

## Fine mapping and marker-assisted selection (MAS) of a low glutelin content gene in rice

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### ABSTRACT

Rice with low glutelin content is suitable as functional food for patients affected with diabetes and kidney failure. The fine mapping of the gene(s) responsible for low glutelin content will provide information regarding the distribution of glutelin related genes in rice genome and will generate markers for the selection of low glutelin rice varieties. Following an SDS-PAGE screen of rice germplasm from Taihu Valley of China, Japonica selection W3660 is identified to be a novel mutant characterized with low glutelin content. For fine mapping the mutant gene for low glutelin content, F<sub>2</sub> and F<sub>3</sub> populations were derived from a cross between W3660 and Jingrennuo. SDS-PAGE analysis of the total endosperm protein showed that the low glutelin content trait was controlled by a single dominant nuclear gene. Genetic mapping, using SSRs, located this gene to chromosome 2, in the region between SSR2-001/SSR2-004 and RM1358. The distances of the two markers to the target gene were 1.1 cM and 3.8 cM respectively. By semi-quantitative RT-PCR analysis, the transcripts of *GluB4/GluB5* genes located within the region do not change. However, *GluB5* gene located proximal to SSR2-001/SSR2-004 was specifically reduced. SSR profiles of seven Japonica varieties were compared with that of W3660 for loci in the relevant genetic region. The markers SSR2-004 and RM1358 were used for marker-assisted selection. The selection efficiencies of SSR2-004 and RM1358 were 96.8% and 92.7% respectively. This provides a standard starting point for the breeding of low glutelin content rice varieties in China.

**Keywords:** fine mapping, low glutelin content, marker-assisted selection, mutant, rice.

### INTRODUCTION

Rice (*Oryza sativa* L.) is considered to be a starchy crop because of its relatively low protein content, which ranges from 7% to 10% of the grain weight. However, rice is an important protein source for human consumption, especially in Asian and Indian subcontinents. The demand for rice as a source of dietary protein is expected to increase significantly along with the world population, which is predicted to double by 2030 [1]. Glutelin is the major digestible component of the rice storage proteins, and accounts for 60% of total seed protein. It is localized in protein body II (PBI) within endosperm cells, while the indigestible prolamine fraction, account-

ing for 5~10% of total seed protein, is contained in PBI. Increasing the glutelin content and lowering prolamine content of rice grains would improve the nutritional value of rice. However, for patients affected with diabetes and kidney failure, the significant consumption of glutelin can lead to exacerbated medical problems. For example, in China there are more than 80 million people who have been diagnosed with diabetes and kidney failure. It is estimated that 35% and 15% of type I and II diabetic patients have complicated renal failure, which leads to serious disturbance of protein metabolism. These patients cannot ingest rice grains with soluble protein content above 4%. Iida *et al* obtained a rice mutant LGC-1, which was characterized as a low glutelin and a high prolamine content crop [2]. As demonstrated by recent studies on diabetic patients, LGC-1 rice functions as an effective supplementary food, especially for those who consume rice grains as their staple food [3]. According to Miyahara *et al* [4], low glutelin

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content trait in LGC-1 was controlled by a single dominant gene, *Lgc-1*, located between RFLP markers XNpb 243 and G365 on chromosome 2. The genetic distance separating the two markers from *Lgc-1* were estimated to be 8.5 cM and 39.1 cM respectively.

We recently identified three lines showing variant glutelin content from an SDS-PAGE screen of rice germplasm from Taihu Valley, China. Among them, Japonica selection W3660 has a phenotype similar to LGC-1 [5]. We are now engaged in an effort to modify the glutelin content of commercial rice varieties by transferring the W3660 trait to adapted material. For this purpose, high throughput DNA markers would be desirable, since SDS-PAGE analysis is tedious and costly to apply in a breeding programme. The object of the present study was to develop molecular markers linked to the low glutelin content trait of W3660.

## MATERIALS AND METHODS

### Plant materials

W3660 was crossed with Jingrennuo, an Indica variety with normal glutelin content, and the resulting F<sub>2</sub> population, consisting of 194 individuals, was used for linkage mapping. The parental lines and the F<sub>2</sub> population were grown in the summer of 2001 in the field at the Rice Research Institute at Nanjing Agricultural University. To finely map the mutant gene, an F<sub>2</sub> plant heterozygous for both the mutant allele and flanking markers was self-pollinated, to construct a derived F<sub>3</sub> population (dF<sub>3</sub>), totaling 442 individuals (Fig. 1). These plants, along with the parental lines were grown, as above, in the summer of 2002. Field management essentially followed normal agricultural conditions.

### Extraction of protein in rice grains and phenotype assays by SDS-PAGE

Mature seeds were harvested from individual F<sub>2</sub> and derived F<sub>3</sub> plants, and bulk-harvested from parental plants for phenotyping by SDS-PAGE. Six seeds were selected randomly from each plant and analyzed for protein profiles by SDS-PAGE. When the protein content segregated from a sample of six seeds per plant, this plant was classified as heterozygous for the mutant allele(s). When no segregation was observed, a further sample of six seeds was analyzed to verify the allelic status as homozygous. Protein extraction was performed as described by Cagampang GB *et al* [6] and Iida *et al* [2, 7]. Briefly, seed was polished, ground, and placed in a 1.5 ml centrifuge tube, to which was added 700  $\mu$ l SDS-Urea extract solution, consisting of 8 M Urea, 4% SDS, 5%  $\beta$ -mercaptoethanol, 20% glycerol, 50 mM Tris-HCl buffer (pH 6.8). The tube was vortexed and incubated overnight at 25°C. After centrifugation at 7000 g for 5 min, 5  $\mu$ l of supernatant was loaded onto an SDS-PAGE gel [8]. We used a 15% polyacrylamide gel for separation, and a 7.5% gel for stacking. Following electrophoresis, gels were stained with CBB-R250.

### DNA extraction

Two to three leaves were taken from each plant before heading and stored at -80°C. DNA was extracted from the frozen leaf tissue according to Dellaporta *et al* [9] with minor modification. Following extraction, the DNA was dissolved in TE buffer (10 mM Tris base, 0.1 mM EDTA). DNA quality and concentration were determined with MBA2000 UV/vis spectrophotometer (Perkin Elmer Co). The stock DNA preparation was diluted to 20 ng/ $\mu$ l for use as templates for PCR amplification.

### SSR marker design

Some of the SSR primers were taken from Temnykh S *et al* [10] and McCouch SR [11]. Others were directly designed from the rice genome sequences according to Rice Genome Program (RGP). For the latter, an *in silico* search for SSR sequences (number of repeats >5) was made in the region defined by linked flanking SSR markers in BAC/PAC clones using SSRIT (The Simple Sequence

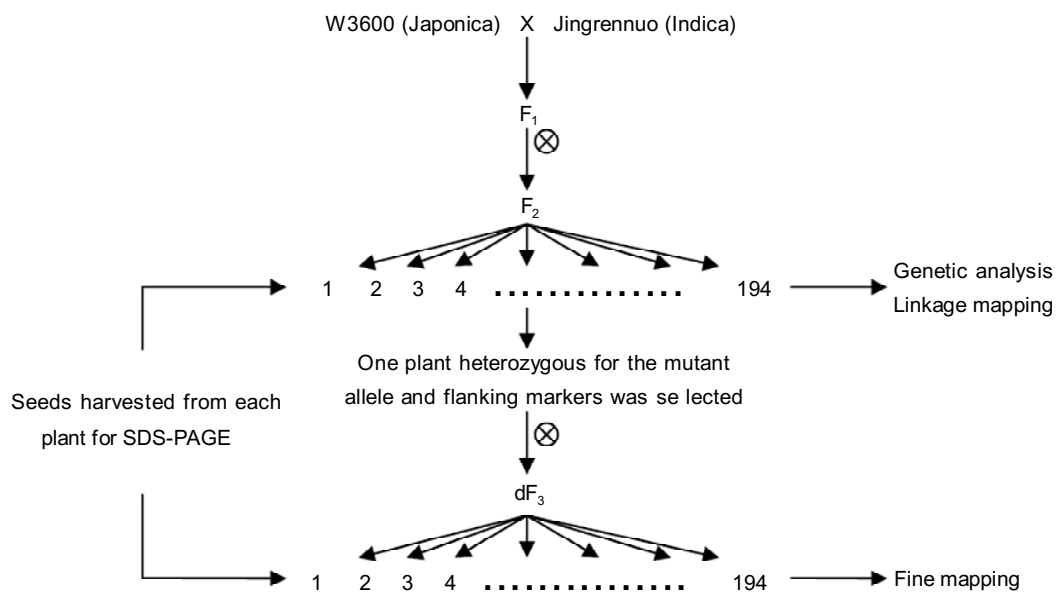


Fig. 1 Schematic diagram of genetic analysis and fine mapping of low glutelin content gene in W3660.

Repeat Identification Tool, <http://www.gramene.org/db/searches/ssrtool>). These SSR and the adjacent sequences of 400~500 bp were BLASTed against the Chinese rice genome database (<http://bfn.genomics.org.cn/rice>) to identify Japonica/Indica polymorphism between Nipponbare and 9311. Polymorphic primers were designed using the program Primer Premier 5.0, and were tested against DNAs of W3660, Jingrennuo and their F<sub>1</sub> hybrid.

### SSR marker analysis

SSR analysis was performed according to Chen *et al* [12]. Each 10 µl PCR reaction contained 10-20 ng of template DNA, 1 × PCR reaction buffer (Mg<sup>2+</sup> free, TaKaRa, Dalian), 1.5 mM of MgCl<sub>2</sub>, 50 µM of dNTPs, 20 µM of primers, and 0.5 U of Taq DNA polymerase (TaKaRa Co). Amplification profiles consisted of 5 min of denaturation at 94°C, 35 cycles of 30 s denaturation at 94°C, 30 s annealing (temperature determined by primer pair sequence), and 60 s extension at 72°C, followed by a final 10 min extension at 72°C. Denatured amplified products were electrophoresed through 8% (w/v) native polyacrylamide gels at 120V for ca.3 h, and visualized by silver staining following Sanguinetti *et al* [13].

### SSR marker-assisted selection

Seven widely grown Japonica varieties (Xiangjing 9707, Xiangjing 119, 9924, Zhendao 88, Wuyujing 3, Zhendao 99, Wuyunjing 7) were used for marker-assisted selection breeding programme. SSR markers linked to mutant gene, identified in this study, were tested for polymorphism between these varieties and W3660. 188 plants with good agronomic performance in the field were selected from an F<sub>2</sub> breeding population derived from the cross Wuyunjing 7 × W3660 for SSR and SDS-PAGE analysis to assess the efficiency of marker-assisted selection. For other crosses or generations, 12 individuals were selected from each of two or three lines. DNA was extracted from young leaves according to the following method. Briefly, tissue was ground in a centrifuge tube in the presence of 40 µl of 0.25 M NaOH until the solution turned green. Then 160 µl of 100 mM Tris-HCl (pH7.6) was added, and the supernatant was diluted 50-fold,

for use as template for SSR analysis. PCR analysis and protein phenotyping was carried out as described above.

## RESULTS

### Phenotype identification and genetic analysis of low glutelin content

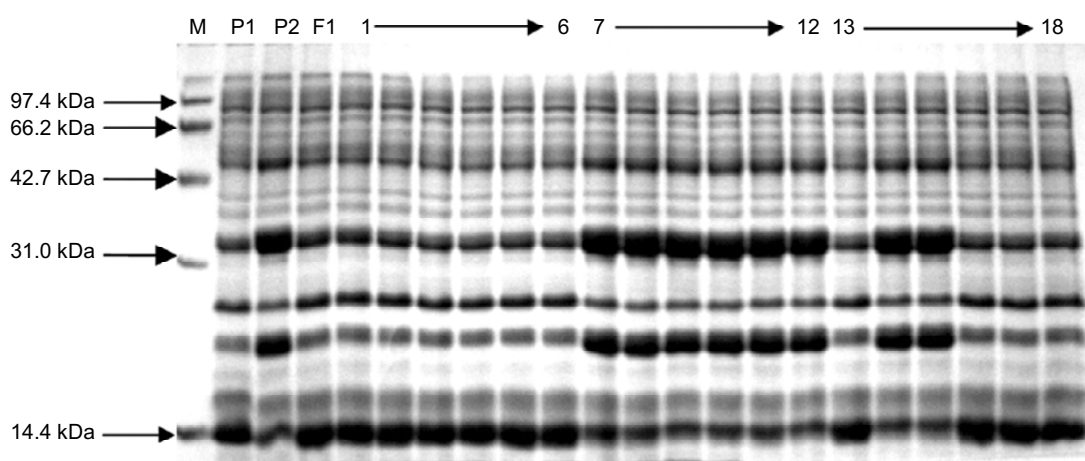
The protein profile revealed by SDS-PAGE analysis indicated that W3660 has less mature glutelin (37-39 kDa and 22-23 kDa) and more prolamine (13 kDa) than Jingrennuo (Fig. 2). The trait segregated in the F<sub>2</sub> and derived F<sub>3</sub> populations from the cross W3660 × Jingrennuo. The low glutelin mutant in W3660 segregated as a monogenic dominant trait, as no intermediate phenotypes were detected. Genotyping results showed the segregation pattern fitted the Mendelian ratio 1:2:1 in both populations (Tab. 1).

### Linkage mapping of mutant gene

In the genome-wide polymorphism screen, 511 SSR loci were tested for W3660, Jingrennuo and their F<sub>1</sub> hybrid. Polymorphic markers evenly distributed across all 12 chromosomes were obtained. Two point linkages indicated an association between loci mapping to chromosome 2 and the target trait. Thereafter, the F<sub>2</sub> population was mapped using ten markers on chromosome 2. According to the linkage map constructed with MAPMAKER/EXP3.0 [14], the mutant gene was located between RM71 and RM424. The marker-mutant gene distances were 16.5 cM and 12.7cM, respectively (Fig. 3).

### Fine mapping of mutant gene

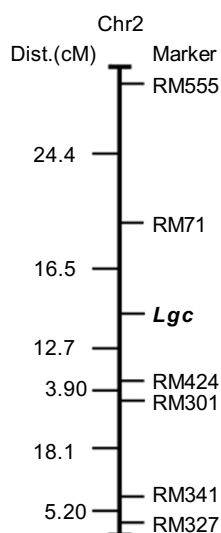
One plant heterozygous for RM71, RM424 and mutant



**Fig. 2** SDS-PAGE analysis of total protein in F<sub>2</sub>,F<sub>3</sub> seeds. M: Mid-range protein molecular weight marker; P<sub>1</sub>: W3660; P<sub>2</sub>: Jingrennuo; 1-6, 7-12, 13-18 are samples randomly selected from plant45, plant131 and plant402 in derived F<sub>3</sub> population. Plant 402 is heterozygous for the mutant allele. For plant45 and plant131 each, another sample of six seeds needs analyzing to verify the allelic status as homozygous.

**Tab. 1** Segregation of low glutelin content in F<sub>2</sub> and derived F<sub>3</sub> populations from the cross of W3660×Jingrennuo

Population	Total	Homozygotes of low glutelin	Homozygotes of low glutelin	Homozygotes of normal type	$\chi^2$ (1:2:1)
F <sub>2</sub>	194	55	94	45	1.21
dF <sub>3</sub>	442	105	227	110	0.43

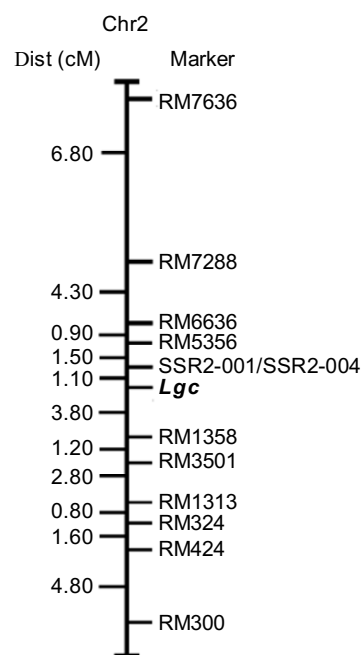
**Fig. 3** Primary mapping results of mutant gene (F<sub>2</sub> population with 194 individuals).

gene was self-pollinated, to generate 442 F<sub>3</sub> progenies. Eleven SSR primer loci were located in the region flanking the target gene within a range of 20 cM, but only two of these - RM7636 and RM7288 - were polymorphic between the parental cultivars. These were added to the linkage map derived from the F<sub>3</sub> progenies. From a further eleven SSR primer pairs mapping between RM7288 and RM324, we obtained five polymorphic assays. Based on an analysis using all these markers, mutant gene was located between RM5356 and RM1358, with marker-mutant gene distances of 2.0 cM and 3.8 cM respectively. By referring to the genomic sequence between these two markers, we were able to design a further four pairs of SSR primers, two of which (Tab. 2), SSR2-001 and SSR2-

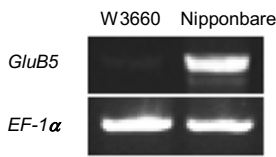
004 were polymorphic with respect to the parental cultivars. SSR2-001 and SSR2-004 co-segregated in the derived F<sub>3</sub> population. Finally we estimated the map location of mutant gene as being 1.1 cM from SSR2-001/SSR2-004 and 3.8 cM from RM1358 (Fig. 4).

#### ***GluB5* gene expression was downregulated in W3660 mutant**

There are six PAC/BAC clones, P0693E08, OSJNBa0033K18, P0521F09, P0411A13, P0017G06, OSJNBa0055M07 covering the region between SSR2-001/

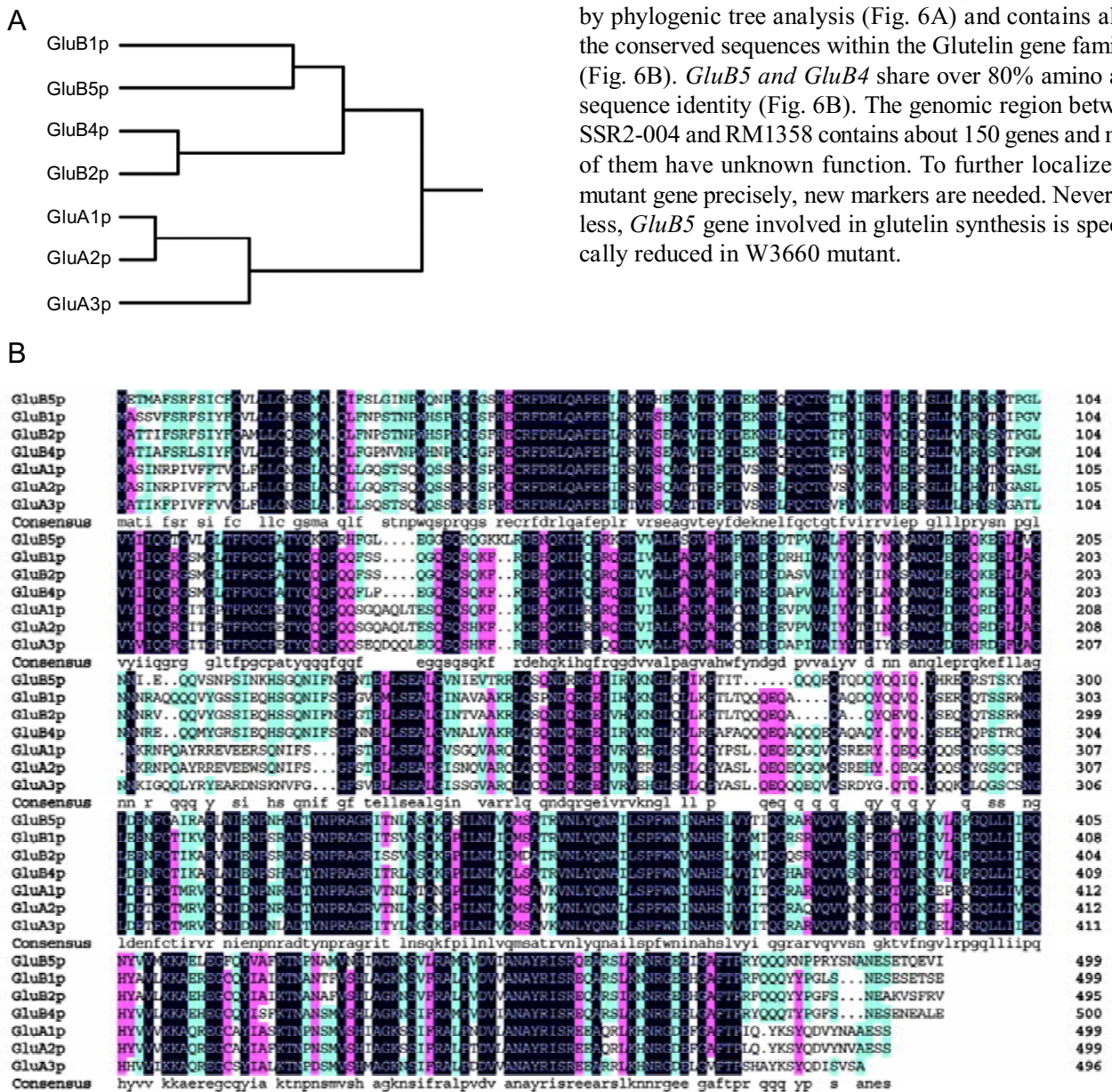
**Fig. 4** Fine mapping results of mutant gene (dF<sub>3</sub> population with 442 individuals).**Tab. 2** Two SSR markers, linked to mutant gene, developed from an *Oryza sativa* var Nipponbare genome database

Primer	SSR motif		Primer Sequence (5'-3')	T <sub>a</sub> (°C)	Size (bp)
SSR2-001	(AT) <sub>25</sub>	Forward	CTGCTTTCCTCTAGTCTGT	54	173
		Reverse	TGCTCATTTGAATTGGGTAT		
SSR2-004	(CTT) <sub>16</sub>	Forward	TTTTCGTATCCACCTTCG	55	143
		Reverse	TTGCTGAGCCCGACATTA		

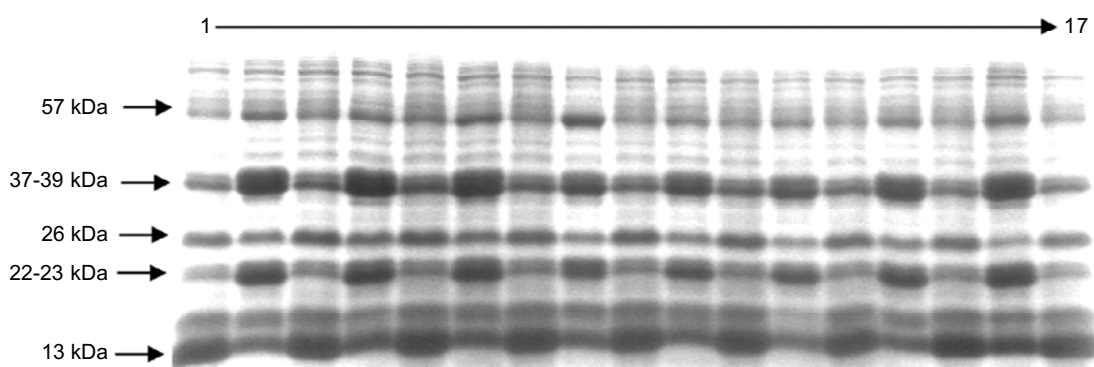


**Fig. 5** Expression analysis of *GluB5* in W3660 and Nipponbare by semi-quantitative RT-PCR. *GluB5* (AY196922) was in the region of 37.0-37.4 cM on chromosome 2. Total RNA was extracted from endosperm of 14 DAF(days after flowering); *EF-1α* was used as a control.

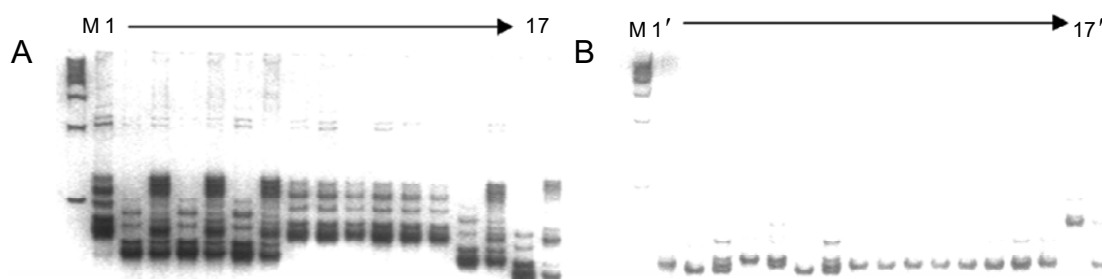
SSR2-004 and RM1358. Two highly interesting genes *GluB4/GluB5* involved in glutelin synthesis are included within the region. We designed primers to analyze the expression of glutelin genes including *GluA-1* (L36819), *GluA-2* (AY585231), *GluA-3* (X54313), *GluB-1* (X54314), *GluB-2* (X54192), *GluB-4* (AF537221) (referred as *GluB4/GluB5* by Kusaba *et al* [15]), *GluB-5* (AY196923) in W3660 mutant by semi-quantitative RT-PCR. The only gene affected in W3660 mutant is *GluB5* (AY196922) (Fig. 5), while transcripts of other Glutelin genes do not change (manuscript in preparation). *GluB5* gene (AY196923) is located on chromosome 2 between 37.0 cM to 37.4 cM. It is most closely related to *GluB4* by phylogenetic tree analysis (Fig. 6A) and contains all of the conserved sequences within the Glutelin gene families (Fig. 6B). *GluB5* and *GluB4* share over 80% amino acid sequence identity (Fig. 6B). The genomic region between SSR2-004 and RM1358 contains about 150 genes and most of them have unknown function. To further localize the mutant gene precisely, new markers are needed. Nevertheless, *GluB5* gene involved in glutelin synthesis is specifically reduced in W3660 mutant.



**Fig. 6** Alignment of *GluB5* (AY196923) with other glutelin genes. (A) Phylogenetic tree of *GluB5* and other glutelin genes; (B) Amino acid sequence alignment between *GluB5* and other glutelin genes. The sequence alignment was done using software DNAMAN.



**Fig. 7** SDS-PAGE analysis of W3660, eight varieties and corresponding  $F_1$  seed. 1 to 17 are seeds of W3660, Xiangjing9707, Xiangjing9707/W3660, Xiangjing119, Xiangjing119/W3660, 9924, 9924/W3660, Zhendao88, Zhendao88/W3660, Wuyujing 3, Wuyujing 3/W3660, Zhendao99, Zhendao99/W3660, Wuyunjing 7, Wuyunjing 7/W3660, Jingrennuo, and W3660/Jingrennuo respectively.



**Fig. 8** Polymorphic analysis between W3660 and 8 varieties with RM1358 (A) and SSR2-004 (B). M represents DNA marker, 1 to 17 and 1' to 17' are W3660, Xiangjing9707, Xiangjing9707/W3660, 9924, 9924/W3660, Wuyunjing 7, Wuyunjing 7/W3660, Zhendao88, Zhendao88/W3660, Wuyujing 3, Wuyujing 3/W3660, Zhendao99, Zhendao99/W3660, Xiangjing119, Xiangjing119/W3660, Jingrennuo, and W3660/Jingrennuo, respectively.

### Marker-assisted selection

SDS-PAGE analysis of the seed protein profiles of W3660 and seven Japonica varieties confirmed that low glutelin content is controlled by a dominant gene in all genetic backgrounds tested (Fig. 7). Of the six SSR markers (RM6639, RM5356, SSR2-001, SSR2-004, RM1358 and RM3501) linked to mutant gene, RM1358 was polymorphic between W3660 and Xiangjing 119, Xiangjing 9707, Wuyunjing 7 and 9924, as was SSR2-004 between W3660 and Xiangjing 9707, Wuyunjing 7 and 9924 (Fig. 8). The other four markers were monomorphic. These results indicated that the two markers flanking mutant gene are appropriate for marker-assisted selection of low glutelin content in the progeny of Japonica varieties. Further validation of RM1358 and SSR2-004 was carried out by genotyping of 188  $F_2$  plants from the cross W3660×Wuyunjing 7 (Tab. 3). This showed that both markers had high selection accuracy (92.7% and 96.8%,

respectively). In a further validation experiment, analysis of  $F_3$  and  $F_4$  plants derived from the cross Wuyunjing 7 × W3660,  $F_5$  plants from Xiangjing119 × W3660,  $F_4$  plants from Xiangjing 9707 × W3660, and  $F_3$  plants from 9924 × W3660, revealed that the protein phenotype of only one individual was inconsistent with genotype based on RM1358 and SSR2-004 scoring. We conclude that the use of RM1358 and SSR2-004 is appropriate for marker-assisted selection of low glutelin content in a Japonica background.

### DISCUSSION

The demand for low-protein rice is expected to increase markedly as the number of at risk patients is increasing annually. Obtaining low-protein cultivars has become a significant new breeding goal to meet the needs of patients on a restricted protein diet. The low-glutelin content line W3660 has good agronomic traits compared with the Japa-

**Tab. 3** Efficiency of RM1358 and SSR2-004 for MAS for low glutelin content trait

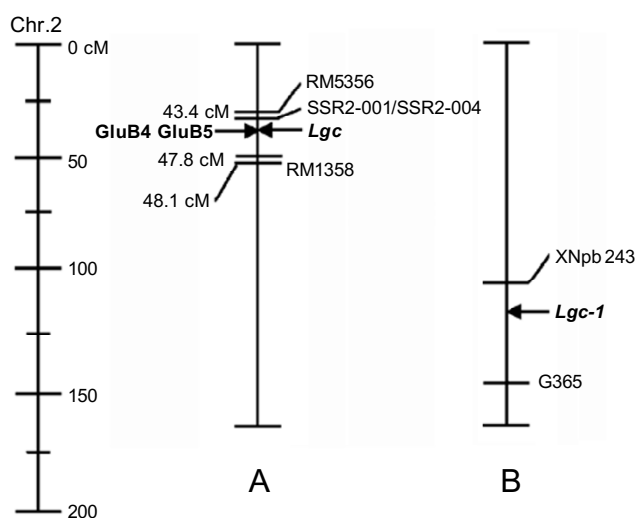
Combination	Generation	Total	Marker	AABB	AaBb	aabb	A_bb	aa B_	AABb	AaBB	Recombination rate
Wuyunjing 7/W3660	F <sub>2</sub>	188	RM1358	55	92	34	1	2	3	1	3.72%
			SSR2-004	57	93	35	0	1	1	1	1.60%
	F <sub>3</sub>	24	RM1358	18	1	5	0	0	0	0	0
			SSR2-004	18	1	5	0	0	0	0	0
	F <sub>4</sub>	24	RM1358	9	13	1	0	0	1	0	4.17%
			SSR2-004	10	13	1	0	0	0	0	0
Xiangjing9707/ W3660	F <sub>4</sub>	24	RM1358	8	10	6	0	0	0	0	0
			SSR2-004	8	10	6	0	0	0	0	0
9924/W3660	F <sub>3</sub>	24	RM1358	16	8	0	0	0	0	0	0
			SSR2-004	16	6	0	0	0	0	0	0
Xiangjing 119/W3660	F <sub>5</sub>	36	RM1358	9	23	3	0	0	1	0	2.78%

A(a), phenotype of plants; A\_, low glutelin content; aa, normal glutelin content; B(b), genotype at SSR marker loci; BB, homozygous for W3660 alleles; Bb, heterozygous; bb, homozygous normal.

nese mutant LGC-1 when cultivated in Jiangsu Province, and is therefore of particular interest as a donor to develop new low glutelin varieties. The fine mapping of the low glutelin content gene in W3660 and the identification of tightly linked SSR markers will enable the use of marker-assisted selection for this trait, and thus will facilitate the breeding of commercial cultivars with low glutelin content. In addition, the mapping of mutant gene provides the basis for its cloning and characterization, as well as the understanding of the biosynthesis of glutelin and the mechanism underlying the reduction of glutelin in W3660.

**Analysis of mapping results in W3660**

Mutant gene in W3660 was initially mapped to a region between 43.4 and 48.1 cM on chromosome 2. Novel markers derived from the whole genome sequence allowed the further definition of the genetic window to between SSR2-001/SSR2-004 and RM1358, with distances of these markers to mutant gene of 1.1 cM and 3.8 cM, respectively (Fig. 9A). It is known that LGC-1 mutant derived from chemical mutagenesis [2] has a phenotype similar to that of W3660. Miyahara K. *et al* [4] used an F<sub>2</sub> population to map *Lgc-1* on rice chromosome 2 between RFLP markers XNpb 243 and G365, with gene-marker distances of 8.5cM and 39.1cM, respectively (Fig. 9B). Kusaba *et al* [15] have recently described the cloning and functional analysis of *Lgc-1*, and have proposed that the gene harbours a tail-to-tail inverted repeat consisting of a functional *GluB4* gene and a truncated *GluB5* gene (AB093593). This structure is thought to produce a GluB hairpin RNA with a dsRNA region. The latter have been implicated as potent inducers of RNA silencing, suggesting that RNA silencing is the



**Fig. 9** Linkage map of the mutant gene in W3660 (A) and *Lgc-1* (B).

mechanism responsible for the low protein phenotype of LGC-1 [15]. The *GluB4* and *GluB5* sequences were used to assign *Lgc-1* to the assembled genomic sequence and to determine its position on the genetic map. RiceBLAST (<http://riceblast.dna.affrc.go.jp/cgi-bin/robo-blast/blast2.cgi?dbname=Japonica>) analysis indicated that *GluB4* and *GluB5* genes located in a region between 43.4 cM and 47.8 cM on chromosome 2. Thus, the mutant gene in W3660 and *Lgc-1* are in the same region.

**W3660 and LGC-1 are two different mutants**

Although the mutant genes in W3660 and *Lgc-1* were

located within the same region, W3660 and LGC-1 were obtained differently and may not necessarily represent same mutation. In *Lgc-1* mutant, most of the glutelin genes including members of *GluB* and *GluA* subfamilies are down-regulated via RNA silencing mechanism mediated by dsRNA formed between *GluB4* and *GluB5* [15]. In W3660 mutant, we only observed decreased transcription of *GluB5* gene, but not other members of Glutelin gene families including *GluB4/GluB5* (Fig. 5 and unpublished data), suggesting that W3660 and LGC-1 are two different mutants. Since *GluB5* coding sequence is located on Chromosome 2 between 37.0 to 37.4 cM, which is outside of our mapped mutation in W3660 on Chromosome 2 between 43.4 to 47.8 cM, then *GluB5* transcription in W3660 probably has defect in transcriptional regulation other than mutations in *GluB5* coding sequence through unknown mechanisms. Except for *GluB4/GluB5*, there are 150 genes found within the genomic region between SSR2-004 and RM1358. However the biological functions of most of these genes are not known. It is thus unclear how the mutations occurring in W3660 were involved in glutelin synthesis. Mechanism of regulating *GluB5* gene expression is probably involved. Although we cannot detect changes in *GluB4/GluB5* gene transcription, the possibility of post-transcriptional regulation and translational regulation on *GluB4/GluB5* cannot be excluded. Nevertheless, *GluB5* gene is specifically reduced in W3660 mutant and is functionally correlated to the low glutelin content phenotype in W3660.

### SSR marker-assisted selection

Marker-assisted selection has become routine for rice in recent years. Its successful use relies on the availability of high throughput markers tightly linked to the trait under selection. In this study, we have identified a number of SSR markers tightly linked to mutant gene in W3660. However, among these only the two loci RM1358 and SSR2-004 were polymorphic between the donor line W3660 and a number of tested Japonica varieties. In general there is a positive relationship between the number of repeats in a microsatellite sequence and the level of polymorphism at the locus. Both of the polymorphic loci were relatively large (the standard allele of RM1358 consists of 24 AG repeats, while SSR2-004 carries 16 CTT repeats), and this may explain why only these loci were informative. We observed single crossing-over events between mutant gene and RM1358 or SSR2-004 in derived F<sub>3</sub> population, respectively, only 16 or 5 progenies. Thus the selection accuracy of both markers was very high, irrespective of genetic background. Although selection based on a single marker is sufficient for breeding purposes, the simultaneous use of both markers should deliver a selection ac-

curacy of 99.8%. In the present study, no double crossing-over events were observed in 442 plants from the derived F<sub>3</sub> progeny of W3660 × Jingrennuo, and 188 plants from the F<sub>2</sub> progeny of Wuyunjing 7 × W3660. In conclusion we suggest that genotyping with RM1358 and SSR2-004 will facilitate the breeding of low glutelin content rice varieties from Japonica × Japonica crosses, while RM1358, SSR2-001, SSR2-004 and RM5356 are suitable for selection in progeny of Indica × Japonica crosses. This provides a standard starting point for the breeding of low glutelin content rice varieties in China.

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