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Effects of Fertilizer Levels on Amount and Quality Contents of Rice Bran Oil in New *japonica* Rice Varieties with Large Embryo in North Western Region of Vietnam

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ABSTRACT

Background: Rice bran oil is produced from rice bran, which contains nutrients such as oryzanol, lecithin, tocopherols and tocotrienol. The potential rice varieties for rice bran oil or brown rice production are the Ja23 and Ja35 lines developed by the Vietnam National of Agricultural. Using nitrogen with different levels of doses has been shown to increase photosynthesis, dry matter accumulation and seed yield in rice plants. Therefore, this study was conducted to evaluate the effects of nitrogen fertilization on grain yield, bran/ rice ratio and the quality of bran, such as lipid and γ-oryzanol content.

Methods: The experiment was conducted during 2021-2022 inside a net house. The experiment was arranged in a completely randomized design (RCD) with five replications for three nitrogen levels and two rice varieties. The rice grains from each cluster were milled to collect bran samples for yield, embryo, aleurone, lipid and γ-oryzanol content analysis.

Result: The rice bran ratio and quality (lipid and γ-oryzanol content) of the Ja35 variety were higher than those of the Ja23 variety at all fertilizer levels. Increasing the nitrogen fertilizer did not increase the rice bran ratio but improved the quality of the rice bran. Therefore, increasing the nitrogen fertilizer level is necessary to increase the rice bran ratio and quality of the rice bran for rice varieties with larger aleurone.

Key words: Aleurone, Embryo, Lipid, Rice, γ-oryzanol.

INTRODUCTION

Rice bran oil is produced from rice bran, which contains nutrients such as oryzanol, lecithin, tocopherols and tocotrienols (Patel and Naik, 2004). The lipid content of rice 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 (Bo *et al*., 2020; Kitta *et al*., 2005). Rice bran includes the aleurone and embryo, which contain about 3/4 of the total lipids in rice grains (Juliano and Bechtel, 1985) reported that the embryo contains up to 34% of the lipid content in rice grains. The size of the rice embryo affects the nutrient content (Lee *et al*., 2019; Hikaru and Takeshi, 1981; Sakata *et al*., 2016) induced 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) identified some quantitative trait loci (QTL) that control the trait of giant embryos on *chromosome 7* and *chromosome 3*. In recent years, some rice varieties with giant embryos have been tested for rice bran oil production in Japan (Maeda *et al*., 2001; Matsushita *et al*., 2008; Ishii, 2013) and Korea (Kim *et al*., 1991; Sakata *et al*., 2016) developed a rice line "MGE13" containing the giant embryo gene Os07g0603700 from the Mizuhochikara rice variety. The potential rice varieties for rice bran oil or brown rice production are the Ja23 and Ja35 lines developed by the Vietnam Academy of Agricultural Sciences from the BC3F8 generation of the cross between the L01050 rice variety carrying qATL7 and the high-yielding Mizuhochikara rice variety. Nutrient supplementation for rice is important in increasing rice bran and brown rice yields. Nitrogen is the main nutrient that helps rice plants grow and develop well (Yoshida, 1972). Using nitrogen with different fertilizer doses has been shown to increase photosynthesis, dry matter accumulation and seed yield in rice plants by increasing nitrogen use efficiency, there by increasing rice yield and quality (Pham *et al*., 2003; Tang

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et al., 2008). Therefore, this study evaluated the effects of nitrogen fertilization on grain yield, bran/rice ratio and the quality of bran, such as lipid and γ-oryzanol content.

MATERIALS AND METHODS

The experiment was conducted on two rice cultivars selected and created by the Vietnam-Japan Plant Research Center of Vietnam National University of Agriculture, Ja 23 promising line was developed by invidual selection at F8 generation from MGE13 population, (an giant embryo mutant line of high yielding variety Mizuhochikara). Ja23 carry homozygous *ge* controlling giant embryo. Ja35 was developed by pedigree selection at BC_3F_8 from backcross population between giant embryo mutant line MGE13 (original from Mizuhochikara) with thick alueron layer variety LO1050. Ja35 carries both gene *ge* controlling giant embryo and QTL *qAT7* related to thick auleron layer. 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 urea Ha Bac (46% N), superphosphate Lam Thao (16% P_2O_5) and potassium chloride (60% K_2O). The soil used in the experiment was collected from rice fields in Chieng Ngan commune, Son La City and was purified to remove impurities.

The experiment was conducted in the 2021 and 2022 Spring crops 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 prepared pots. The experiment consisted of three nitrogen fertilizer formulas: Treatment 1 with 0.5 g of nitrogen per pot (N1-Low fertilizer level); Treatment 2 with 1 g of nitrogen per pot (N2-Medium 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% P_2O_5, 30% N and 30% K_2O. The second application was when the rice plants reached the panicle initiation stage with 50% N and 50% K_2O. The third and final application was the remaining fertilizer when the rice plants started to differentiate their reproductive organs. The experimental soil was composed of 10 g microbial fertilizer (acid Humic 2.5%), 0.5 g of P_2O_5 and 0.5 g of K_2O.

The experiment was arranged in a randomized complete design (RCD) with 5 replications, with 15 pot considered as one replication, for a total of 450 experimental pots. The thickness of the aleurone layer (aleurone and pericarp) was determined by randomly selecting 30 seeds per cluster and measuring using the method of Khin, 2013 #63, in which the rice seeds were cut in half and viewed under a microscope at 40x magnification with a calibrated ruler and images were captured. The images were analyzed using Image J software(https://imagej.nih.gov/ij) to determine the thickness of the aleurone layer.

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 the Vietnam National University of Agriculture. The lipid content in rice bran was determined using the Soxhlet method (Luque de Castro and Priego-Capote, 2010) , while the oryzanol content was determined using the HPLC method (Pallavi, 2017). The data was processed using the GLM (General Linear Model) analysis of variance method and linear regression was determined using Minitab 16.0.2, Crop Stat 7.2. software according to the standard at a significance level of 0.05.

RESULTS AND DISCUSSION

Rice embryo and rice aleurone

The level of nitrogen fertilizer significantly affected the length, width and embryo ratio (DTP/DTH) of rice grains (Table 1). The length, width and dry weight of grains at the N1 level were significantly lower than those at the N2 and N3 levels. The N3 level increased the width of grains and the DTP/DTH more than the N2 level, but there was no significant difference in grain length and weight. Different rice varieties showed differences in grain characteristics, with Ja35 having larger and heavier grains than Ja23 at all nitrogen fertilizer levels. Therefore, the size of rice grains was significantly affected by the amount of nitrogen fertilizer and there was an interaction between fertilizer and a variety of traits in the size of rice grains. Increasing the nitrogen fertilizer level increased the size of grains in all rice varieties. Providing sufficient nitrogen would help rice plants photosynthesize effectively, thereby accumulating and converting products into grains more, leading to larger rice grains and grains(Yoshida, 1972).

The thickness of the aleurone of different rice varieties has a significant difference, with Ja 35 having a thicker aleurone than Ja 23 (Table 1). The average thickness of the aleurone layer in Ja 23 was 23.9 µm at all nitrogen levels, which was lower than that of Ja 35 (25.1 µm). Changing the nitrogen level did not significantly affect the thickness of the aleurone layer in rice grain. However, increasing the nitrogen level from N1 to N3 for the two rice varieties with larger grains had a certain impact on the thickness of the aleurone layer. Increasing the nitrogen level from low to high increased the thickness of the aleurone layer in all experimental rice varieties (Table 1). The aleurone layer is a place to store lipids in the form of triacylglycerols (TAGs) (Tanaka *et al.*, 1977). TAGs mainly comprise phospholipids-a structure composed of glycerol, fatty acids and phosphate groups. Therefore, changing the nitrogen level of fertilizer does not significantly affect the aleurone layer in rice varieties. The analysis results showed that different nitrogen levels did not significantly increase the thickness of the aleurone layer of rice grains. The thickness of the aleurone layer was from 24.3 to 24.8 µm and the low nitrogen level (N1) had a thinner aleurone than the high nitrogen level (N3). Thus, the thickness of the aleurone layer in experimental rice varieties with larger grains is not affected by the amount of nitrogen fertilizer but by the characteristics of the variety.

The area of the embryo in both the wax ripeness and harvesting stages increases when the level of nitrogen fertilizer increases (Cuong *et al*., 2022). Different levels of nitrogen fertilizer had a clear effect on the ratio of embryo area/seed area of both rice varieties (Table 1). Increasing the nitrogen fertilizer level increased the area of the 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, (Lee *et al*., 2019) described a mutation (le) that can be used to breed new rice varieties with large embryo sizes and high nutrient content. Hu *et al*. (2022) reported that a large embryo size will result in a higher ratio of bran and embryo. Therefore, this experiment showed an interaction between the level of nitrogen fertilizer and the embryo area of the rice grain. Increasing the nitrogen fertilizer level significantly increased the embryo area/seed area ratio for all rice varieties.

The weight of the rice grain embryo showed significant changes when the nitrogen fertilizer level was increased (Table 1). Specifically, increasing the fertilizer level led to an increase in the average weight of the embryo from 0.7 mg at low fertilizer levels to 1.0 mg at high fertilizer levels. This is because nitrogen plays a role in the formation of DNA and protein, which are the main components of the grain embryo. Adequate nitrogen supply facilitates embryo development (Hikaru and Takeshi, 1981). The data analysis showed that increasing the nitrogen fertilizer level effectively increased the embryo's weight in all experimental rice varieties in different seasons. Ja35 had a higher embryo weight than Ja23 in both experiments. This indicates that the development of a large embryo requires a large amount of non-structural carbohydrates, which are products of photosynthesis in leaves and part of the carbohydrates from leaves and stems are converted to grains for the development of a large embryo (An *et al*., 2020; Nagasawa *et al*., 2013; Yoshida, 1972). According to Hu (Hu *et al*., 2022), increasing the amount of nitrogen fertilizer applied to both rice varieties with large embryos initially increased the proportions of brown rice, polished rice and whole rice as well as the content of GABA, but then decreased them. Table 2 shows that different levels of nitrogen fertilizer affected the amount of bran obtained after milling. Increasing nitrogen fertilizer increased the weight of the embryo and the thickness of the aleurone (Table 1), leading to an increase in the bran/brown rice proportion for both experimental rice varieties. The Ja35 variety showed a significant difference from Ja23, with an average bran/brown rice ratio of 12.6% for Ja35, which was higher than the 12.0% of Ja23. Therefore, the amount of nitrogen fertilizer clearly impacts the bran/brown rice ratio of different rice varieties. Ja35 had a higher bran/brown rice ratio than Ja23 at all levels of nitrogen fertilizer.

Yield and rice bran oil quality

The average yield of the experimental rice varieties ranged from 24.4 to 46.8 g/pot at different nitrogen fertilizer levels. Increasing the amount of nitrogen fertilizer significantly increased rice yield, with high nitrogen levels resulting in a yield increase of up to 199% compared to low nitrogen levels. There were significant differences in grain yield among different fertilizer levels. However, the yield increase from N1 to N2 was higher than from N2 to N3. The average yield of the Ja35 variety was 44.5 g/cluster, significantly higher than the Ja23 variety at 29.8 g/cluster. The yield was significantly higher when fertilized with 1.5 g/pot (N3) than 0.5 g/pot (N1). These results are similar to those of (Hu *et al*., 2022) on two giant embryo rice varieties, which showed that the yield of both varieties increased with increasing nitrogen levels and the maximum value was recorded at 135 kg Nha^{-1}.

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

Factor		Embryo length (mm)	Embryo width (mm)	[a]Embryo ratio (%)	Embryo weight (mg)	Aleurone (µm)
Nitrogen levels (N)	N1: 0.5 gram N/pot	2.2	1.1	13.1	0.7	24.3
	N2: 1 gram N/pot	2.4	1.2	14.1 (108)	0.9	24.4
	N3: 1.5 gram N/pot	2.5	1.3	14.5 (111)	1.0	24.8
SEm ±		0.03	0.02	0.1	0.02	0.3
CD (P≤0.05)		0.88*	0.07*	0.31*	0.05*	NS
Varieties (V)	V1: Ja35	2.5	1.3	14.3	0.9	25.1
	V2: Ja23	2.3	1.0	13.5	0.8	23.9
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*
Nitrogens × Varieties	0.5 gram N/pot × Ja35	2.4	1.2	13.6	0.8	24.8
	1 gram N/pot × Ja35	2.5	1.3	14.4	1.0	25.2
	1.5 gram N/pot × Ja35	2.7	1.5	14.9	1.0	25.4
	0.5 gram N/pot × Ja23	2.1	1.0	12.6	0.7	23.4
	1 gram N/pot × Ja23	2.3	1.0	13.7	0.9	24.0
	1.5 gram N/pot × Ja23	2.4	1.1	14.1	0.9	24.2
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 levels, respectively. V: Average of varieties; N: Average of nitrogen level. [a]Embryo ratio = Embryo area/seed area. *Significant at P 0.05.

Lipids are the main component of biological membranes, composed of glycerolipids or triacylglycerols in the endoplasmic reticulum (H. U. Kim, 2020). The lipid content in rice bran varies among rice cultivars (Table 2). The Ja35 cultivar has a higher oil content of 24.3% than Ja23 (21.5%). This is due to the larger embryo and thicker aleurone layer in Ja35, resulting in a higher lipid content in the rice bran compared to Ja23. Rawsthorne (2002) showed that the conversion of sugars to lipids in the embryo is related to the product of the cell sap-splitting process. This process breaks down glucose (a product of photosynthesis) to produce the product, so when photosynthesis is active and produces more products, it facilitates the process of sugar splitting and, consequently, the process of lipid synthesis in the embryo. Singh *et al*. (2016) showed that overexpression of Arabidopsis thaliana SFD1/GLY1 increases the number of crystal lipids in genetically modified rice plants. The results in Table 2 show that increasing the nitrogen fertilizer has increased the lipid content in rice bran from 21.7% at N1 to 23.5% at N3. However, there was no significant difference between the average and high nitrogen fertilizer levels. The efficiency of nitrogen fertilization for rice plants in this experiment was evident among different rice cultivars(Hu *et al*., 2022), who identified a tropical japonica rice variety with a massive endosperm mutation that increased the amount of γ-oryzanol. In the two experimental lines, the γ-oryzanol content of the Ja35 line was significantly higher than that of Ja23 at the nitrogen levels used. Specifically, Ja23 only reached 1.9%, 0.1% lower than the Ja35 line (2.0%).

The results in Table 2 show that the amount of γ-oryzanol in rice bran oil is significantly affected by the amount of nitrogen fertilizer. When the amount of nitrogen fertilizer was increased, the amount of γ-oryzanol increased in all rice lines. This value ranged from 1.9-2.0% at different fertilizer levels. The highest amount of γ-oryzanol was observed at the fertilizer level of 1.5 g N/pot, but the difference was insignificant compared to the 1 g N/pot level. There was no significant difference in ã-oryzanol content between different experimental rice crops. Under experimental conditions, the Ja35 strain outperformed the Ja23 strain in terms of lipid content, γ-oryzanol content and the ratio of bran/rice. This strain shows potential for producing brown rice or rice for producing rice bran oil. Changing the nitrogen fertilization rate increased the size and weight of the grains, leading to an increase in the lipid and γ-oryzanol content of the rice bran oil. A nitrogen fertilization rate of 1 gN/pot was found to be as effective as a rate of 1.5 g N/pot and higher than a rate of 0.5 gN/pot.

Linear regression

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 compared to Ja23. The embryo weight of both experimental varieties depends closely on the amount of nitrogen fertilization, with a correlation coefficient of 0.82. However, the dependence of the aleurone layer on the amount of nitrogen fertilization 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 showed a positive correlation and a clear level of dependence (Fig 1) The correlation coefficients (r) for both varieties ranged from 0.63 to 0.71. Ja35 was less affected compared to Ja23. However, the γ-oryzanol content of Ja35 was related to higher levels of nitrogen fertilization than Ja23

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

Factor		Yield/plant (g)	Bran ratio (%)	Lipids (%)	γ-oryzanol (%)
Nitrogen levels (N)	N1: 0.5 gram N/pot	24.4	11.4	21.7	1.9
	N2: 1 gram N/pot	38.4	12.6	23.4	2.0
	N3: 1.5 gram N/pot	48.6 (199)	12.9	23.5	2.0
SEm ±		0.6	0.06	0.22	0.01
CD (P≤0.05)		1.81*	0.18*	0.66*	0.03*
Varieties (V)	V1: Ja35	44.5	12.6	24.3	2.0
	V2: Ja23	29.8	12.0	21.5	1.9
SEm ±		0.5	0.04	0.18	0.009
CD (P≤0.05)		1.48*	0.15*	0.54*	0.03*
Nitrogens × Varieties	0.5 gram N/pot × Ja35	29.2	11.7	22.9	1.9
	1 gram N/pot × Ja35	48.2	13.0	24.9	2.0
	1.5 gram N/pot × Ja35	55.9	13.1	25.0	2.0
	0.5 gram N/pot × Ja23	19.5	11.1	20.5	1.9
	1 gram N/pot × Ja23	28.6	12.2	21.9	1.9
	1.5 gram N/pot × Ja23	41.3	12.7	22.1	1.9
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 levels, respectively. V: Average of varieties; N: Average of nitrogen level.
*Significant at P≤0.05.

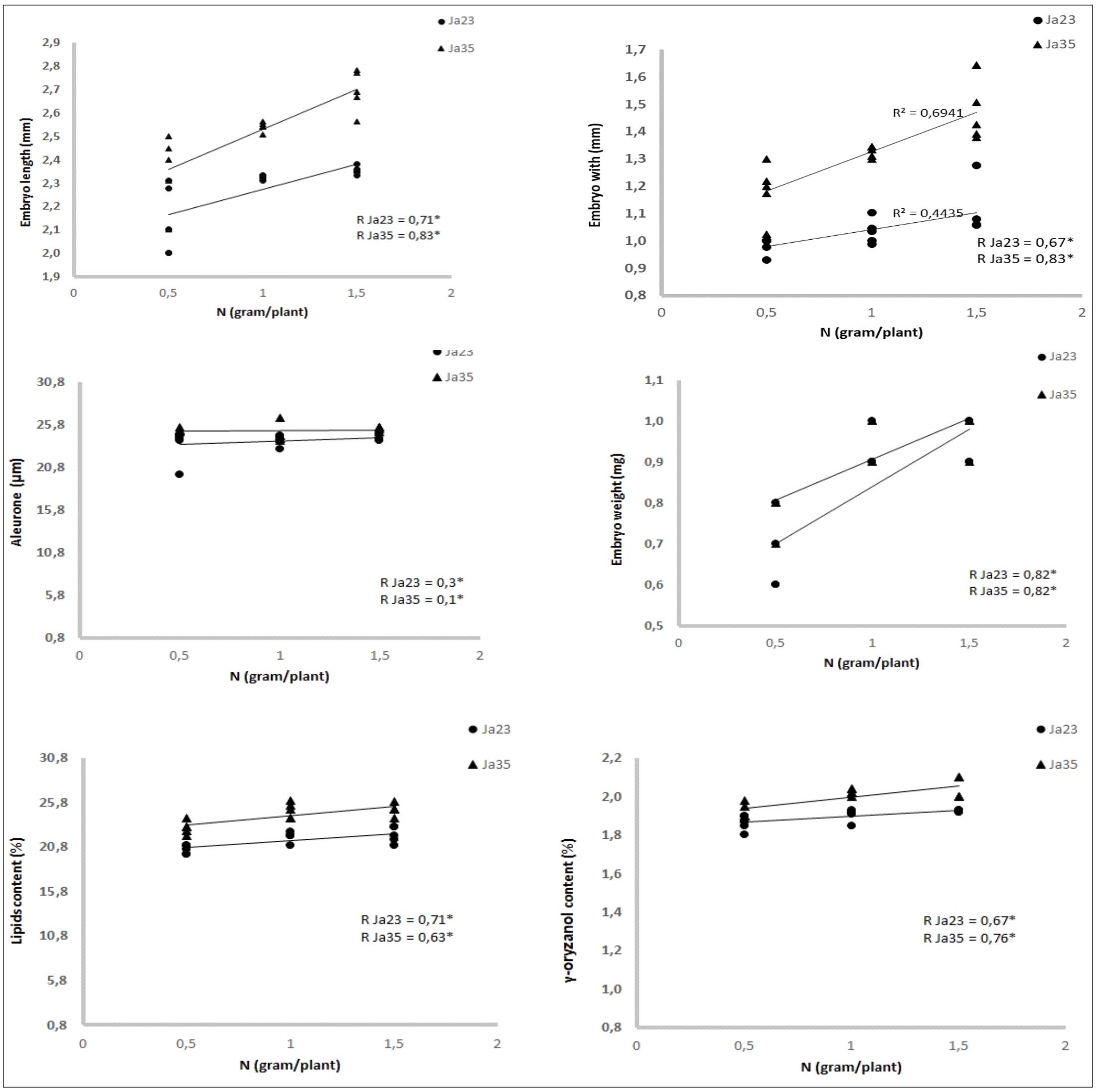

Fig 1 : The linear regression of rice bran components can affect the lipid and γ-oryzanol content through changes in nitrogen fertilization levels . *Significant at P≤0.05.

(r = 0.76). These results indicate that the production of rice can affect the lipid and γ-oryzanol content through changes in nitrogen fertilization levels.The lipid content in rice bran oil of the tested varieties showed a positive correlation and a clear level of dependence. The correlation coefficients (r) for both varieties ranged from 0.63 to 0.71. Ja35 was less affected compared to Ja23. However, the γ-oryzanol content of Ja35 was related to higher levels of nitrogen fertilization than Ja23 (r = 0.76). These results indicate that the production of rice can affect the lipid and γ-oryzanol content through changes in nitrogen fertilization levels.

CONCLUSION

The high nitrogen fertilization level is more effective than the other levels. The fertilization level of 1.5 g N/pot (N3) resulted in the highest individual yield in all rice varieties. Ja35 variety had higher yield than Ja23. Nitrogen fertilizer impacts the size and weight of rice grain embryos. Increasing the amount of nitrogen fertilizer results in larger grain embryos and higher weight. Moderate fertilizer levels affect embryo length and weight similarly to high levels. Ja35 variety has a larger embryo size and weight compared to Ja23 variety. The silk layer of Ja35 is thicker than Ja23. Increasing the nitrogen fertilization rate does not increase the thickness of the silk layer in both rice lines. The correlation between seed size and nitrogen fertilization rate is evident, with the same increasing trend. The thickness of the rice seed's silk layer does not correlate with the amount of nitrogen fertilization. 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 nitrogen fertilizer levels, with Ja35 increasing more than Ja23. There is no significant difference in oil and γ-oryzanol content between high and medium 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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