Rice bran oil, a valuable edible oil extracted from rice bran with a content of 15–22%, is in high demand around the world because of its various health benefits (Lerma-Garcia et al., 2009). Rice bran consists mainly of aleurone layer and embryo fractions. Besides a high lipid content, the embryo consists of high amount of protein and vitamins leading to breeding programs trying to increase the size of the embryo. Satoh and Omura (1981), using the method of mutant egg fertilization with N-methyl-N-nitro-sourea (MNU), created rice giant embryo mutants with two to three times bigger embryo size. Several genes/quantitative trait loci (QTL) controlling for the giant embryo trait have been detected on chromosome 7 (Koh et al., 1996) and chromosome 3 (Taramino et al., 2003). Recently, some giant embryo varieties have released for cultivation to produce oil and functional food in Japan (Maeda et al., 2001; Matsushita et al., 2008; Ishii et al., 2013) and South Korea (Kim et al., 1991).

In a previous report, the promising mutant line “MGE13” with the giant embryo gene Os07g0603700 originating from the high-yielding rice cultivar Mizuhochikara (Miz) was developed (Sakata et al., 2016). The mutant giant embryo had still increased size at 10 days after pollination while the original cultivar had almost developed to its maximum size in the same time period (Itoh et al., 2005). Furthermore, the larger embryo size of mutant type compared to that of the original cultivar rice was found mainly by enhanced cell expansion, but was not significantly related to a larger number of cells in the scutellum (Nagasawa et al., 2013). Moreover, Yang et al. (2013) discovered the relationship between giant rice embryo development and shoot apical meristem (SAM) activity which is controlled by gene ge for both embryonic and post-embryonic (10 days after pollination) growth promoting plant growth parameters such as the growth rate during the vegetative stage, longer leaves, more tillers, and an increased 1,000-grain weight. Furthermore, embryo development was observed in balance with endosperm development (An et al., 2020). The regulation of endosperm development was related to the auxin and abscisic acid signaling pathways from the embryo (Yi et al., 2016; Zheng et al., 2019), in contrast, the embryo development regulated by the apoplastic nutrient pathway including sugar flow from the endosperm which is mainly a photosynthetic product (Du et al., 2018).

Higher nitrogen fertilizer applications have been shown to increase photosynthesis, dry matter accumulation, and grain yield in rice plants (Pham et al., 2003; Tang et al., 2008; Nguyen et al., 2019). Additional nitrogen fertilizer at the time of heading caused the increase of physiological parameters including photosynthesis and amino acid synthesis but reduced the cellulose content in the endosperms (Midori-kawa et al., 2014) as well as was involved in chalky tissue formation, C and N metabolism, and the regulation of ribosomal proteins (Lin et al., 2017).
The hypothesis of this study is that giant embryo development is related to greater plant growth rate during ripening period, due in part to contributions from higher nitrogen accumulation as well as leaf photosynthesis in the giant embryo type than in the original cultivar.

MATERIALS AND METHODS

Plant materials

Two rice genotypes including a giant embryo promising line MGE13, a mutant line by treating the fertilized egg cells of the high-yielding cultivar Mizuhochikara (Miz) with N-methyl-N-nitrosourea (Sakata et al., 2016) and its genetic background cultivar Miz were used in this study. The growth duration of both rice genotypes from sowing to harvesting was 140–145 days in the spring season in Northern Vietnam.

Plant growth and fertilizer application

A pot experiment was conducted in a greenhouse at Vietnam National University of Agriculture, Hanoi, Vietnam in the spring season from February to June in 2020. Seedlings of the two rice genotypes at the 4-leaf stage were individually transplanted into the 5-liter pots containing 5 kg of alluvial soil. The plants were grown in the pots supplied with chemical fertilizer at the treatment of low nitrogen—N1 (0.5 g N pot⁻¹), moderate nitrogen—N2 (1.0 g N pot⁻¹), and high nitrogen levels—N3 (1.5 g N pot⁻¹) with the same basal application of 1.0 g P₂O₅ pot⁻¹ and 1.0 g K₂O pot⁻¹. Urea (46% N), super phosphate (16% P₂O₅), and potassium chloride (60% K₂O) were used in this study. The amounts of nitrogen and potassium fertilizer were applied at the rates of 20%, 50%, and 30% at the basal, the first top-dressing (at the tillering stage), and the second top-dressing (at the panicle initiation stage), respectively, while a 100% dose of phosphorus fertilizer was applied at the basal dressing. The pots were supplied with sufficient water in the greenhouse, which was well ventilated to maintain natural temperature fluctuations.

Sampling and measurements

At the three growth stages (active tillering, heading, and dough-ripening), five plants of each treatment were randomly selected for the measurements of photosynthetic CO₂ exchange rate and stomatal conductance using a portable photosynthesis system (LI-6400, LI-COR, USA) under conditions of the photosynthetic photon flux density at 1,200 μmol m⁻² s⁻¹, air temperature at 30°C and relative humidity at 60–70%. The uppermost fully expanded leaf of each plant was selected for measuring photosynthesis. After the CER measurement, SPAD values were also measured on the same leaves using SPAD-502 (Konica-Minolta, Japan). The measured leaf was dried at 80°C and then used for nitrogen content analysis using the Kjeldahl method. The sampled plants were divided into leaves, culms plus leaf sheaths, and panicles, then oven-dried at 80°C for at least 72 h to determine the dry matter weight.

At the dough-ripening and harvesting stages, total 30 filled grains were randomly selected in each treatment (five plants in each treatment, 6 grains per plant) for measuring the areas of the embryo and endosperm. The grains were dehulled and soaked for at least 24 h in 70% ethanol at room temperature to soften them, and then the grains were cut longitudinally with a razor blade and the cross-sections were photographed and analyzed in ImageJ 1.4.3 software. The embryo/endosperm area ratio was calculated as the embryo area divided by the endosperm area.

The grain yield and yield components of five randomly selected plants of each treatment were assessed at the maturing stage. The number of spikelets per panicle and fertility were the average values of the three tallest tillers below the tallest one of each plant. The 1,000-grain weight was the weight of 1,000 filled grains in each treatment adjusted to 14% moisture content. To determine grain yield per plant, five plants of each treatment were harvested to estimate yield at 14% moisture content.

Data analysis

Data were analyzed via ANOVA using Minitab 16, according to the randomized complete block design to assess difference of genotypes, the effects of nitrogen level and of genotype×nitrogen level interaction. The significance at 5% level was analyzed using Tukey’s test.

RESULTS

Photosynthesis and dry matter accumulation

As the nitrogen fertilizer application was increased from the low level (N1) to the high level (N3), the photosynthetic rate in term of CO₂ exchange rate (CER) increased significantly in both MGE13 and Miz at all the growth stages (Fig. 1). At the heading stage, there was no significant difference in CER between MGE13 and Miz under N1 level, but the CER value was much higher in MGE13 (25.5 μmol m⁻² s⁻¹) than that in Miz (21.4 μmol m⁻² s⁻¹) under N3 level. As the nitrogen fertilizer application level increased from N1 to N2 and N3, the increase of CER values were much higher in MGE13 (55–81%) than those in Miz (40–54%).

At the heading stage, the CER was positively correlated with stomatal conductance (Fig. 2A), SPAD value (Fig. 2B) and leaf nitrogen content (Fig. 2C) in both genotypes under all nitrogen conditions.

Dry weight (DW) increased significantly throughout the growth stages as the nitrogen fertilizer application level was increased in both rice genotypes (Table 1). Under N1 and N2 levels, there were no significant differences in DW between MGE13 and Miz at all the growth stages. However, under N3 level, the DW of MGE13 was significantly higher than that of Miz at all growth stages. The plant growth rate from heading to harvesting (PGR) increased significantly in both MGE13 and Miz as increasing nitrogen level from N1 to N3 (Table 1). There was no significant difference in PGR between MGE13 and Miz under N2 condition but MGE13 showed greater PGR than Miz did under the N3 condition.

Embryo area and embryo/endosperm ratio

At the dough-ripening and harvesting stages, the embryo area was about 1.5-fold bigger in MGE13 than in Miz under all nitrogen conditions (Fig. 3A). At the dough-
ripening stage, higher nitrogen levels resulted in a larger percent increase of embryo area in MGE13 (10–17%) than in Miz (4–5%). At the harvesting stage, the embryo area also increased in both rice genotypes as the nitrogen level was increased from N1 to N2. However, when the nitrogen fertilizer increased from N2 to N3, the embryo area increased significantly in MGE13 only. As the same trend of the dough-ripening stage, the increases of embryo area were also higher in MGE13 (9–13%) than those in Miz (5–6%) when increasing the nitrogen fertilizer application level from N1 to N2 and N3 at the harvesting stage.

The embryo ratio (embryo/endosperm area ratio) was much higher in MGE13 than in Miz under all the nitrogen conditions (Fig. 3B). As the same trend of the embryo area, the embryo ratio significantly increased in both rice genotypes as increasing the nitrogen level from N1 to N2 but the embryo ratio increased significantly in MGE13 only when the fertilizer level increased from N2 to N3 at both dough-ripening and harvesting stages. A significant and positive correlation was found between the embryo area at the harvesting stage and photosynthetic rate at the heading stage in both rice genotypes, but a more positive correlation was observed in MGE13 ($r = 0.96^{**}$) than in Miz ($r = 0.52^*$) (Fig. 4A). Similarly, a more positive correlation was found between the embryo area and plant growth rate during ripening stage in MGE13 ($r = 0.93^{**}$) than in Miz ($r = 0.83^*$).

Yield components and grain yield

The number of panicles per plant was increased in both rice genotypes as the nitrogen level increased (Table 2). Under N1 and N2 conditions, there was no significant difference in the number of panicles per plant between the MGE13 and Miz. However, under N3 condition, the number of panicles was significantly larger in MGE13 than in Miz. As increasing the nitrogen level from N1 to N3, the increase of the number of panicles was much higher in MGE13 (114%) than that in Miz (100%).

The number of spikelets per panicle increased in both rice genotypes as the nitrogen level increased (Table 2). However, there was no significant difference in the number of spikelets per panicle between MGE13 and Miz under each the nitrogen level. The percentage of filled-grain increased as the nitrogen level increased from N1 to N2, but it significantly decreased as increasing the nitrogen level from N2 to N3 in both rice genotypes. There was no significant difference in the 1,000-grain weight between MGE13 and Miz under all the nitrogen levels.

Under the low and moderate nitrogen conditions, there was no significant difference in grain yield in MGE13 and Miz (Table 2). However, under the high nitrogen condition, the grain yield was significantly higher in MGE13 than in Miz. When increasing the nitrogen level from N1 to N2, the increase of the grain yield was slightly higher in MGE13 (80%) than that in Miz (73%). However, when the nitrogen level increased from N1 to N3, the increase of grain yield was much higher in the MGE13 (114%) than that in Miz (89%). Grain yield was positively correlated with the photosynthetic rate at the heading stage (Fig. 5A) and the number of panicles per plant (Fig. 5B) in both genotypes under all nitrogen conditions.

DISCUSSION

The main objective of this study was to examine the effect of the different levels of nitrogen fertilizer application on the relationship of photosynthesis with embryo development and grain yield of a giant embryo mutant line (MGE13) in comparison to its original cultivar (Miz). In all the growth stages, the CO$_2$ exchange rate (CER) increased in both rice genotypes as the nitrogen fertilizer...
application level increased (Fig. 1), which was due to increases in the related parameters including stomatal conductance, SPAD value and leaf nitrogen content (Fig. 2).

MGE13 exhibited higher photosynthetic rate than Miz did under the high nitrogen fertilizer application level (N3) only at the heading stage. This might be due to the fast development of the giant embryo releasing the larger amount of auxin and gibberellin, which urge endosperm development (Nagasawa et al., 2013; Yi et al., 2016; Zheng et al., 2019), as well as increasing the non-structural carbohydrates product translocated from leaves to the endosperm (Yoshida, 1972; Du et al., 2018). This might also be due to the longer period of embryo development after pollination in the giant embryo type for the requested high rate of non-structural carbohydrates translocation (Itoh et al., 2005). In addition, the greater photosynthetic rate in MGE13 might be due to the higher nitrogen use efficiency for both photosynthetic and agronomic parameters (Pham et al., 2003; Tang et al., 2008; Nguyen et al., 2014). Eastmond et al. (1996) indicated that the photosynthetic activity of the developing seed in angiosperms, an activity autonomy due to containing a small number of chloroplasts, is accounted for by the embryo. These authors also showed that the rate of photosynthesis by developing embryos increased from pollination until the onset of desiccation, then declines and stops at maturity. So, the giant rice embryo mutant line MGE13 in this experiment might require the larger amount of nitrogen at the flowering stage for photosynthesis of embryos.

Dry matter accumulation is a critical factor for sucrose translocation into grain formation and grain yield of rice plant (Cao et al., 2020). The grain yield may be contributed by 20–40% of the aboveground biomass before heading and the part of the matter derived from photosynthesis during the grain-filling stage (Zhang et al., 2009). Therefore, the larger amount of dry weight in MGE13, which was due to the greater photosynthetic rate at the heading stage, might contribute to higher grain yield in MGE13 than in Miz under the high nitrogen condition (Pham et al., 2003; Makino, 2011; Nguyen et al., 2019). In addition, the expression of ge related to shoot apical meristem activity might be contributing to dry matter accumulation by causing larger numbers of tillers per plant and/or longer leaves (Satoh and Iwata, 1990; Koh et al., 1996; Yang et al., 2013).

The embryo area was correlated with both photosynthetic rate at the heading stage and the plant growth rate during the ripening stage but there were higher positive correlation coefficients in MGE13 than in Miz (Fig. 4). This indicated that the giant embryo line requested both large amounts of non-structural carbohydrates, a product from leaf photosynthesis, and the other part carbohydrates from leaves and stems translocated into grains for the maintaining balanced relationship of giant embryo and endosperm development (Yoshida, 1972; Nagasawa et al., 2013; An et al., 2020).

Under low and moderate nitrogen conditions, most of the agronomical characters of MGE13 which containing the giant embryo gene ge were similar to those of Miz, which was reported in a previous report (Sakata et al., 2016). Growth analysis showed that there was no significant difference in most of the yield components between two rice genotypes such as the number of spikelets per panicle, the percentage of filled-grain and the 1,000-grain weight under all nitrogen levels (Table 2). However, under the high nitrogen condition, MGE13 exhibited the higher number of panicles per plant than Miz did, this might be due to better tiller formation in the giant embryo type (Yang et al., 2013). In addition, grain yield was crucially
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Table 1  Dry weight and plant growth rate (PGR) in MGE13 and Mizuhokihara (Miz).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Dry weight (g plant⁻¹)</th>
<th>PGR (g plant⁻¹ day⁻¹)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Tillering</td>
<td>Heading</td>
</tr>
<tr>
<td>N</td>
<td>N1</td>
<td>N2</td>
</tr>
<tr>
<td>G</td>
<td>MGE13</td>
<td>MGE13N1</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>24.0</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>35.7</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>0.45</td>
<td>0.52</td>
</tr>
</tbody>
</table>

PGR*: plant growth rate from heading to harvesting
N1, N2 and N3 are low, moderate and high nitrogen level, respectively. Values in each column within each factor followed by the same letter are not significantly different (P ≥ 0.05) by Tukey’s test.

Fig. 3  (A) Embryo area and (B) embryo ratio of MGE13 and Mizuhokihara (Miz) at the dough-ripening and harvesting stages. Vertical bars show the standard error of five replications. Columns labeled with the same letter are not significantly different at each stage (P ≤ 0.05) by Tukey’s test. N1, N2 and N3 are low, moderate and high nitrogen level, respectively. Number in the parentheses indicate the percent of N2 or N3 over the N1 value for the same genotype.

Fig. 4  Correlation of embryo area at harvest stage with CO₂ exchange rate (CER) at heading stage (A) and plant growth rate from heading to harvesting (PGR) (B) in MGE13 (black circle symbol) and Mizuhokihara (Miz) (white circle symbol) under different nitrogen levels. * and **, significant at the 0.05 and 0.01 probability levels, respectively.
associated with number of panicles per plant (Fig. 5B). Therefore, the greater number of panicles per plant might contribute to the higher grain yield in MGE13 under the high nitrogen fertilizer application condition. This finding coincided with previous reports (Pham et al., 2003; Midori-kawa et al., 2014; Nguyen et al., 2019).

In the previous report, MGE13 exhibited both high ratio of rice bran/brown rice and high lipid content in rice bran due to the giant embryo gene (Sakata et al., 2016). This study indicated that the high level of nitrogen fertilizer application would increase embryo area, thereby increase ratio of rice bran/brown rice of MGE13. Therefore, further improvements to photosynthesis and biomass production with sufficient nitrogen application are targets for increase not only in the yield potential but also in the ratio of rice bran/brown rice of giant embryo mutant rice for bran oil production.

ACKNOWLEDGEMENTS

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\[
\text{Table 2 Yield components and grain yield of MGE13 and Mizuhochikara (Miz).}
\]

<table>
<thead>
<tr>
<th>Factor</th>
<th>Number of panicles per plant</th>
<th>Number of spikelets per panicle</th>
<th>Percentage of filled-grain (%)</th>
<th>1,000-grain weight (g)</th>
<th>Grain yield (g plant⁻¹)</th>
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<tbody>
<tr>
<td>N</td>
<td>MGE13</td>
<td>5.7 c</td>
<td>154.2 c</td>
<td>84.7 b</td>
<td>25.9 a</td>
</tr>
<tr>
<td>N</td>
<td>Miz</td>
<td>8.9 b</td>
<td>163.6 b</td>
<td>88.5 a</td>
<td>25.9 a</td>
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<td>G</td>
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<td>177.9 a</td>
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<td>84.6 b</td>
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</tr>
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<td>N×G</td>
<td>MizN3</td>
<td>10.8 b (100)</td>
<td>175.8 a</td>
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N1, N2 and N3 are low, moderate and high nitrogen level, respectively. Values in each column within each factor followed by the same letter are not significantly different (P ≤ 0.05) by Tukey’s test. Number in the parentheses indicate the percent of N2 or N3 over the N1 value for the same genotype.

Fig. 5 Correlation of grain yield with CO₂ exchange rate (CER) at heading stage (A) and number of panicles per plant (B) in MGE13 (black circle symbol) and Mizuhochikara (Miz) (white circle symbol) under different nitrogen levels. **, significant at the 0.01 probability level.

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