QTL analysis on rice grain appearance quality, as exemplifying the typical events of transgenic or backcrossing breeding

Bao Yan1), Rongjia Liu1), Yibo Li1), Yan Wang1), Guanjun Gao1), Qinglu Zhang1), Xing Liu1), Gonghao Jiang2) and Yuqing He*1)

1) National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research (Wuhan) and National Center of Crop Molecular Breeding, Huazhong Agricultural University, Wuhan 430070, China
2) College of Life Science, Heilongjiang University, Haerbin 150080, China

Rice grain shape and yield are usually controlled by multiple quantitative trait loci (QTL). This study used a set of F9–10 recombinant inbred lines (RILs) derived from a cross of Huahui 3 (Bt/Xa21) and Zhongguoxiangdao, and detected 27 QTLs on ten rice chromosomes. Among them, twelve QTLs responsive for grain shape/or yield were mostly reproducibly detected and had not yet been reported before. Interestingly, the two known genes involved in the materials, with one insect-resistant Bt gene, and the other disease-resistant Xa21 gene, were found to closely link the QTLs responsive for grain shape and weight. The Bt fragment insertion was firstly mapped on the chromosome 10 in Huahui 3 and may disrupt grain-related QTLs resulting in weaker yield performance in transgenic plants. The introgression of Xa21 gene by backcrossing from donor material into receptor Minghui 63 may also contain a donor linkage drag which included minor-effect QTL alleles positively affecting grain shape and yield. The QTL analysis on rice grain appearance quality exemplified the typical events of transgenic or backcrossing breeding. The QTL findings in this study will in the future facilitate the gene isolation and breeding application for improvement of rice grain shape and yield.

Key Words: Oryza sativa L., grain shape and weight, QTL, Bt gene.

Introduction

Rice is one of the most important cereal crops and a staple food in Asia. Grain weight is one of the most important components of rice grain yield and is conditioned by quantitative trait locus (QTL). Grain shape, a typical complex quantitative trait, is closely associated with grain weight and usually measured by grain length (GL), width (GW), thickness (GT) and length-to-width ratio (LWR) (Lin and Wu 2003, Yoon et al. 2006). LWR is considered to be an important measure of rice appearance quality since people’s preferences for it are rather different in the rice-producing areas of the world (Wan et al. 2006, 2008). Extensively phenotypic variations have been observed among cultivars, subspecies and species of Oryza sativa L. Hundreds of QTLs for grain shape/weight have been identified and are scattered throughout the whole rice genome (www.gramene.com). Many QTLs can be repeatedly detected across different populations and environments. For example, one major QTL for GL and grain weight was consistently mapped near the centromeric region of rice chromosome 3 using different populations in several independent studies (Li et al. 2004b, Li et al. 1997, Thomson et al. 2003, Xiao et al. 1998, Xing et al. 2002, Yu et al. 1997).

Stable genetic effect on regulating grain weight/shape is critical for a QTL to be utilized in rice breeding. Some QTLs were fine mapped and validated with nearly isogenic lines. A few QTLs associated with grain weight, gw3.1, gw3, gw6, gw8.1, gw9.1 and gwi1, have been identified at accurate intrachromosomal locations (Guo et al. 2009, Li et al. 2004a, Oh et al. 2011, Xie et al. 2006, 2008). GS3, gl-3, qGL7 and qGL7-2 controlling GL were fine mapped at the exact locations on chromosomes 3 and 7 (Bai et al. 2010, Fan et al. 2006, Shao et al. 2010, Wan et al. 2006). qGW5 for GW was narrowed down to a 49.7 kb genomic region on chromosome 5 (Wan et al. 2008).

Grain traits are closely correlated and QTLs for different traits are often mapped in the similar interval. For example, confidence interval of gw3.1 overlapped with that of gl-3 and GS3. Fine mapping of these QTLs provides a basis for further cloning and marker assisted selection breeding.

Several major QTLs controlling rice grain shape were isolated and characterized using map-based cloning method due to the progress in rice functional genomics. GS3, a major QTL for GL, encodes a putative transmembrane protein and is composed of four putative domains. GS3 negatively regulated GL and loss of function or deletion of plant-specific organ size regulation (OSR) domain would result in long grain (Fan et al. 2006, Mao et al. 2010). Three QTLs for GW, GW2, qSW5/GW5 and GS5, have been cloned and

Communicated by S.-N. Ahn
Received February 17, 2014. Accepted June 9, 2014.
*Corresponding author (e-mail: yqhe@mail.hzau.edu.cn)
characterized. GW2 encodes a RING-type protein with E3 ubiquitin ligase activity, which is known to function in the degradation by the ubiquitin proteasome pathway. GW2 E3 ligase is a new negative regulator of cell division and the mutant allele of GW2 promotes spikelet hull cell division, resulting in an increase of grain width and weight (Song et al. 2007). qSW5 and GW5 are the same QTL and have been identified on chromosome 5 (Shomura et al. 2008, Weng et al. 2008). GW5 encodes a novel nuclear protein, which physically interacts with polyubiquitin and is also likely to act in the ubiquitin-proteasome pathway to regulate cell division. Additionally, GW5 is a negative regulator and the mutant allele causes an increase in GW (Weng et al. 2008). GS5 encodes a putative serine carboxypeptidase and functions as a positive regulator of GW. Overexpression of GS5 may promote cell division resulting in an increase of GW (Li et al. 2011). Functional characterization of these genes has provided novel insights to understand genetic and molecular mechanisms regulating grain size. However, the mechanism of regulation of grain size and epistatic interactions between these QTLs are still unclear.

Efficient crop improvement included transgenic and backcrossing breeding. For transgenic breeding, agrobacterium-mediated transformation is being increasingly used in rice. It can realize foreign gene integrating into rice genome and subsequently precisely improve the target trait (Tu et al. 2000a). Backcrossing breeding takes the strategy of transferring donor objective gene into receptor and meanwhile strenuously excluding donor transgenic background via multiple backcrossing. Backcrossing is usually regarded conventional breeding and can be performed more efficiency through marker assisted selection (Chen et al. 2000). In comparison, transgenic breeding can raise some problems such as transgenic silencing and endogenic insertional silencing, while backcrossing breeding needs more crossing generations and can frequently accompany genetic background problems typical of the event of linkage drag.

Here, two parents contrasting in grain shape and yield, with one parent containing the events of transgenic and backcrossing breeding improvement, were selected to develop a RIL mapping population. This study aimed to achieve two points. One was to identify more grain-shape QTLs to help enhancing the understanding of rice seed development, and the other tried to analyze the genetic base underlying the grain shape changes brought by transgenic and backcrossing breeding.

**Materials and Methods**

**Plant materials and field experiments**

A set of RILs population was constructed from an F$_{9:10}$ population consisted of 237 individuals from a cross between Zhongguoxiandao (ZGX) and Huahui 3 (HH 3). ZGX is a high-quality rice which is extensively planted in south China. HH 3 is an improved rice material on the base of an elite indica cytoplasm male sterile (CMS) restorer line Minghui 63. As previously described, firstly the wild-rice-derived dominant gene Xa21 conferring multi-race resistance to bacterial blight and a fused $Bt$ gene $cry1Ab/cry1Ac$ conferring resistance to lepidopteran insects were individually introduced into the same genetic background of Minghui 63. The former (here designated as Minghui 63/ Xa21) was bred from a recurrent backcross between IRBB21 as donor line and Minghui 63 as recurrent parent via marker-assisted selection (Chen et al. 2000). The latter (designated as Minghui 63/Bt) is a marker free transgenic line obtained by biolistic transformation (Tu et al. 2000a). Through crossing the two improved Minghui 63, the two genes were then pyramided into the same recipient plant of Minghui 63 (designated as Huahui 3 or Minghui 63/ Bt&Xa21), showing desirable insect- and disease-resistant phenotypes (Jiang et al. 2004). That is to say, Minghui 63/ Xa21, Minghui 63/Bt and Minghui 63/Bt&Xa21 (HH 3) are near isogenic lines on the background of Minghui 63. The field trials of the lines, including Minghui 63/Xa21, Minghui 63/Bt, the derived RIL population and their two parents ZGX and HH 3, were carried out in the normal season of the years of 2009 and 2010 in Wuhan. The tested materials were planted in a randomized complete block design with two replications. In each block, 24 plants for each line were planted in a two-row plot with a 17 cm distance between plants within a row and 27 cm between rows. The central eight plants in a row in each plot were examined for agronomic traits. Normal cultural practices for growing rice were followed during the course of the experiments.

**Phenotypic measurements**

Harvested rice grains were stored and dried at room temperature for at least 3 months before processing. Five yield-related traits including grain length (GL, in millimeters), grain width (GW, in millimeters), ratio of grain length to width (LWR), grain-thickness (GT, in millimeters) and 1000-grain weight (TGW, in grams) were examined from eight randomly selected plants in the middle of the rows of each line. Ten full-filled rice grains were chosen randomly from each plant for trait measurement. GL was estimated by placing 10 grains length-wise one by one in a straight line along a ruler. These 10 seeds were individually measured for GW and GT using an electronic digital caliper (Guanglu Measuring Instrument Co. Ltd., China) with a precision of 0.1 mm. The averaged GL, GW, and GT as the trait values of that line were used for data analysis. The grain length-to-width ratio (LWR) is equal to GL divided by its GW. TGW was calculated as the grain weight per plant divided by its grain number multiplied by 1000.

**Molecular markers and linkage map**

Fresh leaves were collected from each material for DNA extraction. DNA was extracted using a microisolation method as described by Cho et al. (1996) with minor modifications. Polymorphic simple sequence repeat (SSR) markers or insertion deletion (InDel) markers between the parents
ZGX and HH3 were used to genotype the population. The SSR assay was carried out essentially as described by Wu and Tanksley (1993). The genetic linkage map with RILs was constructed by using MAPMAKER/EXP version 3.0b (1987). The local linkage map based on the NIL-F2 population was developed. Genetic distance was calculated in Haldane function (1919).

Data analysis

The STATISTICA statistical package was used to analyze the t-test, frequency distribution, and correlation coefficients of traits. The chromosomal locations of QTLs were determined by composite interval mapping (CIM). CIM analysis was performed using WinQTLcart 2.0 (Wang and Zeng 2004), and epistatic QTLs analysis was operated by QTLNetwork 2.0 (Yang et al. 2004), and epistatic QTLs analysis was performed using WinQTLcart 2.0 (Wang and Zeng 2004), and epistatic QTLs analysis was operated by QTLNetwork 2.0 (Yang et al. 2008). A threshold of $P \leq 0.005$ and LOD $\geq 2.5$ was used to declare the significant main effect QTL (M-QTL), digenic epistatic QTLs, and QTL $\times$ environment interaction. Contribution rate ($h^2$, %) was estimated as the percentage of variance explained by each locus or epistatic pair in proportion to the total phenotypic variance. QTLs were named following the popular nomenclature but in alphabetic order for QTLs on the same chromosome.

Results

Grain shape and yield performance of Minghui 63 added with Bt and/or Xa21 genes

As mentioned before (Jiang et al. 2004), Minghui 63 added with Bt or Xa21 genes can highly increase the insect- or disease-resistance. However, in the currently used material Minghui 63 possessing Bt with or without Xa21 genes, the grain performance of GL and GW and TGW was significantly decreased when compared to original receptor Minghui 63. Minghui 63 (Xa21) showed a bit better than Minghui 63 in the three traits of GL, GW and TGW, but not reaching a statistical significant level (Table 1). These data suggested that at least the introduction of Bt gene altered the receptor’s grain-related genetic environment. To elucidate the genetic cause thus becomes necessary.

Table 2. Statistics of the five traits in parents of Zhongguoxiandao (ZGX) and Huahui 3 (HH 3) and the derived recombination inbred line (RIL) population observed in 2009 (upper) and 2010 (lower)

| Traits       | Parent (mean ± standard error) | RILs (mean ± standard error) | Range       |
|--------------|-------------------------------|------------------------------|-------------|
| GL (mm)      | HH 3: 9.91 ± 0.11; ZGX 9.61 ± 0.00 | 9.71 ± 0.04; 7.04–10.76     |             |
| GW (mm)      | HH 3: 2.65 ± 0.00; ZGX 2.47 ± 0.00 | 2.77 ± 0.14; 2.03–3.53      |             |
| GT (mm)      | HH 3: 1.89 ± 0.00; ZGX 1.69 ± 0.00 | 2.07 ± 0.12; 1.24–3.40      |             |
| LWR          | HH 3: 3.45 ± 0.05; ZGX 3.35 ± 0.05 | 3.51 ± 0.22; 2.48–4.68      |             |
| TGW (g)      | HH 3: 17.0 ± 0.0; ZGX 15.0 ± 0.0 | 26.2 ± 2.3; 20.7–33.4       |             |

GL, GW and GT are grain length, width and thickness, respectively. LWR, length/width ratio. TGW, 1000-grain weight.

Distribution and correlation of the traits in RILs

In order to elucidate the genetic cases of Bt or Xa21 introduction into Minghui 63 and meanwhile to identify more QTLs responsive for grain shape and yield, a set of RILs population was constructed from an F9:10 population consisted of 237 individuals from a cross between Zhongguoxiandao (ZGX) and Huahui 3 (HH 3, Minghui63/ Bt&Xa21). There were significant differences between two parents on the traits of GL, TGW, and moderate differences on GW, GT and LWR (Table 2). The ZGX grain is significantly longer and heavier than that of HH 3. And due to the similar performance of GW and GT, the heavier TGW of ZGX grain should be mainly raised by the longer GL. Among the population, the five traits exhibited wide variation with the mean values of performance nearly all fallen into the range between that of the two parents. All the five traits in the population exhibited normal distribution patterns similar in the 2 years, indicating a quantitative inheritance underlying these traits (Fig. 1).

The phenotypic correlation coefficients showed the traits of GL, GW, GT and TGW were significantly correlated with each other in the two years ($p < 0.05$) (Table 3). LWR had significant correlation positively with GL and negatively with GW, but with no or small correlation with GT and TGW.

Genetic linkage map

According to the reference genetic maps (http://www.gramene.org/), 1251 SSR and four insertion/deletion (InDel) markers were surveyed and consequently 203 markers were identified to be polymorphic between the two parents ZGX and HH 3. The percentage of marker polymorphism accounted for 16%. Among them, a total of 152 simple sequence repeat (SSR) and the four InDel markers, which evenly distributed on the 12 rice chromosomes, were selected for genotyping the RIL population. The linkage map covers a total of 1680.1 centimorgan (cM) of the 12 rice
chromosomes. The average distance is 10.9 cM between two adjacent markers. The \( Bt \) transgene in the HH 3 is mapped in the interval of RM2503-RM1126 on chromosome 10.

**QTLs for grain shape and yield**

27 QTLs were identified for the five traits evaluated in 2009 and 2010 (Table 4 and Fig. 2), among which 17 QTLs were detected in both years and the other ten QTLs were identified only in one year.

Four QTLs were identified for GL on chromosomes 1, 8, 9 and 10, totally explaining the phenotypic variation of 29.6% in 2009 and 46.7% in 2010 (Table 4), and three QTLs (\( qGL8 \), \( qGL9 \), \( qGL10 \)) of them were identified in both...
QTLs for rice grain appearance quality

The QTL qGL10, flanked by RM25003 and Bt on chromosome 10, had the largest additive effect, explaining the phenotypic variation of 17.7% in 2009 and 28.0% in 2010, and the allele from HH 3 decreased grain length by 0.26 mm and 0.23 mm in 2009 and 2010, respectively (Table 4). The other QTLs increased grain length by HH 3 allele except qGL1.

Six QTLs for GW on chromosomes 2, 3, 4, 10 and 11, totally explained the phenotypic variation of 27.0% in 2009 and 53.5% in 2010. Three (qGW3, qGW10, qGW11a) of them were found in both years (Table 4). One larger effect QTL, qGW10, also flanked by RM1126 and Bt on chromosome 10, co-located with the main effect QTL qGL10 (Fig. 2). The allele of qGW10 from HH 3 decreased grain width, consistent with the effect of qGL10 for grain length.

Analysis of the F_{9:10} population resolved six QTLs for LWR (Table 4). All these QTLs with the alleles of HH 3 decreased the LWR, except qLWR5. All QTLs controlling length/width ratio had small effects explaining 3.5%–9.4% of the phenotypic variance. In total, these QTLs explained 22.7% and 38.5% of the total phenotypic variance in 2009 and 2010, respectively.

For GT, five QTLs were detected and three (qGT3, qGT10, qGT11) of them were detected in two years (Table 4). The QTL qGT10 was identified to possess the largest additive effect in the region of Bt-RM1126 on chromosome 10, explaining 10.2% in 2009 and 23.3% in 2010 of the total phenotypic variation. The qGT10 allele from HH 3 had a decreasing effect on GT in both years. The other four QTLs contributed GT in a percentage of 3.3% to 5.5%.

A total of six QTL for TGW were identified. Of these, the

Table 4. QTLs detected for rice grain size and weight using the F_{9:10} RIL population derived from a cross between Zhongguoxiandao (ZGX) and Huahui 3 (HH 3) by the interval mapping at LOD threshold 2.4

| Trait | QTLs | Chr. | Interval markers | 2009 LOD | A^b (%) | 2010 LOD | A^b (%) |
|-------|------|------|------------------|----------|---------|----------|---------|
| GL    | qGL1 | 1    | RM220–RM243      | 2.96     | -0.07   | 5.55     | 0.10    |
|       | qGL8 | 8    | RM310–RM547      | 2.80     | 0.09    | 4.65     | 0.13    |
|       | qGL9 | 9    | RM566–RM434      | 4.65     | 0.13    | 8.10     | 0.10    |
|       | qGL10| 10   | RM25003–Bt       | 8.04     | -0.26   | 17.65    | -0.23   |
| GW    | qGW2 | 2    | RM262–RM106      | 2.61     | 0.03    | 6.52     | 0.05    |
|       | qGW3 | 3    | RM55–RM7000      | 4.4      | 0.05    | 10.5     | 0.05    |
|       | qGW4 | 4    | RM16434–RM16502  | 4.22     | 0.03    | 9.07     | 0.05    |
|       | qGW10| 10   | Bt–RM1126        | 4.4      | -0.05   | 9.07     | -0.05   |
|       | qGW11a| 11 | RM332–RM116      | 4.13     | 0.04    | 7.12     | 0.04    |
|       | qGW11b| 11 | RM26643–XA       | 4.25     | 0.04    | 6.65     | 0.04    |
| LWR   | qLWR2| 2    | RM324–RM5521     | 2.83     | -0.07   | 8.19     | -0.05   |
|       | qLWR3| 3    | RM55–RM7000      | 2.59     | 0.05    | 4.58     | 0.05    |
|       | qLWR5| 5    | RM169–RM3381     | 2.62     | 0.03    | 3.47     |         |
|       | qLWR10a| 10 | RM1126–RM258     | 4.43     | -0.06   | 7.00     |         |
|       | qLWR10b| 10 | RM467–RM258      | 4.45     | -0.06   | 7.02     |         |
|       | qLWR11| 11 | RM332–RM116      | 3.19     | -0.05   | 5.23     | -0.04   |
| GT    | qGT3 | 3    | RM1373–RM7000    | 3.34     | 0.02    | 5.10     | 0.01    |
|       | qGT4 | 4    | RM261–RM6659     | 3.51     | 0.02    | 3.41     | 0.02    |
|       | qGT5 | 5    | RM593–RM0366     | 3.51     | 0.02    | 3.41     | 0.02    |
|       | qGT10| 10   | Bt–RM1126        | 4.23     | -0.04   | 10.21    | -0.04   |
|       | qGT11| 11   | RM26643–XA       | 3.32     | 0.02    | 5.08     | 0.02    |
| TGW   | qTGW3| 3    | RM55–RM7000      | 7.17     | 0.91    | 15.46    | 0.85    |
|       | qTGW4| 4    | RM16459–RM16502  | 3.51     | 0.42    | 3.41     | 0.42    |
|       | qTGW5| 5    | RM593–RM0366     | 4.35     | -0.54   | 4.65     | -0.61   |
|       | qTGW10| 10 | Bt–RM1126        | 27.57    | -2.05   | 35.54    | -1.89   |
|       | qTGW11a| 11 | RM26092–RM116    | 2.89     | 0.55    | 3.94     |         |
|       | qTGW11b| 11 | RM26643–XA       | 5.80     | 0.71    | 8.47     | 0.66    |

a Chromosome number.

b Additive effects, the positive values indicate the alleles from Huahui 3 increasing the effects.

c h^2, the total phenotypic variation explained by each QTL.

The QTL around the Bt transgene insertion point is marked in boldface.

Table 3. Correlation coefficients among the parameters from 237 RILs derived from the cross of Zhongguoxiandao (ZGX) and Huahui 3 (HH 3) in 2009 (upper half) and 2010 (lower half)

|       | GL (mm) | GW (mm) | LWR | GT (mm) |
|-------|---------|---------|-----|---------|
| GL (mm)^† | 0.15*   | 0.24**  |     |         |
| GW (mm)   | 0.58**  | -0.71** | 0.56** | -0.67** |
| LWR       | 0.44**  | 0.34**  | 0.48** | 0.69**  |
| GT (mm)   | 0.56**  | 0.50**  | 0.66** | 0.71**  |
| TGW (g)   | 0.56**  | 0.50**  | 0.66** | 0.71**  |

* P ≤ 0.05, ** P ≤ 0.01. Abbreviations are the same as in Table 1.

† Correlation coefficients in upper half correspond to the data in 2009 and those in lower half correspond to the data in 2010.
QTLs including \textit{qTGW3}, \textit{qTGW5} and \textit{qTGW10} were detected in both years. The greatest effect QTL was \textit{qTGW10}, flanked by \textit{Bt-RM1126} on chromosome 10 (Fig. 2), explaining 35.5% and 32.3% of the total phenotypic variance (Table 4 and Fig. 2), respectively. The other four QTLs had relatively small effects. Generally, these six QTLs explained 71.4% in 2009 and 53.1% in 2010 of the phenotypic variance, respectively.

Epistatic QTLs and QTL-by-environment interactions among traits

For all traits, 9 pairs of loci were identified showing significant epistatic interactions at the \( p < 0.001 \) level (Table 5). One pair of epistatic interaction was detected for GL, accounting for 1.1% of the phenotypic variation. Three pairs for GW were found, explaining the phenotypic variation of 6.4%. There were four pairs for GT deciphering 4.1% of phenotypic variation. Additionally, one pair of interaction for TGW could explain 0.9% of phenotypic variation. No interaction was found between epistatic QTL and environment for LWR.

Eight QE interactions for all the traits were collectively detected. Two main effect QTLs (\textit{qGT4} and \textit{qGT10}) were detected to significantly interact with environmental factors, and their interaction were also found to further interact with environment was detected for GT. In addition, seven more epistatic QTLs were detected to interact with environment. Data showed that all the detected epistatic QTLs and QTL-by-environment interactions among traits had trivial genetic effects.

Discussion

Rice grain shape or size highly correlated with yield and appearance quality, thus affecting farmers’ economic income. For rice grain size, many QTLs had been previously
mapped, and most QTLs for grain size and weight were mapped on chromosomes 2, 3 and 5. In this study, a total of 27 main-effect QTLs for grain length, grain width, grain thickness and 1000-grain weight were detected on ten rice chromosomes excluding chromosomes 6 and 7. Seventeen of the 27 QTLs were reproducibly detected in two-year trials. Compared to previous researches, the QTL in the region of RM55-RM7000 on chromosome 3, controlling grain width, grain thickness and 1000-grain weight, might be the GS3 gene (Fan et al. 2006, Mao et al. 2010). The QTL qTGW5/qGT5 in the region of RM593-MRG0366 on chromosome 5 might correlate the cloned genes qSW5/GW5 or GS5 (Li et al. 2011, Shomura et al. 2008). The rest QTLs, including qGL1, qLWR5, qGL8, qGL9, qGL10 (qGW10, qGT10 and qTGW10), qGW11a (qTGW11a) and qGW11b (qTGW11b), hadn’t been reported before. Most of these QTLs had good reproducibility in two years.

In our research, two chromosomal regions around the known genes were interestingly found to be implicated with the genetic sites significantly affecting grain shape and yield. The first region is for the QTLs around the site of the known gene Xa21 on chromosome 11, which pleiotropically affected three of the five investigated traits, namely GW, GT and TGW. The QTL allele from HH 3 positively contributed three of the five investigated traits, namely GW, GT and TGW. The QTL allele from donor Minghui63 thus might be very likely caused by the QTL around Xa21. i.e., the introgression of Xa21 gene by backcrossing from donor material into receptor Minghui 63 may contain a donor linkage drag which included QTL alleles positively affecting grain shape and yield. The results exemplified that though the donor genetic background is restrained within a very narrow chromosomal region of 3.8 cM, the risk of genetic linkage drag can in same cases remain and disturb the backcrossing improvement effect. The development and application of molecular markers much closer to objective donor gene and of sufficient number of selectable backcrossing progeny in some breeding cases become very necessary. The QTL allele from donor genome showed the positive role. The Xa21’s disease resistance may act as a good phenotypic marker for facilitating the QTL breeding application.

The second region of RM2503-RM1126 on chromosome 10 was found to be a common site for four major QTLs of qGL10, qGW10, qGT10 and qTGW10. Noticeably, all of these four QTLs steadily played very strong effects on the phenotype in different environments of the two-year trials. Such information highlighted the value and reliability of the QTL founding. In the region of RM2503-RM1126, the Bt transgene in the parent HH 3 was coincidently harbored (Fig. 2). Combined with the case that all the QTLs’ allele from HH 3 had negative additive effects on the grain size and weight, it rationalized that the grain-related QTLs in the interval of RM2503-RM1126 on chromosome 10 might be cohabited with Bt transgene insertion site, or in other word, it is just Bt transgene insertion that strongly disrupted normal function of QTL allele from HH 3 and thus raised the strong declining phenotypic performance (Table 1). The parental material of Minghui 63/Bt used in this study, identical to the transgenic rice T51-1 described by Tu et al. (2000a), was derived from a small number of transgenic plants with Minghui 63 as receptor transformed with a 1.8 kb Bt transgene fragment (Tu et al. 2000a). In 2009, China issued biosafety certificates for Bt rice variety Huihui No.1 and its hybrid Bt Shanyou63. The first field experiments of Bt rice

| Table 5. Epistatic effects and environmental interactions of QTLs identified in the RIL population in 2009 and 2010 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Traits | Ch-In | Interval_i | QTL | Ch-In | Interval_j | QTL | a_i | h^2_i | a_j | h^2_j | a_ij | h^2_ij | a_ei | h^2_ei | a_ej | h^2_ej |
| GL | 9-5 | RM566-RM434 | qGL9 | 10-4 | RM1126-RM467 | -0.20 | 19.9 | -0.06 | 1.05 | | | | | | |
| GW | 4-8 | RM261-RM6659 | qGW4 | 11-8 | XA-RM234 | 0.22 | 2.4 | 0.03 | 5.81 | -0.02 | 0.95 | | | | |
| 3-8 | RM15281-RM487 | | 10-4 | RM1126-RM467 | | | | | | | | | | | |
| 1-10 | RM449-RM395 | | 10-4 | RM1126-RM467 | | | | | | | | | | | |
| GT | 1-3 | RM3252-RM1 | qGT10 | 3-15 | RM222-RM154 | -0.03 | 4.08 | -0.04 | 12.4 | -0.02 | 1.12 | 0.26 | 0.0007 | 0.5 | 0.34 |
| 4-10 | RM16459-RM401 | qGT4 | 10-3 | Bt-RM1126 | 0.02 | 5.18 | 0.01 | 0.5 | 0.0071 | 0.28 | 0.0007 | 0.5 | 0.59 |
| 5-4 | RM593-MRG0366 | qGT5 | 10-3 | Bt-RM1126 | 0.02 | 2.45 | -0.04 | 12.4 | 0.01 | 0.8 | 0.0006 | 0.5 | 0.14 |
| TGW | 5-2 | MRG222-RM413 | | 11-8 | XA-RM234 | -0.5 | 6.62 | 0.24 | 3.55 | -0.3 | 0.85 | 0.002 | 0.02 | 0.02 |

abCh-In and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis.
| a_i and a_j are the additive effects of the test points i and j, respectively. Positive values of a_i and a_j imply that the Huahui 3 genotype has a positive effect on that trait.
| b_i and b_j are the percentages of the phenotypic variations explained by a_i, a_j, a_ij, a_ei and a_ej, respectively.
| d_a ij is the effect of additive-by-additive interaction between points i and j; a positive value indicates that the parental two-locus genotypes have a positive effect on the traits and that the recombinants have a negative effect.
| e_ae i and e_ae j are effects of the environmental interaction of locus i and j, respectively; a positive value implies that the effect in 2010 is larger than in 2009. |
event T51-1, which is the original event of Huihui No.1, were performed in China 1999 (Tu et al. 2000b). This study is the first time to map Bt transgene in Minghui63 Bt material on the rice chromosome 10, and firstly revealed that this Bt insertion linked and affected grain development. The possible endogenous insertional mutagenesis in this study exemplified a typical transgenic breeding problem and suggested that sufficient number of independent transgenic plants should be endeavored to acquire and selection of desirable transgenic event with expected trait performance becomes necessary. In practical transgenic breeding, it is noticeable of a typical case concerned with the position effects of the introduced transgenes on endogenous genes. This is of particular concern for Agrobacterium-mediated transgenic lines in which T-DNA is believed to have a good chance of being integrated into an active chromatin region (Zhong 2001). An et al. (2007) ever mapped 27,621 T-DNA insertion flanked sequences and revealed that T-DNA integration frequency was generally proportionate to chromosome size, and about 45% of the T-DNAs were inserted into the genomic regions and about 55% into the intergenic regions. Wang (2000) ever concluded an empirical transgenic breeding efficiency of 5%–10%, i.e., from 100 independent transgenic lines, only 5–10 transgenic desirable plants could be adequate for breeding application. It is also suggested that an initial number of 300 independent transgenic plants was necessary for a confident and guaranteed breeding improvement (Wang 2000). The mapping result of Bt transgene may also expedite the isolation of the innovative yield-related gene. Based on the Bt insertion, more analysis such as flanking analysis and enlarging segregation population for association mapping should be further performed. Breeding utilization of this QTL will also be emphasized to help improve rice grain shape and yield.

Acknowledgments

This work was partially supported by grants from the National High Technology Research (2012AA101102), the National Nature Science Foundation of China (31171617), the National Program on R&D of Transgenic Plants (2013ZX08001-001, 2013ZX08001-002), the National Agricultural Industry Technique System (CARS-01-03), Nature Science Foundation of Heilongjiang Province (LC201026), the Postdoc Promotion Fund (LBH-Q09020), and Science & Technology Research Project (11551356) in Heilongjiang Province. It was partly supported by the open funds of the National Key Laboratory of Crop Genetic Improvement.

Literature Cited

An, G., D.H. Jeong and S. Park (2007) T-DNA tagging for developmental biology. In: Rice genetics, V., D.S. Brar, D.J. Mackill and B. Hardy (eds.) Proceedings of the Fifth International Rice Genetics Symposium, 19–23 November 2005, Manila, Philippines. Sin-gapore: World Scientific Publishing and Los Baños (Philippines): International Rice Research Institute. pp. 103–116.

Bai, X., L. Luo, W. Yan, M.R. Kovi, W. Zhan and Y. 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.

Chen, S., X.H. Lin, C.G. Xu and Q. Zhang (2000) Improvement of bacterial blight resistance of ‘Minghui 63’, an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Sci. 40: 239–244.

Cho, Y.G., M.W. Blair, O. Panaud and S.R. McCouch (1996) Cloning and mapping of variety-specific rice genomic DNA sequences: amplified fragment length polymorphisms (AFLP) from silver stained polyacrylamide gels. Genome 39: 373–378.

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: 164–171.

Guo, L., L. Ma, H. Jiang, D. Zeng, J. Hu, L. Wu, Z. Gao, G. Zhang and Q. Qian (2009) Genetic analysis and fine mapping of two genes for grain shape and weight in rice. J. Integr. Plant Biol. 51: 45–51.

Haldane, J.B.S. (1919) The combination of linkage values, and the calculation of distances between the loci of linked factors. J. Genet. 8: 299–309.

Jiang, G.H., C.G. Xu, J.M. Tu, X.H. Li, Y.Q. He and Q. Zhang (2004) Pyramiding of insect- and disease-resistance genes into an elite indica, cytoplasm male sterile restorer line of rice, ‘Minghui 63’. Plant Breed. 123: 112–116.

Li, J., M. Thomson and S.R. McCouch (2004a) Fine mapping of a grain-weight quantitative trait locus in the pericentromeric region of rice chromosome 3. Genetics 168: 2187–2195.

Li, J., J. Xiao, S. Grandillo, L. Jiang, Y. Wan, Q. Deng, L. Yuan and S.R. McCouch (2004b) QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (O. sativa L.) and African (O. glaberrima S.) rice. Genome 47: 697–704.

Li, Y., C. Fan, Y. Xing, Y. Jiang, L. Luo, L. Sun, D. Shao, C. Xu, X. 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.

Li, Z., S.R. Pinson, W.D. Park, A.H. Paterson and J.W. Stansel (1997) Epistasis for three grain yield components in rice (Oryza sativa L.). Genetics 145: 453–465.

Lin, L.H. and W.R. Wu (2003) Mapping of QTLs underlying grain shape and grain weight in rice. Mol. Plant Breed. 1: 337–342.

Mao, H., S. Sun, J. Yao, C. Wang, S. Yu, C. Xu, X. Li and Q. Zhang (2010) Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proc. Natl. Acad. Sci. USA 107: 19579–19584.

Oh, J.M., S. Balkunde, P.S. Yang, D.B. Yoon and S.N. Ahn (2011) Fine mapping of grain weight QTL, tgw11 using near isogenic lines from a cross between Oryza sativa and O. grandiglumis. Genes Genomics 33: 259–265.

Shao, G., S. Tang, J. Luo, G. Jiao, X. Wei, A. Tang, J. Wu, J. Zhuang and P. Hu (2010) Mapping of gGL7-2, a grain length QTL on chromosome 7 of rice. J. Genet. Genomics 37: 523–531.

Shomura, A., T. Izawa, K. Ebana, T. Ebihata, H. Kanegae, S. Konishi and M. Yano (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat. Genet. 40: 1023–1028.

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.

Thomson, M.J., T.H. Tai, A.M. McClung, X.H. Lai, M.E. Hinga, K.B. Lobos, Y. Xu, C.P. Martinez and S.R. McCouch (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor. Appl. Genet. 107: 479–493.

Tu, J., G. Zhang, K. Datta, C. Xu, Y. He and Q. Zhang (2000a) Field performance of transgenic elite commercial hybrid rice expressing Bacillus thuringiensis delta-endotoxin. Nat. Biotechnol. 18: 1101–1104.

Tu, J., K. Datta, G.S. Khush, Q. Zhang and S.K. Datta (2000b) Field performance of Xa21 transgenic indica rice (*Oryza sativa* L.), IR72. Theor. Appl. Genet. 101: 15–20.

Wan, X., J. Weng, H. Zhai, J. Wang, C. Lei, X. Liu, T. Guo, L. Jiang, N. Su and J. Wan (2008) Quantitative trait loci (QTL) analysis for rice grain width and fine mapping of an identified QTL allele gw-5 in a recombination hotspot region on chromosome 5. Genetics 179: 2239–2252.

Wan, X.Y., J. Wan, L. Jiang, J.K. Wang, H.Q. Zhai, J.F. Wang, 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, F. (2000) Progress and prospects in the development of transgenic rice breeding. Fujian J. Agric. Sci. 15 (suppl.): 141–144.

Wang, S.C. and Z.B. Zeng (2004) Windows QTL Cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh, NC, 2001–2004. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.

Weng, J., S. Gu, X. Wan, H. Gao, T. Guo, N. Su, C. Lei, X. Zhang, Z. Cheng, X. Guo et al. (2008) Isolation and initial characterization of *GW5*, a major QTL associated with rice grain width and weight. Cell Res. 18: 1199–1209.

Wu, K.S. and S.D. Tanksley (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet. 241: 225–235.

Xiao, J., J. Li, S. Grandillo, S.N. Ahn, L. Yuan, S.D. Tanksley and S.R. McCouch (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative *Oryza rufipogon*. Genetics 150: 899–909.

Xie, X., M.H. Song, F. Jin, S.N. Ahn, J.P. Suh, H.G. Hwang and S.R. McCouch (2006) Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines derived from a cross between *Oryza sativa* and *Oryza rufipogon*. Theor. Appl. Genet. 113: 885–894.

Xie, X., F. 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.

Xing, Z., F. Hua, L. Sun, G. Xu and Q. Zhang (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. Theor. Appl. Genet. 105: 248–257.

Yang, J., C.C. Hu, H. Hu, R.D. Yu, Z. Xia, X. Ye and J. Zhu (2008) QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 24: 721–723.

Yoon, D.B., K.H. Kang, H.J. Kim, H.G. Ju, S.J. Kwon, J.P. Suh, O.Y. Jeong and S.N. Ahn (2006) Mapping quantitative trait loci for yield components and morphological traits in an advanced backcross population between *Oryza grandiglumis* and the *O. sativa japonica* cultivar Hwaseongbyeo. Theor. Appl. Genet. 112: 1052–1062.

Yu, S.B., J.X. Li, C.G. Xu, Y.F. Tan, Y.J. Gao, X.H. Li, Q. Zhang and M.M. Saghai (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc. Natl. Acad. Sci. USA 94: 9226–9231.

Zhong, G.Y. (2001) Genetic issues and pitfalls in transgenic plant breeding. Euphytica 118: 137–144.