Research Paper

Identify QTLs for grain size and weight in common wild rice using chromosome segment substitution lines across six environments

Lan Qi[1,2], Yan Sun[2], Jing Li[2], Long Su[2], Xiaoming Zheng[2], Xiaoning Wang[3], Kaimian Li[1], Qingwen Yang[2] and Weihua Qiao*[2]

[1] *Institute of Tropical Agriculture and Forestry, Hainan University*, Haikou 570228, China

[2] *Institute of Crop Science, Chinese Academy of Agricultural Sciences*, Beijing 100081, China

[3] *Key Laboratory of Crop Genetic Breeding, Hainan Academy of Agricultural Science*, Haikou 571100, China

Grain size and weight are important determinants of rice yield. The identification of beneficial genes from wild rice that have been lost or weakened in cultivated rice has become increasingly important for modern breeding strategies. In this study, we constructed a set of chromosome segment substitution lines (CSSLs) of wild rice, *Oryza rufipogon* with the *indica* cultivar 9311 genetic background. Four grain-related traits, i.e., grain length (GL), grain width (GW), length-width ratio (LWR), and thousand grain weight (TGW), were screened across six environments. A total of 37 quantitative trait loci (QTLs) were identified in these environments and mapped to 12 chromosomes. Sixteen QTLs were detected in at least two environments, and two QTL clusters were observed on Chr. 4 and Chr. 8. Based on a comparative analysis with QTLs identified in previous studies, the CSSLs between *Oryza rufipogon* accessions and 9311 had high genetic diversity. Among the sixteen stable QTLs, seven for TGW, LWR, GL, and GW were not previously identified, indicating potentially novel alleles from wild rice. These CSSLs provide powerful tools for functional studies and the cloning of essential genes in rice; furthermore, we identified elite germplasm for rice variety improvement.

Key Words: *Oryza rufipogon* Griff., chromosome segment substitution lines, grain-related traits, quantitative trait loci.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods in the world; half of the world population depends on rice as the main food resource. The Food and Agriculture Organization predicted that the human population will reach 9 billion in 2050, requiring a 60% increase in food production to feed the population (Alexandratos and Bruinsma 2012). At present, rice breeding is challenged by a yield plateau owing to the narrow genetic background. Common wild rice (*O. rufipogon* Griff.), which is more genetically diverse than *O. sativa* (Sun *et al.* 2001, Wang *et al.* 1992), is considered the direct ancestor of cultivated rice (Wei *et al.* 2012). Transferring genes that control desirable traits from wild relatives to cultivated rice has proven to be an important strategy in rice breeding (McCouch *et al.* 2007, Xiao *et al.* 1996).

Most agronomic traits of rice are controlled by multiple

quantitative trait loci (QTL) (Yamamoto *et al.* 2009, Yano and Sasaki 1997). Genetic populations such as the F_2, BC_1F_2 generations, and some permanent populations, including doubled haploid (Yoshida *et al.* 2002) and recombinant inbred lines, could be used for QTL analyses (Onishi *et al.* 2007). Major QTLs are easily detected from those populations, but minor QTLs are not. Moreover, it is difficult to make repeated observations using temporary populations. Recently, chromosome segment substitution lines (CSSLs) have been developed in rice (Ando *et al.* 2008, Ebitani *et al.* 2005, Kubo *et al.* 2002, Shim *et al.* 2010) for the detection of functionally important QTLs and the identification of elite genetic resources. Each CSSL consists of one or a few homozygous chromosome segments derived from a donor parent in the genetic background of the recurrent parent, and the effect of each substituted segment on a trait can be evaluated without genetic interactions among QTLs. The same CSSL can be simultaneously and repeatedly planted in various locations or in different years for diverse analyses.

The identification of QTLs that control grain production is a primary step in rice cultivar improvement. Nearly 400 QTLs have been detected for grain traits (Huang *et al.* 2013), some of which have been fine mapped (Bai *et al.*

2010, Wan *et al.* 2006, Xie *et al.* 2008). Additionally, some genes related to grain size and weight have been cloned, including *GS3* (Fan *et al.* 2006), *GS5* (Li *et al.* 2011), *GL3.1* (Qi *et al.* 2012), *GW2* (Song *et al.* 2007), and *GW6a* (Song *et al.* 2015).

QTLs that impact breeding must be stable across different environments and in different genetic backgrounds. However, grain-related QTL analyses performed in multiple environments have indicated that environmental conditions contribute substantially to changes in rice grain-related traits. Although the phenotypic characters of wild rice are inferior to those of cultivated rice, modern molecular biology studies have revealed that some genes hidden in wild rice are essential to yield-related trait improvement (Tian *et al.* 2005). Some QTLs associated with rice grain traits have been detected using wild rice introgression lines (Tan *et al.* 2008, Yuan *et al.* 2009). Therefore, the identification of stable QTLs associated with grain size and weight in wild rice is critical for rice breeding.

In this study, we evaluated a set of CSSLs that carry the chromosome segments of *O. rufipogon* from Hainan province of China in the genetic background of the elite *indica* cultivar 9311 to detect QTLs for rice grain size and weight that were not specific to a particular environment and to identify elite genetic resources for yield-related traits. Our results will provide elite materials for rice breeding and map-based cloning of desirable genes.

Materials and Methods

Plant materials

The high-yield *indica* rice variety 9311, which has a sequenced genome and is used as the recipient parent for hybrid rice, was used as the recurrent parent. Wild rice (*O. rufipogon*, laboratory preserved accession number CWR274) from Hainan province, China was used as the donor parent. The F_1 hybrid was backcrossed several times consecutively to 9311. An advanced backcrossed population was generated after three or four rounds of self-pollination. Molecular markers that were polymorphic between the two parents were used to select target lines to construct CSSLs from the BC_3F_1 plants and the subsequent backcross and self-pollination generations.

Field experiments

The parental variety 9311 and 133 CSSLs were grown in six environments (two years × three locations) (**Table 1**)

using the randomized complete block design with two replications. Each plot consisted of rows with 10 plants. Forty plants of each genotype in each plot were planted with a spacing of 10 × 25 cm. Crop management and the control of diseases and insect pests were performed as locally recommended.

Phenotypic evaluation

At maturity, the grains of five plants of each CSSL were harvested and all fully filled grains were selected and dried in an oven at 40°C for 48 h. Then, 200–300 fully filled seeds per CSSL were measured using an automatic seed analyzer (Wanshen Detection Technology Co., Ltd., Hangzhou, China, www.wseen.com). The automatic seed analyzer instrument contained a 5 million pixel resolution camera and a backlight source grass plate. The grain length (GL), grain width (GW), grain length and width ratio (LWR) were automatically calculated via the automatic instrument. Seed weights were detected using an analytical balance (Botoo Electronic Technology Co., Ltd., Shenzhen, China) linked with the automatic seed analyzer, and values were automatically converted to thousand grain weights (TGW), all traits were measured with three replications.

DNA extraction and SSR analysis

DNA was extracted from fresh leaves according to the procedure described by Zheng *et al.* (1995). A polymorphism survey between the parents was conducted using simple sequence repeat (SSR) markers, which were synthesized according to published sequences (McCouch *et al.* 2002, Temnykh *et al.* 2000), and insertion/deletion (InDel) markers, which were developed from a BLASTN alignment between the genome sequences of *japonica* cv. Nipponbare and *indica* cv. 9311. The genomic sequence was obtained from the Gramene website (http://ensembl.gramene.org/Oryza_sativa/Info/Index). Polymerase chain reaction (PCR) primers were designed using Primer Premier 5.0. A 15 μL reaction mixture was applied for PCR. The mixture was composed of 50 ng of template DNA, 0.3 μL of 10 mM each deoxynucleotide triphosphate (dNTP), 0.4 units of *Taq* DNA polymerase, 1.5 μL of 10× PCR buffer with Mg^{2+} (CWbio, Beijing, China), and 1.2 μL of 10 μmol/L forward and reverse primers. Amplification was carried out using the following reaction conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 56°C, and 30 s at 72°C, with a final extension at 72°C for 10 min. PCR products were separated on 8%

Table 1. Test environments in which the CWR274 × 9311 CSSLs populations were evaluated

Environment	Replication	Crop location	Cropping season	Seeding date	Transplanting date
E1	2	Changping, Beijing N40.20°, E115.51°	May–Oct. 2014	April 22, 2014	May 26, 2014
E2	2	Nanjing, Jiangsu N32.03°, E118.47°	May–Oct. 2014	May 15, 2014	June 16, 2014
E3	2	Sanya, Hainan N18.15°, E109.3°	Dec. 2013–May 2014	Dec. 04, 2013	Jan. 05, 2014
E4	2	Changping, Beijing N40.20°, E115.51°	May–Oct. 2015	April 28, 2015	June 02, 2015
E5	2	Nanjing, Jiangsu N32.03°, E118.47°	May–Oct. 2015	May 11, 2015	June 13, 2015
E6	2	Sanya, Hainan N18.15°, E109.3°	Dec. 2014–May 2015	Dec. 01, 2014	Dec. 30, 2015

polyacrylamide denaturing gels, and the bands were revealed by the silver-staining protocol (Panaud *et al.* 1996).

Determination of the physical location of molecular markers

A physical map of the polymorphic SSR and InDel molecular markers was constructed to verify the density and even distribution along the chromosomes. Using the online NCBI BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi), polymorphic markers were physically mapped to all twelve chromosomes of rice Nipponbare pseudomolecules, which were constructed by the International Rice Genome Sequencing Project (2005).

Data analysis and QTL mapping

The construction of graphical genotypes and calculation of the percentage of the total genome in CSSLs were performed using Graphical Genotypes (GGT) (van Berloo 1999). The underlying assumption of the GGT algorithm is that if two neighboring loci come from the same parent, the segment between them is likely to be from the same parental genome; in contrast, if the alleles at a pair of adjacent loci are inherited from different parents, a recombination event is assumed to have happened at the mid-point of the interval.

To explore the genetic basis for the four grain traits in different environments, a procedure of likelihood ratio test combined with stepwise regression (RSTEP-LRT), developed for QTL mapping in non-ideal or ideal CSSL populations was used (Wang *et al.* 2006). In the method of RSTEP-LRT, there are three steps. Firstly, a multiple linear model for phenotypes and adjusted phenotypic values adopted in RSTEP-LRT are summarized as follows. Assuming there were n CSS lines with m marker segments, the averaged phenotypic values for a quantitative trait in interest of the ith CSS line, y_i, is represented by the following linear model,

$$y_i = b_0 + \sum_{j=1}^{m} b_j x_{ij} + e_i \qquad (1)$$

where $i = 0$ (background parent), 1, 2, ..., n, b_0 is the intercept, b_j ($j = 1, ..., m$) is the partial regression coefficient of phenotype on the jth marker segment, x_{ij} is the indicator variable for the jth marker segment in the ith CSS line, and e_i is the random error. Considering the number of marker segments was always larger than the population size in plant genetics, to avoid model overfitting, stepwise regression of bidirectional selection was utilized in model (1) to select important markers associated with the trait in interest, where probabilities for marker segments entering into the model (1) (PIN) was set as 0.001; and probabilities for marker segments moving out of the model (1) (POUT) was set as 0.002 for all the four traits used in this study. Secondly, to test if there is a QTL linked with the jth marker segment, the phenotypic values in model (1) can be adjusted by,

$$\Delta y_i = y_i - b_0 - \sum_{k \neq j} b_k x_{ik} \qquad (2)$$

where Δy_i contained the QTL information on the current segment, and at the same time excluded the genetic effects from the other segments. Then, likelihood ratio test is conducted for the adjusted phenotypic values to determine whether a QTL exists in the testing segment. In this study, 1000 times of permutation test was used to determine LOD thresholds to declare significant QTL ($P < 0.05$), and also to calculate the genome-wide type I error for each QTL. Each time we reshuffled the phenotypic data and conducted the whole process of RSTEP-LRT. We define the P-value based on the permutation test result as 'genome-wide P-value'. For example, if the value of LOD score corresponds to the 100th largest value in the permutation test of 1000 cycles, the genome-wide P-value of the LOD score is regarded as 0.1. When the LOD score of QTL is larger than the maximum of 1000 LOD scores from permutation tests, P-values was set as 0.001, while the LOD score of QTL is smaller than the minimum of 1000 LOD scores from permutation tests, P-values was set as 1. The QTL detection by RSTEP-LRT, the calculation of phenotypic variation explained by each QTL, and the estimation of QTL additive effects, were implemented by software QTL IciMapping v4.1 (Meng *et al.* 2015). QTL nomenclature followed the recommendations of McCouch (2008).

A combined analysis of variance (ANOVA) over the six environments (lines × environments) was used to estimate the variance components, as described by Liu *et al.* (2016). The phenotypic variation of the four grain traits was estimated and a correlation analysis was performed to detect the association between grain traits based on the CSSL population data using SPSS19.0.

Results

Development of CSSLs

A total of 435 SSR markers and 175 InDel markers distributed across the 12 rice chromosomes were examined for polymorphisms between CWR274 and 9311; 119 SSR (27.3%) and 62 InDel (41.3%) polymorphic makers were used for the CSSL analysis (**Supplemental Table 1**). Selection of the CWR274 substitution lines in the 9311 background was achieved using these markers.

To identify the CSSLs, the F_1 plant derived from a cross between CWR274 and 9311 was backcrossed to 9311 three times to produce 324 BC_3F_1 plants. The candidate BC_3F_1 individuals were selected using 181 polymorphic markers based on the following criteria: (i) a single, relatively large chromosomal segment from CWR274 substituted into the target chromosome; (ii) a high level of residual homozygosis of 9311 alleles in non-target chromosomal regions; and (iii) partially overlapping segments in the chosen lines in order to cover all 12 rice chromosomes. These plants were backcrossed continuously by MAS following the same criteria and were then self-pollinated several times. Finally, we selected 36 lines from BC_3F_4, 59 lines from BC_4F_3, and 38 lines from BC_5F_3, to construct the CSSL population. The
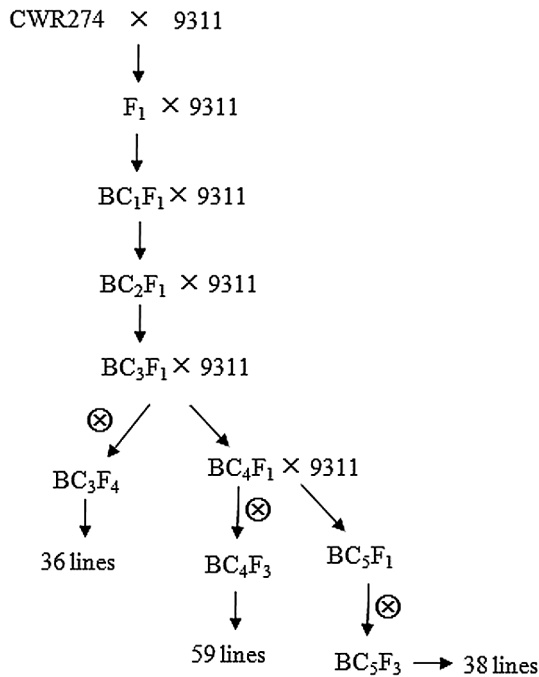
Fig. 1. Breeding scheme for the development of CSSLs carrying CWR274 segments in the 9311 background.

procedure of CSSL development is schematically described in **Fig. 1**.

Genotypes of the CSSLs

Graphical genotypes of the 133 CSSLs were generated using the linkage map of 119 SSR and 62 InDel markers (**Fig. 2**). The substituted chromosome segments covered most of the 12 chromosomes, except for seven gaps on Chr. 1 (defined by RM449 and RM543), Chr. 6 (defined by RM276, RM527 and RM3827), and Chr. 10 (defined by RM216 and RM239). The numbers of substituted chromosome segments among the CSSLs ranged from 1 to 14, with an average of 4.8 segments per CSSL. The roughly estimated lengths of the substituted segments ranged from 0.1 Mb to 7.9 Mb, with an average of 2.06 Mb per segment. The segment coverage was approximately 93.24% of the whole wild rice genome.

Phenotypic evaluations of the four rice grain-related traits in CSSL populations across six environments

The phenotypic variation of the four grain-related traits among the six environments is summarized in **Table 2**. Compared with the recurrent parent 9311, high phenotypic variation was detected in four grain-related traits in the CSSL populations in six environments. The highest degree of variation was observed for TGW, with a range of 24.43 to 39.58 g in E5. The mean TGW for 9311 differed among the six environments, ranging from 31.83 to 33.66 g, and the coefficient of variation ranged from 6.15% to 7.10%. In addition, all four grain-related traits showed continuous distributions and had ultra-parental genetic types in the CSSL

populations, the results show that segment from CWR274 had positive and negative effect in the four traits, suggesting that these traits were quantitatively inherited and polygenic.

Correlations among the rice traits for the six conditions are summarized in **Table 3**. The positive correlations between TGW and the other three grain-related traits were significant ($P < 0.01$), the correlation between GL and GW was not significant.

The results of the ANOVA for TGW, GL, GW, and LWR of the CSSL populations across the six different environments are summarized in **Table 4**. Significant effects of lines, environments, and their interaction (lines × environments) were observed for the four traits. The analysis of variance for grain traits in the CSSL population across two years × three locations is also summarized in **Supplemental Table 2**. Significant effects of CSSL, Site, Year, CSSL × Site, CSSL × Year, Site × Year, and their interaction (CSSL × Site × Year) were observed for the four traits.

QTL analyses for grain traits

Thirty-seven QTLs for the four traits were identified in the six environments and then mapped to twelve chromosomes (**Table 5**, **Fig. 3**). For TGW, the QTL *qTGW4* was consistently detected across three environments, with an average phenotypic variation explained (PVE) of 10.2%, and the wild rice allele at the *qTGW4* locus decreased TGW by an average of 2.24 g. For LWR, *qLWR8* were observed in three environments, with average PVE of 11.13%. *qLWR1.2*, *qLWR1.1*, *qLWR2.2*, *qLWR6*, and *qLWR12* were consistently observed in two environments. For the QTLs of GL, *qGL3.2*, *qGL4*, *qGL12.1*, and *qGL12.2* were consistently detected across two to four environments, and *qGL12.2* was mapped to chromosome 12 with a PVE of 16.54%. The wild rice allele at the *qGL12.2* locus increased GL by an average of 0.28 mm. For the GW QTLs, *qGW8.1*, *qGW9*, *qGW1.2*, *qGW4*, and *qGW8.3* were identified in two to three environments, with a PVE of 5.33–18.16%. For all QTLs that were detected in one or two environments, the performance of 37 QTLs in six environments were listed in **Supplemental Table 3**.

QTL clusters on chromosomes

Of 37 additive effect QTLs affecting four grain traits, 16 were detected under at least two environments and two QTL clusters were observed on Chr. 4 and Chr. 8 (**Fig. 3**). Near the RM280 marker on Chr. 4, one cluster harboring two QTLs (*qTGW4* and *qGL4*) controlled TGW and GL, near the closely linked markers RM126 (*qGW8.1* and *qLWR8*) and InDel8-3 (*TGW8*) on Chr. 8 has two QTLs affected GW and TGW.

Comparison of TGW between CSSLs and 9311 to select an elite resource

Based on the mean improvement in TGW across six environments, to select several high TGW lines for rice breeding, and detect QTLs in these lines that confirm the results
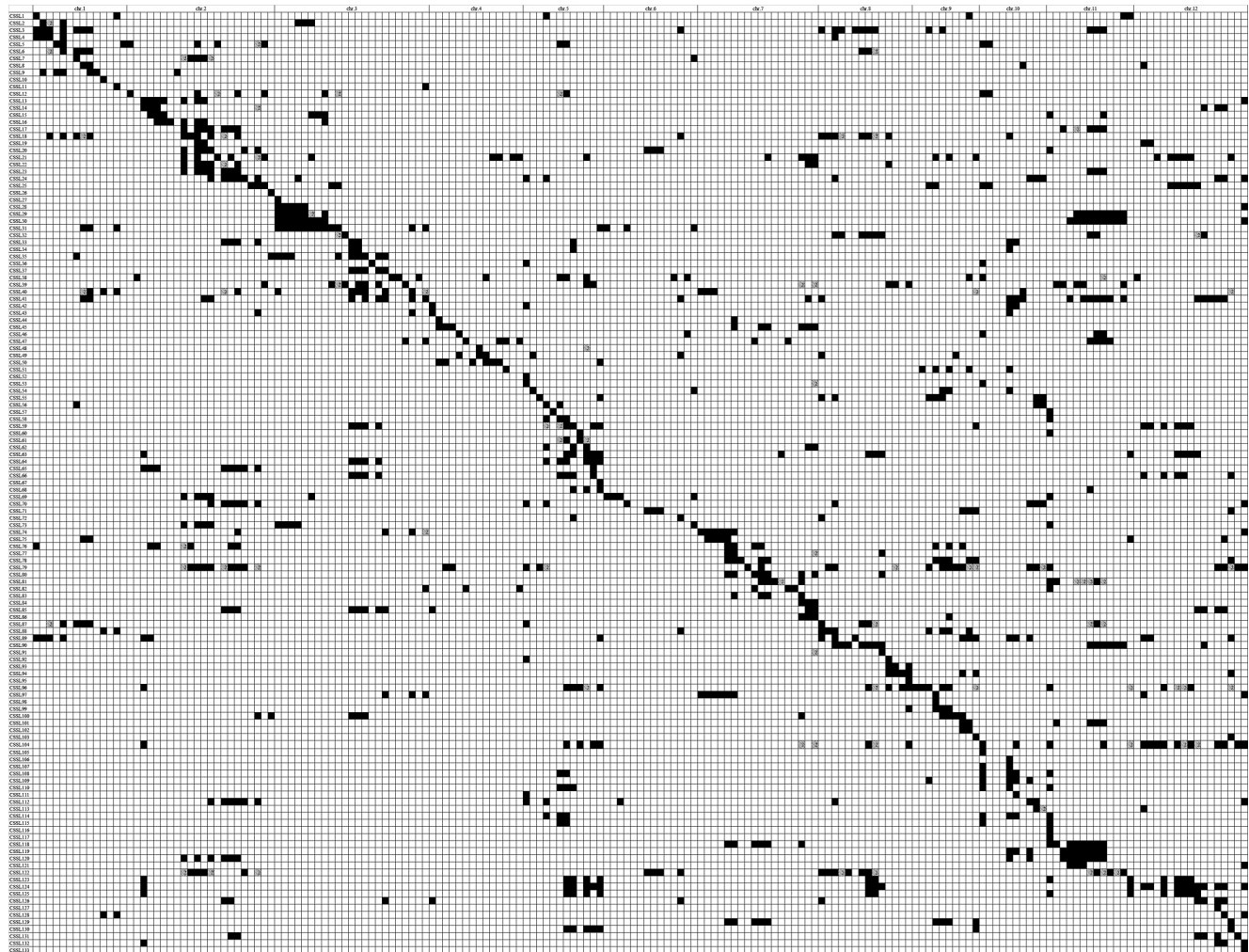
Fig. 2. Graphical representation of the CSSLs developed in this study. Each row represents a candidate CSSL line and each column represents a marker locus. Black bars indicate homozygous regions from CWR274; white bars indicate the 9311 genetic background; gray regions indicate heterozygous regions.

Table 2. Statistics of grain traits of 9311 and CSSLs in the six environments

Trait and environments	9311	CSSLs			Trait and environments	9311	CSSLs		
		Mean ± SD	Range	CV (%)			Mean ± SD	Range	CV (%)
TGW (g)					GL (mm)				
E1	32.89	30.01 ± 2.11	22.97–36.36	7.04	E1	9.77	9.74 ± 0.53	7.97–11.39	5.46
E2	31.83	31.35 ± 1.93	24.28–36.44	6.15	E2	9.69	9.68 ± 0.43	8.26–11.38	4.49
E3	31.94	30.48 ± 2.11	24.00–36.38	6.94	E3	9.62	9.74 ± 0.35	8.52–10.85	3.55
E4	32.36	30.24 ± 2.02	23.57–36.22	6.67	E4	9.46	9.47 ± 0.36	8.19–10.62	3.77
E5	33.66	32.90 ± 2.34	24.43–39.58	7.10	E5	9.58	9.66 ± 0.36	8.32–10.99	3.73
E6	32.5	31.17 ± 2.01	23.38–36.93	6.45	E6	9.57	9.64 ± 0.39	8.21–10.85	4.03
LWR (mm)					GW (mm)				
E1	3.40	3.48 ± 0.22	2.76–4.16	6.40	E1	2.88	2.80 ± 0.09	2.52–3.00	3.33
E2	3.44	3.46 ± 0.20	2.84–4.05	5.89	E2	2.81	2.80 ± 0.09	2.44–3.01	3.29
E3	3.42	3.46 ± 0.17	3.00–4.01	4.82	E3	2.81	2.82 ± 0.10	2.49–3.08	3.43
E4	3.38	3.43 ± 0.17	2.85–4.01	5.09	E4	2.81	2.78 ± 0.10	2.48–3.10	3.66
E5	3.41	3.45 ± 0.17	3.06–4.03	5.05	E5	2.82	2.81 ± 0.10	2.42–3.07	3.64
E6	3.35	3.38 ± 0.17	2.82–3.96	5.13	E6	2.85	2.86 ± 0.09	2.58–3.06	3.23

TGW: thousand grain weight; GL: grain length; LWR: length-width ratio; GW: grain width; CV: coefficient of variation; SD: standard deviation.
Naming of six environments is the same as **Table 1**.

Table 3. Correlations among grain-related traits in combined environments

	TGW	LWR	GL	GW
TGW	1			
LWR	0.165*	1		
GL	0.550**	0.769**	1	
GW	0.382**	−0.662**	−0.033	1

The abbreviations for the four traits (TGW, LWR, GL and GW) are the same as **Table 2**. Asterisks indicate significant correlation coefficients: *, $P \le 0.05$; **, $P \le 0.01$.

Table 4. Analysis of variance for grain traits of the CSSL population across combined six environments

SOD	Line	Environment	Line × Environment	Block (Environment)
df	133	5	665	6
TGW	61.46**	506.51**	5.37**	1.88
LWR	101.33**	99.82**	5.40**	1.1
GL	63.14**	120.86**	4.94**	1.75
GW	52.03**	104.24**	3.39**	46

SOD: sources of difference. *df*: degrees of freedom. The abbreviations for the four traits (TGW, LWR, GL and GW) are the same as those in **Table 2**. Asterisks indicate significance levels: *, $P \le 0.05$; **, $P \le 0.01$.

of the QTL analysis, three highest-TGW CSSLs, CSSL85, CSSL126, and CSSL132, were selected (**Table 6**). Compared to 9311, these CSSLs exhibited increases in TGW by 8.11–10.44%, on average, in the six environments. In addition, four lowest-TGW CSSLs, CSSL21, CSSL31, CSSL35, and CSSL82, showing the most decreases in TGW compared to 9311 in six environments were selected. Compared to 9311, these CSSLs showed apparent decreases in TGW by 17.79–23.85%. Both high-TGW and low-TGW CSSLs carried positive and negative QTLs, respectively. Due to the positive correlation index between TGW and GL, LWR, and GW, the high-TGW CSSLs were selected as elite resources for rice breeding.

Discussion

Previous studies have shown that *O. rufipogon* from southern China is the ancestor of current rice cultivars; more than 20% of alleles in the wild species have been lost during the domestication of rice (Huang *et al.* 2012, Sun et al 2001). The exploitation and utilization of favorable alleles from wild rice that were lost in current cultivars have become increasingly important. Some CSSLs have been utilized to identify new QTLs that may improve existing rice cultivars (Adachi *et al.* 2010, Marzougui *et al.* 2012), and many positive QTLs related to yield have been identified from common wild rice collected from various locations (Fu *et al.* 2010, Furuta *et al.* 2014, Septiningsih *et al.* 2003, Tan *et al.* 2007). In this study, we developed 133 CSSLs harboring chromosomal segments from the donor parent CWR274 collected from Hainan island of China, which has unique morphological and ecological characteristics that are primitive and distinguishable from those of cultivated rice. The common wild rice from that area is rarely used for genomics research, but is an important and unique genetic resource for rice genome and domestication studies (Qi *et al.* 2013).

Graphical genotypes of the 133 CSSLs were generated using a linkage map of 181 molecular markers. There were seven gaps in the population, possibly because MAS was performed from the BC_3 generation, other biological factors, such as genetic hybrid sterility and both the complexity and instability expression of photo-thermo-sensitive genes, offer an explanation for this observation.

Grain size and weight play important roles in the evolution and domestication of cultivated rice. The grains of wild relatives are usually small and round in shape; these traits are often favored under natural selection because they are associated with high fertility and are advantageous for dispersal by natural vectors. Using data for the CSSL population from six environments, a total of 37 QTLs for TGW, GL, GW, and LWR located on 12 chromosomes were identified in this study (**Table 5**).

A combined analysis of variance (ANOVA) of grain traits across years and locations showed that these traits were significantly influenced by genotype and environment, as well as genotype × environment interactions (**Table 4**). The interaction between environmental factors, year and location, also significantly influenced the performance of grain traits (**Supplemental Table 2**). The data indicated that minor QTL with low PVE effects are affected by environmental conditions.

Sixteen major QTLs were detected across more than two environments, among them, seven positive QTLs (*qLWR1.2*, *qLWR8*, *qLWR1.1*, *qLWR6*, *qLWR12*, *qGL12.2*, and *qGL12.1*) for grain size were identified from common wild rice accessions. Most of these QTLs appear to coincide to loci that were described previously (www.gramene.org). Two QTLs for TGW (*qTGW3* and *qTGW5*) were identified and located in the vicinity of QTLs detected in previous reports (Bian *et al.* 2010, Tian *et al.* 2005). Four loci associated with GL and LWR (*qGL3.2*, *qGL4*, *qLWR8*, and *qLWR2.2*) were probably the same locus described by Fan and other research teams (Fan *et al.* 2006, Jing *et al.* 2011, Wan *et al.* 2006). Three GW QTLs (*qGW8.1*, *qGW8.3*, and *qGW9*) may share the same location as those detected in other populations (Aluko *et al.* 2004, Jing *et al.* 2011, Nagata *et al.* 2015, Redona and Mackill 1998). As suggested by Tanksley (1993) and Zhuang *et al.* (1997), QTLs with major effects are more likely to be stable across multiple environments and various genetic backgrounds. For example, *qGL3.2* in the local InDel3-17 on chromosome 3 (**Fig. 3**), which was associated with GL, shared the same location as *GS3*, which has been identified in other interspecific crosses (Fan *et al.* 2006, Zhang *et al.* 2012). These results suggested that our CSSLs are an efficient population for grain-related QTL identification.

More importantly, seven QTLs for TGW, LWR, GL, and

Table 5. QTLs affecting four grain-related traits detected using CSSL population across six environments

Trait	QTL	Marker	Chr.	Environment	LOD Threshold	LOD	PVE (%)	Add
TGW	qTGW4	RM280	4	E2	3.03	3.05	8.17	−1.85
				E5	3.11	3.67	11.18	−2.62
				E6	3.32	3.87	11.26	−2.27
	qTGW5	Indel5-10	5	E3	2.87	3.65	12.38	−1.33
	qTGW3	RM411	3	E1	3.06	5.82	16.53	−3.53
	qTGW8	Indel8-3	8	E1		4.72	12.44	2.50
LWR	qLWR8	RM126	8	E3	3.10	4.54	7.58	0.08
				E4	3.16	6.31	11.14	0.10
				E5	3.14	6.20	14.66	0.11
	qLWR1.1	RM3740	1	E1	3.04	4.59	12.91	0.20
				E6	3.11	3.86	9.19	0.14
	qLWR6	RM30	6	E2	2.97	4.57	13.49	0.15
				E5		3.14	6.74	0.09
	qLWR1.2	RM128	1	E3		3.38	5.69	0.12
				E6		3.49	8.37	0.15
	qLWR2.2	Indel2-9	2	E4		3.60	6.06	−0.06
				E5		5.44	12.69	−0.09
	qLWR12	RM28621	12	E3		3.11	5.08	0.07
				E4		3.51	5.89	0.08
	qLWR3.2	Indel3-11	3	E4		3.58	6.11	−0.10
	qLWR1.3	Indel1-16	1	E4		6.18	10.89	0.14
	qLWR2.1	Indel2-8	2	E3		7.05	12.79	−0.11
	qLWR3.1	Indel3-3	3	E1		3.97	11.06	−0.17
	qLWR3.3	Indel3-17	3	E4		4.32	7.25	−0.16
	qLWR9	RM553	9	E3		4.30	6.99	0.09
GL	qGL12.2	RM28621	12	E2	3.35	6.40	19.87	0.34
				E4	3.14	3.24	7.14	0.17
				E5	3.41	9.70	24.29	0.32
				E6	3.17	5.94	14.85	0.27
	qGL3.2	Indel3-17	3	E3	3.11	5.90	11.94	−0.38
				E4		5.41	12.09	−0.41
				E6		3.02	7.22	−0.35
	qGL4	RM280	4	E4		3.41	7.53	−0.33
				E5		3.76	8.48	−0.35
				E6		3.86	9.30	−0.40
	qGL12.1	RM277	12	E3		5.65	11.39	0.25
				E4		4.19	9.17	0.23
	qGL3.1	Indel3-3	3	E1	2.95	4.28	12.50	−0.43
	qGL2	Indel2-8	2	E3		6.71	14.47	−0.23
	qGL10	RM171	10	E3		5.90	11.90	0.24
GW	qGW4	RM119	4	E4	3.15	3.82	7.66	−0.12
				E5	3.49	6.40	12.62	−0.15
				E6	3.14	3.84	8.41	−0.11
	qGW9	RM553	9	E1	2.84	3.74	7.86	−0.05
				E2	3.32	5.13	11.14	−0.06
				E3	3.08	3.03	8.60	−0.06
	qGW1.2	Indel1-16	1	E4		8.35	18.16	−0.10
				E6		6.90	15.97	−0.09
	qGW8.1	RM126	8	E1		4.71	10.15	−0.05
				E5		6.10	11.96	−0.06
	qGW8.3	Indel8-11	8	E5		3.35	6.26	−0.06
				E6		4.06	8.94	−0.06
	qGW1.1	RM3740	1	E4		4.74	9.67	−0.08
	qGW2	RM110	2	E5		5.94	11.61	−0.20
	qGW3.1	Indel3-26	3	E6		3.33	7.23	0.05
	qGW3.2	RM85	3	E1		4.07	8.70	−0.07
	qGW6	RM589	6	E4		3.27	6.48	0.11
	qGW8.4	RM264	8	E3		3.85	8.39	−0.11
	qGW8.2	RM6008	8	E2		5.75	12.27	−0.19
	qGW11	RM27074	11	E4		3.66	7.53	0.07
	qGW12	RM7119	12	E2		4.20	8.72	0.06

GW that were detected across more than two environments have not previously been identified, indicating potentially novel alleles from common wild rice. Two of the alleles related to GL showed positive effects. One QTL associated with GL (qGL12.2) was stably detected with positive effects in four environments, actually it can be detected
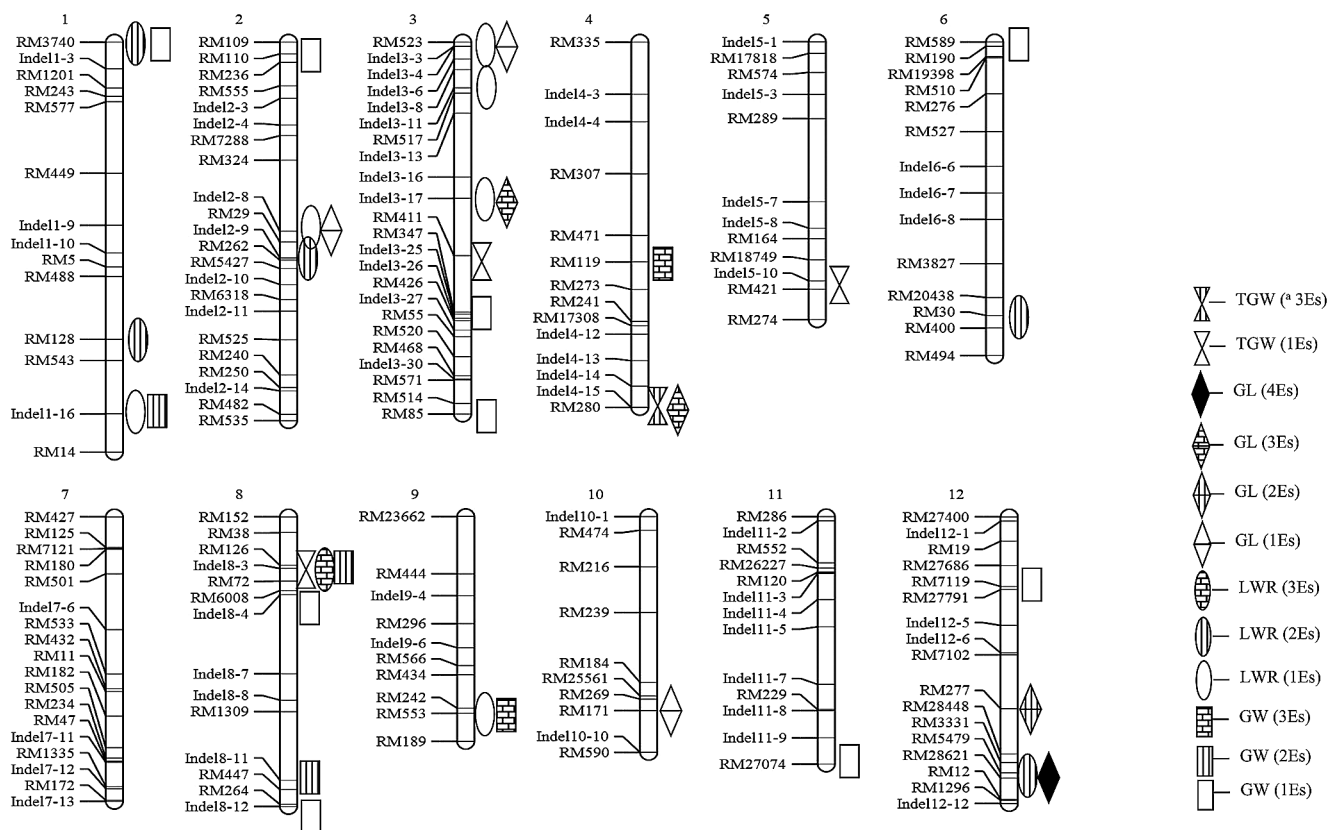
Fig. 3. Map locations of identified QTLs for 1000-grain weight (TGW), grain length (GL), grain width (GW), and length-width ratio (LWR) of the CSSL population, detected in six environments. 4Es represents QTLs detected in four environments, and the same terminology is used for all of the following QTLs; i.e., 1Es represents one environment; 2Es, two environments, etc.

Table 6. Number of high-TGW and low-TGW CSSLs and QTLs included in the substituted segments

Numbers of CSSLs	Thousand grain weight (TGW) (g)						Positive QTL	Negative QTL
	E1	E2	E3	E4	E5	E6		
High-TGW								
85	35.47	36.44	34.07	33.44	37.83	33.98	*qGL12.1, qGL12.2, qLWR12*	
126	33.33	35.7	33.87	36.22	39.58	36.93	*qLWR6, qLWR12, qGL12.2, qGL12.1*	
132	33.78	35.55	34.96	34.09	37.42	35.21	*qGL12.2, qLWR12*	
Low-TGW								
21	28.37	27.15	24	26.9	27.68	26.37		*qLWR3.2, qGL4, qTGW4, qTGW5, qLWR2.2, qLWR2.1, qGL2*
31	25.6	27.03	27.59	25.4	28.13	26.33		*qLWR3.2, qGL3.1, qGL3.2*
35	25.42	26.97	25.22	26.38	26.33	25.66		*qLWR3.1, qLWR3.3, qGL3.2, qGL3.1*
82	27.37	24.28	25.57	23.57	24.43	23.38		*qTGW4, qGL4*

Naming of six environments (E1–E6) is the same as **Table 1**.

in five environments while the threshold was LOD > 2.5 (**Supplemental Table 3**), *qGL12.2* with PVE of 16.54% was near RM28621 on Chr. 12. The wild rice allele at locus *qGL12.2* increased GL by an average of 0.28 mm. These data showed that *qGL12.2* is a novel QTL for modification of rice grain shape.

The traits of GW and GL have high heritability and are stably expressed; however, some QTLs were detected in one or two environments, and minor QTLs providing a small contribution rate were easily affected by environmental conditions. Significant interaction effects of lines and

environments were observed throughout this study. Several QTLs in this study were inconsistent between different environments. Since the CSSL contained more than one introgression segment, the genetic background was also an important factor to influence the stability of QTLs and similar multi-environmental results have previously been reported by Wang *et al.* 2006.

We performed a comparative analysis of high-TGW and low-TGW CSSLs. Compared to 9311, most of the highest-TGW CSSLs carried positive QTL and lowest-TGW CSSLs generally carried negative QTL (**Table 6**); three high-TGW

CSSLs carried the same positive QTL, *qGL12.2*. In future studies, the CSSLs harboring the positive QTLs (*qGL12.2*), such as CSSL132, will be backcrossed to 9311, and secondary F₂ populations will be exploited for fine mapping and positional cloning of the QTL. Given that cultivar 9311 is an elite variety and has been planted on a large scale in China, some elite lines in CSSL population could be used for rice breeding.

Many QTLs affecting related traits were mapped to similar genomic regions (**Fig. 3**); the high trait correlations likely reflected pleiotropic genetic basis. There was no significant correlation between GL and GW, and both were positively correlated with TGW, accordingly, the QTLs associated with GL or GW might affect TGW in the same direction. The correlation was consistent with previous results (Lee *et al.* 2005, Wan *et al.* 2005). Given that LWR was positively affected by GL and negatively affected by GW, three QTL clusters, RM3740 (*qLWR1* and *qGW1.1*) on Chr.1, RM126 (*qLWR8* and *qGW8.1*) on Chr. 8 and RM28621 (*qLWR12* and *qGL12.2*) on Chr. 12, might actually control a single trait, GW or GL.

Although many studies have already reported the development of CSSLs for *O. rufipogon* (Fu *et al.* 2010, Furuta *et al.* 2014, Marri *et al.* 2005), the use of different accessions of *O. rufipogon* may lead to the discovery of novel QTLs. In this study, 37 grain-related QTLs on 12 chromosomes were detected, among which 16 QTLs were detected in at least 2 environments. Seven novel QTLs were identified and *qGL12.2* was stably detected with positive effects in four environments. These novel QTLs identified in wild rice will facilitate fine mapping and functional studies. Our CSSL is not perfect as a single segment substitution line or as NIL; improvements are still required as well as backcrossing with the recipient parent to eliminate genetic noise. However, the advantage of the CSSL in this study was also clear; we have detected several QTLs using this population, and more importantly, this population provides a germplasm resource for rice breeding and a platform for wild rice genomic research.

Acknowledgments

Literature Cited

Adachi, S., Y. Tsuru, M. Kondo, T. Yamamoto, Y. Arai-Sanoh, T. Ando, T. Ookawa, M. Yano and T. Hirasawa (2010) Characterization of a rice variety with high hydraulic conductance and identification of the chromosome region responsible using chromosome segment substitution lines. Ann. Bot. 106: 803–811.

Alexandratos, N. and J. Bruinsma (2012) World agriculture towards 2030/2050: the 2012 revision, FAO, Rome, ESA Working Paper No. 12-03.

Aluko, G., C. Martinez, J. Tohme, C. Castano, C. Bergman and J.H. Oard (2004) QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima*. Theor. Appl. Genet. 109: 630–639.

Ando, T., T. Yamamoto, T. Shimizu, X.F. Ma, A. Shomura, Y. Takeuchi, S.Y. Lin and M. Yano (2008) Genetic dissection and pyramiding of quantitative traits for panicle architecture by using chromosomal segment substitution lines in rice. Theor. Appl. Genet. 116: 881–890.

Bai, X.F., L.J. Luo, W.H. Yan, M.R. Kovi, W. Zhan and Y.Z. Xing (2010) Genetic dissection of rice grain shape using a recombinant inbred line population derived from two contrasting parents and fine mapping a pleiotropic quantitative trait locus *qGL7*. BMC Genet. 11: 16.

Bian, J.M., L. Jiang, L.L. Liu, X.J. Wei, Y.H. Xiao, L.J. Zhang, Z.G. Zhao, H.Q. Zhai and J.M. Wan (2010) Construction of a new set of rice chromosome segment substitution lines and identification of grain weight and related traits QTLs. Breed. Sci. 60: 305–313.

Ebitani, T., Y. Takeuchi, Y. Nonoue, T. Yamamoto, K. Takeuchi and M. Yano (2005) Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of *indica* rice cultivar 'Kasalath' in a genetic background of *japonica* elite cultivar 'Koshihikari'. Breed. Sci. 55: 65–73.

Fan, C., Y. Xing, H. Mao, T. Lu, B. Han, C. Xu, X. Li and Q. Zhang (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor. Appl. Genet. 112: 1164–1171.

Fu, Q., P.J. Zhang, L.B. Tan, Z.F. Zhu, D. Ma, Y.C. Fu, X.C. Zhan, H.W. Cai and C.Q. Sun (2010) Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.). J. Genet. Genomics 37: 147–157.

Furuta, T., K. Uehara, R.B. Angeles-Shim, J. Shim, M. Ashikari and T. Takashi (2014) Development and evaluation of chromosome segment substitution lines (CSSLs) carrying chromosome segments derived from *Oryza rufipogon* in the genetic background of *Oryza sativa* L. Breed. Sci. 63: 468–475.

Huang, R.Y., L.R. Jiang, J.S. Zheng, T.S. Wang, H.C. Wang, Y.M. Huang and Z.L. Hong (2013) Genetic bases of rice grain shape: so many genes, so little known. Trends Plant Sci. 18: 218–226.

Huang, X.H., N. Kurata, X.H. Wei, Z.X. Wang, A.H. Wang, Q. Zhao, Y. Zhao, K.Y. Liu, H.Y. Lu, W.J. Li *et al.* (2012) A map of rice genome variation reveals the origin of cultivated rice. Nature 490: 497–501.

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature 436: 793–800.

Jing, Z.B., Y. Chen, D.J. Pan, Y.Y. Qu, Z.L. Fan, J.Y. Chen and C. Li (2011) Development and evaluation of primary introgression lines of rice by advanced backcross QTL strategy for Gaozhou wild rice (*O. rufipogon*). Rice Genomics Genet. 2: 1–11.

Kubo, T., Y. Aida, K. Nakamura, H. Tsunematsu, K. Doi and A. Yoshimura (2002) Reciprocal chromosome segment substitution series derived from *japonica* and *indica* cross of rice (*Oryza sativa* L.). Breed. Sci. 52: 319–325.

Lee, S.J., C.S. Oh, J.P. Suh, S.R. McCouch and S.N. Ahn (2005) Identification of QTLs for domestication-related and agronomic traits in

an *Oryza sativa × O. rufipogon* BC$_1$F$_7$ population. Plant Breed. 124: 209–219.

Li, Y.B., C.C. Fan, Y.Z. Xing, Y.H. Jiang, L.J. Luo, L. Sun, D. Shao, C.J. Xu, X.H. Li, J. Xiao *et al.* (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. Nat. Genet. 43: 1266–1269.

Liu, X., Z.G. Zhao, L.L. Liu, Y.H. Xiao, Y.L. Tian, S.J. Liu, L.M. Chen, Y. Wang, Y.Q. Liu, S.H. Chen *et al.* (2016) Construction of chromosomal segment substitution lines and genetic dissection of introgressed segments associated with yield determination in the parents of a super-hybrid rice. Plant Breed. 135: 63–72.

Marri, P.R., N. Sarla, L.V. Reddy and E.A. Siddiq (2005) Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*. BMC Genet. 6: 33.

Marzougui, S., K. Sugimoto, U. Yamanouchi, M. Shimono, T. Hoshino, K. Hori, M. Kobayashi, K. Ishiyama and M. Yano (2012) Mapping and characterization of seed dormancy QTLs using chromosome segment substitution lines in rice. Theor. Appl. Genet. 124: 893–902.

McCouch, S.R., L. Teytelman, Y.B. Xu, K.B. Lobos, K. Clare, M. Walton, B.Y. Fu, R. Maghirang, Z.K. Li, Y.Z. Xing *et al.* (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 9: 199–207.

McCouch, S.R., M. Sweeney, J.M. Li, H. Jiang, M. Thomson, E. Septiningsih, J. Edwards, P. Moncada, J.H. Xiao, A. Garris *et al.* (2007) Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa*. Euphytica 154: 317–339.

McCouch, S.R. (2008) Gene nomenclature system for rice. Rice 1: 72–84.

Meng, L., H.H. Li, L.Y. Zhang and J.K. Wang (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J. 3: 269–283.

Onishi, K., Y. Horiuchi, N. Ishigoh-Oka, K. Takagi, N. Ichikawa, M. Maruoka and Y. Sano (2007) A QTL cluster for plant architecture and its ecological significance in Asian wild rice. Breed. Sci. 57: 7–16.

Panaud, O., X. Chen and S.R. McCouch (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol. Gen. Genet. 252: 597–607.

Qi, L., X.N. Wang, J.Z. Zhang, Q.J. Tang, W.D. Meng and X.W. Yan (2013) Study on genetic diversity and differentiation of three wild rice species in Hainan using SRAP markers. J. Plant Genet. Resour. 14: 402–406.

Qi, P., Y.S. Lin, X.J. Song, J.B. Shen, W. Huang, J.X. Shan, M.Z. Zhu, L. Jiang, J.P. Gao and H.X. Lin (2012) The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating Cyclin-T1;3. Cell Res. 22: 1666–1680.

Redona, E.D. and D.J. Mackill (1998) Quantitative trait locus analysis for rice panicle and grain characteristics. Theor. Appl. Genet. 96: 957–963.

Septiningsih, E.M., J. Prasetiyono, E. Lubis, T.H. Tai, T. Tjubaryat, S. Moeljopawiro and S.R. McCouch (2003) Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theor. Appl. Genet. 107: 1419–1432.

Shim, R.A., E.R. Angeles, M. Ashikari and T. Takashi (2010) Development and evaluation of *Oryza glaberrima* steud. chromosome segment substitution lines (CSSLs) in the background of *O. sativa* L. cv. Koshihikari. Breed. Sci. 60: 613–619.

Song, X.J., W. Huang, M. Shi, M.Z. Zhu and H.X. Lin (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat. Genet. 39: 623–630.

Song, X.J., T. Kuroha, M. Ayano, T. Furuta, K. Nagai, N. Komeda, S. Segami, K. Miura, D. Ogawa, T. Kamura *et al.* (2015) Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice. Proc. Nalt. Acad. Sci. USA 112: 76–81.

Sun, C.Q., X.K. Wang, Z.C. Li, A. Yoshimura and N. Iwata (2001) Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theor. Appl. Genet. 102: 157–162.

Tan, L.B., F.X. Liu, W. Xue, G.J. Wang, S. Ye, Z.F. Zhu, Y.C. Fu, X.K. Wang and C.Q. Sun (2007) Development of *Oryza rufipogon* and *O. sativa* introgression lines and assessment for yield-related quantitative trait loci. J. Integr. Plant Biol. 49: 871–884.

Tan, L.B., P.J. Zhang, F.X. Liu, G.J. Wang, S. Ye, Z.F. Zhu, Y.C. Fu, H.W. Cai and C.Q. Sun (2008) Quantitative trait loci underlying domestication- and yield-related traits in an *Oryza sativa × Oryza rufipogon* advanced backcross population. Genome 51: 692–704.

Tanksley, S.D. (1993) Mapping polygenes. Annu. Rev. Genet. 27: 205–233.

Temnykh, S., W.D. Park, N. Ayres, S. Cartinhour, N. Hauck, L. Lipovich, Y.G. Cho, T. Ishii and S.R. McCouch (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 100: 697–712.

Tian, F., D.J. Li, Q. Fu, Z.F. Zhu, Y.C. Fu, X.K. Wang and C.Q. Sun (2005) Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield-related traits. Theor. Appl. Genet. 112: 570–580.

van Berloo, R. (1999) Computer note. GGT. software for the display of graphical genotypes. J. Hered. 90: 328–329.

Wan, X.Y., J.M. Wan, J.F. Weng, L. Jiang, J.C. Bi, C.M. Wang and H.Q. Zhai (2005) Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. Theor. Appl. Genet. 110: 1334–1346.

Wan, X.Y., J.M. Wan, L. Jiang, J.K. Wang, H.Q. Zhai, J.F. Weng, H.L. Wang, C.L. Lei, J.L. Wang, X. Zhang *et al.* (2006) QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. Theor. Appl. Genet. 112: 1258–1270.

Wang, J.K., X.Y. Wan, J. Crossa, J. Crouch, J.F. Weng, H.Q. Zhai and J.M. Wan (2006) QTL mapping of grain length in rice (*Oryza sativa* L.) using chromosome segment substitution lines. Genet. Res. 88: 93–104.

Wang, Z.Y., G. Second and S.D. Tanksley (1992) Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor. Appl. Genet. 83: 565–581.

Wei, X., W.H. Qiao, Y.T. Chen, R.S. Wang, L.R. Cao, W.X. Zhang, N.N. Yuan, Z.C. Li, H.L. Zeng and Q.W. Yang (2012) Domestication and geographic origin of *Oryza sativa* in China: insights from multilocus analysis of nucleotide variation of *O. sativa* and *O. rufipogon*. Mol. Ecol. 21: 5073–5087.

Xiao, J.H., S. Grandllo, S.N. Ahn, S.R. McCouch, S.D. Tanksley, J.M. Li and L.P. Yuan (1996) Genes from wild rice improve yield. Nature 384: 223–224.

Xie, X.B., F.X. Jin, M.H. Song, J.P. Suh, H.G. Hwang, Y.G. Kim, S.R. McCouch and S.N. Ahn (2008) Fine mapping of a yield-enhancing QTL cluster associated with transgressive variation in an *Oryza sativa × O. rufipogon* cross. Theor. Appl. Genet. 116:

613–622.

Yamamoto, T., J. Yonemaru and M. Yano (2009) Towards the understanding of complex traits in rice: substantially or superficially? DNA Res. 16: 141–154.

Yano, M. and T. Sasaki (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol. Biol. 35: 145–153.

Yoshida, S., M. Ikegami, J. Kuze, K. Sawada, Z. Hashimoto, T. Ishii, C. Nakamura and O. Kamijima (2002) QTL analysis for plant and grain characters of sake-brewing rice using a doubled haploid population. Breed. Sci. 52: 309–317.

Yuan, P.R., H.J. Kim, Q.H. Chen, H.G. Ju, S.J. Lee, S.D. Ji and S.N. Ahn (2009) QTL dissection of agronomic and domestication traits using introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*O. sativa* L.) background. J. Crop Sci.

Biotechnol. 12: 245–252.

Zhang, X.J., J.F. Wang, J. Huanga, H.X. Lan, C.L. Wang, C.F. Yin, Y.Y. Wu, H.J. Tang, Q. Qian, J.Y. Li *et al.* (2012) Rare allele of *OsPPKL1* associated with grain length causes extra-large grain and a significant yield increase in rice. Proc. Natl. Acad. Sci. USA 109: 21534–21539.

Zheng, K.L., N. Huang, J. Bennett and G.S. Khush (1995) PCR-based marker assisted selection in rice breeding. IRRI discussion paper series No. 12.

Zhuang, J.Y., H.X. Lin, J. Lu, H.R. Qian, S. Hittalmani, N. Huang and K.L. Zheng (1997) Analysis of QTL × environment interaction for yield components and plant height in rice. Theor. Appl. Genet. 95: 799–808.