QTL mapping of agronomic traits using an RIL population derived from a cross between temperate *japonica* cultivars in rice (*Oryza sativa* L.)

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To employ molecular marker-aided selection for developing *japonica* rice (*Oryza sativa* L.) cultivars with high yielding potential and good eating quality, we carried out QTL analysis for agronomic and grain traits using 231 recombinant inbred lines (RIL) from a cross between two temperate *japonica* cultivars, Suweon365 and Chucheongbyeon. A linkage map with 221 loci, including 134 simple sequence repeats (SSRs), 66 amplified fragment length polymorphisms (AFLPs), and 21 miniature inverted repeat transposable elements (MITEs)-anchored markers, was constructed covering 2,227 cM of the twelve rice chromosomes, with an average distance of 11.0 cM between markers. The proportion of polymorphic bands between parents averaged 19.5%, 12.9% and 42.3% for SSRs, AFLPs and MITEs, respectively. Construction of maps without AFLP or MITE markers indicated that expansion of this map is due to the incorporation of AFLP markers, and this result is consistent with previous reports. Twenty-seven QTLs for ten traits were identified over two years, and fourteen were consistently detected in two consecutive years. When the locations of each QTL detected in this study were compared with those in previous QTLs studies, eight QTLs for culm length, spikelets per panicle and, thickness, length and shape of grain, had not been reported in previous mapping studies, indicating potentially novel alleles of *japonica* rice. Interestingly, a cluster of seven QTLs, including heading date and grain morphology traits, was detected on the long arm of chromosome 7. The markers linked to these QTLs would be valuable in a marker-assisted breeding program for *japonica* cultivars.

Key Words: rice, temperate *japonica*, QTL, MITEs, molecular map.

Introduction

Molecular maps are available for various cross combinations in rice, *Oryza sativa* L. (www.gramene.org), and these maps serve several purposes, including quantitative trait loci (QTL) analysis, marker-aided selection, gene isolation, and evolutionary studies. Of the several marker types, restriction fragment length polymorphism (RFLPs) and simple sequence repeats (SSRs) have been predominantly employed in the construction of molecular maps in rice (*Oryza sativa* L.). Amplified fragment length polymorphism (AFLP) analysis was also used to complement other marker systems because AFLP markers might be derived from any portion of the genome that can be digested with restriction enzymes (Vos *et al.* 1995).

Miniature inverted repeat transposable elements

(MITEs) are currently being developed in several crop species (Wessler 2006). They display a very high copy number of repetitive sequences and even distribution in the rice genome. This feature would facilitate mapping in rice because of the ability to generate a large number of restriction fragments in a single reaction, thus increasing the possibility of identifying polymorphisms and expediting the construction of linkage maps (Casa *et al.* 2000, Wessler 2006).

QTL analysis has been greatly facilitated by the development of molecular markers. Using molecular maps combined with QTL mapping technology, it is possible to characterize and isolate loci controlling quantitative traits, such as heading date (Yano *et al.* 2000), plant panicle trait (Yamagishi *et al.* 2002), yield components (Xiao *et al.* 1996), and grain characters (Redoña and Mackill 1998).

So far, most QTLs have been detected using inter-subspecific and inter-specific crosses (Aluko *et al.* 2004, Tian *et al.* 2006). This is due to the fact that wide crosses possessed a higher level of DNA polymorphism than intra-subspecific crosses and thereby contributed to increase mapping efficiency (Causse *et al.* 1994); however, this

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presented a disadvantage for determining the utility of a QTL for a breeding program that exclusively utilized a *japonica* germplasm. Therefore, the necessity of QTL identification based on the variation of crosses between two related varieties belonging to the same subspecies, especially the *japonica/japonica* cross, has been emphasized using molecular linkage maps based on *japonica* crosses (Fujino *et al.* 2004, Li *et al.* 2005, Tabata *et al.* 2007, Wada *et al.* 2006).

The objective of the present study was to identify QTLs for traits related to yield components and grain morphology using an RIL population derived from a cross between two temperate *japonica* cultivars in rice.

Materials and Methods

Plant materials and trait evaluation

“Suweon365” was crossed as a female parent with “Chucheongbyeol” in 1992. The temperate *japonica* rice Suweon365, derived from a cross between “Seonambyeol” and “Iri353”, is a semi-dwarf, high-yielding and has medium eating quality (National Institute of Crop Science: NICS 1988). Chucheongbyeol or “Akibare” in Japanese (developed from a cross between “Mandainishiki” as the female parent and an F₅ line from a Wakaba/Kinmaze cross as the male parent) was introduced from Japan in 1969. It is late maturing, tall and has good eating quality (RDA 1975). F₁ plants were selfed to produce F₂ seeds and 231 F₈ lines were developed from the resultant F₂ plants by the single-seed descent method. The F₈ recombinant inbred line (RIL) population with the parents were field evaluated in an experimental plot at the National Institute of Crop Science (NICS), Suwon, using a completely randomized block design with two replications for two years (1999 and 2000). Ten traits were measured in the 231 RILs and the parents: days to heading (DTH), culm length (CL), panicle length (PL), panicles per plant (PN), spikelets per panicle (SPP), grain yield (GY), grain thickness (GT), grain length (GL), grain width (GW) and grain length/width ratio (GS). Trait evaluation followed the procedures described in Yoon *et al.* (2006).

For all traits, 10 plants per each line in both years were measured and the average measurements were used for QTL analysis. Grain yield (GY) was evaluated by averaging the grain yield of 10 plants selected from the center of each plot and dried to 10% moisture content. Grain thickness (GT), grain length (GL), grain width (GW) and grain length/width ratio (GS) were measured for 50 grains per plant.

DNA extraction and molecular analysis

DNA was extracted from fresh young leaves of the 231 RILs in bulk following the procedures described in Causse *et al.* (1994). AFLP analysis was performed as described by Vos *et al.* (1995) with minor modifications. Informative combinations from polymorphism screening between the parents were selected for genotyping the RILs. The nomenclature for AFLP markers was derived from the enzyme combination, primer combination and the relative fragment

number. A typical AFLP marker, em241, which is mapped on chromosome 4, indicated that the marker was amplified with the primer combination em24 (e for *EcoRI*, m for *MseI*, 2 for the specific overhang to the *EcoRI* end, 4 for the specific overhang to the *MseI* end, and 1 for the number of the band). General procedures for the transposon display (TD) modified from the AFLP method were performed as described in Casa *et al.* (2000) with the following modifications. Element-specific primers were derived from the long terminal repeat (LTR) sequence of *Dasheng* and *mPing*. Touch-down polymerase chain reaction (PCR) was performed for selective amplification with the following conditions: 94°C for 2 min followed by 25 cycles of 94°C for 30 sec, 65°C for 30 sec (with a 1°C decrease per cycle to 58°C), and 72°C for 60 sec, and additional incubation at 72°C for 5 min. The nomenclature for the MITE-anchored marker was derived from the transposon name and the relative fragment number. PCR products were separated on a 5% polyacrylamide gel and detected by a silver-staining procedure (Promega, USA). Polymorphic bands for each primer were numbered serially in descending order of fragment size from the top to the bottom of the gel image. All bands were treated as dominant markers. Weak bands whose signals were less than 50% of the main band were not scored.

Molecular linkage map construction and QTL analysis

The Mapmaker program was used to establish a molecular map (Lander *et al.* 1987). All pairs of linked markers were identified using the “group” command with an LOD value of 2.0. The “orders” and “compare” commands in Mapmaker were used to identify the most probable marker order within a linkage group. The “ripple” command was used to verify the order. Additional markers were assigned to intervals using the “try” and “ripple” commands. Map distance (cM) was expressed in Kosambi function (Kosambi 1944). Assignment of linkage groups to the respective chromosomes was based on rice maps developed by Temnykh *et al.* (2000). Chi-square tests were performed to examine segregation ratios at the marker loci for deviation from the expected ratio of 1 : 1, and skewing was determined.

The chromosomal locations of QTLs were determined by single-point analysis (SPA) and composite interval mapping (CIM). Primary analysis using SPA was performed using the QGene program (Nelson 1997). In SPA, a QTL was declared if the phenotype was associated with a marker locus at $P < 0.001$ or with two adjacent marker loci at $P < 0.05$. To identify additional QTLs and to increase the resolution of QTL locations, CIM analysis was performed using QTL Cartographer v2.0 (Basten *et al.* 1997). Significance thresholds for CIM were determined using 1,000 permutations for each trait. For CIM, the experiment-wise significance level of $P < 0.01$ corresponded to an average LOD > 3.80, while the level of $P < 0.05$ corresponded to a LOD > 2.52. QTLs detected by SPA or CIM were not always in agreement. The QTL reported in this study was detected by both methods. The proportion of observed phenotypic variation attributable to a

particular QTL was estimated by the coefficient of determination (R^2). The total phenotypic variance explained was estimated by fitting a model simultaneously, including all putative QTLs for the respective trait. The effect of digenic interaction for each trait with two-way ANOVA was analyzed at the significance level of $P < 0.01$ using the SAS program.

Results

Trait evaluation and correlation

The phenotype data of the traits were similar for the two years (Table 1). Trait distributions were contiguous and

showed transgressive segregation with a number of RILs higher or lower than the parent of each trait. These results indicated the involvement of several genes in the control of these traits. Means and ranges for the ten traits are summarized in Table 1. Chucheongbyeon is late maturing, tall and lower yielding with short grains than Suweon365. The mean trait values of the RILs scored similarly to the mid-parent values.

Correlation coefficients among the traits are shown in Table 2. DTH showed significant positive correlations with CL ($p < 0.01$). PN and SPP were negatively correlated. CL and GY showed the highest correlations ($r = 0.368$ – 0.485) among the yield components in both years. Correlation

Table 1. Variation and relationship of agronomic traits of RILs in two years, 1999 and 2000

Traits	1999				2000				Correlation between years ^b
	SS ^a	CC	RILs		SS ^a	CC	RILs		
			Mean	Range			Mean	Range	
DTH (days)	104	114	108±5.0	98–120	106	113	110±5.8	99–126	0.775**
CL (cm)	79.8	89.6	84±8.2	61–102	71.2	88.0	85±8.8	60.5–103	0.652**
PL (cm)	19.9	18.1	19.6±1.2	16.0–23.2	21.7	19.4	20.9±1.3	16.6–24.0	0.246**
PN (no.)	15	17	15.0±2.0	10–22	13	14	13.6±1.6	10–19	0.160**
SPP (no.)	92	88	91±14.5	56–144	113	95	108±14.7	72–155	0.400**
GY (g)	627	580	649±74.5	385–984	543	527	547±68.5	313–801	0.450**
GT (cm)	2.01	2.06	2.06±0.06	1.82–2.20	1.98	2.01	2.00±0.08	1.77–2.08	0.148*
GL (cm)	5.07	4.88	5.02±0.13	4.72–5.38	5.06	4.67	4.89±0.17	4.05–5.31	0.347**
GW (cm)	2.86	2.79	2.80±0.09	2.58–3.00	2.85	2.77	2.78±0.08	2.56–3.03	0.216**
GS	1.82	1.75	1.79±0.06	1.66–1.98	1.77	1.69	1.76±0.07	1.58–1.77	0.368**

DTH Days to heading, CL Culm length, PL Panicle length, PN Panicles per plant, SPP Spikelets per panicle, GY Grain yield, GT Grain thickness, GL Grain length, GW Grain width, GS Grain shape in traits.

^a SS Suweon365, CC Chucheongbyeon

^b *, ** Significant at the 5% and 1% levels, respectively.

Table 2. Correlation coefficients among ten agronomic traits for two years

Traits	Year	DTH	CL	PL	PN	SPP	GY	GT	GL	GW
CL	1999	0.330**								
	2000	0.314**								
PL	1999	0.108	0.254**							
	2000	−0.457**	0.166*							
PN	1999	0.224**	0.101	0.06						
	2000	0.084	0.066	−0.264**						
SPP	1999	−0.104	0.092	0.112	−0.149*					
	2000	−0.175*	0.085	0.402**	−0.385**					
GY	1999	0.144*	0.368**	0.253**	0.074	0.046				
	2000	0.085	0.485**	0.322**	0.039	0.317**				
GT	1999	−0.087	−0.002	0.042	−0.012	0.039	0.005			
	2000	0.185**	0.027	−0.042	−0.125	0.046	0.046			
GL	1999	−0.145*	0.114	0.263**	0.044	−0.088	0.195**	0.077		
	2000	−0.259**	−0.027	0.272**	−0.045	0.088	0.067	0.02		
GW	1999	0.054	−0.006	0.041	−0.029	0.078	0.055	0.256**	0.109	
	2000	−0.347**	−0.036	0.249**	−0.126	0.169*	0.033	0.302**	0.205**	
GS	1999	−0.187**	0.051	0.143*	0.037	−0.064	0.098	−0.12	0.684**	−0.572**
	2000	0.038	0.006	0.047	0.059	−0.055	0.032	−0.205**	0.666**	−0.569**

DTH Days to heading, CL Culm length, PL Panicle length, PN Panicles per plant, SPP Spikelets per panicle, GY Grain yield, GT Grain thickness, GL Grain length, GW Grain width, GS Grain shape.

*, ** Significant at the 5% and 1% levels, respectively.

coefficients of DTH with other traits were different between 1999 and 2000. This might have been due to the cool temperature in early summer in 2000 which affected the heading date and other traits depending on the lines.

Polymorphisms between parents

Three different types of molecular markers, SSR, AFLP and MITE-anchored, were employed for developing the temperate *japonica* map. For SSR, 121 out of 621 markers detected polymorphisms between the parents (19.5%). Forty-eight AFLP primer combinations were screened for the parental polymorphism. Of these, eleven *EcoRI* (+2)/*MseI* (+3) combinations with different overhangs were selected to genotype the RILs. The number of bands generated by each primer combination ranged from 47 to 113 with a mean of 82.5 bands. Of the 908 AFLP bands, 118 were polymorphic (12.9%). This level of polymorphism with SSR and AFLP was lower than that observed between the intersubspecific cross (Cho *et al.* 1998) and similar to that between *japonica* cultivars (Takeuchi *et al.* 2001, Tabata *et al.* 2007). This is attributable to the narrow genetic variation between the parents as both were *japonica* types. On the transposon display (TD), two MITE families were employed. The reactions generated 50–80 bands in the range of 100–700 bp, similar to the AFLP profile. MITE-TD generated 52 polymorphic bands with 14 and 38 bands by *Dasheng* and *mPing* primers, respectively. The average polymorphism ratios in MITE-TD were 28.6% and 51.4% with *Dasheng* and *mPing*, respectively, which were higher than the SSR and AFLP markers.

Segregation distortion of markers

All markers mapped on the molecular map were tested for segregation distortion with a χ^2 test for goodness of fit. Eleven loci (5.2%) deviated from the expected 1 : 1 segregation ratio at $P \leq 0.05$. These loci were located on chromosomes 4, 6, 9, 11 and 12. No complete non-recombinant was observed in this population. Segregation distortion has been documented in several crosses (Causse *et al.* 1994). The lev-

el detected in the present study was lower than that reported in other mapping populations, which is due to the employment of closely related *japonica* cultivars as parents.

Construction of the *japonica* linkage map

A total of 221 SSR, AFLP and MITE-anchored markers have been mapped with the 121 SSR loci as framework markers. All marker positions were in good agreement with previous maps (Causse *et al.* 1994, Temnykh *et al.* 2000). Integration of AFLP and MITE-anchored markers into the SSR map generated a map with 2,227 cM of rice chromosomes with an average interval size of 11.0 cM between markers (Table 3). The genome coverage of the map per chromosome ranged from 76.8% (chromosome 8) to 98.2% (chromosome 11) based on the reported map (Temnykh *et al.* 2000, McCouch *et al.* 2002), and several genomic regions with a low density of markers appeared as large gaps on the map. In particular, chromosome 9 had the shortest distance with the lowest number of loci (nine SSR loci). On the other hand, chromosomes 7, 10, 11 and 12 had the highest number of loci and map distances. AFLP markers were mainly distributed on chromosomes 7, 11 and 12, leading to an expansion of these chromosomes. Clustering of AFLP markers was observed on most of the mapped chromosomes. On the other hand, 21 MITE-anchored markers were evenly distributed among and within chromosomes (Table 3 and Fig. 1).

QTL mapping

Twenty-seven significant QTLs were identified for the ten traits evaluated in 1999 and 2000 (Table 4), which fourteen were detected in both years and the other thirteen were identified in only one of the two years (Fig. 1 and Table 4). No digenic interaction of each trait with two-way ANOVA was detected at the significance level of $P < 0.01$.

Days to heading: Four (*dth6*, *dth7*, *dth8*, and *dth10*) of five putative QTLs were identified in both years. The Chucheongbyeol alleles increased the number of days to heading except for the *dth6* locus.

Table 3. Number of integrated markers and length per chromosome

Marker type	No. amplified fragments		Total	Chromosome											
	Poly-morphic (%)	Mono-morphic		1	2	3	4	5	6	7	8	9	10	11	12
SSR	134 (19.5)	499	134	11	13	11	11	9	11	21	10	9	10	10	8
AFLP*	118 (12.9)	790	66	0	0	0	3	0	0	20	8	0	8	12	15
MITE	52 (42.3)	71	21	0	0	5	2	1	2	2	1	0	6	2	0
No. of markers			221	11	13	16	16	10	13	43	19	9	24	24	23
Total length (cM)			2,227	174.5	171.9	205.6	202.2	131.2	173.3	303.8	185.1	76.7	137.7	209.4	255.4
Ave. length b/n markers			(11.0)	15.9	13.2	13.2	12.6	13.1	13.3	7.1	9.7	8.5	5.7	8.7	11.1
Genome coverage (%)			(Avg. 91.0)	80.1	91.2	87.4	96.0	95.2	97.8	90.1	76.8	82.1	91.1	98.2	95.3

*Selective combination for AFLP analysis: AA/CAT=em12, AC/CAT=em22, TC/CAT=em42, TG/CAT=em52, G/CAT=em62, TA/CAT=em72, TT/CAT=em82, AA/CTG=em14, AC/CTG=em24, TT/CAG=em81, TT/CAA=em86

Fig. 1. The integrated map was developed using SSR, AFLP and MITE-anchored markers. Chromosome numbers are indicated above each chromosome. Designations to the right of the chromosome represent marker names and to the left represent map distances in cM based on the Kosambi function. QTLs detected in the same interval are boxed. QTLs detected in both years are underlined. Centromere region is indicated by black circles on the chromosome.

Culm length: One QTL (*cl10*) was detected in both years. This QTL explained 6.3% of the phenotypic variance with an additive effect of 2.0 cm contributed by the Chucheongbyeon allele. To our knowledge, no QTL associat-

ed with culm length has been reported near this chromosomal region, implying that it might be unique in *japonica* rice.

Panicle length: Five loci were associated with PL. *pl6* and *pl7* shared similar genomic locations to the QTLs

Table 4. Characteristics of putative QTLs for the ten traits detected in RILs

Trait	QTL	CHR	Significant marker	Position (cM) ^a	P	Increased effect	% of PV ^b	Allele effect ^c
Days to heading	<i>dth6^d</i>	6	OSR19	25.9	0.0000	S	9.6	1.5
	<i>dth7^d</i>	7	RM478	363.8	0.0000	C	9.1	-2.0
	<i>dth8^d</i>	8	RM72	37.8	0.0000	C	4.8	-1.0
	<i>dth10^d</i>	10	RM333	131.2	0.0000	C	8.1	-1.0
	<i>dth11</i>	11	RM552	40.0	0.0000	C	5.9	-1.0
							31.1^e	
Culm length	<i>cl10^d</i>	10	em863–RM333	131.2	0.0003	C	6.3	-2.0
Panicle length	<i>pl5^d</i>	5	RM153	0.0	0.0032	S	4.1	0.3
	<i>pl6</i>	6	RM50	54.4	0.0020	S	4.4	0.3
	<i>pl7</i>	7	RM320	224.4	0.0000	S	9.1	0.4
	<i>pl9</i>	9	RM215	76.7	0.0005	C	5.3	-0.3
	<i>pl11^d</i>	11	RM332	25.7	0.0003	S	5.9	0.3
							21.5^e	
Panicles per plant	<i>pn7^d</i>	7	RM47	361.3	0.0000	C	4.4	-0.4
	<i>pn11</i>	11	RM229	127.7	0.0080	C	3.2	-0.4
							5.5^e	
Spikelets per panicle	<i>spp7^d</i>	7	RM505	361.3	0.0043	S	3.7	3.0
	<i>spp10</i>	10	RM474	0.0	0.0000	S	7.9	4.0
	<i>spp11^d</i>	11	em526	154.0	0.0000	S	11.5	5.0
							17.6^e	
Grain yield	<i>gy3</i>	3	RM342C	33.0	0.0174	C	2.1	-32
	<i>gy7^d</i>	7	RM505	347.7	0.0020	S	2.6	38
							4.1^e	
Grain thickness	<i>gt7^d</i>	7	RM478	363.8	0.0000	S	7.0	0.02
Grain length	<i>gl8</i>	8	em123	89.6	0.0003	S	5.0	0.04
	<i>gl12</i>	12	OSR32	71.5	0.0000	S	7.7	0.04
							9.5^e	
Grain width	<i>gw4^d</i>	4	RM241	144.0	0.0000	S	9.8	0.03
	<i>gw7</i>	7	RM478	363.8	0.0000	S	14.1	0.04
	<i>gw12</i>	12	em144	51.3	0.0120	C	4.5	-0.02
							19.5^e	
Grain length/width ratio	<i>gs4^d</i>	4	RM241	144.0	0.0008	C	5.2	-0.02
	<i>gs7</i>	7	RM478	363.8	0.0002	C	5.7	-0.02
	<i>gs12</i>	12	OSR32	71.5	0.0000	S	12.5	0.02
							15.3^e	

For each QTL: The % of PV is the percent of the phenotypic variance explained by the QTL. SS and CC refer to genotypic class defined by single markers: SS (Suweon365) or CC (Chucheongbyeon) is the homozygote, respectively. The allele effect of QTL is the difference in the phenotypic means between SS and CC classes.

^a QTL position from the first marker (cM)

^b Phenotypic variation explained by QTLs

^c Positive value indicated the contribution from the allele of Suweon365 (SS) whereas the negative value is from Chucheongbyeon (CC)

^d Putative QTL detected in both years.

^e The total phenotypic variance explained simultaneously by all putative QTLs.

reported by Mei *et al.* (2005).

Panicles per plant: Two significant QTLs were detected on chromosomes 7 and 11, and the *pn7* locus was detected in both years. The Chucheongbyeon alleles contributed to the increase in panicles per plant at both loci.

Spikelets per panicle: Of the three QTLs detected, two QTLs (*spp7* and *spp11*) were common in both years. At all loci, the Suweon365 alleles increased spikelets per panicle.

Grain yield: Two significant QTLs, *gy3* and *gy7* were associated with grain yield. *gy7* was consistently detected in both years. The *gy7* locus was co-localized with QTLs for grain yield components, including days to heading, panicles per plant and spikelets per panicle, as in the other mapping studies (refer to <http://www.gramene.org>).

Grain thickness: One QTL, *gt7*, was detected in both years. The QTL *gt7* explained 7.0% and 3.9% of the

phenotypic variance in 1999 and 2000, respectively. The *gt7* locus was not reported in previous studies on rice.

Grain length: Two QTLs, *gl8* and *gl12* were associated with grain length (Fig. 1, Table 4). Suweon365 alleles contributed to the increase in grain length at both loci. Neither of the two QTLs was detected in the earlier QTL study (www.gramene.org).

Grain width: Three QTLs were identified for grain width and *gw4* was detected in both years. Although *gw12* was only detected in one year, this QTL explained the largest phenotypic variance (14.1%) among the three QTLs. The Suweon365 alleles at the *gw4* and *gw7* loci increased GW, whereas the Chucheongbyeol allele increased GW at *gw12*.

Grain shape: Three QTLs, *gs4*, *gs7* and *gs12*, affected grain shape. The *gs12* locus was co-localized with *gl12* and *gw12* and the other two loci shared the same region as the QTL for GT on chromosome 7.

Discussion

The linkage map, composed of 221 markers, had a map length of 2,227 cM with an average distance of 11.0 cM between markers (Table 3). This number is relatively larger than that reported in maps constructed from temperate *japonica* rice crosses, such as 1,060 cM (Wada *et al.* 2006), and 891.1 cM (Tabata *et al.* 2007). Construction of maps without AFLP or MITE markers indicated that map expansion is due to the incorporation of AFLP markers, and this result is consistent with previous reports (Ali *et al.* 2000, Lu *et al.* 1997). In this study, AFLP markers were mainly distributed on chromosomes 7, 11 and 12, leading to increased map distance in three chromosomes (approximately 260 cM). Contrary to AFLP, MITE-anchored markers did not contribute to an increase in map distance except for chromosome 3. Several genomic regions with a low density of markers appeared as large gaps on the map. Monomorphic regions are expected in the genome of a population derived from a cross between closely related temperate *japonica* parents. No polymorphic marker on the short arm of chromosome 9 was detected. Kobayashi *et al.* (2007) and Tabata *et al.* (2007) reported similar results for chromosome 9 in temperate *japonica* crosses, suggesting that chromosome 9 may be highly monomorphic and common by descent in *japonica* rice.

A number of QTLs affecting agronomic and grain traits have been mapped previously using populations derived from intra- or inter-subspecific and interspecific crosses (Xiao *et al.* 1996, Redoña and Mackill 1998, Yoon *et al.* 2006). Among the 27 QTLs detected in the present study, nineteen have been detected in previous QTL studies (www.gramene.org). These include the QTLs for days to heading, *dth6* (Yano *et al.* 2000), for panicle length, *pl6* and *pl7*, in the Lemont × Teqing RI population (Mei *et al.* 2005), for grain yield, *gy7* (Tian *et al.* 2006), and for grain width *gw7* (Redoña and Mackill 1998). However, the other eight

QTLs, *cl10* for culm length, *spp10* for spikelets per panicle, *gt7* for grain thickness, *gl8* and *gl12* for grain length and *gs4*, *gs7* and *gs12* for grain shape were identified for the first time in this study, indicating that these QTLs might be specific to temperate *japonica* rice. However, considering that the map showed incomplete genome coverage with gaps in several genomic regions, the number of QTLs detected in this study might be underestimated in the *japonica* genome. In this regard, further QTL analyses using populations derived from other parental combinations are necessary to detect more *japonica*-specific QTLs.

In this study, a cluster of seven QTLs was detected on the long arm of chromosome 7. Two QTLs for SPP and GY were identified near the SSR marker, RM505. The other five QTLs for DTH, PN and three grain traits, GT, GW and GS, were co-localized near SSR markers RM47 and RM478. A QTL for protein content, one of the main components for eating quality, was identified near the SSR marker RM478, using the same RIL population, and this QTL was subsequently confirmed using nearly isogenic lines segregating for the target region flanked by two SSR markers, RM234 and RM3555 (Suh *et al.* 2006, personal communication). In addition to this protein content QTL, this region was confirmed to harbor QTLs for DTH, GW and GT. The finding that Chucheongbyeol alleles were associated with delayed heading and low protein content is consistent with previous reports that late heading lines tend to show low protein content (Kim *et al.* 1994). This result seems to suggest that the co-localization of these two QTLs in this chromosomal region is due to pleiotropy, and high-resolution linkage analysis is currently underway to address this question. Although *gy7* and *spp7* need to be confirmed for their isogenic background, they are stable, considering that they were detected in both years. The availability of linked QTLs for yield, grain quality and shape traits would make it feasible to develop high-yielding *japonica* cultivars with good grain quality.

In this study, MITEs analysis was used to complement other marker systems because a large number of evenly distributed molecular markers can be obtained from a single reaction (Casa *et al.* 2000). The average polymorphism ratio (42.3%) from *Dasheng* and *mPing* is nearly two and three times higher than that of SSR and AFLP markers, respectively. Transposable elements offer several advantages as molecular markers. MITEs are the major component of interspersed repetitive sequences and are evenly distributed in the rice genome (Casa *et al.* 2000, Wessler 2006). The dispersed and abundant insertion loci of many MITEs families offer a rich source of polymorphic bands in mapping populations through exploitation as PCR-based mapping tools. Furthermore, MITEs are frequently associated with genes in plants (Wessler 2006, Kwon *et al.* 2007). These specific characters could be exploited in the development of a powerful marker technique for the mapping and map-based cloning of genes that are related with agronomic important traits, especially in temperate *japonica* cultivars. At present, 221 SSR,

AFLP, and MITE markers have been mapped in this population, providing the foundation for locating genes and QTLs. The map covers more than 91.0% of the rice genome (McCouch *et al.* 2002, <http://www.gramene.org>). The addition of recently developed SSR and MITEs markers is underway to increase the marker density and fill in the gaps.

This population offers a good foundation for the development of a *japonica* map for a *japonica* rice breeding program. As both parents are major cultivars commonly used in breeding programs, a population with marker information would be useful for marker-assisted selection and near-isogenic line development.

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