

Validation of QTLs associated with spikelets per panicle and grain weight in rice

Sang-Min Yeo¹, Yeo-Tae Yun², Dong-Min Kim¹, Chong-Tae Chung² and Sang-Nag Ahn^{1*}

¹Department of Agronomy, Chungnam National University, Daejeon 305-764, Republic of Korea and ²Chungnam Agricultural Research and Extension Services, Yesan 340-861, Republic of Korea

Abstract

In this study, a near-isogenic line (BC₄F₁₀) CR572 developed by introgressing a chromosomal segment from *Oryza rufipogon* (accession no. 105491) into the *Oryza sativa* subsp. *japonica* cv. Hwaseong was found to exhibit a significant increase in the number of spikelets per panicle (SPP) and grain weight compared with the recurrent parent Hwaseong. Quantitative trait locus (QTL) analysis in F₂ generation derived from the cross between CR572 and Hwaseong revealed that two QTLs, *qSPP1* and *qTGW1*, were linked to a simple sequence repeat marker, RM283, on chromosome 1. The additive effect of the *O. rufipogon* allele at *qSPP1* was 13 SPP, and 21.6% of the phenotypic variance was explained by the segregation of RM283. The *qTGW1* QTL explained 19.1% of the phenotypic variance for grain weight. Substitution mapping was carried out with five F₃ lines derived from F₂ plants having informative recombination breakpoints within the target region. Substitution mapping indicated the linkage of *qSPP1* and *qTGW1*. The grain yield of CR572 was 18.2 and 15.8% higher than that of Hwaseong at two locations, respectively, mainly due to the increase in 1000-grain weight and SPP. These results are very useful for QTL cluster transfer by molecular marker-assisted selection in rice breeding programmes and for QTL gene cloning by map-based cloning.

Keywords: 1000-grain weight; QTL; near-isogenic lines; rice; spikelets per panicle

Introduction

Grain weight, which is determined by grain length (GL), grain width (GW) and grain thickness, is an important component of yield. Genes that affect grain size have been identified in rice (Thomson *et al.*, 2003; Fan *et al.*, 2006), and a few genes controlling seed size have been isolated in rice: *GS3*, *GW2*, *qSW5* and *GS5* (Fan *et al.*, 2006; Song *et al.*, 2007; Shomura *et al.*, 2008; Li *et al.*, 2011). *GS3* has a significant effect on seed length, whereas *GW2* has significant effects on both seed width or GW and grain weight in rice.

The number of spikelets per panicle (SPP) strongly influenced by primary and secondary branches is also important for determining yield potential in rice (Zhang *et al.*, 2006). Many studies have detected quantitative trait loci (QTLs) for SPP using various segregating populations (Thomson *et al.*, 2003; Suh *et al.*, 2005; Liu *et al.*, 2010). These QTLs are located across the chromosomes and provide valuable information on the genes that control SPP in different populations.

In a previous study, Hwaseong, a Korean *japonica* rice cultivar, was crossed as a recurrent with *Oryza rufipogon* and QTL analysis was conducted on BC₂F₂ (Cho *et al.*, 2003) and BC₄F₂ (Yun *et al.*, 2010) lines. The *qTGW1* QTL was detected in both generations, whereas the *qSPP1* QTL was detected only in the BC₄F₂ line. The present study was conducted to detect and characterize

*Corresponding author. E-mail: ahnsn@cnu.ac.kr

two QTLs, *qSPP1* and *qTGW1*, using $F_{2,3}$ populations from a cross between Hwaseong and near-isogenic lines (NILs).

Materials and methods

In previous studies, *qSPP1* and *qTGW1* have been detected on chromosome 1 (Cho *et al.*, 2003; Yun *et al.*, 2010). To detect these QTLs, CR572 was crossed with Hwaseong and then allowed to self to generate a F_2 population (380 plants), which was used for QTL analysis. Substitution mapping was carried out to further fine-map the QTLs using F_3 lines. Two populations (F_2 and F_3) and the parents were grown in the field during the summers of 2011–2013 at the Chungnam National University, Daejeon, Korea. F_2 plants were planted 15 cm apart with 30 cm spacing between rows. F_3 plants were planted in a completely randomized block design with three replications. SPP and 1000-grain weight (TGW) were evaluated in F_2 lines. Evaluation of SPP, TGW and GW in F_3 lines was carried out as described by Xie *et al.* (2008). Data were obtained from 15 plants per each line. DNA was extracted from the leaves of F_2 plants using the CTAB method (Causse *et al.*, 1994). Simple sequence repeat (SSR) primers were synthesized according to an available rice genome sequence (<http://www.gramene.org/markers/>). Polymerase chain reaction and silver staining were conducted using the method of Xie *et al.* (2008). Phenotypic means of three genotypes,

Hwaseong and *O. rufipogon* homozygotes and heterozygotes, were compared using Student's *t* test in the F_2 population. QTLs were fine-mapped by comparing the phenotypic means of two genotypes of recombinants within the target region using the SAS statistical package. Paraffin-embedded spikelet tissue sections were prepared to investigate the origins of the observed size differences in spikelets between Hwaseong and CR7201 line following the methods described by Ji *et al.* (2006).

Results and discussion

The SPP and TGW values were higher in CR572 than in Hwaseong, whereas no significant difference was observed for days to heading and spikelet fertility between the parents (data not shown). The possibility of the effect of non-target regions can be excluded because CR572 had no *O. rufipogon* introgression at the non-target regions (Fig. 1(a)). F_2 plants were genotyped with ten SSR markers, and QTL analysis revealed that there was a significant peak near the marker RM522 for TGW and SPP (Fig. 1(b)). The logarithm of odds values for *qSPP1* and *qTGW1* were 19.2 and 8.0, respectively. The *qSPP1* QTL explained 25.1% of the phenotypic variance. The additive effect of the *O. rufipogon* allele at *qTGW1* was 0.52 TGW and 19.1% of the variance could be explained by the QTL.

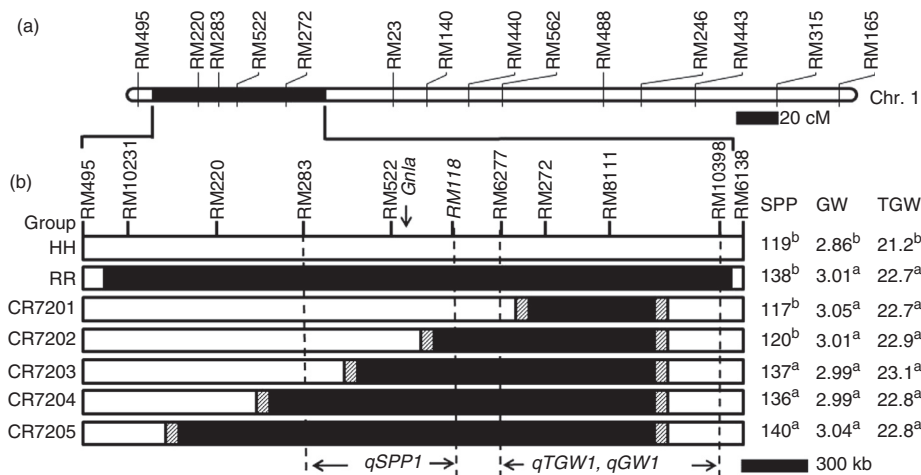


Fig. 1. (a) Single introgression from *Oryza rufipogon* flanked by RM495 and RM23 on chromosome 1 (Chr. 1) in a near-isogenic line (NIL), CR572. (b) Graphical genotypes of the F_3 lines used for the substitution mapping of *qSPP1* and *qTGW1*. The white portions of the graph indicate homozygous Hwaseong chromosomal segments, the black portions indicate homozygous *O. rufipogon* segments, the slashed areas are regions where crossing-over has occurred, and the broken vertical lines define the interval containing QTLs. On the right side of the graphical genotypes, the mean values of three traits for each F_3 line are given. Values that are followed by different letters in each column are significantly different according to Tukey's honestly significant different test at 5%. HH and RR denote Hwaseong and CR572, respectively. The other five F_3 lines (CR7201, CR7202, CR7203, CR7204 and CR7205) are NILs with a single introgression with a different size. The simple sequence repeat marker RM1118 (in italics) was additionally screened to further define the position of *qSPP1*. SPP, spikelets per panicle; GW, grain width; TGW, 1000-grain weight.

To further fine-map *qTGW1* and *qSPP1*, five F_2 plants with informative recombination breakpoints between RM495 and RM6138 were selfed to produce F_3 lines for substitution mapping. Five F_3 lines were evaluated for TGW, SPP, GL, GW and grain yield (YD). The mean phenotypic values of the five traits for each group were compared with those of the controls, Hwaseong and CR572. A comparison delimited the *qTGW1* locus between RM6277 and RM10398 based on the finding that the TGW of the five F_3 lines (CR7201, CR7202, CR7203, CR7204 and CR7205) did not differ significantly from that of CR572 but was higher than that of Hwaseong. Using the same procedure, *qGW1* was found to be located within the same interval. A highly significant correlation was observed between GW and TGW ($r = 0.87$, $P < 0.01$), indicating that the variation in GW was associated with that in TGW at this locus. No major QTL associated with TGW was detected near *qTGW1* in this study, suggesting that *qTGW1* is a new QTL.

For *qSPP1*, CR7201 and CR7202 lines had significantly lower SPP than CR572. The SPP of CR7203, CR7204 and CR7205 lines, which share about 700 kb *O. rufipogon* segment between RM283 and RM1118, did not differ significantly from that of CR572 but was

significantly higher than that of Hwaseong. These results imply that *qSPP1* was located between RM283 and RM1118.

The *qSPP1* QTL shares a similar location with the gene *Gn1A* (grain number 1a) for *OsCKX2* (*Oryza sativa* L. cytokinin oxidase 2), which controls grain number (Ashikari *et al.*, 2005). The *qSPP1* QTL has also been found to be co-localized with *SPP1* controlling the number of SPP (Liu *et al.*, 2010). Because of the low resolution, the relationship of *qSPP1* with *Gn1a* and *SPP1* is not clear.

To test the effect of *qTGW1* and *qSPP1* on yield, we compared the YD of CR572 and Hwaseong at two locations, Daejeon and Yesan, in 2011 and 2012 (data not shown). We found the YD of CR572 to be 18.2 ($P < 0.05$) and 15.8% ($P < 0.05$) higher than that of Hwaseong at the two locations, respectively, mainly due to the increase in TGW and SPP. The *O. rufipogon* segment is of particular interest because the *O. rufipogon* segment was not associated with any change in amylose and protein content. However, additional experiments are needed to determine the effect on other grain quality traits because increased SPP and TGW may have negative effects on *japonica* grain quality.

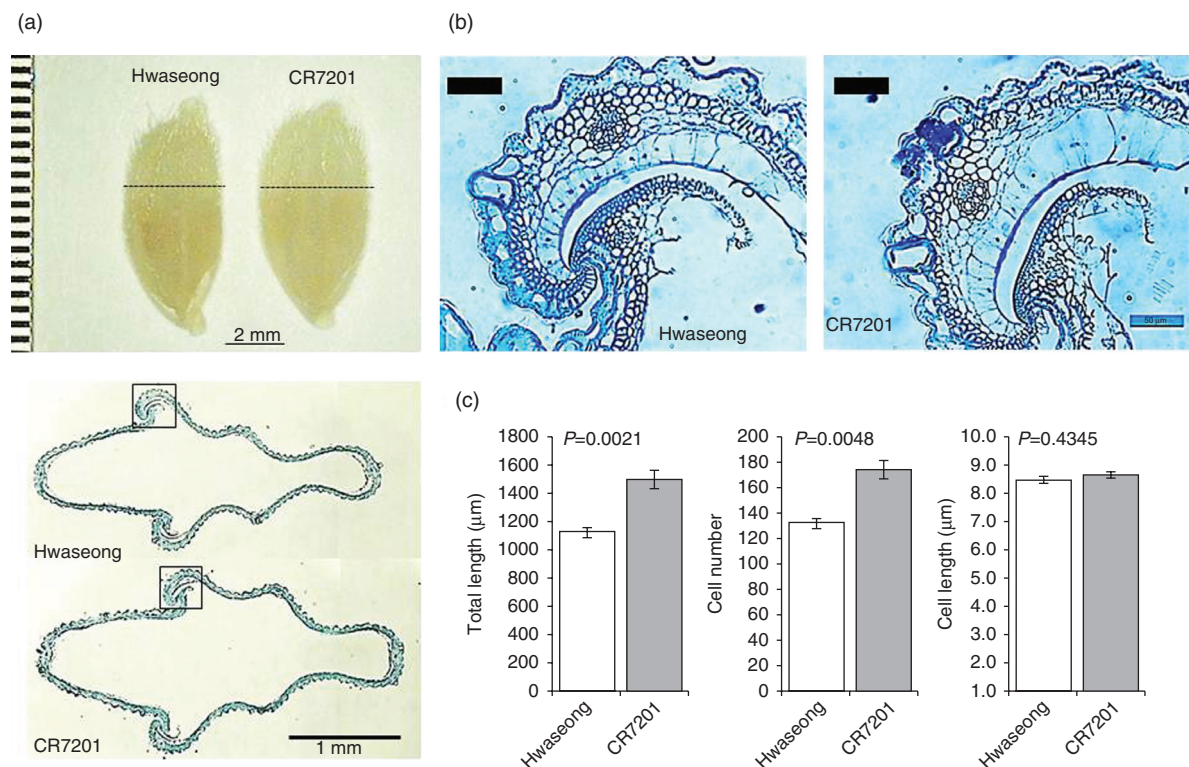


Fig. 2. Histological analyses of spikelet hulls 3 d before heading in Hwaseong and CR7201 (plants in A group in Fig. 1(b)). (a) Top: Spikelets. Bottom: Cross section of spikelet hull. Dotted line indicates position of the cross section. (b) Magnified view of spikelet hull cross section boxed in (a). Scale bar: 50 μm . (c) Comparison of cell number and mean cell length in the outer cell layers of spikelet hulls of Hwaseong and CR7201 ($n = 5$ spikelets).

Before fertilization, the CR7201 spikelet hull was wider than the Hwaseong spikelet hull (Fig. 2), and we compared the cross sections of the central part of the spikelet hull in CR7201 and Hwaseong (Fig. 2(a)). The outer parenchyma cell layer in CR7201 was longer and contained more cells than that in Hwaseong (Fig. 2(b)). These results indicate that the increased width of the CR7201 spikelet hull results from an increase in cell number (Fig. 2(c)).

Several QTLs controlling TGW have been cloned using NILs (Song *et al.*, 2007; Shomura *et al.*, 2008). In the present study, *qTGW1* was found to be responsible for 33.0% of the phenotypic variance. No QTL around the *qTGW1* region has been cloned to date. It would be interesting to clone *qTGW1* to examine the functional relationships of the genes that control TGW and to determine how they interact with other genes/alleles in various genetic backgrounds.

Acknowledgements

This study was supported by the Korean Government (MOEHRD, Basic Research Promotion Fund, KRF-2010-0024118) and the Next-Generation Biogreen 21 Program (Plant Molecular Breeding Center no. PJ008136), Rural Development Administration, Republic of Korea.

References

- Ashikari M, Sakakibara H, Lin SY, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H and Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. *Science* 309: 741–745.
- Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwose J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR and Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138: 1251–1374.
- Cho YC, Suh JP, Choi IS, Hong HC, Baek MK, Kang KH, Kim YG, Ahn SN, Choi HC, Hwang HG and Moon HP (2003) QTLs analysis of yield and its related traits in wild rice relative *Oryza rufipogon*. *Treatises of Crop Research* 4: 19–29.
- Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X and Zhang Q (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theoretical Applied Genetics* 112: 1164–1171.
- Ji HS, Chu SH, Jiang W, Chi YI, Hahn JH, Eun MY, McCouch SR and Koh HJ (2006) Characterization and mapping of a shattering mutant in rice that corresponds to a block of domestication genes. *Genetics* 173: 995–1005.
- Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J, He Y and Zhang Q (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. *Nature Genetics* 43: 1266–1269.
- Liu T, Shao D, Kovi MR and Xing Y (2010) Mapping and validation of quantitative trait loci for spikelets per panicle and 1,000-grain weight in rice (*Oryza sativa* L.). *Theoretical Applied Genetics* 120: 933–942.
- Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S and Yano M (2008) Deletion in a gene associated with grain size increased yields during rice domestication. *Nature Genetics* 40: 1023–1028.
- Song XJ, Huang W, Shi M, Zhu MZ and Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature Genetics* 39: 623–630.
- Suh J, Ahn SN, Cho YC, Kang KH, Choi IS, Kim YG, Suh HS and Hwang HG (2005) Mapping for QTLs for yield traits using an advanced backcross population from a cross between *Oryza sativa* and *O. glaberrima*. *Korean Journal Breeding Science* 37: 214–220.
- Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME and Lobos KB (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. *Theoretical Applied Genetics* 107: 479–493.
- Xie XB, Jin FX, Song MH, Suh JP, Hwang HG, Kim YG, McCouch SR and Ahn SN (2008) Fine mapping of a yield-enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* × *O. rufipogon* cross. *Theoretical Applied Genetics* 116: 613–622.
- Yun YT, Kim DM, Park IK, Chung CT, Seong YK and Ahn SN (2010) Development and evaluation of QTL-NILs for grain weight from an interspecific cross in rice. *Korean Journal Breeding Science* 42: 357–364.
- Zhang YS, Luo LJ, Xu CG, Zhang QF and Xing YZ (2006) Quantitative trait loci for panicle size, heading date and plant height co-segregating in trait-performance derived near-isogenic lines of rice (*Oryza sativa*). *Theoretical Applied Genetics* 113: 361–368.