PERFORMANCE OF NITROGEN-USE EFFICIENT NERICA4 RICE LINES IN INDONESIAN RAIN-FED ECOSYSTEMS

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Abstract. Rice absorbs only 30-40% of N fertilizer application. Therefore, one of the solutions is using a variety of rice that absorbs N more efficiently. This experiment is aimed at determining the yield parameters, NUE performance, and nitrous oxide flux of NERICA4 transgenic lines and NERICA4-wild-type in rain-fed agro-ecosystems at different doses of N fertilizer during a confined field trial. The experiment was conducted in Central Java, Indonesia, during the 2017/2018 rainy season. The experiment used a factorial randomized block design with four replications. Three transgenic lines of NERICA4 (NUE2, NUE9, NUE12) and NERICA4-WT were used as the main plot and N fertilizer rates (60, 120, and 180 kg N/ha) were used as the subplot. The results showed that an increase in the N fertilizer rate generally improved the plant height and the tiller number of NERICA4-NUE lines. The tiller numbers of NERICA4-NUE lines were higher than NERICA4-WT on high N dosages. There was a strong correlation between the N uptake and the grain yield. The analysis showed that all NERICA4-NUE lines use N more efficiently than NERICA4-WT, significantly at 60 kg N/ha with higher total grain than NERICA4-WT. NERICA4-NUE9 tended to emit lower nitrous oxide flux than other NERICA4-NUE and NERICA4-WT.
Keywords: *NERICA4-NUE, nitrogen uptake, nitrogen use efficiency, nitrous oxide flux, yield parameters*

Introduction

Nitrogen is an essential nutrient used by many plants, including rice, for their growth and development. It also plays a role in the formation of DNA, proteins, enzymes, and metabolite products which are involved in synthesizing and transferring energy (Kusano et al., 2011). An initiative by the government of Indonesia increases the productivity of rice crops by subsiding nitrogen fertilizer for farmers. Between 2003 and 2020, the government of Indonesia spent IDR 319.77 trillion (USD 22.12 billion) on fertilizer and seed subsidies. In 2020, the government of Indonesia subsidized 8.88 million tons of fertilizer, which is equivalent to IDR 25.000 billion (Alta et al., 2021). However, rice crops frequently do not absorb fertilizer in rice fields effectively, particularly in rain-fed agro-ecosystems. Low efficiency in using N fertilizers and decreased soil fertility can

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cause low rice productivity. The productivity of rain-fed irrigated rice is still low in Indonesia, ranging between 1.8–3.55 t/ha (Pane et al., 2009; Widyantoro and Toha, 2010; Kasno et al., 2016).

The rice only uses about 30–40% of the available N in the soil. The remaining N, about 60%, is lost from farmlands through erosion, surface run-off, leaching, ground-water pathway and gaseous emissions (NH_3, NO_2, NO, N_2O, and N_2). N losses contributed to the low efficiency of N fertilizer use in rice fields (Mo'allim et al., 2018; Bantacut, 2018; Cheng et al., 2019). Agricultural soils account for more than 50% of total atmospheric N_2O emissions, resulting in significant nitrogen losses from agricultural systems (Du et al., 2022; Smith et al., 2007). Nitrous oxide (N_2O) is one of the greenhouse gases produced by agriculture that could cause global warming and lead to ozone depletion in the stratosphere (Du et al., 2022). N_2O is a potent greenhouse gas (GHG) 268 times stronger than CO_2. N_2O alone contributed 6% of total anthropogenic GHG emissions in 2010 (Islam et al., 2018; Du et al., 2022; Kongchum et al., 2020). The N loss during biological denitrification ranges from 22% to 95%, depending on the soil type (Chew and Pushparajah, 1995). Agriculture is the primary source of more than 50% of ammonia volatilization. Also, the application of N fertilizers to crops accounts for most agriculture-related ammonia volatilization (Li et al., 2017; Cantarella et al., 2018). Therefore, minimizing N losses can reduce environmental pollution and production costs (Sisharmini et al., 2019).

Developing a rice variety that efficiently absorbs N fertilizer could improve the efficiency of N fertilizer. The management of N fertilizer and the timing of N application is generally determined by plant requirements with specific growth stages and is related to N availability in soils (Sun et al., 2017). Among rice varieties, there are differences in the ability to absorb nutrients and exudate elements from roots. The variability of nutrient absorption and nutrient-use efficient use for grain production is affected by morphological and physiological factors among rice varieties.

The study of N-use efficiency (NUE) is necessary to increase crop yield, reduce environmental pollution due to excessive use of N fertilizers, and achieve sustainable agriculture (Yulita et al., 2021). The usage of NUE technologies can considerably reduce the demand for N fertilizer, increase food security, and reduce greenhouse gas emissions from the rice ecosystem (Sapkota et al., 2021).

One of the genes involved in improving nitrogen-use efficiency in plants is alanine aminotransferase (*AlaAT*). AlaAT is a pyridoxal-5′-phosphate-dependent enzyme found in all parts of the plant. This enzyme is active in the leaves, the roots, and other tissues such as the endosperm and the flower (Shrawat et al., 2008; Miyashita et al., 2007; Rocha et al., 2010; McAllister et al., 2013). NUE crops are plants that can efficiently uptake, utilize, and remobilize the available N (Selvaraj et al., 2016). Cereals can absorb only 33% of the nitrogen applied to the soil. They recover less than half of the applied N in grains (Chen et al., 2016). Further improvement of NUE in crops is thus an essential aim in agricultural research and our future food production capabilities (Tiong et al., 2021).

Selvaraj et al. (2016) have produced the NERICA4 (New Rice for Africa 4) transgenic rice lines expressing the *HvAlaAT* gene. These rice lines have been evaluated throughout three growing seasons in two rice-growing ecologies (lowland and upland) in Colombia. The results revealed that the grain yield of NERICA4-NUE transgenic events was significantly higher than sibling nulls and wild-type (WT) controls under different N application rates. This genetic modification could significantly improve dry biomass and grain yields. The analysis showed that the increase in the yield of transgenic rice

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correlated positively with the number of tillers and the number of panicles. However, the performance of the NERICA4-NUE rice has not been studied yet in Indonesia.

The experiment aimed to determine the yield parameters, NUE performance, and nitrous oxide flux of transgenic NERICA4-NUE rice lines and NERICA4-WT in rain-fed agro-ecosystems at different levels of N fertilizer during a confined field trial in Indonesia.

Methodology

Experimental materials

The experiment used four varieties of rice, including three transgenic lines (NERICA4-NUE2, NERICA4-NUE9, and NERICA4-NUE12) and NERICA4-WT.

Experimental design

The field experiment was conducted at Jakenan Experiment Station, Indonesian Agricultural Environment Research Institute, in Pati, Central Java Province, Indonesia, during the 2017-2018 rainy season. It was located at 110°10' E and 6°45'S, an altitude of 12 m above sea level, with a soil type of inceptisols. The soil properties of inceptisols at this site were pH (H_2O) of 5.55, organic C of 0.88%, total N of 0.06%, available P of 22.56 mg/kg P_2O_5, available K of 16.03 mg/kg K_2O, and cation exchange capacity of 11.55 cmol/kg (Wihardjaka, 2018). Based on the meteorological data (2009-2016), the average annual rainfall is 1465 mm, the mean daily solar radiation ranges from 389 to 559 MJ/m^2/month, and the average maximum and minimum temperatures are 34.8 and 23.2 °C, respectively (Wihardjaka et al., 2022).

The experiment used a randomized block in a 4 × 3 factorial design with four replications. The first factor was transgenic lines (V1 = NERICA4-NUE2, V2 = NERICA4-NUE9, V3 = NERICA4-NUE12) and NERICA4-WT (V4), while the second factor was the N fertilizer rate (N1 = 33% N or 60 kg N/ha, N2 = 66% N or 120 kg N/ha, N3 = 100% N or 180 kg N/ha).

The soil was tilled and plotted with a size of 4 × 5 m^2. The perimeter of the plots was outlined by installed barriers using plastic sheets 40 cm in depth (*Fig. 1*). All transgenic NERICA4-NUE and NERICA4-WT seeds were incubated for 24 h and immediately spread in the seedbed. The 21-day-old seedlings were transplanted with a spacing of 20 × 20 cm^2 in each plot. The rice seedling was transplanted on October 26, 2017 and harvested on January 15, 2018 (80 days after transplanting).

Figure 1. *(a) The installation of plastic sheets in each of the experimental plot. (b) The 4 × 5 m^2 experimental plots with various doses of N fertilizer*

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Other fertilizers applied were 45 kg P_2O_5 and 60 kg K_2O per hectare. The SP36 fertilizer was applied 2 days before transplanting (DAT). The KCl fertilizer was applied twice (at 2 DAT and 40 DAT). The N fertilizer in the form of urea was applied three times: a half dose of N at 2 DAT, a quarter dose of N at 30 DAT, and a quarter dose of N at 40 DAT. The carbofuran insecticide 15 kg/ha was applied simultaneously with N fertilizer.

Experimental methods

Data analysis

The parameters observed were grain yield from a harvest area of 1.8 × 2.4 m^2 (= 108 hills); yield components (panicle number, panicle length, 1000-grain weight, number of filled grains, and number of unfilled grains) of two hills per plot; plant height and tiller number of 12 hills in each plot; biomass weight of 4 hills per plot; nitrogen content in soil and plant tissue; nitrous oxide fluxes; and NUE.

The nitrogen content in soil and plant tissue was measured by the Kjeldahl method. The gas samples were taken only 12 and 40 days after transplanting (DAT) using the closed chamber method to determine nitrous oxide flux (*Fig. 2*). In both samplings, gas samples were taken at intervals of 10 min (10, 20, 30, 40, and 50 min) after the placement of the chamber. The nitrous oxide flux was determined using gas chromatography equipped with an electron capture detector (Susilawati et al., 2020).

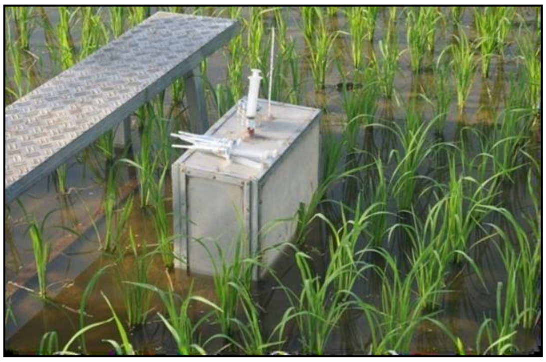

Figure 2. *The closed chamber method was used to collect gas samples for determining nitrous oxide flux*

According to Sudjadi et al. (1987), the value of NUE is computed using the following formula:

$$NUE = \frac{\text{Total N uptake}}{\text{N applied}}\ x\ 100\% \quad \text{(Eq.1)}$$

Statistical analysis

The data was analyzed statistically using univariate analysis of variance (ANOVA) through the SPSS Program. Significantly different means ($p < 0.05$) were tested using the Tukey test at 5% probability. The correlation test was done on the parameters (variables of grain yield, yield components, N uptake, and plant growth).

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Results and discussion

The location of the experiment at Jakenan was the lowland rain-fed agro-ecosystem which was sensitive to drought stress. They planted during the rainy season when there was more water for the plants. *Figure 3* presented the performance of NERICA4-NUE lines, such as NERICA4-NUE12, in confined field trials.

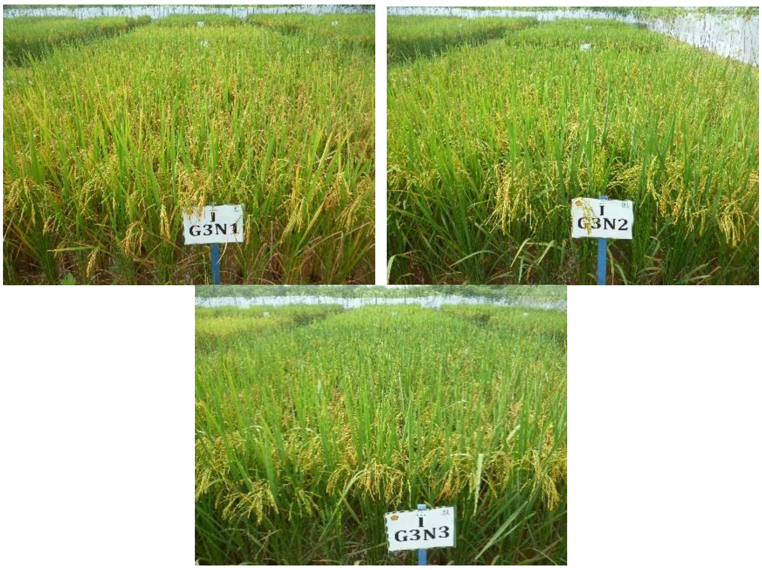

Figure 3. *Performance of NERICA4-NUE12 (G3) at the maturity growth stage at different N levels (N1 = 60 kg/ha, N2 = 120 kg/ha, N3 = 180 kg/ha)*

Rice performance and grain yield

The plant height was only significantly affected by the N level, while the variety of NERICA4 and its interaction with N fertilization were not significant (*Table 1*). *Table 1* showed that the plants provided with high N fertilization treatment were taller than those with low N fertilization treatment. The plant height with fertilization of 120 and 180 kg N/ha was significantly higher than 60 kg N/ha. The increase in plant growth was in line with the rise in the quantity of fertilizer. The plant height at harvesting time ranges from 97-101 cm (dose of 60 kg N/ha), 101-109 cm (dose of 120 kg N/ha), and 108-112 cm (dose of 180 kg N/ha). The results of this study were in line with Sefaoglu et al. (2021) that stated that the higher the N fertilizer application rate is, the taller the plant (mustard) becomes.

The number of tillers, especially the maximum tillers, was only significantly influenced by the varieties of NERICA4 (*Table 1*). Nevertheless, the tiller number was generally higher at 180 kg N/ha than at 60 kg N/ha and 120 kg N/ha. According to Wang et al. (2018) the higher nitrate concentration caused the increase in tiller number in treatments with higher doses of N fertilizer. It enhances the cytokinin pathway and the cell cycle that control tiller growth and root development of rice plants. Plant growth was better in treatments with higher doses of N fertilizer. The increase of N fertilizer rate generally improved the tiller number per hill on all lines of NERICA4-NUE, but the tiller number decreased on NERICA4-WT. NERICA4-NUE2 had the highest tiller number in all treatments of doses of N. The tiller number of NERICA4-NUE lines was more increased than NERICA4-WT, especially with the treatments of 120 and 180 kg N/ha. The increase in N application in the soil did not influence the tiller number of NERICA4-WT.

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The maximum tiller occurred at 30 DAT in the treatment of 60 kg N/ha and at 40 DAT in the treatment of 120 kg N/ha and 180 kg N/ha.

The varieties of NERICA4 and the N level significantly affected the total grain number per panicle and the percentage of filled grain in each panicle (*Table 2*). The increase in N fertilizer rate significantly influenced the yield component, especially the number of total grains per panicle across all varieties and the percentage of filled grain. The number of total grains in NERICA4-NUE was higher than in NERICA4-WT. The increase in N fertilizer did not influence the 1000-grain weight and only enhanced the percentage of filled grain in NERICA4-WT.

Table 1. *The plant height and tiller number of NERICA4-NUE lines and NERICA4-WT across treatments of different doses of N fertilizer*

N dose (kg N/ha)	Line/variety	Plant height (cm)	Tiller number per hill	
			Maximum	Productive
60	NERICA4-NUE2	97.2 ± 3.3 aA	9.7 ± 1.1 bA	9.4 ± 1.2 aA
	NERICA4-NUE9	100.0 ± 1.5 aA	8.8 ± 0.9 abA	8.4 ± 0.3 aA
	NERICA4-NUE12	100.8 ± 1.2 aA	8.9 ± 0.1 aA	8.4 ± 0.2 aA
	NERICA4-WT	99.2 ± 5.6 aA	9.4 ± 0.3 aA	8.8 ± 0.4 aA
120	NERICA4-NUE2	101.8 ± 6.4 aB	11.1 ± 2.1 bA	9.9 ± 1.5 aA
	NERICA4-NUE9	108.3 ± 4.6 aB	10.2 ± 0.4 abA	9.7 ± 0.5 aA
	NERICA4-NUE12	106.4 ± 6.0 aB	9.1 ± 0.8 aA	8.9 ± 1.1 aA
	NERICA4-WT	109.2 ± 3.6 aB	9.3 ± 1.4 aA	8.9 ± 1.3 aA
180	NERICA4-NUE2	108.8 ± 5.4 aB	11.6 ± 0.9 bA	11.0 ± 0.8 aA
	NERICA4-NUE9	111.0 ± 8.1 aB	10.0 ± 0.9 abA	9.5 ± 0.7 aA
	NERICA4-NUE12	108.3 ± 2.0 aB	9.8 ± 0.9 aA	9.8 ± 0.8 aA
	NERICA4-WT	112.0 ± 5.5 aB	9.0 ± 1.1 aA	8.5 ± 0.8 aA
NERICA4 (V)		ns	*	Ns
N level (N)		**	ns	Ns
V X N		ns	ns	Ns

The mean values followed by the same letter for each factor (lowercase letters indicate line/variety, capital letters indicate the dose of N fertilizer) in the same column were not significantly different at 5% Tukey test, * significant at $p < 0.05$, ** significant at $p < 0.01$, ns = not significant $p > 0.05$

Table 2. *The yield components of NERICA4-NUE lines and NERICA4-WT across three levels of N fertilizer*

N dose (kg N/ha)	Line/variety	Weight of 1000 grains (g)	Grains number per panicle	
			Total grains	% filled
60	NERICA4-NUE2	26.38 ± 1.29 aA	927 ± 166 bA	77.6 ± 15.0 aB
	NERICA4-NUE9	27.25 ± 0.23 aA	845 ± 30 bA	74.9 ± 6.3 aB
	NERICA4-NUE12	27.12 ± 1.10 aA	812 ± 90 bA	65.5 ± 6.7 aB
	NERICA4-WT	27.08 ± 1.58 aA	714 ± 52 aA	75.6 ± 9.4 bB
120	NERICA4-NUE2	26.61 ± 2.07 aA	874 ± 529 bB	61.4 ± 13.7 aAB
	NERICA4-NUE9	27.60 ± 1.03 aA	1302 ± 205 bB	72.4 ± 5.0 aAB
	NERICA4-NUE12	27.22 ± 0.66 aA	1033 ± 95 bB	69.2 ± 7.3 aAB
	NERICA4-WT	27.50 ± 1.36 aA	947 ± 190 aB	81.5 ± 4.6 bAB
180	NERICA4-NUE2	25.69 ± 0.86 aA	1263 ± 53 bB	60.0 ± 5.8 aA
	NERICA4-NUE9	27.44 ± 0.52 aA	1179 ± 55 bB	63.6 ± 9.9 aA
	NERICA4-NUE12	26.99 ± 1.09 aA	1312 ± 149 bB	64.9 ± 5.2 aA
	NERICA4-WT	27.23 ± 1.76 aA	947 ± 91 aB	72.0 ± 8.4 bA

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NERICA4 (V)	ns	*	**
N level (N)	ns	**	*
V X N	ns	ns	Ns

The mean values followed by the same letter for each factor (lowercase letters indicate line/variety, capital letters indicate the dose of N fertilizer) in the same column were not significantly different at 5% Tukey test, * significant at p < 0.05, ** significant at p < 0.01, ns = not significant p > 0.05

The NERICA4 varieties and the N level significantly affected grain yield (*Table 3*). The increase in N fertilizer significantly improved the grain yield (moisture content of 14%) of all varieties. The grain yield of NERICA4-NUE12 was higher than NERICA4-WT across various doses of N fertilizer and tended to have the same pattern of improvement (*Fig. 4; Table 3*). The results of this study were in line with the research of Tiong et al. (2021), which stated that the transgenic rice plants expressing *HvAlaAT* showed an upgrade in seed production.

***Table 3**. The grain yield of NERICA4-NUE lines and NERICA4-WT across different levels of N fertilizer*

N dose (kg N/ha)	Line/variety	Grain yield (t/ha)
60	NERICA4-NUE2	3.68 ± 0.60 aA
	NERICA4-NUE9	3.72 ± 0.27 aA
	NERICA4-NUE12	4.50 ± 0.54 bA
	NERICA4-WT	3.58 ± 0.59 aA
120	NERICA4-NUE2	4.38 ± 0.62 aB
	NERICA4-NUE9	4.57 ± 0.57 aB
	NERICA4-NUE12	5.14 ± 0.29 bB
	NERICA4-WT	4.59 ± 0.68 aB
180	NERICA4-NUE2	5.48 ± 0.45 aC
	NERICA4-NUE9	4.40 ± 0.69 aC
	NERICA4-NUE12	5.68 ± 0.37 bC
	NERICA4-WT	5.23 ± 0.61 aC
NERICA4 (V)		**
N level (N)		**
V X N		Ns

The mean values followed by the same letter for each factor (lowercase letters indicate line/variety, capital letters indicate the dose of N fertilizer) in the same column were not significantly different in a 5% level Tukey's test, Grain yield at 14% moisture content, * significant at p < 0.05, ** significant at p < 0.01, ns = not significant p > 0.05

Selvaraj et al. (2016) evaluated the NERICA4-NUE lines (NUE-1, NUE-2, NUE-3, and NUE-6) in three field trials at CIAT, Palmira in Colombia, under rain-fed upland conditions during the dry and rainy season 2012-2014. The results presented that the NERICA4-NUE lines showed up to a 30% yield increase compared to sibling nulls or wild-type under limiting N50% (90 kg/ha). The NERICA4-NUE lines used N more efficiently than sibling nulls or wild-type. Our findings performed that the NERICA4-NUE lines tested at a confined field trial at IAERI under rain-fed conditions during the

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rainy season generally produced higher yields than the NERICA4-WT, so all the lines were assumed to be well-adapted and suitable for cultivation in Indonesia. The NERICA4-NUE2 was one of the genetic materials used by Selvaraj et al (2016). In our experiment, the NERICA4-NUE2 showed no difference in grain yield from the NERICA4-WT. It was different from the result of Selvaraj et al (2016). But there was one promising line, NERICA4-NUE12 performed higher grain yield significantly with NERICA4-WT in all N levels.

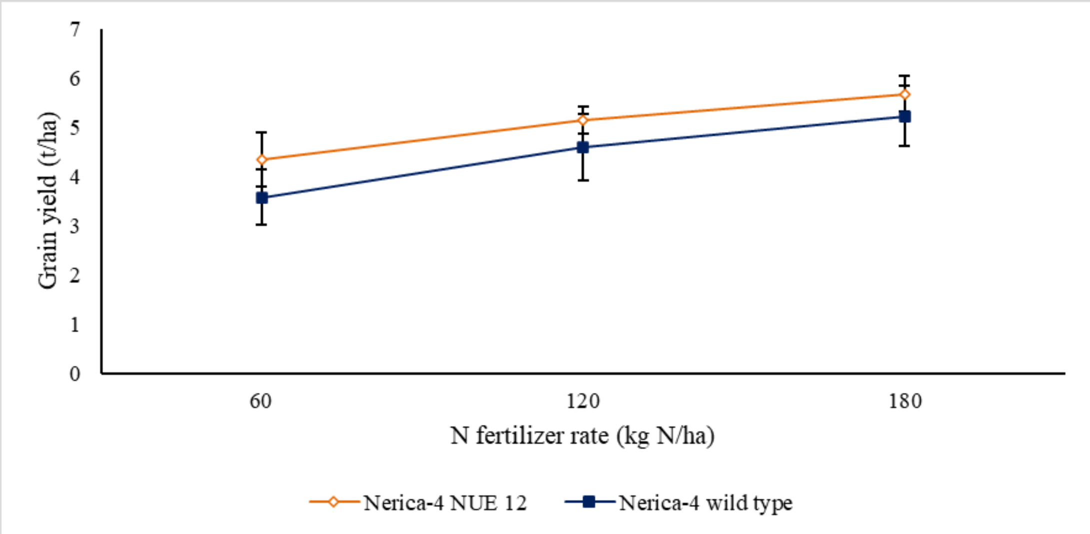

***Figure 4.** The grain yield comparison between NERICA4-NUE 12 and NERICA4-WT across different N levels. Bars represent the means ± s.d. of four replicates. Means labelled with * indicate a significant grain yield difference from WT at p < 0.05*

The plant growth and yield components influenced the grain yield of varieties of NERICA4. The grain yield of varieties of NERICA4 was significantly and positively correlated with N uptake, total grains per panicle, 1000-grain weight, and plant height (*Table 4*). The plant height of the varieties of NERICA4 was also positively correlated with N uptake, total grains per panicle, and 1000-grain weight, each with $p < 0.01$.

***Table 4.** The correlation (rvalue) among yield components and plant growth in NERICA4-NUE lines and NERICA4-WT*

Traits	GY	NU	TG	FG	GW	PH	MT
NU	0.337*						
TG	0.525**	0.202					
FG	-0.087	-0.081	-0.012				
GW	0.323*	0.120	0.238	0.188			
PH	0.719**	0.481**	0.533**	-0.005	0.527**		
MT	0.257	-0.093	0.532**	-0.203	-0.071	0.213	
PT	0.080	-0.072	0.144	-0.152	-0.275	-0.053	0.276

GY = grain yield, NU = N uptake, TG = grain number per panicle, FG = percentage of filled grains per panicle, GW = 1000-grain weight, PH = plant height, MT = number of maximum tillers per hill, PT = number of productive tillers per hill, * significant at $p < 0.05$, ** significant at $p < 0.01$

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N uptake and N use efficiency

The amount of N absorbed by the rice plants is influenced by the existing N in the soil. The plant absorbs the available N in ammonia (NH_4^+) and nitrate (NO_3^-) for its growth and development. The dynamic of the available NH_4^+ in the soil is presented in *Figure 5*. The content of NH_4^+ generally decreased during plant growth. The flooding creates anaerobic conditions in the field, resulting in high ammonium (NH_4^+) as the primary form of available N (Yi et al., 2019). The decrease of NH_4^+ in the soil from the active tillering stage to the maturity stage of the rice plants was caused by an increase in the root volume of the rice. It showed a close relationship between the decrease in soil NH_4^+ content and the increase of root volume of the rice (Dewi et al., 2018). The relatively high ammonium content was detected in NERICA4-NUE9.

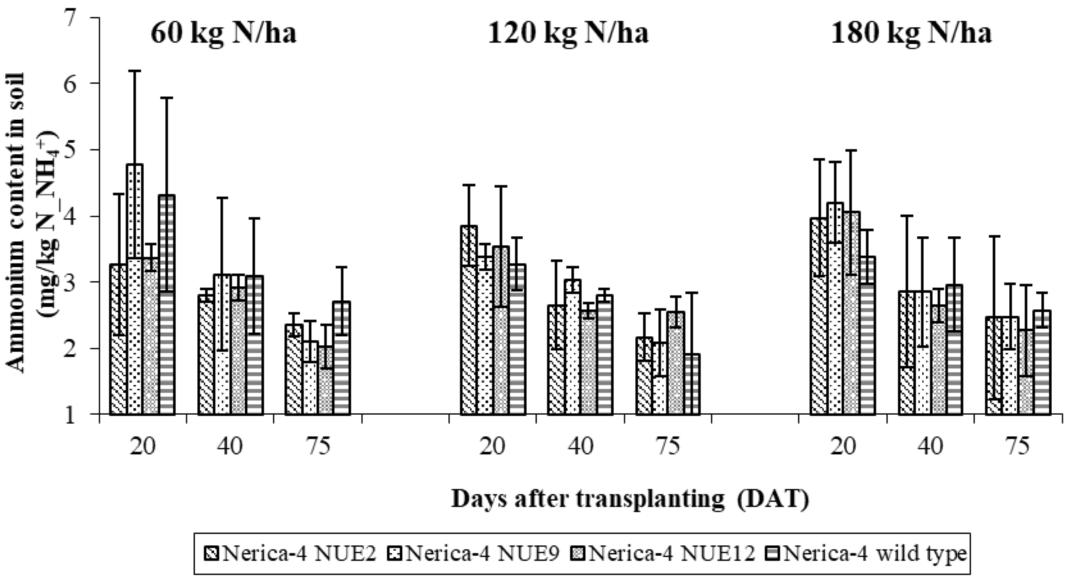

Figure 5. *The ammonium content in soils in which NERICA4-NUE lines and NERICA4-WT were planted across three levels of N fertilization. Bars represent the means ± s.d. of four replicates. Means labelled with * indicate a significant ammonium content difference from WT at $p < 0.05$*

The NERICA4 varieties and the N level significantly affected the total N uptake of rice plants (*Table 5*). The increase in the N fertilizer rate significantly increased nitrogen uptake. It increased by 15.9% at 120 kg N/ha and 22.3% at 180 kg N/ha. The increase in N uptake and use capacity improved the efficiency of N uptake and use in transgenic rice. In the research of Lebedev et al. (2021) and Murchie et al. (2009), rice plants expressing the *HvAlaAT* gene might have impaired pyruvate levels, which influence the level of TCA cycle activity. It can produce more energy to promote N uptake and use and, subsequently, plant growth and development.

The average N uptakes of NERICA4-NUE2, NERICA4-NUE9, NERICA4-NUE12, and NERICA4-WT were 80, 87, 119, and 136 kg N/ha, respectively, at all N doses. The average N uptakes of NERICA4-NUE lines at 60, 120, and 180 kg N/ha are 85, 99, and 122 kg N/ha, respectively, which were lower than those of NERICA4-WT (*Table 5*). The relationship between N uptake and grain yield corresponded with the strong correlation

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(*Tabel 4*). These results corroborated the research of Yang et al. (2019), which stated that a correlation between N uptake and utilization directly affected plant (wheat) yield.

The NERICA4 varieties and the N level significantly affected nitrogen use efficiency (NUE). It was substantially higher at a lower dose of N fertilizer (*Table 5*). NUE value for the NERICA4 varieties at 180 kg N/ha was relatively lower than for the values at 60 and 120 kg N/ha. It indicated that the rice plants effectively absorbed N nutrients from fertilizers at lower N levels. A higher dose of N fertilizer caused partial loss of N from the fertilizer.

Table 5. *The nitrogen use efficiency (NUE) value of NERICA4-NUE lines and NERICA4-WT across different N fertilizer rates*

N dose (kg N/ha)	Line/variety	N uptake (kg N/ha)	NUE value (%)$^{1)}$	Ratio of N uptake and grain yield (kg N/t grains)$^{2)}$
60	NERICA4-NUE2	83.0 ± 25.0 ^{aA}	138 ± 42 ^{aB}	22.7 ± 7.5 ^{aA}
	NERICA4-NUE9	75.3 ± 13.6 ^{aA}	134 ± 15 ^{abB}	22.0 ± 4.2 ^{abA}
	NERICA4-NUE12	98.2 ± 6.8 ^{abB}	164 ± 11 ^{abB}	22.0 ± 2.8 ^{abA}
	NERICA4-WT	134.3 ± 85.3 ^{bA}	174 ± 75 ^{bB}	36.3 ± 19.7 ^{bA}
120	NERICA4-NUE2	80.8 ± 22.2 ^{aAB}	68 ± 18 ^{aA}	18.4 ± 4.6 ^{aA}
	NERICA4-NUE9	102.8 ± 11.1 ^{aAB}	86 ± 18 ^{abA}	22.6 ± 4.9 ^{abA}
	NERICA4-NUE12	113.5 ± 50.3 ^{abAB}	95 ± 42 ^{abA}	22.0 ± 9.5 ^{abA}
	NERICA4-WT	138.8 ± 10.1 ^{bAB}	116 ± 8 ^{bA}	31.0 ± 7.0 ^{bA}
180	NERICA4-NUE2	99.2 ± 19.$^{7\ aB}$	59 ± 8 ^{aA}	19.4 ± 2.9 ^{aA}
	NERICA4-NUE9	122.8 ± 17.3 ^{aB}	73 ± 3 ^{abA}	30.3 ± 3.2 ^{abA}
	NERICA4-NUE12	145.3 ± 9.6 ^{abB}	81 ± 5 ^{abA}	25.7 ± 2.9 ^{abA}
	NERICA4-WT	135.8 ± 11.1 ^{bB}	75 ± 7 ^{bA}	26.3 ± 4.5 ^{bA}
NERICA4 (V)		**	*	**
N level (V)		*	**	ns
V X N		ns	ns	ns

The mean values followed by the same letter for each factor (lowercase letters indicate line/variety, capital letters indicate the dose of N fertilizer) in the same column were not significantly different in a 5% level Tukey's test; 1) NUE = (plant N uptake/N fertilizer use) X 100%; 2) Index NUE = plant N uptake/grain yield; * significant at $p < 0.05$, ** significant at $p < 0.01$, ns = not significant $p > 0.05$

NERICA4 varieties only significantly affected the amount of N nutrients absorbed by the rice plants (*Table 5*). The absorbed N nutrients are used to produce 1 ton grains, known as the index of N uptake. To determine the plant NUE one can use the index of N uptake. A lower index value means the plant can use N nutrients more efficiently in yielding 1 ton of dry grains (14% moisture content). As presented in *Table 5*, NERICA4-NUE2 had the lowest index of N uptake with a sequence of NERICA-WT > NERICA4-NUE9 > NERICA4-NUE12 > NERICA4-NUE2 and index values of 31.2, 25, 23.2, and 20.2 kg N/ton grains, respectively. All lines of NERICA4-NUE utilized N nutrients more efficiently to generate grains than NERICA4-WT. NERICA4-NUE2 and NERICA4-NUE12 used N nutrients more efficiently than NERICA4-WT.

Nitrous oxide flux

One form of N that is lost to the environment is nitrous oxide (N_2O). It is the intermediate product of the nitrification and denitrification processes in plants. The quantity of N_2O

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emissions from the soil depends on the amount of applied N fertilizer, the availability of NH_4^+ and NO_3^- in soil, soil moisture, and temperature. Indirect emission can cause N_2O emissions due to the flow of N from the location of fertilizer application to the surrounding ecosystem through volatilization, leaching, and erosion (Islam et al., 2018).

The observation of N_2O flux was performed in 12 and 40 days after transplanting (DAT). The NERICA4 varieties and the N level did not significantly affect N_2O emissions (*Fig. 6*). However, N_2O emissions from NERICA4-NUE lines were lower than those from NERICA4-WT in 12 DAT and 40 DAT. NERICA4-NUE9 produced the lowest N_2O emissions amongst other NERICA4-NUE.

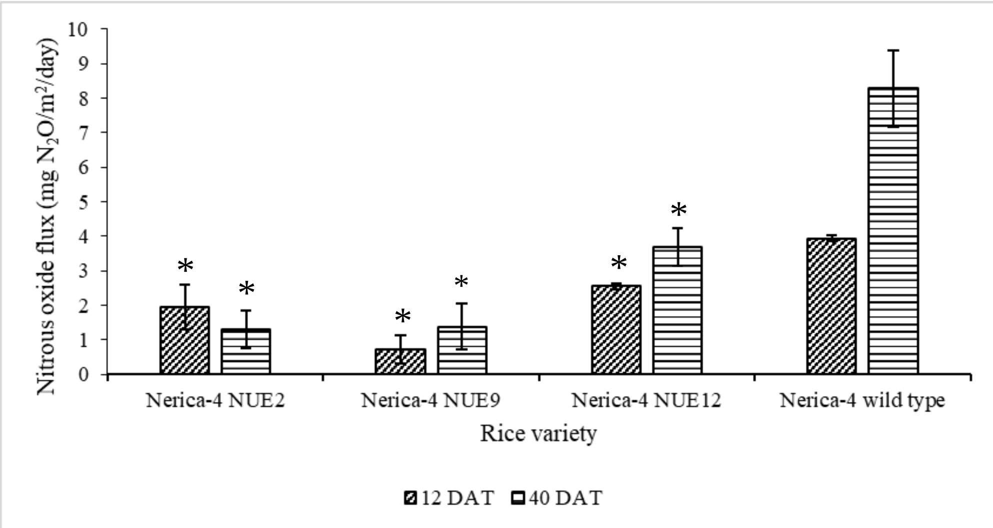

Figure 6. *The nitrous oxide flux of NERICA4-NUE lines and NERICA4-WT in 12 DAT and 40 DAT. Bars represent the means ± s.d. of four replicates. Means labelled with * indicate a significant nitrous oxide flux difference from WT at $p < 0.05$*

Conclusion

Our findings presented that NERICA4-NUE lines were well-adapted to the rain-fed agro-ecosystem in Indonesia. The increase in N fertilizer rate generally improved plant growth parameters (plant height and tiller number) of NERICA4 varieties. The tiller number of NERICA4-NUE lines was higher than that of NERICA4-WT for high N doses (120 and 180 kg N/ha). The grain yield (14% moisture content) of NERICA4-NUE12 was higher than that of NERICA4-WT for all doses of N fertilizer. There was a strong correlation between N uptake and grain yield. NERICA4-NUE12 utilized N nutrients to produce grains more efficiently than others in low N doses. This experiment also found that NERICA4-NUE lines emitted N_2O lower than NERICA4-WT in 12 DAT and 40 DAT. However, further field trial is needed, especially in the dry season, to confirm this finding. The cultivation of NERICA4-NUE lines is potentially more environmentally friendly and economically profitable.

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