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Jiang, Liangrong; Zheng, Jingsheng; Zhang, Zhiyong; Chen, Xiaolong; Chen, Fangyu;
Wang, Tiansheng; Huang, Yumin; Wang, Houcong; Hong, Zonglie; Huang1, Rongyu

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Two independent grain-length mutants mapped to a single region on the long arm of chromosome 2 in rice

Liangrong Jiang[1], Jingsheng Zheng[1], Zhiyong Zhang[1], Xiaolong Chen[1], Fangyu Chen[1], Tiansheng Wang[2], Yumin Huang[1], Houcong Wang[1], Zonglie Hong[3], Rongyu Huang[1]*

1.Xiamen University - School of Life Sciences - Xiamen (Fujian), China.
2.Quanzhou Institute of Agricultural Sciences - Quanzhou (Fujian), China.
3.University of Idaho - Department of Plant, Soil, and Entomological Sciences - Moscow (ID), United States of America.

ABSTRACT: Grain shape in rice is a key determinant of grain appearance, yield and market value, and thus has been widely studied. Rice mutant lines with long-grain phenotypes were previously isolated from an M_2 population derived from mutagenized mature pollen grains that were treated with gamma-ray irradiation for a cultivar Ma85. To understand the genetic basis underlying the long-grain trait, two mutant lines, JF171 and JF178, were crossed with the short-grain parents JF222 and Samba, generating three F_2 populations. Molecular marker-based genetic analysis was employed to detect the major QTLs that affected grain length, grain width, length-to-width ratio and grain weight. Based on the data obtained for the three populations, a joint major QTL for grain length was mapped to a 4.7 Mb region between the SSR makers RM263 and RM318 on the long arm of chromosome 2. The results suggested that the two independent mutations contained in JF171 and JF178 are likely due to alterations in the same genetic region. Furthermore, we developed a BC_2F_2 population using JF178 and Samba and narrowed the region to 0.6 Mb. The results of the current study will be helpful to reveal the genetic basis of the above long-grain mutant lines. In general, our results provide solid genetic data to identify the unknown gene that affects grain length by map-based cloning and practice marker-assisted breeding for long-grain rice cultivars.

Key words: *Oryza sativa* L., grain length, QTL mapping, marker-assisted breeding.

*Corresponding author: huangrongyu@xmu.edu.cn

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INTRODUCTION

Rice is one of the most important cereal crops in the world. To meet the demand of the rapidly growing population, global rice demand should rise from 676 million tons in 2010 to 763 million tons by 2020 and to 852 million tons by 2035 (Khush 2013). Increasing food production on a sustainable track is one of the most prominent challenges facing global food supply systems (Rosegrant and Cline 2003). Among the strategies proposed for improving rice yield and quality, development and application of superior cultivars is the most effective approach. Rice yield is a function of three major components: the number of panicles per plant, number of grains per panicle and grain weight. Grain weight is underlain by grain size and shape. In breeding programs, rice grain shape is measured as grain length, width, length-to-width ratio (L/W ratio) and thickness (Huang et al. 2013). Grain length, width and thickness are positively correlated with grain weight (Tan et al. 2000). In addition to contributing to grain yield, grain shape is also an important quality trait of rice grain products (Unnevehr 1992; Juliano and Villareal 1993).

Most important agronomic traits are genetically controlled by quantitative trait loci (QTLs). The rapid development of DNA markers and genome sequencing has greatly facilitated the discovery and mapping of a number of QTLs for rice grain shape and weight (Huang et al. 2013; Zou and Li 2014). Among them, some loci have been fine mapped or cloned (Huang et al. 2013). The most exciting progress in genetic research on rice grain shape and weight has been the cloning of over twenty genes that are responsible for grain traits, including *GS3*, *GW2*, *qSW5*, *GS5*, *qGL3*, *TGW6*, *GL2*, *GL7*, *GW7*, *GW8* and *GLW7* (Huang et al. 2013; Zhou and Li 2014; Wang et al. 2012; Zhang et al. 2012; Ishimaru et al. 2013; Che et al. 2015; Wang et al. 2015a; Wang et al. 2015b; Si et al. 2016).

The rice cultivar Ma85 has developed non-shattering grains and is resistant to blast disease, but exhibits round grains and low yield. Three decades ago, a set of long-grain mutants from a mutagenized Ma85 population treated with gamma-ray irradiation was isolated (Wang et al. 2003). Using these long grain mutants as breeding parents, a series of long-grain rice cultivars with high yield and fine grain quality have been developed and released for production (Wang et al. 2001; 2011). Despite the extensive use of these long grain mutants in breeding programs, the genetic bases underlying the long grain morphology of these mutant lines are poorly understood. The objectives of this study were to conduct genetic analysis and map the QTLs underlying the grain shape and grain weight traits of two long-grain mutant lines. The knowledge gained from this study will be used in marker-assisted breeding for long-grain cultivars.

MATERIALS AND METHODS

Origin of long grain mutants

The rice (*Oryza sativa* L. ssp. *indica*) cultivar Ma85 was used as the starting material for mutagenesis. JF171 and JF178 were initially selected as independent mutant lines with elongated grains from an M_2 population created by applying mature pollen grains of Ma85 treated with gamma-ray irradiation to male-sterilized flowers (treated with 45 °C water for 5 minutes to kill the pollens) of the same cultivar, Ma85 (Wang et al. 2003).

Construction of initial mapping populations

The two long-grain mutant lines JF178 and JF171 were crossed with the short-grain parents Samba (an *indica* cultivar from Sri Lanka) and JF222 (a selected breeding line) to generate initial mapping populations (Fig. 1a,b; 2a,b; 3f,g). Three F_2 populations consisting of 173 plants for JF222 × JF178, 186 plants for JF178 × Samba and 184 plants for JF171 × Samba, were constructed.

Construction of Secondary Mapping Populations

JF178 was used as the donor parent and Samba as the recurrent parent to generate BC_1F_1, BC_2F_1 and other higher backcrossing populations. Some long grain, Samba-like plants were allowed to self-fertilize to generate BC_2F_2. The BC_2F_2 population of 251 plants was used as a secondary mapping population.

Field trials

All plant materials were maintained in the outdoor field of the Rice Genetics and Breeding Experimental Station at Xiamen University. The plants were grown individually with a row × column space of 22 × 22 cm. Field management of rice growth regarding watering, fertilizer use and disease and insect prevention essentially followed the local agricultural practice. →

Rice Genomic DNA Isolation

Young leaves of rice seedlings were ground using TissueLyser II (Qiagen, manufactured by Retsch, Germany) and genomic DNA was isolated using the CTAB method (Murri and Thompson 1980). The DNA samples were stored at –20 °C in a refrigerator.

Evaluation of the SSR polymorphism and segregation distortion using Bulked Segregant Analysis (BSA)

Preliminary screening of the parents was conducted to identify polymorphism using SSR primers selected from http://archive.gramene.org/markers/ and described by Temnykh et al. (2000; 2001) and McCouch et al. (2002). A total of 535, 550 and 505 pairs of primers were tested for polymorphisms between the parents JF222 and JF178, JF178 and Samba, and JF171 and Samba, respectively, and 112, 242 and 198 pairs showed polymorphism between the parents. For BSA (Tan et al. 1998), rice plants with short grains were selected from each F_2 population, and the corresponding genomic DNA of each plant was taken from the samples. A genomic DNA pool of short grains was prepared by mixing DNA samples of 10 individual plants with extremely short grains. Similarly, a genomic DNA pool of long grains was prepared by mixing DNA samples of 10 individual plants with extremely long grains from each F_2 population. SSR markers that were polymorphic between the two parents of each cross were used to detect polymorphisms between the two DNA pools of each F_2 population. PCR was performed in a mixture of 15 µl of a solution containing 0.33 mmol·L^{-1} dNTP, 20 ng of genomic DNA, 1 U of Taq DNA polymerase, 1 pmol/L primers and 1X PCR buffer. The conditions used for PCR were denaturation at 94 °C for 5 min, 35 cycles of 30 sec at 94 °C, 45 sec at 55 °C and 1 min at 72 °C, followed by 10 min at 72 °C. PCR products were separated on a 6% non-denaturing polyacrylamide gel and observed with 0.1% silver nitrate staining.

Grain trait measurements

When fully mature, the main panicles of individual plants of parental lines and progeny plants were harvested and, dried for 72 hours in an oven at 60 °C. The grain length, width and weight of one-hundred unhulled grains of each line were measured using a Wanshen SC-G seed and kilo-grain weight detection system (Hangzhou Wanshen Detection Technology Co., Ltd., Hangzhou, China). The thickness of the middle position of each grain was measured individually using a Vernier caliper, and ten unhulled grains of each line were measured. Three sets of measurements were performed.

Statistical analysis

SPSS V15.0 software was used to obtain maximum, minimum, mean, and standard deviation. The same software was used to test the data distribution for normality. Correlations between two traits and path coefficients between grain shape traits and 1000 kernel weight were calculated. Figures showing the distribution of frequencies were drawn using Microsoft Office Excel 2007 software.

Linkage map construction and QTL mapping

The SSR markers that were found to be polymorphic during BSA were used to genotype all individual plants in three F_2 populations. Mapmaker EXP3.0 (Lincoln et al. 1992a) was used to analyze the linkage relationships of the SSR markers. Linkage groups of SSR markers were created at a log likelihood ratio (LOD) value of 3.0. A genetic linkage map was drawn using Mapchart 2.1 (Voorrips 2002). Interval mapping for QTLs associated with grain traits was conducted using Mapmaker/QTL 1.1 (Lincoln et al. 1992b) at a threshold of LOD = 3.0.

RESULTS AND DISCUSSION

Grain shape and size traits of F_1 and their distributions in the F_2 populations

The F_1 plants of JF171 × Samba displayed intermediate grain phenotypes between the two parents (Table 1). In the F_2 population, grain length ranged between 6.3 to 11.2 mm, and most individual plants had a grain length between the two parents. The mean value (8.7 ± 0.9 mm) was nearly identical to the median (8.7 mm). Grain width ranged from 2.5 to 3.8 mm, and the 1000-kernel weight varied between 13.5 and 37.5 g, with no transgressive segregation. The mean 1000 kernel weight value (24.5 ± 4.5 g) was nearly identical to the median (24.5 g). All four traits exhibited continuous variation, characteristic of quantitative traits (Fig. 1).

Table 1. Analysis of grain traits detected in three F_2 populations.

Parents & population	Parameters	Traits				
		GL (mm)	GW (mm)	L/W ratio	GT (mm)	KGW (g)
JF171 × Samba						
JF171(♀)	Range	11.5 ± 0.1	3.6 ± 0.0	3.2 ± 0.1	nd	41.9 ± 0.7
Samba(♂)	Range	6.4 ± 0.1	2.5 ± 0.0	2.5 ± 0.1	nd	13.8 ± 0.3
F_1	Range	9.3 ± 0.1	3.2 ± 0.1	2.9 ± 0.1	nd	28.0 ± 0.3
F_2	Range	6.3 – 11.2	2.5 – 3.8	2.3 – 3.6	nd	13.5 – 37.5
	Mean ± SD	8.7 ± 0.9	3.0 ± 0.3	2.9 ± 0.2	nd	24.5 ± 4.5
	Median	8.7	3.1	2.9	nd	24.5
	Kurtosis	–0.45	–0.26	–0.31	nd	–0.36
	Skew	–0.04	–0.20	0.14	nd	0.15
JF222 × JF178						
JF222(♀)	Range	6.4 ± 0.0	2.9 ± 0.0	2.2 ± 0.0	nd	24.5±0.3
JF178(♂)	Range	12.5 ± 0.3	3.0 ± 0.1	4.1 ± 0.1	nd	45.2 ± 1.1
F_1	Range	11.2 ± 0.1	3.1 ± 0.1	3.6 ± 0.1	nd	37.0±0.1
F_2	Range	6.1 – 13.3	2.7 – 4.1	2.1 – 4.3	nd	21.9 – 46.2
	Mean ± SD	10.5 ± 1.1	3.2 ± 0.2	3.3 ± 0.4	nd	33.7 ± 5.5
	Median	10.5	3.2	3.3	nd	34.3
	Kurtosis	0.35	0.28	0.15	nd	– 0.85
	Skew	–0.13	0.48	0.07	nd	–0.04
JF178 × Samba						
JF178(♀)	Range	13.3 ± 0.0	2.9 ± 0.0	4.6 ± 0.0	2.5 ± 0.1	41.7±0.5
Samba(♂)	Range	6.2 ± 0.0	2.2 ± 0.0	2.9 ± 0.1	1.6 ± 0.1	10.5 ±0.2
F_1	Range	9.2 ± 0.1	3.0 ± 0.0	3.4 ± 0.1	2.0 ± 0.1	27.4± 0.3
F_2	Range	6.6 – 12.9	2.0 – 3.4	2.6 – 5.6	1.6 ± 2.4	21.9 – 46.2
	Mean ± SD	9.3 ± 1.3	2.6 ± 0.3	3.6 ± 0.6	2.0 ± 0.1	23.8 ± 5.2
	Median	9.1	2.6	3.5	2.0	24.2
	Kurtosis	–0.07	–0.23	0.08	–0.30	–0.19
	Skew	0.63	–0.09	0.82	0.08	0.28

Abbreviations: nd, not determined; GL, grain length; GW, grain width; L/W ratio, grain length-to-width ratio; GT, grain thickness; KGW, 1000-grain weight.

The F_1 plants of JF222 × JF178 also displayed intermediate grain phenotypes between the two parents (Table 1). The F_2 progeny exhibited transgressive segregation in grain length, L/W ratio and 1000-kernel weight. For grain width, however, most F_2 plants produced wider grains than their parents, showing over-dominance. The mean and median values were similar for all four grain traits, and exhibited typical quantitative heredity as well as a normal distribution (Fig. 2).

The F_1 plants of JF178 × Samba displayed intermediate values for grain length, width, length to width ratio, thickness and 1000-kernel weight, but the grain width of F_1 was close to that of JF178 (Table 1). In the F_2 population, grain length fell between the two parents, and the distribution was somewhat bimodal (Fig. 3a), suggesting the probable of existence of a major QTL for grain length. Chi-square tests showed that the ratio of long grain individuals (grain length ≥ 10.5 mm) to short grain individuals (grain length < 10.5 mm) did not fit a 1:3 or 3:1 ratio. Thus, more than one major QTL for grain length may be involved in this population. Grain width exhibited transgressive segregation and an approximate normal distribution (Fig. 3b). The L/W ratio and 1000-kernel weight both displayed transgressive segregation and a skewed distribution (Fig. 3c, 3d). The grain thickness of F_2 exhibited a normal distribution (Fig. 3e). Genetic analysis of these populations suggested that the rice grain traits were typical quantitative inheritance.

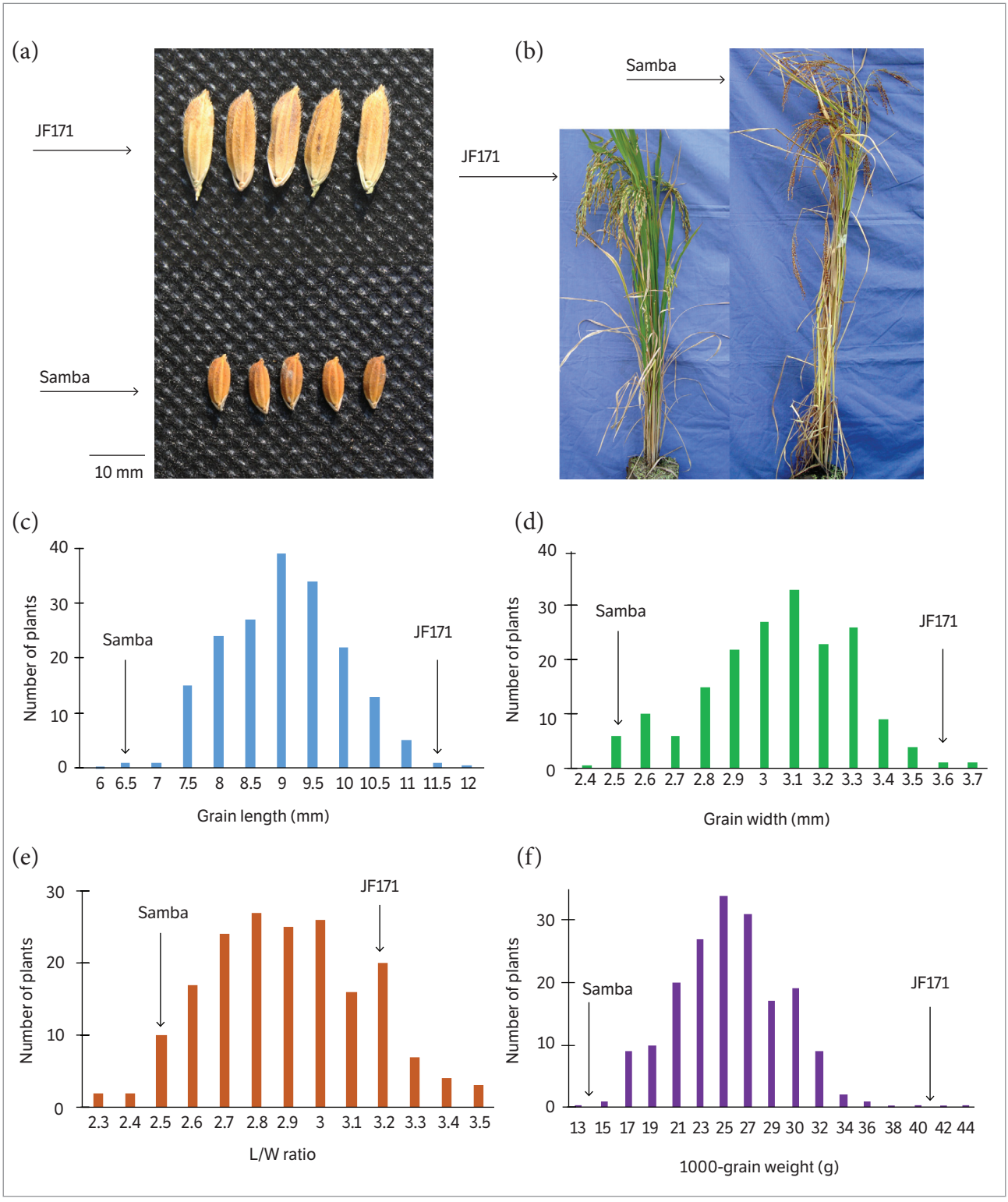

Figure 1. Characterization of grain traits in the JF171/Samba population. Long-grain mutant JF171 was crossed to a very short-grain cultivar, Samba, from Sri Lanka (a-b). Distribution of the grain length in 184 individuals of F_2 population. F_1 plants exhibited median phenotypes in grain traits between the two parents. The F_2 population had median values of grain traits that were very close to those of the F_1 plants, but segregated into individual plants with varied grain traits. Shown are the frequency distributions of F_2 plants in grain length (c), grain width (d), grain length-to-width ratio (e), and 1000 kernel weight (f).

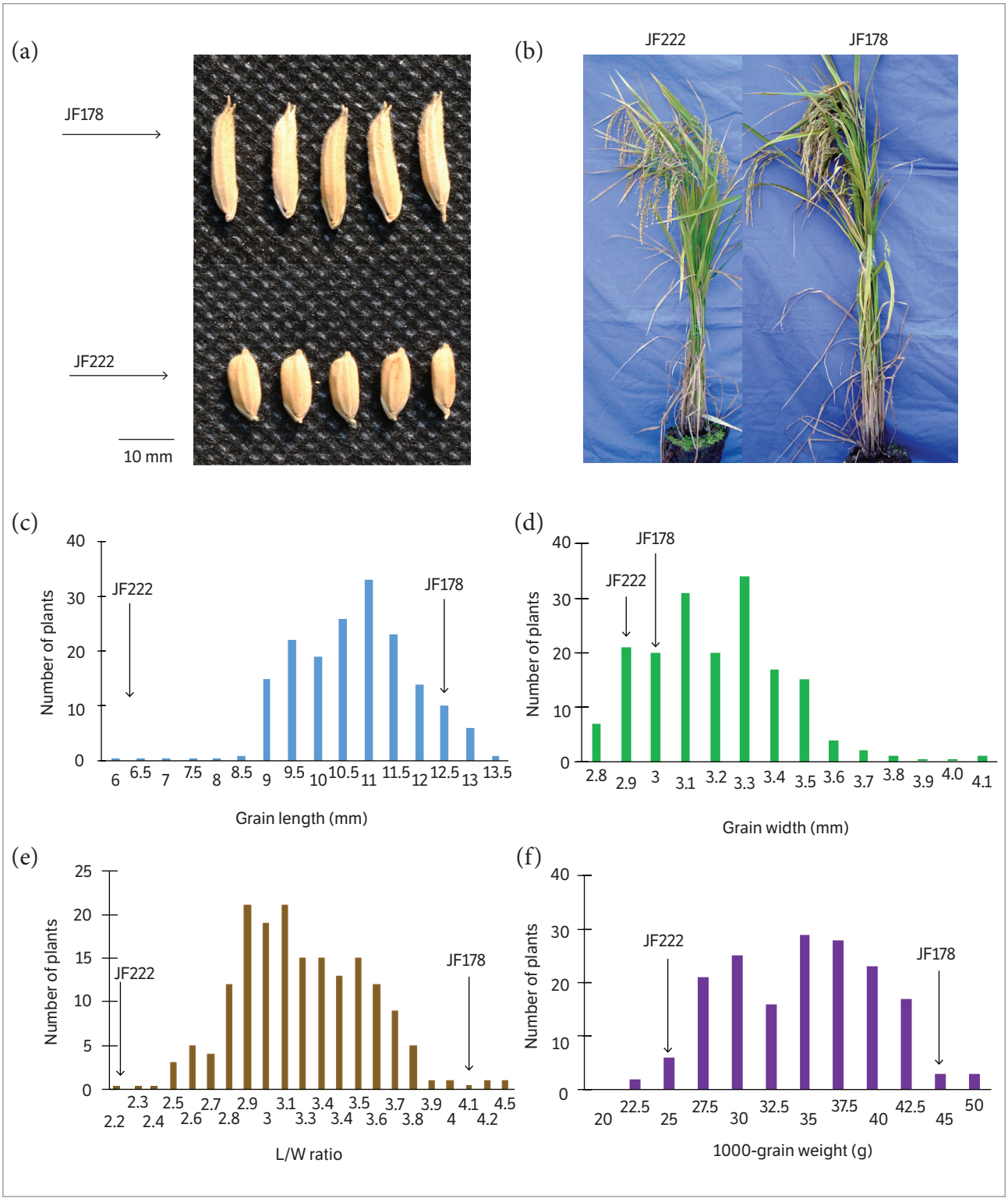

Figure 2. Characterization of grain traits in the JF222/JF178 population. The short-grain breeding line JF222 was crossed to the long-grain mutant JF178 (a-b). The F_1 plants displayed intermediate grain phenotypes between those of the two parents. The F_2 population exhibited partial transgressive segregations in grain length, L/W ratio and KGW, and vigorous transgressive segregation in grain width. Shown are the frequency distributions of grain length (c), grain width (d), grain length to width ratio (e) and 1000 kernel weight (f) in 174 individual plants of the F_2 population.

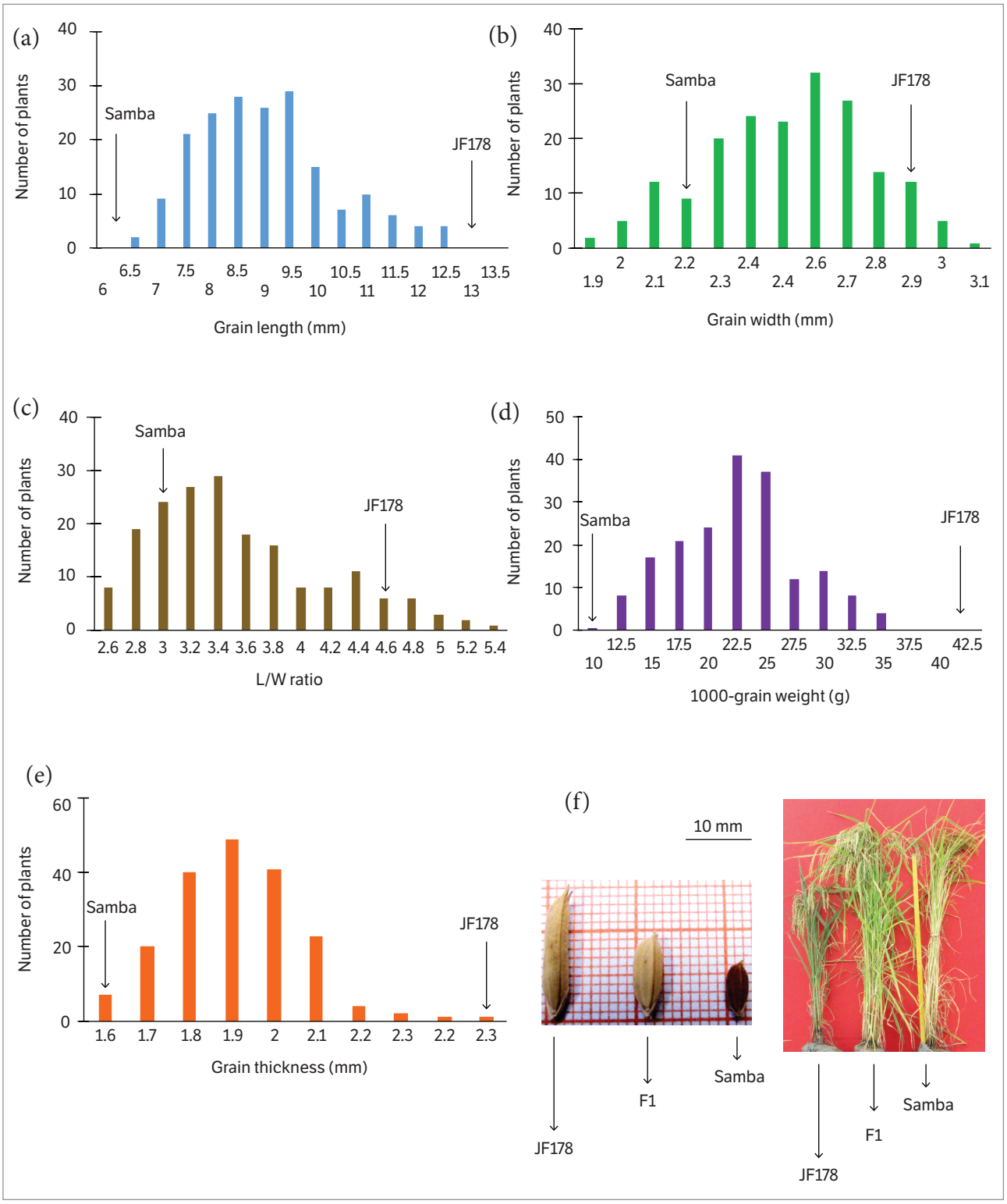

Figure 3. Characterization of grain traits in the JF178/Samba population. The long-grain mutant JF178 was crossed to the very short-grain cultivar Samba. The mean values of grain length, L/W ratio, grain thickness and 1000 kernel weight for F_1 plants were intermediate between those of the two parents, whereas grain width of F_1 was close to that of JF178. In the F_2 population of 186 plants, grain length fell between the two parents and exhibited a bimodal distribution trend (a). Grain width exhibited a certain degree of transgressive segregation and an approximate normal distribution (b). Both the L/W ratio and 1000 kernel weight displayed transgressive segregation and skewed distribution (c-d). Grain thickness did not show a transgressive segregation and exhibited an approximate normal distribution (e). Representative images of grains and plants of JF178, Samba and their F_1 are shown in (f-g).

Analysis of the correlation and path coefficients among grain traits in three populations

The correlations among all grain traits were highly significant in three F_2 populations; the only exception was that the correlation between grain length and grain width was not statistically significant in JF178 × Samba (Table 2). The path coefficients of the 1000-kernel weight with grain length, width, L/W ratio and thickness (exclusevely for the population of JF178 × Samba) suggested that these grain shape traits all contributed to 1000-kernel weight. In JF171 × Samba, grain length and width contributed more to weight than L/W ratio did (Table 2). The path coefficient of the grain length to weight was lower but close to the width to weight. For JF222 × JF178 and JF178 × Samba, the path coefficients showed that the grain length of both contributed more to the 1000 kernel weight than grain width and L/W ratio did (Table 2), suggesting the grain length had a major impact on grain weight in these two populations.

Mapping of QTLs for grain traits in JF171 and JF178

For JF171 × Samba, 198 pairs of SSR polymorphic primers between JF171 and Samba were used to detect polymorphisms between the two genomic DNA pools and 16 were able to detect segregation distortion between the two pools. Nine of the 16 markers were clustered on the long arm of chromosome 2. The remaining seven markers were distributed on chromosomes 1, 6 and 8. A genetic map of these four linkage groups was constructed using the 16 markers and six additional primers derived from the 198 polymorphic primers using Mapmaker Exp 3.0.

For JF222 × JF178, 112 polymorphic SSR markers between parents were used to detect polymorphisms between the two DNA pools. The alleles of 14 loci were not equally distributed between the two pools, five of which were clustered on the long arm of chromosome 2. The remaining 9 were scattered on chromosomes 5, 6, 8 and 10. Linkage maps were constructed by genotyping all 173 F_2 plants using these 14 markers and 9 others derived from the above 112 polymorphic markers.

For JF178 × Samba, 242 markers detected polymorphisms between JF178 and Samba, and 35 of these resided on chromosome 2. Because the major QTLs were mostly found on the long arm of chromosome 2 in the above populations, we focused on this chromosome and only used these 35 markers to screen polymorphisms between the two DNA pools. Twelve markers were found to be polymorphic between the two DNA pools. Seven of them, including RM318 and RM13840 were used to genotype 186 F_2 plants. The partial linkage map of chromosome 2 was constructed.

Table 2. Correlation coefficients of grain traits detected in three F_2 populations.

Populations	Traits	Traits				
		Grain length	Grain width	Grain length-to-width ratio	Grain thickness	1000-grain weight
JF171 × Samba	Grain length	1				
	Grain width	0.609**	1			
	GS	0.628**	-0.231**	1		
	1000-grain weight	0.825**(0.436)[a]	0.772**(0.531)	0.256**(0.105)		1
JF222 × JF178	Grain length	1				
	Grain width	0.364**	1			
	GS	0.783**	-0.289**	1		
	1000-grain weight	0.704**(1.109)	0.680**(0.240)	0.267**(-0.443)		1
JF178 × Samba	Grain length	1				
	Grain width	0.042	1			
	GS	0.809**	-0.544**	1		
	Grain thickness	0.573**	0.419**	-0.241**	1	
	1000-grain weight	0.864**(0.731)	0.394**(0.289)	0.493**(0.012)	0.736**(0.193)	1

** Significance at 1% probability; [a]Values in brackets are direct path coefficients between grain shape traits and KGW. R^2 = 0.796, 0.825 and 0.894 for JF171 × Samba, JF222 × JF178 and JF178 × Samba F2 population, respectively.

QTL mapping was conducted by Mapmaker QTL1.1 in all three populations (Fig. 4a-c). For grain length, a major QTL (*qGL2-1*) was detected between SSR markers RM573 to RM221 (the physical location was 27.6 Mb - 27.9 Mb) on chromosome 2 in JF171 × Samba (Fig. 4a; Table 3). A major QTL (*qGL2-2*) was located in a 1.5 cM interval of RM13838-RM13840 (28.8 Mb - 28.9 Mb) on chromosome 2 in JF222 × JF178, which explained 60.8% variations of the phenotype (Fig. 4B; Table 3). A major QTL (*qGL2-3*) was mapped in a 5.6 cM interval of RM525 - RM318 (28.3 Mb – 29.6 Mb) on chromosome 2 in JF178 × Samba (Fig. 4c; Table 3). Three populations all detected QTLs associated with grain length in neighboring regions (27.6 Mb – 29.6 Mb) on chromosome 2, and it appeared that the long grain length of JF178 and JF171 was due to a mutation in a gene on the long arm of chromosome 2.

For grain width, a major QTL (*qGW2-1*) was located in an interval of 11.2 cM between RM240 and RM318 (29.6 Mb – 31.5 Mb) in JF171 × Samba, 8 cM from the *qGL2-1* (Fig. 4a; Table 3). A major QTL (*qGW2-2*) was found in a 9.2 cM interval between RM263 and RM13838 (25.9 Mb – 28.9 Mb) in JF222 x JF178 (Fig. 4b; Table 3). The major QTL (*qGW2-3*) was located in a 3.5 cM interval between RM13840 and RM525 (28.3 Mb – 28.9 Mb) in JF178 × Samba (Fig. 4c; Table 3). These QTLs for grain width located on neighbouring regions between RM263 and RM318 on the long arm of chromosome 2, corresponded to physical positions of 25.9 Mb to 31.5 Mb, covering the region of QTLs for grain length detected in the current study.

For the 1000-kernel weight, the QTL for the 1000 kernel weight (*qKGW2-1*) was located in the same segment of *qGL2-1* in JF171 × Samba (Figure 4A and Table 3). The QTL for the 1000 kernel weight (*qKGW2-1*) was also located on the same region of *qGL2-2* in JF222 x JF178 (Fig. 4b and Table 3). In JF178 × Samba, the major QTL for grain width (*qKGW2-3*) was located in a 3.5 cM interval between RM13840 and RM525, in the same region as *qGW2-3* (Fig. 4c and Table 3). This suggested that the lower portion of the long arm of the chromosome likely contains a 'super' QTL that has pleiotropic effects on grain shape and grain weight.

Several minor QTLs associated with grain shape and weight also detected in these populations, such as *qGW8-1*, *qKGW6-1*, *qGL10-1*, *qLW2-1* and *qGT2-1* (Table 3). Among these QTLs, *qLW2-1* and *qGT2-1* fell into the same region on chromosome 2 as the major QTLs for grain length, width or grain weight.

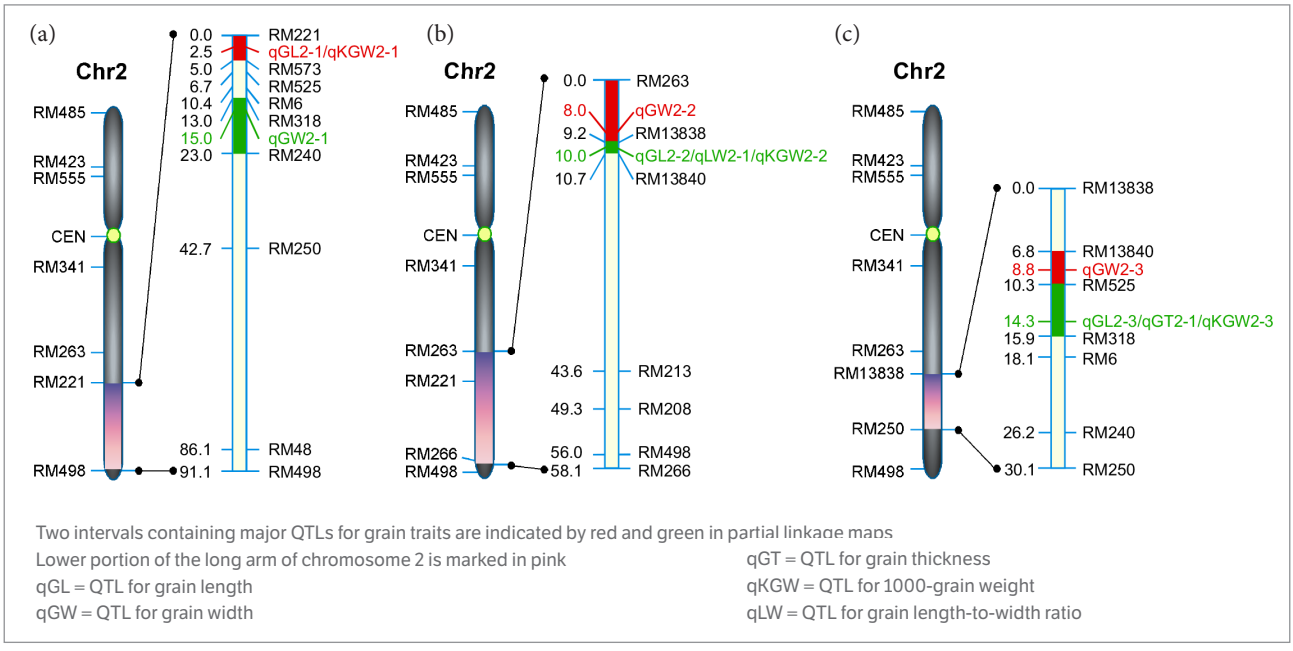

Figure 4. Linkage maps of QTLs for grain traits. A bulked segregant analysis approach was used to analyse segregation data detected using SSR markers. Partial linkage maps in F_2 populations were constructed (Fig. 3 and Fig. 5). Major QTLs for grain length, grain width, grain thickness, L/W ratio, and 1000 kernel weight were identified on the lower portion of the long arm of chromosome 2 in the analysis of the F_2 populations of all three crosses of (a) JF171/Samba, (b) JF222/JF178 and (c) JF178/Samba.

Table 3. Mapping of QTLs for grain shape and grain weight in three F_2 populations and one BC_2F_2 population.

Population	QTL	Chr	Markers interval	Genetic distance (cM)	Physical locations[a] (Mb)	Additive effect	Dominant effect	LOD	V[b] (%)
JF171 × Samba	qGL2-1	2	RM573-RM221	5.0	27.6 - 27.9	-0.62	0.24	10.0	24.8
	qGW2-1	2	RM240-RM318	11.2	29.6 - 31.5	-0.24	0.09	22.8	48.6
	qGW8-1	8	RM264-RM458	18.9	27.3 - 27.6	-0.12	0.02	3.6	10.2
	qKGW2-1	2	RM573-RM221	5.0	27.6 - 27.9	-3.30	1.04	11.5	26.7
	qKGW6-1	6	RM557-RM527	10.9	7.2 - 9.9	1.69	1.11	3.2	8.7
JF222 × JF178	qGL2-2	2	RM13838-RM13840	1.5	28.8 – 28.9	1.13	0.18	35.2	60.8
	qGL10-1	10	RM228-RM496	9.5	22.2 – 22.4	0.45	0.16	3.0	8.0
	qGW2-2	2	RM263-RM13838	9.2	25.9 – 28.9	0.17	0.03	14.5	33.2
	qLW2-1	2	RM13838-RM13840	1.5	28.8 – 28.9	0.19	0.03	7.4	17.9
	qKGW2-2	2	RM13838-RM13840	1.5	28.8 – 28.9	6.02	1.26	35.3	61.0
JF178 × Samba	qGL2-3	2	RM525-RM318	5.6	28.3 – 29.6	1.02	0.06	12.1	26.8
	qGW2-3	2	RM13840-RM525	3.5	28.3 – 28.9	0.17	0.08	9.5	21.7
	qGT2-1	2	RM525-RM318	5.6	28.3 – 29.6	0.10	0.02	10.6	24.2
	qKGW2-3	2	RM525-RM318	5.6	28.3 – 29.6	4.41	1.07	16.3	34.3
JF178 × Samba (BC_2F_2)	qGL2-4	2	RM13840-RM525	1.8	28.3 – 28.9	0.625	0.054	8.02	13.7
	qGW2-4	2	RM13838-RM13840	0.2	28.8 – 28.9	0.186	0.049	29.74	42.1
	qGT2-2	2	RM13840-RM525	1.8	28.3 – 28.9	0.110	0.002	6.89	11.9
	qKGW2-4	2	RM13840-RM525	1.8	28.3 – 28.9	3.430	0.843	16.92	26.7

[a]Physical locations of markers on each chromosome are obtained from the Gramene web site (www.gramene.org). [b]V refers to phenotype variations explained by each QTL. Chr = Chromosome; LOD = Log likelihood ratio.

Fine Mapping of the Major QTLs in a BC_2F_2 Population

To further confirm the presence of the major QTLs for grain traits, we generated an advanced backcross BC_2F_2 population using JF178 as the donor and Samba as the recurrent parent. A total of 251 individual plants from this BC_2F_2 population were analyzed. As shown in Fig. 5, five grain traits all had continuous variations in BC_2F_2. In the BC_2F_2 population, grain length exhibited a bimodal distribution trend (Fig. 5a). Grain width exhibited weak transgressive segregation and an essential normal distribution (Fig. 5b). The L/W ratios displayed transgressive segregation and a skewed distribution (Fig. 5c). The grain thickness exhibited an essential normal distribution (Fig. 5e). The KGW exhibited a skewed distribution (Fig. 5d).

Two gene pools each containing 10 plant individuals from the BC_2F_2 population were constructed. Based on analysis of the JF178/Samba F_2 population, 35 SSR markers that were polymorphic between JF178 and Samba were chosen to detect the two gene pools, and eight of these showed polymorphisms. In addition to these eight markers, four markers that were polymorphic between the two parents were also added. These 12 markers were used to screen the genotypes of the 251 BC_2F_2 plants. The data were collected to construct a partial linkage map covering 65.1 cM of chromosome 2 (Fig. 5g).

Taken together, the results showed that four major QTLs were detected on the lower portion of the long arm of chromosome 2, one locus each for grain length, grain width, grain thickness and KGW (Fig. 5g; Table 3). The major QTL for grain length (*qGL2-4*) was localized in an interval of 1.8 cM between RM525 and RM13840, explaining 13.7% of the variations. The major QTL for grain width (*qGW2-4*) was located in an interval of 0.2 cM between RM13840 and RM13838 and explained 42.1% of the variations. The major QTL for grain thickness (*qGT2-2*) was located in the same segment of *qGL2-4*, and explained 11.9% of the variations. The major QTL for KGW (*qKGW2-4*) was located in the same segment of *qGL2-4* and *qGT2-2*, and explained 26.7% of the variations. These QTL mapping results for the BC_2F_2 population were similar to those obtained using the JF178/Samba F_2 population. However, the BC_2F_2 results →

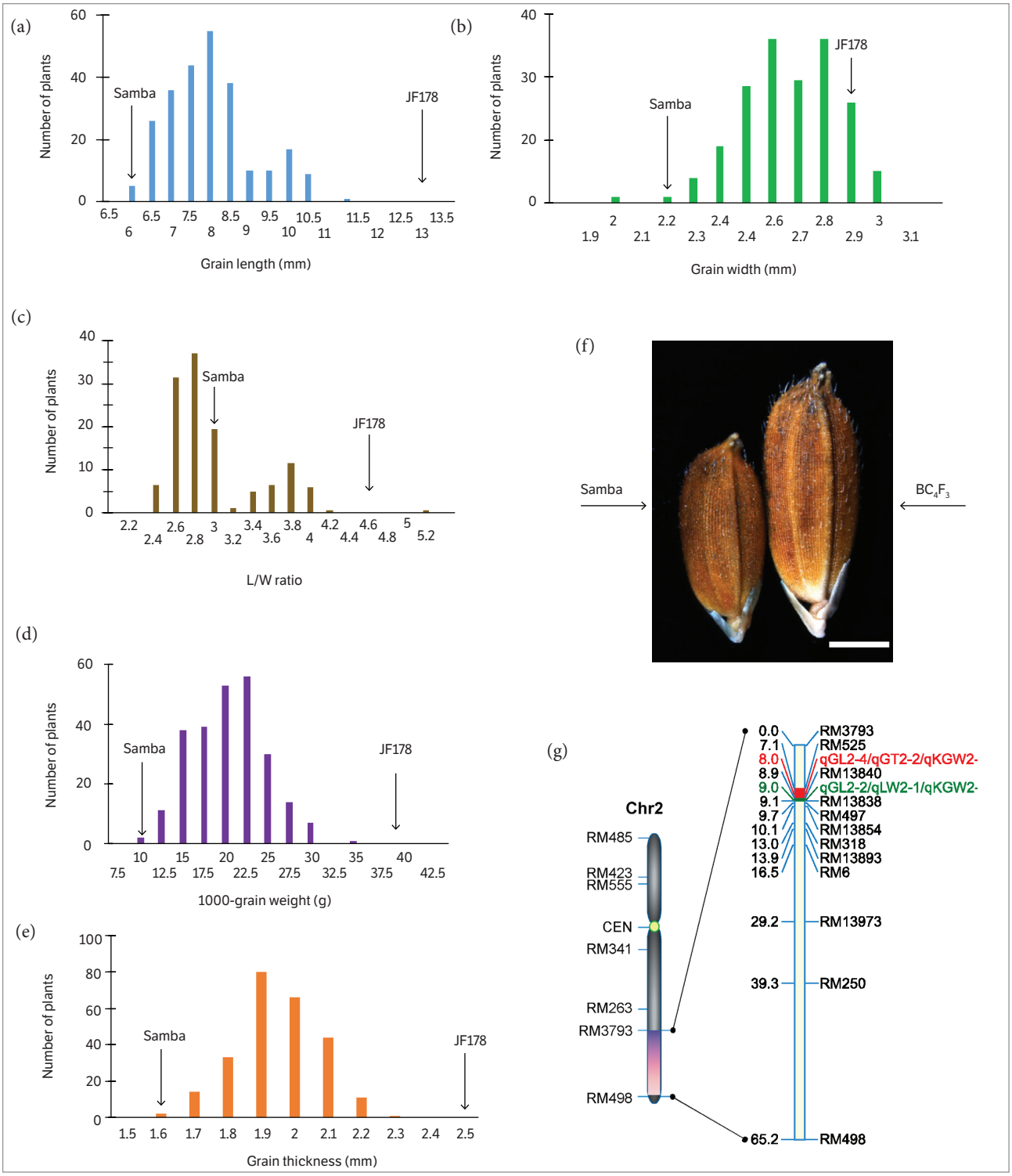

Figure 5. Characterization and mapping of QTLs of grain traits in the JF178/Samba BC_2F_2 population. A total of 251 individual plants from the BC_2F_2 population were analysed. In the population, grain length fell between the two parents and exhibited a bimodal distribution trend (a). Grain width exhibited a weak transgressive segregation and an essential normal distribution (b). The L/W ratio displayed transgressive segregation and a skewed distribution (c). Ggrain thickness and 1000-grain weight exhibited an essentially normal distribution (d-e). Representative images of grains of Samba and its BC_4F_3 near-isogenic lines (f). (g) A partial linkage map in the BC_2F_2 population was constructed and major QTLs for grain shape and weight were mapped to the lower portion of chromosome 2. Two intervals containing major QTLs for grain traits are indicated in red and green. *qGL2-4*, QTL for grain length; *qGW2-4*, QTL for grain width; *qGT2-2*, QTL for grain thickness; *qKGW2-4*, QTL for 1000-grain weight.

were more accurate, and mapped the grain length QTL to a 1.8 cM genetic interval and grain width QTL *qGW2-4* to a 0.2 cM interval between RM13838 and RM13840. The results suggested that the QTLs associated with grain traits may present a good target for gene cloning. In fact, near-isogenic lines were developed (NILs, Fig. 5f) to isolate a major QTL responsible for grain shape from this region.

During further studies on the grain trait QTLs on chromosome 2, we found a report on the cloning and characterization of *GL2* (Che et al. 2015), a QTL for grain length and weight. *GL2*, also located on the long arm of chromosome 2, encodes a growth regulating factor and positively regulates grain length and weight. The QTLs for grain traits mapped in the current study were located in the region between RM263 and RM318 on the long arm of chromosome 2, corresponding to physical position of 25.9 Mb to 29.6 Mb. This region covers the *GL2* locus. Thus, the QTLs detected in the current study are likely to be the same locus as *GL2*, although this must still be confirmed. However, the knowledge gained from this study was helpful to reveal the genetic basis of long grain mutants and was useful in marker-assisted breeding for long-grain rice cultivars.

CONCLUSION

Grain shape traits in rice, including grain length, grain width, length-to-width ratio and grain thickness are key determinants of grain appearance, yield and market values. In the current study, genetic analysis and QTL mapping on rice long-grain mutants showed that several QTLs with large effects on grain shape were contained in these mutants. These QTLs were all located on the region between the SSR markers RM283 and RM318 on chromosome 2 using three F_2 populations. The region of QTL for grain length was narrowed to 1.8 cM and 0.2 cM for grain width in one BC_2F_2 population. Our findings provide information for further understanding grain formation and developmental mechanisms in rice. The tightly linked markers with grain traits identified in the current study will be useful for selection of long-grain cultivars in rice breeding.

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ORCID IDs

L. Jiang
http://orcid.org/0000-0001-7294-0518
J. Zheng
http://orcid.org/0000-0001-7002-7186
Z. Zhang
http://orcid.org/0000-0002-7227-5754
F. Chen
http://orcid.org/0000-0003-1476-5306
X. Chen
http://orcid.org/0000-0001-9949-7990
T. Wang
http://orcid.org/0000-0003-1169-3925
Y. Huang
http://orcid.org/0000-0002-6163-6286
H. Wang
http://orcid.org/0000-0003-3401-7655
Z. Hong
http://orcid.org/0000-0001-9057-4128
R. Huang
http://orcid.org/0000-0002-1812-1957

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