ISSN: 2710-0723

The Effect of Salt-dissolving Growth-promoting Rhizobacteria on the Growth of Pepper

Weiwei Chen[1,2,3,4,5, *], Yuhu Luo[1,2,3,4,5]

[1] Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an 710021, China

[2] Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an 710075, China

[3] Key Laboratory of Degraded and Unused Land Consolidation Engineering, Ministry of Natural Resources, Xi'an 710075, China

[4] Shaanxi Engineering Research Center of Land Consolidation, Xi'an 710075, China

[5] Land Engineering Technology Innovation Center, Ministry of Natural Resources, Xi'an 710075, China

*Corresponding Author: chenweiwei736@126.com

Abstract

Soil salinization is increasing, which seriously threatens the local ecological environment security and affects the development of social economy. Plant growth-promoting rhizobacteria (PGPR) is the bacterial population isolated from rhizosphere soil that has a growth-promoting effect on plants, which is of great significance for improving salt stress. In this study, pepper was used as the research object, and the effect of salt-dissolving and growth-promoting bacteria on pepper plants was studied by pot experiment. A total of 7 treatments were set up, 0 % salt sterility (CK-0 %), 0.5 % salt sterility (CK-0.5 %), 0 % salt-hydrolyzed salt-promoted strains (C-0 %), 0.5 % salt-hydrolyzed salt-promoted strains (C-0.5 %), 1 % salt-hydrolyzed salt-promoted strains (C-1.0 %), 2.0 % salt-hydrolyzed salt-promoted strains (C-2.0 %), 5.0 % salt-hydrolyzed salt-promoted strains (C-5.0 %). The results showed that : (1) The germination rate of pepper seeds inoculated with salt-dissolving and growth-promoting strains was significantly increased, and the salt concentration in the soil had no significant effect on the germination rate of pepper seeds. (2) Inoculation of salt-solubilizing growth-promoting strains and salt concentration in soil also had no significant effect on the plant height of pepper seeds. (3) The fresh weight and dry weight of pepper plants inoculated with salt-solubilizing and growth-promoting strains were significantly increased. This study has important scientific and theoretical significance for the more rational application of Plant growth-promoting rhizobacteria.

Keywords

Plant Growth-promoting Rhizobacteria; Pepper; Soil Salinization; Growth-promoting.

1. Introduction

Soil salinization and secondary salinization is one of the main types of land degradation, which is a global resource and ecological problem. According to statistics, the area of global salinized soil is about 9.52×108 hm2, which is about 10% of the earth's land area. And the area of secondary salinized soil is about 0.77×107 hm2 , 58 % of which occurs in agricultural irrigation area. And about 20% of arable land is affected by salinization, and this proportion is increasing [1]. Soil salinization seriously affects the growth of crops. At present, there are different types

ISSN: 2710-0723

and degrees of soil salinization in all countries of the world, and it is continuing to expand, which seriously hinders the sustainable development and production of agriculture. Although the total phosphorus content in salinized soil is very high, most of it is occluded phosphorus, which is difficult to be absorbed and utilized by plants. Although the extensive use of phosphate fertilizer can temporarily alleviate the problem of phosphorus deficiency, many applied phosphate fertilizers are easy to be chelated by metal ions in the soil after entering the soil, resulting in a decrease in the utilization rate of phosphate fertilizer and a waste of phosphorus resources.

In recent years, with the emphasis on ecological environment protection and sustainable development, the improvement of saline-alkali land by microbial agents has gradually become a hot spot. Microorganisms can improve the internal pore structure of soil, avoid soil agglomeration and enhance soil permeability. In the process of reproduction, microbial colonies will produce substances that are beneficial to plant growth and provide certain nutrients for plant growth and development. Microbial colonies can activate the activity of various enzymes in the soil and repair the soil. At the same time, since microbial colonies provide the nutrients required by the soil, the use of chemical fertilizers can be reduced to avoid the death of beneficial bacteria in the soil. Plant growth-promoting rhizobacteria (PGPR) is the bacterial population isolated from rhizosphere soil that has a growth-promoting effect on plants. In 1978, Kloepper and Schroth et al. isolated plant-beneficial rhizobacteria from potato and introduced the concept of plant growth-promoting rhizobacteria[2]. So far, nearly ten thousand kinds of growth-promoting rhizobacteria have been isolated. These generally promote plant growth through direct or indirect effects. The direct effects mainly include the synthesis of certain substances that have a direct effect on plant growth, such as plant hormones [3], or the production of ACC deaminase to reduce plant ethylene concentration [4], or by fixing nitrogen in the air, dissolving insoluble phosphorus in the soil [5] or the production of siderophores to bind iron, etc., to change the morphology of some nutrients in the soil and improve the absorption of nutrients by plants, or by inducing gene transcription and metabolite biosynthesis in plant cells to change the root microenvironment. Indirect effect refers to PGPR produces a variety of antibiotics to inhibit pathogenic bacteria, such as chitinase, hydrocyanic acid and other antibiotics to inhibit the growth and reproduction of pathogenic bacteria, help plants resist pathogenic microorganisms and promote plant growth and development [7]. Masood et al. [8] found that inoculation with B.citri increased tomato growth, nitrogen uptake, soil $NH4^{+}$ concentration, number of rhizosphere bacteria, soil bacterial gene expression, and soil nitrogenase activity, thereby promoting tomato growth. In 2018, Wang et al.isolated and purified 11 strains with high ACC deaminase activity from the rhizosphere soil of pepper. Under salt stress conditions, these strains have good salt-dissolving and growth-promoting effects [9]. Compared with the uninoculated control, inoculation of ACC deaminase-producing strains under salt stress had a significant positive effect on the growth parameters (germination rate, root and shoot growth and chlorophyll content) of rice, and reduced the production of ethylene [10]. Rhizobacteria are considered to be one of the most effective ways to reduce the use of chemical fertilizers [11], increase crop yield and achieve the desired goal of disease resistance [12]. Although many studies have reported that plant growth promoting rhizobacteria (PGPR) is an environmentally friendly and sustainable agricultural policy, only a small part of them are studying the induction of plant tolerance to salt stress by PGPR. In this paper, the effect of salt-dissolving and growth-promoting bacteria on pepper plants was studied by pot experiment, in order to provide a scientific basis for the more rational application of rhizosphere growth-promoting bacteria.

ISSN: 2710-0723

2. Materials and Methods

2.1. Strains and Substratum

2.1.1. NA-Composite Culture Medium of Soil Extract

Beef extract 3g, protein swelling 5g, NaCl 5g, glucose 1g, agar 15g, soil extract 200 mL, H2O 800 mL, pH : 7.0-7.2, sterilized at 121 °C for 20 minutes. (Preparation of soil extract : 50g of soil samples were collected, added with 200mL of water, cooked at 121 °C for 1 hour, filtered by filter paper and supplemented with water to 200mL).

2.1.2. DF liquid Culture

Component 1: H3BO3 10mg, MnSO4·H2O 11.19mg, ZnSO4·7H2O 124.6mg, CuSO4·5H2O 78.22mg, MoO3 10mg above dissolved in 100mL sterilized distilled water, stored at-4℃;

Component 2: FeSO4·7H2O (100mg) was dissolved in 10mL sterilized distilled water and stored at-4℃;

Take 0.1 mL of components 1 and 2, add KH2PO4 4.0g, Na2HPO4 6.0g, MgSO4·7H2O 0.2g, glucose 2.0g, 2.0g gluonic acid, 2.0g citric acid, (NH4)2SO4 2.0g, H2O1000mL.121℃ was sterilized for 20 min.

2.1.3. ADF Substratum

The (NH4)2SO4 contained in DF liquid medium was replaced by ACC, and ACC could not be sterilized at high temperature. ACC was first prepared into a mother liquor with a concentration of 0.5M, and sterilized by a 0.2μm bacterial filter. The mother liquor was added to the DF liquid medium (without (NH4)2SO4) after high-temperature sterilization and cooling at a rate of 6ml / L. The mother liquor was stored at -20 °C.

2.1.4. DF Solid Culture

Bacto-Agar was added to DF liquid culture at a concentration of 1.8 %. Sterilization at 121 °C for 20 minutes.

2.1.5. ADF Solid Plate

The DF solid culture without (NH4)2SO4 was sterilized and poured into a standard-sized petri dish. After cooling and solidification on an ultra-clean bench, ACC solution was applied to each dish at a dose of 30 μmol.

2.1.6. Strains

ACC method was used to screen plant growth promoting rhizobacteria (PGPR).

2.2. Pot Experiment Design

Preparation and treatment of pepper seeds : Pepper seeds with full and consistent size were selected, disinfected with 70 % alcohol for 5 min, washed three times with sterile water, and then soaked and disinfected with 0.1 % mercury solution for 10-15 min. Finally, the pepper seeds were washed three times with sterile distilled water and dried on filter paper. Each pot was sowed with 10 seeds, and the salt-dissolving and growth-promoting strains were inoculated near the seeds (0.5 g strain suspension was inoculated in each germination box). In addition, no inoculation treatment was set as a control test. After sowing, sodium chloride solution was added to make the salt concentration in the soil 0 %, 0.5 %, 1.0 %, 2.0 % and 5.0 %, respectively. A total of 7 treatments were set up, 0 % salt sterility (CK-0 %), 0.5 % salt sterility (CK-0.5 %), 0 % salt-hydrolyzed salt-promoted strains (C-0 %), 0.5 % salt-hydrolyzed salt-promoted strains (C-0.5 %), 1 % salt-hydrolyzed salt-promoted strains (C-1.0 %), 2.0 % salt-hydrolyzed salt-promoted strains (C-2.0 %), 5.0 % salt-hydrolyzed salt-promoted strains (C-5.0 %). Each treatment was set with 2 replicates, watered once a day, and the water control was consistent. From the 7th day, the germination rate and seedling rate were counted and recorded continuously for 7 days. The growth parameters of pepper, such as plant height, fresh weight and dry weight, were measured when the pepper seedlings grew to the three-leaf stage.

2.3. Statistical Data Analysis

The data of germination rate, plant height, fresh weight and dry weight were statistically analyzed by one-way analysis of variance and Tukey multi-range test (TMRT) of Origin 2015, and $p < 0.05$ was the standard to determine the significant difference between treatments.

3. Results and Analysis

References are cited in the text just by square brackets [1]. (If square brackets are not available, slashes may be used instead, e.g. $p<0.05$.)

3.1. Germination Rate

Rhizosphere salt stress has different effects on plant growth and plant growth-promoting rhizobacteria growth-promoting ability. The results showed that the germination rate of pepper seeds inoculated with salt-dissolving and growth-promoting strains was significantly increased, and the salt concentration in the soil had no significant effect on the germination rate of pepper seeds. Among them, the germination rate of pepper seeds inoculated with 1 % salt concentration in soil and salt-solubilizing and growth-promoting strains was the highest, which was 75 %. The germination rate of pepper seeds in blank control (no salt in soil and no salt-solubilizing and growth-promoting strains) was the lowest, which was 45 %. When the salt concentration in the soil was 0.5 %, the germination rate of pepper seeds inoculated with salt-dissolving and growth-promoting strains was higher than that of uninoculated pepper seeds, which were 70 % and 50 %, respectively. This result is consistent with previous studies, Bal et al. (2013) found that compared with the uninoculated control, the inoculation of ACC deaminase-producing strains under salt stress had a significant positive effect on the growth parameters (germination rate, root cap growth and chlorophyll content) of rice, and reduced the production of ethylene [10].

3.2. Plant Height

Plant height is very important for plants to adapt to different environments. The results showed that the inoculation of salt-dissolving and growth-promoting strains had no significant effect on the plant height of pepper, and the salt concentration in the soil had no significant effect on the plant height of pepper seeds. This result was consistent with the study of He et al. [13], and the application of rhizosphere bacteria alone or in combination did not significantly increase tomato plant height. However, some studies have found that rhizobia can significantly alleviate the adverse effects of salt on mung bean and increase the plant height of mung bean in the presence of tryptophan, the prerequisite substance for IAA synthesis. In 2018, Wang et al.isolated and purified 11 strains with high ACC deaminase activity from the rhizosphere soil of pepper. Under salt stress conditions, these strains have good salt-dissolving and growth-promoting effects [9].

3.3. Fresh Weight and Dry Weight

In this study, the fresh weight and dry weight of pepper plants under salt stress were analyzed. The results showed that the fresh weight and dry weight of pepper plants inoculated with salt-solubilizing and growth-promoting strains were significantly increased. When the salt concentration in the soil was 1 %, the fresh weight and dry weight of the pepper plants inoculated with the salt-dissolving and growth-promoting strains were the highest, 7.4 g and 0.93 g, respectively. Hahm et al.also found that PGPR inoculation significantly increased the height, total fresh weight and total dry weight of pepper plants regardless of growth conditions [14].

ISSN: 2710-0723

4. Conclusion

This study mainly compared the different responses of pepper seeds and pepper plants to salt stress after inoculation with salt-solubilizing and growth-promoting strains. The main conclusions are as follows:

(1) The germination rate of pepper seeds inoculated with salt-dissolving and growth-promoting strains was significantly increased, and the salt concentration in the soil had no significant effect on the germination rate of pepper seeds.

(2) Inoculation of salt-solubilizing growth-promoting strains and salt concentration in soil also had no significant effect on the plant height of pepper seeds.

(3) The fresh weight and dry weight of pepper plants inoculated with salt-solubilizing and growth-promoting strains were significantly increased.

Acknowledgments

This work was supported by the Scientific Research Item of Shaanxi Provincial Land Engineering Construction Group (DJNY2022-18 and 2022-NBYY-05).

References

[1] Ghassemi F, Jakeman AJ, Nix HA. Salinisation of land and water resources: Human causes, extent, manangement and case studies[M]. Canberra, Australia, 1995: 1-3.

[2] Kloepper, J. W. Plant growth-promoting rhizobacteria on radishes presented in part at the Proc.of the 4th Internet. Conf. on Plant Pathogenic Bacter, Station de Pathologie Vegetale et Phytobacteriologie[C], Angers, France, 1978.

[3] Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., SkZ, A. Enhancement of droughtstress tolerance in crops by plant growth promoting rhizobacteria[J], Microbiological Research, 2016, 184, 13-24.

[4] Glick, B. R., Cheng, Z., Czarny, J., Duan, J. Promotion of plant growth by ACC deaminase-producing soil bacteria[J], European Journal of Plant Pathology, 2007, 119, 329-339.

[5] Zhang, X., Li, H., Li, M., Wen, G., Hu, Z. Influence of individual and combined application of biochar, Bacillus megaterium, and phosphatase on phosphorus availability in calcareous soil[J], Journal of Soils and Sediments, 2019, 19, 3688-3698.

[6] Vacheron, J., Desbrosses, G., Bouffaud, M.-L., Touraine, B., Moënne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dyé, F., Prigent-Combaret, C. Plant growth-promoting rhizobacteria and root system functioning[J], Frontiers in Plant Science, 2013, 4.

[7] Raza, W., Ling, N., Yang, L., Huang, Q., Shen, Q. Response of tomato wilt pathogen Ralstonia solanacearum to the volatile organic compounds produced by a biocontrol strain Bacillus amyloliquefaciens SQR-9[J], Scientific Reports, 2016, 6, 24856.

[8] Masood S, Zhao X Q, Shen R F. Bacillus pumilus promotes the growth and nitrogen uptake of tomato plants under nitrogen fertilization[J]. Scientia Horticulturae, 2020, 272.

[9] Wang, W., Wu, Z., He, Y., Huang, Y., Li, X., Ye, B.-C. Plant growth promotion and alleviation of salinity stress in Capsicum annuum L. by Bacillus isolated from saline soil in Xinjiang[J], Ecotoxicology and Environmental Safety, 2018, 164, 520-529.

[10] Bal, H. B., Nayak, L., Das, S., Adhya, T. K. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress[J], Plant and Soil, 2013, 366, 93-105.

[11] Lin, Y., Watts, D. B., Kloepper, J. W., Adesemoye, A. O., Feng, Y. Effect of plant growth-promoting rhizobacteria at various nitrogen rates on corn growth[J], Agricultural Sciences, 2019, 10, 1542-1565.

ISSN: 2710-0723

[12] Kuan, K. B., Othman, R., Rahim, K. A., Shamsuddin, Z. H. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions[J], PloS One, 2016, 11.

[13] Yanhui He, Hugo A. Pantigoso, Zhansheng Wu*, J.M. Vivanco*. Co-inoculation of Bacillus sp. and Pseudomonas putida at different development stages acts as a biostimulant to promote growth, yield and nutrient uptake of tomato. Journal of Applied Microbiology, 2019, 127: 196-207.

[14] Hahm, M.-S., Son, J.-S., Hwang, Y.-J., Kwon, D.-K., Ghim, S.-Y. Alleviation of salt stress in pepper (Capsicum annum L.) plants by plant growth-promoting rhizobacteria[J], Journal of Microbiology and Biotechnology, 2017, 27, 1790-1797.