Genetic Diversity for Lipid Content and Fatty Acid Profile in Rice Bran

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ABSTRACT: Rice (*Oryza sativa* L.) bran contains valuable nutritional constituents, which include lipids with health benefits. A germplasm collection consisting of 204 genetically diverse rice accessions was grown under field conditions and evaluated for total oil content and fatty acid (FA) composition. Genotype effects were highly statistically significant for lipid content and FA profile ($P < 0.001$). Environment (year) significantly affected oil content ($P < 0.05$), as well as stearic, oleic, linoleic, and linolenic acids (all with $P < 0.01$ or lower), but not palmitic acid. The oil content in rice bran varied relatively strongly, ranging from 17.3 to 27.4% (w/w). The major FA in bran oil were palmitic, oleic, and linoleic acids, which were in the ranges of 13.9–22.1, 35.9–49.2, and 27.3–41.0%, respectively. The ratio of saturated to unsaturated FA (S/U ratio) was highly related to the palmitic acid content ($r^2 = 0.97$). Japonica lines were characterized by a low palmitic acid content and S/U ratio, whereas Indica lines showed a high palmitic acid content and a high S/U ratio. The variation found suggests it is possible to select for both oil content and FA profile in rice bran.

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KEY WORDS: Fatty acid composition, genetic variation, oil quality, *Oryza sativa* L., rice bran.

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 not a popular oil worldwide, but it is in steady demand as a so-called "healthy oil" in Asian countries (1). From a nutritional point of view, the interest in rice bran oil has been growing, mainly because of its health benefits, which include a reduction in both serum and LDL cholesterols (2). Nevertheless, the production of rice bran oil is limited by one factor: After milling, the oil is split extraordinarily quickly into FFA by lipase, which makes it unfit for refining and edible use. This process of rancidity development can be avoided either by rapid oil extraction, which involves an *in situ* process, or by inactivating the lipase through a heat treatment, known as a stabilization process. Both alternatives require special installations and equipment, which increase production costs. As a consequence, the utilization of rice bran is restricted mainly to animal feeds. Since rice bran lipids are directly involved in this process, a better knowledge of their presence and properties may help researchers find a solution to this problem. The fat content in rice bran has been reported to range from 12 to 23% (3) or from 16 to 32% (4). Because rice is primarily cultivated to obtain the endosperm or milled rice, little effort has been made to study the quality aspects of rice bran lipids. Information about the variability of lipid content and composition in rice bran is therefore limited. Several studies have reported variations in the FA composition of rice bran oils, but these have included only a reduced number of varieties, mostly of Asian origin (5–8). For such reasons, the characterization of genetically broad rice germplasms for both bran lipid content and FA composition is of special importance in identifying possible sources of variation as well as potentially beneficial genotypes. The aim of the present study was to evaluate a collection of 204 divergent rice cultivars for oil content and FA composition in the bran fraction. Correlations between FA, saturated to unsaturated FA ratio (S/U ratio), and lipid content were also determined.

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MATERIALS AND METHODS

Plant material. In 1999 and 2000, a collection of 204 rice (*Oryza sativa* L.) accessions was grown under field conditions in Beaumont, Texas, using cultural management practices common for the region. The entries represented a genetically diverse rice germplasm collection, including historical and present-day U.S. cultivars, as well as Asian, European, South American, and African cultivars. The genotypes were grown in single plots arranged in a complete randomized design. The plots consisted of six rows, 3.5 m long, spaced 15 cm apart. The within-row spacing was about 10 cm. The plants were kept continuously flooded at about 10 cm standing water. At maturity, the plants were threshed by hand, the grains were dehulled and all broken, and diseased and immature kernels were removed. About 50 g of dehulled kernels was milled for 30 s using a McGill Mill #1 with an 858-g weight in positions 12 and 6 for long- and medium-grain types, respectively. The bran fraction was collected and sieved through an 840-μm sieve. Bran samples were conserved in a freezer under nitrogen until analysis. Surface lipid content was determined by refluxing 5 g of milled rice with petroleum ether in a Goldfish extraction apparatus for 30 min. The solvent was collected and evaporated, and the percentage of surface lipid content was calculated as the mass of the extracted lipid divided by the beginning total milled rice mass. This measurement was used to ensure that all samples were milled within a similar range in degree of milling (i.e., <0.5% surface lipid content).

Analysis of bran oil content. About 400 mg of rice bran was dried for 2 h at 105°C to determine moisture content. The dried rice bran was then used to measure oil content by Soxhlet extraction with light petroleum ether (boiling point 40°C). Extraction time was 12 h. Previous work (9) indicates that rice accessions with a range in milling quality vary in their bran starch content. Poor milling quality lines have greater starch contents, whereas those with superior whole-grain yields have lower starch contents. These varying levels of starch confound data that are expressed on a bran weight basis. To eliminate the effect of different milling qualities on the oil content, the values were corrected to an equivalent of 15% w/w starch content in the bran, using the following formula:

$$\text{corrected oil content (\% w/w)} = \text{oil content as is (\% w/w)} \cdot (100 - 15\%)/(100\% - \%\ \text{of starch in the sample}) \quad [1]$$

Bran oil content was expressed as the percentage (w/w) on a dry weight basis. Analysis was performed with two replications.

FA analysis. The FA composition of rice bran oils was determined by GLC of FAME. FAME were prepared as follows: About 100 mg of rice bran was extracted and transmethylated for 30 min at 20°C with 1 mL of a 0.5 M solution of sodium methylate in methanol. Isooctane (1 mL) and 0.5 mL of 5% (wt/vol) $NaHSO_4$ in water were added, in that order, and samples were mixed. The tubes were then centrifuged for 5 min at 3,822 × *g* and 2 µL of the isooctane phase was injected into the gas chromatograph. Analysis was performed in a Hewlett-Packard gas chromatograph model 5890 Series II equipped with an FID and a fused-silica capillary column (FFAP, 25 m × 0.25 mm × 0.25 µm thickness; Macherey-Nagel GmbH & Co. KG, Düren, Germany). The carrier gas was helium at a pressure of 120 kPa. The oven temperature was programmed as follows: The initial temperature (180°C) was increased linearly to 225°C at a 10°C/min rate, and the final temperature was held for 10.5 min. The injector and detector temperatures were 230 and 250°C, respectively. The samples (2 µL) were injected at a split rate of 45.5:1. FA composition was expressed as the percentage of total FA.

Starch content determination. The method of McClearly *et al.* (10) for determining starch content was modified for rice bran analysis. Fifty milligrams of rice bran was weighed into a test tube and wetted with 200 µL of aqueous ethanol (80% vol/vol). Two milliliters of DMSO was added immediately, and the tubes were stirred with a vortex mixer and placed in boiling water for 5 min. After that, 3 mL of thermostable α-amylase (100 U/mL) in a 4-morpholinepropanesulfonic acid sodium salt (MOPS) buffer (50 mM, pH 7.0) containing calcium chloride (5 mM) and sodium azide (0.02% wt/vol) was added immediately. The tubes were stirred vigorously with a mixer and further incubated for 6 min; all tubes were stirred three times during incubation. The samples were then transferred to a bath at 50°C, and 4 mL sodium acetate buffer (200 mM, pH 4.5) was added, followed by 0.1 mL amyloglucosidase (200 U/mL). Samples were then stirred and incubated for 30 min. After incubation, the sample volume was adjusted to 10 mL and mixed thoroughly. An aliquot of 200 µL of this solution was centrifuged at 2,867 × *g* for 10 min and diluted to 600 µL with distilled water. Three milliliters of glucose determination reagent (12,000 U/L glucose oxidase, 650 U/L peroxidase, and 0.4 mM 4-aminoantipyrine) was added, and the samples were held at 50°C for 20 min. The absorbance at 510 nm was registered for each sample, and starch contents were calculated using a calibration curve developed with glucose standards. Starch content was expressed as the percentage (w/w) on a dry weight basis. Analysis was performed with two replications.

Statistical analysis. Data were statistically analyzed using the nonparametric ANOVA test developed by Kruskal and Wallis (11). All statistical analyses were performed with SAS statistical software (Statistical Analysis System, SAS Institute, Cary, NC). Correlation coefficients were calculated from the means of two seasons.

RESULTS AND DISCUSSION

Genotype and season effects. Table 1 shows the chi-square approximations of the nonparametric ANOVA for oil content and major FA. Genotype effects were highly statistically significant for all traits measured ($P < 0.001$). Seasonal effects (year) significantly affected oil content ($P < 0.05$), as well as stearic, oleic, linoleic, and linolenic acids (all at $P = 0.01$ or lower), but not palmitic acid. The chi-square approximation values attributable to genetic effects overwhelmed those caused by seasonal influences, indicating that most of the variation for these traits was due to genetic differences among the lines. But genotype effects may be overestimated since

TABLE 1
Chi-Square (χ^2) Approximations of the Nonparametric ANOVA for Oil Content and Palmitic (16:0), Stearic (18:0), Oleic (18:1), Linoleic (18:2), and Linolenic (18:3) Acids[a]

		χ^2					
Sources	DF	Oil	16:0	18:0	18:1	18:2	18:3
Genotype	203	339.0***	387.7***	304.2***	348.1***	369.1***	283.4***
Year	1	4.1*	1.5^{NS}	45.1***	15.6***	10.2**	71.5***

[a]Oil content expressed as percentage (w/w) of bran. FA expressed as percentage of total FA. ^{NS} indicates nonsignificance. *, **, and *** indicate significance at $P = 0.05$, 0.01, and 0.001, respectively (Kruskal–Wallis test).

the experimental design used does not allow an estimation of the genotype–year interaction. A study of correlations between the two seasons confirmed that the lines showed consistent values for the studied traits over the two seasons. The correlation coefficients (r) between years were very high, being 0.70, 0.96, 0.82, 0.89, and 0.96 for oil content, palmitic acid, oleic acid, linoleic acid, and the S/U ratio, respectively. This indicates that heritabilities for those traits may be high, particularly for palmitic acid and the S/U ratio.

Variation and distribution of oil content and FA. The genotypes showed a continuous distribution for bran oil content, which ranged from 17.3 to 27.4% w/w (Fig. 1). The wide variation observed was similar to that reported previously (3,4). Most of the lines studied (>75%) showed oil contents above 22% w/w, which represents a lipid content 3- to 24-fold higher than that in oat, wheat, and corn bran. The bran from those species is hardly susceptible to rancidity. Consequently, they find application as food ingredients, which increases their added value. Considering that rice bran has a similar lipase activity, a bran having a higher oil content may be expected to release more FFA than that one having a low oil content. The elevated oil content in rice bran might be at least partially responsible for its higher susceptibility to rancidity. A reduction in oil content may therefore hinder the development of rancidity in rice bran. Further investigations are nevertheless required to confirm this hypothesis.

Two different groups were apparently detected in the distribution for palmitic acid (Fig. 2). One group showed a low palmitic acid content (<17.5%), with a high proportion of genotypes falling into the class with a mean of 16% palmitic acid, whereas the other group displayed similar counts along the whole range (17.5–22%) of palmitic acid content. Using a genetic classification based on random amplified polymorphic DNAs (RAPD) markers (12), we could identify some of the genotypes included in the present study as belonging to the Indica ($n = 3$) or Japonica ($n = 22$) types. An ANOVA was performed between the identified Indica and Japonica cultivars (Table 2). The two groups showed statistically significant differences for palmitic ($P < 0.001$), linoleic ($P < 0.05$), and linolenic ($P < 0.01$) acids, and also for the S/U ratio ($P < 0.001$). Furthermore, comparing the variation in both groups for palmitic acid and for the S/U ratio, the two subspecies could be completely separated from each other. The palmitic acid content ranged from 14.9 to 17.2% in Japonica and from 19.3 to 20.4% in Indica, and the S/U ratio ranged from 0.23 to 0.26 (Japonica) and from 0.29 to 0.31 (Indica). Taira and Chang (13), Taira *et al.* (6), and Taira (7) also divided Indica and Japonica groups based on FA composition. Thus, palmitic acid content and S/U ratio appear to be useful as chemotaxonomical markers for distinguishing Japonica and Indica subspecies. Further studies using genetic information and chemical analysis from a larger number of genotypes are needed to confirm this conjecture.

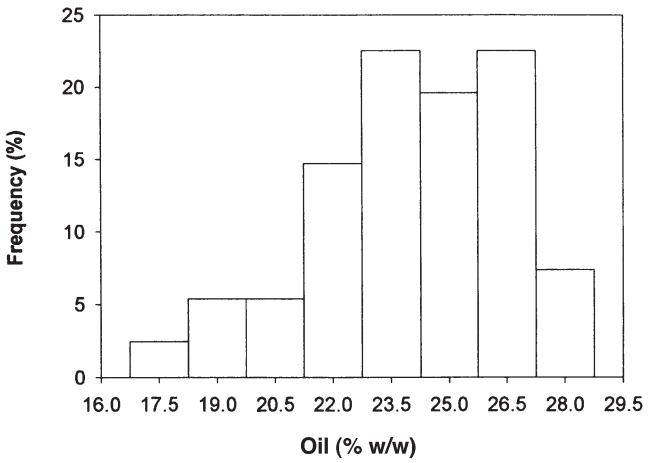

FIG. 1. Frequency distribution of bran oil content (% w/w) in a collection of 204 rice lines.

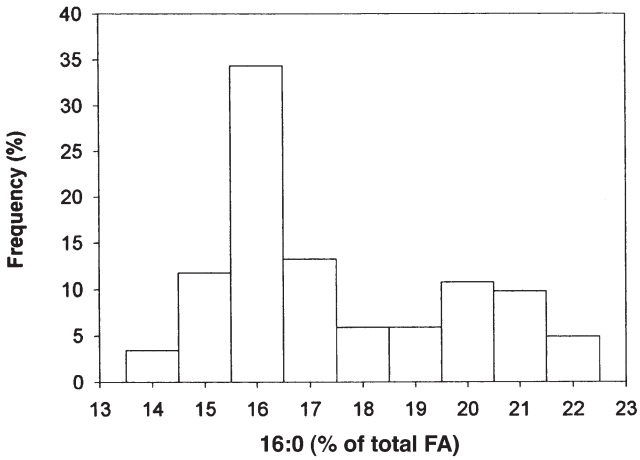

FIG. 2. Frequency distribution of palmitic acid content (% of total FA) in a collection of 204 rice lines.

Wide variation was observed for oleic and linoleic acid contents, which ranged from 35.9 to 49.2% and from 27.3 to 41.0%, respectively (Figs. 3 and 4). Both FA appeared to be normally distributed. Higher contents of monosaturated FA, particularly oleic acid, are desirable since they provide a higher stability in cooking oils. The results indicate that it is possible to increase the oleic acid content and reduce the linoleic acid content by using conventional breeding techniques. The variation for stearic and linolenic acid contents ranged from 1.5–2.9% and from 0.8–1.9%, respectively.

Table 3 presents the lines displaying the highest and the lowest values for oil content, main FA, total saturates, and the S/U ratio among the rice cultivars studied. The lines 'IAC 1201' and 'Scented A' were distinguished by a low palmitic acid content and a high oleic acid content. Thus, they are useful for designing cooking or salad oils in which low saturates and high oleic acid are desired. 'Ngasein' and 'Kataktara' showed the highest values for palmitic acid content as well as for the S/U ratio. Vegetable oils with high contents of saturated FA are desired by the food industry, especially to avoid the need for hydrogenation and transesterification processes in the production of margarines and shortenings. The industrial hydrogenation of oils requires a high energy input and

TABLE 2
Mean Values for Oil Content, Palmitic (16:0), Stearic (18:0), Oleic (18:1), Linoleic (C18:2), and Linolenic (18:3) Acids, and the Saturates to Unsaturates Ratio (S/U ratio) in Selected Indica and Japonica Types[a]

			FA (% of total FA)					
Type	*n*	Oil	16:0	18:0	18:1	18:2	18:3	S/U ratio
Indica	22	24.4^{NS}	19.7***	2.0^{NS}	42.0^{NS}	32.6*	1.6**	0.301***
Japonica	3	23.0	16.2	2.1	42.5	35.7	1.3	0.245

[a]Oil content expressed as percentage (w/w) of bran. ^{NS} indicates nonsignificance. *, **, and *** indicate significance at $P = 0.05$, 0.01, and 0.001, respectively (LSD test).

results in the formation of *trans* FA, which are undesirable for human nutrition. The ingestion of *trans* FA increases LDL to a degree similar to that of saturated FA, but it also reduces HDL; therefore, *trans* isomers are considered more atherogenic than saturated FA (14). Furthermore, in a thermoxidative study using TAG extracted from different sunflower FA mutants, Márquez-Ruiz *et al.* (15) found that a high-palmitic sunflower line (CAS-12) displayed the highest thermal stability, producing half the amount of polar compounds as the conventional line and less than two-thirds that of a high-oleic line. This indicates the high potential of palmitic acid-rich oils for use as frying fats. For such reasons, several breeding programs have been focused on the development of varieties with high contents of saturated FA in major oil crops, particularly high palmitic acid content (16,17). The palmitic acid content in rice bran is as high as in high palmitic acid soybean mutants (15–20%) (18). Therefore, the palmitic acid content in rice bran seems to be a promising breeding target for the production of margarines, shortening, and frying oils. Major alterations in the proportions of individual FA have been achieved in a range of oilseeds using conventional selection, induced mutation, and, more recently, posttranscriptional gene silencing (PTGS). For example, the stearic acid content in cottonseed oil was substantially increased from its normal level of 2 to 40% using PTGS (19). Similar modifications in the contents of certain FA appear to be possible using such techniques in rice.

Analysis of correlations. Starch content showed a significant negative correlation with noncorrected oil content ($r = -0.42$; $P < 0.001$), but it was not correlated with corrected oil content ($r = 0.06$). This indicates that the correction for starch content is quite effective in reducing the effect of the degree of milling on bran lipid content. Corrected oil content was positively correlated with oleic acid and negatively correlated with linoleic acid ($P < 0.001$), suggesting that high bran oil contents are related to elevated contents of oleic acid. Palmitic acid was significantly negatively correlated with oleic and linoleic acids. Oleic and linoleic acids showed a high negative correlation ($r = -0.70$). The S/U ratio was highly positively correlated with palmitic acid ($r = 0.98$) and negatively correlated with oleic and linoleic acids, indicating that higher S/U ratios can be more effectively achieved by increasing the palmitic acid content.

The variation observed for lipid content and FA profile suggests that breeding material is available for modifying oil content and improving lipid quality in rice bran. Divergent values for oil content and FA were consistently identified in some cultivars over the two seasons. Genes from these lines can be introgressed into breeding populations to create rice with the desired oil contents and compositions. Indica lines appear to be a good genetic source for high palmitic acid and Japonica lines for low palmitic acid. Palmitic acid content may also be useful for classifying lines within each group as

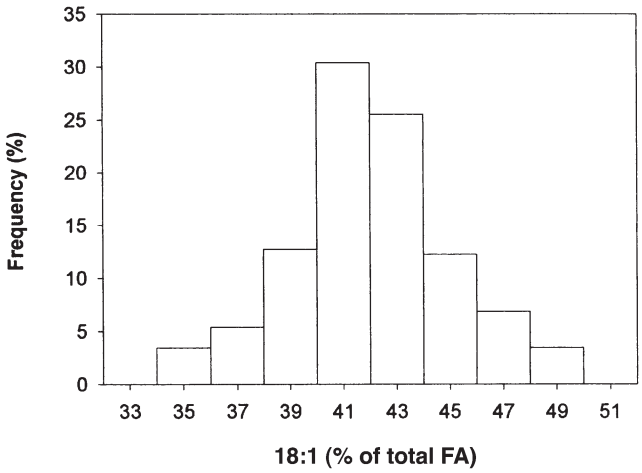

FIG. 3. Frequency distribution of oleic acid content (% of total FA) in a collection of 204 rice lines.

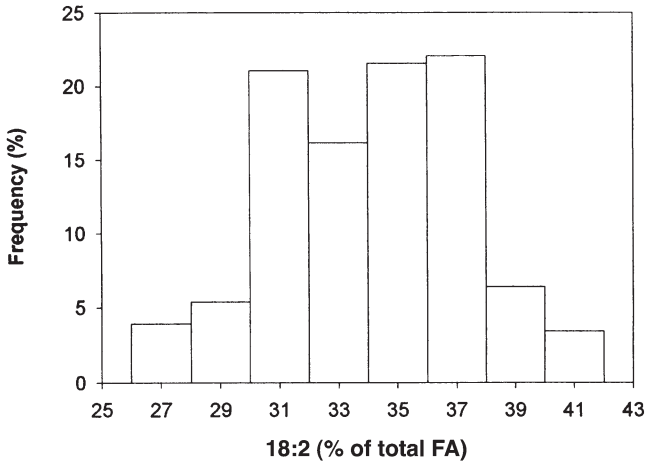

FIG. 4. Frequency distribution of linoleic acid content (% of total FA) in a collection of 204 rice lines.

TABLE 3
Means over Two Environments of the Five Genotypes Showing the Highest and the Lowest Values for Oil Content, FA, Total Saturates, and the S/U Ratio Among the Lines Studied (*n* = 204)

		FA[b] (% of total FA)						
Cultivar/classification	Oil[a] (%)	16:0	18:0	18:1	18:2	18:3	Sat[c]	S/U ratio
High bran oil								
Dawn	27.4	16.1	2.1	42.6	36.1	1.1	19.5	0.24
Newrex	27.1	16.0	2.1	46.2	32.5	1.1	19.4	0.24
Goolarah	27.0	16.7	1.8	42.3	36.0	1.0	19.8	0.25
Basmati Mutant	26.9	21.5	2.0	42.9	30.3	1.2	25.1	0.33
L205	26.8	17.9	2.6	42.8	33.3	1.2	22.0	0.28
Low bran oil								
Banjul	17.3	20.4	2.7	36.9	36.6	1.4	24.5	0.32
Chokoto	17.4	16.9	2.3	40.2	36.9	1.4	20.6	0.26
Kun Shan Wu Shan	18.2	16.3	2.6	44.2	33.3	1.4	20.3	0.25
WC 756	18.3	15.9	1.9	37.9	40.0	1.9	19.4	0.24
Suk Na	18.4	17.6	2.0	42.3	34.4	1.3	21.1	0.27
High palmitic acid								
Ngasein	19.1	22.1	2.5	41.2	31.3	1.0	25.9	0.35
T(N) YU 7	23.9	21.8	1.9	39.8	33.0	1.7	25.0	0.33
Kataktara	23.2	21.8	2.4	41.2	31.6	1.1	25.6	0.34
Basmati Mutant	26.9	21.5	2.0	42.9	30.3	1.2	25.1	0.33
IARI 6627	21.8	21.5	2.3	42.5	30.3	1.2	25.5	0.34
Low palmitic acid								
WC 2936	23.3	13.9	2.1	47.8	32.7	1.1	17.6	0.21
IAC 1201	24.1	13.9	1.8	48.8	32.1	1.1	17.2	0.21
Scented A	20.1	14.4	2.1	48.7	31.0	1.2	18.1	0.22
WC 3777	26.6	14.7	2.4	43.7	35.8	1.0	18.6	0.23
CI 2938-2	25.6	14.8	1.6	45.1	35.0	1.2	17.9	0.22
High oleic acid								
Ai Yeh Lu	25.9	16.3	2.2	49.2	29.3	1.2	19.6	0.24
WC 2685	24.6	18.2	2.1	48.9	27.3	1.2	21.7	0.28
IAC 1201	24.1	13.9	1.8	48.8	32.1	1.1	17.2	0.21
NIRA	24.2	15.9	2.5	48.8	29.5	1.2	19.7	0.25
Scented A	20.1	14.4	2.1	48.7	31.0	1.2	18.1	0.22
Low oleic acid								
Achhame	19.4	17.5	2.2	35.9	40.5	1.6	21.1	0.27
Kakani 2	19.6	17.5	2.2	35.9	40.3	1.6	21.3	0.27
Vary Lava 16	25.1	20.4	1.5	36.0	38.5	1.4	23.5	0.31
Phudugey	25.4	21.0	2.2	36.2	37.6	1.1	24.5	0.33
Banjul	17.3	20.4	2.7	36.9	36.6	1.4	24.5	0.32
High linoleic acid								
Baldo	19.5	16.5	1.8	37.0	41.0	1.4	19.8	0.25
Achhame	19.4	17.5	2.2	35.9	40.5	1.6	21.1	0.27
Palmyra	21.7	15.0	2.0	38.7	40.4	1.6	18.6	0.23
Kakani 2	19.6	17.5	2.2	35.9	40.3	1.6	21.3	0.27
Italiaca Livorno	21.2	16.7	2.2	37.5	40.2	1.4	20.2	0.25
Low linoleic acid								
WC 2685	24.6	18.2	2.1	48.9	27.3	1.2	21.7	0.28
IR262/Khao Dawk	25.4	20.6	2.0	45.8	27.9	1.5	24.0	0.32
Dom Sofid	22.2	19.1	2.3	47.3	27.9	1.3	22.7	0.29
Tehran	22.7	18.6	2.0	47.7	28.2	1.3	22.1	0.28
Jasmine 85	25.9	20.5	2.0	45.1	28.5	1.4	24.3	0.32

[a]Corrected oil content, expressed as percentage (w/w) of bran.
[b]For FA see Table 1; for other abbreviation see Table 2.
[c]Sat, total saturates expressed as percentage of total FA.

a chemotaxonomic marker. Two different alternatives can be proposed for increasing bran utilization: (i) breeding for reduced oil content and a low proportion of linoleic acid to increase rice bran stability, and (ii) breeding for higher bran oil content with an improved FA composition for bran oil extraction. Breeding techniques such as chemical mutagenesis or PTGS may be required to design rice bran oils containing extreme proportions of certain FA.

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