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Research Article
Improvement of Green Soybean Growth and Yield in Alluvial Soil by Endophytic Nitrogen-Fixing Bacteria

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Abstract
Background and Objective: To obtain sustainable agriculture, a biological practice including endophytic microorganisms is the potential to replace traditional farming. The objective of this study was to determine the efficacy of endophytic bacteria on enhancing soil fertility, nitrogen uptake, growth and yield of green soybean. **Materials and Methods:** The experiment was conducted by a completely randomized design with nine treatments and four replications for each. The treatments included (i) 100% of recommendation of fertilizer formula, (ii) 85% N, (iii) 70% N, (iv) 55% N, (v) 85% N plus a mixture of *B. vietnamiensis* ASD-48 and ASD-21, (vi) 70% N plus a mixture of *B. vietnamiensis*, (vii) 55% N plus a mixture of *B. vietnamiensis*, (viii) 0% N plus a mixture of *B. vietnamiensis* and (ix) 0% N. **Results:** The results showed that plant height treated with the supplement of mixed nitrogen-fixing bacteria *B. vietnamiensis* ASD-48 and ASD-21 combined with 85% N obtained equally in treatment with 100% N. The number of pods and pod length were equal between treatment with 100% N and treatment with a mixture of ASD-48 and ASD-21 combined with 85% N. Supplement of a mixture of *B. vietnamiensis* improved soluble-P and NH_4^+, with 10.1 and 15.6%, respectively, at the same level of 85% N. They also enhanced N and K uptake, with an increase of 8.52 and 31.0%, respectively. **Conclusion:** The addition of an endophytic bacterial mixture of nitrogen-fixing *B. vietnamiensis* ASD-48 and ASD-21 enhanced soil fertility, N accumulation and yield.

Key words: Alluvial soil, dyke, *Burkholderia vietnamiensis*, green soybean yield, N_2-fixation, rhizospheric bacteria

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nitrogen is an important nutrient to promote plant growth. It is a component of essential organic compounds, including amino acids (proteins), nucleic acids (DNA and RNA), adenosine triphosphate and nicotinamide adenine dinucleotide in all living cells[1]. It is a very common element in the atmosphere, but unabundant in rocks and soils[2]. Therefore, a large amount of nitrogen fertilizer is applied to improve maximum crop productivity, but its effects on the environment and human health are harmful[3]. Thus, microbial supplement as biofertilizers appears as a way to reduce the amount of nitrogen in the fertilizers. A common type of bacteria used to fix nitrogen from the air is endophytic bacteria. According to Puri *et al.*[4], *Burkholderia vietnamiensis* is a potential candidate to produce biofertilizer for traditional rice varieties in Brazil. The species of *B. vietnamiensis* has been used to enhanced yield in rice[5,6] and sugarcane[7,8]. By the inoculation of this bacteria, the biomass increased by 20% in sugarcane in the field[7]. Besides, soybean is considered to be the main protein source and edible oil around the world. It acts as a source of proteins, vitamin A, C and E, unsaturated fats, thiamine and riboflavin[9]. Vegetable soybean contains many medicinal compounds, such as lactose-free fatty acids, vitamins, pyridoxine, biotin, and isoflavones[10]. It has more protein content than most crops and possesses 63% of high protein meals and 28% of total edible oil in the world[11]. Its maximum seed yield, nitrogen uptake, phosphate uptake and potassium uptake is at about 1,694, 140.39, 9.11 and 50.53 kg ha^{-1} in the alluvial soil of Bihar, respectively[12]. In Japan, the green soybean yield in 2004 was 560 g m^{-2}. The nitrogen level applied for green soybean is suggested to be 30 kg ha^{-1} [13]. Moreover, alluvial soils are common in South-East Asian countries, including Vietnam. Undeposited alluvial soils are alluvial soils that are bordered by dykes to prevent floods. It is usually found in big rivers bank sites. As mentioned previously, nitrogen content tremendously influences the growth of the plant, but the total N content in alluvial soils is 0.71 kg ha^{-1} [14]. Moreover, available N content in the soil declines during plantation[15]. Thus, the necessity of N supplementation has been raised in this study.

This work aimed to determine the impacts of endophytic bacteria (*Burkholderia vietnamiensis*) on soil fertility, growth and yield of green soybean on alluvial soil in dyke.

MATERIALS AND METHODS

Study area: The experiment was conducted at the greenhouse, College of Agriculture, Can Tho University. The experiment took place from September, 2019 to March, 2021.

Materials

Soil: Soil was collected from alluvial soil in dyke in Chau Phu district, An Giang province. Soil properties were showed in Table 1.

Source of bacteria: The bacteria used were the endophytic nitrogen-fixing *Burkholderia vietnamiensis* ASD-48 and ASD-21 isolated alluvial soil in dyke (our preliminary work).

Source of green soybean variety: Mikawashima green soybean was originated from Japan and stored at the College of Agriculture, Can Tho University.

Fertilizers: The fertilizers used in this study consisted of urea (46% N), superphosphate (16% P_2O_5) and potassium chloride (60% K_2O).

Methods

Experimental design: The experiment was conducted by a completely randomized design with nine treatments and four replicates each. A replicate corresponded to a pot with one plant. The treatments were described as follows (i)100% N of RFF, (ii) 85% Nof RFF, (iii) 70% Nof RFF, (iv) 55% N of RFF, (v) 85% N of RFF and mixture of *B. vietnamiensis* ASD-48 and ASD-21, (vi) 70% N of RFF and mixture of *B. vietnamiensis* ASD-48 and ASD-21, (vii) 55% N of RFF and mixture of *B. vietnamiensis* ASD-48 and ASD-21, (viii) 0% N of RFF and mixture of *B. vietnamiensis* ASD-48 and ASD-21 and (ix) 0% N of RFF, without mixture of *B. vietnamiensis.*

Fertilizer preparation: Soil for the green soybean plantation in this study was prepared with the components described in the above design. The inorganic fertilizer formula of recommendation was 60N-60P_2O_5-40K_2O (kg ha^{-1}).

Table 1: Properties of initial soil for green soybean cultivation

Soil properties	Value
pH_{H2O}	5.86±0.05
pH_{KCl}	4.87±0.06
OM (% C)	1.43±0.24
Total N (%)	0.16±0.01
NH_4+(mg NH_4+ kg^{-1})	35.0±7.79
NO_3^- (mg NO_3- kg^{-1})	22.1±0.89
Total P (%)	0.024±0.001
Available P (mg P kg^{-1})	32.1±0.48

Seeds preparation: Green soybean seeds were sterilized by submerging in ethanol 70% for 3 min and sodium hypochlorite 1% for 10 min in order, then rinsed with distilled water. After that, the seeds were incubated in dark conditions for a day to germinate. About 1,000 seeds germinated. Then, they were all soaked in sterile beakers with 63 mL of mix endophytic bacterial suspension of ASD-48 and ASD-21 with bacterial density at 10^8 cell mL^{-1}, and seeds soaked in only distilled water were negative controls. Then, beakers with seeds and the liquid bacterial mixture were covered with aluminium foil, shaken on the shaking machine at 60 rpm for 1 h and let dried under a laminar airflow for 1 h. After being dried, seeds with approximately 6.3×10^6 bacterial cells $seed^{-1}$ in density and seeds without bacterial supplement (the negative control) were planted in different pots.

Parameters of the survey

Soil analysis: A soil sample in each treatment were analyzed, based on the standard methods described by Sparks *et al.*[16]. In brief, in each soil sample, both soil pH_{KCl} and pH_{H2O} were extracted with 1 M KCl and deionized water with a ratio of 1:5. From the extracted solution, Electrical Conductivity (EC) was also measured. Total P was measured by the ascorbic acid method with a spectrometer at a wavelength of 880 nm after phosphate compounds were digested by a mixture of perchloric acid and nitric acid. Then, available P was calculated by the Bray II method. Organic nitrogen was degraded inorganic nitrogen compounds, which were quantified by the Kjeldahl method to achieve total nitrogen (N_{tot}) content. Then, available nitrogen (NH_4^+-N and NO_3^- -N) was extracted by KCl 2M and quantified NH_4^+ by salicylate and NO_3^- by a mixture of vanadium (III) chloride, sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride. Dichromate oxidation in sulfuric acid converted organic C to inorganic by thermal conductivity measurement. Organic matter content was measured by titration with ferrous sulfate heptahydrate.

The evaluation of this experiment was based on the growth, minerals uptake and yield parameters of green soybean. The measurement happened when the plants matured. Different replicates of treatment were included and represented by different pots.

Plant analysis: Stover and seed samples were collected when the mature plants and dried up at 65–70°C for 72 hrs. After that, the samples were separated into small pieces and grounded, then filtered through a 0.5 mm sieve to determine the total nitrogen and phosphate content contained in components of the plant. In short, total nitrogen and phosphate content were, respectively, measured by the Kjeldahl method and the UV-VIS method[17]. Then, the nitrogen and phosphate uptake in each part was calculated from these concentrations.

Agronomic parameters

Plant height (cm): The measurement was from the ground to the peak of the plant. Number of branches (branches), all the branches were counted for each pot. Stem diameter (cm), the measurement consisted of the plant stem top, middle and bottom. The values were transformed into an average value for each pot. Number of leaves (leaves/plant), Leaves were counted in each plant.

Yield components: Number of pods (pods/pot), All the pods were counted in each plant. Pod length (mm), measurement took place between both ends of each pod. Pod diameter (mm), the measurement was determined at the middle of each pod. Pod thickness (mm), the height of the pod was measured at the middle of each seed. Number of seeds (seeds/pod), all the seeds of each pod were counted. Seeds length (mm), measurement took place between both ends of each seed. Seed width (mm), the diameter was measured at the middle of each seed. Seed thickness (mm), the height of the seed was measured at the middle of each seed.

Green soybean yield (g pot^{-1}): The fresh weight of seeds from green soybean pods collected from plants were measured.

Biomass (g pot^{-1}): fresh weight of seed, sheath, stem leaves and root from each pot were measured on a scale. Then, the parts were dried out at the heat of 70°C for 72 hrs to achieve dry biomass weight.

Data analysis: The data shown in this study were inferred from means of four replications in each treatment. The means were processed by SPSS 13.0 software in subjecting to one-way analysis of variance (ANOVA). The significance of differences among means was calculated by Duncan's posthoc test at $p<0.05$.

RESULTS

Effects of potent endophytic nitrogen-fixing bacteria on soil fertility cultivated green soybean: By the addition of the endophytic nitrogen-fixing bacteria, several soil properties were significantly improved (Table 2). Although there were no significant differences in soil total nitrogen and

Table 2: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on soil properties at harvest of green soybean cultivated on in-dyke alluvial soil

Treatments	pH (1:2.5 H_2O)	pH (1:2.5 KCl)	EC (dS m^{-1})	Total P (%)	Available P (mg P kg^{-1})	Total N (%)	Available N (mg NH_4^+ kg^{-1})	Available N (mg NO_3^- kg^{-1})	OM (% C)
100% N-0MEB	5.85	4.85	0.25	0.243	48.1[bc]	0.179	41.9[a]	4.84[b]	1.94
85% N-0MEB	5.73	4.75	0.26	0.252	47.7[bc]	0.171	29.5[c]	4.75[b]	2.22
70% N-0MEB	5.78	4.79	0.27	0.252	47.1[c]	0.174	28.4[d]	4.57[b]	2.31
55% N-0MEB	5.83	4.85	0.22	0.252	47.0[d]	0.179	29.0[cd]	4.63[b]	2.43
85% N-MEB	5.92	4.86	0.26	0.243	52.5[a]	0.175	34.1[b]	6.94[a]	1.96
70% N-MEB	5.80	4.91	0.25	0.256	54.9[b]	0.179	29.2[c]	6.94[a]	2.05
55% N-MEB	5.91	4.83	0.25	0.259	53.0[bc]	0.185	29.9[c]	6.92[a]	1.99
0% N-MEB	5.89	4.81	0.24	0.248	46.0[bc]	0.196	31.2[cd]	6.85[a]	1.71
0% N-0MEB	5.94	4.74	0.25	0.256	44.2[d]	0.199	27.7[e]	4.80[b]	2.06
Significant difference	ns	ns	ns	ns	*	ns	*	*	ns
C.V. (%)	4.07	2.14	15.6	6.88	3.12	8.88	3.33	22.8	8.00

NS: Not significant difference, *: Significant difference at 5%, Numbers in each column with the same following letter are insignificantly different from each other, MEB: A mixture of the two endophytic bacteria *B. vietnamiensis* ASD-48 and ASD-21

phosphate concentrations, the available content of each largely varied among treatments. To be more specific, for available nitrogen content, there were noticeable drops along with the decrease of nitrogen levels applied to the soil. In treatments without bacteria supplement, the drop was from 41.9-28.4 mg NH_4^+ kg^{-1} and in the treatments with bacteria, the decline in NH_4^+ content was from 34.1-29.2 mg NH_4^+ kg^{-1}. But more remarkably, the treatments with bacteria outweighed the treatments without at the same nitrogen levels applied, e.g., the treatment with 85% N of RFF plus bacteria was statistically higher than the treatment with only 85% N of RFF in the amount of NH_4^+, 34.1 compared to 29.5 mg NH_4^+ kg^{-1}, respectively. For available phosphate content, the trends were mostly the same as available nitrogen content in the reduction in products along with the decrease of nitrogen levels and the superior of bacterial applied treatments compared to the treatments without bacteria. However, in this parameter, the treatment with 85% N of RFF plus bacteria (52.5 mg P kg^{-1}) was even significantly higher than the control treatment with 100% N of RFF (48.1 mg P kg^{-1}). These differences were considered to be caused by the bacteria while the treatment with only bacteria dominated the treatment with 0% N of RFF in available nitrogen content (31.2 compared to 27.7 mg NH_4^+ kg^{-1}) and soluble phosphate content (46.0 compared to 44.2 mg P kg^{-1}). Nevertheless, the other parameters, including soil pH values (both pH_{H2O} and pH_{KCl}), electrical conductivity and organic matter were not affected significantly (Table 2). Additionally, the bacterial density among treatments also remarkably changed (Fig. 1). The treatments with bacteria had higher bacterial density in comparison to the treatments without bacteria supplements. Moreover, among treatments without bacteria, the lower the nitrogen levels applied was, the higher the bacterial density was, i.e., the control treatment with 100% N of RFF was the lowest in bacterial density.

Effects of potent endophytic nitrogen-fixing bacteria on nutrient uptake of green soybean cultivated in alluvial soil in dyke: The N, P, K concentration of each component was recorded in Table 3. Moreover, according to Table 4, there was a significant improvement in the biomass accumulated in parts of green soybean by the addition of bacteria to biofertilizers and a remarkable decline in biomass values while the nitrogen levels reduced. At the same nitrogen level, biomass in the treatments with bacteria was always noticeably heavier than it in treatments without bacteria. For instance, in seed, along with the nitrogen levels of 85, 70 and 55% N, the biomass values in treatments with bacteria were 9.03, 8.59 and 8.01 g pot^{-1}, significantly higher than those in the treatments without bacteria supplement, which was 8.67, 8.07 and 7.18g pot^{-1}, respectively. Furthermore, surprisingly that the biomass of the sheath, the stem leaves and the roots in the treatment with 85% N of RFF plus bacteria was even statistically higher than it in the control treatment with 100% N of RFF. The overall uptake of minerals was also affected by nitrogen levels and bacterial supplements (Table 4). For nitrogen levels, the general trend was the decline along with the reduction in the amount of nitrogen applied in the biofertilizers. For bacteria supplements, the general trend was the superior of the treatments with bacteria, in comparison to the treatments without bacterial supplements in the uptake of nitrogen, phosphate and potassium at the equal nitrogen levels (Fig. 2-4). For a detailed evaluation of bacterial influence on minerals content and uptake in green soybean, the nitrogen, phosphate and potassium uptake in different parts of plants was recorded in Table 3 and 4. The nitrogen uptake in seed, sheath, stem, leaves and root had a common trend. The trend was the statistical equivalent between the treatments with bacteria supplementation and the treatments with 15% N levels higher. The difference between the

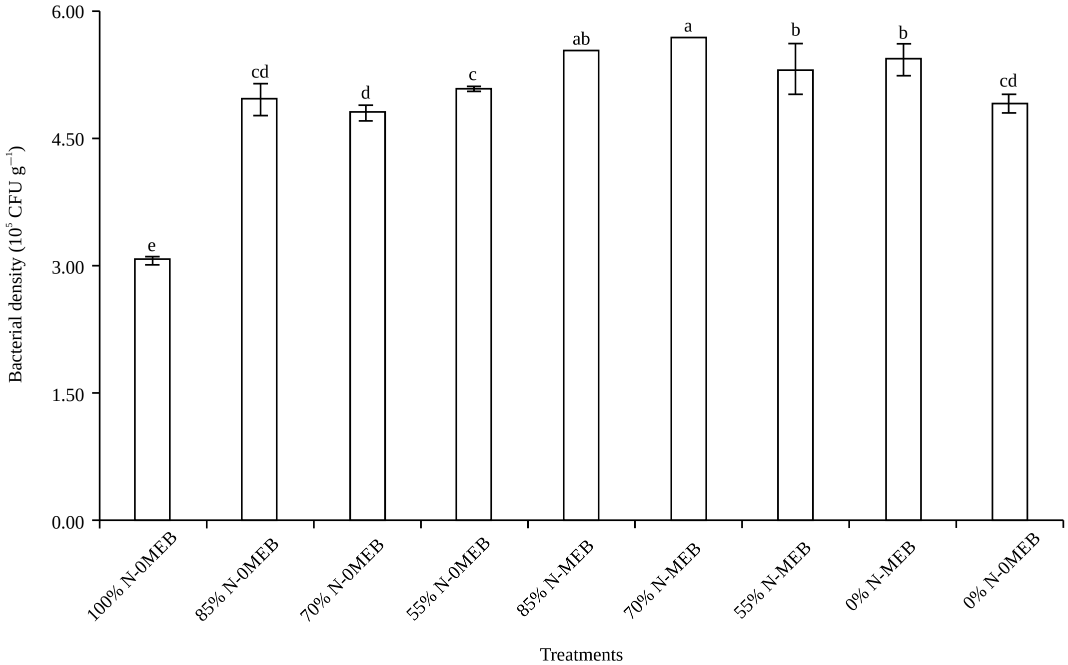

Fig. 1: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on bacterial density in-dyke alluvial soil for green soybean cultivation

Bars with the same lower letter were insignificantly different, Treatments were statistically different at 5%, MEB: A mixture of nitrogen-fixing bacteria *B. vietnamiensis* ASD-48 and ASD-21

Table 3: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on N, P and K contents of green soybean cultivated on in-dyke alluvial soil

	N content (%)				P content (%)				K content (%)			
Treatments	Seed	Sheath	Stem, leaf	Root	Seed	Sheath	Stem, leaf	Root	Seed	Sheath	Stem, leaf	Root
100% N-0MEB	6.32^{bcd}	0.77^{ab}	2.10^{a}	1.19^{a}	0.92	0.54^{c}	1.02^{ab}	0.41^{ab}	1.82^{b}	1.75	1.14	0.77^{ab}
85% N-0MEB	6.00^{bcd}	0.75^{ab}	1.85^{abc}	1.13^{ab}	0.91	0.54^{c}	0.86^{bc}	0.36^{bc}	1.77^{b}	1.73	1.04	0.71^{b}
70% N-0MEB	5.89^{cd}	0.60^{b}	1.47^{c}	0.90^{cde}	0.98	0.60^{bc}	0.76^{c}	0.35^{bc}	2.21^{a}	1.71	1.20	0.73^{ab}
55% N-0MEB	6.02^{bcd}	0.61^{b}	1.56^{bc}	0.77^{de}	0.93	0.61^{bc}	0.96^{ab}	0.31^{c}	2.21^{a}	1.82	1.06	0.59^{b}
85% N-MEB	6.11^{bcd}	0.80^{a}	1.83^{abc}	0.98^{bc}	0.93	0.54^{c}	0.90^{bc}	0.39^{abc}	2.05^{ab}	2.06	1.26	0.99^{ab}
70% N-MEB	6.64^{ab}	0.81^{a}	1.61^{bc}	0.99^{bc}	0.94	0.63^{bc}	0.97^{ab}	0.42^{ab}	2.19^{a}	1.85	1.27	1.08^{ab}
55% N-MEB	7.02^{a}	0.76^{ab}	1.69^{bc}	0.71^{e}	1.00	0.63^{bc}	1.05^{ab}	0.43^{ab}	2.15^{a}	2.01	1.27	0.83^{ab}
0% N-MEB	6.42^{abc}	0.84^{a}	1.89^{ab}	0.91^{cd}	0.91	0.77^{a}	1.12^{a}	0.47^{a}	1.90^{ab}	1.79	1.31	1.21^{a}
0% N-0MEB	5.68^{d}	0.76^{ab}	1.69^{bc}	0.84^{cde}	0.86	0.70^{ab}	0.97^{ab}	0.41^{ab}	1.78^{b}	1.63	1.12	1.12^{ab}
Significant difference	*	*	*	*	ns	*	*	*	*	ns	ns	*
C.V. (%)	7.80	16.8	16.2	14.4	6.82	16.5	14.0	14.0	11.6	13.9	7.94	21.9

NS: Not significant difference, *: Significant difference at 5%, Numbers in each column with the same following letter are insignificantly different from each other. MEB: A mixture of the two endophytic bacteria *B. vietnamiensis* ASD-48 and ASD-21

treatment with bacteria only and the treatment without biofertilizers was significant only at the nitrogen uptake in seed, where nitrogen uptake in the treatment with bacteria only was 392.2 mg pot^{-1}, statistically higher than 307.8 mg pot^{-1} in the other treatment. For phosphate uptake, except for in sheath where there was no significant difference among treatments, in the other parts of green soybean, the trend was similar to nitrogen uptake. However, for phosphate uptake in seed and stem, leaves, there was a significant dominance of the treatment with bacterial only to the treatment without any supplement. Last but not least, for potassium uptake, the trend did not change much from the other minerals uptake. Nevertheless, interestingly, the treatments with 85% N of RFF plus bacteria supplementation was significantly higher than the control treatment with 100% N of RFF for potassium uptake, 179.7 compared to 141.2 mg pot^{-1} in the sheath, 171.8 compared to 141.1 mg pot^{-1} in stem leaves and 15.74 compared to

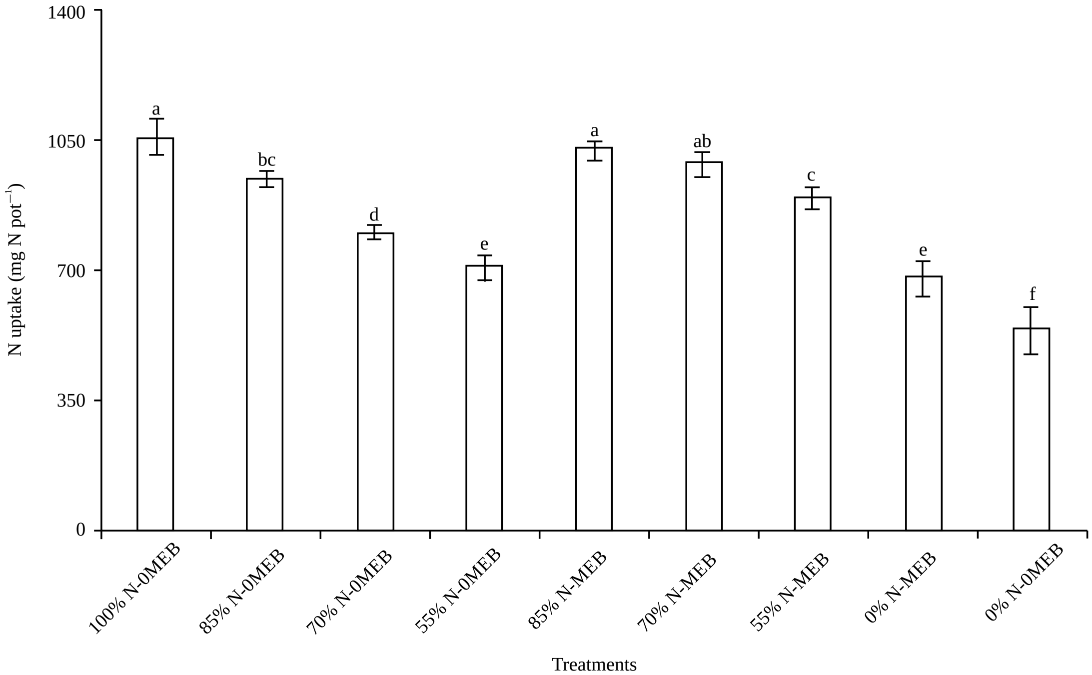

Fig. 2: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on the total nitrogen uptake of green soybean cultivated on in-dyke alluvial soil

Bars with the same lower letter were insignificantly different, Treatments were statistically different at 5%, MEB: A mixture of nitrogen-fixing bacteria *B. vietnamiensis* ASD-48 and ASD-21

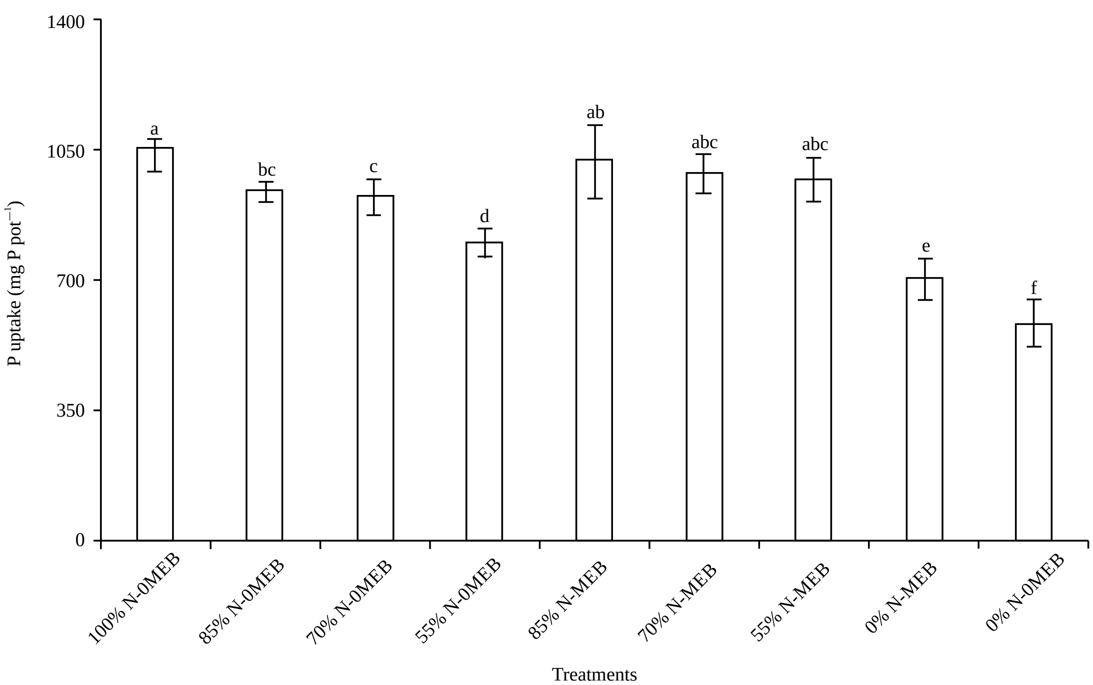

Fig. 3: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on the total phosphate uptake of green soybean cultivated on in-dyke alluvial soil

Bars with the same lower letter were insignificantly different, Treatments were statistically different at 5%, MEB: A mixture of nitrogen-fixing bacteria *B. vietnamiensis* ASD-48 and ASD-21

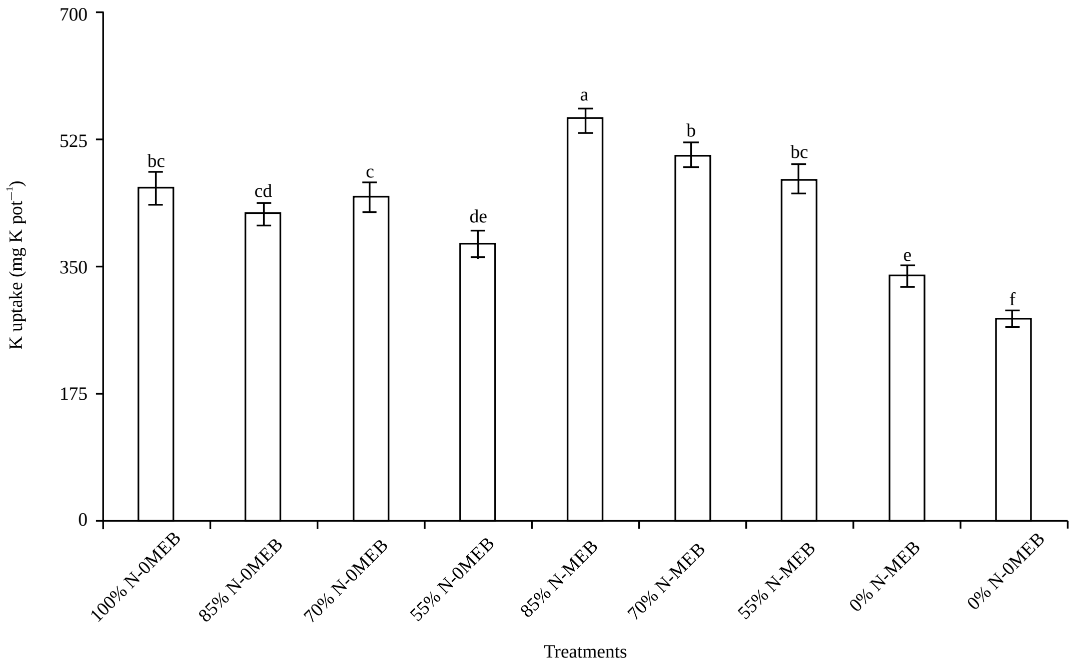

Fig. 4: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on the total potassium uptake of green soybean cultivated on in-dyke alluvial soil

Bars with the same lower letter were insignificantly different, Treatments were statistically different at 5%, MEB: A mixture of nitrogen-fixing bacteria *B. vietnamiensis* ASD-48 and ASD-21

Table 4: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on biomass and N, P and K uptakes of green soybean cultivated on in-dyke alluvial soil

					Uptake (mg pot^{-1})											
	Biomass (g pot^{-1})				N				P				K			
Treatments	Seed	Sheath	Stem, leaf	Root	Seed	Sheath	Stem, leaf	Root	Seed	Sheath	Stem, leaf	Root	Seed	Sheath	Stem, leaf	Root
100% N-0MEB	9.14^{a}	8.04^{b}	12.4^{b}	1.32^{b}	578.2^{a}	61.9^{ab}	261.5^{a}	158.0^{a}	845.4^{a}	43.4	125.8^{a}	5.42^{bc}	166.0^{ab}	141.2^{b}	141.1^{bc}	10.20^{b}
85% N-0MEB	8.67^{b}	7.77^{bc}	12.0^{bc}	1.27^{b}	520.9^{bc}	57.8^{ab}	224.2^{abc}	143.1^{ab}	787.7^{a}	41.8	103.8^{abc}	4.62^{c}	153.9^{b}	134.6^{bc}	124.7^{cd}	8.91^{b}
70% N-0MEB	8.07^{c}	7.11^{d}	11.4^{cd}	1.34^{b}	475.3^{cd}	42.4^{c}	167.0^{de}	118.1^{b}	787.7^{a}	42.7	87.3^{cd}	4.67^{c}	178.1^{b}	121.7^{cd}	137.3^{bc}	9.72^{b}
55% N-0MEB	7.18^{d}	6.30^{e}	9.8^{e}	1.12^{c}	432.3^{de}	38.2^{c}	152.1^{de}	86.3^{c}	664.4^{b}	38.7	94.0^{cd}	3.43^{d}	158.6^{ab}	114.3^{d}	103.1^{de}	6.62^{b}
85% N-MEB	9.03^{a}	8.71^{a}	13.6^{a}	1.59^{a}	551.8^{ab}	69.5^{a}	249.7^{ab}	155.6^{a}	840.5^{a}	47.0	123.2^{ab}	6.13^{ab}	185.7^{a}	179.7^{a}	171.8^{a}	15.74^{a}
70% N-MEB	8.59^{b}	7.63^{c}	12.5^{b}	1.55^{a}	570.9^{ab}	61.5^{ab}	202.3^{bcd}	154.3^{a}	805.7^{a}	47.9	121.4^{ab}	6.46^{a}	188.0^{a}	140.7^{b}	158.8^{ab}	16.80^{a}
55% N-MEB	8.01^{c}	7.43^{c}	11.1^{d}	1.26^{b}	562.4^{ab}	56.2^{b}	187.8^{cde}	89.3^{c}	797.9^{a}	47.2	116.5^{ab}	5.40^{bc}	172.0^{ab}	149.1^{b}	140.0^{bc}	10.48^{b}
0% N-MEB	6.10^{e}	5.11^{f}	9.2^{e}	0.78^{d}	392.2^{d}	43.0^{c}	175.0^{cde}	70.4^{cd}	555.8^{c}	39.7	102.7^{bc}	3.65^{d}	115.8^{c}	91.7^{e}	120.9^{cd}	9.39^{b}
0% N-0MEB	5.41^{f}	4.94^{f}	8.3^{f}	0.67^{e}	307.8^{e}	37.6^{c}	140.8^{e}	56.6^{d}	462.8^{d}	34.9	80.9^{d}	2.76^{d}	96.5^{c}	80.7^{e}	93.5^{e}	7.52^{b}
Significant difference	*	*	*	*	*	*	*	*	*	ns	*	*	*	*	*	*
C.V. (%)	2.93	3.60	4.49	6.59	7.94	17.5	18.7	17.1	7.26	16.9	14.7	15.7	13.7	9.39	14.7	26.1

NS: Not significant difference, *: Significant difference at 5%, Numbers in each column with the same following letter are insignificantly different from each other, MEB: A mixture of the two endophytic bacteria *B. vietnamiensis* ASD-48 and ASD-21

10.20 mg pot^{-1} in roots. Apart from plant biomass and minerals uptake, the concentration of the minerals did not show clear differences among treatments (Table 4).

Effects of potent endophytic nitrogen-fixing bacteria on growth and yield of green soybean cultivated in alluvial soil in dyke: By the addition of bacteria as biofertilizers, the growth of green soybean significantly improved in traits, including plant height, branches number, number of leaves and stem diameter, sharing the parameters shared a common trend. To be more specific, the trend was the noticeably better growth in the treatments added with bacteria in comparison to the treatments without bacteria supplements at the same level of nitrogen applied (Table 5). Especially, in the number of branches, the treatment with 85% N of RFF had a significantly higher value than the control treatment with 100% N of RFF, 6.0 branches compared to 5.0 branches, respectively. However, the significant difference between the treatment with bacterial only and the treatment without any fertilizers appeared only in plant height and leaves

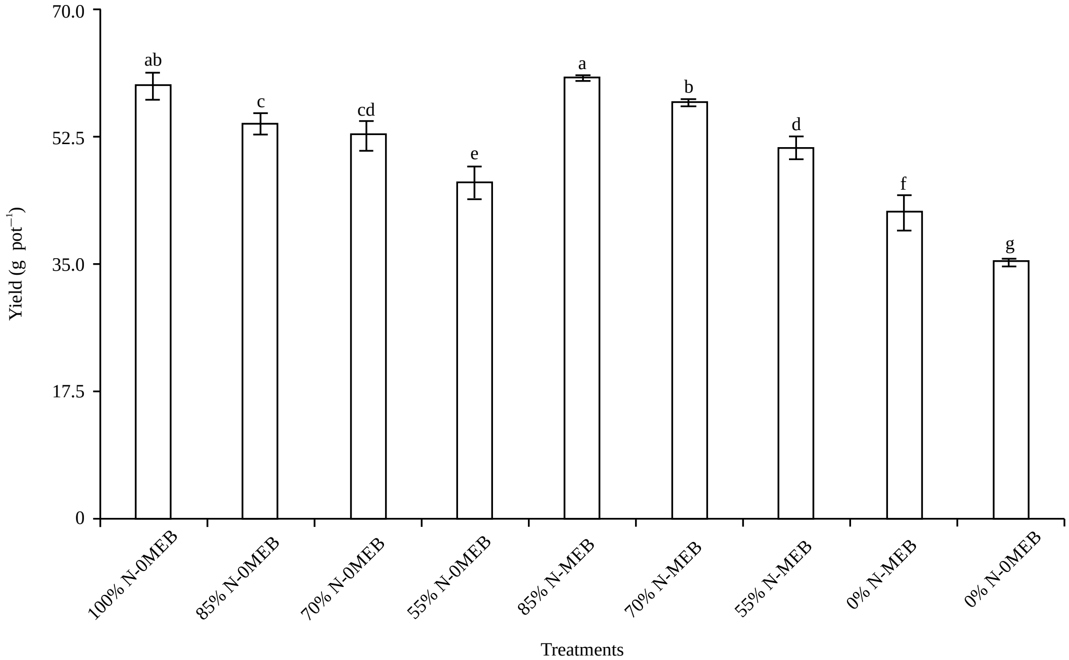

Fig. 5: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on yield of green soybean cultivated on in-dyke alluvial soil

Bars with the same lower letter were insignificantly different, Treatments were statistically different at 5%, MEB: A mixture of nitrogen-fixing bacteria *B. vietnamiensis* ASD-48 and ASD-21

Table 5: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on the growth of green soybean cultivated on in-dyke alluvial soil

Treatments	Plant height (cm)	Number of branches (branches)	Number of leaves (leaves)	Stem diameter (cm)
100% N-0MEB	51.5^{a}	5.0^{bc}	19.2^{ab}	4.25^{bc}
85% N-0MEB	47.3^{b}	5.0^{bc}	18.8^{ab}	4.22^{c}
70% N-0MEB	46.4^{bc}	5.2^{ab}	17.2^{d}	4.16^{c}
55% N-0MEB	45.8^{bc}	4.2^{c}	14.8^{ef}	4.17^{c}
85% N-MEB	50.0^{a}	6.0^{a}	19.6^{a}	4.43^{ab}
70% N-MEB	50.5^{a}	5.2^{ab}	18.4^{bc}	4.47^{a}
55% N-MEB	47.2^{b}	4.8^{bc}	17.4^{cd}	4.23^{bc}
0% N-MEB	45.0^{c}	4.4^{bc}	15.6^{e}	3.64^{d}
0% N-0MEB	43.4^{d}	4.6^{bc}	14.2^{f}	3.81^{d}
Significant difference	*	*	*	*
C.V. (%)	2.34	12.0	4.88	3.57

*: Significant difference at 5%, Numbers in each column with the same following letter are insignificantly different from each other, MEB: A mixture of the two endophytic bacteria *B. vietnamiensis* ASD-48 and ASD-21

number, where the treatment applied with bacteria only had 45.0 cm in height and 15.6 leaves, significantly higher than 43.4 cm in height and 14.2 leaves in the other treatments (Table 5). From the improvement in green soybean growth, the yield of the plants was also enhanced as well and shared the same pattern influenced by bacterial supplementation as the growth of the plants. Overall, the yield in the treatments with bacteria was significantly higher than it in the treatments without bacteria at the same level of nitrogen and the yield decreased along with the reduction in nitrogen level with the highest yield of about 60 g pot^{-1} in the treatment with 85% N of RFF plus bacteria and the control treatment with 100% N of RFF (Fig. 5). For yield components, the characteristics of pods and seeds were checked for changes in this experiment (Table 6). For pod characteristics, all the parameters were significantly changed due to the supplement of bacteria and the reduction of nitrogen levels in biofertilizers. They all followed the same trend in which all the values decreased along with the decline in nitrogen levels and the statistical equivalent between the treatment with 85% N of RFF plus bacteria and the control treatment with 100% N of RFF, e.g., 31.0 compared to 30.8 pods in the number of pods and 56.3 compared to 55.5 mm in the pod length (Fig. 6a). However, in the pod width, in both pod diameter and pod thickness, the

Fig. 6 (a-c): Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21
(a) Number of pods, (b) Length and width and (c) Plant height of green soybean in-dyke alluvial soil

Table 6: Influence of endophytic bacteria of *Burkholderia vietnamiensis* ASD-48 and ASD-21 on yield components of green soybean cultivated on in-dyke alluvial soil

Treatments	Number of pods (pods)	Pod length (mm)	Pod diameter (mm)	Pod thickness (mm)	Number of seeds per pod (seeds)	Seed length (mm)	Seed width (mm)	Seed thickness (mm)
100% N-0MEB	30.8^{a}	55.5^{ab}	12.2^{cd}	8.03^{cd}	2.68	12.7^{b}	9.06	6.43
85% N-0MEB	31.0^{a}	55.7^{ab}	12.3^{cd}	7.73^{de}	2.88	12.8^{b}	8.90	6.40
70% N-0MEB	27.6^{b}	54.7^{abc}	12.0^{d}	7.51^{e}	2.75	12.5^{b}	9.03	6.20
55% N-0MEB	25.8^{b}	52.7^{de}	12.1^{d}	7.44^{e}	2.63	12.7^{b}	8.97	6.38
85% N-MEB	31.0^{a}	56.3^{a}	13.5^{a}	8.74^{a}	2.59	13.5^{a}	9.14	6.86
70% N-MEB	30.0^{a}	55.6^{ab}	12.8^{bc}	8.62^{ab}	2.68	13.6^{a}	9.21	6.57
55% N-MEB	27.2^{b}	54.2^{bcd}	12.9^{ab}	8.55^{ab}	2.66	13.4^{a}	9.50	7.01
0% N-MEB	19.4^{c}	53.4^{cde}	12.4^{bcd}	8.20^{bc}	2.53	12.9^{b}	9.23	6.56
0% N-0MEB	17.0^{d}	52.3^{e}	11.4^{e}	7.57^{e}	2.50	11.7^{c}	8.97	6.31
Significant difference	*	*	*	*	ns	*	ns	ns
C.V. (%)	2.34	2.40	3.47	3.93	4.87	2.64	3.13	6.45

NS: Not significant difference, *: Significant difference at 5%, Numbers in each column with the same following letter are insignificantly different from each other, MEB: A mixture of the two endophytic bacteria *B. vietnamiensis* ASD-48 and ASD-21

values in the control treatment with 100% N of RFF was significantly smaller than them in the treatment with 85% N of RFF plus bacteria, i.e., 13.5 compared to 12.2 mm in diameter and 8.74 compared to 8.03 mm in thickness (Fig. 6b). Moreover, in the treatment with bacteria only, the values statistically outweighing them in the treatment without fertilizer belonged to the number of pods (19.4 compared to 17.0 pods), the pod diameter (12.4 compared to 11.4 mm) and pod thickness (8.2 compared to 7.57 mm). For the seed characteristics, only the seed length significantly changed under the influence of the treatments. In this parameter, the drop of nitrogen level did not cause any significant reduction in length, but the addition of bacteria did make a remarkable difference (Table 5). All of the treatments with both bacteria and nitrogen application had remarkably longer seed in comparison to the treatments with no bacteria at all. Furthermore, the treatment with bacteria only had a significantly higher value in pod length than the treatment without any fertilizers (Fig. 6c).

DISCUSSION

The effect of nitrogen levels applied to fertilizers on the growth and yield of green soybean plants has been studied. The quality of green soybean corresponds positively to the amount of nitrogen applied, i.e., the more nitrogen is, the higher yield gains[18-20]. Sharing the same result as the previous studies, the nitrogen levels in this study also helped in enhancing the growth and yield of the green soybean. The growth of the green soybean was significantly higher in the treatments with high nitrogen application, in comparison to it in treatments with lower nitrogen levels (Table 5, Fig. 6c). The plant was taller at the treatment with 100% N of RFF (51.5 cm), whereas the shortest one was in the treatment with 55% N of RFF (45.8 cm) (Fig. 6c). The number of leaves also decreased in the order of 19.2, 18.8, 17.2 and 14.8 leaves plant, along with the nitrogen decreasing order of 100, 85, 70 and 55% N (Table 5). However, the number of branches and stem diameter did not significantly change under the influence of nitrogen levels, which was similar to the result in the study of Nishioka and Okumura[18]. Moreover, the biomass and minerals uptake increased in parts of the plant as well at higher nitrogen levels. For instance, the seed biomass and nitrogen uptake went down from 9.14-7.18 g pot^{-1} and from 578.2-432.3 g pot^{-1}, respectively, during the drop from 100-55% N in nitrogen levels (Table 4). This was equivalent to the study of Salvagiotti *et al.*[21]. Following the increase in biomass, minerals uptake and growth of the plant, the yield of green soybean was also raised by nitrogen levels. The overall yield decreased from about 60-45 g pot^{-1} along with the reduction in nitrogen levels from 100-55% N applied (Fig. 4). The increase in plant height and yield also happened in the study of Ntambo *et al.*[20]. In detail, the yield components also decline in both quality and quantity when the nitrogen levels dropped from 100 to 55%, which consisted of pods number (30.8-25.8 pods) and pod size (55.5-52.7 mm in length) (Fig. 6b). Nevertheless, the effect of nitrogen levels on pod width and thickness and seeds number and structure were insignificant. The same trend took place in the treatments with bacteria when the nitrogen levels declined.

On the other hand, in this study, the treatments with bacteria did improve in many aspects, compared to the treatment without bacteria (Fig. 6a). The species used in this study has been investigated widely on many cultivars, such as sugarcane[7], rice[22] and turfgrass[23]. For instance, it can reduce 20% of chemical fertilizers for a bean[24] and 25% for rice[25] without any significant changes in yield. This result of this study showed a similar trend, where the endophytic bacteria *B. vietnamiensis* ASD-48 and ASD-21 altered the use of nitrogen fertilizers by more than 15%. To be more specific, in the treatments with the inoculation of the bacteria, almost all the survey parameters had abetter result in comparison to them in the treatment without bacteria at the same level of nitrogen. There were some exceptions, in which the result in both types of treatments was statistically equal. However, the treatment with 85% N of RFF plus bacteria even outweighed the control treatment with 100% N of RFF in branches number (Table 5), in pods width and thickness (Table 6) and biomass and potassium uptake of sheath, stem, leaves and root (Table 4). These strengthened the argument on the ability of bacteria in fertilizers to replace at least 15% of nitrogen fertilizer without changing the growth, yield and minerals uptake of the green soybean (*Glycine max* (L.) Merr.).

CONCLUSION

A mixture of the selected *Burkholderia vietnamiensis* ASD-48 and ASD-21 in fertilizers applied to green soybean enhanced soil fertility as the available nitrogen and phosphate content in soil and replaced 15% of nitrogen fertilizer formula as a recommendation without decreasing the growth, nutrients uptake, growth and yield of the green soybean. The endophytic nitrogen-fixing bacteria strains have a great promise to apply in alluvial soil to meet the sustainable agricultural system.

SIGNIFICANCE STATEMENT

This study discovers the *Burkholderia vietnamiensis* ASD-48 and ASD-21 that can be beneficial for soil fertility to improve green soybean yield. This study will help the farmers reduce 15% of chemical nitrogen fertilizer.

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