








## Application of endophytic bacteria synthesizing indolyl acetic acid isolated from *Krascheninnikovia ceratoides* (L.) Gueldenst in wheat cultivation under saline conditions

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### ABSTRACT

Indolyl acetic acid (IAA) is a biologically active substance that mitigates the effects of stress factors such as drought and salinity in plants. It is known that microorganisms in the microbiome of halophytic plants synthesize biologically active substances, including IAA, to ensure the development of the host plant under conditions of stress factors. *K. ceratoides* is a haloxerophytic plant that grows in saline and arid conditions. Analysis of scientific sources showed that the microorganisms in the endomicrobiome of this plant, their characteristics and potential applications in practice have not been studied. This article presents the results of experiments aimed at determining the effects of temperature, salinity and pH on IAA synthesis in promising strains of endophytic bacteria isolated from the plant *K. ceratoides*. Analysis of the results showed that temperature, salinity and pH affected IAA synthesis in the studied strains. Among the strains, the presence of strains that retain the ability to synthesize ISK at a high level even under the influence of stress factors, the fact that they have a positive effect on the level of germination and some biometric parameters of wheat seeds under salinity conditions, substantiates the possibility of using these strains in the development of promising agrotechnologies.

**Keywords:** indolyl acetic acid, *Krascheninnikovia ceratoides*, endophyte, bacteria, temperature, salinity, wheat.

### INTRODUCTION

Drought stress is one of the agricultural problems that limits plant productivity (Alikulov et al., 2022). In recent years, high temperatures, insufficient precipitation, and nutrient deficiencies have affected the water potential, plant morphology, and physiology of plants growing in arid lands (Muminov et al., 2025). It also reduces the distribution of nutrients and the supply of water-soluble nutrients such as nitrates, sulfates, Ca, Mg, and Si to plants. This is a major obstacle to

increasing agricultural productivity (Halo et al., 2020; Li et al., 2020).

In recent years, there has been increasing interest in isolating endophytic microorganisms from various plants living in arid regions and studying their various plant-stimulating properties (Kondrasheva et al., 2022). For example, research on isolating endophytes from various xerophytic plants is increasing. Priya et al. (2021) conducted research on isolating endophytic microorganisms from xerophytic plants growing in arid regions of India, such as *Helictotrichon schmidii*, *Aristida setaceae*,

*Brachiaria muna*, *Anthoxanthum odoratum*, *Eragrostis atrovirens*, *Agrostis peninsularis*, *Cenchrus setaceus*, *Cenchrus ciliaris*, and *Bothriochloa per-tusa*. Six different endophytic bacteria were isolated from these plants and their plant-stimulating properties were studied (Priya et al., 2021).

Endophytic bacteria *Paenibacillus barengoltzii*, *Bacillus amyloliquefaciens*, *Bacillus thuringiensis*, and *Bacillus cereus* have been isolated from the xerophytic plant *Fagonia mollis*, which is adapted to grow in arid environments. Similarly, endophytic bacteria *Paenibacillus barengoltzii* and *Brevibacillus agri* have been isolated from the xerophytic plant *Achillea fragrantissima* (Forssk) Sch. Bip. and have shown plant tolerance and growth-promoting properties in various stressful environments (Al Kahtani et al., 2020). Phytohormones are signaling molecules that coordinate plant cell activity and control plant growth and development (Singh et al., 2017). One of the most popular phytohormones produced by endophytic microorganisms is indole-3-acetic acid (IAA), which has been shown to increase root and shoot biomass in sugarcane by producing high amounts of IAA via the IPA pathway by *Acetobacter diazotrophicus* (Patil et al., 2011; Yu et al., 2016). In addition, Maheswari et al. (2013) have identified ICA production by members of the genera *Micrococcus*, *Flavobacterium*, and *Serratia*, which are distributed in various tropical cereal legumes.

The plant phytohormone IAA, a derivative of auxin, is found in plants found in all parts of the world. This phytohormone is synthesized via the IPA pathway. Another way to produce indolyl acetic acid is via tryptophan, where in the first step, tryptophan is converted to indole-3-acetamide by tryptophan-2-monooxygenase, and then indole-3-acetamide phohydrolase produces indolyl-3-acetic acid (Mamarasulov et al., 2022). This method is carried out directly by endophytic bacteria found in the internal tissues of plants. These phytohormones are very important for plant growth and development (Jurakulov et al.,

2023). They are especially important for the formation and growth of the root system of the plant.

The aim of this study was to evaluate the effects of temperature, salinity, and pH on the production of indolyl acetic acid by promising endophytic bacterial strains isolated from *K. ceratoides*, and to determine the potential application of bacterial culture suspension treatments to wheat growth under saline conditions.

## MATERIALS AND METHODS

### Research objects

In this study, endophytic bacterial strains stored in the collection of the Laboratory of Molecular Biotechnology of Samarkand State University were used. These strains were isolated by the authors from *Krascheninnikovia ceratoides* (L.) Gueldenst by the corresponding researchers (Table 1).

### IAA synthesis screening

IAA synthesis of endophytic bacteria was determined using the method proposed by Gordon and Weber (1953) (Gordon and Weber, 1951). Endophytic bacteria were grown in King B medium (composition (g/l): peptone-20 g, glycerol-10 g,  $K_2HPO_4$  -1.5 g,  $MgCl_2$  -1.5 g, pH 7.4) containing 5 mg/l L-tryptophan. The cultures were grown for 48 hours at 28 °C in a shaker incubator rotating at 120 rpm. After the incubation period, the cultures were centrifuged at 3000 rpm for 30 minutes at 4 °C. After centrifugation of the cultures, 1 ml of the supernatant was taken into a small test tube and 2 ml of Salkovsky reagent (35 ml of 35 %  $HClO_4$  and 1 ml of 0.5 M  $FeCl_3 \cdot 6H_2O$ ) was added and kept in the dark for 30 minutes. The development of cherry red color in the test tubes indicates the synthesis of indole 3-acetic acid (IAA) by endophytic bacteria. A sterile nutrient medium (King B) without bacterial isolates served as a control (Khramtsova et al., 2006). The amount of IAA was determined by examining it at 530

**Table 1.** Description of the endophytic bacterial collection strains used in the study

Strain	Gene bank account number	References
<i>Priestia megaterium</i> CrEw1004	ON567363	Alikulov et al., 2022; Akramov et al., 2023
<i>Pseudomonas putida</i> CrEw1015	ON567362	
<i>Bacillus subtilis</i> CrEw1018	ON567361	
<i>Brevibacillus parabrevis</i> CrEw1021	ON567360	

nm with a spectrophotometer (EMC-30PC-UV Spectrophotometer, Germany). Solutions of IAA with a concentration of 0–400 µg/ml were used as standards (Mukhtorova et al., 2024).

The effect of temperature on IAA synthesis was evaluated by comparing the optical density of the culture fluid obtained by growing the bacterial strains at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C and 45 °C for 3 days.

The effect of salinity on IAA synthesis was evaluated by comparing the optical density of the culture fluid obtained by growing the bacterial strains in liquid medium containing 1%, 2.5%, 5%, 7.5%, 10% and 12.5% NaCl for 3 days.

The effect of pH on IAA synthesis was evaluated by comparing the optical density of the culture fluid obtained by growing the bacterial strains in liquid medium with a pH value of 4–9 for 3 days.

### **Selection of the norms of bacterial strain suspensions that positively affect seed germination**

Initially, all the most active promising strains were grown in liquid LB medium on a rotating shaker at 120 rpm for 3 days at 28 °C. After the cell culture had grown, it was centrifuged at 3000 rpm and 1 ml of 0.85% NaCl solution was added to the cells and incubated for 4 hours at 28 °C. The resulting cultures were serially diluted to a cell titer of 10<sup>6</sup>, 10<sup>7</sup>, 10<sup>8</sup>, 10<sup>9</sup> cells/ml. A 20% working solution was prepared based on each of the resulting cell titers in distilled water. In this case, 20 wheat seeds were treated separately for 3, 6, 9, 12 hours according to each cell titer. 20 were placed in Petri dishes and left in a thermostat for 7 days at 25 °C. On the 7th day, the germinated seeds were counted and the germination rate (%) of the seeds was determined based on proportioning (Alikulov et al., 2023).

### **Determination of the effect of endophytic bacterial strains on seed germination under salinity conditions**

Initially, cultures of all the most active promising strains were grown according to the above procedure. The resulting cultures were serially diluted to a cell titer of 10<sup>8</sup> cells/ml. Seeds were treated with a 20% solution of the prepared suspension for 6 hours and placed in Petri dishes with medium containing NaCl of different

concentrations (2.5%, 5%, 7.5%, 10%, 12.5% and 15%) in 20-piece containers and left in a thermostat at 25 °C for 7 days. On the 7th day, the germinated seeds were counted and the germination rate (%) of the seeds was determined based on the proportion (Alikulov et al., 2022).

### **Determination of the effect of endophytic bacterial strains on wheat development under salinity conditions**

Seeds were treated with a 20% suspension of bacterial cultures (experiment) and water (control) for 6 hours and sown in 500 ml plastic containers filled with 400 g of sandy soil. Dry seeds were also used as an additional control in the experiment. In the experiment, separate suspensions containing the strains selected as promising strains were used for inoculation. All the plates were prepared in five replicates for each bacterial strain. Three seeds were sown in each plate (total  $n = 15$ ). The experiments were carried out at room temperature and the plants were watered with tap water. The length of the stems and roots was measured after 14 days [Alikulov et al., 2022].

### **Statistical analysis**

Statistical processing and drawing of the results were performed using Microsoft Excel 2013 (USA) computer program. The results of the experiment were statistically summarized by evaluating the arithmetic averages of 5 repeated experiments at the level of statistical reliability of  $p \leq 0.05$ . In the mathematical-statistical analysis, the mean values and deviations of the indicators, as well as the calculation of the probability, were carried out according to the method of (Lakin 1990).

## **RESULTS**

### **Characterization of IAA synthesis properties of endophytic bacteria isolated from *K. ceratoides* and environmental stress factors affecting the process**

The phytohormone production property was high in the endophytic bacterium *P. megaterium* CrEw1004. The formation of a dark pink color in the qualitative reaction indicates that this bacterium produced a large amount of indolyl-3-acetic acid. The endophytic bacterium *B. parabrevis* CrEw1021 produced moderate levels of indolyl acetic acid.

The result is explained by the formation of a light pink color in the Eppendorf flask (Figure 1).

It was also found that the production of indolyl acetic acid by endophytic bacteria *P. putida* CrEw1015 and *B. subtilis* CrEw1018 was lower than the above 2 strains. The ability of these promising endophytic bacterial strains to synthesize IAA in different temperature environments was also evaluated (Table 2).

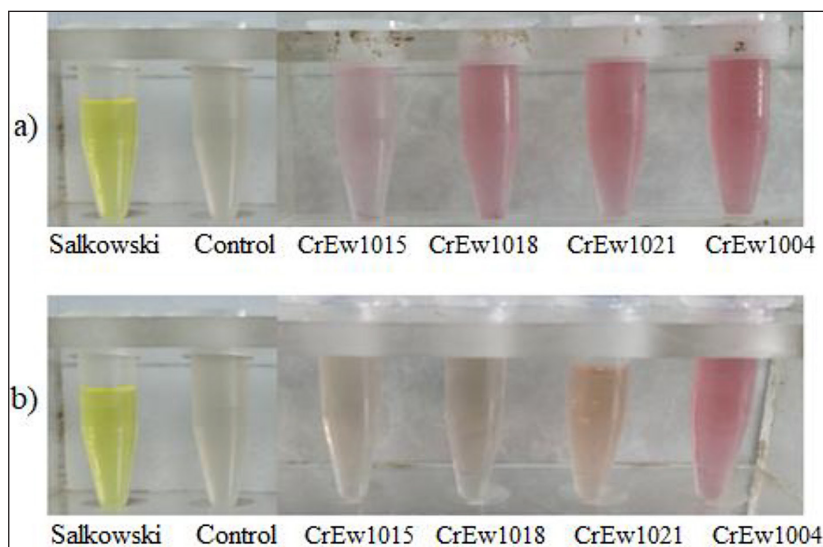
As can be seen from the data presented in this table, it was found that all our strains synthesized indolyl acetic acid to different degrees at different temperatures. If we look at the cross-section of strains, the *P. megaterium* CrEw1004 bacterial strain showed a higher indicator than other bacterial strains at the optimal temperature, i.e. 25 °C and 30 °C. That is, it produced  $131.2 \pm 1 \mu\text{g}$  of IAA at 25 °C and  $139.5 \pm 2 \mu\text{g}$  at 30 °C. This indicator is the highest among other strains. However, at the same time, at 20 °C it synthesized  $72.3 \pm 3 \mu\text{g}$  of IAA, but the highest indicator at this temperature was shown by the *B. parabrevi*s CrEw1021 bacterial strain. This bacterium was found to produce  $79.4 \pm 4 \mu\text{g}$  of IAA. Also, when comparing the amount of IAA produced by bacteria at high temperatures of 35 °C, it was found that the *B. parabrevi*s CrEw1021 bacterial strain produced more IAA than the other strains. When comparing the IAA production of endophytic bacterial strains at the highest temperature in the experiment, it was found that the endophytic bacterial strain *B. subtilis* CrEw1018 synthesized more IAA than the other strains.

When the effect of temperature on the production of IAA by endophytic bacteria was studied, it

was found that at the optimal temperatures for the growth of endophytic bacteria, i.e. 25 °C and 30 °C, the bacterial strain *P. megaterium* CrEw1004 produced high amounts of IAA. Increased temperature stress, i.e., decreasing and increasing temperatures, caused a decrease in the amount of IAA. At the same temperatures, the amount of IAA production in other strains was higher than in the bacterial strain *P. megaterium* CrEw1004.

Salt stress is considered a negative environment for many organism cells. The effects of different concentrations of NaCl salt (1%, 2.5%, 5%, 7.5%, 10% and 12.5%) on the production of IAA of our promising high salinity-tolerant strains were directly evaluated (Figure 2).

The results presented in Figure 2 show that the IAA production activities of endophytic bacterial strains at different percentages of NaCl decreased due to increased salt stress. At 1% salinity, the IAA synthesis property was high in all isolates. The highest indicator was observed in the endophytic bacterial strain *P. megaterium* CrEw1004 at 141.4  $\mu\text{g}$ . In the next places, the bacterial strain *B. parabrevi*s CrEw1021 was proven to synthesize IAA in an amount of 126.2  $\mu\text{g}$ , and the bacterial strain *B. subtilis* CrEw1018 was proven to synthesize IAA in an amount of 113.6  $\mu\text{g}$ . The lowest synthesizing property was demonstrated by the bacterial strain *P. putida* CrEw1015 in an amount of 106.8  $\mu\text{g}$ . Increasing salt stress directly negatively affected the IAA synthesizing property of bacteria. This property was proven in all bacterial strains. Also, the highest rate of ISK synthesis among bacterial strains

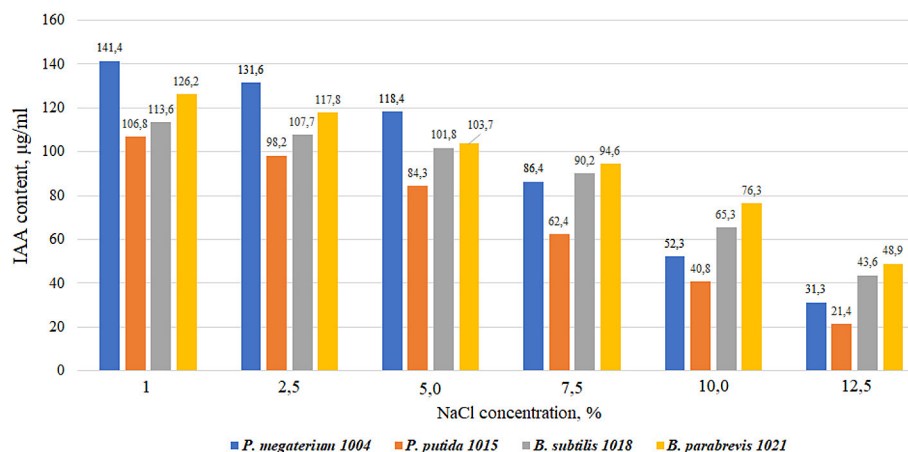


**Figure 1.** IAA production activity of promising strains: (a) IAA synthesis in standard medium; b) IAA synthesis in medium containing 12.5% NaCl



**Table 2.** Effect of temperatures on bacterial IAA synthesis ( $\mu\text{g/ml}$ )

Strain	Temperature, °C					
	20	25	30	35	40	45
<i>Priestia megaterium</i> CrEw1004	72.3 $\pm$ 3	131.2 $\pm$ 1	139.5 $\pm$ 2	102.2 $\pm$ 3	86.8 $\pm$ 1	67.3 $\pm$ 2
<i>Pseudomonas putida</i> CrEw1015	52.8 $\pm$ 2	92.1 $\pm$ 3	108.1 $\pm$ 1	89.2 $\pm$ 3	69.1 $\pm$ 1	48.2 $\pm$ 2
<i>Bacillus subtilis</i> CrEw1018	68.2 $\pm$ 3	104.2 $\pm$ 1	116.3 $\pm$ 3	101.3 $\pm$ 1	91.9 $\pm$ 1	73.3 $\pm$ 2
<i>Brevibacillus parabrevis</i> CrEw1021	79.4 $\pm$ 4	108.6 $\pm$ 2	122.2 $\pm$ 3	119.5 $\pm$ 1	98.2 $\pm$ 2	66.8 $\pm$ 1

**Figure 2.** Effect of NaCl salinity on bacterial IAA synthesis ( $\mu\text{g/ml}$ )

at 7.5% NaCl salinity was shown by the *B. parabrevis* CrEw1021 strain. Its IAA synthesis rate was 94.6  $\mu\text{g}$ . The *B. subtilis* CrEw1018 strain was found to produce IAA at 90.2  $\mu\text{g}$ . The *P. megaterium* CrEw1004 strain, which had the highest rate up to this salinity, had a decrease in IAA synthesis, synthesizing IAA at 86.4  $\mu\text{g}$ . When comparing the IAA synthesis rate at 10% salinity, the highest rate was recorded by the *B. parabrevis* CrEw1021 strain with 76.3  $\mu\text{g}$ , while the lowest rate was recorded by the *P. putida* CrEw1015 strain with 40.8  $\mu\text{g}$ . Also, the amount of IAA synthesis in the 12.5% salinity environment of endophytic bacterial strains was the highest with 48.9  $\mu\text{g}$  for *B. parabrevis* CrEw1021, while the lowest was 21.4  $\mu\text{g}$  for *P. putida* CrEw1015.

At 1% salinity, the highest IAA synthesis was recorded by the endophytic bacterium *P. megaterium* CrEw1004, and the lowest was recorded by the endophytic bacterium *P. putida* CrEw1015. At 10% salt stress, *B. parabrevis* CrEw1021 synthesized the largest amount of IAA, while the *P. putida* CrEw1015 bacterial strain synthesized the smallest amount of IAA.

When analyzing the data presented in Figure 3, it was found that the optimal pH for all bacterial strains to synthesize high amounts of IAA was 7. At this optimal value, the endophytic bacterial strain

*P. megaterium* CrEw1004 synthesized the highest amount of IAA at 140.6  $\mu\text{g}$ , while the lowest value was found for the *P. putida* CrEw1015 strain. This bacterial strain was shown to synthesize 110.1  $\mu\text{g}$  of IAA. It was also found that the bacterial strain *B. subtilis* CrEw1018 synthesized 115.3  $\mu\text{g}$  and the bacterial strain *B. parabrevis* CrEw1021 synthesized 123.6  $\mu\text{g}$  of IAA, respectively.

As the pH of the medium changed towards strong acidity, the ability of the bacterial strains to synthesize IAA in the medium decreased. When the pH of the medium was strongly acidic at 4, the highest indicator was shown by the endophytic bacterial isolate *B. parabrevis* CrEw1021. This strain synthesized 73.8  $\mu\text{g}$  of IAA. While the *P. megaterium* CrEw1004 bacterial strain synthesized 60.3  $\mu\text{g}$  of IAA, the lowest amount was produced by the *P. putida* CrEw1015 strain, which produced 52.4  $\mu\text{g}$ . It was also observed that the synthesis of IAA by endophytic bacterial strains decreased even when the pH was alkaline at 9. The endophytic bacterial strain *P. putida* CrEw1015 showed the highest synthesis of IAA in this medium, at 69.4  $\mu\text{g}$ . It was also proven that the *B. parabrevis* CrEw1021 bacterial strain synthesized the next highest amount of IAA, 66.1  $\mu\text{g}$ .

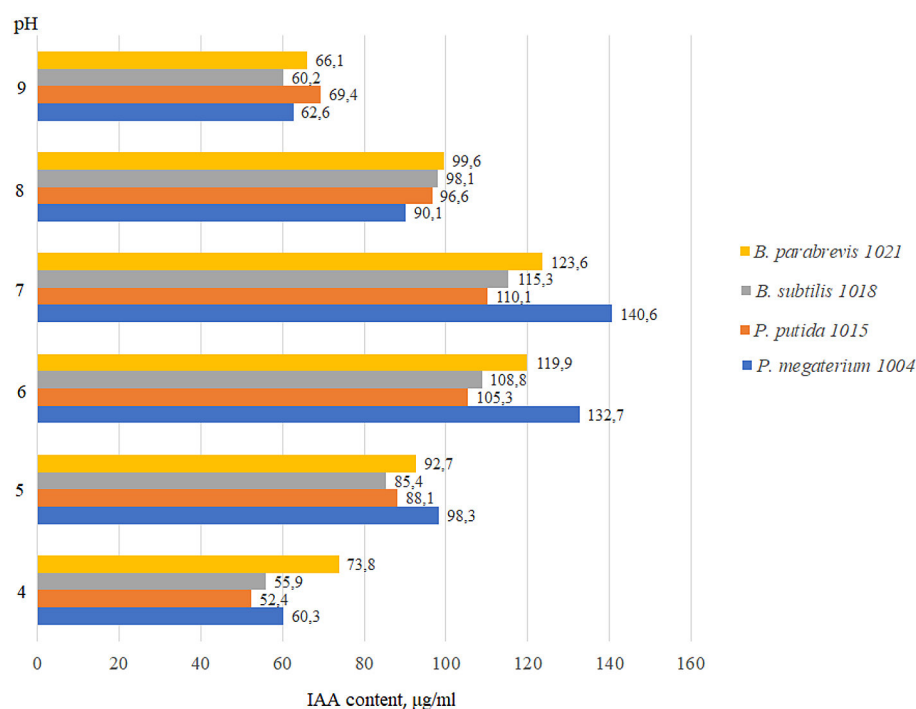


Figure 3. Effect of pH on bacterial IAA synthesis (µg/ml)

Different pH values affected the amount of IAA production by endophytic bacteria. In the optimal pH environment, *P. megaterium* CrEw1004 synthesized a high amount of IAA, while changing the pH environment to alkaline and acidic sides caused a decrease in IAA synthesis.

#### Effect of bacterial suspension treatment on salinity seed germination

During our research, laboratory, vegetative and field experiments were carried out to assess the effect of suspensions of promising strains on the growth and development of wheat plants. The wheat variety “Andijan-2” was used in the experiments. Initially, experiments were conducted to select the parameters of the suspension of bacterial strains that would have a positive effect on seed germination (Table 3).

Looking at the data presented in Table 3, it was observed that the germination properties of seeds were higher in the experimental variants than in the control variants. When analyzing the germination of seeds in working solutions prepared on the basis of cell titers and hours, it was observed that the percentage of germination was higher in the working solution with a cell titer of  $10^8$  cells/ml and when treated for 6 hours. The highest germination indicator belonged to the endophytic bacterial strain *P. megaterium*

CrEw1004, that is, the germination was  $98.5 \pm 0.3$  percent. This indicator was  $85 \pm 0.6$  percent in the control variant treated with sterile water. It was observed that when treated with the bacterial strain *P. putida* CrEw1015, it was  $95 \pm 0.7$  percent, when treated with the bacterial strain *B. subtilis* CrEw1018, it was  $96.5 \pm 0.3$  percent, and when treated with cell suspensions of the bacterial strain *B. parabrevis* CrEw1021, it was  $96.5 \pm 0.3$  percent. When treated with different amounts of cell suspensions of the endophyte bacterium *P. megaterium* CrEw1004 for different hours, the germination rates of the seeds were observed to be at different levels. The highest rate was recorded at  $10^8$  cells/ml and 6 hours, while the lowest rate was  $81.5 \pm 0.3$  percent at  $10^6$  cells/ml and 12 hours. Similarly, the cell titer of the *P. putida* CrEw1015 bacterial strain was  $95 \pm 0.7$  percent when treated for 6 hours, while the lowest rate was  $80 \pm 0.7$  percent. The highest seed germination rate of the *B. subtilis* CrEw1018 bacterial strain was observed when the cell titer was  $10^8$  cells/ml when treated for 6 hours, while the lowest rate was observed when the cell titer was  $10^7$  cells/ml and treated for 12 hours, with  $81.5 \pm 0.9$  percent. The highest germination rate of the *B. parabrevis* CrEw1021 bacterial strain was observed when the seeds were treated for 6 hours at a cell titer of  $10^8$  cells/ml, while the lowest rate was observed at 12 hours at cell titers of  $10^6$ ,  $10^7$ ,

**Table 3.** Effect of seed treatment with promising strains on wheat seed germination, n = 3 (in%)

Strain name	Cell titer, cells/ml	Seed treatment time, hours			
		3	6	9	12
Control	Sterile water	81.5±0.3	85±0.6	81.5±0.9	80±0.3
<i>P. megaterium</i> CrEw1004	10 <sup>6</sup>	83.5±0.7	90±0.5	83.5±0.6	81.5±0.3
	10 <sup>7</sup>	86.5±0.7	91.5±0.6	85±1	83.5±0.3
	10 <sup>8</sup>	95±0.5	98.5±0.3	93.5±0.6	91.5±0.8
	10 <sup>9</sup>	91.5±0.6	93.5±0.3	90±0.6	86.5±0.3
<i>P. putida</i> CrEw1015	10 <sup>6</sup>	88.5±0.8	86.5±1	83.5±0.6	80±0.7
	10 <sup>7</sup>	93.5±0.6	88.5±0.8	90±0.6	83.5±0.6
	10 <sup>8</sup>	91.5±0.8	95±0.7	90±0.6	86.5±0.3
	10 <sup>9</sup>	91.5±0.8	93.5±0.3	85±1	88.5±0.8
<i>B. subtilis</i> CrEw1018	10 <sup>6</sup>	86.5±0.9	90±0.6	90±0.3	83.5±0.8
	10 <sup>7</sup>	90±0.6	91.5±0.9	85±0.6	81.5±0.9
	10 <sup>8</sup>	88.5±0.7	96.5±0.3	88.5±0.7	86.5±1
	10 <sup>9</sup>	86.5±0.3	90±0.6	90±1	85±1
<i>B. parabrevis</i> CrEw1021	10 <sup>6</sup>	86.5±0.7	88.5±0.7	83.5±0.6	81.5±0.7
	10 <sup>7</sup>	88.5±1.2	93.5±0.3	88.5±0.8	81.5±0.3
	10 <sup>8</sup>	93.5±0.3	96.5±0.3	90±1.1	85±1
	10 <sup>9</sup>	90±0.6	91.5±0.7	86.5±0.3	81.5±0.8

10<sup>9</sup> cells/ml, which were 81.5 ± 0.7, 81.5 ± 0.3, 81.5 ± 0.8 percent, respectively.

During the studies, the effect of endophytic bacterial strains on seed germination under salinity conditions was determined (Table 4). Looking at the data presented in Table 4, we can see that the salt stress environment had a negative effect on the germination of plant seeds. It was noted that all promising strains stimulated higher seed germination compared to the control variants in environments with different concentrations of NaCl. The highest result in most salinity environments was recorded by the *P. megaterium* CrEw1004 bacterial strain. At 2.5% NaCl, the seed stimulation property was 95 ± 0.7 and 95 ± 1 percent in the *P. megaterium* CrEw1004 and *B. subtilis* CrEw1018 bacterial strains, respectively.

The *P. putida* CrEw1015 and *B. parabrevis* CrEw1021 bacterial strains recorded 91.5 ± 0.6%. In the control variant, it was slightly lower, 90 ± 0.3%. Similarly, in a 5% salinity environment, the highest indicator was recorded by the *B. subtilis* CrEw1018 bacterial strain with 93 ± 0.6%. The *P. megaterium* CrEw1004 bacterial strain recorded 91.5 ± 0.8%. It can also be seen that the bacterial strain *P. megaterium* CrEw1004 showed 83.5 ± 1.2% at 7.5% salinity, 70 ± 0.6% at 10% salinity, and 58.5 ± 1.2% at 15% salinity. It can be seen that these indicators are higher than those of other endophytic bacterial strains. However, increased salt stress also caused a decrease in seed germination. At 12.5% salinity, the bacterial strains *P. putida* CrEw1015, *B. subtilis* CrEw1018, and *B. parabrevis* CrEw1021 showed 56.5 ± 0.9%, 60 ± 0.9%,

**Table 4.** Effect of treatment with different concentrations of NaCl on wheat seed germination based on promising strains (in %)

Strain name	NaCl concentration, %					
	2.5	5	7.5	10	12.5	15
Control	90±0.3	83.5±0.3	68.5±1.2	58.5±0.3	50±1	43.5±0.7
<i>P. megaterium</i> CrEw1004	95±0.7	91.5±0.8	83.5±1.2	70±0.6	68.5±0.3	58.5±1.2
<i>P. putida</i> CrEw1015	91.5±0.6	88±0.3	75±0.3	63.5±0.9	56.5±0.9	48.5±0.7
<i>B. subtilis</i> CrEw1018	95±1	93±0.6	78.5±0.3	68.5±0.3	60±0.9	51.5±0.9
<i>B. parabrevis</i> CrEw1021	91.5±0.6	86.5±0.3	73.5±0.9	66.5±0.6	61.5±0.7	53.5±0.9

**Note:** Cell titer in bacterial suspension 10<sup>8</sup>, working solution concentration 20%, processing time 6 hours, T = 25 °C.

and  $61.5 \pm 0.7\%$ , respectively. In the studies, the highest rate in the highest salinity environments was recorded by the *P. megaterium* CrEw1004 bacterial strain, followed by the *B. parabrevis* CrEw1021 bacterial strain with  $53.5 \pm 0.9\%$ , the *B. subtilis* CrEw1018 bacterial strain with  $51.5 \pm 0.9\%$ , and the *P. putida* CrEw1015 bacterial strain with  $48.5 \pm 0.7\%$ . The control variant showed  $43.5 \pm 0.7\%$ . Based on the results of these experiments, *P. megaterium* CrEw1004 was studied in the next stages of the study.

### The effect of endophytic bacterial strains on wheat development under salinity conditions

Among the strains, the most active endophytic bacterial strain *P. megaterium* CrEw1004, which stimulated seed germination, was evaluated for its effect on the growth and development of

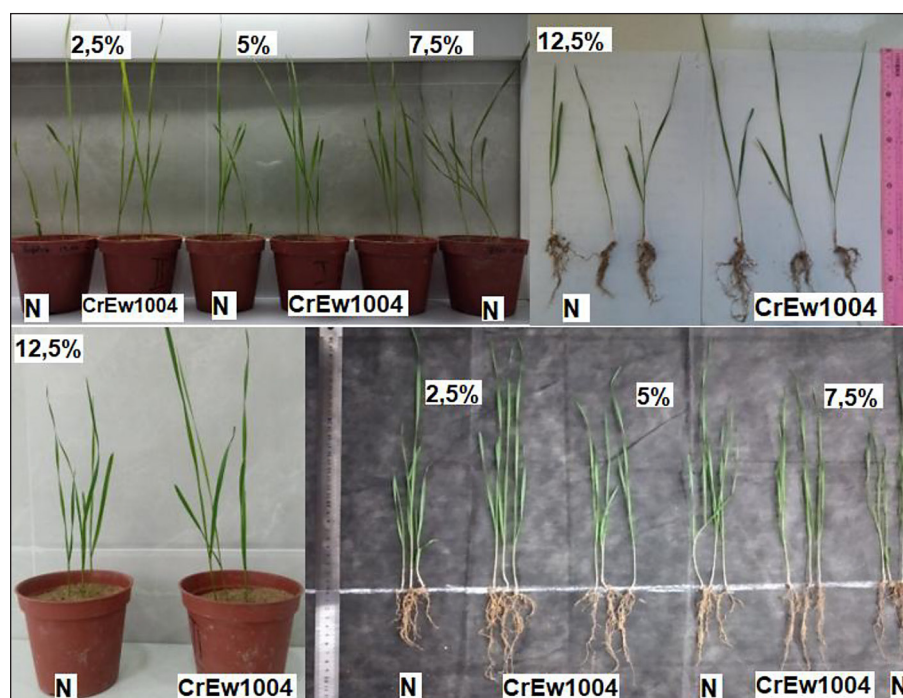
wheat plants and the growth of vegetative organs in vegetative experiments (Table 5). As shown in this table, it can be seen that the treatment of wheat plant seeds with cell suspension of endophytic bacterial strain *P. megaterium* CrEw1004 had a positive effect on the biometric indicators of the plant in different salinity environments compared to the control variant. It was found that the root system of plants planted in a 2.5% salinity environment was 21% higher in the experimental variant than in the control variant, and the plant stem was 12% higher in the experimental variant than in the control variant.

It was observed that the root system of plants planted in a 5% saline environment was 18% longer than the control in the experimental variant, and the stem part was 10% longer than the control. Similarly, the root system of plants planted in a 7.5% saline environment was 20% longer than the control

**Table 5.** Effect of treatment with *P. megaterium* strain CrEw1004 cell suspension on wheat plant growth, cm (vegetative experiments)

Indicators		NaCl concentration, %					
		2.5	5	7.5	10	12.5	15
Root length	Control	10.2±0.9	8.8±0.5	7.4±0.1	6.4±0.3	5.7±0.2	4.3±0.9
	Experience	12.9±0.2	10.7±0.3	9.2±1	8.2±0.2	7.7±0.4	6.5±0.8
Stem length	Control	28.8±0.9	25.7±0.7	22.6±0.4	19.7±0.6	16.5±0.5	13.5±0.7
	Experience	32.8±0.8	28.6±0.4	25.4±0.4	22.6±0.5	19.5±0.6	16.3±0.8

**Note:** Cell titer in bacterial suspension  $10^8$ , working solution concentration 20%, processing time 6 hours.



**Figure 4.** Effect of treatment with *P. megaterium* CrEw1004 on wheat plant growth and development (N-control)



in the experimental variant, and the stem part was 11% longer than the control. The root system of plants planted in a 10% NaCl saline environment was 22% longer than the control in the experimental variant, and the stem part of the plant was 13% longer than the control in the experimental variant.

The root length of plants planted in a 12.5% saline environment increased by 26% in plants treated with bacterial suspension compared to the control, and we can see that the stem part of the plant was 15% longer than the control in the experimental variant. It was observed that the root system of plants planted in a saline environment with 15% NaCl increased by 34% in the experimental variant inoculated with bacteria compared to the control variant, and the development of the plant stem increased by 17% in the experimental variant inoculated with bacteria compared to the control variant (Figure 4).

## DISCUSSION

The plant growth-promoting properties of endophytic bacteria can be used to increase the productivity of cultivated plants in agricultural sectors. Various studies have been conducted in this area, and similar results have been recorded to those proven by world scientists. In particular, Maheswari et al. (2013) isolated about 25 endophytic bacteria from various leguminous plants growing in Indian regions and proved that they synthesize different amounts of ISK.

Phytohormones regulate the defense response of plants against biotic and abiotic stresses, as well as their resistance to various environmental stresses, including salinity (Ryu and Cho, 2015). The response of plants to salinity stress includes a number of changes at the molecular, biochemical and physiological levels (Ahmad et al., 2013). These changes depend on environmental conditions, soil properties and the growth stage of plants.

Studies have shown that the amount of endogenous IAA in plant roots differs depending on the maximum (300 mM NaCl) or minimum (100 mM NaCl) level of salinity (Albacete et al., 2008). Exogenous phytohormones in plants can also reduce the negative effects of salinity (Zahir et al., 2010). Combating salinity stress by exogenous application of phytohormones and their products and balancing the levels of endogenous hormones provides an innovative approach (Ilan-gumaran and Smith, 2017). In experiments, it has

been proven that treating wheat seeds with ISK reduces the harmful effects of salinity stress on wheat growth (Datta et al., 1997). In addition to stimulating root growth, the hormone can also increase its tolerance to salinity (Norboev et al., 2024). IAA helps to maintain the growth of plant leaves, which provides a mitigation of the limitations affecting plant productivity caused by salinity. Also, high concentrations of IAA enhance the protection of bacterial cells from salinity stresses (Bianco et al., 2006). These observations justify the use of IAA-synthesizing bacteria in the cultivation of agricultural crops under salinity and drought conditions.

In most studies, the bacterial seed treatment method is widely used to evaluate the potential of endophytic bacteria isolated from plants to stimulate crop growth. The widespread use of this method in experiments is explained by the fact that most endophytic bacteria are isolated from roots, and the early entry of bacteria into the plant organism through seeds is likely to increase its adaptation capabilities (Rathod et al., 2021). Studies on endophytes conducted in recent decades have shown that endophytes can increase nutrient uptake, stress tolerance, and disease resistance in host plants, resulting in improved yield. Watts et al. (2023) noted that endophytes can provide tolerance to salinity, moisture, and drought conditions, which indicates the possibility of using endophyte-based strategies in crop production in regions affected by stress factors. In addition, endophytes reduce the risks associated with conventional agricultural practices by introducing alternatives to synthetic fertilizers and chemical treatments (Watts et al., 2023).

In experiments on tomato, treatment with *B. amyloliquefaciens* strain MBI600 was shown to increase shoot weight by 15.3% and root length by 20.9% (Samaras et al., 2016). In studies by Shahid et al. (2021), *B. amyloliquefaciens* strain SB-1 was found to have broad antifungal activity through the production of antifungal metabolites such as surfactins, iturins, and fengycins, increased plant growth-related ISK production, and increased root dry weight by up to 96.6% in wheat.

A study by Liu et al. (2020) demonstrated that *B. pumilus* LZP02 strain promoted rice growth by increasing root length, root surface area, and chlorophyll content. In addition, application of *B. pumilus* LZP02 resulted in increased nitrogen, phosphorus, calcium, and magnesium content in rice roots (Liu et al., 2020). Another study demonstrated that *B. pumilus* promoted rice growth under

growth chamber conditions. Treatment with this strain significantly improved growth, root development, and nutrient uptake in 21-day-old rice seedlings compared to the control (Ngo et al., 2019).

## CONCLUSIONS

According to the results of the studies, the synthesis of IAA by endophytic bacteria isolated from *K. ceratoides* is affected by changes in temperature, salinity and pH values. However, among the studied bacterial strains, some strains retained the properties of ISK synthesis under extreme conditions of temperature (*B. subtilis* CrEw1018, *B. parabrevis* CrEw1021), salinity (*P. megaterium* CrEw1004, *B. parabrevis* CrEw1021) and pH (*P. megaterium* CrEw1004, *P. putida* CrEw1015), indicating their potential for use in the cultivation of agricultural crops under stress factors. When growing wheat under salinity conditions, it is recommended to treat the seeds with a 20% bacterial suspension of *P. megaterium* CrEw1004 for 6 hours. The results obtained require further investigation of endophytic bacteria as a source of tools to improve the efficiency of agricultural crop production in saline and arid conditions.

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