Identification of Quantitative Trait Loci for Agronomic Traits in Two Rice Populations Derived from a Cross with a Wide Compatibility Line

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ABSTRACT Hybrid sterility is the major obstacle that can act as a barrier to genetic recombination and limits favorable gene transfer during inter-subspecific crosses in rice. However, hybrid sterility can be overcome by utilization of wide-compatible varieties (WCV). In the present study, two F_2 populations generated from crosses between a wide-compatibility line (HWC-line) and two Korean varieties, Dasan and Hwacheong were evaluated for eight agronomic traits. A total of 157 molecular markers consisted of 116 STS, 40 SSR and 1 FNP were used to create genetic linkage maps spanning all 12 chromosomes. Twenty-one main-effect QTLs (M-QTLs) were identified in HWC-line/Dasan (HD) population. These loci are populated with two QTLs for culm length, one for spikelet per panicle, one for spikelet fertility, four for grain length, five for grain width, five for grain shape, and three for 100 grain-weight. In HWC-line/Hwacheong (HH) population, 17 QTLs were detected including two QTLs for culm length, one for panicle length, one for spikelet fertility, five for grain length, three for grain width, three for grain shape, and two for 100-grain weight. Of the total 34 QTLs found in the two F_2 populations, 9 QTLs associated with culm length, grain length, grain width and grain shape were newly identified in this study. These QTLs will be useful for further understanding the genetic basis of agronomic traits and for developing markers for selection in rice breeding. In addition, HWC-line could be used as a bridge for inter-subspecies crosses overcoming hybrid sterility.

Keywords *Indica*, *Japonica*, QTL analysis, Rice, Wide compatibility

INTRODUCTION

Rice is the most important food crop in Asia. Asian rice (*Oryza sativa* L.) was domesticated from wild relative *Oryza rufipogon* and differentiated into two subspecies, *indica* and *japonica*, on the basis of sterility in F_1 hybrids between them (Oka 1988). Conventional cross hybridization and selection is a widely used strategy for developing new cultivars with important agronomic traits in rice breeding program. Cross hybridization and selection has provided on average about a 1% increase in the annual yield potential of rice since the development of IR8, the first improved semi-dwarf rice variety (Peng *et al.* 2000). However, hybrid sterility in rice has been a major obstacle in the transfer of useful genes by inter-subspecific crosses and lowers the productivity of the hybrid rice. Ikehashi and Araki (1986)

found that this sterility barrier can be overcome by the utilization of a wide-compatibility variety (WCV) carrying the *S5n* allele, such that hybrids of either *indica*/WCV or *japonica*/WCV were highly fertile. Recently, *S5* gene was cloned and its molecular mechanism was elucidated (Chen *et al.* 2008; Yang *et al.* 2012). Therefore, the use of these wide compatibility variety may provide a tool to overcome the sterility barrier between *indica* and *japonica* subspecies.

Development of molecular markers, construction of rice molecular linkage maps (Kurata *et al.* 1994; McCouch *et al.* 2002; Temnykh *et al.* 2000), and the development of statistical models in quantitative genetics greatly facilitate the study of quantitatively inherited complex traits (Tanksley 1993; Zeng 1994). The identification of QTLs provides the first step toward dissecting the molecular basis of complex traits in crops. Recent advance in the positional cloning

Received September 20, 2014; Revised September 24, 2014; Accepted September 24, 2014; Published September 30, 2014

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techniques has allowed it to become one of the most effective means to clone QTLs. So far, more than 15 QTLs including for the growth period, plant architecture, stress tolerance, grain shape and yield of rice have been successfully cloned. Moreover, comparison of the linkage maps among populations within a single or between different rice varieties can give valuable insight into understanding various parameters affecting recombination event, including the direction of crossing and/or parental varieties, genomic differences among parents, and the nature of QTLs and their application in marker-assisted breeding programs (Antonio *et al.* 1996; He *et al.* 2001; Liang *et al.* 2011; Qiao *et al.* 2008).

However, there were only limited studies comparing constructed maps with detected QTLs in different rice populations derived from crosses between WCV and *indica* and/or *japonica* cultivars. Thus, in this study, we constructed molecular linkage maps and detected QTLs for some agronomic traits in two F₂ populations derived from crosses between a WCV line and Dasan (Korean *Tongil-type* cultivar, derived from an *indica* × *japonica* cross and similar to *indica* in its genetic make-up) and Hwacheong (Korean temperate *japonica* cultivar).

MATERIALS AND METHODS

Plant material

The HWC-line, a line showing wide compatibility when crossed to either *indica* or *japonica* cultivars, was found and maintained at the Experimental Farm of Seoul National University, Suwon, Korea (Kim 1999). Each of the two populations comprising 190 F₂ plants derived from the crosses between HWC-line and two Korean cultivars, Dasan (Korean *Tongil-type* cultivar) and Hwacheong (Korean temperate *japonica* cultivar), were used to construct the genetic linkage maps and QTL mapping analysis. In 2010, the F₂ populations including three parental lines were planted at one plant per hill and grown by conventional cultural practices at the Experimental Farm of Seoul National University in Suwon, Korea. The F₂ plants that headed before September 1 were used in this study.

Phenotypic evaluation

A total of eight agronomic traits including culm length (CL), panicle length (PL), spikelet per panicle (SPP), spikelet fertility (SF), grain length (GL), grain width (GW), grain shape (length width ratio, GS), and 100-grain weight (100GW) were measured in two F₂ populations and three parents. CL was measured in centimeters (cm) from the soil surface to the neck of the tallest tiller. PL was measured in cm from panicle neck to panicle tip. SPP was calculated as the average number of spikelet per panicle. SF was calculated as filled grain per total number of spikelet in a panicle, expressed as a percentage. Harvested rice was air-dried and stored at room temperature before measuring. Ten randomly chosen, fully filled grains derived from each parent, and F_{2:3} were used to measure GL, GW and GS at 0.01 mm precision and the values were averaged as the measurements for the plant. The GL and GW were evaluated using digital microscope and measuring software. 100GW was measured in grams as the weight of 100 fully ripened (14% moisture) grains per plant.

Correlation coefficients of eight traits were calculated to determine the correlations between eight traits in the two F₂ populations using PROC CORR in the SAS 9.1. 3 (SAS Institute Inc. 2004).

DNA extraction and molecular marker selection

At the maximum tillering stage, young leaf tissues of parents and each F₂ plant were harvested in field-grown, and genomic DNA was extracted using the modified CTAB method as described in Murray and Thompson (1980).

Numerous Sequence-Tagged Site (STS) markers designed at Crop Molecular Breeding Lab, Seoul National University (unpublished), subspecies-specific STS (SS-STS) markers in Chin *et al.* (2007) and Simple Sequence Repeat (SSR) markers in Temnykh *et al.* (2000) and McCouch *et al.* (2002) were used in this study. One Functional Nucleotide Polymorphism (FNP) marker, qSW5 designed from primer data of Shomura *et al.* (2008) was employed for genotyping *qSW5* allele. The primers sequence of qSW5 are 5'-CGT-CTTGCAACCAACGCCGATGTTATAC-3' (N1212del-F) for forward and 5'-GCATCAGCATCACGCATCTTTC-3' (M23-R) for reverse. Screening of polymorphic STS and

SSR markers among three parents, HWC-line, Dasan and Hwacheong was conducted. The markers that are polymorphic and relatively easy to score were used in the construction of the linkage map to cover the entire genome.

PCR amplification for molecular markers

For PCR amplification of STS and SSR markers, each 10 µl reaction mixture contained 20 ng of template DNA, 0.25 µM of each primer, 1.25 mM of dNTP, 1× of PCR buffer and 0.5 U of *Taq* DNA polymerase. Amplification was carried out in a PTC220 dual 96-well and 384-well thermocycler (MJ Research, USA) as follows: 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C or 58°C, 30 sec at 72°C, and 10 min at 72°C for final extension. PCR products were separated on 3% agarose gel in 0.5× TBE buffer.

For PCR amplification of qSW5 marker, a 50 µl reaction mixture was prepared containing 100 ng of template DNA, 0.4 µM of each primer, 1.6 mM of dNTP Mixture, 2.5 mM of MgCl₂, 5 µl of 10× LA PCR buffer II (Mg²⁺-free) and 2.5 U of TaKaRa LA *Taq* DNA polymerase. Amplification was carried out in a PTC220 dual 96-well thermocycler (MJ Research, USA) as follows: 1 min at 94°C, followed by 30 cycles of 10 sec at 98°C, 3 min 30 sec at 68°C, and 10 min at 72°C for final extension. PCR products were separated on 1% agarose gel in 0.5× TBE buffer.

Linkage map construction and QTL analysis

The molecular linkage map was constructed using the JoinMap 4.0 software (van Ooijen 2006). The Kosambi mapping function (Kosambi 1944) with a minimum LOD (Logarithm of the odds) threshold of 3.0 and a linkage threshold significance of $p=0.05$ was applied to transform

the recombination frequencies into the genetic distances in centiMorgans.

QTL analysis were performed for eight agronomic traits (CL, PL, SPP, SF, GL, GW, GS and 100GW). QTL mapping was conducted independently for each of the two F₂ populations using QTLNetwork (version 2.1) software (Yang *et al.* 2008), which is based on mapping methodology outlined in Yang *et al.* (2007). QTLNetwork 2.1 implements a mixed-model based composite interval mapping (CIM) method and allows to simultaneously detect main-effect and epistatic QTLs in population. Simply, QTLNetwork analyzes the marker intervals, selects the candidate intervals and uses them as cofactors in a one-dimensional genome scan for putative QTL. The mapping procedures are conducted in the framework of a mixed linear model, and an *F*-statistic based on Henderson method 3 is used for hypothesis tests. A total of 1,000 permutation tests were used to calculate the critical *F*-value to control the genomewide type 1 error. An experiment-wise significance level of $p=0.05$ was maintained for QTL detection. For the genome scan, the testing window size, filtration window size and walk-speed were fixed as 10, 10 and 1 cM, respectively. QTL effects and QTL confidence intervals were estimated with a Bayesian method via the summary of the Gibbs samplers (Gibbs sample size=20,000). Detected main-effect QTLs were named according to McCouch *et al.* (1997).

Sequencing of *sd1* locus

In order to identify the variation of *sd1* locus among HWC-line, Dasan and Hwacheong, the coding region of *sd1* was sequenced using designed primer sets (Table 1). The PCR products were purified using a PCR purification

Table 1. The designed primers used in sequencing of *sd1* coding region.

No.	Primer name	Sequence (5'→3')	Amplicon size (HWC-line and Hwacheong/Dasan)	Amplifying region
1	SD1-2F	CAACACAGCGCTCACTTCTC	1023/641bp	exon1, intron1 and exon2
2	SD1ex1-2R	TGGTTTACCATGAAGGTGTCG		
3	SD1ex3F	GGGAATTGTTGTGTGTGCAG	468/479bp	exon3
4	SD1ex3R	GTACAGCGGTAGGGTCCAAA		

kit (iNtRON Biotechnology, Korea) for TA cloning. The purified PCR products were inserted into pGEM-T Easy Vector (Promega, USA) and then transformed into the *E. coli*. The plasmid DNA were extracted using DNA-spin Plasmid DNA Purification Kit (iNtRON Biotechnology, Korea) and further sequenced with an ABI 3700 DNA Sequencer (Applied Biosystems, Inc., CA, USA). The obtained sequences were compared by means of CodonCode Aligner software (version 5.0.2; Codon Code Corporation).

RESULTS

Phenotype of agronomic traits

The plant architecture and grain shape of three parents, HWC-line, Dasan, and Hwacheong, are shown in Fig. 1. HWC-line showed tall culm length and long slender grain shape. The phenotypic data for nine agronomic traits of three parents and eight agronomic traits of two F₂ populations in 2010 are presented in Table 2. The three parents exhibited notable differences in all nine traits. The extent of difference between HWC-line and Hwacheong was larger

Fig. 1. The plant architecture and grain shape of Hwacheong, HWC-line and Dasan.

Table 2. Agronomic traits of three parents and two F₂ populations established in the field in 2010.

Character	Parents						F ₂ populations							
	Hwacheong		HWC-line		Dasan		HWC/DS				HWC/HC			
	Mean	SD ²⁾	Mean	SD	Mean	SD	Mean	SD	Min ^{y)}	Max ^{x)}	Mean	SD	Min	Max
Heading date	Aug. 20		Aug. 12		Aug. 14									
Culm length (cm)	81.2	3.6	93.9	4.12	78.8	2.1	95.9	12.2	60.5	125.0	87.2	8.3	69.0	106.0
Panicle length (cm)	18.7	3.4	23.6	2.2	24.2	1.3	22.6	2.2	15.5	30.5	22.4	2.3	13.2	29.0
Spikelet per panicle	113.8	14.6	146.2	17.4	173.5	39.3	140.9	38.7	49.0	262.0	143.0	25.2	89.7	251.3
Spikelet fertility (%)	93.8	2.8	91.6	1.2	84.8	11.5	65.5	18.8	2.7	96.1	82.6	13.8	8.1	97.6
Grain length (mm)	6.31	0.24	7.47	0.23	7.72	0.25	7.08	0.34	6.06	8.10	6.98	0.29	6.29	7.76
Grain width (mm)	3.31	0.14	3.1	0.13	3.11	0.12	2.94	0.18	2.49	3.39	3.20	0.15	2.82	3.58
Grain shape (GL/GW)	1.91	0.09	2.41	0.1	2.49	0.14	2.43	0.19	2.00	2.98	2.19	0.14	1.82	2.57
100 grains weight (g)	2.4	0.12	2.4	0.09	2.83	0.07	2.27	0.24	1.70	3.10	2.35	0.23	1.61	2.92

²⁾SD is the abbreviation for standard deviation.

^{y)}Min is the abbreviation for minimum.

^{x)}Max is the abbreviation for maximum.

than that of between HWC-line and Dasan for seven agronomic traits except CL and 100GW.

The frequency distribution of phenotype for eight agronomic traits in the two F_2 populations derived from crosses between HWC-line and Dasan (HD), HWC-line

and Hwacheong (HH) are shown in Fig. 2. In the F_2 populations, all traits varied widely with continuous distribution and showed transgressive segregation. The amount of variation was higher in HD population than HH population for CL, SPP, SF, GL, GW and GS. A large

Fig. 2. Frequency distribution of culm length, panicle length, spikelet number per panicle, spikelet fertility, grain length, grain width, grain shape and 100 grains weight in two F_2 populations.

amount of overlapping proportions was observed in the two F_2 populations except SF, GW and GS. For spikelet fertility, the distribution was skewed toward high fertility in HH population. For GW and GS, the distributions were skewed towards each paternal parent in two F_2 populations.

Correlations among eight agronomic traits

Correlation coefficients among eight agronomic traits are presented in Table 3. Significant positive correlations were detected between CL with PL, GL and GW, between PL with SPP, GL and GS in both HD and HH population. In HD population, significant positive correlations were found between CL with 100GW and between GL with GW. SPP negatively correlated with 100GW in HD population with significance. In HH population, significant positive and negative correlations were identified between 100GW with PL and SF, and between SF with GL and GW, respectively. The directions (+ or -) and degree of correlation for most correlations were similar to those observed in previous study (Rahman *et al.* 2007; Thomson *et al.* 2003; Yoon *et al.* 2006).

Polymorphic marker screening

A total of 723 molecular markers (293 STS, 55 SS-STS, 374 SSR and 1 FNP) dispersed throughout the rice genome were used to survey polymorphism among three parents, HWC-line, Dasan and Hwacheong. Of these, 560 (77.5%) markers showed polymorphism between HWC-line and Dasan, 118 (16.3%) markers presented polymorphism between HWC-line and Hwacheong (Table 4).

Linkage map construction

Two F_2 populations obtained from each cross of HWC-line/Dasan and HWC-line/Hwacheong, were used for constructing two genetic linkage maps to identify the QTLs governing eight agronomic traits (Fig. 3 and 4). The total length of all the linkage group in HD was longer than that of the HH population, except chromosome 4 and 7. In HD population, of the total 560 polymorphic markers used to construct a linkage map, 106 markers consisting of 93 STS and 13 SSR were mapped on 12 chromosomes. Whereas in HH population, 58 out of 118 polymorphic markers consisting of 28 STS, 29 SSR and 1 FNP were mapped

Table 3. Correlation coefficients among eight agronomic traits of HD population (Lower) and HH population (Upper).

	Culm length	Panicle length	Spikelet per panicle	Spikelet fertility	Grain length	Grain width	Grain shape	100 grains weight
Culm length		0.28**	0.17*	-0.01	0.22**	0.18*	0.01	0.14
Panicle length	0.39**		0.35**	0.03	0.33**	0.02	0.20**	0.23**
Spikelet per panicle	0.12	0.34**		0.03	0.05	0.06	-0.03	0.05
Spikelet fertility	0.12	-0.14*	-0.07		-0.15*	-0.22**	0.07	0.31**
Grain length	0.25**	0.34**	0.00	-0.11		0.00	0.65**	0.30**
Grain width	0.18*	-0.12	-0.08	0.11	0.18*		-0.75**	0.45**
Grain shape	0.01	0.34**	0.05	-0.13	0.40**	-0.78**		-0.16*
100 grains weight	0.33**	0.12	-0.19**	-0.03	0.51**	0.65**	-0.23**	

* and ** indicate significant at 5% and 1% level, respectively.

Table 4. Number of polymorphic markers in two F_2 populations.

	Total	HWC vs. DS	HWC vs. HC
STS marker	293	213 (72.7%)	51 (17.4%)
SS-STS marker	55	53 (96.4%)	2 (3.6%)
SSR marker	374	293 (78.3%)	64 (17.1%)
FNP marker	1	1 (100%)	1 (100%)
Total	723	560 (77.5%)	118 (16.3%)

Fig. 3. Chromosomal locations of QTLs for seven agronomic traits in the HD population. The number above each chromosome indicates linkage map coverage based on physical position of markers.

Fig. 4. Chromosomal locations of QTLs for seven agronomic traits in the HH population. The number above each chromosome indicates linkage map coverage based on physical position of markers.

on 11 chromosomes with no markers on chromosome 12. The total linkage map length constructed in the HD population was 1942.6 cM with an average distance between two adjacent markers of 18.33 cM, and that in HH population has a total length of 925.53 cM with an average distance of 15.96 cM between adjacent markers. The linkage map coverage of whole genome in HD and HH population were 94.89% and 37.77%, respectively (Fig. 3 and 4).

Significant marker segregation distortion, based on the Chi-square test at $P < 0.05$, was identified in the two F_2 populations, as shown by deviations from the expected 1:2:1 (HWC-line homozygotes:heterozygotes:DS or HC homozygotes) ratio. In the HD population, 20 marker loci (18.9%) of 106 markers used, showed segregation distortion with 16 loci in favor of the DS allele and one marker favoring the HWC-line allele. In the HH population, 21 marker loci (36.2% of the total) displayed distorted segregation, with 17 loci favoring the HWC-line and 4 markers favoring the HC allele. The distorted markers were allocated along nine chromosomes except chromosomes 7, 9 and 10. These markers made clusters of at least three neighboring loci on chromosomes 3 and 12 in the HD population and chromosomes 3, 5 and 11 in the HH population. As shown in the frequency of HWC-line alleles in the HD and HH population (Fig. 5), theoretically, markers in F_2 population should show 50% of each allele from parents, but distorted loci exhibited frequency of HWC-line allele far deviated from 50%.

QTL analysis

A total of 34 main-effect QTLs (M-QTLs) were detected for eight agronomic traits in the two F_2 populations. These M-QTLs were mapped on all 12 chromosomes except chromosomes 8, 11 and 12 (Fig. 3, 4, and Table 5). Of the 34 M-QTLs, four (*qcll.1*, *qgw5*, *q100gw1* and *q100gw5*) were identified in both populations. Three digenic epistatic QTLs (E-QTLs) for culm length and grain shape were identified in HD population, locations of which were found to be on chromosomes 3, 5 and 11 (Table 6).

For culm length (CL), two major M-QTLs, *qcll.1* and *qcll.2* located on chromosome 1 and one E-QTLs located on chromosomes 5 and 11 were identified in HD population. The expressed phenotypic variations by each M-QTL were 37.2% and 39.1%, respectively. In the HH population, *qcll.1* and *qcll.7*, which were located on chromosomes 1 and 7, showed 25.6% and 7.8% of Phenotype Variation Explained (PVE), respectively. The HWC-line allele caused an increase in CL in all M-QTL.

For panicle length (PL), only one M-QTL, *qpl10* on chromosome 10 in the HH population, was detected with 1.0% PVE. The allele of HWC-line decreased PL at this locus. For spikelet per panicle (SPP), one M-QTL, *qspp4*, was mapped on chromosomes 4 in the HD population, and PVE of this QTL was 11.4%. The HWC-line allele increased SPP at this locus. For spikelet fertility (SF), two M-QTLs, *qsfl6* and *qsfl9*, were individually identified on chromosome 6 in the HD population and 9 in HH, and PVEs

Fig. 5. Frequency of HWC-line allele in HD (left) and HH (right) populations. Chromosome numbers are shown on the x-axis while the frequency of HWC-line allele is shown on y-axis.

Table 5. Main-effect QTLs underlying eight agronomic traits in two F₂ populations.

Trait	Chr.	QTL	Marker interval	HWC/DS				HWC/HC				Remarks
				position ²⁾	<i>F</i> ^{y)}	<i>A</i> ^{x)}	<i>PVE</i> (%) ^{w)}	position	<i>F</i>	<i>A</i>	<i>PVE</i> (%)	
CL	1	<i>qcl1.1</i> ^{v)}	S01140-S01160					76.4	33.6	6.59	25.6	novel QTL
	1	<i>qcl1.1</i> ^{v)}	S01143A-RM472	238.7	54.0	5.76	37.2					novel QTL
	1	<i>qcl1.2</i>	RM472-S01157B	269.3	60.4	6.93	39.1					<i>sd1</i> (Sasaki <i>et al.</i> 2002)
	7	<i>qcl7</i>	S07105A-S07118B					166.1	8.2	2.96	7.8	novel QTL
PL	10	<i>qpl10</i>	S10055A-RM5471					5.0	7.4	-0.47	1.0	<i>qPL10-1</i> (Cui <i>et al.</i> 2002)
SPP	4	<i>qspp4</i>	S04065-S04075	81.9	12.1	19.4	11.4					<i>qSPN-4b</i> (Teng <i>et al.</i> 2002)
SF	6	<i>qsfl6</i>	RM276-S06054					17.0	15.9	6.18	11.6	<i>qSF6.2</i> (Reflinur <i>et al.</i> 2012)
	9	<i>qsfl9</i>	S09000A-S09031	0.0	10.5	-7.24	7.8					<i>qSF9.1</i> (Reflinur <i>et al.</i> 2012)
GL	1	<i>qgl1.1</i>	S01038-S01054	103.4	12.2	0.17	11.7					<i>qGL-1</i> (Wan <i>et al.</i> 2005)
	1	<i>qgl1.2</i>	S01143A-RM472	238.7	12.6	0.15	12.7					- (Huang <i>et al.</i> 1997)
	1	<i>qgl1.3</i>	S01160-S01176					119.5	11.3	0.14	10.8	novel QTL
	2	<i>qgl2.1</i>	S02014-S02023					8.0	7.6	-0.08	5.5	<i>qGL-2b</i> (Li <i>et al.</i> 2003)
	2	<i>qgl2.2</i>	S02026-S02039	48.8	8.6	-0.14	7.9					<i>GW2</i> (Song <i>et al.</i> 2007)
	3	<i>qgl3</i>	RM3766-RM218					58.3	17.6	0.14	14.2	<i>gl3a</i> (Xing <i>et al.</i> 2001)
	5	<i>qgl5</i>	qSW5-S05047					4.8	14.6	0.11	6.7	novel QTL
	6	<i>qgl6</i>	S06087-S06105	109.9	14.0	0.18	11.6					<i>gl6</i> (Aluko <i>et al.</i> 2004), <i>qGL-6</i> (Li <i>et al.</i> 2003)
	7	<i>qgl7</i>	S07081B-S07105A					118.8	15.4	0.12	6.5	- (Redona <i>et al.</i> 1998)
GW	1	<i>qgw1</i>	S01160-S01176					121.5	7.5	0.06	2.8	<i>qGW-1</i> (Wan <i>et al.</i> 2005), <i>kw1.1</i> (Li <i>et al.</i> 2004), <i>gw1.1</i> (Septiningsih <i>et al.</i> 2003)
	2	<i>qgw2</i>	S02052-RM341	80.4	13.6	0.10	8.6					- (Cai <i>et al.</i> 2002)
	3	<i>qgw3.1</i>	S03057-RM15007	89.2	11.9	0.06	5.5					novel QTL
	3	<i>qgw3.2</i>	RM15007-S03076B	113.2	13.3	0.04	5.4					novel QTL
	4	<i>qgw4</i>	RM303-RM5503					192.1	7.5	-0.05	4.2	novel QTL
	5	<i>qgw5</i>	S05030A-S05048	59.2	55.3	-0.17	34.0					<i>qSW5</i> (Shomura <i>et al.</i> 2008), <i>GW5</i> (Weng <i>et al.</i> 2008)
	5	<i>qgw5</i>	qSW5-S05047					5.8	90.5	-0.16	47.0	<i>qSW5</i> (Shomura <i>et al.</i> 2008), <i>GW5</i> (Weng <i>et al.</i> 2008)
	9	<i>qgw9</i>	S09082A-S09090C	93.8	6.7	0.05	3.0					novel QTL
GS	2	<i>qgs2</i>	S02026-S02039	54.8	15.1	-0.10	11.4					<i>GW2</i> (Song <i>et al.</i> 2007)
	3	<i>qgs3.1</i>	S03099-S03115	153.1	19.2	-0.09	7.4					<i>qLWR-3</i> (Li <i>et al.</i> 2003)
	3	<i>qgs3.2</i>	RM3766-RM218					58.3	7.9	0.04	4.0	novel QTL
	4	<i>qgs4</i>	RM303-RM5503					193.1	13.1	0.05	7.8	<i>lwr4.1</i> (Li <i>et al.</i> 2004)
	5	<i>qgs5.1</i>	qSW5-S05047					4.8	74.9	0.14	48.1	<i>qSW5</i> (Shomura <i>et al.</i> 2008), <i>GW5</i> (Weng <i>et al.</i> 2008)
	5	<i>qgs5.2</i>	S05048-S05054	69.9	37.5	0.08	29.1					- (Tan <i>et al.</i> 2000)
	5	<i>qgs5.3</i>	S05066-S05077A	102.8	21.5	0.08	21.9					<i>qLWR-5b</i> (Li <i>et al.</i> 2003)
	6	<i>qgs6</i>	S06105-S06124	125.2	8.7	0.06	6.5					- (Hagiwara <i>et al.</i> 2006)

Table 5. Main-effect QTLs underlying eight agronomic traits in two F₂ populations (Continued).

Trait	Chr.	QTL	Marker interval	HWC/DS				HWC/HC				Remarks
				position ²⁾	F ^{y)}	A ^{x)}	PVE(%) ^{w)}	position	F	A	PVE(%)	
100GW	1	<i>q100gw1</i>	S01157B-S01176	299.1	17.4	0.16	13.8					<i>gw1.1</i> (Thomson <i>et al.</i> 2003), <i>qTGW1-3</i> (Hittalmani <i>et al.</i> 2003), <i>gw1.1</i> (Septiningsih <i>et al.</i> 2003), <i>qTGW-1</i> (Hittalmani <i>et al.</i> 2002)
	1	<i>q100gw1</i>	S01160-S01176					121.5	21.5	0.16	15.2	<i>gw1.1</i> (Thomson <i>et al.</i> 2003), <i>qTGW1-3</i> (Hittalmani <i>et al.</i> 2003), <i>gw1.1</i> (Septiningsih <i>et al.</i> 2003), <i>qTGW-1</i> (Hittalmani <i>et al.</i> 2002)
	5	<i>q100gw5</i>	S05030A-S05048	65.2	21.4	-0.15	16.9					<i>qSW5</i> (Shomura <i>et al.</i> 2008), <i>GW5</i> (Weng <i>et al.</i> 2008)
	5	<i>q100gw5</i>	S05038-qSW5					2.0	16.2	-0.12	11.4	<i>qSW5</i> (Shomura <i>et al.</i> 2008), <i>GW5</i> (Weng <i>et al.</i> 2008)
	9	<i>q100gw9</i>	S09069-S09082A	87.2	16.3	0.10	8.2					<i>gw9.1</i> (Thomson <i>et al.</i> 2003), <i>qGW-9</i> (Suh <i>et al.</i> 2005)

²⁾Position is genetic distance (in centiMorgan) of the M-QTL from the first top marker on the chromosome.

^{y)}F represents the F-statistic value.

^{x)}A represents the estimated additive effect of HWC-line allele.

^{w)}PVE(%) is the proportion of additive phenotypic variation explained by each QTL.

^{v)}Same QTL has overlapping marker interval in two populations.

Table 6. Digenic epistatic QTLs for culm length and grain shape in the HD population.

Trait	Chr.	Marker Interval i	Chr.	Marker Interval j	AA ²⁾	PVE (%) ^{y)}	AD ^{x)}	PVE (%)	DA ^{w)}	PVE (%)	DD ^{v)}	PVE (%)
CL	5	S05066-S05077A	11	S11052-RM287	5.2**	2.8	-8.7**	0.1	-1.4	5.2	3.1	0.3
GS	3	S03099-S03115 (<i>qgs3.1</i>)	5	S05048-S05054 (<i>qgs5.2</i>)	-0.02	0.2	0.10**	1.7	-0.10**	2.1	0.066	0.5
	5	S05048-S05054 (<i>qgs5.2</i>)	5	S05066-S05077A (<i>qgs5.3</i>)	0.18**	1.7	0.03	0.0	-0.06	0.0	-0.15*	0.6

²⁾AA represents the estimated additive by additive effect.

^{y)}PVE(%) is the proportion of phenotypic variation explained by each QTL.

^{x)}AD represents the estimated additive by dominance effect.

^{w)}DA represents the estimated dominance by additive effect.

^{v)}DD represents the estimated dominance by dominance effect.

*, ** denote significance levels of $P < 0.05$, 0.001 respectively.

of each locus were 11.6% and 7.8%, respectively. The HWC-line allele had a positive effect on spikelet fertility at these loci.

For grain length (GL), four M-QTLs (*qgl1.1*, *qgl1.2*, *qgl2.2* and *qgl6*) were detected on chromosomes 1, 2 and 6 in the HD population. The PVEs of each locus were 11.7%, 12.7%, 7.9% and 11.6%, respectively. In the HH population,

five M-QTLs (*qgl1.3*, *qgl2.1*, *qgl3*, *qgl5* and *qgl7*) were identified on chromosomes 1, 2, 3, 5 and 7 for GL. The PVEs of each locus were 10.8%, 5.5%, 14.2%, 6.7% and 6.5%, respectively. HWC-line allele increased GL at 7 M-QTLs except *qgl2.1* and *qgl2.2*, located on chromosome 2 in each population.

For grain width (GW), five M-QTLs (*qgw2*, *qgw3.1*,

qgw3.2, *qgw5* and *qgw9*) were detected on chromosomes 2, 3, 5 and 9 in the HD population. The PVEs of each locus were 8.6%, 5.5%, 5.4%, 34.0% and 3.0%, respectively. In the HH population, three M-QTLs for GW, *qgw1*, *qgw4* and *qgw5* were mapped on chromosomes 1, 4 and 5, and each PVE was 2.8%, 4.2% and 47.0%, respectively. The HWC-line allele increased GW at *qgw1*, *qgw2*, *qgw3.1*, *qgw3.2* and *qgw9* loci but decreased GW at *qgw4* and *qgw5*.

For grain shape (GS: grain length to width ratio), five M-QTLs (*qgs2*, *qgs3.1*, *qgs5.2*, *qgs5.3* and *qgs6*) and two E-QTLs, on chromosomes 3, 5 and 6, were identified in the HD population. The PVEs of each M-QTL ranged from 6.5% to 29.1%, explaining 76.3% of the total phenotypic variation of GS. The two E-QTLs for GS were detected to have interactions with three M-QTLs, *qgs3.1*, *qgs5.2* and *qgs5.3*. In the HH population, three M-QTLs, *qgs3.2*, *qgs4* and *qgs5.1* located on chromosomes 3, 4 and 5, were detected and PVEs of each locus were 4.0%, 7.8% and 48.1%, respectively. Accordingly, the HWC-line alleles on *qgs3.2*, *qgs4*, *qgs5.1*, *qgs5.2*, *qgs5.3* and *qgs6* loci were detected to make the grains slender and those on *qgs2* and *qgs3.1* loci rounder.

For 100 grains weight (100GW), three M-QTLs, *q100gw1*,

q100gw5 and *q100gw9* were mapped on chromosomes 1, 5 and 9 in the HD population, and the PVEs of each locus were 13.8%, 16.9% and 8.2%, respectively. In the HH population, *q100gw1* and *q100gw5* were located on chromosomes 1 and 5 for 100GW, and the PVEs of each locus were 15.2% and 11.4%. The HWC-line allele increased 100GW at two loci, *q100gw1* and *q100gw9*, but decreased at *q100gw5*.

Sequence variation of *sd1* locus

The *qcl1.2* contains *sd1* locus cloned by Sasaki *et al.* (2002) (Fig. 6A). We sequenced the coding region of *sd1* among HWC-line, Dasan and Hwacheong (Fig. 6B). Dasan represented 383 bp deletion and 1 bp insertion from exon 1 to exon 2. Furthermore, Dasan showed 1 bp substitution in exon 3 and 11 bp insertion in 3'-UTR. However, HWC-line and Hwacheong had identical sequence in *sd1* coding region.

DISCUSSION

The HWC-line had relatively taller culm length than Dasan or Hwacheong, with long slender shape of grain

Fig. 6. Physical position of markers spanning *qcl1.1* (A) and *sd1* sequence variation among HWC-line, Dasan and Hwacheong (B). Black arrows and box regions represent exons. Green arrows indicate primers used in sequencing.

similar to that of Dasan (Fig. 1 and Table 2). F₁ hybrids derived from crosses between the HWC-line and *indica* or *japonica* cultivars showed moderate fertility. We have previously identified *S5* locus of HWC-line with *S5-n* allele. In this study, our data showed that the peak of continuous distribution of spikelet fertility was around 80% in HWC-line/ Dasan F₂ population, while, it skewed toward 90-100% in the HWC-line and Hwacheong F₂ population (Fig. 2). Moreover, we detected two QTLs for spikelet fertility, *qsf9* and *qsf6*, in the HD population and the HH population, respectively. In the HD population, HWC-line allele of *qsf9* decreased spikelet fertility, while HWC-line allele of *qsf6* increased spikelet fertility in HH population (Table 5). These results imply that HWC-line is more compatible with *japonica* than *indica*, and there are other loci aside from *S5*, such as *qsf9* and *qsf6*, that control hybrid fertility.

A total of 723 molecular markers were screened to detect polymorphism among three parents, HWC-line, Dasan, and Hwacheong. Of the total markers screened, 560 (77.5%) markers in the HD population and 118 (16.3%) in HH were identified as polymorphic between each parent (Table 4). In particular, only two (3.6%, S01140 and S05064) of 55 subspecies-specific STS markers tested exhibited polymorphism in HH population. On the other hand, 53 (96.4%) markers of the 55 subspecies-specific STS markers, except S01140 and S05064, were polymorphic in HD population. The S01140 was tightly linked with *qcl1.1*, which possesses large additive effect of HWC-line allele and high PVE for CL in HH population. The S05064 was found to be located on chromosome 5, which had cluster of grain-related QTLs (*qgl5*, *qgw5.2*, *qgs5* and *q100gw5.2*) making long and slender grain. Thus, S01140 and S05064 alleles of HWC-line are expected to be identical with those of Dasan. These molecular marker screening data, together with the distribution of spikelet fertility in the two F₂ populations, indicate that the genome architecture and genetic composition of HWC-line is similar to *japonica* cultivar. However, three traits related with grain morphology, GL, GW and GS of HWC-line, were almost the same with Dasan, of which genetic make-up is similar to *indica*.

The total length and physical coverage of the molecular

linkage map for the HD population were 1942.6 cM and 94.89%, while in the HH population those were estimated as 925.53 cM and 37.77%. Although the linkage map of HD population covered all 12 chromosomes over the almost entire region, that for the HH population generated incomplete linkage map partially covering 11 chromosomes, leaving out chromosome 12 (Fig. 3 and 4). As the lack of adequate polymorphic molecular markers between the parents, HWC-line and Hwacheong, is mainly attributable to this incomplete linkage map of HH population, newly developed SNP marker systems and recently available high-throughput genotyping technologies (Chen *et al.* 2014; Huang *et al.* 2009; Thomson *et al.* 2012) are expected to provide more extensive polymorphic molecular markers and new genotyping platform to complete the linkage map of HH population.

Some markers used in this study displayed significant segregation distortion and clustered on chromosomes 3, 5, 11 and 12. The chromosomal location of these distorted marker clusters matched to what have been previously reported (Chin *et al.* 2011; Harushima *et al.* 2001; 2002; Xu *et al.* 1997). In the HD population, 19 of the distorted 20 markers, with the exception of one on chromosome 8, were skewed in favor of the Dasan (*indica*) alleles, a phenomenon consistent to those observed in previous studies (Chin *et al.* 2011; Harushima *et al.* 2002; Xu *et al.* 1997). Of distorted 21 markers in the HH population, 17 were distorted towards the HWC-line alleles, with remaining four markers favoring the Hwacheong alleles. Thus, the result of segregation distortion in HH population suggests that HWC-line may contain some chromosomal regions that render the line to closely resemble *indica* cultivar.

We identified QTLs underlying eight agronomic traits of HWC-line using two F₂ populations derived from HWC-line with Dasan and Hwacheong. A total of 34 M-QTLs with significant additive effects (Table 5) and 3 E-QTLs (Table 6) with epistatic effects were detected in this study. In order to examine if these QTLs are whether novel or previously reported, physical position of these detected QTLs were compared with existing QTLs using Gramene (www.gramene.org) and Q-TARO (Yonemaru *et al.* 2010) database. A total of nine novel QTLs were identified as a result. In the HD population, 21 M-QTLs and 3 E-QTLs were detected

for CL, SPP, SF, GL, GW, GS and 100GW. Of these M-QTLs, 2 for CL and 2 for GS were newly identified in this study. 17 M-QTLs were detected for CL, PL, SF, GL, GW, GS and 100GW in the HH population. Of these, 6 M-QTLs for CL, GL, GW and GS were newly identified. Notably, one novel M-QTL for CL overlapped in both HD and HH populations.

Furthermore, two M-QTLs for CL, *qcl11.1* and *qcl7* loci were newly identified, and one pair of E-QTLs detected for CL in HD population, by contrast, had a low PVE, suggesting that M-QTLs are stronger in determining CL in this population. All HWC-line alleles for CL showed positive additive effect and provided tall culm length to HWC-line. Of these QTLs for CL, *qcl1.1* showed large additive effect and high PVE, and are considered to be the same locus in both HD and HH populations. We compared the physical position of markers with QTLs for CL on chromosome 1 (Fig. 6A). The physical interval of *qcl1.1* in HH population includes those of *qcl1.1* and *qcl1.2* in HD population. The *qcl1.2* contains *sd1* locus cloned by Sasaki *et al.* (2002). We identified *sd1* sequence variation among HWC-line, Dasan and Hwacheong (Fig. 6B). Dasan showed some variation, but HWC-line and Hwacheong had identical sequence in *sd1* coding region. The *sd1* is candidate gene of *qcl1.2*, but not *qcl1.1*. These marker physical positions and *sd1* sequence variation imply *qcl1.1* is putative novel QTL for CL. Fine mapping of *qcl1.1* is in progress. Also, a total of nine QTLs were detected for GL. Of these, two QTLs, *qgl1.3* and *qgl3*, have not been identified in previous QTL studies, indicating putative novel QTLs for GL. It is notable that seven QTLs for GL, except *qgl2.1* and *qgl2.2*, increased GL with maternal allele. This indicates that these HWC-line alleles made long grain shape of HWC-line. For GW, a total of seven QTLs were identified and four QTLs, *qgw3.1*, *qgw3.2*, *qgw4* and *qgw9*, were newly identified. For GS, one (*qgs3.2*) of the eight total QTLs were newly detected, and two E-QTLs for GS detected in the HD population, showed interaction with three M-QTLs. Of these E-QTLs, the interaction of *qgs5.2* and *qgs5.3* had large effect but low PVE. This result suggests that M-QTLs may contribute most phenotypic variation to GS in the HD population.

Several QTLs controlling different traits were identified in similar chromosomal position, which suggests a pleiotropic genetic effect of a single QTL. In this study, a total of eight clusters of QTLs controlling different traits were detected closely at a distance of less than 7 cM and their confidence intervals overlapped (Table 5, Fig. 3 and 4). Two loci for CL and GL, *qcl1.1* and *qgl1.2*, were located at the same site of chromosome 1, and those affected in the same direction. This might contribute to significant positive correlation observed between CL and GL in the HD population. Seven clusters of QTLs controlling grain related traits, GL, GW, GS and 100GW, and the direction of their effects were highly correlated with one another (Table 3). Especially, *qgw5* and *q100gw5* in the HD population and *qgl5*, *qgw5*, *qgs5.1* and *q100gw5* in the HH population were considered to have strong pleiotropic genetic effect for grain-related traits by *qSW5/GW5* locus.

Taken together, all these data obtained by the present study suggest that HWC-line genome is similar to *japonica* cultivar even though HWC-line contains chromosomal portion resembling *indica* cultivar. In particular, the grain shape related traits, GL, GW, and GS of HWC-line were almost identical to those of Dasan, which is similar to *indica* cultivar, and the QTLs for GL, GW and GS identified in this study explained this phenomenon, confirming that the HWC-line alleles contributed to long and slender grain shape.

In conclusion, the QTLs identified in this study will be able to provide basic information on putative functional genes related to some agronomic traits and facilitate breeding new rice cultivar by MAS. Furthermore, the HWC-line could be utilized for inter-subspecies crosses heading toward hybrid breeding.

ACKNOWLEDGMENTS

This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ008125), Rural Development Administration, Republic of Korea.

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