agronomy

MDPI

Article

Characterization of *flo4-6*, a Novel *cyOsPPDKB* Allele Conferring Floury Endosperm Characteristics Suitable for Dry-Milled Rice Flour Production

Su-Kyung Ha [1], Hyun-Sook Lee [1], Seung Young Lee [1,2], Chang-Min Lee [1], Youngjun Mo [1,2,*] and Ji-Ung Jeung [1,*]

[1] National Institute of Crop Science, Rural Development Administration, Wanju 55365, Republic of Korea
[2] Department of Crop Science and Biotechnology, Jeonbuk National University, Jeonju 54896, Republic of Korea
* Correspondence: yjmo@jbnu.ac.kr (Y.M.); jrnj@korea.kr (J.-U.J.)

Abstract: Rice cultivars with floury endosperm provide a useful raw material for producing dry-milled rice flour, helping to enhance the processed rice food industry. To expand the genetic resources for breeding floury endosperm rice cultivars, we developed Samkwang(SA)-flo3 (SK-flo3), a floury endosperm mutant line derived from the chemical mutagenesis of Samkwang, an elite Korean *japonica* rice cultivar. Compared with Samkwang, SK-flo3 showed significantly lower grain hardness, which is suitable for producing dry-milled flour without the soaking and drying processes required in regular wet rice milling. The dry-milled flour of SK-flo3 exhibited excellent physicochemical properties with less damaged starch and finer flour particles relative to Samkwang. Genetic analyses revealed a G-to-A point mutation in exon 7 of *cyOsPPDKB*, substituting glycine with aspartic acid as a causative mutation for the floury endosperm of SK-flo3. We named this allele *flo4-6* and developed a molecular marker to efficiently transfer it to commercial rice cultivars. Our results provide useful genetic resources and information for developing specialty rice cultivars for high-quality rice flour production with reduced milling costs.

Keywords: floury endosperm; rice flour; Samkwang(SA)-flo3; mutant

check for updates

Citation: Ha, S.-K.; Lee, H.-S.; Lee, S.Y.; Lee, C.-M.; Mo, Y.; Jeung, J.-U. Characterization of *flo4-6*, a Novel *cyOsPPDKB* Allele Conferring Floury Endosperm Characteristics Suitable for Dry-Milled Rice Flour Production. *Agronomy* **2023**, *13*, 1306. https://doi.org/10.3390/agronomy13051306

Academic Editor: Jianbo Wang

Received: 12 February 2023
Revised: 25 April 2023
Accepted: 4 May 2023
Published: 6 May 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Rice is a major cereal crop consumed as a staple by nearly four billion people globally [1]. However, rice consumption in many Asian countries has been gradually decreasing over the past few decades because of dietary diversification and globalization and the increasing consumption of wheat, meat, dairy products, vegetables, and fruits [2]. In South Korea, annual rice consumption per capita declined drastically from 116.3 kg in 1991 to 56.9 kg in 2021 [3]. On the other hand, rice consumed as processed food, such as bread, noodles, cookies, liquor, and plant-based meat, has been continuously increasing, with rice flour accounting for approximately 70% of the total rice used for processed food [4]. To further boost rice consumption and secure rice production infrastructure, the Korean government has launched policy initiatives to enhance the processed food industry using rice flour to produce various food products [5].

Unlike the dry milling process commonly used to make wheat flour, rice grains generally require a wet milling process for pulverization because of their high grain hardness. This entails the complicated procedures of soaking rice grains for softening prior to milling and the subsequent drying and sterilizing of the milled flour, which leads to high production costs as well as environmental issues surrounding wastewater [6]. Hence, developing rice cultivars suitable for dry milling has been suggested as one of the crop improvement approaches to boost rice consumption by providing improved raw materials for the processed rice food industry [7].

Endosperm mutants induced by chemicals or irradiation have been popularly utilized in rice to diversify grain characteristics. Among the different classes of endosperm mutants, such as 'white core', 'floury', 'waxy', 'dull', 'sugary', and 'shrunken' [8], floury mutants with loosely packed starch granules across the entire endosperm area are especially useful for producing dry-milled flour because their soft endosperm breaks easily without soaking and produces flour with a finer particle size and less damaged starch compared to regular rice cultivars [7,9]. Using such induced mutations with floury endosperm characteristics, several rice cultivars, including Suweon542, Hangaru, and Shingil, were released in Korea as specialty cultivars for dry-milled rice flour production [10–12]. Recently, the causal mutation underlying the floury endosperm characteristics of Suweon542 was revealed as a G-to-A SNP in exon 7 of *cyOsPPDKB*, a missense mutation replacing glycine with aspartic acid, and named *flo4-4* (formerly *flo7*(*t*)) [12,13]. Through molecular breeding, *flo4-4* was deployed to develop new rice cultivars for producing dry-milled flour, such as Baromi2 (formerly Garumi2), with improved disease resistance and AromaT with black pericarp and fragrance [14,15]. In Korea, these specialty rice cultivars with an induced *cyOsPPDKB* allele for dry-milled flour production are referred to as "Garussal" ("garu" means flour, and "ssal" means rice in Korean).

Rice floury endosperm mutants also provide valuable genetic materials for studying the molecular mechanisms underlying grain development and starch synthesis. To date, over 20 endosperm mutants named *flo* (floury) have been characterized [7,16]. These include induced mutations in genes that are directly involved in starch accumulation, such as *SSIIIa* (*flo5*), encoding a soluble starch synthase [17]; *AGPL2* (*flo6*), encoding an ADP-glucose pyrophosphorylase [18]; *Ugp1* (*flo8*), encoding a UDP-glucose pyrophosphorylase [19]; and *GLYI7* (*flo15*) encoding a glyoxylase I [20]. While most *flo* mutants exhibit a milky-white appearance in the entire or partial region of the endosperm, their effects on grain quality traits, such as amylose content, amylopectin chain length distribution, protein, and lipid contents, as well as agronomic traits, such as plant height and grain yield components, are highly variable [7]. To utilize the floury endosperm characteristics in rice breeding, it is necessary to improve the *flo* allelic diversity and evaluate the effects of different mutant alleles on major agronomic traits and grain quality.

While Baromi2 carrying *flo4-4* is an early maturing rice cultivar for dry-milled flour production well-adapted to the double cropping system in Korea [14], mid-late maturing rice cultivars with floury endosperm are also required to increase yield and expand adaptability. In this study, we conducted agronomic and genetic analyses on Samkwang(SA)-flo3 (SK-flo3), a floury endosperm mutant line induced by sodium azide with the genetic background of the Korean mid-late maturing elite cultivar, Samkwang (SK). We identified *flo4-6* as the novel *cyOsPPDKB* allele responsible for the floury endosperm characteristics of SK-flo3 and evaluated its utility for breeding rice cultivars for dry-milled rice flour production.

2. Materials and Methods

2.1. Plant Material

Samkwang(SA)-flo3 is a floury endosperm mutant line derived from sodium azide (SA) treatment on a mid-late maturing non-glutinous *japonica* rice cultivar, Samkwang (SK). In brief, after harvesting a single panicle from each M_1 plant, the ear-to-row method was used to advance generations until the M_7 generation. A total of 5000 M_8 lines were established as a mutant stock for forward genetics screens. Out of the six mutant lines screened as floury endosperm mutants, a line with the pedigree 'Samkwang(SA)-M1-3810-1-2-6-1-1', with good agronomic performance comparable to wild-type, was designated as Samkwang(SA)-flo3 (SK-flo3) for further analyses.

2.2. Evaluation of Agronomic Traits and Grain/Flour Physicochemical Properties

A preliminary yield trial (PYT) was conducted to evaluate the major agronomic traits and grain/flour physicochemical properties of SK-flo3 in the experimental field of the National Institute of Crop Science (NICS), Rural Development Administration (RDA),

Wanju (35°84′ N 127°05′ E), South Korea, in 2020. The seeds of the SK-flo3 and wild-type SK were sown on April 30 and were transplanted on May 30. Each plot, consisting of eight rows (spaced by 30 cm) with thirty hills (spaced by 15 cm) per row and three plants per hill, was planted in two replications. For local adaptability trials (LATs), SK-flo3 was designated as Jeonju672 and evaluated in two different locations, Suweon (37°27′ N 126°98′ E) and Hwaseong (37°22′ N 127°04′ E), in 2021 and 2022. The seeds were sown on April 25 and were transplanted on May 25 with the planting density the same as the PYT described above in a randomized complete block design with three replications per variety. The plants were cultivated and evaluated according to the RDA's standard evaluation methods for rice [21]. The heading date was determined when 40% of the plants in a plot showed emerged panicles. Culm length, panicle length, and tiller number were measured from 10 random hills in the middle rows of each plot. Kernels per panicle and 1000-grain weight of brown rice were measured using grains harvested from three random hills in each plot. Brown rice yield per plot was measured from the dehulled kernels of grains harvested from 80 hills.

The grain hardness of brown rice was determined by measuring the pressure at the grain breakage point using a 50 mm probe attachment of TA.XT Plus (Stable Micro Systems, Godalming, Surrey, UK) under the conditions of 0.4 mm/s and 40.0 g for test speed and trigger force, respectively. After tempering at 15% moisture, the dehulled kernels were milled using the Buhler MLU-202 laboratory mill (Buhler AG, Uzwil, Switzerland). The particle size distribution of the flour was determined using an LSI 32320 laser diffraction particle size analyzer (Beckman Coulter, CA, USA).

2.3. Genetic Analyses, Sequencing, and Molecular Marker Development

To study the genetics underlying the floury endosperm of SK-flo3, an F_2 population (n = 173) derived from the cross between SK-flo3 and SK was used. Based on the visual endosperm phenotype of the F_2 seeds, a chi-squared test was conducted to determine the goodness of fit of the observed phenotype to the expected segregation ratio. An additional F_2 population (n = 167) derived from the cross between SK-flo3 and Baromi2, a floury endosperm rice cultivar carrying *flo4-4* [13], was used for an allelism test.

As the genetic analyses revealed that SK-flo3 and *flo4-4* (*cyOsPPDKB* carrying a G-to-A SNP in exon 8) [13] were allelic, *cyOsPPDKB* (Os05g0405000-2) was sequenced from SK-flo3 and wild-type SK. The amplification and Sanger sequencing of *cyOsPPDKB* were conducted using the customized sequencing service at Macrogen (Daejeon, Republic of Korea). To develop a molecular marker for the novel SNP in *cyOsPPDKB* identified from SK-flo3, a derived cleaved amplified polymorphic sequence (dCAPS) marker with a restriction enzyme *RsaI* was designed using dCAPS Finder 2.0 [22]. Polymerase chain reaction (PCR) was performed in a total volume of 20 µL containing 10 ng of template DNA, 5 pmol of each primer, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, and 1U of Taq polymerase. PCR was performed under the following conditions: one cycle at 95 °C for 5 min, followed by 40 cycles of 95 °C for 20 s, 58 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products digested with *RsaI* were separated on a 2% agarose gel.

3. Results

3.1. Major Agronomic Traits of SK-flo3

Major agronomic and yield-related traits of SK-flo3 (designated as Jeonju672 in LAT) were evaluated and compared with its wild-type cultivar, SK (Figure 1, Table 1). In PYT conducted in Wanju in 2020, there was no significant difference in heading date, culm length, panicle length, and tiller number between SK-flo3 and SK (Table 1). However, kernels per panicle of SK-flo3 (87) were 13.9% fewer than those of SK (101). In addition, due to its floury endosperm with loosely packed starch granules, the 1000-grain weight of the brown rice of SK-flo3 (18.5 g) was 13.1% lighter than that of SK (21.3 g) (Figure 1b, Table 1). As a result, the brown rice yield of SK-flo3 (445 kg/10 a) was 17.7% lower than that of SK (541 kg/10 a). The reduced yield of SK-flo3 relative to wild-type SK was also

observed in LAT conducted in two different locations in 2021 and 2022, where the brown rice yield of SK-flo3 was 13.0–17.5% lower than that of SK.

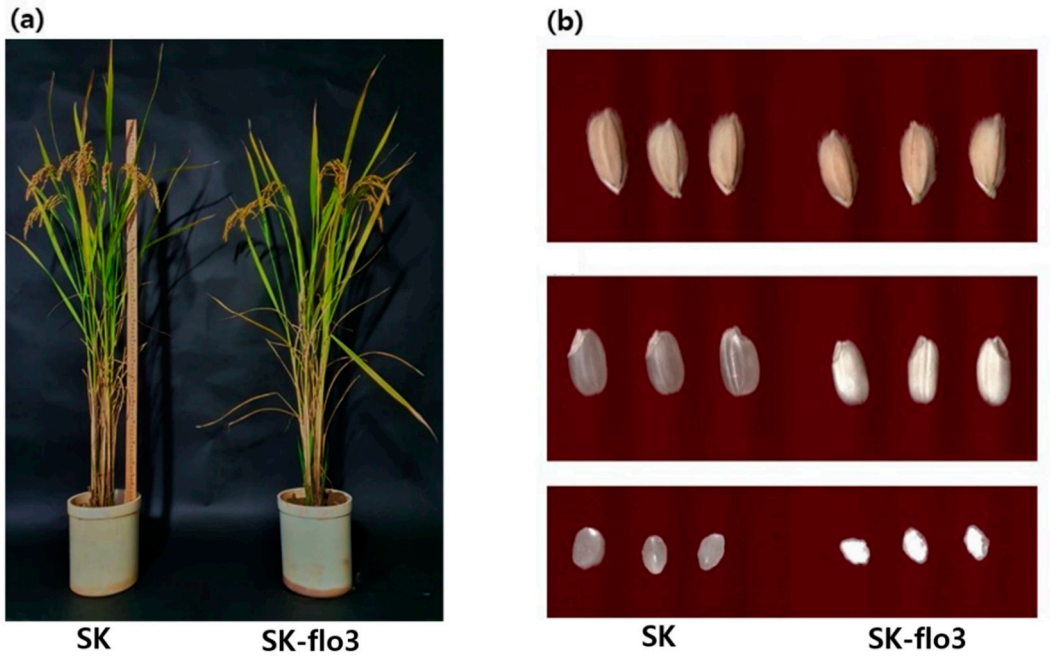

Figure 1. Phenotypic comparison between Samkwang(SA)-flo3 (SK-flo3), a floury endosperm mutant line, and Samkwang (SK), the wild-type cultivar. Plants at maturity (**a**) and hulled rice, brown rice, and their cross-sections (**b**).

Table 1. Comparison of major agronomic traits between Samkwang(SA)-flo3 (SK-flo3, designated as Jeonju672 in LAT) and Samkwang (SK).

Trial	Location	Variety	HD (mm/dd)	CL (cm)	PL (cm)	TN (No.)	SN (No.)	TGW (g)	BRY (kg/10 a)	Yield Decrease (%)
PYT	Wanju	SK-flo3	8/14	76 ± 1.7	19 ± 0.7	15 ± 1.8	87 ± 20.6 *	18.5 ± 0.62 *	445 ± 24.0 *	–17.7
		SK	8/13	77 ± 2.1	20 ± 0.3	14 ± 2.9	101 ± 12.5	21.3 ± 0.52	541 ± 19.6	
LAT	Suweon	SK-flo3	8/15	86 ± 5.1	19 ± 0.6	14 ± 1.3	119 ± 13.4	18.9 ± 0.56 **	591 ± 49.6 *	–13.0
		SK	8/16	88 ± 4.1	20 ± 1.6	15 ± 1.8	121 ± 13.8	21.9 ± 0.70	679 ± 66.3	
	Hwaseong	SK-flo3	8/16	80 ± 5.4	17 ± 1.3 *	13 ± 1.5	94 ± 15.9	21.9 ± 2.66	491 ± 44.6 *	–17.5
		SK	8/15	81 ± 3.5	19 ± 1.2	13 ± 0.9	109 ± 16.8	23.4 ± 0.77	595 ± 94.9	

PYT: preliminary yield trial (2020); LAT: local adaptability trial (2021 and 2022); HD: heading date; CL: culm length; PL: panicle length; TN: tiller number per plant; SN: spikelet number per panicle; TGW: 1000-grain weight of brown rice; BRY: brown rice yield. * $p < 0.05$; ** $p < 0.01$. The p values are from Student's t-tests for PYT and 2-way (year × variety) ANOVAs for LAT.

3.2. Identification of the Causal Mutation for the Floury Endosperm of SK-flo3

To study the genetics underlying the floury endosperm phenotype of SK-flo3, an F_2 population was derived from the cross between SK-flo3 and wild-type SK. The F_2 population exhibited a 3:1 segregation ratio of the transparent endosperm phenotype to the floury endosperm phenotype (130 transparent:43 floury; chi-square p-value 0.965), indicating that the mutant phenotype is controlled by a single recessive gene. To further test whether the causal mutation of SK-flo3 is allelic to *flo4-4*, a previously reported floury endosperm mutant carrying a missense mutation in *cyOsPPDKB* [13], we crossed SK-flo3 with Baromi2, a Korean rice cultivar carrying the *flo4-4* allele. All individuals in the F_2 population (n = 167) exhibited the floury endosperm, suggesting that SK-flo3 is allelic to *flo4-4*.

Sequencing *cyOsPPDKB* (Os05g04045000-2) from SK-flo3 and SK revealed a G-to-A SNP in exon 7 from SK-flo3 relative to SK, which substitutes glycine (G) at position 354

with aspartic acid (D) (Figure 2). The ORF sequence of *cyOsPPDKB* was identical between SK and Nipponbare, and the G-to-A SNP in exon 7 was the only nucleotide variation of *cyOsPPDKB* between SK and SK-flo3 (GenBank ID: MW349590.1). We designated this mutation as *flo4-6*, a novel induced *cyOsPPDKB* allele conferring the floury endosperm phenotype. To distinguish *flo4-6* from the wild-type allele, a dCAPS marker was developed with the *RsaI* restriction enzyme, which digests the PCR amplicon from SK-flo3 into two fragments (175 bp and 43 bp) while leaving that of the wild-type SK intact (218 bp) (Table 2, Figure 3a).

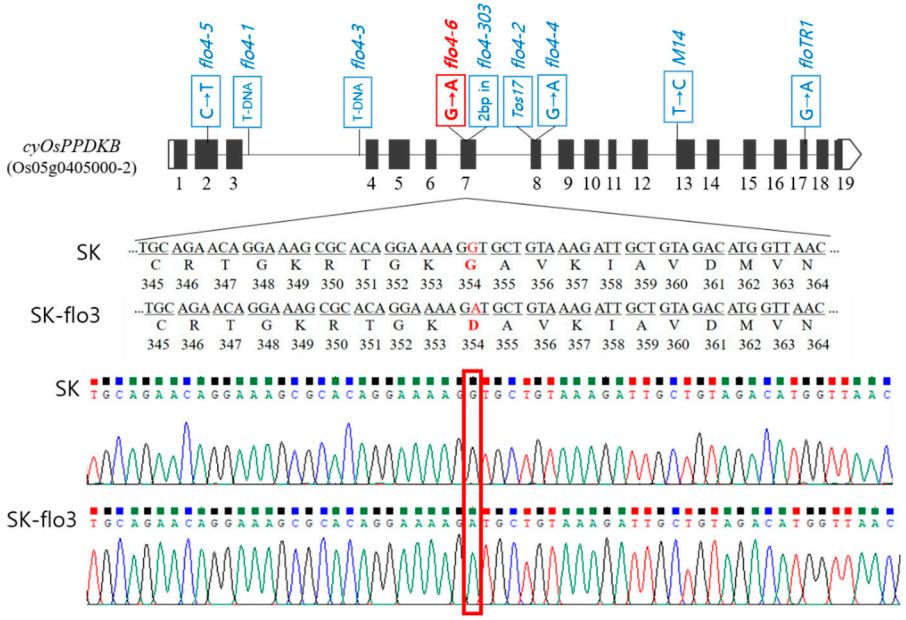

Figure 2. The structure of *cyOsPPDKB* and the sequence near the mutation site in SK-flo3 relative to wild-type SK. The G-to-A mutation in exon 7 of SK-flo3 is highlighted in red boxes. Previously reported allelic mutants of *cyOsPPDKB* are indicated in blue boxes, i.e., *flo4-1* through *flo4-3* [23], *flo4-4* [13], *flo4-5* [24], *M14* [25], *floTR1* [26], and *flo4-303* [27].

Table 2. dCAPS marker for distinguishing *flo4-6* of SK-flo3 from wild-type *cyOsPPDKB* of SK.

Primer Sequence (5′ to 3′)	Restriction Enzyme	Fragment Size
F: GATTTGAAACGTCTTGATCATGC R: AGCATGAACTTGGTCACCTTCTGTCTACAGCAATCTTTACAGTA	*RsaI*	SK-flo3: 218 bp (intact) SK: 175 bp and 43 bp (digested)

Underlined: tail sequence added for size discrimination.

Genotyping the SK/SK-flo3 F_2 population with the dCAPS marker clearly classified three different genotypes—the homozygous wild-type *cyOsPPDKB* from SK, the homozygous *flo4-6* from SK-flo3, and the heterozygous genotype (Figure 3a). All $F_{2:3}$ seeds derived from the homozygous wild-type and homozygous *flo4-6* F_2 plants exhibited transparent and floury endosperm, respectively, while those derived from the heterozygous plants showed a 3:1 ratio of transparent seeds to floury seeds (Figure 3b). The co-segregation pattern confirmed that the G-to-A mutation in exon 7 of *cyOsPPDKB* (*flo4-6* allele) is responsible for the floury endosperm phenotype of SK-flo3.

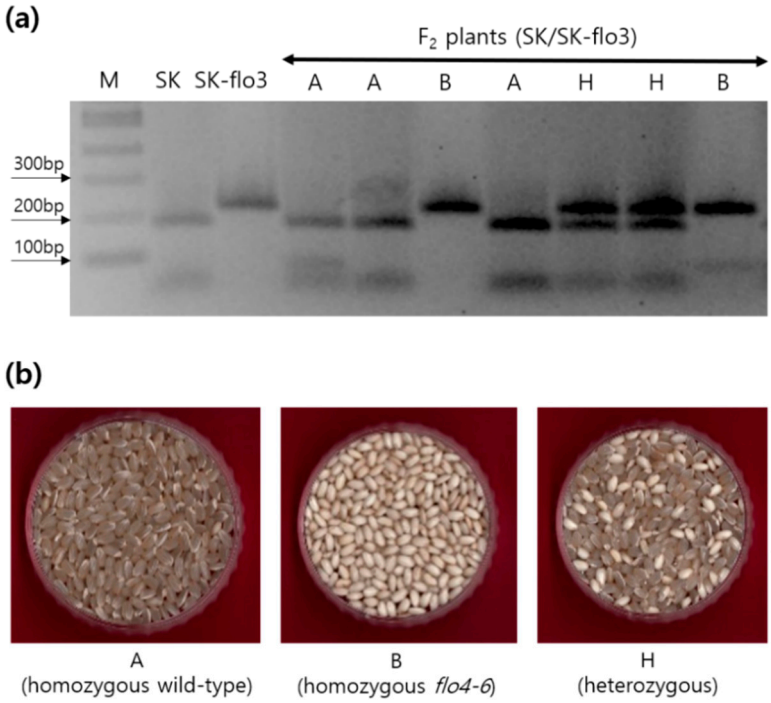

Figure 3. Co-segregation analysis of the *cyOsPPDKB* genotype and endosperm phenotype. (**a**) Genotyping SK, SK-flo3, and the F_2 plants derived from the SK/SK-flo3 cross using the dCAPS marker for *flo4-6*. (**b**) Endosperm phenotype of the $F_{2:3}$ seeds according to the *cyOsPPDKB* genotype of F_2. M indicates a size marker. A, B, and H indicate the homozygous wild-type (SK), the homozygous *flo4-6* (SK-flo3), and the heterozygous genotype, respectively.

3.3. Dry Milling Properties of SK-flo3

To evaluate SK-flo3 as raw material for producing dry-milled rice flour, we compared the physicochemical properties of rice grains and dry-milled flour of SK-flo3 with those of SK and Baromi2. Baromi2, formerly known as Garumi2 [28], is a major Korean rice cultivar carrying *flo4-4* used for dry-milled flour production.

Grain hardness is an important factor determining dry milling suitability because the grains with low hardness produce fine flour particles preferred for enhancing end-use quality [29]. The grain hardness of brown rice of SK-flo3 was 3.0 kg, which was 67% lower than that of SK (9.2 kg) and as soft as that of Baromi2 (3.1 kg) (Table 3). The mean particle size of dry-milled flour of SK-flo3 (65.3 μm) was 28% lower than that of SK (91.1 μm) and similar to that of Baromi2 (61.5 μm). The damaged starch content of the dry-milled flour was 6.0% in SA-flo3, which was one-half the value of SK (12.0%) but slightly higher than that of Baromi2 (4.9%). There was no significant difference in the lightness and ash content of dry-milled flour among SK-flo3, SK, and Baromi2. Overall, our analyses suggest that SK-flo3 and its novel *cyOsPPDKB* allele, *flo4-6*, provide a useful genetic resource for developing rice cultivars suitable for dry-milled flour production.

Table 3. Physicochemical properties of rice grains and dry-milled flour.

Variety	Grain Hardness (kg)	Dry-Milled Rice Flour					
		Mean Particle Size (μm)	Damaged Starch (%)	Amylose (%)	Protein (%)	Lightness (CIE Value)	Ash (%)
SK-flo3	3.0 ± 0.34 [a]	65.3 ± 0.86 [a]	6.0 ± 0.09 [a]	15.6 ± 0.55 [a]	6.2 ± 0.06 [a]	92.6 ± 1.82 [a]	0.64 ± 0.01 [a]
SK	9.2 ± 0.82 [b]	91.1 ± 0.73 [b]	12.0 ± 0.13 [b]	18.0 ± 1.08 [b]	6.4 ± 0.10 [a]	93.2 ± 1.49 [a]	0.67 ± 0.01 [a]
Baromi2	3.1 ± 0.33 [a]	61.5 ± 1.36 [a]	4.9 ± 0.02 [c]	17.0 ± 1.33 [b]	6.5 ± 0.45 [a]	91.2 ± 0.03 [a]	0.58 ± 0.01 [a]

Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

4. Discussion

In this study, we developed a rice mutant line SK-flo3 with floury endosperm through chemical mutagenesis. Analyses of agronomic and physicochemical traits demonstrated that SK-flo3 provides a useful genetic resource for breeding cultivars suitable for dry-milled rice flour production. Through genetic analyses, we identified *flo4-6* as a novel mutated *cyOsPPDKB* allele causative of the floury endosperm phenotype of SK-flo3 and developed a dCAPS marker for efficiently transferring *flo4-6* during molecular breeding. In this section, we discuss the practical use of *flo4-6* in rice breeding programs, the comparison of *flo4-6* with previously identified *flo4* alleles, and future research prospects regarding rice starch biosynthesis.

Samkwang, the wild-type cultivar of SK-flo3, is a high-quality medium-late maturing *japonica* rice cultivar recorded as having the largest seed production of government-certified seeds in Korea from 2017 to 2020 [30]. As SK-flo3 retains the elite genetic background of Samkwang, it represents useful breeding material to develop rice cultivars for dry-milled flour production with high quality. Our study demonstrated that SK-flo3 possesses excellent dry milling properties, such as low grain hardness, fine flour particle size, and low damaged starch content, which are comparable to Suweon542 [12], a previously developed floury endosperm mutant in the genetic background of Namil, a Korean *japonica* rice cultivar with early maturity. Yield reduction is a common drawback of floury endosperm cultivars because the loosely-packed starch granule structure of the floury endosperm reduces grain weight. In PYT (2020) and LAT (2021 and 2022) conducted in three different locations, SK-flo3 showed a 13.0–17.7% yield reduction relative to wild-type SK. As the yield reduction observed in SK-flo3 is more severe than that observed in Suweon542, which showed only a 6% decrease relative to wild-type Namil [12], breeding efforts are underway to improve the yield potential of SK-flo3 by crossing it with high-yielding *japonica* cultivars. Similar breeding efforts have proven successful in breeding Baromi2, which was developed by crossing Suweon542 with Jopyeong to improve disease resistance and preharvest sprouting tolerance and is currently used as a main rice cultivar for producing dry-milled flour in Korea [14].

The causal mutation for the floury endosperm of SK-flo3 was revealed as an amino acid substitution mutation in exon 7 of *cyOsPPDKB*. We named this *flo4-6* as a novel induced allele of *flo4* [23]. Including *flo4-6*, nine different *flo4* alleles have been reported so far (Figure 2, Table 4). These include three insertional mutant alleles induced by transgenic approaches (*flo4-1*, *flo4-2*, and *flo4-3*), four chemically mutagenized alleles (*flo4-4*, *flo4-5*, *flo4-6*, and *floTR1*), and two radioactively mutagenized alleles (*M14* and *flo4-303*) (see Table 4 for the relevant references). All nine *flo4* mutants exhibit a white non-translucent appearance throughout the endosperm with a loosely packed starch granule structure, resulting in a decrease in grain weight of 6–16% (Table 4). These characteristics are also observed in other floury endosperm mutants carrying induced mutations in different *flo* genes, such as *FLO2*, *AGPL2*, *FLO10*, *AlaAT1*, *NDUFA9*, *NPPR3*, and *CMDH* (reviewed in [7]). Unlike the common endosperm appearance and reduced grain weight, physicochemical traits showed wide variation among different *flo4* mutants (Table 4). For example, the changes in amylose content relative to the wild type ranged from a 46% decrease in *floTR1* to a 5% increase in *flo4-4*, and those in the protein content ranged from an 18% decrease in *flo4-4* to a 16% increase in *floTR1*. Considering the *flo4* mutants were developed under different genetic backgrounds and carry a number of random background mutations from chemical or radioactive mutagenesis, it is currently unclear whether the wide variation in the physicochemical traits are the effects of different *cyOsPPDKB* alleles or whether there are other underlying genetic causes. Developing near-isogenic lines (NILs) carrying different *flo4* alleles in the identical genetic background would help answer this question. The NILs are also required for evaluating the more precise effects of different *flo4* alleles on agronomic, physicochemical, and milling properties. This will provide breeders with information that can be used when choosing proper *flo4* alleles. Further evaluation on the quality of processed food made from rice flour, such as bread, noodles, and cookies,

would also facilitate the utilization of the floury endosperm rice cultivars, as recently demonstrated by *floTR4*, which confers enhanced bread loaf volume as well as dry milling properties [26].

Table 4. Allelic mutants of *cyOsPPDKB* characterized in rice.

Mutant	Mutagen (Wild-Type)	Effect (Position [z])	GW	AC	PC	LC	HD	PS	DS	Ref.
flo4-1	T-DNA (Dongjin)	insertional (intron 3)	6%↓	15%↓	~5%↑ [y]	~30%↑ [y]	-	-	-	[17,23]
flo4-2	Tos17 (Hwayoung)	insertional (exon 8)	7%↓	15%↓	~5%↑ [y]	~35%↑ [y]	-	-	-	[17,23]
flo4-3	T-DNA (Hwayoung)	insertional (intron 3)	-	-	-	-	-	-	-	[23]
flo4-4 [*flo7(t)*]	sodium azide (Namil)	Gly→Asp (exon 8)	16%↓	5%↑	18%↓	-	56%↓	25%↓	47%↓	[12,13]
flo4-5	sodium azide (Namil)	Ser→Phe (exon 2)	11%↓	=	15%↓	107%↑	55%↓	21%↓	45%↓	[24,31]
M14	gamma-ray (Kitaake)	Leu→Phe (exon 13)	11%↓	13%↓	=	-	-	-	-	[25]
floTR1	sodium azide (Tsugaruroman)	Gly→Asp (exon 17)	11%↓	46%↓	16%↑	-	-	50%↓ [x]	27%↓	[26]
flo4-303	gamma-ray (Hoshinoyume)	frame-shift (exon 7)	10%↓	14%↓	-	-	-	38%↓ [x]	40%↓	[27]
flo4-6	sodium azide (Samkwang)	Gly→Asp (exon 7)	6–14%↓	13%↓	=	-	67%↓	28%↓	50%↓	this study

GW grain weight; AC amylose content; PC protein content; LC lipid content; HD hardness of rice grain; PS particle size of rice flour; DS damaged starch content; ↑ increase, ↓ decrease, and = equivalent relative to wild-type; - unavailable. [z] See Figure 2 for the positions of the causal mutations. The exon/intron position is based on the Os05g0405000-2 annotation for *cyOsPPDKB*. [y] PC and LC changes in *flo4-1* and *flo4-2* relative to wild-type were estimated based on the bar graphs in the figures of the relevant references. [x] median particle size for *floTR1* and *flo4-303*, while the rest are the mean particle size.

Allelic variation in *cyOsPPDKB* also provides useful material for gene function studies. Pyruvate orthophosphate dikinase (PPDK) encoded by the *PPDK* gene is an enzyme that produces pyruvate and ATP from PEP, PPi, and AMP [32]. Rice *OsPPDKB* produces two different transcripts—a chloroplastic *PPDKB* (*chOsPPDKB*; Os05t0405000-01) and a cytoplasmic *PPDKB* (*cyOsPPDKB*; Os05t0405000-02). Of these, *cyOsPPDKB* is expressed highly in the endosperm and is considered to play an important role in compensating for the lack of ATP under anaerobic conditions in the developing endosperm [32]. The current study, as well as previous research, demonstrates that *cyOsPPDKB* plays a crucial role in rice endosperm starch synthesis because not only the knockout mutations (*flo4-1*, *flo4-2*, *flo4-3*, and *flo4-303*) but also the missense mutations (*flo4-4*, *flo 4-5*, *flo 4-6*, *M17*, and *flo-TR*) substituting an amino acid in different protein regions exhibit the similar floury endosperm appearance (Figure 2, Table 4). As these mutants show wide variation in other physicochemical properties, such as amylose and protein contents, a more precise molecular genetics study is required to further elucidate the function of *cyOsPPDKB* in rice endosperm development. In addition to the mutant *flo4* alleles, creating an allelic variation with an identical genetic background using a gene editing tool, such as CRISPR-Cas9, would help elucidate the molecular function of *cyOsPPDKB* and evaluate its role in grain development.

5. Patents

Patent number; 10-2020-0181310 (application date: 22 December 2020).

Author Contributions: Conceptualization, J.-U.J.; investigation, S.-K.H., H.-S.L., S.Y.L. and C.-M.L.; writing—original draft preparation, S.-K.H. and S.Y.L.; writing—review and editing, Y.M.; supervision, Y.M. and J.-U.J.; project administration, J.-U.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Institute of Crop Science, Rural Development Administration of South Korea (PJ016831012022).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fukagawa, N.K.; Ziska, L.H. Rice: Importance for Global Nutrition. *J. Nutr. Sci. Vitam.* **2019**, *65*, S2–S3. [CrossRef] [PubMed]
2. Pingali, P. Westernization of Asian Diets and the Transformation of Food Systems: Implications for Research and Policy. *Food Policy* **2007**, *32*, 281–298. [CrossRef]
3. KOSTAT. The 2021 Results of Grain Consumption Survey. 2021. Available online: http://kostat.go.kr/portal/korea/kor_nw/1/4/8/index.board (accessed on 24 December 2022).
4. Kim, M.H. Review on Rice Flour Manufacturing and Utilization. *J. Biosyst. Eng.* **2013**, *38*, 103–112. [CrossRef]
5. Jeong, O.Y.; Park, H.S.; Baek, M.K.; Kim, W.J.; Lee, G.M.; Lee, C.M.; Bombay, M.; Ancheta, M.B.; Lee, J.H. Review of Rice in Korea: Current Status, Future Prospects, and Comparisons with Rice in Other Countries. *J. Crop Sci. Biotechnol.* **2021**, *24*, 1–11. [CrossRef]
6. Chiang, P.-Y.; Yeh, A.-I. Effect of Soaking on Wet-Milling of Rice. *J. Cereal Sci.* **2002**, *35*, 85–94. [CrossRef]
7. Mo, Y.; Jeung, J.-U. The Use of Floury Endosperm Mutants to Develop Rice Cultivars Suitable for Dry Milling. *Plant Biotechnol. Rep.* **2020**, *14*, 185–191. [CrossRef]
8. Satoh, H.; Omura, T. New Endosperm Mutations Induced by Chemical Mutagens in Rice *Oryza sativa* L. *Jpn. J. Breed.* **1981**, *31*, 316–326. [CrossRef]
9. Araki, E.; Ashida, K.; Aoki, N.; Takahashi, M.; Hamada, S. Characteristics of Rice Flour Suitable for the Production of Rice Flour Bread Containing Gluten and Methods of Reducing the Cost of Producing Rice Flour. *Jpn. Agric. Res. Q.* **2016**, *50*, 23–31. [CrossRef]
10. Won, Y.-J.; Ahn, E.-K.; Jeong, E.-G.; Chang, J.-K.; Lee, J.-H.; Jung, K.-H.; Hyun, U.-J.; Cho, Y.-C.; Oh, S.-K.; Yoon, M.-R.; et al. An Opaque Endosperm Rice Cultivar, 'Hangaru', Suitable for Exclusive Dry-Milling Rice Flour Production. *Korean J. Breed. Sci.* **2019**, *51*, 134–139. [CrossRef]
11. Cho, Y.; Baek, M.; Park, H.; Cho, J.; Ahn, E.; Suh, J.; Jeung, J. 'Shingil (Milyang317)', Tongil-Type Variety Specialized for Rice Flour. *Korean J. Breed. Sci.* **2020**, *72*, 58–72. [CrossRef]
12. Mo, Y.; Jeung, J.-U.; Shin, Y.-S.; Park, C.S.; Kang, K.-H.; Kim, B.-K. Agronomic and Genetic Analysis of Suweon 542, a Rice Floury Mutant Line Suitable for Dry Milling. *Rice* **2013**, *6*, 37. [CrossRef] [PubMed]
13. Wang, H.; Mo, Y.; Im, D.E.; Jang, S.G.; Ham, T.H.; Lee, J.; Jeung, J.U.; Kwon, S.W. A New SNP in *CyOsPPDK* Gene is Associated with Floury Endosperm in Suweon 542. *Mol. Genet. Genom.* **2018**, *293*, 1151–1158. [CrossRef] [PubMed]
14. Ha, S.K.; Kim, B.-K.; Hwang, W.-H.; Mo, Y.; Jeong, J.-M.; Lee, D.-K.; Kim, W.-J.; Kim, J.-J.; Jeong, J.-U. Early Maturing Rice Variety "Baromi2" with a Floury Endosperm and Suitable for Dry-Milling of Rice Grain. *Korean J. Breed. Sci.* **2022**, *54*, 433–441. [CrossRef]
15. Ha, S.K.; Mo, Y.; Jeong, J.-M.; Lee, H.-S.; Kim, J.; Seo, W.-D.; Jeong, J.-U. Agronomic and End-Use Quality Analysis of "AromaT", a Black Rice (*Oryza sativa* L.) Variety with Floury Endosperm. *Korean J. Crop Sci.* **2022**, *67*, 9–16. [CrossRef]
16. Yang, H.; Wang, Y.; Tian, Y.; Teng, X.; Lv, Z.; Lei, J.; Duan, E.; Dong, H.; Yang, X.; Zhang, Y.; et al. Rice *FLOURY ENDOSPERM22*, Encoding a Pentatricopeptide Repeat Protein, Is Involved in Both Mitochondrial RNA Splicing and Editing and Is Crucial for Endosperm Development. *J. Integr. Plant Biol.* **2023**, *65*, 755–771. [CrossRef]
17. Ryoo, N.; Yu, C.; Park, C.S.; Baik, M.Y.; Park, I.M.; Cho, M.H.; Bhoo, S.H.; An, G.; Hahn, T.R.; Jeon, J.S. Knockout of a Starch Synthase Gene *OsSSIIIa/Flo5* Causes White-Core Floury Endosperm in Rice (*Oryza satva* L.). *Plant Cell Rep.* **2007**, *26*, 1083–1095. [CrossRef]
18. Zhang, D.; Wu, J.; Zhang, Y.; Shi, C. Phenotypic and Candidate Gene Analysis of a New Floury Endosperm Mutant (*Osagpl2-3*) in Rice. *Plant Mol. Biol. Report.* **2012**, *30*, 1303–1312. [CrossRef]
19. Long, W.; Dong, B.; Wang, Y.; Pan, P.; Wang, Y.; Liu, L.; Chen, X.; Liu, X.; Liu, S.; Tian, Y.; et al. *FLOURY ENDOSPERM8*, Encoding the UDP-Glucose Pyrophosphorylase 1, Affects the Synthesis and Structure of Starch in Rice Endosperm. *J. Plant Biol.* **2017**, *60*, 513–522. [CrossRef]
20. You, X.; Zhang, W.; Hu, J.; Jing, R.; Cai, Y.; Feng, Z.; Kong, F.; Zhang, J.; Yan, H.; Chen, W.; et al. *FLOURY ENDOSPERM15* Encodes a Glyoxalase I Involved in Compound Granule Formation and Starch Synthesis in Rice Endosperm. *Plant Cell Rep.* **2019**, *38*, 345–359. [CrossRef]
21. RDA. *Manual for Standard Evaluation Method in Agricultural Experiment and Research*; RDA Press: Suweon, Republic of Korea, 2012; pp. 317–338, ISBN 978-8-948-01649-9.
22. Neff, M.M.; Turk, E.; Kalishman, M. Web-Based Primer Design for Single Nucleotide Polymorphism Analysis. *Trends Genet.* **2002**, *18*, 613–615. [CrossRef]

23. Kang, H.G.; Park, S.; Matsuoka, M.; An, G. White-Core Endosperm *floury endosperm-4* in Rice Is Generated by Knockout Mutations in the C4-Type Pyruvate Orthophosphate Dikinase Gene (*OsPPDKB*). *Plant J.* **2005**, *42*, 901–911. [CrossRef]
24. Wang, H.; Ham, T.H.; Im, D.E.; Lar, S.M.; Jang, S.G.; Lee, J.; Mo, Y.; Jeung, J.U.; Kim, S.T.; Kwon, S.W. A New SNP in Rice Gene Encoding Pyruvate Phosphate Dikinase (PPDK) Associated with Floury Endosperm. *Genes* **2020**, *11*, 465. [CrossRef] [PubMed]
25. Zhang, L.; Zhao, L.; Lin, L.; Zhao, L.; Liu, Q.; Wei, C. A Novel Mutation of *OsPPDKB*, Encoding Pyruvate Orthophosphate Dikinase, Affects Metabolism and Structure of Starch in the Rice Endosperm. *Int. J. Mol. Sci.* **2018**, *19*, 2268. [CrossRef] [PubMed]
26. Muroyama, R.; Ito, H.; Takahashi, S.; Kang, D.J.; Hamada, S. Biochemical Analysis of a Novel Allele of the *OsPPDKB* Gene Associated with Floury Endosperm. *J. Cereal Sci.* **2022**, *107*, 103529. [CrossRef]
27. Matsuba, S.; Maruyama-Funatsuki, W.; Umemoto, T.; Kato, H.; Kuroki, M.; Yokogami, N.; Ikegaya, T.; Shimizu, H.; Iriki, N. The Induced Mutant Allele *flo4-303* Confers Floury Characteristics on the Japonica Rice Cultivar 'Hoshinoko'. *Breed. Sci.* **2022**, *72*, 383–388. [CrossRef] [PubMed]
28. Jeong, J.-U.; Kim, B.-K.; Hwang, W.-H.; Mo, Y.; Jeong, J.-M.; Lee, D.-K.; Ha, S.K.; Kim, W.-J.; Kim, J.-J. New Rice Cultivar, Garumi1 and Garumi2 Suitable for Dry Milling. Korea Intellectual Property Rights Information Service. 2019. Available online: http://kpat.kipris.or.kr/kpat/biblioa.do?method=biblioFrame&link=Y&applno=1020200066078&pub_reg= (accessed on 7 February 2023).
29. Ashida, K.; Iida, S.; Yasui, T. Morphological, Physical, and Chemical Properties of Grain and Flour from Chalky Rice Mutants. *Cereal Chem.* **2009**, *86*, 225–231. [CrossRef]
30. Jeong, J.-U.; Shin, Y.-S. Evaluations on the Namil(SA)-Flo1, a Floury Japonica Rice Line, for Dry Milling Process to Produce Rice Flour. *Korean J. Crop Sci.* **2011**, *56*, 57–63. [CrossRef]
31. Korea Seed & Variety Service. Available online: https://www.seed.go.kr/sites/seed_eng/index..do (accessed on 2 January 2023).
32. Lee, S.K.; Jeon, J.S. Review: Crucial Role of Inorganic Pyrophosphate in Integrating Carbon Metabolism from Sucrose Breakdown to Starch Synthesis in Rice Endosperm. *Plant Sci.* **2020**, *298*, 110572. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.