

QTL Mapping of Genes Related with Grain Chemical Properties Based on Molecular Map of Rice

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ABSTRACT

This study was conducted to investigate the chromosomal locations and effects of quantitative trait loci (QTL) associated with chemical properties of rice (*Oryza sativa* L.). One hundred sixty four recombinant inbred lines (MGRILs) of F₁₁ were derived from the cross between Milyang 23, Tongil type, and Gihobyeo, japonica type. They were evaluated for 7 traits of chemical property in rice. Transgressive segregation was observed for all traits examined. Eight significant QTLs were detected (LOD \geq 2.0) for five traits, including two QTLs for amylose content, two QTLs for potassium content, one QTL for ratio of magnesium to potassium, one QTL for fat content and two QTLs for ash content. Phenotypic variation explained by each QTL ranged from 7.2% to 14.4%. However, no significant QTL was detected for magnesium and protein contents. In amylose content and ash content M alleles originated from Milyang 23 were responsible for increasing these traits and J alleles originated from Gihobyeo also responsible for increasing these traits. Pleiotropic effects of single QTLs on different traits are observed.

Key words : rice, molecular map, QTLs, recombinant inbred lines, chemical property.

Many traits in plants are quantitatively inherited. The advent of molecular marker technology has enabled investigation of the inheritance of quantitative traits (Tankley, 1993). These molecular techniques have been widely used to map quantitative trait loci (QTLs) in such crops as maize (Beavis et al., 1991; Stuber et al., 1987), tomato (Paterson et al., 1991), rye (Plaschke et al., 1993). In rice, several research groups have made a saturated genetic map (McCouch et al., 1988; Causse et al., 1994; Saito et al., 1991) and analyzed QTLs associated with agronomic traits including yield and its components (Lin et al., 1996; Wu et al., 1996; Xiao et al., 1996). Also, previous efforts have been focused on major quantitative genes associated with plant morphological traits such as plant height and days to heading (Cho et al., 1994; Li et al., 1995). There has been great interest in improvement of the eating quality of rice. Many researchers have worked on relation-

ships between eating quality and chemical properties in cooked rice using Near Infra-Red analysis (Yang, 1994; Lim, 1994). It has been suggested that low content of amylose, protein, and high ratio of Mg/K are related with good eating quality. However it is not clear yet to determine the effects of chemical properties on eating quality.

The present study was conducted to localize QTLs associated with chemical properties of rice and to estimate the contribution of each QTL in rice genome.

MATERIALS AND METHODS

Plant materials

Two elite rice cultivars, Milyang 23 (Tongil type) and Gihobyeo (japonica type) were crossed in 1988 at National Institute of Agricultural Science and Technology, Korea. Milyang 23 has poor eating quality with high amylose content. In contrast, Gihobyeo has good eating quality with low amylose content. Development of a population from F₁ plants was conducted by the Single Seed Descent (SSD) method. The population F₉ generation was transplanted at National Honam Agricultural Experiment Station (NHAES), southwest side of Korea, in 1993. The materials (F₁₁) used in this experiment were harvested at NHAES in 1995.

Analysis of chemical properties

Bulked seeds from each line were dehulled. Samples of brown rice were dried at 40°C for 48 hrs. The contents of amylose, protein, magnesium, potassium, lipid and ash were analysed by NIR (near infrared system, USA). Using an algorithm called SELECT, samples were divided into calibration and validation sets (Shenk & Westerhaus, 1991).

RFLP map and linkage analysis

Total DNA extraction, restriction endonuclease digestion, electrophoresis, Southern blotting, hybridization and

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autoradiography were carried out as described by McCouch et al. (1988). A total of 132 probes were used, including rice genomic DNA (RG), rice cDNA (RZ), barley cDNA (BCD), rice random genomic DNA (G), callus cDNA (C), *NatI*-linking clone (L), YAC end clones (Y), and cDNA clones made in Korea (KCD). All of these clones except KCD provided from Dr S. D. Tanksley at Cornell University and from Japan Rice Genome Research Program. The program MAPMAKER (Lander et al. 1987) was used to establish an RFLP map at a minimum LOD value of 3.0 and recombination fraction expressed in centiMorgan which was calculated by Kosambi mapping function (Kosambi, 1944). The order of markers was established using multiple point analysis at a LOD of 3.0.

QTL analysis

The analysis of QTL associated with each trait was performed using two different procedures: one-way analysis of variance (ANOVA) using PROC GLM procedure in Statistical Analysis System (SAS), (SAS Institution, 1988) and interval mapping in MAPMAKER/QTL (Lander & Bostein, 1989). The one-way ANOVAs involved testing the significance of the associations between marker genotypes and trait values at each locus. In interval mapping, a LOD score of 2.0 was used as the threshold for detecting QTL. The proportion of the total phenotypic variation explained by each QTL was cal-

culated as an iR^2 value (iR^2 = ratio of the sum of squares explained by the QTL to the total sum of squares).

RESULTS AND DISCUSSION

Transgressive segregation of traits

The frequency distribution of phenotypes for each trait in the F_{11} MGRILs is shown in Fig. 1. All traits approximately fit normal distributions. Milyang 23 and Gihobyeo had almost identical values for fat content, and cohesiveness of cooked rice. However, phenotypic values of some F_{11} MGRILs ranged beyond parents (e.g., transgressive segregants) for all traits. The occurrence of such transgression could be associated with the interactions of complementary QTL alleles from two parents. However in this study, it could not be concluded that our results of segregation were due to either complementary gene effects or overdominance of a major gene. These factors should be verified by further studies.

Correlation of traits

Correlations between traits were evaluated by regressing phenotypic values of one trait on those of another traits. The correlation coefficients among traits are presented in Table 1. For most of the correlations, the direction (+ or -) and the degree of correlations were consistent with other observations (Yang, 1994).

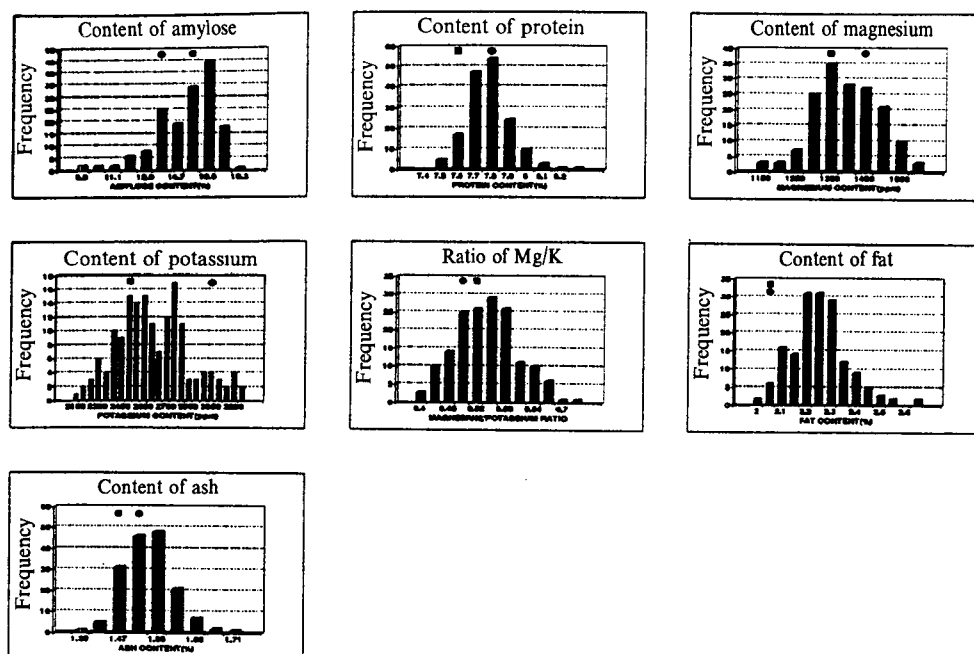


Fig. 1. Frequency distributions of chemical properties of brown rice in F_{11} MG RILs.

■ : Milyang 23 ● : Gihobyeo

Table 1. Correlation coefficients among chemical properties.

	AMY	PRO	Mg	K	Mg /K	Fat	Ash
Amylose (AMY)		-0.515**	-0.062	-0.638**	0.430**	-0.462**	-0.379**
Protein (PRO)			-0.048	0.514**	-0.401**	0.396**	0.372*
Magnesium (Mg)				-0.157	0.677**	-0.192*	-0.268**
Potassium (K)					-0.824**	0.628**	0.776**
Mg /K						-0.562**	-0.721**
Fat							0.816**
Ash							

*, ** : Significant at 5% and 1% level, respectively.

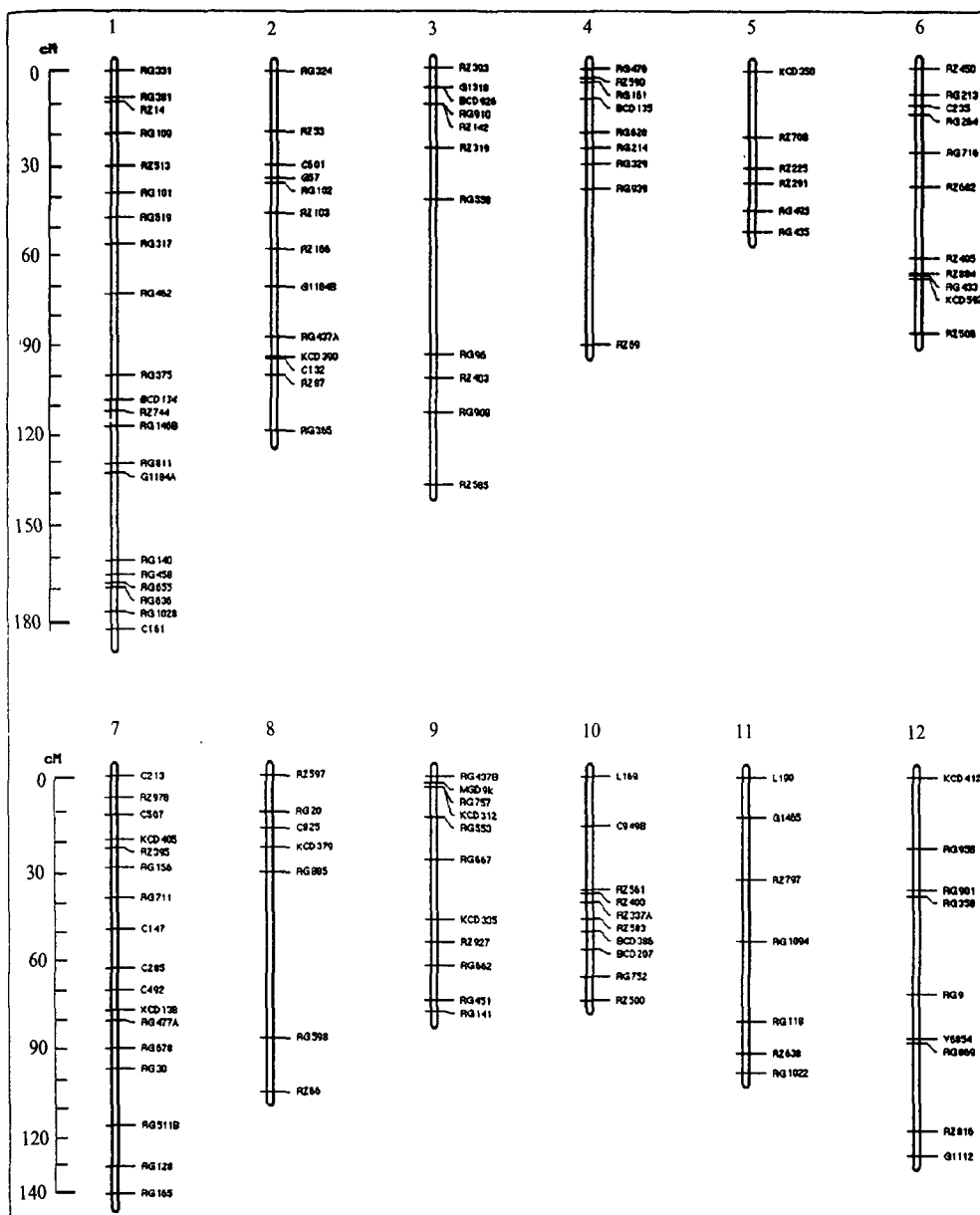


Fig. 2. RFLP linkage map of rice.

Table 2. Characteristics of QTLs associated with chemical properties of rice.

Trait	QTL name	Chromosome number	Interval	%Var [†]	Phenotypic effect [‡]
Amylose	AMY1	7	KCD405 – RZ395	8.7	-1.02
	AMY2	8	RZ597 – RG20	11.7	1.00
Potassium	POT1	6	C235 – RG264	10.2	0.11
	POT2	7	KCD405 – RZ395	7.3	0.05
Mg /K	MgK1	7	KCD405 – RZ395	14.4	-0.05
Fat	FAT1	7	KCD405 – RZ395	7.2	0.07
Ash	ASH1	7	KCD405 – RZ395	12.5	0.04
	ASH2	8	RG598 – RZ66	7.2	-0.03

† : Percentage of variance explained by each QTL.

‡ : The signs; + (omitted) and -, preceding phenotypic effect, represent that the J alleles in homozygous state had higher phenotypic effects than the respective M alleles in homozygous state, and that the J alleles in homozygous state had lower phenotypic effects than the respective M alleles in homozygous state respectively. (M, J alleles represent the alleles of the locus originated from Milyang 23 and Gihbyeon, respectively)

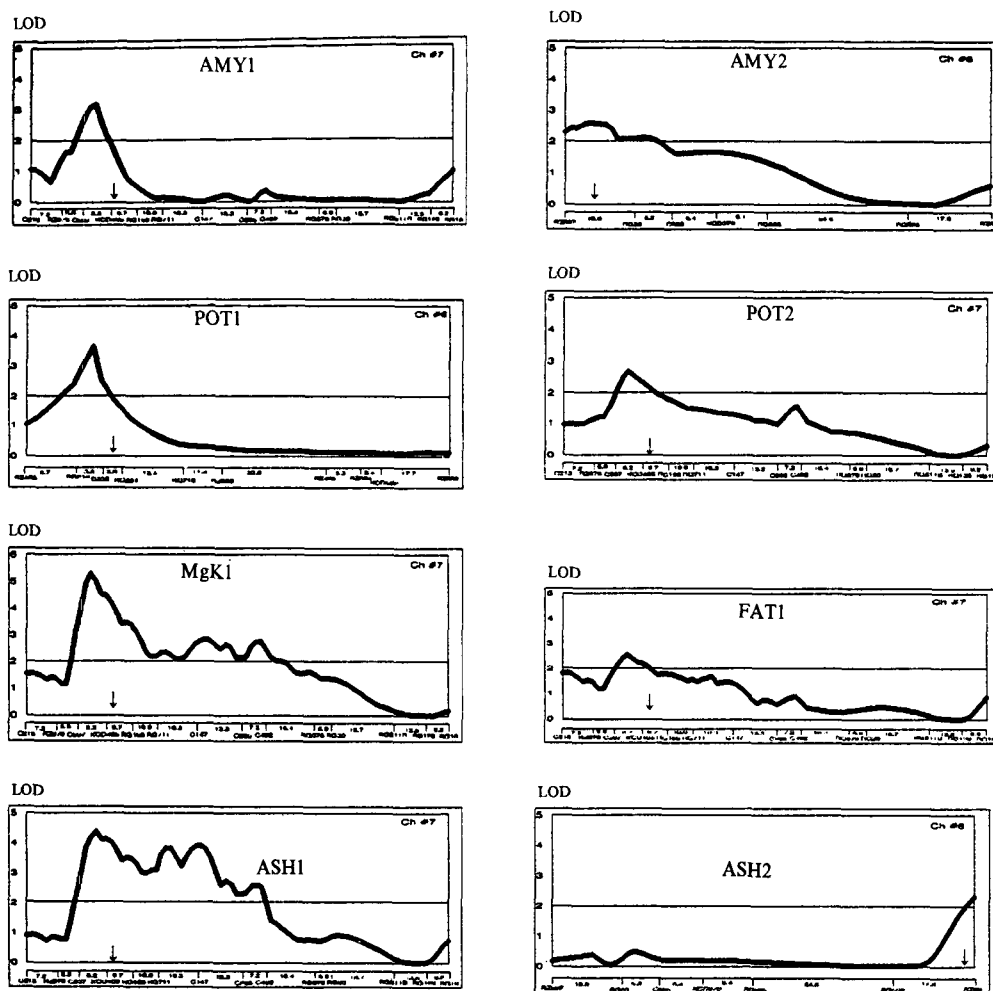


Fig. 3. QTL likelihood plots indicating LOD scores and chromosome numbers for grain chemical traits. Arrows show the position of QTL.

QTLs for traits

For each trait, the association of QTLs with DNA markers was assessed by single-point analysis and interval mapping. Both analyses gave similar results in identifying QTLs for each trait. Only the results from the interval mapping analysis are presented here. The QTLs for each trait are shown in Table 2 and their QTL likelihood plots are shown in Fig. 3.

Amylose content

Two QTLs were detected (AMY1, AMY2) for amylose content and their chromosomal locations were on chromosomes, 7 and 8. In AMY1, M alleles originated from Milyang 23 were responsible for increasing the amylose content. In AMY2, J alleles originated from Gihobyeo were also responsible for increasing amylose content. These two QTLs together explained 20.4% of the total phenotypic variation.

Potassium content

Two QTLs on chromosomes 6 and 7 significantly affected potassium content. Increase of potassium content was caused by J alleles at both QTLs. These two QTLs together explained 17.5% of the total phenotypic variation.

Ratio of magnesium to potassium

Only one QTL on chromosome 7 affected the Mg/K ratio. M alleles were associated with an increase of the Mg/K ratio at MgK1. This QTL explained 20.7% of the total phenotypic variation.

Fat content

Only one QTL was detected on chromosome 7 for content of fat. J alleles at this QTL increased the fat content. This QTL explained 7.2% of the total phenotypic variation.

Ash content

Two QTLs were identified on chromosomes 7 and 8 for content of ash. M alleles at ASH1 increased content of ash. J alleles at ASH2 also increased the ash content. These two QTLs together explained 19.7% of the total phenotypic variation.

Protein and magnesium content

No significant QTLs were detected for protein and magnesium content. More saturated frame map is thought to be required to detect QTLs for these traits.

Correlated traits often have QTLs sharing similar genomic location

As reported by Paterson et al. (1991) and Xiao et al. (1996), QTLs associated with traits which were correlated were mapped to the same chromosomal locations. The same trend was observed in our study. For example, fat content was highly correlated with ash content ($r=0.816$) and their QTLs were found at the almost same location of chromosome 7. In addition their allelic effects at each QTL were the same for each trait. Ash content and Mg/K ratio were showed a highly negative correlation ($r=-0.721$). QTLs associated with ASH2 and Mg/K ratio were mapped to similar locations in chromosome 7. In this case, the QTLs associated with both traits had opposite effects. Trait correlations may result from either pleiotropic effects of single genes or tight linkages of several genes controlling the traits (Lin et al., 1996). Pleiotropy was suggested at several chromosomal regions in our study. For example, QTLs associated with amylose, potassium, fat, and ash content as well as the Mg/K ratio were detected between KCD405-RZ395 on chromosome 7. This result suggested that genetic correlation among these traits might be due to pleiotropy or linkage of genes.

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