Farm conditions (dry)	Control 2: Treated with H_2O	*P. chlororaphis* HAST17
Number of sprouted plants (plants/m^2) (n=5)	105.4±2.9	109.6±3.3	112.9±1.6*
Stem length (cm) (n=10)	74.7±2.8	78.6±4.0	83.4±2.9
Stem diameter (cm)	1.6±0.2	1.7±0.1	2.1±0.1**
Root mass in the 0-60 cm soil layer (g/plant) (n=3)	10.2±0.1	10.4±0.3	11.0±0.1**
1000-seed mass (g) (n=5)	102.6±3.9	106.8±3.2	112.4±4.1*
Yield (kg/m^2) (n=3)	0.31±0.01	0.32±0.01	0.37±0.01*
Additional yield compared to the control (kg/m^2)	-	C1-+0.01	C1- +0.06; C2- +0.05

Note: *Statistically significant at $p < 0.05$, ** at $p < 0.01$.

31.6%, dry root weight by 7.8%, and 1000-seed weight by 9.5% in cotton under saline conditions. This resulted in an additional yield of 500 kg per hectare. The results indicated the need to develop a technology for producing crop growth promoters based on the bacterial strain *P. chlororaphis* HAST17.

DISCUSSION

The majority of plants grown in stress conditions develop various morphological, physiological, and biochemical adaptations to adverse environmental factors. Among these, some adaptations were dependent on the mechanisms of interaction between microorganisms found in the endomicrobiota of vegetative organs of plants and the host plant organism. It was of greater importance for plant nutrition and protection from adverse factors (Cardarelli *et al*., 2012). The effectiveness of plant-bacterial relationships directly depends on how early and late the bacteria appear in the plant organism (Akramov *et al*., 2025).

Therefore, in most studies, the ability of bacteria to stimulate crop growth and development receives assessment by inoculating bacterial strains into seeds. This is typical due to the early entry of bacteria into the plant organism through seeds, which develops conditions for better manifestation of its biological properties (Cardarelli *et al*., 2012). In previous years, extensive research has been progressive on the application of endophytic bacteria to improve seed germination. Similar experiments from Rudolph *et al*. (2015) demonstrated the use of bacterial strains *P. fluorescens and P. putida* considerably improved wheat seed germination (Rudolph *et al*., 2015).

Bacteria in the plant endomicrobiota may have beneficial effects on crop production by increasing stress tolerance. Bacteria may also improve crop growth and yield while reducing biohazards associated with mineral fertilizers through their ecological purity (Watts *et al*., 2023). The effects of bacteria *Pseudomonas* sp. selected as promising strains in the presented study on the growth and development of crops have reached in-depth scrutiny in several past studies. In previous studies, they found the bacterial strain *P. simiae* WCS417 enhanced the growth of *Arabidopsis thaliana* and improved lateral root formation (Pieterse *et al*., 2021), and the *P. fluorescens* SS101 strain could increase the biomass of *Nicotiana tabacum* (Park *et al*., 2015). Moreover, *P. chlororaphis* MA 432 increased the yield of cereal plants, including barley, oats, wheat, and rye, by protecting them from various plant pathogenic fungi (Johnsson *et al*., 1998). Finally, *P. fluorescens* inhibited the growth of the pathogenic fungus *Ralstonia solanacearum* in tomatoes, ensuring stable plant growth and development (Raza *et al*., 2016).

The bacterium *P. chlororaphis* is applicable in the cultivation of major crops and also used as soil and seed treatment agents in horticultural crops. These bacterial strains are also beneficial as biocontrol agents against some fungal pathogens by producing phenazine-type antibiotics (Thomas *et al*., 2000).

CONCLUSIONS

According to the results, endophytic bacteria isolated from *Halocnemum strobilaceum* stimulate the growth and development of cotton under saline conditions. The bacterial strain *Pseudomonas chlororaphis* HAST17 revealed the optimum potential to stimulate crop growth and development under salinity conditions compared with other strains. The study recommends that the bacterial strain *P. chlororaphis* HAST17 can be effective as a seed treatment stimulant for growing cotton under salt-stress conditions.

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