Identification of Quantitative Trait Loci Associated with Grain Shape Using Cheongchenong/Nagdong Double Haploid Lines in Rice

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ABSTRACT We performed a molecular marker-based analysis of quantitative trait loci (QTLs) for the traits that determine appearance quality of the grains using 120 double haploid (DH) lines developed by anther culture from F₁ hybrid of a cross between Cheongcheong (*Oryza sativa* L. ssp. Indica) and Nagdong (*Oryza sativa* L. ssp. Japonica). The traits studied included grain length, grain width, grain thickness, length to width ratio and thousand grain weight. This experiment was conducted with two replications of 2013 and 2014. A linkage map with 217 DNA markers was constructed spanning across 2,067.1 centiMorgans (cM) at an average interval of 9.5 cM between markers and covering 12 rice chromosomes. Interval mapping procedure was used to identify the QTLs controlling five grain traits and QTLs detected were further confirmed using composite interval mapping. A total of 24 QTLs affecting grain appearances were identified and mapped on all the twelve chromosomes for 2 years at grain quality. Nine of the 24 QTLs were reproducibly detected in two year trials. Major QTL for grain length was detected on chromosome 5 in 2013 with a phenotypic variation of 64% and chromosome 7 in 2014 that explained 55% of the phenotypic variation. The QTL findings in this study will in future facilitate the gene isolation and breeding application for improvement of rice grain shape and yield.

Keywords Quantitative trait loci, Double haploid, Grain quality, Rice

INTRODUCTION

Rice is one of the most important cereal crops and a staple food for 40% of the world population. More than 90% of rice is produced and consumed in Asia (Khush 2004). The current world population of 7.4 billion is expected to reach 8.5 billion by 2030 and rice production must increase by 50% in order to meet the growing demand. High yield is an important consideration for commercial and private producers, but demand for superior grain quality is increasingly a priority for international export markets in all rice producing areas of the world (Juliano *et al.* 1990). Grain yield in rice is determined by three major components; number of panicles per plant, number of grains per panicle and grain weight (He *et al.*

1999; Huang *et al.* 2013). Grain weight is the most reliable trait of rice grain yield and is conditioned by quantitative trait locus (QTL). Grain shape, a typical complex quantitative trait, is closely associated with grain weight and usually measured by grain length, width, thickness and length-to-width ratio (Tan *et al.* 2000). Besides its contribution to grain yield, grain shape is an important quality trait that has a major impact on the market values of rice grain 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 (Juliano and Villareal 1993). Since these characteristics related to grain appearance are quantitatively inherited, it is difficult for

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breeders to efficiently achieve improvement by using convectional selection methods (Qin *et al.* 2008). The identification of major quantitative trait loci (QTLs) for grain shape and grain weight is an important objective of rice genetic research and breeding programs, this has led to development of DNA markers and linkage maps which have provided new opportunities for genetic improvement of rice grain quality with physical and chemical properties (Cho *et al.* 2010). Quantitative traits like the grain size in rice has been studied extensively and QTLs governing this important trait identified (Redoña and Mackill 1998; Tan *et al.* 2000). Redoña and Mackill (1998) found seven, four and three QTLs for grain length, width and shape respectively and reported that the grain length/width ratio was mostly controlled by loci on chromosome 3 and 7 that coincided with the most important QTLs identified for grain length and grain width. Similarly in this study a locus on chromosome 7 and was found to have a major control on the grain length, grain width was majorly controlled by a locus on chromosome 2. The length/width ratio was controlled by loci on chromosome 2 and 7, as reported by Redoña and Mackill (1998), these loci coincided with the ones identified for grain length and width. In the present study, we performed a molecular marker-based analysis of QTLs for the traits that determine appearance quality of the grains using 120 double haploid (DH) lines developed by anther culture from F₁ hybrid of a cross between Cheongcheong (*Oryza sativa* L. ssp. Indica) and Nagdong (*Oryza sativa* L. ssp. Japonica). The traits studied included grain length, grain width, grain thickness, length to width ration and thousand grain weight. The objective of this study was to determine the genetic control of these traits in this cross in order to formulate a strategy for improving the appearance quality of this hybrid.

MATERIALS AND METHODS

Materials and field arrangement

A population of 120 DH lines derived from a cross between Cheongcheong and Nagdong (CNDH) was used in this study. This was conducted on the experimental farm of Kyungpook National University in 2013 and 2014.

Thirty plants were planted in each row with 15 cm between plants within each row and 30 cm between rows. The parental lines of Cheongcheong and Nagdong were planted in ten plots as controls.

Phenotypic measurements

Grains were harvested from different replications and kept at room temperature for at least three months before testing. Brown rice was used in this study. Five yield-related trait including grain length, grain width, ratio of grain length to width, grain-thickness, and 1,000-grain weight were examined from twenty randomly selected unbroken rice grains. Grains of each DH lines and parents were lined up lengthwise along a vernier caliper (Mitutoyo, Kawasaki, Japan) to measure length, after which the grains were arranged in the breadth to measure the width and thickness. The values were averaged and used as the measurements of length, width and thickness of individual grains. The length width ratio of the grains was calculated as grain length divided by width, a measurement which reflects the shape of the grains. The weight of 1,000-grain weight was measured using the Electronic scale (Ohaus, Parsippany, NJ, USA). All experiments were replicated at least three times, and all data were analysed using the SPSS program (IBM SPSS Statistics ver. 22; IBM Co., Armonk, NY, USA).

Quantitative trait loci analysis

A genetic map consisting of 217 DNA markers was constructed using MAPMAKER/EXP ver. 3.0. Distances between the markers were presented in centiMorgans (cM) using the Kosambi function. The completed genetic map used by the Plant Molecular Breeding Laboratory at the school of Applied Biosciences in Kyungpook National University. To identify the putative QTLs, composite interval mapping was employed using WinQTL Cartographer 2.5. A logarithm of odds deviation (LOD) value of 3.0 was used as the significant threshold for claiming a QTL at $P < 0.05$. QTL mapper 1.6 was used to detect the main effects QTLs, epistatic interactions and their environmental interaction with a threshold of $P \leq 0.005$. The QTLs 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 prefixes RM were named according to Temnykh *et al.* (2000; 2001). The whole genome spanned across 2,067.1 cM with an average interval of 9.5 cM. The frequency distribution and correlation coefficients of traits were analyzed using the SPSS program (IBM SPSS Statistics ver. 22).

RESULTS

Phenotypic evaluations

Phenotypic grain length and ratio of grain length to width of the female parent Cheongcheong were more than the male parent Nagdong for the two years. Grain length for Cheongcheong was 0.83 mm and 0.96 mm more than Nagdong in 2014 and 2013, respectively. Ratio of grain length/width was 0.41 mm and 0.46 mm more than that of Nagdong for 2014 and 2013, respectively. The Cheongcheong grain was significantly longer and heavier than that of Nagdong for the two years. Due to almost similar performance of grain width and grain-thickness, the heavier 1,000-grain weight of Cheongcheong grain was mainly raised by the longer grain length, as shown in Table 1.

Among the DH population, the five traits exhibited wide variation with the mean values of performance nearly falling into the range between that of the two parents. All the five traits showed high broad heritability in both years.

The phenotypic distributions for the five traits in DH population are shown in Fig. 1. Trait distributions were normal distribution confirming quantitative inheritance of the traits studied.

Phenotypic values of the traits studied showed both significantly positive and negative correlations ($P < 0.001$) as shown in Table 2. Highly significant correlations were observed between grain width and grain thickness ($r=0.767^{**}$, $r=0.784^{**}$) or the ratio of grain length to width ($r=-0.811^{**}$, $r=-0.827^{**}$) for the two years respectively. Grain weight showed significantly positive correlation to the grain width ($r=0.583^{**}$, $r=0.558^{**}$) and grain thickness ($r=0.677^{**}$, $r=0.603^{**}$), but negative correlation to the ratio of grain length to width ($r=-0.110^{**}$, $r=-0.080$), respectively for the two years. Negative correlations also existed in both grain length and grain thickness.

Simple sequence repeat (SSR) map construction

A linkage map of 217 SSR markers from 12 linkages groups was constructed (Fig. 2).

Quantitative trait locus mapping traits

A total of 24 QTLs affecting grain appearances were identified and mapped on all the twelve chromosomes (Table 3), 47 QTLs were mapped in 2013 and 14 QTLs were mapped in 2014 (data not shown).

Among them five QTLs, *qGR2*, *qGL7*, *qGR7*, *qGR12*, and *qTWG8*, were located on the same regions of

Table 1. The grain phenotypes of 120 DH lines from a cross of Cheongcheong and Nagdong.

Grain trait	Year	Parent		DH ²⁾ population		
		Cheongcheong	Nagdong	Range	Mean	Heritability
Grain length (mm)	2014	5.74±0.15	4.91±0.09	4.27-7.26	5.41±0.18	98.0
	2013	5.88±0.14	4.92±0.12	4.75-7.37	5.31±0.16	100.0
Grain width (mm)	2014	2.66±0.15	2.80±0.09	2.14-3.05	2.66±0.11	100.0
	2013	2.71±0.07	2.87±0.07	2.12-3.15	2.74±0.09	99.4
Grain thickness (mm)	2014	1.96±0.07	2.00±0.10	1.61-2.19	1.90±0.09	95.0
	2013	1.99±0.06	2.04±0.05	1.65-2.22	1.94±0.08	98.2
Ratio of length to width (%)	2014	2.16±1.63	1.75±1.49	1.59-3.32	2.05±1.93	99.5
	2013	2.17±0.02	1.71±0.54	1.57-3.17	2.04±1.76	97.8
Grain weight (g)	2014	23.50±0.41	21.00±1.26	15.00-26.50	20.94±3.13	94.0
	2013	23.83±0.29	22.0±0.50	29.50-16.50	22.37±2.86	100.0

Values are presented as mean±standard deviation, range, or percentage only.

²⁾DH: double haploid.

Fig. 1. Frequency distributions of five traits associated with grain appearance in Cheongcheong/Nagdong doubled haploid population. 'C' and 'N' indicate mean values of Cheongcheong and Nagdong in 2013 and 2014, respectively.

Table 2. Correlation analysis among five grain traits of 120 DH lines from CNDH from a cross Cheongcheong and Nagdong.

Grain trait	GL ²⁾		GW		GT		GR		TGW	
	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013
GL	1.00	1.00								
GW	-0.425**	-0.441**	1.00	1.00						
GT	-0.255**	-0.337**	0.767**	0.784**	1.00	1.00				
GR	0.864**	0.859**	-0.811**	-0.827**	-0.563**	-0.632**	1.00	1.00		
TGW	0.317**	0.367**	0.583**	0.558**	0.677**	0.603**	-0.110	-0.080	1.00	1.00

**These marks mean significant at the 1% level.

²⁾DH: double haploid, GL: grain length, GW: grain width, GT: grain thickness, GR: ratio of length to width, TGW: thousand grain weight.

chromosomes under the two years. The Cheongcheong alleles contributed to 36 QTLs for grain length, ratio of length to width and thousand grain weight in 2013 whereas the remaining 11 QTLs were contributed by Nagdong

alleles for grain width, weight, thickness, grain length and ratio of length to width. In 2014, Cheongcheong alleles contributed 8 QTLs for grain length and ratio of length to width whereas Nagdong alleles contributed to 6 QTLs for

Fig. 2. The genetic regions associated with the grain length for 2 years in Cheongcheong/Nagdong doubled haploid. cM: centiMorgans, GL: grain length, GW: grain width, GT: grain thickness, GR: Ratio of grain length/width, TGW: 1,000-grain weight.

ratio of length to width and thousand grain weight (data not shown).

Grain length

Three QTLs; *qGL2*, *qGL5*, *qGL7* were identified in 2014 for grain length at the regions RM1211 on chromosome 2, RM5311 on chromosome 5, RM21582 on chromosome 7 and *qGL5*, *qGL7* and *qGL9* (RM23914) were identified in 2013. Two QTLs, *qGL5* and *qGL7* were detected in both years at the same loci. The QTL located on chromosome 7 (*qGL7*) RM21582 showed the largest effect in 2014 with an LOD score of 5.18 and phenotypic variation of 55%, while

in 2013, QTL located on chromosome 5 (*qGL5*) RM5311 showed the largest effect on the grain length, with an LOD of 10.11 and explaining 64% of the total phenotypic variation. Cheongcheong alleles showed increased effect on grain length for all the QTLs, (*qGL2*, *qGL5*, and *qGL7*) in both years except *qGL9* whose Nagdong alleles increased the grain length effects.

Grain width

Three QTLs were mapped for the grain width, *qGW1* (RM12285), *qGW3* (RM15448), and *qGW10* (RM24934) in 2013 and one QTL in 2014; *qGW2* (RM12895). The

Table 3. The main effect quantitative trait loci (QTLs) of the grain appearance for 2 years in Cheongcheong/Nagdong doubled haploid population.

Characteristic	QTLs	Chromosome	Peak closed marker ^{z)}	LOD ^{y)}	Add (%) ^{x)}	R ² (%) ^{w)}	Increasing ^{v)} effect
Grain length	<i>qGL2</i>	2	RM1211	3.17	17	50	Cheongcheong
	<i>qGL5</i>	5	RM5311	10.11	24	64	Cheongcheong
	<i>qGL7</i>	7	RM21582	7.86	21	63	Cheongcheong
	<i>qGL9</i>	9	RM23914	3.34	-13	62	Nagdong
Grain width	<i>qGW1</i>	1	RM12285	3.44	-7	50	Nagdong
	<i>qGW2</i>	2	RM12895	6.64	-24	-31	Nagdong
	<i>qGW3</i>	3	RM15448	3.59	-8	55	Nagdong
	<i>qGW10</i>	10	RM24934	3.01	-6	51	Nagdong
Grain thickness	<i>qGT2</i>	2	RM12895	3.36	-4	-33	Nagdong
	<i>qGT10</i>	10	RM12895	3.36	-4	33	Nagdong
Ratio of grain length/width	<i>qGR1</i>	1	RM12285	6.39	9	68	Cheongcheong
	<i>qGR2</i>	2	RM12895	49.33	22	69	Cheongcheong
	<i>qGR3</i>	3	RM15063	12.84	11	69	Cheongcheong
	<i>qGR4</i>	4	RM16429	3.34	-4	61	Nagdong
	<i>qGR6</i>	6	RM527	3.53	4	63	Cheongcheong
	<i>qGR7</i>	7	RM21582	16.15	8	61	Cheongcheong
	<i>qGR8</i>	8	RM223	11.49	9	68	Cheongcheong
	<i>qGR9</i>	9	RM24002	3.45	6	62	Cheongcheong
	<i>qGR10</i>	10	RM25128	5.50	5	63	Cheongcheong
	<i>qGR11</i>	11	RM167	6.96	5	63	Cheongcheong
	<i>qGR12</i>	12	RM8216	7.72	6	63	Cheongcheong
Thousand grain weight	<i>qTGW1</i>	1	RM1297	4.23	-1.07	48	Nagdong
	<i>qTGW2</i>	2	RM6	3.94	1.0	45	Cheongcheong
	<i>qTGW8</i>	8	RM23230	4.04	0.99	41	Cheongcheong

^{z)}Those within the significance threshold on each border of the quantitative trait locus (QTL) range.

^{y)}Logarithm of odds deviation.

^{x)}Positive values of the additive effect indicate that alleles from Cheongcheong are in the direction of increasing the traits.

^{w)}R² the proportion of evaluated phenotype variation attributable a particular QTL was estimated by the coefficient of determination.

^{v)}Increase allele is the source of the allele causing an increase in the measured trait.

additive effect of a single QTL ranged from 6% to 24%. In all the putative QTLs, alleles for increased grain width were from Nagdong. In 2014, all the three QTLs were detected on chromosome 2 with *qGW2* (RM12895) showing the largest effect with an LOD score of 6.64, phenotypic variation of 31% and increasing additive effect of 24% from Nagdong allele. QTLs were detected on chromosome 1, 3, and 10. *qGW3* (RM15448) on chromosome 3 showed the largest effect with an LOD score of 3.59, phenotypic variation of 55% and an increasing additive effect of 8% from Nagdong allele.

Grain thickness

Two QTLs were detected for grain-thickness, *qGT2* and *qGT10*, on chromosome 2 and 10 in 2014 and 2013 respectively. Both QTLs had an LOD score of 3.36, additive effect of 4% and phenotypic variations of 33%. They both showed increased effect from Nagdong alleles.

Ratio of grain length/width

Eleven QTLs were identified for the ratio of grain length to width on all the chromosomes except chromosome 5. Ten of them showed increased effect from Cheongcheong alleles and the remaining one showed increased effect from

Nagdong alleles. These QTLs were detected 11 chromosome with an LOD score ranging from 3.34 to 49.33 and additive effects ranging from 4% to 22%. One QTL was detected on chromosome 4 with an LOD score of 3.34 and an additive effect of 4% from Nagdong allele with phenotypic variation of 61% in. One QTL was detected on chromosome 7 for both years at RM21582, its additive increasing effect was from Cheongcheong allele. *qGR10* and *qGR11* were detected on chromosomes 10 and 11 respectively in 2013 and they both showed increasing effect from Cheongcheong allele. QTL *qGR12* (RM8216) was detected in both years with an additive increasing effect from Cheongcheong allele. Three QTLs showed increased effects from Cheongcheong allele and one from Nagdong allele. *qGR2* (RM12895), *qGR7* (RM21582), and *qGR12* (RM8216) were repeatedly detected across the two years.

One thousand-grain weight

Three QTLs were mapped for 1,000-grain weight, consisting of one QTL identified in both years and three QTLs only in 2013 and 2014. A QTL *qTGW8* detected for two years was located on chromosome 8 at RM23230 at an average LOD value of 4.04 and 3.15 for 2013 and 2014 respectively. The *qTGW1*, and *qTGW2* were mapped on chromosome 1, and 2 with LOD scores of 4.23, 3.94 and phenotypic variations of 48%, 45%, respectively. *qTGW2* and *qTGW8* showed increased additive effect from Cheongcheong allele and *qTGW1* from Nagdong allele.

DISCUSSION

Grain shape is the main attribute that determine the appearance quality of the rice grains. Rice grain shape or size is highly correlated with yield and appearance quality. The present study has characterized the genetic bases of the five studied QTLs traits which are related to the appearance quality of rice grains using populations from a cross between the parents Cheongcheong and Nagdong. In this study a total of 24 QTLs affecting grain appearances were identified and mapped on all the twelve chromosomes, 47 QTLs were mapped in 2013 and 14 QTLs were mapped in 2014. Five of the 24 QTLs were reproducibly detected in

two year trials. The number of QTLs per trait identified in the present study ranged from one to six except for ratio of grain length to width which exhibited a total of 11 QTLs from eleven chromosomes in both years. When two or more QTLs were found for the same trait, alleles with similar effect either increasing or decreasing at all loci could be present in the two parents in two different ways: dispersed (one parent having increasing allele at some loci but decreasing allele at others and vice versa for the other parent; Xu *et al.* 2002) or associated (one parent having all increasing alleles and the other having all decreasing alleles.) Allele association resulted in significant differences between the parents. For instance, all the alleles from Cheongcheong at three QTLs of grain width had decreasing effects, whereas all the alleles from Cheongcheong at three QTLs of grain length loci in 2014 had increasing effects. As a result a significant phenotypic difference was found between the two parents for these two traits. In contrast three and eleven QTLs were found for 1,000-grain weight and ratio of grain length to width respectively and cumulatively they exhibited high phenotypic variations; however QTLs alleles with increasing effects at these loci were dispersed between the two parents. The increasing and decreasing allelic effects from each parent counteracted each other, resulting in no significant difference between the parents. This has been supported by the fact that numerous QTLs have been identified using two parents without a significant phenotypic difference (Xu *et al.* 2002). The major QTLs for grain length in this study were mapped at chromosome 5 and 7 in both years. QTL of grain length were commonly located on the chromosome 1 to 8 and 10 in the earlier researches using different mapping population consisting of RIL from a cross Zhenshan97/Shanyou63 or Lemont/Teqing or Zhenshan97/Mighui63 or Asominori/IR24 (Tan *et al.* 2000; Xu *et al.* 2004; Ge *et al.* 2005; Wan *et al.* 2005; Zheng *et al.* 2007). Rabiei *et al.* (2004) reported a QTL of *qgl2* flanked by RM434 and RM341, *qGL2* was also mapped at RM1211 in this study. Tan *et al.* (2000) earlier identified QTL on chromosome 7 that affected grain length. Further the locus identified on chromosome 7 in this study *qGL7* (RM21582) was found to be different locus compared to the earlier mapped. The QTLs of grain width

located on chromosome 2 had been reported in earlier researches. For *qwi2.1* and *qwi2.2*, Rabiei *et al.* (2004) and Xu *et al.* (2004) had identified the same chromosomal regions as identified in the present study. The *qgw2-2* averagely explaining 31% of phenotype variation with increasing effect from Nagdong, was regarded as a major QTL which could be useful in marker assisted selection. Zhang *et al.* (2004) presented two QTLs, *qgw-2a* and *qgw-2b* in the intervals of GA120-G357 and CT565-RG171, which were detected in two different environmental conditions with higher LOD values and contributions of phenotype variation explained. It is however difficult to confirm whether those two QTLs were located on the same position as *qgw2-1* and *qgw2-2*. QTLs *qgw1* and *qgw10* were new loci associated with grain width that were detected in the present study in the year 2013 trial. QTLs for grain thickness have already been mapped on eleven chromosomes except chromosome 7 (Yoshinda *et al.* 2002; Xu *et al.* 2004; Jiang *et al.* 2005; Zheng *et al.* 2007). In this study two QTLs were identified on chromosome 2 and 10 in 2014 and 2013 respectively and they both had an increasing effect from Nagdong. Detection of a major QTL controlling grain-thickness on chromosome 2 by several researchers was found to be similar to *qGT2* locus at RM12895, suggesting that it is important and prevalence in other populations. Twenty-four QTLs were identified for the ratio of ratio of grain length to width on all the chromosomes except chromosome 5 in 2013 and six QTLs on chromosomes 2, 7, and 12 in 2014. Five QTLs were reproducibly repeated over the two years of the study. The highest LOD achieved in 2013 was 49.33 which was detected on RM 12895 on chromosome 2 (*qGR2*). This indicates that the markers in 2013 were more precise and these markers can be used in identifying the marker gene. There was a large difference on the number of QTLs of ratio of grain length to ratio identified in the two years; this again might have been caused by difference in various environmental conditions experienced like weather, soil aeration, soil nutrition etc.

Several QTLs associated with the ratio of length to width have been reported earlier. Yan *et al.* (2003) reported a major QTL for length and width ratio on chromosome 7 and minor QTLs on chromosomes 2 and 6. However in this

study, major QTLs were detected on chromosome 2 in both years. Ratio of grain length to width was mostly controlled by loci on chromosomes 2 and 7 that coincided with the most QTLs identified for width and length. Three QTLs were mapped for 1,000-grain weight; consisting of one QTL identified in both years and two QTLs only in 2013. The major QTL was detected on chromosome 8 in 2014 accounting for 31% of the total phenotypic variation. In 2013, *qtgw1* was the major one with an LOD of 4.23 and a total phenotypic variation of 48%. The QTL identified by Redoña and Mackill (1998) on chromosome 8 might be allelic to a locus near RM23230 in this study. However other QTLs by Redoña and Mackill (1998), Xiao *et al.* (1996), and Lin *et al.* (1996) on chromosome 4 were not associated with any of the markers detected. QTLs detected on chromosome 3 were found to affect four traits of the five investigated traits namely; grain width, ratio of grain length to width, grain-thickness, and 1,000-grain weight (Yan *et al.* 2014). In this study, the QTLs around chromosome 2 pleiotropically affected four of the five investigated traits namely; grain length, grain width, grain-thickness, and ratio of grain length to width. This explains that marker aided introgression of this QTL can be used in developing genetic backgrounds in rice breeding for these traits. Transgressive segregates were observed for all the traits examined in the current study (Fig. 1). For those traits in which two or more significant QTLs were detected, both parents were found to possess QTL alleles which increased phenotypic values (Table 3). The occurrence of such transgression could be directly associated with the inheritance of complementary QTL alleles from the two parents. For example four significant QTLs were detected for grain length, Cheongcheong alleles were associated with an increase in grain length at three of the four QTLs and Nagdong alleles on the other remaining one. As demonstrated by Veldboom and Lee (1994), correlated traits often have QTLs mapping on the same chromosomal locations. The same trend was observed in the current study, for example, grain length and ratio of grain length to width were highly correlated ($r=0.864$ and 0.859 in 2014 and 2013, respectively) and had one QTL with large effects which were found at the same map locations. grain width and ratio of grain length to width showed highly negative

correlation ($r=-0.811$ and $r=-0.827$ for 2014 and 2013, respectively) and each had three similar QTLs in similar locations. In this case, the QTLs acting on both traits had opposite effects. 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 would be a more effective approach in breeding for these traits in rice. The results presented in the present study should facilitate rice breeding, especially for improved hybrid rice quality.

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