Rice Science, 2014, 21(3): 127−132

DOI: 10.1016/S1672-6308(13)60175-4

Molecular Improvement of Grain Weight and Yield in Rice by Using *GW6* Gene

LI Yuan-yuan[1, #], TAO Hong-jian[1, #], ZHAO Xiang-qian[2], XU Jie[1], LI Geng-mi[1], HU Shi-kai[1], DONG Guo-jun[1], SHI Zheng-yuan[1], WU Li-wen[1], HU Jiang[1], YE Guo-you[2], GUO Long-biao[1]
([1]*State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China;* [2]*Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute (IRRI), College, Los Banos 4031, Laguna, the Philippines;* [#]*These authors contributed equally to this paper*)

Abstract: Molecular design breeding is one of straightforward approaches to break yield barriers in rice. In this study, *GW6* gene for grain length and width from Baodali was transferred into an indica recurrent parent 9311 and a japonica variety Zhonghua 11 (ZH11) using marker-assisted backcross (MAB). One and three introgression lines were selected for phenotypic analysis from 9311 and ZH11 genetic backgrounds, respectively. SSL-1, an improved 9311 near isogenic line with *GW6* performed 11%, 19% and 6.7% higher of grain length, 1000-grain weight and single plant yield, respectively, as compared with 9311. All the three improved ZH11-*GW6* lines, R1, R2 and R3, had more than 30% increase in grain weight and about 7% higher in grain yield. Seed plumpness of R1, R2 and R3 was improved synchronously because the three ZH11-*GW6* lines contained *GIF1* (*Grain Incomplete Filling 1*), a dominant grain filling gene. Thus, *GW6* has high potential in increasing the yield of inbred lines through MAB, making it an important genetic resource in super hybrid rice breeding. This study provides insights in the utilization of *GW6* for large grain and high yield rice breeding via molecular design breeding.
Key words: rice; *GW6* gene; molecular marker-assisted selection; yield

Rice is one of the most important crops in the world, and is also a model monocot species for molecular biology research. Successful utilization of semi-dwarf gene and heterosis are characterized as two 'green revolutions' which led to big breakthroughs in rice yield in China. In 1990s, new plant type and super rice breeding sponsored by International Rice Research Institute (IRRI, the Philippines) and rice breeders in China contributed to dramatic yield increase. Sequenced rice genome has provided new technologies and tools in functional genomics, transcriptomics and proteomics of important agronomic traits in rice. Up to now, a lot of functional genes related to rice yield and other important agronomic traits have been cloned (Guo et al, 2009; Miura et al, 2010; Ikeda et al, 2013). In addition, genomic-based genotyping platforms and re-sequencing on huge germplasms and genetic populations greatly promote the efficiency of genes/QTL mapping and cloning (Gao et al, 2013; Han and Huang, 2013). With the development of rice functional genomics, new technology platforms have been proved successful in improving complex agronomic traits such as yield, grain quality and disease resistance, which are inefficiently selected using traditional breeding methods (Liu et al, 2003). These strategies will be beneficial not only in QTL/gene cloning and functional identification, but also in molecular design breeding (Gou et al, 2008).

Grain weight, one of the three yield components, is an integrated index for grain length, width and thickness. Grain length and width is a complex trait controlled by multiple genes. At least 89 QTLs for rice grain weight or related traits distributing on the 12 chromosomes have been detected (Yu et al, 1997; Xing et al, 2001; Brondani et al, 2002; Xu et al, 2002; Thomson et al, 2003). A series of genes, *GS3* (Fan et al, 2006), *GW2* (Song et al, 2007), *qSW5* (Shomura et al, 2008), *qGW8* (Wang S K et al, 2012), *Ghd7* (Xue et al, 2008), and *qGL3* (Zhang et al, 2012) for grain weight or shape have been isolated via map-based cloning strategies. Recently, some single chromosome substitution lines or pyramided lines containing two desirable QTLs/genes for grain weight, panicle length or panicle shape have been developed, such as HJX74-*GS3*-*GW8*,

Received: 13 January 2014; **Accepted:** 24 Februry 2014
Corresponding author: GUO Long-biao (guolongbiao@caas.cn)

9311-*IPA1*, 9311-*DEP1*-*Gn1* and Koshihikari-*Gn1*-*sd1*, which have shown high efficiency in grain shape, quality and yield improvement in rice marker-assisted breeding (Wang et al, 2008; Yang et al, 2010; Liu et al, 2012; Huang et al, 2009; Ashikari et al, 2006). Baodali, an elite indica rice variety, has a 1000-grain weight of about 48.8 g due to a major QTL *GW6* for grain length and weight. This gene has been fine-mapped to 4.7 cM region with two flanking SSR markers, RM7179 and RM3187, on chromosome 6 (Ma et al, 2006; Guo et al, 2009). In order to investigate the genetic effects of *GW6* under different backgrounds and pyramiding effects of *GW6* and *GIF1*, 9311 (indica, male parent for super hybrid rice) and Zhonghua 11 (ZH11) (japonica, recurrent parent with *GIF1*) were used as parents and the introgression lines 9311-*GW6* and ZH11-*GW6*-*GIF1* were developed using marker-assisted backcross (MAB). Grain lengths and weights of 9311-*GW6* and ZH11-*GW6*-*GIF1* increased significantly with no considerable weakness in other major agronomic traits, which would be beneficial in the utilization of *GW6* for grain length and weight improvement in rice molecular design breeding.

MATERIALS AND METHODS

Marker-assisted backcross

Baodali was used as a donor parent, and 9311 and ZH11 were used as recurrent parents. F_1 generations derived from Baodali/ZH11 and Baodali/9311 were screened using two flanking markers, RM7179 and RM3187, for *GW6* detection. Screened plants with *GW6* were backcrossed four times with the recurrent parent, ZH11. In addition to the flanking markers, foreground screening of BC_4F_1 was carried out using additional markers RM7193, RM20190 and RM20197 within the QTL region. After selfing, one 9311-*GW6* near isogenic line (NIL) named as SSL-1 and three ZH11-*GW6* NILs named as R1, R2 and R3 were selected for phenotypic analysis (Fig. 1).

DNA extraction and PCR amplification

Total genomic DNA was isolated by CTAB with some modifications (Murray and Thompson, 1980). PCR reaction was carried out in a final volume of 25 μL containing 50 ng template DNA, 2.5 μL of 10 × reaction buffer (with 2.0 mmol/L Mg^{2+}), 0.2 mmol/L dNTP mixture, 0.2 μmol/L each primer, and 1.0 U *Taq* DNA polymerase. Thirty-five cycles were carried out with an initial 5 min period at 94 °C followed by

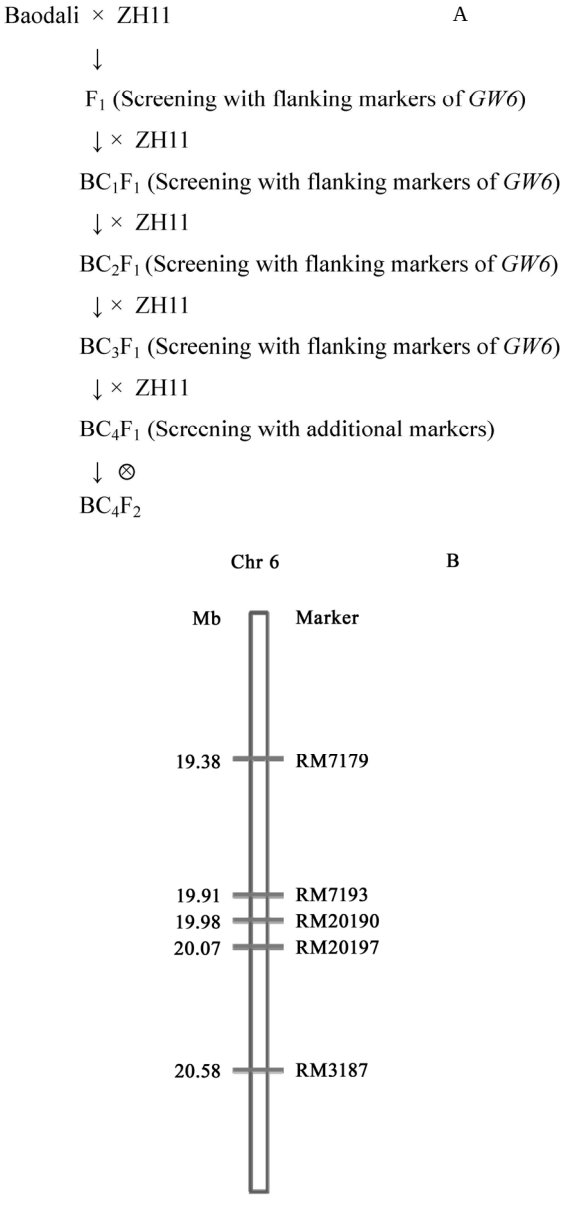

Fig. 1. Molecular assistant backcross.

A, Diagram of molecular assistant backcross; B, Relative distance of the markers on chromosome 6 (Chr 6) (partial).

ZH11, Zhonghua 11.

cycles of 1 min at 94 °C, 1 min at 58 °C and 2 min at 72 °C and a final 10 min extension at 72 °C. PCR products were separated on 3.0%–5.0% agarose gels based on the lengths of the fragments and stained with ethidium bromide for visualizing DNA bands.

Field experiments and data collection

Development of introgression lines were conducted at the farms of China National Rice Research Institute (CNRRI), Hangzhou and Lingshui, China, during 2009–2011. Baodali, 9311, ZH11 and the four developed

introgression lines were grown for agronomic trait analysis at Hangzhou in 2011. And 21-day-old seedlings were transplanted into five row plots with one seedling per hill. Each row within a plot consisted of seven plants with a spacing of 20 cm between plants and 20 cm between rows. Field plots were laid out in a random completed block design with three replications. Pest, insect and water managements were performed on the basis of CNRRI experimental farm practices. Three typical plants in the middle of plot were harvested individually. Number of panicles per plant, number of grains per panicle, grain length, grain width, 1000-grain weight and single plant yield were measured.

RESULTS

Development of ZH11-*GW6* NIL

A total of 142 randomly distributed SSR markers were used for background selection in BC_4F_1 population with ZH11 as the recurrent parent. One plant with heterozygous locus in *GW6* and homozygous loci in all 142 background markers was identified and harvested. BC_4F_2 population was genotyped with additional three polymorphic markers (RM7193, RM20190 and RM20197) within *GW6* region to detect recombinants. Selected recombinants of ZH11-*GW6* NILs were named as R1, R2 and R3.

Further genotyping using high density markers showed that these three NILs were not single segment substitution lines. Two, two and one additional chromosome segments from donor were detected in R1, R2 and R3, respectively, beside the targeted *GW6* region. The common region of foreground in the three NILs was within the interval of RM20190–RM20197.

Pyramiding of *GW6* and *GIF1* in ZH11

Os04g33740 of ZH11 was sequenced in other studies in China (Wang et al, 2008). It is interesting that ZH11 contained the dominant grain filling gene, *GIF1*. Using two flanking markers RM142 and RM6997, plants with homozygous *GIF1* locus were selected for further backcrossing in BC_2F_1. Three ZH11-*GW6* NILs with *GIF1* was confirmed by PCR and sequencing (Fig. 2). Therefore, the three developed NILs could be considered as pyramided lines of *GW6* and *GIF1* under the ZH11 background.

Morphological features of ZH11-*GW6*-*GIF1*

Compared with the recurrent parent, ZH11, no significant differences in days to flowering, plant height, number of tillers per plant and number of grains per panicle were observed in BC_4F_2 population. The segregation of plants with long grains and short grains fitted a ratio of 3:1. This indicated that *GW6* is controlled by a dominant gene (Fig. 3-A, B).

Average grain lengths of the three ZH11-*GW6*-*GIF1* lines, R1, R2 and R3, were 10.5, 10.6 and 10.3 mm, respectively, whereas the grain length of ZH11 was 7.6 mm. Average grain widths of the three NILs

Fig. 2. Comparison and detection of Zhonghua 11 (ZH11) and 9311 with their near isogenic lines (NILs) of *GW6*.

A, ZH11 and ZH11-*GW6* plants; B, ZH11-*GW6* grains are larger than ZH11 grains, and ZH11-*GW6* brown rice is larger than ZH11 brown rice; C, *GIF1* gene locus detection of ZH11-*GW6* lines R1, R2 and R3 (Q22 is from Baodali); D, 9311 and 9311-*GW6* introgression line SSL-1; E, Seeds of Baodali, 9311 and 9311-*GW6* introgression line SSL-1.

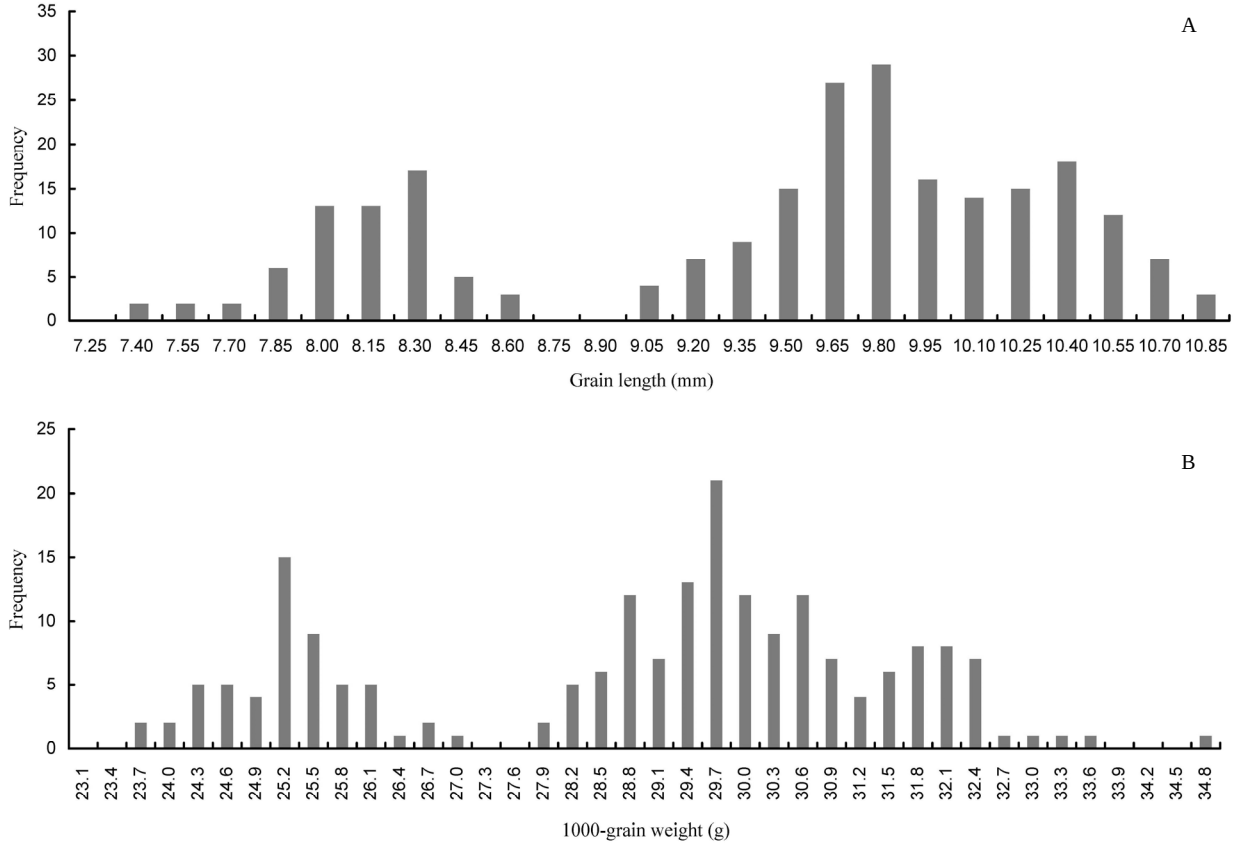

Fig. 3. Distributions of grain length (A) and 1000-grain weight (B) in BC_4F_2 generation.

increased by 0.8 (36.2%), 0.9 (38.6%) and 0.7 (31.3%) mm, respectively, leading to significantly higher grain weights than that of the recurrent parent. Additionally, seed plumpness of the three NILs was higher than that of the donor parent, Baodali. This was contributed by the *GIF1* gene of ZH11. Numbers of grains per panicle of the three improved lines were slightly higher than that of ZH11. All the above resulted in single plant yield increase, although there was a slightly decrease in number of tillers per plant (Table 1).

Development of 9311-*GW6* NIL

Similarly, 9311-*GW6* NIL was developed. The plant with the longest grain in BC_1F_1 population of Baodali/9311//9311 was selected for three successive backcrosses at Hangzhou and Lingshui, China. One plant with the most similar genetic background with 9311 and heterozygous locus in *GW6* was selected in BC_4F_2 generation. Growth duration and grain number per panicle of the selected plant were similar to that of 9311. Fixed introgression line 9311-*GW6* was developed after selfing, named as SSL-1.

Morphological features of 9311-*GW6* (SSL-1)

Average grain lengths of SSL-1 and 9311 were 10.7 and 9.5 mm, respectively, that is, about 11% increase

Table 1. Agronomic traits of ZH11-*GW6* and 9311-*GW6*.

Rice material	Grain length (mm)	Grain width (mm)	No. of grains per panicle	Panicle length (cm)	No. of tillers per plant	1000-grain weight (g)	Single plant yield (g)	Plant height (cm)	Growth duration (d)
R1	10.50 ± 0.50	4.00 ± 0.20	124.1 ± 1.0	20.70 ± 0.61	10.2 ± 0.5	33.50 ± 1.32	28.90 ± 0.95	114.50 ± 0.66	122.0 ± 1.7
R2	10.60 ± 0.36	4.10 ± 0.26	121.4 ± 1.5	20.40 ± 1.44	10.6 ± 0.5	34.10 ± 0.79	29.10 ± 0.61	114.10 ± 0.46	124.0 ± 1.0
R3	10.30 ± 0.26	3.90 ± 0.36	122.7 ± 0.6	20.30 ± 0.61	10.4 ± 0.5	32.30 ± 0.66	28.60 ± 0.78	116.30 ± 0.44	119.0 ± 1.7
ZH11	7.60 ± 0.53	3.20 ± 0.26	116.2 ± 1.1	19.70 ± 0.61	11.1 ± 0.6	24.60 ± 0.98	27.60 ± 1.13	114.60 ± 1.11	124.0 ± 1.7
SSL-1	10.70 ± 0.26	3.10 ± 0.26	156.1 ± 1.0	23.20 ± 1.06	8.3 ± 0.4	40.50 ± 0.56	35.40 ± 0.56	131.20 ± 0.56	131.0 ± 2.0
9311	9.59 ± 0.12	2.84 ± 0.22	148.3 ± 2.0	21.97 ± 1.96	10.1 ± 0.4	34.00 ± 0.46	33.20 ± 0.30	111.30 ± 0.46	130.0 ± 1.0
Baodali	12.80 ± 0.72	3.60 ± 0.10	143.7 ± 1.5	22.60 ± 1.44	6.9 ± 0.7	48.80 ± 1.31	31.70 ± 0.95	121.40 ± 0.79	127.0 ± 2.0

ZH11, Zhonghua 11; R1, R2 and R3 are three ZH11-*GW6* lines; SSL-1, 9311-*GW6* near isogenic line.
Data are expressed as mean ± *SD*.

was observed. Compared with 9311, average grain weight of SSL-1 increased from 34.0 to 40.5 g. Panicle length and number of grains per panicle increased slightly with no apparent weakness in other panicle architecture traits. Therefore, about 6.6% higher single plant yields were obtained (Table 1). These results suggested that *GW6* is a useful potential gene for high yield when conducting molecular design breeding in rice.

DISCUSSION

Marker-assisted selection (MAS) technology is an effective approach for crop improvement. With the rapid development of high-throughput genotyping and gene cloning, molecular design plays an increasingly important role in QTL pyramiding. MAB and MAS-QTL pyramiding have enabled quick and accurate selection of lines with the target trait (Chen et al, 2000; Ashikari and Matsuoka, 2006). MAS-QTL pyramiding approach is based on a strategy to efficiently accumulate beneficial QTLs in a single line. Some studies have pyramided major disease tolerance genes for durable or multiple resistance in rice (Singh et al, 2001; Pei et al, 2011; Suh et al, 2013). A novel QTL/gene pyramiding scheme based on marker-assisted and phenotype selection (MAPS) was reported, which allows to pyramid 24 QTLs at a single hybridization without massive cross work (Zong et al, 2012).

Many QTLs related to rice yield have been mapped in different mapping populations and some of them have been cloned and functionally characterized. Further, a few have been widely used in rice breeding in China, such as *GS3*, *GW5* and *GW8*. Molecular design breeding or QTLs/genes pyramiding using linked or functional markers has become a straightforward approach for improving plant type and yield. A pyramided NIL (*qHD8* + *GS3*) under Zhenshan 97 background has higher yield potential and longer grain than Zhenshan 97 (Wang P et al, 2012). Compared with HJX74, pyramided line HJX74-*GS3*-*GW8* has better grain quality, eating quality and higher yield as well (Wang S K el al, 2012). Some lines containing eight yield-related positive QTLs developed by pyramiding based on MAPS have shown increased panicle and spikelet size compared to the parent variety 9311 (Zong et al, 2012).

A major QTL, *GW6* for grain length and weight, was fine-mapped by Guo et al (2009). In this study, 9311-*GW6* (SSL-1) and ZH11-*GW6* (R1, R2 and R3) NILs were developed using MAB, which laid a foundation for gene cloning and function analysis. Significant increases in grain length, grain width, grain weight and single plant yield were observed under both 9311 and ZH11 genetic backgrounds. It is noteworthy that only slight weakness in other major agronomic traits was observed (Table 1). It implied that *GW6* might be used in both indica and japonica rice breeding. Furthermore, *GW6* is controlled by a single dominant gene which was confirmed in BC_4F_2 population using ZH11 as the recurrent parent (Fig. 3-A, B). Thus, *GW6* has a high potential to increase the yield of hybrid rice.

It is interesting that ZH11 contains the *GIF1* gene and all the three NILs (R1, R2 and R3) have this gene as well (Fig. 2). The three NILs can be considered as pyramided lines of *GW6* and *GIF1*. Grain weight and single plant yield of pyramided lines were about 30% and 5% higher than those of the recurrent parent. Thus, it is possible to improve grain weight and seed plumpness together using *GW6* and *GIF1* via MAB. Additive effect is common in pyramiding lines (Ando et al, 2008). *GW6* increased the 1000-grain weight by about 19% under the 9311 background, however, more than 30% higher grain weights were observed in the three ZH11-*GW6*-*GIF1* NILs (Table 1). This result suggests the presence of their additive effect, epistatic interaction or genetic background effect, which will be confirmed in further study.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Science Foundation of China (Grant No. 31271700) and National Basic Research Program of China (Grant No. 2013CBA01405).

REFERENCES

Ando T, Yamamoto T, Shimizu T, Ma X F, Shomura A, Takeuchi Y, Lin S Y, Yano M. 2008. Genetic dissection and pyramiding of quantitative traits for panicle architecture by using chromosomal segment substitution lines in rice. *Theor Appl Genet*, **116**(6): 881–890.

Ashikari M, Matsuoka M. 2006. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends Plant Sci*, **11**(7): 344–350.

Brondani C, Rangel N, Brondani V, Ferreira E. 2002. QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theor Appl Genet*, **104**(6/7): 1192–1203.

Chen S, Lin X H, Xu C G, Zhang Q F. 2000. Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line

of hybrid rice, by molecular marker-assisted selection. *Crop Sci*, **40**(1): 239–244.

Fan C C, Xing Y Z, Mao H L, Lu T T, Han B, Xu C G, Li X H, Zhang Q F. 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**(6): 1164–1171.

Gao Z Y, Zhao S C, He W M, Guo L B, Peng Y L, Wang J J, Guo X S, Zhang X M, Rao Y C, Zhang C, Dong G J, Zheng F Y, Lu C X, Hu J, Zhou Q, Liu H J, Wu H Y, Xu J, Ni P X, Zeng D L, Liu D H, Tian P, Gong L H, Ye C, Zhang G H, Wang J, Tian F K, Xue D W, Liao Y, Zhu L, Chen M S, Li J Y, Cheng S H, Zhang G Y, Wang J, Qian Q. 2013. Dissecting yield-associated loci in super hybrid rice by resequencing recombinant inbred lines and improving parental genome sequences. *Proc Natl Acad Sci USA*, **110**(35): 14492–14497.

Guo L B, Cheng S H, Qian Q. 2008. Progress and prospects of breeding by gene design in rice. *Chin J Rice Sci*, **22**(6): 650–657. (in Chinese with English abstract)

Guo L B, Ma L L, Jiang H, Zeng D L, Hu J, Wu L W, Gao Z Y, Zhang G H, Qian Q. 2009. Genetic analysis and fine mapping of two genes for grain shape and weight in rice. *J Integr Plant Biol*, **51**(1): 45–51.

Han B, Huang X H. 2013. Sequencing-based genome-wide association study in rice. *Curr Opin Plant Biol*, **16**: 133–138.

Huang X Z, Qian Q, Liu Z B, Sun H Y, He S Y, Luo D, Xia G M, Chu C C, Li J Y, Fu X D. 2009. Natural variation at the *DEP1* locus enhances grain yield in rice. *Nat Genet*, **41**(4): 494–497.

Ikeda M, Miura K, Aya K, Kitano H, Matsuoka M. 2013. Genes offering the potential for designing yield-related traits in rice. *Curr Opin Plant Biol*, **16**(2): 213–220.

Liu J, Tao H J, Shi S, Ye W J, Qian Q, Guo L B. 2012. Genetics and breeding improvement for panicle type in rice. *Chin J Rice Sci*, **26**(2): 227–234. (in Chinese with English abstract)

Liu S P, Li X, Wang Z Y, Li X H, He Y Q. 2003. Gene pyramiding to increase the blast resistance in rice. *Mol Plant Breeding*, **1**(1): 22–26. (in Chinese with English abstract)

Ma L L, Guo L B, Qian Q. 2006. Germplasm resources and genetic analysis of large grain in rice. *Chin Bull Bot*, **23**(4): 395–401. (in Chinese with English abstract)

Miura K, Ikeda M, Matsubara A, Song X J, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M. 2010. *OsSPL14* promotes panicle branching and higher grain productivity in rice. *Nat Genet*, **42**(6): 545–549.

Murray M G, Thompson W F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucl Acids Res*, **8**(19): 4321–4325.

Pei Q L, Wang C L, Liu P Q, Wang J, Zhao K J. 2011. Marker-assisted selection for pyramiding disease and insect resistance genes in rice. *Chin J Rice Sci*, **25**(2): 119–129. (in Chinese with English abstract)

Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yano M. 2008. Deletion in a gene associated with grain size increased yields during rice domestication. *Nat Genet*, **40**(8): 1023–1028.

Singh S, Sidhu J S, Huang N, Vikal Y, Li Z, Brar D S, Dhaliwal H S, Khush G S. 2001. Pyramiding three bacterial blight resistance genes (*Xa5*, *Xa13* and *Xa21*) using marker assisted selection into indica rice cultivar PR106. *Theor Appl Genet*, **102**(6/7): 1011–1015.

Song X J, Huang W, Shi M, Zhu M Z, Lin H X. 2007. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat Genet*, **39**(5): 623–630.

Suh J P, Jeung J U, Noh T H, Cho Y C, Park S H, Park H S, Shin M S, Kim C K, Jena K K. 2013. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice*, **6**: 5.

Thomson M J, Tai T H, McClung A M, Lai X H, Hinga M E, Lobos K B, Xu Y, Martinez C P, McCcouch S R. 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.

Wang E T, Wang J J, Zhu X D, Hao W, Wang L Y, Li Q, Zhang L X, He W, Lu B R, Lin H X, Ma H, Zhang G Q, He Z H. 2008. Control of rice grain-filling and yield by a gene with a potential signature of domestication. *Nat Genet*, **40**: 1370–1374.

Wang P, Xing Y Z, Li Z K, Yu S B. 2012. Improving rice yield and quality by QTL pyramiding. *Mol Breeding*, **29**(4): 903–913.

Wang S K, Wu K, Yuan Q B, Liu X Y, Liu Z B, Lin X Y, Zeng R Z, Zhu H T, Dong G J, Qian Q, Zhang G Q, Fu X D. 2012. Control of grain size, shape and quality by *OsSPL16* in rice. *Nat Genet*, **44**(8): 950–954.

Xing Y Z, Tan Y F, Xu C G, Hua J P, Sun X L. 2001. Mapping quantitative trait loci for grain appearance traits of rice using a recombinant inbred line population. *Acta Bot Sin*, **43**(8): 840–845. (in Chinese with English abstract)

Xu J L, Xue Q Z, Luo L J, Li Z K. 2002. Genetic dissection of grain weight and its related traits in rice (*Oryza sativa* L.). *Chin J Rice Sci*, **16**(1): 6–10. (in Chinese with English abstract)

Xue W Y, Xing Y Z, Weng X Y, Zhao Y, Tang W J, Wang L, Zhou H J, Yu S B, Xu C G, Li X H, Zhang Q F. 2008. Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nat Genet*, **40**(6): 761–767.

Yang T F, Zeng R Z, Zhu H T, Chen L, Zhang Z M, Ding X H, Li W T, Zhang G Q. 2010. Effect of grain length gene *GS3* in pyramiding breeding of rice. *Mol Plant Breeding*, **8**(1): 59–66. (in Chinese with English abstract)

Yu S B, Li J X, Xu C G, Tan Y F, Gao Y J, Li X H, Zhang Q F, Maroof M A. 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proc Natl Acad Sci USA*, **94**(17): 9226–9231.

Zhang X J, Wang J F, Huang J, Lan H X, Wang C L, Yin C F, Wu Y Y, Tang H J, Qian Q, Li J Y, Zhang H S. 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**(52): 21534–21539.

Zong G, Wang A H, Wang L, Liang G H, Gu M H, Sang T, Han B. 2012. A pyramid breeding of eight grain-yield related quantitative trait loci based on marker-assistant and phenotype selection in rice (*Oryza sativa* L.). *J Genet Genom*, **39**: 335–350.