

## Effect of *Gluconacetobacter diazotrophicus* inoculation and reduced nitrogen fertilizer on yield and growth parameters of rice varieties

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**ABSTRACT:** This study aimed to investigate effects of nitrogen (N) fertilizer rates and inoculation of rice seeds with N-fixing bacterium *Gluconacetobacter diazotrophicus* on the growth parameters and yield of OM5451 and OM6976 rice varieties in the Vietnamese Mekong Delta region. Nitrogen fertilizer rates of 50 kg N.ha<sup>-1</sup> and 100 kg N.ha<sup>-1</sup> were used, with latter reflecting farmer practice. Three rice seed inoculation methods were also employed: Seeds soaked in water for 24 hours and allowed to stand for 30 hours (control) (B<sub>0</sub>); Seeds soaked in water for 24 hours and inoculated with *G. diazotrophicus* for 30 hours (B<sub>1</sub>); Seeds soaked with *G. diazotrophicus* in water for 24 hours and allowed to stand for 30 hours. Applying 50 kg N.ha<sup>-1</sup> without combining with NFB bacterium in this experiment generally resulted in less tillers, shorter plants, a lower SPAD index, and lower grain yield. Combining *G. diazotrophicus* bacterium with reduced N fertilizer of 50 kg N.ha<sup>-1</sup> demonstrated rice growth and yield may be maintained in both varieties compared to 100 kg N.ha<sup>-1</sup>. These results providing a firm foundation for future research of adding NFB to paddy soils to decrease the N fertilizer requirement.

Index terms: alluvial soils, nitrogen-fixing bacterium, paddy soil, seed, Vietnam.

**RESUMO:** Este estudo teve como objetivo investigar os efeitos das doses de nitrogênio (N) do fertilizante e da inoculação de sementes de arroz com a bactéria fixadora de nitrogênio *Gluconacetobacter diazotrophicus* sobre os parâmetros de crescimento e produtividade das variedades de arroz OM5451 e OM6976 na região do Delta do Mekong Vietnamita. Foram utilizadas doses de nitrogênio fertilizantes de 50 kg N.ha<sup>-1</sup> e 100 kg N.ha<sup>-1</sup>, sendo que a última reflete a prática do agricultor. Três métodos de inoculação de sementes de arroz também foram empregados: sementes embebidas em água por 24 horas e deixadas em repouso por 30 horas (controle) (B<sub>0</sub>); Sementes embebidas em água por 24 horas e inoculadas com *G. diazotrophicus* por 30 horas (B<sub>1</sub>); Sementes embebidas com *G. diazotrophicus* em água por 24 horas e deixadas em repouso por 30 horas (B<sub>2</sub>). A aplicação de 50 kg N.ha<sup>-1</sup> sem combinação com a bactéria NFB neste experimento geralmente resultou em menos perfilhos, plantas mais curtas, índice SPAD mais baixo e menor rendimento de grãos. A combinação da bactéria *G. diazotrophicus* com fertilizante de N reduzido de 50 kg N.ha<sup>-1</sup> demonstrou que o crescimento e produtividade do arroz podem ser mantidos em ambas as variedades em comparação com 100 kg N.ha<sup>-1</sup>. Esses resultados fornecem uma base sólida para pesquisas futuras de adição de NFB aos solos de arroz para diminuir a necessidade de fertilizante N.

Termos para indexação: solos aluviais, bactéria fixadora de nitrogênio, solo de arroz, sementes, Vietnã.

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## INTRODUCTION

The Vietnamese Mekong Delta (VMD) region is the largest rice cultivation area in Vietnam (GRISP, 2013). It accounts for more than half of domestic rice production and approximately 90% of rice is exported (Pandey et al., 2010; Wassmann et al., 2010). In the intensification of cultivation of rice, farmers have traditionally applied more fertilizer to increase rice yield. In parallel with this the risk of loss of fertilizer through runoff, leaching, and evaporation due to the excessive fertilizers has become major problem in agricultural production systems (Can, 2002). According to Ha and Bo (2013), about 40–60% of applied fertilizers is lost from improper application leading to acidification, eutrophication, and greenhouse gas emissions.

Nitrogen (N) is an essential element for rice growth by promoting root development, flowering, as well as the uptake of other nutrients (Weil and Brady, 2017). A large amount of chemical N fertilizers have therefore often been applied to gain high-crop yield (Galloway et al., 2004). However, losses of excess N can result in many problems in the rice production system, including high input cost and environmental pollution with  $N_2O$  and  $NH_3$  gas emission (Wulf et al., 2002). Recently, some studies showed that the application of Neb26 compounds belonging to the group of urea inhibitor products (Giang et al., 2017) or combining N fertilizer with green manure (Ding et al., 2018) could slightly increase N use efficiency. However, these methods have not been completely effective in improving N use efficiency, growth, and rice yield. Giang et al. (2017) reported that the rice yield in the treatment with a 25% reduction in Neb26 N fertilizer (5.3 tons.ha<sup>-1</sup>) was not significantly different to the treatment receiving 100% inorganic N fertilizer (5.3 tons.ha<sup>-1</sup>). The rice yield was, however, lower in the treatment to which 50% Neb26 N fertilizer (5.0 tons.ha<sup>-1</sup>) was applied compared with the control treatment (Giang et al., 2017).

A biological nitrogen source should therefore be considered as an alternative approach to this problem. Nitrogen-fixing bacterium (NFB) are the most important microorganisms contributing to natural processes to fix  $N_2$  from the atmosphere into the inorganic N compounds usable by plants (Kennedy and Islam, 2001). *Gluconacetobacter diazotrophicus* is the NFB and member of the family *Acetobacteraceae* (Mehnaz and Lazarovits, 2017). This bacterium was originally isolated in sugarcane (Chawla et al., 2014), but it has also been found in natural endophytic association with potatoes (Luna et al., 2012), rice (Jha et al., 2009), and other host plants (Chawla et al., 2014). As an endophytic bacterium *G. diazotrophicus* needs to enter the seeds to be effective. Soaking seeds in the bacterium is the preferred method of application of NFB in rice. There has been no other reported use of *G. diazotrophicus* on rice seeds in investigating the growth of paddy rice.

The underlying hypothesis of this study is that the application *G. diazotrophicus* bacterium to rice seed will create a symbiotic relationship within the host plants enabling it to obtain N *via* a biological process in fixing the  $N_2$  gas from the air to the soil as a substitute for chemical N fertilizer. The aims of this study were to evaluate the difference in the tillers, plant height, SPAD index, biomass, and yield under different N fertilizer rates and *G. diazotrophicus* addition compared to standard treatments on alluvial soil in Vinh Long province of Vietnam.

## MATERIAL AND METHODS

### *Experimental site and soil characteristics*

The experiment was conducted in the paddy soil of a rice with the mono-rice farming system which is continuously cropped three times per year at Tra On district, Vinh Long province, which is in the VMD region (9°57'13" N, 105°56'54" E). The soil was classified as Mollic Gleysols, it was slightly acidic (pH 5.7), soil organic carbon (26.8 kg C.ha<sup>-1</sup>), and total N (1.00 kg N.ha<sup>-1</sup>) were considered very low level for the paddy field. The soil was rich in total P (2.36 kg P.ha<sup>-1</sup>) and available soil-P (13.0 mg.kg<sup>-1</sup>) was considered average.

### Experimental design and treatments

The field trial was laid out in a split-plot design with four replicates. To the main plots N fertilizer was applied at two rates: 50 kg N.ha<sup>-1</sup> (N<sub>50</sub>) and 100 kg N.ha<sup>-1</sup> (N<sub>100</sub>) with the latter reflecting the recommended rate for rice. To the sub-plots three seed inoculation methods were applied: Seeds soaked in water for 24 hours and allowed to stand for 30 hours (control) (B<sub>0</sub>); seeds soaked in water for 24 hours, mixed with *G. diazotrophicus* and allowed to stand for 30 hours (B<sub>1</sub>); Seeds soaked with *G. diazotrophicus* in water for 24 hours and allowed to stand for 30 hours (B<sub>2</sub>). A germination test of coated rice seeds was implemented at 25 °C under laboratory conditions. The content of *G. diazotrophicus* in the treatments was 1.0 × 10<sup>9</sup> CFU.g<sup>-1</sup>.

### Field Management

Each experimental plot had an area of 42 m<sup>2</sup> (6 m × 7 m), separated by the bunds (30 cm wide × 30 cm high). Bunds were covered with a plastic sheet installed to a depth of 50 cm below the soil surface to minimize seepage between adjacent plots and from the surrounding field. Rice cultivars used for this experiment were OM5451 and OM6976 with the dominant characteristics being high-yield and short-duration growth period of 90–97 days.

Fertilizer was applied to the trial at a rate of 50 or 100 kg N.ha<sup>-1</sup>, 30 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup>, and 30 kg K<sub>2</sub>O.ha<sup>-1</sup> as recommended by the Cuu Long Rice Research Institute, Vietnam. This application was in the form of urea, superphosphate, and potassium chloride. All phosphorus fertilizer was applied at sowing time. Nitrogen was applied 10, 20, and 40 days after sowing (DAS) 20%, 40%, and 40% of the total rate, respectively. Potassium fertilizer was split into two equal application 20 and 40 DAS.

### Measured crop parameters

Measurements of the number of tillers, plant height, leaf chlorophyll content (SPAD index), and grain yield were collected at various growth stages of the crop. The number of tillers and rice height were measured at tiller initiation (20 DAS), active tillering (30 DAS), and panicle initiation (45 DAS). These parameters were determined from samples collected in an area of 0.25 m<sup>2</sup> (0.5 m × 0.5 m) within the experimental plot. SPAD index was recorded at 30 DAS, 60 DAS, and 75 DAS.

For determination of grain yield, rice was harvested from an area of 5 m<sup>2</sup> of each plot. Grain was separated, air-dried, and then weighed. The final grain yield measured at 14% moisture.

Germination was calculated according to the following equation (Mori et al., 2012):

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated after innoculation}}{\text{Number of seeds tested}} \times 100$$

### Statistical analysis

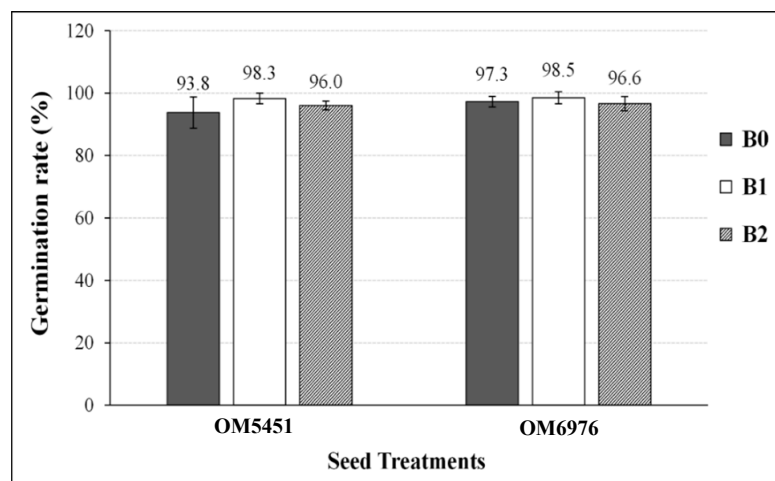
The data collected from the experiment were statistically analyzed with Genstat using a general analysis of variance. A P-value < 0.05 was considered a statistically significant difference.

## RESULTS AND DISCUSSION

The percentage of seed germination of varieties OM5451 and OM6976 under the various treatments are shown in Figure 1. Seedling emergence, which is a complex process in rice cultivation system, is controlled by many factors including water, light, temperature and phytohormones (Huang et al., 2018). In this study, the seed inoculation was implemented under laboratory conditions meaning that seed emergence was mainly depended on rice variety (Ju et al., 2000). The germination of rice seeds could be used to evaluate seedling growth and grain yield (He and Yang, 2013). On average, there were no significant differences in the germination rate between treatments (P > 0.05). The germination rate was

consistently above 93.0% in both varieties. The result of this study indicates that applying NFB to coat the seeds did not affect the germination of rice seed.

The number of tillers is an important factor influencing rice yield. Under normal conditions the number of tillers is influenced by agricultural practices and the environment (Huang et al., 2013). A recent study reported that the application of N fertilizer results increased the number of tillers in rice cultivation (Zhong et al., 2003). Another study, however, indicated that reducing N fertilizer did not affect rice tillers (Sathiya and Ramesh, 2009). For the variety OM5451 at 30 DAS in the current experiment, treatments with 50 kg N.ha<sup>-1</sup> and 100 kg N.ha<sup>-1</sup> and no bacterium had less tillers than the respective treatments with the bacterium (Table 1). These results may support the hypothesis that when *G. diazotrophicus* is applied to the seed a 50% reduction N may be possible whilst maintaining the number of tillers. This trend did not carry through to 45 DAS, however, with no significant differences between N<sub>50</sub> × B<sub>0</sub> and N<sub>50</sub> × B<sub>2</sub>, or between any of the 100 kg N.ha<sup>-1</sup> treatments. The interaction of N fertilizer rate and seed treatment methods (N × B) also resulted in a significant effect on the number of OM6976 tillers at 30 DAS (Table 1). The results show that the number of tillers was highest in N<sub>50</sub>



B<sub>0</sub>: Rice seeds soaked in water for 24 hours, incubated 30 hours; B<sub>1</sub>: Rice seeds soaked with water for 24 hours, incubated with NFB for 30 hours; B<sub>2</sub>: Rice seeds soaked with NFB for 24 hours, incubated for 30 hours

Figure 1. The seedling emergence of OM5451 and OM6976 varieties under different seeds incubation treatments.

Table 1. The number of tillers (tillers per m<sup>2</sup>) over N rates and seed treatment methods of two rice varieties.

Treatments	OM5451			OM6976		
	20 DAS	30 DAS	45 DAS	20 DAS	30 DAS	45 DAS
N <sub>50</sub> × B <sub>0</sub>	421 <sup>a</sup>	726 <sup>a</sup>	581 <sup>a</sup>	427 <sup>b</sup>	526 <sup>a</sup>	498 <sup>a</sup>
N <sub>50</sub> × B <sub>1</sub>	489 <sup>ab</sup>	895 <sup>b</sup>	654 <sup>b</sup>	373 <sup>b</sup>	653 <sup>bc</sup>	551 <sup>abc</sup>
N <sub>50</sub> × B <sub>2</sub>	454 <sup>ab</sup>	964 <sup>c</sup>	600 <sup>a</sup>	417 <sup>b</sup>	728 <sup>c</sup>	593 <sup>bcd</sup>
N <sub>100</sub> × B <sub>0</sub>	455 <sup>ab</sup>	888 <sup>b</sup>	722 <sup>c</sup>	334 <sup>b</sup>	517 <sup>a</sup>	526 <sup>ab</sup>
N <sub>100</sub> × B <sub>1</sub>	500 <sup>b</sup>	1031 <sup>cd</sup>	719 <sup>c</sup>	388 <sup>b</sup>	735 <sup>c</sup>	642 <sup>d</sup>
N <sub>100</sub> × B <sub>2</sub>	522 <sup>b</sup>	1080 <sup>d</sup>	723 <sup>c</sup>	562 <sup>a</sup>	578 <sup>ab</sup>	626 <sup>d</sup>
F-test	*	*	*	*	*	*
LSD	72.4	95.7	53.2	73.7	101	90.6
CV (%)	11.1	13.8	14.9	21.6	17.5	12.9

Means in a column for each rice variety followed by the same letter are not significantly different. In the analysis of variance, \* means P < 0.05; N and B treatments are explained in the text.

$\times B_2$  and  $N_{100} \times B_1$  treatments at 30 DAS (728 and 735 tillers, respectively), although these were not significantly different from each other or from  $N_{50} \times B_1$ , again providing support for the hypothesis that when *G. diazotrophicus* is applied to the seed a 50% reduction N may be possible whilst maintaining the number of tillers. It is also of note in this context that at 45 DAS  $N_{50} \times B_2$ ,  $N_{100} \times B_1$  and  $N_{100} \times B_2$  were not significantly different from each other.

Plant height is another important indicator of yield, and some significant differences were observed with respect to the variety OM5451 (Table 2). At 20 DAS,  $N_{50} \times B_0$  was significantly lower than  $N_{50} \times B_1$  and  $N_{100} \times B_2$  which were not different from each other. The corresponding pairs of  $N_{50}$  and  $N_{100}$  treatments at 30 DAS were different from each other, with the  $N_{100}$  treatments producing a consistently taller plant (Table 2). The same trend was observed at 45 DAS with the  $N_{50}$  treatments not different from each other, but different from the  $N_{100}$  treatments which were also not different from each other. The results of this experiment showed no significant differences between any of the OM6976 treatments at any number of DAS (Table 2).

Soil Plant Analysis Development (SPAD) is another important index which reflects the chlorophyll content of leaves with a higher chlorophyll content leading to better photosynthetic assimilation and plant performance (Ghosh et al., 2020) and rice yield (Nawaz et al., 2017). The SPAD value is therefore considered a reliable parameter for predicting the N status of rice, as affected by various natural or anthropogenic factors (Yuan et al., 2016). This ability of the bacterium to supply the host plant with fixed N (Hala, 2020) without the formation of nodules, could promote plant growth in terms of increased tiller number, chlorophyll content (SPAD), and yield (Chawla et al., 2014). Our results showed that  $N_{50}$  treatments with the inclusion of *G. diazotrophicus* bacterium were not statistically different, and in one case better, than the equivalent  $N_{100}$  treatments in both OM5451 and OM6976 varieties (Table 3). For OM5451, at 30 DAS, the results showed that SPAD in the  $N_{50} \times B_0$  was significantly lower than the  $N_{100} \times B_0$ ,  $B_1$ , and  $B_2$  treatments (Table 3). However, SPAD in the treatment which combined 50 kg N.ha<sup>-1</sup> with *G. diazotrophicus* did not differ significantly compared with the treatment received 100 kg N.ha<sup>-1</sup>  $B_0$  and  $B_2$ . Similar results were observed at 60 DAS with  $N_{50} \times B_1$  and  $B_2$  not significantly different from  $N_{100} \times B_1$  and  $B_2$  (Table 3). At 70 DAS, however,  $N_{50} \times B_2$  had a significantly higher SPAD than any of the  $N_{100}$  treatments. There were no significant interactions between N application rates and seed treatment with *G. diazotrophicus* on the SPAD index at 30 DAS in OM6976 (Table 3) and at 60 DAS  $N_{50} \times B_0$  and  $B_1$  were not significantly different from all of the  $N_{100}$  treatments which may suggest that OM6976 is a less N responsive variety. The combination effect of N fertilizer and rice seed treatment using NFB on OM6976 significantly increased rice leaf SPAD at 70 DAS. The application 50 kg N.ha<sup>-1</sup> without *G. diazotrophicus* resulted in a significantly lower SPAD than the treatment received 100 kg N.ha<sup>-1</sup> without *G. diazotrophicus*, as expected. The combination of  $N_{50}$  and the  $B_2$  seed

Table 2. Rice height (cm) over N rates and seed treatment methods of two rice varieties.

Treatments	OM5451			OM6976		
	20 DAS	30 DAS	45 DAS	20 DAS	30 DAS	45 DAS
$N_{50} \times B_0$	25.7 <sup>a</sup>	39.5 <sup>ab</sup>	49.0 <sup>a</sup>	28.2	45.2	59.0
$N_{50} \times B_1$	27.6 <sup>b</sup>	39.8 <sup>abc</sup>	50.0 <sup>a</sup>	29.0	45.3	57.5
$N_{50} \times B_2$	26.4 <sup>ab</sup>	38.4 <sup>a</sup>	50.4 <sup>a</sup>	28.6	44.0	58.4
$N_{100} \times B_0$	26.7 <sup>ab</sup>	41.2 <sup>cd</sup>	55.2 <sup>b</sup>	30.3	45.3	60.7
$N_{100} \times B_1$	26.8 <sup>ab</sup>	41.8 <sup>d</sup>	54.4 <sup>b</sup>	29.4	43.8	59.0
$N_{100} \times B_2$	27.7 <sup>b</sup>	40.8 <sup>bcd</sup>	53.8 <sup>b</sup>	28.7	43.2	60.5
F-test	*	*	*	ns	ns	ns
LSD	1.31	1.61	2.25	2.41	4.15	4.93
CV (%)	3.95	3.75	5.39	5.88	6.19	11.1

Means in a column for each rice variety followed by the same letter are not significantly different. In the analysis of variance, ns means  $P > 0.05$ , \* means  $P < 0.05$ ; N and B treatments are explained in the text.

treatment method produced a significantly larger SPAD than  $N_{50} \times B_0$  and was not different from  $N_{100} \times B_0$ , a result which supports the hypothesis but which needs to be considered in the context of  $N_{50} \times B_0$ ,  $N_{50} \times B_2$  and  $N_{100} \times B_0$  not being different from  $N_{50} \times B_1$ ,  $N_{100} \times B_1$  and  $N_{100} \times B_2$  (Table 3). The results are therefore inconclusive with respect to the benefit to SPAD from the addition of *G. diazotrophicus* although they do suggest that further investigation is warranted as the 50% reduction in N fertilizer is not detrimental to SPAD as may be naturally expected.

Nitrogen—a macronutrient for rice and other host plants—is closely related to grain yield in rice (Esfahani et al., 2008). Biological N fixation that is process converts the inert dinitrogen gas of the air ( $N_2$ ) to reactive N that becomes available to forms of the plant is probably the most important biochemical reaction in the Earth (Weil and Brady, 2017). The *G. diazotrophicus* bacterium is the rice growth-promoting rhizobacteria, and the amount of N fixed in the root environment in the agricultural system applying NFB range in 20–30 kg N.ha<sup>-1</sup> per year (Weil and Brady, 2017). In our study, *G. diazotrophicus* bacterium was applied in seeds incubation stage to increase the N fixation capacity with the N fixed enabling a 50% reduction in N fertilizer. This study hypothesized that 50 kg N.ha<sup>-1</sup> with the addition of *G. diazotrophicus* bacterium would maintain rice yield compared with applying 100 kg N.ha<sup>-1</sup> for the rice varieties OM5451 and OM6976.

Table 3. SPAD index over N rates and seed treatment methods of two rice varieties.

Treatments	OM5451			OM6976		
	30 DAS	60 DAS	70 DAS	30 DAS	60 DAS	70 DAS
$N_{50} \times B_0$	28.1 <sup>a</sup>	31.7 <sup>a</sup>	31.9 <sup>bc</sup>	34.0	35.1 <sup>ab</sup>	33.4 <sup>a</sup>
$N_{50} \times B_1$	28.8 <sup>ab</sup>	31.8 <sup>ab</sup>	31.9 <sup>bc</sup>	34.2	35.0 <sup>ab</sup>	34.8 <sup>ab</sup>
$N_{50} \times B_2$	29.2 <sup>ab</sup>	31.3 <sup>ab</sup>	33.4 <sup>c</sup>	34.8	32.5 <sup>a</sup>	37.1 <sup>b</sup>
$N_{100} \times B_0$	30.8 <sup>bc</sup>	34.5 <sup>c</sup>	28.1 <sup>a</sup>	35.3	36.9 <sup>b</sup>	37.4 <sup>b</sup>
$N_{100} \times B_1$	33.2 <sup>c</sup>	32.7 <sup>ab</sup>	30.5 <sup>b</sup>	35.8	36.2 <sup>ab</sup>	35.9 <sup>ab</sup>
$N_{100} \times B_2$	30.9 <sup>bc</sup>	33.5 <sup>ab</sup>	30.4 <sup>b</sup>	36.7	38.4 <sup>b</sup>	35.9 <sup>ab</sup>
F-test	*	*	*	ns	*	*
LSD	2.48	2.90	1.64	3.36	4.08	3.18
CV (%)	7.30	6.09	6.39	6.12	8.55	7.63

Means in a column for each rice variety followed by the same letter are not significantly different. In the analysis of variance, ns means  $P > 0.05$ ; \* means  $P < 0.05$ ; N and B treatments are explained in the text.

Table 4. Rice yield (tons.ha<sup>-1</sup>) over N rates and seed treatment methods of two rice varieties.

Treatments	OM5451	OM6976
$N_{50} \times B_0$	5.70	5.38 <sup>a</sup>
$N_{50} \times B_1$	5.61	5.33 <sup>a</sup>
$N_{50} \times B_2$	5.93	5.44 <sup>ab</sup>
$N_{100} \times B_0$	5.98	5.64 <sup>ab</sup>
$N_{100} \times B_1$	6.19	6.04 <sup>b</sup>
$N_{100} \times B_2$	6.34	5.71 <sup>ab</sup>
F-test	ns	*
LSD	0.78	0.68
CV (%)	8.53	8.32

Means in a column for each rice variety followed by the same letter are not significantly different. In the analysis of variance, ns means  $P > 0.05$ ; \* means  $P < 0.05$ ; N and B treatments are explained in the text.

There were, however, no significant differences in yield between the two N fertilizer rates and three seeds inoculation methods for the variety OM5451 (Table 4). In OM6976, the application of 50 kg N.ha<sup>-1</sup> with *G. diazotrophicus* according to the B<sub>2</sub> treatment method resulted a yield which was not significantly different from the 100 kg N.ha<sup>-1</sup> treatments (Table 4). Treatments N50 × B<sub>0</sub> and N50 × B<sub>1</sub> were significantly different from N100 × B1 but not different from other treatments. From the observed results it is not possible to say that NFB when combined with N fertilizer at 50 kg N.ha<sup>-1</sup> is the reason for sustained yield in the OM6976 rice variety compared to 100 kg N.ha<sup>-1</sup> (Table 4) but the results indicate that further investigation is justified.

## CONCLUSIONS

Rice seeds coated *G. diazotrophicus* forming a symbiotic relationship in the root environment and could supplied amount of N fixed for rice demand. The application 50 kg N.ha<sup>-1</sup> without combine with NFB bacterium resulted in reduce the number of tillers, plant height, SPAD index, and grain yield. Combining *G. diazotrophicus* bacterium with reduced nitrogen fertilizer of 50 kg N.ha<sup>-1</sup> demonstrated the maintaining rice growth and rice yield in both OM5451 and OM6976 varieties. The application of 50 kg N.ha<sup>-1</sup> combined *G. diazotrophicus* bacterium can save 50% amount of N fertilizer. More field experiments with *G. diazotrophicus* should be carried in different soil types and cropping seasons to fully estimate the effect of this bacterium on the rice growth in the VMD region.

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