SALT TOLERANT RHIZOBACTERIA MITIGATING THE EFFECTS OF SALINITY STRESS ON GROWTH OF SOYBEAN (*Glycine max* (L.) Merr.) ON SALINITY SOIL

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Abstract

Salinity is a serious abiotic stress that adversely affects plants. Soybean (Glycine max (L.) Merr.) is not a salt tolerant crop and salt stress is a major problem for its productivity. One way to mitigate salt stress is to inoculate with salt-tolerant growth-promoting rhizobacteria. The purpose of this study was to isolate local strains of salt-tolerant rhizobia and phosphate-solubilizing bacteria and to study their ability to promote soybean growth on saline soils. In our study, isolates of rhizobia and phosphate-solubilizing bacteria were isolated from the rhizosphere and nodules of soybean growing on saline soil. Their growth under salt stress conditions was studied and high salt-tolerant isolates were selected: three isolates of rhizobia and four phosphate-solubilizing bacteria. Under salt stress, isolates of rhizobacteria were studied for nodulation and phosphate solubilization, and active strains were selected. This strains were identified by the molecular genetic method of Sanger. It was found that rhizobia RH21, RH26, RH28 refers to Bradyrhizobium japonicum, phosphate solubilizing bacteria to Pseudomonas koreensis FT4, Bacillus pumilus FM12 and Bacillus sp. FC11. The inoculation with rhizobacteria mitigated of the salt stress was showed. This is demonstrated by an increase in indicators of growth of inoculated plants. The co-inoculation with two strains rhizobacteria (RH28 and FT4) most effectively mitigated salt stress: the roots weight was increased by 3 times, shoot by 2.7 times and number of nodules by more than 3 times compared with control without salinity. Our study showed that inoculation with local strains of salt-tolerant rhizobacteria increased resistance to salt stress, improved growth, and promoted ecological adaptation of soybeans to saline soils.

Keywords: *salt-tolerant rhizobacteria, rhizobia, phosphate-solubilizing bacteria, salt stress, soybeans, inoculation, plant growth*

1. INTRODUCTION

Climate change significantly affects the productivity of agricultural crops. One of the negative factors of this influence is the increase salinity of agricultural lands (Asad, 2021). The annual increase in the area of saline soils at a rate of 10 % per year poses a serious threat to agricultural production (Singh, 2022). Salt soils are a strong abiotic stress for plants, which can lead to the loss of up to 70 % of crop yields (Nawaz et al., 2020; Chele et al., 2021). To adapt crops to a climate change and maintain food security, it is necessary to apply new environmentally friendly and progressive methods to increase the sustainability of agricultural production.

Soybean (*Glycine max* (L.) Merr.) is one of the strategic crops that provides food for many countries of the world. On a global scale, soybeans are comparable in importance to wheat, rice and corn. The high content of high-grade protein in terms of amino acid composition (up to 45-48 %) and high-quality oil (up to 25 %) determine its wide application (Wijewardana et al., 2019). Currently, soybean sown area is 102 million ha and production is 261.3 million tons (Younesabadi et al., 2021). However, soybeans are not salt-tolerant crops, and soil salinity is the main limitation of its production in many regions of the world (Ilangumaran et al., 2021). Salt stress slows down soybean growth, causes leaf chlorosis, reduces protein accumulation, inhibits nodule formation and atmospheric nitrogen fixation, which leads to a significant decrease in its productivity (Otie et al., 2021). Due to changing climate, farmers are interested in increasing the yield of soybeans on saline soils. The increase in the cost of agricultural inputs and the loss of soil fertility also encourage the search for sustainable strategies.

One of the main elements of plant nutrition is nitrogen, its deficiency causes a significant delay in plant growth, slows down flowering, pod set, and causes a weakening of immunity (Anas et al., 2020). Phosphorus is an equally important nutrient for crops; it accelerates the growth and development of plants, the process of their maturation, and increases the cold resistance and resistance of crops to pests and diseases (Wiel et al., 2018). Mineral nitrogen and phosphorus fertilizers are used to increase the yield of soybeans. The use of high doses of fertilizers allows to achieve high yields, but at the same time causes significant damage to soil health, water pollution and other negative effects (Li et al., 2021).

An alternative way to improve the nitrogen and phosphorus nutrition of plants is the use of microorganisms that contribute to the enrichment of plants with biological nitrogen and increase the availability of insoluble mineral phosphates for plants. Inoculation with beneficial microorganisms is a promising way to improve salt tolerance and plant productivity. However, the use of biofertilizers often turns out to be ineffective, which is associated with the inability of microbes of industrial imported preparations to saline soils (Wekesa et al., 2022).

Various salt-tolerant rhizobacteria that stimulate plant growth have been isolated from extremely alkaline, saline, and sodic soils (Kumar et al., 2020). Many of them are known to mitigate biotic and abiotic stress and increase crop productivity in saline soils. The aim of this study was to isolate local strains of salt-tolerant rhizobia and phosphate-solubilizing bacteria and to study their ability to promote soybean growth in saline soils.

The scientific novelty of our study lies in the fact that local strains of salt-tolerant rhizobia and phosphate-solubilizing bacteria adapted to the soil and climatic conditions of Kazakhstan will be isolated. New local strains of salt-tolerant rhizobia will be able to actively form nodules on soybean roots, actively fixing atmospheric nitrogen, phosphate-solubilizing bacteria will mobilize phosphates into plant-accessible forms and increase nitrogen and phosphorus nutrition of plants on saline soils. The isolated strains are natural representatives of soils and will not enter into antagonistic relationships with soil microflora. The practical significance of the study lies in the fact that the use of local salt-tolerant rhizobacteria will increase soybean resistance to salt stress and allow soybean to be grown on saline soils, which is important for sustainable agriculture.

2. MATERIALS AND METHODS

2.1 Research Objects

The objects of the study were rhizobia isolated from nodules on roots of soybean (*Glycine max* (L.) Merr.) variety "Eureka" and phosphate solubilizing bacteria isolated from soybean plants rhizosphere. Soybean plants were collected in the flowering phase in July 2021. In this phase of soybean growth, the nodules were well formed and visible. Soil samples for the isolation of phosphate-solubilizing bacteria were collected in the soybean rhizosphere in the Almaty region of the south-east of Kazakhstan in accordance with the International Standard (ISO-17.4.4.02-2017). In the experiments, soybean seeds of the variety "Eureka" were used. This soybean variety is mid-season, drought-resistant, recommended for cultivation in the south-east of Kazakhstan, and it's the most widely used by farmers. Seeds were provided by Kazakh Research Institute of Agriculture and Plant growing LLC, Almalybak village, Karasai district, Almaty region, Kazakhstan (department of fodder, oil crops, and corn).

2.2 Type of Soil and Salinity

The samples of soil and soybean plants were collected in a saline field in the Almaty region of Kazakhstan (N 43°39'36,24'', E 77°58'87.21"), 365 m above sea level. Soil type - gray soil, low humus content - 0.92 %, easily hydrolyzed nitrogen 57.3 mg/kg soil, mobile phosphorus 20.9 mg/kg soil and mobile potassium 448.0 mg/kg. The content of salts in the water extract of the soil: 1.062 %, the pH 9.3 is alkaline. According to the content of HCO_3 ions, the plots where the soil and soybean plants were collected belong to weakly alkaline soils (0.02 %).

2.3 Isolation of Rhizobia

To isolate rhizobia, large soybean plants of the variety "Eureka" with a large number of nodules on the roots in the flowering phase were selected. Large nodules were separated from the roots and sterilized by 70 % ethanol for 2 min, then 2 % sodium hypochlorite (NaOCl) for 5 min and washed with sterile table water. The nodules were crushed, transferred to Petri dishes with Maze medium with the addition of 10% NaCl, smeared with a spatula over the surface of the medium to obtain individual colonies (Albdaiwi et al., 2019). The composition of the Maze medium, g/l: K_2HPO_4 - 1.0; $MgSO_4$ - 0.3; sucrose - 10.0; NaCl - 100.0, decoction of 100 g of peas, pH 6.8-7.0, table water - 1000 ml. Petri dishes were incubated in a thermostat at 28° C for 7-9 days. When grown on Maze's medium, rhizobia formed translucent colorless or white mucous colonies.

2.4 Isolation of Phosphate-solubilizing Bacteria

Phosphate-solubilizing bacteria were isolated on NBRIP (National Botanical Research Institute's phosphate growth medium) supplemented with 10 % NaCl (Mohamed et al., 2019). To isolate bacteria, 10 g of soil was diluted with 90 ml of distilled sterile water and mixed on a shaker at 180 rpm for 2 hours at 28°C. Microorganisms were inoculated from dilutions in Petri dishes with NBRIP agar medium, then incubation at 28°C and kept until halo zones around bacterial colonies (3-5 days).

2.5 Nodulation of Rhizobia

For study of nodulation, rhizobia were grown in Iswaran's medium. Medium composition (g/l): sucrose - 10.0; K_2HPO_4-0,5; $MgSO_4 \times 7H_2O$ - 0,2; calcium gluconate - 1.5; $FeCl_3$ - 0.01; pH 6.8 - 7.0 on a rotary shaker at 180 rpm at a temperature of 28° C for 5 days. Seeds before sowing were sterilized with 70 % ethanol for 2 min, then with 2 % sodium hypochlorite solution (NaOCl) for 5 min and washed with sterile table water. The seeds were inoculated with a suspension of bacteria at a concentration of 10^8 cells/ml for two hours at 23° C. Then the seeds were sown in vegetative vessels with sterile vermiculite, Knopp's liquid medium was used to nutrient the plants. Before sowing, 250 ml of water or NaCl solution with a concentration of 250 and 500 mM was added to each vessel. Soybean seeds without inoculation with rhizobia, soaked in sterile water served as a negative control, seeds inoculation with rhizobia without NaCl served as a positive control. The number and weight of nodules were counted after 45 days of cultivation. The experiments were carried out in five replicates.

2.6 Nitrogenase Activity of Rhizobia

The nitrogenase activity of rhizobia was determined by the acetylene method (ARA) (Das & De, 2018). Rhizobacteria were grown on Iswaran's medium under aeration conditions to a concentration of 10^8 cells/ml. The bacterial suspension was placed in 10 ml vials; acetylene was injected to a concentration of 10 % (w/v). The cultures were incubated in an acetylene atmosphere for 1.5 h, gas samples were taken with a 1 ml syringe from the vessel, and the presence of ethylene was determined on an Agilent Technology 7890B gas chromatograph (USA) with a flame ionization detector. The experiments were carried out in three replicates.

2.7 Phosphate-solubilizing Activity of Bacteria

Phosphate-solubilizing activity of bacteria was studied by Segi's modified method (Segi, 1983). To the NBRIP medium containing insoluble tricalcium phosphate in the form of a fine precipitate, was added 10 % NaCl. In the NBRIP medium holes 7 mm in diameter were pierced with a sterile drill and 0.5 ml of a bacterial suspension with a concentration of 10^8 cells/ml was placed. As a control, sterile NBRIP medium was placed in the holes. Dishes were incubated at 28°C for 3 days until the formation of transparent halo zones around the holes. Phosphate mobilizing activity of bacteria was assessed by the diameter of halo zones in mm. Experiments were carried out in five replicates.

2.8 Screening salt-tolerance of isolates of rhizobacteria

The isolates of rhizobacteria were tested for resistance to salt stress at different NaCl concentrations according to the protocol (Krishnamoorthy et al., 2022). Rhizobia were grown on Iswaran's medium, phosphate solubilizing bacteria on NBRIP medium. NaCl was added to the media at concentrations of 100, 250, and 500 mM. Bacterial growth was recorded by optical density at 540 nm on a spectrophotometer (PD-303, Apel, Japan). Next, the growth of isolates was assessed, where is the complete absence of growth, + the presence of growth, the addition of + indicates the intensity of growth. Experiments were carried out in five replications.

2.9 Biocompatibility of Rhizobia and Phosphate-solubilizing Bacteria

The biocompatibility of phosphate-solubilizing bacteria and rhizobia was studied by the perpendicular streak method (Netrusov & Kotova, 2020). Rhizobia were streaked in the center of a Petri dishes on Maze agar medium, phosphate mobilizing bacteria were inoculated perpendicular to the streak of rhizobia. After 3-5 days, the presence or absence of zones of growth inhibition of phosphate-mobilizing bacteria from the central streak was studied. Bacterial strains without antagonism grew in the immediate vicinity of the central streak.

2.10 Plant Growth Promotion Activity of Rhizobacteria

To study the effect of rhizobacteria on germination and development of soybean plants, phosphate-solubilizing bacteria and rhizobia were grown separately. Then the suspensions of bacteria were mixed in a ratio of 1:1. Seeds of soybean variety "Eureka" were sterilized as described and inoculated before sowing with a co-culture of rhizobacteria with a concentration of 10^8 cells/ml for two hours at 23° C. Then they were sown in vegetative vessels with a volume of 5000 ml, five seeds per vessel. In the experiments, highly saline soil of the Almaty region of Kazakhstan was used. Soil type - grey soil, low humus content - 0.85 %, easily hydrolyzed nitrogen 54.6 mg/kg soil, mobile phosphorus 18.7 mg/kg soil, mobile potassium 452.0 mg/kg soil. The content of salts in the water extract of the soil is 1.066 %, the pH 9.2 is alkaline. Uninoculated seeds served as control. The germination was determined by counting the number of germinated seeds in variants and control. To assess the growth-stimulating activity after 60 days, the number and dry weight of nodules, dry weight of roots and shoots (drying to obtain a constant weight) were determined. The experiment was repeated 3 times and was carried out in an open area under conditions close to the field.

2.11 Statistical analysis

All experiments were repeated 3-5 times. Statistical processing of the obtained results was carried out using the STATISTICA 10.0, var. 6 software package (Borovikov, 2016). Results show mean values (M) and standard errors of means (±SEM) (Gomez & Gomez, 1984).

3. RESULTS

From the nodules on soybean roots 24 isolates of rhizobia were isolated, from the samples of soybean rhizosphere soil 32 isolates of phosphate-solubilizing bacteria were isolated. The isolates were screened for their ability to grow under saline conditions (100, 250, 500 mM NaCl). The dates of the twenty most salt-tolerant isolates rhizobia and phosphate-solubilizing bacteria are shown in Table 1.

Table 1. Screening for salinity tolerance of the isolates rhizobia and phosphate-solubilizing bacteria

	Concentration NaCl (mM)		
Isolates	**100**	**250**	**500**
	Rhizobia		
MA12	+	-	-
MA34	+	-	-
RH28	++	-	-
RH37	+++	+++	+
RH42	++++	+++	+

RH26	++++	++++	+++
RH21	++++	++++	+++
RH28	++++	++++	+++
RH28	++++	-	-
RH22	+++	-	-
Phosphate solubilizing bacteria			
FL27	++	-	-
FY36	+++	-	-
FT2	+++	+++	+
FT34	++	-	-
FT4	++++	+++	+++
FM9	++++	+++	+++
FC11	++++	++++	+++
FM12	++++	++++	+++
FY30	+++	++	+
FY23	+++	++	+

Note: Qualitative assessment: - indicates absence of the growth; indicates presence of the growth; additional + indicates intensity of the growth exhibited by the isolates

Not all of the bacterial isolates grew at high salinity It was found (Table 1). At a concentration of 100 mM NaCl all isolates grew and their growth differed significantly. But only three isolates of rhizobia RH21, RH26; RH28 and four isolates of phosphate solubilizing bacteria FM12, FM9, FC11, FT4 grew at a concentration of 500 mM NaCl. These isolates were selected as the most salt tolerant.

One of the main indicators when using rhizobia to improve the nitrogen nutrition of legumes is their ability to form nodules on soybean roots. In this regard, the effect of salt stress on the ability of rhizobia to nodulate, nitrogenase activity, dry weight of roots and nodules was studied. Three strains of rhizobia RH21, RH-6, RH28 were used in the experiments, which showed active growth under high salt stress (Table 2).

Table 2. Nodulation and nitrogenase activity of rhizobia isolates

Isolates	Number of nodules per plant (pcs)	Dry weight of nodules (mg/plant)	Root dry weight (g/plant)	Nitrogenase activity (µmole C_2H_4/ml/h)
without NaCl				
Control	0	0	9.3	0
RH26	28.5	233.2	25.0	6.25
RH21	29.5	252.5	26.8	6.31
RH28	30.3	263.4	30.7	6.34
250 mM NaCl				

Control	0	0	7.2	0
RH26	23.1	148.2	23.6	5.63
RH21	24.6	151.5	24.2	5.73
RH28	25.3	158.4	27.9	5.85
		500 mM NaCl		
Control	0	0	4.1	0
RH26	21.5	130.1	19.7	5,09
RH21	21.6	131.6	19.9	5.17
RH28	23.9	138.1	23.7	5.22

Note: Values are means ± standard error of the mean (±SEM), n = 5; The standard deviation is given, indicate significant differences based on *Student–Newman–Keuls* test (SNK-test) at $p < 0.05$.

Table 2 shows that salt stress significantly affects soybean plants. Inoculation with salt-tolerant rhizobia mitigated salt stress. This is confirmed by the fact that in the variants without inoculation under high salt stress (500 mM NaCl), the weight of the roots decreased by 55 % or more, in the variants with inoculation only by 21-22 %. At the same time, the number of nodules on the soybean roots decreased by only 21-23 %, the activity of nitrogen fixation by 17-18 % compared with variants without salt stress (p <0.05) (positive control). It was found that the RH28 isolate had the highest activity of nodulation and nitrogen fixation.

To assess the ability of bacteria to mobilize phosphates under salt stress, bacteria were grown in NBRIP medium containing 100, 250, and 500 mM NaCl for five days. The results are presented in Table 3.

Table 3. Phosphate solubilizing activity of bacteria under salt stress

Isolates	**Zone of solubilization (mm)**			
	Concentration NaCl (mM)			
	0	**100**	**250**	**500**
FT4	37.4	33.4	28.1	26.1
FM9	32.6	26.6	21.9	15,8
FM12	36.2	30.2	26.8	24.8
FC11	36.2	32.5	27.7	

Note: Values are means ± standard error of the mean (±SEM), n = 5; The standard deviation is given, indicate significant differences based on SNK-test at $p < 0.05$.

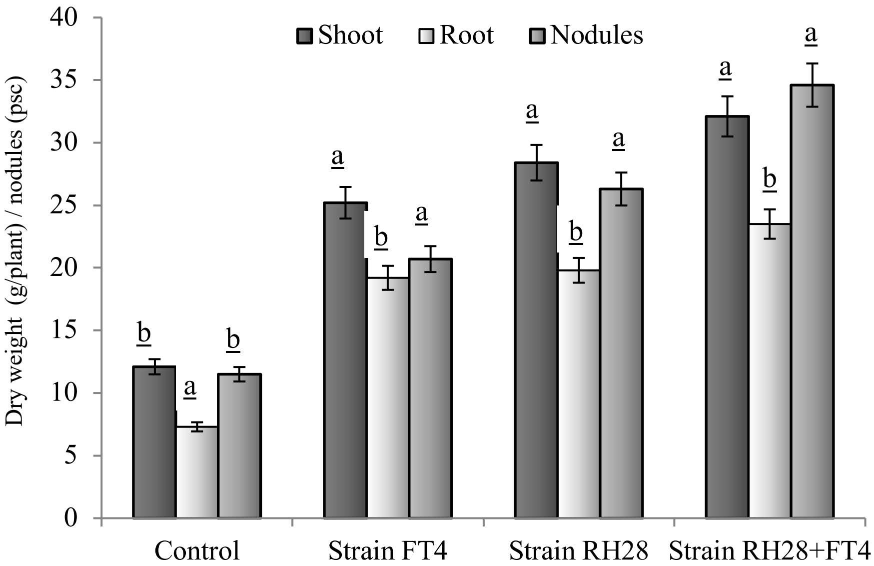
Table 3 shows that the bacteria had a high ability to mobilize phosphates under salt stress conditions. The phosphate mobilization zone at high salinity (500 mM NaCl) varied from 15.8 to 26.1 mm (p <0.05). It was shown that strain FM9 had the lowest activity of phosphate solubilization (15.8 mm), strains FT4, FM12 and FC11 were characterized by high activity of phosphate mobilization. This indicates that they can be used as potential microbial inoculants for agriculture on saline soils. The maximum zone of phosphate mobilization at a high degree of salinity was noted in strain FT4 (26.1 mm).

Identification of rhizobacteria by the molecular genetic method of Sanger showed that rhizobia strains RH21, RH26, RH28 refers to *Bradyrhizobium japonicum*, phosphate-solubilizing bacteria refer to *Bacillus pumilus* FM12 and *Bacillus sp.* FC11.

Since the joint use of rhizobacteria is planned, the biocompatibility of strains of phosphate solubilizing bacteria and rhizobia was studied. It was found that the strains are biocompatible and did not have antagonism. Based on the data, two of the most active and biocompatible strains of rhizobia *Bradyrhizobium japonicum* RH-28 and phosphate-solubilizing bacteria *Pseudomonas koreensis* FT4 were selected.

Experiments on the effect of seed inoculation with individual strains and co-culture of these strains on the growth shoots, roots and nodulation were carried out. In the experiments, high saline soil was used. Figure 1 shows effect of individual strains and their co-culture on the dry weight of shoot, roots and nodulation.

Fig. 1. Effect of inoculation on the dry weight of shoots, roots and nodules in highly saline soils



Control without inoculation, soybean variety "Eureka". Columns represent mean values while bars represent standard deviation (n = 5). Different letters show statistically significant different values ($p < 0.05$) between treatments as evaluated from SNK-test

It has been established that salt stress adversely affects the growth and development of soybean plants. Inoculation with salt-tolerant rhizobacteria significantly alleviates stress (Figure 1). This is evidenced by an increase in indicators of plant growth (dry weight of the root, stem and number of nodules) in inoculated crops. It was shown that inoculation with RH-28 rhizobia increased the dry weight of roots by 2.7 times, stems by 2.3 times, and the number of nodules by 2.3 times compared with control without inoculation. Phosphate-solubilizing bacteria also had a positive effect on growth rates, but their influence was less effective. The maximum positive effect on soybean plant growth was found with co-inoculation with two strains RH28 and FT4. At the same time, the dry weight of roots increased by 3.2 times, stems by 2.7 times, and the number of nodules more than three times compared to the control (Figure 1). We can be said that co-inoculation with two strains most effectively mitigated salt stress.

4. DISCUSSION

Soil salinity is one of the main limiting factors in agricultural and a major problem affecting crops production (Vaishnav et al., 2020; Dewi et al., 2022). More than 50 % of cultivated and irrigated land in the world is subject to salinization and degradation (Machado et al., 2017). Climate change, excessive use of groundwater and mineralized water for irrigation exacerbates this process (Dotaniya et al., 2022). Soil salinity reduces the productivity of many crops, including soybean (Kamran et al., 2019). Salt-tolerant PGPR (Plant Growth Promotion Rhizobacteria) form an integral part of the soil microbiota, interacting with crops to mitigate the harmful effects of stress, improve growth, and support plants survive under inhibitory levels of salt stress (Garciay et al., 2019). Therefore, the role of salt-tolerant bacteria is extremely significant in the modern agricultural system, subject to many biotic and abiotic stresses. This emphasizes the need to identify strains of rhizobacteria that will increase crops productivity in various environmental conditions. In this regard, the finding and isolation of microorganisms that can mitigate salt stress has great research potential, and the development of inoculants based on them for crops growing on saline soils is a promising strategy for improving their productivity.

The rhizobia and phosphate-solubilizing bacteria in the rhizosphere of plants have beneficial properties for plants and play an important role in their nutrition. However, not all rhizobacteria can actively grow and reach their potential on saline soils. In our study, from plants and the rhizosphere of soybean growing on saline soils, local isolates of rhizobacteria were isolated: rhizobia from nodules on the roots of soybean, phosphate-solubilizing bacteria from soils of the rhizosphere. A total of 24 isolates of rhizobia and 32 isolates of phosphate-solubilizing bacteria were isolated. The ability of the isolates to grow under conditions of high salt stress was studied. It was shown that only three isolates of rhizobia and four phosphate-solubilizing bacteria actively grew at high salinity. This is consistent with Khan et al. (2019), who showed that out of 126 isolates from the soil rhizosphere, only five strains were highly resistant to salt stress. Also, this is confirmed by Mahmood et al. (2019), who found that out of 152 isolates, 50 % showed resistance to soil salinity, but only two were selected as highly salt tolerant.

One of the main symbiotic characteristics of rhizobia for use in agriculture is ability to form nodules. We studied the nodulation of rhizobia isolates to under high salt stress (500 mM NaCl). It was founded that salt stress had a negative effect on root growth and formation of nodules. High salt stress reduced root weight by 55 % or more. The similar results with the culture of *Asparagus officinalis* L. were obtained in researches by Guo et al. (2022), who found that root biomass decreased by 96-71 % under high salt stress. Our study showed that inoculation with salt-tolerant rhizobia mitigated the salt stress of plants and the weight of the roots decreased by only 21-22%, the number of nodules by 21-23%, and the activity of nitrogen fixation by 17-18% compared with the control without salt stress. This is consistent with a number of researches founded that inoculation mitigated salt stress in tomatoes, rice. alfalfa and increased root biomass (Haque et al., 2022; Shultana et al., 2020; Irshad et al., 2021).

In the rhizosphere of plants there are various microorganisms that can promote their growth. One such of them are phosphate solubilizing bacteria. These bacteria are able to mobilize insoluble phosphates into a forms available to plants, improve phosphorus nutrition, and promote plants growth (Tian et al., 2021). However, growth and phosphate mobilization have been shown to be significantly reduced under salt stress (Dey et al., 2021). Our study found that FT2, FT4, FM9, FM12, FC11 isolates not only actively grow under conditions of high salt stress (500 mM NaCl), but also efficiently mobilize phosphates. The three isolates FT4, FM12 and FC11 were the most active. Compared to their activity of mobilize phosphates under non-saline conditions, their activity decreased by only 28-30 %. This is an important factor for growing soybean in saline soils, as these bacteria will be successful as inoculants due to their ability to survive high salt stress.

The identification of active strains by the Sanger molecular genetic method based on sequencing of the 16S rRNA nucleotide sequence was carried out. It was found that rhizobia RH21, RH26, RH28 refers to *Bradyrhizobium japonicum*, phosphate solubilizing bacteria to *Pseudomonas koreensis* FT4, *Bacillus pumilus* FM12 and *Bacillus* sp. FC11.

In our study, inoculation with rhizobacteria significantly mitigate the negative effect of salt stress on soybean plants. This is confirmed by an increase in growth indicators in inoculated plants. The researchers have found this fact for different crops (Khan et al., 2019; Ayuso-Calles et al., 2021; Ha-Tran et al., 2021). We found the maximum positive effect on soybean plant growth during co-inoculation with two strains of rhizobia RH-28 and phosphate-solubilizing bacteria FT4. Inoculation with two strains increased the dry weight of roots by 3 times, stems by 2.7 times, and the number of nodules by more than three times compared with control. Similar results under salt stress were found by Kumawat et al. (2021) with beans, which showed that inoculation with two strains of *Rhizobium* sp. and *Enterococcus mundtii* increased the yield of beans under salt stress by 8.92 % and Ullah et al. (2017) with wheat co-inoculation with two strains of *Rhizobium leguminosarum* and *Mesorhizobium ciceri* increased grain productivity up to 48% compared to control without inoculation. The inoculation with strains of *Azospirillum brasilense* and *Paenibacillus dendritiformis* of melon and tomato increased the biomass of roots by 78-102 %, shoots by 37-57 % (Gopalakrishnan et al., 2022) under salinity was shown. We can say that in our study, the co-inoculation with two strains was highly effective at mitigating salt stress and had a positive influence on the growth and development of soybean plants.

The use of aboriginal strains of rhizobacteria to mitigate salt stress has the added benefit of easily acclimatizing the bacteria to soil and climate conditions, which ultimately enhances interactions between plants and microbes. This study showed that inoculation with local strains of salt-tolerant rhizobacteria induces stress tolerance, improves growth, and promotes ecological adaptation of soybeans. Co-inoculation of aboriginal strains of rhizobia and phosphate-solubilizing bacteria has high potential for sustainable and ecological agriculture. On the base of isolated strains, it is possible to develop inoculants for cultures growing on saline soils, which is a promising strategy for improving crops productivity.

5. CONCLUSIONS

Our results indicate a high prospect of using salt-tolerant rhizobacteria in soybean cultivation on saline soils. It has been shown that the application of local salt-tolerant rhizobacteria (co-inoculation of rhizobia and phosphate-solubilizing bacteria) mitigates salt stress. The high plant growth promoting activity of isolated salt tolerant rhizobacteria, increased nitrogen and phosphorus nutrition of plants under salt stress conditions indicate the possibility of their use to increase the productivity of soybean. The results obtained allow us to propose new approaches to increasing the productivity of soybean or other leguminous plants under saline conditions.

However, research on the interaction between PGPR and crops in the presence of soil salinity regimes is still in at an early stage. The inoculation with microbes may work as a putative mechanism for reducing salinity stress in salt-sensitive crops. Therefore, a wide range of research is needed in this area and use of PGPR and other symbiotic microorganisms can be useful in developing strategies for sustainable agriculture in saline soils.

FUNDING

The work has been completed in the framework of the grant project IRN AP09259080.

ACKNOWLEDGEMENTS

This study used soybean (*Glycine max* (L.) Merr.) variety "Eureka". Seeds of soybean were kindly provided by Kazakh Research Institute of Agriculture and Plant growing LLC **(**department of fodder, oil crops, and corn). Almalybak village, Karasai district, Almaty region, Kazakhstan.

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