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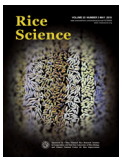

QTL-Seq Identified a Major QTL for Grain Length and Weight in Rice Using Near Isogenic F_2 Population

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Abstract: Mapping and isolation of quantitative trait loci (QTLs) or genes controlling grain size or weight is very important to uncover the molecular mechanisms of seed development and crop breeding. To identify the QTLs controlling grain size and weight, we developed a near isogenic line F_2 (NIL-F_2) population, which was derived from a residual heterozygous plant in an F_7 generation of recombinant inbred line (RIL). With the completion of more than 30× whole genome re-sequencing of the parents, two DNA bulks for large and small grains, a total of 58.94 Gb clean nucleotide data were generated. A total of 455 262 single nucleotide polymorphisms (SNPs) between the parents were identified to perform bulked QTL-seq. A candidate genomic region containing SNPs strongly associated with grain length and weight was identified from 15 to 20 Mb on chromosome 5. We designated the major QTL in the candidate region as *qTGW5.3*. Then, *qTGW5.3* was further validated with PCR-based conventional QTL mapping method through developing simple sequence repeat and Insertion/Deletion markers in the F_2 population. Furthermore, recombinants and the progeny tests delimited the candidate region of *qTGW5.3* to 1.13 Mb, flanked by HX5009 (15.15 Mb) and HX5003 (16.28 Mb). A set of NILs, selected from the F_2 population, was developed to evaluate the genetic effect of *qTGW5.3*. Significant QTL effects were detected on grain length, grain width and 1000-grain weight of H12-29 allele with 1.14 mm, -0.11 mm and 3.11 g, which explained 99.64%, 95.51% and 97.32% of the phenotypic variations, respectively.

Key words: grain length; grain weight; QTL-seq; quantitative trait locus; near-isogenic line; rice; single nucleotide polymorphism; recombinant inbred line; *qTGW5.3*

Uncovering the molecular mechanism of seed development is vital for the formation of rice yield. Grain size and weight, as the phenotypes of rice seed, are closely related and controlled by quantitative trait loci (QTLs) (Zuo and Li, 2014; Huang and Qian, 2017). Up to now, 13 QTLs for grain size or weight have been isolated and characterized in detail. Grain size, which can be specified by grain length, width and thickness, is predominantly regulated by the cell proliferation or/and the expansion of maternal integument and zygotic tissues (Li and Li, 2016). *GS3*, *GL3.1*, *GW2*, *GW5*, *GW6a* and *OsLG3* determine grain size through regulating cell proliferation in spikelet hulls while *TGW6* does in endosperm (Li and Li, 2016; Duan et al, 2017; Yu et al, 2017). *GLW7* and *qNPT1*/*WTG1* can change grain size in rice by regulating cell expansion, whereas *GS2*, *GS5*, *GL7*/*GW7* and *GW8* influence grain size combining the regulation of cell division and cell expansion (Li and Li, 2016; Yi et al, 2016; Huang et al, 2017; Wang

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et al, 2017). Cloning and functional analysis of these QTLs have thrown light on regulatory mechanism of seed development. However, it keeps unclear how these influence factors function in the genetic network to regulate grain size and weight in rice.

QTLs identified from primary mapping populations are usually located in the extended marker intervals, which are mainly caused by the noise from the complex genetic background. Development of near isogenic lines (NILs) can block the noise of genetic background and make the target QTL exhibit the characteristics of a single gene. Several QTLs related to yield traits have been successfully isolated using NILs in rice (Yan et al, 2011; Song et al, 2015; Wang et al, 2015).

Bulked segregant analysis (BSA) is used as a kind of elegant method for rapid identification of molecular markers tightly linked to the causal gene (Giovannoni et al, 1991; Michelmore et al, 1991). Rapid development of next-generation sequencing technologies has boosted the combination of BSA and whole genome re-sequencing of DNA pool to identify candidate genomic region for the target gene or QTL, which generates several efficient techniques for gene or locus mapping, such as SHOREmap, X-QTL, Next Generation Mapping, MutMap, QTL-seq and SLAF-seq (Schneeberger et al, 2009; Ehrenreich et al, 2010; Austin et al, 2011; Schneeberger and Weigel, 2011; Abe et al, 2012; Sun et al, 2013; Takagi et al, 2013). Among these techniques, QTL-seq that combines BSA with next-generation sequencing has successfully identified QTLs responsible for the phenotypic difference in rice (Takagi et al, 2013; Daware et al, 2016; Ogiso-Tanaka et al, 2017), cucumber (Lu et al, 2014) and tomato (Illa-Berenguer et al, 2015).

In this study, an NIL-F_2 population was developed from a residual heterozygous plant of an F_7 generation of recombinant inbred line (RIL) according to the trait-performance strategy and residual heterozygous line method (Zhang et al, 2006; Shao et al, 2010). Residual heterozygous plant derived from RIL has a heterozygous segment at the target QTL region in a homozygous background (Yamanaka et al, 2005; Cheng et al, 2007). Based on the marker genotypes in the NIL-F_2 population, homozygous plant at the target QTL region is selected to generate NILs (Bai et al, 2012). In this study, QTL-seq was conducted to identify the candidate genomic region determining grain length and weight in an NIL-F_2 population. A major QTL controlling grain size and weight was detected in the region from 15 to 20 Mb on chromosome 5, designated as *qTGW5.3*. Then, *qTGW5.3* was further validated, and its candidate region was narrowed down to an interval of 1.13 Mb by screening the recombinants and their progeny test. Finally, a set of NILs was constructed to estimate the genetic effect of *qTGW5.3*.

MATERIALS AND METHODS

Genetic materials and field experiments

An *indica*/*indica* rice cross was made between a pair of varieties with contrasting grain size, Hui 12-29 (H12-29) and Fuhui 212 (FH212). The parental line FH212 is a small grain variety while H12-29 shows large grain. F_2 progenies derived from the H12-29/FH212 cross were further developed into RILs by single-seed descent method. Eighteen F_7 generation plants of each RIL were planted in 2014 in Zhejiang Province, China. One inbred line exhibited a significant segregation of grain length among the individual plants. Within the inbred line, we found 13 individual plants with large grain and 5 plants with small grain. Self-pollinated seeds of the five plants with large grain were further developed into advanced genetic populations in 2015 in Zhejiang Province, China. Among the five populations, three populations showed obvious segregation of small and large grains, while the remaining two populations are consistent with large grain. We chose one segregating population containing 176 plants as an NIL-F_2 population for mapping and validation of the target QTL controlling grain length and weight.

Measurement of yield traits

Harvested rice from the paddy field was air-dried. Seven traits including number of panicles per plant (NPP), number of spikelets per panicle (NSP), number of filled grains per panicle (NGP), 1000-grain weight (TGW), grain length (GL), grain width (GW) and grain yield per plant (GY) were measured by manual or SC-G seed counting and grain weighting device (Wanshen Ltd, Hangzhou, China).

Extraction of genomic DNA and construction of two DNA bulks

Based on the phenotypic investigation, two DNA bulks for QTL-seq were made through the selection of the extreme individuals from the NIL-F_2 population. A total of 35 individuals with large grains were sampled as large grain bulk (L-bulk), and 35 individuals with