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Comparison and analysis of QTLs for grain and hull thickness related traits in two recombinant inbred line (RIL) populations in rice (*Oryza sativa* L.)

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Abstract

Grain traits are major constraints in rice production, which are key factors in determining grain yield and market values. This study used two recombinant inbred line (RIL) populations, RIL-JJ (*japonica/japonica*) and RIL-IJ (*indica/japonica*) derived from the two crosses Shennong 265/Lijiangxintuanheigu (SN265/LTH) and Shennong 265/Luhui 99 (SN265/LH99). Sixty-eight quantitative trait loci (QTLs) associated with 10 grain traits were consistently detected on the 12 chromosomes across different populations and two environments. Although 61.75% of the QTLs clustered together across two populations, only 16.17% could be detected across two populations. Eight major QTLs were detected on the 9, 10 and 12 chromosomes in RIL-JJ under two environments, a novel QTL clustered on the 10 chromosome, *qGT10*, *qBT10* and *qTGW10*, have a higher percentage of explained phenotypic variation (PVE) and additive effect; 15 major QTLs were detected on the 5, 8, 9, and 11 chromosomes in RIL-IJ under two environments, a novel clustered QTL, *qGT8* and *qTGW8*, on the 8 chromosome have a higher additive effect. Finally, the analysis of major QTL-BSA mapping narrowed the *qTGW10* to a 1.47-Mb region flanked by simple sequence repeat markers RM467 and RM6368 on chromosome 10. A comparison of QTLs for grain traits in two different genetic backgrounds recombinant inbred line populations confirmed that genetic background had a significant impact on grain traits. The identified QTLs were stable across different populations and various environments, and 29.42% of QTLs controlling grain traits were reliably detected in different environments. Fewer QTLs were detected for brown rice traits than for paddy rice traits, 7 and 17 QTLs for brown rice out of 25 and 43 QTLs under RIL-JJ and RIL-IJ populations, respectively. The identification of genes constituting the QTLs will help to further our understanding of the molecular mechanisms underlying grain shape.

Keywords: paddy rice, brown rice, hull thickness, genetic backgrounds, QTL mapping, rice (*Oryza sativa* L.)

1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops in particular for Asia country, contributing more than 50% of staple calorie intake (Khush 2005; Xing and Zhang 2010; Huang *et al*. 2013). Grain shape is a major constraint in rice production, as it is a key factor in deter-

mining yield and market values (Xu *et al*. 2004; Kato *et al*. 2011; Wang *et al*. 2012; Che *et al*. 2015; Duan *et al*. 2015; Hu *et al*. 201). An understanding of genetic mechanisms underlying grain shape is of importance for molecular biologists and plant breeders alike, and is currently the focus of considerable research (Ashikari and Matsuoka 2006; Miura *et al*. 2011; Gao *et al*. 2016). Determinants of rice grain yield include a number of sub-traits: spike number per plant, grain number per panicle and grain weight. Grain weight is further divided into grain length (GL), grain width (GW), grain length and width rate (GLW), grain thickness (GT), and 1 000-grain weight (TGW). These traits are well-known to be controlled by quantitative trait loci (QTLs) and affected by the environment (Ashikari and Matsuoka 2006; Weng *et al*. 2008; Luo *et al*. 2009; Liu *et al*. 2010; Liang *et al*. 2012; Huang *et al*. 2013; Wang *et al*. 2015; Gao *et al*. 2016). The three grain sub-traits vary depending on rice variety, with different varieties under different genetic controls (Li *et al*. 2004; Bai *et al*. 2011; Maryam *et al*. 2012). On the basis of grain shape, rice is primarily classified into long, medium and short grain categories corresponding to GL:GW ratios of >3.0, 2.1–2.9 and <2.0, respectively. There are significant differences between grain traits and the genes that control them in *O. sativa* subspecies *indica* and *japonica*. In general, the former has a larger GLW (>3.0), while the latter has a shorter and rounder shape (GLW<2.5). Over the last two decades, numerous genes and QTLs associated with these traits have been identified using numerous mapping populations (http://archive.gramene.org/qtl/); some of these traits, such as *GW2* (Song *et al*. 2007), *GS2* (Che *et al*. 2015; Duan *et al*. 2015; Hu *et al*. 2015), *GS3* (Fan *et al*. 2006; Takano-kai *et al*. 2011), *GW5* (Weng *et al*. 2008), *qSW5* (Shomura *et al*. 2008), *GS5* (Li *et al*. 2011), *GS6* (Guo *et al*. 2009), *GW8* (Xie *et al*. 2006), *DEP1* (Huang *et al*. 2009), SRS1/*DEP2* (Abe *et al*. 2010), *qGL7/qSS7* (Bai *et al*. 2010; Qiu *et al*. 2012; Shao *et al*. 2012), *SRS3* (Kitagawa *et al*. 2010), *SRS5* (Segami *et al*. 2012), *D2* (Hong *et al*. 2003), *tgw11* (Oh *et al*. 2011), and *GIF1* (Wang *et al*. 2008, 2010), have been finely mapped. Comprehensive analysis suggests that genetic background, population size, map density, and environment have a significant effect on QTL mapping success, with population genetic background especially important (Wan *et al*. 2006; Song *et al*. 2007; Huang *et al*. 2013). QTL accuracy is generally depended upon genetic diversity in the parent, heritability of traits and density of genetic markers. Although comparative analysis is an effective way to compare large quantities of QTL data, QTL from different experimental studies are usually derived from individual populations under varied environments and use different analytical methods; this hampers alignment of multiple QTLs across different data sets (Thabuis 2004; Ashikari and Matsuoka 2006; Xing and Zhang 2010; Wang *et al*. 2012; Mei *et al*.

2013; Pu *et al*. 2014; Deng *et al*. 2015). In this study, two RIL populations with diverse grain traits and genetic backgrounds were used to detect QTLs for paddy rice (unhulled) and brown rice (hulled) grain traits across two environments. The two sets of RIL populations were derived from crosses between a common female parent, Shennong 265 (SN265) was a super high yield *japonica* cultivar in north which have a ideal plant type, short erect panicle and two male parents, *japonica* cultivar Lijiangxingtuanheigui (LTH) and *indica* cultivar Luhui 99 (LH99). The former (LTH) was a high altitude area *japonica* cultivar and there have significant difference in grain traits with temperate *japonica* rice (SN265), and LH99 was a important restorer line for *indica* hybrid rice cross and was extremely different from two *japonica*. The RIL-JJ (*japonica/japonica*) and RIL-IJ (*indica/japonica*) populations have significant difference in grain traits and genetic background so that we used them as research materials in order to discuss its influence for QTLs of grain traits and detect novel QTLs. The objectives of this study were (1) to detect QTLs for paddy and brown rice grain traits and hull thickness (HT); (2) to compare the number and distribution of QTLs in paddy and brown rice; and (3) to perform a systematic analysis and confirm whether genetic background affects rice grain traits.

2. Materials and methods

2.1. Mapping populations and field experiment

Two sets of RIL populations, developed from the crosses SN265×LTH (RIL-JJ) and SN265×LH99 (RIL-IJ), were used in this study (Jiang *et al*. 2012; Zhang *et al*. 2014). The two RIL-JJ and RIL-IJ populations, consisting of 96 and 158 lines, the RIL-JJ were planted during the rice-growing season at the experimental farm of Shenyang Agricultural University, Hainan (in 2012) and Shenyang (in 2013), China; the RIL-IJ were planted during the rice-growing season at the experimental farm of Shenyang Agricultural University, respectively, in 2012 and 2013. The field management followed normal agricultural practice. 25-d-old seedlings of each line were transplanted into a three-row plot with eight plants per row and spacing of 30 cm×13.3 cm. Three replications of each line were transplanted per sowing.

2.2. Measuring grain traits

For trait measurements, 20 fully filled rice grains were randomly selected from three spikes per line. These 20 seeds were individually measured for paddy and brown rice length (GL and BL), width (GW and BW) and thickness (GT and BT) using an electronic digital vernier caliper (Mitutoyo, Japan) with a precision of 0.01 mm. We remove the hulls