

Varietal Differences of Rice (*Oryza sativa* L.) Genotypes for Aleurone Traits Contributing to Lipid Content

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Abstract

The increasing demand for oil is more than the increasing production of oil seed crops. Rice bran oil has been used extensively in Japan, Korea, China, Taiwan, Thailand and Pakistan. Interest in rice oil is increasing because it is identified as "healthy oil" that reduces serum cholesterol. In rice seed, the lipid content deposited in the aleurone layer and embryo has been reported from 17.5 to 21.7%. To explore the useful genetic resources for aleurone traits closely related to the lipid content, 333 varieties were investigated. Brown rice seeds were cut in cross around center with razor blade and the outermost region of the cut surface stained with oil red O were measured with a fluorescence microscope. As a result, there were wide variations in stained area among varieties and many varieties with large stained area were found in Japonica group. Twenty varieties were selected according to their stained area to cover the whole range among varieties used and confirmed their thickness of aleurone layer by using micro thin section that was made by Leica cryostat with slight modification of the Kawamoto's film method (2008). The photos of sections were taken under a light microscope. The aleurone traits were measured by using Image J software. The amount and content of triacylglycerol (TAG) of brown rice without embryo were also measured. Both amount and content of TAG were significantly correlated with the area, the average thickness and the percentage of aleurone layer. These results suggest that the aleurone traits will be able to use as good indicators for selection of rice varieties with high TAG amount and content.

Keywords: aleurone layer, lipid content, rice bran oil, triacylglycerol, varietal differences.

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important food crops, primarily in East and Southeast Asia. Rice bran is a valuable by-product of rice milling that contains a high concentration of nutritional compounds including edible lipids. Rice bran oil is used in foods, feed and industrial applications. More recent efforts have emphasized the nutritional benefits of rice bran oil. It can aptly be concluded that rice bran oil is an important future source for edible and essential oils in all over the world.

Rice bran oil has been extensively used in Asian countries such as Japan, Korea, China, Taiwan, Thailand and Pakistan (Kahlon, *et al.*, 1992). The use of rice bran oil in Japan, where it is the largest volume domestically produces vegetable oil, is as a frying oil (Orthofer, 2005). In brown rice, the lipid content is from 2.3 to 3.9% (Juliano, 1977, Fujino, 1978). Embryos and aleurone layers in rice are major tissues to deposit lipids and the content in these tissues has been reported from 17.5 to 21.7%. The content in rice embryos and aleurone layers is equivalent to that of soybean and cotton seeds. The physiological role played by the aleurone layer is to provide a reserve site for minerals to deposit, which are crucial as an essential nutrient when seeds germinate

Tanaka *et al.*, 1973). In the cells, oil bodies and aleurone particles are most predominant and seem to play a more important physiological function than starchy endosperm.

Because rice is primarily used for food, starchy endosperm or milled rice is more important. Little effort has been made to study about rice bran layer. There is some information about the thickness of aleurone layer of some varieties. Genes for enlargement of embryos and aleurone layers can contribute to an increase in the oil content of rice grains (Omura and Satoh, 1981). For such reason, characterization of genetically broad rice germplasms for thickness of aleurone layer is of special importance in identifying potential varieties. Therefore, the objectives of this study were to evaluate the thickness of aleurone layer and to compare it with the storage lipid of the seed.

Materials and Methods

Half-seed staining method

All experiments were conducted at Kyushu University in 2010-2011. Three hundred and thirty three rice varieties including 176 of IRRI Core Collection, 59 of World Rice Collection and 96 of Core Collection stored at Kyushu University, were used. The brown kernels were cut in half around center with razor blade and then stained with oil red O for 5 -10 minutes and then rinsed well in 70% ethanol for 3 - 4 times. The total cut surface area and the area of the stained region were measured by using a fluorescence microscope (BZ 9000, Keyence Co. Ltd Osaka, Japan). Then the percentage of the stained area and the average thickness of the stained region can be calculated by using the following formula.

$$\text{The percentage of the stained area (\%)} = \frac{\text{the area of the stained region } (\mu\text{m}^2)}{\text{the total cut surface area } (\mu\text{m}^2)} \times 100$$

$$\text{The average thickness of the stained region } (\mu\text{m}) = \frac{\text{the area of the stained region } (\mu\text{m}^2)}{\text{the circumference } (\mu\text{m})}$$

Sectioning method

To confirm that the relationship between the thickness of the stained region and the real thickness of the aleurone layer, we selected 20 varieties according to their stained area to cover the whole range among varieties tested. The measurements were made by using micro thin section that was made by Leica cryostat with slight modification of the method of Kawamoto (2009). The sections were stained with Hematoxylin and Eosin and examined under a light microscope. The aleurone traits were measured using Image J software. Then the percentage of the aleurone layer and the average thickness of aleurone layer were calculated by the same formulae in half-seed staining method.

Measurement of Triacylglycerol (TAG)

The storage lipids (triacylglycerol: TAG) of selected varieties were analyzed. Hundred brown kernels without embryos were milled (Degree of milling: 90%) in a small-scale rice mill. The lipids were extracted from the rice bran by the method of Folch *et al.* (1957). The TAG content was measured using enzyme assay kits (Triglyceride E test; Wako Pure Chemicals Osaka, Japan).

Results and Discussion

In each trait, the varieties were significantly different. Varietal groups were also significantly different in all traits except in percentage of stained area. Most of the *indica* varieties have smaller cut surface area. The smallest one was found in wild rice and the largest one in

tropical *japonica*. The mean of total cut surface area was 4.01 mm². There was wide variation from 0.07 to 0.35 mm² for area of the stained region and the mean was 0.18 mm². There were many varieties with large stained area in *japonica* group (Figure1). The smallest stained area was found in wild rice. Some varieties with large stained area were also found in *indica*, intermediate hybrid and tropical *japonica*. Although the percentage of stained area (%) was significantly different among varieties, the varietal groups were not significant and the mean was 4.45 %. In *japonica* group, many varieties with large thickness of stained region were found (Figure1). But there were also some *indica* varieties with large thickness of stained region. The mean of the thickness of stained region was 20.15µm. The results revealed that the varieties with smallest ones were found in *indica* and wild groups.

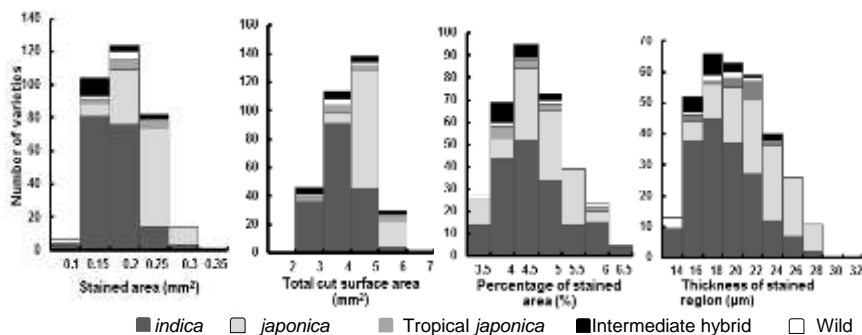
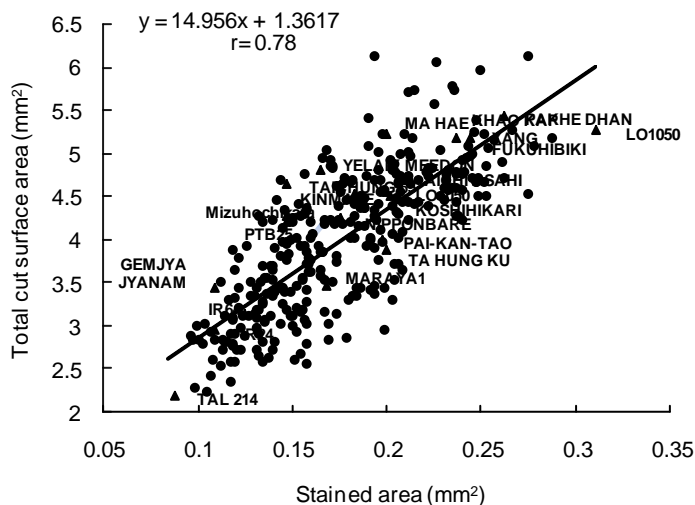


Figure 1. Frequency distribution of aleurone traits of different varietal groups.

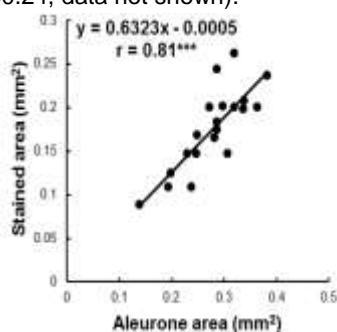


▲ indicates the selected varieties to check the aleurone layer, *** indicates the significance at 0.001 level.

Figure 2. The relationship between the stained area (mm²) and total cut surface area (mm²) of 333 rice varieties.

We selected 20 varieties according to their value of stained area to cover the whole range of varieties tested (Figure 2). The total cut surface area was nearly the same in half seed staining method and sectioning (Table.1). The mean of aleurone area was 0.27 mm² and the range was 0.12 - 0.42 mm² (Table 1). It was larger than the area of stained region. This may be due to the fact

that the stained region may become thinner than the real one depending on the time taken to wash the seed in 70% ethanol after staining. The correlation between these two parameters was significantly high ($r=0.81^{***}$) (Figure 3). We can, therefore, use the area of stained region as a good indicator for selecting varieties for thickness of aleurone layer. The range of the thickness of aleurone layer was 22.88 - 48.78 μm (Table 1). The thickness of aleurone layer was larger than and significantly correlated with that of stained region ($r=0.59^{**}$, data not shown). The percentage of aleurone layer was higher than that of the area of stained region and the correlation between these two parameters was not significant ($r=0.24$, data not shown).



*** indicates significance at 0.001 level.

Figure.3. The relationship between the stained area (mm^2) and the aleurone area (mm^2) of 20 rice varieties.

Table 1. Mean with the standard deviation of aleurone traits and Triacylglycerol (TAG) content of 20 rice varieties

	Sectioning method	Half-seed staining method
Total cut surface area (mm^2)	4.19 \pm 0.99 (1.93 - 5.60)	4.24 \pm 0.86 (1.97 - 6.00)
Aleurone area (mm^2)	0.27 \pm 0.07 (0.12 - 0.42)	0.18 \pm 0.05 (0.07 - 0.32)
Percentage of aleurone area (%)	6.45 \pm 0.95 (0.20 - 9.00)	4.14 \pm 0.83 (2.30 - 6.49)
Thickness of aleurone layer (mm)	35.26 \pm 6.12 (22.88 - 48.78)	19.23 \pm 4.28 (10.00 - 29.14)
Amount of TAG (mg) in 100 seed	18.78 \pm 7.91 (6.89 - 41.1)	
Content of TAG (mg /g of seed)	8.19 \pm 2.3 (5.02 - 14.72)	

The values in parentheses indicate the range of each parameter.

The amount of TAG increased with increasing aleurone area. The range of amount of TAG without embryo in 100 brown seed ranged from 6.89 to 41.1 mg among 20 varieties tested. It was highly correlated with the area ($r=0.71^{***}$) and thickness of aleurone layer ($r=0.67^{**}$) (Figure 4). However, the percentage of aleurone area was not correlated with the amount of TAG. The content of TAG also increased with increasing the aleurone area. The range of content of TAG without embryo in 1g of brown kernel was from 5.02 to 14.72 mg/g among 20 varieties tested. It was highly

correlated with the area ($r=0.61^{**}$), percentage ($r=0.58^{**}$) and average thickness of aleurone layer ($r=0.68^{**}$) (Figure 4).

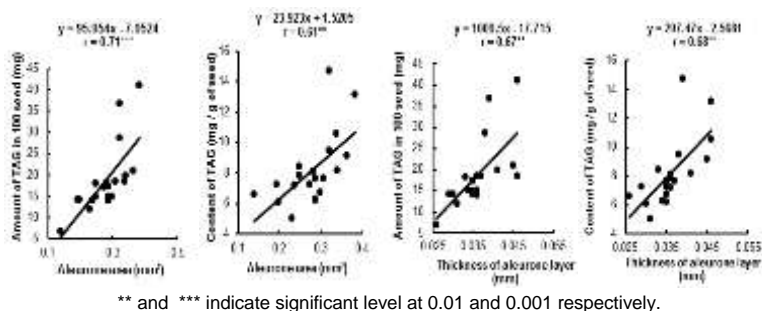


Figure 4. Relationship between TAG and aleurone traits of 20 rice varieties.

Conclusions

There was wide variation in aleurone traits among the genetically diverse rice varieties. The correlation between the aleurone area by sectioning method and the stained area by half-seed staining method was significantly high. Therefore, the stained area could be used as a good indicator for selecting varieties for thickness of aleurone layer. Furthermore, there were high correlation between aleurone area and aleurone thickness, and TAG. So, selection of varieties with high lipid content could be done without measuring directly lipid content of the varieties. A great potential was obtained to produce rice bran oil and it did not require any special cultivation since it is a by-product of the rice milling process.

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