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Fine mapping and candidate analysis of *Osgl*: a gene regulating grain shape and quality in *japonica* rice

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Abstract

Grain size is one of the important agronomic traits that determines *japonica* rice (*Oryza sativa* subsp. *japonica*) yield. Map-based cloning and genetic analysis of grain size-related genes have an impact on elucidating the genetic regulation mechanism of rice yield formation. In this study, the *Osgl* gene was identified by map-based cloning. The mutant has significant increase in grain length, grain width and grain length and width ratio, yield per plot, panicle length, number of grains per panicle and seed setting rate compared to the wild type (WT). There was no noticeable difference in grain thickness and 1000-grain weight between the *Osgl* mutant and wild type. Taken together, the result signifies that the *Osgl* mutant regulates organ size grain length possibly due to cell proliferation. Starch granules in the *Osgl* mutant are closely arranged and regular polyhedron-like, while the composite starch particles contained in the white, opaque parts of the wild-type endosperm are loosely arranged, and the shape changes to spherical or ellipsoid. Genetic analysis showed that the *Osgl* mutant was controlled by a recessive single gene and was finely located in chromosome 3 through map-based cloning within a physical distance of 40 kB. Sequencing analysis revealed that A to G substitution in the 4th exon of *Os03g0407400* gene resulted in an amino acid change glycine to argenine in PEBP-like domain. Taken together, *Osgl* mutant has a pleiotropic effect on grain yield and grain quality.

Key words: gene mapping, grain shape, grain quality, *Oryza sativa* subsp. *japonica*.

Introduction

Rice is a cereal food crop widely grown and consumed. About 3 billion people in the world feed on rice as the staple food. As the population continues to grow and the arable land continues to decrease, food production is still facing tremendous pressure (Xing, Zhang, 2010). Grain shape is an important factor as well as indicator to measure the appearance quality of rice. The main indicators of rice grain shape include grain length, grain width and aspect ratio (Zeng et al., 2017). Among them, grain length has significant positive correlation with the 1000-grain weight (Huang et al., 2013). Grain length is a quantitative trait controlled by multiple genes (Qian et al., 2016). There are many reports on the identification of grain length quantitative trait locus (QTL) in more than 20 different populations. The existence of grain length QTL has been detected on all 12 chromosomes of rice (Huang et al., 2013). To date, many genes controlling grain length have been detected and cloned in rice through map-based cloning. All prominent examples: *GS3* (Mao et al., 2010), *GL3.1* (Qi et al., 2012), *GL3.3* (Hu et al., 2018), *LG3* (Xiong et al., 2018) and *LG3b* (Yu et al., 2018), were mapped in chromosome 3. In addition, multiple signalling pathways were reported to regulate grain size such as G-protein, ubiquitin-proteasome, mitogen-activated protein and transcriptional factor together with phytohormones (Zuo, Li, 2014; Li et al., 2019).

A lot of studies have been done on grain shape regulation in rice, and many pointed out that grain size is determined by cell proliferation or cell expansion (Xing, Zhang, 2010; Hu et al., 2015; Feng et al., 2016). *GL3.1/qGL3* is a grain weight and length QTL located in chromosome 3. Two research teams have completed the cloning of this gene (Qi et al., 2012; Zhang et al., 2012). Located within the range of 20 and 47 kB, respectively, *GL3.1* encodes serine/threonine protein phosphatase *OsPPKL1*, which accelerates cell division by affecting protein phosphorylation in spikelets resulting in longer grains (Zhang et al., 2012). *OsPPKL1* contains two Kelch domains, of which the rare allelic variation *qgl3* in AVLDT conserved region situated in second Kelch domain results in a long-grain phenotype (Hu et al., 2012). Sequencing of 94 cultivars revealed that only two cultivars carried long-grain alleles of *GL3.1/qGL3* indicating that it is a rare allele (Zhang et al., 2012). *TGW3/qTGW3/GL3.3* is located in chromosome 3, which

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mainly controls grain weight and length. *GL3.3* encodes a protein kinase *OsGSK5/OsSK41* of the *GSK3/SHAGGY*-Like family. Functional loss of this gene will increase rice grain length and grain weight and is a negative controller for grain length and grain weight.

The protein encoded by *TGW3* is located in the nucleus and cytoplasm. The tissue-specific expression shows that *TGW3* is mostly expressed in an immature panicle and can synergistically change the size and number of cells in the glume. Further research found that this gene and locus GS3 have a genetic interaction effect, and the superposition of the two can cause a significant increase in rice grain shape. The *GS3-TGW3* genotype combination is commonly used in the breeding of large-grained *japonica* rice (*Oryza sativa* subsp. *japonica*) cultivars, but the application of this combination has not been found in *indica* rice (*Oryza sativa* L. *indica*) ones, so this locus can be used in cultivated *indica* rice cultivars (Hu et al., 2018; Xia et al., 2018; Ying et al., 2018).

At present, excellent rice quality is not only an important research direction for breeders but also a major aspect of consumer concern (Zeng et al., 2017). People usually divide the quality of rice into appearance, milling, cooking and eating and nutritional one. Among them, cooking and eating quality directly affects the taste of rice, which is the most important one of many quality indicators. It is generally closely related to physical and chemical indicators such as amylose content, gelatinization temperature, gel consistency and rapid viscosity value.

In the study, new long-grain *Osgl* mutant, derived from *japonica* rice cultivar 'Zhenong 34' treated with ethyl methenesulfonate (EMS), was reported. The aims were: to examine the morphological difference between the *Osgl* mutant and wild type (WT); to analyse grain quality parameters between the *Osgl* mutant and WT; to identify the candidate gene for grain length through map-based cloning approach.

Materials and methods

Experimental material. Japonica rice (*Oryza sativa* subsp. *japonica*) cultivar 'Zhenong 34' was mutated by ethyl methanesulfonate (EMS), and long-grain mutant was screened from it. After multiple generations of continuous selfing, organ size grain length with stable mutant phenotype was obtained and named *Osgl*. Genetic analysis was obtained based on cross between 'Zhenong 34' and the mutant, respectively. All the seeds were planted at a paddy experimental field of Zijingan campus, Zhejiang University, Hangzhou, China.

Measurement of agronomic and grain quality traits. In 2018, 10 *japonica* rice plants from the middle row of *Osgl* mutant and 10 from the wild type (WT) were selected at the plant maturity stage. The grain length, width and thickness were measured by an electronic digital display Vernier caliper, and fully filled grains were used for measuring the 1000-grain weight. The degree of chalkiness, amylose content, protein content, starch content and alkali spreading values were measured, respectively, according to the national standard NY/T 593-2013 (Cooking rice variety quality).

Rapid viscosity analysis (RVA). The viscosity change of rice flour was measured with RVA Viscosity Tester (Perten, Sweden), and the parameters were set according to the AACC Methods 61-01.01 and 61-02 (Determination of the pasting properties of rice with the rapid Visco Analyser). The first-level parameters read by the instrument were peak, hot paste, cool paste, pasting temperature as well as peak time; the second-level parameters were breakdown, setback along with consistency viscosity. Each sample was measured three times, and the average value was taken.

Determination of protein content and protein fractionation. Crude protein was evaluated with a Kjeltec 2300 Auto analyzer (Foss AB, Sweden). Protein value was computed based on 6.25 nitrogen conversion factor, while rice grain protein fractions were measured according to Kumamaru et al. (1988) with slight modifications. Briefly, 1.0 g flour was successively extracted with 50 mM phosphate buffer (pH 6.8). The same buffer was used to measure albumin content, while buffer with 0.5 M NaCl was used for measuring globulin, and 60% *n*-propanol was used for evaluating prolamin. Furthermore, 0.1 M NaOH was used for measuring glutelin content. The sample was homogenized by adding 10 ml solvent, kept at room temperature for 30 min and centrifuged at 12,000 rpm for 10 min to separate extract from residues.

Fatty acid composition of Osgl mutant and wild type (WT). Fatty acid methyl ester (FAME) was prepared according to the method of Verma and Srivastav (2017) with a little modification for extraction of fatty acid from rice flour. Then the extracted fatty acids were subjected to gas liquid chromatography (GLC) with gas chromatograph Varian CP 3800 (USA) equipped with a flame ionization detector (FID), and fused silica capillary column (50 mm × 0.25 mm), coated with CP-SIL 88 as the stationary phase. The oven temperature was programmed at 200ºC for 13 min. The injector and FID were at 250ºC. A reference standard FAME mix (Supelco Inc.) was analysed under the same operating conditions to determine the peak identity. The FAMEs were expressed as relative area percentage.

Cytological observation. Developing spikelets at the heading stage were collected and put in formaldehyde acetic acid (5% glacial acetic acid, 5% formaldehyde as well as 70% ethyl alcohol) overnight. Followed by dehydrating the samples in ordered ethanol successions cured with dimethyl benzene, slot in paraffin, segmented rotating microtome. In addition, it was stained by means of sarranine plus toluidine blue. The samples were perceived and photographed with Nikon ECLIPSE TI-SR (Japan). Whereas for scanning electron microscopy (SEM) investigation, developing panicles were set inside glutaraldehyde overnight at 4°C temperature as well as cleaned in phosphate buffer (0.1 M, pH 7.0), post-fixed with 1% OsO_4 for 1.5 h and cleaned, dried up through ordered ethanol successions (30, 50, 70, 80, 90, 95 and 100 %), further desiccated and photographed using Hitachi TM-1000 SEM (Japan). While for the chalky grains sharp blade razor was used to scratch the edges of the mature seeds of both WT and mutants and gently break apart from the middle. The other side was cut with a blade, and the unnatural cross-section of the sample gently adhered to the stage with double-sided and photographed with Hitachi S-3400N (Japan). All samples were processed and observed at Zhejiang University, China.

Gene mapping and candidate gene prediction. The total DNA of each individual plant leaf was extracted with CTAB (cetyltrimethyl ammonium bromide) method, respectively equal amount was polled according to the bulk segregant analysis (BSA) method. For genetic analysis, the mutant phenotype and WT plants from the F_2 segregation population were counted at 25 days after heading. To detect the separation ratio, chi-squared test was used (Yu et al., 2018). To determine the location of the *Osgl* gene, a total of 300 SSR (simple-sequence repeat) markers and 50 InDel (insertion/deletion polymorphism) markers were used to select the linkage markers based on the BSA method, described by Su et al. (2017). Then map-based cloning was used to fine map the *Osgl* gene. For designing new polymorphic primers based on the DNA sequence differences between (japonica) and (indica), databases NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Gramene (http://www.gramene.org/), and software *DNASTAR* (DNASTAR Inc. USA) and *Primer 5* (Source Forge, USA) were used (Yu et al., 2018). The primers used for gene mapping are listed in Table 1. Database Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/) was used to obtain the function of candidate genes in the region, then the candidate genes of mutant and WT

Table 1. List of *Oryza sativa* subsp. *japonica* primers used for fine mapping *Osgl* gene

Marker	Forward primer (5′→3′)	Reverse primer (5′→3′)
R3M23	AGCAAAGCTGGAACGAAGAG	TAAATTACGCCGTGTCAACG
M3MS1	GCAACCAAGTCCACGCTAAT	TAGCCGAAGATCAGCCTCCT
M3M4	GATTATTGGAGACGGGACGA	GACGGCATGACCACTCTTTT
M3M1	AGCTTTGGTGTCGTTCTGCT	CCGACTTGGAGAGAATGGAA
M3M12	GCTGTGTTGTCCTTTGCTGA	CCAATAAACCCCACTGCAAC
M3M6	AAGAACGACTACGCGCATCT	CCATCGCTCTCTTTCCTCAG
M3M2	CAACACCAGCAACGAACAAC	ACGAGGGATTATCAGCCATT
M3M5	CGGTATGCCAAGTTGAATGA	TTGCCGCAGTAAACAAGAAG
M3M17	TGCCCATCTCCCTCGTTTAC	TGTTCGTTGCTGGTGTTG
M3M20	CCTTCAGTAAGAGAGATGTG	AGTTGATGGTTTTGTGGGAT
M3M21	TCTGCTTCICGGTTATCTGTA	TTAGGTCCCTTTTCTCGTCC
M3M22	CAACACCAGCAACGAACAAC	ACGAGGGATTATCAGCCATT

were sequenced and NCBI blast was used (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to analyse the results and determinate the mutation site.

The polymerase chain reaction (PCR) system was 10 µL and included the components: 1 µL 10 × PCR buffer (Mg^{2+} plus), 0.1 µL dNTPs (2.5 mM), 1 µL primer (10 µM), 1 µL DNA template, 0.1 µL Taq enzyme (5U μL^{-1}), add double distilled H_2O to make up to 10 µL. The PCR program was: pre-denaturation at 94°C for 5 min, 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, 35 cycles and a final extension at 72°C for 4 min. The product after electrophoresis was detected on 8% polyacrylamide gel and silver staining.

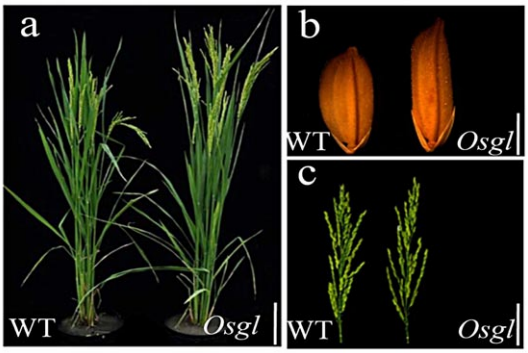
Gene expression analysis. Total RNA from leaves and other tissues was extracted using reagent TRIzol, and RNA concentration was checked by agarose gel electrophoresis and micro spectrophotometer Nano Drop 2000 (Thermo Fisher Scientific Inc.). For gene expression, Prime Script RT Reagent Kit (Takara Bio Inc.) with gDNA eraser and SYBR Green Premix Ex Taq II (Takara Bio Inc.) were used. The reaction system was 20 µL, and it was performed on a fluorescent quantitative PCR machine Light Cycler 480 (Roche, Switzerland). The PCR amplification program was: 95°C 5 min (95°C 10 s, 60°C 30 s) × 45 cycles; dissolution curve reaction conditions: 95°C 30 s, 65°C 1 min, 95°C continuous. Cooling at the end of the reaction was 40°C for 30 s. After the PCR completed, as reference rice gene *OsActin1* was used. The relative amount of gene expression was calculated using the comparative 2-ΔΔCT method (Yu et al., 2018).

Statistical analysis. The data are presented as the mean ± standard deviation (SD). The software *SPSS Statistics*, version 16.0 (IBM Inc., USA) was used for all statistical analyses. Statistical significance was determined for independent biological samples using *Student's t*-test. Differences were considered significant at $P < 0.05$; an asterisk (*) was presented.

Results

Comparison of agronomic and yield traits of Osgl mutant and wild type (WT). A plant with organ size grain length (*Osgl*) mutation was identified through ethyl methane sulfonate (EMS) mutagenesis with rice cultivar 'Zhenong 34'. *Osgl* mutation was identified from a screen of mutants in M2 generation. Phenotypic comparison between the *Osgl* mutant with WT showed contrasting difference: plant height of *Osgl* mutant was slightly decreased, and plant architecture was upright and compact (Figure 1a). The panicle of *Osgl* mutant was also compact and longer than that of the WT (Figure 1b).

Phenotypic comparison between the *Osgl* mutant and WT shows contrasting difference: there is significant increase in plant height, grain length, grain length to width ratio in *Osgl* mutant compared with the WT. The number of grains per panicle and seed setting rate were all lowered in *Osgl* mutant compared to the WT with no noticeable difference in 1000-grain weight ($P \leq 0.05$) between the *Osgl* mutant and WT (Table 2).



Note. The WT and *Osgl* mutant at anthesis stage (scale bar – 10 cm) (a), full-grown grains of WT and *Osgl* mutant (scale bar – 1 mm) (b) and full-grown panicles (scale bar – 5 cm) (c).

Figure 1. Phenotypic characterization of organ size grain length (*Osgl*) of *Oryza sativa* subsp. *japonica* wild type (WT) and *Osgl* mutant

Osgl gene regulates the growth of spikelet hull as a result of promoting cell division and cell expansion. The grain length and width of the spikelet hull were larger in *Osgl* mutant compared with the WT. Consistent with this, the length of the outer parenchyma cell layer and the number of cells were significantly increased in *Osgl* mutant. Taken together, these data indicate that *Osgl* mutant regulates grain size by promoting cell proliferation (Figure 2).

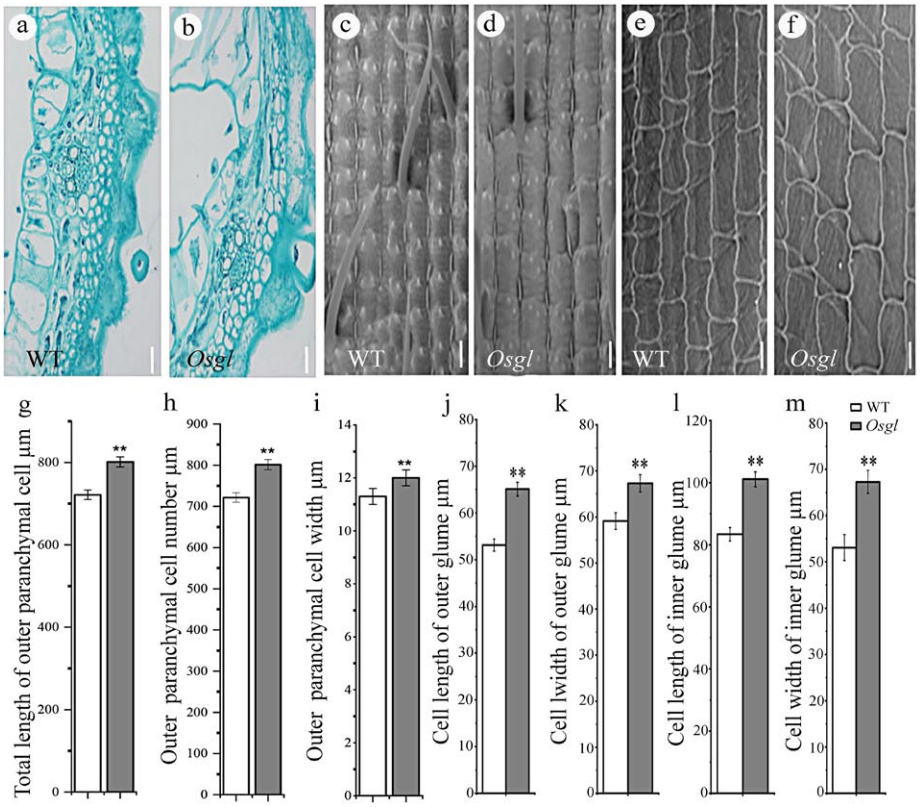
Genetic analysis and map-based cloning of Osgl gene. There were 385 medium-grain plants and 144 long-grain phenotype in F_2 population of *Osgl*/Zhenong 34 (WT). Segregation ratio of WT to Osgl mutant fitted Mendel ratio 3:1 ($x^2 = 0.73 < x^2_{0.05} = 3.84$, n = 408). This result specified that the long-grain trait was controlled as a result of a single recessive gene. Mapping analysis was conducted using F_2 population resulting from the crossing between the *Osgl* mutant to cultivar 'Zhenongda 104'. Candidate gene mapped in chromosome 3 between InDel markers R3M23 and M3MS1 (Figure 3a). A set of 541 individuals with long grains and some markers were used. Target locus narrowed to 40 kB interval between markers M3M12 and M3M6 (Figure 3b).

A total of 5 recognized genes were dispersed in the region on Rice Genome Annotation Project (RGAP) catalogue as well as the gene function interpretations were found on http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/. The genes are *Os03g0407000*, *Os03g0407100*, *Os03g0407400*, *Os03g0407900* and *Os03g0408101*. To find the candidate gene among them, all the genes were sequenced, and from all the sequence no difference was found between the *Osgl* mutant and WT with the exception of *Os03g0407400*, which was found single base substitution of G to A mutation in the 4th exon of *Osgl* mutant, which changes amino acid glycine to argenine in PEBP (phosphatidylethanolamine-binding protein)-like domain (Figure 3d, Table 3).

Table 2. Comparison of grain yield and other agronomic traits between *Oryza sativa* subsp. *japonica* wild type (WT) and *Osgl* mutant

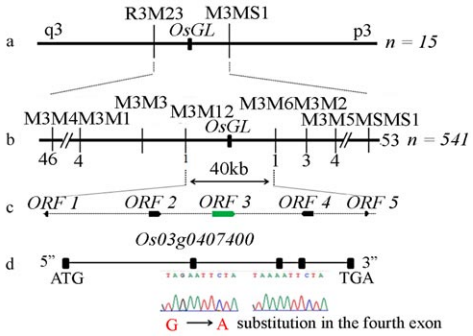
Traits	WT	*Osgl*
Grain length mm	8.03 ± 0.10	9.19 ± 0.17**
Grain width mm	2.34 ± 0.17	2.39 ± 0.7
Grain thickness mm	2.02 ± 0.7	1.94 ± 0.03
Grain length to width ratio	2.72 ±0.14	3.92 ± 0.11**
1000-grain weight g	26.07 ± 0.12	25.92 ± 0.16
Panicle length cm	22.5 ± 0.61	21.42 ± 0.95
Grain per panicle	225.33 ± 10.02	216.33 ± 12.8
Plant height cm	60.2. ± 2.15	45.8. ± 2.2**
Seed setting rate	85.02 ± 3.6	73.00 ± 4.7**
Yield per plot kg	85.02 ± 3.6	73.00 ± 4.7**

** – significant at $P \leq 0.01$



Note. Comparison of WT and *Osgl* mutant total length of paranchyma cell, number and width of paranchyma cell (l, m)

Figure 2. Scanning electron microscopy (SEM) cross-section of *Oryza sativa* subsp. *japonica Osgl* mutant (a, b) and wild type (WT) (c, f), comparison of *Osgl* mutant and WT total cell length, width and number of paranchyma cell (g, i), SEM of external (j, k) and internal (l, m) glume surface (scale bar – 200 and 100 µM)



Primary mapping of *Osgl* gene in chromosome 3 with 15 individual F_2 plants (a); the *OsGL* gene mapping to a distance of 40 kB using 541 F2 individuals (b); 5 genes distributing within the region (c); the single based substitution of G to A mutation of *Os03g0407400* gene (d)

Figure 3. The *Oryza sativa* subsp. *japonica Osgl* gene mapping

Annotation of *Os03g0407400* gene showed that it encoded a protein with plant-specific organ size regulation, which is among the G-protein signalling pathways.

Mutational confirmation of OsGL gene. To validate the mutation site, the cleaved amplified polymorphic sequence (CAPS) marker with *Eco*RI restriction enzymes site primer was designed. After the digestion *Eco*RI cut the mutant sequence, and a clear co-segregation was observed between the *Osgl* mutant, WT and F_2 population. The results show consistency between the *Osgl* mutant and WT. Also, the significant association involving *Osgl* gene and G to A mutation confirmed the contribution of G to A mutation in *Osgl* gene. For this reason, the improvement of markers on the basis of single nucleotide polymorphisms (SNPs) could differentiate alleles conferring *Osgl* mutant and WT (Figure 4). Hence, the *Os03g0407400* gene, named as *Osgl*, was the gene responsible for the grain length as well as organ size.

Table 3. Annotated *Oryza sativa* subsp. *japonica* genes in the mapping region

Gene locus	Putative function	Sequence difference
Os03g0407000	Von Willebrand factor, type A domain-containing protein	no difference
Os03g0407100	A protein of unknown function DUF1677, plant family protein	no difference
Os03g0407400	Protein with plant-specific organ size regulation (OSR) domain, a transmembrane region, TNFR/NGFR (tumor necrosis factor receptor/nerve growth factor receptor) family cysteine-rich domain and VWFC (von Willebrand factor type C) module, regulator of grain size and organ size	single base change of G to A mutation in the 4th exon
Os03g0407900	Similar to serine/threonine-protein kinase-like	no difference
Os03g0408101	Hypothetical protein	no difference

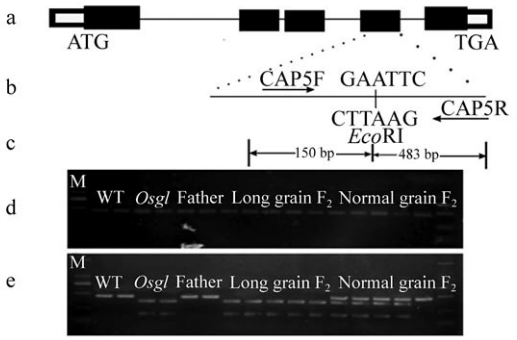
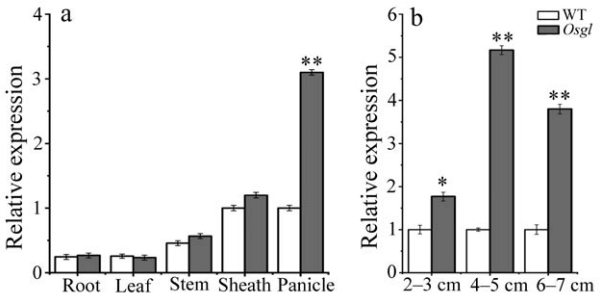


Figure 4. Cleaved amplified polymorphic sequence (CAPS) marker: gene structure (a), sequence change and restriction enzyme (b), CAPS undigested (c) and CAPS digested (d) gel results of *Oryza sativa* subsp. *japonica*



Note. OsActin gene was used as the control, and the values of expression levels in the WT were set to 1 (n=3); data are presented as mean±SD.

Figure 5. Relative expression of *Osgl* gene in *Oryza sativa* subsp. *japonica*

Gene expression pattern analysis of OsGL gene. The quantitative real-time polymerase chain reaction (qRT-PCR) was used to explore the expression pattern of *OsGL* gene (Figure 5). *OsGL* gene was considerably expressed not only in panicle but in all the other tissues tested such as root, stem, sheath and leaf (Figure 5a). In addition, difference of expression level was observed all through panicle developmental process, and the expression pattern shows that there was noticeable expression at 2–3 cm; however, significant expression was observed at 4–5 cm (Figure 5b), and the expression kept decreasing as the panicle matured. These results were consistent with gene biological function of organ size grain regulation.

Performance of grain quality traits in Osgl mutant and WT. To further study the possibility, by which *Osgl* mutant allele results in change of grain quality traits such as amylose content, starch, gelatinization temperature, gel consistency and chalkiness degree as well as the chalkiness percentage, the grain quality traits were compared between the *Osgl* mutant and WT. There was a significant increase in alkali spreading value, protein content and gel consistency in *Osgl* mutant; however, the degree of chalkiness and starch content were significantly lower in *Osgl* mutant compared with the WT. There was no noticeable variation between the *Osgl* mutant and WT for amylose content. Results of our experiment indicated that *Osgl* mutant could have a pleiotropic effect on both grain size and grain quality traits in rice (Figure 6).

Morphological observation of starch structure of *Osgl* mutant and WT. Earlier studies revealed that chalky endosperm usually is filled with loosely packed as well as spherical starch granules, while transparent endosperm usually has closely packed polyhedral starch granules (She et al., 2010). Using SEM, the morphological properties of chalky grain were observed in both *Osgl* mutant and WT. The observed result shows contrasting changes in microstructure between the *Osgl* mutant and WT from the upper as well as bottom parts of translucent grains and from the upper translucent part of the white part of the grains (Figure 7). Composite starch particles present in the endosperm of *Osgl* mutant are closely arranged and regular polyhedron-like (Figure 7b, d and f), while the composite starch particles contained in the white, opaque parts of the wild-type endosperm are loosely arranged, and the shape changes to spherical or ellipsoid (Figure 7a, c and e). The results indicate that the morphological structure and arrangement of starch particles in the endosperm of the WT are defective.

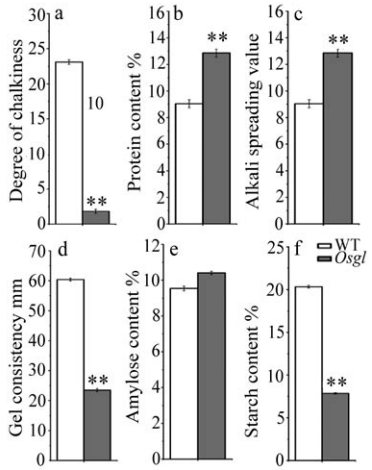
Rapid viscosity analysis shows contrasting difference in breakdown and setback viscosities between the *Osgl* mutant and WT. Final, setback and consistence viscosities were much higher than the wild-type grain, while peak and breakdown viscosities in *Osgl* mutant grains were even lower than the WT; *t*-test showed that the differences were significant (Table 4).

Results of our experiment are consistent with Cheng et al. (2002), who reported that rice grain with elevated breakdown viscosity, peak viscosity with low trough viscosity, final viscosity, setback viscosity, consistence viscosity and pasting temperature as well as peak viscosity time had a very soft texture with superb eating quality. The result indicated that the chalkiness had a stronger influence on cooking and eating quality (Table 4).

Performance of four protein fraction components between the *Osgl* mutant and WT was observed. The result shows a slightly higher content of glutelin in *Osgl* mutant, whereas prolamin and globulin contents were considerably higher in *Osgl* mutant, but the significance was not remarkable based on the *t*-test. Glutelin content in *Osgl* mutant was considerably decreased by 0.984% in WT compared with the *Osgl* mutant (Table 5).

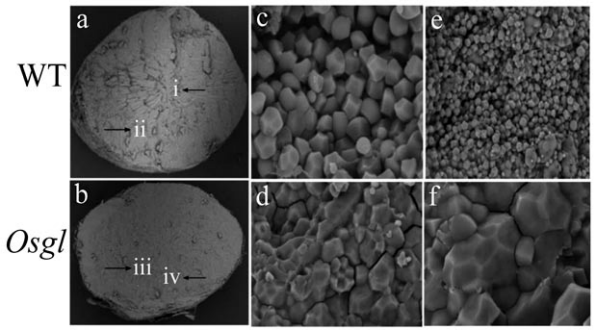
Taken together, the results demonstrate that high content of chalkiness in the WT had little effect on prolamin, globulin and albumin contents with a huge influence on glutelin content, signifying that high chalkiness content affects the nutritional quality in *Osgl* mutant (Table 5).

Comparison of Osgl mutant and WT fatty acid composition. Comparative results for fatty acids between the *Osgl* mutant and WT revealed that the concentration of linoleic acid was high in the WT. While the concentrations of palmitic, stearic and oleic acids were high in *Osgl* mutant, the arachidic and docosanoic acids concentration was low and insignificant in WT and *Osgl* mutant (Figure 8).



** – significant at 0.05

Figure 6. Measurement of *Oryza sativa* subsp. *japonica* grain quality traits: degree of chalkiness (a), protein content (b), alkali spreading value (c), gel consistency (d), amylose content (e) and starch content (f)



Note. Traverse piece of polished rice (a, b) (scale bars – 100 μm), starch granules within the inner endosperm cell (c, d) (scale bars – 3 μm; the sign within i and ii illustrate the location of images), the starch granule contained in endosperm cells (e, f) (scale bars – 3 μm); the arrows in iii and iv showed the location of imagery.

Figure 7. Scanning electron microscopy (SEM) of *Oryza sativa* subsp. *japonica* grain cross-section and physiological changes of wild type (WT) and *Osgl* mutant

Table 4. Rapid viscosity analysis (RVA) profile of *Oryza sativa* subsp. *japonica* wild type (WT) and *Osgl* mutant

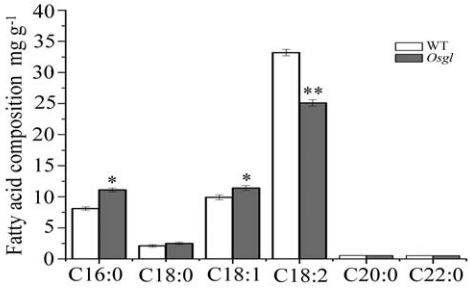
RVA characteristics	WT	*Osgl*	*t*-value
Peak viscosity	228.50	234.33	3.259*
Trough viscosity	167.33	164.17	1.642
Breakdown viscosity	61.17	70.17	3.056*
Final viscosity	306.13	298.00	3.192*
Setback viscosity	77.63	63.67	3.389*
Consistence viscosity	138.79	133.83	2.902*
Peak viscosity time min	6.33	6.27	0.612
Pasting temperature °C	79.95	80.80	1.401

* – significant at 0.05

Table 5. Comparison of protein fraction components (mg g^{-1}) of *Oryza sativa* subsp. *japonica* wild type and *Osgl* mutant

	Albumin	Globulin	Prolamin	Glutelin
Wild type	0.398 ± 0.020	0.519 ± 0.030	1.117 ± 0.031	7.347 ± 0.022
Osgl mutant	0.392 ± 0.027	0.525 ± 0.019	1.120 ± 0.023	7.420 ± 0.027
t-value	0.275	0.288	0.146	3.635*

* – significant at 0.05



Saturated fatty acids: C16:0 – palmitic, C18:0 – stearic, C18:1 – oleic, C18:2 – linoleic; unsaturated fatty acids: C20:0 – arachidic, C22:0 – docosanoic

Figure 8. Fatty acid composition of *Oryza sativa* subsp. *japonica* wild type (WT) and *Osgl* mutant

Discussion

Although improving grain shape together with grain yield is the main goal due to the projected population growth, the main concern will be rice grain quality. Several mutants are known to produce long and slender grain shape, but their mechanism is still uncertain. Because of this, more planting materials from diverse genetic backgrounds need to be exploited with a view to enhancing our knowledge on molecular mechanisms. Here, by ethyl methane sulfonate (EMS) mutagenesis, organ size grain length (*Osgl*) mutant was isolated from *japonica* rice cultivar 'Zhenong 34'. The *Osgl* gene was mapped in chromosome 3. *Osgl* gene encoding protein with plant-specific organ size regulation, which is among the G-protein signalling pathways controlling organ grain size.

To date, almost all the genes identified as well as the QTLs are co-ordinately controlled by cell proliferation and cell expansion which may set an upper limit for final grain size. Therefore, it is likely that the long-grain phenotype of *Osgl* mutant was due to increased cell number longitudinally, which may be the reason why *Osgl* mutant grains are longer than WT. In other reports (Mao et al., 2010; Qi et al., 2012), for instance, loci *GS3*, *qGL3/GL3.1* as well as *BG1* control grain size due to increased cell number in spikelet's hull. Interestingly, *GL7* results in producing slender grains due to cell length increase longitudinally and decreasing cell size transversely (Wang et al., 2015).

Chalky rice grain differed from transparent grain in terms of morphological, physicochemical, textural and thermal characteristics (Lin et al., 2016). As seen in the current study, SEM examination signifies that the shape of starch granule in transparent part is habitual polygon with few inter-granule gaps, whereas the chalky portion is irregular with loose arrangement. There is considerable disparity in starch granule structure, shape and arrangement consequences in chalkiness generation. Taken together, the result shows lower starch density in chalky part of the grains compared with the transparent part. This will possibly explain the reason why chalky grains have lower amylose content compared with transparent grains (Xi et al., 2016; Zhang et al., 2019).

Amylose content plays an imperative role in shaping cooking, eating and pasting characteristics like consistency, taste, stickiness, grain elongation, hardness, gel consistency along with gelatinization temperature in rice (Liu et al., 2014). Besides amylose content, other factors were also reported to influence rice cooking quality *viz.* proteins and amylopectin (Xu et al., 2018). In *Osgl* mutant, low amylose content was observed compared with the WT (Figure 6a), which is consistent with the earlier report (Xu et al., 2018), while in *Osgl* mutant, the content of amylose was slightly higher compared with the WT (Figure 6a–d). These results signify that differences in rice grain texture occur in both the medium as well as long-grain cultivars. Thus, intermediary, or low, gelatinization temperature is preferred to be a good quality cultivar. Similar results were observed in *Osgl* mutant, which has intermediary gelatinization temperature (Figure 6a–d). Understanding the relations between amylose content and gelatinization temperature along with pasting properties will be essential to describe eating and coking quality of many rice cultivars and will also have considerable influence on grain quality breeding (Xu et al., 2015).

Earlier research (Hori et al., 2016; Kim et al., 2017) elucidated that cooking and eating quality in rice is always related to the rapid viscosity analysis (RVA) characteristic. RVA breakdown is caused as a result of interruption of gelatinized starch granule composition, or due to differentiation of gelatinized starch granules and the viscosity, the disruption can be completely or partially (Rithesh et al., 2018). Rapid viscosity profile is an indirect indicator of rice-eating quality, and viscosity is used to find a particular feature in rice cultivar. Different RVA profiles represent diverse characteristics of rice cooking. For instance, high setback viscosity with small breakdown viscosity is an indicator that the brown rice will be very hardened when cooked, while higher breakdown and small setback viscosity is an indicator that the cooked rice will be very soft and sticky (Lin et al., 2016). Similar result was obtained in mutants that have high peak viscosity and break down viscosity with low set back viscosity than WT, which indicates that *Osgl* mutant is much softer than WT.

High concentrations of protein fraction component, albumin, globulin and glutelin with prolamin separately or in combination have a significant effect on RVA characteristics (Baxter et al., 2010; 2014). The content of globulin is very low in *Osgl* mutant that suggests slightly otherwise no connection with RVA, which may be due to the fact that globulins were synthesized in rice grain in early development (Nakamura et al., 2016). It is likely that globulin takes part in maintaining protein formation (Tong et al., 2014), which is the reason why its contents are very small and each rice grain protein component has a distinctive role in grain texture (Paula, Conti-Silva, 2014). Prolamin is undigested by human beings (Kubota et al., 2010); therefore, rice with low prolamin content will have soft texture. Also, rice viscosity characteristics change due to aging, or it can be a result of protein composition change (Zhou et al., 2010). All the protein fractions show different quantity of aqueous solubility, which might likely influence grain quality by influencing the rates of starch hydration at some point all through cooking time.

Fatty acids composition in the current study shows palmitic, stearic, oleic and linoleic acids to be present in mutants and WT, while the content of arachidic and docosanoic acids was very low in *Osgl* mutant and WT (Figure 8). The result is similar to that of (Concepcion et al., 2018); both revealed that palmitic and oleic along with linoleic acids are the major fatty acids present in rice.

Conclusion

The map-based cloning is a potential approach for gene discovery, which provides information for dissecting the genetic and molecular basis of grain size and for improving the grain size. *OsGL* is a promising gene with pleiotropic effect on grain length and appearance quality and can be utilized in genomic-assisted breeding for *japonica* rice (*Oryza sativa* subsp. *japonica*) cultivar improvement.

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Grūdų formą ir kokybę reguliuojančio japoninio ryžio *Osgl* geno tikslus kartografavimas ir kandidatinių genų analizė

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Santrauka

Vienas svarbių agronominių požymių, lemiančių japoninio ryžio (*Oryza sativa* subsp. *japonica*) grūdų derlių, yra grūdų dydis. Genolapiu pagrįstas su grūdų dydžiu susijusių genų klonavimas ir genetinė analizė yra svarbūs ryžių derliaus formavimosi genetinio reguliavimo mechanizmo nustatymui. *Osgl* (organo dydžio grūdo ilgis) mutanto augalo architektūra buvo vertikali ir kompaktiška, o grūdo ilgis, grūdų plotis ir grūdo ilgio bei pločio santykis gerokai padidėjo. Palyginus su laukiniu tipu, taip pat gerokai padidėjo mutanto grūdų ilgis, plotis, grūdų ilgio ir pločio santykis, derlius iš laukelio, šluotelės ilgis, grūdų skaičius šluotelėje ir sėklų užsimezgimo greitis. *Osgl* mutanto ir laukinio tipo augalo grūdų storis ir tūkstančio grūdų masė pastebimai nesiskyrė. *Osgl* mutanto krakmolo granulės yra glaudžiai išsidėsčiusios ir taisyklingos daugiakampio formos; krakmolo sudėtinės dalelės, esančios baltose, neskaidriose laukinio tipo endospermo dalyse, yra laisvai išsidėsčiusios, o jų forma sferinė arba elipsoidinė. Genetinė analizė parodė, kad *Osgl* mutantą valdo vienas recesyvinis genas, esantis 3 chromosomoje, 40 kbB fiziniame atstume tarp genolapyje naudotų žymeklių. Sekvenavimo analizė parodė, kad *Os03g0407400* geno ketvirtajame egzone įvyko A-G pakeitimas, dėl kurio į PEBP (fosfatidiletanolaminą surišantį baltymą) panašioje srityje aminorūgštis glicinas pasikeitė į argininą.
Apibendrinant galima teigti, kad *Osgl* mutantas turi pleiotropinį poveikį grūdų derliui ir grūdų kokybei. Galimai dėl ląstelių proliferacijos pokyčių jame keičiasi grūdų ilgis.

Reikšminiai žodžiai: genų kartografavimas, grūdų forma, grūdų kokybė, *Oryza sativa* subsp. *japonica*.