Effects of Microbial Fertilizers on Soil Improvement and Wheat Growth Characteristics in Saline-alkali Land

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Abstract [**Objectives**] This study was conducted to further enrich the research on saline-alkali land improvement, and explore the effects of biological bacterial fertilizers containing *Bacillus subtilis* and *Bacillus velezensis* HM-3 in saline-alkali land improvement and crop growth promotion. [**Methods**] Wheat was planted in saline-alkali land in Huanghua City, Hebei Province, and a mixed application experiment was carried out using biological agents from Hemiao Biotechnology Co., Ltd. [**Results**] Compared with the field of control check (CK), water-soluble salts and pH value in the experimental fields decreased, and living bacteria count in the soil increased. Meanwhile, the economic characters of wheat in the experimental fields showed excellent performance, with yields increasing by 39.09% and 27.49% compared with the CK. It could be seen that the application of biological bacterial fertilizers achieved obvious effects of improving saline-alkali soil and increasing wheat yield. [**Conclusions**] In this study, the effects of biological bacterial fertilizers on saline-alkali land and wheat growth characters were clarified, providing some technical support and theoretical guidance for wheat planting in Huanghua saline-alkali land.

Key words Saline-alkali land; Soil improvement; Wheat; Yield
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Saline-alkali land is an area with high content of salt and alkaline substances in soil. It is characterized by high salt concentration on the surface, poor soil structure, low water content and extreme soil pH[1], which will destroy the stability of crop cell membrane[2-4], reduce the root activity and photosynthetic function of crops[5] and limit the production of crops. Soil salinization has become an increasingly serious global problem. According to statistics, the total area of saline-alkali land in China is about 99.13 million hm^2, of which about 36.7 million hm^2 has certain utilization value[6-8]. The utilization of improved saline-alkali land can effectively relieve the bearing pressure of cultivated land and guarantee food security, and scientific and reasonable fertilization is the key to improve saline-alkali land and ensure high yield and high quality of crops.

In recent years, China has made remarkable achievements in the control and improvement of saline-alkali land, mainly through measures such as hydraulic engineering improvement, chemical improvement, physical improvement and biological improvement[9-12]. These methods mainly focus on mechanical ploughing, surface covering, application of soil improvers, utilization of underground saline water resources and planting salt-tolerant plants. However, there are still many shortcomings in these methods, such as being greatly affected by natural factors including rainfall, requiring physical measures such as leaching when using chemical improvers and relying more on fresh water resources, and the study on salt-tolerant mechanism and variety research and development of salt-tolerant plants are not deep enough, which affects the sustainable utilization of saline-alkali land.

Among them, biological measures stand out because of their most ecological and economic benefits. Studies show that microbial agents can not only improve soil quality and repair saline-alkali land, but also promote crop growth to some extent[13-15]. Zhang *et al.*[16] found that the application of compound microbial agents (effective strains include *Bacillus megaterium*, *Bacillus licheniformis* and *Trichoderma viride*) was beneficial to soil improvement and safflower growth in saline-alkali land, and achieved the dual effects of restoring soil and increasing crop yield. Among them, Bacillus has good biocontrol effect and can further improve crop disease resistance. Hou *et al.*[17] found that lactic acid bacteria compound preparations could effectively reduce the pH of saline-alkali soil and increase the number of actinomycetes and nutrient content in soil, and had good improvement effect. However, at present, the microbial improvement of saline-alkali land is still in the primary stage, and it is difficult to meet the needs of saline-alkali land treatment because of the high cost of applying more compound microbial agents. In this study, *Bacillus velezensis* HM3, a

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growth-promoting strain showing excellent tolerance in saline-alkali environment and both saline-alkali land improvement and growth-promoting effects, was applied to wheat in saline-alkali land in Huanghua City, Hebei Province in combination with bio-organic fertilizers in the planting process, so as to explore the effects of applying different kinds of fertilizers in improving saline-alkali land and promoting crop growth, as well as the salt tolerance and growth promotion effect of selected strains in actual production. This study achieved the dual effects of restoring soil and increasing crop yield, providing a theoretical basis for further utilization of saline-alkali land.

Materials and Methods

Time and location

Experimental time The experiment was carried out from October 2023 to June 2024.

Experimental location The experiment was carried out in Houxianzhuang Village, Huanghua City, Cangzhou City, Hebei Province (E: 17.394 642°, N: 38.327 108°).

Conditions of experimental field

Basic conditions of experimental field The soil type was loam. The previous crop was maize. Controlled release fertilizer for maize was applied at a rate of 600 kg/hm^2 as the base fertilizer, and the yield of maize was 2 250 kg/hm^2.

Soil nutrient status in experimental field Soil nutrient status in sample plot 1: Organic matter 11.7 g/kg, ammonium nitrogen 30.269 mg/kg, available phosphorus 33.614 mg/kg, available potassium 82.354 mg/kg, pH 8.32.

Soil nutrient status in sample plot 2: Organic matter 13.5 g/kg, ammonium nitrogen 39.237 mg/kg, available phosphorus 36.577 mg/kg, available potassium 89.456 mg/kg, and pH value 7.91.

Soil nutrient status in sample plot 3 (control): Organic matter 11.8 g/kg, ammonium nitrogen 33.956 mg/kg, available phosphorus 39.237 mg/kg, available potassium 80.307 mg/kg, pH 8.24.

Experimental fertilizers

The experimental fertilizers were a series of biological agents produced by Qinhuangdao Hemiao Biotechnology Co., Ltd., namely microbial inoculum for seed dressing, granular bio-organic fertilizer as the base fertilizer and powder bio-organic fertilizer No.1, No.2 and No.3 for root irrigation. Main technical indexes of bio-organic fertilizers were as follows: containing *Bacillus subtilis*, *B. velezensis* HM3, *etc.*, effective viable bacteria count ≥20 million/g, organic matter ≥ 40%.

Crop variety

Crop variety: Jiemai 19.

Experimental methods

Experimental design There were three treatments in the experiment. Treatment 1 was sample plot 1, with an area of 0.67 hm^2, sown with 300 kg/hm^2 of wheat seeds. Treatment 2 was sample plot 2, with an area of 0.91 hm^2, sown with 300 kg/hm^2 of wheat seeds. Treatment 3 was sample plot 3 with an area of 0.67 hm^2, sown with 300 kg/hm^2 of wheat seeds.

Fertilization situation of various treatments Wheat seeds were sown in the experimental field on October 17, 2023. The fertilization schemes of various treatments were as follows:

Treatment 1: The seed dressing agent was mixed with wheat seeds at a rate of 0.1 kg/kg. Diammonium phosphate was applied at a rate of 420 kg/hm^2 as the base fertilizer. On November 10, 2023, root irrigation was carried out with 50.145 kg/hm^2 of powder bio-organic fertilizer No.1 at the seedling stage. On February 27, 2024, 90.375 kg/hm^2 of powder bio-organic fertilizer No.2 was used to irrigate the roots at the re-greening stage. On April 26, 2024, root irrigation was carried out with Hemiao No.8 at a rate of 450 g/hm^2.

Treatment 2: Diammonium phosphate was applied at a rate of 420 kg/hm^2 as the base fertilizer. On March 15, 2024, 48.72 kg/hm^2 of powder bio-organic fertilizer No.3 was used to irrigate the roots at the re-greening stage. On April 26, 2024, root irrigation was carried out with Hemiao No.8 at a rate of 450 kg/hm^2.

Treatment 3: Diammonium phosphate was applied at a rate of 450 kg/hm^2 as the base fertilizer.

Yield was measured on June 7, and other management measures in the experimental field were consistent.

Item determination

Determination of soil physical and chemical indexes Before planting, soil samples were collected on October 12, 2023, and after planting, soil samples were collected on June 6, 2024. All the data was average values measured after five-point sampling and mixing.

Soil available nitrogen was determined by the alkaline hydrolysis diffusion method. Soil available phosphorus was determined by the sodium bicarbonate extraction-molybdenum antimony colorimetric method. Soil available potassium was determined by the ammonium acetate leaching-flame photometry. Soil total salt was determined by the drying mass method. pH value was determined by the glass electrode method.

Determination of soil microbial communities The soil samples before planting were collected on October 12, 2023, and the soil samples after planting were collected on May 29, 2024. The living bacteria count before and after planting was determined by the plate count method.

Determination of wheat growth status Wheat plant samples were collected on June 6. Plant height (cm), plant uniformity, stem diameter (mm), fibrous root number (root), fibrous root length (cm), effective tiller number (number per plant), ear length (cm), number of ears per unit area, number of grains per ear and 1 000-grain were measured on June 7 and 8, and their average values were recorded.

Wheat was harvested on June 7, 2024. The investigation method was five-point sampling. During yield measurement, 20 m^2 was harvested from each sampling point in each treatment to obtain yield, and the unit yield was calculated.

Data processing

Excel 2010 and DPS 7.05 were used for processing the data.

Results and Analysis

Effects of fertilization on soil salinity and alkalinity

In order to explore the effects of fertilization on soil salinity and alkalinity, samples were collected before and after planting to determine the total amount of soil water-soluble salts and soil pH value. As shown in Table 1, the total amount of water-soluble salts in treatment 1 decreased by 15%, and that in treatment 2 decreased by 5.56%; and the pH value of treatment 1 decreased from 8.32 to 7.76, and that of treatment 2 decreased from 7.91 to 7.45, that is to say, the two values decreased by 6.73 and 5.82%, respectively. It could be seen that after fertilization treatment, compared with the CK, the soil pH value decreased. It could be clearly seen that compared with the CK showing no change in the total amount of water-soluble salts before and after planting, the values of experimental groups both decreased, indicating that soil salinity and alkalinity changed after fertilization treatment.

Table 1 Soil test of saline-alkali wheat fields

Treatment	Treatment 1			Treatment 2			Treatment 3 (CK)		
Sample	Before planting g/kg	After harvesting g/kg	Increasing or decreasing rate//%	Before planting g/kg	After harvesting g/kg	Increasing or decreasing rate//%	Before planting g/kg	After harvesting g/kg	Increasing or decreasing rate//%
Organic matter	11.7	15.44	31.97	13.5	19.65	45.56	11.8	9.4	-20.34
Water-soluble salts	2	1.7	-15	1.8	1.7	-5.56	1.8	1.9	5.56
pH	8.32	7.96	-5.52	7.91	7.45	-5.82	8.24	8.21	-0.36

Effects of fertilization on soil nutrient contents In order to explore the effects of fertilization on soil nutrient contents, samples were collected before and after planting to determine ammonium nitrogen, available phosphorus and available potassium contents in the soil. It can be seen from Table 2 that the ammonium nitrogen contents in the experimental fields after planting were, respectively, 24.544 and 29.314 mg/kg, which were 18.91 and 25.29 mg/kg lower than that before planting, while the ammonium nitrogen content in the ammonium nitrogen content in the field of the CK was only 10.86 mg/kg lower than that before planting. The experimental results showed that the content of ammonium nitrogen decreased and the conversion effect of ammonium nitrogen was strong. The application of microbial fertilizers enabled microorganisms to root in the soil and continuously metabolize during their growth process, and their secretions could degrade soil organic matter, regulate soil nutrient status, and enhance the nitrogen-retention capacity of saline-alkali soil.

Table 2 Determination of nitrogen, phosphorus and potassium contents in soil of saline-alkali wheat fields

Treatment	Treatment 1			Treatment 2			Treatment 3 (CK)		
Sample	Before planting mg/kg	After harvesting mg/kg	Increasing or decreasing rate//%	Before planting mg/kg	After harvesting mg/kg	Increasing or decreasing rate//%	Before planting mg/kg	After harvesting mg/kg	Increasing or decreasing rate//%
Ammonium nitrogen	30.269	24.544	-18.91	39.237	29.314	-25.29	33.956	30.269	-10.86
Available phosphorus	33.614	28.731	-14.53	36.577	20.569	-43.77	39.237	28.75	-26.73
Available potassium	82.354	95.922	16.48	89.456	95.324	6.56	80.307	70.119	-12.69

Meanwhile, from Table 3, it can be seen that the application of seedling fertilizers to wheat cultivated in saline-alkali land could increase the number of beneficial microorganisms in the soil, and the total contents of bacteria, fungi and total numbers of in the experimental field increased by more than 190.05%, 104.57% and 159.71%, respectively. The experimental results showed that the treatment with microbial fertilizers significantly improved the microbial ecological environment of the soil, promoted the growth of beneficial microorganisms in the soil, increased the diversity of soil organisms, and formed a stable soil ecosystem.

Table 3 Microbial community testing items of Huanghua saline-alkali wheat fields

Treatment	Treatment 1			Treatment 2			Treatment 3 (CK)		
Sample	Before planting cfu/g soil	After harvesting cfu/g soil	Increasing or decreasing rate//%	Before planting cfu/g soil	After harvesting cfu/g soil	Increasing or decreasing rate//%	Before planting cfu/g soil	After harvesting cfu/g soil	Increasing or decreasing rate//%
Total count of bacteria	8.41×10^5	13.8×10^5	64.09	5.91×10^5	12.19×10^5	104.74	5.69×10^5	5.22×10^5	-8.26
Total count of fungi	0.85×10^3	4.71×10^3	454.11	1.12×10^3	4.88×10^3	335.71	3.24×10^3	3.87×10^3	19.44
Total count of actinomycetes	1.38×10^4	1.56×10^4	13.04	9.66×10^4	11.17×10^4	15.63	1.43×10^4	1.93×10^4	34.97

Effects of fertilization on biological characters of wheat in saline-alkali land at the re-greening stage

According to field investigation, wheat applied with tested fertilizers grew vigorously, which was reflected by dark green and shiny leaves and strong plants. It can be seen from Table 4 that the agronomic characters of wheat in treatment 1 and treatment 2 were better than those in the CK, and the proportions of vigorous seedlings in the experimental fields were, respectively, 63.6% and 65.6%, which were greater than that in the CK. Moreover, wheat plants in the experimental fields also had good biological characters, among which the average plant height in the experimental fields was more than 15 cm, which was 3 cm higher than that in the CK. The total numbers of tillers in the experimental fields were 7.92 million and 7.965 million tillers/hm^2, respectively, while that in the field of the CK was only 7.395 million tillers/hm^2. The experimental results showed that the application of microbial fertilizers for seedlings had a good promotion effect on the economic characters of wheat in saline-alkali land, reflecting the excellent effect of microbial fertilizers for seedlings in promoting crop growth.

Table 4 Effects of different treatments on biological characters of wheat at the re-greening stage

Treatment	Number of leaves on main stem (leaf number)	Plant height cm	Secondary roots per plant//roots	Number of tiller per plant//tillers	Total number of tillers 10^4 tillers/hm^2	Number of large tillers with three leaves 10^4 tillers/hm^2	Classification of wheat seedlings//% Vigorous plants	Class 1	Class 2	Class 3
Treatment 1	6.7	15.3	16.3	2.7	792.0	424.5	63.6	17.4	5.8	13.2
Treatment 2	6.4	15.6	14.4	2.5	796.5	457.5	65.2	15.3	7.7	11.8
Treatment 3 (CK)	5.8	12.6	8.2	1.6	739.5	384.0	59.3	18.6	9.2	12.9

Effects of different treatments on wheat yield

Table 5 shows the statistical results of field investigation and laboratory test. The ear number of treatment 1 was as high as 5 407 230 ears/hm^2, which was 613 590 ears/hm^2 higher than that of the CK. Compared with treatment 3, the numbers of grains per ears in treatment 1 and treatment 2 increased by 4 and 2 grains, respectively, and the 1 000-grain weights increased by 2.8 and 2.9 g, respectively. The results showed that on the basis of conventional fertilization, the number of grains per ear and 1 000-grain weight could be increased by applying seedling fertilizers.

The application of seedling fertilizers increased wheat yield. It can be seen from Table 6 that the average yield of treatment 1 increased by 1 514.75 kg/hm^2 compared with treatment 3, with an increasing rate of 39.09%. Compared with treatment 3, the average yield of treatment 2 increased by 1 072.50 kg/hm^2, with an increasing rate of 27.49%.

Table 5 Statistical results of field investigation and laboratory test

Treatment	Density ears /hm^2	Number of grains per ear//ears	1 000-grain weight//g
Treatment 1	5 407 230	35.0	30.2
Treatment 2	5 310 690	33.0	30.3
Treatment 3 (CK)	4 793 640	31.0	27.4

Table 6 Statistical results of wheat yield

Treatment	Plot yield//kg/20 m^2 I	II	III	IV	V	Mean	Equivalent yield//kg/hm^2	Comparing treatment 1 with treatment 3 kg/hm^2	%	Comparing treatment 2 with treatment 3 kg/hm^2	%
1	13.43	11.03	12.51	9.66	7.60	10.85	5 425.65	101.65	39.09	71.50	27.49
2	12.31	11.13	11.45	8.63	6.19	9.94	4 973.55	–	–	–	–
3	10.20	8.64	9.35	6.06	4.74	7.80	3 900.9	–	–	–	–

Paired statistical analysis among treatments

Paired statistical analysis of treatments 1 and 3 Table 7 shows the results of paired statistical analysis of treatments 1 and 3.

Table 7 Results of paired statistical analysis of treatments 1 and 3

Replicate	Treatment 1 X1	Treatment 3 X2	di X1-X2	di-d	(di-d)^2
1	13.43	10.2	3.23	0.18	0.03
2	11.03	8.64	2.39	−0.66	0.44
3	12.51	9.35	3.16	0.11	0.01
4	9.66	6.06	3.60	0.55	0.30
5	7.6	4.74	2.86	−0.19	0.04
x (mean), d	10.85	7.80	3.05		
Total	54.23	38.99			0.82

Standard deviation of single observation: $Sd = 0.45$; mean standard deviation $Sd1 = 0.45/2.236 = 0.20$; $t = 3.05/0.2 = 15.25$; the degree of freedom $n-1=5-1=4$, and according to table lookup, $t_{0.05}=2.78$, $t_{0.01}=4.60$.

Because $t=15.3>t_{0.01}=4.60$, the difference level between treatment 1 and treatment 3 was extremely significant, indicating that fertilizers from Hemiao Biotechnology Co., Ltd. had an extremely significant effect.

Paired statistical analysis of treatment 2 and treatment 3 Table 8 shows the results of paired statistical analysis of treatments 2 and 3.

Standard deviation of single observation: $Sd = 0.44$; mean standard deviation $Sd1 = 0.44/2.236 = 0.20$; $t = 2.14/0.2 = 10.70$; the degree of freedom $n-1=5-1=4$, and according to table lookup, $t_{0.05}=2.78$, $t_{0.01}=4.60$.

Because $t=10.70>t_{0.01}=4.60$, the difference level between treatment 2 and treatment 3 was extremely significant, indicating that fertilizers Hemiao Biotechnology Co., Ltd. had an extremely significant effect.

Paired statistical analysis of treatments 1 and 2 Table 9 shows the results of paired statistical analysis of treatments 2 and 3.

Table 8 Results of paired statistical analysis of treatments 2 and 3

Replicate	Treatment 2	Treatment 3	di	di-d	(di-d)^2
	X1	X2	X1-X2		
1	12.31	10.2	2.11	−0.03	0.00
2	11.13	8.64	2.49	0.35	0.12
3	11.45	9.35	2.10	−0.04	0.00
4	8.63	6.06	2.57	0.43	0.18
5	6.19	4.74	1.45	−0.69	0.48
x (mean), d	9.94	7.80	2.14		
Total	49.71	38.99			0.79

Table 9 Results of paired statistical analysis of treatments 2 and 3

Replicate	Treatment 1	Treatment 2	di	di-d	(di-d)^2
	X1	X2	X1-X2		
1	13.43	12.31	1.12	0.22	0.05
2	11.03	11.13	−0.10	−1	1.00
3	12.51	11.45	1.06	0.16	0.03
4	9.66	8.63	1.03	0.13	0.02
5	7.6	6.19	1.41	0.51	0.26
x (mean), d	10.85	9.94	0.90		
Total	54.23	49.71			1.35

Standard deviation of single observation: $Sd = 0.58$; mean standard deviation $Sd1 = 0.58/2.236 = 0.26$; $t = 0.90/0.26 = 3.46$; the degree of freedom n − 1 = 5 − 1 = 4, and according to table lookup, $t_{0.05} = 2.78$, $t_{0.01} = 4.60$.

Because $t = 3.46 > t_{0.05} = 2.78$, the difference level between treatment 1 and treatment 3 was extremely significant, indicating that powder fertilizers from Hemiao Biotechnology Co., Ltd. had an extremely significant effect.

Conclusions and Discussion

Biological improvement methods taking microorganisms as the core are an emerging agricultural measure that utilizes functional microorganisms to improve microbial communities of soil environment, enhance soil structure, and alleviate the salt stress effect of plants through various mechanisms[18-19]. These methods increase the number and types of beneficial microorganisms in the soil, promote microbial activities and reproduction, and provide a good micro-ecological environment for microorganisms in saline-alkali environment[16]. Microorganisms can also secrete extracellular polysaccharides to make the soil structure more stable[20], rapidly improve the soil aggregate structure from the microscopic point of view, and make the soil loose, leading to reduced fixation of phosphorus and potassium elements in the soil and improved permeability and nutrient preservation ability, which further improve the nutrient content of crops[21] and promote their growth and development. On the one hand, microorganisms can decompose organic substances in soil and release nutrients such as nitrogen, phosphorus and potassium for absorption and utilization by plants. It can not only improve the utilization rate of soil nutrients[22], but also secrete hormones and organic compounds related to plant cell growth and root development, and stimulate soil biological activities to activate soil nutrients and improve crop stress resistance[23]. On the other hand, for areas with poor soil conditions such as saline-alkali land, the application of biological agents and biological fertilizers can significantly improve the buffering performance of soil[24]. They reduce the contents of harmful ions and pH value in the soil through metabolic activities of microorganisms, making the soil more suitable for crop growth. Wang *et al.*[25] found that microbial fertilizers significantly reduced soil pH and total salt content, and microbial fertilizers containing Bacillus was beneficial to the improvement of saline-alkali soil. Other experiments of Peng *et al.*[26] showed that the mixed fertilizers of Bacillus subtilis, Bacillus megaterium and other strains had the ability to restore soil pH value, which is consistent with the research results of this study. The results of this study showed that the screened Bacillus strains could not only improve saline-alkali land, but also promote the growth of wheat in saline-alkali land.

In this study, the effects of biological bacterial fertilizers on the improvement of saline-alkali land and wheat growth traits were clarified. The results showed that the total amounts of water-soluble salts in the experimental fields decreased by 15% and 5.56%, respectively; the pH decreased to 7.76 and 7.45, respectively; and the living bacteria count in the soil increased, showing a good improvement effect. Meanwhile, in terms of wheat growth indexes, the yield increased by 39.09 and 27.49% respectively, and the ear number of plot 1 was as high as 5 407 230 ears/hm^2. The biological bacterial fertilizers also promoted excellent economic characters of wheat, showing a good growth-promoting effect on wheat planting in saline-alkali land. In this study, biological bacterial fertilizers significantly reduced the contents of ammonium nitrogen, available phosphorus and available potassium after wheat planting, and reduced soil pH and water-soluble salt content. The experimental results showed that applying fertilizers from Hemiao Biotechnology Co., Ltd. to wheat cultivated in saline-alkali land could effectively improve soil and reduce soluble-salt content and alkalinity. They could greatly increase the number of beneficial microorganisms in the soil, make wheat plants grow healthily, and greatly improve the proportion of vigorous seedlings, the number of grains per ear and 1 000-grain weight. They could greatly increase the yield of wheat, with an average yield increase of more than 30% per hectare, that is, an extremely significant yield-increasing effect. It shows that the application of fertilizers from Hemiao Biotechnology Co., Ltd. was beneficial to soil improvement and wheat growth in saline-alkali land, achieving the dual effects of restoring soil and increasing crop yield, and also providing a theoretical basis for further utilization of saline-alkali land.

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