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Haplotype Analysis of the *GL7/GW7/SLG7* Gene and Its Application in Improving the Grain Quality of Three-Line Hybrid Rice

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Abstract

Improving grain quality is second only to enhancing grain yield in breeding hybrid rice. Yet, rice grain quality, especially milling and appearance quality, is facing increasing threats from global warming due to climate change, leading to a relatively slow progress in high-quality rice breeding. Identifying additional grain quality genes is an effective way to combat against the threats on rice grain quality. In the present study, we used the germplasm from 3,000 Rice Genomes Project for genome-wide association study, and identified *GL7/GW7/SLG7* as a major QTL besides *GS3* and *GW5* for grain shape. Among nine haplotypes of *GL7* (H1-9), H1-H4, which harbored an 11-bp deletion, were designated as the functional *GL7* allele and were primarily present in *Geng/Japonica* (*GJ*) rice. We developed KASP markers for *GL7* major haplotypes (H1 and H2), and established a breeding system assisted by the markers to effectively improve *Xian/Indica* (*XI*) hybrids for grain quality. Yuehesimiao (YHSM) and Yixiang 1 A (YX1A) are widely applicated *XI* restorer line and sterile line in three-line hybrids. The improved hybrid YX1A^{*GL7*}/YHSM^{*GL7*} exhibited longer grain, higher ratio of grain length-to-width and larger rate of head rice, but lower chalkiness rate and degree than that of any other hybrids and both parents. Furthermore, the improved hybrid with *GL7* had statistically same yield of grains with all other hybrids, indicating no penalty of grain yield while improving grain quality. The *GL7* haplotype along with its marker KASP-S2 and breeding strategy resulted from this study could be valuable sources for developing *XI* hybrids with high quality and high yield of grains in rice.

Keywords Oryza sativa, Grain Quality, GL7/GW7, Hybrid Rice

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Introduction

Rice (*Oryza sativa* L.) as one of the most important food crops provides calories and nutrients for half of the world's population. As the largest rice production and consumption country, China has made dramatic contributions to global food security by developing and commercializing hybrid rice (Cheng et al. 2007a, b; Bai et al. 2018). With the improvement of living standards, the quest for grain quality is growing, and rice varieties with superior quality are attracting tremendous attention and exerting high economic value (Fitzgerald et al. 2009). As a result, improving grain quality has nowadays become one of the most significant goals in breeding practices for hybrid rice.

Rice grain quality is a complex trait that comprises milling quality, appearance quality, eating and cooking quality (ECQ) and nutritional quality (Zhou et al. 2020). Head rice rate, grain shape and grain chalkiness, amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) which reflect milling quality, appearance quality, and ECQ, respectively, are the main rating indexes of rice grain quality (Zhou et al. 2020). Rice varieties with high quality are usually endowed with high head rice yield (milling quality), low chalkiness (appearance quality), good taste (ECQ) or high nutrition (Zhu et al. 2024). Accordingly, rice breeding efforts primarily focus on increasing the head rice rate, decreasing grain chalkiness and balancing AC, GC and GT of hybrid rice.

The enormous progress made in rice functional genomics has greatly assisted in understanding the genetic and molecular mechanism of rice grain quality (Qin et al. 2021; Zhao et al. 2022; Ren et al. 2023). GS3, GW5/GSE5, GL3.3, Chalk5 and WCR1 are reported to be major genes responsible for appearance quality by influencing grain shape and chalkiness in rice (Fan et al. 2006; Weng et al. 2008; Li et al. 2014; Liu et al. 2017; Xia et al. 2018; Huang et al. 2021; Wu et al. 2022). Wx and ALK are major genes to determine ECO with minor effects on milling quality by controlling the content of amylose and amylopectin (Gao et al. 2003; Tian et al. 2009; Zhou et al. 2021b). Favorable alleles of gs3, GW5, chalk5, Wx^b and ALK^{TT} are highly associated with superior grain quality, demonstrating their tremendous roles in improving grain quality of rice (Zeng et al. 2017; Ren et al. 2023; Yang et al. 2023a; Gao et al. 2024).

Molecular breeding has largely accelerated breeding process because of precisely targeting functional genes in rice (Wang et al. 2005). Genetic manipulation of *GS3*, *GW5* and *Wx* with assistance of molecular selections has successfully improved overall grain quality in rice (Huang et al. 2021, 2022; Kim et al. 2021; Mao et al. 2021; Jiang et al. 2022). Nevertheless, along with global warming, the continuous rise of the temperature worldwide is a big challenge to rice grain quality, especially for milling

and appearance quality (Liu et al. 2024). Meanwhile, constantly pursuing high yield in breeding has given birth to hybrid rice with large and wide grains, which often show poor appearance quality compared to varieties with slender grain shape (Gong et al. 2017). What's more, a large proportion of Asian consumers, including Southern Chinese, Indians and Philippines, have strong preferences for long and slender grains (Yang et al. 2023a, b). Therefore, such market demand for rice in such wide areas is accelerating the breeders to pay a special attention to appearance quality for extra-long and slender grains in new varieties of hybrid rice.

GS3 and GW5 are the major genes for rice grain shape diversity and most XI hybrid varieties carry the superior allele of gs3GW5. Thus, diversifying the source of grain shape genes in XI is important for continuously pursuing hybrid varieties with high quality of grains. In the present study, we identified a QTL GL7/GW7/SLG7 for grain shape besides GS3 and GW5 through genome-wide association study, and demonstrated GL7 effectively improved grain quality. Three pairs of KASP (Kompetitive Allelespecific PCR) markers were developed for functional GL7 allele through haplotype analysis. By molecular selection of these KASP markers, we introduced GL7 into a restorer line Yuehesimiao (YHSM) and maintainer Yixiang 1B (YX1B) in three-line hybrid system of XI rice. The appearance quality of YHSM^{GL7} and YX1B^{GL7} was significantly improved with longer and slender grains, and lower chalkiness in comparison with the original YHSM and YX1B. Four hybrids of YX1A/YHSM, YX1A/ YHSM^{GL7}, YX1A^{GL7}/YHSM and YX1A^{GL7}/YHSM^{GL7} were generated by crossing YHSM and YHSM^{GL7} with YX1A and YX1A^{GL7}, respectively, for phenotypic comparison. Grain yields per plant of four hybrids were significantly higher than their parents. Chalkiness rate and degree of YX1A^{GL7}/YHSM^{GL7} were significantly lower than YX1A/YHSM, leading YX1A^{GL7/}YHSM^{GL7} to show a higher rate of head rice vield. Also, YX1AGL7/YHW-M^{GL7} had a slightly improved ECQ over YX1A/YHSM. Our results showed a great potential of GL7 on improving grain quality and diversifying genetic resources in hybrid breeding, especially for XI rice.

Materials and Methods

Plant Materials and Genotyping

In this study, four inbred lines were used to study GL7 for breeding purposes, including two restorer lines, Yuehesimiao (YHSM) and Taiguoxiaoxiangzhan (TGXXZ), and two maintainer lines, Yixiang 1B (YX1B) and Shu 6B (S6B), which genotypes for quality genes were identified from resequencing data. During breeding process, allele types of GL7 were determined using KASP-S2 marker (Supplementary Table 2). Genetic background analysis of the improved YHSM^{GL7} and YX1B^{GL7} was based on resequencing data. The number of differential SNPs across whole genome between the original and improved lines (YHSM vs. YHSM^{GL7} and YX1B vs. YX1B^{GL7}) was calculated in 100-kb intervals. The R package 'RIdeogram' was used to generate karyograms for comparing the genetic background differences (Hao et al. 2020).

An 11-bp deletion was tightly linked to the functional *GL7* allele, and most varieties with the *GL7* genotype harbored three SNPs variation. Basing on the three SNPs (S2, S5 and S7), three pairs of KASP primers were developed (http://www.snpway.com:8339/), and validated with LGC standard protocol, where 50 ng DNA were used as template. The fluorophores of KASP PCR were read using BMG LABTECH FLUOstar Omega SNP Microplate reader with its recommended procedure. Two replicates of each sample at least were amplified using KASP assay protocol to select proper KASP markers for specifically and stably distinguishing functional *GL7* allele from non-functional *gl7* allele.

Grain Quality Assessment

Ten plants in the middle of plot were harvested from each material to assess agronomic traits, including plant height, effective panicle number, panicle length, grain number per plant, filled grain number, grain length, grain width and grain weight per plant (grain yield per plant) and thousand grain weight. For milling quality, 25 g of fully filled seeds was taken from each sample for dehulling to yield brown rice, and further milling to yield white rice, where the weight of brown rice and white rice were recorded as M1 and M2, respectively. Then, 10 g of white rice was taken from each sample and placed on a MICROTEH Scanner (MRS- 9600TFU2L) and a Wanshen SC-E Rice Appearance Quality Analyzer (Wanshen Testing Technology Co., LTD, Hangzhou, China) for determining chalkiness rate, chalkiness degree, transparency degree and head rice rate (H0). The rate of brown rice, milled rice and head rice was calculated with formula M1/25*100%, M2/25*100% and H0*M2/25, respectively.

ECQ traits were collected from 10 g of polished flour filtered by 100-mere, and 100 g of head rice for each sample. Apparent amylose content (AAC), gel consistency (GC), viscosity characteristics and taste scores were assessed with previously described methods (Zhou et al. 2021b). The lipid of both white and brown rice was extracted and measured by GC-MS method (Zhou et al. 2021a). The storage protein was extracted and measured with the method recommended by Chen et al. (2018). Each reported trait of grain quality in the study was averaged from six replicates at least.

Genome-Wide Association Analysis

Genotypic data for the association panel were downloaded from 3,000 Rice Genomes Project (https://iric.irri. org/projects/3000-rice-genomes-project) provided by the International Rice Research Institute (IRRI) (Sanciangco et al. 2018). We retrieved a dataset including 2.3 million biallelic InDels and 29 million biallelic SNPs. These datasets were merged using PLINK software for subsequent analyses on association. Phenotypic data for the ratio of grain length-to-width were downloaded from website (https://doi.org/10.7910/DVN/HGRSJG) (Sanciangco et al. 2018). After filtering out variants with minor allele frequency (MAF) ≤ 0.05 and missing rate > 20%, a total of 4,514,775 high-quality variants (4,044,849 SNPs and 469,926 InDels) were retained for calculating population structure matrix (Q) and kinship matrix (K) for conducting genome-wide association studies (GWAS), which were performed using the mixed linear model (MLM) implemented in the EMMAX software (Kang et al. 2010). GWAS were performed separately in all accessions, the XI group, and the GJ group, based on previous subgroup classification information (Wang et al. 2018). The significance threshold was determined based on Bonferroni correction set at 8.07×10^{-7} . Candidate genes in the target regions were identified based on the Nipponbare reference genome, considering genes to locate within ±100 kb of the most significant SNPs (Kawahara et al. 2013).

Haplotype and Phylogenetic Analysis

The imputed genotypic data for the variants in GL7 were downloaded from RiceVarMap (http://ricevarmap.nc pgr.cn/) (Zhao et al. 2021). There were 68 variants sites located 2 kb upstream of the transcription start site and within the coding sequence of the gene. Twenty-four SNPs and five InDels with $MAF \ge 0.05$ were selected for haplotype analysis among 3000 varieties, yielding nine major haplotypes, which number of varieties in rice subpopulations were summarized. The information on each subpopulation was modified with the published population structure (Wang et al. 2018). In order to evaluate the evolution of GL7, a phylogenetic tree was constructed using all the polymorphic sites with MAF \ge 0.005 in the locus. The phylogenetic tree for GL7 was constructed using UPGMA method by Mega7.0 software (Kumar et al. 2016). The genotypes of GL7 in wild rice were obtained from the Rice Super Pan-genome Information Resource Database (http://www.ricesuperpir.com/).

Statistical Analysis

The ratios of grain length-to-width among varieties with different *GL7* genotypes was compared using one-way ANOVA. Phenotypic differences between YHSM and YHSM^{*GL7*}, and between YX1B and YX1B^{*GL7*} were assessed using a two-tailed *t*-test. Phenotypic differences

among YX1A/YHSM, YX1A/YHSM^{GL7}, YX1A^{GL7}/ YHSM, and YX1A^{GL7}/ YHSM^{GL7} were compared using Duncan's multiple comparison test in the R package 'agricolae'(de Mendiburu and de Mendiburu 2019). All statistical analyses were conducted in R software (Ihaka and Gentleman 1996). Boxplots and histograms were created using the boxplot and barplot functions in R, respectively.

Results

GL7 Is a Major Gene for Rice Grain Shape

A genome-wide association study of the grain lengthto-width traits was performed basing on the published genotype and phenotype data from 3,000 Rice Genomes Project (3 K-RGP)(Wang et al. 2018). Three significant associations were identified, among which GW5/GSE5was outstanding, followed by GS3 and GL7/GW7/SLG7(Fig. 1a; Supplementary Table 1; Supplementary Table



Fig. 1 Identification and haplotype analysis of *GL7*. **a**) Manhattan plots and **b**) quantile–quantile plots depicting GWAS results using a mixed model for grain length-to-width ratio in rice. **c**) Gene structure, **d**) haplotype analysis and **e**) phylogenetic analysis of *GL7* in varieties from 3 K-RGP. Effects of *GL7* allele (-) and *g*/7 allele (TATCACTTTCT, namely the 11-bp insertion) on grain length-to-width ratio in all varieties **f**) and the *GJ* group **g**). The variants in (**d**) named as S and ID represent SNP and InDels variations respectively, and those marked in red were used to design molecular markers

2; Supplementary Fig. 2a-d). In order to investigate the haplotype and evolutionary history of GL7, we filtered out variants with MAF < 0.05 and obtained 29 variants (24 SNPs and 5 InDels) for haplotype analysis of GL7 in the 3 K-RGP varieties. A total of 9 major haplotypes (H1-9) were identified across all the samples (Fig. 1c-d). An 11-bp deletion in the fourth InDel (Del 4) (Fig. 1cd), which was previously reported to be closely linked to the functional *GL7* genotype (Wang et al. 2015b), was detected in haplotypes H1-H4 but was absent in H5-H8 (Fig. 1c). Therefore, we classified H1-H4 as GL7 allelic type and H5–H8 as gl7 allelic type. H2 was the predominant haplotype of GL7 allelic type, with 90% of its occurrence in Geng/Japonica (GJ) rice (Fig. 1d). Except for the 11-bp deletion, three SNPs (S2, S5 and S7) namely A-C-A were present in 79.8% GL7 allele (Fig. 1d). By comparing the ratio of grain length-to-width between GL7 allelic accessions and gl7 allelic accessions, we observed no significant differences (Fig. 1f; Supplementary Fig. 2f). However, within the GJ subgroup, those varieties with the *GL7* allele, harboring either the 11-bp deletion or the A-C-A SNPs, exhibited significantly higher grain lengthto-width ratio than those with the *gl7* allele (Fig. 1g; Supplementary Fig. 2h). A significant effect on grain length-to-width ratio was observed for the A-C-A SNPs in XI accessions (Supplementary Fig. 2e, g), indicating these three SNPs could be used for distinguishing GL7 allele.

Phylogenetic study on GL7 based on previously reported subgroup classification information (Wang et al. 2018) demonstrated that nearly all tropical Geng/Japonica (GJ-trp) rice possessed GL7 allelic type, whereas most temperate Geng/Japonica (GJ-tmp), Aus, and Xian/Indica (XI) rice had gl7 allelic type (Fig. 1d-e). Among the 28 O. rufipogon (Ruf) accessions, 8 carry the 11 bp deletion. The branching information in the evolutionary tree indicated that *GL7* allele originated within the *GJ* branch (Fig. 1e), suggesting that it was likely first introgressed from wild rice (Ruf) into GJ-trp, and subsequently introduced into some varieties of *GJ-tmp* and *XI* during breeding process. Taking these together, we inferred that GL7 allele was predominantly present in *GJ-trp* rice, which was largely responsible for significant variation of grain shape in GI varieties. On the other hand, the mere occurrences of GL7 in XI rice suggested its potential application in grain shape improvement in breeding practices (Fig. 1e).

Molecular Breeding System for Using *GL7* to Improve Grain Quality of *XI* Rice

To effectively use *GL7* on improving grain quality in *XI*, we established a breeding system with marker-assisted selection (MAS) for *GL7*. First, we selected the restorer line Taiguoxiaoxiangzhan (TGXXZ) and the maintainer line Shu 6B (S6B) as donor parents because previously sequencing study proved them with *GL7* allele (Fig. 2a). The *GL7* alleles in TGXXZ and S6B belonged to the



Fig. 2 MAS design of *GL7* on improving three-line hybrid rice grain quality. **a**) The genotypes of major rice grain quality genes of tested parental lines. **b**) The fluorescence calling of effective *GL7* KASP marker. Red, blue, and green dots represent homozygous *GL7*, homozygous *gl7*, and heterozygous genotypes, respectively. **c**) Molecular design breeding schedule of YHSM^{GL7}, YX1B^{GL7} and YX1A^{GL7}

H2 haplotype (Supplementary Fig. 1). Meanwhile, we selected the widely commercialized restorer Yuehesimiao (YHSM) and maintainer Yixiang 1B (YX1B) as recipient parents, both of which carried the gl7 allele belonging to H6 and H7 haplotype, respectively (Supplementary Fig. 1a). The genotypic information on the genes essential for grain quality in each of the four parents is shown in Fig. 2a.

Our breeding strategy was designed as shown in Fig. 2c. The GL7 allele was introduced from TGXXZ and S6B into YHSM and YX1B, respectively through hybridization. Then, after three to four generations of backcrossing and three generations of selfing where marker-assisted selection for GL7 allele was conducted in each generation, the improved YHSM^{GL7} and YX1B^{GL7} for GL7 allele were generated. Subsequently, continuously backcrossing male sterile (MS) line YX1A with YX1B^{GL7} produced the improved MS YX1AGL7. Finally, YHSM and YHSMGL7 were crossed with YX1A and YX1A^{GL7}, respectively to yield four F1 hybrids: YX1A/YHSM, YX1A/YHSMGL7, YX1A^{GL7}/YHSM and YX1A^{GL7}/YHSM^{GL7}(Fig. 2c). We evaluated each of the F1 hybrids for all the traits essential for grain yield and quality to assess functional roles of *GL7* in hybrid breeding for *XI* rice.

Three pairs of KASP markers for GL7 were developed with the specific SNP sites (S2, S5 and S7) from H1 and H2 functional GL7 haplotype (Fig. 1d; Supplemental Table 2; Supplementary Fig. 2). Genotypic identification revealed that the KASP marker (7-5290) at S2 worked the best, effectively distinguishing between homozygous GL7, heterozygous GL7/gl7 and homozygous gl7

Using *GL7* to Effectively Improve Elite Parents of Hybrid for Grain Quality

YHSM, a restorer line known for its good quality, high vield and lodging resistance, is a medium grain type of variety since its head rice is ~6.0 mm long with 2.9 for the ratio of grain length-to-width (Table 1). In order to make YHSM a slenderer variety, we crossed YHSM with TGXXZ which harbors the functional GL7 allele (Fig. 2a), and backcrossed to YHSM continuously YHSM^{GL7} through MAS of GL7 (Figs. 2b and 3a-b). Consequently, the seed length, head rice length and ratio of grain lengthto-width were significantly increased, but seed width was significantly decreased in YHSM^{GL7} over its original YHSM, and those changes had no effect statistically on grain yield per plant (Fig. 3c and f-h; Supplementary Fig. S1, Table 2). Furthermore, those morphological improvements in YHSM^{GL7} made both chalkiness rate and degree less than that in its original YHSM (Fig. 3d and i-j; Table 1). However, those traits essential for milling quality, eating and cooking quality were statistically similar between YHSM^{GL7} and YHSM (Fig. 3e; Table 1). These results suggested that GL7 could effectively improve grain appearance quality without a penalty to grain yield.

YX1B, the maintainer for YX1A that is popularly accepted because of high combining ability in the cytoplasmic male sterility (CMS) or three-line system, has long been known for its supreme quality of grains with

Table 1 Impact of different genotypes of GL7 on the grain quality traits

Trait	VICM			VY1B							
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	gl7	GL7	P-value	gl7	GL7	P-value	gl7/gl7	GL7/gl7	gl7/GL7	GL7/GL7	
BRR (%)	79.3±0.38	75.08 ± 0.36	3.4×10 ⁻⁶	78.3 ± 0.5	75.6 ± 0.4	1.2×10^{-4}	78.7±0.8a	77.1±0.5a	77.6±0.6a	77.1±0.5a	
HRR (%)	59.23±1.29	59.19 ± 0.55	0.95	58.2 ± 0.6	57.7 ± 1.1	0.51	$54.8 \pm 2.1 b$	53.3±1.8b	51.1±1.5b	58.1±1.0a	
HGL (mm)	6.0 ± 0.24	6.5 ± 0.34	0.01	7.0 ± 0.42	7.2 ± 0.63	0.38	6.6±0.48b	6.8±0.52b	6.82±0.57b	$7.02 \pm 0.51a$	
HGW (mm)	2.07 ± 0.05	1.77 ± 0.05	1.3×10^{-5}	2.27 ± 0.06	2.20 ± 0.06	2.1×10^{-3}	2.25±0.06a	2.09±0.11b	$2.12 \pm 0.05b$	$2.06 \pm 0.07 b$	
CR (%)	4.05 ± 0.84	2.45 ± 0.36	7.6×10^{-4}	7.11±0.52	6.58 ± 0.54	8.0×10^{-3}	4.66±0.72a	4.42±0.73a	3.67±0.80ab	$2.55 \pm 0.72b$	
CD (%)	1.15 ± 0.25	0.59 ± 0.25	3.8×10^{-3}	2.42 ± 0.32	1.92 ± 0.44	5.3×10^{-3}	1.46±0.18a	1.42±0.28a	1.33±0.29a	0.74±0.21b	
AAC (%)	15.9 ± 0.1	14.6 ± 0.8	0.56	14.2 ± 0.5	14.7 ± 0.5	0.45	15.8±1.2a	15.6±0.6a	16.1±1.0a	$16.3 \pm 0.5a$	
GC (mm)	67.9±2.3	69.7 ± 2.6	0.55	77.4 ± 2.6	77.7 ± 2.6	0.88	81.7±1.0a	78.9±1.9a	78.5±2.5a	$79.8 \pm 2.0a$	
GT(℃)	70.5 ± 0.9	70.6 ± 1.1	0.93	69.7 ± 1.1	70.1 ± 0.5	0.68	70.1±1.1a	70.9±0.6a	70.4±1.1a	71.6±0.6a	
Арр	6.90 ± 0.07	6.78 ± 0.08	0.06	7.6 ± 0.2	7.3 ± 0.2	0.04	8.6±0.1a	8.6±0.1a	8.4±0.2a	8.6±0.1a	
Tas	6.78 ± 0.15	6.65 ± 0.09	0.13	7.2 ± 0.3	6.9 ± 0.2	0.08	8.1±0.2a	7.8±0.3a	7.9±0.2a	8.1±0.3a	
Hardness	3.07 ± 0.64	2.74 ± 0.16	0.66	2.39 ± 0.58	3.03 ± 0.88	0.34	2.38±0.34a	2.45±0.19a	2.63±0.38a	$2.37 \pm 0.21a$	
Viscosity	0.39±0.114	0.23 ± 0.05	0.12	0.30 ± 0.08	0.23 ± 0.09	0.37	0.36±0.08a	0.27±0.08a	0.38±0.06a	$0.33 \pm 0.13a$	
Balance	0.13 ± 0.04	0.08 ± 0.03	0.12	0.13 ± 0.04	0.07 ± 0.01	0.05	0.15±0.01a	0.11±0.03a	0.15±0.04a	0.14±0.06a	
Elasticity	0.87 ± 0.06	0.88 ± 0.01	0.77	0.87 ± 0.04	0.90 ± 0.02	0.35	0.90±0.02a	0.89±0.02a	0.88±0.02a	$0.90 \pm 0.01a$	
Cos	81.4±0.8	82.1±1.0	0.42	83.2 ± 1.6	82.2±1.2	0.17	88.8±0.6a	88.4±0.7a	87.7±0.7a	89.2±0.8a	

Note: YHSM, Yuehesimiao; YX1B, Yixiang 1B; YX1A, Yixiang 1 A; BRR, Brown rice rate; HRR, Head rice rate; HGL, Head rice grain length; HGW: Head rice grain width; CR: Chalkiness rate; CD: Chalkiness degree; AAC: Apparent amylose content; GC: Gel consistency; GT: Gelatinization temperature; App: Appearance score of cooked rice; Tas: Taste Score of cooked rice. Cos: Comprehensive score of cooked rice. *P*-value was calculated using *T*-test. Significance a, ab, b was calculated basing multiple comparison using Duncan's multiple comparison test



Fig. 3 Introducing of *GL7* improved the grain quality of YHSM. **a**) The plant morphology of YHSM and YHSM^{*GL7*} lines (scale bar = 20 cm). **b**) The genome sequence information of YHSM^{*GL7*} The blue lines indicate the sequences introgressed into YHSM. **c**) The seed of morphology of YHSM and YHSM^{*GL7*} lines (scale bar = 1 cm). **e**) The genome sequence information of YHSM^{*GL7*} lines indicate the sequences introgressed into YHSM. **c**) The seed of morphology of YHSM and YHSM^{*GL7*} lines (scale bars from left to right were 1 cm and 0.5 cm, respectively). **d**) The appearance of YHSM and YHSM^{*GL7*} head rice (scale bar = 1 cm). **e**) The RVA properties of YHSM and YHSM^{*GL7*} lines. The grain length **f**), grain width **g**), grain length-width ratio **h**), chalkiness rate **i**) and chalkiness degree **j**) of YHSM and YHSM^{*GL7*} lines

Trait	YHSM			YX1B			YX1A/YHSM			
	gl7	GL7	P-value	gl7	GL7	P-value	gl7/gl7	GL7/gl7	gl7/GL7	GL7/GL7
PH (cm)	95.7 ± 4.0	97.4 ± 4.6	0.56	98.0 ± 3.4	96.1 ± 5.3	0.46	108.0±5.7a	109.2±5.7a	106.7±7.4a	110.6±5.1a
EPN	5.8 ± 1.4	5.7 ± 1.6	0.88	5.1 ± 1.6	5.3 ± 1.5	0.76	6.9±2.2a	6.5±2.7a	6.2±1.6a	7.5±3.1a
PL (cm)	24.0 ± 1.1	24.4 ± 0.4	0.38	27.2 ± 1.2	27.3 ± 1.5	0.93	27.8±0.9ab	$28.5 \pm 0.8a$	27.9±0.3a	$28.3 \pm 0.5a$
GNPP	193 ± 30	194 ± 19	0.90	128±8	120±17	0.18	178±18a	196±21a	165±11a	171±41a
SSR (%)	92 ± 1.7	92.6 ± 1.4	0.41	86.7 ± 3.2	83.4 ± 4.4	0.06	88.6±4.6a	90±1.7a	$88.5 \pm 2.5a$	86.8±3.8a
GN	1026 ± 309	1012 ± 244	0.91	564 ± 189	525 ± 138	0.59	1085±330a	1121±396a	1014±232a	1096±446a
GL (mm)	9.07 ± 0.14	9.64 ± 0.1	3.6×10^{-9}	10.11 ± 0.12	10.77 ± 0.09	1.3×10 ⁻¹¹	9.56±0.19c	9.9±0.14b	9.99±0.21b	$10.47 \pm 0.14a$
GW (mm)	2.85 ± 0.05	2.52 ± 0.04	1.9×10 ⁻¹²	3.07 ± 0.04	3.00 ± 0.04	6.2×10^{-4}	$3.05 \pm 0.04a$	$2.90 \pm 0.08 b$	$2.93 \pm 0.04 b$	$2.88 \pm 0.04 b$
LTW	3.21 ± 0.06	3.88 ± 0.07	9.0×10 ⁻¹⁵	3.30 ± 0.03	3.63 ± 0.04	4.0×10^{-14}	3.16±0.07c	$3.42 \pm 0.14b$	$3.42 \pm 0.1b$	3.59±0.06a
TGW (g)	23.9 ± 0.4	21.1 ± 0.3	3.5×10^{-13}	31.1 ± 0.7	31.9±0.8	0.03	28.1±0.7b	27.1±0.3c	29.8±1a	$28.6 \pm 0.4 b$
GY (g)	24.5 ± 7.3	21.4 ± 5.1	0.28	17.6±6	16.7 ± 4.4	0.73	$30.4 \pm 8.8a$	$30.3 \pm 10.5a$	$30.7 \pm 7.5a$	$31.3 \pm 12.7a$

Table 2 Impact of different genotypes of GL7 on the yield related traits

Note: YHSM, Yuehesimiao; YX1B, Yixiang 1B; YX1A, Yixiang 1 A; PH, Plant height; EPN, Effective panicle number; PL, Panicle length; GNPP: Grain number per panicle; SSR: Seed setting rate; GN: Grain number per plant; GL: Grain length; GW: Grain width; LTW: Grain length-width ratio; TGW: Thousand grain wight; GY: Grain yield per plant. *P*-value was calculated using *T*-test. a, ab, b, c was calculated basing multiple comparison using Duncan's multiple comparison test

fragrance and high combining ability. For example, hybrid variety Yixiangyou 2115 (YXY2115) derived from YX1A has become a check variety annually in the regional rice variety test program for Upper Yangtze River Area in China because of its outstanding performance. YX1B carries gs3GW5 allele, making its head rice \sim 7.0 mm long and ratio~2.8 for grain length-to-width (Fig. 2a). By crossing YX1B with Shu 6B which has functional GL7 allele (Fig. 2a) and backcrossing YX1B continuously with their offsprings under MAS for GL7 in each cross, we successfully transferred GL7 from Shu 6B to YX1B. The resulting YX1B^{GL7} was tremendously improved for grain quality over YX1B (Fig. 4a-b). Compared to YX1B, YX1B^{GL7} had significantly longer grain, and greater ratio of grain length-to-width, but similar traits influencing grain yield (Fig. 4; Supplementary Fig. 1; Table 2), and significantly lower rate and degree of grain chalkiness, but similar traits influencing grain quality on cooking and eating (Fig. 4d-e; Table 2).

Using *GL7* to Effectively Improve Elite Hybrids for Grain Quality

We used the improved YX1A^{GL7} generated from its improved maintainer YX1B^{GL7} and its original YX1A to cross the improved restorer YHSM^{GL7} and its original YHSM, yielding YX1A/YHSM, YX1A/YHSM^{GL7}, YX1A^{GL7}/YHSM and YX1A^{GL7}/YHSM^{GL7} for agronomic comparison (Fig. 2c). Four hybrids uniformly and significantly had much higher yield per plant than their parental restorer and maintainer from inbreeding, (Fig. 5; Supplementary Fig. 1; Table 2), proving great heterosis on yield in three-line hybrid rice. Among four hybrids, YX1A^{GL7}/YHSM^{GL7} had the longest grain and highest ratio of grain length-to-width, but the lowest width of grain (Fig. 5c and f-h; Table 2), making it the slenderest shape of grain. Adversely, the grain chalkiness rate and degree of YX1A^{GL7}/YHSM^{GL7} was the lowest among four hybrids (Figs. 3d and 5d; Table 1). However, YX1AGL7/ YHSM^{GL7} had slightly higher rate and degree of grain chalkiness than inbred YHSM^{GL7}, which can be explained by that hybrid variety usually has relatively poorer grain quality than conventionally inbred variety. Similarly with grain shape, YX1AGL7/YHSMGL7 had the highest rate of head rice, which was significantly higher than those of other three hybrids, which might be due to lower rate and degree of grain chalkiness (Table 1) because chalky grains are easily broken during milling process. Other traits related to yield including plant height and grain number were not significantly different among four hybrids (Fig. 5a-b; Table 2), so did as the traits related to cooking and eating quality (Fig. 5e. Table 1). All these results uniformly proved that GL7 had profound effects on improving grain quality without any penalty on yield in hybrid rice, especially for XI rice.

Discussion

GL7/GW7/SLG7 was a major gene responsible for slender rice grain and enhancing the GL7 expression could significantly increase grain length, decrease grain width and chalkiness without yield penalty (Wang et al. 2015a, b; Zhou et al. 2015). In the present study, we identified GL7 as a major grain length-width ratio QTL besides GS3 and GW5 (Fig. 1a; Supplementary Table 1). Haplotype analysis revealed that GL7 originated from GJ-trp rice and underwent selection within this subgroup. GS3, the major gene for cultivated rice grain shape, has undergone strong selection during domestication and the gs3 long grain allele was originated from GJ and subsequently introgressed into XI during breeding process (Takano-Kai et al. 2009). Unlike GS3, the mutations in GW5/GSE5 originally occurred in wild rice (O. rufipogon), which is now widely present in XI and GJ subpopulations, indicating the broad utilization of *GW5* in breeding (Duan et al. 2017; Zhou et al. 2017). Cultivated rice varieties display a



Fig. 4 Introducing of *GL7* improved the grain quality of YX1B. **a**) The plant morphology of YX1B and YX1B^{*GL7*} lines (scale bar = 20 cm). **b**) The genome sequence information of YX1B^{*GL7*}. The blue lines indicate the sequences introgressed into YX1B. **c**) The seed of morphology of YX1B and YX1B^{*GL7*} lines (scale bars from left to right were 1 cm and 0.5 cm, respectively). **d**) The appearance of YX1B and YX1B^{*GL7*} head rice (scale bar = 1 cm). **e**) The RVA properties of YX1B and YX1B^{*GL7*} lines. The grain length **f**), grain width **g**), grain length-width ratio **h**), chalkiness rate **i**) and chalkiness degree **j**) of YX1B and YX1B^{*GL7*} lines

wide diversity in grain shape. Dissecting the genetic basis of grain shape and uncovering the evolutionary history of related genes will not only deepen our understanding of rice domestication but also provide valuable genetic resources for modern rice breeding aimed at achieving desired traits. In the present study, we found that GL7 was rare in XI rice and demonstrated a model to introgress GL7 into XI rice. With marker-assisted selection (MAS) of GL7, we successfully introduced GL7 into the backbone restorer line YHSM, the backbone maintainer line YX1B, and the corresponding sterile line YX1A. The grain shape and



Fig. 5 Introducing of *GL7* improved the grain quality of hybrid rice. The plant morphology **a**) (scale bar = 20 cm), panicle morphology **b**) (scale bar = 5 cm), seed morphology **c**) (scale bar = 1 cm in the left and 0.5 cm in the right), head rice appearance **d**) (scale bar = 1 cm) and RVA properties **e**) of YX1A/YHSM, YX1A/YHSM^{GL7}, YX1A^{GL7}/YHSM and YX1A^{GL7}/YHSM^{GL7} hybrid lines. The histogram of grain length **f**), grain width **g**), grain length-width ratio **h**), chalkiness rate **i**) and chalkiness degree **j**) of YX1A/YHSM, YX1A/YHSM^{GL7}, YX1A^{GL7}/YHSM and YX1A^{GL7}/YHSM, SM^{GL7}, YX1A^{GL7}/YHSM and YX1A^{GL7}/YHSM, SM^{GL7}, YX1A^{GL7}/YHSM and YX1A^{GL7}/YHSM, SM^{GL7}, YX1A^{GL7}/YHSM, SM^{GL7}, YX1A^{GL7}/YHSM, SM^{GL7}, SM

appearance quality of YHSM^{GL7}, YX1B^{GL7} and YX1A^{GL7}/ YHSM^{GL7} were significantly improved, demonstrated by longer grains, greater ratio of grain length-to-width and greater rate of head rice yield, but lower rate and degree of grain chalkiness than their parents and other hybrids. Yet, the effect of *GL7* on thousand-grain weight (TGW) was different between YHSM and YX1B. The TGW was significantly decreased in YHSM^{GL7} but slightly increased in YX1B^{GL7} compared to their original parents (Table 2), which might be caused by the complexity of TGW and

the genomic diversity of YHSM and YX1B (Fan et al. 2006; Weng et al., 2008; Zhou et al. 2015). Our study on GL7 shed light on genetic diversity of grain shape in cultivated rice, and provided promising targets for breeding hybrid rice with high quality of grain.

The variation of grain chalkiness is always accompanied with a variation of amylose content in grain. Previous studies show that knock-out of either WCR1 or bZip60 significantly increased the rate of grain chalkiness and decreased amylose content, and *Chalk5* effectively regulates the rate of grain chalkiness along with a change of amylose content in rice (Li et al. 2014; Wu et al. 2022; Yang et al. 2022). In our study, we found that GL7 significantly decreased the rate and degree of grain chalkiness, but the amylose content was not significantly changed (Table 1). In order to figure out the reason for the effect of GL7 on grain chalkiness, we tested the storage components in rice grain (Supplemental Table 3). The total content of storage protein was significantly changed between YHSM^{GL7} and YHSM, but similar among others. Furthermore, wherever GL7 existed, albumin content significantly elevated. The lipid content and lipid composition in grains were not uniformly changed, but YX1B^{GL7} had a significantly lower content of oleic acid (C18:1) and linoleic acid (C18:2). Starch, protein and lipids are the major components in rice grain, which content and composition are major determinants of grain quality (Xia et al. 2022). It is widely acknowledged that the chalky/ opaque endosperm part was due to the loosely packed of starch granules and protein bodies, where an accumulation of storage protein has effect on rice grain chalkiness (Xi et al. 2014; Guo et al. 2024). Besides, the phytohormones also affect the formation of grain chalkiness (Lin et al. 2017; Xie et al. 2021). Taking all these together, as a major gene to regulate grain shape, GL7 might function through affecting the plant hormones to regulate spikelet development, endosperm filling and the accumulation of storage protein to control grain shape and grain chalkiness in rice.

Conclusion

In this study, we identified GL7/GW7/SLG7 as a major grain shape QTL besides GS3 and GW5. Haplotype analysis revealed nine haplotypes (H1-H9) of GL7, among which H1-H4 harbored an 11-bp deletion were designated as the functional GL7 allele and were primarily present in GJ rice. We further established a breeding system with MAS of GL7, and through this system, we successfully introduced the GL7 allele into three-line backbone parents YX1B and YHSM. The grain appearance quality of YX1B^{GL7}, YHSM^{GL7} and hybrid YX1A^{GL7}/ YHSM^{GL7} were significantly improved with increased grain length and grain length-to -width ratio, and decreased grain width and grain chalkiness rate and ratio compared to their original parents and intercrosses. Our results provide genetic information on the diversity of cultivated rice grain shape and demonstrated the potential of *GL7* on improving grain quality, especially XI rice.

Abbreviations

ECQ	Eating and cooking quality
AC	Amylose content
GC	Gel consistency
GT	Gelatinization temperature
KASP	Kompetitive allele-specific PCR
GWAS	Genome wide association study

- MLM Mixed linear model
- MAF Minor allele frequency
- SNP Single nucleotide polymorphism MAS Marker-assisted selection
- CMS Cytoplasm male sterility
- erropiusini nule sterinty

Supplementary Information

The online version contains supplementary material available at https://doi.or g/10.1186/s12284-025-00786-4.

Supplementary Material 1

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Author Contributions

Professor H.Z., X. W. and X. D. designed and supervised the study; D. X. and P. X. performed the experiments; S. Zheng., L. X., Y. Y., Z. Y., S. D. and Y. L. assisted in the traits measuring and photograph taking. D. X. and H. Z. analyzed the data. P. X. provided the plant materials. Y. W. assisted in the KASP genotyping experiment. D. X. wrote the manuscript, P. X., X. M. and H. Z. edited and revised. All authors read and approved the final manuscript.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests

The authors declare no competing interests.

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