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Construction of a chromosome-assigned, sequence-tagged linkage map for the radish, *Raphanus sativus* L. and QTL analysis of morphological traits

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The radish displays great morphological variation but the genetic factors underlying this variability are mostly unknown. To identify quantitative trait loci (QTLs) controlling radish morphological traits, we cultivated 94 F₄ and F₅ recombinant inbred lines derived from a cross between the rat-tail radish and the Japanese radish cultivar ‘Harufuku’ inbred lines. Eight morphological traits (ovule and seed numbers per silique, plant shape, pubescence and root formation) were measured for investigation. We constructed a map composed of 322 markers with a total length of 673.6 cM. The linkage groups were assigned to the radish chromosomes using disomic rape-radish chromosome-addition lines. On the map, eight and 10 QTLs were identified in 2008 and 2009, respectively. The chromosome-linkage group correspondence, the sequence-specific markers and the QTLs detected here will provide useful information for further genetic studies and for selection during radish breeding programs.

Key Words: radish, STS, linkage map, chromosome assignment, QTL.

Introduction

The radish, *Raphanus sativus* L. ($2n = 18$), is an important vegetable, especially in Asia (see Kaneko *et al.* 2007 for a review), and is also used as fodder and oil plants. Its hypertrophied root with hypocotyl is mainly used for human consumption. Young pods are also eaten as a vegetable in tropical Asia. Although nomenclature is complex, cultivated radishes are classified into three main types based on edible parts: fodder and oilseed radish (convar. *oleifer* Alef.), seed-pod or rat-tailed radish (convar. *caudatus* (L.f.) Pistr.) and small- and large-rooted radish (convar. *sativus* L.) (Crisp 1995). Wide morphological variations are observed in the roots of local and commercial radish cultivars. Despite such importance, few studies have been undertaken to analyze the genetic factors that determine the variability of agronomic traits in radish. The radish genome is closely related to the *Brassica* genomes carried by the vegetable and oilseed plants, *Brassica rapa*, *B. nigra*, *B. oleracea* and the amphidiploid species (*B. juncea*, *B. napus* and *B. carinata*). Linkage maps of *Brassica* species have been extensively studied

and numerous genetic loci for qualitative and quantitative traits have been identified (see Schmidt and Bancroft 2011 for a review). The whole genome data are now available for *B. rapa* (Wang *et al.* 2011). In contrast, limited number of genetic maps has been constructed for radish (Bett and Lydiat 2003, Budahn *et al.* 2009, Kamei *et al.* 2010, Li *et al.* 2011, Nakatsuji *et al.* 2011, Shirasawa *et al.* 2011, Tsuru *et al.* 2005, 2008, Xu *et al.* 2012), few of which have included agronomic trait loci.

In addition to the molecular genetic map, cytogenetic study can provide information complementary to that from genetic and physical maps. A complete set of disomic rape-radish chromosome-addition lines has also been developed (Budahn *et al.* 2008) and the position of a locus for resistance against the beet cyst nematode has been determined using the chromosome-addition lines (Budahn *et al.* 2009). However, the cytogenetic data have not yet been integrated to sequence-specific molecular markers of radish.

Molecular markers provide a powerful tool for gene mapping and efficient selection of the genotypes having desired agronomic traits. This strategy is known as marker-assisted selection (MAS). Among the molecular markers, simple sequence repeats (SSRs), tandem repeats of short DNA sequences from one to six base pairs, are useful tools. Because of their high level of polymorphism, ubiquity and

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co-dominance, SSRs have become a valuable source of molecular markers in genetic analysis (Jones *et al.* 2009). SSRs have also been useful to detect the conserved order of genes among related genomes, referred to as “synteny”. Although we have previously developed 417 SSRs, including 293 genomic SSRs, from the rat-tailed radish, Japanese cultivar ‘Harufuku’ and their F₁ plant (Nakatsuji *et al.* 2011), most of our SSRs have not yet been employed for mapping studies. The rat-tail radish and ‘Harufuku’ have several differences in vegetative and reproductive morphology traits (e.g. ovule and seed numbers, plant shape, leaf pubescence and root formation). Such the differences between the two radishes allowed us to investigate genetic loci controlling their morphologies.

The objective of this study was the construction of a radish linkage map containing radish SSR and sequence-tagged site (STS) markers, assignment of the linkage groups (LGs) to the chromosomes and mapping of quantitative trait loci (QTLs) for vegetative and generative characters.

Materials and Methods

Plant materials

A population of 94 F₄ and F₅ recombinant inbred lines (RILs) was developed from a cross between the rat-tail radish and the Japanese radish ‘Harufuku’ inbred lines (Saya-S and Haru-S, respectively) using a single-seed-descent of the F₁ plant after four generations of selfings. The rat-tail radish is grown in Southeast Asia for its edible young seed-pods (Kitamura 1958). Instead of many seed-pods and larger plant top, the rat-tailed radish forms no fleshy root (Supplemental Fig. 1A). Its leaves are erect without pubescence on the surface. The ‘Harufuku’ is classified into Spring radish, which has a white, wedge-shaped root (Furusato and Miyazawa 1958) (Supplemental Fig. 1B). It has hairy, prostrate leaves. Although these two cultivars are classified as different con-varieties (*caudatus* and *sativus*), they intercrossed freely. Both parental lines were supplied by the NARO Institute of Vegetable and Tea Science, Tsu, Japan. The parental lines Saya-S and Haru-S used in this study are highly homozygous by five or six successive selfings. A commercial rat-tail radish cultivar (Chia Tai Co. Ltd., Bangkok, Thailand) (Supplemental Fig. 1A) was used for field tests instead of the original parental line Saya-S because of the severe inbred depression in the Saya-S. Note that the ‘Harufuku’ inbred line Haru-S used in this study had shorter main root and more lateral roots than the original cultivar (Supplemental Fig. 1B), probably being an aspect of inbred depression.

Field tests

The population of 94 F₄ or F₅ families as well as their parental lines was used for investigation of eight agronomic traits (Table 1). For scoring the ovule (*OV*) and seed numbers (*SE*) per silique, each F₄ RIL was grown in a heated greenhouse under a natural photoperiod until June 2008. For the other agronomic traits, F₅ RILs were cultivated under

natural conditions in an open field of the Kyoto Prefectural University Farm (Seika, Kyoto, Japan). Six plants per each RIL were cultivated from mid-September to mid-December. The field test was performed once in 2008 and 2009, respectively. Plant shape (*PS*) and pubescence (*Pub*) were categorized visually on 1–5 scales two months after sowing (Table 1 and Supplemental Fig. 2). Fresh weights for the following parts were measured at harvest (three months after sowing). After measurement of whole plant weight (*WW*), plant tops were removed to measure upper part weight (*UW*). The remainder (hypocotyls and roots) was taken as whole root weight (*RW*). The whole root was separated into main and lateral roots to measure main root weight (*MW*) and lateral root weight, respectively. The lateral root weight trait was excluded from further analysis because the variation was small (data not shown). After removal of extreme outliers, the mean score of the six F₅ plants for each line was used as data for each F₄ line.

Genotyping, linkage mapping, synteny detection and QTL analysis

Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) from a bulk of leaf tissue consisting on average of 13 F₅ plants for each line, because the initial DNA extracted from F₄ plants by the CTAB method was not of sufficient quality for AFLP analysis. We confirmed no significant incongruence in genotypes between the F₄ and the bulked F₅ DNAs for each line with tested markers (data not shown). AFLP was performed according to method described by Tsuru *et al.* (2008). SSR primers from *B. rapa* (Suwabe *et al.* 2006) and radish (Nakatsuji *et al.* 2011, Wang *et al.* 2007, this study) were randomly picked up to screen polymorphic markers between the parental lines. PCR amplification and electrophoresis were carried out as described previously (Saito *et al.* 2006). The published STS markers for *B. rapa* and radish (Kamei *et al.* 2010, Li *et al.* 2011, Niikura and Matsuura 1998, Saito *et al.* 2006) were also used. New STS markers were developed from the sequences of *Arabidopsis*, *B. rapa* and radish at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/nucleotide/>) and the Radish-Database (Supplemental Fig. 3, <http://radish.plantbiology.msu.edu/index.php/Sequences:All>) (their accession nos. AB630374-AB630382 and AB611697-AB611700).

Segregation of each marker in the bulked F₅ families was scored and treated as a genotype of the F₄ line. Markers deviating significantly ($P < 0.001$) from the expected segregation ratio were excluded from the analysis. A linkage map was constructed using JoinMap ver. 3.0 (Van Ooijen and Voorrips 2001) as described previously (Saito *et al.* 2006). To detect potential synteny, nucleotide sequences of the mapped sequence-tagged markers were searched for the *Arabidopsis* genome data using a BlastN algorithm under the default parameter sets at the NCBI. QTL analysis was performed using MapQTL 6 software (Van Ooijen 2009) as described previously (Kubo *et al.* 2010). The significant

logarithm of odds (LOD) thresholds of each QTL ($\alpha = 0.05$) were estimated by 1000 permutations. They ranged from 2.75 (*UW* in 2009) to 3.00 (*OV*).

Chromosomal assignment

A complete set of disomic rape-radish chromosome-addition lines (Budahn *et al.* 2008) was used for assignment of the chromosomes corresponding to the radish LGs. Each disomic addition line has one of the nine pairs of the radish-derived chromosomes (pairs AA-II) on the synthetic *B. napus* background (Peterka *et al.* 2004). Thirty six markers (33 radish SSRs, one radish STS and two *B. rapa* SSRs) were screened for PCR, using the genomic DNAs of the nine disomic addition lines as well as their parental lines as templates. Loci which yielded radish-specific PCR products in the particular chromosome addition line (Supplemental Fig. 5, arrowheads) were assigned to one of the nine radish chromosomes (Supplemental Fig. 5, letters).

Results

Analysis of phenotypic variation

Phenotypic variation of eight agronomic traits examined in RILs and the parental lines are shown in Supplemental Fig. 4 and summarized in Table 1. The mean scores of the rat-tailed radish were larger than those of the Haru-S in *OV*, *PS*, *WW* and *UW* in each year (and presumably also in *SE*, see Discussion) (Table 1). It was *vice versa* in *Pub*, *RW* and *MW*. The majority of RILs were roughly segregated into two categories of pubescence (*Pub*), presence (categories 2–5) and absence (category 1), whose distribution was biased towards the presence of pubescence (Supplemental Fig. 4D). Since the segregation ratio, divided into the above two categories of *Pub* as a qualitative trait, was significantly deviated at $P < 0.0001$ from an expected segregation ratio for RIL

population, we conducted the QTL analysis for *Pub* as below. For the other traits, RILs did not show a discrete segregation, suggesting that these traits are controlled by multiple genes. The distributions of RILs for weight traits (*WW*, *UW*, *RW* and *MW*) were shifted towards larger values in 2009 than seen in 2008 (Supplemental Fig. 4E–4H, gray and black bars, respectively) probably because of an environmental effect. In root weight traits (*RW* and *MW*), RILs with transgressive weight exceeding the values for the Haru-S (Supplemental Fig. 4G–4H, pink arrows) were found. In *OV*, transgression beyond the value for the rat-tailed radish also occurred (Supplemental Fig. 4A, green arrow).

Linkage mapping, LG assignment using the disomic chromosome addition lines and synteny to *Arabidopsis*

The map comprised nine LGs, including 163 radish AFLPs, 97 radish SSRs (75 genomic SSRs and 22 EST-SSRs), 37 radish STSs, 15 *B. rapa* SSRs, seven *B. rapa* STSs and three *Arabidopsis* STSs (Fig. 1). Of these, 10 SSR and 25 STS markers were newly developed or modified in this study (Table 2). The total map length was 673.6 cM, with an average marker interval of 2.1 cM. A total of 36 markers were tested for assignment of the present LGs to radish chromosomes using the disomic addition lines (Budahn *et al.* 2008) (Supplemental Fig. 5 for examples). Of these, 19 markers (18 radish SSRs and one radish STS) were assigned to the radish chromosomes (Fig. 1, gray boxes). There was no incongruence between the markers and the assigned chromosomes. Thus, we also refer each LG to our previous nomenclature (Chrs A–I) as well as LG numbers (R1–R9) given by Li *et al.* (2011) (Fig. 1, parentheses). Nucleotide sequences of 138 sequence-tagged markers showed homology to *Arabidopsis* (data not shown). Most of the homologies to the five *Arabidopsis* chromosomes were divided into many segments and dispersed throughout the present

Table 1. List of traits analyzed and the phenotypic values of the F₄ or F₅ population and the parental lines

Trait	Abbreviation	Year	Mean (\pm SD) in RIL	Range in RIL	Mean (\pm SD) in rat-tail radish	Mean (\pm SD) in Haru-S
Ovule number per silique	<i>OV</i>	2008	9.93 \pm 2.07	5.00–16.25	9.40 \pm 0.80	6.60 \pm 0.49
Seed number per silique	<i>SE</i>	2008	3.09 \pm 1.00	1.14–5.55	N.D. ^c	3.04 \pm 1.51
Plant shape	<i>PS</i>	2008	3.1 \pm 0.8	1–5 ^a	5	1
		2009	3.2 \pm 0.7	1–5 ^a	5	1
Pubescence	<i>Pub</i>	2008	2.9 \pm 1.3	1–5 ^b	1	5
		2009	2.9 \pm 1.3	1–5 ^b	1	5
Whole plant weight (g)	<i>WW</i>	2008	598 \pm 236	142.6–1516	982 \pm 311	429 \pm 173
		2009	1267 \pm 458	426.7–2890	2486 \pm 1140	1038 \pm 252
Upper part weight (g)	<i>UW</i>	2008	416 \pm 173	119.8–1142	879 \pm 268	232 \pm 86.9
		2009	1005 \pm 385	341.7–2518	2346 \pm 1096	716 \pm 174
Whole root weight (g)	<i>RW</i>	2008	180 \pm 119	16.8–648	103 \pm 46.5	197 \pm 88.4
		2009	249 \pm 174	26.0–801	135 \pm 63.9	320 \pm 83.4
Main root weight (g)	<i>MW</i>	2008	148 \pm 96.3	15.7–534	87.5 \pm 43.3	175 \pm 76.3
		2009	239 \pm 165	26.0–744	123 \pm 63.3	314 \pm 84.8

^a 1 = prostrate; 2 = semi-prostrate; 3 = intermediate; 4 = open; 5 = erect, see Supplemental Fig. 2.

^b 1 = no hair; 2 = sparse; 3 = intermediate; 4 = abundant; 5 = very abundant hair like Haru-S.

^c N.D., not determined.

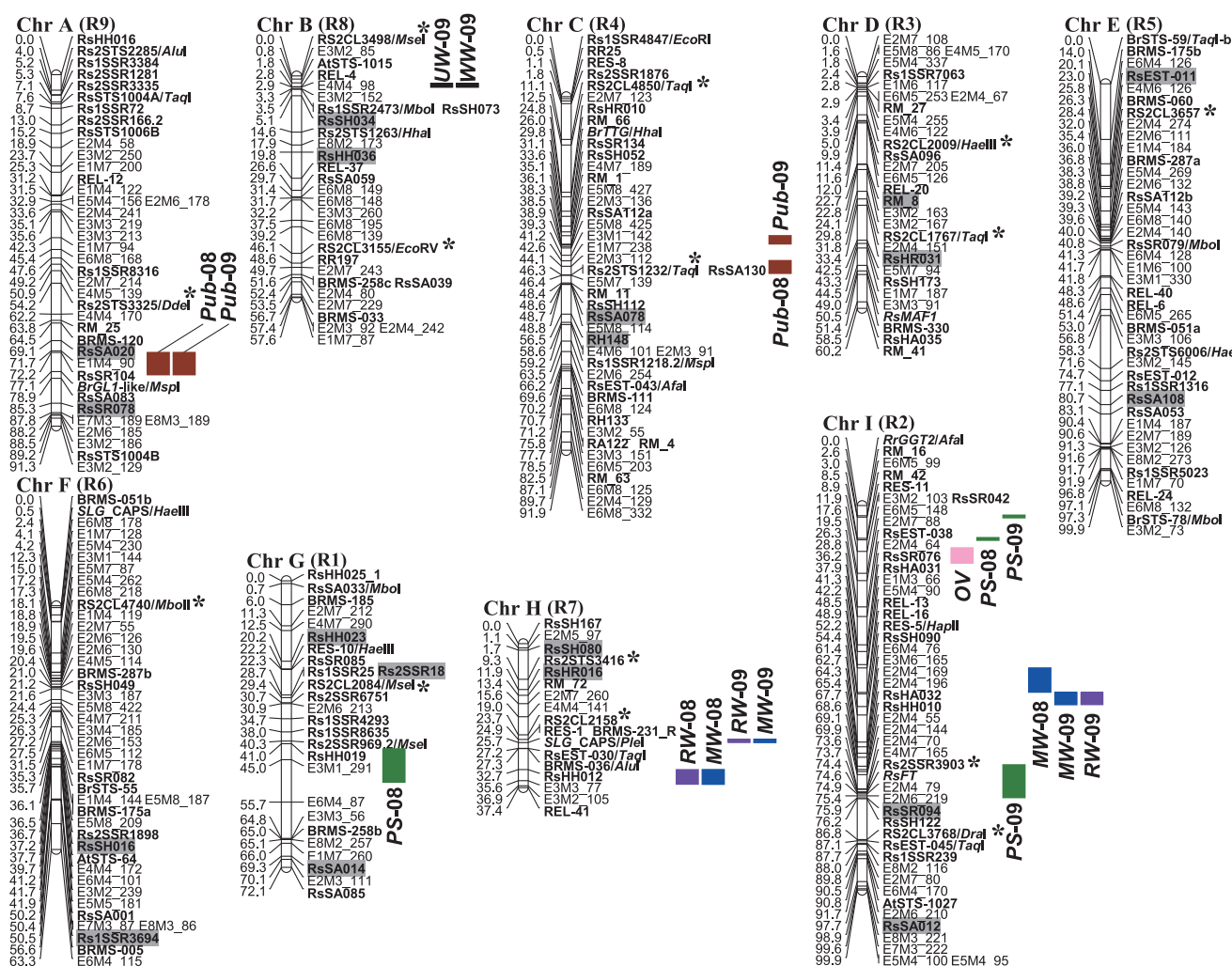


Fig. 1. A linkage map of radish and the locations of QTLs for eight agronomic traits. Linkage groups were referred to chromosome numbers (Chrs A–I) (Budahn *et al.* 2008) and LG numbers (R1–R9) (Li *et al.* 2011). Marker names and genetic distances (cM) are shown on the right and left of each LG, respectively. SSR and STS markers are indicated with bold-face type. Markers used for the assignment to radish chromosomes and LG numbers are indicated with gray boxes and asterisks, respectively. Locations of QTLs are indicated with differentially-colored, vertical thick bars, the lengths of which correspond to the map positions in Table 3. The QTLs detected in 2008 and 2009 are represented with suffix numbers such as “-08” and “-09”, respectively.

radish map. Relatively large clusters were found in all radish LGs, which may represent syntenes to *Arabidopsis*.

QTL analysis

On the present map, QTLs were identified for five and six traits in 2008 and 2009, respectively (Table 3 and Fig. 1, thick bars). The phenotypic variations explained by each QTL varied from 9.3% to 37.7% (Table 3). A single *OV* QTL was detected on Chr I (Fig. 1, pink thick bar). The *Saya-S* allele resulted in an increase of the *OV*. *PS* QTLs were detected on Chr G in 2008 and on the lower part of Chr I in 2009 (Fig. 1, green thick bars). On the upper part of Chr I, *PS* QTL was located within the vicinity in each year. The QTLs on Chr G and on the upper part of Chr I had additive effects of the same direction; the *Saya-S* allele increased the *PS* scale, whereas the QTL on the lower part of Chr I had an

opposite effect (Table 3). *Pub* QTLs were detected on Chrs A and C in both years (Fig. 1, brown thick bars). The QTLs on Chrs A and C explained a proportion of phenotypic variance of 34.4–37.7% and 24.2–25.3%, respectively and the *Haru-S* allele resulted in an increase of *Pub*. QTLs for *WW* and *UW* were identified at an overlapped region on Chr B in 2009 (Fig. 1, black thick bars). The QTL alleles affecting an increase of *WW* and *UW* would come from the *Saya-S*. *RW* QTLs were detected on Chr H in both years and on Chr I in 2009 (Fig. 1, blue thick bars). The *Haru-S* allele increased *RW* (Table 3). *MW* QTLs were found on Chrs H and I in both years (Fig. 1, blue thick bars). Three of the *MW* QTLs overlapped with the *RW* QTLs. These QTLs explained 14.6–33.9% of phenotypic variation. Like the case in *RW*, the *Haru-S* allele increased the *MW*.

Table 2. List of SSR and STS markers developed and modified in this study

Marker name ^a	Marker type	Repeat motif		Primer sequence (5'-3')	Product size (bp) ^b	GenBank accession no.
<i>Radish genomic SSR markers</i>						
REL-4	SSR	(CTT) ₆	Forward	GATACGGAGACAAGAAGAAG	237	AB630374
			Reverse	CGGACAGAAGAGAAAAAGAC		
REL-12	SSR	(GAA) ₂₅	Forward	CTTAACACCAGGAATCC	330	AB630375
			Reverse	GCCTCTGAAATCCTCCAATC		
REL-13	SSR	(CA) ₁₃	Forward	CTAGCAATGCATACCAAACAG	166	AB630376
			Reverse	AACTTGGTCGTTGAGCAG		
REL-16	SSR	(TG) ₁₃	Forward	ACAGCAACGTTTTCAAGTGCTC	197	AB630377
			Reverse	CTCACATGCAATGCAATGCATAC		
REL-20	SSR	(TC) ₈	Forward	CCTTCATTCTTTGCTTCTTC	162	AB630378
			Reverse	TTGTTTCTTCCCAGAAAGCC		
REL-37	SSR	(GA) ₁₆	Forward	GAAAACGGGAATCAGATACAAC	94	AB630379
			Reverse	CTCATTCACTTTCTTCGCCA		
REL-40	SSR	(AC) ₁₂	Forward	TGATAGCAAAGGCAGAGGTGGC	176	AB630380
			Reverse	CAAGAAACCTGTCGCAAGGTACAG		
REL-41	SSR	(CA) ₆	Forward	CGTTCATATCAACTTTTTGAGCGAG	154	AB630381
			Reverse	TTATGCCAGCCTTAGCTGC		
<i>SSR and STS markers developed from radish ESTs</i>						
RES-8	SSR	(T) ₁₅ GT C(A) ₂₁	Forward	GGATAACAATTACCTCGATCA	206	AF248491
			Reverse	GGAAACCAAATACCACAAAAG		
RES-10/ <i>Hae</i> III	STS (CAPS)	–	Forward	CTTCCTAGTAGCTAACTC	195	AF263920
			Reverse	AACAGTCATGACACGTGG		
<i>RrGGT2/Afa</i> I	STS (CAPS)	–	Forward	CTATCTCCAGGCACTGGAATCG	431	EX761507
			Reverse	ATCGTCCCTCCGGCGATTGG		
RsEST-011	STS (indel)	–	Forward	ATCATGTCTACGTGCGGAAACT	353	AF051121
			Reverse	ACCATTACATCACACCCATGC		
RsEST-012	STS (indel)	–	Forward	GTACCAAGTTCCCCAGTTATGC	294	AF051120
			Reverse	GATGCCAACAAGGACTTGTAA		
RsEST-030/ <i>Taq</i> I	STS (CAPS)	–	Forward	CTATTCCTTTACCCGTTTCT	303	T25178
			Reverse	CTGACAGACTCTTGCTTCTCAG		
RsEST-038	STS (indel)	–	Forward	GATGTATCAGGTGGTGAAAT	192	EL738632
			Reverse	TACACCACCAGAAAAGTCCAC		
RsEST-043/ <i>Afa</i> I	STS (CAPS)	–	Forward	ATCATGGTCCGATACACAAATC	350	EL738626
			Reverse	TGCATTGAAACCAAAAGACC		
RsEST-045/ <i>Taq</i> I	STS (CAPS)	–	Forward	GCAGAAATTTCCGCTGTAGTTC	275	EL738624
			Reverse	TGACAAAATACTTCTGCAAGC		
<i>RsFT</i>	STS (indel)	–	Forward	TAGGTTTTCTGTTATATCACTGCT	103	AB611697
			Reverse	AAATCGAGTCTCTGAAAGTTTTAC		
<i>RsMAF1</i>	STS (indel)	–	Forward	GTCAGGATCCTTGATCGATATGG	81	FD958357
			Reverse	GCTCAGAGATTTTGACTGAAGAT		
RsSTS1004A/ <i>Taq</i> I	STS (CAPS)	–	Forward	ATTTCAATCCACTCAAGCTGAATCT	522	AB611698
			Reverse	TTTTATCTTGATGGAAGTCTAACC		
RsSTS1004B	STS (indel)	–	Forward	TCTAAGCCCATGGTTATGAGAC	154	AB611699
			Reverse	AAGGAAAGGCCCACTTGAATA		
RsSTS1006B	STS (indel)	–	Forward	CCCTAACGCACAAGTAATTA	191	AB611700
			Reverse	TAATCCGTGTAGCAGAACAATA		
RsISSR7063	SSR	(CT) ₇	Forward	GGACAAGTTTATACTTTAAGTG	223	RS1CL7063Contig1 ^c
			Reverse	GATTTTTGCACAAGTTTTCTAGAG		
Rs2STS1232/ <i>Taq</i> I	STS (CAPS)	–	Forward	AGCCTGGTAAGCATCTCCAA	468	RS2CL1232Contig1 ^c
			Reverse	TGACCATGGTGGGAGTGAAT		
Rs2STS1263/ <i>Hha</i> I	STS (CAPS)	–	Forward	GATCCAAGGTTCCATGCCCATG	366	RS2CL1263Contig1 ^c
			Reverse	GACCCGGCAGCATTTCTGCTCTA		
Rs2STS2285/ <i>Alu</i> I	STS (CAPS)	–	Forward	TGTTCTTCAGGGGAGTTTGATT	489	RS2CL2285Contig1 ^c
			Reverse	CCTTGGTCTTCTAGTTGCGTTT		
Rs2STS3325/ <i>Dde</i> I	STS (CAPS)	–	Forward	CTAGCTCCAGGGAGTCAATTCA	517	RS2CL3325Contig1 ^c
			Reverse	CCATCTGAGTAACGAGTGCG		

Table 2. (continued)

Marker name ^a	Marker type	Repeat motif		Primer sequence (5'-3')	Product size (bp) ^b	GenBank accession no.
Rs2STS3416	STS (indel)	–	Forward	TACCAAAGAAGAGACCCTTATG	451	RS2CL3416Contig1 ^c
			Reverse	TCTAAGAACCCTTGATCTTTGT		
Rs2STS6006/ <i>Hae</i> III	STS (CAPS)	–	Forward	CACAGCCATCACTTGCAGATCC	539	RS2CL6006Contig1 ^c
			Reverse	ATGCTGCTACTGTACACCGGGT		
<i>Markers developed from Brassica rapa genomic DNA</i>						
<i>BrGL1-like/Msp</i> I	STS (CAPS)	–	Forward	GAACAGATAACCAAGTCAAG	310	AC232566
			Reverse	TTAAACCAGAAAGCGTTACTGC		
<i>Marker modified from the previously reported B. rapa SSR</i>						
BRMS-036_ <i>R</i> / <i>Alu</i> I	STS (CAPS)	–	Forward	GGTCCATTCCTTTTGCATCTG	141	AB630382
			Reverse	GGGTAACAAACATTTCTTAACCTCTC		
BRMS-231_ <i>R</i>	STS (indel)	–	Forward	CGGTGTATTTCCATTTATGCGTTT	180	CU695294
			Reverse	TTCTGGACTTCAATTAGAGATGGTC		
<i>Markers developed from Arabidopsis genome data</i>						
AtSTS-64	STS (indel)	–	Forward	CTCTCGGGTAGTTTACCTGACTTC	640	At3g05990 ^d
			Reverse	GAACCTGGCCAGTTAGGTTATTGT		
AtSTS-1015	STS (indel)	–	Forward	GTTGGTTAGGCTTAAACAGATG	850	At1g74860 ^d
			Reverse	TCCTACAATATCTTCAGCTTCTC		
AtSTS-1027	STS (indel)	–	Forward	TAAAACCTATCATCTCCGTACC	880	At1g71950 ^d
			Reverse	GCACAACCTGAATCACACC		

^a In CAPS markers, restriction enzymes used for polymorphism detection are indicated after slashes (/).

^b Sizes are estimated by agarose gel electrophoresis or predicted from the sequences used for primer design.

^c For markers designed from radish cDNA contig data, the cDNA contig nos. are shown instead of GenBank accession nos. (their sequences are shown in Supplemental Fig. 3).

^d For markers designed from *Arabidopsis* genome data, the locus tags are shown instead of GenBank accession nos.

Table 3. Map positions and effect of QTLs detected in RILs

Trait	Year	Chr	Map position in cM (peak) ^a	Marker interval ^b	LOD ^c	R ² (%) ^d	Additive effect ^c
<i>OV</i>	2008	I	10.9–14.9 (11.9)	RES-11–E6M5_148	3.5	11.8	0.79
<i>PS</i>	2008	G	42.0–50.7 (45.0)	RsHH019–E6M4_87	3.8	10.8	0.31
		I	8.5–8.9 (8.5)	RM_42–RES-11	6.4	20.9	0.40
	2009	I	2.6–3.0 (2.6)	RM_16–E6M5_99	3.5	16.9	0.32
		I	67.4–75.9 (68.6)	E2M4_196–RsSR094	3.2	11.9	–0.27
<i>Pub</i>	2008	A	72.2–78.1 (77.1)	RsSR104–RsSA083	13.6	37.7	–0.86
		C	42.6–46.1 (44.1)	E1M7_238–Rs2STS1232/ <i>Taq</i> I	9.7	24.2	–0.68
	2009	A	72.2–78.1 (77.1)	RsSR104–RsSA083	12.8	34.4	–0.82
		C	36.1–38.3 (38.3)	RM_1–E5M8_427	10.1	25.3	–0.69
<i>WW</i>	2009	B	1.8–2.8 (2.8)	AtSTS-1015–REL-4	3.8	18.7	213.34
<i>UW</i>	2009	B	1.8–2.8 (2.8)	AtSTS-1015–REL-4	3.6	17.8	175.33
<i>RW</i>	2008	H	34.7–35.6 (35.6)	RsHH012–E3M3_77	3.7	9.3	–51.95
	2009	H	24.9–25.7 (24.9)	RES-1– <i>SLG</i> _CAPS/ <i>Ple</i> I	11.6	33.9	–113.01
		I	48.5–51.9 (48.9)	REL-13–RES-5/ <i>Hap</i> II	6.6	16.8	–82.24
<i>MW</i>	2008	H	32.7–36.6 (35.6)	RsHH012–E3M2_105	8.4	25.1	–53.52
		I	42.2–48.5 (46.2)	E5M4_90–REL-13	5.4	14.6	–42.84
	2009	H	24.9–25.7 (24.9)	RES-1– <i>SLG</i> _CAPS/ <i>Ple</i> I	11.7	33.9	–107.27
		I	48.5–51.9 (48.9)	REL-13–RES-5/ <i>Hap</i> II	6.9	17.3	–79.06

^a Region above the LOD threshold, in which the peak position is in parentheses.

^b Smallest marker interval flanking a region above the LOD threshold.

^c Scores at the LOD peaks.

^d Phenotypic variation explained by each QTL at the LOD peak.

Discussion

Recently, we made radish linkage maps using F_1 and F_2 populations (Budahn *et al.* 2009, Kamei *et al.* 2010, Nakatsuji *et al.* 2011, Tsuru *et al.* 2005, 2008). These studies included limited number of specific markers derived from radish. Here we located 134 radish-specific markers (97 SSRs and 37 STSs) on the map using RILs. The map consisted of nine LGs, which corresponds to the haploid chromosome number of radish. Based on the anchor markers, correspondence of LGs between this study and Li *et al.* (2011) has been accomplished (Fig. 1, asterisks and parentheses). Through these maps, LGs from different studies (Kamei *et al.* 2010, Nakatsuji *et al.* 2011, Shirasawa *et al.* 2011) can also be assigned (data not shown). The total length of our map (673.6 cM) was shorter than those of Li *et al.* (2011) and Shirasawa *et al.* (2011). Assumed from the positions of anchor markers and the distributions of syntenic regions, the present map was roughly estimated to cover >70% of the above two maps (data not shown). Since our map still includes anonymous AFLPs and STSs that did not hit to *Arabidopsis* locus, the value might be underestimated. Thus, our map is considered to cover a significant fraction of the radish genome.

Two recent reports (Li *et al.* 2011, Shirasawa *et al.* 2011) involved many more markers and gave a more detailed picture of *Arabidopsis* synteny than this study. However, the present map has the following two advantages over the previous maps. First, each LG on the present map was assigned to a radish chromosome (Fig. 1, gray boxes). Previous work of chromosome-LG assignment was done with anonymous RAPD markers (Budahn *et al.* 2009). In this study, the assignment has been completed with radish-specific markers (Supplemental Fig. 5). This allows us to conduct easier and more concrete chromosome-LG assignment than in many other radish maps. Although Akaba *et al.* (2009) have made monosomic rape-radish addition lines, no assignment to radish linkage map has been reported. The chromosomal correspondence to the set of addition lines in Akaba *et al.* (2009) and ours should be considered in the future. Second, by using the present map, several QTLs for morphological traits were detected. Some of the QTLs showed low consistency between years probably because of an environmental effect. Indeed, the weight traits in 2008 were mostly smaller than those in 2009 (Supplemental Fig. 4E–4H). Low precipitation during the primary growth of radish could be a reason for such difference, because the monthly total precipitation in October, 2008 was lower than that of the climate normal (averages of 1997–2005) (data not shown). Unfortunately, the seeds of RILs were insufficient to assess the possibility of environmental effects and genotype-environment interaction by planting several fields. In contrast to the QTLs with low consistency, QTLs that were reproducible in the two years suggest the presence of loci with seemingly stable effects. The markers linked to the stable QTLs could provide practical tools for selecting rad-

ish lines. The details of QTLs are further described below.

The ovule and seed number traits in our radish population were quantitative. Although the *SE* remains to score in the rat-tailed radish inbred line Saya-S, presumably the Saya-S had more *SE* than the Haru-S, assumed from a report describing that seed numbers in rat-tailed radishes were ≥ 8 (Yazawa *et al.* 1953). In *Arabidopsis*, both of the *OV* and *SE* traits were also quantitative, in which QTLs overlapped on chrs 1 and 2 (Alonso-Blanco *et al.* 1999). In wild radish *R. raphanistrum*, the ovule number had a positive effect on the seed number (Conner *et al.* 1996). Unlike these cases, in this study, only an *OV* QTL has been found whereas no *SE* QTL has been detected. Such discrepancy might be because the number of ovules becoming seeds may be influenced by factors such as the ovule number itself, the quantity and quality of pollen transferred, nutrients and photosynthate (Lee 1988).

Two QTLs for pubescence were found in radish. There is no report of a pubescence locus on a radish map, whereas many reports of *B. rapa* studies (Ågren and Schemske 1992, Kubo *et al.* 2010, Li *et al.* 2009, Lou *et al.* 2007, Nozaki *et al.* 1997, Song *et al.* 1995, Teutonico and Osborn 1994, Zhang *et al.* 2009) have indicated that the pubescence is controlled by a single locus with the proposal of a few candidate genes. In the present case, a marker made from the *B. rapa* candidate gene (*BrGL1*) was mapped at the peak position of the *Pub* QTL on Chr A (Table 3 and Fig. 1). This gene is a good candidate for a *Pub* gene in our population; however, we have no information regarding the second *Pub* gene in the radish. The *Pub* QTLs found in this study are potentially used for breeding a pest-resistant radish line, as utilized in *Brassica* crops (Ågren and Schemske 1992).

The plant shape (e.g. leaf angle) is an important trait for cultivation because it largely affects planting density and effectiveness of receiving solar energy. This trait has extensively been studied in maize and rice (Tian *et al.* 2011 and references therein), whereas little is known for Brassicaceae crops. In the *PS* QTLs identified here, the rat-tail radish allele would influence the plant shape into an erect form (Table 3). Although this trait might be environmentally variable, these QTLs could be useful for the selection of radish lines with a more compact plant shape, thus improving canopy.

Root formation is one of the most important traits for the Asian radish cultivars, because a thick and long cylindrical root is indispensable for Asian cultivars. In the present population between rat-tail and Japanese radishes (Saya-S and Haru-S), most *MW* QTLs were associated with *RW* QTLs (Table 3 and Fig. 1). This clearly demonstrated that thickness of the main root contributed to the total root weight. In this study, we have found two potential QTL clusters for root formation. We previously identified four genomic regions of root-related QTLs using an F_2 population between Chinese and Japanese radishes (Tsuru *et al.* 2008). No significant overlap was detectable in the positions of root QTLs between the previous and the present studies (data not

shown). The root formation QTL might act in a cultivar-specific manner.

In conclusion, we have constructed a chromosome-assigned, sequence-tagged linkage map of the radish, and have identified several QTLs for morphological traits. The present data will provide useful information for further genetic studies and for selection during radish breeding programs. As an example, the QTL detected in radish could be introduced into *Brassica* crops via homoeologous recombination as in the case of the fertility restorer gene (Sakai *et al.* 1996). Genetic markers closely linked to the loci are powerful tools for detecting recombination in interspecific hybrids. For this purpose, the present chromosome-assigned, sequence-tagged linkage map of the radish would be useful for MAS and chromosome manipulation not only in the radish, but also in a wide range of *Brassica* crops.

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