DNA RESEARCH 7, 93–101 (2000)

Mapping of Genes for Cooking and Eating Qualities in Thai Jasmine Rice (KDML105)

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(Received 24 September 1999; revised 4 February 2000)

Abstract

Thai jasmine rice, KDML 105, is known as the best quality rice. It is known not only for its aroma but also for its good cooking and eating qualities. Amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) are important traits determining rice quality. A population of recombinant inbred lines (RIL) derived from KDML105×CT9993 cross was used to study the genetic control of AC, GC and GT traits. A total of 191 markers were used in the linkage map construction. The 1605.3 cM linkage map covering nearly the whole rice genome was used for QTL (define QTL) analysis. Four QTLs for AC were detected on chromosomes 3, 4, 6 and 7. These QTLs accounted for 80% of phenotypic variation explained (PVE) in AC. The presence of one major gene as well as several modifiers was responsible for the expression of the trait. Two QTLs on chromosome 6 and one on chromosome 7 were detected for GC, which accounts for 57% of PVE. A single gene of major effect along with modifier genes controls GC from this cross. The QTLs in the vicinity of waxy locus were major contributors in the expression of AC and GC. The finding that the position of QTLs for AC and GC were near each other may reflect tight linkage or pleiotropy. Three QTLs were detected, one on chromosome 2 and two on chromosome 6, which accounted for 67% of PVE in GT. Just like AC and GC, one major gene and modifier genes governed the variation in GT resulting from the KDML105×CT9993 cross. Breeding for cooking and eating qualities will largely rely on the preferences of the end users.

Key words: Amylose Content (AC); Gel Consistency (GC); Gelatinization Temperature (GT); Gene Mapping; Quantitative Trait Loci (QTL)

1. Introduction

The development of DNA marker technology has been useful in the construction of genetic maps of various organisms.[1,2] Genetic maps reveal the location of the genes along the chromosome, the number of genes that influence a trait and the effects of the genes in the expression of the trait. Mapping of genes would help explain gene function, regulation and expression as well as providing information of genome evolution. Differences in DNA cutting sites or nucleotide sequences in a particular allelic locus is the basic requirement in linkage analysis that would define the genetic distances between polymorphic traits.

The rice genome has been widely used for genetic map construction and for locating genes of various agronomic importance.[3–6] Rice is the major energy source and staple food of more than half of the world's population. It also has various industrial uses such as in baby food products, rice noodle making, and brewing to name a few. Starch is the major constituent of the rice endosperm (90% dry matter). Amylose content (AC) of starch reveals the appearance and texture of rice and therefore affects the cooking and eating qualities of rice. Also, starch physical properties such as gelatinization temperature (GT) and gel consistency (GC) are responsible in differences in rice cooking and processing behaviors.

Knowing and understanding the genetic bases of AC, GC and GT are major goals in rice breeding programs, particularly concerning rice grain quality. Studies on AC were often related to the study of waxy gene in rice.[7–10]

Communicated by Satoshi Tabata

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Several investigators have reported on the inheritance of AC, GC and GT. High AC was incompletely dominant to low AC and is controlled by one gene of major effect and several modifiers.[11,12] Two dominant complementary genes were reported to control high AC.[13] A difference of 2.5% AC is controlled by few genes of small or approximately equal effects.[11–13] GC is controlled by a single gene with major effect along with several minor genes and modifiers.[14] Likewise a single gene was found to control GT.[13]

Khao Dawk Mali 105 (KDML105), the Thai jasmine rice, is a famous variety that is soft, tender and fluffy when cooked. Such exceptionally good qualities of KDML resulted from its low AC, low GT and medium GC. Although these three traits have been studied,[15,16] the inheritance and molecular-marker based analysis of AC, GC and GT in this variety has not yet been done.

The objective of the study was to map the QTLs for AC, GC and GT by using RILs of KDML105 crossed with CT9993 as the mapping population. Information about molecular markers that are found tightly linked to the QTLs that control AC, GC and GT in rice will facilitate breeding strategies in improving rice grain cooking and eating quality traits.

2. Materials and Methods

2.1. Plant materials

One hundred forty-one recombinant inbred lines (RILs) derived from F_8 of a cross between the two most divergent parents, KDML105 and CT9993-5-10-1-M, were used for analysing quantitative control of amylose content (AC), gel consistency (GC) and gelatinization temperature (GT). KDML105, a jasmine rice, has good cooking qualities with 16.78% AC, medium GC and low GT. The CT9993, an upland japonica rice from Center of International Tropical Agriculture (CIAT), has intermediate cooking quality with 24.04% AC, hard GC and high GT. The RI plants used in this study headed and ripened and were harvested in the same season to minimize variation in heading date.

2.2. Phenotyping of AC, GC and GT

Amylose content (%) was measured following the procedure of Juliano[17] with some modifications. Digesting tubes were used in place of volumetric flasks to prevent upsetting. The samples were boiled for 10 min in the digesting tubes to completely disperse the powder. The optical density of the amylose-iodine blue color was measured at 620 nm using a spectrophotometer.

The dispersion of 100 mg of milled rice flour wetted with 0.2 ml of 95% ethanol containing 0.025% (w/v) thymol blue in 11×100 mm culture tubes in 2 ml of 0.2 N KOH was used to measure GC. The sample was mixed vigorously. Tubes were covered with glass marbles before subjecting them to boiling water bath. After 5 min the tubes were removed and were mixed again and cooled in ice water bath for 20 min. The cooled tubes were then laid horizontally against a ruled graphing paper and gel length was measured after 1 hour. The gel consistency values were classified as soft (61–100 mm), medium (41–60 mm) or hard (26–40 mm).

Milled rice derived from 10-grain samples were used to determine GT by incubating the grains in 15 ml of 1.7% KOH at room temperature for 23 hr. The degree of spreading was measured using the following seven-point semi-quantitative rating scale: 1, grain not affected; 2, grain swollen; 3, grain swollen, collar incomplete and narrow; 4, grain swollen, collar complete and wide; 5, grain split or segmented, collar complete and wide; 6, grain dispersed, merging with collar and 7, grain completely dispersed and intermingled. Alkali spreading values correspond to GT as follows: 1–2, high (74.5–80°C); 3, high intermediate; 4–5, intermediate (70–74°C) and 6–7 low (<70°C).

2.3. RFLP, SSR and AFLP analyses

Molecular marker technologies such as RFLP, SSR and AFLP were used in order to construct the map and analyze the QTL. Total genomic DNA of the two parents and the 141 RI lines were isolated following the procedure of McCouch.[3] Parental DNAs were digested with seven restriction enzymes: *Dra* I, *Xba* I, *Eco*RI, *Eco*RV, *Hin*dIII, *Bgl* III and *Bam*HI. Rice genomic and cDNA clones as well as oat cDNA clones provided by the Rice Genome Project, Japan and Cornell University, U.S.A. that showed polymorphism with the specific enzyme used to digest the parental DNAs were used in the RI population. Digested DNAs were transferred to a nylon membrane and were hybridized with labeled probes. Hybridization was done at 65°C overnight. Probe labeling, DNA hybridization and chemiluminescent detection were carried out with a DIG system (Boehringer Mannheim) according to the manufacturer's instructions.

Thirty-six SSR markers (including the waxy marker) that gave polymorphism between the parents were also used in the mapping population. SSR was performed following the technique of Chen et al.[18] Amplified products were loaded onto 4.5% polyacrylamide gels and were detected by silver staining.

AFLP was performed according to the procedure of Vos.[19] Fifty-two were detected to be polymorphic between the parents and the primer combinations that yielded the polymorphic loci were used to survey the RI population. PCR products were electrophoresed in 4.5% polyacrylamide gel and were detected by silver staining.

2.4. Linkage map construction and QTL analysis

MAPMAKER EXP.3.0[20] software using the Haldane map function was used for linkage map construction in

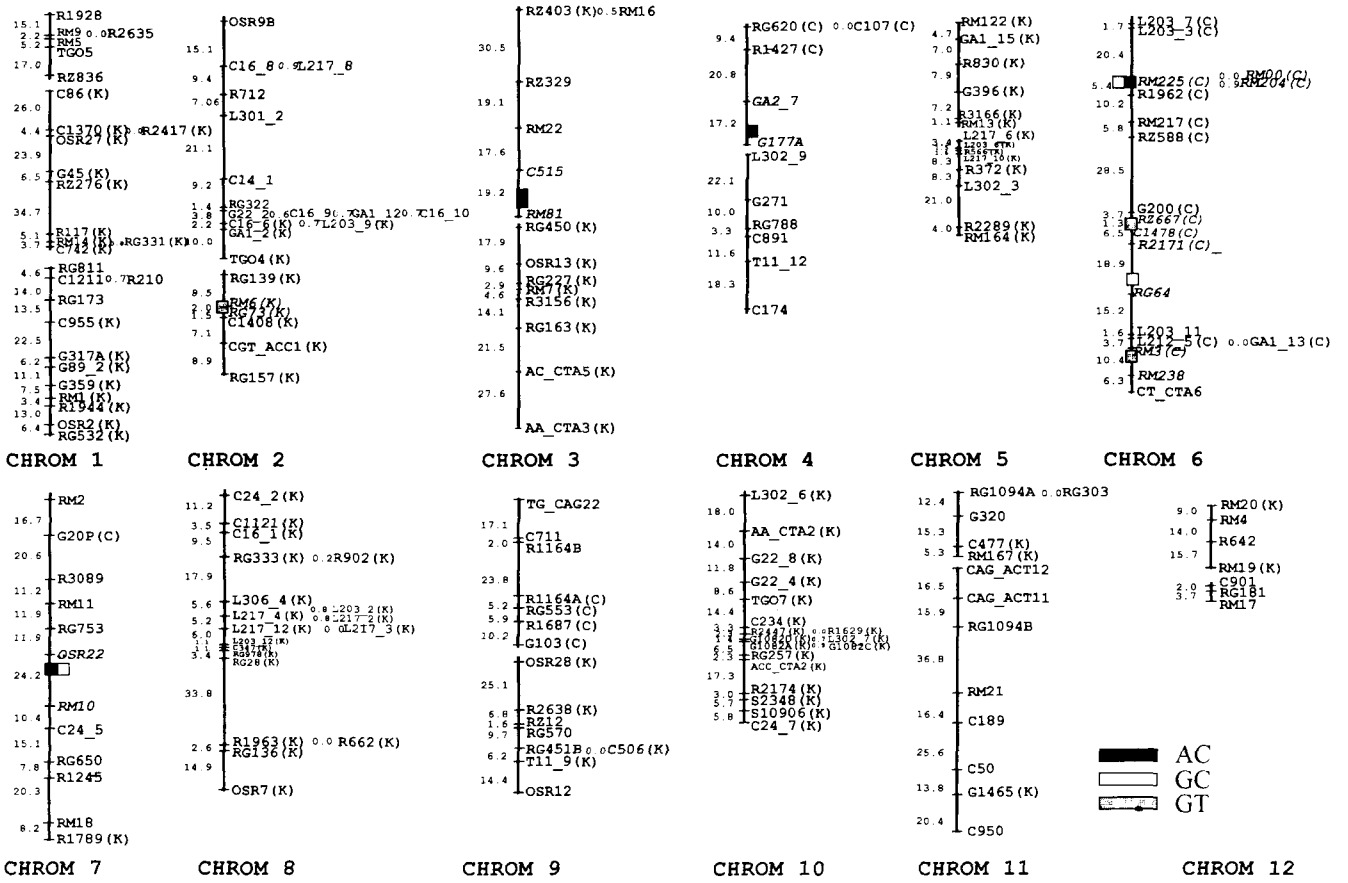

Figure 1. Framework map of recombinant inbred population from the KDML105/CT9993 cross. Skewed markers are identified by (K) for KDML105 and (C) for CT9993. Mapping locations of the QTLs identified for AC, GC and GT are also indicated in the framework map with the marker intervals written in italics.

the RIL population of the CT9993/KDML105 cross. The linkage map was constructed using 191 markers (103 RFLPs, 36 SSRs and 52 AFLPs). The linkage groups were assigned to their corresponding chromosomes according to previous maps.[5,6]

QTL analysis was performed with the software package MQTL.[21] Both simple interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures were used for QTL detection. Each data set was analyzed with 1000 permutations, a 5 cM walking speed and a Type I error rate of 5%. The significant threshold (a LOD score of 2.4 or above) was used to declare the presence of a QTL. Twenty-seven background markers were specified as cofactors in the sCIM. An association of markers with AC, GC, and GT was analyzed using simple regression, multiple regression and the ANOVA analysis procedure in STATGRAPHIC (version 2.1). QTL×QTL interactions were also analyzed using the mentioned statistical procedures.

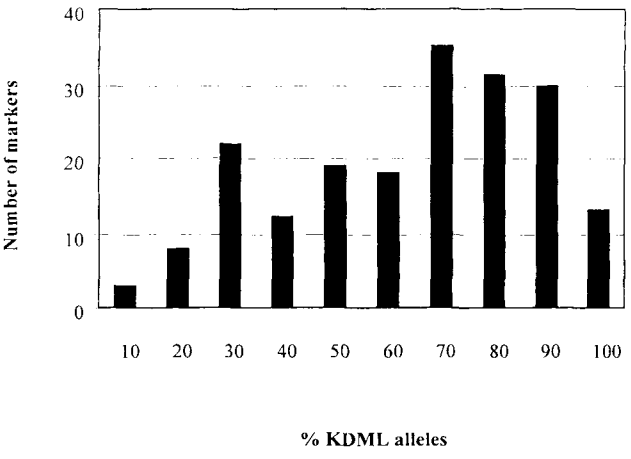

Figure 2. Distribution of percent KDML alleles for the 191 molecular markers.

3. Results

3.1. Map construction

The 191-marker based map comprises a total map distance of 1605.3 cM (Fig. 1). The average marker inter-

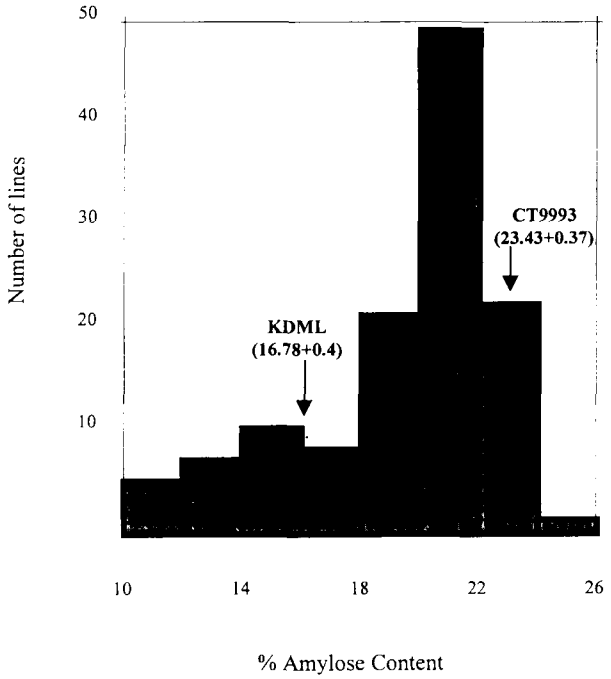

Figure 3. A. Amylose content (%) in the KDML105 × CT9993 RI population.

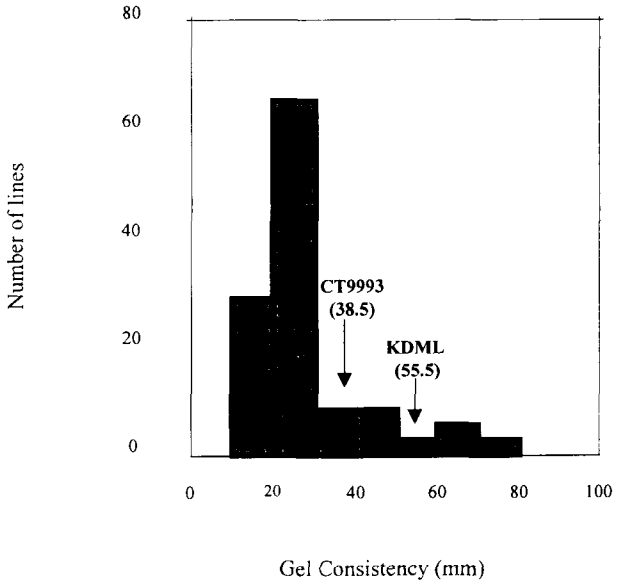

Figure 3. B. Gel consistency (nm) in the KDML105 × CT9993 RI population.

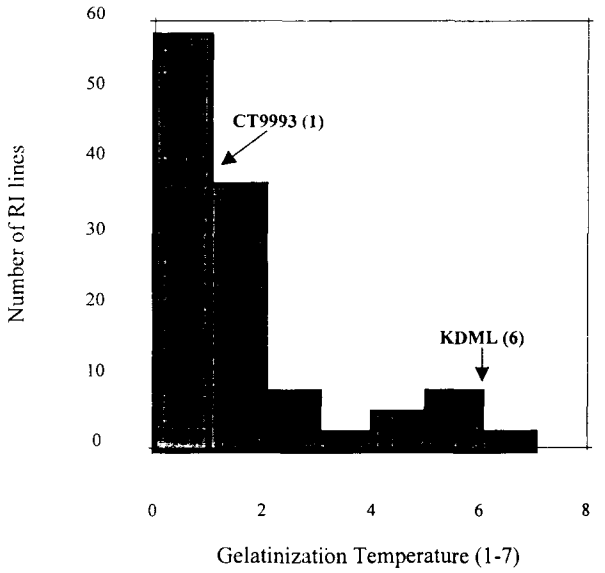

Figure 3. C. Gelatinization temperature (1–7) in the KDML105 × CT9993 RI population.

val is 11.5 cM. Marker orders are consistent with published maps.[5,6] The percentage of KDML105 allele for each marker was calculated and is shown in Fig. 2. The percentage of indica allele of 96 markers ranged from 61–90%. Taking the population as a whole, it carried 64% of KDML loci and 36% of CT9993 loci indicating unequal amount of genetic material has been transmitted from the parents to the progenies. Of the 191 marker loci, 120 showed significant segregation distortion ($p \leq 0.05$). The distorted markers were not randomly distributed. They showed some systematic pattern on the genome. KDML105 alleles were over-represented at 97 loci mainly on chromosomes 1, 2, 3, 5, 8, 10, 11 and 12 (Fig. 1). Chromosomes 3, 8 and 10 have prominent distortions as evidenced by the X^2 values greater than 63.66 ($P < 0.01$). On chromosomes 4, 6, and 7, segregation distortions were in favor of CT9993 alleles (Fig. 1). Frequency of distribution favoring both alleles occurred equally on chromosome 9 (Fig. 1). Segregation distortion is commonly found in RI populations derived from indica × japonica crosses.[22] Percent heterozygosity was not fairly equal among the codominant markers representing the 12 rice chromosomes, ranging from 0.71% for RG73 of chromosome 2 to 16.31% of R3166 of chromosome 5. On the average, a high level of residual heterozygosity (6.61%) was observed as compared with the expected (0.78%) for F_8 RI lines.

3.2. Trait performance

The two parents were significantly different in AC ($P \ll 0.01$). The AC of KDML105 was 16.78±0.47% and the AC of CT9993 was 23.43±0.31%. The AC of the RIL population was well distributed ranging from 10.83% to 24.86%. Dull endosperm was also observed in some of the progeny. The frequency distribution of progenies did not show discrete classes (Fig. 3A) indicating that this trait was quantitatively inherited. The GC of KDML105 is intermediate (55.5 mm gel dispersion) while CT has a hard GC (38.5 mm gel dispersion). The dispersion of 100 mg of milled rice flour was used to determine GC. Transgres-

Table 1. Means and standard deviation of AC, GC and GT in the progenies of KDML 105 × CT9993 and their parents.

	KD	CT	RI Population		
			Mean	SD	range
AC (%)	16.78	23.43	20.02	3.24	11 -24
GC (mm)	Intermediate (55 5)	hard (38.5)	27.89	13.3	9-75
GT (1-7)	Low (6)	high (1)	1.78	1.42	1-6.5

sive segregants with a low GC value were predominant in the population. Continuous distribution indicates the quantitative inheritance for GC (Fig. 3B). The frequency distribution of GT in the progeny did not show discrete classes (Fig. 3C). Most of the RI lines have high GT values (showing intact or slightly swollen grains) based on alkali spreading values. The mean and distribution properties of AC, GC and GT are shown in Table 1.

3.3. QTL analysis

There were 22 low AC transgressive segregants with AC values lower than KDML105. These data suggested that both parents possessed some alleles for low AC phenotypes and a unique profile of alleles from the QTLs responsible for the expression of AC may be required for a low AC, as exemplified by the transgressants. Four QTLs for AC were detected on chromosomes 3, 4, 6 and 7 using the SIM and sCIM procedures of the software MQTL. The chromosome 3 and 4 QTLs were mapped to the *RM81-C155* and *G177A-GA2-7* intervals, respectively. The CT9993 allele in both loci lowered AC in the progeny. These QTLs accounted for 11.28% (chromosome 3) and 15.99% (chromosome 4) of PVE in AC. The major QTL was located on chromosome 6 in the vicinity of the wx locus. This QTL accounted for 58.69% of PVE. KDML105 contributed a low AC allele at this locus. The smallest-effect QTL on chromosome 7 was mapped to the *RM10-OSR22* interval. This QTL accounted for 9.18% of PVE. Again, the KDML105 allele at this locus lowered AC. All QTLs detected in this cross accounted for 80.16% of the AC variation observed in the RIL population (Table 2).

Significant interactions between QTL_3 and QTL_4, and between QTL_6 and QTL_7, were detected. Sources of alleles on the $QTL_3 \times QTL_4$ and $QTL_6 \times QTL_7$ interaction were important. Only progeny having CT9993 alleles on chromosomes 3 and 4 (Fig. 4A) and KDML105 alleles on chromosome 6 and 7 showed the low AC phenotypes (Fig. 4B). Two allelic compositions, $KD_{QTL3} \times KD_{QTL4}$, $CT_{QTL3} \times KD_{QTL4}$, showed the same level of AC in the progeny. The allelic composition of the $QTL_6 \times QTL_7$ interaction contributed to different levels of AC in the progeny. The average AC values of the $KD_{QTL6} \times KD_{QTL7}$, $KD_{QTL6} \times CT_{QTL7}$, $CT_{QTL6} \times KD_{QTL7}$, and $CT_{QTL6} \times CT_{QTL7}$ genotypes were 15.1, 16.8, 20.6, and 21.7, respectively (Fig. 4B). The 22 transgressive segregants with AC lower than KDML105 have the allelic profile of CT_{QTL3}, CT_{QTL4}, KD_{QTL6}, and KD_{QTL7}.

Three QTLs for GC accounted for 57.46% of phenotypic variation were detected on chromosomes 6 and 7. Two QTLs on chromosome 6 were mapped to the vicinity of the *waxy* locus and to the *RG64-R2171* interval. KDML105 alleles at the *waxy* locus raised the GC value but at the *RG64-R2171* interval lowered the GC value. The chromosome 7 QTL was mapped to the *RM10-OSR22* interval with KDML105 significantly associated with soft GC phenotype (high GC value). The chromosome 6-1 (near *waxy*), 6-2 (near *RG64*) and 7 QTLs accounted for 53.75%, 11.36%, and 13.55% of the PVE (Table 2). Transgressive segregation in favor for low GC was observed.

Significant interactions were observed between the QTLs on chromosomes 6 (near *RG64*) and 7 and between the two QTLs on chromosome 6. Only the progenies with KDML alleles on QTL_7 and with CT9993 alleles on $QTL_{6\text{-}2}$ showed high GC values (corresponding to soft GC) (Fig. 4C). The same is true with the progeny having KDML alleles at $QTL_{6\text{-}1}$ and with CT9993 alleles at $QTL_{6\text{-}2}$ (Fig. 4D).

Three QTLs explaining 67% of phenotypic variation in GT were detected, one on chromosome 2 and two on chromosome 6. The chromosome 2 QTL was located in the *RG73-RM6* interval. The KDML105 allele at this locus lowered the GT score which accounted for 12.22% of the PVE. Two QTLs located on chromosome 6 in the *C1478-RZ667* ($QTL_{6\text{-}1}$) and *RM3-RM238* ($QTL_{6\text{-}2}$) intervals accounted for 60.30% and 8.57% of the PVE, respectively. The CT9993 allele in the *C1478-RZ667* interval lowered the GT value but the allele in the *RM3-RM238* interval raised GT (Table 2).

Interactions between $QTL_2 \times QTL_{6\text{-}1}$ and $QTL_{6\text{-}1} \times QTL_{6\text{-}2}$ were significant. $KD_{QTL6\text{-}1} \times CT_{QTL6\text{-}2}$ and $CT_{QTL2} \times KD_{QTL6\text{-}1}$ raised the GT score (Figs. 8 and

Table 2. Chromosomal location, allele phase and effect expressed as percent phenotypic variance explained for amylose content (AC), gelconsistency (GC) and gelatinization temperature (GT) in KDML 105 × CT9993.

Character	Marker interval	Chromosome	PVE (%)	Total PVE	Effect (allelic phase)
AC	RM81- C155	3	11.28	80.16	1.86 (CT)
	G177A- GA2-7	4	15.99		0.63 (CT)
	Waxy-RM204	6	58.69		4.48 (KD)
	OSR22- RM10	7	9.18		0.96 (KD)
GC	Waxy – RM225	6	53.10	57.46	16.98 (KD)
	RG64 – R2171	6	10.50		5.18 (CT)
	OSR22 – RM10	7	12.77		8.20 (KD)
GT	RG73 – RM6	2	12.22	66.69	0.24 (KD)
	C1478 – RZ667	6	60.30		2.21 (CT)
	RM3 – RM238	6	8.57		0.68 (KD)

9). The presence of $QTL_{6\text{-}1}$(near *C1478*) was important in lowering GT, even though KDML coming from both $QTL_{6\text{-}2}$ and QTL_7 were present (Figs. 4E and 4F). It is noted that chromosome 6 contains QTLs responsible for rice grain qualities.

4. Discussion

Rice grain quality is usually evaluated according to its suitability for a specific end user. AC, GC and GT are inherent characteristics of rice that determine cooking and eating qualities, as well as processing properties of rice. AC, GC and GT were mapped in the present study to better understand the role of each trait in relation to rice quality.

The abortion of male and/or female gametes was reported to cause segregation distortion in indica-japonica crosses.[23] Gametophytic genes (*ga*) were reported to be responsible for the gametic selection during fertilization favoring indica alleles. Such genes were located on chromosomes 8 and 11.[23] These genes could be responsible for the distortion observed on chromosomes 8 and 11 although chromosome 11 has minimal marker distortion. Distortions in these chromosomes were also observed in a mapping study using double haploid lines.[24] Sterility genes were found on chromosomes 2, 3, 6, 7, 11 and 12.[23,24] The distorted segregation in these chromosomes from the KDML105/CT9993 cross was likely due to effects of these genes. A study conducted by McCouch et al.,[3] showed segregation distortions on chromosome 3 favoring the japonica alleles. Restoration fertility gene was located on chromosome 10[5,25]; this gene might account for the unequal allele distribution on chromosome 10 of this cross. There were no reported sterility genes, *ga* and other genes responsible for sterility mapped on chromosome 5. It would be interesting to find out the sterility mechanism in this region using this cross. To establish the linkage maps, information from previously established maps were obtained. The order of markers showed no major disagreement with the order of markers described in the previous studies.[5,6] In different crosses, map distances could change but it does not affect the ability to detect QTLs. The residual heterozygosity could be due to lower fertility of the plants homozygous for particular segments, therefore causing unintentional selection for heterozygotes during propagation of the RILs.

Several investigators studied the inheritance pattern of AC. One major gene governed the inheritance of AC in an F_2 population derived from a low/intermediate AC cross.[11] Strong evidence of transgressive segregants indicating the presence of modifier genes was observed in F_3. The same result was also observed in a cross between high and low AC.[13] A single gene of major effect is responsible for differentiating low and intermediate AC parents, differing only by 6–12% in AC. The occurrence of transgressive segregants was due to modifier genes.[12] The QTL data confirm the multi-locus control of AC in KDML105 and provide some evidence for a low AC allele in CT9993. These results supported the presence of transgressive segregation in the KDML105 × CT9993 population. KDML105 contributed low AC alleles at QTLs on chromosomes 6 and 7, while CT9993 contributed the low AC alleles at QTLs on chromosomes 3 and 4. The largest-effect QTL was located at the waxy locus. Major QTL for AC was mapped on chromosome 6 in the vicinity of *wx* gene.[15] The relationship of the KDML105 chromosome 6 QTL to the *wx* gene remains to be determined. Three small-effect QTLs detected on

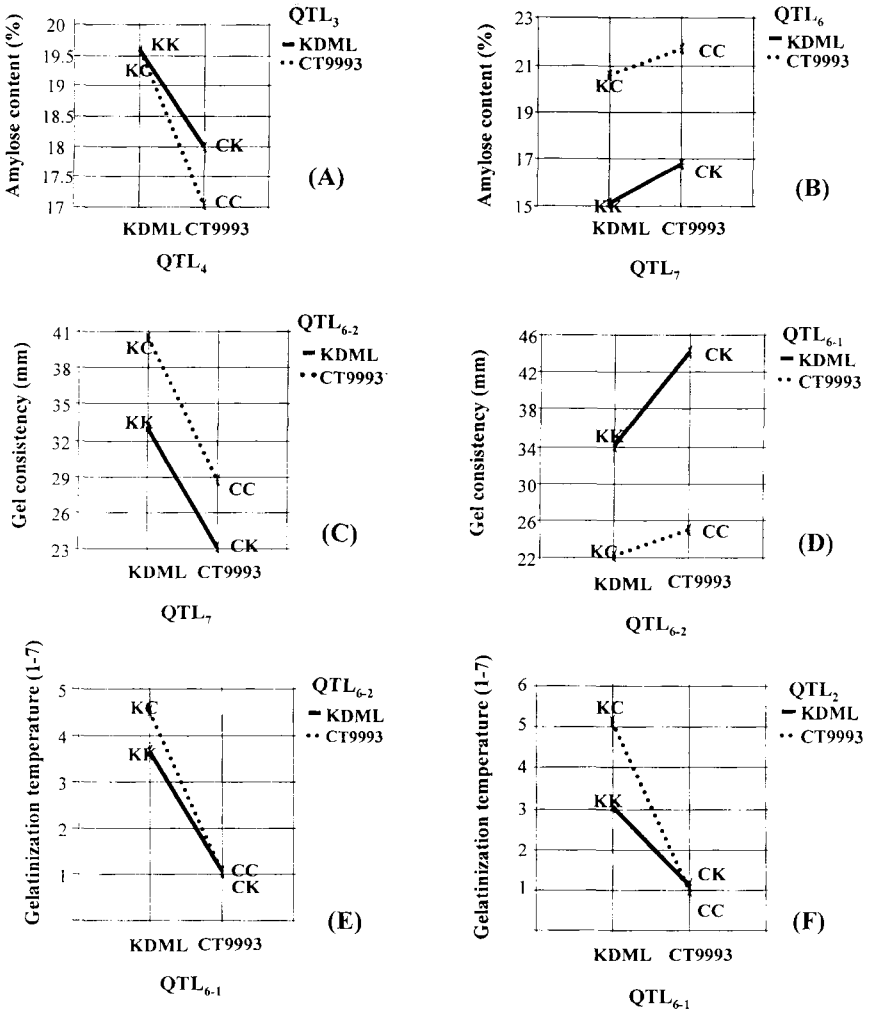

Figure 4. Plots for two locus interactions between QTLs for AC, GC and GT. (A and B) QTL interactions for AC between QTL_4 (*G177A*) and QTL_3 (*RM81*) and between QTL_7 (*OSR22*) and QTL_6 (*waxy*). (C and D) QTL interactions for GC between QTL_7 (*OSR22*) and QTL_{6-2} (*RG64*) and between QTL_{6-1} (*waxy*) and QTL_{6-2} (RG64). (E and F) QTL interactions for GT between QTL_{6-1} (*C1478*) and QTL_{6-2} (*RM3*) and between QTL_{6-1} (*C1478*) and QTL_2 (*RG73*). KK, KC, CK and CC refer to the allelic composition of the RI lines with reference to the QTLs mentioned.

chromosomes 3, 4, and 7 and the epistatic interaction of $QTL_3 \times QTL_4$ and $QTL_6 \times QTL_7$ may indicate the complexity of genetic control of AC in this germplasm. However, a larger population is required for estimating higher order QTL × QTL interaction.

A major gene controlled GC with multiple allelic form in different populations derived from crosses between hard and soft, hard and medium, and medium and soft. The expression of this gene was influenced by modifiers.[14] Major QTL in this experiment was detected in the vicinity of the *wx* gene. The coincidence of QTLs in the vicinity of the *wx* gene for AC and GC may be due to pleiotropy or linkage. Two minor QTLs were detected on chromosomes 6 and 7. The chromosome 7 QTL and the QTL reported by He[15] might be allelic and still need to be resolved. The small-effect QTL on chromosome 6 mapped to the *RG64-R2171* interval was not reported in other genetic materials. This QTL should be of considerable value and utility.

Amylopectin rather than amylose appeared to be the major contributor to gel consistency of the starch.[26] Starch Branching Enzyme III (SBE III) is responsible in the formation of amylopectin.[27] The gene coding for SBE III was mapped on chromosome 2 in which we did not find a QTL in our mapping population. Transgressive segregants with GC lower than CT9993 was observed extensively. This evidence was due to allelic interaction within each of the three QTLs. The specific configuration of alleles at three loci was important to get high GC. This finding indicates the difficulty of manipulating GC in rice improvement.

Genetic analysis of alkali spreading score to determine GT using a cross between low and intermediate GT was reported.[13] It was reported that the trait was controlled

by one gene of major effect. In the KDML105 × CT9993 cross the QTL data confirm the multi-locus control of GT in KDML105. The largest-effect QTL was detected on chromosome 6, near C1478 and two small-effect QTLs were detected on chromosomes 2 and 6. Epistatic interactions with the major QTL indicate the complexity of this trait.

4.1. Role of the wx *gene*

Amylose content, that is often studied through the *waxy* gene of rice, can be used to infer the waxy allele that is present in chromosome 6 of rice.[7,10] Rice strains can be classified as those carrying Wx^a, which is predominant in indica rice and have high AC or Wx^b, which is found in japonica rice and has low AC.[7] The QTL in chromosome 6 near the *waxy* locus gives low AC and it can be inferred that the Wx^b allele may be present. Evidence supports the regulation in the amount of AC that relates to the differential regulation of the *wx* gene, which plays a major part in the production of amylose.[10,28,29]

4.2. Potential of KDML105

KDML105 has a good potential in producing good quality rice. The QTLs near the waxy locus have KDML alleles conferring low AC and soft GC. The QTL near *C1478* that is also controlled by KDML allele resulted in low GT. Although the interaction of KDML allele (from the QTLs mentioned) with the other QTL alleles for each trait is necessary to give good AC, GC and GT profiles, KDML alleles still have the greatest contribution. More extensive studies of the intramolecular and/or intermolecular interactions of AC (the major determinant of rice eating quality) with other components of rice grain, such as protein, lipid and non-starch polysaccharides, will be of great importance in analyzing the texture of cooked rice.[30] Studying the other rice texture determinants in KDML will allow an extended knowledge on the properties of KDML in terms of rice grain quality. This can make KDML105 an excellent source of genetic material for an effective breeding program in improving rice grain quality traits that are suitable for end users.

Acknowledments: We are thankful to Dr. Theerayut Toojinda for his help in the data analysis and critical reading of the manuscript. The Rockefeller Foundation graduate scholarship program supported this research.

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