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Research Article

Enhancement of Green Soybean Growth and Yield in Alluvial Soil by Potent N_2-fixing Rhizospheric Bacteria

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Abstract

Background and Objective: Nitrogen fertilizer causes adverse effects on the environment aspect, so biological nitrogen sources should be applied in agriculture. The objective of the study was to evaluate the efficacy of N_2-fixing rhizospheric bacteria in improving soil fertility, nitrogen (N) uptake, growth and yield of green soybean (*Glycine max* (L.) Merr). **Materials and Methods:** The pot experiment was conducted with 9 treatments (i) 100% N of recommendation of fertilizer formula (RFF), (ii) 85% N, (iii) 70% N, (iv) 55% N, (v) 85% N and a mixture of *Enterobacter cloacae* ASD-07 and ASD-28, (vi) 70% N and a mixture of ASD-07 and ASD-28, (vii) 55% N and a mixture of ASD-07 and ASD-28, (viii) 0% N and ASD-07 and ASD-28, and (ix) 0% N, without bacteria, with four replications in in-dyke alluvial soil collected from Chau Phu District, An Giang Province. **Results:** The use of a mixture of the two N_2-fixing rhizospheric strains as biofertilizers at 85% N level improved NH_4^+ content and N uptake with an increase of 3.1 mg kg^{-1} and 95.8 mg pot^{-1}, respectively. They also produced higher values in growth including plant height, number of leaves, stem diameter and yield components including pod number, pod diameter, seed length, seed diameter and seed thickness. **Conclusion:** The application of a mixture of the two N_2-fixing rhizospheric strains resulted in replacing 15% N of RFF, but it still improved the yield.

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

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

INTRODUCTION

Nitrogen (N) is one of the major influences on the growth and yield of plants. N is rich in the atmosphere in the form of N_2 which plants cannot take. In soil, the major forms of available nitrogen that plants can take are usually NO_3^- (aerobic soils) and NH_4^+ (wetland)[1]. Moreover, a great loss in the amount of nitrogen in the soil can be caused by gases emission (NH_3 and N_2O) and leaching[2]. A large amount of nitrogen fertilizer is used to maximize crop productivity every year[3]. However, the improper use of nitrogen fertilizer can lead to negative effects on the environment such as soil and air pollution[4,5]. Therefore, biological approaches appear as an alternative method to solve these obstacles. Rhizospheric bacteria have been used as a nitrogen source to supply for plants to promote their growth and yield[6,7]. For instance, there have been studies of yield promoting rhizospheric bacteria applied on chili[8], maize[9], strawberry[10], cabbage[11] and potato[2]. Furthermore, green soybean is the main protein and edible source in the world. It contains many valuable nutrients[12,13]. The yield and minerals uptake of soybean is 1,694 kg ha^{-1} for maximum yield, 140.39 kg ha^{-1} for N uptake, 9.11 kg ha^{-1} for phosphate (P) uptake and 50.53 kg ha^{-1} for potassium (K) uptake[14]. The suggested N supplementation for soybean is 30 kg ha^{-1} [15]. In Vietnam and other South-East Asian countries, alluvial soils are abundant along the big river banks. However, to prevent floods, the lands are bordered by dykes, leading to the formation of undeposited alluvial soils. Moreover, the N content in alluvial soils is low, about 0.71 kg ha^{-1} [16]. This number is not sufficient for plant growth and may decline during the plant cycle[17]. Ultimately, the need for an efficient and environmental-friendly nitrogen source has been raised in this study.

This research aimed to determine the influence of a rhizosphere N_2-fixing bacteria, *Enterobacter cloacae* ASD-07 and ASD-28, on soil fertility, growth and yield of green soybean cultivated on in-dyke alluvial soil.

MATERIALS AND METHODS

Study area: The experiment was carried out at the greenhouse, College of Agriculture, Can Tho University. This experiment was conducted from September, 2019-March, 2021.

Materials

Soil: Alluvial soil in dyke in Chau Phu District, An Giang Province was chosen to be the experiment soil. Its properties were shown in Table 1.

Source of bacteria: The bacteria source in this study were the rhizospheric N_2-fixing *Enterobacter cloacae* ASD-07 and ASD-28 isolated from alluvial soil in dyke in An Giang province (our preliminary work).

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

Commercial fertilizers: The fertilizer used in this study was 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. Each treatment had four replications. Each replicate was represented by a pot with one plant. The treatments consisted of (i) 100% N of recommended fertilizer formula (RFF), (ii) 85% N of RFF, (iii) 70% N of RFF, (iv) 55% N of RFF, (v) 85% N of RFF and mixture of *E. cloacae* ASD-07 and ASD-28, (vi) 70% N of RFF and mixture of *E. cloacae* ASD-07 and ASD-28, (vii) 55% N of RFF and mixture of *E. cloacae* ASD-07 and ASD-28, (viii) 0% N of RFF and *E. cloacae* ASD-07 and ASD-28 and (ix) 0% N of RFF, without mixture of *E. cloacae* ASD-07 and ASD-28.

Soil and fertilizer preparation: Soil for the green soybean plantation in this study was prepared with the properties described in Table 1. The inorganic fertilizer was formulated as 60 N-60 P_2O_5-40 K_2O in the recommendation.

Seeds preparation: Green soybean seeds were sterilized by being submerged in ethanol 70% for 3 min and sodium hypochlorite 1% for 10 min and were rinsed with distilled water then. Next, the seeds were left germinated in dark incubation for 24 hrs. The germination happened in about 1,000 seeds. After that, the germinated seeds were soaked in sterile beakers with 63 mL of rhizospheric bacterial suspension of *Enterobacter cloacae* ASD-07 and ASD-28 at the density of 10^8 cell mL^{-1}. Seeds soaked in only distilled water served as a negative control. Then, beakers containing germinated seeds and liquid bacterial mixture were covered with aluminium foil and shaken at 60 rpm for 1 hr by a shaker. After that, they were left dried under a laminar airflow for an hour. Then, seeds containing approximately 6.3×10^6 bacterial cells per seed and seeds without bacterial supplementation (the negative control) were planted in different pots.

Table 1: Initial soil properties of alluvial soil in dyke in Chau Phu District, An Giang Province

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

Values are the mean of three replications and±standard deviation

Parameters of survey

Soil analysis: A soil sample at harvest crop in each treatment was analyzed, based on the standard methods described by Sparks *et al.*[18]. To be more specific, in each soil sample, both soil pH_{KCl} and pH_{H2O} were extracted by KCl 1 M or deionized water (1:5 ratio). Then, Electrical Conductivity (EC) was also measured from the previous extracted solution. Before total P was measured by the ascorbic acid method with a spectrometer at 880 nm wavelength, phosphate compounds were discomposed by perchloric acid and nitric acid mixture. From that, available P was calculated following the Bray II method. Total nitrogen content was measured by the Kjeldahl method based on inorganic nitrogen compounds degraded from organic nitrogen. After that, available nitrogen (NH_4^+-N and NO_3^--N) was extracted by KCl 2 M and quantified in NH_4^+ by salicylate and in NO_3^- by the mixture of vanadium (III) chloride, sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride. Organic carbon was converted into inorganic carbon by being oxidized by dichromate sulfuric acid with thermal conductivity measurement. Organic matter (OM) content was titrated by ferrous sulfate heptahydrate for quantification. Bacterial density was counted based on the most probable number method.

Plant analysis: The evaluation of this experiment consisted of the growth, the minerals uptake and the yield and its components of green soybean. The measurement took place at the physiological maturity stage for each pot. The parameters were checked as followed: Stover and seed samples were collected when the plants matured, then dried up at 65-70°C for 72 hrs. Next, the dried samples were cut into small pieces, grounded and sieved by a 0.5 mm net for the determination of total N and P content in parts of the plant. Briefly described, total N and P content quantified measured by the Kjeldahl method and the UV-VIS, atomic absorption spectroscopy methods[19], respectively. Finally, the uptake of these in each part was inferred from their concentrations.

Agronomic parameters: Plant height (cm): The length from the ground to the peak of the plant was measured at the physiological maturity stage. The number of branches (branches): All the branches were counted in each pot. Stem diameter (cm): The diameter values of the plant stem top, middle and bottom were measured. The values were then transformed into an average value for each pot. The number of leaves (leaves plant^{-1}): Leaves were counted in each pot.

Yield components: The number of pods (pods pot^{-1}): The pods were counted in each pot. Pod length (mm): A segment from both ends of each pod was measured. Pod diameter (mm): The diameter at the middle of each pod was measured. Pod thickness (mm): The height of the pod in the middle of each pod was measured. The number of seeds (seeds/pod): The seeds of each pod were counted. Seeds length (mm): A seed was measured from both ends. Seed width (mm): The diameter in the middle of each seed was measured. Seed thickness (mm): The height of each seed in the middle of it was measured.

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

Biomass (g pot^{-1}): Fresh weight of seeds, 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 means of four replications in each treatment. The means were conducted by SPSS 13.0 software in subjecting to a one-way Analysis of Variance (ANOVA). Significant differences among means were conducted by Duncan's posthoc test at $p<0.05$.

RESULTS

Effects of potent nitrogen-fixing rhizospheric bacteria on soil fertility cultivated soybean: Throughout a season of bacterial application, at the harvest stage, soil properties

Table 2: Influence of nitrogen-fixing rhizospheric bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the improvement of soil fertility on alluvial soil in dyke

Treatments	pH_{H2O} (1:2.5)	pH_{Kcl} (1:2.5)	Electrical conductivity ($dS\ m^{-1}$)	Total N (% N)	Available N (mg NH_4^+ kg^{-1})	Available N (mg NO_3^- kg^{-1})	Total P (% P)	Soluble P (mg P kg^{-1})	Organic matter (% C)
100% N-0MRB	5.89	4.85	0.27	0.26	42.6^{a}	4.92^{d}	0.23	49.7^{bc}	2.30
85% N-0MRB	5.81	4.76	0.27	0.25	36.0^{c}	5.07^{d}	0.24	48.2^{bc}	2.44
70% N-0MRB	5.84	4.76	0.29	0.24	34.0^{d}	4.89^{d}	0.24	47.5^{c}	2.43
55% N-0MRB	5.69	4.90	0.28	0.26	35.5^{cd}	4.88^{d}	0.24	45.5^{d}	2.66
85% N-MRB	6.02	4.89	0.29	0.26	39.1^{b}	6.76^{bc}	0.24	66.7^{a}	2.55
70% N-MRB	6.04	4.83	0.32	0.25	36.1^{c}	6.63^{c}	0.24	49.8^{b}	2.23
55% N-MRB	5.82	4.80	0.31	0.25	36.4^{c}	7.06^{ab}	0.24	48.0^{bc}	2.50
0% N-MRB	6.01	4.89	0.28	0.24	34.9^{cd}	7.33^{a}	0.24	48.6^{bc}	2.39
0% N-0MRB	6.00	4.80	0.28	0.27	30.9^{e}	5.04^{d}	0.24	44.2^{d}	2.39
Significant difference	NS	NS	NS	NS	*	*	NS	*	NS
CV (%)	4.08	2.17	15.6	1.00	3.12	4.90	12.5	3.33	8.02

Numbers with the same following letter were insignificantly different in the same column, NS: Not significantly different, *Significantly different at 5%, phosphate and potassium fertilizers were applied as a recommendation, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

changed noticeably (Table 2). Although there was no statistical change in the total content of nitrogen and phosphate in soil, the available concentration significantly varied among treatments. For available nitrogen content, there were remarkable differences among treatments in the amount of NH_4^+ and NO_3^- in soil. The NH_4^+ concentration in soil reduced along with the decline in nitrogen levels applied to the fertilizer from 42.6 >36.0>34.0~35.5>30.9 mg NH_4^+ kg^{-1} in the control treatment with 100% N of RFF and treatments with 85, 70, 55 and 0% N of RFF, respectively (Table 2). Additionally, these values in the treatments with bacteria *E. cloacae* ASD-07 and ASD-28 were significantly higher than that of in the treatment without bacteria at the same nitrogen levels, including 85% N (39.1 compared to 36.0 mg NH_4^+ kg^{-1}), 70% N (36.1 compared to 34.0 mg NH_4^+ kg^{-1}) and 0% N (34.9 compared to 30.9 mg NH_4^+ kg^{-1}). For another available nitrogen concentration, NO_3^- the content was not statistically changed by the influence of inorganic nitrogen fertilizer application, ranging from 4.88-5.07 mg NO_3^- kg^{-1} in treatments without bacteria (including the treatment with no fertilizer applied), but was significantly affected by the bacteria application. With the supplementation of bacteria, the NO_3^- concentration in soil rose remarkably and was higher than every treatment with no bacterial supplement. Surprisingly, in the treatment with bacteria, the nitrate concentrations had a rising trend following the decrease in nitrogen levels in the fertilizer. In detail, the value peaked at the treatment with bacteria only (7.33 mg NO_3^- kg^{-1}) and bottomed at the treatment with 85% N of RFF (6.76 mg NO_3^- kg^{-1}) and the treatment with 70% N of RFF (6.63 mg NO_3^- kg^{-1}). For available phosphate content, the reduction of values happened following the decline of nitrogen levels in both treatments with and without bacteria. However, while applying bacteria of *E. cloacae* ASD-07 and ASD-28, the soluble phosphate content was raised in comparison with that of treatments without bacteria supplements at the same nitrogen levels. Interestingly, the soluble phosphate concentration in the treatment with 85% N of RFF plus bacteria was even statistically higher than that in the control treatment with 100% N of RFF, 66.7 mg P kg^{-1} compared to 49.7 mg P kg^{-1}, respectively (Table 2). In addition, the bacteria population certainly received an impact from bacteria application, i.e most of the treatments with bacteria possessed remarkably higher bacterial density than the treatments without bacteria did, except for the treatment with 85% N of RFF plus bacteria, whose number was statistically equivalent to the value in the treatment with 55% N of RFF. Impressively, levels the lower nitrogen application was, the higher bacterial density was, in another word, the bacterial densities increased along with the reduction in nitrogen fertilizer level (Fig. 1). Nevertheless, both nitrogen levels and bacteria supplementation did not have a significant influence on other parameters, including pH_{H2O}, pH_{KCl}, EC and OM.

Effects of potent nitrogen-fixing rhizospheric bacteria on N uptake of green soybean cultivated in alluvial soil in dyke: Based on the biomass (Table 3) and concentrations in their components (Table 4), their uptakes were indicated in Table 5, the minerals uptake among stovers of green soybean was severely affected by the application of nitrogen fertilizer and supplementation of bacteria of *E. cloacae* ASD-07 and ASD-28. Overall, the pattern was the reduction of the amount of minerals uptake following nitrogen level decline and the higher uptake content in treatments with bacteria in comparison to treatments without bacteria at the same nitrogen levels. For total N uptake, the trends were shown in Fig. 2. Furthermore, for the total uptake of P and K, the treatment with 85% N of RFF plus bacteria statistically outweighed the control treatment with 100% N of RFF (Fig. 3 and Fig. 4). For detailed evaluation, minerals uptake in stovers

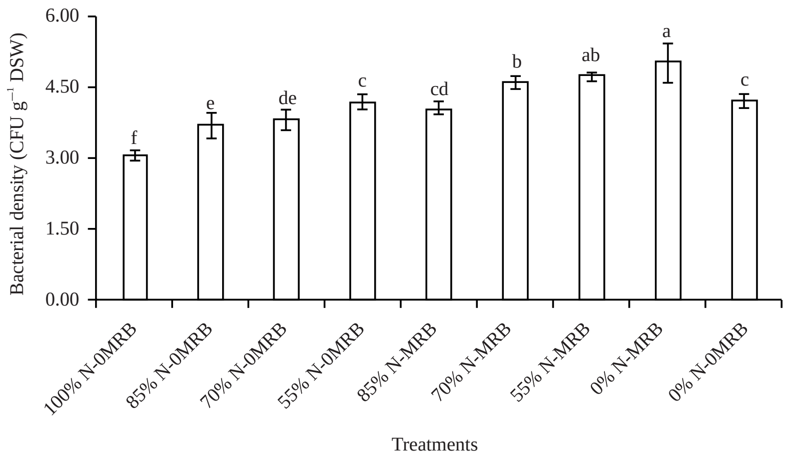

Fig. 1: Influence of nitrogen-fixing rhizospheric bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on bacterial density in alluvial soil in dyke

Columns with the same lower letter were insignificantly different, treatments were statistically different at 5%, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28 and DSW: Dry soil weight

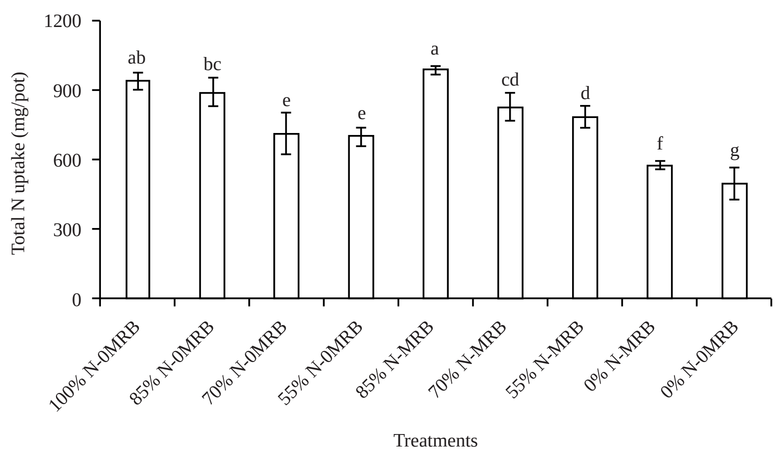

Fig. 2: Influence of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the total N uptake in green soybean cultivated on alluvial soil in dyke

Columns with the same lower letter were insignificantly different, treatments were statistically different at 5%, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

of green soybean was measured. In roots, the minerals uptake had the same trend as the total minerals uptake. The drops, in the order of nitrogen levels reduction from 100% N, 85% N, 70-55% N, were from 14.9~14.7>10.0>8.22 mg pot^{-1} for N uptake, from 5.54~5.26~4.68>3.34 mg pot^{-1} for P uptake and from 9.94~9.12~8.6>6.4 mg pot^{-1} for P uptake, the differences were significant at 5%. It was interesting that P and K uptake in roots was statistically higher in the treatment with 85% N of RFF plus bacteria than in the control treatment with 100% N of RFF. However, the difference between the treatment with bacteria only and the treatment without any supplement was insignificant in P uptake in the root. In stem leaves, the trend was similar to in roots. However, surprisingly, in P uptake in stem leaves, the treatment with 85% N of RFF (151.6 mg pot^{-1}) statistically outweighed the control treatment with 100% N of RFF (117.5 mg pot^{-1}), but the treatment with bacteria only was insignificantly different from the treatment with no fertilization. In sheath and seeds, the minerals uptake followed the same pattern. However, the treatment with 0% N of RFF plus bacteria significantly outweighed the treatment with 0% N of RFF plus no bacteria only in N and P uptake in seeds (Table 5). The biomass forming in stovers was also influenced by treatments. Overall, the common trend was the reduction in biomass of roots, stem leaves, sheath and seeds along

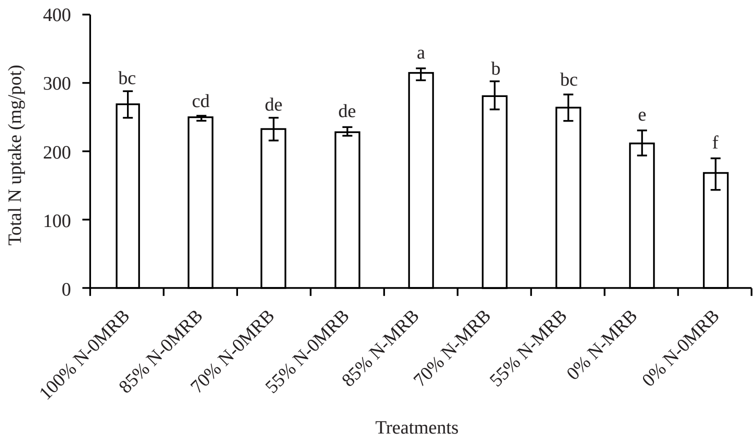

Fig. 3: Influence of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the total P uptake in green soybean cultivated on alluvial soil in dyke

Columns with the same lower letter were insignificantly different, treatments were statistically different at 5%, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

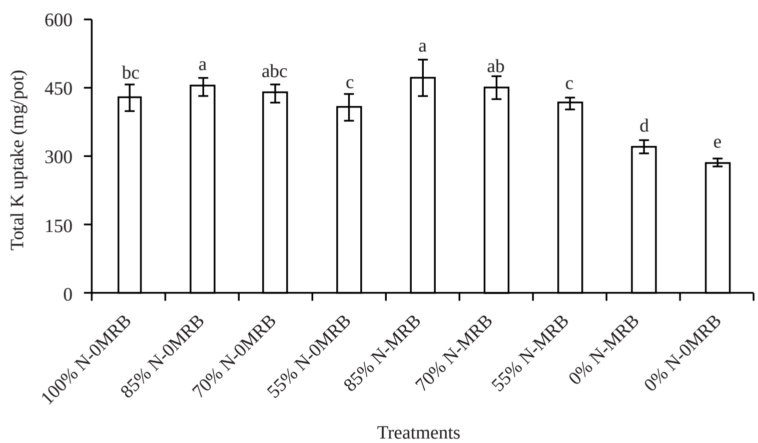

Fig. 4: Influence of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the total K uptake in green soybean cultivated on alluvial soil in dyke

Columns with the same lower letter were insignificantly different, treatments were statistically different at 5%, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

with the decline in nitrogen levels applied to fertilizer and the significant superiority of the treatments supplied with *E. cloacae* ASD-07 and ASD-28 to the treatments without them (Table 3). For further evaluation, the decrease in biomass from the control treatment with 100% N of RFF to the treatment with 55% N of RFF was from 1.37-1.06 g pot^{-1} in roots, from 11.9-10.2 g pot^{-1} in stem leaves, from 8.26-6.93 g pot^{-1} in the sheath and from 9.60-8.02 g pot^{-1} in seeds. Although the bacterial treatments were statistically higher than the non-bacterial treatments in biomass of stovers, the treatment with bacteria significantly differed from the treatment with no fertilization only in roots (0.79 compared to 0.65 g pot^{-1}) and in seeds (6.02 compared to 5.60 g pot^{-1}) (Table 3). Nevertheless, in roots and seeds, there is significantly greater biomass in the bacterial treatment with 85% N of RFF (1.60 g pot^{-1}) in comparison to the biomass in the control treatment with 100% N of RFF (1.37 g pot^{-1}). Unlike minerals uptake and biomass, the only N concentration in stovers of green soybean showed clear trends in significant differences among treatments, whereas P and K concentrations had an unclear influence on nitrogen levels and bacteria supplementation. For nitrogen content in stovers, remarkable differences appeared in roots and stem leaves, where the content reduction along nitrogen levels decline from 100% N of RFF to 55% N of RFF was from 1.10 to 0.77% and from 2.18 to 1.62%, respectively. Additionally, for nitrogen concentration

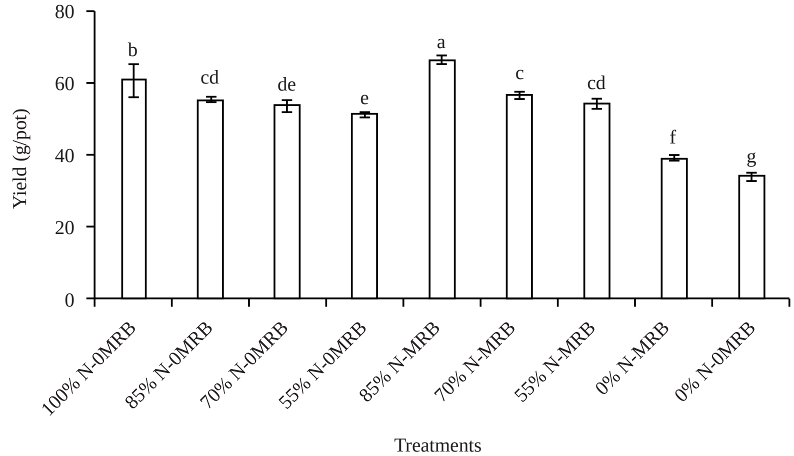

Fig. 5: Influence of nitrogen-fixing rhizospheric bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on yield of green soybean cultivated on alluvial soil in dyke

Columns with the same lower letter were insignificantly different, treatments were statistically different at 5%, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

Table 3: Influence of nitrogen-fixing rhizosphere bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on dry biomass of green soybean cultivated on alluvial soil in dyke

	(g pot^{-1})			
Treatments	Roots	Stem leaves	Sheath	Seeds
100% N-0MRB	1.37^{b}	11.9^{b}	8.26^{ab}	9.60^{a}
85% N-0MRB	1.39^{b}	11.3^{c}	8.34^{ab}	9.30^{a}
70% N-0MRB	1.23^{c}	11.4^{bc}	7.48^{cd}	8.12^{b}
55% N-0MRB	1.06^{c}	10.2^{d}	6.93^{d}	8.02^{b}
85% N-MRB	1.60^{a}	13.9^{a}	8.51^{a}	9.50^{a}
70% N-MRB	1.35^{b}	11.4^{bc}	7.84^{bc}	8.20^{b}
55% N-MRB	1.02^{d}	11.4^{bc}	7.54^{cd}	7.86^{b}
0% N-MRB	0.79^{e}	8.50^{e}	5.35^{e}	6.02^{c}
0% N-0MRB	0.65^{f}	8.2^{e}	4.94^{e}	5.60^{d}
Significant difference	*	*	*	*
CV (%)	6.09	3.71	6.37	3.72

Numbers with the same following letter were insignificantly different in the same column, NS: Not significantly different, *Significantly different at 5%, phosphate and potassium fertilizers were applied as a recommendation, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

in stem leaves, the value of the treatment with bacteria only was 1.75%, significantly different from the value of 1.61% in the treatment without fertilization (Table 4).

Effects of potent nitrogen-fixing rhizospheric bacteria on growth and yield of green soybean cultivated in alluvial soil in dyke: The green soybean growth was largely improved by the addition of bacterial strains *E. cloacae* ASD-07 and ASD-28 and reduced by nitrogen levels among treatments. Both factors, bacteria supplementation and nitrogen fertilization contributed to the development of green soybean in traits, including plant height and leaves number (Table 6). For instance, along with the decrease of nitrogen levels from 100-55% N, the height reduced in order 51.4-45.2 cm and leaves were less from 18.6-15.4 leaves. At the nitrogen level of 85% N of RFF, the plants in the treatment with bacteria application (50.8 cm and 19.4 leaves) were significantly taller and leafier than those in the treatment without (48.0 and 17.6 cm) and statistically equal to the control treatment with 100% N of RFF (51.4 cm and 18.6 leaves). Moreover, in these two characteristics, the treatment with bacteria only had a plant height of 46.8 cm and a leave number of 15.2 leaves, significantly different from the treatment with no application whose values were 45.6 cm and 13.4 leaves, respectively. However, the effect of nitrogen fertilization and bacteria supplementation on the traits of branch number and stem diameter among treatments was neither insignificant nor unclear. From the improvement in plant growth, the yield and its components were enhanced as well. Nevertheless, the influences of inorganic nitrogen fertilizer and bacteria on yield components were different in different traits. The number of pods was affected by both bacteria addition and inorganic

Table 4: Influence of nitrogen-fixing rhizospheric bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the N, P and K content in roots, stem leaves, sheath and seeds of green soybean plants cultivated on alluvial soils in dyke

	N (%)				P (%)				K (%)			
Treatments	Roots	Stem leaves	Sheath	Seeds	Roots	Stem leaves	Sheath	Seeds	Roots	Stem leaves	Sheath	Seeds
100% N-0MRB	1.10^{a}	2.18^{a}	0.73^{a}	6.29	0.40	1.00^{ab}	0.56^{b}	1.02^{a}	0.72	1.02	1.49^{c}	1.79
85% N-0MRB	1.06^{a}	2.13^{a}	0.76^{a}	6.15	0.38	0.89^{b}	0.56^{b}	1.02^{a}	0.66	1.01	1.90^{ab}	1.81
70% N-0MRB	0.81^{cd}	1.58^{c}	0.60^{b}	5.89	0.38	0.85^{b}	0.61^{b}	1.04^{a}	0.70	1.01	2.21^{a}	1.81
55% N-0MRB	0.77^{cd}	1.62^{c}	0.61^{b}	6.02	0.31	0.99^{ab}	0.61^{b}	1.02^{a}	0.61	0.95	2.21^{a}	1.88
85% N-MRB	0.93^{b}	2.10^{a}	0.71^{ab}	6.52	0.42	1.10^{a}	0.64^{b}	1.04^{a}	0.76	0.93	1.81^{b}	1.81
70% N-MRB	0.86^{bc}	1.99^{ab}	0.68^{ab}	6.51	0.39	1.10^{a}	0.67^{b}	1.20^{a}	0.80	1.07	2.00^{ab}	1.94
55% N-MRB	0.74^{de}	1.95^{ab}	0.71^{ab}	6.35	0.38	1.10^{a}	0.67^{b}	1.07^{a}	0.78	0.95	2.03^{ab}	1.86
0% N-MRB	0.65^{ef}	1.75^{b}	0.60^{b}	6.47	0.43	1.11^{a}	0.84^{a}	1.14^{a}	0.77	1.09	2.10^{ab}	1.79
0% N-0MRB	0.58^{f}	1.61^{c}	0.60^{b}	5.95	0.40	0.93^{ab}	0.81^{a}	0.85^{c}	0.60	1.05	1.95^{ab}	1.77
Significant difference	*	*	*	NS	NS	*	*	*	NS	NS	*	NS
CV (%)	8.49	11.3	11.6	6.55	16.3	12.6	14.3	10.5	17.2	13.7	11.0	5.73

Numbers with the same following letter were insignificantly different in the same column, NS: Not significantly different, *Significantly different at 5%, phosphate and potassium fertilizers were applied as a recommendation, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

Table 5: Influence of nitrogen-fixing rhizospheric bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the nutrients uptake in roots, stem leaves, sheath and seeds of green soybean cultivated on alluvial soil in dyke

	Uptake (mg pot^{-1})											
	N				P				K			
Treatments	Roots	Stem leaves	Sheath	Seeds	Roots	Stem leaves	Sheath	Seeds	Roots	Stem leaves	Sheath	Seeds
100% N-0MRB	14.9^{a}	258.9^{ab}	60.8^{ab}	604.3^{a}	5.54^{b}	117.5^{bc}	46.6^{abc}	98.1^{a}	9.94^{bc}	121.3^{ab}	122.3^{b}	172.4^{a}
85% N-0MRB	14.7^{a}	240.8^{b}	63.3^{a}	571.9^{ab}	5.26^{b}	100.6^{cd}	46.9^{abc}	94.9^{a}	9.12^{bc}	114.2^{abc}	158.5^{a}	168.7^{a}
70% N-0MRB	10.0^{c}	179.7^{c}	44.6^{cd}	478.4^{d}	4.68^{bc}	97.4^{d}	45.3^{bc}	84.9^{b}	8.60^{c}	115.1^{abc}	164.9^{a}	147.3^{b}
55% N-0MRB	8.22^{d}	166.2^{cd}	42.1^{d}	483.0^{cd}	3.34^{de}	101.2^{cd}	42.1^{c}	81.8^{b}	6.40^{de}	96.5^{cde}	153.2^{a}	150.4^{b}
85% N-MRB	15.0^{a}	292.2^{a}	60.3^{ab}	619.2^{a}	6.70^{a}	151.6^{a}	54.6^{a}	98.8^{a}	12.2^{a}	129.0^{a}	154.4^{a}	172.3^{a}
70% N-MRB	11.6^{b}	227.4^{b}	52.9^{bc}	533.7^{bc}	5.22^{b}	125.3^{b}	51.8^{ab}	98.4^{a}	10.9^{ab}	122.4^{ab}	155.7^{a}	159.4^{ab}
55% N-MRB	7.50^{d}	222.8^{b}	53.5^{bc}	498.7^{cd}	3.92^{cd}	124.4^{b}	50.5^{ab}	83.7^{b}	8.02^{cd}	107.4^{bcd}	153.0^{a}	146.0^{b}
0% N-MRB	5.10^{e}	150.1^{cd}	32.0^{e}	389.5^{e}	3.34^{de}	95.2^{d}	44.6^{bc}	68.5^{c}	5.96^{e}	93.5^{de}	112.7^{bc}	108.1^{c}
0% N-0MRB	3.74^{f}	132.3^{d}	29.6^{e}	333.0^{f}	2.56^{e}	76.6^{e}	39.9^{c}	47.7^{d}	3.86^{f}	85.8^{e}	95.9^{c}	99.1^{c}
Significant difference	*	*	*	*	*	*	*	*	*	*	*	*
CV (%)	8.83	13.0	13.9	7.49	13.9	11.8	12.0	8.84	18.2	12.9	12.1	7.07

Numbers with the same following letter were insignificantly different in the same column, *Significantly different at 5%, phosphate and potassium fertilizers were applied as a recommendation,MRB: Mxture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

Table 6: Influence of nitrogen-fixing rhizospheric bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the growth of green soybean cultivated on alluvial soil in dyke

Treatments	Plant height (cm)	Branches number (branches)	Leaves number (leaves)	Stem diameter (cm)
100% N-0MRB	51.4^{a}	5.2^{a}	18.6^{ab}	4.37^{ab}
85% N-0MRB	48.0^{c}	5.0^{a}	17.6^{bc}	4.13^{c}
70% N-0MRB	48.6^{bc}	4.6^{ab}	17.2^{c}	4.25^{abc}
55% N-0MRB	45.2^{f}	4.0^{b}	15.4^{d}	4.22^{bc}
85% N-MRB	50.8^{a}	5.2^{a}	19.4^{a}	4.45^{a}
70% N-MRB	49.5^{b}	4.6^{a}	18.0^{bc}	4.42^{ab}
55% N-MRB	46.5^{de}	4.8^{ab}	16.0^{d}	4.42^{ab}
0% N-MRB	46.8^{d}	4.4^{ab}	15.2^{d}	3.59^{d}
0% N-0MRB	45.6^{ef}	4.4^{ab}	13.4^{e}	3.40^{d}
Significant difference	*	NS	*	*
CV (%)	1.69	13.5	5.21	3.58

Numbers with the same following letter were insignificantly different in the same column, NS: Not significantly different, *Significantly different at 5%, phosphate and potassium fertilizers were applied as a recommendation, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

nitrogen application, while pod width (both diameter and thickness) and seeds' length, diameter and thickness were under the influence of only bacterial factor and the number of seeds per pod and pod length received an insignificant impact from both factors. Due to the influence of bacteria and nitrogen levels, the number of pods reduced from 32.2-25.8

Table 7: Influence of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28 on the yield and its components of green soybean cultivated on alluvial soil in dyke

Treatments	Pods number (pods)	Pod length (mm)	Pod diameter (mm)	Pod thickness (mm)	Seeds number per pod (seeds)	Seed length (mm)	Seed diameter (mm)	Seed thickness (mm)
100% N-0MRB	32.2^{a}	55.6^{a}	12.5^{b}	7.94^{b}	2.68	12.3^{d}	9.00^{b}	6.16^{b}
85% N-0MRB	30.4^{b}	55.9^{a}	12.5^{b}	7.89^{b}	2.98	13.2^{b}	9.12^{b}	6.29^{b}
70% N-0MRB	26.6^{d}	55.0^{ab}	12.2^{b}	7.65^{b}	2.75	12.8^{bcd}	9.03^{b}	6.20^{b}
55% N-0MRB	25.8^{d}	53.1^{cd}	12.5^{b}	7.93^{b}	2.63	13.0^{bc}	9.00^{b}	6.28^{b}
85% N-MRB	33.0^{a}	55.2^{ab}	13.3^{a}	8.97^{a}	2.70	13.9^{a}	9.88^{a}	7.28^{a}
70% N-MRB	28.8^{c}	54.4^{abc}	13.1^{a}	7.94^{b}	2.70	13.0^{bc}	9.25^{b}	6.53^{b}
55% N-MRB	27.0^{d}	54.7^{abc}	12.5^{b}	7.95^{b}	2.75	13.3^{b}	9.09^{b}	6.54^{b}
0% N-MRB	21.6^{e}	53.5^{bcd}	12.4^{b}	9.00^{a}	2.63	13.9^{a}	9.90^{a}	7.39^{a}
0% N-0MRB	18.8^{f}	52.3^{d}	11.8^{c}	7.94^{b}	2.50	12.5^{cd}	8.93^{b}	6.25^{b}
Significant difference	*	*	*	*	NS	*	*	*
CV (%)	3.90	2.38	1.82	3.81	8.11	3.34	3.08	6.15

Numbers with the same following letter were insignificantly different in the same column, NS: Not significantly different, *Significantly different at 5%, phosphate and potassium fertilizers were applied as a recommendation, MRB: Mixture of nitrogen-fixing bacteria *Enterobacter cloacae* ASD-07 and ASD-28

pods along with the reduction from 100-55% N and the number of pods in treatment with bacteria only (21.6 pods) was more than that in the treatment with no fertilization (18.7 pods). Additionally, the treatment with 85% N of RFF plus bacteria had a statistically equal pod number to the control treatment with 100% N of RFF. Because of the impact of bacteria supplementation, the results of the treatments with bacteria were all significantly improved or at least equal in comparison to the treatments without bacteria inoculation in parameters of pod width, seed length and width (Table 7). From such improvements in yield components, the yield rose significantly to bacteria supplementation. This led to the significant superiority of the treatment with 85% N of RFF plus bacteria (66.1 g pot^{-1}) compared to the control treatment with 100% N of RFF (60.5 g pot^{-1}) in yield. Moreover, the decrease in results following the nitrogen levels cut-off also happened in yield. From 100-55% N of RFF, the yield dropped from 60.5-51.1 g pot^{-1} (Fig. 5).

DISCUSSION

In this study, the supplementation of rhizosphere bacteria significantly improved the soil fertility as available N and P (Table 2). The NH_4^+ and available P contents increased by 13.7 mg NH_4^+ kg^{-1} and 13.3 mg P kg^{-1} in comparison to the case of no N application with and without bacteria. Similar results have also been shown in the studies of Ku *et al.*[20] and Wang *et al.*[21], where the availability of N, P and K rose under the influence of a strain of rhizosphere bacteria. To be more specific, although the total content of both N and P did not statically change under the influence of both bacterial supplementation and levels of inorganic nitrogen applied in soil, ranging from 0.24-0.27% and from 0.23-0.24%, respectively, the available contents of those minerals varied among treatments. With the application of inorganic nitrogen fertilizer, the control treatment with 100% N of RFF certainly had the highest ammonium content (42.6 mg NH_4^+ kg^{-1}), significantly different from other treatments. However, at the nitrogen levels of 85, 70 and 0% N of RFF, the treatments with bacteria possessed a remarkably higher soil ammonium content than the treatments without bacteria did. Moreover, surprisingly, the inorganic nitrogen fertilizer did not show any significant impact on the concentration of soil nitrate content. Therefore, the difference in nitrate content in soil among treatments depended on the bacterial supplement. All the treatments without bacteria were statistically equal to each other in nitrate concentration, ranging from 4.88-5.07 mg NO_3^- kg^{-1} and significantly lower than all treatments with bacteria in this property as well. This indicated the ability of the bacteria *E. cloacae* to oxidize nitrogen compounds into nitrate, correspondent to the study of Wan *et al.*[22]. It is more interestingly, the more inorganic nitrogen applied was, the lower efficacy of the bacterial strains to fix free nitrogen compound, where the treatment with only bacteria had a significantly better amount of nitrate than the treatment with bacteria plus either 85 or 70% N of RFF (Table 2). For available phosphate, at the same nitrogen levels, the treatments with bacteria always outweighed the treatments without bacteria supplementation and the content reduced along with the decline of nitrogen levels applied to biofertilizers. It was more surprising that the treatment with 85% N of RFF was even significantly higher than the control treatment with 100% N of RFF in the concentration of soluble phosphate (Table 2). Ultimately, from the changes in soil properties, the bacterial strains of *E. cloacae* ASD-07 and ASD-28 had the capability of improving soil available nutrients for plants. From that improvement, the minerals uptake, biomass, growth and yield of green soybean were also enhanced (Fig. 1, Tables 3, 5). The minerals uptake, including N, P and K, in roots, stem leaves, sheath and seeds, rose under the influence of inorganic nitrogen fertilizer levels and bacteria supplements (Table 5, Fig. 2-4). The boost in minerals uptake by *E. cloacae* is also following Safirzadeh *et al.*[23] and Tsegaye *et al.*[24]. Furthermore, it was a surprise that N uptake in the treatment with 85% N of RFF plus bacteria was statically equivalent to that in the control treatment with 100% N of RFF in total (Fig. 2) and each biomass component of green soybean (Table 3). This became statistically superior in the total P, K uptakes (Figs. 3, 4). In addition, for biomass, the trend was similar to minerals uptake, but for biomass in roots and sheath, the treatment with bacteria plus 85% N of RFF had significantly better values than the control one did, 1.60 compared to 1.37 g pot^{-1} and 13.9 compared to 11.9 g pot^{-1}, respectively (Table 3). Plant growth traits shared the common trend with the equivalent in the results between the treatment with bacteria plus 85% N of RFF and the control treatment with 100% N of RFF and the superior numbers of treatments with bacteria to those of treatments without bacteria, except for the number of branches, which received no significant influence from both factors (Table 6). Moreover, the improvement by the bacteria supplement happened in almost yield components, except for the number of seeds per pod. While that nitrogen fertilization occurred only in the number of pods and pod length (Table 7). Finally, these results led to a better yield in treatments with bacterial strains *E. cloacae* ASD-07 and ASD-28 in comparison to treatments without them at the common nitrogen level. Moreover, the yield in the treatment with bacteria and 85% N of RFF was significantly higher than that in the control treatment with 100% N of RFF plus no bacteria supplementation (Fig. 5). Altogether, in this study, there was evidence for the potent rhizospheric N_2-fixing bacterial strains of *E. cloacae* ASD-07 and ASD-28 in reducing 15% of inorganic nitrogen fertilizer without decreasing the productivity of green soybean (*Glycine max* (L.) Merr).

CONCLUSION

The application of the rhizospheric bacterial strains of N_2-fixation *Enterobacter cloacae* ASD-07 and ASD-28 as biofertilizers for green soybean improved the available soil nutrients for plants, including N and P, enhanced N, P and K uptake and replaced 15% of nitrogen fertilizer without making a decline in biomass, minerals uptake, growth and yield. These N_2-fixing bacterial strains had a great potential to apply to alluvial soil in dyke for a sustainable agricultural system.

SIGNIFICANCE STATEMENT

This study discovers the N_2-fixing rhizospheric bacteria *Enterobacter cloacae* ASD-07 and ASD-28 that can be beneficial for soil fertility to improve green soybean yield. This study will help the farmers reduce 15% of chemical nitrogen fertilizer.

REFERENCES

1. Krapp, A., 2015. Plant nitrogen assimilation and its regulation: A complex puzzle with missing pieces. Curr. Opin. Plant Biol., 25: 115-122.
2. Wang, Z., Y. Li, L. Zhuang, Y. Yu and J. Liu, 2019. A rhizosphere-derived consortium of *Bacillus subtilis* and *Trichoderma harzianum* suppresses common scab of potato and increases yield. Comput. Struct. Biotechnol. J., 17: 645-653.
3. Plett, D.C., K. Ranathunge, V.J. Melino, N. Kuya, Y. Uga and H.J. Kronzucker, 2020. The intersection of nitrogen nutrition and water use in plants: New paths toward improved crop productivity. J. Exp. Bot., 71: 4452-4468.
4. Wang, X., J. Fan, Y. Xing, G. Xu and H. Wang, 2018. The effects of mulch and nitrogen fertilizer on the soil environment of crop plants. Adv. Agron., 153: 121-173.
5. Wang, R., J. Min, H.J. Kronzucker, Y. Li and W. Shi, 2019. N and P runoff losses in China's vegetable production systems: Loss characteristics, impact, and management practices. Sci. Total Environ., 663: 971-979.
6. Morris, J.J., R.E. Lenski and E.R. Zinser, 2012. The black queen hypothesis: Evolution of dependencies through adaptive gene loss. mBio, Vol. 3. 10.1128/mBio.00036-12.
7. Rogers, C. and G.E.D. Oldroyd, 2014. Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. J. Exp. Bot., 65: 1939-1946.
8. Yanti, Y., F.F. Astuti, T. Habazar and C.R. Nasution, 2016. Screening of rhizobacteria from rhizosphere of healthy chili to control bacterial wilt disease and to promote growth and yield of chili. Biodiversitas J. Biol. Divers., Vol. 18. 10.13057/biodiv/d180101.
9. Salvo, L.P.D., G.C. Cellucci, M.E. Carlino and I.E.G. de Salamone, 2018. Plant growth-promoting rhizobacteria inoculation and nitrogen fertilization increase maize (*Zea mays* L.) grain yield and modified rhizosphere microbial communities. Appl. Soil Ecol., 126: 113-120.
10. Mikiciuk, G., L. Sas-Paszt, M. Mikiciuk, E. Derkowska and P. Trzciński *et al.*, 2019. Mycorrhizal frequency, physiological parameters, and yield of strawberry plants inoculated with endomycorrhizal fungi and rhizosphere bacteria. Mycorrhiza, 29: 489-501.

11. Qiao, C., C.R. Penton, W. Xiong, C. Liu and R. Wang *et al*., 2019. Reshaping the rhizosphere microbiome by bio-organic amendment to enhance crop yield in a maize-cabbage rotation system. Appl. Soil Ecol., 142: 136-146.
12. Alghamdi, S.S., M.A. Khan, E.H. El-Harty, M.H. Ammar, M. Farooq and H.M. Migdadi, 2018. Comparative phytochemical profiling of different soybean (*Glycine max* (L.) merr) genotypes using GC-MS. Saudi J. Bio. Sci., 25: 15-21.
13. Shilpashree, N., S.N. Devi, D.C. Manjunathagowda, A. Muddappa and S.A.M. Abdelmohsen *et al*., 2021. Morphological characterization, variability and diversity among vegetable soybean (*Glycine max* L.) genotypes. Plants, Vol. 10. 10.3390/plants10040671.
14. Raj, R.K., K.K. Sinha, S. Kumari, A.K. Choubey, A. Pandit and D.K. Yadav, 2020. Effect of nutrient and weed management on yield, nutrient uptake and quality of soybean [*Glycine max* (L.) Merrill] in alluvial soil of Bihar. J. Pharmacogn. Phytochem., 9: 1586-1589.
15. Szpunar-Krok, E., A. Wondołowska-Grabowska, D. Bobrecka-Jamro, M. Jańczak-Pieniążek, A. Kotecki and M. Kozak, 2021. Effect of nitrogen fertilisation and inoculation with *Bradyrhizobium japonicum* on the fatty acid profile of soybean (*Glycine max* (L.) merrill) seeds. Agronomy, Vol. 11. 10.3390/agronomy11050941.
16. Ju, X.T., X.J. Liu, F.S. Zhang and P. Christie, 2006. Effect of long-term fertilization on organic nitrogen forms in a calcareous alluvial soil on the North China Plain. Pedosphere, 16: 224-229.
17. Pal, S.K., D. Saha and A.K. Mukhopadhyay, 1987. Influence of cropping and N-fertilization on changes in different forms of nitrogen in an alluvial soil. Plant Soil, 101: 137-139.
18. Sparks, D.L., A.L. Page, P.A. Helmke, R.H. Loeppert and P. N. Soltanpour *et al*., 1996. Methods of Soil Analysis: Part 3 Chemical Methods, 5.3. Soil Science Society of America, America, ISBN: 9780891188254, Pages: 1390.
19. Temminghoff, E.E.J.M. and V.J.G. Houba, 2004. Plant Analysis Procedures. 2nd Edn., Springer Netherlands, Germany, ISBN-13 978-90-481-6717-3, Pages: 180.
20. Ku, Y., G. Xu, X. Tian, H. Xie, X. Yang and C. Cao, 2018. Root colonization and growth promotion of soybean, wheat and Chinese cabbage by *Bacillus cereus* YL6. PLoS ONE, Vol. 13. 10.1371/journal.pone.0200181.
21. Wang, J., R. Li, H. Zhang, G. Wei and Z. Li, 2020. Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. BMC Microbiol., Vol. 20. 10.1186/s12866-020-1708-z.
22. Wan, W., D. He and Z. Xue, 2016. Removal of nitrogen and phosphorus by heterotrophic nitrification-aerobic denitrification of a denitrifying phosphorus-accumulating bacterium *Enterobacter cloacae* HW-15. Ecol. Engineer., 99: 199-208.
23. Safirzadeh, S., M. Chorom and N. Enayatizamir, 2019. Effect of phosphate solubilising bacteria (*Enterobacter cloacae*) on phosphorus uptake efficiency in sugarcane (*Saccharum officinarum* L.). Soil Res., 57: 333-341.
24. Tsegaye, Z., D. Bekele, S. Chaniyalew, A. Feleke, T. Alemu and F. Assefa, 2021. The effects of plant growth-promoting bacteria (PGPB) inoculation on growth, yield and grain nutrient uptake of two teff varieties under greenhouse condition. Preprints, 10.20944/preprints202103.0046.v1.