Seed Biostimulant MGW9 (SB-MGW9) Biopriming Improves Salt Tolerance during Maize Seed Germination

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Original article

Keywords: Salt stress, Seed biostimulant, Biopriming, seed germination, seed vigour, Zea mays, Bacillus sp.

DOI: https://doi.org/10.21203/rs.3.rs-266586/v1

Abstract

Crop performance is seriously affected by high salt concentrations in soils. To develop more new seed pre-sowing treatment technologies it is crucial to improve the salt tolerance of seed germination. Here we isolated and identified the strain *Bacillus sp*. MGW9 and developed the seed biostimulant MGW9 (SB-MGW9) by the strain. Effect of seed biopriming with SB-MGW9 in maize (*Zea mays* L.) under saline condition were studied. The results showed that the *Bacillus sp*. MGW9 has the characteristics of salt tolerance, nitrogen fixation, phosphorus dissolution, indole-3-acetic acid production and the like. Seed biopriming with SB-MGW9 enhanced the performance of maize during seed germination under salinity stress to improve the germination energy, germination percentage, shoot/seedling length, primary root length, shoot/seedling fresh weight, shoot/seedling dry weight, root fresh weight and root dry weight. SB-MGW9 biopriming also alleviates the salinity damage to maize by improving relative water content, chlorophyll content, proline content, soluble sugar content, root activity, activities of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase, decreasing the malondialdehyde content. Especially, the field seedling emergence of maize seeds in saline-alkali soil can be improved by SB-MGW9 biopriming. Therefore, maize seed biopriming with SB-MGW9 can be an effective approach to resist the inhibitory effects of salinity stress and promote seed germination and seedling growth.

Introduction

Soil salinization is an increasingly serious agricultural problem in the world. It seriously affects the growth and development of crops, resulting in serious loss of productivity. As a result of poor irrigation water, over-fertilization and desertification processes, cultivated soils around the world have become more saline and alkaline. More than 800 million hectares of land worldwide are currently affected by salt stress (Ramadoss et al. 2013). Maize (*Zea mays* L.) is an important global cereal crop whose production needs to be increased to meet the food needs of a growing world population (Tilman et al. 2011). Nevertheless, the growth of maize and grain quality can be severely affected by salinity, drought, high temperature and other adverse environmental conditions (Sabagh et al. 2020). Salt stress is emerging as a particular constraint to global crop production, and it is estimated that it will affect about 20% of the world's irrigated land and will lead to a loss of up to 50% of the land by the middle of the twenty-first century (Mahajan and Tuteja 2005; Zhu 2001). Sodium chloride (NaCl), as the main form of soil salinity, can lead to crop yield reduction or even death by making root water uptake more difficult, and lead to plant poisoning by accumulating high concentrations of Na^+ and Cl^- in plants (Deinlein et al. 2014; Paul and Lade 2014; Yu et al. 2020; Zhu 2001). High concentrations of salt lead to a combination of ionic imbalance and hypertonic effects, at biochemical and molecular levels (Munns 2002; Tester and Davenport 2003). For example, salt stress can lead to chloroplast damage, decreased photosynthetic rate and increased photorespiratory rate, accumulation of reactive oxygen species (ROS) and decreased enzyme efficiency (Hoshida et al. 2000; Teixeira and Pereira 2007). However, most plants have developed the ability to reduce the negative effects of salinity through regulation and compartmentalization of ions, synthesis of compatible solutes, induction of antioxidant enzymes, induction of phytohormones, and alteration of photosynthetic pathways (Rojas-Tapias et al. 2012).

Various methodologies are in vogue to develop stress-tolerant varieties, either through conventional breeding or through transgenic technology. Alternatively, however, simpler and more economical practices are competing to solve this problem. Seed priming is a farmer-friendly technique recommended by many researchers for better establishment and growth even under adverse conditions (Filippou et al. 2013a; dos Santos Araújo 2021). It is well known that different environmental stresses often activate similar cell signaling pathways and cellular responses, and seed priming can activate these signaling pathways early in growth and lead to faster plant defense responses. Different seed priming methods employed to mitigate stress tolerance as reported by many researchers are main as follows: hydropriming, halo- and osmopriming, matrix priming, thermopriming, biopriming, drum priming, priming using growth regulators, nutrient priming and redox priming.

In recent years, microorganism and its engineering technology play an important role in helping plants to resist abiotic stress and improving crop yield and quality, which has become an effective way to alleviate plant growth stress and has broad application prospects (de Vries et al. 2020; Mahanty et al. 2016; Venkateswarlu et al. 2008). In particular, the use of some microbial agents such as *Azospirillum*, *Bacillus*, *Gliocladium*, *Pseudomonas*, *Rhizobium*, *Trichoderma*, and other biopriming treatment on seeds to improve seed viability or vigour is very worthy of further study.

Some progress has been made in seed biopriming, and the growth-promoting ability of microorganisms may be highly specific to certain plant species, cultivars and genotypes (Bashan 1998; Moeinzadeh et al. 2011; dos Santos Araújo 2021). In order to reduce the toxic effects of high salt on plant growth, some plant growth promoting bacterias (PGPBs) have been developed to improve the salt tolerance of plants (Ali et al. 2014). PGPBs are considered to be microorganisms that can grow in, on or around plant tissues, stimulating plant growth by a variety of mechanisms, such as synthesis of phytohormones, fixation of non-symbiotic nitrogen, dissolution of inorganic phosphate and mineralization of organic phosphate and/or other nutrients, and antagonism against phytopathogenic microorganisms (Esitken et al. 2010). Shahid et al. (2011) observed that treatment of seeds with *T. viride* improved germination and vigour of chickpea. Under salt stress, *Bacillus subtilis* and *Pseudomonas fluorescens* could significantly increase the fresh weight, dry weight, photosynthetic pigments, proline, total free amino acids and crude protein content of radish roots and leaves (Mohamed and Gomaa 2012). Under 320mM NaCl stress, the root elongation and dry weight of *Hallobacillus sp.* SL3 and *Bacillus halodenitrificans* PU62 were increased by more than 90% and 17.4%, respectively, compared with those of uninoculated wheat seedlings (Ramadoss et al. 2013). Many research findings suggest that that seed biopriming with different beneficial microorganisms can not only improve seed quality, but also improve seedling vigour and resistance to abiotic and biotic stresses, thus providing an innovative crop protection tool for sustainable improvement of crop yields.

In recent years, a kind of agricultural product called plant biological stimulants can help crops resist abiotic stress, which has attracted much attention (Akhtar et al. 2008; Hamel and Plenchette 2007; Harrier and Watson 2004; van der Heijden et al. 2004). By definition, a plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content (du Jardin 2015). There is very little research using seed biostimulant to improve the germination and emergence ability of crop seeds under abiotic and biotic stresses. Our purpose was to isolate and identificate the salt-tolerant beneficial strains for the development of seed biostimulant, and to study the effects of bio-priming on seed germination and seedling emergence of different maize varieties under salt stress by pre-sowing treatment with the probiotics as bio-initiators, so as to provide a basis for the research on improving seed quality.

Materials And Methods

Isolation and cultivation of strains

Bacterial strains were isolated from the extremely arid soil samples near the Great Wall of Ming Dynasty in Shandan County of Gansu Province (100.88E, 38.84N). Soil samples were taken and were placed in sterile sealed bags and stored at -20 °C in August 2017. The soil sample is diluted by 10-fold gradient dilution method. Weigh 20 g of soil sample, mix it well and grind it, pour it into a triangular flask containing 80mL of sterile water, shake it well and mix it well, put it into a triangular flask, add 80mL of sterile water, take the supernatant and dilute it into soil suspension with the concentration of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. The strain was isolated by dilution-spreading plate method, and 200μL of diluent was spread on beef extract peptone agar medium containing 10 different concentrations of NaCl (5, 7, 8, 9, 10, 11, 12, 13, 14 and 15% (w/v)) (agar 15-25 g per litre, peptone 10.0 g, sodium chloride 5.0g, beef extract 3.0g, pH 7.4-7.6) and six replicates per concentration. After 6 to 7 days of culture at 28 °C, the colonies with different morphological characteristics were selected on the plate, and the single colony was purified on the beef extract peptone agar plate by plate streaking method. The candidate bacterial strains were numbered and maintained in strain preservation tube with 25% (V/V) glycerol and stored at -80 °C. In this work, we selected candidate bacterial strain MGW9 for further study as follows.

Screening for salt-tolerance level and growth promoting characteristics of strain MGW9

To a flask containing 50 mL of nutrient broth, NaCl was added to give a final salt concentration of 5, 7, 8, 9, 10, 11, 12, 13, 14 and 15% (w/V). The strain MGW9 of active growth were then added to each flask and incubated on a rotating shaker at 30 °C with 180 rpm. Bacterial growth was determined as OD_{600nm} to determine salt tolerance again.

The strain MGW9 was streaked and inoculated into a nitrogen-free culture medium containing 0-15% of NaCl, cultured in a dark incubator at 30 °C for 4-6 days, the growth condition of the strain MGW9 was observed, and whether the strain had the nitrogen-fixing capacity was detected according to the presence or absence of colonies on a plate, and each NaCl concentration is repeated for three times.

The strain MGW9 was placed on bacterial inorganic and organic phosphorus media containing 0-15% NaCl and incubated at 30 °C for 7 days, respectively, and the strain was observed for the presence of transparent circles, i.e phosphate-solubilizing circles, with three replicates per NaCl concentration. If a clear area appears around the colony indicating that it has the property of dissolving phosphate. The diameter of phosphate-solubilizing zone (D) and colony diameter (d) were measured, and the phosphate-solubilizing ability of strain MGW9 was qualitatively tested by D/d. According to the method described by Li et al. (2019b), the phosphate solubilization index (PSI) = (colony diameter + halo zone diameter)/colony diameter.

According to the method of indole-3-acetic acid (IAA) production test described by Li and Jiang (2017), King's B medium containing 100 mg ml^{-1} L-tryptophan and 0-15% NaCl was used to screen for IAA production. The culture supernatant of the candidate strain was mixed with Salkowski reagent at a ratio of 1:1 ($V: V^{-1}$). The pink mixture indicates the generation of IAA and its density was recorded at OD_{530nm}. The concentration of IAA produced was estimated from a standard curve of IAA in the range of 0-100 μg mL^{-1}.

Note: according to the salt-tolerance level of strain MGW9, we choose the maximum salt concentration to study its growth-promoting characteristics.

Identification of strain MGW9

The strain MGW9 was cultured on beef extract peptone agar medium at 28 °C for 48 h with 200 rmp with three replicates, and then its morphological characteristics were observed by microscope. Genomic DNA of the strain MGW9 was extracted by bacterial genomic DNA rapid isolation kit (Sangon Biotech (Shanghai) Co., Ltd., China), and identified according to the complete 16s rDNA sequence. The 16s rDNA was amplify by PCR using a forward 27F primer (5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse 1492R primer (5'-GGTTACCTTGTTACGACTT-3'). The sequencing of PCR reaction products was completed by Qingdao Pacino Gene Biotechnology Co., Ltd. Sequence homology of nucleotides was compared using the blast search program. The tightly related sequences were aligned by Clustalx using MEGA version 5.1 software package, and the phylogenetic tree was constructed by Neighbor Joining (NJ) method. The bootstrap replications (1000) were used as statistical support for nodes in the phylogenetic tree.

The 16S rRNA gene sequence of strain MGW9 was deposited in the NCBI GenBank under accession number MW663489.

Seed priming using the seed biostimulant MGW9 (SB-MGW9)

Seeds of hybrid maize 'Zhongdi 175' (ZD175), 'Zhengdan 958' (ZD958) and 'Denghai 605' (DH605) were used. Pure maize seeds were randomly selected from each sample for the following experiments.

i) Thousand-seed weight (TSW) test: the TSW was measured using 500 seeds in each of the three replicates and then converted to thousand seed weight (Li et al. 2019a).

ii) Seed moisture content (SMC) test: the seeds were ground and dried at 130 ± 0.5 °C for 4 hours (h) and the moisture content basis was calculated from the fresh weight (ISTA 2007).

iii) Seed water absorption test: 100 maize seeds of the three maize varieties were measured the initial weight, then soaked in sterile water, taken out every 2 h, wiping off floating water on the surfaces of the seeds, measuring the weight and calculating the water absorption of the seeds at different time points. And the water absorption characteristic equation of maize seed was obtained by curve fitting analysis of the average water absorption of three maize seed samples at each time point.

iv) Preparing SB-MGW9: the strain MGW9 was inoculated into a beef extract peptone liquid culture medium and cultured in a fermentation tank at the stirring speed of 150 r/minute (min), the culture temperature of 28 °C and the ventilation rate of 1.5 L/min for 48-60 h, and the number of the MGW9 is adjusted to be 1.0×10^8-1.5×10^8 cfu/mL, and the pH value of the bacterial liquid is adjusted to be 7.0-8.0.

v) Seed priming with SB-MGW9: two-factor randomized block design was used in the experiment. Soaking time for the factor A, set two different time: 3 and 6 h. Moisturizing time was factor B, which was divided into two different time: 12 and 24 h. After treatment, the primed seeds were air dried at 25 °C to near their original moisture contents. Factors A and B were randomly divided into 4 treatments, and the untreated group (no priming) was used as control (C). Treatment 1 (T1) means the seeds soak for 3 h, and moisturize for 12 h; Treatment 2 (T2) means the seeds soak for 3 h, and moisturize for 24 h; Treatment 3 (T3) means the seeds soak for 6 h, and moisturize for 12 h; Treatment 4 (T4) means the seeds soak for 6 h, and moisturize for 24 h.

Germination and seedling growth test

The pure seeds are randomly selected for standard germination test. The seed surface was sterilized with 1% NaClO (w/v, Beijing Chemical Reagent Company, Beijing, China) for 10 minutes, then washed three times with distilled water and air dried for use. The seeds are germinated by adopting a rolling paper germination. Firstly, two pieces of germinating paper (Anchor Paper Co., St Paul, MN, USA) are stacked and moistened by 100 mmol/L NaCl solution, and the redundant water on the paper is removed by a towel; Secondly, the primed seeds are alternately placed on a germination paper bed, the directions of the seed holes are consistent, the paper bed is rolled up and placed into a self-sealing bag, the seed hole ends are vertically placed into a Versatile Environmental Test Chamber (MGC-350HP, Shanghai Yiheng Technology Instrument Co., Ltd., Shanghai, China) with three replicates of 100 seeds and incubated at 25 ± 0.5 °C and on an illumination cycle of 12 hours of light and 12 hours of darkness. Each treatment was repeated 3 times, with 100 seeds per repetition (Jiang et al. 2016). The no priming seeds were used as control. The germination energy (GE) and germination percentage (GP) were measured on the 4th and 7th day after the experiment was established. GP is the normal seedling number on the 7th day after seed planting (Li et al. 2019a). While counting the GP, 10 seedlings with uniform size were randomly selected to measure six indices, including shoot/seedling length (SL), primary root length (PRL), shoot/seedling fresh weight (SFW), shoot/seedling dry weight (SDW), root fresh weight (RFW) and root dry weight (RDW). For SDW and RDW, the plant tissue (shoot/seedling or root) were dried at 105 ± 0.5 ℃ for 8 h.

Assay for biochemical index

After passing through a 2 mm sieve, the sand was sterilized and placed in plastic pots (volume 150 ml) of 100 g of sterilized sand per pot. The content of deionized water in the sterilized sand is 10% (V: W). Salt treatment was carried out by supplementing deionized water with NaCl at a final concentration of 100 mM. After the primed seeds are placed in the sand bed, the pot is sealed with transparent preservative film. Seeds not primed were used as control. There were three replications for each treatment and 15 seedlings per replication. The relative water content (RWC) of the leaf samples was determined, expressed as a percentage, referring to the method of Ghahfarokhi et al. (2015). The chlorophyll content was measured by SPAD502 Plus meter. The level of lipid peroxidation was determined by the content of malondialdehyde (MDA), the content of proline was determined by extraction with 3% 5-sulfosalicylic acid at room temperature, and the content of soluble sugar was measured by anthrone-sulfuric acid method (Zhu et al. 2010). The root activity was determined by triphenyltetrazolium chloride (TTC) method (Li et al. 2015). Approximately 500 mg of a fresh leaf sample was homogenize in 10 ml of a 0.05 M phosphate buffer (pH 7.8) solution and centrifuge at 10000 × g for 10 min (Li et al. 2019b). The supernatant was then collected and stored at 4 ℃ for use. The activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) were measured (Chen and Asada, 1992; Ghahfarokhi et al. 2015; Zhu et al. 2010).

Field seedling emergence test

For field seedling emergence (FSE), the samples were sown at the saline-alkali land in Binzhou (soil salt content is 0.61%, pH is 7.72), Dongying (soil salt content is 0.54%, pH is 7.63), and Weifang (soil salt content is 0.56%, pH is 7.57) experimental base, Shandong, China, in 2020. In this study, the row spacing, row spacing and line length were 0.06, 0.06 and 0.60 m, respectively. The seeds were sown using the single seed sowing method with 10 seeds per row and 10 rows per repetition (continuous row, 100 seeds) in three repetitions. The arrangement of seeds was designed by the method of partition comparison. In June, the field emergence test was completed. FSE was measured at three-leaf stage of maize. FSE (%) = [FSE-Binzhou (%) + FSE-Dongying (%) + FSE-Weifang (%)]/3.

Statistical analysis

Data were analyzed by a one-way analysis of variance (ANOVA) using the SAS statistical software package (SAS Institute, 1999), followed by the calculation of the lowest significant differences (LSD). The data was used to make a comparison and statistical analysis by SPSS 11.0, and Excel. The work was completed in the Seed Science and Engineering Laboratory of Qingdao Agricultural University from March to September 2020.

Results

Isolation, identification and characteristics of strain MGW9

According to the morphological characteristics, 19 strains of salt-tolerant bacteria were isolated from soil samples. Among all the isolated strains, the strain MGW9 not only has the salt tolerance of 12% NaCl, but also has good nitrogen fixation, phosphorus dissolution and indole-3-acetic acid (IAA) production performance. The characteristics of the strain MGW9 cultured under the condition of 12% NaCl mainly include the following three aspects: i) Nitrogen fixation: the strain MGW9 was streaked on nitrogen-free medium and cultured in dark incubator at 30 ℃ for 4–6 days, and MGW9 colonies were found on the plate; ii) Phosphorus dissolution: the transparent circle of MGW9 could be observed on the 3rd day after inoculation in organic phosphate and inorganic phosphate medium, and the size of transparent circle tended to be stable until the 7th day. When the transparent zone was stable, the ratio of the diameter of dissolving phosphorus zone (D) to the diameter of bacterial colony (d) was 1.7 in organic phosphorus medium, and 1.95 in inorganic phosphorus medium; iii) IAA production: the standard curve equation of IAA concentration and absorbance change was $Y = 0.025X + 0.001$ ($R^2 = 0.985$, Y represents absorbance, X represents IAA concentration). IAA production of the strain MGW9 in the king's B medium was 19.24 mg/L.

Blast search and phylogenetic analysis at the National Center for Biotechnology Information (NCBI) of the United States showed that the strain MGW9 had 99.0% sequence homology with *Bacteria WSB-1* (KJ950500.1). Based on its morphology including gram-positive staining, the cells are rod-shaped and 16s rDNA genetic sequence (1424bp), the strain was identified as *Bacillus sp.* MGW9. The strain MGW9 has been preserved in the China General Microbiological Culture Collection Center on November 6, 2019; CGMCC No. 18690; The test result of the collection center is that the strain MGW9 is alive and is recommended to be classified as *Bacillus sp.* (Fig. 1).

Figure 1 here

TSW, SMC and water absorption characteristics of seed samples from three maize varieties

The thousand seed weight (TSW) and seed moisture content (SMC) of seed samples from three maize varieties were 342.7-361.3g and 11.3–11.8% (lower than the safe water content 13%), respectively. The water absorption curve equation of maize seed was $Y = K(6.519X - 0.224X^2 + 2.879)$, K is coefficient of variation. In the seed imbibition stage, the water absorption rate of the three varieties of seed samples showed a trend of first fast and then slow change, and the change became stable after 12 hours (Fig. 2).

Figure 2 here

Effects of seed biopriming with SB-MGW9 on maize seed germination under normal and saline conditions

Compared to normal condition, the germination energy (GE) and germination percentage (GP) of three maize varieties sample seeds were significantly decreased under salt stress, the GE of ZD175, ZD958 and DH605 decreased by 5%, 20.5% and 14.5%, respectively, and the GP of ZD175, ZD958 and DH605 decreased by 10%, 11.6% and 8.5%, respectively. Compared with the control, the GE and GP of seeds after priming treatments were higher than the control, and the priming effect was different under different germination environment. In the normal germination environment, except ZD958, the GE and GP of ZD175 and DH605 sample seeds after biopriming were not significantly different from the control ($P < 0.05$). And there was no significant difference in the GE among different priming treatments of the same variety, and the GP was the same (Fig. 3a, b). This may be related to the fact that the priming effect was not obvious when the initial level of seed vigour of sample was high. Under salinity stress, the GE and GP of seeds after priming treatments were significantly higher than that of the control ($P < 0.05$) except the GP of ZD175-T1, and the priming effects of different priming treatments were different (Fig. 3c, d). Comprehensively analyzing the GE and GP of different priming treatments, the results showed that T3 had the best seed biopriming effect (Fig. 3).

Figure 3 here

Effects of seed biopriming with SB-MGW9 on maize seedling growth under salinity stress condition

Under salinity stress, the six seedling growth indices after seed biopriming were higher than the control. The suitable seed biopriming treatment with SB-MGW9 was different for different varieties. The suitable treatments of ZD175 were T2 and T3, and the suitable treatments of ZD958 were T3 and T4, and DH605 were T2 and T3 (Table 1). Comprehensive consideration of the six indices of seedling growth, the most suitable treatment for the three maize varieties was T3. Compared with the control, the shoot/seedling length (SL), primary root length (PRL), shoot/seedling fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW) of ZD175 increased by 49.3%, 50.7%, 58.0%, 70.0%, 61.1% and 61.5%, respectively, ZD958 increased by 49.2%, 49.2%, 35.0%, 25.0%, 57.3% and 83.3%, respectively, DH605 increased by 44.1%, 46.5%, 60.0%, 62.5%, 48.9% and 61.5% respectively (Table 1).

Table 1
Influence of seed biopriming with SB-MGW9 on the SL, PRL, SFW, SDW, RFW and RDW in maize under salinity stress

Varieties	Treatments	SL	PRL	SFW	SDW	RFW	RDW
		(cm)	**(cm)**	**(g/10S)**	**(g/10S)**	**(g/10S)**	**(g/10S)**
ZD175	C	2.21 ± 0.02d	5.06 ± 0.13c	1.00 ± 0.01c	0.10 ± 0.02b	0.95 ± 0.06c	0.13 ± 0.01c
	T1	2.88 ± 0.10b	6.90 ± 0.62b	1.37 ± 0.07b	0.13 ± 0.02ab	1.24 ± 0.13b	0.17 ± 0.01b
	T2	3.05 ± 0.08b	7.85 ± 0.60a	1.46 ± 0.16ab	0.16 ± 0.03a	1.40 ± 0.07ab	0.21 ± 0.02a
	T3	3.30 ± 0.14a	8.08 ± 0.53a	1.58 ± 0.12a	0.17 ± 0.04a	1.53 ± 0.12a	0.21 ± 0.02a
	T4	2.67 ± 0.11c	7.48 ± 0.51ab	1.28 ± 0.14b	0.15 ± 0.01a	1.32 ± 0.12b	0.20 ± 0.01a
ZD958	C	1.93 ± 0.14c	5.11 ± 0.10d	0.80 ± 0.08d	0.08 ± 0.01c	0.82 ± 0.12c	0.12 ± 0.01d
	T1	2.38 ± 0.19ab	6.65 ± 0.36c	1.01 ± 0.03c	0.09 ± 0.02bc	1.13 ± 0.06ab	0.16 ± 0.02c
	T2	2.21 ± 0.07bc	7.22 ± 0.17b	1.15 ± 0.04b	0.11 ± 0.01ab	1.07 ± 0.05b	0.18 ± 0.02bc
	T3	2.73 ± 0.34a	7.73 ± 0.05a	1.08 ± 0.06bc	0.10 ± 0.002ab	1.29 ± 0.18a	0.22 ± 0.01a
	T4	2.52 ± 0.25ab	7.58 ± 0.51ab	1.29 ± 0.04a	0.12 ± 0.004a	1.22 ± 0.07ab	0.19 ± 0.02ab
DH605	C	1.98 ± 0.16c	4.54 ± 0.15d	0.90 ± 0.03d	0.08 ± 0.01c	0.92 ± 0.07b	0.13 ± 0.01d
	T1	2.27 ± 0.31bc	5.85 ± 0.19c	1.14 ± 0.04c	0.10 ± 0.03bc	1.26 ± 0.14a	0.18 ± 0.02c
	T2	2.59 ± 0.23ab	6.90 ± 0.22a	1.30 ± 0.10b	0.12 ± 0.002ab	1.45 ± 0.24a	0.23 ± 0.01a
	T3	2.68 ± 0.17a	6.65 ± 0.32b	1.44 ± 0.14a	0.13 ± 0.01a	1.37 ± 0.28a	0.21 ± 0.02ab
	T4	2.45 ± 0.10ab	6.34 ± 0.23b	1.22 ± 0.01bc	0.12 ± 0.004ab	1.21 ± 0.05ab	0.20 ± 0.01bc

According to Duncan's multiple range test, different letters in the same column indicate significant differences between treatments at the 0.05 level. SL = shoot/seedling length; PRL = primary root length, SFW = shoot/seedling fresh weight, RFW = root fresh weight, SDW = shoot dry weight; RDW = root dry weight; S = seedling (s).

Effects of seed biopriming with SB-MGW9 on the RWC, Chl content, MDA content, proline content, soluble sugar content and root activity of maize seedlings under salinity stress

Under salinity stress, compared with the control, the relative water content (RWC), chlorophyll (Chl) content, proline content, soluble sugar content and root activity of the seedlings of the three maize varieties after seed biopriming were significantly increased except for the malondialdehyde (MDA) content ($P < 0.05$). By comparing and analyzing the data of six biochemical indices, it can be seen that different seed biopriming treatments have different seed biopriming effects. According to the content of MDA, the suitable seed biopriming treatments were T3 and T4, but there was no significant difference between T3 and T4. And from the other five indices, the suitable seed biopriming treatments for the three maize varieties were T2 and T3, and there was also no significant difference between T2 and T3. Comprehensive consideration of the six biochemical indices data, the suitable seed biopriming treatment for the three maize varieties was T3. Compared with the control, the RWC, chlorophyll, proline, soluble sugar content and root activity of ZD175-T3 increased by 9.1%, 12.3%, 49.1%, 37.2% and 25.0% respectively, and those of ZD958-T3 increased by 7.3%, 9.3%, 59.9%, 29.4% and 15.9% respectively, and those of DH605-T3 increased by 5.0%, 12.3%, 56.9%, 24.0% and 14.3% respectively. In addition, the MDA content in seedling of ZD175-T3, ZD958-T3 and DH605-T3 decreased by 32.2%, 24.3% and 29.4%, respectively (Fig. 4).

Figure 4 here

Effects of seed biopriming with SB-MGW9 on the SOD, CAT, POD and APX activities of maize seedlings under salinity stress

Compared with the control, the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) in maize seedlings increased significantly after seed biopriming with SB-MGW9 ($P < 0.05$). Comprehensive analysis of the four enzyme activity data, different seed biopriming treatments had different priming effects. The suitable seed biopriming treatments for the three maize varieties were T2 and T3, and there were no significant differences in the four enzyme activity indices between T2 and T3. The most suitable treatments for different maize varieties were T3 for ZD175 and DH605, and T2 for ZD958. Compared with the control, the SOD, CAT, POD and APX activities of ZD175-T3 were increased by 42.1%, 23.4%, 36.1% and 63.9% respectively; the SOD, CAT, POD and APX activities of ZD958-T2 were increased by 26.8%, 19.6%, 43.9% and 94.9% respectively; the SOD, CAT, POD and APX activities of DH605-T3 were increased by 47.0%, 20.7%, 33.5% and 27.2% respectively (Fig. 5).

Figure 5 here

Effects of seed biopriming with SB-MGW9 on the saline-alkali field seedling emergence of maize

The results showed that the field seedling emergence (FSE) of maize seeds after biopriming treatment increased significantly compared with the control ($P < 0.05$), and different maize varieties and biopriming treatments had different biopriming effects. According to the results of Binzhou-FSE, the suitable seed biopriming treatments for ZD175 were T3 and T4, for ZD958 and DH605 were T2 and T3; The suitable seed biopriming treatments were T2 and T3 for ZD175, T3 and T4 for ZD958 and DH605 according to the results of Dongying-FSE; and the results of Weifang-FSE show that the suitable seed biopriming treatments of ZD175 and DH605 were T2 and T3, ZD958 were T3 and T4. According to the results of FSE which is the average of FSE- Binzhou, FSE-Dongying and FSE-Weifang, the suitable seed biopriming treatments for ZD175 and ZD958 were T3 and T4, and for DH605 were T2 and T3. Comprehensive considering the results of these indices, the most suitable seed biopriming treatment for the three maize varieties was T3. Compared to the control, FSE-ZD175-T3, FSE-ZD958-T3 and FSE-DH605-T3 increased by 8.2%, 8.9% and 6.7%, respectively (Fig. 6).

Figure 6 here

Discussion

Soil salinity is an increasingly serious global problem, as salt hampers plant growth and development and reduces crop yield. Seed germination and early seedling growth are critical stages in plant establishment and production and are very sensitive to salt stress. The harmful effect of NaCl on seed germination and seedling emergence are caused by the decrease of water use efficiency and nutrient supplement ability when sodium accumulates in soil and the toxic effect of sodium and chloride ions on plants (Parida and Das 2005; Munns and Tester 2008; Deinlein et al. 2014; van Zelm et al. 2020). This study indicated that 100mmol/L NaCl solution as germination solution had obvious salt stress effect on the seed germination and seedling growth of three maize varieties ZD175, ZD958 and DH605. The germination energy (GE) and germination percentage (GP) of that seed under the salt stress condition are respectively reduce by 12.4–20.5% and 8.5–11.6% compared with the control (no stress). Therefore, it is of great practical significance for agricultural production to study the technical methods of improving seed vigour under salt stress in order to alleviate the adverse effects of salt stress on seed germination and seedling emergence.

Various methods have been used to improve crop resistance to stress, including conventional breeding methods such as selective hybridization, mutation breeding, polyploid breeding, genetic engineering and so on (Jisha and Puthur 2015), but seed priming as a simple, economical and effective method is more popular with farmers which can stimulate seed germination, enhance morphological parameters, and improve plant growth and development under abiotic stress (Jisha et al. 2013; Rhaman et al. 2020a, 2020b). dos Santos Araújo et al. (2021) suggest that seed priming with H_2O_2 can improve the salt tolerance of maize plants by protecting chloroplast ultrastructure and regulating primary metabolites. Pill et al. (1991) used polyethylene glycol 8000 (PEG), synthetic seawater (INO) and $NaNO_3$ to priming tomato and asparagus seeds, and the results showed that $NaNO_3$ priming for tomato seeds and INO priming for asparagus seeds could effectively improve seed germination under salinity stress. In addition, seed priming has been reported to improve the salt tolerance of maize (Li and Jiang 2017), wheat (Fercha et al. 2014), cucumber (Passam and Kakouriotis 1994), *Brassica* spp. (Sharma and Kumar 1999), muskmelon (Yeaoung et al. 1996) and other crops.

A recent trend in sustainable development is the use of beneficial microorganisms to increase the nutrient use efficiency of field crops without compromising soil health (Meena et al. 2017). Biopriming is an emerging and promising seed and/or seedling treatment tool for inducing systemic resistance to abiotic and biotic stresses in treated crop. It is a process of biological treatment of seeds refers to the process of combining seed hydration and inoculation with beneficial organisms to protect seeds (Rakshit et al. 2015). In most cases, microbial inoculants such as rhizospheric or endophytic microorganisms (bacteria or fungi) that promote plant growth are used (Ogireddy et al. 2019; Rakshit et al. 2015). As with other seed priming techniques, this technique has proven to be of paramount importance in improving seed quality and performance as well as plant growth (Aliye et al. 2008; Rajkumar et al. 2010, 2012). Bano and Fatima (2009) found that co-inoculation with plant growth-promoting *Rhizobium* and *Pseudomonas* species resulted in some positive adaptative responses of maize plants under salinity. Panuccio et al. (2018) showed that biopriming with *Rosmarinus officinalis* L. and *Artemisia* L. leaf extracts could improve the germination percentage and germination indexes of maize seeds under salinity stress. Among different beneficial microorganisms, *Trichoderma* is the most culturable fungi, has been widely used in the field of agriculture as plant symbiont. Lalitha et al. (2012) found that seed treatment with *T. viride* enhanced root length and seed germination in mustard. Sureshrao et al. (2016) also reported that seed bio-priming enhances rhizospheric colonization of *T. viride* in rice.

Plant biological stimulant is a new concept put forward in recent years. It is applied to plants for the purpose of enhancing nutritional efficiency, abiotic stress tolerance and/or crop quality traits, irrespective of their nutritional content. By definition (du Jardin 2015), beneficial microorganisms are one of the important sources for the development of plant biostimulant products. For beneficial fungi, some of these have been extensively studied and used for their biopesticidal and biocontrol (inducer of disease resistance) abilities and have been exploited by the biotechnology industry as sources of enzymes (Mukherjee et al. 2012; Nicolás et al. 2014). Many plant responses have been demonstrated to be fungal induced including increased abiotic stress tolerance, nutrient use efficiency and enhanced plant growth (Colla et al. 2015; Shoresh et al. 2010). Based on these effects, these fungal endophyte can be considered as biostimulants. For beneficial bacteria, it can interact with plants in all possible ways. There are mainly two types of symbiotic endosymbionts and symbiotic rhizospheric plant growth-promoting rhizobacteria (PGPRs) when they are used as biostimulants. PGPRs are multifunctional, affecting all aspects of plant life, including nutrition and growth, morphogenesis and development, responses to biotic and abiotic stresses, and interactions with other organisms in agroecosystems (Babalola 2010; Berendsen et al. 2012; Berg et al. 2014; Bhattacharyya and Jha 2012; Philippot et al. 2013). Some of these functions are usually performed by the same organism, some are strain-specific, and others depend on synergy in the bacterial community. At present, there are few reports on the combination of plant biological stimulants and seed biopriming to improve seed vigour. Therefore, in this study, *Bacillus sp.* MGW9 was isolated and purified from extremely arid soil samples by salt-tolerant screening combined with morphological and molecular identification. Based on the characteristics of salt tolerance, nitrogen fixation, phosphorus solubilization and indole-3-acetic acid (IAA) production of *Bacillus sp.* MGW9, the strain was used to develop the seed biostimulant MGW9 (SB-MGW9).

Here, the objective of this study was to investigate the effects of SB-MGW9 biopriming on seed germination and seedling growth of maize under salt stress. Related reports show that some microorganisms can improve the growth performance of plants under stress environment by providing plant hormones, soluble phosphate, fixed nitrogen, and other substances (Hayat et al. 2010; Ji et al. 2014), and the characteristics of the strain are similar to *Bacillus sp.* MGW9. Some researchers began to pay attention to the application of microorganisms in seed pre-sowing treatment because of the ability of beneficial microorganisms to inhibit diseases, better crop germination ability and vitality. We set up four seed biopriming treatments according to the water absorption characteristics of maize seeds (Fig. 2), including seed soaking time and moisturizing time. The results of germination test showed that the germination energy (GE) and germination percentage (GP) of three maize varieties under normal and salt stress conditions were increased after seed biopriming treatment (Fig. 3), but the GE and GP of ZD175 (T1, T2, T3 and T4) and the GP of DH605 (T1, T2, T3 and T4) were not significantly different from the control under normal condition (Fig. 3a). However, under saline condition, the GE and GP of T2, T3 and T4 of ZD175 and the four seed biopriming treatments of ZD958 and DH605 were significantly higher than that of the control ($P < 0.05$) (Fig. 3b). Our experimental results showed that SB-MGW9 biopriming could better improve the GE and GP of maize during maize seed germination under NaCl stress, and if the initial level of seed vigour is high and the seeds germinate under normal condition, the SB-MGW9 biopriming effect may not be significant. According to the results of seedling growth test, biopriming with SB-MGW9 could increase the shoot/seedling length (SL), primary root length (PRL), shoot/seedling fresh weight (SFW), root fresh weight (RFW), shoot/seedling dry weight (SDW) and root dry weight (RDW) of maize seedlings under salt stress. This may be due to the earlier completion of metabolic activities before germination in the priming process (dos Santos Araújo et al. 2021; Panuccio et al. 2018), and this advantage of priming seeds leads to the improvement of seed germination and seedling growth.

The leaf relative water content (RWC) is often used to judge the water status of plants, and is considered as a relevant attribute to screen the salt tolerance of crops, and the decrease of RWC is harmful to the growth and development of seedlings (Suriya-arunroj et al. 2004). In the present study, SB-MGW9 biopriming can improve RWC, which showed SB-MGW9 may play an important role in promoting seedlings to absorb water and alleviate the negative effects of salt stress on maize seedling growth (Fig. 4a).

Leaf chlorophyll content, as an important physiological parameter, can well reflect the degree of plant stress. Kalajietal et al. (2016) suggested that salt effects and priming could be better understood by studying photosynthesis-related parameters in chloroplasts. Some studies have shown that salt stress can reduce the content of chlorophyll in many plants, such as cucumber (Tiwari et al. 2009) and green bean (Yasar et al. 2008). In this work, leaf chlorophyll reduction has a negative effect on plant photosynthesis, which may be one of the important reasons for slow growth of maize seedlings. In case of salt stress, the presence of Fe ions in soil solution is severely reduced (Lemanceau et al. 2009), and the synthesis of a chlorophyll precursor of 5-aminolevulinic acid is inhibited (Santos 2004). The decrease of photosynthetic activity may be related to the decrease of chlorophyll enzyme (Santos 2004), chloroplast yield (Perur et al. 1961)), chloroplast size (Terry and Abadia 1986) and low concentration of Rubisco protein (Timperio et al. 2007). However, compared with non-bioprimed seeds, it was observed that suitable SB-MGW9 biopriming treatment could significantly increase the chlorophyll content of maize seedlings under salt stress (Fig. 4b).

Salt stress often induces the increase of reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}) and hydroxyl radical (·OH) in plants, resulting in oxidative damage to plants (Hyodo et al., 2017). Malondialdehyde (MDA) is a kind of lipid peroxidation product, which is considered to be one of the important indices of oxidative damage to cell membrane caused by ROS (Parida and Das 2005). Our results suggest that salt stress induces an increase in MDA content in maize seedlings, suggesting that the presence of salt stress may enhance membrane lipid peroxidation, leading to increased membrane permeability, electrolyte extravasation, and ultimately damage to the cell membrane system. However, ZD175-T3, ZD958-T4 and DH605-T3 significantly decreased by 32.2%, 27.1% and 29.4%, respectively, compared to non-bioprimed seeds (Fig. 4c). This indicated that maize seedlings had stronger tolerance to oxidative stress after seed biopriming with SB-MGW9. This is similar to the result that the content of MDA in mycorrhizal inoculated maize plants is lower than that in non-mycorrhizal plants under temperature stress (Zhu et al. 2010). In addition, proline is a good osmotic agent and radical scavenger to stabilize subcellular structure, which can quench single O^{2-} or directly react with OH (Filippou et al. 2013b). Under stress conditions, proline accumulation may be due to increased synthesis and decreased degradation, which helps to maintain cell water status and protect cell membranes and proteins (Kishor and Sreenivasulu 2014). Bano and Fatima (2009) showed that microorganisms (*Rhizobium* and *Pseudomonas*) introduced in the rhizosphere can improve water use efficiency of maize plants, induce the synthesis of osmotic regulators such as proline, and help maintain the integrity of cell membranes. This is consistent with the results of this study, under salt stress, the proline content of biopriming maize seedlings was significantly higher than that of non-biopriming seedlings (Fig. 4d).

In plants, carbohydrate metabolism is involved in key processes in response to abiotic stresses, with key roles in carbon storage, osmotic homeostasis, osmoprotectants and free radical scavenging (Gangola and Ramadoss 2018). Soluble sugars are important osmolytes in plant cells, and their accumulation contributes to the regulation of osmotic stress in plant cells and results in the preservation of biomolecules and membranes (Bohnert and Sheveleva 1998). Gandonou et al. (2012) reported a significant increase in soluble sugar content in sugarcane leaves and roots under salt stress. Borrelli et al. (2018) have shown that carbohydrate stores in wheat plants under salt stress are quickly mobilized, releasing soluble sugars that act as compatible solutes under stress. In this work, our results showed that the soluble sugar content increased significantly after seed biopriming with SB-MGW9, which may help to alleviate osmotic stress (Fig. 4e). Similarly results were observed by Feng et al. (2002) who found have shown that the colonization of arbuscular mycorrhizal fungi could significantly increase the soluble sugar content of salt-treated maize seedlings, indicating that these plants have a higher osmotic adjustment capacity. And dos Santos Araújo et al. (2021) showed that H_2O_2 priming could increase the contents of six sugars and polyols in maize plants under salt stress to alleviate osmotic stress. Root activity is a general indicator of the ability of roots to absorb water and nutrients. The decrease of root activity is harmful to the growth and development of maize seedlings. The increase of root activity was beneficial to absorb more water and nutrients in the process of maize seed germination and seedling formation. In our study, an increase in root activity was observed by seed biopriming with SB-MGW9 (Fig. 4f).

Abiotic stresses, including salinity, are often interrelated, either individually or in combination. They lead to excessive production of reactive oxygen species (ROS) in plants, such as superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH), which cause damage to biochemical processes and eventually lead to oxidative stress (Hyodo et al. 2017). However, plants have evolved a series of defensive measures, including the use of their own antioxidant enzymes and non-antioxidant metabolites to eliminate ROS (Feng et al. 2002). The common antioxidant enzymes include SOD, POD, CAT and APX, which play an important role in the process of scavenging ROS. SOD catalyzes the conversion of O^{2-} to H_2O_2 and O_2, while POD and CAT can scavenge H_2O. And APX can protect chloroplasts and other cellular components from H_2O_2 and hydroxyl damage. Some studies have found that under abiotic stress, inoculation of some beneficial bacteria (such as: *Bacillus subtilis SU47*, *Glomus etunicatum*) can improve plant antioxidant enzyme activity to alleviate the negative effects of stress on plant growth (Upadhyay et al. 2011; Zhu et al. 2010). In our study, compared with non-bioprimed seeds, the activities of SOD, POD, CAT and APX in maize seedlings were significantly increased after biopriming treatment ($P < 0.05$), indicating that SB-MGW9 biopriming may improve the antioxidant defense capacity of maize seedlings under salt stress (Fig. 5).

In addition, the field seedling emergence (FSE) of seeds after bio-priming was measured in saline and alkaline soil of Binzhou, Dongying and Weifang, respectively in this work. From the results of FSE determination, although the biopriming effects of different treatments on seed samples of three maize varieties were different, on the whole, compared with the seeds without biopriming treatment, the FSE of priming seeds was significantly improved (Fig. 6). The results further indicated that SB-MGW9 biopriming could effectively improve the salt stress resistance of maize at the stage of seed germination and seedling.

In this study, we've isolated and identified the strain *Bacillus sp*. MGW9 (CGMCC No. 18690) with the characteristics of salt tolerance, nitrogen fixation, phosphorus solubilization, IAA production and so on. We've also developed the SB-MGW9, and demonstrated that maize seeds biopriming with SB-MGW9 can resist the inhibitory effects of NaCl stress and promote seed germination and seedling growth. According to our current research results, we argue that the use of SB-MGW9 biopriming to improve salt stress resistance of maize seed germination may be to improve the antioxidant capacity of plants, increase the RWC, the content of chlorophyll, proline, soluble sugar, root activity and other aspects of the comprehensive role to promote plant growth.

The results indicated that the suitable biopriming treatments of SB-MGW9 for the three maize varieties were T2 (the seeds soak for 3 h, and moisturize for 24 h) and T3 (the seeds soak for 6 h, and moisturize for 12 h), and *SB-MGW9* may be the promising technique to decrease the deleterious effects of salt stress for maize seed germination and seedling. In addition, based on the current research results, the molecular regulation mechanism of SB-MGW9 biopriming to improve maize seed vigour and whether spraying SB-MGW9 on maize seedlings can improve the effect of salt resistance etc. need to be further studied.

Declarations

Declaration of competing interest

All authors declare that no potential conflict of interest.

Acknowledgements

The authors are grateful to Professor Yanming Zhao of Qingdao Agricultural University for helping us to get soil samples in Gansu Province.

Authors' contributions

HL, HY and YL performed the experiment, analyzed the data and wrote the draft. HZ and LL provided important research assistance to this study. JW and XJ designed the research and made revision for the manuscript.

Funding

This research was supported by the National Key Research and Development Program of China (2017YFD0701203, 2017YFD0101202, 2018YFD0300604, 2019YFE0120400), National Natural Science Foundation of China (31801906、31601386, 31671621), Special Fund for Agro-scientific Research in the Public Interest (201303002), National System (Maize) of Modern Industrial Technology (nycytx-02), National Science and Technology of China (2014BAD16B07), Project of Shandong Province Higher Educational Science and Technology Program (J18KA121), Natural Science Foundation of Shandong Province (ZR2017LC026), Applied Basic Research Programs of Science and Technology Foundation for Youths of Qingdao (19-6-2-36-cg).

Availability of data and materials

The authors declare that all the data and materials used in this study are available.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All authors agree with the contents of the manuscript and its submission to the journal.

Competing interests

The authors declare that they have no competing interests.

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