

Pasting properties, grain-filling characteristics and allelic variation linked to the grain quality in diverse rice

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Abstract Physical appearance and eating and cooking qualities (ECQ) encompassed in rice quality traits have pivotal roles in the new varieties approved, dictating market value. In this regard, it was increasingly important to evaluate various physiological mechanisms, physicochemical properties and the genetic basis involved in controlling grain quality. The investigation in this study showed a large variation in the phenotypic diversity of grain appearance and ECQ among 48 rice accessions. A significant correlation was observed between RVA-pasting

properties and multiple grain-quality properties, and attributes of *Setback* (*SB*) and consistency (*Cons*) service as the primary keys in starch pasting. Grain-filling characteristics were surveyed and the principal differences of the trends of rice grain filling were found. The filling rate and duration exhibited significant influence on grain quality. Significant marker-trait association for grain shape, chalkiness and ECQ were explored integrally, proving optional valuable loci can be used for alleles pyramiding. Three SNP markers, GBSSI-G/T, GBSSI-4-IF and SSIIa-GC/TT, can straightforwardly be used for polygenic pyramiding by carrying alleles of T/T, C/C and TT/TT, satisfying for genotype selection for ECQ properties of low-AC, high-GC, and low-GT.

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Abbreviations

AC	Amylose content
AS	Area size of grain
ASV	Alkali spreading value
CS	Circularity
DEC	Degree of endosperm chalkiness
GC	Gel consistency
GL	Grain length
GT	Gelatinization temperature
GW	Grain width
KGW	1000-grain weight

LWR	Grain length to width ratio
MAS	Marker assisted selection
PL	Perimeter length of grain
PGWC	Percentage of grain with chalkiness

Introduction

It is still an important target to develop new rice varieties with various grain qualities to meet diverse purposes worldwide. There are various aspects of rice grain quality, including grain appearance (grain shape and endosperm chalkiness), milling quality, nutritional quality, and eating and cooking qualities (ECQ) (Hsu et al. 2014). Moreover, the international rice market is highly segmented because consumers have very specific preferences (Cuevas et al. 2016), which vary depending on the geographical location (Calin-gacion et al. 2014). Furthermore, different products require rice with specific starch properties. A wide range of variation in rice starch characteristics is therefore required to meet the varied needs of processors and consumers (Asante et al. 2013).

Appearance is one of the crucial properties of rice grain affecting the market acceptability significantly (Tanabata et al. 2012). Grain shape and chalkiness, mostly conditioned by quantitative multiple loci, have attracted significant attention in rice genetic research. Until now, more than 100 QTLs have been identified for grain-size variation in rice (Anand et al. 2015) and many QTLs for chalkiness or related components were also identified (Zhao et al. 2015). Eating and cooking quality refers to the sensory perception of consumers of the cooked rice and is associated with such characteristics as glossiness, flavor, and stickiness (Hsu et al. 2014). Amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) were accepted as the three most prominent physical and chemical characteristics of starch for ECQs (Bian et al. 2014), which are mainly controlled by the starch properties of the rice. While the cooking characteristics reflect the chemical reaction that occurs during cooking of the rice grain, including hydration, gelatinization, length of cooking time, kernel elongation, and volume expansion (Hsu et al. 2014). However, ECQ properties are complicated, and many diverse aspects must be considered to evaluate grain quality (Hsu et al. 2014). The granules swell and burst, the

semi-crystalline structure is lost, and the smaller amylose molecules start leaching out of the granule, forming a network that holds water and increases the mixture's viscosity (Khan et al. 2013). Accordingly, the physicochemical properties of rice starch have been used as an indirect index of ECQ, and lots of studies have applied starch viscosity and thermodynamic properties as additional properties to evaluate grain quality (Hsu et al. 2014). The starch pasting viscosity of rice flour, a pasting curve, is generated in a standard temperature program of "heat–hold–cool–hold", to reveal the pasting properties of rice starch in the cooked grain (Wang et al. 2013).

Grain filling is actually a process of starch accumulation. It has been reported that there are 33 major enzymes involved in the metabolism of carbohydrates in developing rice endosperm (Yang and Zhang 2010). The *GIF1* (*GRAIN INCOMPLETE FILLING 1*) was cloned as a cell-wall invertase gene affecting the rate of grain filling and the mutant form of *GIF1* showed slower grain filling than the *WT*, accompanied by markedly more grain chalkiness as a result of abnormally developed and loosely packed starch granules (Wang et al. 2008). Many genes related to starch synthesis and carbohydrate metabolism were significantly up- or down regulated in *gif1* grains. These studies suggested that grain-filling was involved in the biosynthesis of starch, which was the key determinant of grain quality. Further studies are needed to investigate the effects of grain-filling properties on rice grain quality forming regularity.

Intensive genetic studies have pinpointed the genes that control grain the appearance and ECQ traits. Advances in genetic studies have developed molecular techniques, thereby allowing marker-assisted breeding for improved these grain qualities in rice (Phing Lau et al. 2016). Based on genetic understanding and availability of markers for such traits, MAS can facilitate the development of new varieties with high-grain quality. Despite recent advances were conducive to our understanding of grain quality regulation in rice, the effects of alleles of finely mapped QTLs or cloned genes and the influence of the combinations of alleles from multiple genes remain largely unknown (Lee et al. 2015). It is necessary to test their effects in different genetic backgrounds (Zhao et al. 2015).

In this study, the grain quality traits in the diverse rice germplasm were comprehensively investigated. Their relationship with RVA-pasting properties was

assessed. Differential grain-filling patterns among diverse genotypes were surveyed and their influence on grain quality were explored. We conducted the allelic association analysis of multiple QTL/genes for grain quality traits widely. Furthermore, the effects of allele combinations of the significant markers were examined, and the potential regression models with genotype selection for rice ECQ improvement were developed.

Materials and methods

Plant material

A total of 48 rice accessions (PSB Rice Germplasm Collection) from various regions in China (45) and three other countries (3) were used in this study (Table 1). Specifically, the 45 accessions from 14 provinces of China represent the genetic ecological diversity of rice in China. There are three subspecies in this rice panel, namely *indica*, *japonica* and *javanica* (Table 1). All accessions were used for DNA extraction, genotyping and association analysis, while the subgroup of 18 rice accessions (code #PSB01–PSB18) was also used to test starch-pasting viscosity and survey grain-filling characteristics. The experiment was conducted at the experimental field of South China Agriculture University, Guangdong Province, China, during the rice-growing season (August–December) of 2015.

Phenotypic evaluation for grain-quality traits

Images of the more than 100 mature grains were captured on a CanoScan 5600F (Canon, Japan) scanner with the supplied software without image enhancement, and the grain-shape parameters of GW, GL, LWR, CS, PL, and AS were measured precisely using SmartGrain Software (Tanabata et al. 2012). The chalkiness parameters were measured with an automatic machine (JMW12) for three replicates, according to Xu et al. (2012). Two metrics were used to describe grain chalkiness, as previously described (Zhao et al. 2016). These were the percentage of grains with chalk (PGWC) and the degree of endosperm chalkiness (DEC), which is the ratio of total chalky area to the total kernel area of all sampled grains.

Dried rice grains were stored at room temperature for one month prior to the evaluation of grain ECQ traits, as described by Su et al. (2011). Briefly, samples were boiled for 10 min in the volumetric flasks to completely disperse the grain powder, and the optical density of the amylose-iodine blue was measured at 620 nm using a spectrophotometer (Leng et al. 2014). GC was measured using 100 mg of milled rice flour. The flour was first wetted with 0.2 ml of 95% ethanol containing 0.025% (w/v) thymol blue in 11 × 100 mm culture tubes, followed by adding 2 ml of 0.2 N KOH, and mixed vigorously. Tubes were covered with glass marbles, heated in a boiling water bath for 8 min, mixed again and kept in an ice water bath for 20 min. Finally, the tubes were laid horizontally against a ruled graphing paper and the gel length was measured after 1 h (Su et al. 2011). The ASV was determined by incubating six milled grains in 10 ml of 1.7% KOH at 28 °C for 23 h. The degree of spreading was rated using the following 7-point semi-quantitative criteria: (1) grain not affected; (2) grain swollen; (3) grain swollen, collar incomplete and narrow; (4) grain swollen, collar complete and wide; (5) grain split, collar complete and wide; (6) grain dispersed, merging with collar; and (7) grain completely dispersed and intermingled (Su et al. 2011). It classified as low GT for ASV 5.5–7.0, intermediate GT for ASV 3.5–5.4 and high GT for ASV ≤ 3.4 (Tuaño et al. 2016). For all the phenotypic parameters, more than two biological replicates for each genotype were assayed.

Assessment of RVA-pasting viscosity parameters

Rice pasting properties were determined using a Rapid Visco Analyser (RVA-TecMaster; Newport Scientific, Sydney, Australia), according to Standard Method AAC61-02 released by the American Association of Cereal Chemists. Flour (3 g, 12% m.b.) was mixed with 25 g of double-deionized water in the RVA sample can (Kong et al. 2014). The Rice Method 1 program was used with heating and cooling cycle set as (1) holding at 50 °C for 1 min, (2) heated to 95 °C in 3.8 min, (3) holding at 95 °C for 2.5 min, (4) cooling to 50 °C in 3.8 min, (5) holding at 50 °C in 1.4 min. The RVA paddle speed was at 960 rpm for the first 10 s of the test, after which the speed was at 160 rpm. The peak (PV), hot paste (HPV), cool paste (CPV) viscosities and their derivative parameters breakdown (BD = PV–HPV), setback (SB = CPV–

Table 1 Origin and group of rice germplasm used in this study

ID	Accessions	Source	Sub-species	Q ₁	Q ₂
PSB 01	Yuzhenxiang	Hunan, China	Indica	0.582	0.418
PSB 02	02428	Jiangsu, China	Japonica	0.446	0.554
PSB 03	Huahang 31	Guangdong, China	Indica	0.600	0.400
PSB 04	Wushansimiao	Guangdong, China	Indica	0.598	0.402
PSB 05	Shennong 9816	Liaoning, China	Japonica	0.400	0.600
PSB 06	Basmati 370	Pakistan	Indica	0.599	0.401
PSB 07	Kasalath	India	Indica	0.549	0.451
PSB 08	Huakuanzhan	Guangdong, China	Indica	0.600	0.400
PSB 09	Xiangyaxiangzhan	Guangdong, China	Indica	0.597	0.403
PSB 10	Francis	United States	Javanica	0.572	0.428
PSB 11	Yunjing 22	Yunnan, China	Japonica	0.400	0.600
PSB 12	TaifengB	Guangdong, China	Indica	0.581	0.419
PSB 13	Jingxiangnuo	Guangdong, China	Indica	0.597	0.403
PSB 14	Wanlinuo	Guangdong, China	Indica	0.584	0.416
PSB 15	Hongwanxian 924	Jiangxi, China	Indica	0.595	0.405
PSB 16	Guiyu 9	Guangxi, China	Indica	0.600	0.400
PSB 17	Longjing 21	Heilongjiang, China	Japonica	0.400	0.600
PSB 18	Meixiangzhan 2	Guangdong, China	Indica	0.600	0.400
PSB 19	Xiangwanxian 13	Hunan, China	Indica	0.598	0.402
PSB 20	Liufeng 003	Guangxi, China	Indica	0.600	0.400
PSB 21	Luhui 8258	Sichuan, China	Indica	0.600	0.400
PSB 22	Jiuqixiang	Guangdong, China	Indica	0.600	0.400
PSB 23	Shengbashimiao	Guangdong, China	Indica	0.585	0.415
PSB 24	Nongguizhan	Guangdong, China	Indica	0.600	0.400
PSB 25	Zaojingzhan	Guangdong, China	Indica	0.600	0.400
PSB 26	Hanghui 1173	Guangdong, China	Indica	0.600	0.400
PSB 27	Qiu B	Guangxi, China	Indica	0.587	0.413
PSB 28	Huazhan	Zhejiang, China	Indica	0.600	0.400
PSB 29	Chenghui 727	Sichuan, China	Indica	0.600	0.400
PSB 30	9311	Jiangsu, China	Indica	0.591	0.409
PSB 31	Yunnanhonggu	Yunnan, China	Indica	0.600	0.400
PSB 32	Dalihongnuo	Yunnan, China	Indica	0.400	0.600
PSB 33	Shennong 016	Liaoning, China	Japonica	0.400	0.600
PSB 34	Wuyunjing 23	Jiangsu, China	Japonica	0.400	0.600
PSB 35	Nanjing 46	Jiangsu, China	Japonica	0.400	0.600
PSB 36	Yongyou 8	Zhejiang, China	Japonica	0.400	0.600
PSB 37	W 030	Jiangsu, China	Japonica	0.400	0.600
PSB 38	Xiangyinong 1	Guangdong, China	Indica	0.599	0.401
PSB 39	Chan Chengzhan	Guangdong, China	Indica	0.600	0.400
PSB 40	Yuebiao 5	Guangdong, China	Indica	0.600	0.400
PSB 41	Guangyuanzhan 15	Guangdong, China	Indica	0.600	0.400
PSB 42	Hengfeng B	Guangdong, China	Indica	0.591	0.409
PSB 43	Kaipingxiaoyouli	Guangdong, China	Indica	0.579	0.421
PSB 44	Ruanzhan	Guangdong, China	Indica	0.600	0.400
PSB 45	Pengdao 2	Guangdong, China	Indica	0.600	0.400

Table 1 continued

ID	Accessions	Source	Sub-species	Q ₁	Q ₂
PSB 46	Huangguangruanzhan	Guangdong, China	Indica	0.600	0.400
PSB 47	Guihefeng	Guangxi, China	Indica	0.600	0.400
PSB 48	Sanglandao	Hainan,China	Indica	0.600	0.400

PV), and consistency (Cons = CPV–HPV) were recorded with the ThermoLine for Windows software (Version 1.2). The pasting temperature (PT) was calculated according to Bao's method (Bao 2008). The viscosity was measured in rapid visco units (RVU) (Wang et al. 2013).

Sampling and determination of grain-filling pattern

In brief, 100 grains from the middle of five panicles open-pollinated on the same day were chosen and obtained. And each variety was sampled at 5, 9, 15, 20, 25, 30, and 35 days after fertilization (DAF), as described by Wang et al. (2012). Subsequently, wet weight, dry weight, and moisture content were investigated. Grain-filling processes of were fitted by Richards' growth equation, as described by Zhu et al. (1988) with CurveExpert1.4 program (Daniel Hyams, Washington, USA): $W = A/(1 + Be^{-kt})^{1/N}$, where W is the grain weight, A is the final grain weight, t is the time after anthesis, and B , k , and N are coefficients determined by regression. The grain-filling rate (R) was calculated as the derivative of Richards' growth equation: $R = AkBe^{-kt}/N(1 + Be^{-kt})^{(N+1)/N}$ (Richards 1959). Other parameter values were estimated subsequently, including day of reaching maximum grain-filling rate (D_{max}), grain weight of reaching maximum grain-filling rate (W_{max}), maximum grain-filling rate (R_{max}), average grain-filling rate (R_{mean}), initial grain filling rate (R_0) and I (percentage of W_{max} to A). The active grain-filling period (D) was defined as the period during which W constituted to 90% of A . D_3 was defined as the day which W constituted 99% of A , representing the total-grain filling duration. The duration of gradual increasing stage (T_c), rapid increasing stage (T_m), and slow increasing stage (T_l), and their correspondent grain-filling rate (R_c , R_m , R_l), were defined as described by Feng et al. (2011).

Molecular marker genotyping

The rice genomic DNA was extracted using the CTAB method and quantified using the NanoDrop ND-1000 Spectrophotometer. Markers for 20 starch synthesis genes, 41 fine mapped grain shape and related traits QTLs/genes, and 54 chalkiness QTLs/genes described by Zhao et al. (2015) were used for genotyping all the rice accessions. Three types of PCR-based markers, i.e., simple sequence repeats (SSRs), sequence tagged sites (STSs) and single nucleotide polymorphism (SNP) were used. PCR amplification was performed in a 15 μ l reaction mixture containing 7.5 μ l 2 \times AceTaq Master Mix, 5 pM of each primer and 1 μ l gDNA (~50 ng). The PCR conditions were: initial denaturation at 95 °C for 5 min, 33 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C or 57 °C for 30 s, and extension at 72 °C for 45 s. As a final step, the reaction mixture was incubated at 72 °C for 5 min. The PCR products were separated using the Fragment Analyzer CE system (Advanced Analytical Technologies, Inc.), according to the manufacturer's instructions. Alleles were visualized and discriminated using PROSize 2.0 software (Advanced Analytical Technologies, Inc.).

Statistical analyses and marker-trait association

PowerMarker 3.25 (Liu and Muse 2005) was used to estimate the number of alleles per locus, the diversity index, and the polymorphism information content. The genetic similarity coefficient between each variety was calculated using NTSYS-pc 2.10. The similarity matrix was employed to construct a dendrogram using the un-weighted pair-group method arithmetic average (UPGMA) algorithm and nested clustering (SHAN) routine to determine the genetic relationships among the materials. Population structure was estimated with the program Structure V2.3.2, as described by Zheng et al. (2015). The hypotheses for 2–10

subpopulations (K) with an admixture model and correlated allelic frequencies were tested, the length of burn-in period equal to 10,000 iterations and a run of 100,000 replications of Markov Chain Monte Carlo after burn in. Ten runs of the Structure program were performed and an average likelihood value, $\text{LnP}(D)$, across all runs was calculated for each K . The most likely number of clusters (K) was selected by comparing the logarithmized probabilities of data $\text{LnP}(D)$ and ΔK , according to Evanno et al. (2005). The general linear model method implemented by TASSEL V3.0 was used for association analysis between markers and rice-quality traits. The percentages of admixture (Q matrix) were used as covariates. The P value, determining whether a marker is associated with a trait was set at 0.01. To discover the effects of allelic combinations, the allelic variations of the associated markers were converted to dummy variables, and employed into stepwise regression using SAS statistical software. The mean and standard deviation of the phenotype were calculated subsequently. In this study, typical carrier is the representative material harboring each allele of the significant association loci, and possessing the desirable value for the corresponding trait among all materials containing (Guo et al. 2016).

Results

Phenotypic diversity of grain quality and RVA-pasting properties

Twelve properties related to rice grain quality, including nine grain appearance traits and three key ECQ parameters were evaluated in the 48 rice germplasm (Table S1). As expected, the diverse rice panel revealed a wide range variation for all the evaluated traits (Table S1; Fig. 1). The longest grain length was recorded as 12.46 mm for Yuzhenxiang, while 02428 had the shortest grain length of 7.10. The widest grain was recorded as 3.70 for 02428, while Kaipingxiaoyouli had the slenderest grain of 1.62. The other four parameters (LWR, CS, PL, and AS) were examined for grain shape too. Rice varieties can be broadly classified into three broad categories, as follows: slender (LWR > 4.26, CS < 0.46), medium (LWR 3.12–4.21, CS 0.47–0.55) and bold (LWR < 2.37, CS > 0.67). The KGW traits appeared to be normally

distributed, but the distributions of PGWC and DEC were seriously skewed (Fig. 1). For ECQ traits, AC, GC and ASV averaged 16.86%, 24.50 mm and 5.46 respectively. The coefficient of variation ranged from 5.89 to 27.47%. Clearly, four categories based on AC can be broadly classified: waxy-type (AC < 5%), low-type (AC 10–14%), medium-type (AC 14–21%), and high-type (AC > 21%). ASV of starch endosperm gives information about gelatinization temperature, and three GT grades can be classified based on the ASV (Table S1). An analysis of the correlation between overall grain-quality traits, given in Table S2, indicates a strong correlation among grain appearance quality, while the association of ECQ was relatively weak with other traits, except for a very significant correlation between AC and GC ($r = -0.6455$) (Table S2).

A broad diversity of pasting viscosity properties was observed among the selected rice accessions, with an average of 348.2 RVU of PV (peak viscosity), 171.7 RVU of HPV (hot paste viscosity), 309.6 RVU of CPV (cool paste viscosity), 176.5 RVU of BD (breakdown), -38.6 RVU of SB (setback), 137.9 RVU of Cons (consistency), 68.3 of PT (pasting temperature) and 5.4 min of peak-time (Table S3). Among all RVA profile characteristics, SB was found to have maximum dispersion. In order to explore the relationship between the grain-quality traits and pasting properties, the correlation analysis was conducted and is shown in Table 2. Several crucial significant correlations between AC and pasting properties were detected, including PV, CPV, BD, SB and Cons, while the GC was related to SB and Cons. ASV was closely related to PT, as expected. The result indicates clearly that the RVA profile was largely in response to the ECQ properties.

Effect of grain-filling pattern on grain quality

Grain-filling characteristics across 18 diverse genotypes were surveyed and their pattern was fitted with the Richards model. The main dynamic parameters of the grain-filling process are shown in Table 3. Marked differences of the trends of rice grain filling were found among the 18 different genotypes. For instance, the R_0 (initial grain-filling rate) ranged from 0.099 (Xiangyaxiangzhan) to 0.555 (Hongwanxian 924), the D (active grain-filling period) ranged from 19.322 days (Guiyu 9) to 32.503 days (02428), and the R_{\max} ranged from 0.106 (Kasalath) to 0.213 (Shennong

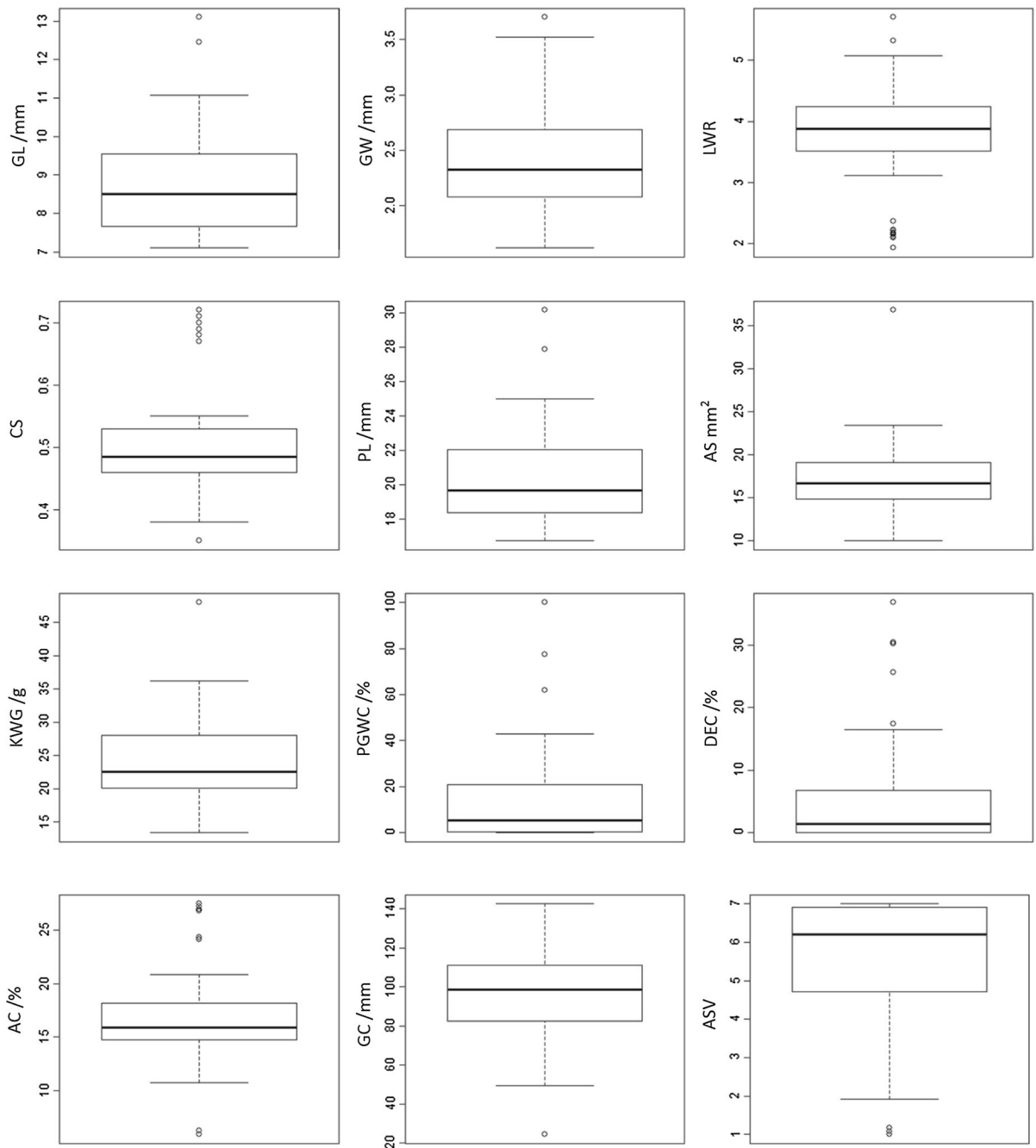


Fig. 1 Box plots of grain quality traits among 48 rice accessions. Box edges represent the upper and lower quartile with median value shown as bold line in the middle of the box.

Whiskers represent 1.5 times the quartile of the data. Individuals falling outside the range of the whiskers shown as open dots

9816). Based on the values of R_{mean} (mean grain-filling rate), all rice varieties can be broadly divided into three categories, including high-type ($R_{\text{mean}} > 0.12$), medium ($R_{\text{mean}} 0.09\text{--}0.12$) and low-type ($R_{\text{mean}} < 0.09$),

and their representative fitting curves are displayed in Fig. 2. The filling process was divided into three different stages, and wide individual differences were found among most of the genotypes (Fig. S1).

Table 2 Correlation among major grain ECQ traits and RVA features

Traits	Peak viscosity	Hot paste viscosity	Cool paste viscosity	Breakdown	Setback	Consistency	Pasting temperature	Peak time
AC	-0.622*	0.168	0.538*	-0.651**	0.794**	0.689**	-0.079	-0.187
GC	0.392	-0.02	-0.408	0.354	-0.547*	-0.665**	-0.088	0.003
ASV	0.164	0.328	0.294	-0.074	0.085	0.065	-0.910**	0.104

*, ** indicate significant differences in Student's *t* test at $P < 0.05$ and $P < 0.01$, respectively

Furthermore, correlation analysis indicated that grain shape (GW, LWR and CS) was mainly affected by D_3 (total grain-filling duration), T_m (duration of rapid increasing stage) and T_l (duration of slow increasing stage), while the grain weight was associated primarily with the population grain-filling rate parameters (such as R_0 , R_{max} and R_{mean}) (see Table S4). PGWC and DEC were found to be most relevant to the D (active grain-filling duration) and D_{max} (day of reaching maximum grain-filling rate), respectively, and the day of inflexion point of the grain-filling curve (D_l and D_2) was also shown to be significantly associated with DEC, indicating that the point and duration of staging exerted a significant influence on the endosperm chalkiness of rice grain. For ECQ, AC was associated with multiple grain-filling rate parameters. Only D_3 (total grain-filling duration) was detected as being related to traits of GC. Parameter of ASV were related to several factors, although the coefficient of correlation was minor. In addition, the effect of the filling pattern was detected to be significant on three milling quality traits, which play key roles in rice processing. Specifically, R_{mean} , R_{max} , R_e and R_m were all exhibited to be enormously positive correlated (r 0.699–0.828) with brown rice rate (BRR).

Genetic structure and marker-trait association analysis

A total of 154 markers were polymorphic and could be distinguished among the 48 accessions. And the genotype results were supplied in Table S1. The structure analysis with these markers showed that the log-likelihood increased with the elevation of model parameters K , and the ΔK value was much higher for the model parameter $K = 2$ than for the other values of K . Population structure data based on the Q matrix for each accession are summarized in Table 1, and the

accessions could be divided into two subpopulations: POP1 and POP2 (Fig. S2). The UPGMA tree made with these 154 markers showed that all the rice accessions could be assigned into two large groups (at the position of coefficient of genetic similarity of 0.62), representing *indica* and *japonica* subspecies, respectively (Fig. 3), while the variety of Kasalath was classified by itself into a subgroup of *indica* group, at the coefficient of genetic similarity of 0.71.

Marker-trait association mapping of overall grain-quality parameters were performed based on the Q+K model. Markers significantly associated with grain shape and grain weight are shown in Table S5, and the numbers of loci associated with GL, GW, LWR, CS, PL, AS and KGW were 7, 5, 6, 9, 7, 8, and 9, respectively. Among these markers, RM22020 on Chr07 identified up to six parameters and five markers were significantly associated with five parameters: MID06029, RM14820, RM3466, SSIIc-3-F and SSIIIb-F (Table S5). This indicates that these markers had relatively high-reliable effects on grain shape and weight. The marker had the strongest association with GL, GW, LWR, CS, PL, AS and KGW was SSIIIb-F, InDel1, InDel1, RM3466, SSIIIb-F, SSIIc-3-F and SSIIIb-F, respectively. Furthermore, SSIIIb-F was found to have higher contribution of the phenotypic variance (0.377 for AS, 0.357 for GL, 0.357 for PL and 0.233 for KGW) among these significant loci (Table S5).

Markers significantly associated with grain chalkiness properties are shown in Table S6, and the numbers of loci associated with PGWC and DEC were 16 and 17, respectively. Unexpectedly, all of the same sixteen markers were identified to be associated with both PGWC and DEC simultaneously, accounting for 9.12 (RM17947) –24.07% (RM27073) and 7.49 (RM17947) –24.17% (RM5586) of the phenotypic variance, respectively. RM5586 was tightly linked to the *GIF1* (*GRAIN INCOMPLETE FILLING 1*) gene

Table 3 Grain-filling parameters derived based on Richards equation for different rice genotypes

Accessions	A (g 100 ⁻¹ grain ⁻¹)	R ₀	R _{max} (g 100 ⁻¹ grain ⁻¹ days ⁻¹)	R _{mean} (g 100 ⁻¹ grain ⁻¹ days ⁻¹)	W _{max} (g)	I (%)	D _{max} (days)	D (days)	R ²
Yuzhenxiang	2.722	0.122	0.150	0.096	1.678	0.616	13.631	28.408	0.9998
02428	2.939	0.145	0.137	0.090	1.589	0.541	12.783	32.503	0.9983
Huahan 31	2.288	0.160	0.143	0.093	1.331	0.582	12.234	24.627	0.9990
Wushansimiao	2.345	0.177	0.154	0.100	1.334	0.569	12.449	23.414	0.9978
Shennong 9816	3.306	0.479	0.213	0.144	1.445	0.437	9.789	22.991	0.9996
Basmati 370	2.928	0.176	0.184	0.120	1.640	0.560	11.868	24.382	0.9992
Kasalath	1.998	0.397	0.106	0.072	0.873	0.437	9.906	27.831	0.9951
Huakuanzhan	3.596	0.417	0.182	0.123	1.548	0.430	9.630	29.151	0.9987
Xiangyaxiangzhan	2.042	0.099	0.123	0.074	1.459	0.715	16.664	27.415	0.9977
Francis	2.450	0.108	0.128	0.081	1.560	0.637	14.927	30.174	0.9969
Yunjing 22	3.155	0.466	0.169	0.115	1.347	0.427	10.019	27.541	0.9972
TaifengB	2.771	0.185	0.141	0.094	1.424	0.514	9.786	29.573	0.9993
Jingxianguo	3.086	0.185	0.190	0.125	1.686	0.546	10.759	24.758	0.9998
Wanlimuo	2.303	0.215	0.161	0.106	1.250	0.543	11.340	21.696	0.9929
Hongwanxian 924	2.100	0.555	0.148	0.100	0.910	0.433	6.311	20.970	0.9967
Guiyu 9	2.480	0.449	0.191	0.128	1.133	0.457	9.170	19.322	0.9999
Longjing 21	2.944	0.434	0.177	0.119	1.293	0.439	9.569	24.669	0.9955
Meixiangzhan 2	2.067	0.244	0.139	0.092	1.063	0.514	11.017	22.431	0.9997

A the final value of growth, R₀ initial grain filling rate, R_{max} maximum grain-filling rate, D_{max} day of reaching maximum grain filling rate, W_{max} grain weight of reaching maximum grain filling rate, I percentage of W_{max} to A, R_{mean} mean grain filling rate, D active grain filling duration (90%), R² the determination coefficient of the equation

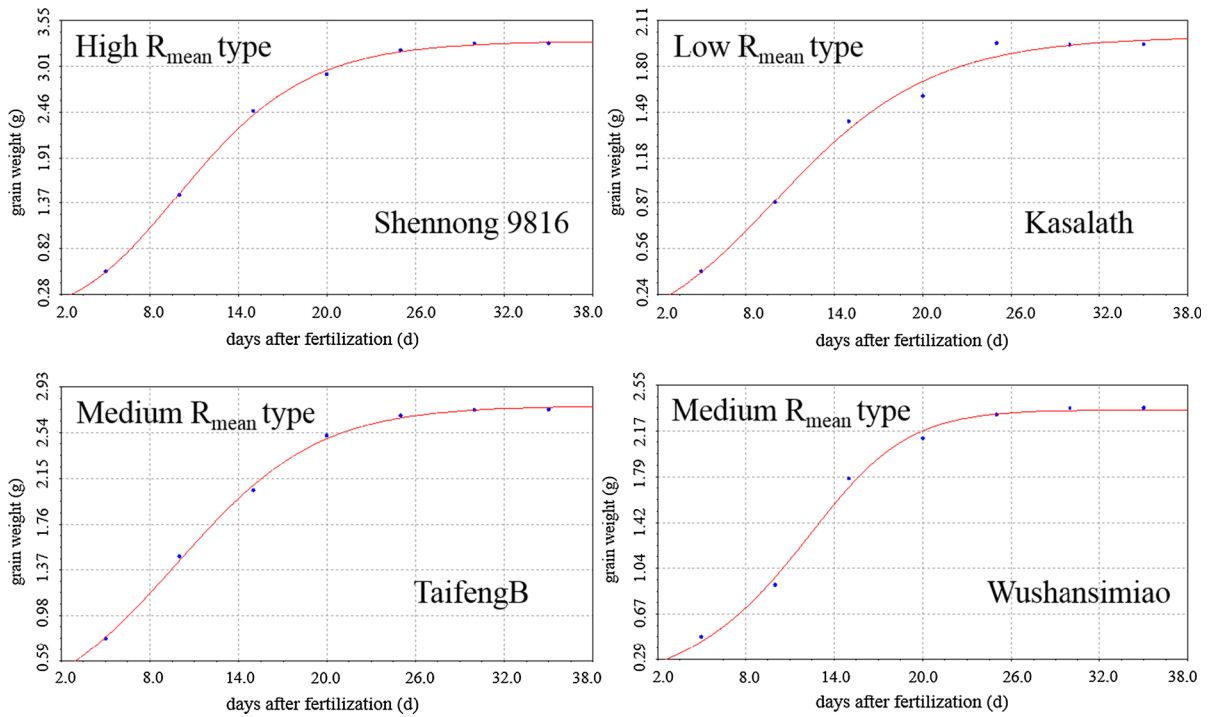


Fig. 2 The representative grain filling processes fitted with the Richards model. Three types of rice grain-filling pattern based on R_{mean} (mean grain-filling rate) were indicated at the upper-

left corner of each curves. While their representative rice varieties were indicated at the lower-right-corner

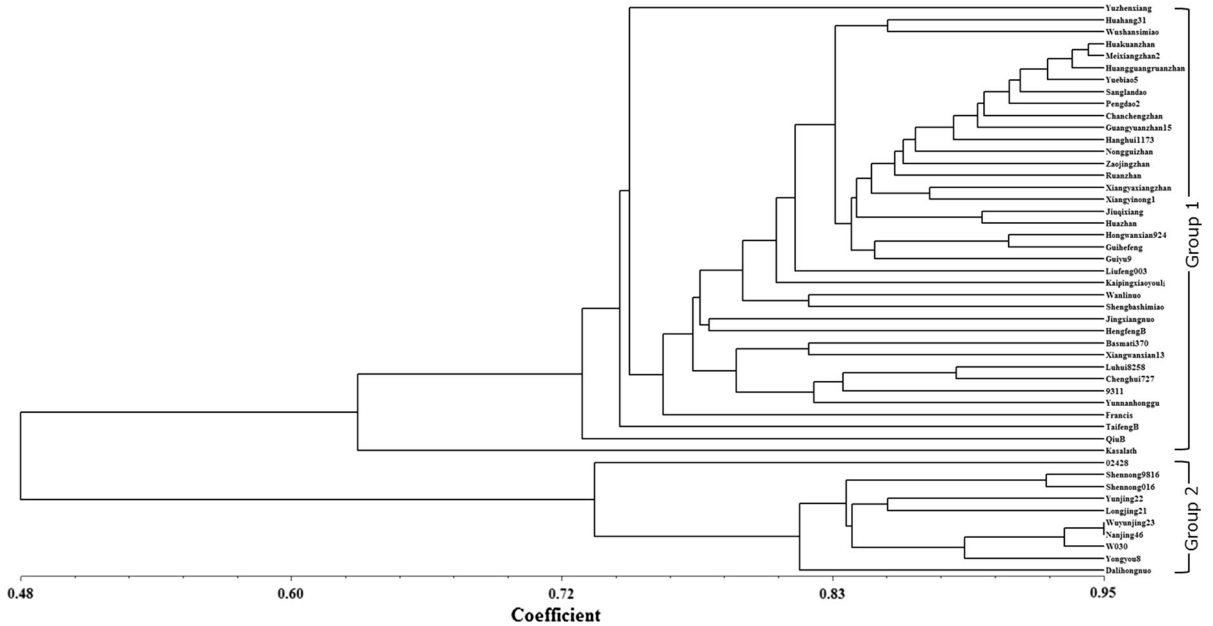


Fig. 3 Dendrogram of 48 rice accessions constructed on the basis of similarity coefficients in UPGMA analysis

and RM27073 was around the QTL of *PGWC-11a*. Additionally, markers of RM6745 linked to QTL of *qCH10(t)*, RM10398 linked to QTL of *qGW-1*, nkssr04-19 linked to QTL of *qGN-1*, and RM3872 linked to QTL of *qPGWC-3* were detected to be significant, accounting for more than 15% of the phenotypic variance for PGWC, while RM5586 and RM27073 were also determined to account than 15% of the phenotypic variance for DEC (Table S6).

Markers significantly associated with ECQ properties were shown in Table 4, and the numbers of loci associated with AC, GC and ASV were 14, 7 and 4, respectively. The marker of GBSSI-G/T was found to have strongest association with AC and GC, accounting for 61.14% (P value 3.90×10^{-11}) and 41.9% (P value 2.80×10^{-7}) of the phenotypic variance, respectively. While the markers of SSIIa-GC/TT and SSIIa-F on *SSIIa* gene had the strongest association with ASV and accounted for 75.6 and 74.5% of the phenotypic variance with P value of 9.66×10^{-16} and 2.92×10^{-15} .

Analysis of allelic effects for ECQ and development of regression equations

The ECQ has got heightened attention in practices of rice breeding all over the world. The elite alleles and genotype carriers were extremely important in improving target traits with the expected phenotypic effects. Given that, the allele effects of significant loci associated with eating cooking quality and their typical carrier materials are summarized and shown in Table 5. To provide the underlying quantitative genetics basis to support marker-assisted precise design for improved ECQ of rice, the effects of allelic combinations and equation models for ECQ based on the stepwise regression was analyzed (see Table S7). The rice germplasm collection were therefore grouped into various classes according to specific allelic combinations. For example, 37 germplasm had an ASV ranging from 4.67 to 7.00, with a mean value of 6.43, in the presence of allelic combination of SSIIa-

Table 4 The marker loci associated with eating and cooking traits

Traits	Marker loci	Chr	Position (Mb)	F value	P value	R ²
Amylose content	GBSSI-G/T	6	17.7	74.928	3.90E-11	0.614
	GBSSI-4-IF	6	17.7	20.914	4.13E-07	0.479
	BeIIb-1-F	2	19.36	23.242	1.67E-05	0.335
	BeIIb-3-F	2	19.36	23.242	1.67E-05	0.335
	MS40671	5	5.34	7.353	4.39E-04	0.333
	RM16	3	23.08	9.963	2.70E-04	0.307
	BeIIb-2-F	2	19.36	9.930	2.76E-04	0.306
	RM481	7	2.87	4.542	3.87E-03	0.297
	GBSSI-F	6	17.7	17.308	1.41E-04	0.273
	GBSSI-3-IF	6	17.7	7.954	1.13E-03	0.261
	BEIIb-IF	2	19.36	15.411	2.94E-04	0.251
	SSI-2-F	6	3.08	9.603	3.34E-03	0.173
	RM15206	3	16.77	9.506	3.49E-03	0.172
	RM15937	8	30.94	7.680	8.09E-03	0.143
Gel consistency	GBSSI-G/T	6	17.7	36.383	2.80E-07	0.419
	GBSSI-4-IF	6	17.7	13.339	2.96E-05	0.354
	SSI-1-F	6	3.08	4.595	7.08E-03	0.227
	SSI-2-F	6	3.08	12.511	9.51E-04	0.204
	RMG2762.HAU2762	2	22.46	5.356	8.28E-03	0.183
	RM15206	3	16.77	10.552	2.20E-03	0.178
	SSIIIb-IF	4	31.54	7.979	7.03E-03	0.141
ASV	SSIIa-GC/TT	6	6.75	146.341	9.66E-16	0.756
	SSIIa-F	6	6.75	137.215	2.92E-15	0.745
	RI0399	5	3.29	6.339	3.81E-03	0.221
	RM10316	1	5.29	5.235	9.13E-03	0.190

Table 5 Allele effects of principal significant loci associated with ECQ and their typical genotype carriers

Traits	Significant associated locus			Allele	A_i	Typical carrier materials
	Gene	Locus	D_e			
AC (%)	<i>GBSSI (Wx)</i>	GBSSI-G/T (0.61)	10.64	T	-1.33	W 030, Ruanzhan
				G	9.31	Kasalath, TaifengB
		GBSSI-4-IF (0.48)	10.16	C	-1.06	Wushansimiao, Pengdao 2
				T	9.10	Nongguizhan
	<i>BEI1b</i>	BeI1b-1-F (0.33)	2.25	131-bp	-0.56	Wushansimiao, Basmati 370
				121-bp	1.69	Kasalath, 9311
		BeI1b-3-F (0.33)	2.25	451-bp	-0.56	Huahang 31, Nanjing 46
				391-bp	-0.69	TaifengB, 9311
GC (mm)	<i>GBSSI (Wx)</i>	GBSSI-G/T (0.42)	50.88	T	-0.36	Huakuanzhan, Shennong 016
				G	-44.52	Kasalath, Nongguizhan
		GBSSI-4-IF (0.35)	50.67	C	5.12	Kaipingxiaoyouli
				T	-45.55	TaifengB
	<i>SSI</i>	SSI-1-F (0.23)	72.73	198-bp	2.38	Shennong 016, Nanjing 46
				220-bp	-70.35	Kasalath
		SSI-2-F (0.20)	57.23	71-bp	2.38	Nanjing 46, Yongyou 8
				65-bp	-54.85	Qiu B, Kasalath
GT	<i>SSIIa (alk)</i>	SSIIa-GC/TT (0.76)	4.06	TT	0.93	Nongguizhan, Ruanzhan
				GC	-3.13	Chenghui 727, Jiuqixiang
		SSIIa-F (0.75)	4.35	114-bp	0.73	Hongwanxian 924, Meixiangzhan 2
				106-bp	-3.62	Hengfeng B, Huazhan
	<i>qPGWC-5</i>	RI0399 (0.22)	1.55	355-bp	0.43	Guihefeng, Wushansimiao
				386-bp	-1.12	Huazhan, Yunnanhonggu
<i>Gn1a</i>	RM10316 (0.19)	2.83	147-bp	-2.44	Hengfeng B; Yunnanhonggu	
			145-bp	0.39	TaifengB, Meixiangzhan 2	
			142-bp	-0.83	Chenghui 727	

The number in parentheses in the column of "Locus" indicates the explained percentage of total phenotypic variation by the locus. D_e represents the difference phenotypic extreme value between alleles. A_i represents the allelic phenotypic effect

GC/TT (TT) and SSIIa-F (II). This indicated that the similarity in rice GT (represented by ASV) could be attributed to certain allele combination, even in the presence of different genetic backgrounds. Regression models were also provided for prediction of rice AC, GC and ASV (Table S7), which can be used for molecular breeding of rice with desired grain quality.

Discussion

The acceptance of new rice genotypes by producers and consumers hinges not only on their potential for higher yields. Recent emphasis has also been on premium-value genotypes that have the ability to

satisfy consumer preferences for grain quality (Anacleto et al. 2015). The holistic understanding of the physicochemical properties and genetic variability of grain quality and attributes thus was necessary to enable breeding programs of rice quality to efficiently tap the huge genetic diversity. The diverse rice panel, collected from different ecological regions, revealed significant variability in both grain appearance and quality traits, as evident from various statistical parameters. Furthermore, the relationship between the grain-quality traits and pasting properties was explored, and attributes of *setback* (SB) and consistency (Cons) were revealed to serve as the primary keys in starch pasting, making a major impact on the performance of AC and GC.

To our knowledge, previous studies about the effects of grain-filling pattern among different genotypes on rice grain quality are relatively few, and their interrelation is little understood. The present data, for the first time, shows a close association between differentiation of grain quality and the grain-filling process. The grain filling duration was detected to be very significantly and negatively correlated with grain-shape parameters. D_3 (total grain filling duration), T_m (duration of rapid increasing stage), and T_l (duration of slow increasing stage) were found to be associated with more than three parameters (GW, LWR and CS). Seed development depends on the accumulation of storage starch and storage protein during grain filling (Wang et al. 2015), and our result suggested the filling duration of round and wide rice grain usually lasts even longer, which may contribute to increased endosperm wide. The grain weight was associated primarily with population grain-filling rate parameters (such as R_0 , R_{max} and R_{mean}), which was consistent with previous research (Wang et al. 2008). Fujita et al. (1984) also found that both the grain-filling rate and the duration of the period of grain filling differed among rice varieties and were positively and significantly correlated with the grain size. This suggested the grain-filling rate was low in small-grain varieties, and generally increased with increased grain size. Our data revealed that the point and duration of staging exerted significant influence on the endosperm chalkiness of rice grain, and PGWC and DEC were found to be most relevant to the D (active grain-filling duration) and D_{max} (day of reaching maximum grain-filling rate), respectively. However, few significant correlations were detected to be involved in eating and cooking properties. Further investigation is needed to understand their relationship during grain development in rice, combined with characteristics of the biosynthesis of enzymes involving sucrose-to-starch conversion and endogenous plant-growth regulators (Wang et al. 2012).

Properties of AC, GC, and GT of rice grains have been extensively studied. Overall, 20 starch synthesis genes, 41 fine mapped grain shape and related traits QTLs/genes, and 54 chalkiness QTLs/genes were summed up by Zhao et al. (2015). The consensus among many studies was that different alleles of the QTLs or genes have different impacts on the grain-quality traits under their control (Anacleto et al. 2015; Huang et al. 2013; Kharabian-Masouleh et al. 2012;

Lee et al. 2009, 2015; Tian et al. 2009). However, the effects of overall molecular markers of these QTL/genes and the combined influence of multiple alleles on grain quality remain to be investigated. *Indica* population of breeding lines has been used for attempting to identify marker-trait associations and elucidate this issue (Zhao et al. 2015). The genetic background of this population is composed of advanced breeding lines, however, is relatively narrow, and may not be widespread enough for mining of unique variation. In our candidate-gene association mapping study, intra-subspecies and intra-varietal heterogeneity of a number of rice accessions have been tested and exploited. A more significant effect, thus, was identified for most identical characteristics. For instance, InDel1 and RM21945 around the qGL7-2 were detected to contribute allelic effects of 15.4 and 17.7% on LWR in this study, while only 3.34–8.11 and 5.25–6.05% in the previous study. We also arrived at an effect of GBSSI-4-IF up to 47.9 and 35.4% on AC and GC, respectively, and similar results were reported by Tian et al. (2009) and Biselli et al. (2014), while the R^2 were only 18.09–25.52 and 3.53–10.94% in the previous study (Zhao et al. 2015). The discrepancies in different reports were due to the limitation of the heterogeneity, which depends on the genetic ecological diversity and genetic backgrounds (Kadirvel et al. 2015; Yang et al. 2014).

The results of our study suggested that the wide variation on ECQ properties was caused by the genetic variation and haplotype of alleles involved in starch synthesis. RM22020 on Chr07 identified up to six parameters and five markers were significantly associated with five parameters, including MID06029, RM14820, RM3466, SSIIC-3-F and SSIIIB-F, indicating that these markers had relatively high-reliable effects on grain shape and weight. The marker had the strongest association with GL, GW, LWR, CS, PL, AS and KGW was SSIIIB-F, InDel1, InDel1, RM3466, SSIIIB-F, SSIIC-3-F and SSIIIB-F, respectively. The numbers of loci associated with PGWC and DEC were 16 and 17 respectively, clearly indicating that the chalkiness properties depend on a high complexity genetic pattern. The formation of rice chalkiness proves to be a complicated physiological process and tightly relates to “source-sink” of rice, dynamics of grain filing, biosynthesis and accumulation of starch in endosperm (Zhou et al. 2009). As a complicated quantitative trait, the manipulative network and

formative mechanisms of rice chalkiness remain unclear. The associated markers revealed in this study may promote progressive improvement strategy of decreased rice chalkiness in breeding practice. According to Lestari et al. (2015), the rapid evaluation of eating quality at early breeding generations in breeding programs for better eating quality is of great importance. The desirable attributes of ECQ can be combined with appropriate AC, GC and GT to develop new rice varieties with suitable ECQ properties, according to the regional preferences. Our study demonstrated that the marker GBSSI-G/T accounted for 61.14 and 41.9% of the phenotypic variance of AC and GC, respectively, and SSIIa-GC/TT and SSIIa-F on SSIIa gene accounted for 75.6 and 74.5% of the phenotypic variance for GT. Similar results was obtained by previous research (Bao et al. 2006; Kharabian-Masouleh et al. 2012; Mo et al. 2014; Tran et al. 2013; Yang et al. 2014), and confirmed this marker set may be effectively utilized as a simplified tool to predict ECQ properties and MAS strategies. These results provided optional valuable loci that can be used for alleles pyramiding, together with available donors. For instance, three markers, including GBSSI-G/T, GBSSI-4-IF and SSIIa-GC/TT, can straightforwardly be used for polygenic pyramiding by carrying alleles of T/T, C/C and TT/TT, with the aim of satisfying genotype selection for ECQ with properties of low-AC, high-GC, and low-GT. In this case, Yunjing 22, Zaojingzhan and Guihefeng can be used as donors affording desirable alleles. A model regression equation was formulated to estimate palatability of *indica* rice in Indonesia (Lestari et al. 2015). However, due to limitations of population (24 *indica* rice varieties) and genetic similarity, the result seems to be insufficient for broader regional practical application. In this study, a highly significant correlation was observed between ECQ properties diagnosed by the regression equation (Table S7) and evaluation of chemical method, demonstrating that this model can be employed as a good candidate diagnostic tool to generally predict the ECQ of rice varieties.

In addition, our results declared that allelic combination related to differential performance can be identified through association analysis and the effect can be attributed to alleles present in a given panel of individuals, in accordance with the previous studies (Lee et al. 2015; Mo et al. 2014; Oliveira Borba et al. 2010; Xu et al. 2013; Zhao et al. 2015). This allows

breeders to trace the most favorable combinations in all elite lines and cultivars. This is especially important for rice grain-quality traits, since the improving lines could be genotyped at an early stage, which is normally evaluated after harvesting.

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Authors contribution T.G. Z.C. and H.W. designed the overall experiment and edited the manuscript. L.C. X.Y. and W.G. did genotyping, phenotyping, data analysis and wrote this manuscript. J.Y. L.W. S.C. Z.G. and J.Z. assisted in data analysis and participated in preparation of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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