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Fine mapping of *qWCR7*, a grain chalkiness QTL in rice

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Abstract Chalkiness is one of the key determinants of rice quality and is a highly undesirable trait for breeding and marketing. In this study, *qWCR7*, a major quantitative trait locus (QTL) of white-core rate (WCR), was genetically validated using a BC_3F_2 segregation population and further fine mapped using a near isogenic line (NIL) population, of which both were derived from a cross between the donor parent DL208 and the recurrent parent ZS97. *qWCR7* was finally narrowed to a genomic interval of ~ 68 kb, containing seven annotated genes. Among those, two genes displayed markedly different expression levels in endosperm of NILs. Transcriptome analysis showed that the synthesis and accumulation of metabolites played a key role in chalkiness formation. The contents of storage components and expression levels of related genes were detected, suggesting that starch and storage protein were closely related to white-core trait. Our findings have laid the foundation of map-based cloning of *qWCR7*, which may have potential value in quality improvement during rice breeding.

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Introduction

Rice grain quality is a multi-faceted trait including appearance quality, cooking and eating quality (ECQ), processing quality, nutritional quality, and hygiene quality (Zhou et al. 2020). Among them, appearance quality is mainly determined by grain shape, translucency, and chalkiness, which are immediately noted to consumers and thus are the key factors affecting the commercial value (Fitzgerald et al. 2009). Grain chalkiness refers to the opaque region in endosperm and is classified into white-belly, white-core, white-back, and floury endosperm, according to its position (Lisle et al. 2000; Zhou et al. 2009b; Siebenmorgen et al. 2013). Except for its role in decreasing appearance quality, chalkiness also has a negative effect on ECQ and processing quality. Therefore, grain chalkiness is highly unpopular for marketing, and reducing chalkiness has become an important goal in improving grain quality (Cheng et al. 2005; Misra et al. 2019).

Chalky endosperm is often accompanied by changes in the morphology and arrangement of starch granules in rice and maize (Wang et al. 2008; Li et al. 2014; Zhang et al. 2018). Scanning electron microscope analysis has revealed that round and loosely packed starch granules are filled in chalky endosperm,

which is completely different from polyhedral and densely packed starch granules in transparent endosperm; the loose arrangement of starch granules leads to the presence of air spaces and finally opaque phenotype (Kang et al. 2005; Fujita et al. 2007; Guo et al. 2011). Moreover, chalky grains are easier to break in the milling process due to reduced grain density, resulting in the decrease of head rice yield (Zhao and Fitzgerald 2013; Li et al. 2014). Hence, cultivating rice varieties with low chalkiness rate may be a potential strategy to enhance milling yield.

Previous reports have shown that rice chalkiness is a complex quantitative trait dominated by polygenes and environment (Siebenmorgen et al. 2013; Jagadish et al. 2015; Kaneko et al. 2016; Yun et al. 2016; Tang et al. 2019). In the past 20 years, numerous quantitative trait loci (QTL) for chalkiness have been mapped across all 12 pairs of rice chromosomes, but only a few QTL have been fine mapped or cloned (Zhou et al. 2009a; Li et al. 2014; Gao et al. 2016; Zhu et al. 2018). Zhou et al. (2009a) detected two QTL associated with the percentage of chalky grain using a chromosome segmental substitution line (CSSL) population of PA64S and 9311 and narrowed *qPGWC-7* to a 44 kb DNA segment. Gao et al. (2016) detected a major QTL controlling area of chalky endosperm *qACE9* from a recombinant inbred lines' (RILs) population of PA64S and 9311 and fine mapped it into an interval of 22 kb region. Zhu et al. (2018) mapped a QTL for percentage of chalky grain *qPCG1* in an interval of ~139 kb using residual heterozygous lines (RHLs) of Xieqingzao B and Zhonghui 9308. Yang et al. (2021) fine mapped two QTL for grain chalkiness on chromosomes 9 and 11, *qPGC9* and *qPGC11* respectively, using two single-segment substitution lines (SSSLs), 11–09 with substitution segment from Basmati 370 and HP97-11 with substitution segment from *Oryza glaberrima*. *Chalk5* is the firstly cloned major QTL positively controlling white-belly and encodes a vacuolar H^+-translocating pyrophosphatase (V-PPase) with inorganic pyrophosphate (PPi) hydrolysis and H^+-translocation activity (Li et al. 2014). Yet, more investigations are needed to unearth the genetic basis of chalkiness.

In a previous study, we repeatedly detected *qWCR7* for white-core rate (WCR) on chromosome 7 using a RIL population derived from a cross between two rice cultivars (ZS97 and DL208) (Peng et al. 2014). In this study, we performed genetic validation with a BC_3F_2 segregating population and fine mapping with a near isogenic line (NIL) population, of which both were derived from the same cross between DL208 and the recurrent parent ZS97. Furthermore, we conducted transcriptome analysis in endosperm to analyze the causes of chalkiness formation. We also observed starch granule morphology of white-core grain by scanning electron microscopy. Finally, we analyzed the effect of *qWCR7* on storage components by detecting the contents (oil, protein, and starch) and expression levels of related genes.

Materials and methods

Population and field experiment

A NIL population of *qWCR7* was developed from a cross between *indica* cultivar DL208 (the donor parent) and ZS97 (the recurrent parent). To verify the genetic effect of *qWCR7*, a BC_3F_1 plant carrying the heterozygous region of *qWCR7* was selected and self-pollinated to generate the BC_3F_2 population consisting of 180 plants. To conduct fine mapping of *qWCR7*, a total of 6300 NIL-F_2 individuals were used to screen recombinants in the *qWCR7* region, which were subjected to progeny test analysis.

The BC_3F_2 population and NIL-F_2 population were planted under natural field conditions at the experimental station of Huazhong Agricultural University at Wuhan (N 30.49°, E 114.36°), Hubei province, in 2013 and 2014, respectively. The progenies of recombinants were grown at Lingshui (N 18.51°, E 110.04°), Hainan province, in 2015. Twelve 30-day-old seedlings of each line were transplanted with 16.5 cm spacing in single row plots in the field; rows were 26.4 cm apart. Field management followed local practices. Ten plants from the middle of each row were harvested individually for trait measurement.

Phenotyping

Harvested seeds from each plant were air dried and stored at room temperature for 3 months before further phenotyping. White-core rate was defined as percentage of white-core grains in random samples of more than 100 dehulled grains from each plant through visual assessment. Flour ground from milled grain was used for measuring total starch

content, amylose content, and storage proteins. Total starch content was detected by anthrone colorimetry using a professional Kit (Grace Biotechnology, G0548W, Suzhou). Detection of amylose content was based on Iodine colorimetry (Bao et al. 2006). Measurement of glutelin, prolamin, globulin, and albumin contents in the flour was based on previously published methods (Kumamaru 1988). The brown rice was ground into flour to analyze total lipid content using gas chromatograph-mass spectrometry (GC–MS) (Wu et al. 2005).

Marker development and genotyping

All mapping primers were designed by referring to the genomes of ZS97 and MH63 (http://ricevarmap.ncpgr.cn/) (Zhao et al. 2015) and Rice EST Data Base (http://redb.ncpgr.cn/). Genomic DNA was extracted from young leaves, according to the cetyltrimethylammonium bromide (CTAB) procedure (Murray and Thompson 1980). PCR amplification reaction was performed at an initial 94 °C for 5 min, followed by 32 cycles of 30 s at 94 °C, 30 s/1 min at 55 °C, and 30 s at 72 °C, and then 72 °C for 5 min. The PCR products were identified by sequencing or using 4% non-denaturing polyacrylamide gel electrophoresis (PAGE) and visualized using silver staining. The polymorphic simple sequence repeat (SSR) or insertion/deletion (InDel) markers within the QTL interval were applied to identify the genotype of individuals (Supplementary Table 1).

The parental lines ZS97 and DL208 were sequenced by the illumine HiSeq2000 that generate 100 bp paired-end reads. The paired-end reads of the two parental lines were aligned against the rice reference genome (IRGSP 7.0) using BWA software (Li and Durbin 2009). SNPs were identified using SAMtools and BCFtools. Only alignments with mapping quality ≥ 40 were used, and bases with base quality ≥ 10 were used to identify SNPs. Only the reads uniquely mapped to the genome sequence were retained for further analysis. Genotypes of 4,726 varieties (http://ricevarmap.ncpgr.cn/) were used to impute missing genotypes of the parents using Beagle software (Browning et al. 2018).

Transcriptome assembly and functional annotation

Endosperm samples (two biological replicates) at the 14th day after flowering (DAF) were used for RNA sequencing by Hiseq-PE150 (Novogene Biotech. Company, Tianjin). Raw data were processed using NGS QC Toolkit (Patel and Jain 2012), and the reads containing ploy-N and the low-quality reads were removed to obtain the clean reads. The read counts of each gene were obtained by featureCounts and the fragments per kilobase of transcript per million fragments mapped (FPKM) value of each gene was calculated using R script. Differentially expression genes were identified using the DESeq2 (with replicates). P value ≤ 0.05 and $|\mathrm{Log_2FoldChange}| \geq 1$ determined as significantly differentially expression genes DEGs were selected and analyzed by GO (Gene Ontology) function and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment by clusterProfiler of R package.

Scanning electron microscopy (SEM)

Milled rice grains were transversely cut in the midsection and coated with gold under vacuum conditions. Starch granule morphology at the central and peripheral parts of the endosperm was examined with a scanning electron microscope (JSM-6390LV, JEOL) at an accelerating voltage of 10 kV and spot size of 30 nm. SEM analysis involved at least three biological replications of mounted specimens. All procedures were carried out according to the manufacturer's protocol.

RNA extraction, reverse transcription, and qRT-PCR

Total RNA was extracted from plant tissues using an RNA extraction kit (TRIzol, Invitrogen). First-strand cDNA was synthesized in 20 μl of reaction medium containing 2 μg of RNA and 200 U of M-MLV reverse transcriptase (Promega). Quantitative RT-PCR was performed on a QuantStudio6 Flex machine using SYBR Green PCR reagent according to the manufacturer's instructions. All assays were performed with at least three biological and three technical replications. The rice *actin1* gene served as the internal control to normalize gene expression.

Primers of the genes involved in metabolism of storage components were used for qRT-PCR assays (Supplementary Table 2).

Statistical analysis

QTL analysis of the BC_3F_2 population was performed by WinQTLCart 2.5 and Mapmaker/Exp3.0 (Lander et al. 1987; Zeng 1994). The significance of differences for two sets of data was performed using two-tailed *t* test at a $P<0.05$ level.

Results

Genetic validation of QTL effects of qWCR7

In a previous study, *qWCR7* was located between markers RM445 and RM418 on chromosome 7 using a RIL population derived from a cross between DL208 and ZS97 (Peng et al. 2014). To validate the genetic effect of *qWCR7*, we developed a segregating population of 180 BC_3F_2 plants with ZS97 being the recurrent parent and DL208 the donor parent. The phenotypic variation of WCR in the population showed continuous bimodal distribution (Fig. 1). Meanwhile, we added an InDel marker WB1693 and analyzed the effect of the QTL. *qWCR7* was located between markers WB1693 and RM445, explaining 81.40% of the phenotypic variance (Table 1). This result indicated that *qWCR7* was a major genetic factor conferring WCR variation, and the ZS97-derived allele increased the WCR.

Table 1 Genetic effect of *qWCR7* in the BC_3F_2 population

Trait	QTL	Interval	LOD	A	V (%)
WCR	*qWCR7*	WB1693-RM445	35.33	26.96	81.40

QTL, quantitative trait locus. *LOD*, logarithms of odds. *WCR*, white-core rate. *A*, additive effect, the positive value means that the ZS97 allele increases trait value. *V*, variance, phenotypic variation explained by the QTL

Fine mapping of qWCR7

To improve the mapping resolution of *qWCR7*, we developed a NIL-F_2 population consisting of 6,300 individuals, from which a total of 45 recombinants were identified between WB1693 and RM445. Subsequently, 5 polymorphic indel markers were developed within the target region based on publicly available rice genome sequences (http://ricevarmap.ncpgr.cn/) and used in genotyping of recombinants (Fig. 2a). We undertook progeny tests to determine the *qWCR7* genotype of each recombinant (Supplementary Table 3). *qWCR7* showed incompletely dominant and was eventually narrowed to a genomic interval of ~ 68 kb flanked by markers M1711 and M1718 (Fig. 2a). To evaluate the effect of *qWCR7* on chalkiness, we used a pair of NILs, $NIL^{qWCR7-ZS97}$ and $NIL^{qWCR7-DL208}$, which carried the *qWCR7* locus from ZS97 and DL208, respectively, in the background of ZS97. Compared with $NIL^{qWCR7-DL208}$, $NIL^{qWCR7-ZS97}$ displayed ~ 24% higher WCR value (Fig. 2b).

According to the annotation information of Nipponbare (MSU Rice Genome Annotation Project), the target region of *qWCR7* contains seven annotated

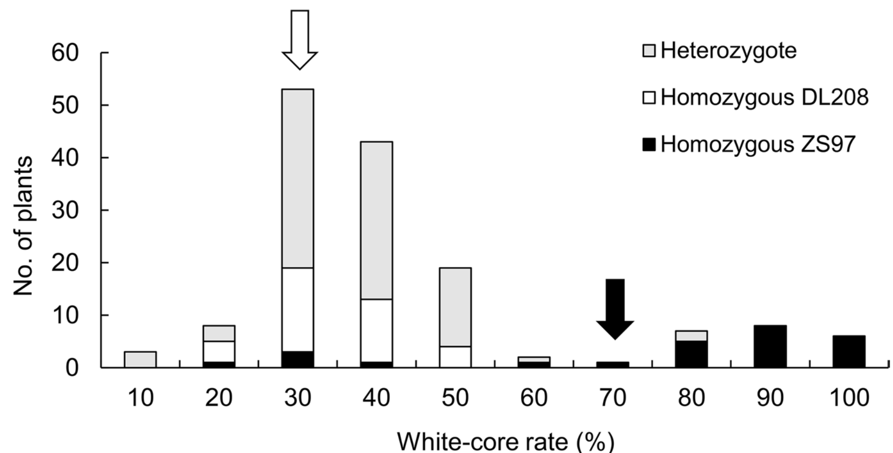

Fig. 1 Frequency distribution of WCR in 180 BC_3F_2 population. Arrows indicate the average values of homozygous ZS97 (black) and homozygous DL208 (white), respectively

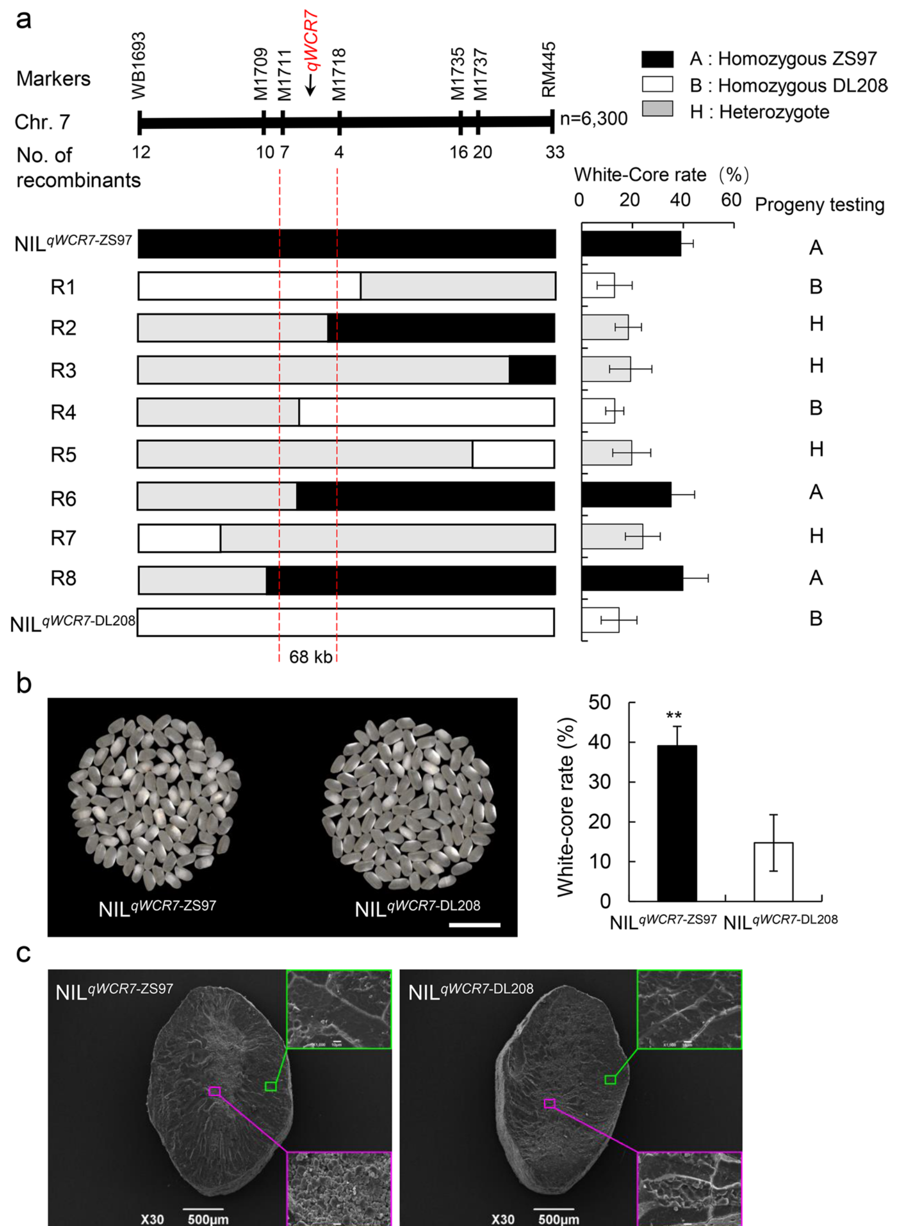

Fig. 2 Fine mapping of *qWCR7* for grain chalkiness. **a** Substitution mapping of *qWCR7*. Fine mapping of the *qWCR7* region using NIL-F2 population consisting of 6,300 individuals. The number of recombinants indicates the number of recombinants between the target site and flanking molecular markers. Genotypes and phenotypes of recombinants, each of which was confirmed by progeny test. Black, white, and gray blocks represent the genotypes of homozygous ZS97, homozygous DL208, and heterozygote, respectively. **b** The head rice appearance and WCR values of the NIL$^{qWCR7-ZS97}$ and NIL$^{qWCR7-DL208}$. Scale bar: 10 mm. Significant difference were based on two-tailed *t* test, $^{**}P \leq 0.01$. Error bars, s.e.m. **c** Scanning electron microscopic analysis of transverse sections of mature seeds from the NILs. Magenta and green rectangles represent the center and the edge positions of endosperm. Scale bars: 500 μm (entire grain), 10 μm (boxes)

genes (*LOC_Os07g29220*, *LOC_Os07g29224*, *LOC_Os07g29230*, *LOC_Os07g29240*, *LOC_Os07g29280*, *LOC_Os07g29290*, *LOC_Os07g29300*). Among these genes, *LOC_Os07g29220*, *LOC_Os07g29230*, and *LOC_Os07g29290* encode a putative cyclopropane-fatty-acyl-phospholipid synthase, an LTP family protein precursor and an expansin precursor, respectively, whereas the remaining genes encode expressed proteins. We further compared the sequence variations of these candidate genes including the 2 kb promoter and the coding sequence between ZS97 and DL208 (Table 2). The result showed that the variations occurred in the promoter, intron, and 5′ and 3′ untranslated regions (UTR).

Many studies have shown that the formation of chalkiness is related to the morphology and arrangement of starch granules. Then, we examined the starch granule morphology of mature seeds using scanning electron microscopy. Results showed that starch granules of white-core grains from NIL$^{qWCR7-ZS97}$

Table 2 The sequence variations of the candidate genes between ZS97 and DL208

Gene	SNP/InDel	Variation position	ZS97	DL208
LOC_Os07g29220	Chr7_17114083	Promoter	AT	A
	Chr7_17114517	Promoter	T	G
	Chr7_17115626	Promoter	CAACG	C
	Chr7_17115909	5′ UTR	TGGCGGC	TGGC
	Chr7_17119343	Intron	C	T
	Chr7_17119430	Intron	T	A
LOC_Os07g29224	Chr7_17124436	Promoter	C	TTGCTACGAGAGGCGC
	Chr7_17125972	Promoter	T	G
	Chr7_17125992	Promoter	G	C
	Chr7_17127037	Intron	GT	G
	Chr7_17127319	3′ UTR	A	ATCGAAGAAGAATTCAGCATGGAA AGATAGGTACCAATGGCCTGAC
	Chr7_17128151	3′ UTR	A	G
	Chr7_17129427	3′ UTR	CTT	CT
	Chr7_17129544	3′ UTR	A	G
LOC_Os07g29230	Chr7_17130315	Promoter	CA	C
	Chr7_17130662	Promoter	AATGACTTAACT	A
	Chr7_17130675	Promoter	TATGTGCAGTTAAGTTA	T
LOC_Os07g29240	Chr7_17135875	Promoter	ACTT	ACT
	Chr7_17137537	Intron	A	G
	Chr7_17144605	3′ UTR	T	TCCATGCCACTGTAAATGTGGCAA ATCAGAAAAATGCCACAACTA TTTGCGTTATCGGTTAGATGCCAC TAAAAATTTTTAAAATTAGAA
LOC_Os07g29280	Chr7_17159074	Promoter	T	C
	Chr7_17159101	Promoter	A	G
	Chr7_17159242	Promoter	C	CAAAAATAATATTTTTAATAATTTT
LOC_Os07g29290	Chr7_17164567	Intron	GT	G
	Chr7_17164735	Intron	GTTT	GT
	Chr7_17165583	5′ UTR	CAG	CAGAG

SNP, single-nucleotide polymorphism. *InDel*, insertion/deletion. Reference genome, Nipponbare

were small, round, and loosely packed in the center of endosperm, which were notably different from polyhedral and densely packed starch granules from NIL$^{qWCR7-\mathrm{DL208}}$; however, no difference was observed in the edge of endosperm of both the grains (Fig. 2c). This result indicated that altered morphology and spatial distribution of starch granules caused white-core endosperm.

Transcriptome analysis in endosperm

To detect putative differentially expressed genes (DEGs) affecting WCR, we performed transcriptome analysis in the 14th day after flowering (DAF) endosperm of NIL$^{qWCR7-\mathrm{ZS97}}$ and NIL$^{qWCR7-\mathrm{DL208}}$, which were denoised as 14EZ and 14ED, respectively. DEGs were identified using the following criteria: *P* value ≤ 0.05 and $|\mathrm{Log_2Fold\ Change}| \geq 1$. There were 1849 DEGs in 14ED vs 14EZ (1010 downregulated and 839 upregulated) (Fig. 3a and b). Gene Ontology (GO) enrichment analysis of DEGs showed that the most enriched genes participated in "biological process," followed by "molecular function" and "cellular component." The "biological process" category was dominated by photosynthesis (GO:0,015,979), epigenetic (GO:0,040,029), DNA metabolic process (GO:0,006,259), cell cycle (GO:0,007,049), flower development (GO:0,009,908), cell differentiation

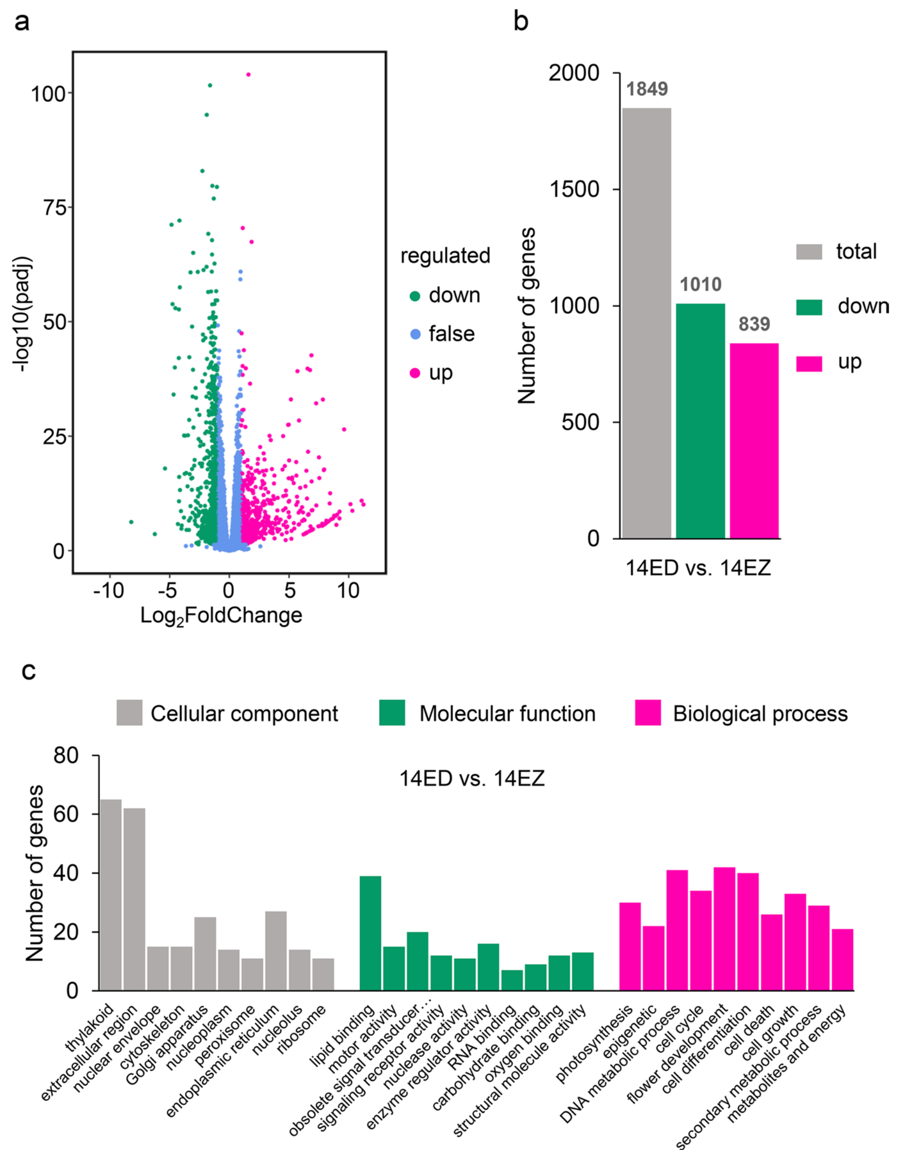

Fig. 3 DEGs and Gene Ontology (GO) enrichment analysis. **a** and **b** DEGs obtained by 14ED vs. 14EZ. DEGs were differentially expressed with statistical significance (P value ≤ 0.05 and $|\mathrm{Log_2FoldChange}| \geq 1$). **c** Comparison of GO classifications of DEGs in 14 DAF endosperm

(GO:0,030,154), cell death (GO:0,008,219), cell growth (GO:0,016,049), secondary metabolic process (GO:0,019,748), and metabolites and energy (GO:0,006,091). The "molecular function" category was dominated by lipid binding (GO:0,008,289), motor activity (GO:0,003,774), and obsolete signal transducer activity (GO:0,004,871). In the "cellular component" category, thylakoid (GO:0,009,579), extracellular region (GO:0,005,576), and nuclear envelope (GO:0,005,635) were prominently represented (Fig. 3c). The above results showed that lipid binding, photosynthesis, and metabolic regulation may be tightly related to white-core.

To identify metabolic pathways in which DEGs were involved, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. DEGs mainly belonged to metabolic pathways, biosynthesis of secondary metabolites, starch and sucrose metabolism, photosynthesis, ABC transporters, phenylpropanoid biosynthesis, fatty acid elongation, plant–pathogen interaction, plant hormone signal transduction etc. (Fig. 4a). Further analysis exhibited that the total number of DEGs related to metabolic pathways, biosynthesis of secondary metabolites, and starch and sucrose metabolism accounted for a very large proportion, indicating that

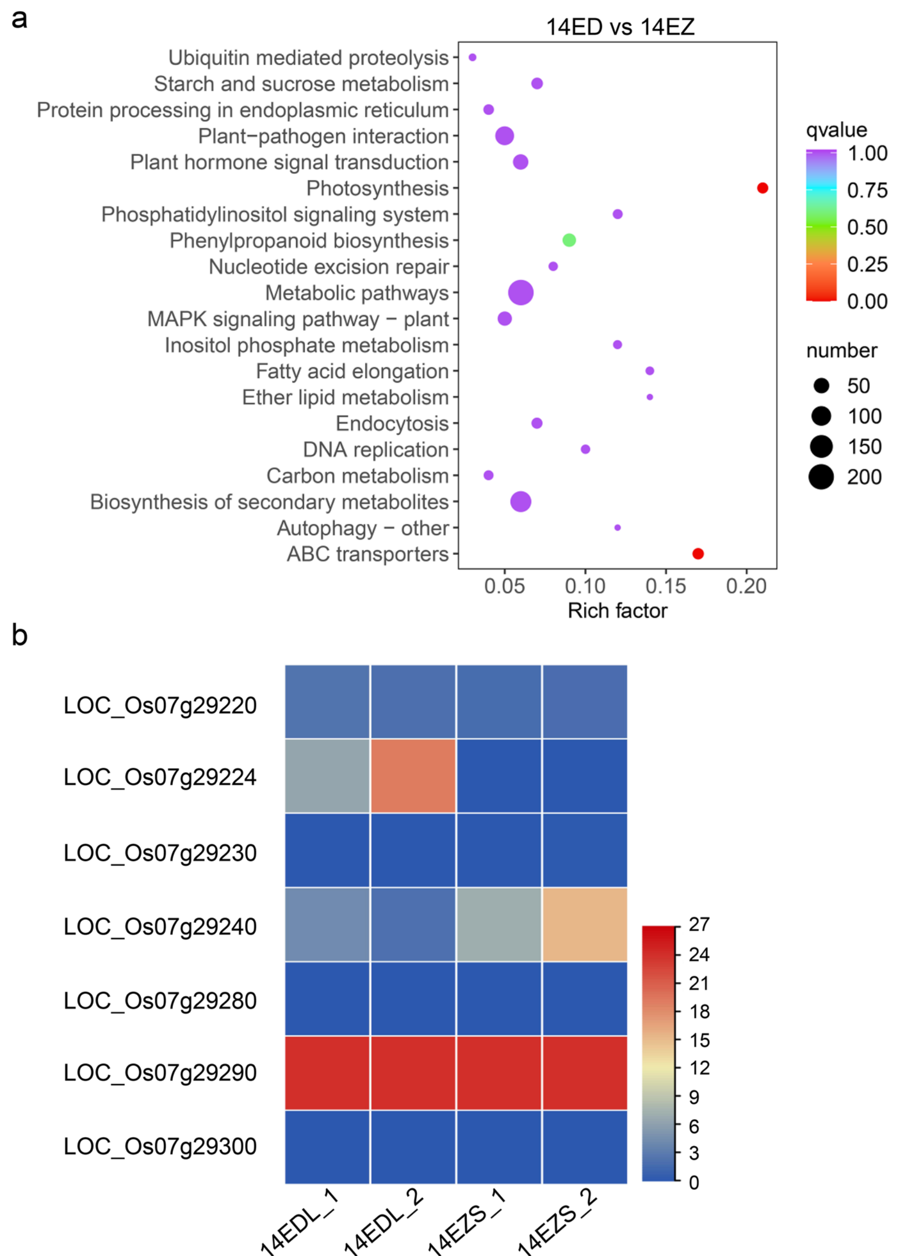

Fig. 4 KEGG pathway assignments and expression analysis of the putative genes at *qWCR7* locus. **a** KEGG pathway enrichment analysis of DEGs by 14ED vs. 14EZ. The major 20 categories are shown. **b** Expression patterns of the 7 candidate genes in 14 DAF endosperm of NILs, which are shown as fragments per kilobase of transcript per million fragments mapped (FPKM)

the accumulation of metabolites such as starch and protein may plays an important role in the process of chalkiness formation.

In order to analyze the transcription level of the 7 putative genes in the *qWCR7* region, we extracted their transcriptome data of endosperm. As shown in Fig. 4b, *LOC_Os07g29230*, *LOC_Os07g29280*, and *LOC_Os07g29300* were not expressed in 14 DAF endosperm, and the expression level of *LOC_Os07g29290* was the highest. Importantly, both *LOC_Os07g29224* and *LOC_Os07g29240* had markedly different expression levels between NIL$^{qWCR7-ZS97}$ and NIL$^{qWCR7-DL208}$, suggesting that they might be the causal gene underlying *qWCR7*.

Effects of qWCR7 on rice grain storage components

Chalkiness formation is closely related to changes in the contents of storage components such as oil, protein, and starch (Kang et al. 2005; Li et al. 2014). To detect the effects of *qWCR7* on storage components, we investigated the contents in the mature grains of NIL$^{qWCR7-\mathrm{ZS97}}$ and NIL$^{qWCR7-\mathrm{DL208}}$. Firstly, the oil composition consisting of various fatty acids was detected using GC–MS. Among identified fatty acids, myristic acid (C14:0), stearic acid (C18:0), and arachidic acid (C20:0) showed markedly higher values in NIL$^{qWCR7-\mathrm{ZS97}}$ than in NIL$^{qWCR7-\mathrm{DL208}}$. However, no difference was observed in other fatty acids accounting for more than 96% of the oil concentration between the two NILs, leading to similar content of the total oil (Fig. 5a). Subsequently, we detected storage protein contents including prolamin, glutelin, albumin, globulin, and total protein. Compared with NIL$^{qWCR7-\mathrm{DL208}}$, NIL$^{qWCR7-\mathrm{ZS97}}$ displayed significantly higher contents in glutelin but lower content in albumin. The content of total protein showed

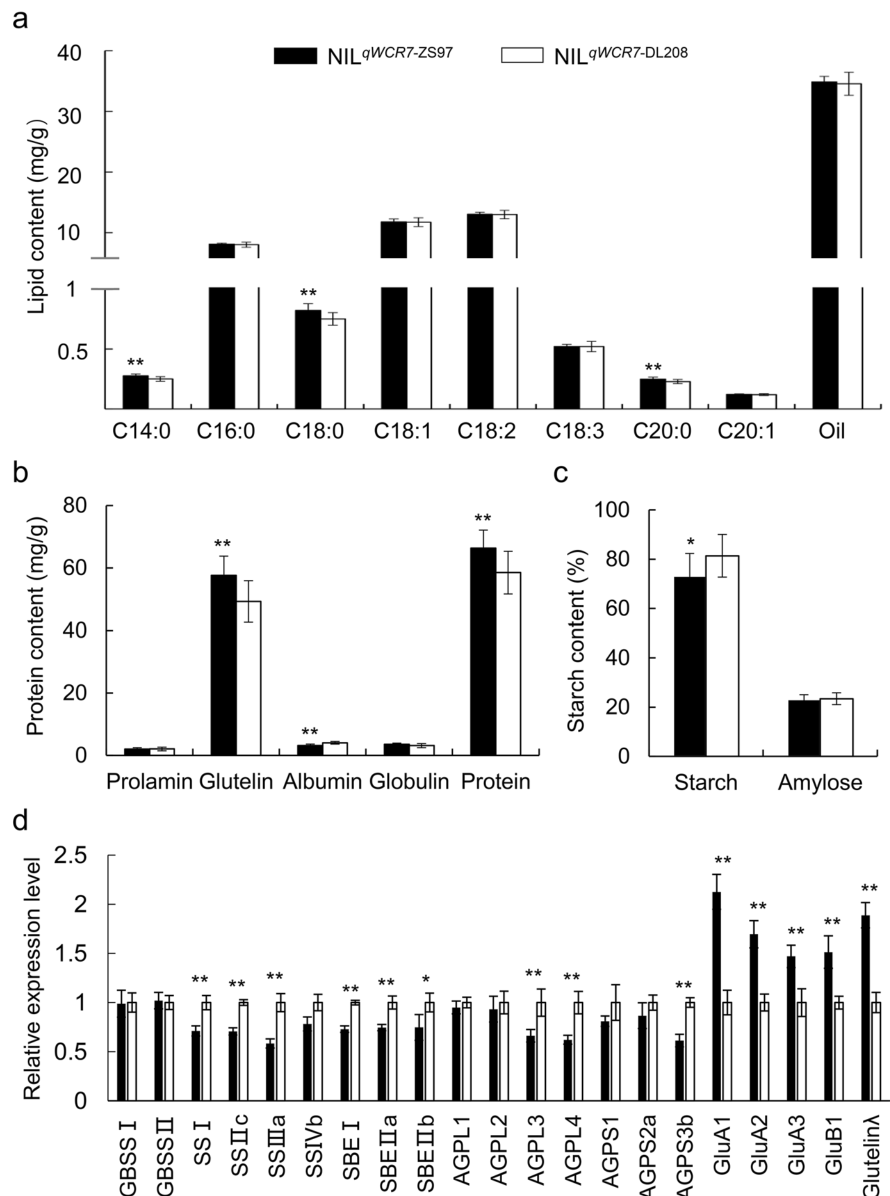

Fig. 5 The content of stored components in NIL$^{qWCR7-\mathrm{ZS97}}$ and NIL$^{qWCR7-\mathrm{DL208}}$. **a** Lipid content in brown rice. C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, eicosenoic acid; oil, total lipid content. **b** Protein content in milled rice. **c** Starch content in milled rice. In **a**–**c**, the number of NIL$^{qWCR7-\mathrm{ZS97}}$ and NIL$^{qWCR7-\mathrm{DL208}}$ was 11 and 12 plants, respectively, and each sample consisted of at least 300 grains. Significant differences were based on two-tailed *t* tests, $*P \le 0.05$, $**P \le 0.01$. Error bars, s.e.m. **d** The effects of *qWCR7* on transcription levels of genes related to storage components in 7 DAF endosperms. Endosperm samples were from different plants. Significant differences were based on two-tailed *t* tests ($n=4$), $*P \le 0.05$, $**P \le 0.01$. Error bars, s.e.m

higher value in NIL$^{qWCR7-\mathrm{ZS97}}$, which was related to the extremely high proportion of glutenin in storage protein (Fig. 5b). Then, we determined contents of total starch and amylose in milled rice. The amylose contents were similar between NIL$^{qWCR7-\mathrm{ZS97}}$ and NIL$^{qWCR7-\mathrm{DL208}}$, but the total starch content of NIL$^{qWCR7-\mathrm{ZS97}}$ was significantly lower than that of NIL$^{qWCR7-\mathrm{DL208}}$ (Fig. 5c).

In addition, we examined the transcription level of genes related to storage components in 7 DAF endosperm. Relative expression levels of starch synthesis genes *GBSSI*, *GBSSII*, *SSIVb*, *AGPL1*, *AGPL2*, *AGPS1*, and *AGPS2a* displayed no difference, whereas that of *SSI*, *SSIIc*, *SSIIIa*, *SBEI*, *SBEIIa*, *SBEIIb*, *AGPL3*, *AGPL4*, and *AGPS3b* were generally higher in NIL$^{qWCR7-\mathrm{DL208}}$ than in NIL$^{qWCR7-\mathrm{ZS97}}$. The levels of glutelin synthesis genes *GluA1*, *GluA2*, *GluA3*, *GluB1*, and *Glutelinλ* were markedly lower in NIL$^{qWCR7-\mathrm{DL208}}$ than in NIL$^{qWCR7-\mathrm{ZS97}}$ (Fig. 5d). The above results suggested that *qWCR7* had pleiotropic effects on storage components by affecting the expression levels of key genes related to starch and storage protein synthesis, which may influence the formation of white-core.

Discussion

Chalkiness is one of the key determinants of rice appearance quality. Chalky grains are also easier to crack during polishing due to the loose arrangement of starch granules of opaque endosperm, leading to a decreased head rice yield (Li et al. 2014). Moreover, the market value of rice largely depends on chalky grain rate and broken kernel rate, and chalkiness has a direct or indirect impact on both aspects (Fitzgerald et al. 2009). Therefore, cultivating varieties with low chalkiness not only helps to improve quality, but also contributes to marketable yield in rice.

Until now, a lot of QTL for rice quality have been mapped, but only a few QTL/genes related to chalkiness have been fine mapped or cloned. The main reason is that chalkiness phenotype is greatly influenced by numerous genetic factors and external stress (Nevame et al. 2018; Deng et al. 2021). To obtain genetically stable QTL of chalkiness, researchers often used different populations under multiple environmental conditions (Zhou et al. 2009a; Gao et al. 2016; Yun et al. 2016; Zhu et al. 2018). The percentage of chalky grains was significantly different in Hainan and Zhejiang province using three populations, and the phenotypic variance of *qPCG1* ranged from 6.8 to 21.9% (Zhu et al. 2018). Previously, *qWCR7* was detected in RILs (ZS97/DL208) and F_2 (ZS97/WG97) populations in multiple years, and the phenotypic variance ranged from 12.5 to 19.9% (Peng et al. 2014). To reduce the interference of genetic background, we constructed a NIL population of high generation in this study to fine map *qWCR7* in two environments (Figs. 1 and 2). Compared with the RILs and F_2 populations, the genetic effect of *qWCR7* was more stable and greater in the NIL population (Table 1). Taken together, it is necessary to verify the reliability of chalkiness QTL in a variety of environmental and genetic backgrounds, which would facilitate the following utilization in rice breeding. At the same time, high-quality NIL population also plays an important role in fine mapping of minor QTL.

With the global warming, high temperature has become an important factor to promote the production of chalkiness in rice (Zhao and Fitzgerald 2013; Nevame et al. 2018). Lanning et al. (2011) reported that there is a significant positive correlation between night temperature and chalkiness rate during grain filling. Lyman et al. (2013) showed that high temperature stress increased percentages of chalky and broken kernels. Nevame et al. (2018) concluded that high temperature affected chalkiness formation by grain filling rate, carbohydrate biosynthesis, protein formation/degradation, redox homeostasis, and cell rescue/defense pathways. In our study, we found that the WCR of NILs in Wuhan was significantly higher than that in Lingshui (Figs. 1 and 2). Then we compared the average value of the maximum temperature of the two locations in grain filling stage and found that the temperature in Wuhan (30 °C) was significantly higher than that in Lingshui (20 °C). Thus, we speculated that chalkiness variation of the two locations is probably related to environmental conditions especially the temperature during grain filling stage.

Numerous studies have reported that chalkiness formation is influenced by the synthesis and accumulation of storage substances in endosperm (Fujita et al. 2007; Ryoo et al. 2007; Wang et al. 2008; Li et al. 2014). *Flo5* encoded a soluble starch synthase SSIIIa involved in the biosynthesis of amylopectin, and its mutant displayed a white-core floury endosperm (Fujita et al. 2007; Ryoo et al. 2007).

Wang et al. (2008) identified a cell-wall invertase gene *GIF1* required for carbon partitioning, and its mutant *gif1* showed markedly less sugar content and more grain chalkiness. In this study, the GO function and KEGG pathway analysis showed that a large number of DEGs participated in "secondary metabolic process" and "metabolites and energy" biological processes through "metabolic pathways," "biosynthesis of secondary metabolites," and "starch and sucrose metabolism" pathways, suggesting that the synthesis and accumulation of metabolites such as starch and protein may play a key role in chalkiness formation (Figs. 3c and 4a). We further detected the contents of starch and storage protein in NILs, and higher WCR was accompanied by lower starch content and higher protein content (Fig. 5b and c). Expression analysis showed that the difference of total starch was not induced by amylose but may be caused by amylopectin which is synthesized by soluble starch synthase (SS), starch branching enzyme (SBE), and ADP-glucose pyrophosphorylase (AGP) (Fig. 5d). In addition, we found that glutelin was the key storage protein causing the difference of total protein in NILs (Fig. 5b and d). Therefore, we concluded that the changes of these storage substances were closely related to the formation of chalkiness.

In summary, our results revealed that *qWCR7* was a key genetic factor conferring WCR variation and was narrowed to a genomic interval of ~ 68 kb. Transcriptome analysis showed that the synthesis and accumulation of metabolites played a key role in chalkiness formation. The detection of storage components and expression levels of related genes exhibited that starch and storage protein were tightly related to white-core trait. Thus, our findings showed that *qWCR7* may have potential value in the improvement of rice grain quality.

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Author contribution Bian Wu conducted most of the experiments, including genetic effect validation, fine mapping, expression analysis, electron microscopy, transcriptome analysis, and detection of storage substances. Duo Xia, Hao Zhou, and Shiyuan Cheng contributed to some statistical analysis. Yipei Wang and Minqi Li contributed to detection of storage substances. Guanjun Gao, Qinglu Zhang, and Xianghua Li participated in field management and logistics. Yuqing He designed and supervised the study. Yuqing and Bian Wu analyzed the data and wrote the paper.

Declarations

Ethics approval The experiments comply with the ethical standards in the country in which they were performed.

Conflict of interest The authors declare no competing interests.

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