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Research Article

The effect of halotolerant bacteria isolated from saline soil on growth and yield of maize in saline soil

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Abstract

Salinity is a common problem of abiotic stress in the world. Salinity stress causes yield loss in cultivated crops, such as maize. The yield of maize exposed to salinity stress can be increased with the application of some beneficial microorganisms. Three isolates of halotolerant bacteria from saline fields can potentially be used as biostimulants (plant growth-promoting rhizobacteria). A field experiment to study the effect of halotolerant bacteria isolates application on the growth and yield of maize (*Zea mays* L.) in saline soil was arranged in a randomized block design with a combination of isolate types and frequency applications, and it was repeated three times. In this study, four bacterial strains used were SN13 (*Streptomyces* sp.), SN22 (*Bacillus megaterium*), SN23 (*Bacillus* sp.) and SN26 (*Bacillus aryabhattai*) isolated from the soil of saline-prone regions of Lamongan, in coastal East Java, Indonesia. Results indicated that an application of halotolerant bacteria was able to improve the yield and nutrient uptake of maize in saline soil. However, the application of halotolerant bacteria significantly improved leaf total chlorophyll content (105.94%), plant dry weight (56.14%), grain weight per cob (108.11%) and had a positive trend in increasing N uptake (61.19%), and Na uptake (73.09%) compared to control. It is concluded that the application of halotolerant bacteria is able to alleviate the salinity stress of maize in saline soil.

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Introduction

Saline soil can decrease the growth and yield of crops because of its high concentration of soluble salt. Saline soil is widely distributed in the world and accelerated naturally through the expansion of intensive irrigation and combined with high evaporation rates (Turan and Tripathy, 2012). The salinity level is classified according to the electrical conductivity (EC) value. A saline soil is identified as having EC of more than 4 dS m^{-1} (≈ 40 mM NaCl) and exchangeable sodium of less than 15%. The value of EC is affected by the concentration of salt, porosity, permeability, moisture, and temperature of the soil, as well as the composition of clay. The determination of soil salinity can be a laboratory test on EC of soil water extract, Concentration of soluble ions contained by soil water and total dissolved ions (Hardie and Doyle, 2012). The excessive level of sodium in the soil altered soil pH and exchangeable ions, as well as the alteration in soil structure and nutritional imbalance (Qadir and Schubert, 2002). Salinity respectively defined not only as the excessive concentration of salt but also as excessive sodium (Na^+) and pH. It is caused by high water-soluble salts, i.e., Na^+, K^+, Ca^{2+}, Mg^{2+}, CO_3^{2-}, Cl^- HCO_3^- and SO_4^{2-} concentrations in soil (Daliakopoulos et al., 2016). Salinity stress affects both of morphology and physiology of a plant. Salinity causes water and osmotic stress, limiting plants from absorbing the water and harming plants (Hnilickova et al., 2017). Utilizing saline soils needs innovative technology to optimize land productivity.

Microbial technology, such as halotolerant bacteria application, may be a proper strategy to reduce the harmful effect of salinity stress. Genetically, plant tolerance to salinity stress has a wide variation. They can be grouped as sensitive, moderate-sensitive, moderate-tolerance and tolerance (Grieve et al., 2014). Nutrient imbalance occurs when a plant exhibits salinity stress. Plants accumulate more Na in the tissue while other nutrient essentials, such as low N and K contents (Leksmy et al., 2013). Currently, many bacteria genera such as Bacillus, Pseudomonas Azospirillum, Rhizobium, Alcaligenes, Clostridium, Thiobacillus, Serratia, Streptomyces and Klebsiella are potential plant growth-promoting bacteria in saline soils (Arora et al., 2012).

Bacteria identified as *Pseudomonas* spp., *Bacillus subtilis* and *Bacillus megaterium* not only reduce the salt content and pH but also increase the organic content of saline soil (Fu et al., 2018). Nutrient availability is crucial for plant life. *Bacillus* sp. has been reported to tolerate heavy metals and salinity and can protect cells from oxidative damage by producing the catalase enzyme (Rayavarapu and Padmavathi, 2016). *B. aryabhattai* was able to produce organic acids and was able to increase dissolved Zn (Ramesh et al., 2014). *Streptomyces* sp. was a potential agent for increasing plant growth and controlling plant disease (Sousa et al., 2008). Inoculation of *B. megaterium* bacteria can increase plant growth by increasing dissolved phosphorus (Zou et al., 2010).

The positive effect of rhizobacteria isolated from the unstressed environment on a plant grown in saline soil has been widely reported. However, the information on the impact of halotolerant bacteria in endemic saline is very limited. This research was conducted with a hypothesis that different combinations and different frequency applications of halotolerant bacteria affected the yield and nutrient uptake of maize in saline soil

Materials and Methods

Experimental design

Four isolates of bacteria SN13, SN22, SN23 and SN26, were isolated from the rhizosphere area of weeds growing in the saline soil in Sidomukti Village, Brondong District, Lamongan Regency of East Java. They were cultured and evaluated at the Bacteriology Laboratory of Pests and Plant Disease Department, Brawijaya University (i.e., salinity tolerance, hypersensitivity, nitrogen-fixing, and IAA production test). This research was conducted using a randomized block design with three replicates for each treatment. The treatments were consortium isolates and frequency of application. There were 10 treatment combinations, namely: B0 = control; B1 = SN 13-22-26 consortium, applied once; B2 = SN 13-22-26 consortium, applied two times; B3 = SN 13-22-26 consortium, applied three times; B4 = SN 22-23-26 consortium, applied once; B5 = SN 22-23-26 consortium, applied two times; B6 = SN 22-23-26 consortium, applied three times; B7 = SN 13-22-23-26 consortium, applied once; B8 = SN 13-22-23-26 consortium, applied two times; and B9 = SN 13-22-23-26 consortium, applied three times.

Field experiment

The field experiment took place in the saline soil on agricultural land located at Sidomukti Village, Brondong District, Lamongan Regency of East Java. The soil properties of the initial test revealed EC 4.12 dS m^{-1}, pH 7.1, CEC 37.8 me 100 g^{-1}, organic C and total N 1.39 and 0.16%, respectively. Whereas P, K, and Na contents were 8.61 ppm, 0.4 and 1.68 me 100 $g^{-1,}$ respectively. Maize seeds of the BISI-18 variety were planted with a planting distance of 65 x 20 cm. The ungrown plants were replaced maximum until two weeks after planting (WAP). Before planting, maize seeds were soaked with halotolerant bacteria of 5 mL L^{-1} for 120 minutes. In addition, some seeds were soaked with distilled water for the control treatment. Plants were watered once a day at initial growth and every two days in the next. N-P-K fertilizers with doses of 326.09 (N)-333.3 (P_2O_5)-200 (K_2O) kg ha^{-1} were applied as urea, SP 36 and KCl. Fertilizers were applied in three splits (as basal fertilizer, 3-4 WAP, and 7 WAP). Organic pesticide from *Azadirachta indica* extract that was used for controlling pests and diseases was sprayed with a concentration 5 mL L^{-1}.

Halotolerant bacteria application

The first application of halotolerant bacteria was seed treatment (soaking) for all treatments and re-applied at 2 WAP for application two times and one more application at 4 WAP for application three times. Re-applications at vegetative growth were done through soil drenching with 30 mL of 30 mL L^{-1} solutions near the plant. To prevent solar radiation's negative effects, halotolerant bacteria were applied in the afternoon.

Growth and yield measurements

Data of leaf area index (LAI) and plant dry weight were recorded from four representative plants of each treatment at 12 WAP. Likewise, grain weight per cob was recorded from eighteen representative plants of each treatment. Chlorophyll content in the leaves of maize was measured non-destructively using chlorophyll meter SPAD-502 at 8 WAP. To estimate total chlorophyll content, its value was converted according to Cerovic et al. (2012) by the following equation:

Total chlorophyll ($mg\ g^{-1}$) = 82.2 (x) / (1.35-(x)) (x = SPAD value).

Plant biomass (dried material) was used for laboratory tests to determine nutrient content (total N, P, K and Na). Total N was determined by the semi-micro Kjeldahl method. P was determined by a Spectro photometer using a standard curve. K and Na were determined using a Flame photometer. Nutrient uptake was calculated by multiplying the nutrient content (in%) of each treatment with plant dry weight.

Statistical analysis

Data observed were analyzed using ANOVA (F test) at a 5% error level and followed by Least Significant Differences (LSD) at a 5% error level. The correlation and regression analysis between variables was performed using SPSS 16.

Results

Growth and yield of maize

The result showed that halotolerant bacteria application increased LAI and plant dry weight as well as grain weight (Table 1). Plant dry weight (DW) increased up to 113.47% over the control. The total chlorophyll content of maize leaves increased by the application of halotolerant bacteria in all treatments compared to the control treatment (Figure 1).

Table 1. Growth and yield of maize affected by isolate bacteria combination and frequency application of halotolerant bacteria in saline soil.

Treatments	LAI	Plant DW (g plant^{-1})	Grain Weight (g cob $^{-1}$)
Control	1.13 ± 0.39	49.04 ± 9.00 a	6.53 ± 0.46 a
SN 13+SN 22+SN 26 (once)	1.43 ± 0.18	49.22 ± 5.81 a	27.32 ± 15.05 b
SN 13+SN 22+SN 26 (2 times)	1.49 ± 0.46	60.44 ± 24.89 ab	26.97 ± 5.04 b
SN 13+SN 22+SN 26 (3 times)	1.38 ± 0.15	66.30 ± 35.40 ab	15.60 ± 2.32 ab
SN 22+SN 23+SN 26 (once)	1.92 ± 0.38	89.66 ± 3.02 b	34.73 ± 8.49 bc
SN 22+SN 23+SN 26 (2 times)	1.42 ± 0.42	55.88 ± 5.02 a	32.13 ± 13.40 bc
SN 22+SN 23+SN 26 (3 times)	1.60 ± 0.31	73.65 ± 21.67 ab	24.53 ± 2.88 b
SN 13+SN 22+SN 23+SN 26 (once)	1.97 ± 0.25	78.22 ± 14.52 ab	23.32 ± 2.17 b
SN 13+SN 22+SN 23+SN 26 (2 times)	1.56 ± 0.10	68.48 ± 3.36 ab	15.46 ± 0.20 ab
SN 13+SN 22+SN 23+SN 26 (3 times)	2.43 ± 0.41	121.04 ± 3.28 b	40.64 ± 8.58 c
LSD (5%)	ns	38.9	11.68

Notes: means ± standard deviations accompanied by the same letters do not significantly differ at LSD 5%. ns: not significant; LAI: Leaf Area Index; DW: Dry Weight.

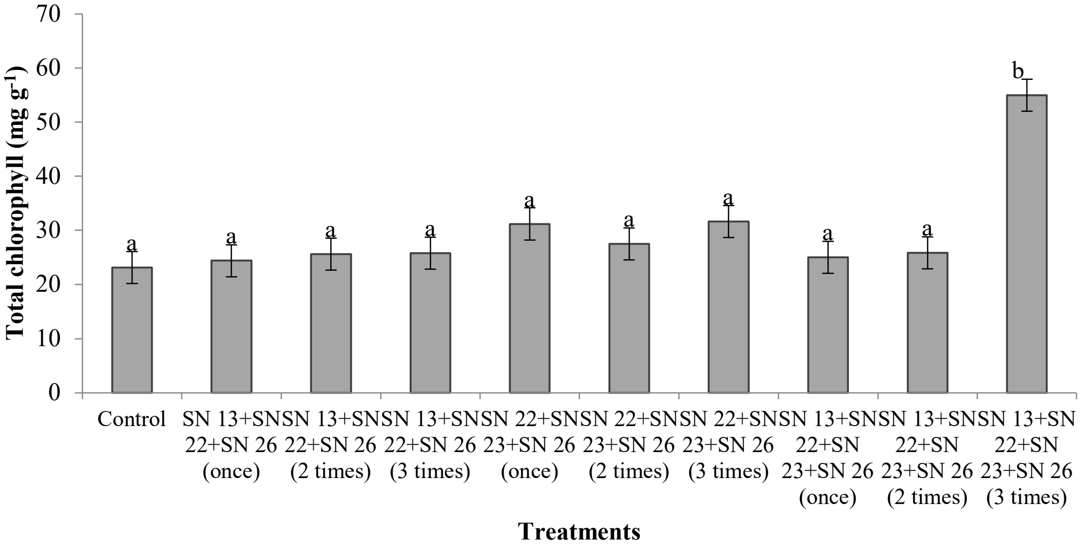
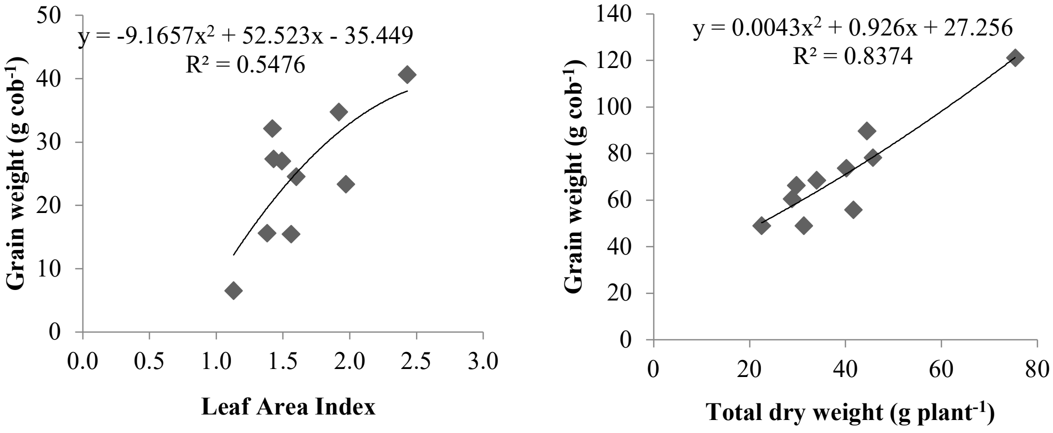


Figure 1. Effect of halotolerant bacteria on total chlorophyll of maize.

Grain weight per cob per plant increased by 108.11% application of halotolerant bacteria compared to the control treatment. Leaf area index had a positive correlation to grain weight at the significant level of 0.01 (r = 0.781**), and plant dry weight had a positive correlation with grain weight at the significant level of 0.05 (r = 0.716*). The value of the regression coefficient (R^2) on the leaves area index was 55.23%, and the plant dry weight was 71.6% (Figure 2). It shows that LAI and plant DW had straight rate effects on grain weight per cob, which means if the leaves area index or dry weight of the plant increased, grain weight would also increase.

Nutrient uptake

Generally, the results of this study indicated that nutrient uptake by application of halotolerant bacteria increased compared to the control treatment. Even statistically, P and K uptake were affected by the application of halotolerant bacteria; the P and K values had a positive trend. This analysis shows that more nutrients accumulated in the shoot increased yield.



A B

Figure 2. Regression (a) leaf area index and grain weight; (b) plant dry weight and grain weight.

Correlation and regression analysis results between nutrient uptake and yield component showed strong relationships. At the significant level of 0.01, grain weight was strongly correlated with N uptake (r = 0.777***), P uptake (r = 0.79**), and K uptake (r = 0.784**). The value of the regression coefficient (R^2) of each nutrient was 55.3%, 49,3% and 58.4% for N, P, and K, respectively (Figure 3). These results showed that more nutrients accumulated in the shoot would increase grain weight per cob.

Discussion

Maize growth is influenced by genetics and environment as supporting factors. Genetic factors have a potential effect on plant growth. While the environment is a supporting factor in optimizing the genetic potential of maize. Inappropriate environmental conditions, such as land with high salt concentrations, may inhibit maize growth. Saline stress conditions may inhibit the expansion and division of a cell, as well as stomatal closure, inhibit leaf expansion, and stimulate senescent acceleration of leaves due to excessive accumulation of toxic ions (Rajendran et al., 2009). The use of microbial technology, such as the use of halotolerant bacteria, can be an effort to develop saline land technology innovations so that plants can grow optimally in conditions that are not suitable. The application of halotolerant bacteria enhanced the growth, yield and nutrient uptake of maize in saline soil. Bacteria cannot only adapt to an unfavorable environment (i.e., saline soil) but can also alleviate salinity stress experienced by plants. Indole acetic acid (IAA) is one of the phytohormones which stimulates growth. Previous research revealed that bacteria SN 13, SN 22 and SN 23 potentially produced IAA in the amount of each 4.83, 10.08, dan 2.10 ppm (Aini et al., 2022). Bacterial inoculation can increase plant growth and germination rates, plant response to stress from external factors and protect plants from disease (Gholami et al., 2009).

Plant growth-promoting rhizobacteria (PGPR) can produce phytohormones such as IAA, which can increase root growth and produce a larger area of the root surface, allowing plants to absorb more nutrients because of the broad range of roots to get nutrients, the cytokinin hormone that can stimulate cell division, cell extension and expansion of plant tissue, and gibberellin (Akhtar et al., 2012). Bacteria such as Azospirillum were able to provide N elements and produce phytohormones such as IAA, and were able to decompose organic cellulose, amylose, and organic materials containing a number of fats and proteins in the soil.

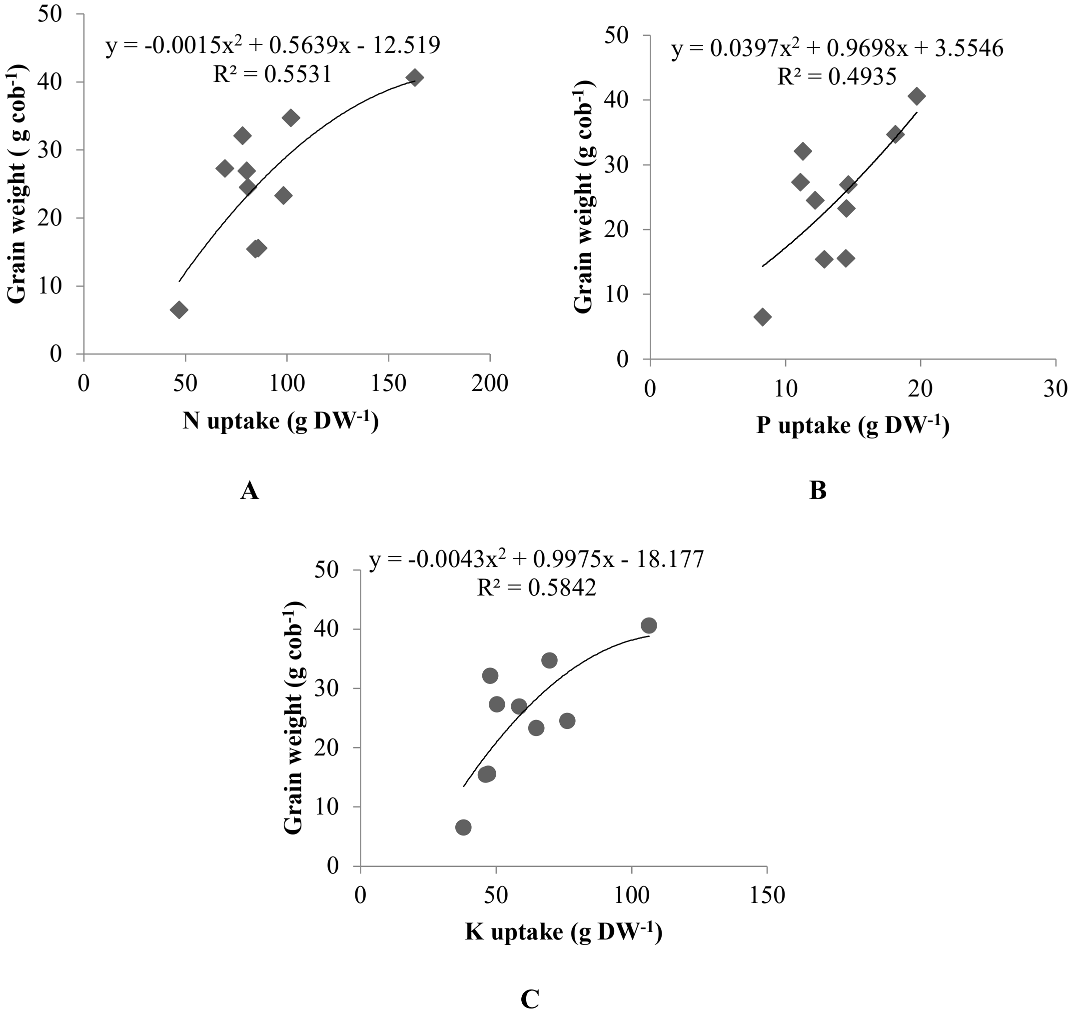


Figure 3. Regression (a) N uptake and grain weight; (b) P uptake and grain weight; (c) K uptake and grain weight.

Egamberdiyeva (2005) stated that *Pseudomonas* spp. produce biologically active compounds that may have the ability to possibly survive under ecological stress, such as heat and salinity stress, as well as low nutrient-poor soil. Some *Pseudomonas* isolates are known to dissolve organic and inorganic phosphates and can produce phytohormones such as IAA that have an essential function in the development of plant organs such as roots.

The results of research conducted by Rayavarapu and Padmavathi (2016) showed that *Bacillus* sp. was tolerant to heavy metals and salinity and played a role in plant cell protection from oxidative damage by producing catalase enzyme. Ramesh et al. (2014) stated that *B. aryabhattai* inoculation could produce organic acids and increase dissolved Zn in the growing media. Some *Pseudomonas* strains produce chelating agents called siderophores with high affinity, which can increase plant growth through increased iron solubility in the area of plant rhizosphere and can reduce the adverse effects of pathogens (Sivasakthi et al., 2014). The research by Sousa et al. (2008) showed that *Streptomyces* sp. has good adaptability and can stand extreme environments such as acid soils and saline soils. This ability indicates that *Streptomyces* sp. has the potential as a potential agent for increasing plant growth and controlling plant disease. Inoculation of *B. megaterium* can increase plant growth by increasing dissolved phosphorus (Zouet et al., 2010).

Growth and yield can be evaluated to know plant responses under environmental stress. In this research, growth and yield were affected by the application of halotolerant bacteria. In the leaf area index, the control treatment gave the lowest average leaf area index due to the salt stress conditions that were able to reduce plant growth. Salinity stress on maize plants caused a decrease in leaf area and leaf number caused by low water and nutrient supply and excessive Na^+ and Cl^- in plant tissue. It inhibited cell differentiation at the growing point. The application of isolates of halotolerant bacteria increased an average leaf area index of 132.17% compared to those without giving halotolerant isolates. Inoculation of halotolerant bacteria could increase chlorophyll content up to 73.64% over the control treatment.

The increase of chlorophyll content by the bacterial isolates inoculation is thought to be related to the role of the rhizosphere bacteria as a biofertilizer and fixation of nitrogen. Inoculation by halotolerant bacteria can increase nutrient uptake, reaching 61.19% compared to the control treatment. Nitrogen is a nutrient that can affect the chlorophyll content in plant leaves. High nitrogen levels can increase the green pigment of plant leaves so that the leaves will appear greener. This is in accordance with the statement of Nadeem et al. (2006) that the chlorophyll content in maize decreases in salt stress conditions, but the inoculation of rhizobacteria enhanced the leaf chlorophyll content of maize. The high chlorophyll content of plants increases the rate of photosynthesis, which then increases the plant's leaf area. The higher the plant's leaf area, the greater the reception of sunlight, thereby increasing the dry weight of the plant because the chemical energy produced is also greater.

The application of halotolerant bacteria increased the grain weight of maize. The low yield of maize in the control treatment was caused by salt stress conditions. According to Li and Li (2017), salt stress might affect every stage of plant life, including germination as well as vegetative and reproductive growth. Likewise, Farooq et al. (2015) reported that salt stress could affect nutritional disorders and oxidative damage, both of which can reduce the growth and development of maize productivity. The statement is supported by the results of EC observations that have been carried out, wherein the control treatment, EC values were obtained with an average of 4.1 dS m^{-1} with the highest Na soil content among the other treatments, which was 1.08 me 100 g^{-1}. The results of the study of Radic et al. (2007) show that increased NaCl concentrations negatively affect the germinability and development of corn, and the crop yield will also decrease. These results are consistent with the study of Gholami et al. (2009) that plants inoculated with PGPR bacteria were generally able to produce higher grain weight compared to treatment without bacterial inoculation.

The increase in grain weight was influenced by an increase in the weight of a hundred seeds and seed number per cob of maize. Giving *Azospirillum* sp. Bacteria and *Pseudomonas* sp. were significantly able to increase grain weight up to 44% compared to the control treatment. In the field experiment, the inoculation of PGPR strains increased all observational parameters. The beneficial effect of giving PGPR on the yield and growth of several plants, such as wheat, corn, and soybeans, is related to its increasing N_2 fixation ability, phosphate absorption capacity and phytohormone production (Gholami et al., 2009). Under salinity stress, lowering nutrient uptake is one of the causes of decreasing yield. The results of this research indicated that halotolerant bacteria application increased macronutrient uptake in shoots and tended to reduce sodium uptake. PGPR might colonize roots and support plants to absorb macronutrients (Zameer et al., 2016). Inoculation of halotolerant bacteria isolates increased the total N and total contents of soil P, K, and organic C, respectively, reaching 59.96%, 84.87%, 25.50%, and 97.60%. This is in line with the statement of Arora et al. (2016) that the administration of inoculated halotolerant bacteria can reduce Na content, which can increase the organic C content, enzyme activity, and levels of available N and P to facilitate plant growth.

Conclusion

Application of halotolerant bacteria, SN 13, SN 22, SN 23 and SN 26, with a variety of combinations and frequencies, could alleviate the salinity stress of maize in saline soil. The potential of bacteria to increase yield and nutrient uptake was affected by their ability to produce phytohormones and increase nutrient uptake.

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