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Growth promotion of maize by the use of salt-tolerant phosphate solubilizing bacteria coupled with tailor-made chemical fertilizer

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Abstract

The study aims to examine the effects of *Bacillus megaterium* A12ag, a salt-tolerant phosphate solubilizing bacteria (PSB), on maize germination and growth under various levels of electrical conductivity (EC). At an EC of 3 ds/m, seeds inoculated with the bacterium increased the germination percentage, root and shoot dry weight, and root and shoot length by 8.1, 9, 6, 23, and 21%, respectively, compared to the uninoculated control. The effects of the salt-tolerant PSB coupled with tailor-made fertilizer (TMF) on maize growth, including shoot height, root length, leaf greenness, leaf number/plant, and shoot and root dry weight in pot experiments, were also assessed. The experiments consisted of six treatments: 1) uninoculated (C), 2) inoculated with the salt-tolerant PSB (B), 3) inoculated with the salt-tolerant PSB coupled with tailor-made chemical fertilizer (B+TMF), 4) inoculated with the salt-tolerant PSB coupled with chemical fertilizer at the typical rate applied by the local agriculturists (B+AF), 5) applied only tailor-made chemical fertilizer, TMF, and 6) applied only chemical fertilizer at the typical rate applied by the local agriculturists (AF). SimCorn software was employed to formulate the TMF. The software recommended using the 81 kg N/ha, 23 kg P/ha, and 69 kg K/ha. The B+TMF showed the highest performance in promoting maize growth, while there was no significant difference between the growth of maize with AF and TMF. This study highlights potential strategies of using the salt-tolerant PSB coupled with tailor-made chemical fertilizer to concurrently reduce the application of chemical fertilizers while promoting plant growth.

Keywords: Saline soil, Salt-tolerant bacteria, Plant-growth promoting bacteria, Tailor-made fertilizer

1. Introduction

Maize (*Zea mays* L.) is one of the globally grown crops, cultivated over 197 million hectares of land [1]. It can be cultivated under a wide diversity of soil types, climates, and management practices. In 2021, the maize growing area in Thailand was around 1.129 million ha with total maize production of 5.34 million tons [2]. Thai maize farmers apply chemical fertilizer at a rate of 100-375 kg/ha depending on traditional maize growing practices in each region. Most of them use chemical fertilizers to increase maize yields, although they are aware that such continued use also decreases soil fertility [3]. Continuous maize cropping with traditional practices not only leads to a deterioration of the soil quality but also increases the production cost by 25% [4]. To address the issue of continuously increasing chemical fertilizer prices and the adverse effects from the overuse of chemical fertilizers, tailor-made chemical fertilizer (TMF) technology has been proposed by Prof. Dr. Tassanee Attanand and her research team to improve the efficient use of chemical fertilizers which aim at increasing crop yield with decreasing usage, maintaining soil quality, and reducing the crop production cost. TMF technology is computer software that is used to formulate fertilizer for a site-specific crop by considering the soil type, soil fertility status, growing conditions, and fertilization practices [5]. The achievements of TMF practices have been reported elsewhere [6,7].

Phosphorus is the second most essential plant nutrient after nitrogen, which influences plant growth and development [8]. Phosphorus deficiency results in crooked and missing rows as the kernels twist and produce

small auricles in maize [9]. The phosphorus content in most soil is relatively low and the chemical phosphorus fertilizers applied to the soil are immobilized rapidly through precipitation with several cations, and then become unavailable to plants [10]. The Northeast of Thailand not only faces the problem of low soil fertility but also high soil salinity. The availability of phosphorus is reduced in saline soils not only because of ionic strength effects that reduce the activity of phosphate but also because phosphate concentrations in the soil solution are sorpted in the form of calcium-bound P [11,12]. Phosphate solubilizing bacteria (PSB) play a key role in solubilization of fixed soil phosphorus by the production of organic acids, phosphatases, chelating compounds, and siderophores [13,14]. Their roles in promoting plant growth are well reported [15-17]. Extensive work has been reported on the need to use salt-tolerant PSB to promote plant growth in saline soils because they are highly adapted to grow in saline environments and highly compatible with other rhizosphere microbes [18-21]. *Bacillus* is the most attractive genus for using as bioinoculants not only because of its ability in forming endospore but also its salt tolerance trait [22-24]. It is therefore of great interest to determine the performance of *Bacillus megaterium* A12ag, salt-tolerant PSB which were isolated from saline soil and selected from our previous work [25] for their ability to solubilize insoluble phosphate, on the enhancement of seed germination and seedling growth under various salinities (0, 3, and 6 dS/m). The effects of using only salt-tolerant PSB, using salt-tolerant PSB coupled with TMF, and using salt-tolerant PSB coupled with chemical fertilizer at the typical rate applied by the local agriculturists, were also evaluated.

2. Materials and methods

2.1 Effects of *Bacillus megaterium* A12ag on germination and seedling growth

B. megaterium A12ag, a salt-tolerant PSB, used in this work was obtained from our previous work [25]. It was grown in trypticase soy broth at 35°C for 48 h in a shaker (New Brunswick Scientific Model C24, USA.) at 150 rpm. Inoculum was prepared by centrifugation of bacterial suspension at 10,000g for 3 min, using a Sorvall RC 5C centrifuge, Dupont, U.S.A., and diluted to obtain 1×10^8 CFU/ml by adjusting their optical density at 600 nm to approximately 1.0 using sterile phosphate buffer (0.1 M, pH 7.0). Effects of a salt-tolerant PSB on germination and growth of maize (Brixgar 99 from Star Agritech Company Limited) were evaluated using the paper towel method according to the International Seed Testing Association [26]. In brief, the maize seeds were surface disinfected by immersion in 1% sodium hypochlorite for 5 min and washed 5 times with sterilized distilled water. The disinfected seeds were immersed in the bacterial inoculum (1×10^8 CFU/ml) or phosphate buffer (0.1 M, pH 7.0) for 30 min and used as inoculated and uninoculated treatments, respectively. They were then placed in folded paper towels moistened with 20 ml of saline waters (electrical conductivity at 0, 3, 6 ds/m [27]) and placed in plastic tubs. Seven days after planting, the germinations were observed. Then, the length and dry weight of the root and shoot of the seedlings were determined. Seed germination was defined as radical emergence to 1 mm. The germination percentage was calculated using the following equation:

$$\text{Germination percentage} = (\text{number of germinated seeds} / \text{number of total seeds}) \times 100$$

2.2 Effects of *B. megaterium* A12ag and tailor-made chemical fertilizer on maize growth

The pot experiments were conducted at Maha Sarakham province, which was situated between latitude 16°20'N and longitude 103°12'E. The experimental design was a randomized complete block with six treatments of different fertilizer applications: 1) uninoculated (C), 2) inoculated with the salt-tolerant PSB (B), 3) inoculated with the salt-tolerant PSB coupled with TMF (B+TMF), 4) inoculated with the salt-tolerant PSB couple with chemical fertilizer at the typical rate applied by the local agriculturists (B+AF), 5) applied only TMF, (TMF), and 6) applied only chemical fertilizer at the typical rate applied by the local agriculturists (AF). The soils used for growing maize in pot experiments were Yang Talat series and were obtained from the rice field of the Faculty of Technology at Na Si Nuan campus, Mahasarakham University, Thailand, at 0-30 cm depth. They were air-dried and sieved through a 2 mm sieve. The pots were disinfected with 20% sodium hypochlorite followed by tap water twice. Then, 5 kg of soil was loaded into a 12-inch diameter pot. Three seedlings were planted in each pot by dropping well. Each pot was watered 300 mL a day. The least growing one was pulled out when the seedlings were 1 week old.

In the inoculated treatment, 10 mL of bacterial inoculum (1×10^8 CFU/ml) was inoculated into the seed wells on the first and 14th days of planting. In treatments using tailor-made fertilizer technology, the chemical fertilizer applied was formulated according to the software SimCorn [28] which was developed by Prof. Dr. Tassanee Attanand and her research team. For the soil used in this study, the software recommended using chemical fertilizer 46-0-0 at the rate of 68.8 kg/ha, 18-46-0 at the rate of 50 kg/ha, and 0-0-60 at the rate of 118.8 kg/ha, on the first day of planting, and using 46-0-0 at the rate of 87.5 kg/ha when the corn was 14 days old. In treatments using the chemical fertilizer at rate typically applied by the local agriculturists, chemical

fertilizer formula 15-15-15 was applied at the rate of 312.5 kg/ha and 156.3 kg/ha on the first day of planting, and when the corn was 14 days old, respectively.

Maize plants aged 14 and 21 days were withdrawn for growth evaluation. The parameters evaluated were the height of the stem, length of the root, leaf greenness, and the number of leaves per plant. For dry weight determination, the plant materials were dried at 80°C for 24 h to a constant dry weight. The leaf greenness was determined using the soil plant analysis development (SPAD) chlorophyll meter (SPAD-502 Konica Minolta Sensing, Inc., Japan) and recorded in the SPAD unit. They were air-dried at room temperature and then analyzed for pH, electrical conductivity (EC), soil texture class, organic matter, total nitrogen, available phosphorus, and available potassium according to the methods described by the Division of Soil Analysis [29]. The methods used for the determination of organic matter, total nitrogen, available phosphorus, and available potassium were chromic acid wet oxidation, Kjeldahl, colorimetric, and flame photometer, respectively [29].

2.3 Statistical Analysis

All treatments were conducted with three replicates. One-way analysis of variance (ANOVA) was conducted to determine any significant differences between the treatments at $P < 0.05$. The significant differences between treatment means were determined using Duncan's multiple range test and significance was declared at $P < 0.05$. All statistical analyses were performed using SPSS Software version 22.0.

3. Results and discussion

3.1 Effect of *B. megaterium* A12ag on germination and seedling growth

Phosphorus is an essential element for the emergence of plant seeds. The embryos need phosphorus to develop a root system and perform physiological processes [30]. Phosphorus deficiency in seeds adversely affects seed germination, seedling establishment, and plant growth [31]. The potential of the salt-tolerant PSB, *B. megaterium* strain A12ag, to promote maize growth was therefore of interest. The germination and seedling growth were laboratory assayed in order to evaluate the effects of *B. megaterium* A12ag on maize seeds at various salinities (EC at 0, 3, and 6 dS/m). The results indicate that the inoculated treatment (+PSB) had beneficial effects on germination percentage, and seedling growth in terms of root/shoot dry weight, and root/shoot length as compared to the uninoculated treatments (-PSB) (Table 1). The majority of phosphorus in the maize seeds is accumulated in the form of phytate, which is insoluble and located in the scutellum [32]. The activities of PSB in producing enzymes such as phytase and phosphatases facilitate the hydrolysis of phytate in the inoculated seeds, resulting in the release of the phosphate anion, inositol, and micronutrients that contribute to seedling development [33]. Therefore, the higher germination percentage and improved seedling growth observed could infer a higher phosphorus availability in the inoculated seeds than in the uninoculated treatments. At EC of 3 dS/m, the inoculation with *B. megaterium* A12ag provided the highest increase in the germination percentage, root and shoot dry weight, and root and shoot length compared to the uninoculated, showing increases of 8.1, 9, 6, 23, and 21%, respectively. The effects of +PSB on maize seed germination in the EC 6 dS/m were greater than in the absence of salt stress (EC 0 dS/m) as compared with -PSB which could be due to the adverse effects of high salinity on maize seeds and stimulation of salt-tolerant PSB activities [34,35]. Increasing electrical conductivity led to a decrease in germination percentage and seedling growth in both inoculated and uninoculated treatments, which resulted from plant salinity stress. Salinity affects almost all stages of plant development by interfering with essential nutrient uptakes, preventing plant water uptake, and triggering toxic effects caused by salt ions [36,37]. However, at the same electrical conductivity level studied, the inoculated treatments significantly increased the germination rate as compared to the uninoculated treatments, which confirmed the positive effects of the salt-tolerant PSB.

Table 1 Effect of *B. megaterium* A12ag on germination and seedling growth of maize seeds grown under diverse salinity.

EC (dS/m)	Germination (%)		Root dry weight (mg/plant)		Shoot dry weight (mg/plant)		Root length (cm)		Shoot length (cm)	
	+PSB	-PSB	+PSB	-PSB	+PSB	-PSB	+PSB	-PSB	+PSB	-PSB
0	96.0 ± 0.3 ^{Aa}	94.0 ± 0.4 ^{Ba}	13.1 ± 0.4 ^{Aa}	12.1 ± 0.5 ^{Ba}	20.6 ± 0.5 ^{Aa}	19.4 ± 0.5 ^{Ba}	8.0 ± 0.2 ^{Aa}	7.2 ± 0.2 ^{Ba}	8.0 ± 0.1 ^{Aa}	7.2 ± 0.2 ^{Ba}
3	93.0 ± 0.3 ^{Ab}	86.0 ± 0.4 ^{Bb}	12.0 ± 0.4 ^{Ab}	11.0 ± 0.4 ^{Bb}	18.5 ± 0.5 ^{Ab}	17.5 ± 0.5 ^{Bb}	7.0 ± 0.2 ^{Ab}	6.5 ± 0.2 ^{Bb}	6.7 ± 0.2 ^{Ab}	5.7 ± 0.3 ^{Bb}
6	87.0 ± 0.3 ^{Ac}	84.0 ± 0.5 ^{Bc}	10.8 ± 0.3 ^{Ac}	9.9 ± 0.4 ^{Bc}	17.3 ± 0.4 ^{Ac}	16.4 ± 0.3 ^{Bc}	6.2 ± 0.2 ^{Ac}	5.7 ± 0.2 ^{Bc}	5.6 ± 0.2 ^{Ac}	4.7 ± 0.3 ^{Bc}

+PSB = treatments inoculated with PSB, -PSB = treatments uninoculated with PSB. Each value represents the mean of three replicates per treatment.

ABC Values with the same letter within rows of each parameter indicate no significant difference with $P \geq 0.05$.

abc Values with the same letter within columns indicate no significant difference with $P \geq 0.05$.

3.2 Effect of *B. megaterium* A12ag and tailor-made chemical fertilizer on maize growth

The pot experiments were conducted to determine the effects of *B. megaterium* A12ag coupled with the tailor-made chemical fertilizer on maize growth, in comparison with the use of chemical fertilizer by a traditional practice of the local maize farmers. The physical and chemical properties of soils used for pot experiments are shown in Table 2. As compared to the soil interpretation manual [38] and tropical soil manual [39], soils used for pot experiments were non-saline and had extremely low total nitrogen, low available phosphorus, and low available potassium. In addition, the soil texture was categorized as loamy sand. From interviewing the local maize farmers about the rate of chemical fertilizer used, the first fertilizer application was a complete fertilizer (15-15-15) at the rate of 312.5 kg/ha, which was done simultaneously with sowing. In the second fertilizer application, the same fertilizer formula at the rate of 156.3 kg/ha was applied 2 weeks after sowing. The maize plants were subjected to different treatments and their shoot height, root length, leaf number per plant, leaf greenness (SPAD unit), shoot dry weight, and root dry weight were measured at two different planting periods: 14 days and 21 days (Table 3).

Table 2 The physical and chemical properties of soils used in the pot trials.

Characteristics	Value	Interpretation
Sand (%)	80.8 ± 1.2	
Silt (%)	14.0 ± 0.85	
Clay (%)	5.3 ± 0.04	
Soil texture	Loamy sand	Coarse soil*
pH (1:1 H ₂ O)	5.2 ± 0.2	Low (4.5-6.0)*
Electrical conductivity (1:5) (dS/m)	0.10 ± 0.00	Non saline (0-2)**
Organic matter (%)	0.73 ± 0.007	Low (0.5-1.0)*
Total nitrogen (%)	0.05 ± 0.003	Low (0.050-0.075)*
Available phosphorus (mg/kg)	2.97 ± 0.09	Extremely low (<3.0)*
Exchangeable potassium (mg/kg)	22.0 ± 0.9	Extremely low (<30)*

* Classification of soil according to Peverill et al. [38].

** Classification of saline soil according to Landon [39].

Table 3 Growth parameters of maize plants under various treatments.

Planting period (days)	Treatment symbol*	Shoot height (cm)	Root Length (cm)	Leaf number/plant	leaf greenness (SPAD unit)	Shoot dry Weight (g/plant)	Root dry Weight (g/plant)
14	C	4.6 ± 0.3 ^d	6.4 ± 0.5 ^c	3.7 ± 0.0 ^{bc}	26.6 ± 0.8 ^d	0.083 ± 0.013 ^c	0.0232 ± 0.004 ^b
	B	6.5 ± 0.1 ^b	7.9 ± 0.5 ^b	5.0 ± 0.0 ^a	31.6 ± 0.7 ^c	0.227 ± 0.014 ^c	0.0318 ± 0.004 ^{ab}
	B+TMF	8.5 ± 0.2 ^a	9.7 ± 0.4 ^a	5.0 ± 0.0 ^a	38.1 ± 0.8 ^a	0.396 ± 0.017 ^a	0.0440 ± 0.005 ^a
	B+AF	7.2 ± 0.3 ^b	8.8 ± 0.4 ^{ab}	5.0 ± 0.0 ^a	35.8 ± 0.7 ^b	0.300 ± 0.016 ^b	0.0421 ± 0.006 ^a
	TMF	5.5 ± 0.3 ^c	8.0 ± 0.4 ^b	4.0 ± 0.0 ^b	35.3 ± 0.8 ^b	0.131 ± 0.015 ^d	0.0356 ± 0.005 ^{ab}
	AF	5.4 ± 0.3 ^c	7.8 ± 0.5 ^{bc}	4.0 ± 0.0 ^b	34.1 ± 0.8 ^b	0.128 ± 0.016 ^d	0.0322 ± 0.006 ^{ab}
21	C	5.7 ± 0.4 ^d	8.2 ± 0.5 ^c	4.0 ± 0.0 ^d	35.9 ± 0.8 ^d	0.295 ± 0.019 ^d	0.0464 ± 0.005 ^{cd}
	B	7.0 ± 0.3 ^{cd}	8.9 ± 0.6 ^{bc}	5.0 ± 0.0 ^{cd}	38.6 ± 0.7 ^c	0.422 ± 0.017 ^{cd}	0.0816 ± 0.006 ^{bc}
	B+TMF	12.4 ± 0.3 ^a	12.0 ± 0.6 ^a	7.0 ± 0.0 ^a	46.4 ± 0.8 ^a	1.739 ± 0.019 ^a	0.1409 ± 0.005 ^a
	B+AF	10.5 ± 0.3 ^b	11.4 ± 0.6 ^{ab}	6.3 ± 0.0 ^b	43.7 ± 0.7 ^b	0.890 ± 0.020 ^b	0.0965 ± 0.005 ^b
	TMF	7.8 ± 0.4 ^c	9.5 ± 0.5 ^b	5.3 ± 0.0 ^c	42.8 ± 0.7 ^b	0.691 ± 0.016 ^c	0.0789 ± 0.004 ^{bc}
	AF	7.5 ± 0.3 ^c	9.4 ± 0.6 ^b	5.3 ± 0.0 ^c	41.5 ± 0.7 ^b	0.629 ± 0.018 ^c	0.0747 ± 0.007 ^{bc}

*Treatment symbol: C, uninoculated; B, inoculated with the salt-tolerant PSB; B+TMF, inoculated with the salt-tolerant PSB coupled with TMF; B+AF, inoculated with the salt-tolerant PSB coupled with chemical fertilizer at the typical rate applied by the local agriculturists; TMF, applied only TMF; AF, applied only chemical fertilizer at the typical rate applied by the local agriculturists.

Each value represents the mean of three replicates per treatment. ^{abc} Values with the same letter within columns of each planting period indicate no significant difference with $P \geq 0.05$.

The growth of maize during the 14-day and 21-day planting periods yielded consistent outcomes on plant growth traits across all investigated parameters in the experimental treatments. Due to the fact that maize feeds on a lot of nutrients [40,41], treatments applying fertilizer showed higher maize growth in all parameters examined than the B and C treatments in which no fertilizer was applied. Higher maize growth was observed in the treatment that solely applied the salt-tolerant PSB than in the uninoculated treatment (control). These results might be due to the PSB activities in providing more available phosphorus for plant [13,14] and increased rate of uptake of phosphate by plant roots [42]. After a 21-day planting period, it was shown that the *B. megaterium* A12ag inoculation coupled with the tailor-made chemical fertilizer (B+TMF) significantly promoted maize growth in all aspects compared to the other treatments. These findings confirmed the beneficial effects of *B. megaterium* A12ag on plant development, which could be caused by the salt-tolerant PSB raising phosphorus availability for maize plants [43,44]. Interestingly, the B+AF treatment had higher nitrogen and phosphorus levels than the B+TMF treatment, nevertheless, the results for promoting maize growth were less effective. The higher potassium level in the B+TMF treatment (0-0-60 at a rate of 118.8 kg/ha) compared to the B+AF treatment (15-15-15 at a rate of 312.5 kg/ha) may be the cause of these results since potassium plays major roles in plant metabolism, like photosynthesis and respiration, translocation of sugar during some protein formations, regulation of osmotic pressure, and improvement of plant resistance against stress [45]. The findings were in

line with those of a study by Kubar et al. [46], which found that dry weight and shoot length of maize were improved by a sufficient potassium level. Additionally, a well-balanced diet is essential for enhancing maize growth and yield [47]. Thus, these findings suggest that the specially tailored fertilizer used in the B+TMF treatment has a better nutritional balance than the B+AF treatment.

4. Conclusion

The present study investigated the effect of incorporating the salt-tolerant PSB, *B. megaterium* A12ag, coupled with the tailor-made chemical fertilizer, on the growth of maize plants. The results revealed that incorporating the salt-tolerant PSB and the tailor-made chemical fertilizer yielded the most significant enhancements in maize plant growth. Consequently, employing this integrated approach holds promising prospects for promoting the growth of maize plants grown in nutrient-deficient soils. Additionally, this strategy is also a potentially effective way to balance nutrient requirements for plant growth, lower the costs of chemical fertilizers, and lessen the adverse effects of fertilizer overuse.

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