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Validation of *qGS10*, a quantitative trait locus for grain size on the long arm of chromosome 10 in rice (*Oryza sativa* L.)

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Abstract

Grain size is a major determinant of grain weight and a trait having important impact on grain quality in rice. The objective of this study is to detect QTLs for grain size in rice and identify important QTLs that have not been well characterized before. The QTL mapping was first performed using three recombinant inbred line populations derived from *indica* rice crosses Teqing/IRBB lines, Zhenshan 97/Milyang 46, Xieqingzao/Milyang 46. Fourteen QTLs for grain length and 10 QTLs for grain width were detected, including seven shared by two populations and 17 found in one population. Three of the seven common QTLs were found to coincide in position with those that have been cloned and the four others remained to be clarified. One of them, qGS10 located in the interval RM6100–RM228 on the long arm of chromosome 10, was validated using $F_{2:3}$ populations and near isogenic lines derived from residual heterozygotes for the interval RM6100–RM228. The QTL was found to have a considerable effect on grain size and grain weight, and a small effect on grain number. This region was also previously detected for quality traits in rice in a number of studies, providing a good candidate for functional analysis and breeding utilization.

Keywords: grain size, quantitative trait locus, residual heterozygote, rice (Oryza sativa L.)

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops, feeding half of the world's population. Grain yield

in rice is determined by three components, i.e., number of panicles per plant, number of grains per panicle and grain weight. Grain size is a major determinant of grain weight, and a trait having important impact on the market value of rice grain. Long and slender grains are preferred in the major segment of the international market, whereas short and round grains are favored in northern China, Korea and Japan (Calingacion *et al.* 2014). In addition, slender grains are more likely to have lower grain chalkiness thus a better appearance quality (Wang *et al.* 2005).

Over the last two decades, a large number of quantitative trait loci (QTLs) for grain size and grain weight in rice were detected and some of them were cloned since 2006. *GS3*, a major negative regulator controlling grain length and weight is the first QTL cloned for grain size (Fan *et al.* 2006). Eight more QTLs were cloned up to date, including *GL3.1/qGL3*

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(Qi et al. 2012; Zhang et al. 2012), *TGW6* (Ishimaru et al. 2013), *GW6a* (Song et al. 2015), and *GW7/GL7* (Wang S K et al. 2015; Wang Y X et al. 2015) determining grain length and weight, and *GW2* (Song et al. 2007), *qSW5/GW5* (Shomura et al. 2008; Weng et al. 2008), *GS5* (Li Y et al. 2011), and *GW8* (Wang et al. 2012b) responsible for grain width and weight.

It has been commonly applied that QTLs exhibiting major and consistent effects in primary mapping populations were first targeted for cloning. As a result, QTLs that have been cloned for yield traits in rice, either those for grain size and grain weight described above, or others associated with grain number (Ikeda *et al.* 2013; Yan *et al.* 2013), all showed large effects for the trait under study. Because few QTLs of this kind is available, it is not uncommon that different groups separately make great efforts on the same QTL (Shomura *et al.* 2008; Weng *et al.* 2008; Qi *et al.* 2012; Zhang *et al.* 2012; Ikeda *et al.* 2013; Wang S K *et al.* 2015; Wang Y X *et al.* 2015). Diversifying rice crosses in constructing populations for primary QTL mapping may facilitate the detection of new QTLs and alleviate the shortage of candidate QTLs for cloning.

In the present study, QTL mapping for grain size in rice was performed using three primary populations, followed by the validation of one QTL region. Fourteen QTLs for grain length and 10 QTLs for grain width were detected in three recombinant inbred line (RIL) populations derived from the *indica* rice crosses Zhenshan 97/Milyang 46 (ZM), Xieqing-zao/Milyang 46 (XM) and Teqing/IRBB lines (TI). One QTL shared by different populations and located in a region that was away from those that have been cloned was selected for validation. Two lines of the TI population were crossed to develop an $F_{2:3}$ population and three sets of near isogenic lines (NILs). The target QTL, *qGS10* located in the interval RM6100–RM228 on the long arm of chromosome 10, was validated to have a considerable effect on grain size and grain weight.

2. Materials and methods

2.1. Plant materials

The three RIL populations used in this study have been reported by Mei *et al.* (2013). Both the female and male parents of the TI population are *indica* rice restorer lines, of which the male parent included six IRBB lines (IRBB50, IRBB51, IRBB52, IRBB54, IRBB55, and IRBB59) that are NILs in the genetic background of IR24 (Huang *et al.* 1997a). The numbers of RILs included in the TI population are 122 for Teqing/IRBB52, 77 for Teqing/IRBB50, two for Teqing/IRBB50, and one each for Teqing/IRBB51, Teqing/

IRBB54 and Teqing/IRBB55. The female parents of the ZM and XM populations, Zhenshan 97 and Xieqingzao, are maintainer lines of the commercial three-line *indica* rice hybrid Shanyou 10 and Xieyou 46, respectively, and the common male parent Milyang 46 is the restorer line of the two hybrids (Mei *et al.* 2013). In the rice zone of middle-lower reaches of Yangtze River in China, Zhenshan 97 and Xieqingzao are used as early-season rice, and Milyang 46, Teqing and IR24 are grown as middle-season rice.

Development of secondary populations for the validation of *gGS10* were described below and illustrated in Fig. 1. Two lines of the TI population, having distinct phenotypes in arain size and different genotypes in the interval RM6100-RM228 on the long arm of rice chromosome 10, were selected and crossed. 120 F₂ plants were produced and assayed using the four markers in the qGS10 region, i.e., RM6100, RM3773, RM3123, and RM228. Plants that were heterozygous in all the four marker loci were identified as residual heterozygotes (RHs) for qGS10. They were then subjected to genotyping with 122 polymorphic SSR markers located in other regions. One plant was selected, remained to be heterozygous in the target region and identified to be heterozygous and homozygous at 19 and 103 marker loci in the background, respectively. This plant was selfed to produce a F_2 -type population and then a F_{23} -type population.



Fig. 1 Construction of a residual heterozygote (RH)-derived F_{23} population and three sets of near isogenic lines (NILs).

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