Eurasian Journal of Soil Science 2025, 14(3), 198 - 207

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

The effectiveness of bio-treatment on licorice (*Glycyrrhiza glabra*) productivity and soil restoration in saline ecosystems

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Article Info

Received : 04.11.2024

Accepted : 10.03.2025

Available online: 15.03.2025

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Abstract

Licorice (Glycyrrhiza glabra) is a highly valued medicinal plant, widely used in the pharmaceutical industry, therefore its natural habitat is dwindling sharply in the Aral Sea region. Considering the essential role of this salt-tolerant halophyte for ecosystem functions, urgent actions are needed to help restore degraded landscapes. The experiment was conducted during vegetation seasons 2022 and 2023 in saline lands (EC~10-12 dS m^{-1}) of Karakalpakstan using a split-plot design with an RCBD arrangement. The effects of seed bio-treatments, i.e. BIST, Zamin, and Geogumat on the root yield of licorice and its quality as well as microbial community composition in the root rhizosphere were studied in abandoned saline land. Results indicate that the Geogumat application increased the seed germination by 24.3%, root biomass by 37% and glycyrrhizin content by 12.7%. Similarly, Zamin and BIST also significantly enhanced these parameters compared to the control under soil salinity stress. It has been found that licorice as a legume interacted with N_2-fixing microbes, thereby significantly increased NPK availability in the soil. The root and shoot biomass increased in response to the seed bio-treatments, most likely because of improved soil microbial activity. The presented eco-friendly research endeavors in this study might be considered as a significant solution to convert abandoned saline lands into sustainable agricultural production, thereby reducing the negative impacts of climate change and restoring ecosystem functionality.

Keywords: Licorice, biofertilizers, saline soil, arid environment, beneficial bacteria, ecosystem sustainability.

Introduction

Land degradation remains a crucial issue for Central Asia, requiring sustained collaborative efforts to enhance soil health, improve land management, and strengthen resilience to climate impacts. Meanwhile, the increasing severity of soil salinization is leading to serious ecological, health, economic and social challenges, particularly in the Aral Sea regions (Ahn et al., 2024).

doi : https://doi.org/10.18393/ejss.1657415

www : https://ejss.fesss.org/10.18393/ejss.1657415

Publisher : Federation of Eurasian Soil Science Societies

e-ISSN : 2147-4249

Transforming salt-affected lands into productive agricultural systems can be achieved through the introduction of salt-tolerant crops and innovative land management practices. Various eco-friendly strategies have been tested in arid regions such as Karakalpakstan, to encourage the widespread adoption of soil restoration practices. Over the past 30 years, the proportion of strongly and moderately saline lands in the region has expanded from 38.5% to 58.4% due to the Aral Sea tragedy. Furthermore, hundreds of tons of salt are carried annually by wind and water from abandoned saline areas to adjacent irrigated farmland, increasing the risk of soil salinization. Currently, approximately 91% of irrigated land is affected by salinity, with 48% classified as highly saline (12-16 dS/m) (Rustamova et al., 2023). As a result, significant efforts directed towards developing and implementing new management strategies to enhance crop yield and soil health in salt-affected and marginal fields (Nurbekov et al., 2023).

During the Soviet era, agricultural production in Karakalpakstan was largely concentrated on cotton and wheat cultivation. The absence of organic matter incorporation back into soils and cultivation of non-legumes associated with the overuse of chemicals has had detrimental implications, such as reduced humus content and micronutrient levels in the soil.

The use of halophyte legumes in saline soil restoration is an innovative and cost-effective approach of reclaiming abandoned irrigated lands. It has been proved that developing highly productive fodder systems through the introduction of palatable halophytes can enhance the productivity of saline soils, providing revenue to farmers with limited resources. Halophytes are ecologically and physiologically distinct salt-loving plants that may produce significant green biomass and grain yields under saline conditions (Fozi et al., 2024).

Licorice (Glycyrrhiza glabra) is a perennial herbaceous halophyte, inherently more resilient to salt and drought stresses than many other desert plants originated in Central Asian rangelands. This halophyte plant grows well in fragile ecological environments because of its deep root system. It has a significant commercial value due to its strong nutritional profile. Therefore, it is widely used in medicine, cosmetics, and industry. The demand for licorice grown in Uzbekistan has gradually increased since 2000, owing to its pharmacological qualities, which include glycyrrhizin and oleanane-type triterpene saponins. As a result, its native habitat declined rapidly in the deltas of the Amudarya and Syrdarya rivers in Uzbekistan (Khaitov et al., 2021).

Although licorice is known to be drought and salt-tolerant, the plant's reaction to these stresses differs according to its genotype and developmental stage. Major constraints are salt stress and water deficiency at the early licorice vegetation stages that can interfere with herb seed germination, impact seedling development, and potentially cause death through long-term suppression (Bao et al., 2024).

Soil beneficial bacteria facilitate plant growth, as a component of climate-resilient agriculture, has significant promise for rehabilitating abandoned saline lands. Licorice with soil beneficial bacteria can resist environmental stresses, improve soil's physical and chemical characteristics, enrich it with organic matter, and restore soil biological activity (Begmatov et al., 2020). These proactive measures support sustainable farming by increasing nutrient availability, improving soil health, and enhancing crop productivity, while also balancing environmental and ecological objectives. A key priority is improving soil health with a strong emphasis on sustainable natural resources management and resilience. Furthermore, the application of modern biotechnologies generate added value by creating a more suitable healthy environment for plant survival.

However, while extensive research has been conducted on bio-treatments for leguminous and medicinal plants, studies specific to licorice grown in saline soils are limited. Even though the region is suitable for long-term sustainable licorice production, licorice faces abiotic and biotic challenges, especially during the initial growth period. Therefore, this study aimed to examine the effectiveness of bio-treatment agents on licorice productivity such as root yield and phytochemical content as well as soil microbiological activity, revitalizing the saline dryland ecosystem.

Material and Methods

Climate and soil characteristics

The trial was established in agro-climatic zones in Nukus district at the Experimental Station of the Institute of Agriculture and Agrotechnologies of Karakalpakstan during the 2022-2023 vegetation seasons. The climate of the area is full of contrast. It is an arid Karakalpakstan area with very hot (> 40^{o}C) and dry summer in July-August and severe cool winters (< -20^{o}C) in January (Figure 1). This region experiences notable temperature variations throughout the year. There has also been an increasing trend in the number of sunny days with high temperatures in addition to a general trend of increasing degrees of aridity and salinity. Even daily temperature fluctuations can be substantial with hot days and cool nights. The extreme environmental conditions pose difficulty for year-round cropping, which is aggravated by very little rainfall, averaging around 90-120 mm annually.

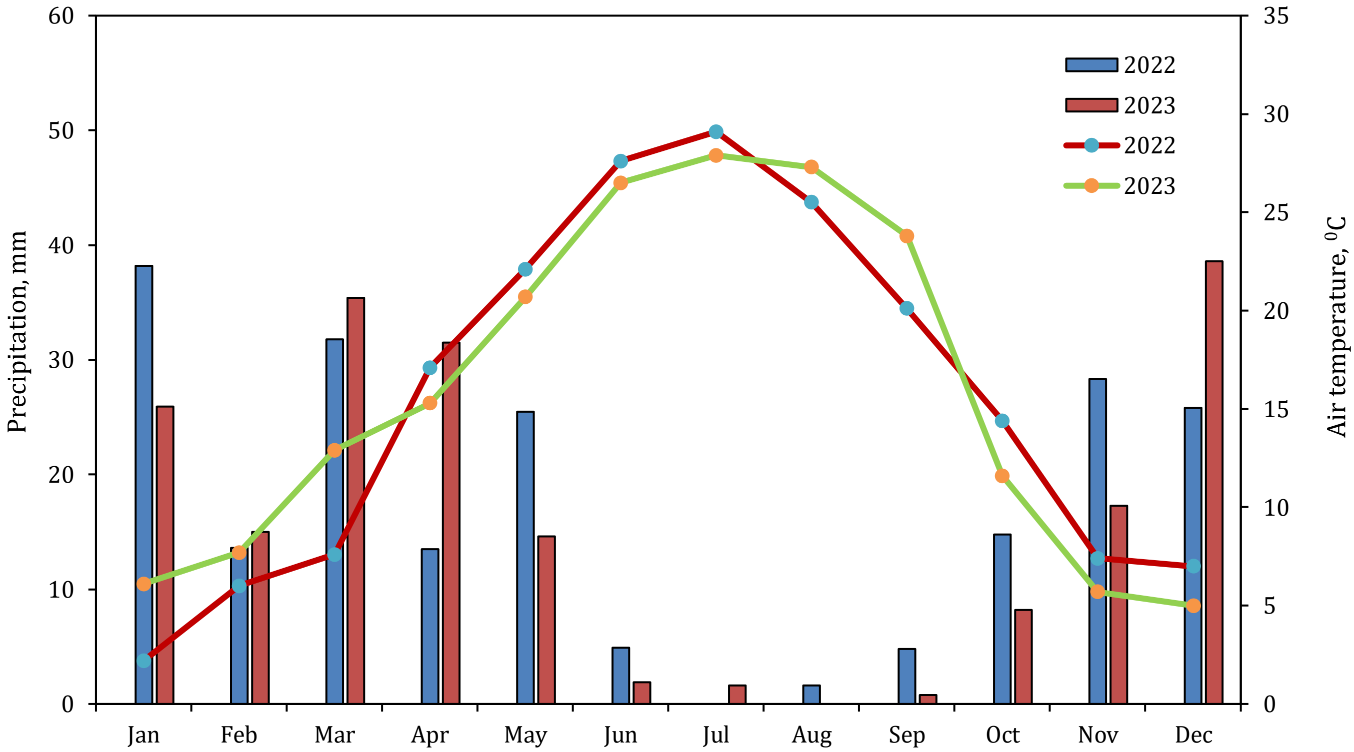

Figure 1. Climate records in Karakalpakstan, air temperature (in curves) and precipitation data (in columns) during 2022-2023.

Soil in this area is highly saline with an electrical conductivity (EC) ranging from 12 to 16 dS m^{-1}. The pH of the soil was in the range of 8-8.5 and had loam texture at the top 0- 17 cm and Silt loam at 17-150 cm soil depth (USDA classification). The humus content in the arable layer is 0.626%, in the lower horizons 0.277%, and in the parent rock 0.172%. The dominant cations were Na^{+} and Mg^{2+} with wide differences in their concentrations. The levels of sodium absorption ratio (SAR) were in the range of 4.38 to 11.43, suggesting that groundwater is marginal in its characteristics.

The experimental field was divided into small sections (0.03-0.05 ha) to carry out the salt leaching process with 2500-3200 m^3/ha water. The field was leached three times as per the traditional cropping practice (the first one was on February 21 and the second one was on March 14). The third and last leaching activities were carried out at the beginning of April. After the leaching activity, the experimental plot was deep plowed (30 cm depth) and chiseled one more time and root residues were removed manually. Mineral fertilizers were ($N_{100}P_{140}K_{80}$) uniformly applied to the fields before seed planting. Usually, after the irrigation of the field soil gets harder in clay loam plots. Therefore, it is necessary to use organic manure to make soil softer if available. Considering the local climatic conditions, weeding will be carried out by hand during the crop vegetation period when required. The high salinity levels in the soil and groundwater enforce the need to apply furrow irrigation, also known as flooding irrigation. Furrow irrigation was applied three times during the vegetation period at a norm of 800-1000 m^3 identical to all plots, totaling 2400-3000 m^3 per hectare.

Biofertilizers and their use

Bacterial fertilizers BIST (prepared on the base of Pseudomonas putida Pp-1 consists 1×10^7 CFU/mL), Zamin (Azotobacter Chroococcum consists 5×10^9 CFU/mL) were provided by the Institute of Microbiology, Academy of Science of Uzbekistan.

Geogumat consisted of a consortium of beneficial soil bacteria including more than 20 microorganisms, i.e. Bacillus megaterium, Bacillus mucilaginosus, Basillus Subtilis and etc. It is liquid of 12% organic fertilizer and formed by microelements. There is humic acid at least 32% and Fulfa and other organic acids consist of at least 25.0%.

Licorice seeds (cv. Tong shabnami) were provided by Botanika Scientific Research Institute, Tashkent, Uzbekistan.

When the ground warmed up sufficiently under the sun, seeding began on April 22–24. Before starting experiments, the seeds were sorted by eliminating broken, small and infected seeds. Surface-sterilized licorice seeds in the solution of 75mL chloride + 25mL water for 2-3 min, thoroughly rinsed 5 times with sterile water.

Then the inoculation process continued for 30 min with appropriate biofertilizers following the standard procedure before planting the seeds in the experimental plots. In order to avoid cross-contamination between the biofertilizers all the required protection measures were taken into consideration. The seed planting procedure was conducted manually in this case study at standards of 10 kg/ha. To increase germination and produce robust seedlings, the field was irrigated following the seeding procedure. Before second furrow irrigation, the norm remaining of N and 20% of P and K fertilizers were delivered after weeding and cultivation activities.

Experiment design

The selected field was abandoned from agricultural production for the last 15 years, hence heavily infested with wild vegetation. A lot of effort in terms of heavy machinery like bulldozer was used to bring back this field into shape for crop cultivation. The experimental field with a size of 135 x 100 m was laser leveled at the beginning of the land preparation process.

The experiment was set out in three replications using a randomized complete block design (RCBD) with a split-plot treatment structure. The experiment's total land area was 0.12 hectares, of which each plot measured 96 square meters (4.8 m x 20 m, or 8 rows, each measuring 0.6 m in width and 20 m in length). Each plot's accounting area measured 24 square meters. These field trials were carried out in compliance with the "Methods of Field Experiments" (UzPITI, 2007).

Data collection and chemical analysis

The parameters of seed yield, root mass, and fodder productivity were measured on a 1 square meter area in each plot. Two phases of plant density were measured: following seedling emergence and at the end of the vegetation period. The 25 tagged plants in each plot were subjected to agronomic measurements, including plant height, weight, and the quantity of leaves and pods.

The number of leaves, plant height, biomass of plants, particular leaf weights (leaf dry weight, leaf: shoot dry ratio, shoot dry matter), leaf area, and chlorophyll content were all measured during the field trials. At the end of each season, root mass was calculated by using the monometer method which is conducted by digging up to one meter and then drying in a drier apparatus for 72 hours at 700C. Using specialized equipment, 20 samples on 1 cm2 were taken to determine the leaf area.

Plant extracts, i.e., ash, glycyrrhizic acid, extractive compounds and flavonoids were determined by spectrophotometry in the Mettler_Toledo laboratory complex. Extraction method of glycyrrhizic acid analysis was as follows: samples extracted with 70% ethanol or methanol-water (50:50 v/v) using ultrasonic or Soxhlet extraction. Hydrolysis with HCl was done to release glycyrrhizic acid from glycosides. The absorbance was measured at 254 nm using HPLC-UV for direct UV absorption.

The selection of actinomycetes, ammonifiers, and oligonitrophils as indicators of soil microbial activity is based on their critical ecological roles in nutrient cycling, soil fertility, and organic matter decomposition. Several microbiological analyses were conducted to determine the microbial activity of soil samples at the beginning and end of the experiment. The serial dilution plate method was employed to observe total numbers of actinomycetes, ammonifiers and oligonitrophils by counting colony-forming units (CFUs) on agar plates (25-30^{o}C) during 5-7 days. The medium used for the enumeration of total numbers of spores and micromycetes was soil agar.

Data analysis

A one-way ANOVA tool (CropStat statistical software program) was used for statistical analysis of collected data during the 2022-2023 vegetation seasons. Tukey's least significant difference (LSD) test was employed to determine the significance of mean value differences and all experiments were conducted in duplicate. Microsoft Office Excel 2007 was used to create the graphics.

Results

Effect of biofertilizers on soil nutrient and microbial content

The application of the biofertilizers as a seed treatment for licorice cultivation significantly increased soil humus content and total form N and P as compared to the control group (Table 1).

Table 1. Soil chemical analysis.

Soil depth (cm)	Soil ECe (dS/m)	Soil pH	Exchangeable Na percentage (ESP)	Total forms (%)		Exchangeable forms (mg/kg)		
				Humus	N	NO_3	P_2O_5	K_2O
Before the experiments								
0-15	15.6b	6.1	0.4	0.6a	0.010	12.2c	16.2b	522a
15-30	17.2a	6.5	0.4	0.6a	0.015	10.3c	10.8c	500b
30-50	17.8a	7	0.8	0.5b	0.001	7.12c	5.12e	484c
The BIST plot								
0-15	12.5d	7.1	0.7	0.7a	0.012	18.6b	18.4a	518a
15-30	13.4c	6.8	0.8	0.6a	0.014	15.1b	6.67	324d
30-50	13.8c	6.4	0.8	0.5b	0.009	12.3c	12.4c	226f
The Zamin plot								
0-15	11.1e	7.6	0.16	0.6a	0.009	17.0b	16.2b	572a
15-30	11.9e	7.6	0.19	0.6a	0.016	14.6b	10.8	500b
30-50	13.1cd	7.7	0.3	0.5b	0.009	10.1c	8.3d	494c
The Geogumat plot								
0-15	9.2 g	7.7	0.1	0.7a	0.031	22.8a	19.6a	548a
15-30	10.2f	7.4	0.99	0.7a	0.019	16.1b	14.3c	341d
30-50	11.8e	7.5	0.8	0.5b	0.006	11.7c	9.4d	263e

Means of three replications (n = 3) separated by lowercase letters (a and g) in each column are significantly different at *P*≤5% according to the Tukey's LSD test.

The exchangeable NO_3, P_2O_5 and K_2O contents were enhanced significantly with the application of the licorice seed treatments, thereby increasing soil fertility parameters.The effect was more pronounced at the Geogumat biotreated plot, exhibiting the highest soil fertility index. Whereas soil salinity indicators decreased significantly in the plots where licorice seeds were planted with bacterial inoculations. The highest effect was seen in the Geogumat plot followed by Zamin and BIST treatments.

Likewise, soil chemical properties were substantially affected by the seed bio-treatments. Particularly, exchangeable forms of NO_3 value increased by 52.5, 39.3 and 144.3% respectively compared to the control value at 0-15 cm soil horizon under BIST, Zamin and Geogumat bio-treatments. Similarly, P_2O_5 and K_2O parameters were higher significantly than that of the control group. In terms of humus content, there was some increase even though it did not reach to a significant point during the two-vegetation period.

As Table 2 shows, the seed bio-treatments increased soil microbial activity, i.e., ammonifiers, spores, oligonitrophils, micromycetes, actinomycetes. The application of Geogumat produced the greatest number of the aforementioned soil bacterial communities, followed by BIST and Zamin bio-treatments.

The humus content of the soil significantly improved as a result of these beneficial soil interventions. Similarly, the application of bio-treatments resulted in the greatest levels of total N and P. Increased nutrient availability keeps the ameliorative properties of the soil intact and revitalizes the ecology of the soil. Significant favorable connections were found between the bio-treatments utilized in this experiment and the soil's N cycle. In turn, exchangeable NO_3, P_2O_5, and K_2O concentrations are closely correlated with increasing the overall form of N in the soil.

Table 2. Quantity of microorganisms in the licorice root rhizosphere as affected by the bio-treatments (averaged across two growth seasons).

Treatments	Ammonifiers	Oligonitrophils	Micromycetes	Actinomycetes
At the beginning of the experiment				
	$5.2x10^6$d	$3.1x10^4$d	$5.5x10^3$c	$4.2x10^4$d
At the vegetation period				
Control	$4.5x10^6$c	$4.5x10^5$c	$9.1x10^3$d	$7.5x10^4$c
BIST	$6.3x10^7$b	$2.7x10^6$b	$3.7x10^4$b	$3.4x10^5$b
Zamin	$3.5x10^6$c	$5.2x10^5$c	$1.5x10^4$d	$3.5x10^5$b
Geogumat	$7.5x10^8$a	$3.5x10^7$a	$7.5x10^4$a	$4.5x10^5$a

Means of three replications (n = 3) separated by lowercase letters (a and c) in each column are significantly different at *P*≤5% according to the Tukey's LSD test.

Early research supported a similar phenomenon, demonstrating that certain macronutrients are stoichiometrically related to preserving ecosystem balance (Aziz et al., 2013). This study demonstrated how the bacterial inoculant, when combined with legumes, increased the population and diversity of soil microbes in salt soils. A large number of soil microorganisms, which are essential to the nutrient cycling and ecosystem functioning of the soil, determine the majority of the accessible nutrient content of the soil (Hammerschmiedt et al., 2021). The seed inoculation produced the highest microbial activity, indicating that the bacterial injection improves the biological condition of the soil.

Licorice fodder yield parameters

Table 3 shows the seed germination and yield production under salt stress. The seed germination increased by 34.1, 44.6 and 57.2% under BIST, Zamin and Geogumat treatments, respectively. Likewise, the fodder yield index was significantly higher in the seed biotreated plots as compared to the control.

Table 3. Effect of biofertilizers on seed germination, fodder yield and quality parameters.

Treatments	2022				2023		
	Seed germination, %	Fodder yield	Food units	Digestible protein	Fodder yield	Food units	Digestible protein
Control	42.3d	143.3c	89.8c	22.0c	189.0d	112.2c	23.8c
BIST	56.7c	185.5b	98.2b	23.3b	220.4c	122.7b	25.1b
Zamin	61.2b	196.3ab	98.6b	24.8ab	231.4b	123.2b	25.2b
Geogumat	66.5a	211.4a	104.3a	25.7a	266.7a	128.6a	25.6a

Means of three replications (n = 3) separated by lower case letters (a and d) in each column are significantly different at *P*≤5% according to the Tukey's LSD test.

The highest increase in fodder yield was observed in the Geogumat variable, followed by Zamin and BIST, exhibiting 47.5, 36.9 and 29.4% increases as compared to the control group. This mode was seen in the values such as food unit and digestible protein in the plant shoot which were significantly higher than that of the control in the 2022 season.

Table 4. Effect of different biofertilizers on licorice fodder productivity and quality.

Treatments	Protein	Oil	Cellulose	Ash	Ca
Control	18.0c	17.0c	26.8c	7.07c	2.00c
BIST	18.9b	17.9b	27.5b	8.00b	2.08b
Zamin	19.1b	18.0ab	28.5ab	8.93a	2.10b
Geogumat	21.1a	18.2a	29.0a	8.94a	2.20a

Means of three replications (n = 3) separated by lowercase letters (a and c) in each column are significantly different at *P*≤5% according to the Tukey's LSD test.

A similar trend was observed in terms of fodder protein, oil, cellulose, ash and Ca parameters. Whenever, the highest parameters were monitored in the Geogumat treatment, followed by Zamin and BIST variables.

Under the Geogumat application, the difference in the fodder protein was substantially improved, while the increase was 16.5% higher than that of the control. While Zamin and BIST increased fodder protein parameters by 6.1 and 5%, exhibiting a significant difference as compared to the control values.

The absolute contents of fodder oil, cellulose, ash and Ca demonstrated an increasing trend under the applied bio-treatments, reaching to significant level in most cases.

Effect of biofertilizers on the root yield and phytochemical contents

Analysis of variance revealed that the used licorice seed bio-treatments led to a significant increase in root yield and its quality indicators (Table 5). The average root yield in the Geogumat, Zamin and BIST treatments were elevated by 37, 23 and 16%, respectively than that of the control. Similarly, secondary metabolites were produced significantly higher with the seed bio-treatments under saline environmental conditions.

Table 5. Licorice root yield and phytohemical changes due to biofertilizers

Treatments	Average root yield, t ha^{-1}	Total ashes, %	Glycyrrhiza acid, %	Extractive substances,%	Flavonoids, %	Quality, %
Control	30.0d	4.89b	7.00b	39.50b	2.00b	0.175b
BIST	34.8c	5.15a	7.81a	40.05b	2.23ab	0.187a
Zamin	36.9b	4.95b	7.82a	40.08b	2.24ab	0.180b
Geogumat	41.1a	5.23a	7.89a	41.20a	2.29a	0.191a

Means of three replications (n = 3) separated by lowercase letters (a and d) in each column are significantly different at *P*≤5% according to the Tukey's LSD test.

Meanwhile, the contents of glycyrrhizin were higher in the biotreated variables, reaching a significant level as compared to the control. Particularly, the maximum glycyrrhiza acid was achieved under Geogumat, followed by Zamin and BIST seed treatments, exhibiting 12.7, 11.7 and 11.5% increases over the control values. This two-year analysis found that greater licorice root components, i.e. total ashes, extractive substances, flavonoids parameters were achieved with the seed bio-treatment applications.

Discussion

The effectiveness of licorice inoculation in saline environment

Soil degradation in the Aral Sea region is caused by a number of reasons, including (i) improper irrigation and badly maintained drainage systems, (ii) waterlogging and subsequent salinization, and (iii) salt dispersion from dust storms due to the Aral sea crisis, that can reach a distance of 500 km (Begmatov et al., 2020; Makhanova and Ibraeva, 2025). Fine particles of sodium bicarbonate, sodium chloride, and sodium sulfate are carried by the wind and have an adverse effect on agricultural lands, crops and natural vegetation. Excessive amounts of these salts have a dangerous impact on soil health and further deteriorate plant growth and development, lowering crop production and harming food security (Chernyh et al., 2024). Considering local environmental characteristics some prerequisites must be taken into account for crop selection, such as adaptability, pest and disease resistance, marketability and profitability, accessible technology, and agricultural systems to reduce a high risk of climate change, soil salinity, and drought. Whereas, applied on-farm management techniques should provide profitability and sustainability.

Licorice production in abandoned lands in this area as a model of green technologies can contribute to a more sustainable agricultural system, promoting soil health and productivity. In addition to the theoretical discussion of how biofertilization affects licorice quality under salt stress, this study exhibited practical justification for the restoration of soil health and ecosystem functions. In degraded dryland systems, biotic obstacles, such as reduced beneficial soil microbial populations, and abiotic barriers, such as less moisture and nutrient availability and soil erodibility, frequently restrict ecosystem recovery (Chaudhary et al., 2020; Esmaeili et al., 2024).

Although the demand for active soil restoration is increasing, little is known about how these methods might be applied most effectively to enhance soil health in degraded drylands across environmental gradients. To restore soil health in degraded drylands, soil-based restoration strategies have become more popular in recent decades (Faist et al., 2020; Román et al., 2021). Nevertheless, the effectiveness of various soil treatments in encouraging the restoration of soil function has varied. According to a recent study, biocrust inoculation improved aggregate stability but straw barriers and soil tackifiers did not, suggesting that some treatments may be more effective than others at enhancing soil health (He et al., 2021).

However, investigations on the role of bioagents in stimulating and alleviating salt stress for licorice production are important. Therefore, the effectiveness of up-scaling of licorice with bioinoculants was practiced in saline abandoned lands of Karakalpakstan. Despite the fact that licorice is an ingenious perennial legume that can withstand drought and saline pressures, crop producers do not cultivate it in subsistence farming systems. Achieving these aims and scaling up necessitates a change in the current environmental situation. If thorough salinity alleviation methods are not performed prior to planting, the salinization damage will be catastrophic for licorice species with medium- and high salt tolerance. Biological solutions are a practical answer to soil salinization restriction that will increase productivity and revenue generation. Previous studies also exhibited a tendency for the increase of licorice root yield quality parameters initially under salt and water stresses and then falling rapidly as the stress increased (Zhao and Li, 2023).

The planning method involved categorizing agricultural land based on production, taking into account a number of elements related to soil qualities and fertility. It is apparent that current agricultural intervention should be supported, and markets established accordingly.

In addition to the improvement of seed germination, root system development, and seedling growth, the used biofertilizers increased crop output by 15% to 20% and increased tolerance to environmental stressors. They also increased the efficiency of mineral fertilizers and the quality of the soil. The Geogumat application had the highest overall output as determined by total root biomass with a 30% increase over the control. This suggests that beneficial bacteria might be especially useful for encouraging the growth of licorice roots. A significant increase in yield was also observed in the compost-treated group, indicating that organic matter promotes the growth of root biomass.

With an average rise of 25%, licorice plants treated with bio-treatments demonstrated a considerable increase in root length when compared to the control group. This discovery emphasizes how biofertilizers can promote root development, most likely because of better nutrient uptake. Although it turned out that plants in the nitrogen-fixing bacteria group also had higher root biomass.

Nutrients turnover

Licorice requires fertilization as a fundamental factor to survive, grow and produce high-quality raw materials. Since licorice is a nitrogen-demanding crop, especially in its early stages, the N fixation ability provides an efficient alternative to synthetic fertilizers. Plant growth may still be stunted if fertilizers are delivered in low or excess amounts of what is needed (Khaitov et al., 2022).

The findings of this study demonstrated that the presence of salt-tolerant bacteria in licorice rhizosphere triggered the synthesis of a nitrogen fixation mechanism, which further enhanced plant tolerance to water and salt stresses and thereby enhanced the production of alkali and metabolites, so making a significant contribution to agroecology and development. Various studies have demonstrated the benefits of bio-treatment in improving growth parameters and enhancing plant resilience against biotic and abiotic stresses.

Licorice root exudates can promote the growth of beneficial bacteria by providing a carbon source. Certain beneficial bacteria, such as Bacillus and Pseudomonas, use these compounds to boost their growth and metabolism, which in turn can lead to improved nutrient cycling. The nutrient availability in the soil was greatly impacted by the seed inoculation applications. The amount of N and humus content in the soil primarily reflects the favorable relationship between licorice and microbes. The appropriate match between the two organisms, as well as anthropogenic and environmental factors, determine the fixed amount of N (Li et al., 2022).

Licorice's organic compounds can interact with soil minerals, enhancing nutrient solubility. Beneficial bacteria work with these compounds to break down organic matter, releasing nitrogen, phosphorus, and other essential nutrients that plants can absorb more efficiently. As this study shows, the induction of a nitrogen fixation mechanism in the presence of salt-tolerant bacteria reduced water and salt stress and enhanced the synthesis of alkali in medicinal plants, thereby improving soil health and agroecology. Some compounds in licorice can suppress pathogenic organisms, reducing disease pressure on plants. Beneficial bacteria work synergistically with these compounds to outcompete harmful pathogens, creating a healthier root-zone environment.

According to Abdiev et al. (2019) and Aşık and Arıoğlu (2020), this approach was previously well-documented and demonstrated that symbiotic N_2 fixation in legumes with competent rhizobia plays a crucial role in compensating for missing soil nitrogen (N) and potentially overcoming nutrient deficit (Khaitov et al., 2024). When combined, the revitalization of beneficial soil bacteria greatly benefited plant health and soil biological processes.

Biofertilizers are known to boost root-based crops by promoting nutrient uptake and increasing root length and surface area, which is consistent with studies in other leguminous and medicinal plants. This process enhances the soil microbiome's biodiversity, fostering a balance where beneficial microbes dominate over harmful ones. As it turned out this technique has a great value in alleviating nutritional deficiency in degraded lands.

Enhanced soil health and microbial diversity

When used with beneficial bacteria, licorice's organic material can enhance soil aggregation. The bacteria help bind soil particles together, forming stable soil aggregates that improve aeration, root penetration, and water retention (Dahnoun et al., 2024). This combination improves root establishment, reduces soil erosion and salinity stress. Licorice roots and plant residues contribute organic matter that, when broken down by beneficial bacteria, boosts soil organic carbon (Dang et al., 2021). This improves soil fertility, resilience to climate extremes, and the overall carbon sequestration potential of the soil (Li et al., 2018).

Integrating licorice extracts or residues with beneficial bacteria could be a sustainable soil management approach, enhancing nutrient cycling, soil fertility, and crop resilience against diseases and stress factors. The highest number of soil beneficial bacteria achieved with the seed bio-treatments suggests that this technique might be a key factor in restoring soil microbial activity. Soil study conducted at the end of the vegetation season showed that the use of biofertilizers enhanced the amount of organic matter and soil microbial activity, both of which are factors in better soil health. This implies that bio-treatment improves the soil environment and promotes plant growth, resulting in a more sustainable licorice cultivation system. Also, this study suggests that plant productivity is positively correlated with various groups of beneficial soil microbes interacting with the root rhizosphere.

The current study facilitated predictive knowledge in improving the ecological contexts in which soil-based restoration treatments may be most effective for enhancing soil health within the wide range of restoration outcomes. Numerous studies demonstrated the effect of salt-tolerant bacteria beneficial bacteria on medicinal plants under a stressful environment and assessed the change in the pharmacological value of the host plant's metabolites influenced by this flora (Verma et al., 2020; Sharma et al., 2022). The findings also indicated a positive impact on agroecology and soil health due to the presence of salt-tolerant bacteria in legume's rhizosphere that stimulate the nitrogen fixation mechanism, thereby enhancing alkali production in medicinal plants. Chua et al. (2019) discovered that microbial diversity had a greater influence on soil health, whereas effects may vary among ecosystems. According to Luna et al. (2016), organic additions including microbial compounds improved several elements of soil health more effectively than other approaches. According to other research, soil microorganisms significantly influence the success of restoration initiatives, supporting ecosystem functions and services (Wang et al., 2018).

The microbial activity was noticeably higher in the Geogumat group than in the control group, indicating increased root exudates, which may support greater rhizosphere microorganisms. Given this, microbes living in the rhizosphere of licorice can form a mutualistic association and coordinate their involvement in plant adaptations to stress tolerance. The plant growth dynamics treated with the organic substances improved substantially, suggesting the seed bio-treatments enhanced nutrient availability. It is important to point out that licorice cultivation technology with the appropriate seed treatment agents could also be commercialized in the future. As turned out in this study, these strategies ensure sustainable resource utilization, increasing crop areas and improving soil health without compromising water deficiency problems.

Conclusion

This study showed the importance of providing adequate seed bio-treatment to maximize licorice root yield while improving soil health, especially in abandoned saline lands. A more pronounced effect was observed when Geogumat was applied as a seed bio-treatment, generating the highest root yield of 41.1 ton ha^{-1} which was 37% higher than the control group. Along with Geogumat, Zamin and BIST seed treatments also played a crucial role in improving soil quality parameters, soil bacterial populations and nutrient availability, likely due to increased soil microbial diversity, nutrient turnover and better root-soil interactions under soil salinity.

Soil restoration with these biological approaches contributes to ecosystem recovery in drylands, preventing irreversible soil degradation in these fragile desert areas. In addition to recovering soil health. This approach also plays a critical role in supporting biodiversity, wildlife habitats and the health of the surrounding ecosystem. Further actions should focus on the integration of this cost-effective organic practice into large-scale agricultural production, applying it for salt-affected soil reclamation, and promoting its benefits to crop producers.

Acknowledgements

This study was carried out within the GEF-funded project entitled "Food Systems, Land Use and Restoration Impact Program in Uzbekistan" (FOLUR). The Food and Agriculture Organization of the United Nations (FAO) has been implementing this national project since 2022 at the demonstration sites in Uzbekistan with a target to improve land use and restoration, and crop yield in the arid areas.

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