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Khin, Ohn Mar
Graduate school of Bioresource and Bioenvironmental sciences, Kyushu University

Sato, Masao
Faculty of Agriculture, Kyushu University

Li-Tao, Tong
Graduate school of Bioresource and Bioenvironmental sciences, Kyushu University

Matsue, Yuji
Faculty of Agriculture, Kyushu University

他

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Close Association between Aleurone Traits and Lipid Contents of Rice Grains Observed in Widely Different Genetic Resources of *Oryza sativa*

Ohn Mar Khin[1], Masao Sato[2], Tong Li-Tao[1], Yuji Matsue[2], Atsushi Yoshimura[2] and Toshihiro Mochizuki[2]

([1]Graduate school of Bioresource and Bioenvironmental sciences, Kyushu University, Fukuoka 812-8581, Japan; [2]Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan)

Abstract: We investigated 321 varieties to identify the aleurone traits that are closely related to the lipid content of rice. Brown rice seeds were cut crosswise near the center with a razor blade and the cut surface was stained with Oil Red O, and then observed under integrated fluorescence microscope (BZ9000). We found wide variations among varieties in the area stained, but the *japonica* group contained many varieties with a large stained area. We selected 17 representative varieties covering the whole range of stained areas and confirmed the thickness of aleurone layer using Kawamoto's film method (micro-thin sections that were created with cryomicrotome). Aleurone traits were examined under a light microscope and measured. We found a strong correlation between the area of the aleurone layer and the stained area (r = 0.799*) and between the thickness of the aleurone layer and the thickness of the stained region (r = 0.543*). The area of the stained region therefore provides a fast and effective indicator for selecting varieties for the thickness of the aleurone layer. We also measured the amount (mg per 100 seeds) and proportion of triacylglycerols (TAGs) to 1 g of seed (mg g^{-1}) in brown rice seeds without embryos. The amount and proportion of TAG to 1 g of seed were both significantly correlated with the area, average thickness and percentage of aleurone layer. This suggests that the aleurone traits will be good indicators for the selection of varieties with high levels of TAG.**

Key words: Aleurone layer, Genotypic differences, Lipid, Rice bran oil, Triacylglycerol.

Rice (*Oryza sativa* L.) is one of the world's most important food crops. More than half of the people in the world eat rice as a main part of their diet, particularly in East and Southeast Asia. Rice bran is a valuable byproduct of rice milling that contains a high concentration of nutritional compounds, including edible lipids. The definition of rice bran proposed by FAO (1994) is "a byproduct of polishing brown rice, comprising the pericarp, aleurone layer, embryo and some endosperm" and this rice bran is the source of rice bran oil. Rice bran oil has been used in traditional foods, but recently its nutritional benefits are gathering concern. Although it is used in foods, livestock feed and industrial application, only high-quality oil is used in cooking, as a salad oil and a cooking oil. Rice bran oil has been used extensively in Asian countries such as Japan, Korea, China, Taiwan, Thailand and Pakistan (Kahlon et al., 1992). The main use of rice bran oil in Japan, where it accounts for the largest volume of domestically produced vegetable oil, is as a frying oil, because its flavor is preferred over alternative oils (Orthoefer, 2005).

Rice bran oil is a minor constituent of rough rice compared with the carbohydrate and protein contents (Orthoefer, 2005). The lipid content of rice grains is 2.3 to 3.9% (w/w) (Fujino, 1978). The embryo and the aleurone layer are major tissues where lipids accumulate, and the lipid content of these tissues has been reported to reach 17.5 to 21.7% (w/w) (Okuno, 1997). The lipid content of rice embryo and aleurone layer is equivalent to that of soybean and cotton seeds (Okuno, 1997). These lipids are not only an essential energy source for germination and growth of the plants, but also an important nutritional source for humans. Lipids are usually stored as triacylglycerols (TAGs) that are found primarily in seeds and pollen, where they serve as energy and carbon stores (Somerville et al.,

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Abbreviation: TAG, Triacylglycerol.

2000). Oil bodies are present in rice embryo and aleurone cell layers (Tanaka et al., 1977), and are mainly composed of TAGs. Based on the analysis of acyl ester bonds, embryos contained 34% of the total TAGs in the rice seed, and thus possessed less oil content than the aleurone layer (Lawrence et al., 1998).

Because rice is primarily eaten as a food, a starchy endosperm is more important than the content of rice bran oil. Thus, there have been few studies on the rice bran layer. Genes responsible for the enlargement of embryo and aleurone layer can increase the oil content of rice grains (Omura and Satoh, 1981). The characterization of a genetically broad range of rice germplasms for aleurone traits is of particular importance in identifying potential varieties.

Although Nagato et al. (1960) and Hoshikawa (1967) studied the number of aleurone cell layers and their thickness, little information is available to support the selection of potential varieties for rice improvement. The objectives of the present study are to evaluate the aleurone traits of many genetically diverse rice varieties and to study the correlation between the lipid content of brown rice excluding the embryo and its aleurone traits. However, the measurement of aleurone traits and lipid is time-consuming and laborious. Thus, we developed a fast and easy method for the measurement of aleurone traits and confirmed the correlation between the results obtained by this method and aleurone traits obtained by the cross-sectioning method. We then determined the relationship between the aleurone traits and lipid contents.

Materials and Methods

1. Half–cut seed method

We examined 321 rice varieties including 178 *indica* rice, 111 *japonica* rice, 14 tropical *japonica* rice and 18 intermediate rice accessions stored at the Institute of Genetic Resources, Faculty of Agriculture, Kyushu University. Three to five brown kernels of each variety were cut in half crosswise with a razor blade and then stained with Oil Red O dye, which is commonly used to stain lipids. Oil Red O stock solution was prepared by dissolving 0.5 g of Oil Red O (Sigma) in 100 mL of isopropanol. Oil Red O working solution was prepared with 6 mL of Oil Red O stock solution and 4 mL of distilled water. The half-cut seeds were stained in Oil Red O working solution for 5 to 10 min. They were then rinsed well in 70% ethanol three or four times. By this method, the outermost region of the half-cut seed was stained red (Fig. 1). The outermost region of the rice seed comprises the pericarp and some of the aleurone area. In a rice seed, the lipids accumulate primarily in the aleurone layer and the embryo, so we assumed that the outermost stained region represented the region of the aleurone layer.

We measured the total cut surface area which shows the seed size and the area of the stained region using an integrated fluorescence microscope (BZ9000, Keyence Co. Ltd., Osaka, Japan). Because we used non-fluorescent Oil Red O for staining, we could observe the lipids using the bright field observation mode. As compared with a standard optical microscope, a clear full-focus image could be obtained with extra light inside BZ9000. The half-cut stained seeds were placed on the stage and covered with a cover slide. Then the specimen was examined by using 4 × objective lens and the image was captured. Using the captured image, the aleurone traits were measured using a BIOREVO BZ9000 Analyzer. We then calculated the percentage of the stained area in the cut surface and the

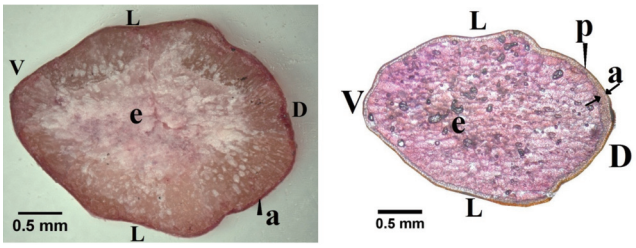

Fig. 1. Half-cut seed method (left) and cross-sectioning method (right). a, Aleurone layer; e, Endosperm; p, Pericarp; D, L, and V mean dorsal, lateral and ventral sides, respectively, of the rice seed.

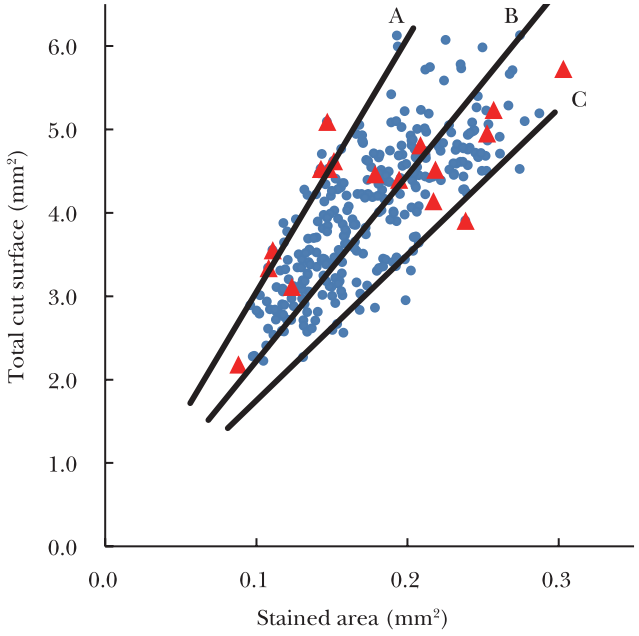

Fig. 2. Relationship between the stained area (mm^2) on the cut surface and the total cut surface area (mm^2) of 321 rice varieties. ▲ indicates the 17 varieties selected to check the relationship between the stained area and the aleurone layer. Line A represents the 30 varieties with the lowest percentage of stained areas, B represents the mean for all varieties, and C represents the 30 varieties with the highest percentage of stained areas.

average thickness of the stained region as follows. The circumference of the cut surface is the outer length of the stained region. Assuming that the difference between the outer and inner lengths of the stained region is negligible, the average thickness of the stained region can be estimated by dividing the area of the stained region with the circumference of the cut surface (outer length of the stained region).

Percentage of the stained area (%) = [area of the stained region (mm^2)/the total cut surface area (mm^2)] × 100

Average thickness of the stained region (μm) = [area of the stained region (mm^2)/circumference of the cut surface (mm)] × 1000

2. Cross-Sectioning method

The seeds used in this step were obtained from the Kyushu University farm, where they were harvested in 2010. To examine the relationship between the stained region and the aleurone layer, we selected 17 representative varieties covering the whole range of stained areas by the half-cut seed method (Fig. 2). The measurements were made using micro-thin sections obtained with a cryomicrotome (CM 1100 cryostat; Leica Co. Ltd., Heidelberg, Germany) with a slight modification of Kawamoto's film method (Kawamoto, 2009). The seeds of the selected varieties were cut in half crosswise with a razor blade; the cut was made approximately at the center of the seed to make sure the samples that we used for measurement were obtained from around the central region. The half-cut seed was placed in an embedding well, with the cut surface facing the floor of the well, the well was filled with Super cryoembedding medium (SCEM) (Leica Microsystems, Tokyo, Japan), and the sample was frozen inside the cryochamber. The samples were then sectioned with a uniform speed to produce 10-μm thick sections.

Before collecting each section, we placed a strip (1 cm × 2 cm) of adhesive film (Cryofilm type 2C-10) (Leica) on the cut surface of frozen specimen in the cryomicrotome and then produced the section. The section on adhesive film was placed on a pre-cooled glass slide, facing upwards and was air-dried for 5 to 10 min, stained with Carrazzi's hematoxylin solution for 5 to 10 min, and rinsed with distilled water for 1 min. The section was then counterstained with 0.2% eosin Y for 30 s, rinsed with distilled water for 30 s and then rinsed with 70% ethanol. We used hematoxylin and eosin to distinguish clearly between the endosperm and aleurone cell layers. Aleurone cell layers were not stained with eosin. By this method, the endosperm was stained pink and the aleurone layer was stained purple (Fig.1). The stained section was mounted on a glass slide with Super cryomounting medium (Leica). Excess mounting solution was removed using filter paper. We examined the sections under a light microscope to determine the following aleurone traits: the total area of cut surface, the aleurone area and the thickness of the aleurone layer in ventral, lateral and dorsal region. We measured these parameters using version 1.4.5 of the ImageJ software (http://rsbweb.nih.gov/ij/). All of the measurements used 5 to 10 seeds of each variety. We calculated the percentage of the aleurone layer and the average thickness of the aleurone layer using the following formulas. Assumption for the calculation of average thickness of the aleurone layer was the same as described in half-cut seed method.

Percentage of the aleurone area (%) = [area of the aleurone layer (mm^2)/the total area of cut surface (mm^2)] × 100

Average thickness of the aleurone layer (μm) = [area of the aleurone layer (mm^2)/circumference of the cut surface without pericarp (mm)] × 1000

3. TAG measurements

We used the same accessions used for the cross-sectioning analysis for TAG measurement. We analyzed the storage lipids (TAG) in each of the selected varieties. We milled 100 brown kernels without embryos (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 rice bran was homogenized with 50 mL of chloroform–methanol mixture (2:1, *v*/*v*). The mixture was filtered through filter paper (Toyo No.2 paper) into a graduated cylinder and the filtrate volume was recorded. We then added 10 mL of deionized water to the graduated cylinder, and allowed the mixture to separate into two layers. The lipid–chloroform layer was collected in a round-bottom flask, and the solvent was removed under reduced pressure and nitrogen with a rotary evaporator at 40°C. The lipid samples were transferred into 25-mL volumetric flask containing hexane, and stored at −20°C until they were analyzed. The TAG was measured using enzyme assay kits (Triglyceride E test; Wako Pure Chemicals, Osaka, Japan). The measurement was performed three times for each variety.

Results

We observed wide variations in all of the measured traits among the varieties analyzed by the half-cut seed method (Table 1). The mean of the total cut surface area was 4.01 mm^2 and the range was from 1.97 to 6.38 mm^2 (Table 1, Fig. 2). The area of the stained region ranged from 0.07 to 0.33 mm^2 and the mean was 0.18 mm^2 (Table 1, Fig. 2). The mean percentage of the stained region was 4.4% and the range was from 2.3 to 7.5% (Table 1). The mean of the thickness of the stained region was 20.1 μm and the range was from 10.0 to 35.6 μm (Table 1).

Most of the *indica* varieties have a smaller cut surface area than the other varieties. The largest was found in tropical *japonica* (Fig. 3). In the *japonica* group, many

Table 1. Mean with standard deviation of the aleurone traits of the different varietal groups determined by the half-cut seed method.

	Total cut surface area (mm^2)	Stained area (mm^2)	Percentage of stained region (%)	Average thickness of stained region (μm)
indica (178)	3.61 ± 0.72 (2.12 – 6.08)	0.16 ± 0.04 (0.07 – 0.32)	4.4 ± 0.9 (2.3 – 7.5)	19.0 ± 3.9 (10.5 – 34.5)
Intermediate (18)	3.76 ± 1.03 (2.59 – 6.00)	0.15 ± 0.05 (0.08 – 0.28)	4.1 ± 0.69 (2.6 – 5.7)	18.3 ± 3.6 (11.5 – 27.6)
japonica (111)	4.63 ± 0.61 (1.97 – 6.27)	0.21 ± 0.05 (0.07 – 0.33)	4.5 ± 0.80 (2.5 – 6.8)	22.2 ± 4.2 (10.0 – 35.6)
Tropical *japonica* (14)	4.3 ± 0.98 (2.56 – 6.38)	0.19 ± 0.04 (0.12 – 0.29)	4.4 ± 0.67 (3.2 – 6.2)	20.2 ± 2.4 (15.2 – 24.1)
Mean (321)	4.01 ± 0.86	0.18 ± 0.05	4.4 ± 0.85	20.1 ± 4.2
Range	(1.97 – 6.38)	(0.07 – 0.33)	(2.3 – 7.5)	(10.0 – 35.6)
Varieties (*F*value)	45.91***	7.84***	5.06***	4.57***
Varietal groups (*F*value)	18.37***	1.98^{ns}	1.42^{ns}	0.20^{ns}

The values in parentheses indicate the range of values for each parameter. *, *** and ns indicate significance at $p < 0.05$, $p < 0.001$, and non-significant, respectively.

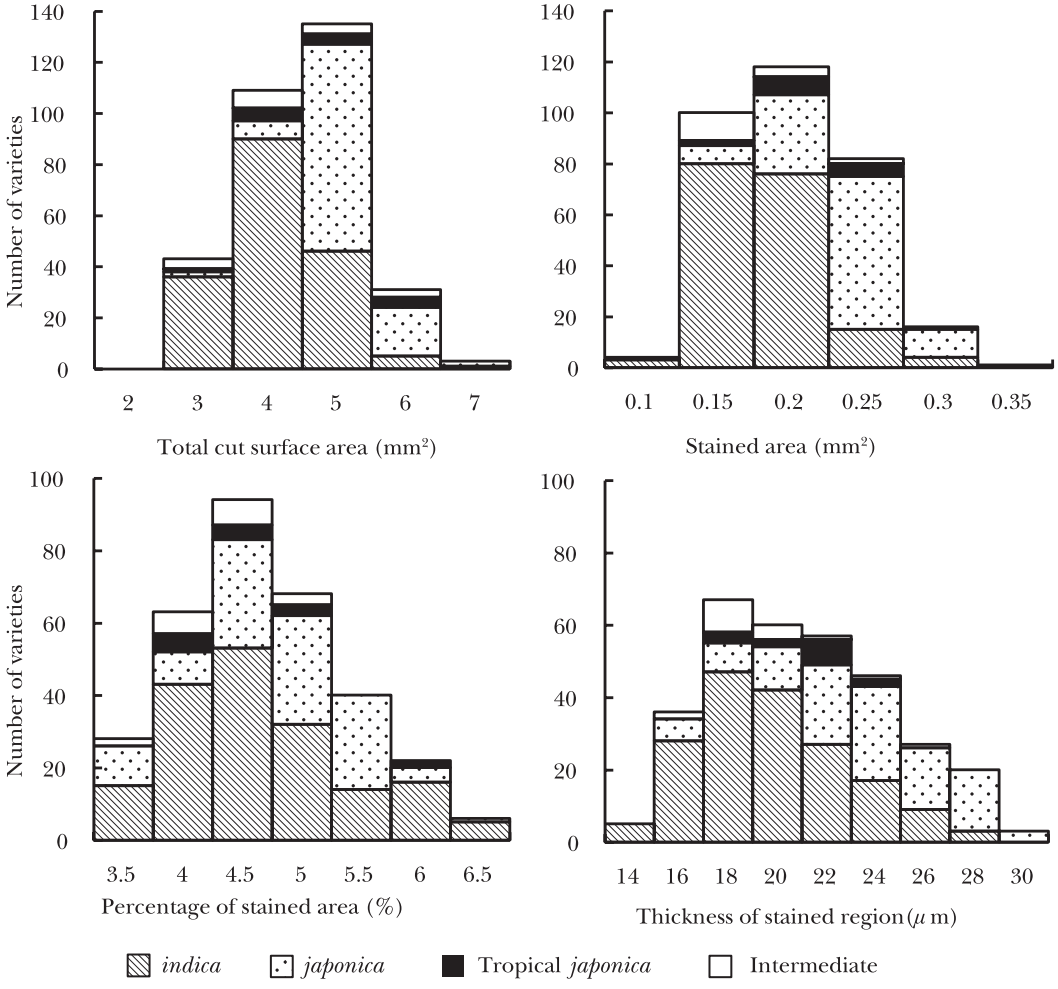

Fig. 3. Frequency distribution of the aleurone traits in different varietal groups.

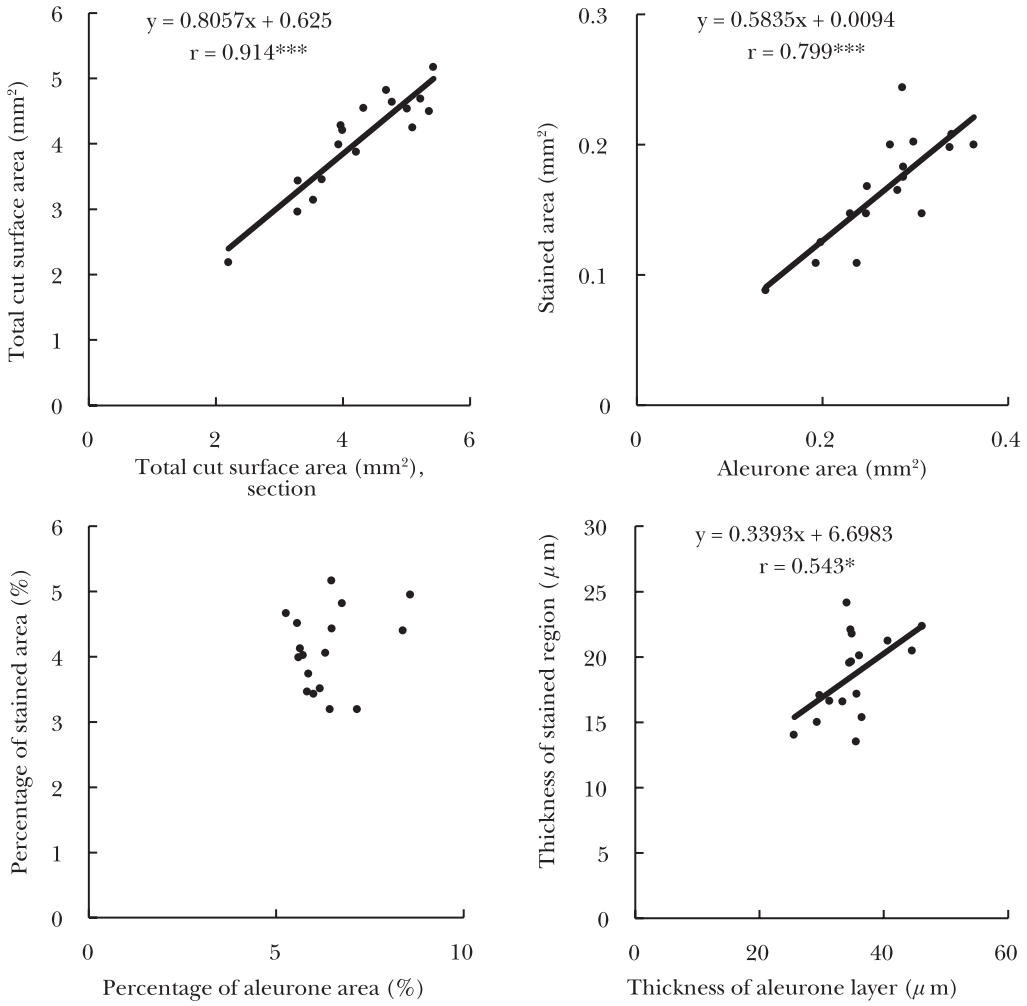

Fig. 4. The relationship between the aleurone traits in the 17 rice varieties determined by the half-cut seed method and the cross-sectioning method. * and *** indicate significance at $p < 0.05$ and $p < 0.001$, respectively.

varieties had a large stained area (Fig. 3). The largest stained area was found in *japonica* (Table 1). In *indica*, intermediate, and tropical *japonica*, some varieties also had a large stained area. The average thickness of the stained region varied significantly with the variety, but not with the varietal group (Table 1). In the *japonica* group, many varieties with a large thickness of stained region were found (Fig. 3). However, some *indica* varieties also had a thick stained region.

In the half-cut seed method, the outermost region was stained red but the aleurone layer could not be distinguished from the other tissues. We selected 17 representative varieties covering the whole range of stained areas to compare the measurements obtained by the half-cut seed with those obtained by the cross-sectioning methods (Fig. 4). In the cross-sectioning method, only the aleurone layer was measured; the outer pericarp regions were not included in the measurement. The mean total cut surface area was similar in the two methods: 4.25 mm^2 in the half-cut seed method and 4.1 mm^2 in the cross-sectioning method (Table 2). The mean of the aleurone area was 0.26 mm^2 and the range was from 0.12 to 0.41 mm^2 with the cross-sectioning method, and the mean of the stained area was 0.18 mm^2 and the range was from 0.07 to 0.32 mm^2 with the half-cut seed method (Table 2). The aleurone area measured by the cross-sectioning method was larger than the area of the stained region measured by the half-cut seed method. However, the correlation between these two parameters was significantly high (r = 0.799***, Fig. 4). The thickness of aleurone layer ranged from 22.9 to 48.8 μm with the cross-sectioning method, and that of the stained region ranged from 10.0 to 29.1 μm with the half-cut seed method (Table 2). Although, the thickness of aleurone layer obtained by the cross-sectioning method was clearly larger than the thickness of the stained region obtained by the half-cut seed method, the two parameters were significantly correlated (r = 0.543*, Fig. 4). The mean percentage of the aleurone layer obtained by the cross-

Table 2. Mean with the standard deviation of the aleurone traits of the 17 rice varieties determined by the two methods.

	Cross-Sectioning method	Half-cut seed method
Total cut surface area (mm^2)	4.07 ± 0.99 (2.05 – 5.57)	4.25 ± 0.89 (2.0 – 6.00)
Aleurone area (mm^2)	0.26 ± 0.07 (0.12 – 0.41)	0.18 ± 0.06 (0.07 – 0.32)
Percentage of aleurone area (%)	6.4 ± 0.95 (5.1 – 9.0)	4.2 ± 1.01 (2.3 – 6.5)
Average thickness of aleurone layer (μm)	34.4 ± 5.9 (22.9 – 48.8)	19.0 ± 4.8 (10.0 – 29.1)

The values in parentheses indicate the range of values for each parameter.

Table 3. The aleurone traits of the 17 rice varieties determined by the cross-sectioning method.

Varieties	Total cut Surface area(mm^2)	Aleurone area (mm^2)	Percentage of aleurone area (%)	Average thickness of aleurone layer (μm)	Aleurone thickness around dorsal region(μm)	Aleurone thickness around ventral region(μm)	Aleurone thickness around lateral region(μm)	Amount of TAG in 100 seeds (mg)[a]	Proportion of TAG to the 1 g of seed (mg g^{-1})[b]
Ta hung ku	4.21	0.27	6.5	34.7	53.7	29.9	35.2	17.2	7.28
PTB25	3.99	0.25	6.2	33.4	55.3	28.7	36.7	18.2	8.45
Pai-kan-tao	3.93	0.34	8.6	46.2	85.8	54.9	49.1	18.5	10.57
Gemjya jyanam	3.29	0.24	7.2	35.6	65.0	28.7	35.8	13.9	7.21
Yelaik meedon	4.69	0.28	5.9	35.7	49.5	36.4	38.9	18.4	8.11
Aichi asahi	5.22	0.34	6.5	40.7	66.7	28.1	39.0	19.8	8.19
Maraya	3.67	0.25	6.8	36.1	75.4	28.5	30.4	14.9	7.84
Fukuhibiki	5.42	0.29	5.3	34.1	74.1	25.5	31.8	14.9	6.26
Nipponbare	5.09	0.29	5.6	34.5	71.3	24.1	29.3	14.1	6.23
Koshihikari	5.36	0.29	5.6	34.9	78.5	25.5	33.9	14.9	6.75
Kinmaze	5.01	0.29	5.7	34.8	64.8	24.3	33.9	17.4	7.66
IR64	3.53	0.19	5.6	29.7	41.6	22.9	30.2	14.3	6.08
IR24	3.29	0.19	5.9	29.3	46.2	23.5	30.7	14.2	7.24
Mizuhochikara	3.97	0.23	5.8	31.3	51.9	27.3	31.4	11.9	5.02
LO-1050	4.32	0.36	8.4	44.6	105.1	40.3	53.6	20.9	9.17
Taichung 65	4.77	0.31	6.4	36.5	77.8	31.3	39.3	18.5	7.65
TAL-214	2.19	0.12	5.6	25.6	58.1	20.1	28.0	6.9	6.63
Mean	4.07	0.26	6.4	34.4	65.7	28.8	35.3	15.6	7.34
STD	0.99	0.07	0.95	5.93	18.28	8.65	7.67	3.5	1.3
Minium	2.05	0.12	5.1	22.9	38.2	16.2	23.6	6.4	4.82
Maximum	5.57	0.41	9.0	48.8	124.4	59.0	56.7	21.3	11.05
F value	61.02***	25.19***	5.97***	19.22***	8.92***	14.01***	12.06***	37.80***	25.14***

*** indicates significance at $p < 0.001$. [a) b)] Seeds without embryos were used.

sectioning method was 6.4%, versus that of stained region obtained by the half-cut seed method was 4.2% (Table 2). The two parameters were not significantly correlated (r = 0.237). The thickness of aleurone layer varied significantly with the variety and also differed with the position in the rice seed (Table 3). The aleurone layer was thicker in the dorsal region, than in the ventral and lateral regions in all 17 varieties. The average thickness was similar to the thickness in the ventral and lateral regions and smaller than the thickness in the dorsal region. This is because the aleurone layer was thicker in the dorsal region and thinner in the ventral and lateral regions. Furthermore, the dorsal region is only a small part of the whole section, and lateral and ventral regions occupy a large part of the whole section.

The amount of TAG in 100 brown seeds without embryo

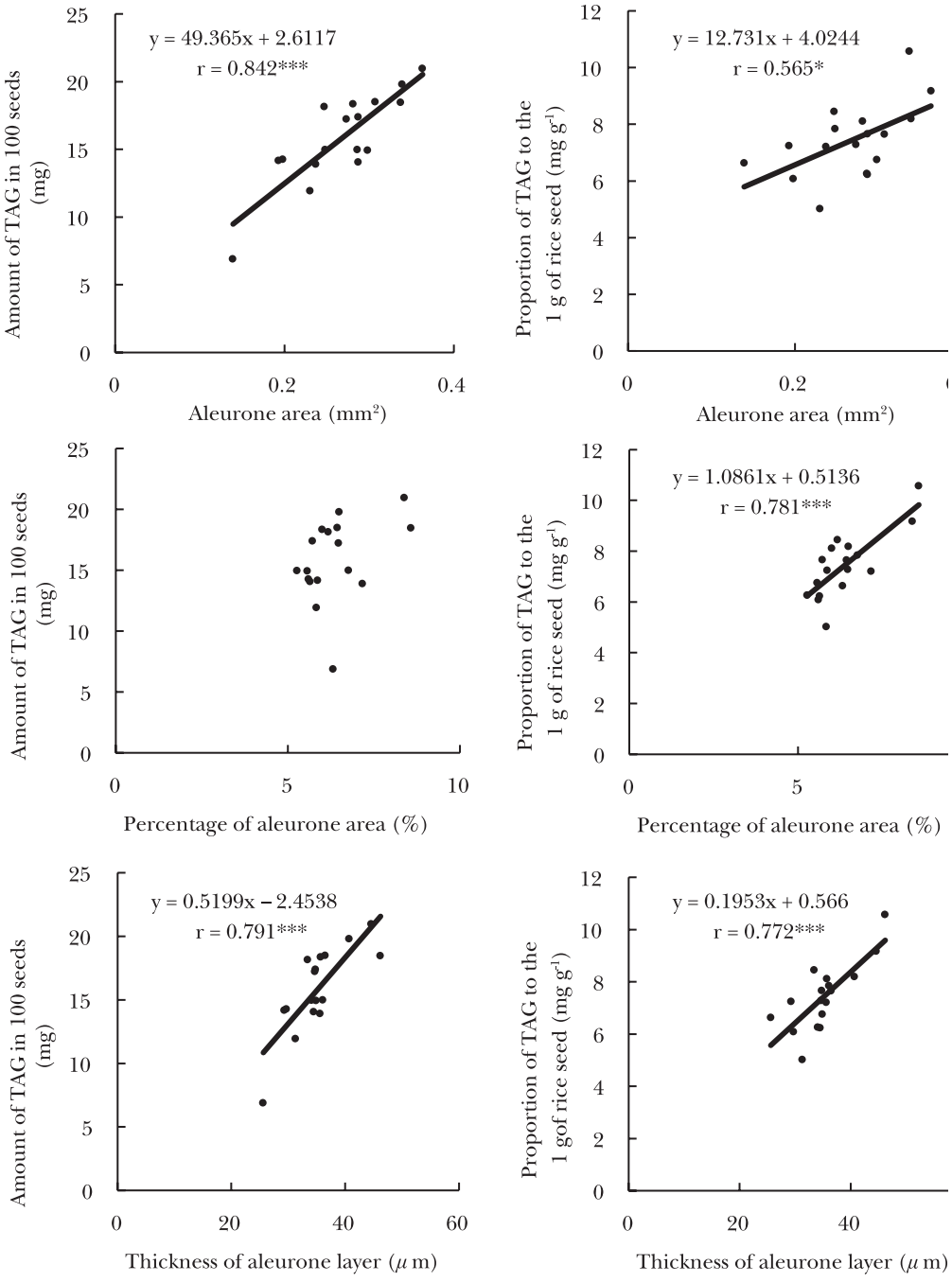

Fig. 5. Relationship between TAG and the aleurone traits in the 17 rice varieties.
* and *** indicate significance at $p < 0.05$ and $p < 0.001$, respectively.

positively correlated with the aleurone area. The amount of TAG ranged from 6.4 to 21.3 mg among the 17 varieties tested (Table 3). It was significantly correlated with the area (r = 0.842***) and the thickness (r = 0.791***) of the aleurone layer (Fig. 5). However, it was not significantly correlated with the percentage of aleurone area (r = 0.402). The proportion of TAG to 1 g of seed also positively correlated with the aleurone area. The proportion of TAG to 1 g of seed ranged from 4.8 to 11.1 mg g^{-1} (Table 3) among the 17 varieties tested. It was significantly correlated with the area (r = 0.565*), percentage (r = 0.781***) and average thickness (r = 0.772***) of aleurone layer (Fig. 5).

Discussion

In the present study, we measured the aleurone traits by the half-cut seed method and the cross-sectioning method. The former method is easy and fast, because half-cut seeds are easy to prepare and the stained regions can be easily measured. Although, the aleurone layer could not be distinguished from the pericarp, we assumed that the

stained region represented the aleurone region because Oil Red O stains lipids, which are concentrated in the aleurone layer. However, to confirm this assumption, we compared the results of this analysis with the results obtained by the more precise cross-sectioning method, in which the aleurone layer can be clearly distinguished from other tissues. We found that the aleurone layer was larger than the stained area obtained by the half-cut seed method. The stained area was, on the average, 30% smaller than the aleurone area measured by the cross-sectioning method. Similarly, the average thickness of the stained region measured by the half-cut seed method was 45% smaller than the thickness of the aleurone layer measured by the cross-sectioning method. Although the measurements by the half-cut seed method were taken based on the outermost region of the seed including the pericarp, the aleurone area in the cross-sectioning method was still larger than the stained area obtained by the half-cut seed method. This may be due to the fact that the stained region may become thinner than the real aleurone area, with the time taken to wash the half-cut seeds in 70% ethanol after staining. However, the correlation between the area of the aleurone layer and the stained area was significant and high ($r = 0.799^{***}$), and the significant correlation between the average thickness of the aleurone layer and that of the stained region was also detected ($r = 0.543^{*}$) (Fig. 4). Thus, it is still reasonable to assume that the stained area represents the aleurone area, despite the discrepancy between the areas measured by the two methods. Therefore, we can use the area of the stained region obtained by the half-cut seed method as a quick and reasonably reliable indicator for selecting varieties based on the thickness of the aleurone layer.

There have been several studies on the number of cell layers in the aleurone layer and thickness of aleurone layer (Nagato et al., 1960; Hoshikawa, 1967) but few on the aleurone area and the average thickness of aleurone layer. In the present study, the aleurone area, the percentage of aleurone layer and the average thickness of aleurone layer were measured in genetically diverse rice varieties. Hoshikawa (1967) reported that the number of cell layers in the aleurone layer did not differ with the variety, the varietal groups classified according to grain size, nor between glutinuous and non-glutinuous varieties. However, we observed a wide variation in the aleurone area and in the thickness of the aleurone region among the tested varieties. The *japonica* varieties had a larger stained area than the other varieties, and most of the *indica* varieties had a smaller stained area. Our finding agrees with that of Cho (1956), who reported that Japanese rice had more aleurone cells than *indica* rice.

Our results showed that the thickness of the aleurone layer depended on the position of measurement in the seed and that the thickness varied significantly with the variety. We found that, the aleurone layer was thickest in the dorsal region in all varieties. Nagato et al. (1960) reported similar results. Most previous reports described the number of aleurone cell layers without measuring their thickness. Juliano and Aldama (1937) reported that the aleurone layer of a Philippine rice variety consisted of from one to as many as five layers of cells. Del Rosario et al. (1968) also found up to five layers in the rice aleurone and found that the aleurone layer was thicker at the dorsal side than at the ventral side. Wang et al. (2004) also stated that more aleurone cell layers formed along the surface layer of the dorsal endosperm, whereas the other surface layer of the endosperm formed only one aleurone cell layer.

Although Mizuhochikara and TAL 214 had a similar aleurone thickness at all positions, the aleurone area of Mizuhochikara was larger than that of TAL 214 because the seeds of TAL-214 were smaller (Table 3). Furthermore, the seeds of LO 1050, Taichung 65 and Mizuhochikara were nearly the same in size, but greatly differed in the thickness and the area of the aleurone region (Table 3). Our results demonstrate that some varieties had a thicker aleurone layer only in the dorsal region than the other varieties. Some varieties had a thicker aleurone layer at all positions than the other varieties. From this point of view, both the thickness and the area of the aleurone layer were important parameters for the selection of varieties for aleurone traits.

TAG amount and the proportion of TAG to 1 g of seed both positively correlated with the aleurone area and aleurone thickness and the correlation between them was also high. The percentage of aleurone layer was correlated only with the proportion of TAG to 1 g of seed. The correlation of the aleurone area with TAG amount was higher than that with the proportion of TAG to 1 g of seed. The correlation of the percentage of aleurone layer with the proportion of TAG to 1 g of seed was significant although that with TAG amount was not. However, the average thickness was significantly correlated with both the amount and the proportion of TAG to 1 g of seed. In this experiment, we focused on the aleurone area. However, TAG was extracted from the whole seed without embryo. Therefore, we also studied the correlation between aleurone volume and TAG. The aleurone volume (mm^3) [aleurone area (mm^2) × seed length (mm)] was significantly correlated with both TAG amount ($r = 0.92^{***}$) and the proportion of TAG to 1 g of seed ($r = 0.534^{*}$) (data not shown). Therefore, we can select varieties with a large amount of TAG by selecting varieties with a large aleurone area and thickness and can select varieties with a high proportion of TAG to 1 g of seed by selecting those with a large aleurone area, percentage and thickness.

In conclusion, we found wide variation in aleurone traits among genetically diverse rice varieties. The correlation between the aleurone area determined using the cross-

sectioning method and the stained area determined using the half-cut seed method was high and significant. Therefore, the stained area obtained by the half-cut seed method, which is fast and easy, can be used as an indicator for selecting varieties based on the thickness of the aleurone layer. We also found high and significant correlations between several aleurone traits and amount and proportion of TAG to 1 g of seed. Using this method, it is possible to select varieties with a high lipid content without directly measuring their lipid content.

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