Asia-Pacific Journal of Science and Technology: Volume: 28. Issue: 04. Article ID.: APST-28-04-01. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Graduate Studies,
Khon Kaen University, Thailand

Silicate solubilizing bacteria enhanced salt tolerance capacity and improved rice yield cultivated in salt-affected soil

Nghia K. Nguyen[1,*] and Duong V.H. Tran[2]

[1]Deparment of Soil Science, College of Agriculture, Can Tho University, Can Tho City, Vietnam
[2]Deparment of Agriculture and Aquaculture, Bac Lieu Technical Economic College, Bac Lieu City, Vietnam
[*]Corresponding author: nknghia@ctu.edu.vn

Received 23 May 2022
Revised 14 July 2022
Accepted 4 August 2022

Abstract

Saltwater intrusion and increased soil salinity in the Vietnam Mekong Delta are reducing cultivated rice grain yield. The element silicon is known to improve the salt tolerance of rice grown in saline soils. This study evaluated the efficacy of silicate solubilizing bacteria (SSB) on the growth and yield of MTL480 rice cultivar grown in salt-affected soil under greenhouse conditions in two consecutive seasons. Five SSB, *Ochrobactrum ciceri* TCM_39 (TCM_39), *Microbacterium neimengense* MCM_15 (MCM_15), *Klebsiella aerogenes* LCT_01 (LCT_01), *Citrobacter freundii* RTTV_12 (RTTV_12), and *Olivibacter jilunii* PTST_30 (PTST_30) were isolated from bamboo, sugarcane, rice planted soils, earthworm intestine, and earthworm feces as bacterial sources for this study. Results showed that soil treatments inoculated with SSB enhanced the salt tolerance capability and yield of rice plants considerably compared to non-inoculated treatments. Three SSB inoculated treatments-TCM_39, RTTV_12, and a mixture of all five SSBs had significantly higher levels of the number of silicate solubilizing bacteria in soil and increased soluble silicate concentration in soil, silicate content in rice stem, K^+/Na^+ ratio in the rice stem and filled rice grain weight per pot compared to other treatments including the positive control (100% NPK recommended). Moreover, soil soluble silicon concentration strongly correlated with other soil parameters. This suggests that these five bacteria have a high potential for bio-product development to protect rice when grown in salt-affected soils.

Keywords: Rice cultivation, Salt-affected soil, Salt tolerance ability, Silicate, Silicate solubilizing bacteria

1. Introduction

Soil salinity negatively effects rice photosynthesis, nutrient uptake and assimilation, and grain yield [1]. A changing climate, rising sea level, land subsidence, and increasing variability in the Mekong River and distributaries flows are expanding the salinity intrusion zone and contamination of fresh water aquifers in the Vietnam Mekong Delta [2]. While rice is cultivated in waterlogged environments, it is sensitive to soil salinity with a saturated paste electric conductivity (ECSPE) threshold of 3 dS/m [1]. Soil is considered saline at an ECSPE value greater than 4 dS/m [1]. Thus, it is urgent to find new technologies to manage rice cultivation on increasingly salt-affected soils, especially in shrimp-rice farming systems. It is well known that silicon (Si) is agronomically an essential element for rice production and has beneficial effects in mediating abiotic plant stress such as salt sensitivity [1,3]. Si influences plant uptake and transport of macronutrients and micronutrients as well as improves crop growth and development [4]. It helps mitigate the negative effects of salinity stress on rice plants via enhancing antioxidant enzymes and cell membrane structural and functional maintenance activities. An abundant element in the earth's crust, Si is most often found in the form of insoluble silicates-aluminum silicate, magnesium silicate, iron silicate, calcium silicate, sodium, and potassium silicates [5]. However, for plants to benefit from Si, the element has to be in soluble form for transport to roots, leaves, stems and hulls [3,6]. Weathering processes can produce a dissolvable form of Si, ortho-silicic acid (H_4SiO_4) that is readily available for plant use [5]. The reservoir of soil Si is effected by (i) soil parent material, (ii) sediment

movement and soil erosion, (iii) crop harvest and residue removal without Si replenishment, and (iv) weathering processes and other biogeochemical conditions that limit/accelerate Si cycling and distribution [4,7]. The rice plant absorbs typically about 230-470 kg/ha in each cropping season with Si accumulating in rice biomass; however, Si removed at harvest depletes the store of soil Si [4,7]. Cropping systems in tropical and subtropical soils have low available Si content and Si fertilizers can be used to meet crop annual Si needs for growth and productivity [7].

The presence of microbial activity in rock and mineral weathering processes promotes Si dissolution, thereby increasing soluble Si in soil and plant availability [5]. A number of studies have suggested silicate solubilizing microbe-based bio fertilizers as potential regulators of the Si biogeochemical cycle and to maintain ortho-silicic acid in the soil [1,5,7,8]. Prior research has used silicate solubilizing bacteria (SSB) combined with fly ash made from a thermal power station and found that it increased the number of filled grains in a panicle and rice grain yield [8]. Another experiment in the laboratory showed that soil inoculated with five different SSB (*Ochrobactrum ciceri* TCM_39 (TCM_39), *Microbacterium neimengense* MCM_15 (MCM_15), *Klebsiella aerogenes* LCT_01 (LCT_01), *Olivibacter jilunii* PTST_30 (PTST_30), and *Citrobacter freundii* RTTV_12 (RTTV_12)) enhanced rice height, number of roots, and dried biomass when cultured in liquid Hoagland medium with and without NaCl (0.3%) [9].

Previous studies have focused on the role of Si fertilizers on plant growth and productivity. Research on the effects of Si fertilizer and SSB applications to increase salt tolerance while enhancing growth and yield of rice has been very limited. A 2022 synthesis and review of salinity effects on rice and strategies to secure crop productivity lists inoculation with growth promoting bacteria as a promising management tactic to reduce salt stress [1]. They further recommend that more research is needed to identify plant growth promoting bacteria and better understand the impacts on production and the environment [1]. In this paper we present an experimental study conducted to evaluate the efficacy of five SSB and compare 7 Si fertilizer treatments to the use of Nitrogen, Phosphorus, Potassium (NPK) fertilizers only on salt tolerance capability, growth, and yield of MTL 480 rice cultivar grown on salt-affected soil under greenhouse conditions.

2. Materials and methods

2.1 Source of bacteria

TCM_39, MCM_15, LCT_01, RTTV_12, PTST_30 was isolated from samples of bamboo, sugarcane, rice planted soils, earthworm intestine and feces and used as bacterial sources for this study. These materials have been found to have a high diversity of silicate solubilizing bacteria and were selected for SSB isolation and evaluation. Five SSB demonstrated the highest soluble Si concentrations (33.84-52.02 mg/L) and were selected for this experiment. Prior laboratory experiments have indicated some degree of efficacy for these five SSBs on enhancement of germination ratio, growth, and biomass of rice plants in both salinity and normal conditions [10,11].

2.2 Rice cultivar

The rice cultivar MTL 480 was used for this study. This rice cultivar originated from the Mekong Delta Development Research Institute, Can Tho University, Vietnam. Geneticly, this is a high salinity tolerant rice variety with a growing period between 94 and 97 days. Average plant height, weight of 1,000 rice grains, and potential yield of this rice cultivar are 90 cm, 26-27 g, and 6-8 t/ha, respectively [12].

2.3 Preparation of bacterial source

Each bacterial strain was enriched in a sterilized 100 mL flask containing 20 mL tryptic soy broth (TSB) medium for 3 days. The TSB medium composition in 1 L included 30 g tryptone soya broth in 1 L of distilled water. After the incubation, the bacterial culture was aseptically transferred into a sterilized 50 mL Falcon tube, centrifuged at 6,000 rpm in 5 mins, the supernatant was discarded, and the tube refilled with 20 mL sterilized distilled water. This procedure was repeated three times. Then, the bacterial pellet was adjusted to obtain the density of 10^8 colony forming unit (CFU)/mL.

2.4 Preparation of MTL 480 rice cultivar seeds

The MTL 480 rice cultivar used in the experiment was prepared as follows: first, the rice seeds were sterilized in a NaClO 1% solution for 10 minutes, then the NaClO solution was discarded and ethanol 70% was added and the seed soaked for 1 min, then washed four times with sterilized distilled water. Afterward, the seeds were soaked in bacterial suspension (prepared in part 2.3) for 24 hours. Rice seeds in the control treatment

(without bacterial inoculation) were prepared only with sterilized distilled water. The incubated seeds were transferred into a petri dish containing filter paper; this filter paper was conditioned with 10 mL of sterilized distilled water to maintain humidity. The petri dish containing the rice seeds was placed in the dark under laboratory conditions until rice seeds germinated and grew about 1 cm.

2.5 Fixation of bacteria in used coal

Used coal was ground and sieved with a sieve (2 x 2 mm diameter). Then, the sterilized ground coal was aseptically transferred into a 250 mL flask containing 100 mL liquid soil extract medium with magnesium trisilicate 0.05% added. Simultaneously, an aliquot of 2 mL bacterial suspension prepared in section 2.3 was added to this flask to obtain the amount of 10^{11} CFU/mL. The sample flasks were shaken by an orbital shaker at 100 rpm for 24 hours under laboratory conditions [13]. Finally, the coal containing bacteria was harvested with bacterial density as follows: TCM_39, 12 x 10^9 CFU/g; RTTV_12, 6 x 10^9 CFU/g; PTST_30, 11 x 10^9 CFU/g; LCT_01, 11 x 10^9 CFU/g; and MCM_15, 8 x 10^9 CFU/g.

2.6 Soil sample preparation

Salt-affected soil from a rice field in a rice-shrimp farming system at Long Hai ward, Phuoc Long district, Bac Lieu province, Vietnam, was collected for the greenhouse house experiment. In the field, soil sample was collected by using shovel at a depth 10-15 cm beneath the top layer of soil in a random zig-zag manner, at 20 places. The total soil sample about 500 kg was collected and mixed well by shovel. Then an aliquot of the soil samples collected was used for soil property analyses, including soil pH, EC, phosphorous, nitrogen (NH_4^+, and NO_3^-), and the concentration of silicate solubilizing bacteria. Soil properties were pH 7.80, EC 4.16 (mS/cm), available P 0.0059-0.0153 (mg/L), NH_4^+ 0.544-1.083 (mg/L), NO_3^- 0.014-0.058 (mg/L), and bacterial density 4.99 (log 10 CFU/mL). Soluble Si concentration in the soil was 16.9 g/kg and was interpreted as a low value [14] indicating that Si fertilizer amendments could increase the plant available Si in the soil. Next, 5 kg of dry soil was transferred into 36 experimental pots (30 cm height x 30 cm diameter). Then, tap water was added to each pot at a level of 10 cm above the soil surface and soaked for seven days. Finally, the soil was stirred and leveled before the rice seedling was transplanted into the pot.

2.7 Experimental design

The experiment was conducted using a randomized design with nine treatments, four replicates, and two consecutive cropping seasons (Summer-Autumn, and Autumn-Winter season) in the greenhouse condition at the Soil Science Department, College of Agriculture, Can Tho University, Vietnam. The treatments are listed in (Table 1). as follows:

Table 1 Nine treatments in two consecutive cropping seasons under the greenhouse.

Treatment	Characteristic
Treatment 1 (T1)	Control (without fertilizer and bacteria)
Treatment 2 (T2)	NPK fertilizer (43-68-45)
Treatment 3 (T3)	NPK+Si (100 kg/ha)
Treatment 4 (T4)	NPK+Si+LCT_01
Treatment 5 (T5)	NPK+Si+RTTV_12
Treatment 6 (T6)	NPK+Si+PTST_30
Treatment 7 (T7)	NPK+Si+MCM_15
Treatment 8 (T8)	NPK+Si+TCM_39
Treatment 9 (T9)	NPK+Si+MIX (mixture of the five silicate solubilizing bacteria-SSB)

$CaSiO_3$ (Ca 17.4% and SiO_2 19%) as Si fertilizer source was applied at the dose of 100 kg/ha. NPK fertilizer was urea (46% N), superphosphate (16% P_2O_5), and potassium chloride (60% K_2O) based on the recommended chemical fertilizer formula of 43N-68P_2O_5-45K_2O. This fertilizer was applied four times during the growing period at 0, 10, 20, and 40 days after transplant [15]. SSB fixed in the used coal carrier material was applied one day prior to transplant by mixing 50 g of used coal containing bacterial cells to a depth of 0-10 cm in each pot (approximately 1% dried soil weight in pot). The final soil bacterial density was as follows: TCM_39, 12 x 10^7 CFU/g; RTTV_12, 6 x 10^7 CFU/g; PTST_30, 11 x 10^7 CFU/g; LCT_01, 11 x 10^7 CFU/g; and MCM_15, 8 x 10^7 CFU/g. Experimental pot water management used the wet-dry alternative method. This pot water management

was maintained up to seven days before the reproductive phase. Weed and insect pressures were managed by traditional methods such as removing a weed and catching insects by hand.

The salt concentration in the soil was held at a level of 0.4% during the experimental period by using a salt meter to determine salinity in the soil solution once every three days. In the second season experiment, soil in each treatment pot from the first season was retained and reused. The soil was saturated with water to a mud consistency at 10 cm depth of soil surface and treatment applications of NPK and Si fertilizers and SSB replicated the first season treatments. The techniques for the preparation of the rice cultivar, SSB, and rice cultivation also replicated the same management practices as in the first cropping season.

2.8 Collected parameters

2.8.1 Number of silicate solubilizing bacteria in soil

One gram of soil was weighed and put into 100 mL jar containing 99 mL buffer phosphate. This jar was shaken orbitally for an hour under laboratory conditions. A diluted series with dilution coefficient 10 was prepared and vortexed as well. An aliquot of 100 µL of bacterial suspension of 10^0, 10^{-1}, and 10^{-2} was spread on a soil extract agar (SEA) medium surface. The SEA medium component in 1 L included glucose 1.0 g; K_2HPO_4 0.5 g; soil extract 100 mL [16]; agar 20 g; distilled water 900 mL; magnesium trisilicate 0.25%; pH 7.0-7.2. All of the samples of agar plates were incubated in 30°C incubator for 24 hours and then counted for colony forming units exhibited on the agar medium to determine the bacterial number in the liquid medium (CFU/mL) [17].

2.8.2 Soluble Si concentration in soil

Soluble Si concentration in soil on day 0, 15, 30, 45, 60, and 90 was determined following the method of Pereira et al. [18]. Briefly, a 10 g soil sample was transferred into a plastic bottle, and 50 mL Na_2CO_3 (10 g/L) and 50 mL NH_4NO_3 (16 g/L) were added and shaken at 60 rpm for 1 hour, then left standing in the laboratory for 5 days. The soluble Si concentration in soil solution was measured by Molybdenum Blue Colorimetric method [19]. An aliquot of 1 mL of sample was transferred into 50 mL Falcon, then 2.5 mL ammonium acetate 20%-, and 1-mL ammonium molybdate 0.3 M were added, vortexed for 5 s, and the sample left standing 5 min for stabilization. Then, 0.5 mL acid tartaric 20%, 0.5 mL reducing solution (reducing solution components included 2 g Na_2SO_3, 0.4 g $C_{10}H_9NO_4S$, and 25 g $NaHSO_3$ in 250 mL distilled water), and 2 mL acid acetic 20% were added into the Falcon, and the sample was kept standing under laboratory conditions for 60 mins. Finally, the sample was measured by spectrophotometer at 815 nm wavelength.

2.8.3 Si concentration in rice stem

Si concentration in the rice stem at harvest time was determined according to the method by Wei-min et al. [20]. Briefly, the rice stem and leaf were dried at 70°C for 48 h, then ground and sieved to 0.5 mm diameter. Next, a 0.1 g sample was transferred to a 50 mL Falcon tube; after that, 3 mL NaOH 50% was added to the tube which was then autoclaved at 120°C for 20 mins. The sample was then filled with 50 mL of distilled water. Finally, the Molybdenum Blue Colorimetric method determined Si concentration in sample solution [19].

2.8.4 K^+/Na^+ ratio in rice stem

The K^+/Na^+ ratio in the rice stem was evaluated as described by Chapman et al. [21]. The rice stem and leaf were dried at 70°C for 48 h, then ground and sieved to 0.5 mm diameter. Next, a 0.3 g sample was digested with 3.3 mL digestion solution (digestion solution component including 18 mL distilled water, 100 mL concentrated H_2SO_4, and 6 g salicylic acid) at 180°C in 1 h, cooled; after that, the 1 mL H_2O_2 30% was amended into the mixture, then the sample was heated for 5-10 mins; and the whole procedure was repeated until the sample solution was decolored entirely. The sample solution was then filled with 50 mL of distilled water. Finally, the K^+/Na^+ content was determined by atomic absorption spectrophotometer, and the K^+/Na^+ ratio was counted.

2.8.5 Filled rice grain weight in pot

The weight of the filled rice grain grown in each treatment pot was determined by weighing the entire filled grain in the pot and converting into 14% moisture content.

2.9. Data analysis

The data were analyzed through one-way analysis of variance (ANOVA), Duncan Multiple Range Test, and correlation using statistical package for the social science (SPSS) 22.0 software.

3. Results and discussion

3.1 Number of silicate solubilizing bacteria in soil

The number of silicate solubilizing bacteria in the soil in seasons 1 and 2 is presented in (Table 2). In the first season, the density of silicate solubilizing bacteria in the soil of all treatments increased gradually from day 0 to day 45. The highest number of silicate solubilizing bacteria was reached at day 45 when bacterial numbers varied between 5.20 and 5.56 $\log_{10}$ CFU.g^{-1} soil. After day 45 the number of silicate solubilizing bacteria declined steadily. The number of silicate solubilizing bacteria in the T9 treatment which was a mixture of all 5 bacterial strains was significantly higher than all other treatments (T1-T8) in any sampling period (p<0.05). Treatments T4-T8 inoculated with a single SSB revealed a non-significant difference in bacterial density compared to each other; as well with T2 and T3 treatments without SSB application (p>0.05). Likewise, the number of silicate solubilizing bacteria in soil in the second season was similar to the first season. Three treatments, T5 (NPK+Si+RTTV_12), T8 (NPK+Si+TCM_39), and T9 (NPK+Si+MIX) had a higher number of silicate solubilizing bacteria in soil than the other treatments at any sampling time.

Season 2 soil baseline 0 and end period 90 days of SSB in all treatments were higher than in season 1 baseline 0 This means SSB adapted and was growing in the soil after two consecutive crops, and applications of SSB in the soil boosted the density of SSB. Furthermore, in T1, T2, T3 (control, NPK and NPK+Si the treatments without inoculation of SSB) it appeared that existing SSB in the soil increased over time. However, the numbers of these SSB were not sufficient to solubilize silicate for rice uptake in salt-affected soil conditions because the rice plants can absorb unlimited soluble Si from the soil. This suggests the applications of the isolated SSB to the soil to enhance the numbers of SSB in soil as well as soluble Si concentration in soil for rice to uptake can lead to an improvement of rice salt-tolerance ability, growth, and yield. Further, Si concentration in soil, Si concentration in the rice stem, K^+/Na^+ ratio in the rice stem and filled grain weight in the experimental pots were significantly improved by treatments with SSB inoculation as compared to treatments without SSB inoculation. Future studies
are needed to move from experimental pots to field conditions to determine the efficacy of SSB on salt-tolerance ability, growth, and yield of rice plants as well as the farmer returns in salt-affected soil regions. Few studies have explored the application of SSB to improve salinity tolerant capacity and grain yield of rice when grown on salt-affected soil.

3.2 Soluble Si concentration in soil

Soluble Si concentration in soil on days 0, 15, 30, 45, 60, and 90 after seeding of the treatments in the first and second season in (Table 3). show that soluble Si concentration in soil of the treatments strongly varied. In the first season, especially on days 15, 45 and 90, the highest values fluctuated ranging 30.9-47.8 g.kg^{-1}, 8.78-103 g.kg^{-1}, and 29.0-83.4 g.kg^{-1}, respectively. SSB amended treatments T5, T8, T9 (RTTV_12, TCM_39 and MIX) always achieved the highest soluble Si concentration in soil in any sampling period (p<0.05). Treatments applied with other SSB, T4, T6, T7 (LCT_01, PTST_30, and MCM_15) showed no significant available Si concentration in soil of season 1 compared with the other treatments without SSB application. This same trend of soluble Si concentration in soil treatments in the first season was seen in the second season data. Further, the available Si content in soil increased in season 2. The treatments inoculated with SSB had a significantly higher soluble Si concentration in soil than those without SSB applications. This might be because SSB can produce organic acids like citric acid, oxalic acid, keto acid, hydroxyl carboxylic acid, tartaric acid, gluconic acid, acetic acid, 2-keto-gluconic acid, alkalis or polysaccharide [22,23] which function in silicate bio-solubilization to form available H_4SiO_4 for the plant to uptake. Especially, in the 1st season crop, the difference of available Si soil content in three out of six treatments with SSB was not significant as compared to that of the treatments without SSB T1, T2, T3 (control, NPK, and NPK+Si).

Table 2 Number of silicate solubilizing bacteria in soil in the first and second season under the greenhouse condition.

No.	Treatment	Number of silicate solubilizing bacteria in soil (log_{10} CFU.g^{-1} soil)											
		Days after seeding (season 1)						Days after seeding (season 2)					
		0	15	30	45	60	90	0	15	30	45	60	90
T1	Control	4.40	4.50^{e}	4.56^{c}	5.20^{d}	4.96^{f}	4.95^{e}	4.95^{e}	5.03^{e}	5.12^{d}	5.19^{d}	5.15^{e}	5.13^{e}
T2	NPK	4.40	4.52^{de}	4.58^{c}	5.32^{c}	5.24^{e}	5.23^{d}	5.23^{d}	5.23^{d}	5.25^{c}	5.30^{c}	5.25^{d}	5.24^{d}
T3	NPK+Si	4.40	4.54^{de}	4.64^{c}	5.38^{bc}	5.28^{de}	5.25^{d}	5.25^{d}	5.25^{cd}	5.26^{c}	5.45^{b}	5.38^{bc}	5.36^{bc}
T4	NPK+Si+LCT_01	4.40	4.54^{de}	4.83^{b}	5.41^{bc}	5.28^{de}	5.26^{d}	5.26^{d}	5.26^{cd}	5.27^{c}	5.40^{b}	5.35^{c}	5.33^{c}
T5	NPK+Si+RTTV_12	4.40	4.60^{d}	4.85^{b}	5.43^{bc}	5.41^{b}	5.41^{ab}	5.41^{ab}	5.45^{a}	5.45^{ab}	5.63^{a}	5.57^{a}	5.56^{a}
T6	NPK+Si+PTST_30	4.40	4.56^{de}	4.89^{b}	5.48^{ab}	5.46^{ab}	5.41^{ab}	5.41^{ab}	5.41^{ab}	5.41^{ab}	5.47^{b}	5.45^{b}	5.43^{b}
T7	NPK+Si+MCM_15	4.40	4.7^{c}	4.86^{b}	5.44^{abc}	5.32^{cd}	5.30^{cd}	5.30^{cd}	5.33^{bc}	5.39^{b}	5.43^{b}	5.40^{bc}	5.37^{bc}
T8	NPK+Si+TCM_39	4.40	4.81^{b}	4.94^{b}	5.42^{bc}	5.39^{bc}	5.35^{bc}	5.35^{bc}	5.40^{ab}	5.44^{ab}	5.62^{a}	5.56^{a}	5.53^{a}
T9	NPK+Si+MIX	4.40	5.03^{a}	5.42^{a}	5.56^{a}	5.49^{a}	5.46^{a}	5.46^{a}	5.49^{a}	5.49^{a}	5.63^{a}	5.58^{a}	5.57^{a}
F		ns	*	*	*	*	*	*	*	*	*	*	
CV (%)		0.88	3.74	5.23	2.23	2.93	2.88	2.67	2.34	2.83	2.76	2.73	

Notes: *a significant difference at the 5% level in the same column and numbers followed by the same letters are not a significant difference at the 5% level by Duncan test.

Table 3 Soil soluble Si concentrations in the 1 and 2 crops under the greenhouse condition.

No.	Treatment	Soluble Si concentration (g.kg^{-1} dried soil)											
		Days after seeding (season 1)						Days after seeding (season 2)					
		0	15	30	45	60	90	0	15	30	45	60	90
T1	Control	22.0	30.9^{e}	4.2^{f}	8.78^{f}	5.39^{f}	29.0^{h}	29.0^{h}	97.1^{h}	26.7^{g}	67.2^{g}	30.0^{f}	56.7^{f}
T2	NPK	22.0	31.2^{e}	7.46^{e}	12.1^{e}	7.66^{e}	33.4^{g}	33.4^{g}	116^{g}	40.6^{f}	83.9^{f}	42.5^{e}	69.2^{e}
T3	NPK+Si	22.0	46.2^{a}	8.19^{de}	16.6^{d}	9.29^{de}	42.2^{f}	42.2^{f}	124^{f}	46.1^{f}	89.4^{f}	46.7^{e}	85.8^{d}
T4	NPK+Si+LCT_01	22.0	35.9^{d}	8.55^{d}	16.6^{d}	9.29^{de}	54.7^{e}	54.7^{e}	224^{c}	113^{c}	139^{d}	75.8^{c}	140^{b}
T5	NPK+Si+RTTV_12	22.0	38.5^{c}	17.3^{b}	42.1^{c}	22.3^{a}	60.6^{d}	60.6^{d}	230^{bc}	124^{b}	153^{c}	96.7^{b}	144^{b}
T6	NPK+Si+PTST_30	22.0	34.3^{d}	8.19^{de}	16.6^{d}	10.9^{cd}	64.3^{d}	64.3^{d}	207^{d}	102^{d}	131^{d}	80.0^{c}	123^{c}
T7	NPK+Si+MCM_15	22.0	42.0^{b}	8.19^{de}	42.7^{c}	11.9^{c}	69.4^{c}	69.4^{c}	146^{e}	73.9^{e}	106^{e}	59.2^{d}	85.8^{d}
T8	NPK+Si+TCM_39	22.0	46.6^{a}	11.5^{c}	44.3^{b}	18.4^{b}	83.4^{a}	83.4^{a}	235^{b}	127^{b}	167^{b}	92.5^{b}	140^{b}
T9	NPK+Si+MIX	22.0	47.8^{a}	27.4^{a}	103^{a}	21.6^{a}	75.3^{b}	75.3^{b}	252^{a}	141^{a}	189^{a}	113^{a}	161^{a}
F		ns	*	*	*	*	*	*	*	*	*	*	*
CV (%)		0.0	16.4	60.1	84.2	47.0	32.8	32.8	31.8	46.3	31.8	38.4	32.6

Notes: *a significant difference at the 5% level in the same column and numbers followed by the same letters are not a significant difference at the 5% level by Duncan test.

However, in the 2nd crop season, soluble Si concentration in soil of all of the treatments with SSB were significantly higher than that of the treatments without SSB. The reason could be because SSB had adapted well and continued to increase silicate solubilization [24]. The soil soluble Si increased on days 15, 45 and 90, but decreased on days 30 and 60 in both crops. This could reflect the plant growth cycle. When the SSB were inoculated in the soil on day 0, SSB had already adapted well and proliferated gradually, increasing the soluble Si content in the soil. However, during this period (15 days after transplant) the root biomass of rice plants was low and only a small amount of soluble Si content in the soil by bacteria was uptaken by the rice plants, so the amount of soluble Si content in the soil at this stage was high. Then, in the vegetative stage (30 days after seedling), the biomass of roots and stems of the rice grew and needed more nutrients; hence more soluble Si was taken up by the rice plants and the soluble Si in the soil decreased accordingly. Moreover, during the time period of 30-45 days after transplant, there could be a symbiotic relationship between rice roots and the numbers of SSB in the soil, contributing to an enhancement in the numbers of SSB due to more rice root exudates released into the soil, stimulating a higher bacterial population, especially SSB; thus, an increase of soluble Si concentration in the soil.

At the reproductive stage (45-60 days after transplant), rice could take up a large amount of available Si content to form panicles and seeds, and as a result, the soluble Si content in the soil was exhausted again. However, soluble Si content in the soil increased by 90 days because growth had slowed and it was the harvesting stage, and the plant reduced its Si uptake. These findings are consistent with the study by Ma et al. [25] that showed Si strongly influenced the enhancement of rice biomass and yield, specifically in the reproductive stage, and at this stage, rice plants take up a large amount of soluble Si from soil.

3.3 Si concentration in rice stem

Si plant uptake capacity in the rice stem of the treatments in the first and second crop is presented in (Figure 1). The results show that most of the treatments applied with SSB had a higher amount of Si concentration in rice stem than those without SSB application for both seasons ($p<0.05$). Seasons 1 and 2 treatments with SSB had a higher Si concentration uptake and accumulation in the rice stem than the treatments without SSB application ($p<0.05$). The Si concentration in the rice stem of the treatments with SSB application in the first season varied between 41.3 and 65.0 g/kg, while the Si values in the treatments without SSB application were between 35.9 and 40.9 g/kg. In the second season, the treatments applied with SSB accumulated Si in the rice stem, ranging between 50.0 and 75.3 g/kg Si content in the rice stem in the treatment T9 (MIX) had the highest value, 75.3 g/kg, followed by T8 (TCM_39), T5 (RTTV_12), T6 (PTST_30), T4 (LCT_01), and T7 (MCM_15) with Si values between 50.0 and 65.3 g/kg. One explanation for these findings is that the silicate solubilizing efficacy of SSB was increased when there were several strains of SSB present; with each single strain stimulated by the presence of other strains [26]. Si concentration in the rice stem significantly differed between the group of treatments with and without SSB applications. The treatments with SSB application had significantly higher Si in the rice stem compared to the treatments without SSB application ($p<0.05$). The treatment with NPK+Si had a significantly higher Si content in the rice stem than the treatment with only NPK and the control ($p<0.05$). These findings are consistent with Peera et al. [8] and Kang et al. [27] which also showed that application of SSB into the soil assisted in increasing available Si concentration in soils for rice uptake; as a consequence, Si concentration in rice stem was boosted. Additionally, in season 1, although Si concentration in the rice stem in most of the treatments with SSB was significantly higher than that in the treatments without SSB, the filled grain weight in the experimental pot was not significantly different among many of the treatments with SSB and without SSB. Until the second season, there were both significant differences including Si concentration in the rice stem and filled grain weight in pot. Thus, although Si concentration in the rice stem played an important role in formation of the filled grain, there seem to be other factors that are affecting grain weight. This is a knowledge gap that should be addressed in future studies.

3.4 K+/Na+ ratio in rice stem

K+/Na+ ratio in the rice stem of the treatments at harvest time in the first and second season are presented in (Figure 2). and show significant differences ($p<0.05$). The treatments containing SSB had a higher K+/Na+ ratio in the rice stem than the treatments without SSB application for both seasons. The K+/Na+ ratio of the treatments in the first season was between 0.24 and 0.61. The treatments with SSB application varied from 0.42 to 0.61, while other treatments without SSB application fluctuated between 0.24 and 0.34. In the second season, the treatments amended with SSB had a higher K+/Na+ ratio in the rice stem and varied from 0.48 to 0.61, while control treatments were between 0.24 and 0.34. Moreover, the treatments without SSB were significantly different from each other ($p<0.05$). These results were consistent with Peera et al. [8] and Kang et al. [27] who found that SSB applications helped to enhance soluble Si concentration in soil for rice uptake and that Si content in the rice stem increased in the SSB inoculated treatments.

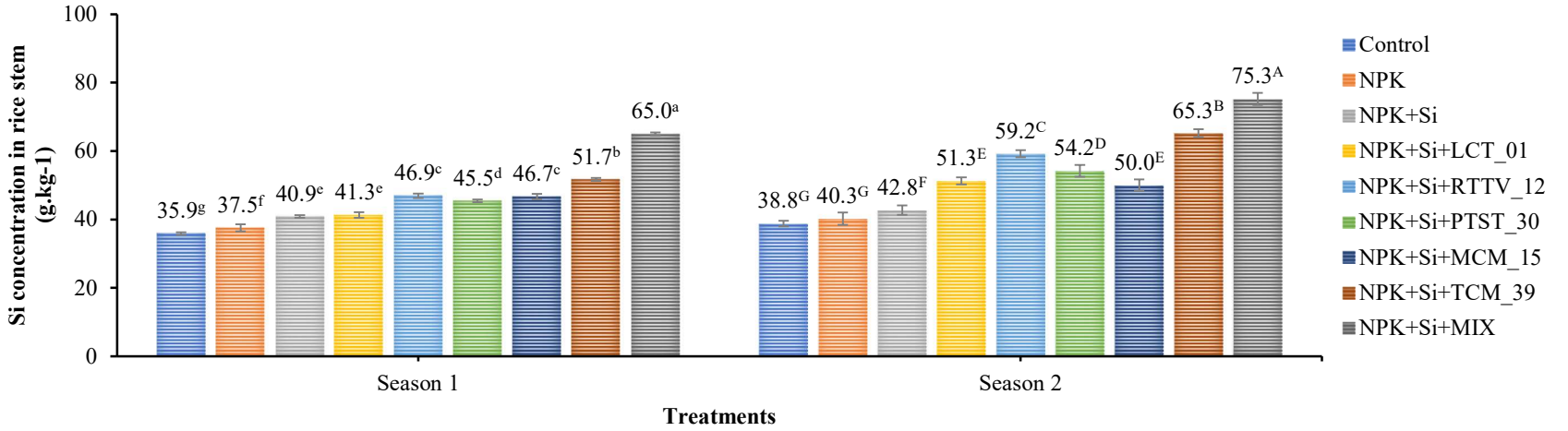

Figure 1 Si concentration in rice stem when cultivated in salt-affected soil at harvesting time in the first and second season under the greenhouse condition
Note: numbers followed by the same capital or lowercase letters in the same group are not a significant difference at the 5% level by Duncan test.

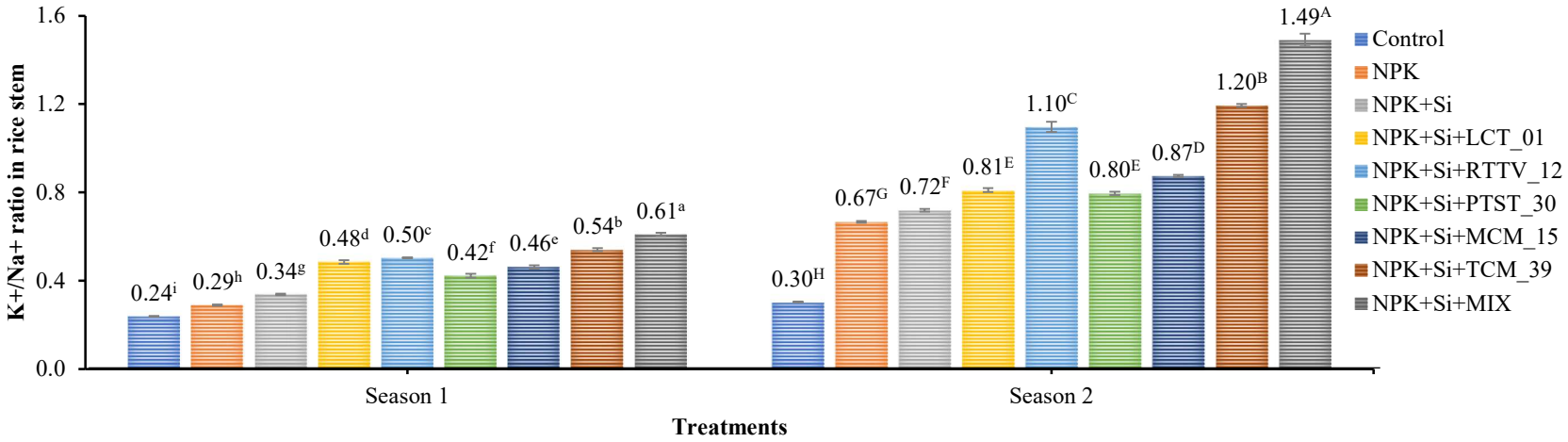

Figure 2 K^+/Na^+ ratio in rice stem of the treatments when cultivated in salt-affected soil at the harvesting time in the first and second season.
Note: numbers followed by the same capital or lowercase letters in the same group are not a significant difference at the 5% level by Duncan test.

The application of Si has been found to enhance K+ transport and absorption but reduce Na+ transport and absorption from root to stem in rice, barley, sugarcane, and chicken pea *(Cicer arietinum* L.) under salinity conditions [28-30]. These mechanisms have been explained by several studies. First, the Na+/H+ antiporter has been found to play an important role in maintaining a low Na+ concentration by removing Na+ from the cytosol or compartmentalizing it in vacuoles [31]. The tonoplast Na+/H+ antiporter is involved in Na+ compartmentation, and it driven by a H+-ATPase and H+-pyrophosphatase (H+-PPase) in the tonoplast [32]. The activities of plasma membrane H+-ATPase and H+-PPase in the tonoplast under salt stress increase significantly when Si is amended to the plants. The enhancement in activities of plasma membrane H+-ATPases and H+-PPase might facilitate Na+ export from the cell and Na+ compartmentation in vacuoles through the tonoplast Na+/H+ antiporter. Moreover, Si improves K+ uptake by ameliorating H+-ATPase activity in both hydroponics and soil. Thus, under salinity conditions, Si has potential to mitigate Na+ level and boost K+ level in the cytoplasm by stimulating H+-ATPase activities on the plasma membrane and tonoplast and H+-PPase activities on the tonoplast. Both Si-enhanced exodermal development and Si deposition on the exodermis contribute to alleviate loading of salt ions into the xylem of rice roots and mitigate salt ion accumulation in rice shoots [33].

3.5. Filled rice grain weight in pot

Filled rice grain weight in the treatment pots at 14% moisture in the first crop is presented in (Figure 3). which shows that filled rice grain weight in the treatment pots were significantly different. In the first crop SSB treatments, T8 (NPK+Si + TCM_39) and T9 (NPK+Si + MIX) had the significantly highest filled rice grain weight in the pots with values varying from 12.9 to 13.3 g/pot, respectively, as compared to other treatments (p<0.05) which varied from 6.0 to 11.8 g/pot. Filled rice grain weight with SSB application in treatment pots T6 (NPK+Si + PTST_30), T7 (NPK+Si + MCM_15), and T4 (NPK+Si + LCT_01) were 11.5, 11.1 and 10.9 g/pot, respectively. They were not significantly different from each other and other treatments without SSB application. The control treatment had the lowest value of filled rice grain weight (6.0 g/pot). In the second season, filled rice grain weight showed that most of the treatments with SSB applications obtained a higher filled rice grain weight in the pot than in the other treatments without the SSB amendment. The filled rice grain weight in the treatment pots T5 (RTTV_12), T8 (TCM_39), and T9 (MIX) were 16.4, 19.5, and 20.3 g/pot, respectively (p<0.05); higher than other treatments. Among the treatments without SSB, the control with no fertilization had the lowest filled rice grain weight in the pot (7.74 g/pot), followed by NPK (11.3 g/pot) and NPK+Si (11.8 g/pot). These differences were not significant (p>0.05). We conclude that treatments of NPK+Si + SSB ameliorated filled rice grain weight in the SSB treatment pots. This can be explained by SSB biologically dissolving the silicate in the soil and transforming insoluable Si into a soluable form available to plants. The soluble Si concentration in soil was taken up by rice plants, increased plant biomass and enhanced rice growth and yield because Si availability was no longer limited. Additionally, there was a very noticeable point in season 1 where most of the treatments with and without SSB had filled grain yield in treatment pots that were not significantly different, except for the treatments with SSB T8 and T9 (TCM_39 and MIX). SSB T8 and T9 seemed to boost salt-tolerance capacity, growth and yield of rice. Among bacteria, inoculation of TCM_39 and MIX showed the highest efficacy over season 1 suggesting they could adapt and proliferate better than the other bacterial strains, and the combination of the single strains enhanced adaptation and growth capability greater than a single strain. In season 2 the significant differences for filled grain yield in treatment pots with and without SSB was clear. The treatments with SSB had higher filled grain yield than the treatments T1, T2, and T3 without SSB (control, NPK, NPK+Si). This could be due to SSB gradually solubilizing the silicate and increasing adaptation. The increase in soluble Si concentration in the soil improved rice plant uptake and seemed to reinforce salt-tolerance capacity, growth, and yield. The positive correlations between soluble Si concentration in soil with Si concentration in rice stem, K+/Na+ ratio in rice stem, numbers of SSB in soil, and filled grain weight of rice per pot are shown in (Table 4). Over two consecutive seasons, the treatment with NPK+Si only filled grain weight of rice per pot was not significantly different from the treatment with NPK. The application of only Si fertilizer did not improve the salt-tolerance ability, growth, and yield of rice plants over both seasons. However, salt-tolerance ability, growth, and yield of rice plants was enhanced when combined with SSB. This result was consistent with Peera et al. [8] findings which showed that SSB inoculation and fly ash applications increased grain and straw yield compared with control treatments without microbial applications.

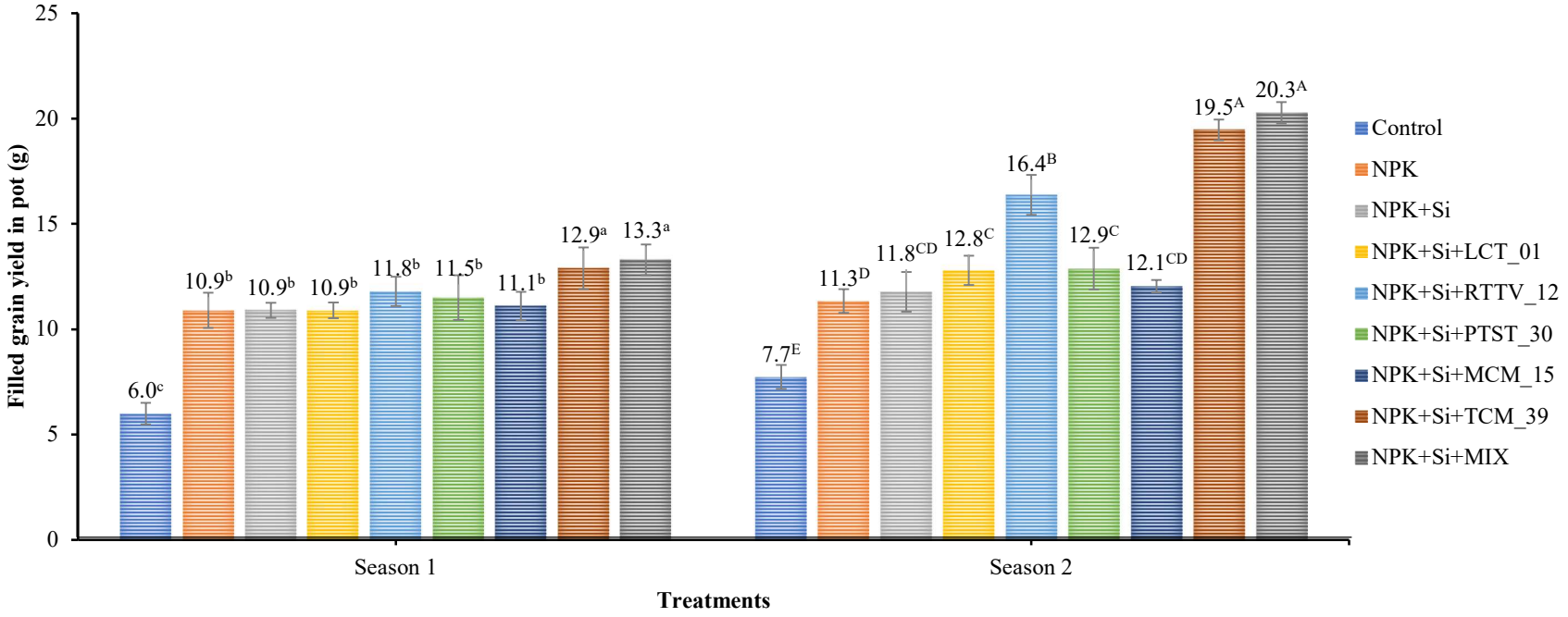

Figure 3 Filled rice grain weight in the pot of the treatments when cultivated on salt-affected soil at harvesting time in the first and second season under the greenhouse.
Note: numbers followed by the same capital or lowercase letters in the same group are not a significant difference at the 5% level by Duncan test.

3.6 Correlation analysis

The results of correlation analysis presented in (Table 4). indicate that soluble Si concentration in the soil had a significantly positive correlation with Si concentration in the rice stem, K/Na ratio, SSB, and filled rice grain weight per treatment pot, respectively. The correlation coefficient values (r) of these correlations were 0.94, 0.95, 0.93, and 0.94, respectively. This means that increasing silicate solubilizing bacterial numbers in soils enhances the soluble Si concentration in the soil and increase Si plant availability. An increase in soluble Si improved biomass and increased rice plant uptake of K^+ compared to Na^+ (since Si accumulation in rice cells can prevent the transfer of Na^+ into rice cells). As a result, the filled rice grain weight per pot was significantly enhanced when cultivated on salt-affected soils under greenhouse conditions.

Table 4 A correlation between soluble Si concentration in soil with some other soil parameters **(**correlation coefficient (r)).

.	SiRS	K+/Na+	SSB	FGW
SSi	+ 0.94**	+ 0.95**	+ 0.93**	+ 0.94**

*Notes: ** a significant correlation at 1%; SiRS: Si concentration in rice stem; K/Na: K^+/Na^+ ratio in rice stem; SSB: numbers of SSB; FGW: Filled grain weight of rice per pot; SSi: Soluble Si concentration in soil. These values were mean values of two seasons.

4. Conclusion

Salinity currently affects almost 20% of cultivated soils globally and 20-30% of irrigated agriculture and is predicted to increase to affecting more than 50% of total arable land by 2050 [1]. In the Vietnam Delta, salinity significantly reduces rice yields which over time will impact Vietnam food security, farmer livelihoods, and economic export balance of trade. Our research on silicon soluable bacteria is an important technology for improving rice plant growth and increasing productivity in saline soils. Laboratory and greenhouse experiments reveal that five SSB, namely TCM_39, MCM_15, LCT_01, PTST_30, and RTTV_12, isolated from bamboo, sugarcane, rice soil, earthworm's feces, and earthworms intestine, respectively, can enhance the salt tolerant capacity of rice and improve the yield of MTL 480 rice cultivar effectively. In particular, the treatments inoculated with either a mixture containing all five SSBs, or TCM_39, or RTTV_12 induced significantly higher numbers of silicate solubilizing bacteria in soil, and increased soluble silicate concentration in soil, silicate concentration in the rice stem, K^+/Na^+ ratio in rice stem, and filled rice grain weight per pot compared to other treatments including the recommended 100% NPK without bacteria inoculation. Further, soluble silicon concentration in the soil showed a strong positive correlation with other parameters, including Si concentration in the rice stem, K^+/Na^+ ratio, numbers of silicate solubilizing bacteria in soils, and filled rice grain weight per pot. These SSB have a high potential to be used in bio-products to improve salt tolerant capability, growth, and yield of rice plants when cultivated in salt-affected soils under the uncertainties of climate change, rising sea levels and saltwater intrusion into the Vietnam Mekong Delta.

5. References

[1] Korres, NE, Loka, DA, Gitsopoulos, TK,Varanasi, VK, Chachalis, D, Price A, Slaton, NA. Salinity effects on rice, rice weeds, and strategies to secure crop productivity and effective weed control A review. Agron Sustain Dev. 2022;2:581-26.

[2] International Center for Tropical Agriculture (CIAT). The drought and salinity intrusion in the Mekong River Delta of Vietnam. Ha Noi: CGIAR System Organization; 2016.

[3] Ma JF, Yamaji N. A cooperative system of silicon transport in plants. Trends Plant Sci. 2015;20(7):435-442.

[4] Ma JF, Yamaji N. Functions and transport of silicon in plants. Cell Mol Life Sci. 2008;65:3049-3057.

[5] Raturi G, Sharma Y, Rana V, Thakral V, Myaka B, Salvi P, et al. Exploration of silicate solubilizing bacteria for sustainable agriculture and silicon biogeochemical cycle. Plant Physiol Biochem. 2021;166:827-838.

[6] Datnoff LE, Deren CW, Snyder GH. Silicon fertilization for disease management of rice in Florida. J Crop Prot. 1997;16:525-531.

[7] Meena VD, Dotaniya ML, Coumar V, Rajendiran S, Ajay, Kundu S, et al. A case for silicon fertilization to improve crop yields in tropical soils. Proc Natl Acad Sci. 2014;84:505-518.

[8] Peera SKPG, Balasubramaniam P, Mabendran PP. Effect of fly ash and silicate solubilizing bacteria on yield and silicon uptake of rice in Cauvery Delta Zone. Environ. Ecol. 2016;34:1966-1971.

[9] Tran Vo Hai Duong, Dao Thi The, Nguyen Khoi Nghia. Efficiency of five isolated silicate solubilizing bacteria on germination rate, growth and biomass of rice under the condition supplemented with and without NaCl. Can Tho Univ J Sci. 2018;54:227-234.

[10] Tran Vo Hai Duong, Nguyen Khoi Nghia. Isolation and selection of silicate solubilizing bacteria from many various habitats. TNU J Sci Technol. 2018;180:9-14.
[11] Tran Vo Hai Duong, Nguyen Khoi Nghia. Evaluation of some biophysical characteristics of five silicate solubilizing bacteria isolated from different ecosystems. Vietnam J Sci Technol. 2019;17:775-784.
[12] Mekong Delta Development Research Institute. The rice cultivar characteristics, https://mdi.ctu.edu.vn/dao-tao/quy-khuyen-hoc/35-dac-tinh-giong. [accessed 12 September 2021].
[13] Nguyen Khoi Nghia, Do Hoang Sang, Nguyen Thi Kieu Oanh, Nguyen Thi To Quyen, Lam Tu Lang, Duong Minh Vien. Biodegradation of the pesticide Propuxur in soil by Paracoccus sp. P23-7 immobilized on biochar. Can Tho Univ J Sci. 2015;40:90-98.
[14] Haysom MBC, Chapman LS. Some aspects of the calcium silicate trials at Mackay. Proc Qld Soc Sugar Cane Technol. 2013;42:117-122.
[15] Rao GB, Susmitha P. Silicon management in rice. Int J Chem Stud. 2017;5:1359-1361.
[16] Bold HC. The morphology of *Chlamydomonas chlamydogama* sp. nov. Bull Torrey Bot Club. 1949;76:101-108.
[17] Vasanthi N, Saleena LM, Raj SA. Evaluation of media for isolation and screening of silicate solubilizing bacteria. Int J Curr Res. 2013;5:406-408.
[18] Pereira HS, Korndorfer GH, Moura WF, Correa GF. Silicon extractors available in slag and fertilizer. Rev Bras Ciênc Solo. 2003;27:265-274.
[19] Hallmark CT, Wilding LP, Smeck. Chemical and Microbiological Properties. In: Page AL, editor. Methods of Soil Analysis. 1st ed. Madison: Elsevier; 1982. p. 263-274.
[20] Wei-min D, Ke-qin Z, Bin-wu D, Cheng-xiao S, Kang-le Z, Run C, et al. Rapid determination of silicon content in rice. Rice Sci. 2005;12:145-147.
[21] Chapman HD, Pratt PF. Methods of plant analysis. In Chapman HD, Pratt PF, editors. Methods of Analysis for Soils, Plants and Water. Division of Agricultural Sciences: University of California; 1982. p. 60-64.
[22] Sheng XF. Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus. Soil Biol Biochem. 2005;37:1918-1922.
[23] Sheng FX, Zhao F, He YL, Qiu G, Chen L. Isolation and characterization of silicate mineral solubilizing Bacillus globisporus Q12 from the surface of weathered feldspar. Can J Microbiol. 2008;54:1064-1068.
[24] Lau JA, Lennon JT. Rapid responses of soil microorganisms improve plant fittness in novel environments. Proc Natl Acad Sci. 2012;109:1-5.
[25] Ma JF, Nishimura K, Takahashi E. Effect of silicon on the growth of rice plant at different growth stages. J Soil Sci Plant Nutr. 1989;35:347-356.
[26] Bargaz A, Lyamlouli K, Chtouki M, Zeroual Y, Dhiba D. Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Front Microbiol. 2018;9:1-25.
[27] Kang SM, Waqas M, Shahzad R, You YH, Asaf S, Khan MA, et al. Isolation and characterization of a novel silicate-solubilizing bacterial strain Burkholderia eburnea CS4-2 that promotes growth of japonica rice (Oryza sativa L. cv. Dongjin). J Soil Sci Plant Nutr. 2017;63:233-241.
[28] Liang Y, Zhang W, Chen Q, Liu Y, Ding R. Effect of exogenous silicon (Si) on H+-ATPase activity, phospholipids and fluidity of plasma membrane in leaves of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot. 2006;57:212-219.
[29] Ashraf M, Afzal M, Ahmed R, Mujeeb F, Sarwar A, Ali L. Alleviation of detrimental effects of NaCl by silicon nutrition in salt-sensitive and salt-tolerant genotypes of sugarcane (*Saccharum officinarum* L.). Plant Soil. 2010;326:381-391.
[30] Garg N, Bhandari P. Interactive effects of silicon and arbuscular mycorrhiza in modulating ascorbate-glutathione cycle and antioxidant scavenging capacity in differentially salt-tolerant Cicer arietinum L. genotypes subjected to long-term salinity. Protoplasma. 2016;253:1325-1345.
[31] Yue Y, Zhang M, Zhang JC, Duan LS, Li ZH. SOS1 gene overexpression increased salt tolerance in transgenic tobacco by maintaining a higher K+/Na+ ratio. J Plant Physiol. 2012;169:255-261.
[32] Zhu JK. Plant salt tolerance. Trends Plant Sci. 2001;6:66-71.
[33] Zhu Y, Gong H. Beneficial effects of silicon on salt and drought tolerance in plants. Agron Sustain Dev. 2014;34:455-472.