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The Potential of Bacterial Strains of *Luteovulum sphaeroides* W22 and W47 for Producing δ-Aminolevulinic Acid to Improve Soil Quality, Growth and Yield of Saline-Irrigated Rice Cultivated in Salt-Contaminated Soil

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Abstract: The present study aimed to identify the abilities of the δ-aminolevulinic acid (ALA) producing purple non-sulfur bacteria (PNSB), *Luteovulum sphaeroides* W22 and W47, to reduce the Na^+ concentration, and to ameliorate the soil fertility, nutrients uptake, growth and yield of rice on the salt-contaminated soil. A two-factor experiment was conducted following a completely randomized block design. The factors were the frequency of applying saline irrigation (zero, one, two, three and four times) and the ALA-producing PNSB supplementation (applying only W22, only W47 and mixed W22 + W47). The results revealed that supplying the PNSB mixture not only reduced the proline content but also increased the plant height, number of panicles per pot, percentage of filled seeds, contents of NH_4^+, PO_4^{3-}, total N, P uptake and grain yield. The mixed PNSB application also reduced the Na content and the total Na uptake in plants. *L. sphaeroides* W22 and W47 decreased the proline content by 31.3% and increased the grain yield by 27.2% in the condition of applying 5‰ saline irrigation four times.

Keywords: δ-aminolevulinic acid; purple non-sulfur bacteria; rice; saline irrigation; salt-contaminated soil

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1. Introduction

According to the climate change scenario by the year of 2030, the rising of the sea water will result in the saline water intruding to the domestic fields about 10 km along Hau River and coastal areas, which severely damages the rice cultivation in Vietnam [1]. Furthermore, salt-contaminated soils affect plant physiology via osmosis stress, ionic stress and nutrition imbalance [2], resulting in a reduction in rice yield [3,4]. Therefore, in order to minimize the damage caused by the saline water intrusion, numerous approaches have been applied, including lime fertilizations [5], organic supplementations [6], saline washing [7], planting [8] and bacterial supplementations [4,9]. Among these approaches, the one utilizing bacteria is a promising solution because bacteria are able to secrete compounds, such as exopolymeric substances (EPS) and δ-aminolevulinic acid (ALA), which help plants to overcome saline stress [9–12]. In particular, purple non-sulfur bacteria (PNSB) have been proven to be capable of providing ALA [13], a precursor of chlorophyll, antioxidant enzymes and other metabolites, which can limit adverse effects caused by abiotic stress [14,15]. Moreover, bacteria that can produce ALA, at first, are examined on an artificially salt-contaminated soil created by adding NaCl 0.25% [16]. Further, δ-aminolevulinic acid is naturally synthesized following two pathways. The first one is the C4 pathway, depending on the participation of succinyl-coenzyme A (CoA) and glycine because the ALA synthesizing enzyme is coded by two genes, *hemA* and *hemT*. The other pathway is called C5, which comprises three enzymic reactions from glutamate [17]. In addition, ALA plays a role in increasing the photosynthetic efficiency and promoting the

development of rice, even when a low ALA concentration from 1 to 5 mg ALA L^{-1} is applied [15]. Thus, the PNSB have the potential to help rice tolerate conditions of high Na^+ concentrations [9]. Additionally, ALA-producing bacterial strains possess some benefits to provide nutrients to plants, such as nitrogen (N) fixation, phosphorus (P) and potassium (K) solubilization and synthesis of several other plant-growth-promoting substances, such as 3-indole acetic acid (IAA) and siderophores [4,10,12,13]. Consequently, the present study was carried out in order to ascertain the ability of ALA-producing purple non-sulfur bacterial strains to reduce the Na content and to enhance the growth, nutrients uptake and yield of rice cultivated in the salt-contaminated soil, as well as to improve the soil fertility.

2. Materials and Methods

In the experiment, the salt-contaminated soil was collected at the depth of 0–20 cm in An Thuan commune, Thanh Phu district, Ben Tre province. The collected soil was dried up, separated from plant residues, crushed, mixed well and transferred to a black plastic pot whose large bottom × small bottom × height was 23 cm × 17 cm × 18 cm in an amount of 8 kg before being put into application. Then, the soil was supplied with 2 L of tap water to create muds before sowing. The chemical and physical properties of the saline soil are briefly described in Table 1.

Table 1. Properties of the soil at the beginning of the crop in Thanh Phu, Ben Tre.

Soil Properties	Unit	Value
pH_{H_2O}	-	6.48 ± 0.15
pH_{KCl}	-	6.42 ± 0.18
EC	mS cm^{-1}	6.12 ± 1.17
N_{total}	% N	0.15 ± 0.08
NH_4^+	mg kg^{-1}	49.7 ± 1.60
P_{total}	% P	0.017 ± 0.003
$P_{available}$	mg kg^{-1}	22.6 ± 3.12
Al-P	mg kg^{-1}	122.3 ± 5.67
Fe-P	mg kg^{-1}	90.4 ± 7.19
Ca-P	mg kg^{-1}	127.1 ± 6.39
CEC	meq 100 g^{-1}	15.1 ± 1.17
Na^+	meq 100 g^{-1}	4.25 ± 0.21
K^+	meq 100 g^{-1}	1.56 ± 0.27
Mg^{2+}	meq 100 g^{-1}	10.8 ± 1.08
Ca^{2+}	meq 100 g^{-1}	2.25 ± 0.24
Salt	%	5.21 ± 0.06
Silt	%	38.29 ± 3.82
Clay	%	56.60 ± 2.46

Mean ± standard deviation (n = 4).

Bacteria source: The ALA-producing *Luteovulum sphaeroides* W22 and *L. sphaeroides* W47 were isolated and screened from the rice-shrimp saline soil in Thanh Phu, Ben Tre, with accession numbers of MW819907 and OQ819480 [9].

Fertilizer formula: Types of fertilizers used in the current study included urea (46% N), super phosphate (16% P_2O_5) and potassium (60% K_2O).

Experiment design: Experiment 1 was conducted according to a completely randomized block design with 2 factors. Therein, the first factor (A) was frequencies of applying saline irrigation (none, one, two, three and four times); the other one (B) was the supplementation of ALA-producing purple non-sulfur bacteria (the single strain of *L. sphaeroides* W22, the single strain of *L. sphaeroides* W47 and a mixture of *L. sphaeroides* W22 and W47). In this research, we focused on the differences between single and mixed strains, so the negative control was not designed. However, we mentioned it in the following experiment. Experiment 2 was carried out in the saline soil following the same design as the other experiment with 3 treatments, including (i) no saline irrigation applied, (ii) applying saline irrigation but no bacteria applied and (iii) applying both saline irrigation and the mixture

of *L. sphaeroides* W22 and W47. Experimental pots were placed in the greenhouse in the Agricultural Research and Practice Station, College of Agriculture, Can Tho University. In the greenhouse, the light and dark hours per day were 11 and 13, respectively; the temperature was 36 °C (10.029783 N, 105.767414 E).

Bacterial inoculation: The inoculation of bacteria was referred to from the studies by Khuong et al. [4,9,11]. A single strain of the bacteria was supplied in an amount of 4 mL and a density of 1×10^8 CFU mL^{-1}, while, in the bacterial mixture, the volume of application was 2 mL for each strain. The bacterial supplementation was conducted 4 times at 25, 35, 45 and 60 days after sowing (DAS); i.e., the final bacterial density was approximately 20×10^4 CFU g^{-1}.

Seeds preparation: The rice used in the current study was the OM5451 rice cultivar purchased from the Cuu Long Delta Rice Research Institute, Vietnam. Its seeds were sterilized by ethanol and sodium hypochlorite 1%. Then, they were rinsed by sterile deionized water to ensure that the seeds were free from bacteria. After that, they were incubated for 24 h to germinate. Finally, 6 rice seeds were sowed directly into each pot.

Fertilization: It followed a recommended formula of $90N–60P_2O_5–30K_2O$ (kg ha^{-1}). For the P fertilizer, it was applied 100% to the ground. For the N fertilizer, it was divided into 3 portions of 30–40–30% and applied each portion at 10, 20 and 45 DAS, respectively. For the K fertilizer, it was separated in 2 halves and fertilized at 10 and 45 DAS with each half.

Irrigation: Tap water was applied continuously at 3–5 cm height during the experiment, except when the saline irrigation was in use.

Saline irrigation: A solution of NaCl at a dose of 4‰was used in experiment 1 and at a dose of 5‰ in experiment 2, and 10 mL of saline irrigation was poured into each pot at 10, 20, 30 and 40 DAS.

Soil analysis: Soil samples at the beginning and at the end of the crop were drilled, collected and brought back to the laboratory. They were left to dry, removed from plant residues and crushed through a 0.5 mm and 2.0 mm sieve. The methods for the soil analysis were summarized as follows: either pH_{H_2O} or pH_{KCl} was extracted with a ratio of 1:2.5 (soil–water) or 1:2.5 (soil-KCl 1 M), respectively, and measured by a pH meter. The solution from the pH_{H_2O} extraction was reused to measure the electrical conductivity (EC) by an EC meter. To determine the total N content, the samples were digested by a mixture of $H_2SO_{4saturated}$: $CuSO_4$: Se with a ratio 100:10:1, and the Kjeldahl method (Velp UDK129, VELP Scientifica Srl, Italy) was applied to distill samples before they were titrated by H_2SO_4 0.01 N. The blue phenol method was used to determine the amount of the available N at the 640 nm wavelength. The total P content was turned into inorganic forms by a mixture of $H_2SO_{4saturated}$—$HClO_4$; the inorganic solution was revealed in colors by ascorbic acid at the 880 nm wavelength. The available P content was determined by the Bray II method. It was extracted from the soil by HCl 0.1 N and NH_4F 0.03 N with soil–water ratio of 1:7, and the colorimetric method was conducted by ascorbic acid at the 880 nm wavelength. The unavailable P compounds, including ferrous phosphate (Fe-P), aluminum phosphate (Al-P) and calcium phosphate (Ca-P), were extracted by solutions, including NaOH 0.1 M, NH_4F 0.5 M and H_2SO_4 0.25 M, respectively, then detected by ascorbic acid and a spectrometer (UV–Vis 1800 Shimadzu) at the 880 nm wavelength. The cation exchange capacity (CEC) was determined by being extracted from the soil by $BaCl_2$ 0.1 M and titrated by EDTA 0.01 N. Concentrations of K^+, Na^+, Ca^{2+} and Mg^{2+} from the CEC extracted solution were measured by an atomic absorption spectrometer ICP–OES (Icap 6300 Duo Thermo, Thermo Fisher Scientific Inc., Waltham, MA, USA) at wavelengths of 766.5, 589.0, 422.7 and 285.2 nm, respectively [18].

Biochemical properties of rice: The proline content in plants was analyzed at 45 DAS. Leaf and stem samples were collected to determine their moisture by drying stem and leaves of immature rice at 70 °C for 72 h. Proline was examined by the Ninhydrin method of Bates et al. [19], which proceeded as follows: 0.5 g of the fresh rice samples were weighed and put into a 13 mm × 100 mm tube, and then 10 mL of sulfosalicylic acid 3% was added to completely digest the samples, which were then put on a reciprocal shaker in 30 min

and centrifuged at 3000 rpm for 15 min. After the debris was removed, the clear extract was collected. Then, 2.0 mL of the sample extract was reacted with 2.0 mL of Ninhydrin and 2 mL of glacial acetic acid in a tube, well mixed and covered. The samples were put into an incubator for 1 h at 100 °C. They were cooled in ice water, reacted with 4 mL of toluene, shaken for 15–20 s and measured on the atomic absorption spectrophotometer at the 520 nm wavelength.

N, P, K and sodium (Na) content in plants: Concentrations of N, P, K and Na in plants were measured according to the method of Walinga et al. [20]. Collected stems, leaves and seeds were dried up. Subsequently, they were milled well and used to analyze the concentrations of N, P, K and Na in stems, leaves and seeds. The samples were digested by a mixture of saturated H_2SO_4 and salicylic acid. The amount of N in the samples was determined by the Kjeldahl distilling method. The P proportion was measured at the 880 nm wavelength by the spectrometer. Concentrations of K and Na were detected by the atomic absorption spectrometer. Biomass in stem, leaves and seeds was checked by drying the stovers at 70 °C for 72 h and weighing them afterward.

Growth, yield components and grain yield: All growth and agronomic parameters were measured following the description of IRRI [21]. The growth parameters included the plant height and panicle length, which were checked on 8 plants or panicles per pot when it was at 90 DAS. The plant height was measured from the ground to the peak of a plant. The panicle length was measured from the neck to the end of a panicle. Yield components were measured as follows: the panicle number per pot: the number of panicles of a pot was counted; the seeds number per panicle: the total of seeds was counted on 8 panicles of a pot; the filled seeds percentage: it was equal to number of filled seeds divided by the total number of seeds; the 1000-seed weight: the weight of 1000 seeds in each treatment was determined. The actual grain yield: the seed weight and moisture of each pot was measured at harvesting and converted into the weight at 14% humidity.

Statistical Analysis: The numeric data were subjected to a two-way analysis of variance (ANOVA) by the SPSS software, version 13.0. The Duncan's post hoc test was utilized for comparing the differences between means of treatments at 5% significance. A relationship between the bacterial density of the PNSB, Na exchange content, Na content in seeds, yield and proline content produced in rice was evaluated by the correlation analysis. Correlations between the bacterial density and the Na exchange content, between the bacterial density and the Na content in seeds, between the Na exchange content and the proline content, between the Na content in seeds and the proline content, between the bacterial density and proline content and between the bacterial density and the yield were expressed by the Pearson correlation coefficient (R^2). The r value ranging between −1 and +1 indicated linearity between 2 variances, and, when it was equal to 0, there was no correlation between variances. Values, including +1, +0.8, +0.5 and +0.1, showed proportionally high, moderate and low correlations, whereas those including −0.8, −0.5 and −0.1 showed inversely high, moderate and low correlations, respectively [22].

3. Results

3.1. Influences of Both the ALA-Producing PNSB and the Frequency of the Saline Irrigation on the Proline Content and Features of Rice Cultivated in Salt-Contaminated Soil, and on the Soil Properties

3.1.1. Proline Content, Growth, Yield Components and Grain Yield of Rice Planted on Salt-Contaminated Soil

Proline content: Proline concentrations differed at 5% significance under impacts of both factors. To be more specific, the treatments applied with the saline irrigation had higher proline accumulations in plants than the ones without saline irrigation. The proline contents in the treatments applied with the saline irrigation four, three, two, one time and none were 5.63 > 4.91 > 4.06 > 3.73 > 2.77 μmol g^{-1} DW, respectively. Further, supplying the single strain of *L. sphaeroides* W22 had a proline content at 4.91 μmol g^{-1} DW, higher than that in the treatment supplied with the single strain of *L. sphaeroides* W47, 4.01 μmol g^{-1} DW. Nevertheless, the treatments supplied with the mixture of both *L. sphaeroides* W22 and W47 resulted in the lowest proline concentration, 3.74 μmol g^{-1} DW (Table 2).

Table 2. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria and the frequency of applying the saline irrigation on the proline content, growth, yield components and grain yield in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Factors		Proline Content	Plant Height	Panicle Length	Panicle Number Pot^{-1}	Seeds Number Panicle^{-1}	Filled Seeds Ratio	1000-Seed Weight	Grain Yield
		(μmol g^{-1} DW)	(cm)	(cm)	(Panicles)	(Seeds)	(%)	(g)	(g pot^{-1})
Frequencies of applying saline irrigation (A)	0	2.77 ± 0.65 ^{e}	78.6 ± 2.95 ^{a}	19.9 ± 0.81 ^{a}	11.4 ± 0.90 ^{a}	65.5 ± 3.99 ^{a}	83.9 ± 5.74 ^{a}	25.1 ± 2.22 ^{a}	18.9 ± 1.63 ^{a}
	1	3.73 ± 0.62 ^{d}	77.5 ± 2.27 ^{a}	19.5 ± 0.58 ^{ab}	11.4 ± 0.90 ^{a}	64.0 ± 3.90 ^{a}	85.4 ± 8.26 ^{a}	24.5 ± 0.83 ^{a}	18.1 ± 1.50 ^{b}
	2	4.06 ± 0.75 ^{c}	76.1 ± 3.54 ^{a}	19.2 ± 0.69 ^{ab}	11.0 ± 1.21 ^{a}	66.7 ± 5.37 ^{a}	76.9 ± 7.88 ^{b}	24.8 ± 1.22 ^{a}	17.8 ± 1.79 ^{b}
	3	4.91 ± 0.87 ^{b}	75.8 ± 4.15 ^{a}	19.0 ± 0.82 ^{b}	11.0 ± 1.12 ^{a}	67.0 ± 4.81 ^{a}	75.3 ± 7.56 ^{b}	25.2 ± 2.22 ^{a}	17.5 ± 1.40 ^{b}
	4	5.63 ± 0.80 ^{a}	72.6 ± 5.74 ^{b}	18.7 ± 1.16 ^{b}	9.92 ± 0.67 ^{b}	62.3 ± 5.82 ^{a}	76.9 ± 6.63 ^{b}	24.5 ± 2.23 ^{a}	15.3 ± 1.00 ^{c}
Bacterial Inoculant (B) (2×10^5 CFU g^{-1})	W22	4.91 ± 1.17 ^{a}	75.6 ± 3.39 ^{b}	19.1 ± 0.65 ^{a}	10.5 ± 0.76 ^{b}	64.7 ± 4.58 ^{a}	76.9 ± 7.42 ^{b}	24.5 ± 1.20 ^{a}	16.0 ± 1.17 ^{b}
	W47	4.01 ± 1.48 ^{b}	74.6 ± 4.05 ^{b}	19.3 ± 1.17 ^{a}	10.6 ± 0.94 ^{b}	66.4 ± 6.11 ^{a}	78.4 ± 6.41 ^{b}	24.8 ± 2.03 ^{a}	18.1 ± 1.77 ^{a}
	W22 + W47	3.74 ± 0.58 ^{c}	78.0 ± 4.89 ^{a}	19.4 ± 0.85 ^{a}	11.8 ± 1.06 ^{a}	64.2 ± 4.05 ^{a}	83.8 ± 9.15 ^{a}	25.1 ± 2.10 ^{a}	18.5 ± 1.73 ^{a}
F (A)		*	*	*	*	ns	*	ns	*
F (B)		*	*	ns	*	ns	*	ns	*
F (A × B)		*	ns	ns	ns	ns	ns	ns	ns
CV (%)		7.63	5.12	4.56	6.59	6.79	8.33	7.43	7.78

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance; DW: dry weight.

Rice growth and yield: All rice growth and yield components reduced according to the increase in the frequency of applying saline water, except for the number of seeds per panicle and the 1000-seed weight. For instance, the plant height, panicle length, panicles number per pot and filled seed ratio dropped from 78.6 to 72.6 cm, 19.9 to 18.7 cm, 11.4 to 9.92 panicles and 83.9 to 76.9%, respectively, from zero times to four times of applying saline water. This led the yield to decline from 18.9 to 15.3 g pot^{-1}. In the meantime, the bacteria supplementation had an opposite trend. In the treatments supplied with the mixture of both ALA-producing *L. sphaeroides* W22 and W47, plant heights were the highest and valued at 78.0 cm tall, significantly higher than those in the treatments supplied with a single strain of either *L. sphaeroides* W22 or W47, which were 75.6 and 74.6 cm tall, respectively. The panicle length fluctuated from 19.1 to 19.4 cm in treatments supplied with the PNSB. Furthermore, supplying the mixture of the ALA-producing *L. sphaeroides* W22 and W47 contributed to an increase to 11.8 panicles pot^{-1} in the number of panicles per pot, which had more panicles than the treatments supplied with a single strain of either *L. sphaeroides* W22 or *L. sphaeroides* W47 (10.5–10.6 panicles pot^{-1}). The treatments supplied with the mixture of both *L. sphaeroides* W22 and W47 had a filled seeds percentage of 83.8% which was higher than those in the treatments supplied with either the single strain of *L. sphaeroides* W22 (76.9%) or the single strain of *L. sphaeroides* W47 (78.4%). Nonetheless, the seeds number per panicle and 1000-seed weight remained statistically unchanged under the effects of the bacterial supplementation and valued averagely at 65.1 seeds panicle^{-1} and 24.8 g, respectively. Ultimately, the grain yield in the treatments supplied with the single strain of *L. sphaeroides* W22 (16.0 g pot^{-1}) was lower than those in the treatments supplied with either the single strain of *L. sphaeroides* W47 or the mixture of both *L. sphaeroides* W22 and W47 (18.1 and 18.5 g pot^{-1}) (Table 2).

3.1.2. Chemical Properties of Salt-Contaminated Soil

The values of the pH_{H_2O} and pH_{KCl} among treatments changed insignificantly according to both factors. The means of pH_{H_2O} and pH_{KCl} were 6.08 and 5.49. The EC values changed at 5% significance regarding the frequency of applying the saline irrigation. In detail, the treatments without applying the saline irrigation had an EC value at 1.85 mS cm^{-1}, which was lower than those in the treatments applied with the saline irrigation from one to four times (2.55–2.64 mS cm^{-1}). In the treatments supplied with the single strain of *L. sphaeroides* W22, the EC value was 2.60 mS cm^{-1} and 5% significantly higher than those

in the treatments supplied with either the single strain of W47 or the bacterial mixture of the W22 and W47, valuing at 2.32 and 2.40 mS cm^{-1}, respectively (Table 3).

Table 3. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria and the frequency of applying the saline irrigation on chemical properties of salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Factors		pH_{H_2O}	pH_{KCl}	EC	N_{total}	NH_4^+	P_{total}	$P_{available}$
				(mS cm^{-1})	(%)	(mg kg^{-1})	(%)	(mg kg^{-1})
Frequencies of applying saline irrigation (A)	0	6.16 ± 0.22 ^{a}	5.36 ± 0.32 ^{a}	1.85 ± 0.18 ^{b}	0.090 ± 0.006 ^{a}	6.20 ± 1.44 ^{a}	0.046 ± 0.012 ^{a}	11.8 ± 3.20 ^{a}
	1	6.11 ± 0.16 ^{a}	5.31 ± 0.25 ^{a}	2.55 ± 0.22 ^{a}	0.086 ± 0.012 ^{a}	6.73 ± 1.84 ^{a}	0.047 ± 0.008 ^{a}	11.1 ± 2.63 ^{a}
	2	6.13 ± 0.24 ^{a}	5.83 ± 0.90 ^{a}	2.54 ± 0.45 ^{a}	0.083 ± 0.017 ^{a}	6.42 ± 1.34 ^{a}	0.045 ± 0.007 ^{a}	11.8 ± 3.33 ^{a}
	3	5.99 ± 0.21 ^{a}	5.49 ± 0.23 ^{a}	2.64 ± 0.48 ^{a}	0.089 ± 0.008 ^{a}	6.31 ± 0.68 ^{a}	0.043 ± 0.007 ^{a}	12.4 ± 1.11 ^{a}
	4	6.04 ± 0.32 ^{a}	5.47 ± 0.35 ^{a}	2.62 ± 0.33 ^{a}	0.081 ± 0.013 ^{a}	6.92 ± 1.05 ^{a}	0.064 ± 0.090 ^{a}	12.4 ± 0.96 ^{a}
Bacterial inoculant (B) (2×10^5 CFU g^{-1})	W22	6.06 ± 0.31 ^{a}	5.40 ± 0.37 ^{a}	2.60 ± 0.48 ^{a}	0.089 ± 0.012 ^{a}	6.67 ± 0.90 ^{b}	0.064 ± 0.068 ^{a}	9.70 ± 2.20 ^{b}
	W47	6.14 ± 0.20 ^{a}	5.66 ± 0.73 ^{a}	2.32 ± 0.48 ^{b}	0.085 ± 0.010 ^{a}	7.42 ± 0.89 ^{a}	0.041 ± 0.005 ^{a}	13.1 ± 1.73 ^{a}
	W22 + W47	6.04 ± 0.19 ^{a}	5.42 ± 0.29 ^{a}	2.40 ± 0.36 ^{b}	0.084 ± 0.014 ^{a}	7.46 ± 1.24 ^{a}	0.041 ± 0.009 ^{a}	12.9 ± 1.68 ^{a}
F (A)		ns	ns	*	ns	ns	ns	ns
F (B)		ns	ns	*	ns	*	ns	*
F (A × B)		ns	ns	*	ns	*	ns	*
CV (%)		3.98	8.78	11.9	10.2	12.6	11.3	12.1

Factors		Al-P	Ca-P	Fe-P	CEC	K^+	Na^+	Ca^{2+}	Mg^{2+}
		(mg kg^{-1})			(meq 100 g^{-1})				
Frequencies of applying saline irrigation (A)	0	29.2 ± 11.8 ^{a}	68.8 ± 5.74 ^{a}	86.6 ± 6.42 ^{b}	23.1 ± 2.07 ^{a}	7.18 ± 1.71 ^{a}	3.30 ± 0.34 ^{d}	3.93 ± 0.47 ^{a}	7.88 ± 1.30 ^{a}
	1	26.3 ± 8.00 ^{a}	68.4 ± 4.90 ^{a}	84.2 ± 3.23 $^{b\,c}$	24.3 ± 3.02 ^{a}	6.86 ± 1.65 ^{a}	4.17 ± 0.30 ^{c}	3.95 ± 0.71 ^{a}	7.67 ± 1.02 ^{a}
	2	24.2 ± 8.34 ^{a}	70.4 ± 11.4 ^{a}	91.0 ± 8.81 ^{a}	24.5 ± 2.87 ^{a}	7.53 ± 0.81 ^{a}	4.80 ± 0.42 ^{b}	3.56 ± 0.88 ^{a}	8.04 ± 1.15 ^{a}
	3	25.6 ± 10.5 ^{a}	65.4 ± 10.5 ^{a}	94.6 ± 9.80 ^{a}	24.8 ± 3.24 ^{a}	7.14 ± 1.30 ^{a}	5.05 ± 0.34 ^{a}	3.46 ± 0.83 ^{a}	8.29 ± 1.26 ^{a}
	4	27.3 ± 8.56 ^{a}	63.7 ± 10.4 ^{a}	81.8 ± 6.37 ^{c}	23.8 ± 5.03 ^{a}	7.89 ± 1.32 ^{a}	5.22 ± 0.29 ^{a}	3.88 ± 0.60 ^{a}	8.38 ± 0.72 ^{a}
Bacterial inoculant (B) (2×10^5 CFU g^{-1})	W22	32.0 ± 11.5 ^{a}	68.6 ± 13.2 ^{a,b}	87.9 ± 5.35 ^{a}	23.4 ± 4.16 ^{a}	6.93 ± 1.61 ^{b}	4.71 ± 0.74 ^{a}	3.86 ± 0.61 ^{a}	7.93 ± 1.17 ^{b}
	W47	25.1 ± 6.53 ^{b}	69.7 ± 5.11 ^{a}	85.9 ± 6.52 ^{a}	23.8 ± 2.77 ^{a}	6.88 ± 1.28 ^{b}	4.45 ± 0.88 ^{b}	3.54 ± 0.73 ^{a}	7.66 ± 1.04 ^{b}
	W22 + W47	22.3 ± 6.74 ^{b}	63.7 ± 5.73 ^{b}	89.1 ± 12.0 ^{a}	25.1 ± 2.77 ^{a}	8.15 ± 0.86 ^{a}	4.36 ± 0.71 ^{b}	3.86 ± 0.79 ^{a}	8.57 ± 1.10 ^{a}
F (A)		ns	ns	*	ns	ns	*	ns	ns
F (B)		*	*	ns	ns	*	*	ns	*
F (A × B)		ns	*	*	ns	ns	*	ns	*
CV (%)		34.5	11.5	5.40	15.1	18.5	6.20	19.7	11.9

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance; EC: electrical conductivity; CEC: cations exchange capacity.

The total N and P contents, according to the two factors, did not differ significantly and valued averagely at 0.086 and 0.049%. The results in Table 3 indicated that the NH_4^+ content was not significantly different when the frequency of applying the saline irrigation rose and was 6.52 mg NH_4^+ kg^{-1} on average. However, in the treatments supplied with ALA-producing *L. sphaeroides* W22 and W47, the NH_4^+ content peaked at 7.46 mg NH_4^+ kg^{-1}, different at 5% significance from those in the treatments supplied with a single strain of the W22, whose NH_4^+ content was the lowest (6.67 mg NH_4^+ kg^{-1}). The increasing times of applying the saline irrigation did not significantly affect the amount of the available P in the soil, whose mean was 11.9 mg P kg^{-1}, while, in the treatments supplied with the single strain of W22, the available P content was 9.70 mg P kg^{-1} and lower than those in the treatments supplied with either the single strain of W47 (13.1 mg P kg^{-1}) or the mixture of the W22 and W47 (12.9 mg P kg^{-1}). Treatments with different numbers of times of applying the saline irrigation did not show significant differences in concentrations of Al-P and Ca-P (Table 3). However, there were 5% significant differences in Fe-P content according to different saline irrigation applying times. The treatments applied with the saline irrigation two or three times had corresponding Fe-P contents of 91.0 and 94.6 mg P kg^{-1}, higher than those in the treatments applied with the saline irrigation zero and one time, with 86.6 and 84.2 mg P kg^{-1}, respectively. A low Fe-P content was recorded in the treatments applied with the saline irrigation four times, with 81.8 mg P kg^{-1}. With the supplementation of the ALA-producing PNSB, the treatments supplied with the single strain of W22 had higher Al-P content (32.0 mg P kg^{-1}) than the treatments supplied with the single strain of W47 or the mixture of W22 and W47, corresponding to 25.1 and 22.3 mg P kg^{-1}.

The concentration of Ca-P in the treatments supplied with the single strain of W47 was 69.7 mg P kg^{-1}, higher than those in the treatments supplied with the bacterial mixture of the W22 and W47, 63.7 mg P kg^{-1}. However, the Fe-P content was not statistically different between treatments influenced by the bacterial factor and measured at 87.6 mg P kg^{-1} on average (Table 3).

The CEC and Ca^{2+} content was insignificantly different under the influences of both factors and averagely valued at 24.1 meq CEC 100 g^{-1} and 3.75 meq Ca^{2+} 100 g^{-1}. For the frequency of applying the saline irrigation, K^+ and Mg^{2+} contents among treatments changed insignificantly and were 7.32 meq K^+ 100 g^{-1} and 8.05 meq Mg^{2+} 100 g^{-1}. The treatments without applying the saline irrigation had the lowest Na^+ content, 3.30 meq Na^+ 100 g^{-1}. The treatments applied with the saline irrigation one to four times had a rising Na^+ content in an order as follows: 4.17 < 4.80 < 5.05~5.22 meq Na^+ 100 g^{-1}. However, concentrations of K^+, Na^+ and Mg^{2+} in the treatments supplied with the mixture of *L. sphaeroides* W22 and W47 were higher at 5% significance than those in the treatments supplied with the single strain of W22 or the single strain of W47 (Table 3). The more times the saline irrigation was applied, the lower the bacterial density was. The bacterial density was 4.43 > 4.19 > 4.08 > 3.96 > 3.83 log MPN g^{-1} DSW along with the increasing frequency of applying the saline irrigation. In addition, supplying the mixture of W22 and W47, the bacterial density was 4.45 log MPN g^{-1} DSW, 5% significantly higher than those in the treatments supplied with the single strain of either W22 or W47, which was in a range of 3.82–3.97 log MPN g^{-1} DSW (Figure 1).

3.1.3. Biomass, N, P, K and Na Contents and Uptakes in Seeds and in Stem Leaves of Rice

N, P, K and Na concentrations in seeds: The N, P, K and Na contents in seeds varied at 5% significance regarding both factors. The treatments applied with the saline irrigation four times had the N content in seeds at 1.59%, which was higher than those in the treatments applied with the saline irrigation 0–3 times (1.38–1.43%). In addition, P and Na contents in seeds in the treatments applied with the saline irrigation three and four times were correspondingly 0.359–0.360% and 0.110–0.116%, which were higher than 0.297–0.312% and 0.077–0.101% in the treatments applied with the saline irrigation 0–2 times. Nevertheless, the K content in the treatments without applying the saline irrigation and the ones applied with the saline irrigation once were 0.227 and 0.241%, higher than those in the treatments applied with the saline irrigation 2–4 times, with the K concentration ranging roughly 0.174–0.183%. For the bacterial factor, the treatments supplied with the mixture of *L. sphaeroides* W22 and W47 possessed the highest N and P concentrations, which were correspondingly 1.53 and 0.373%. In the treatments supplied with a single strain of either W22 or W47, the N content was 1.37 and 1.42%, and the P content was 0.297 and 0.313%. On the contrary, the treatments supplied with the mixture of the W22 and W47 had lower K content in seeds than those in the treatments supplied with the single strain of W22 (Table 4).

N, P, K and Na concentrations in stem, leaves: Both factors had insignificantly different N and P contents in stem leaves, which fluctuated from 1.27 to 1.45% and from 0.196 to 0.243% for the frequency of applying the saline irrigation, and from 1.32 to 1.48% and from 0.200 to 0.222% for the supplementation of the PNSB. Furthermore, the frequency of applying the saline irrigation did not affect the K contents, which valued approximately 1.42–1.60%. However, the treatments applied with the saline irrigation one to four times had higher Na content (0.623–0.807%) than those in the treatments without the saline irrigation (0.546%). In addition, the treatments supplied with a single strain of *L. sphaeroides* W22 or W47 had the K content in stem leaves at 1.68 and 1.57%, respectively, which were higher than those in the treatments supplied with the mixture of the W22 and W47 (1.30%). Additionally, the Na content peaked in the treatments applied with the saline irrigation four times (0.807%). The lowest Na content was recorded in the treatments without the saline irrigation (0.546%). In addition, the treatments supplied with the single strain of W22 had a Na content of 0.751%, which was higher than those in the treatments supplied

with either the single strain of W47 or the mixture of the W22 and W47 (0.639 and 0.620%, respectively) (Table 4).

Table 4. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria and frequency of applying the saline irrigation on N, P, K and Na contents in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Factors		Concentration (%)							
		N		P		K		Na	
		Seeds	Stems, Leaves	Seeds	Stems, Leaves	Seeds	Stems, Leaves	Seeds	Stems, Leaves
Frequencies of applying saline irrigation (A)	0	1.40 ± 0.15 ^{b}	1.27 ± 0.20 ^{a}	0.312 ± 0.052 ^{b}	0.243 ± 0.070 ^{a}	0.227 ± 0.055 ^{a}	1.54 ± 0.27 ^{a}	0.077 ± 0.015 ^{c}	0.546 ± 0.080 ^{d}
	1	1.43 ± 0.18 ^{b}	1.39 ± 0.27 ^{a}	0.308 ± 0.060 ^{b}	0.202 ± 0.051 ^{a}	0.241 ± 0.053 ^{a}	1.60 ± 0.37 ^{a}	0.095 ± 0.010 ^{b}	0.623 ± 0.159 ^{c}
	2	1.38 ± 0.15 ^{b}	1.39 ± 0.34 ^{a}	0.297 ± 0.058 ^{b}	0.199 ± 0.042 ^{a}	0.174 ± 0.065 ^{b}	1.42 ± 0.33 ^{a}	0.101 ± 0.010 ^{b}	0.698 ± 0.112 ^{b}
	3	1.40 ± 0.11 ^{b}	1.40 ± 0.31 ^{a}	0.360 ± 0.081 ^{a}	0.196 ± 0.063 ^{a}	0.183 ± 0.060 ^{b}	1.48 ± 0.43 ^{a}	0.110 ± 0.016 ^{a}	0.677 ± 0.072 $^{b\,c}$
	4	1.59 ± 0.19 ^{a}	1.45 ± 0.26 ^{a}	0.359 ± 0.058 ^{a}	0.207 ± 0.042 ^{a}	0.183 ± 0.052 ^{b}	1.54 ± 0.64 ^{a}	0.116 ± 0.004 ^{a}	0.807 ± 0.078 ^{a}
Bacterial Inoculant (B) (2×10^5 CFU g^{-1})	W22	1.37 ± 0.15 ^{b}	1.34 ± 0.29 ^{a}	0.297 ± 0.059 ^{b}	0.200 ± 0.050 ^{a}	0.235 ± 0.065 ^{a}	1.68 ± 0.39 ^{a}	0.104 ± 0.011 ^{a}	0.751 ± 0.02 ^{a}
	W47	1.42 ± 0.14 ^{b}	1.32 ± 0.25 ^{a}	0.313 ± 0.054 ^{b}	0.222 ± 0.067 ^{a}	0.196 ± 0.060 ^{b}	1.57 ± 0.42 ^{a}	0.100 ± 0.021 $^{a\,b}$	0.639 ± 0.13 ^{b}
	W22 + W47	1.53 ± 0.19 ^{a}	1.48 ± 0.28 ^{a}	0.373 ± 0.062 ^{a}	0.206 ± 0.048 ^{a}	0.175 ± 0.045 ^{b}	1.30 ± 0.36 ^{b}	0.095 ± 0.020 ^{b}	0.620 ± 0.14 ^{b}
F (A)		*	ns	*	ns	*	ns	*	*
F (B)		*	ns	*	ns	*	*	*	*
F (A × B)		ns	ns	*	*	ns	*	ns	*
CV (%)		9.81	20.6	13.7	26.2	27.1	24.6	11.0	10.6

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance.

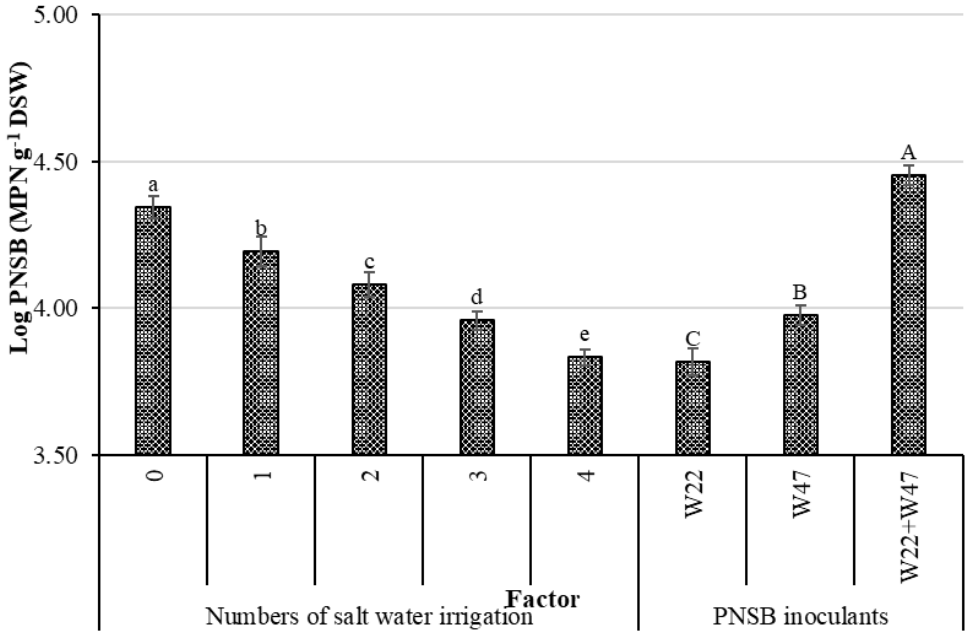

Figure 1. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria and the frequency of applying the saline irrigation on the bacterial density in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition. Note: different uppercase and lowercase letters indicate significant differences for PNSB density by PNSB inoculants and numbers of salt water irrigation, respectively.

Dry biomass: Biomass values in seeds and stems and leaves in different treatments were different from each other at 5% significance according to both factors. In particular, the treatments applied with the saline irrigation four times led to lower biomass in seeds, stems and leaves and valued at 11.7 and 12.0 g pot^{-1}. The treatments without the saline irrigation had biomass in seeds and stems and leaves at 14.3 and 16.2 g pot^{-1}, higher than those in the treatments applied with the saline irrigation two to three times, whose results were 13.3 and 13.1 g pot^{-1} and 14.2 and 14.0 g pot^{-1}, respectively. In addition, the biomass in seeds and stems and leaves in the treatments supplied with the mixture of *L. sphaeroides* W22 and W47 was the highest, valued at 14.8 and 15.6 g pot^{-1}. In the meantime, in the treatments supplied with a single strain of either W22 or W47, the biomass in seeds and stems and leaves was 12.6 and 12.5 and 13.9 and 13.7 g pot^{-1}, respectively (Table 5).

Table 5. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria and the frequency of applying the saline irrigation on the biomass and N, P, K and Na uptakes in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Factors		Biomass (g pot^{-1})		Uptake (g pot^{-1})							
				N		P		K		Na	
		Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves
Frequencies of applying saline irrigation (A)	0	14.3 ±1.70 a	16.2 ± 1.30 a	0.202 ± 0.038 a,b	0.200 ± 0.030 a	0.044 ± 0.010 a b	0.039 ± 0.011 a	0.032 ± 0.008 a	0.245 ± 0.042 a	0.0109 ± 0.001 b	0.0882 ± 0.012 a
	1	14.1 ±1.66 a	15.6 ± 1.41 b	0.203 ± 0.042 a	0.217 ± 0.054 a	0.044 ± 0.008 a b	0.031 ± 0.008 a	0.033 ± 0.005 a	0.247 ± 0.059 a	0.0133 ± 0.002 a	0.0956 ± 0.019 a
	2	13.3 ±1.28 b	14.2 ± 1.44 c	0.184 ± 0.028 b	0.198 ± 0.047 a	0.040 ± 0.006 b	0.028 ± 0.006 a	0.023 ± 0.008 b	0.207 ± 0.048 a b	0.0133 ± 0.002 a	0.0983 ± 0.013 a
	3	13.1 ±1.21 b	14.0 ± 1.00 c	0.183 ± 0.025 b	0.196 ± 0.049 a	0.048 ± 0.010 a	0.027 ± 0.010 a	0.024 ± 0.008 b	0.201 ± 0.055 b	0.0143 ± 0.002 a	0.0943 ± 0.007 a
	4	11.7 ±0.89 c	12.0 ± 1.91 d	0.188 ± 0.029 a	0.172 ± 0.025 a	0.042 ± 0.005 a b	0.025 ± 0.005 a	0.021 ± 0.007 b	0.186 ± 0.081 b	0.0134 ± 0.001 a	0.0964 ± 0.012 a
Bacterial Inoculant (B) (2×10^5 CFU g^{-1})	W22	12.6 ±0.83 b	13.9 ± 0.99 b	0.174 ± 0.023 b	0.186 ± 0.040 b	0.037 ± 0.007 b	0.027 ± 0.008 a	0.030 ± 0.009 a	0.234 ± 0.060 a	0.0131 ± 0.001 a b	0.1037 ± 0.012 a
	W47	12.5 ±1.07 b	13.7 ± 1.64 b	0.177 ± 0.019 b	0.180 ± 0.030 b	0.039 ± 0.006 b	0.031 ± 0.012 a	0.026 ± 0.009 a	0.213 ± 0.049 a	0.0124 ± 0.002 b	0.0866 ± 0.013 b
	W22 + W47	14.8 ±1.66 a	15.6 ± 2.34 a	0.225 ± 0.027 a	0.227 ± 0.045 a	0.055 ± 0.007 a	0.032 ± 0.008 a	0.025 ± 0.008 a	0.207 ± 0.074 a	0.0138 ± 0.002 a	0.0934 ± 0.002 b
F (A)		*	*	ns	ns	*	ns	*	*	*	ns
F (B)		*	*	*	*	*	ns	ns	ns	*	*
F (A × B)		*	*	ns	ns	*	ns	ns	*	ns	ns
CV (%)		5.08	3.82	11.0	16.0	13.8	25.7	25.9	25.1	12.7	12.1

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance.

N, P, K and Na uptakes: The N uptake in the treatments applied with the saline irrigation ranged from 0.183 to 0.203 g pot^{-1} in seeds and from 0.172 to 0.217 g pot^{-1} in stems and leaves. With the bacterial supplementation, the N uptake in the treatments supplied with the single strain of either *L. sphaeroides* W22 or W47 was 0.174 and 0.177 g pot^{-1} in seeds and 0.186 and 0.180 g pot^{-1} in stems and leaves, significantly lower than those in the treatments supplied with the mixture of the W22 and W47, valuing at 0.225 and 0.227 g pot^{-1} (Table 5). In addition, the treatment without the saline irrigation had the total N uptake of 0.41 g pot^{-1}, equivalent to those in the treatments applied with the saline irrigation one, two and three times, but it was higher than those in the treatments applied with the saline irrigation four times (0.36 g pot^{-1}). The treatments supplied with the mixture of the ALA-producing W22 and W47 possessed higher total N uptake (0.452 g pot^{-1}) at 5% significance than those in the treatments supplied with a single strain of W22 or W47, with 0.360 and 0.357 g pot^{-1}, respectively (Figure 2).

The P uptake in seeds fluctuated from 0.042 to 0.048 g pot^{-1}. In addition, in the treatments supplied with the mixture of *L. sphaeroides* W22 and W47, the P uptake in seeds was 0.055 g pot^{-1}, 5% significantly different from those in the treatments supplied with a single strain of either W22 or W47, which were correspondingly 0.037 and 0.039 g pot^{-1}. However, the P uptake in stems and leaves differed insignificantly for the both factors and averagely valued at 0.030 g pot^{-1} (Table 5). The total P uptake in the treatments without applying the saline irrigation was the highest with the result at 0.084 g pot^{-1}. In the meantime, the treatments applied with the saline irrigation one to four times resulted in the total P uptake from 0.067 to 0.075 g pot^{-1}. In the treatments supplied with the mixture of W22 and W47, the total P uptake valued at 0.086 g pot^{-1}, significantly higher than those in the treatments supplied with a single strain of W22 or W47, corresponding to 0.065 and 0.067 g pot^{-1} (Figure 2).

The K uptake in seeds and in stems and leaves in the treatments without applying the saline irrigation (0.032 and 0.245 g pot^{-1}) was statistically equal to those in the treatments applied with the saline irrigation once (0.033 and 0.247 g pot^{-1}). However, it was higher at 5% significance than those in the treatments applied with the saline irrigation three and four times, which resulted in 0.024 and 0.021 g pot^{-1} in seeds and 0.021 and 0.186 g pot^{-1} in stem leaves (Table 5). Similarly, the treatments without the saline irrigation and the ones applied with the saline irrigation once had the total K uptake of 0.282 and 0.281 g pot^{-1}, which were significantly different from those in the treatments applied with the saline irrigation three to four times (0.207–0.255 g pot^{-1}). Nevertheless, supplying either a single strain or the mixture of the PNSB had equivalent K uptake values in seeds and in stems and leaves

and statistically unchanged total K, whose means were 0.027, 0.218 and 0.245 g pot^{-1} (Table 5 and Figure 2).

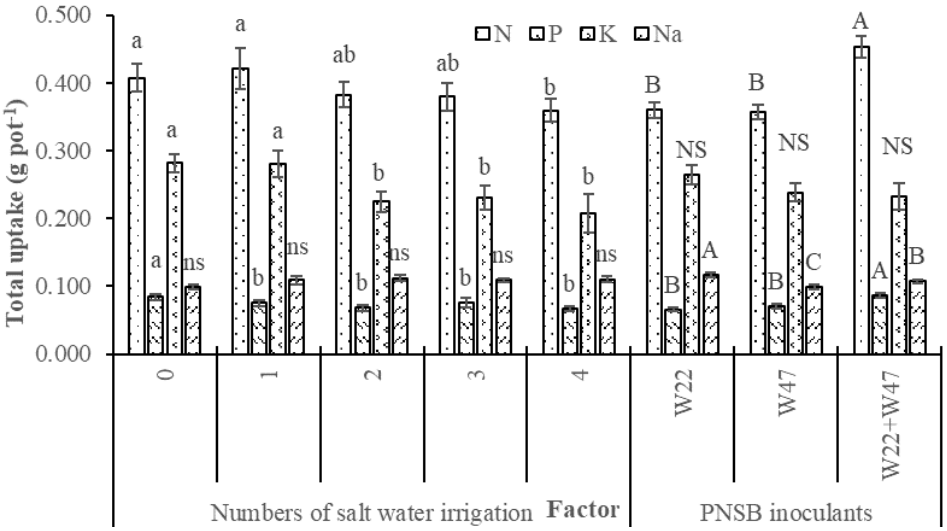

Figure 2. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria and the frequency of applying saline irrigation on total N, P, K and Na uptakes in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition. Note: 0: no saline irrigation; 1: saline irrigation once; 2: saline irrigation twice; 3: saline irrigation 3 times; 4: saline irrigation 4 times; W22: supplying with the single strain of *L. sphaeroides* W22; W47: supplying with the single strain of *L. sphaeroides* W47; W22 + W47: supplying with the mixture of W22 and W47. The comparisons were between bars with the same pattern. The different lowercase letters indicate significant differences among the frequencies of saline irrigation, and the uppercase ones indicate significant differences between the supplementations of the PNSB. NS/ns: not significant.

The Na uptake in seeds in the treatments without applying the saline irrigation was the lowest, with a value of 0.0109 g pot^{-1}, while the treatments applied with the saline irrigation one to four times had equivalent Na uptake in seeds and ranged approximately 0.0133–0.0143 g pot^{-1}. Regarding the bacterial supplementation, the treatments supplied with the mixture of *L. sphaeroides* W22 and W47 had the Na uptake in seeds at 0.0138 g pot^{-1}, which was higher than those in the treatments supplied with the single strain of W47, 0.0124 g pot^{-1}. However, leveling up the frequency of applying the saline irrigation did not affect the Na uptake in stems and leaves, whose mean was approximately 0.0882–0.0983 g pot^{-1}. The treatments supplied with the single strain of the ALA-producing W22 had the Na uptake in stems and leaves valuing 0.1037 g pot^{-1}, higher than those in the treatments supplied with either the single strain of W47 or the mixture of both the W22 and W47 (Table 5). Nevertheless, the total Na uptake changed insignificantly under the influence from different saline irrigation frequencies and was averagely 0.108 g pot^{-1}. The treatments supplied with the single strain of the ALA-producing W22 had total Na uptake of 0.117 g pot^{-1}, higher at 5% significance than those in the treatments supplied with the mixture of the W22 and W47, 0.107 g pot^{-1}. In addition, the treatments supplied with the single strain of W47 had the lowest total Na uptake (0.099 g pot^{-1}) (Figure 2).

3.1.4. Interactions between the δ-Aminolevulinic Acid Producing Purple Non-Sulfur Bacteria and Na^+ Content in the Soil, Na Content in Seeds and Proline Content

The bacterial density interacted with the concentrations of Na^+ in the soil and Na contents in seeds, with correlation coefficients of 0.4810 and 0.5876, respectively (Figure 3A,B). This indicated that, the higher the bacterial density was, the lower Na^+ in the soil and Na in seeds was. Nevertheless, there was a close correlation between the Na concentration and the proline content. In detail, the correlation coefficient between the exchangeable Na^+ in the soil and proline was 0.6362 (Figure 4A) and was 0.6185 between the Na content in seeds

and proline (Figure 4B). In other words, the proline content and Na content were positively correlated. Moreover, increasing the density of the bacteria led to a decrease in the proline content, with a correlation coefficient of 0.6922 (Figure 5A), while a high bacterial density contributed to an increase in rice grain yield, r = 0.6113 (Figure 5B).

3.2. Influences of the Mixture of the ALA-Producing PNSB on the Proline Content and Features of Rice Cultivated in Salt-Contaminated Soil, and on the Soil Properties

3.2.1. Influences of the Mixture of the δ-Aminolevulinic Acid Producing Purple Non-sulfur Bacteria on the Proline Content, Growth, Yield Components and Grain Yield of Rice Cultivated in Salt-Contaminated Soil

Proline content: The treatment applied with the saline irrigation and without supplying the ALA-producing PNSB had the highest proline concentration (4.39 μmol g^{-1} DW). The treatments without applying the saline irrigation and no bacterial supplementation and the ones applied with the saline irrigation and supplied with the mixture of the *L. sphaeroides* W22 and W47 had an equivalent proline concentration, with 2.95 and 3.02 μmol g^{-1} DW, respectively (Table 6).

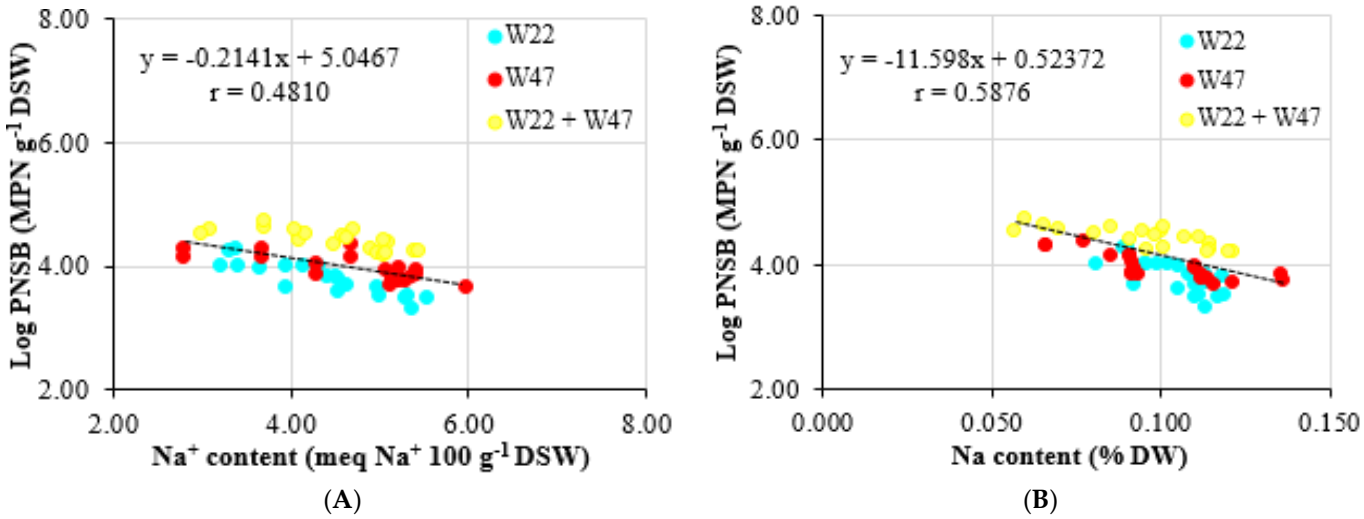

Figure 3. The linear correlations between PNSB soil population and (**A**) soil Na^+ concentration ($p < 0.05$); (**B**) and rice Na concentration ($p < 0.05$).

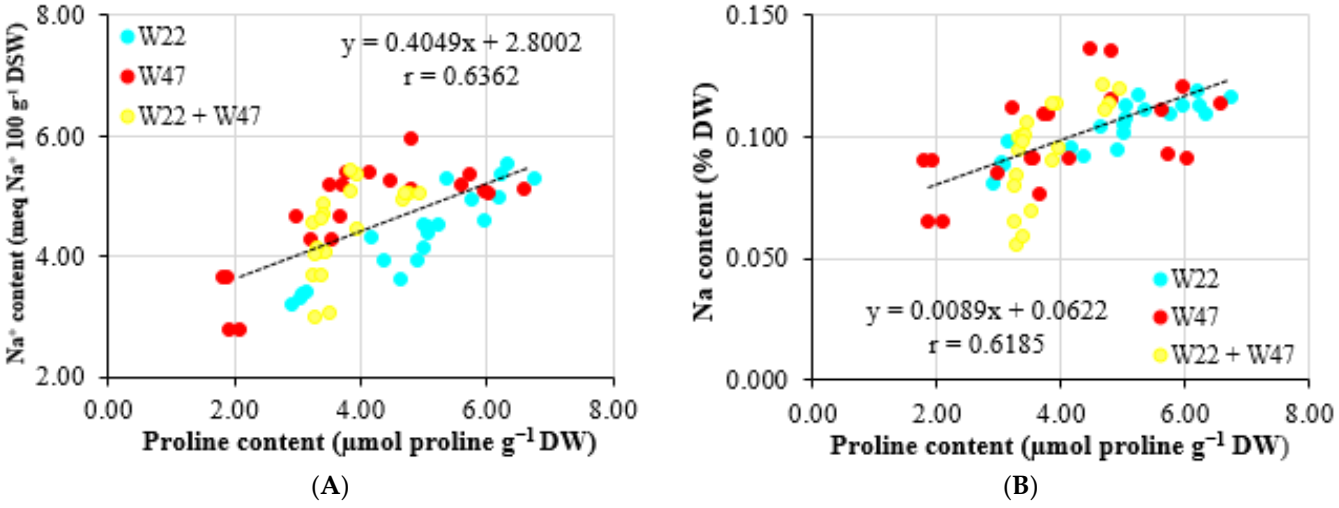

Figure 4. The linear correlations (**A**) between the soil Na^+ concentration and the leaves, stems proline content ($p < 0.05$); and (**B**) between the seed Na concentration and the leaf, stem proline content ($p < 0.05$).

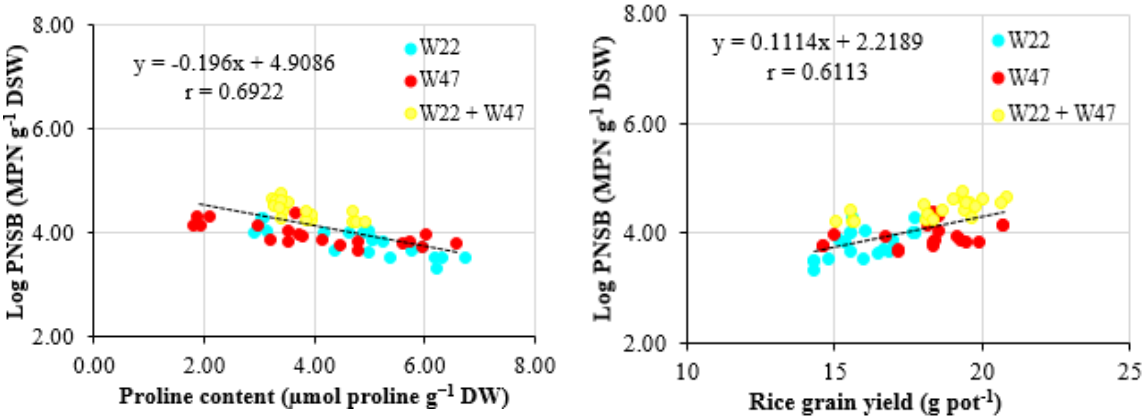

Figure 5. The linear correlations between the PNSB soil population and (**A**) the leaves, stems proline content ($p < 0.05$) and (**B**) the grain yield ($p < 0.05$). Note: W22: supplying with the single strain of *L. sphaeroides* W22; W47: supplying with the single strain of *L. sphaeroides* W47; W22 + W47: supplying with the mixture of the W22 and W47; DSW: dry soil weight; DW: dry weight.

Table 6. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria on the proline content, growth, yield components and grain yield in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Treatments	Proline Content	Plant Height	Panicle Length	Panicles Number Pot^{-1}	Seeds Number Panicle^{-1}	Filled Seeds Percentage	1000-Seed Weight	Grain Yield
	(µmol g^{-1} DW)	(cm)	(cm)	(Panicles)	(Seeds)	(%)	(g)	(g pot^{-1})
NI–NB	2.95 ± 0.066 ^{b}	82.0 ± 1.33 ^{a}	19.0 ± 0.82 ^{a}	13.8 ± 0.96 ^{a}	72.7 ± 1.44 ^{a}	82.7 ± 1.91 ^{a}	24.5 ± 0.28 ^{a}	17.4 ± 0.97 ^{a}
I–NB	4.39 ± 0.170 ^{a}	73.8 ± 0.39 ^{b}	16.2 ± 0.62 ^{c}	8.75 ± 0.50 ^{c}	61.2 ± 1.37 ^{b}	71.7 ± 1.14 ^{c}	24.1 ± 0.35 ^{a}	11.3 ± 1.09 ^{c}
I–B	3.02 ± 0.120 ^{b}	81.3 ± 0.42 ^{a}	17.8 ± 0.43 ^{b}	11.5 ± 0.58 ^{b}	75.4 ± 6.49 ^{a}	74.6 ± 0.90 ^{b}	24.3 ± 0.66 ^{a}	14.4 ± 0.53 ^{b}
F	*	*	*	*	*	*	ns	*
CV (%)	3.55	1.02	3.84	5.70	5.63	2.01	1.70	6.24

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance. NI–NB: no saline irrigation and no bacteria; I–IB: saline irrigation and no bacteria; I–B: saline irrigation and bacteria applied.

Plant growth and yield: The plant height and the number of seeds per panicle in the treatments without either the saline irrigation or the bacterial supplementation and the ones with both the saline irrigation and the mixture of *L. sphaeroides* W22 and W47 were better than those in the treatments applied with the saline irrigation but not supplied with bacteria. The highest results in the panicle length, panicle number per pot and filled seed percentage were recorded in the treatments without either the saline irrigation or bacteria. However, those in the treatments applied with the saline irrigation and the mixture of W22 and W47 were higher than those in the treatments applied with the saline irrigation but no bacteria. This resulted in the same trend in the grain yield, which peaked in the treatment without either the saline water or the bacteria (17.4 g pot^{-1}), and it showed dominance of the treatment applied with the saline irrigation and the mixture of W22 and W47 (14.4 g pot^{-1}) over the one applied with the saline irrigation but no bacteria (11.3 g pot^{-1}). However, the 1000-seed weight did not change under influences of both factors (Table 6).

3.2.2. Influences of the Mixture of δ-Aminolevulinic Acid Producing Purple Nonculture Bacteria on Chemical Characteristics of the Salt-Contaminated Soil

Supplying the mixture of the ALA-producing *L. sphaeroides* W22 and W47 contributed to an improvement in the soil aquatic pH and concentrations of NH_4^+, available P and exchangeable K, which were sequentially 6.16 and 18.3 mg NH_4^+ kg^{-1}, 24.1 mg P kg^{-1} and 8.65 meq K^+ 100 g^{-1} in comparison with the treatments without either the saline ir-

rigation or bacteria and the ones applied with the saline irrigation but without applying bacteria. Values of EC and exchangeable Na^+ content in the treatments applied with both the saline irrigation and the mixture of W22 and W47 were correspondingly 2.42 mS cm^{-1} and 3.90 meq Na^+ 100 g^{-1}, lower than those in the treatments applied with the saline irrigation but without applying bacteria, resulting in 2.73 mS cm^{-1} and 4.77 meq Na^+ 100 g^{-1}, and higher than those in the treatments without either the saline irrigation or bacteria, whose result was 1.73 mS cm^{-1} and 1.89 meq Na^+ 100 g^{-1}, respectively. In the meantime, the CEC fluctuated between 18.0 and 19.6 meq CEC 100 g^{-1}. In addition, the treatments supplied with the bacteria had a bacterial density of 5.76 MPN g^{-1} DSW, significantly higher than those in the treatments without applying bacteria, ranging from 1.96 to 2.08 MPN g^{-1} DSW (Table 7).

Table 7. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria on chemical properties of salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Treatments	pH_{H_2O}	EC	NH_4^+	$P_{available}$	CEC	K^+	Na^+	Log PNSB
		(mS cm^{-1})	(mg kg^{-1})	(mg kg^{-1})	(meq 100 g^{-1})			(MPN g^{-1})
NI–NB	5.71 ± 0.088 ^{c}	1.73 ± 0.051 ^{c}	12.9 ± 0.70 ^{b}	16.7 ± 0.67 ^{b}	18.0 ± 1.02 ^{a}	6.42 ± 0.15 ^{b}	1.89 ± 0.078 ^{c}	2.08 ± 0.11 ^{b}
I–NB	5.95 ± 0.079 ^{b}	2.73 ± 0.130 ^{a}	13.2 ± 0.17 ^{b}	16.6 ± 0.71 ^{b}	19.6 ± 0.24 ^{a}	6.41 ± 0.45 ^{b}	4.77 ± 0.190 ^{a}	1.96 ± 0.09 ^{b}
I–B	6.16 ± 0.083 ^{a}	2.42 ± 0.064 ^{b}	18.3 ± 0.44 ^{a}	24.1 ± 0.80 ^{a}	19.0 ± 1.36 ^{a}	8.65 ± 0.34 ^{a}	3.90 ± 0.088 ^{b}	5.76 ± 0.33 ^{a}
F	*	*	*	*	ns	*	*	*
CV (%)	1.57	3.91	3.14	4.33	4.73	5.28	3.85	4.78

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance. NI–NB: no saline irrigation and no bacteria; I–IB: saline irrigation and no bacteria; I–B: saline irrigation and bacteria applied.

3.2.3. Influences of the Mixture of the δ-Aminolevulinic Acid Producing Purple Non-sulfur Bacteria on the Biomass, N, P, K, Na Concentrations and Uptakes in Seeds and in Stem Leaves

N, P, K and Na concentrations: Contents of N, P and K in seeds in the treatments without either the saline irrigation or bacteria and in the ones applied with both the saline irrigation and the mixture of *L. sphaeroides* W22 and W47 were correspondingly 1.64, 0.397 and 0.240% and 1.56, 0.361 and 0.229%, higher than those in the treatments applied with the saline irrigation but no bacteria (1.22, 0.277 and 0.164%, respectively). Likewise, in the treatments without either the saline irrigation or bacteria and the ones applied with both the saline irrigation and the mixture of the W22 and W47, N and K concentrations in stems and leaves were higher than those in the treatments with the saline irrigation but no bacteria applied. On the contrary, the Na content in the treatments without either the saline irrigation or bacteria was 0.090% in seeds and 0.585% in stems and leaves. In the meantime, in the treatments applied with both the saline irrigation and the mixture of the W22 and W47, it was 0.072 and 0.662%, respectively, which was lower than those in the treatments applied with the saline irrigation but no bacteria applied (0.152 and 0.882%) (Table 8).

Table 8. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria on N, P, K and Na contents in seeds and in stems and leaves in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Treatments	Concentration (%)							
	N		P		K		Na	
	Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves
NI–NB	1.64 ± 0.018 ^{a}	1.51 ± 0.11 ^{a}	0.397 ± 0.012 ^{a}	0.229 ± 0.024 ^{a}	0.240 ± 0.008 ^{a}	1.75 ± 0.07 ^{a}	0.090 ± 0.054 ^{b}	0.585 ± 0.007 ^{c}
I–NB	1.22 ± 0.041 ^{c}	1.27 ± 0.07 ^{b}	0.277 ± 0.006 ^{b}	0.199 ± 0.024 ^{a}	0.164 ± 0.018 ^{b}	1.07 ± 0.11 ^{b}	0.152 ± 0.017 ^{a}	0.882 ± 0.013 ^{a}
I–B	1.56 ± 0.057 ^{b}	1.42 ± 0.11 ^{a}	0.361 ± 0.039 ^{a}	0.226 ± 0.025 ^{a}	0.229 ± 0.007 ^{a}	1.69 ± 0.13 ^{a}	0.072 ± 0.022 ^{b}	0.662 ± 0.007 ^{b}
P	*	*	*	ns	*	*	*	*
CV (%)	2.78	5.34	6.56	12.6	5.59	4.64	5.97	5.47

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance. NI–NB: no saline irrigation and no bacteria; I–IB: saline irrigation and no bacteria; I–B: saline irrigation and bacteria applied.

Dry biomass: The biomass in seeds and stems and leaves peaked in the treatments without either the saline irrigation or bacteria and was recorded as 13.6 and 16.5 g pot^{-1},

respectively. Subsequently, the treatments applied with both the saline irrigation and the mixture of *L. sphaeroides* W22 had W47 had dry seeds and stem and leaves biomass correspondingly at 10.9 and 13.3 g pot^{-1}, and the lowest result was found in the treatments applied with the saline irrigation but no bacteria, whose dry biomass was 8.68 g pot^{-1} in seeds and 10.8 g pot^{-1} in stems and leaves (Table 9).

Table 9. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria on the biomass and N, P, K and Na uptake in seeds and in stems and leaves in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition.

Factors	Biomass (g pot^{-1})		Uptake (g pot^{-1})							
			N		P		K		Na	
	Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves	Seeds	Stem, Leaves
NI–NB	13.6 ± 0.37 ^{a}	16.5 ± 0.21 ^{a}	0.224 ± 0.007 ^{a}	0.250 ± 0.018 ^{a}	0.054 ± 0.002 ^{a}	0.038 ± 0.004 ^{a}	0.033 ± 0.0005 ^{a}	0.289 ± 0.010 ^{a}	0.0122 ± 0.009 ^{a}	0.0967 ± 0.0010 ^{a}
I–NB	8.68 ± 0.19 ^{c}	10.8 ± 0.45 ^{c}	0.106 ± 0.003 ^{c}	0.137 ± 0.007 ^{c}	0.024 ± 0.001 ^{c}	0.021 ± 0.003 ^{c}	0.014 ± 0.0021 ^{c}	0.115 ± 0.013 ^{c}	0.0132 ± 0.005 ^{a}	0.0948 ± 0.0010 ^{a}
I–B	10.9 ± 0.17 ^{b}	13.3 ± 0.36 ^{b}	0.170 ± 0.008 ^{b}	0.189 ± 0.013 ^{b}	0.039 ± 0.004 ^{b}	0.030 ± 0.003 ^{b}	0.025 ± 0.0014 ^{b}	0.224 ± 0.013 ^{b}	0.0078 ± 0.003 ^{c}	0.0881 ± 0.0007 ^{b}
P	*	*	*	*	*	*	*	*	*	ns
CV (%)	2.22	3.06	3.23	5.02	5.72	13.4	5.89	3.91	7.27	6.28

Note: in the same column, numbers with different following letters were significantly different from each other at 5% (*); ns: no significance. NI–NB: no saline irrigation and no bacteria; I–IB: saline irrigation and no bacteria; I–B: saline irrigation and bacteria applied.

N, P, K and Na uptakes: N and P uptakes in seeds and in stems and leaves in the treatments applied with both the saline irrigation and bacteria were higher than those in the treatments applied with the saline irrigation but no bacteria applied. However, they were lower than those in the treatments without either the saline irrigation or bacteria. In detail, in the treatments applied with both the saline irrigation and the bacteria, N and P uptakes were 0.170 and 0.039 g pot^{-1} in seeds and 0.189 and 0.030 g pot^{-1} in stems and leaves, and higher than those in the treatments applied with the saline irrigation but no bacteria applied. Nevertheless, they are lower than those in the treatments without either the saline irrigation or bacteria, with 0.224 and 0.054 g pot^{-1} in seeds and 0.250 and 0.038 g pot^{-1} in stem leaves. In contrast, the Na uptake in the treatments without either the saline irrigation or bacteria and in the ones applied with the saline irrigation but no bacteria were higher than those in the treatments applied with both the saline irrigation and bacteria, i.e., with 0.0122–0.0132 compared with 0.0078 g pot^{-1} in seeds and 0.0948–0.0967 compared with 0.0881 g pot^{-1} in stems and leaves (Table 9). Thereby, the total N, P and K uptakes followed the same trend as their uptakes in stovers and are shown in Figure 6.

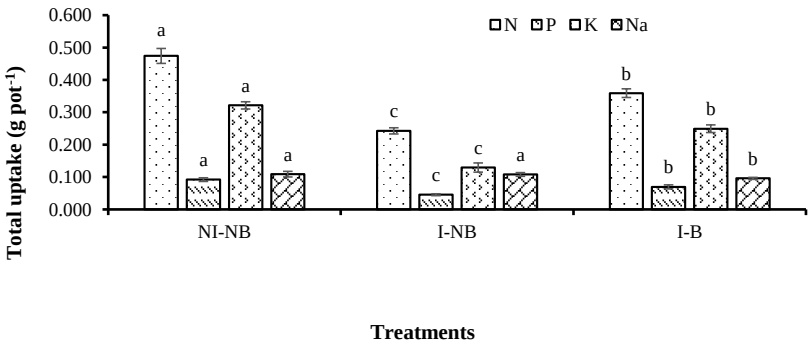

Figure 6. Influences of the δ-aminolevulinic acid producing purple non-sulfur bacteria and the saline irrigation on the total N, P, K and Na uptakes in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under the greenhouse condition. Note: NI–NB: no saline irrigation and no bacteria; I–IB: saline irrigation and no bacteria; I–B: saline irrigation and bacteria applied. The comparisons were between bars with the same pattern. The different lowercase letters indicate significant differences among treatments.

4. Discussion

Supplying the mixture of *L. sphaeroides* W22 and W47 was highly promising in enhancing a reduction in stress caused by salinity because the accumulation of the proline content within a plant appeared to be lower (Table 2). This was also stated by a lower proline content in the treatments supplied with the mixture of the W22 and W47 (3.02 μmol g^{-1} DW) compared with that in the control treatments without bacteria (4.39 μmol g^{-1} DW) (Table 6). When the frequency of applying the saline irrigation increased to three and four times, the proline content in the treatments supplied with the mixture of W22 and W47 was lower than that in the treatments supplied with each individual strain (Figure S1, Supplementary Data). Lutts et al. [23] claimed that, in rice, the accumulation of proline is a sign of being damaged rather than having the ability to tolerate salinity; that is, saline-tolerant plants accumulate less proline than saline-susceptible ones. According to Siddique et al. [24], proline is a beneficial amino acid, gathered in cultivars living in different stressful environments, including drought, heavy metals contamination and salinization. Table 2 indicates that, the more often applying the saline irrigation was, the higher the accumulated proline became. This can be interpreted that the proline production was meant to tolerate saline stress, which agreed with the study by Bhusan et al. [25], where the proline concentration increased in NaCl 25 mM treated conditions in comparison with the no-saline-treated ones. From the above, the bacteria in the current study were able to lessen the stress on rice plants suffered from the saline condition. In other words, the bacteria were suitable to aid plants that were grown in highly saline conditions. Therefore, a close correlation between the soil bacterial density and the proline content was observed, with a correlation coefficient of r = 0.6922 (Figure 5A). Moreover, ALA seemed to be the key to help plants fight against saline stress. ALA has been observed for a capacity in lower salt concentration in soil and induces salt resistance in crops [10,14]. The significance was that supplying the mixture of ALA-producing *L. sphaeroides* W22 and W47 highly negatively correlated to the Na^+ content in the soil (r = 0.6362) (Figure 4A) and in seeds (r = 0.6185) (Figure 4B).

Table 2 presents that the plant height and panicle length decreased as the frequency of applying the saline irrigation increased. In detail, the plant height and panicle length decreased in the treatments applied with the saline irrigation four times by 7.63 and 6.03% compared with those in the treatments without the saline irrigation. Previous studies demonstrated that saline concentrations in te water influenced the agronomic parameters more severely in the vegetative and reproductive stages than in the filling and ripening stages [26]. This result was consistent with the study by Gerona et al. [3], where the panicle length decreased from 27.9 to 17.3 cm in CSR28 rice in a condition of 10 dS m^{-1} salinity. Prodjinoto et al. [27] also stated that treatment with NaCl resulted in a reduction in plant height from 62.7 to 33.3 cm compared with that in the control treatment without NaCl. However, the percentage of differences in the current study was not as high as the two previous ones; this may be due to differences in rice cultivars (salt-sensitive ones versus the normal one in the study) or the salt concentrations. Salt-contaminated soil or water changes morphologies and modifies metabolism in rice by restricting their development [28–30]. However, supplying the ALA-producing bacteria contributed to a reduction in damages on rice growth caused by NaCl because ALA adjusts crucial physiological processes in plants cultivated in saline conditions [31]. In the current study, the plant height significantly increased in the treatments supplied with the mixture of W22 and W47 compared with those supplied with a single strain of either W22 or W47 (Table 2). Kantachote et al. [32] proved that plants in the treatments supplied with a biofertilizer containing bacteria of *R. palustris* TK103 were taller than those in the control treatment. In addition, the treatments supplied with bacteria of *R. palustris* TK103, PP803 or TN114 ameliorated the panicle length. However, supplying a single strain of either W22 or W47 resulted in an equivalent panicle length, fluctuating between 19.1 and 19.4 cm (Table 2). Noticeably, the panicles in the treatments supplied with the mixture of W22 and W47 (17.8 cm) were longer than those in the treatments without bacterial supplementa-

tion (16.2 cm) (Table 6). This revealed the role of W22 and W47 in the improvement in rice growth.

Applying the saline irrigation during the vegetative stage adversely affects the yield components and grain yield of plants [33]. To be more specific, rice is restricted in the biomass, plant height, number of panicles, 1000-seed weight and ratio of filled seeds [34,35]. The results in Table 2 indicate that the yield components, including the number of panicles per pot and the percentage of filled seeds, decreased as the frequency of applying the saline irrigation increased. In detail, the number of panicles per pot started to drop with applying the saline irrigation twice, while, with applying the saline irrigation once, the percentage of filled grain decreased compared with no applied saline irrigation. This result was in accordance with the studies by Hakim et al. and Thitisaksakul et al. [36,37], where the number of panicles per pot and the ratio of filled seeds were also decreased by saline irrigation at 4 dS m^{-1}, in comparison with the control treatment. Moreover, the percentage of filled seeds in the CSR28 rice under the 10 dS m^{-1} saline condition was lower than that in the control treatment, with 71% compared to 90.2% [3]. However, 1000-seed weight in the current study had not been significantly affected by the saline irrigation application (Table 2). Meanwhile, in the study by Bhusan et al. [25], the saline-susceptible rice (BRRI dhan 29) in treatments applied with 25 mM NaCl had lower seed weight per pot compared with that in the treatment without irrigated salinity. This could indicate that the OM5451 rice cultivar used in the current study was neither venerable nor tolerant to salinity. Moreover, during the supplementation of the bacterial mixture, the number of panicles per pot and the percentage of filled seeds increased in comparison with those in the treatments supplied with a single strain of either W22 or W47. This was in accordance with studies by Khuong et al. [11,13,38], where supplying bacteria of *Rhodopseudomonas* spp., also a PNSB, also contributed to improvements in the number of panicles per square meter and the number of filled seeds per panicle in comparison with the control treatment without bacteria. However, the number of seeds per panicle was equivalent to both individual and mixed supplementation (Table 2), while, in the study by Kantachote et al. [32], supplying bacteria of *R. palustris* PP803 enhanced the number of seeds per panicle in comparison with the control treatment. This could be because the KDML 105 rice variety used in this study has been identified to have salt-tolerance genotype [39]. The result in Table 6 also presents that supplying the mixture of W22 and W47 increased the number of panicles per pot, the number of seeds per panicle and the percentage of filled seeds by 23.9, 19.0 and 3.89%, respectively, resulting in a yield improvement.

Applying the saline irrigation 1–4 times at different vegetative and reproductive stages of rice reduced its yield by 5.76–25.9% in comparison with when no saline irrigation was applied. In particular, the lowest grain yield was recorded in the treatment applied with the saline irrigation four times (Table 2). This result was in accordance with the study by Baxter et al. [40], where grain yields in different rice varieties all declined at a dose of 2.4 dS m^{-1}. The bacteria of ALA-excreting *R. palustris* TN114 and PP803 have been determined to be capable of supporting plants to overcome saline stress [14,41]. The bacteria of *L. sphaeroides* have been proven to be capable of providing ALA [9,13,42] to enable rice to tolerate saline stress [43,44] for improving rice grain yield. To be more specific, the treatments applied with either the single strain of W47 or the mixture of the W22 and W47 resulted in higher yield than those in the treatments applied with the single strain of W22 (Table 2). This result was also consistent with the study by Khuong et al. [38], where supplying a bacterial mixture provided better yield in comparison with the control treatments and the ones applied with a bacterial strain individually. In the current study, both the single strain of W47 or the mixture of ALA-producing W22 and W47 supported rice growth in saline conditions. Kang et al. [45]; Naeem et al. [46]; Nunkaew et al. [14]; Wongkantrakorn et al. [44] proved that low ALA concentrations (0.01–30 mg L^{-1}) are still capable of stimulating plant development in order to minimize the damage caused by salinity. Under the condition of cultivating in saline paddy fields, the maximum ALA content synthesized by the *R. palustris* TK103, PP803 and P1 strains was in the range of 1.4–1.7 mg ALA L^{-1}, leading to higher growth

and yield [16]. The result of the current study also certified that the *Luteovulum* genus belonging to the PNSB group enhanced rice yield by 21.5% when the saline irrigation was applied four times at 5‰ concentration (Table 6). This result was also proven via the correlation between bacterial densities and rice grain yield, with the correlation coefficient (r = 0.6113) (Figure 5B).

The results in Table 3 illustrate that the salt-contaminated soil had pH_{H_2O} at roughly 5.99–6.16 and pH_{KCl} at 5.31–5.83, and it was evaluated as acidic according to the categorization of Shannon et al. [47]. The EC was in the range of 1.85–2.64 mS cm^{-1}, classified to be from moderate to high based on the evaluation of Shannon et al. [47]. The proline content started to rise when the EC reached 2.5 dS m^{-1} [40]. High pH and salinity were considered to be a remarkable obstacle for cultivating plants on saline soils, especially rice vulnerable to salinity [48]. The above result revealed that the pH_{H_2O} was slightly moderate acidity and pH_{KCl} was intermediate, which altogether allowed sustainable growth. However, the frequency of the saline irrigation and the supplementation of the bacteria had not influenced pH_{H_2O} and pH_{KCl} yet, while the EC rose when the saline irrigation was applied in comparison with the no saline irrigation. Nonetheless, the treatments supplied with either the mixture of *L. sphaeroides* W22 and W47 or the single of W47 contributed to lowering the EC, in comparison with supplying the single strain of W22 (Table 3), with a correlation coefficient between bacterial densities and Na^+ concentrations (r = 0.4810) (Figure 3A). In the same line, supplying the bacterial mixture increased the pH by 0.21 and decreased the EC by 0.31 mS cm^{-1} in comparison with the no bacteria case (Table 7).

The frequency of applying the saline water and the supplementation of the bacteria did not affect the concentration of total N and total P in the soil. However, supplying the bacterial mixture improved the concentrations of NH_4^+ and available P in comparison with those in supplying the bacteria individually. This was also expressed via a higher amount of NH_4^+ and available P by 5.10 and 7.50 mg kg^{-1} in the treatments supplied with the mixture of both the W22 and W47 in comparison with the case of supplying with no bacteria (Table 7). This can be explained regarding that the strains of W22 and W47 were capable of fixing N and solubilizing P [9]. Thus, the concentration of NH_4^+ and available P all rose. Moreover, this can be expressed through a decreased unavailable P content due to its being solubilized into available forms. The PNSB have been determined to be capable of fixing N and solubilizing P [13,15,38,49].

Although the CEC content was statistically unchanged between the supplementation of either the mixture of *L. sphaeroides* W22 and W47 or a single strain of them, the treatments supplied with either the mixture of the W22 and W47 or the single strain of W47 had lower Na^+ concentrations than those in the treatments supplied with the single strain of W47. Further, in the present study, the correlation relationship between bacterial densities and Na^+ concentrations was also determined, with r = 0.4810 (Figure 3A). Likewise, supplying the bacterial mixture helped to increase K^+ and Mg^{2+} concentrations in comparison with the case of using a single strain (Table 3). Thus, supplying the mixture of both the W22 and W47 helped to reduce Na^+ and enhance K^+ and Mg^{2+} in comparison with supplying a single strain of either W22 or W47 (Table 7). This can be interpreted that the bacteria of W22 and W47 secrete EPS to immobilize Na^+ [9]. The reason is that EPS contains functional groups, including –OH and -HOOC, which are able to bind with Na^+ ions [13,48,50]. Furthermore, the PNSB group has demonstrated K solubilizing capacity [51], leading to a higher K^+ concentration in the treatments with bacteria than those in the ones without bacteria (Table 7).

The result in Table 4 highlights that increasing the frequency of applying the saline irrigation changed N, P, K and Na concentrations in seeds. In detail, the N, P and Na contents increased in the treatments applied with the saline irrigation four times. However, applying the saline irrigation from two times above led to a reduction in the K content in seeds. Huang et al. [52] stated that two rice varieties, saline-tolerant rice (Jinyuan85) and saline-susceptible rice (Liaojing763), had decreased N contents in stems and leaves when being treated with NaCl. In addition, the Na content increased along with an increase in

the number of times of applying the saline irrigation. Meanwhile, supplying the ALA-producing PNSB increased N and P concentrations in seeds. To be more specific, supplying the mixture of both *L. sphaeroides* W22 and W47 had higher N and P contents in seeds in comparison with the treatments supplied with the bacteria individually. Figure 3B also illustrates the correlation between bacterial densities and accumulated Na contents (r = 0.5876); that is, the bacteria prevented Na from accumulating within the rice. In addition, supplying the mixture of the W22 and W47 enhanced N, P and K concentrations in stems, leaves and in seeds, except for the P content in stems and leaves. It also restricted the Na content in stems, leaves and seeds in comparison with the results in the treatments without bacteria (Table 8).

Applying the saline irrigation four times reduced the total N, P and K uptakes and maintained the Na uptake. However, supplying the mixture of *L. sphaeroides* W22 and W47 increased the total uptakes of N and P and reduced the total Na uptake in comparison with supplying a single strain of either W22 or W47 (Figure 2). Moreover, when the mixture of W22 and W47 was applied, the total N, P and K uptakes increased by 32.4, 34.7 and 48.1%, respectively, and the total Na uptake decreased by 11.2% compared with those in the treatments applied with the saline irrigation four times at 4‰ but no bacteria applied (Figure 2). This demonstrated that the bacteria of W22 and W47 had fixed N and solubilized P and K [13], resulting in an enhancement in the concentrations of NH_4^+, available P and exchangeable K (Table 7). They also increased in the N, P and K uptakes (Figure 2), resulting in higher growth and yield components of rice, and ultimately grain yield (Table 6). This result was consistent with the study by García Morales et al. [53], where mineral concentrations in the soil or in highly nutritious solution may lead to lower N uptake in rice.

5. Conclusions

The study has demonstrated that *L. sphaeroides* W22 and W47 were able to reduce not only the soil salinity but also the stress on rice caused by salinity and to increase the performance of rice plants cultivated in salt-cultivated soil. Supplying the mixture of the bacteria showed the best performance compared to the treatments with an individual strain. It contributed to ameliorating the plant height by 9.2%, the concentrations of NH_4^+, available P and exchanging K by 27.4, 31.0 and 25.9%, the total uptake of N, P and K by 32.4, 34.7 and 48.1%, respectively, and the grain yield by 27.4%, as well as limiting the concentrations of the proline and the exchanging Na by 31.3% and 18.3%, respectively, and the total Na uptake by 11.2% compared with those in the treatment applied with saline irrigation at 4‰ but no bacteria.

Moreover, the saline irrigation has been certified to negatively affect the growth of rice, which demonstrated the status occurring in the south of Vietnam. Applying the saline irrigation once resulted in a lower percentage of filled seeds, while applying it three times reduced the panicle length. When the saline irrigation was applied four times, the plant height and the number of panicles per pot decreased in comparison to those in the treatments without applying the saline irrigation. The grain yield also dropped under saline conditions (15.3–18.1 g pot^{-1}) compared with that in the treatment without applying the saline irrigation (18.9 g pot^{-1}).

Based on the remarkable performance of the mixture of *L. sphaeroides* W22 and W47, it is possible to perform as a biofertilizer in actual field conditions in salt-contaminated regions in Vietnam, where rice plants are heavily damaged.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051409/s1, Figure S1: Influences of δ-aminolevulinic acid producing purple non-sulfur bacteria and frequencies of applying saline irrigation on proline content in rice cultivated in salt-contaminated soil in Thanh Phu, Ben Tre under greenhouse condition.

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