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Genetic analysis of rice grain quality

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Abstract The inheritance of grain quality is more complicated than that of other agronomic traits in cereals due to epistasis, maternal and cytoplasmic effects, and the triploid nature of endosperm. In the present study, an established rice DH population derived from anther culture of an *indica/japonica* hybrid was used for genetic analysis of rice grain quality. A total of five parameters, amylose content (AC), alkali-spreading score (ASS), gel consistency (GC), percentage of grain with a white core (PGWC) and the square of the white core (SWC), were estimated for the DH lines and the parent varieties. For each parent, the value of each parameter was relatively stable in three locations, Beijing, Hangzhou and Chengdu, while the differences between the parents were significant for all five parameters. AC showed a bimodal distribution, and the distribution of ASS was skewed toward the value of JX17, while the other three parameters displayed continuous distributions among the DH lines with partially transgressive segregations. For AC, a minor and a major gene were found on chromosomes 5 and 6 respectively. The major gene, which should be an allele of *wx*, explained 91.9% of the total variation. For GC, two QTLs were identified on chromosomes 2 and 7 respectively. For ASS, a minor and a major gene were both located on chromosome 6. The major gene should be the same locus as the alkali degeneration gene (*alk*). Genetic linkage between *alk* and *wx* was found in QTL mapping. For PGWC, two QTLs were located on chromosomes 8 and 12. Only a minor QTL was found for SWC on chromosome 3. The results and the molecular markers presented here may be useful in rice breeding for grain quality improvement.

Key words Rice · Cooking quality · Appearance quality · DH population · QTL

Introduction

As a pivotal crop in cereals, rice provides the staple food for more than 50% of the world's people. Its higher yield and better taste are two major subjects for many breeding programs but, in contrast to disease and insect resistance, grain yield and quality are both controlled by quantitative trait loci (QTLs) showing continuous phenotypic variation in rice progeny (Yano and Sasaki 1997). It is thus difficult for breeders to improve rice grain yield and quality using conventional methods, due to a lack of discrete phenotypic segregation in the progeny. As rice grain quality is an endosperm trait, its inheritance can be more complicated because the genetic expression of an endosperm trait in cereal seeds is conditioned not only by the triploid endosperm genotype, but also by the diploid maternal genotype and any additional possible cytoplasmic differences (Pooni et al. 1992; Zhu and Weir 1994; Mo 1995).

Rice grain quality includes the milling, appearance, cooking and nutritional qualities. Among these, people pay more attention to the appearance and cooking quality (Huang et al. 1998). The appearance quality is often judged in China by the percentage of grain with a white core and a square of white core. The cooking

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quality is judged by the amylose content, alkali spreading score and gel consistency. The inheritance of rice cooking quality has been widely studied (Mckenzie and Rutger 1983; Sano et al. 1986; Pooni et al. 1992; Zhu and Weir 1994; Mo 1995). Pooni et al. (1992) suggested that amylose content might be related to the effects of the maternal plant or cytoplasm, whereas Xu et al. (1995) reported that rice amylose content was mainly controlled by the triploid endosperm genotype without any cytoplasmic effect. Shi et al. (1997) suggested that direct seed effects, maternal effects and cytoplasmic effects were the main factors in controlling amylose content, alkali spreading score and gel consistency respectively, and also detected strong genotype × environment interaction effects for these traits. So far, several genetic models have been developed for analyzing the inheritance of endosperm traits. Pooni et al. (1992) proposed a model for analyzing the direct seed effects and maternal/cytoplasm effects of endosperm traits. Zhu and Weir (1994) put forward an endosperm model for the analysis of the cytoplasmic and maternal effects. Mo (1995) presented a mating design, and a corresponding statistical method, with which the genetic effects of the endosperm and maternal genotype, as well as effects due to a cytoplasm difference, can be independently tested. Though the above-mentioned models were used to identify various genetic parameters, except for the *wx* gene (Khush et al. 1984; Sano et al. 1986), the gene loci for rice grain quality have not been located on the chromosomes. Furthermore, the heterozygotic individuals in F_2 or F_3 progeny, which were usually used in the aforementioned research, cannot provide a large number of genetically identical seeds for the accurate analysis of rice grain quality.

A doubled-haploid (DH) population, derived from the anther culture of an *indica*/*japonica* hybrid, was developed in our laboratory for QTL identification (Lu et al. 1996; Tan et al. 1996; He et al. 1997; Liu et al. 1997; He et al. 1998). Such populations have several advantages over F_2 or F_3 populations in mapping QTLs controlling grain quality. First, the homozygotic genotype of each pure DH line can be copied by the triploid endosperm of appropriate seeds with a different dosage. This would simplify the QTL mapping of grain quality regardless of the maternal and cytoplasmic effects. Second, DH lines make it possible to replicate phenotypic measurements, so that minor genes controlling grain quality can be detected. Finally, each DH line could supply enough genetically identical seeds for grain quality analysis. In the present study, the established DH population, and its molecular linkage map, were used to analyze and identify the major and minor genes for rice cooking quality, including amylose content, alkali spreading score and gel consistency, as well as rice appearance quality, including the percentage of grain with a white core and a square of white core.

Materials and methods

Plant materials

A doubled-haploid (DH) population consisting of 132 pure lines was established in our laboratory via anther culture of an F_1 hybrid between ZYQ8 (*indica*) and JX17 (*japonica*) (Lu et al. 1996; He et al. 1998). For each DH line, 40 plants were grown in two rows in the field of the experimental farm of Sichuan Agricultural University (Chengdu), with the parent plants being grown between every tenth DH line as the control, in the summer of 1997. The parents were also planted in Beijing and Hangzhou.

Rice grain quality evaluation

The parameters of rice cooking quality were analyzed with the following approaches: amylose content (AC) by the method of Perez and Juliano (1978), gel consistency (GC) according to the report of Cagampang et al. (1973), and alkali spreading score (ASS) based on the procedure of Little et al. (1958). Two parameters of rice quality were estimated as follows. (1) The percentage of grain with a white core (PGWC): the ratio of the number of white-core kernels to the total number of observed kernels. (2) The square of the white core (SWC): the ratio of the square of the white core to the square of the white-core kernel. All above parameters were measured with three replications for each sample of the DH lines and the parents.

RFLP map construction and QTL detection

Rice total DNA was extracted, digested, and hybridized as described by McCouch et al. (1988). PCR amplification and primer sequences of microsatellites followed the techniques of Chen et al. (1997). A total of 243 RFLP and microsatellite markers distributed over all 12 rice chromosomes were selected to construct a rice linkage map using Mapmaker/EXP Version 3.0 (Lander et al. 1987; Lincoln et al. 1993 a). Interval QTL mapping was carried out using the software Mapmaker/QTL Version 1.1 with a LOD threshold of 2.0 for declaring the presence of putative QTLs (Lander et al. 1989; Lincoln et al. 1993 b). In addition, the additive effect and the percentage of variation explained by individual QTLs were also estimated.

Results

The rice grain quality performance of the parents in three environmental conditions

Significant differences were observed on all five rice grain quality parameters between the parents, ZYQ8 and JX17, at three locations, Beijing, Hangzhou and Chengdu (Table 1). Though conditions of weather, soil and cultivation were quite different among these three places, the values of the five parameters were still relatively stable for each parent in the three trials. For each parameter, the difference between ZYQ8 and JX17 might be distinct in value but similar in direction. As compared with JX17, ZYQ8 was associated with a higher amylose content, a lower alkali spreading score, a softer gel consistency and a more-white core. The cooking quality parameters, AC, GC and ASS, showed less variation than the appearance of the quality parameters, PGWC and SWC, in the parents.

The variation of rice grain quality among the DH lines

The five parameters were estimated for the DH lines and the parents grown in Chengdu (Fig. 1). The segregation for AC in DH lines showed a bimodal distribution, corresponding to the AC of ZYQ8 and JX17. The two categories for AC gave a good fit to a 1:1 ratio with a slightly transgressive segregation. This suggested that AC is controlled by some minor modifying genes as well as a major one. The distribution of GC ranged from 30 to 98 and the mean value was just close to the value of ZYQ8, indicating that this trait is controlled by at least two non-allelic QTLs. The distribution of ASS skewed toward the value of JX17, and the scores in 70% of the DH lines were higher than 6, which supported a major gene control of this trait. More than 50% of the DH lines showed transgressive segregation for PGWC with a range from 2% to 100%. The SWC of DH lines was focused in a range of 10–20%, though a few lines gave SWC scores of more than 50%.

Table 1 Comparison of rice grain quality in three locations between ZYQ8 and JX17

Parents	Locations	AC	GC	ASS	PGWC	SWC
ZYQ8	Chengdu	21.55	70	3.4	50	15
	Beijing	22.64	72	3	68	15
	Hangzhou	22.10	65	3.3	33	7.5
JX17	Chengdu	15.73	95	6.8	4	1
	Beijing	15.31	85	6	38.5	1.5
	Hangzhou	16.40	96	7	20	2.25

QTL detection

A rice linkage map was constructed using this DH population, and 243 markers evenly distributed over all 12 rice chromosomes were selected for QTL

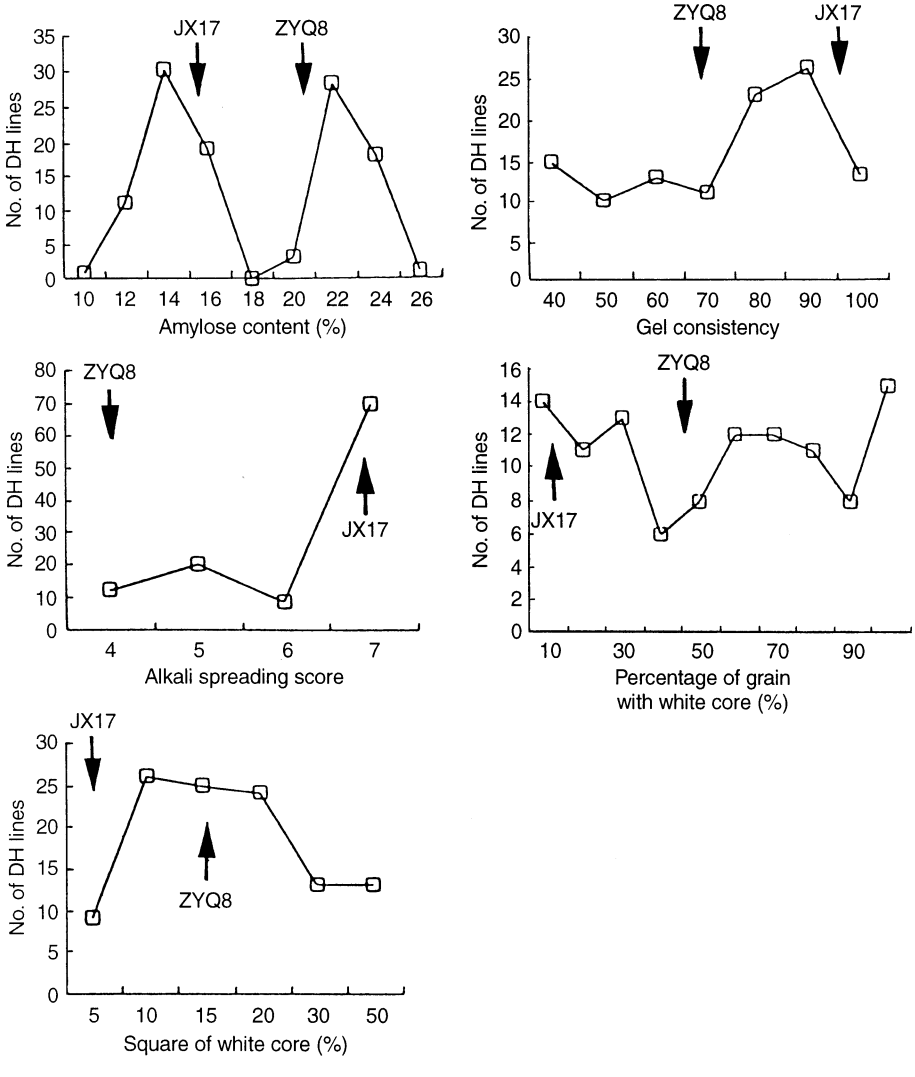

Fig. 1 Distributions of rice grain quality characters in the ZYQ8/JX17 DH population. The values of ZYQ8 and JZ17 are shown by *arrows*

Table 2 QTL locations and biometrical parameters for rice grain quality in ZYQ8/JX17 DH population. QTL nomenclature follows that of McCouch et al. (1997)

Traits	Locus	Chromosomes	Marker interval	LOD score	Additive effect	Variance explained
AC	qAC-5	5	RG573–C624	2.67	−3.32	11.8
	Wx	6	Wx	28.39	− 8.52	91.1
GC	qGC-2	2	RG171–G243A	4.14	17.60	20.2
	qGC-7	7	TCT122–RG769	3.26	17.00	14.2
ASS	qASS-6	6	CT201–RZ450	6.19	1.23	24.6
	alk	6	CT506–C235	27.04	2.33	82.4
PGWC	qPGWC-8	8	G187–RZ66	3.67	− 29.45	21.9
	qPGWC-12	12	CT462–RG574	2.15	− 20.14	10.0
SWC	qSWC-3	3	CT211A–G1318	2.19	7.08	8.8

identification for rice grain quality with Mapmaker/QTL software. The QTL positions and their biometrical parameters are shown in Table 2 and Fig. 2.

A major gene and a QTL were detected for AC on chromosomes 6 and 5. The major gene on chromosome 6 that explained 91.1% of the total variation should be an allele of *wx* (*Wx*). The QTL, qAC-5, explained 11.8% of the total variation. ZYQ8 had positive effects at these two loci. Two QTLs, qGC-2 and qGC-7, were found for GC on chromosomes 2 and 7 which explained 20.2% and 14.2%, respectively, of the total variation. The positive additive effects of these two loci were both contributed by JX17. A major gene and a QTL related to ASS were both located on chromosome 6. The major gene should be the same locus as the *alk* (alkali degeneration) gene which accounted for 82.4% of the total variation, while the QTL, qASS-6, could be responsible for 24.6% of the total variation. JX17 alleles can increase the ASS value at these two loci.

Two QTLs were detected for PGWC. The QTL, qPGWC-8, on chromosome 8 explained 21.9% of the total variation with an additive effect of 29.45%, while the other one, qPGWC-12, on chromosome 12, accounted for 10.0% of the total variation with an additive effect of 20.14%. ZYQ8 contributed both of the positive effects at these two loci for PGWC. Only a minor QTL, qSWC-3, was found for SWC on chromosome 3 and explained 8.8% of the variance. Though the SWC of ZYQ8 was higher than that of JX17, the qSWC-3 affecting SWC in the progeny was from JX17.

Discussion

The inheritance of grain quality is complicated due to epistasis, cytoplasmic effects, and the triploid nature of endosperm (Pooni et al. 1992; Zhu and Weir 1994; Mo 1995). In the present study, in order to simplify genetic analysis, a permanent mapping population, the ZYQ8/JX17 DH population, was used for analyzing the major and minor genes controlling rice grain quality. We identified both a major gene and a minor gene for AC and ASS, and a number of QTLs with minor effects for GC, PGWC and SWC, respectively, but did not detect the presence of a digenic interaction between QTLs. Similar results had also been obtained by a tentative test of rice grain quality in this DH population in 1992 (data not shown).

A series of studies with the ZYQ8/JX17 DH population have been carried out in our laboratory to identify the QTLs for some important traits in rice (Lu et al. 1996; Tan et al. 1996; He et al. 1997; Liu et al. 1997; He et al. 1998). In 1996 we reported the comparative mapping of QTLs for agronomic traits of rice across three environmental conditions and found that QTL by environment interaction was trait-dependent. QTLs for spikelets and grains per panicle were less sensitive to the environment than heading date and plant height (Lu et al. 1996). In the present study, we also found that some grain quality traits, like AC, GC and ASS, were more stable than the other traits, such as PGWC and SWC, in the parents that we tested in three locations. The obvious lack of linkage between the mapped QTLs for yield and grain quality suggested that these two important agronomic traits can be combined together.

AC is a key factor in determining rice cooking quality and market class. It was reported that AC in non-waxy rice was controlled by a single dominant gene with a major effect and a number of modifying genes with minor effects (Bollich and Webb 1973; Mckenzie and Rutger 1983). Kumar et al. (1987) showed that, in non-waxy varieties, AC was determined by a multiple allelic series at the *wx* locus. Sano et al. (1986) detected two different alleles, Wx^a and Wx^b, at the *wx* locus in non-waxy rice varieties. Wx^a drastically enhanced the quantitative level of Wx protein as well as AC in comparison with Wx^b. Wx^b was predominant in the *japonica* type of rice, while most strains of the *indica* type had Wx^a (Sano et al. 1986). Bligh et al. (1995) found a microsatellite locus closely linked to the *Wx* gene. Wang et al. (1995) reported that AC in rice endosperm was related to the post-transcriptional regulation of the *Wx* gene. Recently, Ayres et al. (1997) identified seven *Wx* microsatellite alleles which together explained 82.9% of the total variation in AC of

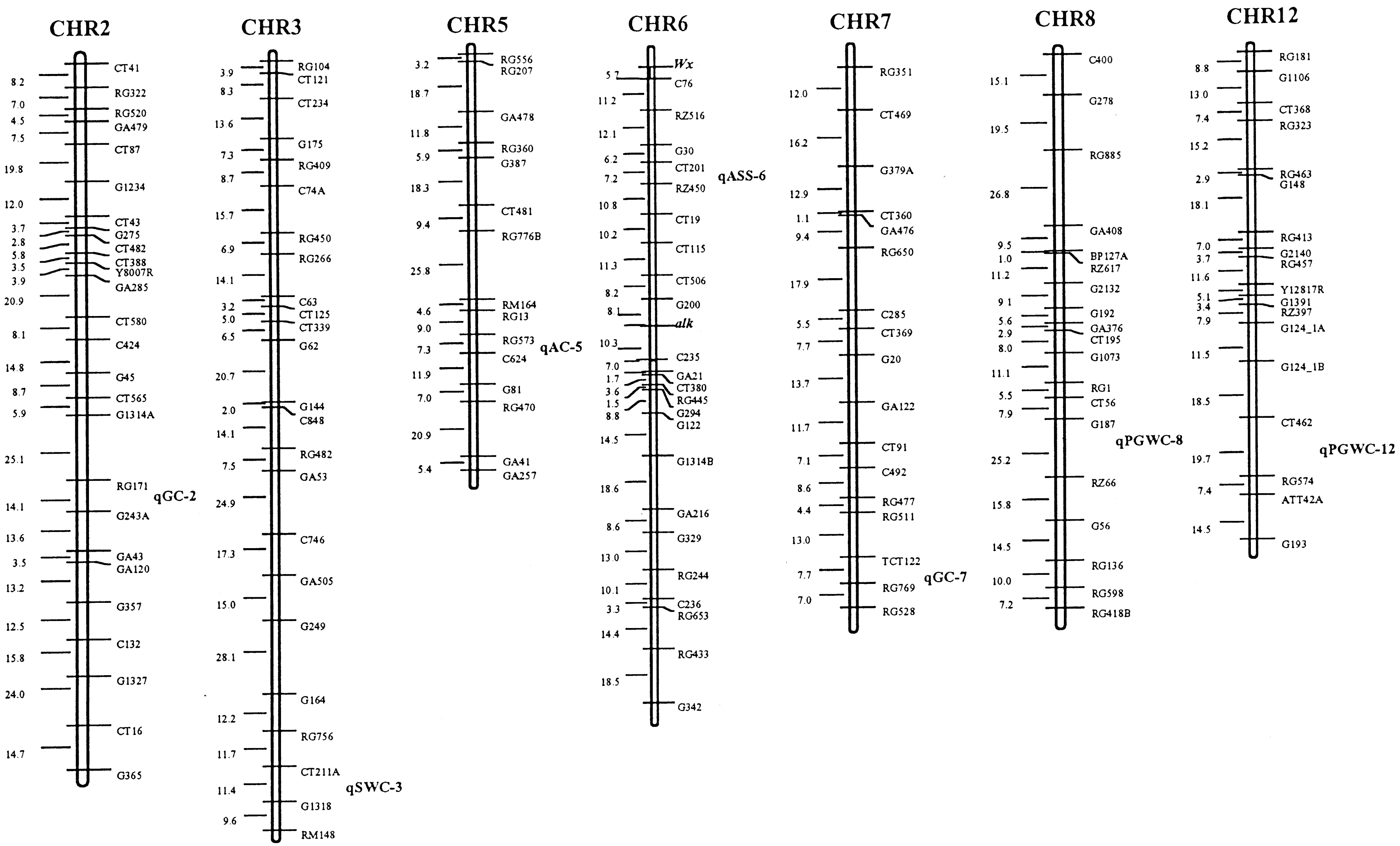

Fig. 2 Some rice chromosomes associated with rice grain quality in the ZYQ8/JX17 DH population. The scale in Kosambi centiMorgans (cM) is shown on the left of each chromosome. The major gene and QTL positions for rice grain quality are shown on the right of chromosome in *bold style*

the 89 non-waxy rice cultivars which they tested. In the present study, ZYQ8 is an intermediate AC variety and JX17 is a low AC variety. The major gene for AC detected here is an allele of *wx* and, therefore, ZYQ8 and JX17 should have Wx^a and Wx^b alleles respectively. On the other hand, there is a wide diversity of AC in non-waxy *indica* or *japonica* varieties, suggesting a series of alleles at the Wx^a or Wx^b locus. However, Yano et al. (1988) reported that the low AC of null mutants was determined by single recessive genes which were non-allelic to the *wx* gene. Maybe the minor QTL, qAC-5, that we detected is one of them.

Only a few papers reported the inheritance of other rice cooking qualities (Mckenzie and Rutger 1983; Shi et al. 1997). Shi et al. (1997) suggested that the cooking quality traits were mainly controlled by genetic effects, but they also noted genotype × environment interaction effects. Mckenzie and Rutger (1983) reported that a major gene controlling AC was linked with a gene for ASS. The *alk* and qASS-6 found in the present study were also linked with *Wx*. Because the ASS of grain is partially determined by its AC level, it should be further studied whether qASS-6 is the same as *Wx* or whether a gene family located in the interval from *Wx* to *alk* on chromosome 6 is responsible for rice cooking quality. qGC-2 and qGC-7 were two detected gene loci for GC, suggesting that GC is also determined by some loci with relatively major effects.

Some researchers have investigated the inheritance of rice appearance quality traits (Ebata and Tashiro 1973; Takeda and Saito 1983) and found that PGWC and other quality traits are dependent not only on the genotypic but also on an environmental influence, and are controlled by some minor genes with additive effects. This conclusion is in accordance with the results of the present study.

Grain quality is an economically important character in rice varieties. Any knowledge of the genetic mechanism of rice grain quality will be of benefit to rice breeders. Therefore, the major and minor genes affecting rice grain quality, identified in the present study, should accelerate the process of breeding new rice varieties with both a higher yield and a better quality.

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