Malaysian Journal of Soil Science 2025 Vol. 29: 115-123

Combining Endophytic Bacteria with Reduced Nitrogen Fertilizer Application Improves Soil Characteristics and Rice Productivity in the Mekong Delta Region of Vietnam

Vu Van Long[1]*; Le Bich Tuyen[2]; Tran Van Dung[3]; Huynh Van Tien[4]; Le Van Cuong[5] & Nguyen Thi My Linh[6]

[1]*Faculty of Natural Resources - Environment, Kien Giang University, Kien Giang, 91752, Vietnam*
[2]*Faculty of Food Sciences and Health, Kien Giang University, Kien Giang, 91752, Vietnam*
[3]*Faculty of Soil Science, College of Agriculture, Can Tho University, Can Tho, 94100, Vietnam*
[4]*Institute of Food and Biotechnology, Can Tho University, Can Tho, Vietnam*
[5]*Faculty of Forestry, Vietnam National University of Forestry – Southern Campus, Dongnai, Vietnam*
[6] *Faculty of Architecture-Construction and Environment, Nam Can Tho University, Can Tho, Vietnam*

**Corresponding author: vvlong@vnkgu.edu.vn*

ABSTRACT

The study was carried out to evaluate the effects of combining *Gluconacetobacter diazotrophicus* bacteria and reduced nitrogen (N) fertilizer on rice growth, grain yield, and soil properties. The field experiment was conducted at the triple rice cultivation in the Vietnamese Mekong Delta region. It was laid out in a split-plot design, with *G. diazotrophicus* as the primary parameter and different N fertilizer rates as the sub-factor. Two methods were applied for rice seeds included: inoculation with *G. diazotrophicus* (G_d) and without *G. diazotrophicus* (G_o). Four N fertilizer rates: 0 kg N ha^{-1} (N_0), 50 kg N ha^{-1} (N_{50}), 75 kg N ha^{-1} (N_{75}), and 100 kg N ha^{-1} (N_{100}). The study results revealed that applying *G. diazotrophicus* combined with reduced N fertilizer can maintain the tiller number, height, biomass, and grain yield, while significantly increasing the SPAD value. Furthermore, the combination of *G. diazotrophicus* with reduced N fertilizer resulted in a significant increase in the soil pH, NH_4^+-N, and available phosphorus. In contrast, soil EC, soil organic matter, and total N were maintained. Further research on the applying *G. diazotrophicus* bacteria combined with reduced N and P fertilizers to enhance soil characteristics, and improve N use efficiency, and grain yield in the Vietnamese Mekong Delta region is recommended.

Keywords: *Gluconacetobacter diazotrophicus*, growth-promoting bacteria, nitrogen-fixing bacteria, paddy soil, rice seeds inoculation

INTRODUCTION

The Mekong Delta region is the largest rice production area in Vietnam (Qui *et al*., 2024). It accounts for more than 90% of Vietnam's annual rice exports (Anh *et al*., 2020). In paddy rice cultivation, nitrogen (N) is the most important nutrient for rice growth, grain yield, and the uptake of other elements (Gu and Yang, 2022). According to Galloway *et al*. (2004), N fertilizer has been applied in a large amount to increase yield in the rice cultivation system. Previous studies have estimated that rice uses approximately 50% of N fertilizer and the remaining N fertilizer is lost through runoff, the fluxes of NH_3 and N_2O (Dung *et al*., 2021; Long *et al*., 2024). Excessive application of N fertilizer can negatively affect rice production, resulting in high costs, becoming an ecosystem problem, and contributing to global warming potential (Wang *et al*., 2017; Ding *et al*., 2018).

Numerous studies have been conducted on paddy soils to improve yield and N use efficiency, including the application of slow-release fertilizers (Geng *et al*., 2015), ure inhibitors (Klimczyk *et al*., 2021; Skorupka and Nosalewicz, 2021), and inorganic fertilizers combined with organic fertilizers (Ismael *et al*., 2021; Wang *et al*., 2022). However, the application of these methods did not result in a significantly positive grain yield response or N use efficiency.

Next to plant photosynthesis, biological N fixation is considered the most important biochemical reaction on Earth (Weil and Brady, 2017). In this process, dinitrogen gas (N_2) is transformed into bioavailable ammonium forms. According to Soumare *et al.* (2020), biological N fixation is carried out exclusively including archaea and bacteria (Azotobacter, Azospirillum, Bacillus, and Clostridium). *Gluconacetobacter diazotrophicus* (formerly *Acetobacter diazotrophicus*), a member of the *Acetobacteraceae* family, is recognized as an endophytic bacterium that promotes plant growth (Mehnaz and Lazarovits, 2017; Saranraj *et al*., 2021). This bacterium was originally isolated from sugarcane in Brazil (Cavalcante and Dobereiner, 1988). Recently, it was also isolated from paddy rice (Muthukumarasamy *et al*., 2005; Jha *et al*., 2009; Long *et al*., 2024). In the paddy rice systems, *G. diazotrophicus* can provide up to 50–70% of the N needed for rice growth (Reddy *et al*., 2017; Dung *et al*., 2021; Long *et al*., 2024).

We hypothesized that the inoculation of rice seeds with *G. diazotrophicus* bacteria can establish a symbiotic relationship with rice plants, allowing them to convert N_2 gas to bioavailable ammonium as an alternative to inorganic N fertilizer. The objectives of the present study were carried out to explore rice growth, grain yield, and soil properties under inoculation with and without *G. diazotrophicus* in a paddy rice system in the Mekong Delta region of Vietnam.

MATERIALS AND METHODS

Experimental site and soil characteristics

The experiment was conducted in the triple rice area in Mong Tho A commune, Chau Thanh district, Kien Giang province (10°02′27.6″N, 105°09′17.7″E). The soil is classified as Eutric Gleysols, according to FAO-WRB (2014). At the 0–20 cm soil depth, the soil pH varied around 5.88, and the soil electrical conductivity (1.24 mS cm^{-1}) did not affect rice growth. Soil texture was classified as clay with clay and silt contents were 60.4% and 39.1%, respectively. Soil organic matter (7.95%) and available N (73.9 mg NH_4^+-N kg^{-1}) were considered at a high level for the paddy field. The other soil characteristics are shown in Table 1.

Table 1: Soil characteristics at the beginning of the experiment

Soil characteristics	Value
Sand (%)	0.51
Silt (%)	39.1
Clay (%)	60.4
$pH_{1:2.5}$	5.88
$EC_{1:2.5}$ (mS cm^{-1})	1.24
Soil organic matter (%)	7.95
Total N (%)	0.19
Available N (mg NH_4^+-N kg^{-1})	73.9
Total P (% P_2O_5)	0.04
Available P (mg P kg^{-1})	14.1
CEC (cmol kg^{-1})	18.8
Exchangeable K (cmol kg^{-1})	0.23
Exchangeable Na (cmol kg^{-1})	0.52
Exchangeable Ca (cmol kg^{-1})	9.47
Exchangeable Mg (cmol kg^{-1})	8.00

Experimental design, treatments, and management

The field experiment was conducted on two consecutive crops, using a split-plot design with three replicates for each treatment. The N-fixing bacteria (NFB) was used as the main plot, and N fertilizer rates were used as the sub-plot. Two NFB methods were: Rice seeds were inoculated with *G. diazotrophicus* (G_d) or not inoculated with *G. diazotrophicus* (G_o). Four N fertilizer rates: 0 kg N ha^{-1} (N_0); 50 kg N ha^{-1} (N_{50}); 75 kg N ha^{-1} (N_{75}), and 100 kg N ha^{-1} (N_{100}), which is the farmer's practice.

The method of *G. diazotrophicus* inoculation was described by Long et al. (2024). Rice seeds were soaked in water for 24 hours, then incubated for 24 hours. Afterwards, the rice seeds were soaked in a *G. diazotrophicus* bacteria solution (10^8 CFU mL^{-1}) for 1 hour, and finally incubated for 4–6 hours before sowing.

In the paddy field, 24 treatment plots (30 m^2/plot) were separated by bunds, which were covered with a plastic sheet installed to a depth of 50 cm from the soil surface. Rice variety Dai Thom 8, which has a growth time ranging from 90–95 days, was used in this study. Phosphorus fertilizer was applied at 60 kg P_2O_5 ha^{-1} and potassium fertilizer was 30 kg K_2O ha^{-1}. Phosphorus fertilizer was applied all before sowing, and urea fertilizer was top-dressed at 10 (20% N), 20 (40% N), and 45 days (40% N) after sowing (DAS), while potassium fertilizer was applied at 20 (50% K_2O) and 40 (50% K_2O) DAS.

Soil, plant sampling and analyses

At the start of the experiment, soil samples were taken at a depth of 0–20 cm to determine the soil properties. After two cropping seasons, soil samples were taken to evaluate the soil pH, EC, soil organic matter, total N, NH_4^+-N, and available P at the harvest stage. The rice height, number of tillers, and SPAD were measured at 20, 45, 60, and 75 DAS in the second cropping season. The chlorophyll content was measured by SPAD-502 plus chlorophyll meter (Konica Minolta, Tokyo, Japan). Rice biomass and yield were collected for each treatment at the harvest of the second cropping season. Rice yields were calculated in 5 m^2 plant samples for each plot and determined at 14% moisture content.

The soil texture was determined by using the pipette method, which is described by Kroetsch and Wang (2008). Soil pH and EC were extracted with distilled water (1:2.5 ratio). Soil organic matter content was obtained by the Walkley-Black method (Walkley and Black, 1934). Soil total N was analyzed by the Kjeldahl digestion. Soil mineral NH_4^+-N was analyzed by the phenate method after extracting soils with 2M KCl at a 1:10 ratio. Soil P availability was determined by the Olsen-P method (Olsen *et al.*, 1982). Soil total P concentration was measured by alkaline digestion followed by molybdate colorimetric measurement (Murphy and Riley, 1962). Exchangeable K^+, Na^+, Ca^{2+}, and Mg^{2+} cations were analyzed from the $BaCl_2$-compulsive exchange method (Hendershot and Duquette, 1986).

Statistical analysis

Statistical analyses of the data were performed using RStudio software (version 2024. 04.1). The splitplot function from the Doebioresearch package was used to perform analysis of variance (Popat and Banakara, 2020). When significant treatment effects were detected, a least significant difference (LSD) test was used to compare the treatment means at the 5% significance level.

RESULTS AND DISCUSSION

The number of tillers

The effects of NFB combined with N fertilizer rates on the number of active tillers are shown in **Table 2**. In general, the results showed that the application of *G. diazotrophicus* combined with N rates did not significantly affect the number of active tillers at 20, 45, and 75 DAS ($p>0.05$). However, the treatment with no applied N fertilizer (110 tillers) resulted in a significantly higher than that in the treatment applied 75 kg N ha^{-1} (88.7 tillers) at 65 DAS ($p<0.05$). In addition, applying 50 kg N ha^{-1} (101 tillers) or 100 kg N ha^{-1} (96.8 tillers) could maintain the number of tillers. The results demonstrated that combining *G. diazotrophicus* and reduced N fertilizer also significantly affected the tiller number at 65 DAS ($p<0.05$).

The number of tillers is an important factor influencing rice grain yield (Wang *et al*., 2017). Deng *et al*. (2015) reported that active tillers have a positive association with rice biomass and grain yield. Besides, N fertilizer was a significantly affect on the promotion of tillers in the poor soil and did not significant affect on the rich soil (Huang *et al*., 2013; Deng *et al*., 2015). In this study, soil experiment was determined as rich in available N (73.9 mg NH_4^+-N kg^{-1}), therefore reducing N fertilizer 0–75 kg N ha^{-1} did not significantly affect the number of active tillers. Recently, some studies also reported that reducing N fertilizer by 50% did not significantly affect the tiller number in the rice cultivation systems (Dung *et al*., 2021; Long *et al*., 2024).

Table 2: The tiller number in the *G. diazotrophicus* combined with N fertilizer rates treatment

Treatments	The number of active tillers			
	20 DAS	**45DAS**	**60 DAS**	**75 DAS**
G_o	66.0	89.7	105	115
G_d	81.1	87.2	93.3	114
N_0	70.0	91.2	110^{a}	121
N_{50}	70.5	76.8	101^{ab}	108
N_{75}	73.5	104	88.7^{b}	121
N_{100}	80.2	82.0	96.8^{ab}	106
F-test				
G	*ns*	*ns*	*ns*	*ns*
N	*ns*	*ns*	*	*ns*
G × N	*ns*	*ns*	*	*ns*

Means in a column followed by the same letter are not significantly different, means followed by different letters (a, b) are significantly different at $p< 0.05$. In the analysis of variance, ns means $p >0.05$, * means $p <0.05$; G_d, G_o are rice seeds inoculated with/without *G. diazotrophicus*, respectively; N_0, N_{50}, N_{75}, N_{100} are the treatments applied with 0, 50, 75, and 100 kg N ha^{-1}, respectively.

Rice height

In general, the inoculation of rice seeds with *G. diazotrophicus* bacteria did not significantly affect rice height during the rice growth stages (**Table 3**). Similarly, the results also indicated that rice height in the treatments applied with 0, 50, and 75 kg N ha^{-1} did not differ significantly, either among the treatments applied with 50, 75, and 100 kg N ha^{-1} at 20, 60, and 75 DAS, except at 45 DAS. Applying 0 or 75 kg N ha^{-1} resulted in significantly higher rice height than it in the treatment applied 50 kg N ha^{-1} ($p<0.05$), but did not differ significantly compared to the treatment applied 100 kg N ha^{-1} ($p>0.05$) at 45 DAS. Additionally, combining *G. diazotrophicus* bacteria with reduced N fertilizers (0–75 kg N ha^{-1}) demonstrated that rice height can be maintained.

Nitrogen is an essential nutrient for rice growth and it is recommended in many rice-growing regions to increase rice production (Noor, 2017; Ghoneim *et al*., 2018). According to Qiu *et al*. (2020), the application of N fertilizer stimulates the elongation of rice stem internodes, resulting in increased plant height. However, several studies reported that the rice height significantly increased when applied with a higher 190 kg N ha^{-1} (Yoseftabar, 2013; Ghoneim *et al*., 2018; Zhang *et al*., 2020). On the other hand, Long *et al*. (2024) indicated that the application of 50–75 kg N ha^{-1} did not significantly affect rice height compared with the treatment applied 100 kg N ha^{-1}.

SPAD index

Table 4 shows the SPAD values during the rice growth stages. The results indicated that applying *G. diazotrophicus* did not significantly affect the SPAD index at any of the rice-growing stages (p>0.05). In addition, the SPAD values in the treatments applied with 0, 50, and 75 kg N ha^{-1} did not differ significantly, either among the treatments applied with 50, 75, and 100 kg N ha^{-1} at 20 and 45 DAS (p>0.05). However, applying 50 kg N ha^{-1} resulted in a significantly lower SPAD index than the treatments applied 0, 75, and 100 kg N ha^{-1} at 60 DAS (p<0.05). At 75 DAS, the application of 75 kg N ha^{-1} significantly decreased the SPAD value compared to the treatment applied 100 kg N ha^{-1} (p<0.05). There was no significant difference in SPAD of the treatments applied 0–50 kg N ha^{-1} compared to the treatment applied 100 kg N ha^{-1}. The results of this study demonstrated that the combination of *G. diazotrophicus* with reduced N fertilizer significantly increased the SPAD value at all the rice growth stages (p<0.05).

Table 3: The rice height in the *G. diazotrophicus* combined with N fertilizer rates treatment

Treatments	Rice height (cm)			
	20 DAS	45DAS	60 DAS	75 DAS
G_o	40.9	74.6	94.1	98.5
G_d	40.7	74.5	95.4	98.7
N_0	42.1	76.2^{a}	92.0	98.2
N_{50}	41.1	72.0^{b}	94.7	97.9
N_{75}	40.3	75.9^{a}	95.8	99.8
N_{100}	39.7	74.2^{ab}	96.6	98.4
F-test				
G	*ns*	*ns*	*ns*	*ns*
N	*ns*	*	*ns*	*ns*
G × N	*ns*	*	*	*ns*

Means in a column followed by the same letter are not significantly different, means followed by different letters (a, b) are significantly different at p< 0.05. In the analysis of variance, ns means p >0.05, * means p <0.05; G_d, G_o are rice seeds inoculated with/without *G. diazotrophicus*, respectively; N_0, N_{50}, N_{75}, N_{100} are the treatments applied with 0, 50, 75, and 100 kg N ha^{-1}, respectively.

Table 4: SPAD index in the *G. diazotrophicus* combined with N fertilizer rates treatment

Treatments	SPAD value			
	20 DAS	45DAS	60 DAS	75 DAS
G_o	34.3	35.9	39.4	44.9
G_d	35.5	35.3	39.4	41.9
N_0	35.5	34.9	40.5^{a}	41.8^{ab}
N_{50}	33.0	35.9	36.6^{b}	43.6^{ab}
N_{75}	37.6	36.4	40.0^{a}	39.8^{b}
N_{100}	33.8	35.2	40.7^{a}	48.3^{a}
F-test				
G	*ns*	*ns*	*ns*	*ns*
N	*ns*	*ns*	*	*
G × N	*	*	*	*

Means in a column followed by the same letter are not significantly different, means followed by different letters (a, b) are significantly different at p< 0.05. In the analysis of variance, ns means p >0.05, * means p <0.05; G_d, G_o are rice seeds inoculated with/without *G. diazotrophicus*, respectively; N_0, N_{50}, N_{75}, N_{100} are the treatments applied with 0, 50, 75, and 100 kg N ha^{-1}, respectively.

Soil Plant Analysis Development (SPAD), which is a low-cost method for determining chlorophyll in leaves, is designed to predict the N status of rice leaves (Lin *et al*., 2010; Yang *et al*., 2018; Hou *et al*., 2021). According to Yuan *et al*. (2016), the SPAD index depends on many factors, such as N and Mg contents, especially the available N in the soil (Maltese *et al*., 2020; Wadas and Dziugieł, 2020). The available N in the study soil ranged in a high level, which explains why the rice in the treatment applied 0–75 kg N ha^{-1} was not deficient in N nutrient. In paddy fields, a recent study reported that the application of G. diazotrophicus can provide up to 70% N for rice (Reddy *et al*., 2017). Similarly, several studies have reported that the combination of G. diazotrophicus with reduced N fertilizer can maintain the N uptake capacity and improve the SPAD value in rice leaves (Dung *et al*., 2021; Long *et al*., 2024).

Rice biomass and grain yield

Rice biomass in the treatment applied *G. diazotrophicus* and reduced N fertilizer ranged from 8.42 to 9.43 tons ha^{-1} (**Table 5**). The results showed no significantly different in rice biomass among the treatments of rice seeds without/inoculated with *G. diazotrophicus,* neither among the treatments applied with 0, 50, 75, and 100 kg N ha^{-1} (p>0.05). Besides, the application of *G. diazotrophicus* combined with N fertilizer (G × N**)** did not significantly affect rice biomass.

The rice yield in the second cropping system ranged from 4.47 to 4.87 tons ha^{-1} (**Table 5**). Similarly, the results showed no significant differences in grain yield between the treatments with and without G. *diazotrophicus*, nor among the treatments applied with 0, 50, 75, and 100 kg N ha^{-1}.

According to Reddy *et al*. (2017), *G. diazotrophicus* bacteria can supply up to 50–70% of the N demands of rice plants. Recently, several studies also indicated that applying NFB bacteria combined with reduced N fertilizer can maintain rice biomass and grain yield (Dung *et al*., 2021; Long *et al*., 2024). The results of this study demonstrated that the combination of *G. diazotrophicus* bacteria can increase the N uptake capacity, promote rice growth, biomass, and grain yield under low-N fertilizer conditions through the biological N fixation process.

Table 5: Rice biomass and grain yield in the *G. diazotrophicus* combined with N fertilizer rates treatment

Treatments	Rice biomass (tons ha^{-1})	Grain yield (tons ha^{-1})
G_o	8.42	4.72
G_d	9.83	4.58
N_0	8.85	4.77
N_{50}	9.54	4.87
N_{75}	8.47	4.47
N_{100}	9.65	4.50
F-test		
G	*ns*	*ns*
N	*ns*	*ns*
G × N	*ns*	*ns*

G_d, G_o are rice seeds inoculated with/without *G. diazotrophicus*, respectively; N_0, N_{50}, N_{75}, N_{100} are the treatments applied with 0, 50, 75, and 100 kg N ha^{-1}, respectively.

Soil characteristics

After two consecutive cropping seasons, the results showed that the soil pH, EC, SOM, total N, and available N in the treatments applied with or without *G. diazotrophicus* did not differ significantly (p>0.05), either among the treatments applied with 0, 50, 75, and 100 kg N ha^{-1} (**Table 6**). However, the treatments applied with 0 or 100 kg N ha^{-1} resulted in significantly higher soil pH than the treatment applied 75 kg N ha^{-1} (p<0.05). Besides, combining *G. diazotrophicus* bacteria with reduced N fertilizer resulted in significantly increased soil pH, NH_4^+-N, and available P (p<0.05).

Gluconacetobacter diazotrophicus is recognized as a growth-promoting bacterium capable of N fixation, solubilization of soil phosphates, or increasing soil pH, leading to an increased N and P availability (Gelsomino *et al*., 2006; Yadav *et al*., 2009; Chawla et al., 2014). Similarly, recent studies have shown that applying *G. diazotrophicus* bacteria could improve the soil pH, available N, and available P in the soil (Yadav *et al*., 2009; Paredes-Villanueva *et al*., 2020; Long *et al*., 2024).

Table 6: Soil characteristics in the *G. diazotrophicus* combined with N fertilizer rates treatment

Treatments	Soil characteristics					
	pH	**EC ($mS\ cm^{-1}$)**	**SOM (%)**	**Total N (%)**	**NH_4^+-N ($mg\ kg^{-1}$)**	**Avai. P ($mg\ kg^{-1}$)**
G_o	4.92	0.34	4.96	0.21	42.6	9.63
G_d	4.88	0.33	5.25	0.18	41.5	10.6
N_0	4.92[a]	0.34	5.10	0.19	43.1	10.5
N_{50}	4.88[ab]	0.32	5.21	0.23	42.1	8.89
N_{75}	4.86[b]	0.35	5.20	0.18	40.6	10.0
N_{100}	4.94[a]	0.34	4.92	0.19	42.4	11.2
F-test						
G	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
N	*	*ns*	*ns*	*ns*	*ns*	*ns*
G × N	*	*ns*	*ns*	*ns*	*	*

Means in a column followed by the same letter are not significantly different, means followed by the different letters (a, b) are significantly different at $p < 0.05$. In the analysis of variance, ns means $p > 0.05$, * means $p < 0.05$; G_0, G_o are rice seeds without/incubated with *G. diazotrophicus*, respectively; N_0, N_{50}, N_{75}, N_{100} are the treatments applied with 0, 50, 75, and 100 kg N ha^{-1}, respectively.

CONCLUSION

This study was conducted to assess the effects of *G. diazotrophicus* combined with different N fertilizer rates on rice growth, biomass, grain yield, and soil properties in the rice cultivation system. After two consecutive cropping seasons, applying *G. diazotrophicus* could maintain the tiller number, rice height, SPAD value, biomass production, and rice yield under N fertilizer application rates of 0, 50, 75, and 100 kg ha^{-1}. In addition, combining *G. diazotrophicus* bacteria with reduced N fertilizer resulted in increased soil pH, NH_4^+-N, and available soil P, while soil EC, soil organic matter, and total N were maintained. Further research on the application of *G. diazotrophicus* combined with reducing N and P fertilizers, improving N and P use efficiency, increasing rice yield, and enhancing soil properties in the Vietnamese Mekong Delta region is recommended.

ACKNOWLEDGEMENTS

This research was funded by The Vietnamese Ministry of Education and Training (No. B2023-TKG-01). We extend our gratitude to Mr. Pham Sang Tay, Mr. Che Thanh Thien, and Mr. Vo Trung Duong for their support in managing the field experiment in Chau Thanh District, Kien Giang Province, Vietnam.

REFERENCES

Anh, D.T., Tinh, T.V., & Vang, N.N. 2020. The domestic rice value chain in the Mekong Delta. *In:* Cramb, R. (Ed.): White gold: The commercialisation of rice farming in the lower mekong basin. 375–395.

Cavalcante, V.A. & Dobereiner, J. 1988. A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant and Soil* 108: 23–31.

Chawla, N., Phour, M., Suneja, S., Sangwaan S., & Goyal, S. 2014. *Gluconacetobacter diazotrophicus*: An overview. *Res. Environ. Life Sci* 7: 1–10.

Deng, F., Wang, L., Ren, W.-J., Mei, X.-F., & Li, S.-X. 2015. Optimized nitrogen managements and polyaspartic acid urea improved dry matter production and yield of indica hybrid rice. *Soil and Tillage Research* 145: 1–9.

Ding, W., Xu, X., He, P., Ullah, S., Zhang, J., Cui, Z., & Zhou, W. 2018. Improving yield and nitrogen use efficiency through alternative fertilization options for rice in china: A meta-analysis. *Field Crops Research* 227: 11–18.

Dung, T.V., Tan, D.B., Khanh, T.H., Gale, D., & Long, V.V. 2021. Effect of *Gluconacetobacter diazotrophicus* inoculation and reduced nitrogen fertilizer on yield and growth parameters of rice varieties. *Journal of Seed Science* 43: e202143029.

FAO-WRB. 2014. World reference base for soil resources. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

Gelsomino, A., Badalucco, L., Landi, L., & Cacco, G. 2006. Soil carbon, nitrogen and phosphorus dynamics as affected by solarization alone or combined with organic amendment. *Plant and Soil* 279: 307–325.

Geng, J., Sun, Y., Zhang, M., Li, C., Yang, Y., Liu, Z., & Li, S. 2015. Long-term effects of controlled release urea application on crop yields and soil fertility under rice-oilseed rape rotation system. *Field Crops Research* 184: 65–73.

Ghoneim, A.M., Gewaily, E.E., & Osman, M.M. 2018. Effects of nitrogen levels on growth, yield and nitrogen use efficiency of some newly released egyptian rice genotypes. *Open agriculture* 3(1): 310–318.

Gu, J. & Yang, J. 2022. Nitrogen (n) transformation in paddy rice field: Its effect on n uptake and relation to improved n management. *Crop and Environment* 1(1): 7–14.

Hendershot, W.H. & Duquette, M. 1986. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Science Society of America Journal* 50(3): 605–608.

Hou, W., Shen, J., Xu, W., Khan, M.R., Wang, Y., Zhou, X., Gao, Q., Murtaza, B., & Zhang, Z. 2021. Recommended nitrogen rates and the verification of effects based on leaf spad readings of rice. *PeerJ* 9: e12107.

Huang, M., Yang, C., Ji, Q., Jiang, L., Tan, J., & Li, Y. 2013. Tillering responses of rice to plant density and nitrogen rate in a subtropical environment of southern china. *Field Crops Research* 149: 187–192.

Ismael, F., Ndayiragije, A., & Fangueiro, D. 2021. New fertilizer strategies combining manure and urea for improved rice growth in mozambique. *Agronomy* 11(4): 783.

Jha, B., Thakur, M.C., Gontia, I., Albrecht, V., Stoffels, M., Schmid, M., & Hartmann, A. 2009. Isolation, partial identification and application of diazotrophic rhizobacteria from traditional indian rice cultivars. *European Journal of Soil Biology* 45(1): 62–72.

Klimczyk, M., Siczek, A. & Schimmelpfennig, L. 2021. Improving the efficiency of urea-based fertilization leading to reduction in ammonia emission. *Science of the Total Environment* 771: 145483.

Kroetsch, D. & Wang, C. 2008. Particle size distribution. In: M. R. Carter, Gregorich, E.G (Ed.): *Soil sampling and methods of analysis*, CRC Press and Taylor and Francis Group: Boca Raton, Florida: 713–725.

Lin, F.F., Deng, J.S., Shi, Y.Y., Chen, L.S.,& Wang, K. 2010. Investigation of spad meter-based indices for estimating rice nitrogen status. *Computers and Electronics in Agriculture* 71: S60–S65.

Long, V.V., Duong, V.T., Tuyen, L.B., Dung, T.V., Nghia, N.K., Nam, T.S., Viet, N.Q., Tien, H. V., & Cuong, L. V. 2024. Gluconacetobacter diazotrophicus bacteria combined nitrogen fertilization promotes rice yield and soil quality in the paddy field in the Vietnamese Mekong Delta region. *Malaysian Journal of Soil Science* 28: 359–368.

Maltese, N.E., Maddonni, G.A., Melchiori, R.J.M., Ferreyra, J.M., & Caviglia, O.P. 2020. Crop nitrogen status of early-and late-sown maize at different plant densities. *Field Crops Research* 258: 107965.

Mehnaz, S. & Lazarovits, G. 2017. Gluconacetobacter azotocaptans: A plant growth-promoting bacteria. *In:* Mehnaz s. (Ed.) rhizotrophs: Plant growth promotion to bioremediation. Microorganisms for Sustainability, vol 2. Springer, Singapore.

Murphy, J. & Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica chimica acta, 27: 31-36.

Muthukumarasamy, R., Cleenwerck, I., Revathi, G., Vadivelu, M., Janssens, D., Hoste, B., Gum, K., Park, K.-D., Son, C.Y.,& T. Sa, 2005. Natural association of gluconacetobacter diazotrophicus and diazotrophic acetobacter peroxydans with wetland rice. *Systematic and applied microbiology* 28(3): 277-286.

Noor, M.A., 2017. Nitrogen management and regulation for optimum nue in maize–a mini review. *Cogent Food & Agriculture* 3(1): 1348214.

Olsen, S.R., Sommers, L.E. & Page, A. 1982. Phosphorus. *In:* Page, A. L. (Ed.): Methods of soil analysis part 2 chemical and microbiological properties,. Madison, American Society of Agronomy, Soil Science Society of America. .

Paredes-Villanueva, J., Del Rosario, J., Urcia-Pulido, M., & Zavaleta-Armas, J. 2020. Plant growth promoter collection of gluconacetobacter diazotrophicus from northern coast of peru. *Scientia Agropecuaria* 11(1): 15–21.

Popat, R. & Banakara, K. 2020. Doebioresearch: Analysis of design of experiments for biological research. Available online: https://CRAN.R-project.org/package=doebioresearch (accessed on 10 September 2024).

Qiu, J., Wu, Z., Jiang, T.-H., Liu, F., & Zhang, Z.-J. 2020. Effects of nitrogen reduction at different growth stages on rice population production characteristics and preliminary analysis of nitrogen reduction strategies. *Journal of Yangzhou University, Agricultural and Life Sciences Edition* 41(3): 52–58.

Qui, N.V., Khoa, L.V., Phuong, N.M., Vien, D.M., Dung, T.V., Linh, T.B., Khanh, T.H., Thuong, B.T., Tran, V.T.T., & Nghia, N.K. 2024. Effects of rotating rice with upland crops and adding organic amendments, and of related soil quality on rice yield in the Vietnamese Mekong Delta. *Agronomy* 14(6): 1185.

Reddy, I.N.B.L., Kim, B.-K., Yoon, I.-S., Kim, K.-H., & Kwon, T.-R. 2017. Salt tolerance in rice: Focus on mechanisms and approaches. *Rice Science* 24(3): 123–144.

Saranraj, P., Jayaprakash, A., Devi, V., Al-Tawaha, A., & Al-Tawaha, A. 2021. Isolation and nitrogen fixing efficiency of gluconacetobacter diazotrophicus associated with sugarcane: A review. *IOP Conference Series: Earth and Environmental Science*. IOP Publishing: 012171.

Skorupka, M. & Nosalewicz, A. 2021. Ammonia volatilization from fertilizer urea—a new challenge for agriculture and industry in view of growing global demand for food and energy crops. *Agriculture* 11(9): 822.

Soumare, A., Diedhiou, A.G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., & Kouisni, L. 2020. Exploiting biological nitrogen fixation: A route towards a sustainable agriculture. *Plants* 9(8): 1011.

Wadas, W. & Dziugieł, T. 2020. Changes in assimilation area and chlorophyll content of very early potato (solanum tuberosum l.) cultivars as influenced by biostimulants. *Agronomy* 10(3): 387.

Walkley, A. & Black, I.A. 1934. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* 37(1): 29–38.

Wang, C., Ma, X., Shen, J., Chen, D., Zheng, L., Ge, T., Li, Y., & Wu, J. 2022. Reduction in net greenhouse gas emissions through a combination of pig manure and reduced inorganic fertilizer application in a double-rice cropping system: Three-year results. *Agriculture, Ecosystems & Environment* 326: 107799.

Wang, H., Hu, R., Chen, X., Zhong, X., Zheng, Z., Huang, N., & Xue, C. 2017. Reduction in nitrogen fertilizer use results in increased rice yields and improved environmental protection. *International Journal of Agricultural Sustainability* 15(6): 681–692.

Wang, Y., Lu, J., Ren, T., Hussain, S., Guo, C., Wang, S., Cong, R., & Li, X. 2017. Effects of nitrogen and tiller type on grain yield and physiological responses in rice. *AoB Plants* 9(2): plx012.

Weil, R. & Brady, N.C. 2017. The nature and properties of soils (15^{th} ed.). Pearson Education Limited: Edinburgh Gate, Harlow, Essex CM20 2JE, England. 1104 pages.

Yadav, R., Suman, A., Prasad, S., & Prakash, O. 2009. Effect of *Gluconacetobacter diazotrophicus* and trichoderma viride on soil health, yield and n-economy of sugarcane cultivation under subtropical climatic conditions of india. *European journal of Agronomy* 30(4): 296–303.

Yang, H., Yang, J., Li, F., & Liu, N. 2018. Replacing the nitrogen nutrition index by spad values and analysis of effect factors for estimating rice nitrogen status. *Agronomy Journal* 110(2): 545–554.

Yoseftabar, S. 2013. Effect nitrogen management on panicle structure and yield in rice (*Oryza sativa L.*). *International Journal of Agriculture and Crop Sciences* 5(11): 1224–1227.

Yuan, Z., Ata-Ul-Karim, S.T., Cao, Q., Lu, Z., Cao, W., Zhu, Y., & Liu, X. 2016. Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings. *Field Crops Research* 185: 12–20.

Zhang, J., Tong, T., Potcho, P.M., Huang, S., Ma, L.,& Tang, X. 2020. Nitrogen effects on yield, quality and physiological characteristics of giant rice. *Agronomy* 10(11): 1816.