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Genetic Mapping of QTLs for Grain Dimension in Rice Grown in Two Asian Countries

¹Ke Xiao, ¹Hailong Zuo, ¹Yanjun Dong, ²Lijun Luo, ²Hanwei Mei and ³M. Matsuo

¹College of Life and Environment Sciences, Shanghai Normal University,
Shanghai, 200234, China

²Shanghai Agro-biological Gene Center, Shanghai, 201106, China

³Faculty of Agricultural, Miyazaki University, Miyazaki City, 889-2192, Japan

Abstract: Grain dimension is one of importantly agronomic traits in rice, which can affect the yield, the processing habit of grain and the market value of rice. This study was conducted to identify Quantitative Trait Loci (QTLs) for Grain Length (GL), Grain Width (GW) and grain Length-width Ratio (LWR), which mainly represent grain dimension in rice, using recombinant inbred (RI) population derived from a cross between a *japonica* variety, Asominori and an *indica* variety, IR24, grown in Japan (2002) and China (2005). Continuous distributions and transgressive segregations of GL, GW and LWR in rice were observed in the RI population, suggesting that grain dimension was quantitatively inherited trait. A total of ten QTLs for grain dimension were mapped to chromosomes 1, 2, 3, 5, 7, which explained 7.0~36.6% of total phenotypic variation and five out of them were commonly detected in the both countries. The results and the tightly linked molecular markers that flank the five common QTLs will be useful in rice-breeding for grain dimension improvement in Asian countries.

Key words: Quantitative Trait Locus (QTL), grain dimension, molecular marker, rice (*Oryza sativa* L.)

INTRODUCTION

Rice is one of the most important food supplies in Asia countries including China and Japan. Improving the quality of rice grain is always the primary object of breeding program all over the world. Grain dimension, which mainly represent the Grain Length (GL), Grain Width (GW) and Length-width Ratio (LWR), is a important component of rice grain quality, because it not only affects yield, milling quality but also determines market value in rice. Generally, in the processing of rice, the grains with suitable dimension or shape can get higher percent of milling-rice than those with excessive length or width, which are easily broken in the milling processing. Moreover different consumer groups may prefer various dimensions of the rice. Consumers in USA, southern China and most of the Asian countries generally prefer long and slender rice while in Japan and Sri Lanka and northern China they may prefer short and bold grain cultivars. Thus, the grain dimension is a major trait which needs to be improved in the rice breeding research. In past, rice dimension traits were reported to be quantitatively inherited trait (Huang *et al.*, 1997; Tan *et al.*, 2000; Li *et al.*, 2003) detected 12 Quantitative Trait Loci (QTLs) for GL, GW and LWR and Tan *et al.* (2000) found a major GL QTL on chromosome 3 and a GW

QTL on chromosome 5 using the RI population derived from Zhenshan/Ming63. It is believable that the identification of Quantitative Trait Loci (QTLs) for rice dimension and the tightly linked molecular markers that flank those QTLs are necessary to breed rice varieties with elite dimension by the marker-assisted selection. However, the previous QTL mapping for rice-grain dimension were often performed in a certain country, so the utilization of the resultant QTLs is relatively limited. In this study, we used Recombinant Inbred Lines (RILs) derived from a *japonica* variety, Asominori and an *indica* variety, IR24 and grew them in Japan and China in different years to detect stably expressed QTLs to provide basic information for marker-assisted selection to breed elite rice variety in Asia countries.

MATERIALS AND METHODS

Plant materials: Recombinant Inbred (RI) lines, kindly provided by professor A. Yoshimura of plant breeding laboratory, Agricultural faculty of Kyushu University, Japan were developed by single seed descent from the progeny of combination of a cross of *japonica* cultivar Asominori from Japan with *indica* cultivar IR24 developed by IRRI. In past, one hundred sixty-five F₆ lines were obtained from 227 original F₂ individual plants. From

these, 71 lines were randomly selected and used for mapping. The restriction fragment length polymorphism (RFLP) map covering 1275 cM in entire rice chromosomes was constructed with 375 markers from the F6 and F7 generations (Tsunematsu *et al.*, 1996). In the study, we used a subset of 289 RFLP markers without overlapping for all loci from the original genetic map (Tsunematsu *et al.*, 1996) to map QTLs affecting grain dimension in rice, for which the average interval distance between pair of markers was 4.4 cM.

Field experiment and measure of grain dimension: The germinated seeds of 68 RI lines along with its parents, Asominori (P1) and IR24 (P2) were sown on 15, May, 2002 (Miyazaki, Japan) and on 15, May, 2005 (Shanghai, China), respectively. After 30 (2002, Japan), 25 days (2005, China), all seedlings were transplanted to Experiment Farm of both Miyazaki University (Miyazaki, Japan) and Shanghai Normal University (Shanghai, China) with single seedling per hill spaced at 10 by 15 cm, respectively. Each plot included three lines with 5 plants per line (Japan) and one line with 4 plants per line (China), respectively. The other managements followed the local conventional methods. All experiments were conducted in randomized complete design with two replicates (Japan) and one replicate (China). At about 40 days after heading, well-ripened rice-grains from plants were harvested and dried. Then 20 grains (Japan) and 10 grain (China) were randomly selected and used to measure the values of three traits viz. Grain Length (GL) and width (GW) and grain Length-width Ratio (LWR), which mainly represent in rice-grain dimension. Average values for each line were used for QTL analyses.

Detection of QTLs: Two methods were simultaneously used to identify significant marker locus-trait association: single marker analysis and Composite Interval Mapping (CIM) analysis. Both analyses were performed by QTL Cartographer computer program software version 2.5. In single marker analysis, the linkages between respective marker loci and putative QTLs are determined. When F-value exceeded a critical value of less than 0.01 probability level, the QTLs were considered to be significant. CIM analysis was applied to trait mean and marker data to more precisely identify the locations of QTL (Zeng, 1994). The locus with a LOD threshold more than 2.6 was to be declared the presence of a putative QTL. In this study, only the QTL detected by both methods were listed. In addition, the additive effect and percentage of variation explained by an individual QTL were also estimated. The QTL were named according to the suggestions of McCouch *et al.* (1997).

RESULTS

Frequency distribution of GL, GW and GWR and their relationships: Figure 1 showed the values of the both parents and the frequency distributions for GL, GW and LWR in RI population. Obviously, IR24 had longer and slender grain dimension than Asominori and they had significant difference in grain dimension. Continuous variation and transgressive segregations of three traits studied suggested that GL, GW and LWR were quantitatively inherited traits. In addition, correlation coefficients (Table 1) (0.88 for GL; 0.78 for GW and 0.90 for LWR, respectively) for the same trait between both countries were significant at 1% level, which showed the expression of grain dimension were relatively stable in rice. Furthermore, their significant correlations (Table 1) among three traits in both Japan and China showed that there existed genetic-relationship among them.

QTLs for grain dimension: Five QTLs for GL were detected on chromosomes 1, 3, 5 and 7, respectively (Table 2 and Fig. 2) and tentatively named qGL-1, qGL-3, qGL-5-1, qGL-5-2 and qGL-7. Among them, qGL-3 with the largest effects (LOD>7.8) was commonly detected in both countries in the same location of markers between C80 and C1677 and explained 21.4~23.6% of the total phenotype variation. However, the other four QTLs were detected in only one country (qGL-1 on chromosome 1 and qGL-5-1 on chromosome 5 in only Japan; qGL-5-2 on chromosome 5 and qGL-7 on chromosome 7 in only China). In addition, the all QTL alleles from IR24 increased the GL expect qGL-1.

Three QTLs for GW were identified and mapped to chromosomes 2, 3 and 5 (Table 2 and Fig. 2), tentatively designated as qGW-2, qGW-3 and qGW-5. Among them, qGW-2 and qGW-5 were commonly detected in both countries. The qGW-5 with the largest effect (LOD>7.9) was located in interval markers between Y1060L and R569, which explained 28.7~36.6% of the total variation and qGW-2, located near to G1340 on chromosome 2, accounted for 8.8~10.2% of the total variation. qGW-3, near G1316 on chromosome 3, was detected in only Japan, which explained 8.8% of total variation. Further the Asominori alleles in all GW QTLs increased GW.

Table 1: Correlation coefficients among the three traits associated with grain dimension

		China (2005)			Japan (2002)		
		GL	GW	LWR	GL	GW	LWR
China	GL						
	GW	-0.25*					
	LWR	0.81**	-0.76**				
Japan	GL	0.88**	-0.25**	0.74**			
	GW	-0.4**	0.78**	-0.74**	-0.35**		
	LWR	0.78**	-0.62**	0.90**	0.82**	-0.82**	

* and ** indicate the significant difference at the 0.05 and 0.01 level, respectively

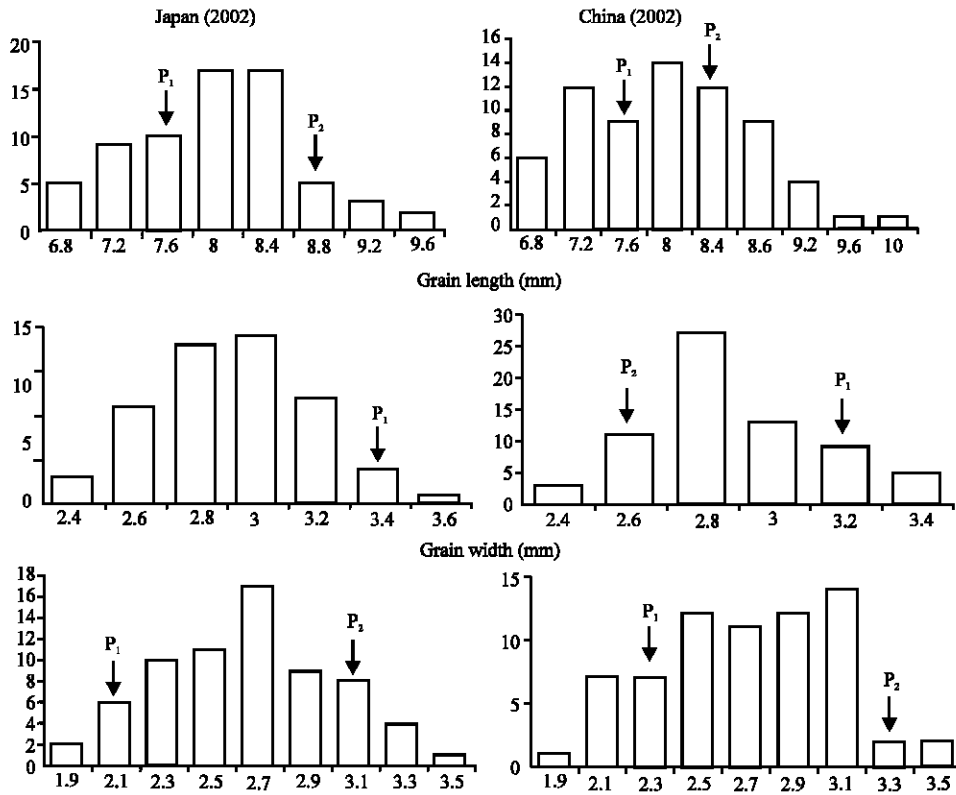


Fig. 1: Frequency distribution of Grain Length (GL) Grain Width (GW) and Grain Length-width Ratio (GLWR). Arrowheads indicate the mean of Asominori (P₁) and IR24 (P₂)

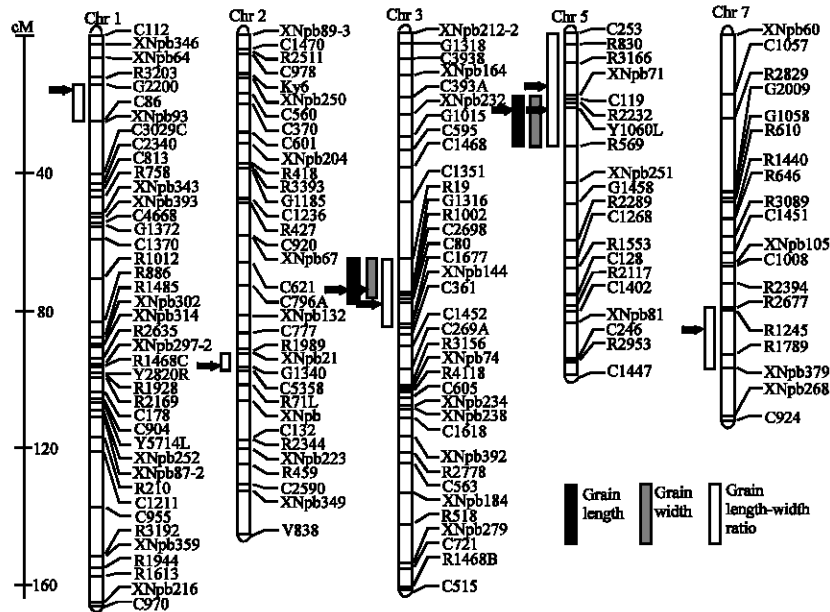


Fig. 2: Locations of QTLs for Grain Length (GL), Grain Width (GW) and grain Length-width Ratio (LWR). The length of the bars indicate range the genomic region with probability <0.01 of QTLs, based on single marker analysis. Black arrowheads indicate the location of peak LODs for QTLs detected

Table 2: QTLs associated with Grain Length (GL), Grain Width (GW) and grain Length-width Ratio (LWR) based on Composite Interval Mapping (CIM) and single marker analysis methods

	QTL ^a		Markers ^b		Value	(%) ^d	Effect (mm) ^e
Japan (2002)							
GL	qGL-1	1	<i>G2200-C86</i>	0.009	3.5	10.0	0.44
	qGL-3	3	<i>C80-C1677</i>	0.000	8.3	23.6	-0.68
	qGL-5-1	5	<i>Y1060L-R569</i>	0.000	2.7	7.0	-0.39
GW	qGW-2	2	<i>G1340-C535B</i>	0.003	3.8	8.8	0.15
	qGW-3	3	<i>R19-G1316</i>	0.000	3.6	8.9	0.15
	qGW-5	5	<i>Y1060L-R569</i>	0.000	11.2	36.6	0.31
LWR	qLWR-3	3	<i>R19-G1316</i>	0.000	10.6	22.4	-0.19
	qLWR-5	5	<i>Y1060L-R569</i>	0.000	13.3	27.0	-0.20
China (2005)							
GL	qGL-3	3	<i>C80-C1677</i>	0.000	7.8	21.4	-0.36
	qGL-5-2	5	<i>R3166-XNpb71</i>	0.000	3.7	11.0	-0.27
	qGL-7	7	<i>R1245-R1789</i>	0.000	5.6	16.8	-0.33
GW	qGW-2	2	<i>G1340-C535B</i>	0.010	3.3	10.2	0.08
	qGW-5	5	<i>Y1060L-R569</i>	0.000	7.9	28.7	0.13
LWR	qLWR-3	3	<i>R19-G1316</i>	0.000	6.6	17.7	-0.17
	qLWR-5	5	<i>Y1060L-R569</i>	0.000	10.9	28.4	-0.21

^aQTLs in black letters were commonly detected in Japan and China. ^bMarkers in italic letters indicate the nearest marker linked to putative QTL. ^cValues indicate probability that the putative QTL is unlinked to the nearest marker by single marker analysis method. ^dVariance explained by the QTL. ^ePositive values indicates Asominori alleles are in the direction of increasing traits

Two QTLs for LWR were found on chromosome 3 and 5 (Table 2 and Fig. 2), tentatively named qLWR-3 and qLWR-5, which were commonly detected in both countries. In detail, qLWR-3, located between R19 and G1316 on chromosome 3 and qLWR-5, located between Y1060L and R569 on chromosome 5, explained 17.7~27.0% and 27.0~28.4% of the total phenotype variation, respectively. In addition, the both alleles from Asominori reduced the LWR.

DISCUSSION

In this study, we reported the results of QTL mapping for grain dimension in rice, using the recombinant inbred lines derived from *japonica* Asominori and *indica* IR24 with 289 RFLP markers in two Asian countries (China and Japan). Resultantly, ten QTLs associated with grain dimension in rice were detected in the study. It was noted that five common QTLs (qGL-3, qGW-2, qGW-5, qLWR-3, qLWR-5) for grain dimension detected in both Asian countries showed that the five QTLs can be stably expressed in different environments. Those results could explain the results of significant correlation coefficients (Table 1) for the same trait between Japan and China. However, the other five QTLs (qGL-1, qGL-5-1, qGL-5-2, qLWR-3, qLWR-5) were detected in only one country, suggesting that the expressions of those QTLs are easily affected by environmental factors. From those results, it could be concluded that the five common QTLs will be more valuable for improvement of grain dimension in Asia.

In addition, the *indica* parent, IR24, had increasing alleles for grain dimension at qGL-3, qGL-5-1, qGL-5-2, qGL-7, but decreasing alleles at qGL-1, qGW-2, qGW-3,

qGW-5, qLWR-3, qLWR-5, while Asominori alleles had the opposite effects. These results could explain the transgression and continuous distributions for grain dimension in the RI population. More interestingly, both qGW-3 for GW and qLWR-3 for LWR were located on the same genomic position and linked to qGL-3 for GL on chromosome 3. And in chromosome 5, we found three QTLs (qGL-5-1, qGW-5 and qLWR-5) on the same genomic position and another QTL (qGL-5-2) in the near region. This supported the results of significantly ($p < 0.05$) high correlations among GL, GW and LWR (Table 1).

To date, there have been several other reports on QTL analysis of rice dimension (Huang *et al.*, 1997; Tan *et al.*, 2000; Li *et al.*, 2003). In comparing with the QTLs detected in this study, the QTLs for GL and GW on chromosome 3 reported by Huang *et al.* (1997) were tightly linked/allelic to the QTLs detected in this study; the major QTLs on chromosome 3 and chromosome 5 reported by Tan *et al.* (2000) might be coincided with the QTLs detected in our study. However, Li *et al.* (2003) also found few QTLs on chromosome 6, 12 for grain dimension but they didn't detect the QTLs for GL on chromosome 3 and 5, in which there existed the major QTLs for grain dimension in our studies. This indicated that QTLs determining grain dimension varied with cultivars studied in rice. More recently, a grain weight QTL, *gw3.1*, was mapped on chromosome 3 (Thomson *et al.*, 2003) and narrowed down to a 93.8-kb region (Li *et al.*, 2004), which might be near qGL-3, qGW-3 and qLWR-3 detected in our study, which suggested that the grain dimension is related to the yield in rice.

In summary, based on our studies, we can confirm that there existed at least five stably expressed QTLs associated with grain dimension in two Asian countries.

In addition, the closely linked molecular markers that flank the QTLs detected in this study should be very useful for marker-assisted breeding to select rice variety with suitable dimension in Asia.

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