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Effects of Fertilizer Levels on Amount and Quality Contents of Rice Bran Oil in New Japonica Rice Varieties with Giant Embryo in Northwest Region of Vietnam

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ABSTRACT: T*he experiment was conducted at Tay Bac University to evaluate the effects of different nitrogen fertilizer levels (low - 0.5g N, moderate - 1g N, and high - 1.5g N per pot with a diameter of 0.03m2 containing 5kg of rice-planting soil) on grain yield, rice bran ratio, oil content and quality in rice bran of two newly-bred rice varieties with large embryo, Ja35 and Ja23, and was performed in the summer-autumn crop 2021 and the spring crop 2022. The results show that the grain yield of both experimental rice varieties increases as the nitrogen fertilizer level increases from low to high. The grain yield of the Ja35 variety is higher than that of the Ja23 variety at all nitrogen fertilizer levels. The rice bran ratio and quality (lipid and γ-oryzanol content) of the Ja35 variety are higher than those of the Ja23 variety at all fertilizer levels. Increasing the amount of nitrogen fertilizer do not increase the rice bran ratio but improve the quality of rice bran (oil content in the bran and γ-oryzanol content in the bran oil), in which the Ja35 variety has more oil content and γ-oryzanol in the bran oil than the Ja23 variety. Therefore, increasing the nitrogen fertilizer level is necessary to increase the rice bran ratio and quality for rice varieties with thick testa.*

KEYWORDS: *Rice, Nitrogen fertilizer, rice embryo, larger embryo, lipids content, γ-oryzanol content*

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I. INTRODUCTION

Rice bran oil is produced from rice bran, and contains nutrients such as oryzanol, lecithin, tocopherols, and tocotrienols (Patel & Naik, 2004). The lipid content varies from 1.72% to 3.84% in brown rice and from 0.09% to 1.52% in white rice depending on different genotypes, cultivation conditions, and extraction methods (Kitta et al, 2005; Wang et al, 2006). Rice bran includes testa and embryo, and contains about 3/4 of the total lipids in rice grains (Juliano & Bechtel, 1985). Lawrence et al, (1998) states that the embryo contains up to 34% of the lipid content in rice grains. The large size of rice embryo increases the nutrient content (Gileung Lee et al, 2019). Satoh and Omura (1981) induce mutations using N-methyl-N-nitrosourea (MNU) to create giant rice embryos with sizes 2 to 3 times larger than those of normal rice. Koh et al (1996) identify some genes/ quantitative trait loci (QTL) that control the trait of giant embryos on chromosome 7 and chromosome 3 (Taramino et al, 2003). In recent years, some rice varieties with giant embryos have been experimentally grown for rice bran oil production in Japan (Maeda et al., 2001; Matsushita et al., 2008; Ishii et al., 2013) and Korea (Kim et al, 1991). Sakata et al., (2016) bred the rice variety "MGE13" containing the giant embryo gene Os07g0603700 from the Mizuhochikara rice variety (Miz). The potential rice varieties, Ja23 and Ja35, are bred by Vietnam National University of Agriculture from the BC3F8 generation of a backcross between the L01050 rice variety carrying qATL7 and the original high-yielding variety, Mizuhochikara. These are promising rice lines for production of rice bran oil or brown rice. At the same time, with the breeding process, addition of nutrients to rice is an important factor in increasing the amount of rice bran, and brown rice yields. Among the nutrients, nitrogen is the main nutrient that helps rice plants grow and develop well (Yoshida, 1981). Using nitrogen with different fertilizer doses has been shown to increase photosynthesis, dry matter accumulation, and grain yield of rice plants by increasing the efficiency of use of nitrogen fertilizers, thereby increasing the yield and quality of rice (Cuong Van Pham et al, 2004; Hanh Thi Tang et al, 2008; Nguyen Hong Hanh et al, 2019).

Therefore, this study is conducted to evaluate the effects of nitrogen fertilizer levels on grain yield, bran/rice ratio, and bran quality such as lipid and γ-oryzanol content in rice bran under the influence of different levels of nitrogen fertilization.

II. RESEARCH METHODOLOGY AND CONTENT

2.1. Experimental objects and materials

The experiment was conducted on two rice varieties newly bred by the Vietnam-Japan Plant Research Center of Vietnam National University of Agriculture, Ja35 and Ja23, which were selected from the BC3F8 generation of a hybrid combination between the L01050 rice variety with thick testa, and the high-yielding variety, Mizuhochikara. The experimental materials were plastic pots with a volume of 5 litres and a diameter of 25 cm, and the fertilizers used in the experiment included Ha Bac Urea (46% N), Lam Thao Superphosphate (16% P2O5), and potassium chloride (60% K2O). The soil used in the experiment was collected from rice fields in Chieng Ngan commune, Son La City, and was purified to remove impurities

2.2. Experimental design and monitoring criteria

The experiment was conducted in the summer-autumn crop 2021 and the spring crop 2022 inside a net house at Tay Bac University. The seeds were soaked and incubated until they germinated, then planted in seed trays. After the plants had three leaves, they were transplanted into the prepared pots. The experiment consisted of three nitrogen fertilization treatments: Treatment 1 with 0.5 g of nitrogen per pot (N1 - low fertilizer level); Treatment 2 with 1 g of nitrogen per pot (N2 - moderate fertilizer level); Treatment 3 with 1.5 g of nitrogen per pot (N3 - high fertilizer level). Each pot contained 5 kg of soil. The first application of fertilizer (before planting) was a combination of 100% P2O5, 30% N, and 30% K2O. The second application was when the rice plants reached the panicle initiation stage with 50% N and 50% K2O. The third application was the remaining fertilizer when the rice plants started to differentiate their reproductive organs. The experimental soil was composed of 10g Song Gianh microbial fertilizer, 0.5g of P2O5, and 0.5g of K2O. The experiment was arranged in a completely randomized design (CRD) with 5 replications, with each pot considered as one replication, and a total of 120 experimental pots. Ten seeds were randomly selected from each cluster at harvest to separate seed embryos and measure embryo length and width. The seed embryos were then dried to a constant weight to determine embryo weight. The thickness of testa layer (aleurone and pericarp) was determined by randomly selecting 10 seeds per cluster to measure using the half-cut-seed method of Khin et al (2013), in which the rice seeds were cut in half and viewed under a microscope at 40x magnification and photographed with a calibrated ruler on the same focal length. The images were analyzed using Image J software (https://imagej.nih.gov/ij) to determine the thickness of testa.

The rice grains from each cluster were milled to collect bran samples for analysis of lipid and γ-oryzanol content at Tay Bac University and Vietnam National University of Agriculture. The lipid content in rice bran was determined using the Soxhlet method (M.D. Luque de Castro, F. Priego-Capote, 2010), while the oryzanol content was determined using the HPLC method (Pallavi Nemgonda Patil, 2017).

2.3. Data analysis method

The data was processed using the GLM (General Linear Model) analysis of variance method, and linear regression was determined using Minitab 16.0.2 software according to the Tukey standard at a significance level of 0.05.

III. RESULTS AND DISCUSSION

3.1. Effect of different nitrogen levels to rice embryo and aleurone.

The level of nitrogen fertilizer significantly affects the embryo length and width, and the ratio of embryo area to seed area (the EA/SA ratio) of rice lines (Table 1). The length, width, and dry weight of embryo at the N1 level are significantly lower than those at the N2 and N3 levels. The N3 level increases the embryo width and the embryo ratio more than the N2 level, but there is no significant difference in embryo length and weight. Different rice varieties show differences in embryo characteristics, Ja35 has larger and heavier embryo than Ja23 at all nitrogen fertilizer levels. Therefore, the size of embryo is significantly affected by the amount of nitrogen fertilizer, and there is an interaction between fertilizer and line/variety characteristics on the rice embryo size. Increasing the nitrogen fertilizer level increases the size of embryo in all rice varieties. Sufficient supply of N will help rice plants to photosynthesize effectively from which the process of accumulation and conversion of products into grains is greater, helping to have larger embryos and seeds (Yoshida, 1981).

The thickness of testa of different rice varieties has a significant difference, Ja35 has a thicker testa layer than Ja23 (Table 1). On average, at different levels of nitrogen fertilization, the average thickness of testa layer of Ja23 is 23.9 µm, which is lower than that of Ja35 (25.1 µm). Changing the nitrogen level does not significantly affect the thickness of aleurone layer in rice grain. However, increasing the nitrogen level from N1 to N3 for the two rice varieties with larger embryos have a certain impact on the thickness of testa layer. Increasing the nitrogen level from low to high increases the thickness of testa layer in all experimental rice varieties (Table 1). The testa layer is a place to store lipids in the form of triacylglycerols (TAGs) (Tanaka et al, 1977). TAGs are mainly composed of phospholipids, a structure including glycerol, fatty acids, and phosphate groups. Therefore, changing the nitrogen level (N) does not significantly affect the testa layer in rice varieties. The analysis results show that different nitrogen levels do not significantly increase the thickness of testa layer of rice grains. The thickness of testa layer is from 24.3 to 24.8 µm, and the low nitrogen level (N1) has a thinner testa layer than the high nitrogen level (N3). Thus, the thickness of testa layer in experimental rice varieties with large embryos is not affected by the amount of nitrogen fertilizer but by the characteristics of varieties.

The area of embryo in both the wax ripeness and harvesting stages increases when the level of nitrogen fertilizer increases (Cuong Van PHAM et al, 2022). Different levels of nitrogen fertilizer have a clear effect on the ratio of embryo area/seed area of both rice varieties (Table 1). Increasing the nitrogen fertilizer level increases the area of embryo, leading to an increase in the ratio of embryo area/seed area. Among the two new rice varieties, Ja35 had a higher embryo area/seed area ratio than Ja23. Gileung Lee et al (2019) described a mutation (le) that can be used to breed new rice varieties with large embryo sizes and nutrient-rich seeds. Ming-Hsuan Chen et al (2020) reported that a large embryo size will result in a higher ratio of bran and embryo. Therefore, this experiment showes an interaction between the level of nitrogen fertilizer and the embryo area of rice grains. Increasing the level of nitrogen fertilizer significantly increases the ratio of embryo area/seed area for all rice varieties.

Table 1: Rice embryo and rice aleurone at different nitrogen levels

Factor		Embryo length (mm)	Embryo width (mm)	a Embryo ratio (mm^2)	*Embryo weight (mg)*	*Aleurone* (µm)
N	N1	*2.2 b*	*1.1 c*	*13.1c*	*0.7b*	*24.3ns*
	N2	*2.4 a*	*1.2 b*	*14.1b(108)*	*0.9a*	*24.4ns*
	N3	*2.5 a*	*1.3 a*	*14.5a* (111)	*1.0a*	*24.8ns*
SEm		*0.03*	*0.02*	*0.1*	*0.02*	*0.3*
CD($P_{0.05}$)		*0.88*	*0.07*	*0.31*	*0.05*	*0.9*
G	Ja35	*2.5a*	*1.3 a*	*14.3a*	*0.9a*	*25.1a*
	Ja23	*2.3b*	*1.0 b*	*13.5b*	*0.8b*	*23.9b*
SEm		0.02	0.02	0.09	0.01	0.25
CD($P_{0.05}$)		0.07	0.06	0.25	0.04	0.72
N x G	N1x Ja35	2.4b	1.2bc	13.6c	0.8b	24.8ab
	N2x Ja35	2.5a	1.3ab	14.4ab	1.0a	25.2ab
	N2x Ja35	2.7a	1.5a	14.9a	1.0a	25.4a
	N1x Ja23	2.1c	1.0d	12.6d	0.7c	23.4b
	N1x Ja23	2.3bc	1.0cd	13.7c	0.9a	24.0ab
	N1x Ja23	2.4b	1.1cd	14.1bc	0.9a	24.2ab
SEm		*0.04*	*0.03*	*0.15*	*0.03*	*0.42*
CD($P_{0.05}$)		*0.13*	*0.99*	*0.44*	*0.08*	*1.25*

N1, N2 and N3 are low, moderate and high nitrogen level, respectively. G: average of varieties; N: average of nitrogen level. [a] Embryo ratio = embryo area/seed area. ns: no significant;

Embryo weight of rice varieties shows significant changes when the nitrogen fertilizer level is increased (Table 1). Specifically, increasing the nitrogen fertilizer level leads to an increase in the average weight of embryo from 0.7 mg at the low fertilizer level to 1.0 mg at the high fertilizer level. This is because nitrogen plays a role in the formation of DNA and protein, which are the two main components of embryo. Adequate supply of nitrogen facilitates the development of embryo (Yoshida, 1981). The data analysis results show that increasing the nitrogen fertilizer level is effective in increasing the weight of embryo in all experimental rice varieties in different rice crops. Ja35 has a higher embryo weight than Ja23 both experimental crops. This indicates that the varieties with giant embryo require a large amount of non-structural carbohydrates, which are products of photosynthesis in leaves, and another part of the carbohydrates from leaves and stems to be converted into seeds for development of large embryo (Yoshida, 1972; Nagasawa et al, 2013; An et al, 2020).

3.2. Effect of nitrogen fertilizer levels on yield, lipid and oryzanol content in rice bran oil.

Mingming Hu et al (2022) stated that when increasing the amount of nitrogen fertilizer applied to both varieties of rice with giant embryos, the proportions of brown rice, milled rice, and whole rice as well as the content of GABA initially increase, but then decrease. Table 2 shows that different levels of nitrogen fertilizer affect the amount of bran obtained after milling. Increasing levels of nitrogen fertilizer increases the weight of embryo and the thickness of testa layer (Table 1), leading to an increase in the ratio of bran/brown rice for both experimental rice varieties. The Ja35 variety shows a significant difference compared to Ja23. Specifically, the average bran/brown rice ratio of Ja35 is 12.6% which is higher than that of Ja23 which only reaches 12.0%. Therefore, the amount of nitrogen fertilizer has a clear impact on the bran/brown rice ratio of different rice varieties. Ja35 has a higher bran/brown rice ratio than Ja23 at all levels of nitrogen fertilizer.

The average yield of the experimental rice varieties ranges from 24.4 to 46.8 gr/pot at different levels of nitrogen fertilizer. Increasing the amount of nitrogen fertilizer significantly increases rice yield; the high nitrogen levels result in a yield 199% higher than the low nitrogen levels. There are significant differences in grain yield among different fertilizer levels. However, the yield when increasing the amount of fertilizer from N1 to N2 is higher than that when increasing from N2 to N3. The average yield of the Ja35 variety is 44.5 g/cluster, which is significantly higher than the Ja23 variety at 29.8 g/cluster. The yield is significantly higher when fertilized with 1.5 g/pot (N3) compared to 0.5 g/pot (N1). This result is similar to the study of Mingming Hu et al (2022) on two rice varieties with giant embryo, which shows that the yield of both varieties increases with increasing nitrogen levels, and the maximum value is recorded at 135 kg Nha^{-1}.

Table 2: Effects of nitrogen levels on rice bran, lipids content and γ-oryzanol

Factor		Yield/plant (g)	Bran ratio (%)	Lipids (%)	γ-oryzanol (%)
N	N1	24.4 c	11.4c	21.7b	1.9b
	N2	38.4 b	12.6b	23.4a	2.0a
	N3	48.6 a (199)	12.9a	23.5a	2.0a
SEm		*0.6*	*0.06*	*0.22*	*0.01*
CD($P_{0.05}$)		*1.81*	*0.18*	*0.66*	*0.03*
G	Ja35	44.5 a	12.6a	24.3a	2.0a
	Ja23	29.8 b	12.0b	21.5b	29.8b
SEm		*0.5*	*0.04*	*0.18*	*0.009*
CD($P_{0.05}$)		*1.48*	*0.15*	*0.54*	*0.03*
N x G	N1x Ja35	29.2 d	11.7d	22.9b	1.9b
	N2x Ja35	48.2 b	13.0ab	24.9a	2.0a
	N3x Ja35	55.9 a	13.1a	25.0a	2.0a
	N1x Ja23	19.5 e	11.1e	20.5c	1.9b
	N2x Ja23	28.6 d	12.2c	21.9bc	1.9b
	N3x Ja23	41.3 c	12.7b	22.1b	1.9b
SEm		*0.87*	*0.09*	*0.22*	*0.011*
CD($P_{0.05}$)		*2.5*	*0.25*	*0.94*	*0.05*

N1, N2 and N3 are low, moderate and high nitrogen level, respectively..G: average of varieties; N: average of nitrogen level.

Lipids are the main component of biological membranes, composed of glycerolipids or triacylglycerols in the endoplasmic reticulum (Hyun Uk Kim, 2020). The lipid content in rice bran varies among rice varieties (Table 2). The Ja35 variety has an oil content of 24.3%, higher than Ja23 (21.5%). The reason is that Ja35 has larger embryo and thicker testa layer than Ja23, which leads to a higher lipid content in the rice bran compared to Ja23. The research by Stephen Rawsthorne (2002) shows that the metabolism to lipids in the embryo is related to the product of glycolysis in the cytoplasm. This process breaks down glucose (a product of photosynthesis) to produce products, so when photosynthesis produces many products, it facilitates the process of glycolysis and, as a result, the biosynthesis and metabolism of lipids in the embryo is more extensive. Vijayata Singh et al (2016) points out that overexpression of Arabidopsis thaliana SFD1/GLY1 increases the plastid lipid content in genetically-modified rice plants. The results in Table 2 show that increasing the level of nitrogen fertilizer increases the lipid content in rice bran from 21.7% at N1 to 23.5% at N3. However, there is no significant difference between the moderate and high levels of nitrogen fertilizer. The effect of increasing the amount of nitrogen fertilizer for rice plants in this experiment is clearly shown for different rice lines and varieties.

Ming-Hsuan Chen et al (2020) identifies a tropical japonica rice variety with a giant embryo mutation that increases the content of γ-oryzanol. In the two experimental rice lines, the γ-oryzanol content of Ja35 is significantly higher than that of Ja23 at the nitrogen levels used. Specifically, Ja23 only reaches 1.9%, which is 0.1% lower than Ja35 (2.0%).

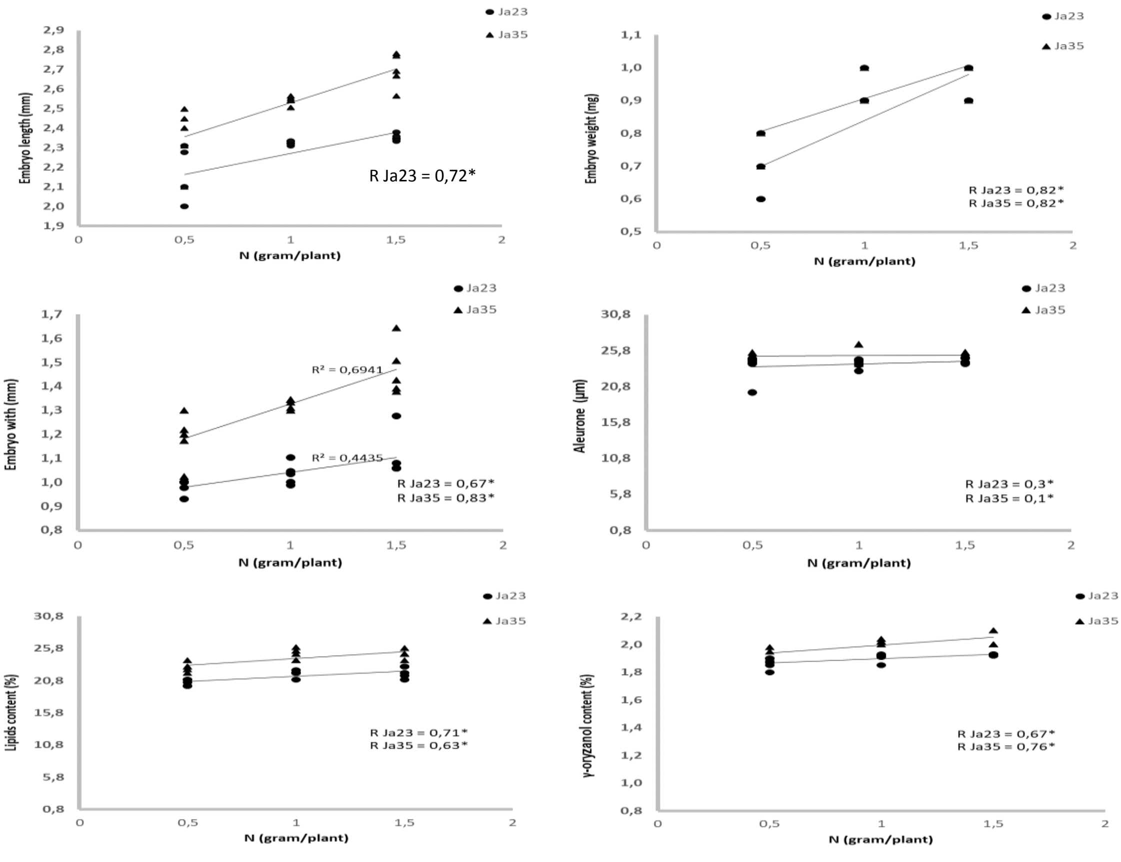
The results in Table 2 show that the content of γ-oryzanol in rice bran oil is significantly affected by the amount of nitrogen fertilizer. When the amount of nitrogen fertilizer is increased, the content of γ-oryzanol increases in all rice lines. This value ranges from 1.9-2.0% at different fertilizer levels. The highest content of γ-oryzanol is observed at the fertilizer level of 1.5 gN/pot, but the difference is not significant compared to the 1 gN/pot level. There is no significant difference in γ-oryzanol content between different experimental rice crops.

Thus, under experimental conditions, Ja35 outperforms Ja23 in terms of lipid content, γ-oryzanol content, and the ratio of bran/brown rice. This is a promising rice line in production of brown rice, or rice production for processing rice bran oil. Changing the nitrogen fertilization level increases the size and weight of embryos, leading to an increase in the lipid and γ-oryzanol content in rice bran oil. The nitrogen fertilization level of 1 gN/pot is found to be as effective as the level of 1.5gN/pot, and higher than the level of 0.5 gN/pot.

3.3. Linear regression between fertilizer nitrogen amount and some factors related to the quality of rice bran.

The relationship between the amount of nitrogen fertilizer and the embryo size of rice varieties has a positive and strong correlation. The correlation coefficient (r) for embryo length ranges from 0.72 to 0.83, and for embryo width ranges from 0.67 to 0.83. The Ja35 variety has a stronger correlation with nitrogen fertilization level than Ja23. The embryo weight of both experimental varieties depends closely on the amount of nitrogen fertilizer when the correlation coefficient (r) reaches 0.82. However, the dependence of the thickness of testa layer on the amount of nitrogen fertilizer for both varieties is not clear, only ranging from 0.1 to 0.3.

The lipid content in rice bran oil of the tested varieties shows a positive correlation and a clear level of dependence. The correlation coefficients (r) for both varieties ranges from 0.63 to 0.71. Ja35 is less affected than



Ja23. However, the γ-oryzanol content of Ja35 is more correlated to the levels of nitrogen fertilizer than Ja23 (r = 0.76). The aforementioned results indicate that production of rice can affect the lipid and γ-oryzanol content through changes in nitrogen fertilization levels.

IV. CONCLUSION

1. The high level of nitrogen fertilization is more effective than the other levels. The fertilization level of 1.5 g/pot (N3) results in the highest individual yield in all rice varieties. Ja35 variety has higher yield than Ja23.
2. Nitrogen fertilizer has an impact on the size and weight of rice embryos of rice lines. Increasing the amount of nitrogen fertilizer results in larger embryos and higher weight. The moderate level of fertilizer has similar effects on embryo length and weight as the high level. Ja35 variety has larger embryo size and weight than Ja23 variety.

3. The testa layer of Ja35 is thicker than that of Ja23. Increasing the nitrogen fertilization level does not increase the thickness of testa layer in both rice varieties.
4. The correlation between the embryo size and the nitrogen fertilization level is very clear and they have the same increasing trend. The thickness of testa layer of rice grain has no correlation with the amount of nitrogen fertilizer.
5. Ja23 contains less oil in its bran and less γ-oryzanol in its oil compared to Ja35 at all levels of fertilizer. The oil content in rice bran and the γ-oryzanol content in the oil of both rice varieties increase with increasing the nitrogen fertilizer levels, Ja35 increases more strongly than Ja23. There is no significant difference in oil and γ-oryzanol content between high and moderate levels of nitrogen fertilization. Increasing the amount of nitrogen fertilizer increases the oil content by 1.8% and the γ-oryzanol content by 0.1%.

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