

Genetic Mapping of QTLs that Control Grain Characteristics in Rice (*Oryza sativa* L.)

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We performed a molecular marker-based analysis of quantitative trait loci (QTLs) for traits that determine the quality of the appearance of grains, using 120 doubled-haploid (DH) lines developed by another culture from the F₁ cross between 'Cheongcheong' (*Oryza sativa* L. ssp. Indica) and 'Nagdong' (*Oryza sativa* L. ssp. Japonica). The traits studied included length, width, and thickness of the grains, as well as length-to-width ratio and 1,000-grain weight. The objective of this study was to determine the genetic control of these traits in order to formulate a strategy for improving the appearance of this hybrid. Within the DH population, five traits exhibited wide variation, with mean values occurring within the range of the two parents. Three QTLs were identified for grain length on chromosomes 2, 5, and 7. Three QTLs were mapped for grain width on chromosome 2: qGW2-1, qGW2-2, and qGW2-3. Six chromosomes were identified for the grain length-to-width ratio; four of these were on chromosome 2, whereas the other two were on chromosomes 7 and 12. One QTL influencing 1,000-grain weight was identified and located on chromosome 8. The results presented in the present study should facilitate rice-breeding, especially for improved hybrid-rice quality.

Key words : Doubled haploid, grain quality, QTL, rice

Introduction

Rice is one of the most important cereal crops and a staple food in Asia. Grain yield of rice is determined by three major components: number of panicles per plant, number of grains per panicle, and grain weight [2]. Grain weight is the most reliable trait of rice yield and is determined by a quantitative trait locus (QTL). Grain shape, a typically complex quantitative trait, is closely associated with grain weight, and usually determined by grain length (GL), width (GW), thickness (GT) and the length to width ratio (LWR) [11]. Besides its contribution to yield, grain shape is an important quality trait that has a major impact on the market value of rice products. A long, slender grain of rice is generally preferred by consumers in southern China, the USA, and South and Southeast Asian countries, whereas consumers in Japan,

Korea, and northern China prefer a rice grain that is short and round [4]. Since characteristics related to grain appearance are quantitatively inherited, it is difficult for breeders to efficiently improve them using conventional selection methods [8]. The identification of major QTLs for grain shape and weight is an important objective for enhancing genetic knowledge and breeding programs for rice. This has led to the development of DNA markers and linkage maps that have provided new opportunities for genetic improvement of grain quality. Quantitative traits, such as grain size, have been studied extensively, and QTLs governing this important trait have been identified [10, 11, 17]. Redoña and Mackill [10] found seven, four, and three QTLs for GL, GW, and grain shape (GS), respectively, and reported that the LWR was primarily controlled by loci on chromosomes 3 and 7, which coincided with the most important QTLs identified for GL and GW. Similarly, a locus on chromosome 7 primarily controlled GL, whereas GW was primarily controlled by a locus on chromosome 2. The LWR was controlled by loci on chromosomes 2 and 7 [10]; these loci coincided with the ones identified for GL and GW. We performed a molecular marker-based analysis of QTLs for the traits that determine quality of appearance of grains using 120 doubled haploid (DH) lines developed by anther culture

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from F₁ hybrids from a cross between 'Cheongcheong' (*Oryza sativa* L. ssp. Indica) and 'Nagdong' (*Oryza sativa* L. ssp. Japonica). The traits studied included GL, GW, GT, LWR, and weight of 1,000 grains (TGW). The objective of our study was to determine the genetic control of these traits in this hybrid in order to formulate a strategy for improving grain appearance.

Materials and Methods

Phenotypic measurements

A population of 120 DH lines derived from a cross between 'Cheongcheong' (*Oryza Sativa* L. ssp. Indica) and 'Nagdong' (*Oryza Sativa* L. ssp. Japonica) was used in this study. Our study was conducted on the experimental farm of Kyungpook National University in 2014. Seventeen plants were planted in each row with 15 cm between plants within rows and 30 cm between rows. The parents 'Cheongcheong' and 'Nagdong' were planted in ten plots as controls. After harvesting, rice grains were kept for at least three months before testing. Brown rice was used in this study. Five yield-related traits, including GL (mm), GW (mm), LWR, GT (mm), and TGW (g) were examined for 20 randomly selected unbroken rice grains. We measured GL, GW, and GT for each DH and parent grain using a Vernier calliper (Mitutoyo, Japan). The values were averaged and used as the measurement for GL, GW, and GT of individual grains. The LWR of grains was calculated as GL divided by GW, a measurement that reflects the shape of the grain. The TGW was obtained using an electronic scale (Ohaus, USA).

QTL analysis

A genetic map consisting of 217 DNA markers was constructed using MAPMAKER/EXP version 3.0. Distances between the markers were presented in centiMorgans (cM) using the Kosambi mapping function. We used the completed genetic map at the Plant Molecular Breeding Laboratory in the school of Applied Biosciences at Kyungpook National

University. To identify the putative QTLs, composite interval mapping was employed using WinQTLCart Cartographer 2.5. An LOD value of 3.0 was used as the threshold for obtaining a QTL at $p < 0.05$. QTL mapper 1.6 was used to detect the main effects of QTLs, epistatic interactions, and their environmental interactions with a threshold of $p \leq 0.005$. The QTL locations identified in the current research were compared with previously reported QTLs affecting rice grain size and related traits using a genomic database. The markers were named using the prefix RM following Temnykh et al. [12, 13]. The whole genome spanned across 2067.1 cM with an average interval of 9.5 cM. The frequency distribution and correlation coefficients of traits were analyzed using SPSS (IBM SPSS Statistics, version.22, North Carolina, USA).

Results

Phenotypic values of the traits were significantly different ($p < 0.001$) between the parents. LWR exhibited a strong positive correlation. Negative correlations existed in both GW and GT (Table 1). GL and GS of the female parent of 'Cheongcheong' were 0.83 mm and 0.41 mm greater than those of the male parent of 'Nagdong,' respectively, whereas GW of 'Cheongcheong' was 0.14 mm less than that of 'Nagdong.' The 'Cheongcheong' grain is significantly longer and heavier than that of 'Nagdong.' Because of the similar performance of GW and GT, the greater TGW of 'Cheongcheong' grains was mainly caused by the longer GL, as shown in Table 2.

Within the DH population, the five traits exhibited wide variation with nearly all mean values falling into the value ranges of the two parents. Phenotypic distributions for the five traits in the DH population are shown in Fig. 1. Trait distributions were continuous and normal, confirming quantitative inheritance of the traits studied.

A linkage map of 217 SSR markers from 5 linkage groups was constructed (Fig. 2).

Table 1. Correlation coefficients among 5 grain traits of 120 DH lines from a cross of 'Cheongcheong' and 'Nagdong'

Grain traits	GL 1	GW 2	GT 3	LWR 4	TGW 5
Grain length (GL 1)	1.00				
Grain width (GW 2)	-0.425**	1.00			
Grain thickness (GT 3)	-0.255**	0.767**	1.00		
Ratio of length to width (LWR 4)	0.864**	-0.811**	-0.563**	1.00	
Thousand grain weight (TGW 5)	0.317**	0.583**	0.677**	-0.110	1.00

** means significant at 1% level of probability.

Table 2. The Grain phenotypes of 120 DH lines from a cross of ‘Cheongcheong’ and ‘Nagdong’

Grain traits	Parents		DH population			
	Cheongcheong	Nagdong	Means	Range	Means	Heritability (%)
Grain length	5.74±0.15	4.91±0.09	5.32±0.12	4.27-7.26	5.41±0.18	98.0
Grain width	2.66±0.15	2.80±0.09	2.73±0.12	2.14-3.05	2.66±0.11	100.0
Grain thickness	1.96±0.07	2.00±0.10	1.98±0.09	1.61-2.19	1.9±0.09	95.0
Ratio of length to width	2.16±1.63	1.75±1.49	1.96±1.56	1.59-3.32	2.05±1.93	99.5
Grain weight	23.50±0.41	21.00±1.26	22.25±0.84	15.00-26.5	20.94±3.13	94.0

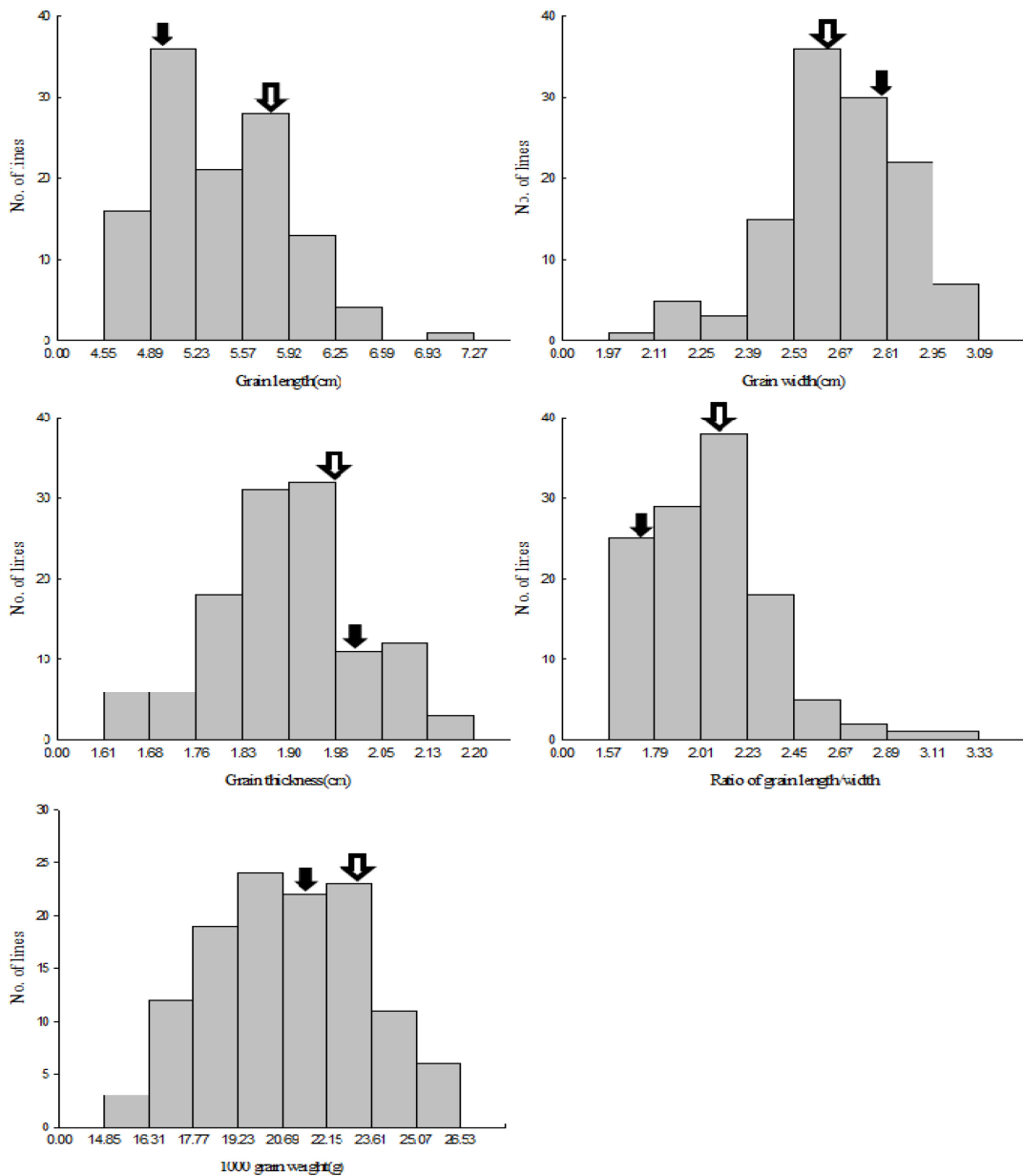


Fig. 1. Frequency distribution of the grain appearance in a population of 120 DH lines derived from a cross between ‘Cheongcheong’ and ‘Nagdong’. White arrow represents for ‘Cheongcheong’, and black arrow for ‘Nagdong’.

Three QTLs were identified for GL on chromosomes 2, 5, and 7 (Table 3, Fig. 2). The QTL located on chromosome

7 (qGL7), RM21582, exhibited the greatest effect on the GL with an LOD score of 5.18, and explained 55% of the total

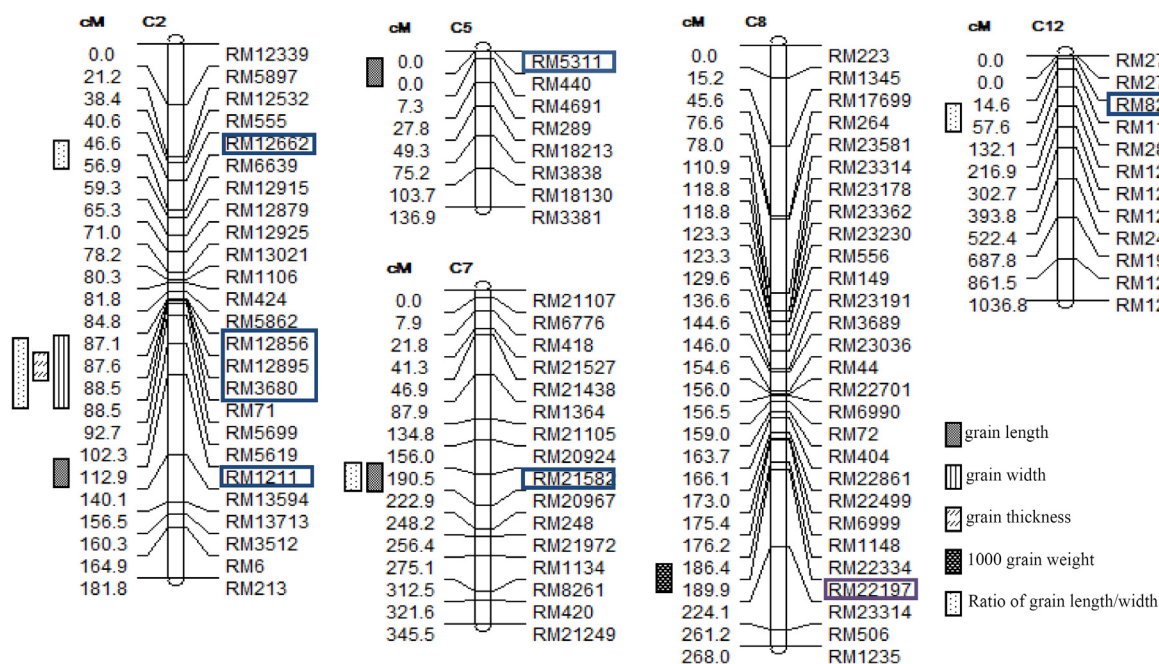


Fig. 2. The genetic regions associated with the grain length in a population of 120 DH lines derived from a cross between ‘Cheongcheong’ and ‘Nagdong’. Mapped markers for 5 rice grain traits are boxed along with the symbol of associated trait in opposite side. cM : centi Morgan, C2 : chromosome 2, C5 : chromosome 5, C7 : chromosome 7, C8 : chromosome 8, C12 : chromosome 12.

Table 3. The main effect QTLs of the grain appearance in a population of 120 DH lines derived from a cross between ‘Cheongcheong’ and ‘Nagdong’

Characteristics	QTLs	Chr.	Interval markers ^a	LOD ^b	Add ^c (%)	R ^{2d} (%)	Increasing Effect
Grain length	qGL2	2	RM1211	3.17	17	50	Cheongcheong
	qGL5	5	RM5311	3.45	17	53	Cheongcheong
	qGL7	7	RM21582	5.18	19	55	Cheongcheong
Grain width	qGW2-1	2	RM12856	3.74	-19	-24	Nagdong
	qGW2-2	2	RM12895	6.64	-24	-31	Nagdong
	qGW2-3	2	RM3680	4.98	-22	-28	Nagdong
Grain thickness	qGT2	2	RM12895	3.36	-4	-33	Nagdong
Ratio of grain length/width	qLWR2-1	2	RM12662	7.51	-15	-61	Nagdong
	qLWR2-2	2	RM12856	7.48	20	65	Cheongcheong
	qLWR2-3	2	RM12895	13.51	22	58	Cheongcheong
	qLWR2-4	2	RM3680	10.88	21	60	Cheongcheong
	qLWR2-7	7	RM21582	5.07	9	59	Cheongcheong
1000 grain weight	qLWR2-12	12	RM8216	3.15	9	62	Cheongcheong
	qTGW8	8	RM22197	3.15	-10.5	-31	Nagdong

^aInterval markers are those within the significance threshold on each border of the QTL range, ^bLOD means logarithm of odds deviation, ^cPositive values of the additive effect indicate that alleles from ‘Cheongcheong’ are in the direction of increasing the traits, ^dR² the proportion of evaluated phenotype variation attributable a particular QTL was estimated by the coefficient of determination, ^eIncrease allele is the source of the allele causing an increase in the measured trait.

phenotypic variation. The QTL on chromosome 5 (qGL5), RM5311, explained 53% of phenotypic variation, with an LOD score of 3.45. Another QTL on chromosome 2 (qGL2), RM1211, explained 50% of the phenotypic variation, with

an LOD score of 3.17. The additive effect of a single QTL ranged from 17% to 19%. Overall, three QTLs, alleles from ‘Cheongcheong’ exhibited an increasing effect on GL.

Three QTLs were mapped for GW on chromosome 2;

qGW2-1, qGW2-2, and qGW2-3. Among them, the QTL with the largest effect was qGW2-2 with an LOD score of 6.64. It explained 31% of the phenotypic variation and had an additive effect of 24% for increased GW. QTLs qGW2-1 and qGW2-3 explained 24% and 28% of the phenotypic variation, with LOD scores of 3.74 and 4.98, respectively. All of the putative QTL alleles for increasing GW were from 'Nagdong'. The additive effect of a single QTL ranged from 19% to 24%.

Six chromosomes were identified for LWR. Four out of the six were on chromosome 2, whereas the other two were on chromosomes 7 and 12. The two QTLs on chromosome 2, qLWR2-3 and qLWR2-4, had particularly high LOD values of 13.51 and 10.88, and additive effects of 22% and 21%, respectively. However, both of these QTLs were 'Cheongcheong' alleles and were responsible for an increase in LWR. The other four QTLs explained less than 20% of the additive effect. 'Nagdong' alleles at qGR2-1 (chromosome 2) caused an increase in GR, whereas in the other QTLs (alleles from 'Cheongcheong') were responsible for increases in GS.

One QTL was mapped for GT on chromosome 2 at RM 12895. The LOD score was 3.36 explaining 33% of the phenotypic variation. The additive effect of 4% for increased GT was realized and the putative QTL alleles for increased GT were from 'Nagdong'.

One QTL influencing TGW was identified and was located on chromosome 8. The LOD score was 3.15 and showed an additive effect of 10.5% on the trait that explained 31% of the total phenotypic variation. The putative QTL alleles for increased TGW were from 'Nagdong'.

Discussion

Grain shape is the main attribute that determines the quality of appearance of rice grains. Rice grain shape and size are highly correlated with yield and quality of appearance. Many QTLs have been previously mapped for rice grain size, and most QTLs for grain size and weight were mapped on chromosomes 2, 3, and 5. We characterized the genetic basis of the five studied QTL traits that are related to the quality of appearance of rice grains in populations from a cross between the parents 'Cheongcheong' and 'Nagdong'. For successful QTL mapping, large differences between trait values for the parents are essential. In this study, the parents 'Cheongcheong' and 'Nagdong' differed significantly for all the traits studied (Table 2).

Fourteen main-effect QTLs for GL, GW, GT, and TGW were detected on five rice chromosomes; 2, 5, 7, 8, and 12. The percentage of phenotypic variation explained by each QTL ranged from 24 to 65%, with an average of 47.9%. Among these 14 QTLs, nine of them accounted for phenotypic variation greater than 45%, whereas only four QTLs explained more than 60% of the phenotypic variation (Table 3). Each trait studied was governed by one or two major QTLs that explained a large percentage of phenotypic variation and several minor QTLs explaining smaller percentages of phenotypic variance. Similar findings have been previously reported in regards to a major locus similar to the qGW2 locus on chromosome 2 that controlled GW. Qin et al. [8] identified two major QTLs controlling GW on chromosomes 2 (RM 318-2038) and 6 (6008-6011) that accounted for 11% and 12% of phenotypic variation, respectively. The locus detected on chromosome 2 in this study (qGW2) is likely the same locus described in earlier studies [3, 9, 10, 15, 16]. Similarly Tan et al. [11] identified a QTL on chromosome 7 that affected GL. Further, the locus identified on chromosome 7 in this study, qGL7 (RM 21582), for GL was a different locus compared to the one mapped by Yan et al. [16]. Yan et al. [15] reported a major QTL for LWR on chromosome 7, and two minor QTLs on chromosomes 2 and 6. However, in this study, two major loci were detected on chromosome 2, qLWR2 (RM12856) and 12 qLWR12 (RM8216), for the LWR. Previous detection of a major QTL controlling GT on chromosome 2 was similar to the qGt2 locus at RM12895, suggesting it is important and prevalent in other populations. However, Yan et al. [15] detected QTLs on chromosome 3 that affected four of the five traits investigated, namely: GW, LWR, GT, and TGW. In our study, the QTLs around chromosome 2 pleiotropically affected four of the five investigated traits; GL, GW, GT, and LWR. Grain shape measured using the LWR was mostly controlled by loci on chromosomes 2 and 7 that coincided with the most important QTLs identified for width and length. One locus associated with TGW was detected at chromosome 8, accounting for 31% of the phenotypic variation. The QTL identified by Redoña et al. [10] on chromosome 8 might be allelic to a locus near RM22197. However, other QTLs on chromosome 4 were not associated with any of the markers detected in the present study [1, 6, 10, 14]. It is surprising that no QTL for TGW, GL, or GW was detected on chromosome 3 in our study. A QTL associated with GW or GL has been reported in the locus on rice chromosome 3 in many different populations

[5, 7, 10, 14]. This study identified several QTLs from both parents that control the size and shape of rice grains. Marker aided introgression of these QTLs into desired genetic backgrounds will be a more effective approach in breeding for these traits in rice. The results presented herein should facilitate rice breeding, especially for improved hybrid rice quality and appearance.

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초록 : 벼의 낱알 특성에 관여하는 양적형질유전자좌 분석

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미립 품질 향상을 위하여 미립 형태를 결정하는 특성을 위한 분자유종기술을 확립하기 위하여 미립과 관련된 양적형질 유전자좌를 탐색하고, 이들 환경요인과 상호작용 효과를 분석한 결과는 다음과 같다. 인디카 품종인 '청청'과 자포니카형인 '낙동'이 교배된 조합 F₁의 약배양에 의해 양성된 120 계통(DH 집단)과 217개의 DNA 마커를 이용하여 전체 길이가 2,067cM이고, 마커간 평균거리가 9.5cM인 유전자 지도를 작성하였다. 미립형태 관련 유전자좌 분석에서 미립의 외형인 길이, 폭, 두께, 장폭비, 천립중과 관련하여 14개의 QTL이 탐색되었다. 현미의 미립길이 관련 3개의 QTL (qGL2, qGL5, qGL7), 미립 폭 관련 3개의 QTL (qGW2-1, qGW2-2, qGW2-3), 미립 두께 관련 1개의 QTL (qGT2), 장폭비 관련 6개의 QTL (qLWR2-1, qLWR2-2, qLWR2-3, qLWR2-4, qLWR7, qLWR12) 및 천립중 관련 1개의 QTL (qTGW8)이 선발되었다. 미립 장폭비 관련 4개의 QTL은 미립길기와 미립두께에서 동일한 염색체 상에서 확인되었다. 본 연구에서 구명된 QTL 마커들은 쌀 품종개량을 위하여 이용될 수 있을 것이라 판단된다.