

# Physicochemical Characteristics and QTL Mapping Associated with the Lipid Content of High-Lipid Rice

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## ABSTRACT

This study was conducted to examine the physicochemical characteristics and perform QTL mapping of genetic factors associated with the lipid content of rice. A rice strain with a high lipid content, “P31-2-2-2-B-B”, was developed from mutants of “Dongjin” created by T-DNA insertion. The lipid content of “P31-2-2-2-B-B” brown rice was 4.42% whereas that of the donor cultivar “Dongjin” was 2.56%. The total fatty acid content of the high-lipid mutant brown rice was 7.82% and that of “Dongjin” was 3.43%. The unsaturated fatty acid composition of the mutant brown rice was 2.73% oleic acid, 2.74% linoleic acid, and 0.34% linolenic acid. In contrast, the fatty acid composition of the donor cultivar “Dongjin” was 1.30% oleic acid and 0.99% linoleic acid. The percentage of unsaturated fatty acid to total fatty acid in the high-lipid mutant was higher (74.3%) than that of “Dongjin” (66.8%). Continuous frequency distribution and transgressive segregation of the lipid content were observed in the F<sub>3</sub> family (seeds) derived from a cross between the high-lipid mutant “P31-2-2-2-B-B” and a *tongil-type* cultivar “Samgang”. This result implied that the lipid content was a quantitative trait controlled by a polygene. Additionally, the broad sense heritability of lipid content was estimated to be 89.6% based on analysis of the F<sub>3</sub> seeds. A significant QTL, *qRLC5*, was identified on chromosome 5 with a LOD score of 2.37, and was flanked by 5007 and 5014. Results of the present study should be useful for improving rice nutritional quality through marker-assisted selection.

**Keywords:** Lipid Content; Fatty Acid Content; Quantitative Trait Loci (QTLs); Rice (*Oriza Sativa* L.)

## 1. Introduction

Rice is a staple cereal crop for more than half the world's population. In most Asian countries including South Korea, 60% - 70% of the daily nutrients are supplied by rice. However, rice consumption has been declining over the years in Asia. It is important to improve the chemical composition and physical properties of rice in order to encourage rice consumption and diversify the use of this grain. Milled rice contains 372 Kcal per 100 g, and is mostly composed of carbohydrates, proteins, and fats [1]. In addition, rice contains nutrients such as numerous minerals and vitamins. Rice lipid is mainly concentrated in the embryo, bran, and aleurone layer. Brown rice is rich in oleic acid, linoleic acid, and various trace components such as  $\gamma$ -oryzanol, sterols, tocopherol, and tocotorienol [2,3]. In general, lipid content amounts to 1.6% - 3.02% at 14% moisture in brown rice [4-6]. The lipids found in rice, especially unsaturated fatty acids,

greatly influence grain appearance and quality [3,6-11]. Changes in the crude fat content of rice are associated with aging and deterioration of rice. Oxidation reduces both the total lipid and free fatty acid contents, which are related to the quality of stored rice [6,9,12]. Lipids derived from rice enhance the immune system and help to overcome fatigue. Additionally, these lipids reduce serum and LDL cholesterol while elevating HDL cholesterol levels [13-15]. Thus, lipids from rice have been identified as food-derived functional compounds. Heritability of the lipid content in rice is relatively high (60.90% - 68.25%) [7], and has been reported to be a quantitative trait controlled by a polygene [10,11,16]. Hu *et al.* [10] detected three QTLs (Quantitative Trait Loci) on chromosomes 1, 2, and 5 associated with the lipid content of rice. Cho *et al.* [17] identified 12 QTLs on chromosomes 1, 3, 4, 5, 7, 8, and 12. Liu *et al.* [6] found 14 QTLs related to lipid content on chromosomes 1, 3, and 5 - 9. Yu *et al.* [18] detected four QTLs controlling the lipid content of brown rice on chromosomes 3, 5, 6, and 8. Further-

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more, Qin *et al.* [3] found QTLs associated with the lipid content of brown rice on chromosomes 1, 2, 3, and 5 - 9.

In the present study, we analyzed the physicochemical properties and fatty acid composition of a high-lipid mutant rice strain, "P31-2-2-2-B-B". In addition, we identified that QTLs influence lipid content. The results of our investigation may be used as a basis to further develop varieties of high-lipid rice.

## 2. Materials and Methods

### 2.1. Plant Materials

The selected high-lipid rice strain by the bulk and pedigree breeding method, "P31-2-2-2-B-B", derived from "P31" was used for this study. "P31" is a lipid mutant line derived from "Dongjin" by T-DNA insertion from POSTECH (located in Pohang of South Korea) in 2005. Donor cultivar "Dongjin" was also included in our analyses for comparison purposes. Experimental trials were carried out in the experimental plots of Kyungpook National University (KNU) in Gunwi (South Korea) from 2007 to 2010. One plant per hill was transplanted with 30 cm between rows and 15 cm between plots. Field management was conducted according to the normal cultivation practices recommended by Rural Development Administration (RDA) of South Korea with an application for fertilizer (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) at the rate of 90, 45, and 57 kg·ha<sup>-1</sup>.

### 2.2. Analysis of Rice Chemical Content

Damage, red, green, and broken grains of the brown rice were removed before the test. The amylose, lipid, protein, and starch contents of the brown rice for three replication per each line was determined with near-infrared spectroscopy (NIRS) using a spectrophotometer (Foss 6500). Crude fat and fatty acid contents were analyzed by the Local Innovation Center of KNU. To extract crude fat from the rice, the Soxhlet extraction method was used with ethyl ether as a solvent. To recover fatty acids, a methanol:heptane:benzene:2,2-dimethoxypropane:H<sub>2</sub>SO<sub>4</sub> solution (37:36:20:5:2, v:v:v:v) was added to 300 mg of each of rice powder sample. After heating to 80°C, the single phase was cooled to room temperature. Fatty acid composition was measured using a previously described method [19] which involved preparing fatty acid methyl esters by direct transmethylation and analyzing them with a gas chromatography instrument (Agilent 6890 N). Detailed running conditions for the GC were also previously described [20].

### 2.3. Analysis of Genetic Factors Associated with Lipid Content

To evaluate the broad sense heritability of lipid content, the F<sub>3</sub> family, which consisting of 170 plants derived

from a cross between japonica variety "P31-2-2-2-B" and tongil-type cultivar "Samgang" cultivated in a greenhouse at KNU during the winter of 2010, was evaluated. Lipid contents of the rice were evaluated by NIRS using a spectrophotometer (Foss 6500). T-test of F<sub>3</sub> family was calculated by the program of SAS 9.1.

### 2.4. QTL Analysis to Identify Factors Associated with Lipid Content

The mapping population consisted of 90 lines (F<sub>2</sub> population), derived from a cross between "P31-2-2-2-B" (japonica) and "Samgang" (Tongil-type). The molecular marker data were essentially the same as described in Qin *et al.* [3], which consisted of 56 simple sequence repeats (SSR) and 116 sequence tagged site (STS) markers covering 12 chromosomes. A genetic linkage map with one SSR and eight STS markers was created using MAPMAKER/EXP version 3.0 [21]. WinQTL cart 2.5 [22] and QTLMAPPER version 1.6 [11] were used for the QTL analysis. Composite interval mapping (CIM) was operated for the whole genome scanning on QTLs detection by one thousand permutation test at a 0.05 significant level at a threshold of LOD2.0 [22]. QTL nomenclature used in our study was previously described by McCouch *et al.* [23].

## 3. Results

### 3.1. Analysis of Rice Chemical Content

Chemical content of the high-lipid mutant "P31-2-2-2-B-B" rice determined by NIRS is shown in **Table 1**. The amylose and starch contents of "P31-2-2-2-B-B" brown rice (22.57% and 73.39%, respectively) were higher than those of the donor cultivar "Dongjin" (20.32% and 71.44%, respectively). Lipid level in the high-lipid mutant was about 1.6 times higher (3.00%) than that of "Dongjin" (1.83%). Crude fat content of the high-lipid mutant 'P31-2-2-2-B-B' rice determined by the Soxhlet method was about 1.7 times higher (4.42%) than that of "Dongjin" rice (2.56%). The fatty acid compositions of "P31-2-2-2-B-B" and "Dongjin" were measurably different (**Table 2**). The oleic acid and linoleic acid contents of "P31-2-2-2-B-B" were 2.73% and 2.74% respectively, which was about 2.1 and 2.8 times higher than the levels found in "Dongjin" (1.30% and 0.99%, respectively). The linolenic acid content of "P31-2-2-2-B-B" was 0.34%; this was not measured in the donor cultivar "Dongjin". The ratio of unsaturated fatty acids to total fatty acids in "P31-2-2-2-B-B" was relatively higher (74.3%) than that of "Dongjin" (66.8%).

### 3.2. Analysis of Genetic Factors Associated with Lipid Content

Continuous frequency distribution and transgressive

**Table 1. Chemical contents of the high-lipid mutant “P31-2-2-2-B-B” and “Dongjin” brown rice.**

Cultivar	Chemical content (%) <sup>a</sup>			
	Amylose	Lipid	Protein	Starch
P31-2-2-2-B-B	22.57 ± 0.75 <sup>b</sup>	3.00 ± 0.16	8.05 ± 0.54	73.39 ± 0.62
Dongjin	20.32 ± 0.44	1.83 ± 0.08	8.25 ± 0.15	71.44 ± 0.73

<sup>a</sup>Measured by NIRS. <sup>b</sup>Data are presented as the mean ± SD.

**Table 2. Crude fat contents of the high-lipid mutant “P31-2-2-2-B-B” and “Dongjin” brown rice.**

Cultivar	Crude fat content (%) <sup>a</sup>	Ratio
P31-2-2-2-B-B	4.42 ± 0.17 <sup>b</sup>	1.73
Dongjin	2.56 ± 0.28	1.00

<sup>a</sup>Measured by the Soxhlet method. <sup>b</sup>Data are presented as the mean ± SD.

segregation of lipid content were observed in seeds from the F<sub>3</sub> family derived from a cross between the high-lipid *japonica* variety “P31-2-2-2-B-B” strain and *tongil-type* cultivar “Samgang” (Figure 1). The approximately normal distribution was observed, indicating quantitative inheritance of lipid content. T-test results showed that there were significant differences in lipid content between the two parental lines. A lipid content value ranged from 1.72% to 3.29% in the F<sub>3</sub> has relatively high broad sense heritability (89.6%; Table 3).

### 3.3. QTL Analysis of Factors Affecting Lipid Content

A QTL specific for lipid content was detected on chromosome 5. QTL a *qRLC5* was located on chromosome 5 between markers 5007 and 5014 with an LOD score of 2.37, accounting for 20.0% of the phenotypic variation. At this locus, alleles from the “P31-2-2-2-B-B” parent strain increased the lipid content by 0.12% (Figure 2, Table 4).

## 4. Discussion

The lipid content of rice is low and most is composed of unsaturated fatty acids. Additionally, according to the previous report, the lipid content of rice material increases the immune system and improves HDL level [13-15]. In the present study, crude fat content of the high-lipid mutant “P31-2-2-2-B-B” determined by the Soxhlet method was about 1.7 times higher than that of “Dongjin” (Table 5). Differences in composition between the two rice strains observed in this analysis (Table 5) are similar to the ones found with NIRS. The total fatty acid level in “P31-2-2-2-B-B” was greater (7.82%, about 2.3 times) than that of “Dongjin” (3.43%). Similarly, “P31-2-2-2-B-B” had a high concentration of total unsaturated fatty acids (5.81%) which was about 2.5-times higher

**Table 3. Fatty acid composition of the high-lipid mutant “P31-2-2-2-B-B” and “Dongjin” brown rice.**

Fatty acid composition (%)	P31-2-2-2-B-B (% total)	Dongjin (% total)
Saturated fatty acids		
Palmitic acid, C <sub>16:0</sub>	1.55 ± 0.02 <sup>a</sup> (19.8)	0.78 ± 0.04 (22.7)
Stearic acid, C <sub>18:0</sub>	0.46 ± 0.006 (5.9)	0.36 ± 0.02 (10.5)
Subtotal	2.01 ± 0.03 (25.7)	1.14 ± 0.06 (33.2)
Unsaturated fatty acids		
Oleic acid, C <sub>18:1</sub>	2.73 ± 0.04 (34.9)	1.30 ± 0.06 (37.9)
Linoleic acid, C <sub>18:2</sub>	2.74 ± 0.04 (35.0)	0.99 ± 0.08 (28.9)
Linolenic acid, C <sub>18:3</sub>	0.34 ± 0.004 (4.3)	-
Subtotal	5.81 ± 0.08 (74.3)	2.29 ± 0.14 (66.8)
Total	7.82 ± 0.11 (100)	3.43 ± 0.20 (100)

<sup>a</sup>Data are presented as the mean ± SD.

**Table 4. Lipid content and heritability estimated from the analysis of F<sub>3</sub> seeds produced by a cross between the high-lipid “P31-2-2-2-B-B” mutant rice and *tongil-type* cultivar “Samgang”.**

Cultivar	Lipid content (%)
P31-2-2-2-B-B (P <sub>1</sub> )	3.25 ± 0.05 <sup>**</sup>
Samgang (P <sub>2</sub> )	2.08 ± 0.01
F <sub>3</sub> seeds	
Mean ± SD	2.32 ± 0.13
Range	1.72 - 3.29
H <sub>B</sub> <sup>2</sup>	89.6

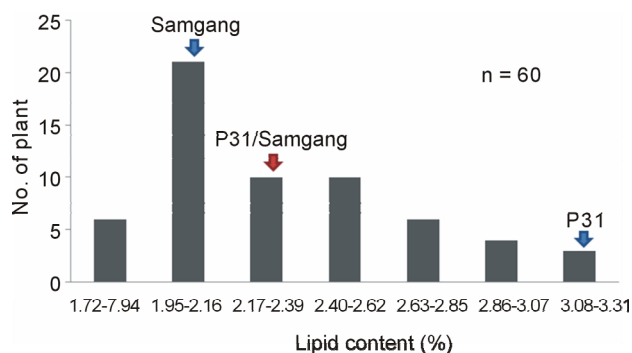
Data are presented as the mean ± SD. <sup>\*\*</sup>Indicates significant difference at a level of 1%.  $H_B^2 = \{V_{F_3} - 1/2(V_{P_1} + V_{P_2})\} / V_{F_3} * 100\%$ .

**Table 5. QTL locations and biometrical parameters associated with the lipid content of rice.**

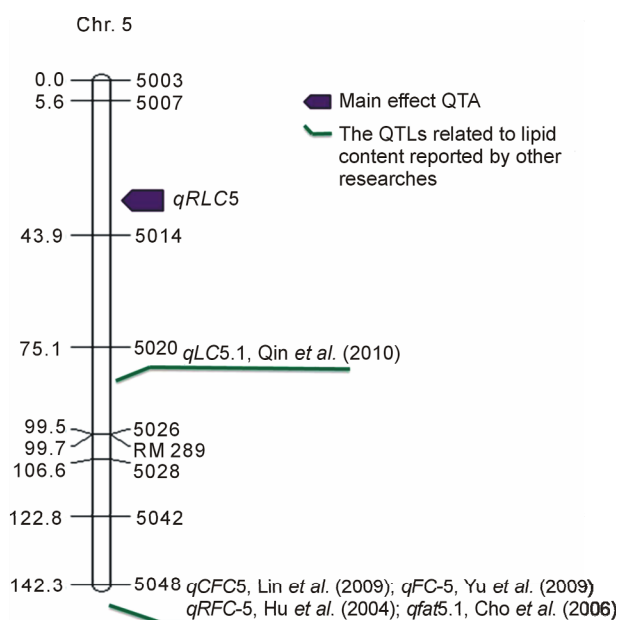
QTL	Interval markers	LOD	R <sup>2</sup> (%) <sup>a</sup>	A <sup>b</sup>	Increasing allele
qRLC5	5007-5014	2.37	20.0	0.12	P31-2-2-2-B-B

<sup>a</sup>R<sup>2</sup>: Percentage of phenotypic variation explained. <sup>b</sup>A: a positive value indicates the genotype from the parent “P31-2-2-2-B-B” toward increasing the trait value.

than that observed in “Dongjin” (Table 2). The percentages of each fatty acid relative to the total fatty acid levels in “P31-2-2-2-B-B” were 19.8% for palmitic acid, 34.9% for oleic acid, and 35.0% for linoleic acid. Similar results were reported by the USDA [24], Choi *et al.* [25], and Koshen [26]. However, the percentage of linolenic acid in the high-lipid strain observed in the current study was higher (4.3%) than that previously reported by the USDA (1.5%; 1998). In this study, lipid content was a quantitative trait controlled by a polygene as previously



**Figure 1.** Frequency distribution of lipid contents in  $F_3$  seeds from a cross between the high-lipid “P31-2-2-2-B-B” mutant and *tongil-type* cultivar “Samgang”.



**Figure 2.** Chromosomal locations of QTLs associated with the lipid content of rice.

reported by Kang *et al.* [16], Chen *et al.* [9], and Hu *et al.* [10]. However, Qi *et al.* [7] reported that the broad sense heritability was 60.90% - 68.25%. Compared to this previous study, the result (89.6%) obtained in our investigation should be higher due to the different genotype and smaller number of samples. Thus, more stable heritability will be estimated from the larger number of samples in a future study. The interval distance of *qRLC5* between 5007 and 5014 observed in the present study was relatively wide (38.3 cM). This was due to a small number of markers. If we were to perform this analysis using more polymorphic markers, the distance of between the *qRLC5* markers would be narrow. As shown in **Figure 2**, *qRLC5* was detected at a location different from the one reported by Qin *et al.* [3]. Additionally, previous studies were detected to analyze QTL of lipid content after marker 5048 loci [6,10,17,18]. Therefore, fine mapping of chromo-

some 5 could enable us to identify QTLs with high average LOD scores that affect lipid content. In the present study, we analyzed the physicochemical properties of a high-lipid mutant strain and identified QTLs that influence lipid content. This result may be used as a basis to further develop varieties of high-lipid rice and improve rice nutritional quality through marker-assisted selection.

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