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Dissecting the Genetic Basis of Extremely Large Grain Shape in Rice Cultivar ‘JZ1560’

Jie-Zheng Ying [a,b], Ji-Ping Gao [a], Jun-Xiang Shan [a], Mei-Zhen Zhu [a], Min Shi [a], Hong-Xuan Lin [a,*]

[a] *National Key Laboratory of Plant Molecular Genetics and National Center for Plant Gene Research (Shanghai), Shanghai Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China*
[b] *National Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China*

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ABSTRACT

Rice grain shape, grain length (GL), width (GW), thickness (GT) and length-to-width ratio (LWR), are usually controlled by multiple quantitative trait locus (QTL). To elucidate the genetic basis of extremely large grain shape, QTL analysis was performed using an F_2 population derived from a cross between a *japonica* cultivar ‘JZ1560’ (extremely large grain) and a contrasting *indica* cultivar ‘FAZ1’ (small grain). A total number of 24 QTLs were detected on seven different chromosomes. QTLs for GL, GW, GT and LWR explained 11.6%, 95.62%, 91.5% and 89.9% of total phenotypic variation, respectively. Many QTLs pleiotropically controlled different grain traits, contributing complex traits correlation. *GW2* and *qSW5*/*GW5*, which have been cloned previously to control GW, showed similar chromosomal locations with *qGW2-1*/*qGT2-1*/*qLWR2-2* and *qGW5-2*/*qLWR5-1* and should be the right candidate genes. Plants pyramiding *GW2* and *qSW5*/*GW5* showed a significant increase in GW compared with those carrying one of the two major QTLs. Furthermore, no significant QTL interaction was observed between *GW2* and *qSW5*/*GW5*. These results suggested that *GW2* and *qSW5*/*GW5* might work in independent pathways to regulate grain traits. ‘JZ1560’ alleles underlying all QTLs contributed an increase in GW and GT and the accumulation of additive effects generates the extremely large grain shape in ‘JZ1560’.

KEYWORDS: Rice; Quantitative trait locus; Additive effect; Grain shape

1. 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. These variations have been investigated by developing a number of mapping populations derived from the crosses between a great diversity of rice materials. 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

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Abbreviations: GL, grain length; GW, grain width; GT, grain thickness; LWR, length-to-width ratio; QTL, quantitative trait locus.

[*] Corresponding author. Tel: +86 21 5492 4129, fax: +86 21 5492 4015.
E-mail address: hxlin@sibs.ac.cn (H.-X. Lin).

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mapped near the centromeric region of rice chromosome 3 using different populations in several independent studies (Li et al., 1997; Yu et al., 1997; Xiao et al., 1998; Xing et al., 2002; Thomson et al., 2003; Li et al., 2004b). 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 *tgw11*, have been identified at accurate intrachromosomal locations (Li et al., 2004a; Xie et al., 2006, 2008; Guo et al., 2009; Oh et al., 2011). *GS3*, *gl-3*, *qGL7* and *qGL7-2* controlling GL were fine mapped at the exact locations on chromosomes 3 and 7 (Fan et al., 2006; Wan et al., 2006; Bai et al., 2010; Shao et al., 2010). *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 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 traits. However, the mechanism of regulation of grain size and epistatic interactions between these QTLs are still unclear.

Identification of more QTLs and characterization of genes underlying the QTLs will be very helpful in enhancing the understanding of rice seed development, which requires more QTLs to be mapped and cloned. Here, two parents contrasting in grain shape were selected to develop an F_2 mapping population to dissect the genetic basis of extremely large grain shape in rice.

2. MATERIALS AND METHODS

2.1. Construction of an F_2 population and field trials

The parental line, ‘Fengaizhan-1’ (‘FAZ1’), is a small grain *indica* cultivar, and the other parental line, ‘Jizi1560’ (‘JZ1560’), is an extremely large grain *japonica* cultivar. An F_2 population derived from a cross of ‘FAZ1’ × ‘JZ1560’ was constructed for QTL mapping as described in our previous study (Ying et al., 2012). One hundred and forty-five plants along with both parents were planted in a paddy field in Shanghai, East China.

2.2. Measurements of grain traits

Twenty fully filled grains were selected from each F_2 plant and their parents for the measurements of grain traits including GL, GW and GT. GL was evaluated by lining up the selected 20 grains one by one along a ruler. GW and GT were measured for each grain individually. LWR was calculated by dividing GL by GW.

2.3. QTL detection and data analysis

A genetic linkage map with 143 markers, covering 12 chromosomes and spanning 1451.70 cM, was constructed as described in our previous study (Ying et al., 2012). QTLs for grain traits were mapped with the information from Mapmaker by composite interval mapping (CIM) using Windows QTL Cartographer 2.5 (Wang et al., 2006). QTL analysis was performed with 1000 permutations at the 0.05 probability level. The LOD threshold was fixed at a LOD score of 3.0 following 500 permutation tests for each trait. Multiple interval mapping (MIM) approach was conducted to analyze QTL interaction between QTLs for single trait in the computer program Windows QTL Cartographer 2.5 (Wang et al., 2006). In this study, the Bayesian Information Criterion (BIC) default value $c(n) = \ln(n)$ was used to search QTL epistatic interaction at a walk speed of 1 cM.

Correlation coefficients were calculated to determine correlations between traits in F_2 populations using PROC CORR in the SAS program (SAS Institute, 1999).

3. RESULTS

3.1. Phenotypic variation and correlations among grain traits

The mean values of each grain trait, including GL, GW, GT and LWR, are shown in Table 1. ‘JZ1560’ has an extremely large grain size of 64.6 g/1000 grains, in contrast to ‘FAZl’ with 19.3 g/1000 grains (Fig. 1). A wide variation of GW was observed from 2.5 to 4.5 mm in the F_2 population. All the grain traits in the F_2 population showed continuous segregation and no significant transgressive segregation except GL and LWR was observed, indicating that they are controlled by