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Nitrogen use efficiency of rice inoculated with free-living nitrogen-fixing bacteria

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Abstract Nitrogen is an essential element that promotes vegetative growth, which is usually limited to the uptake of plants. In this study, rice plants' efficiency to utilize nitrogen was investigated in field conditions in Phitsanulok province, northern region, Thailand. Biofertilizers containing five free-living nitrogen-fixing bacteria were used to combine with chemical fertilizer (CF, 16-20-0) at different rates (187.50 kg/ha and 93.75 kg/ha). The results showed that the number of tillers per hill, total nitrogen in rice shoot and Nitrogen Partial Factor Productivity were significantly different ($p \leq 0.05$) among all treatments. The maximum shoot height (98.80 ± 3.83 cm) was found in the NK5-5½CF treatment. The highest grain yield (4.71 t/ha) was observed in the treatment NK12-1½CF. NK12-1 combined with ½CF increased the shoot height to 4.71 cm, three tillers per hill and 0.6 t/ha (14.60%) of grain yield compared to control. The highest 1000-grain weight was produced in the NK 5-5 ½CF treatment (29.17 g), and the lowest was observed in control (27.64 g). All treatments were significantly different in Nitrogen Partial Factor Productivity ($p < 0.05$) but not in Nitrogen Harvest Index. In conclusion, biofertilizers applied enhanced rice growth, but had no effects on the yield components. NK12-1 was the most effective on nitrogen use efficiency. Also, NK5-5 and NK12-1 were capable of reducing half of the chemical nitrogen fertilizer input.

Keywords: Biofertilizer, Nitrogen-fixing bacteria, Nitrogen use efficiency, Growth and yield of rice

Introduction

Rice (*Oryza sativa* L.) is an important food crop for more than half of the world's population. Rice production must be increased to meet the growing global population (Huang *et al*., 2018). In the early 1960s to 1990s, rice consumption per year increased from 85 to 103 kg per person in Asia. During the same period, global rice consumption per capita increased from 50 to 65 kg per year (Mohanty, 2013). In Asia during 2009, the most per capita rice consumption per annum (173.3 kg) was Bangladesh, then Cambodia (160.3 kg),

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followed by Vietnam (141.2 kg) (GRiSP, 2013). To satisfy consumer demands, rice production must be improved. The proper nutrition management is a crucial way to be considered. Nitrogen element is a criticle component for plant growth. A total of 1 ton of grain yield of rice requires about 14.7 kg of nitrogen. Nitrate (NO_3-) and ammonium (NH_4+) are the main sources of nitrogen for rice crops (Dobermann and Fairhurst, 2000).

However, nitrogen that exists in the soil is usually limited by the uptake of plants. Thus, a large amount of nitrogen from external sources must be applied to the field. Application of excessive nitrogen causes serious environmental pollution (soil acidification, air pollution and water eutrophication). It leads to low nitrogen use efficiency (NUE) due to rapid loss of ammonia volatilization, denitrification, erosion, leaching and run-off. For instance, nitrate leaches into groundwater and drains into rivers. It causes human health problems when the water containing over 10 mg/L of nitrate is used for drinking. Thailand is an agricultural country whose farmers produce large amount of rice. The excessive industrial fertilizers being used in this country has caused numbers of illnesses and deaths to farmers (Ngampimol, 2008). To increase NUE in rice production, researchers have developed various nutrient management technologies (Choudhury and Kennedy, 2005, Wu *et al*., 2016). NUE is being used as an agricultural solution to achieve crop productivity, cost reduction, and environmental pollution mitigation (Huang *et al*., 2018).

Biological nitrogen fixation technology is being used to minimize the application of chemical fertilizer in paddy fields. This biological nitrogen fixation can be free-living or symbiotic nitrogen fixing-bacteria (Raimam *et al*., 2007). These bacteria interact with nitrogen elements and enhance plant growth and production (Mohammadi and Sohrabi, 2012). Biofertilizer products containing *Pseudomonas* spp., *Candida* spp., *Bacillus subtilis* and *Bacillus amyloliquefaciens* applied at different nitrogen fertilizer rates were found to increase grain and straw yields of rice (Cong *et al*., 2009). Microbial biofertilizer was suggested to be good to reduce chemical fertilizer. Based on the above, the study aimed to evaluate the effects of applying selected free-living nitrogen-fixing bacteria on NUE with the hypothesis that nitrogen-fixing bacteria might improve the NUE of rice in the paddy field.

Materials and methods

Experimental design

The field trial was performed on the clay loam soil at Tumbon Wat Prik, Muang district, Phitsanulok province, lower northern Thailand (16°44'20.7"N, 100°12'53.9"E) from July 2016 to December 2016. The experimental field was arranged in a Randomized Complete Block Design (RCBD) including four replications of eight treatments. Each plot's size was 3 m × 4 m, applied with biofertilizers, chemical fertilizer, and without any fertilizer (control) (Table 1). Biofertilizers contained five free-living nitrogen-fixing bacteria including NK3-1, NK5-5, NK8-4, NK12-1 and NK 12-2, previously isolated from sheath blade of different local rice varieties in the south of Thailand. These isolates were examined for their abilities for Indole-3-Acetic Acid (IAA) production and nitrogen-fixing capability (Table 2).

Table 1. Application of different fertilizers in the treatments

Treatments	Treatment code	Biofertilizer code	Chemical fertilizer (16-20-0) (kg/ha)
T_1	Control	–	–
T_2	CF	–	187.50
T_3	NK3-1½CF	NK3-1	93.75
T_4	NK5-5½CF	NK5-5	93.75
T_5	NK8-4½CF	NK8-4	93.75
T_6	NK12-1½CF	NK12-1	93.75
T_7	NK12-2½CF	NK12-2	93.75
T_8	NK3-1CF	NK3-1	187.50

Table 2. Bacterial isolates used in the study

Code	Rice variety	Phytonic habitat	Indole-3-acetic acid (μM/ml)	Nitrogenase activity (nmol C_2H_4/hr/ml)	Isolated place
NK3-1	Hom Jampah	Sheath	77.50	0.216	Singhanakorn, Songkla
NK5-5	Hom Nil	Sheath	52.10	0.185	Singhanakorn, Songkla
NK8-4	Kawn	Sheath	109.60	0.158	Singhanakorn, Songkla
NK12-1	Mail	Sheath	216.50	0.167	Singhanakorn, Songkla
NK12-2	Mail	Sheath	51.50	0.228	Singhanakorn, Songkla

Inoculation of rice seeds and data collection

Seeds of rice cultivar Phitsanulok 2 were obtained from Phitsanulok Rice Research Centre, Thailand. For biofertilizer inoculation, seeds were surface-sterilized with 3% hydrogen peroxide for 3 min, rinsed five times with distilled water and inoculated with biofertilizer containing free-living nitrogen-fixing bacteria in the treatments T3-T8 (1g seed:1g biofertilizer). After incubation for 24 h, the rice seeds were sown in nursery trays. The field soil was ploughed and flooded for five days and then puddled before transplantation. After 20 days of germination, the seedlings were transplanted to a spacing of 25 cm × 25 cm hill with one seedling per hill. At 43 days after transplantation (DAT), chemical fertilizer (16-20-0) at 93.75 kg/ha (half dose, ½CF) was applied to T3-T7 and at 187.50 kg/ha (full dose, CF) was applied to T2 and T8. Every 14 days, shoot height (cm), and the number of tillers per hill were recorded. At 76 DAT, chlorophyll level in the leaves was measured using a chlorophyll meter (SPAD-502, Konica-Minolta, Japan). The obtained values (x) were then applied to determine leaf-blade nitrogen concentration using the equation, y = 0.079x - 0.154; y is the leaf-blade nitrogen concentration, and x is the measured SPAD value. After harvest, the data of panicle length (cm), panicle number, thousand grain weight (g), grain yield (t/ha) and grain sterility (%) were collected by sampling ten tillers per plot. The grain yield was collected from the centre of the plot within the area of 5.5 m^2. Two guard rows were left in each plot of the experimental field. The nitrogen content of all the treatments were calculated to determine the NUE (Lu *et al*., 2012).

- Nitrogen harvest index (NHI, %) = (N in grain/N in grain and straw) × 100
- Nitrogen partial factor productivity (NPFP, g grain/g Nf) = grain yield with added N/amount of applied N

Before transplantation and after harvest, a composite soil sample was collected from each plot. The sample was air-dried and pulverized to pass through a 2-mm mesh sieve. The processed soil was measured for pH (soil 1: water 1) using a pH meter. Total nitrogen was determined using the Kjeldahl method (Bremner,1960) and available phosphorus through the Bray II method (Bray and Kurtz, 1945). Exchangeable potassium was analysed using ammonium acetate extraction (Schollenberger, 1945) and soil organic matter was determined using the potassium dichromate and sulfuric acid digestion methods (Walkley and Black, 1934). Rice grain and shoot dry biomass samples were analysed for their total nitrogen using the Kjeldahl method (Bremner,1960), and total phosphorus was determined using yellow vanadate-molybdate phosphoric acid. The samples were digested (Nitric acid and perchloric acid ratio, 4:1) and measured through an Atomic Absorption Spectrophotometer (AAS) to determine the amount of total potassium (Jumpen Onthong, 2012).

Statistical analysis

Analysis of variance of all data were performed using F test, and all treatment means were compared using Duncan Multiple Range Test (DMRT) at p = 0.05 level.

Results

Rice growth parameters

The rice growth, including shoot height, number of tillers per hill and shoot dry weight, were measured. The obtained results demonstrated a significant difference only in the number of tillers per hill. Chemical fertilizer alone (T2) produced the highest number of tillers but not significantly different from those in other CF treatments, except for the control and NK12-2½CF treatments. All biofertilizers, except for NK12-2 combined with ½CF, promoted tillering similary to the use of CF in T2 ($p \leq 0.05$, Table 3). Biofertilizer, in combination with different doses of CF, influenced shoot height. These results showed that the highest shoot height (98.80 cm) was obtained in the treatment of biofertilizer NK 5-5, which enhanced the shoot height to 6.44 cm compared to control. Biofertilizer NK12-2, NK3-1 combined with a full dose of CF, NK12-1 resulted in shoot height to 97.90 cm, 97.12 cm and 97.07 cm, respectively. Biofertilizer NK5-5 influenced the shoot height over the treatments with the application of full doses of CF.

The number of tillers per hill (18) was obtained in the treatment NK3-1CF, significantly different from the control and NK12-2½CF treatment. There were 17 tillers per hill in both the biofertilizer treatments (NK12-1½CF and CF). The lowest number of tillers per hill was found in control (14 tillers per hill). Biofertilizer NK3-1, in combination with a full dose of CF significantly increased the rice tillers over the control.

All treatments showed higher shoot dry weight than control. Biofertilizer NK 5-5 increased 0.9 t/ha of shoot dry weight over control. Biofertilizer NK 3-1 combined with CF produced 4.35 t/ha, gaining 0.3 t/ha compared to biofertiliser NK3-1 and chemical fertilizer alone (0.2 t/ha).

Table 3. Effect of free-living nitrogen-fixing bacteria on the shoot height, number of tillers per hill and shoot dry weight

Treatments	Shoot height (cm)	Number of tillers per hill	Shoot dry weight (t/ha)
Control	92.36 ± 5.76	14 ± 3.18[c]	3.68 ± 3.04
CF	95.63 ± 6.56	17 ± 2.07[ab]	4.04 ± 2.76
NK3-1½CF	94.14 ± 4.32	16 ± 0.41[abc]	4.16 ± 2.28
NK5-5½CF	98.80 ± 3.83	16 ± 0.45 [abc]	4.59 ± 4.76
NK8-4½CF	94.20 ±10.01	16 ± 2.50 [abc]	4.17 ± 5.55
NK12-1½CF	97.07 ± 1.55	17 ± 1.05 [ab]	4.26 ± 2.94
NK12-2½CF	97.90 ± 4.87	15 ± 1.29 [bc]	3.84 ± 2.72
NK3-1CF	97.12 ± 5.13	18 ± 0.36 [a]	4.35 ± 1.78
F-test	ns	*	ns
CV (%)	5.68	11.10	14.70

* = significant difference ($p \leq 0.05$); ns = no significant difference ($p > 0.05$); the probability at 5% level by the Duncan Multiple Range Test (DMRT).

Yield components

The observed results showed that the yield components including grain yield, thousand grain weight, panicle number, panicle length, and filled grain were not significantly different ($p > 0.05$, Table 4) in all the treatments. The highest grain yield was found in the treatment NK 12-1½CF at 4.71 t/ha, which was higher by 14.60 % and 13.49 % over control and the full dose treatment of CF. Biofertilizer NK3-1CF produced 4.65 t/ha of grain yield which increased 12.05 % over the treatment of full dose of chemical fertilizer and 13.14% as compared to the control. The highest thousand grain weight was produced by the biofertilizer NK 5-5 (29.17 g), and the lowest was observed in control (27.64 g). The treatment of control mostly produced low yield components as compared to all treatments.

Table 4. Effect of free-living nitrogen-fixing bacteria on the rice yield components

Treatments	Panicle length (cm)	Panicle number	Grain yield (t/ha)	Thousand grain weight (g)	Filled kernel (%)	Unfilled kernel (%)
Control	27.86 ±0.90	13 ±1.73	4.11 ±0.48	27.64 ±0.88	74.57 ±1.08	25.43 ±1.08
CF	28.91 ±1.26	14 ±1.50	4.15 ±0.32 (0.97†)	27.56 ±0.71	73.88 ±2.87	26.13 ±2.87
NK3-1½CF	28.51 ±0.95	13 ±0.95	4.53 ±0.20 (10.22†, 9.16‡)	27.93 ±1.21	73.82 ±0.82	26.18 ±0.82
NK5-5½CF	28.73 ±0.79	15 ±1.25	4.60 ±0.25 (11.92, 10.84)	29.17 ±2.22	71.99 ±1.89	28.01 ±1.89
NK8-4½CF	28.65 ±1.31	14 ±2.62	4.49 ±0.57 (9.25, 8.19)	27.40 ±0.98	72.14 ±2.16	27.86 ±2.16
NK12-1½CF	28.27 ±0.51	14 ±1.50	4.71 ±0.23 (14.60, 13.49)	28.44 ±1.07	71.42 ±4.57	28.58 ±4.57
NK12-2½CF	28.36 ±0.16	13 ±1.25	4.31 ±0.52 (4.87, 3.86)	28.09 ±0.40	73.08 ±4.19	26.92 ±4.19
NK3-1CF	27.76 ±1.61	15 ±0.81	4.65 ±0.36 (13.14, 12.05)	27.72 ±1.33	70.99 ±1.86	29.02 ±1.86
F-test	ns	ns	ns	ns	ns	ns
CV (%)	3.49	10.97	15.01	4.28	3.72	9.94

ns = no significant difference at 5% level by the Duncan Multiple Range Test (DMRT); †, ‡ = numbers in parentheses are the increased percentages of grain yield over control and a full dose of chemical fertilizer treatment.

Chlorophyll content and NUE

Nitrogen content of rice leaves was calculated from SPAD values (Table 5). The results showed that the highest leaf-blade nitrogen concentration (2.90%) was obtained in the NK3-1 treatment combined with a full dose of chemical fertilizer which increased 0.09% over applying chemical fertilizer alone and 0.38% as compared to control.

NUE is important to understand the amount of nitrogen fertilizer applied to absorb efficiently and avoid nitrogen loss. In this study, the value of NHI was not significantly different ($p > 0.05$, Table 5), but NPFP was significantly different among all treatments. At the half dose of chemical nitrogen fertilizer rates (15 kg/ha), NHI was higher than the treatment applied with full CF doses except for the NK5-5 treatment combined with a half dose of CF. Chemical fertilizer and biofertilizer did not increase the percentage of NHI. It was not influencing on NHI as the treatment without fertilizer was the highest. The nitrogen rates of 15 kg/ha increased NPFP around 150 kg grain/kg N, which was significantly higher than the nitrogen rates of 30 kg/ha.

Table 5 Effect of free-living nitrogen-fixing bacteria on NUE and leaf-blade nitrogen concentration

Treatments	NUE			Chlorophyll content
	NHI **(%)**	NPFP **(**g grain/g N_f**)**	Measured SPAD value	Leaf-blade nitrogen content **(%)**
Control	66.83 ± 4.96	-	35.48	2.68 ± 0.17
CF	61.76 ± 6.44	137.92 ± 19.63^b	37.06	2.81 ± 0.12
NK3-1½CF	62.60 ± 4.09	301.30 ± 25.22^a	36.14	2.73 ± 0.14
NK5-5½CF	61.02 ± 5.46	307.21 ± 31.02^a	36.89	2.79 ± 0.23
NK8-4½CF	65.35 ± 5.43	299.36 ± 69.85^a	36.23	2.74 ± 0.11
NK12-1½CF	63.56 ± 6.82	313.79 ± 28.05^a	35.87	2.71 ± 0.16
NK12-2½CF	63.73 ± 4.35	286.82 ± 63.73^a	35.63	2.69 ± 0.08
NK3-1CF	58.68 ± 3.84	154.98 ± 22.09^b	38.23	2.90 ± 0.13
F-test	ns	*	–	ns
CV (%)	8.31	31.44	–	5.45

* = significant difference ($p < 0.05$); ns = no significant difference ($p > 0.05$); the probability at 5% level by the Duncan Multiple Range Test (DMRT**).**

Soil and plant analysis

Before transplantation, the soil analysis results demonstrated that the total nitrogen content, soil organic matter, and available phosphorus were not significantly different among all treatments (Table 6). Total nitrogen of 0.23% to 0.25%, soil organic matter of 2.94% to 3.06% and available phosphorus of 1.33 mg/kg to 6.43 mg/kg were found in the soil. After harvest, total nitrogen in the soil at harvest did not differ from total soil nitrogen before transplantation.

The total nitrogen was not significantly different in the rice grain among all treatments, but it was significantly different in the rice straw (Table 7). A total of 2.54 kg/ha of nitrogen removal by the plant (grain and straw) was the highest in the biofertilizer NK3-1 treatment combined with full dose of chemical fertilizer, followed by the biofertilizer NK12-1 (2.38 kg N/ha) treatment combined with half dose of chemical fertilizer. The lowest nitrogen uptake (1.91kg N/ha) was observed in control, and total phosphorus and potassium were not significantly different in both rice grain and straw.

Discussion

In this study, all biofertilizers increased the shoot height compared to control. Even though, there was no significant difference in applying full doses of chemical fertilizer in T8 compared with T2 and T3 treatments, but there were similar results due to the other treatments were applied biofertilizer incombination with half dose of chemical fertilizer. Biofertilizers containing free-living nitrogen-fixing bacteria provide nitrogen compound for the rice plant to increase the shoot height, which was fixed from the atmosphere by microorganisms (Galloway *et al*., 2004). Nitrogen element is a criticle component for plant growth. It promotes plant by developing shoot, tillers and leaves (Dobermann and Fairhurst, 2000). Biofertilizers enhanced the shoot height during the vegetative growth compared to the plants without any inoculation of biofertilizer (control and CF). These biofertilizers may contribute to the continuous supply of nitrogen in the early growth stages where beneficial association occurs with the rice plants. This result agrees with the findings of Aon and Singh where nitrogen-fixing bacteria stimulated the growth of rice seedlings (Aon *et al*., 2015; Singh *et al*., 2015). *Bacillus* spp. expressing nitrogenase activity up to 1 nmol C_2H4 mg protein/h were found to promote sunflower growth (Ambrosini *et al*., 2016). Biofertilizers used in this study were also capable of producing IAA which stimulated the shoot height through cell enlargement, cell division, stem and root elongation (Shahab *et al*., 2009).

Biofertilizer NK3-1, in combination with a full dose of CF significantly increased the rice tillers over the control. This is in agreement with the observations of Yuvaraj and Singh who reported that the combination of biofertilizer (*Azotobactor* and *Azospirillum*) with inorganic fertilizer significantly increased rice tillers (Yuvaraj, 2016; Singh *et al*., 2015). Biofertilizer NK12-1 produced an equal number of tillers per hill compared to the treatment involving full doses of CF. It was revealed that biofertilizer NK12-1 was capable of increasing applied NUE in rice. As compared to control, biofertilizer NK12-1 significantly increased the number of tillers per hill. According to the previous study, inoculation with strains of diazotroph (*Azotobactor* spp.) enhanced the chlorophyll content, total root length, and biomass production. Yousuf indicated that the heterotrophic *Bacillus* spp. expressing nitrogenase activity were able to fix atmospheric dinitrogen. *Enterobacter* spp. isolated from rice rhizosphere had a positive contribution to fix the nitrogen gas, as indicated by the nitrogenase activity (Yousuf *et al*., 2017).

Although, shoot dry weight was not significantly different ($p > 0.05$) in all the treatments, it increased as compared to control which agrees with the results of Cong who reported that biofertilizer products containing *Pseudomonas* spp, *Candida* spp, *Bacillus subtilis* and *Bacillus amyloliquefaciens* applied at different rates of nitrogen on rice, could increase the grain and straw yield (Cong *et al*., 2009). The shoot dry weight was increased due to the plant’s height, or the number of tillers per hill was stimulated. The biofertilizers containing free-living nitrogen-fixing bacteria in this study were capable of enhancing the plant's height, the number of tillers per hill and shoot dry weight through nitrogen fixation and IAA production. Many studies reported that selected microorganisms promoted the growth of plants. For instance, Purwanto illustrated that *in vitro* inoculation of *Rhizobium* sp. LM-5 increased chlorophyll content, root length and biomass of rice seedlings (Purwanto *et al*., 2017). *Bacillus pumilus* and *Bacillus subtilis* produced IAA and nitrogenase to improve the growth and yield of cauliflower under field conditions (Kaushal and Kaushal, 2015).

The nitrogen-fixing biofertilizer could improve the grain yield and reduce the dosage of chemical fertilizer by increasing the natural nitrogen fertilizer into the rice plants to stimulate the growth and yield while the treatments applied biofertilizer incombination with ½CF provided higher grain yield than full dose treatment of CF. Many reports described that the inoculation of biofertilizer (*Glomus mosseae*, *Herbaspirillum seropedicae*) to rice seedlings significantly enhanced the rice yield over uninoculated control (Subashini *et al*., 2007; Banayo *et al*., 2012; Hoseinzade *et al*., 2016).

In this case, biofertilizers did not affect thousand grain weight which agrees with the observations of Kecskés (Kecskés *et al*., 2016). Moreover, Beutler also reported that nitrogen-fixing bacteria contributed to increased growth but did not stimulate the yield (Beutler *et al*., 2016). The effect of biofertilizers on panicle length and panicle number of rices were not significantly different. However, a low value of panicle length and number of tillers per hill were enhanced by the application of biofertilizer, as reported by Islam (Islam *et al*., 2012), where the application of biofertilizer increased the panicle length over control. The treatment of control produced the highest percentage of filled grain (74.57%), which showed that biofertilizer did not influence grain sterility. In this study, the environmental conditions may affect grain sterility in the paddy field (Peterson *et al*., 1974; Abeysiriwardena *et al*., 2010).

All biofertilizers in this study contributed to increase NPFP by providing natural nitrogen to the field. The rates of application of nitrogen influenced NUE. NPFP declined due to increase in the nitrogen input. NPFP normally varies from 40 to70 kg/kg N, and higher than 70 kg/kg N noted only at low nitrogen rates (Che *et al*., 2015; Ayadi *et al*., 2016).

Biofertilizer NK12-1, combined with a half dose of chemical fertilizer enhanced the rice growth notably over the treatment involving a full dose of chemical fertilizer. The biofertilizer inoculation could reduce chemical fertilizer application in the paddy field (Orona-Castro *et al*., 2013). Free-living biological nitrogen fixation is a key process improving soil fertility and producing nitrogen in many terrestrial ecosystems (Cleveland *et al*., 1999; Wakelin *et al*., 2010). It can contribute to the availability of nitrogen in the soil by 50-150 kg/ha which relates to cereal crops like sunflower, potato, maize, different flowers, mustard, rice, wheat, cotton, etc. (Chowdhury and Mukherjee, 2006) and generate nitrogen content in the soil (Watanabe, 1984).

In conclusion, the biofertilizer products containing free-living nitrogen-fixing bacteria were observed to enhance rice growth significantly. Biofertilizer NK5-5 increased the plant height up to 6.44 cm, whereas biofertilizer NK12-1 enhanced four tillers per hill as compared to control. Although the yield components were not significantly different, biofertilizer NK12-1 contributed to promoting the grain yield to 0.6 t/ha. Biofertilizers NK5-5 and NK12-1 reduced half of the chemical fertilizer and maintain sustainable soil nitrogen better than the control. Also, biofertilizer NK12-1 was the most effective on nitrogen use efficiency and rice growth.

Table 6. Soil chemical analysis before transplantation and after harvest

Treatments	Before transplantation					After harvest				
	Total N (%)	Available P (mg/kg)	Exchangeable K (mg/kg)	OM (g/kg)	pH	Total N (%)	Available P (mg/kg)	Exchangeable K (mg/kg)	OM (g/kg)	pH
Control	0.25	6.43	70.77[a]	2.96	5.56[a]	0.22	6.43	31.49	2.95	5.72[a]
CF	0.24	3.37	68.66[ab]	2.99	5.39[ab]	0.23	6.31	38.97	2.83	5.41[ab]
NK 3-1½CF	0.23	3.78	59.73[abc]	3.06	5.38[ab]	0.24	4.83	42.49	3.06	5.42[ab]
NK 5-5½CF	0.24	5.20	58.33[abc]	3.01	5.30[b]	0.23	7.18	40.55	3.03	5.36[ab]
NK 8-4½CF	0.23	1.33	55.86[bc]	2.94	5.42[ab]	0.25	5.32	40.07	3.02	5.26[b]
NK 12-1½CF	0.25	3.67	54.76[bc]	2.94	5.31[b]	0.24	5.44	38.83	2.98	5.03b[c]
NK 12-2½CF	0.25	2.76	49.24[c]	2.95	5.24[b]	0.24	6.68	44.92	3.12	4.73[c]
NK 3-1CF	0.25	4.49	47.97[c]	2.98	5.30[b]	0.23	1.98	45.54	3.02	5.10[b]
F-test	Ns	ns	*	ns	*	ns	ns	ns	ns	*
CV (%)	6.78	68.04	25.21	4.01	3.02	5.16	76.63	19.00	5.73	6.72

* = significant difference ($p < 0.05$); ns = no significant difference ($p > 0.05$); the probability at 5% level by Duncan Multiple Range Test (DMRT).

Table 7. Effect of free-living nitrogen-fixing bacteria on the nutrients of rice grain and straw

Treatments	Rice grain analysis			Rice straw analysis			Total nitrogen uptake (kg N/ha)
	Total N (mg/kg)	Total P (%)	Total K (%)	Total N (mg/kg)	Total P (%)	Total K (%)	
Control	310.33 ±20.69	1.01 ±1.01	0.13 ±0.07	170.33 ±26.80[c]	1.94 ±1.24	0.83 ±0.37	1.91
CF	331.34 ±26.94	3.17 ±1.77	0.18 ±0.03	210.00 ±34.50[ab]	1.64 ±0.85	0.78 ±0.18	2.23
NK3-1½CF	312.67 ±12.04	1.76 ±1.53	0.12 ±0.07	203.00 ±19.24[abc]	1.33 ±0.87	0.67 ±0.16	2.26
NK5-5½CF	310.33 ±20.69	1.55 ±0.53	0.12 ±0.02	200.67 ±24.69[bc]	1.17 ±0.90	0.77 ±0.18	2.34
NK8-4½CF	326.67 ±13.19	2.65 ±1.84	0.11 ±0.03	186.67 ±22.86[bc]	1.52 ±0.64	0.82 ±0.14	2.26
NK12-1½CF	319.66 ±4.67	2.09 ±0.95	0.12 ±0.00	203.00 ±32.66[abc]	1.45 ±0.79	0.81 ±0.11	2.38
NK12-2½CF	326.67 ±22.86	2.58 ±1.52	0.12 ±0.05	205.33 ±22.86[abc]	1.63 ±0.80	0.70 ±0.02	2.21
NK3-1CF	322.00 ±5.39	2.60 ±1.82	0.09 ±0.01	240.33 ±14.00[a]	2.21 ±0.27	0.72 ±0.18	2.54
F-test	ns	ns	ns	*	ns	ns	–
CV (%)	5.41	61.92	38.82	14.51	49.68	23.92	–

* = significant difference ($p < 0.05$); ns = no significant difference ($p > 0.05$); the probability at 5% level by the Duncan Multiple Range Test (DMRT).

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