Genetic Analysis for Cooking and Eating Quality of Super Rice and Fine Mapping of a Novel Locus \textit{qGC10} for Gel Consistency

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Rice (\textit{Oryza sativa} L.) is an important cereal that provides food for more than half of the world’s population. Besides grain yield, improving grain quality is also essential to rice breeders. Amylose content (AC), gelatinization temperature (GT) and gel consistency (GC) are considered to be three indicators for cooking and eating quality in rice. Using a genetic map of RILs derived from the super rice Liang-You-Pei-Jiu with high-density SNPs, we detected 3 QTLs for AC, 3 QTLs for GT, and 8 QTLs for GC on chromosomes 3, 4, 5, 6, 10, and 12. \textit{Wx} locus, an important determinator for AC and GC, resided in one QTL cluster for AC and GC, \textit{qAC6} and \textit{qGC6} here. And a novel major QTL \textit{qGC10} on chromosome 10 was identified in both Lingshui and Hangzhou. With the \textit{BC4F2} population derived from a CSSL harboring the segment for \textit{qGC10} from 93-11 in PA64s background, it was fine mapped between two molecular markers within 181 kb region with 27 annotated genes. Quantitative real-time PCR results showed that eight genes were differentially expressed in endosperm of two parents. After DNA sequencing, only \textit{LOC_Os10g04900}, which encodes a F-box domain containing protein, has 2 bp deletion in the exon of PA64s, resulting in a premature stop codon. Therefore, \textit{LOC_Os10g04900} is considered to be the most likely candidate gene for \textit{qGC10} associated with gel consistency. Identification of \textit{qGC10} provides a new genetic resource for improvement of rice quality.

\textbf{Keywords:} cooking and eating quality, gel consistency, QTL analysis, fine mapping, rice

\section*{INTRODUCTION}

Rice is one of the most important crops served as staple food for more than half of world population. Rice planting area has reached 30.18 million hectares in 2018, and rice production was about 202.70 million tons, accounting for 25.80\% of total grain output. It is a milestone in the history of rice breeding that the application of hybrid rice in 1970s (Li et al., 2016). The development of hybrid rice in China has made great contribution to the world's rice yield. Recently, besides pursuing of
high yield, more attention has been paid to rice quality. Liang-You-Pei-Jiu, the super hybrid rice from the cross of the indica variety 93-11 and the light-thermo-sensitive genic male sterile line PA64s was a successful example with 10.5 tons per hectare in 2000 (Gao et al., 2013). However, compared to rice production, the development of quality breeding was relatively stunted (Su et al., 2011). Therefore, with better living conditions, in order to meet the demands of people, rice quality needs to be improved by breeders.

Rice quality includes various characteristics when it is being produced, processed, sold, cooked, and ate. In general, it involves processing quality (also called milling quality), appearance quality, cooking and eating quality, and nutritional quality. High-quality rice included 14 indicators, among which the milled rice rate, chalky degree, eating quality and amylose content were grading indexes while roughness, chalky rate, grain shape, gel consistency were indexes for reference. The amylose content (AC), gelatinization temperature (GT) and gel consistency (GC) are three major indicators for physical and chemical characteristics of the starch in rice endosperm which directly affected cooking and eating quality (Sun S.-Y. et al., 2005).

GC, being a kind of colloidal property of rice starch and referring to viscosity of 4.4% rice glue, is an important indicator of rice quality. It can be divided into three categories: soft (>60 mm), adhesive (40–60 mm) and hard GC (<40 mm). Rice with hard GC is rough and fluffy, and becomes hard and dry after cool; while rice with soft GC is soft and elastic, and still keeps soft after cooling. Usually, the larger GC, the softer, stickier, better taste and shinier rice (Wang et al., 2007).

Previous studies indicated natural genetic variations for rice quality (Tian et al., 2009). As a quantitative trait, GC is suitable for QTL analysis and its genetic basis is also complicated. So far, 22 QTLs for GC have been reported on chromosomes 1, 2, 3, 6, 7, and 10. He et al. (1999) detected two major QTLs for GC on chromosomes 2 and 7, which explain 20.2 and 14.2% of the genetic variation, respectively. Huang et al. (2000) used rice varieties with high GC and middle GC as two parents to construct the recombinant inbred lines (RILs) and detected two major QTLs for GC on chromosome 3. A major QTL for GC was identified by State Key Laboratory of Rice Biology, China National Rice Research Institute with double haploid (DH) population came from TN1 and CJ6, and further fine mapped and confirmed to be the Wx gene (Su et al., 2011). Because the Wx gene was reported to controlling AC, and GC was found negatively correlated with AC (Zhang et al., 2002; Sun Y. et al., 2005), therefore, both GC and AC were controlled by Wx. However, association analysis showed the Wx gene only account for nearly 40% of phenotypic variation for GC (Tian et al., 2009). And some varieties with same level of AC exhibited different GC (Sun Y. et al., 2005). A gene responsible for GT, ALK, was also reported as a modifier gene for GC in rice (Gao et al., 2011). Thus, other QTLs/genes are to be mapped and isolated to clarify the genetic basis for GC.

Although QTL mapping for rice quality initiated in the 1990s, few studies on fine mapping of QTLs for cooking and eating quality have been reported. Here, we detected QTLs for cooking and eating quality of super rice and fine mapped a novel QTL qGC10 for GC, which will help further cloning of the QTL/gene and improvement of rice quality.

MATERIALS AND METHODS

Cultivation and Management of Experimental Materials

A RIL from a cross between 93-11 and PA64s were used in this study for cooking quality traits analysis (Gao et al., 2013). Total of 1779 high-quality polymorphic SNP markers were used to construct the linkage map with 1568.21 cM (Supplementary Data S1). The parents 93-11 is an indica variety and the PA64s is the indica type light-thermo sensitive genic male sterile line. A total of 116 and 102 RILs were cultivated in Hangzhou (HZ) in 2011 and Lingshui (LS) in 2011, 2012, respectively. Each line was grown in triplicate in a randomized block design. The plot size was four rows of six plants with 20 × 20 cm spacing. Field management and chemical input for disease and pest control followed the standard procedures to avoid yield loss during the whole growth duration. Mature seeds of each line (3 biological replications) and two parents (6 biological replications) were harvested 30 days after heading and dried in an electro-thermal incubator (ZXDP-A2160, Shanghai) at 30°C for 72 h. The dried seeds (20 g) were shucked and polished.

Development of Fine Mapping Population

To develop a chromosome segment substitution line (CSSL) containing the qGC10 for GC detected both in Lingshui and Hangzhou on chromosome 10, a line of RILs with 93-11 genotype in the qGC10 region was selected to backcross with recurrent parent PA64s. Two markers IND-1 and IND-6 (Supplementary Table S1) were used for marker assisted selection (MAS) of each generation. As a result, a BC1F1 line was constructed exhibiting heterozygous across the entire qGC10 region with genetic background of PA64s. After self-crossing, a BC1F2 population was obtained for fine mapping of qGC10. A NIL carrying homozygous allele of 93-11 in the target QTL region between markers IND-4 and SNP-1 (Supplementary Table S1), designated NIL-qGC10, was also developed with PA64s background.

Measurement of Cooking and Eating Quality in Rice

Apparent Amylose content (AAC) was measured following the procedure of Perez and Juliano with some modifications (Perez and Juliano, 1978). Absorbance of the starch solution was determined at 620 nm using the spectrophotometer. Gelatinization temperature (GT) was indirectly estimated via the alkali digestion test (Little et al., 1958). Six whole-grain, milled rice samples were placed in triplicate square plastic boxes containing 10 mL 1.7% potassium hydroxide (KOH). The boxes were incubated for 23 h at 30°C. Grain appearance and disintegration were visually rated after incubation based on the standard numerical scale and expressed with alkali spreading

http://archive.gramene.org/db/qtl/
value (ASV). Gel Consistency (GC) was evaluated according to Cagampang et al. (1973). Rice starch becomes rice paste glue after dilute alkali and heat treatment, after cooling in the horizontal tube has a certain degree of extension (Tran et al., 2011). Technical measurements were performed for each sample in triplicate.

DNA Isolation and PCR Analysis
Genomic DNA of the parents and BC_{4}F_{2} individuals was extracted from fresh leaves using the CTAB method (Aline et al., 2009). DNA amplification was performed using a Gene Amp PCR system 9700 thermo cycler. PCR was performed in a 15 µL reaction mix including 25 ng genomic DNA, 2 µL of each primer, 1.0 µL 10 × PCR buffer, 0.1 mmol/L dNTP, 0.2 µL 5 U/µL Taq DNA polymerase (Tiangen Biotech, Beijing, China) and 1.5 µL ddH2O. Amplification conditions consisted of an initial denaturation at 94°C for 5 min, 40 cycles of 94°C for 30 s, 55–60°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min, and saving at 15°C forever.

Statistical Analyses and QTL Analysis
All statistical analyses were completed using the SAS (Statistical Analyses and QTL Analysis) v8.01. QTL analysis was performed with the MultiQTL package using the maximum likelihood interval mapping approach for the RIL-selfing population. For major-effect QTLs, the LOD threshold was obtained based on a permutation test (1000 permutations, P = 0.05) for each dataset. QTLs were named according to McCouch et al. (1997).

Design of Markers for Fine Mapping
Primers were designed in qGC10 region on the basis of insertions/deletions (InDels) and SNPs identified between 93-11 and PA64s (Supplementary Table S1) (Gao et al., 2013). Genotypes of SNP markers were screened by high-resolution dissociation curve analysis system (LightScanner 96, Idaho Technology Inc.).

Determination of RVA
Pasting properties of starch granules were analyzed by RVA model 3-D (Newport Scientific, Sydney, NSW, Australia), a programmed heating and cooling cycle was followed, as described by Toyoshima et al. (1997) and analyzed with Thermal Cycle for Windows (TCW). Duplicate measurements were performed for each sample of each line (3 biological repetitions). The main steps and the relevant parameters: Three gram flour of each

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**TABLE 1** | Phenotypic variation of cooking and eating quality traits in the parents.

| Harvested location | Traits     | Parents | RIL population |
|--------------------|------------|---------|----------------|
|                    |            | 93-11   | PA64s          |                |
| Lingshui (2011)    | AAC (%)    | 17.29 ± 1.01 | 23.09 ± 0.26** | 19.41 ± 3.76   | 13.53 | 28.82 |
|                    | GT (ASV)   | 6.8 ± 0.3  | 4.0 ± 0.0**    | 6.57 ± 0.69    | 4.3  | 7.0   |
|                    | GC (mm)    | 92.30 ± 2.37 | 37.50 ± 2.00** | 80.65 ± 14.02  | 28.00 | 100.00|
| (2012)             | GC (mm)    | 90.83 ± 2.44 | 36.50 ± 1.66** | 82.28 ± 13.32  | 28.00 | 100.00|
| Hangzhou (2011)    | AAC (%)    | 17.70 ± 1.12 | 20.30 ± 0.28*  | 18.27 ± 6.05   | 4.36 | 30.82 |
|                    | GT (ASV)   | 6.5 ± 0.0   | 5.0 ± 0.0**    | 5.68 ± 1.33    | 3.0  | 7.0   |
|                    | GC (mm)    | 85.30 ± 2.25 | 32.10 ± 1.01** | 65.96 ± 13.25  | 30.5 | 99    |

AAC, apparent amylose content; GT, gelatinization temperature; GC, gel consistency. Mean ± SD. * and ** indicate at 5 and 1% significant level compared to 93-11 according to t-test (n = 6), respectively.

**TABLE 2** | Correlation coefficients between AC, GT, and GC.

|          | AAC vs. GT | AAC vs. GC | GT vs. GC |
|----------|------------|------------|-----------|
| Lingshui (2011) | 0.101      | −0.035     | −0.264**  |
| Hangzhou (2011)  | 0.032      | −0.314**   | −0.213*   |

AAC, apparent amylose content; GT, gelatinization temperature; GC, gel consistency. * and ** indicate at 5 and 1% significant level, respectively.

**FIGURE 1** | Phenotype of amylose content (AAC, A), gelatinization temperature (ASV, B) and gel consistency (C) in parents 93-11 and PA64s. The error bar for each value represents mean ± SD (n = 6). * and ** indicate at 5 and 1% significant level compared to 93-11 according to t-test (n = 6), respectively.
sample at 14% moisture was weighed into aluminum canister, then 25 mL distilled water was added. A paddle was placed in the canister to mix the sample. The RVA disperses the samples by rotating the paddle at 960 rpm for the first 10 s of the test, after which the viscosity is sensed using a constant paddle rotation speed of 160 rpm. The idle temperature is set to 50°C and the following 12.5 min test profiles were run: (1) 50°C held for 1.0 min, (2) the temperature is linearly ramped up to 95°C until 4.8 min, (3) the temperature is held at 95°C until 7.5 min, (4) the temperature is linearly ramped down to 50°C at 11 min, (5) held at 50°C until 12.5 min (Yan et al., 2005). The determination was repeated two times per sample. RVA curve was described by six parameters: peak viscosity, holding strength, breakdown, final viscosity, consistence and setback. All the viscosity parameters were expressed in Rapid Viscosity Units (RVU).

| Trait       | Chromosome | LOD | Genetic position (cM) | SE  | PEV (%) | Marker          |
|-------------|------------|-----|-----------------------|-----|---------|-----------------|
| AC-LS-2011  | 3          | 3.6 | 76.21–103.25          | −2.00 | 2.4     | SNP3-191–SNP3-273 |
| AC-LS-2011  | 6          | 19.8| 0.00–11.53            | −4.77 | 35.7    | SNP6-1–SNP6-27   |
| AC-HZ-2011  | 6          | 33.3| 0.00–1.64             | −6.59 | 64.9    | SNP6-1–SNP6-11   |
| GC-LS-2011  | 4          | 2.6 | 69.52–172.06          | −7.27 | 6.4     | SNP4-130–SNP4-261|
| GC-LS-2011  | 5          | 3.0 | 127.31–143.37         | 8.03  | 7.9     | SNP5-142–SNP5-157|
| GC-LS-2011  | 6          | 3.4 | 0.00–7.93             | 9.68  | 11.4    | SNP6-1–SNP6-19   |
| GC-LS-2011  | 6          | 3.8 | 8.07–18.39            | 10.63 | 13.8    | SNP10-9–SNP10-21 |
| GC-LS-2011  | 6          | 3.3 | 0.00–11.53            | 9.26  | 12.3    | SNP6-1–SNP6-27   |
| GC-LS-2011  | 6          | 3.9 | 0.00–34.09            | 9.88  | 14.0    | SNP10-1–SNP10-36 |
| GC-HZ-2011  | 6          | 3.2 | 0.00–9.52             | 9.27  | 11.2    | SNP6-1–SNP6-23   |
| GC-HZ-2011  | 6          | 2.5 | 0.00–18.39            | 9.08  | 12.2    | SNP10-1–SNP10-21 |
| GT-LS-2011  | 6          | 7.6 | 25.49–71.14           | 1.27  | 23.4    | SNP6-50–SNP6-96  |
| GT-LS-2011  | 12         | 3.1 | 69.74–125.01          | 0.93  | 12.6    | SNP12-95–SNP12-143|
| GT-HZ-2011  | 6          | 10.6| 25.49–71.14           | 0.84  | 41.8    | SNP6-50–SNP6-96  |

FIGURE 2 | Location of QTLs for AC, GT, and GC on SNP map. Number indicates genetic distance (cM) along each chromosome.
RESULTS

Phenotypic Variation of Cooking and Eating Quality in the Parents and RILs

Significant differences existed in AAC, GT and GC between two parents in both Lingshui and Hangzhou (Table 1 and Figure 1). A total of 116 and 102 RILs, together with their parents from Hangzhou and Lingshui were investigated respectively for the three indexes for cooking and eating quality. For each trait, the distribution of the RIL population appeared continuously with transgressive characteristic in the two environments. Nearly normal continuous distributions of phenotypic values were observed from LS population for AAC and GC and HZ population for GC, which indicated that AAC and GC were controlled by poly-genes (Supplementary Figure S1). In HZ population, AAC and GT showed bimodal distribution of phenotypic values, which indicated that AAC and GT were controlled by a major gene and some minor genes (Supplementary Figure S1).

Correlation Analysis of Three Traits

Based on Pearson’s correlation analysis, trait correlations between cooking and eating quality parameters showed that GC exhibited significantly negative correlation to AAC and GT in HZ and GT in LS (Table 2). The correlation between AAC and GC was negative and highly significant in HZ while little in LS. Similarly, GC showed a significantly negative correlation to GT in both LS and HZ. There was no significant correlation between AAC and GT, whether in LS or HZ. These findings supported that GC is an important factor affecting cooking and eating quality.

Identification of QTLs for Cooking and Eating Quality Traits

A linkage map with high density SNP markers covering 12 chromosomes has been established to detect QTLs for rice cooking and eating quality traits, with a total distance of 1568.21 cM and the average distance of about 0.88 cM between the markers. A total of 14 QTLs, including 3 QTLs for AC, 3 QTLs for GT and 8 QTLs for GC were identified on chromosomes 3, 4, 5, 6, 10, and 12 (Table 3 and Figure 2). One QTL cluster

TABLE 3 | Phenotypic variation of cooking and eating quality in the parents and RILs.

| Variety/Line | Peak viscosity | Holding strength | Final viscosity | Breakdown | Consistency | Setback |
|--------------|----------------|-----------------|----------------|-----------|-------------|---------|
| 93-11        | 222.17 ± 1.65\(^a\) | 145.09 ± 1.65\(^b\) | 245.33 ± 0.35\(^b\) | 77.09 ± 3.30\(^a\) | 100.25 ± 1.29\(^c\) | 23.17 ± 2.00\(^c\) |
| PA64s        | 203.29 ± 1.12\(^c\) | 166.54 ± 1.82\(^a\) | 288.00 ± 0.59\(^a\) | 36.75 ± 0.71\(^b\) | 121.46 ± 1.23\(^b\) | 84.71 ± 0.53\(^a\) |
| CSSL-qGC10   | 208.25 ± 0.96\(^b\) | 126.92 ± 3.30\(^c\) | 269.79 ± 7.48\(^a\) | 81.33 ± 4.24\(^a\) | 142.88 ± 10.78\(^a\) | 61.55 ± 6.54\(^b\) |

Breakdown is equal to the difference between peak viscosity and holding strength, Consistency is equal to the difference between final viscosity and holding strength, Setback is equal to the difference between final viscosity and peak viscosity. Data are means (SD) and duplicate measurements were performed for each sample (n = 3). Different superscript letters represent significant differences (P < 0.05) by t-test.

Correlation Analysis of Three Traits

Based on Pearson’s correlation analysis, trait correlations between cooking and eating quality parameters showed that GC exhibited significantly negative correlation to AAC and GT in HZ and GT in LS (Table 2). The correlation between AAC and GC was negative and highly significant in HZ while little in LS. Similarly, GC showed a significantly negative correlation to GT in both LS and HZ. There was no significant correlation between AAC and GT, whether in LS or HZ. These findings supported that GC is an important factor affecting cooking and eating quality.

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for AC and GC was detected on chromosome 6. The qAC6 covering the Wx gene (Fan et al., 2005), detected in both LS and HZ between markers SNP6-1 and SNP6-27, SNP6-1 and SNP6-11 on chromosome 6, explained 35.7 and 64.9% of phenotypic variation, respectively. Meanwhile, the qGT6 in LS and HZ covering the ALK gene (Gao et al., 2011), were identically mapped between SNP6-50 and SNP6-96 on chromosome 6, explained 23.4 and 41.8% of total phenotypic variation in LS and HZ, respectively. The major QTL, qGC6, was preliminary mapped on chromosome 6 in the interval of SNP6-1 and SNP6-19 in LS in 2011, SNP6-1 and SNP6-27 in LS in 2012, SNP6-1 and SNP6-23 in HZ in 2011 accounting for 11.4, 12.3, and 11.2% of phenotypic variance, individually. The qGC10 was identified on chromosome 10 for three times with explained phenotypic variation of 11.4, 12.3, and 11.2% in LS in 2011, 2012 and HZ in 2011.

Meanwhile, PA64s had the smallest breakdown value and the largest setback value, which is in agreement with the previous reports (Zheng et al., 2018).

**Fine Mapping of qGC10**

For fine mapping of qGC10, a CSSL CSSL-qGC10 with PA64s background was constructed to develop BC4F2 population by backcrossing to PA64s and self-pollination. Based on the resequencing data of 93-11 and PA64s, several InDel markers were designed to screen the population (Supplementary Table S1). According to the GC value, lines with high GC (>60 mm) and low GC (<40 mm) were selected for fine mapping. Combining the phenotype and genotype analysis, qGC10 was finally delimited in 181 kb region between markers IND-4 and SNP-1 on chromosome 10 (Figure 4A).

**Candidate Genes Determination at the qGC10 Locus**

According to Rice Genome Annotation Project Database3, there were 27 annotated genes in 181 kb target region for qGC10 on chromosome 10 (Table 5 and Figure 4B). Among them, 8 genes differentially expressed in endosperm between 93-11 and PA64s were sequenced and 2 bp deletion (G84G85) was detected in the exon of LOC_Os10g04900 in PA64s (Figures 4C, 5 and Supplementary Figure S2), resulting in a premature stop codon and a protein with only 28 amino acids. And LOC_Os10g04900 is highly expressed in the panicle (Supplementary Figure S3). Therefore, LOC_Os10g04900 was considered the most likely candidate gene for qGC10 locus associated with GC.

3http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/
High density genetic linkage map combined with different populations facilitate QTL identification and fine mapping. With different genetic populations, such as F2 population, double haploid (DH) population, RILs, CSSLs and near-isogenic lines (NILs), hundreds of genomic regions associated with cooking and eating quality traits have been identified in rice (Su et al., 2011). Among them, CSSLs and NILs are effective materials for QTL mapping and positional cloning owing to all lines have the same background without complex variations in genetic background (Zhou et al., 2009; Anis et al., 2019).

AC, GT, and GC are considered to be three vital traits that determine cooking and eating quality of rice (Tan et al., 1999). As quantitative traits, they are determined mainly by genetic factors and affected by environmental conditions (Fan et al., 2005). Series of QTLs have been reported for AC, GT and GC (Sun S.-Y. et al., 2005; Su et al., 2011; Ponce et al., 2018; Yang et al., 2018). However, only few QTLs have been fine mapped or cloned. In the study, we used a RIL population derived from a cross between PA64s and 93-11 to identify QTLs for AC, GT under two environments, and GC under three environments. Some QTLs were environmentally dependent, such as qAC3, qGT12, qGC4 and qGC5 detected in LS in 2011, whose phenotypic variances explained (PEV) were relatively lower. However, stable QTLs detected in different environments, for example, a novel major QTL qGC10 detected in both LS (2011 and 2012) and HZ (2011) were suitable for fine mapping further.

In Hangzhou, GC showed negative but significant correlation with AC (Table 2), which indicated that AC affect GC in cooked rice. Both in Lingshui and Hangzhou, GC was also negatively correlated with GT. These results suggested glossy sticky rice texture tend to have high AC and undergo fast breakdown of starch molecules (Su et al., 2011; Pang et al., 2016), supporting the fact that indeed GC was the important factor affecting cooking quality traits (Bao et al., 2006; Gao et al., 2011).

Notably, chromosome 6 contained most of QTLs identified here responsible for AC, GT and GC, where located one QTL cluster for AC and GC. We also detected a major QTL for AC, qAC6, located on the same locus of previously reported gene on chromosome 6 and several other modifiers (Lanceras et al., 2000; Septiningsih et al., 2003; Aluko et al., 2004; Takeuchi et al., 2007). GT was controlled by a major QTL on chromosome 6 covering the ALK gene (He et al., 1999; Lanceras et al., 2000; Umemoto et al., 2002; Gao et al., 2003), where the qGT6 mapped here. The qGT12 for GT identified on chromosome 12 in our study was also reported previously (Liu et al., 2014). Therefore, our results were consistent with previous studies that GT was controlled by ALK and other minor QTLs.

### DISCUSSION

TABLE 5 | Candidate genes at the qGC10 locus.

| Gene ID | Annotation |
|---------|------------|
| LOC_Os10g04890 | Expressed protein |
| LOC_Os10g04900 | OsFBX364 - F-box domain containing protein, expressed |
| LOC_Os10g04910 | Expressed protein |
| LOC_Os10g04920 | Expressed protein |
| LOC_Os10g04930 | Hypothetical protein |
| LOC_Os10g04940 | Hypothetical protein |
| LOC_Os10g04950 | Expressed protein |
| LOC_Os10g04960 | OsFBX365 - F-box domain containing protein, expressed |
| LOC_Os10g04970 | Expressed protein |
| LOC_Os10g05000 | OsFBX366 - F-box domain containing protein, expressed |
| LOC_Os10g05010 | Expressed protein |
| LOC_Os10g05020 | Cytochrome P450, putative, expressed |
| LOC_Os10g05030 | Expressed protein |
| LOC_Os10g05040 | Transposon protein, putative, CACTA, En/Spm sub-class, expressed |
| LOC_Os10g05050 | Expressed protein |
| LOC_Os10g05069 | Lyosomal alpha-mannosidase precursor, putative, expressed |
| LOC_Os10g05088 | GDSL-like lipase/acylhydrolase, putative, expressed |
| LOC_Os10g05110 | Retrotransposon protein, putative, unclassified |
| LOC_Os10g05120 | Retrotransposon protein, putative, unclassified, expressed |
| LOC_Os10g05130 | Expressed protein |
| LOC_Os10g05140 | Hypothetical protein |
| LOC_Os10g05150 | KAZ2 - Kazal-type serine protease inhibitor precursor, putative, expressed |
| LOC_Os10g05160 | Expressed protein |
| LOC_Os10g05170 | OsWAK100 - OsWAK receptor-like cytoplasmic kinase |
| LOC_Os10g05180 | 26S proteasome regulatory subunit S5A, putative, expressed |
| LOC_Os10g05190 | Transposon protein, putative, CACTA, En/Spm sub-class, expressed |
| LOC_Os10g05200 | OsFBX367 - F-box domain containing protein, expressed |

FIGURE 5 | Relative expression of 8 genes in the endosperm of 93-11 and PA64s. The error bar for each value represents mean ± SD (n = 3). ** indicate at 1% significant level compared to PA64s according to t test.
Several major QTLs for GC have been reported, such as \( qGC6 \), whose mapped interval including the \( Wx \) gene. Previous studies showed that \( Wx \) also regulated GC in rice, and in turn affected grain quality (Su et al., 2011). In our experiments, one QTL for GC, \( qGC6 \), detected on chromosome 6, was co-located with \( qAC6 \). This QTL cluster covered the \( Wx \) gene, which play an important role in controlling AC. \( Wx \) is not only the key gene for AC, but also a modifier gene for GC in rice (Tian et al., 2009). Besides, a novel major QTL, \( qGC10 \) for GC was mapped on chromosome 10. We further fine mapped the \( qGC10 \) within 2.37–2.55 Mb region with a BC\(_4\)F\(_2\) population. In the target region, 8 genes differentially expressed in endosperm were sequenced and only \( LOC\_Os10g04900 \), encoding a F-box domain containing protein, was found to have 2 bp insertion/deletion in the exon between two parents, 93-11 and PA64s (Figures 4C and Supplementary Figure S2). Quantitative real-time PCR results showed that \( LOC\_Os10g04900 \) is highly expressed in the panicle (Supplementary Figure S3). Previous study have found that \( LOC\_Os10g04900 \) was expressed in the milk stage grains by analyzing expression pattern in different developmental stages in rice (Yang et al., 2012). In other crops, its homologous proteins were also found expressed in seeds of Barley (\( Hordeum vulgare \), BAK07103) and Sorghum (\( Sorghum bicolor \), XP_002461331)\(^4\). Hence, \( LOC\_Os10g04900 \) is considered the most likely candidate gene for the \( qGC10 \) locus associated with gel consistency and to be confirmed further by complementation test.

CONCLUSION

Cooking and eating quality is an economically vital trait for rice. Understanding of genetic mechanism underlying cooking and eating quality will be beneficial for breeding of new rice varieties with high yield and good quality. Total of 3 QTLs for AC, 3 QTLs for GT and 8 QTLs for GC were detected on chromosomes 3, 4, 5, 6, 10, and 12 using high-resolution genetic map of RILs derived from super rice Liang-You-Pei-Jiu. A novel major QTL for GC, \( qGC10 \) was identified on chromosome 10 in rice. With the BC\(_4\)F\(_2\) population derived from the CSSL-6, 10, and 12 using high-resolution genetic map of RILs derived from super rice Liang-You-Pei-Jiu. A novel major QTL for GC, \( qGC10 \) was identified on chromosome 10 in rice. With the BC\(_4\)F\(_2\) population derived from the CSSL-4, 5 and ZG analyzed the data. AZ and ZG wrote the manuscript. All authors read and approved the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00342/full#supplementary-material

REFERENCES

Aline, B., Silva, R. M., Henrique, R. G., De, Q. S. J. R., Andrade, B. E. D., and Ann, V. E. (2009). Ctab methods for dna extraction of sweetpotato for microsatellite analysis. Sci. Agric. 66, 529–534. doi: 10.1590/S0103-90162009000400015

Aluko, G., Martinez, C., Tohme, J., Catano, C., Bergman, C., and Oard, J. H. (2004). QTL mapping of grain quality traits from the interspecific cross Oryza sativa \( \times \) O. glaberrima. Theor. Appl. Genet. 109, 630–639. doi: 10.1007/s00122-004-1668-y

Anis, G. B., Zhang, Y., Islam, A., Zhang, Y., Cao, Y., Wu, W., et al. (2019). \( RDWN6XB \), a major quantitative trait locus positively enhances root system architecture under nitrogen deficiency in rice. BMC Plant Biol. 19:12. doi: 10.1186/s12870-018-1620-y

Bao, J., Corke, H., and Sun, M. (2006). Nucleotide diversity in starch synthase IIa and validation of single nucleotide polymorphisms in relation to starch gelatinization temperature and other physicochemical properties in rice. Theor. Appl. Genet. 113, 1171–1183. doi: 10.1007/s00122-006-0355-6
Cagampang, G. B., Perez, C. M., and Juliano, B. O. (1973). A gel consistency test for the eating quality of rice. *Sci. Food Agric.* 24, 1589–1594. doi: 10.1002/jsfa.2740242114

Fan, C. C., Yu, X. Q., Xing, Y. Z., Xu, C. G., Luo, L. J., and Zhang, Q. F. (2005). The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled-haploid line population. *Theor. Appl. Genet.* 110, 1445–1452. doi: 10.1007/s00122-005-1975-y

Gao, Z., Zeng, D., Cheng, F., Tian, Z., Guo, L., Su, Y., et al. (2011). ALK, the key gene for gelatinization temperature, is a modifier gene for gel consistency in rice. *Integr. Plant Biol.* 53, 756–765. doi: 10.1111/j.1744-7909.2011.01065.x

Gao, Z., Zeng, D., Cui, X., Zhou, Y., Yan, M., Huang, D., et al. (2003). Map-based cloning of the ALK gene, which controls the gelatinization temperature of rice. *Sci. China C Life Sci.* 46, 661–668. doi: 10.1360/03cy00099

Gao, Z., Zhao, S., He, W., Guo, L., Peng, Y., Wang, J., et al. (2013). Dissecting yield-associated loci in super hybrid rice by sequencing recombinant inbred lines and improving parental genome sequences. *Proc. Natl. Acad. Sci. U.S.A.* 110, 14492–14497. doi: 10.1073/pnas.1306579110

He, P., Li, S., Qian, Q., Ma, Y., Li, J., Wang, W., et al. (1999). Genetic analysis of rice grain quality. *Theor. Appl. Genet.* 98, 502–508. doi: 10.1007/s001220051098

Huang, Z., Tan, X., Xu, C., and Vanavichit, A. (2000). Molecular mapping QTLs for gel consistency in rice (*Oryza sativa* L.). *Sci. Agric.* Sin. 31, 1–5.

Jia, L., Ding, X., Wang, P., and Deng, X. (2008). Rice RVA profile characteristics and correlation with the physical/chemical quality. *Acta Agron. Sin.* 34, 790–794. doi: 10.3724/sp.j.1006.2008.00790

Lanceras, J. C., Huang, Z., Navikul, O., Vanavichit, A., Ruanjaichon, V., and Tragoonrung, S. (2000). Mapping of genes for cooking and eating qualities in Thai Jasmine rice (KDM1015). *DNA Res.* 7, 93–101. doi: 10.1093/dnares/7.2.93

Li, D., Huang, Z., Song, S., Xin, Y., Mao, D., Lv, Q., et al. (2016). Integrated analysis of phenome, genome, and transcriptome of hybrid rice uncovered multiple heterosis-related loci for yield increase. *Proc. Natl. Acad. Sci. U.S.A.* 113, E6026–E6035. doi: 10.1073/pnas.1610115113

Little, R. R., Hilder, G. B., and Dawson, E. H. (1958). Differential effect of dilute alkali on 25 varieties of milled white rice. *Cereal Chem.* 35, 111–126.

Liu, X., Zhu, K., Zhang, C., Hong, R., Sun, P., Tang, S., et al. (2014). Mapping of minor QTLs for rice gelatinization temperature using chromosome segment substitution lines from *Indica* 9311 in the *Japonica* background. *Acta Agron. Sin.* 40, 1740–1747.

McCouch, S. R., Chen, X., Panaud, O., Tennykh, S., Xu, Y., Cho, Y. G., et al. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Mol. Biol.* 35, 89–99. doi: 10.1023/A:100571431474

Pang, Y., Ali, J., Wang, X., Franje, N. J., Revilleza, J. E., Xu, J., et al. (2016). Relationship of rice grain amylose, gelatinization temperature and pasta properties for breeding better eating and cooking quality of rice varieties. *PLoS One* 11:e0168483. doi: 10.1371/journal.pone.0168483

Perez, C. M., and Juliano, B. O. (1978). Modification of the simplified amylose test for milled starch. *Starch* 30, 424–426. doi: 10.1002/star.19780301206

Ponce, K. S., Ye, G., and Zhao, X. (2018). QTL identification for cooking and eating quality in *indica* rice using multi-parent advanced generation intercross (magic) population. *Front. Plant Sci.* 9:868. doi: 10.3389/fpls.2018.00868

Sabouri, A., Rabiei, B., Toorchi, M., Aharizad, S., and Ali, M. (2012). Mapping quantitative trait loci (QTL) associated with cooking quality in rice (*Oryza sativa* L.). *Aust. J. Crop Sci.* 6, 808–814.

Septimingham, E. M., Trijatmiko, K. R., Moeljopawiro, S., and McCouch, S. R. (2003). Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* Variety IR64 and the wild relative *O. rufipogon*. *Theor. Appl. Genet.* 107, 1419–1432. doi: 10.1007/s00122-003-1376-z

Su, Y., Yao, R., Hu, S., Yang, Y., Gao, Z., Zhang, G., et al. (2011). Map-based cloning proves qGC-6, a major QTL for gel consistency of *japonica*/*indica* cross, responds by Waxy in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 123, 859–867. doi: 10.1007/s00122-011-1632-6

Sun, S.-Y., Hao, W., and Lin, H.-X. (2005). Identification of QTLs for cooking and eating quality of rice grain. *Rice Sci.* 13, 161–169.

Sun, Y., Yan, L., Dong, C., Wang, P., Huang, X., and Deng, X. (2005). Genetic relationship among Wx gene, AC, GC and GT of rice. *Acta Agron. Sin.* 31, 535–539.

Takeuchi, Y., Nonoue, Y., Ebihara, T., Suzuki, K., Aoki, N., Sato, H., et al. (2007). QTL detection for eating quality including glossiness, stickiness, taste and hardness of cooked rice. *Breed. Sci.* 57, 231–242. doi: 10.1270/bibs.57.231

Tan, Y., Ji, Y., Xu, S., Xing, Y., Xu, C., and Zhang, Q. (1999). The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. *Theor. Appl. Genet.* 99, 642–648. doi: 10.1007/s001220051279

Tian, Z., Qian, L., Liu, Q., Yan, M., Liu, X., Yan, C., et al. (2009). Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21760–21765. doi: 10.1073/pnas.0912961106

Toyoshima, H., Okadome, H., Ohhtsubo, K., Suto, M., Horisue, N., Inatsu, O., et al. (1997). Cooperative test on the small-scale rapid method for the gelatinization properties test of rice flours with a Rapid-Visco-Analyser (RVA). *Nippon Shokuhin Kagakukaishi* 44, 579–584. doi: 10.3136/nskk.44.579

Tragoonrung, S. (2000). Mapping of genes for cooking and eating qualities in *japonica*-type and *indica*-type rice varieties. *Theor. Appl. Genet.* 104, 1–8. doi: 10.1007/s001220200000

Umemoto, T., Yano, M., Sato, H., Shomura, A., and Nakamura, Y. (2002). Mapping of a gene responsible for the difference in amylopectin structure between *japonica*-type and *indica*-type rice varieties. *Theor. Appl. Genet.* 110, 519–525. doi: 10.1007/s00122-001-1604-x

Yang, G., Yan, K., Wu, B., Wang, G., Gao, Y., and Zheng, C. (2012). Genomewide analysis of intronic microRNAs in rice and *Arabidopsis*. *J. Genet.* 91, 313–324. doi: 10.1016/j.jger.2011.12.004

Zhang, X., Wang, Y., Shi, C., Bao, G., and Ye, S. (2002). Progress of study on grain quality in *japonica* rice using multi-parent advanced generation intercross (magic) population. *Front. Plant Sci.* 9:688. doi: 10.3389/fpls.2018.00868

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