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QTL Mapping for Hull Thickness and Related Traits in Hybrid Rice Xieyou 9308

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Abstract: We conducted a quantitative trait locus (QTL) analysis of 165 rice recombinant inbred lines derived from a cross between Zhonghui 9308 (Z9308) and Xieqingzao B (XB) in Hainan and Hangzhou, China. Grain thickness (GT), brown rice thickness (BRT), hull thickness (HT) and milling quality were used for QTL mapping. HT was significantly and positively correlated with GT and BRT. Twenty-nine QTLs were detected with phenotypic effects ranging from 2.80% to 21.27%. Six QTLs, *qGT3*, *qBRT3*, *qBRT4*, *qHT6.1*, *qHT8* and *qHT11*, were detected repeatedly across two environments. Inherited from XB, *qHT6.1*, *qHT8* and *qHT11* showed stable expression, explaining 9.92%, 21.27% and 10.83% of the phenotypic variances in Hainan and 9.61%, 6.40% and 6.71% in Hangzhou, respectively. Additionally, the QTL cluster between RM5944 and RM5626 on chromosome 3 was probably responsible for GT and milling quality. The cluster between RM6992 and RM6473 on chromosome 4 played an important role in grain filling. Three near isogenic lines (NILs), X345, X338 and X389, were selected because they contained homozygous fragments from Zhonghui 9308, corresponding to *qHT6.1*, *qHT8* and *qHT11*, respectively. The hull of XB was thicker than those of X345, X338 and X389. In all the lines, *qHT6.1*, *qHT8* and *qHT11* that regulated rice HT were stably inherited with obvious genetic effects.
Key words: rice; hull thickness; milling quality; QTL mapping

Rice is not only a model organism for monocotyledons, but also an important food crop. Rice grain consists of a hull reciprocally hooked by the lemma and palea, inner and outer bran layers, and brown rice (Yang, 2005). And the grain weight is determined by the volume and shape of the hull that acts in a sink capacity (Venkateswarlu and Visperas, 1987). Rice hull plays important roles in preventing damage and maintaining humidity during the development of brown rice (Zhou et al, 2003; Abebe et al, 2004). Additionally, it has been reported that the percent of hull is negatively correlated with the milling quality (Jongkaewwattana and Geng, 2001). Thus, the shape, capacity and thickness of the hull are important aspects affecting yield and milling quality. However, there has been little regard for rice hull thickness (HT) in previous studies.

The developing rice hull consists of an outer epidermis, skin tissues, soft tissues and endepidermis, and its thickness is mainly determined by the soft tissues (Yang, 2005). No genetic assays for genes or QTL controlling HT have been conducted previously. There are two types of cloned genes for grain size and hull mutants that might relate to rice HT. Several genes regulating grain size have been cloned, such as *DTH8* (Wei et al, 2010), *GS3* (Fan et al, 2006), *SRS5* (Segami et al, 2012), *GIF1* (Wang et al, 2008), *gw5* (Weng et al, 2008), *GW2* (Song et al, 2007), *GS5* (Li et al, 2011), *qGW8* (Wang et al, 2012) and *qSW5* (Wan et al, 2005). Among these, most of the genes act in regulating cell division or extension, resulting in the alteration of grain size. For example, *DTH8/GHD8* encodes a putative HAP3 subunit of the CCAAT-box-binding transcription factor that regulates yield, plant height and flowering time (Zhang et al, 2006; Wei et al, 2010). *GS3* was reported to encode 232 amino acids with a putative PEBP-like domain, a transmembrane region, a putative TNFR/NGFR family cysteine-rich domain and a VWFC module. It is involved in regulating seed length, stigma length and stigma exsertion (Fan et al, 2006; Noriko et al, 2011). These two genes might

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also function in regulating rice hull development. Genetic studies of rice hulls always focused on rice hull mutants affecting flower development. Specifically, a palea formation controlling gene, *depressed palea 1* (*dp1*), which regulates palea formation and floral organ number, has been cloned on chromosome 6 (Jin et al, 2011). The *DP1* gene encodes a nuclear-localized AT-hook DNA binding protein, resulting in a primary defect in the main structure of the palea. The s*tunted lemma palea 1* (*slp1*) rice mutant displays severely degenerated lemmas/paleae, and *SLP1* is localized at a 46.4-kb genomic region containing three putative genes, *OsSPL16*, *OsMADS45* and *OsMADS37* (Wang et al, 2011). Two other genes, *G1* and *OsMADS1*, were also reported to regulate rice hull development (Jeon et al, 2000; Yoshida et al, 2009). Additionally, three genes controlling hull color, *black hull 4* (*Bh4*), *gh1* and *gh2* were reported (Tobias and Chow, 2005; Zhu et al, 2011; Hong et al, 2012).

Until now, little study associated with rice HT has been conducted. The objective of this study was to establish a measuring method for HT and conduct a genetic assay on rice HT, grain thickness (GT) and brown rice thickness (BRT) using QTL analysis. Additionally, the relationships of HT, GT, BRT with milling quality, brown rice rate (BR), milled rice rate (MR) and head rice rate (HR) were also used for association analysis and QTL mapping.

MATERIALS AND METHODS

Rice materials

A total of 165 recombinant inbred lines (RILs) derived from Xieyou 9308, the cross between Zhonghui 9308 (Z9308, a typical indica restorer) and Xieqingzao B (XB, an indica maintainer with 25% genes from japonica, http://www.ricedata.cn/variety/), were used. The trials were conducted in the experimental fields of the China National Rice Research Institute, Hangzhou (30.3° N, 20.2° E), Zhejiang Province, and Lingshui (18.2° N, 108.9° E), Hainan Province, China, in 2011, respectively. RILs and the two parental lines were sown in December 2011 and grown under natural conditions in Hainan. Field trials were conducted in randomized complete blocks with two replicates. The F_1 hybrid of Z9308 × XB was backcrossed with Z9308 three times to produce the BC_4F_1 population. The BC_4F_2 lines were selected from the BC_4F_1 selfing population by marker-assisted selection (MAS). Using gained near isogenic lines (NILs) based on the background of XB, rice hull QTLs were validated in the BC_4F_2 generation.

Phenotypic evaluation

Forty days after heading, four plants from the individual lines were harvested. Two panicles from each plant were selected for the evaluation of GT, BRT and HT. Grains in the middle of the panicle were threshed and dropped into water, and finally the submerged grains were gathered. Ten grains were selected randomly for evaluation. The remaining harvested panicles from each line were mixed and threshed for milling quality analysis. The water content of the sample was approximately 12% as measured by moisture analyzer (MB35, Switzerland). Milling quality, containing BR, MR and HR was measured against a national reference standard. GT and BRT were measured using a Vernier Caliper (TESA CAL IP67, SWISS) and the HT was calculated as follows:

$$HT = \sum (GT - BRT) / (2 \times 10).$$

Scanning electron microscope analysis

For the two parental lines, spikelets were sampled from the middle of the panicle every 5 d after the heading day until 25 days after heading (DAH). A scanning electron microscope (SEM) examination was conducted following the protocol reported by Li et al (2010) with some modifications. In brief, fresh rice spikelets from the two parents were fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.0). The fixed samples were rinsed three times for 15 min each time with phosphate buffer, and they were fixed overnight with 1% OsO_4 in phosphate buffer at 4 °C, and then washed three times for 15 min each time in the phosphate buffer and dehydrated through an ethanol series of 50%, 70%, 80%, 90%, 95% and 100% for 15 min with each step. Subsequently, they were incubated in 1:1 ethanol-isoamyl acetate mixture for 30 min and transferred to pure isoamyl acetate for 1 h. Finally, the samples were dried to a critical point with liquid CO_2 and then coated with gold-palladium before mounted for observation. They were photographed under an SEM (Hitachi TM-1000 Tabletop Microscope, Japan). The thickness of the palea and lemma in the photos was determined using Adobe Photoshop CS6. The dynamic graph was drawn with Microsoft Office Excel 2007.

QTL analysis

QTL analysis was conducted using the composite