

Original Article



Improving Effect of Microbial Agent on Saline-Alkali Soil and Promoting Growth of Wheat Seedling

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Abstract:

This study investigates the effects of microbial agents on the improvement of saline-alkali soil and the growth-promoting effects on wheat seedlings. Pot experiments were conducted with five treatments: a blank control, JK-2, JK-3, HM-7, and a composite microbial agent. The nitrogen, phosphorus, potassium content, and pH value of the soil before and after treatment were measured. Wheat indicators such as plant height, maximum leaf length, fresh weight, and dry weight were compared and analyzed. Additionally, a field experiment was conducted. The results showed significant changes in the nitrogen, phosphorus, and potassium contents, as well as a notable decrease in pH values after the application of microbial agents. The effect of increasing ammonium nitrogen content was ranked as composite agent > JK-2 > blank control > JK-3 > HM-7; the effect of increasing available phosphorus content was composite agent > JK-3 > HM-7 > JK-2 > blank control; the effect of increasing available potassium content was JK-2 > HM-7 > composite agent > JK-3 > blank control; and the decrease in pH was composite agent > JK-2 > JK-3 > HM-7 > blank control. Among these, the composite microbial agent increased ammonium nitrogen, available potassium, and available phosphorus by 172.23%, 90.42%, and 192.38%, respectively, compared to the blank group, and reduced the pH by 0.35, showing a good improvement effect on saline-alkali soil. The application of single microbial agents did not show significant growth-promoting effects on wheat seedlings, while the composite microbial agent showed significant differences. Wheat plant height, maximum leaf length, fresh weight, and dry weight increased by 22.02%, 18.97%, 33.7%, and 34.7%, respectively, compared to the blank control. Moreover, the field experiment demonstrated that the composite microbial agent significantly increased wheat yield. The composite microbial agent promoted the release and transformation of soil nutrients through its unique biological activity, thereby improving soil fertility. Additionally, its role in reducing soil pH helps improve the soil environment of saline-alkali land, creating more favorable conditions for plant growth, and shows a clear growth-promoting effect on wheat, providing data support for its application in saline-alkali soil improvement.

Keywords: saline-alkali soil; microbial agents; soil improvement; wheat; growth-promoting effect

Introduction

Saline-alkali land (soil) is a general term for salinized, alkalized, and saline-alkali soils,

referring to soils where soluble salts have accumulated excessively, thereby inhibiting or

damaging the growth and development of crops. According to the data from the Second National Soil Census, China has 1.6×10^8 hm² of saline soil, 8.6×10^5 hm² of alkaline soil, and about 1.8×10^7 hm² of different types of salinized and alkalinized soils. Nearly 20% of the country's arable land has become saline-alkali, covering an area of 7.6×10^6 hm²^[1-2]. Due to the increasingly sharp contradiction between land, population, and food, along with the improper use of fertilizers and pesticides, once fertile land is gradually losing its nutrients and becoming barren, making arable land a scarce resource. As a rich reserve of land resources, saline-alkali land holds tremendous development potential. To date, approximately 80% of saline soils remain underutilized, and their development is crucial to ensuring food security, fostering national economic growth, and effectively advancing the rural revitalization strategy^{Error! Reference source not found.}. Therefore, how to effectively improve saline-alkali land, enhance its soil quality, and increase agricultural productivity has become an important research topic in both agriculture and ecology.

The main restoration technologies for existing saline-alkali land currently include hydraulic engineering techniques, physical measures, chemical measures, and biological measures. Among these, biological measures have gained widespread recognition and application in agriculture due to their high efficiency, cleanliness, and safety. These measures primarily involve the careful selection and planting of salt-tolerant plant varieties and the utilization of microbial resources. As a new type of biological fertilizer, microbial agents have shown great potential in the improvement of saline-alkali land. For example, a study by Wang Guoli and colleagues from the Chinese Academy of Agricultural Sciences demonstrated that treatment with ACCC19743 microbial agent effectively reduced soil pH, increased the relative abundance of Proteobacteria and Bacteroidetes, improved

chlorophyll content in sunflower leaves, and promoted its growth and development, making it recommended for use in severely saline-alkali lands in the Hetao irrigation area^{Error! Reference source not found.}. A team led by Professor Yu Qilin from Nankai University developed a microbial agent using synthetic biology techniques to increase soybean yield and resistance to stress. This agent significantly enhanced soybean adaptability and resistance to saline-alkali land and also showed good results in crops like rice and corn^{Error! Reference source not found.}. The team led by Professor Yang Guoping from Northern University for Nationalities applied microbial liquid organic fertilizer through full-cover spraying to achieve high yields in salt-tolerant maize planting, transforming the warm spring farm into a medium-yield improved land^{Error! Reference source not found.}. Experimental results show that the active microorganisms contained in these agents can symbiotically interact with other microorganisms in the soil. Through biological processes such as nitrogen fixation and phosphorus solubilization, they improve the nutritional status of the soil, promoting plant growth and development. Microbial agents can also improve soil structure, further enhancing soil quality. The application of microbial agents can achieve biological restoration of saline-alkali land, reduce dependence on chemical fertilizers, decrease environmental pollution, and promote the sustainable development of agriculture.

The Heilong River Basin is located in Hebei Province, China, roughly situated between the Ziya River Basin and the Zhangwei River Basin. It includes 50 counties (cities, districts) across six cities: Hengshui, Xingtai, Handan, Cangzhou, Baoding, and Shijiazhuang, covering an area of 34,000 square kilometers. This region is a key economic zone in China, an important national agricultural planting area, and one of the regions most affected by salinity. Salinized soil has become a major limiting factor and obstacle to

agricultural development and sustainable growth in the Heilonggang River Basin. The extremely low nutrient content in saline-alkali soils leads to the excessive use of chemical fertilizers in agricultural production, which exacerbates the salinization. With the introduction of the national agricultural development strategy “Two Reductions and One Increase” and the growing environmental awareness, the use of chemical fertilizers has been restricted. Consequently, the use of salt-tolerant microbial agents to improve the fertility of saline-alkali soils and enhance plant salt tolerance has gained increasing attention, becoming a key research direction in soil improvement.

This study collects saline-alkali soil from the Heilonggang River Basin and conducts pot experiments to investigate the improvement effects of several microbial agents on saline-alkali soil and their growth-promoting effects on wheat seedlings. Additionally, a field experiment using microbial agents in saline-alkali soil is conducted, aiming to provide new materials for soil improvement in the Heilonggang River Basin and offer data support for the application of microbial agents in saline-alkali soil amelioration.

1 Materials and Methods

1.1 Experimental Soil

The tested saline-alkali soil belongs to the severe saline-alkali soil in the Heilonggang Basin of Xingtai City, Hebei Province, China, located at N37.21° and E114.93°. Soil layers of 5 to 20 cm were scooped up, bagged, and taken back to the laboratory for future use.

1.2 Experimental Strain

Antagonistic bacterium HM-7: The Microbiology Laboratory of Xingtai University isolated an antagonistic bacterium HM-7 from the soil, which exhibits antagonistic activity against cucumber wilt disease. It was identified as *Bacillus amyloliquefaciens*, a species of biocontrol bacillus. This bacterium has a broad antibacterial

spectrum and can inhibit diseases in various crops, including cucumber, thereby enhancing plant disease resistance^[7-8].

Antagonistic strains JK-2 and JK-3: These antagonistic strains, isolated by the Microbiology Laboratory of Xingtai University, are capable of inhibiting a variety of plant pathogenic fungi.

1.3 Preparation of Bacterial Suspension

After the bacterial strains were activated and inoculated into beef extract peptone liquid medium, they were shaken and cultured for 24 hours. The optical density (OD) at 600 nm was measured, resulting in OD₆₀₀ (JK-2) = 0.982, OD₆₀₀ (JK-3) = 0.875, and OD₆₀₀ (HM-7) = 0.761. Subsequently, JK-2, JK-3, and HM-7 were mixed in a 1:1:1 ratio to prepare a composite microbial agent.

1.4 Wheat Planting Experiment

Full wheat seeds were selected, disinfected by soaking in 75% alcohol, and then washed three times with sterile water. The seeds were placed in a petri dish lined with gauze and incubated in the dark at 26°C. When the sprouts reached a length of 0.5 cm, they were planted. The wheat was cultivated in nutrient pots measuring 3 cm in length, 3 cm in width, and 5 cm in height. Eight seeds were sown per pot, with 10 pots per treatment. The control group was treated with sterile water, while the experimental groups were treated with JK-2, JK-3, HM-7, or the composite microbial agent, for a total of five treatments. Each treatment was repeated three times. Irrigation was performed every five days, adding 700 µl of the corresponding microbial agent or water to each pot. Regular irrigation was conducted based on the drought conditions during the wheat plant's growth period to meet the water requirements for growth. No fertilizers were applied throughout the experiment to ensure uniform management conditions across all treatments^[9].

1.5 Sample Collection and Determination

Methods

At the end of the experiment, the soil from the nutrient pots was removed, and the residual wheat root systems were separated. The soil was then classified, mixed, labeled, and air-dried. The nitrogen, phosphorus, potassium content, and pH of the soil were measured using a soil nutrient rapid tester.

Twenty days after wheat planting, measurements were taken for the above-ground plant height, maximum leaf length, chlorophyll content, fresh weight of the whole plant, and dry weight of the whole plant ^[10].

Plant height: The wheat plant height was measured using a ruler, with the starting point at the base of the stem and the endpoint at the growing point of the main stem.

Maximum leaf length: The longest leaf was measured using a scale, with the starting point at the base of the leaf and the endpoint at the leaf tip.

Fresh weight of the whole plant: The wheat seedlings were washed thoroughly with clean water, allowed to air dry, and then weighed using an electronic balance to determine the fresh weight of the whole plant.

Dry weight of the whole plant: The wheat seedlings, after measuring the fresh weight, were placed in an oven at 105°C for 30 minutes, then dried at 80°C until fully dry, and the dry weight was measured using an electronic balance.

1.6 Wheat Field Experiment

Plump wheat seeds were selected and planted in subplots (2m × 2m) in the field. The control group was irrigated with clean water, while the experimental groups were treated with JK-2, JK-3, HM-7, and a compound microbial agent, resulting in a total of five treatments. Each treatment was replicated three times. Irrigation was applied every three months. Regular irrigation was carried out based on the drought

conditions during the wheat plant growth period to meet the water requirements of the wheat. No fertilization was applied throughout the experiment to ensure consistent management practices. The agronomic characteristics of the wheat under different treatments were observed.

1.7 Data Processing and Analysis

All the experimental data of soil samples were recorded and plotted using Microsoft Excel 2010 software. The determination data of physical and chemical properties of soil were statistically analyzed by SPSS 27.0 software.

2 Results and Analysis

2.1 The Contents of Nitrogen, Phosphorus and Potassium and Ph of the Soil under Different Treatments

2.1.1 The Influence of Different Microbial Agent Treatments on the Content of Ammonium Nitrogen in Soil

The effect of different microbial inoculants on ammonium nitrogen content in saline-alkali soils is shown in Table 1 and Figure 1. The results indicate that the ammonium nitrogen content in soils treated with compound microbial agent and JK-2 was significantly higher than that in the control group, while the ammonium nitrogen content in soils treated with JK-3 and HM-7 was slightly lower than the control group. Specifically, the nitrogen content in the saline-alkali soil treated with the compound microbial agent increased by 172.23%; the nitrogen content in the soil treated with JK-2 increased by 168.72%; the nitrogen content in the soil treated with JK-3 decreased by 18.28%; and the nitrogen content in the soil treated with HM-7 decreased by 10.37%. The effect on increasing ammonium nitrogen content in the soil was found to be in the order of: compound microbial agent > JK-2 > control group > JK-3 > HM-7 ($P < 0.05$). This suggests that the compound microbial agent and JK-2 can promote nitrogen release and transformation in the soil, thereby improving soil fertility, while

JK-3 and HM-7 have some inhibitory effects on soil. the transformation and release of nitrogen in the

Table 1: Nitrogen, phosphorus, potassium content and pH in saline soil under different treatments

Treatments	Ammonium nitrogen content/(mg/kg)	Effective phosphorus content/(mg/kg)	Effective potassium content/(mg/kg)	pH
Control	57.11±0.41b	23.74±0.70e	8.56±0.07e	8.86a
Composite agent	155.47±1.33a	69.41±1.03a	16.30±0.65c	8.51e
JK-2	153.86±0.57a	37.19±0.30d	24.09±0.29a	8.62d
JK-3	46.67±1.25c	58.04±1.35b	11.07±1.08d	8.69c
HM-7	51.19±0.42d	49.87±0.77c	20.73±1.22b	8.75b

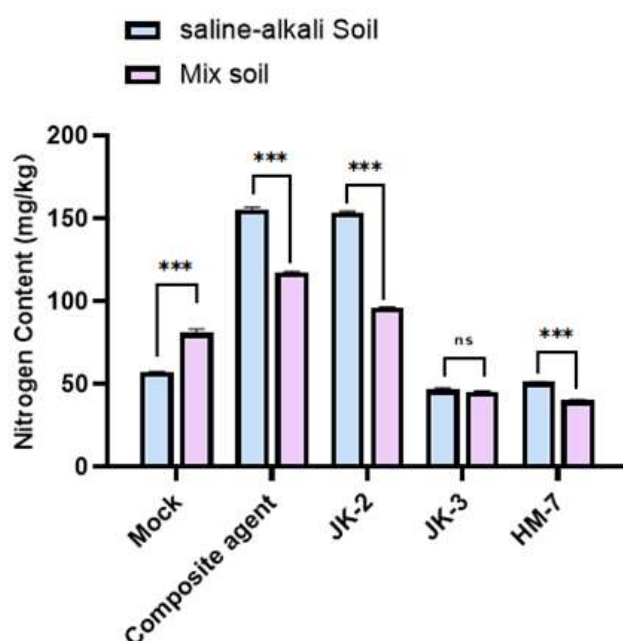


Fig. 1 Ammonium nitrogen content in soil with different microbial agents.

2.1.2 The Influence of Different Microbial Agent Treatments on the Content of Available Phosphorus in Soil

The effect of different microbial inoculants on available phosphorus content in saline-alkali soils is shown in Table 1 and Figure 2. The results indicate that the available phosphorus content in soils treated with microbial inoculants was significantly higher than that in the control group. Specifically, the available phosphorus in the saline-alkali soil treated with the compound microbial agent increased by 192.38%; the available phosphorus in the soil treated with JK-2

increased by 56.66%; the available phosphorus in the soil treated with JK-3 increased by 144.48%; and the available phosphorus in the soil treated with HM-7 increased by 110.06%. The effect on increasing available phosphorus content in the soil was found to be in the order of: compound microbial agent > JK-3 > HM-7 > JK-2 > control group ($P < 0.05$). This suggests that all the microbial inoculants can promote the release and transformation of phosphorus in the soil, thereby improving soil fertility, with the compound microbial agent having the most significant promoting effect.

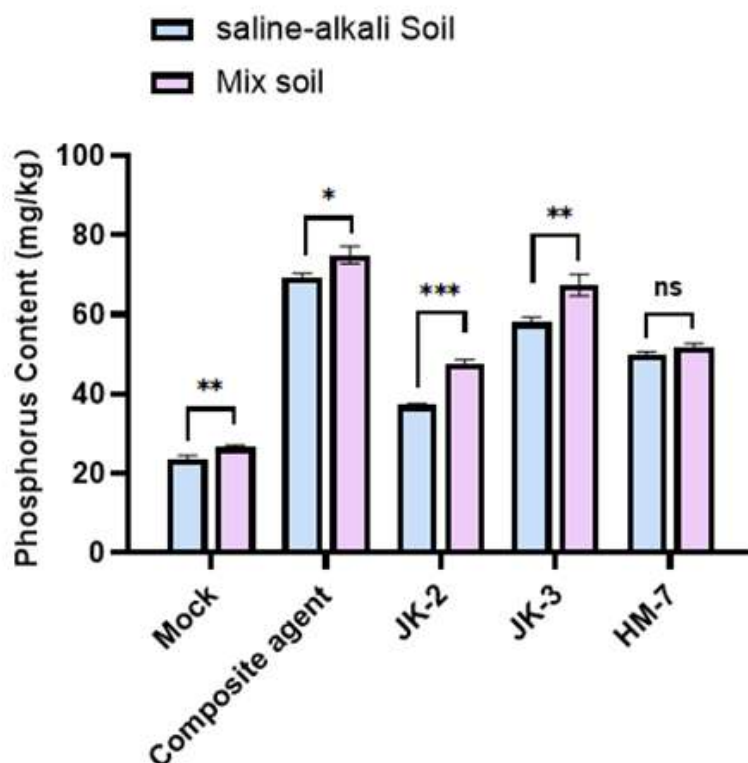


Fig. 2 Effective phosphorus content in soil with different microbial fertilizers applied.

2.1.3 The Influence of Different Microbial Agent Treatments on the Content of Available Potassium in Soil

The effect of different microbial inoculants on available potassium content in saline-alkali soils is shown in Table 1 and Figure 3. The results indicate that the available potassium content in soils treated with microbial inoculants was significantly higher than that in the control group. Specifically, the available potassium in the saline-alkali soil treated with the compound microbial agent increased by 90.42%; the available

potassium in the soil treated with JK-2 increased by 181.43%; the available potassium in the soil treated with JK-3 increased by 29.32%; and the available potassium in the soil treated with HM-7 increased by 142.17%. The effect on increasing available potassium content in the soil was found to be in the order of: JK-2 > HM-7 > compound microbial agent > JK-3 > control group ($P < 0.05$). This suggests that all the microbial inoculants can promote the release and transformation of potassium in the soil, thereby improving soil fertility.

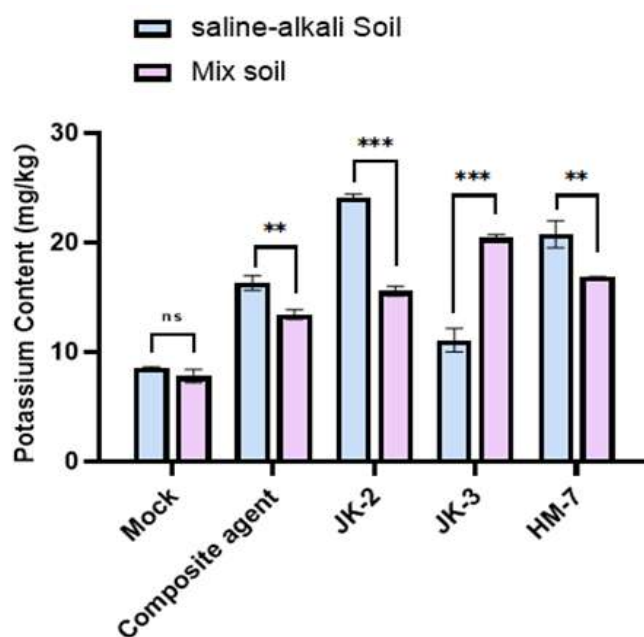


Fig. 3 Effective potassium content in the soil applied with different microbial agents.

2.1.4 The Influence of Different Microbial Agent Treatments on Soil pH

The changes in pH after treatment with different biological inoculants are shown in Table 1 and Figure 4. The experimental results indicate that the pH of the soil in all biological inoculant treatment groups decreased, and the pH of the soil in the treatment groups was significantly lower than that in the control group. Among the various microbial inoculants applied, the treatment with

the compound microbial agent showed the greatest decrease in soil pH, with a reduction of 0.35. The second greatest reduction was observed with JK-2, which decreased by 0.24. The observed treatment effects were as follows: compound microbial agent > JK-2 > JK-3 > HM-7 > control group ($P < 0.05$). This suggests that microbial inoculants have a role in reducing soil alkalinity, which helps improve the soil environment in saline-alkali lands.

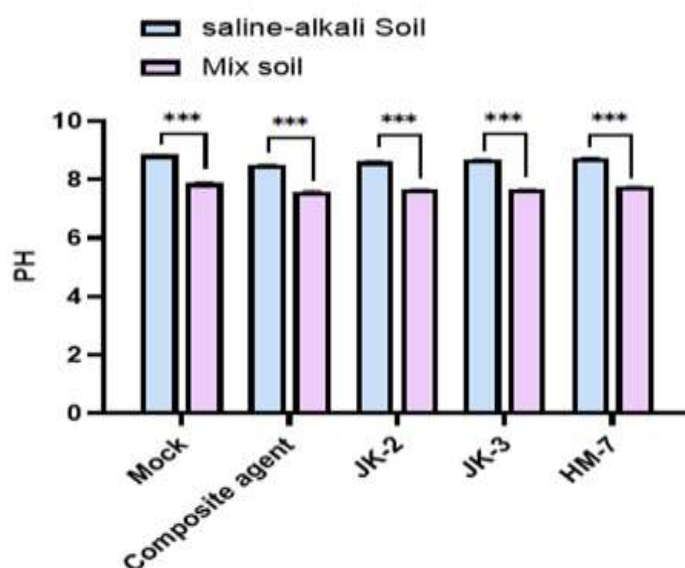


Fig. 4 pH of soil treated with different microbial agents.

2.2 The Effects of Different Treatments on Wheat Growth

The results of the various wheat indicators under different treatments are shown in Table 2. It can be seen that after treatment with the compound microbial agent, the wheat seedlings had significantly higher plant height, maximum leaf length, fresh weight, and dry weight compared to those under other treatments. Specifically, plant height increased by 22.02%, maximum leaf length grew by 18.97%, fresh weight increased by

33.7%, and dry weight increased by 34.7%. Although the wheat seedlings treated with single microbial agents showed higher values for all indicators compared to the control group, statistical analysis revealed no significant differences. This indicates that the single microbial agents did not show a promotive effect on wheat growth, whereas the compound microbial agent treatment significantly affected wheat growth and development, exhibiting a promotive effect on the wheat seedlings.

Table 2 Role of different treatments on the growth of wheat seedlings

Treatments	Plant height/(cm)	Maximum leaf length/(cm)	Fresh weight/(g)	Dry weight/(g)
Control	21.0943±2.5685b	17.0335±2.7695b	0.1356±0.0290b	0.0138±0.0061b
HM-7	22.5832±1.8816b	17.7060±1.8203b	0.1381±0.0106b	0.0147±0.0054b
JK-2	22.1831±2.3598b	17.6729±2.2806b	0.1364±0.0191b	0.0142±0.0026b
JK-3	22.4337±2.6369b	17.6397±2.5137b	0.1379±0.0029b	0.0141±0.0039b
Composite agent	25.7356±2.7112a	20.2659±2.2910a	0.1813±0.01370a	0.0186±0.0049a

2.3 Effects of Different Treatments on Agronomic Traits of Wheat

The agronomic traits of wheat under different treatments were investigated, and it was found that compared with the control, several different treatments had little effect on plant height, ear length, ear width, grain length and grain width of wheat, and there was no significant difference as shown in Fig5 and 6. However, the compound

fungicide treatment could significantly increase the number of tillers, yield per plant and 1000-grain weight of wheat as shown in Fig7, while the other three treatments were not much different from the control, suggesting that the compound fungicide could significantly increase wheat yield. Because the treatment of compound bacterial agent had little effect on grain length and width, but the 1000-grain weight was large, which may enhance the grain inclusions to a certain extent.



Fig. 5 Field phenotype with different microbial agents.

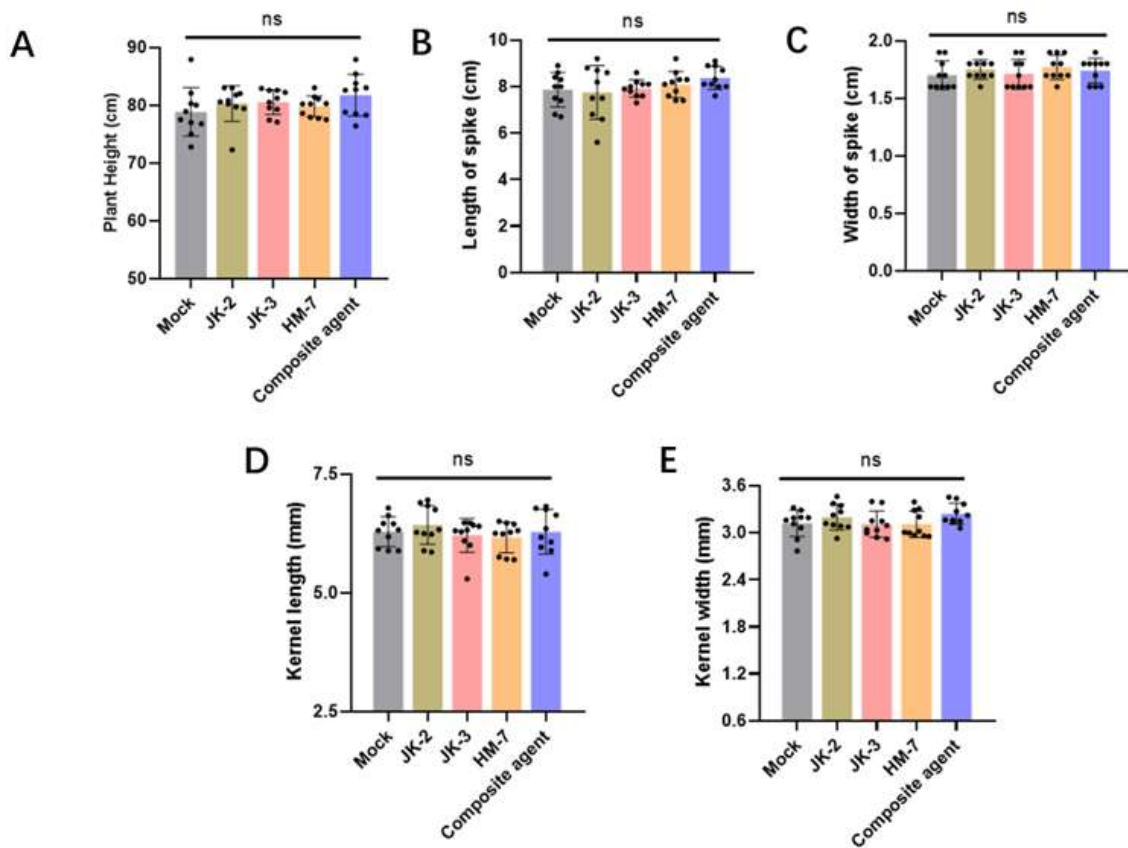


Fig. 6 Agronomic traits with different microbial agents.

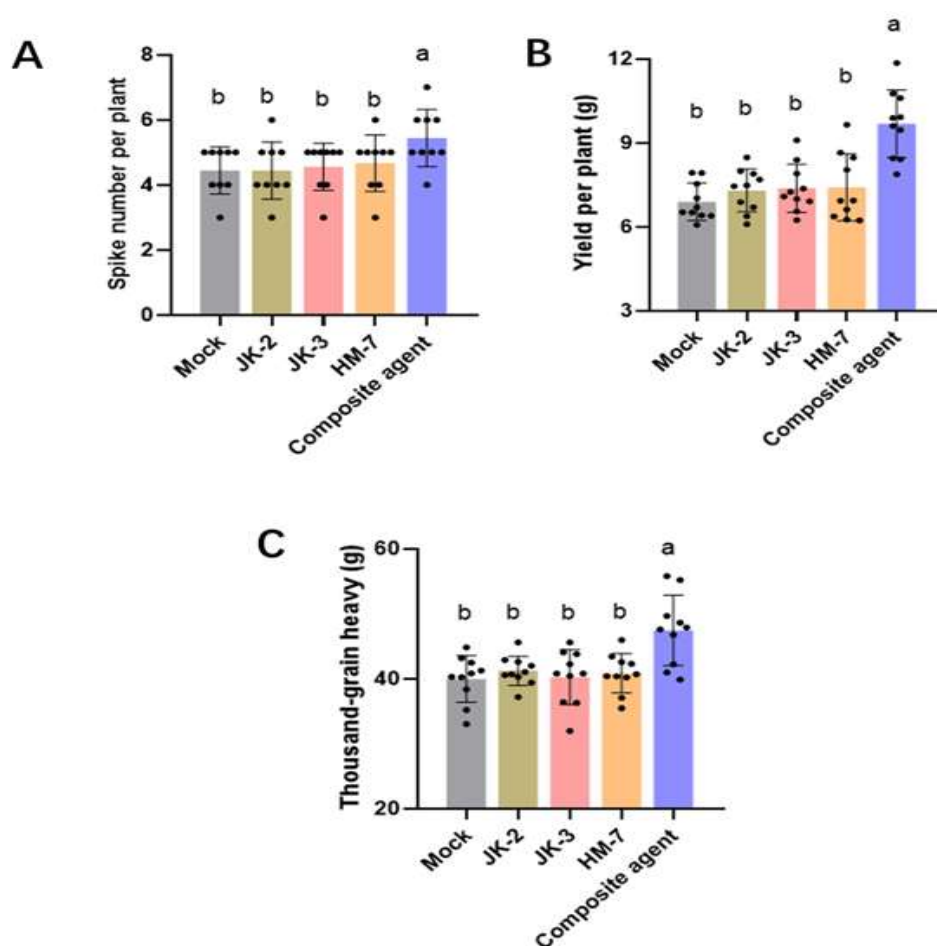


Fig. 7 Yield agronomic traits with different microbial agents.

3 Conclusion and Discussion

Through comparative experiments, it was found that after applying the microbial composite agent, the contents of ammonium nitrogen, available phosphorus, and available potassium in saline-alkali soils significantly increased. Specifically, the ammonium nitrogen content increased by 172.23%, available potassium increased by 90.42%, and available phosphorus increased by 192.38%. Additionally, this microbial agent effectively reduced the pH of the soil. This indicates that the microbial composite agent promoted the release and transformation of nitrogen, phosphorus, and potassium in the soil to varying degrees, improved soil fertility, and reduced soil pH, thereby creating more favorable conditions for plant growth. The microbial composite agent shows good potential for application in the improvement of saline-alkali

land and provides effective technical support for the sustainable utilization of such land.

The experiment found that the microbial composite agent had a stronger effect on the pH, ammonium nitrogen, and available potassium content of saline-alkali soil than individual microbial agents, with the only exception being available phosphorus, where the effect was not as significant as that of JK-2 and HM-7. Furthermore, in terms of the impact on ammonium nitrogen content, treatments with the single agents JK-3 and HM-7 resulted in lower levels compared to the control group, and the specific reasons for this require further investigation.

The results of the plant growth-promoting experiment on wheat showed that the use of these three functional microbial agents individually did not have a significant effect on promoting wheat

growth. However, the experimental group using the composite microbial agent showed significant improvements ($P < 0.05$) in various indicators, such as plant height, fresh weight, dry weight, and root growth, compared to the other groups. For instance, the fresh weight and plant height increased by 33.7% and 22.02%, respectively, compared to the control group. These findings highlight the high efficiency of the composite microbial agent in promoting wheat growth. The reason may lie in the different functional effects of the three microbial agents on wheat growth, and their strategic combination leads to complementary and synergistic effects, thus unleashing the full potential of each agent. However, the specific mechanisms require further research for clarification. Liu Pengfei *et al.* studied salt-tolerant growth-promoting bacterial strains using maize as an indicator crop through greenhouse pot experiments. They found that, in mildly saline-alkali soils, the strain FY2 (*Bacillus amyloliquefaciens*) significantly altered the diversity and richness of the soil bacterial community. Compared to the CK treatment, the relative abundance of unclassified bacterial phyla increased by 36.16%, Bacteroidetes increased by 51.75%, and Acidobacteria increased significantly by 35.71%. In moderately saline-alkali soils, the application of strains XS8 (*Micrococcus luteus*) and SXB1 (*Bacillus subtilis*) did not significantly affect the diversity and richness of the soil bacterial community but significantly increased the relative abundance of Proteobacteria. They also found that the application of salt-tolerant growth-promoting strains significantly changed the functional composition of the soil bacterial community, especially influencing nitrogen cycle-related functions. In mildly saline-alkali soils, the application of strain FY2 significantly reduced soil denitrification activity, while in moderately saline-alkali soils, the growth-promoting strains XS8 and SXB1 significantly increased soil nitrate respiration and nitrogen respiration functions. These findings provide scientific guidance and

theoretical support for the improvement of saline-alkali land and increased crop yield using salt-tolerant microbial growth-promoting strains ^[11]. The effects of the composite microbial agent on soil microbial diversity and nitrogen, phosphorus, and potassium cycling functions require further investigation.

There has been extensive research on microbial agents in improving saline-alkali soils and promoting plant growth. For example, Marwa *et al.* studied a bio-organic fertilizer that effectively improves soil fertility in saline-alkali land and enhances soybean yield and quality ^[12]. Yin Zhirong *et al.* studied the effects of microbial fertilizers on the improvement of the physicochemical properties of native saline-alkali land and the promotion of wolfberry growth ^[13]. Pang Ning studied the growth-promoting effects of composite microbial agents on rice and their role in improving saline-alkali soils ^[14]. Hafez *et al.* used organic amendments combined with rhizobia (*Azospirillum brasilense*) to restore and enhance the fertility of Egyptian saline-alkali soils ^[15]. These experimental results were conducted in pot experiments, and their effectiveness in field applications still needs further verification. Some microbial agents for improving saline-alkali soils have already been widely promoted across the country. For instance, Professor Yang Guoping's team from the Northern University for Nationalities, with the support of alkali-tolerant microbial agents, achieved successful salt-alkali resistant maize planting, which has been promoted in saline-alkali fields in Shandong, Inner Mongolia, Xinjiang, and Gansu ^[6]. However, overall, microbial agents still face issues such as inconsistent effectiveness, limited strain adaptability, unclear mechanisms, high application costs, and a lack of long-term efficacy evaluation, which limit their widespread use. Further research, optimization, and observation by scientists are needed.

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Author Contributions

R.T. and J.X. contributed equally to the composition of the manuscript. X.Z. and H.Z. contributed to the conceptualization of the manuscript. H.Z., S.W. and J.F. prepared the manuscript figures.

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Data availability

The data are available in the article. All authors have no interest disputes.

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