Analysis of Organ-specific Regulation of DNA Methylation in *Brassica rapa* by MSAP.

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Summary

DNA methylation is an important epigenetic modification regulating gene expression. In this study, we investigated differences of DNA methylation status between leaves, stamens, and pistils by methylation sensitive amplification polymorphism (MSAP) analysis in *Brassica rapa*. The difference of DNA methylation status was the largest between leaves and stamens/pistils. About half of the differentially methylated sequences were genic regions, but their expression levels did not differ between organs. The percentage of genic regions detected by MSAP analysis was higher than that of the total length of the genic regions in the genome, suggesting that genic regions are differentially methylated between organs in *B. rapa*.

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Key words : DNA methylation, MSAP, *Brassica rapa,* organ-specificity

Gene expression is regulated not only genetically by the nucleotide sequences of genes but also epigenetically by DNA methylation and histone modifications. In mammalian cells, cytosines at CG sites are methylated, while those at CNG (N is either A, T, C, or G) sites and CHH (H is either A, T, or C) sites as well as CG sites are methylated in plants. DNA methyltransferases important for regulation of the DNA methylation are widely conserved in living organisms, but not detected in yeast and nematode (Chan *et al.,* 2005). In *Arabidopsis thaliana*, *DDM1* (decrease in DNA methylation 1), which is an SWI2/SNF2 subfamily chromatin remodeling factor, is also required for DNA methylation as are four DNA methyltransferases, i.e., *MET1* (methyltransferase 1), *CMT3* (chromomethylase 3), *DRM1* (domains rearranged methylase 1), and *DRM2* (Finnegan and Kovac, 2000). Some hypomethylated mutants show morphological abnormalities, indicating that gene regulation by DNA methylation is required for normal development (Ronemus *et al.,* 1996, Kakutani *et al.*, 1996). Ruiz-Garcia *et al.* (2005) have revealed that the DNA methylation level in *A. thaliana* changes throughout its life cycle and have suggested developmental regulation by DNA methylation. A genome-wide decrease in DNA methylation has been observed in the endosperm compared with the embryo (Gehring *et al.*, 2009, Hsich *et al.,* 2009).

Recent studies have revealed that agriculturally important traits were regulated by some epigenetic modification (Manning *et al.*, 2006, Hauben *et al.*, 2009, Martin *et al.*, 2009). In *Brassica rapa*, three DNA methyltransferase genes, i.e., *BrMET1a, BrMET1b,* and *BrCMT*, and two genes for chromatin remodeling factors, i.e., *BrDDM1a* and *BrDDM1b,* have been isolated, and hypomethylated plants with an RNAi construct of *BrDDM1* (*ddm1*-RNAi plants) have been obtained (Fujimoto *et al.*, 2006, 2008, Sasaki *et al.*, 2011). Comprehensive information on the DNA methylation status has been obtained in some organisms. However there is little information on the DNA methylation status of each tissue in *B. rapa*, which includes various vegetables, although expression of recessive alleles of *SP11/SCR* in *B. rapa* have been revealed to be regulated by tissue-specific DNA methylation (Shiba *et al.*, 2006, Tarutani *et al.*, 2011).

In this study, we performed MSAP (methylation-sensitive amplification polymorphism) analysis to investigate the epigenetic regulation in *B. rapa*. MSAP is a modified method of AFLP (amplification fragment length polymorphism) analysis using *Hap* II/*Msp* I instead of *Mse* I, in which the difference of DNA methylation at 5'-CCGG-3' sites can be detected as polymorphism (Xiong *et al.*, 1999). *Hap* II and *Msp* I are isoschizomers which recognize CCGG sites, but their respective sensitivities to DNA methylation differ. *Hap* II cannot perform digestion when either cytosine is fully methylated, while *Msp* I can digest $C^{5m}CGG$ but not $^{5m}CCGG$. We detected sequences whose DNA methylation is differentially regulated between different organs.

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MATERIALS AND METHODS

Plant materials

An F_1 cultivar of *Brassica rapa*, 'Osome' (Takii Seed Co. Kyoto, Japan), was used. Genomic DNAs were isolated from leaves, stamens, and pistils by the DNeasy Plant Mini Kit (Qiagen, USA). RNAs were isolated from leaves, stamens, and pistils by the SV Total RNA Isolation Kit (Promega, USA), and used for RT-PCR. Stamens and pistils were taken from flower buds 3-5 mm in length.

MSAP analysis

MSAP analysis was performed following Xiong *et al.* (1999). DNAs (100 ng) were digested with *Hap* II/*Eco* RI or *Msp* I/*Eco* RI (TaKaRa Bio, Shiga, Japan). Oligonucleotides used in MSAP analysis are shown in Table 1. PCR products were electrophoresed in 5% polyacrylamide gel containing 8.5 M urea. After the electrophoresis, DNA fragments were stained by the silver staining method. MSAP analysis was repeated twice using the same primer pair, and reproducible

Table 1. Adaptors and primers used for MSAP analysis

Adaptors			
Eco RI-ad-F	5'-CTCGTAGACTGCGTACC	*Hap* II/*Msp* I-ad-F	5'-GATCATGAGTCCTGCT
Eco RI-ad-R	5'-AATTGGTACGCAGTCTAC	*Hap* II/*Msp* I-ad-R	5'-CGAGCAGGACTCATGA
Pre-selective promers			
Eco+0	5'-GACTGCGTACCAATTC	HM+0	5'-ATCATGAGTCCTGCTCGG
Selective primers			
Eco+CAA	5'-GACTGCGTACCAATTC*CAA*	HM+CAA	5'-ATCATGAGTCCTGCTCGG*CAA*
Eco+CAC	5'-GACTGCGTACCAATTC*CAC*	HM+ CAC	5'-ATCATGAGTCCTGCTCGG*CAC*
Eco+CAG	5'-GACTGCGTACCAATTC*CAG*	HM+ACG	5'-ATCATGAGTCCTGCTCGG*ACG*
Eco+CAT	5'-GACTGCGTACCAATTC*CAT*	HM+TAG	5'-ATCATGAGTCCTGCTCGG*TAG*
Eco+ACG	5'-GACTGCGTACCAATTC*ACG*	HM+TGC	5'-ATCATGAGTCCTGCTCGG*TGC*
Eco+TAC	5'-GACTGCGTACCAATTC*TAC*	HM+GAT	5'-ATCATGAGTCCTGCTCGG*GAT*
Eco+GTA	5'-GACTGCGTACCAATTC*GTA*		
Eco+CGT	5'-GACTGCGTACCAATTC*CGT*		
Eco+ATG	5'-GACTGCGTACCAATTC*ATG*		
Eco+TGC	5'-GACTGCGTACCAATTC*TGC*		
Eco+GCA	5'-GACTGCGTACCAATTC*GCA*		
Eco+AGC	5'-GACTGCGTACCAATTC*AGC*		

1. Underlined sequences indicate complementary sequences between forward (F) and reverse (R) sequences of adaptors.
2. Italic trinucleotides indicate added sequences to pre-selective primers for selective amplification.

Table 2. Primer pairs used in RT-PCR and bisulfite sequencing analysis

target sequences	primer pairs for RT-PCR	primer pairs for bisulfite sequencing
MSAPt-4(4)	F; 5'- CAGCCAGAAAGCGTCTATAG-3'	F; 5'-AAAAGGGAAGTTTGAGAAGYAAAAT-3'
	R; 5'-TGTGCAGTGGATATCATGATAG-3'	R; 5'-ARTTTACRATAAAATTAAAACCAAC-3'
MSAPt-12	F; 5'-GAATGCTTTCACAGACCATCGTG-3'	F; 5'-AATTTGATTAAAYAGGYATYGATGG-3'
	R; 5'-AAGCTCGGTCATTGAACCCAG-3'	R; 5'-CCARCACATARCACACCRCCATCATC-3'
MSAPt-13	F; 5'-GACATCTGAACAAAGCGTTGGC-3'	F; 5'-AGAYAGAGAGATAGTGAGAAYTGGG-3'
	R; 5'-GAGATAGTGAGAACTGGGCCTCC-3'	R; 5'-CTRACACCACARAAARCCCAAAACC-3'
MSAPt-19	F; 5'-CCACGTGGTGCCATTTGGTC-3'	F; 5'-ATGGYAGGTYTTGAGTTGGGGAGAT-3'
	R; 5'-GCACTCTGAGTGCGTGTGCC-3'	R; 5'-CTCCATACCRTTTTCCACATCCCC-3'
MSAPt-54(1)	F; 5'-ACCTCGGCTTTTGTAAACAGC-3'	F; 5'-TGTTYGAGAGGAGATAYTAAGGGGG-3'
	R; 5'-AATTTCGGAAGAAGGGAATGG-3'	R; 5'-AAACTAAARTCCCRACCATTCTCTCTC-3'

polymorphic bands were counted.

Characterization of polymorphic bands

Bands showing polymorphism were cut from polyacrylamide gels and heated with 50 μl of PCR buffer at 65℃ for 2 hours. DNAs were reamplified by PCR using HM+0/Eco+0 primers. The PCR condition was 94℃ for 1 minute, followed by 45 cycles of 94℃ for 30 seconds, 58℃ for 30 seconds, and 72℃ for 1 minute, and final extension at 72℃ for 3 minutes. Reamplified PCR products were cloned using pGEM-T Easy System I (Promega) and the nucleotide sequences were determined with a CEQ 2000XL DNA Analyzer (Beckman Coulter, USA). The sequence data were analyzed using Sequencher (Gene Codes Corporation, USA), and BLAST search of DDBJ was performed (http://blast.ddbj.nig.ac.jp/top-j.html).

To characterize the flanking sequences, suppression PCR was performed following Siebert *et al.* (1995). Amplified PCR products were cloned and sequenced.

RT-PCR

cDNAs were synthesized using the First-Strand cDNA Synthesis Kit (GE healthcare, UK). Expression of genes differentially methylated between organs was analyzed by RT-PCR using total RNA as a template with primer pairs listed in Table 2. The actin gene was used as a control. The RT-PCR condition was 94℃ for 1 minute followed by 28-35 cycles of 94℃ for 30 seconds, 55℃ for 30 seconds, and 72℃ for 1 minute, and final extension at 72℃ for 3 minutes.

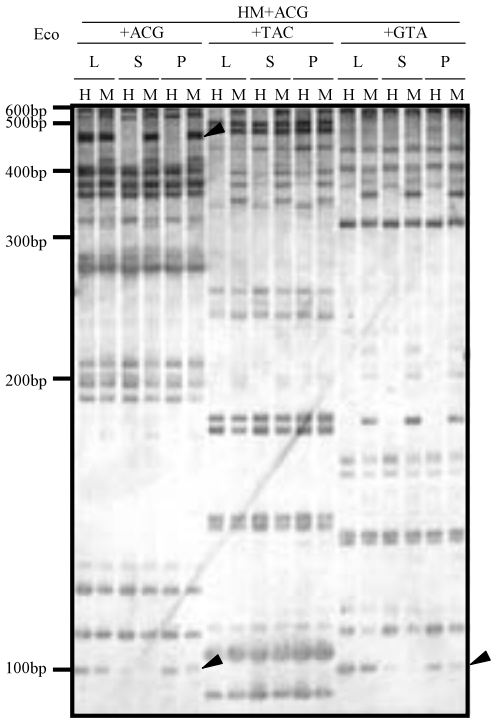

Figure 1. Polymorphic bands in MSAP analysis between leaves, stamens, and pistils. HG+ACG and Eco+NNN (N; A, T, C, or G) indicate primer name. H, *Hap* II/*Eco* RI digestion; M, *Msp* I/*Eco* RI digestion. Arrowhead shows polymorphic bands between organs. L, leaves; S, stamens; P, pistils.

Table 3. The numbers of polymorphic bands between leaves, stamens, and pistils in MSAP

leaf		stamen		pistil		No. of sites	
H	M	H	M	H	M		
+	+	-	+	-	+	9	
-	-	+	+	+	+	8	
+	+	-	-	-	-	5	
+	-	-	-	-	-	4	reproductive tissue specific
+	+	+	-	+	-	4	
-	-	-	+	-	+	2	
-	+	+	+	+	+	1	
+	+	-	-	+	+	3	stamen specific
+	+	+	-	+	+	1	
+	+	+	+	-	+	8	
-	+	-	+	+	+	2	pistil specific
-	-	-	-	+	+	1	
-	-	-	-	+	-	1	
-	-	+	-	+	+	1	others
+	+	-	-	+	-	1	
						51	

H, *Hap* II digestion; M, *Msp* I digestion; +, band present; -,band absent

Bisulfite sequencing

Genomic bisulfite sequencing analysis was performed as described by Paulin *et al.* (1998) using DNAs independently sampled for MSAP analysis. Genomic DNAs (1 μg) extracted from leaves, stamens, and pistils were digested with *Eco* RI and *Sal* I in 200 μl of the reaction mixture. After ethanol precipitation, DNAs were dissolved in 20 μl of water. After being heated at 94℃ for 10 minutes and then cooled on ice, DNAs were denatured by the addition of 2.2 μl of 3 N NaOH and incubated at 37℃ for 30 minutes. The denatured DNAs were dissolved in 208 μl of urea/bisulfite solution (7.5 g of urea (Wako, Osaka, Japan) and 7.6 g of sodium metabisulfite (MERCK, Germany) dissolved in 20 ml of water, adjusted to pH 5.0) and 12 μl of 10 mM hydroquinone (SIGMA, USA) and overlaid with mineral oil. The samples were subjected to 30 cycles of 95℃ for 30 seconds and 55℃ for 15 minutes, followed by 55℃ for 15 hours in a PCR instrument. After the reaction, DNAs were purified using the Gene Clean Kit (Q-BIOgene, USA) and eluted with 20 μl of water. For the desulfonation, 3 N NaOH was added and the DNAs were incubated at 37℃ for 15 minutes. After ethanol precipitation, DNAs were eluted with 20 μl of water. PCR was performed in 50 μl of reaction mixture containing 5 μl of DNA as a template. The PCR condition was 94℃ for 2 minutes followed by 40 cycles of 94℃ for 20 seconds, 50℃ for 30 seconds, and 72℃ for 1 minute, and a final extension at 72℃ for 10 minutes. The primers employed are listed in Table 2. The PCR products were gel-purified and cloned. Ten to twelve independent clones were sequenced.

RESULTS

MSAP analysis was performed to detect sequences differentially methylated between organs using DNAs extracted from leaves, stamens, and pistils. In MSAP analysis using 72 primer pairs, 51 of 1861 bands showed polymorphism between organs (Fig. 1, Table 3). Thirty-three polymorphisms (64.7%) were observed between the vegetative organ (leaf) and reproductive organs (stamen and pistil). There were 12 polymorphisms specific to pistils (23.5%) and four specific to stamens (7.8%). Of these polymorphic bands, 29 bands were sequenced, and BLAST search of DDBJ revealed 23 of these sequences to be similar to the known sequences, i.e., 13 putative genic regions, five transposon-like sequences, one microsatellite sequence, and four sequences with no annotation (Table 4).

Bisulfite-sequencing analysis was performed to detect DNA methylation of these sequences. Flanking sequences of MSAPt-4(4), -12, -13, -19, and -54(1), which are parts of the putative genic regions showing polymorphism between different organs in MSAP, were characterized by suppression PCR. Bisulfite-sequencing analyses revealed differentially methylated cytosines between organs at CCGG sites of sequences of MSAPt-4(4), -12, and -19 (Fig. 2 and Table 5). A 197 bp sequence of MSAPt-4(4), which corresponds to the 10^{th} to 11^{th} intron of the putative serine carboxypeptidase 1 gene of *A. thaliana*, contained 42 cytosines, i.e., three in a CG context and 39 in a non-CG context. Only two cytosines in the CG context were methylated, and one of them was the recognition site of *Hap*II and *Msp*I used in MSAP analysis, CCGG. These methylation statuses differed between organs, and methylation levels of cytosines in leaves were higher than those in other organs (Fig. 2A) consistent with MSAP analysis, indicating more methylation of the CCGG site in leaves than in stamens and pistils. The methylation status of a 303 bp sequence of MSAPt-12 containing 58 cytosines, which corresponds to the 10^{th} to 12^{th} exon of a putative protein gene (homologous to At3g45045 of *A. thaliana*), differed in two CG contexts between organs (Fig. 2B). A 287 bp flanking sequence of MSAPt-19, which corresponds to the 3^{rd} exon to the 3^{rd} intron of a hypothetical protein gene (homologous to At4g14850 of *A. thaliana*), contained 51 cytosines, 13 of which are of the CG context and 38 of which are of the non-CG context. There were nine methylated sites, all of which were of the CG context. Of these sites, five differed in DNA methylation status between organs (Fig. 2C). Consistent with MSAP analysis, the target site was highly methylated in stamens. However, the methylation status in pistils was the same as that in leaves. In MSAPt-54(1), methylation levels did not differ between organs at the CCGG site recognized by MSAP analysis, but some cytosines were differentially methylated between organs (Fig. 2D, Table 5). A 270 bp flanking sequence of MSAPt-54(1), which corresponds to putative trehalose-6-phosphate synthase, contained 23 methylated cytosines including 14 in the CG context and 9 in the non-CG context. In this sequence, all the cytosines differentially methylated between organs were in the non-CG contexts, mainly in the CHH contexts. A 278 bp flanking sequence of MSAPt-13 contained 103 cytosines, but there was no methylated cytosine in any of the samples. Expression levels of these four genes analyzed by RT-PCR did not show the negative correlation between DNA methylation and expression levels (Fig. 3).

DISCUSSION

Since MSAP analysis, which can detect differences of DNA methylation in the whole genome using methylation-sensitive and -insensitive restriction endonucleases, does not require information of genome sequences, it is suitable for analyzing the difference of DNA methylation in Brassica, whose genome sequencing has not been completed. Many studies have revealed differences of DNA methylation by MSAP analysis in various plants (Xiong *et al.*, 1999, Xu *et al.*, 2004, Jaligot *et al.*, 2004, Labra *et al.*, 2004, Salmon *et al.*, 2005, 2008, Smykal *et al.*, 2007), but few reports have shown quantitative differences of methylation levels at each locus detected by MSAP analysis.

In the present study, we applied MSAP analysis to investigate the sequences having different methylation levels between vegetative organs and reproductive organs in *B. rapa*. The bisulfite sequence method revealed differences of methylation levels in three organs, but these differences did not completely correspond to the results of MSAP analysis.

Table 4. Annotation of sequences showing organ-specificity of DNA methylation in MSAP

band name	band pattern								
	leaf		stamen		pistil				
(MSAPt)	H	M	H	M	H	M	length(bp)	gene	Southern blot*
4(2)	-	-	+	+	+	+	320	microsatellite sequence	-
4(4)	-	-	+	+	+	+	232	Serine carboxypeptidase I	+
6	+	+	+	+	-	+	209	putative retroelement pol polyprotein	-
8	+	+	-	-	-	-	400	A. thaliana chloroplast genomic DNA, photosystem II G protein	+
10	-	-	+	+	+	+	294	no hit	+
11	+	+	-	-	-	-	141	B. rapa subsp. pekinensis clone KBrH070I10,complete sequence, B. oleracea var. alboglabra EST	+
12	-	+	-	+	+	+	250	putative protein similarity to KIAA1094 protein, Homo sapiens	+
13	-	-	-	-	+	-	246	Arabidopsis thaliana cDNA clone:RAFL07-17-L13	+
17(1)	+	+	+	+	-	+	284	A intact retrotransposon, Centromeric Retrotransposon of Brassica 1(CRB1)	+
17(2)	+	+	-	+	-	+	238	no hit	+
18	+	+	+	+	-	+	269	Brassica oleracea Contig C, complete sequence	-
19	+	+	-	-	-	-	179	A. thaliana mRNA for hypothetical protein, complete cds, clone;RAFL14-50-J09	+
27	+	+	+	-	+	-	209	Arabidopsis thaliana chloroplast genomic DNA,ATPase alpha subunit	+
28(2)	+	+	+	+	-	+	285	Brassica rapa subsp. pekinensis clone KBrB080J15, complete sequence.	+
28(3)	+	+	+	+	-	+	221	Brassica rapa subsp. pekinensis clone KBrB010M19, complete sequence	+
29(1)	+	+	-	+	-	+	439	Brassica napus putative diacylglycerol acyltransferase mRNA, complete cds.	+
30	+	-	-	-	-	-	247	ethylene responsive element binding factor-related	+
34	+	+	+	-	+	-	490	Arabidopsis thaliana chloroplast genomic DNA	+
35	-	-	+	+	+	+	111	B. napus mitochondorial DNA, ribosomal protein S12	+
37	+	-	-	-	-	-	173	A. thaliana putative disease resistance protein gene	+
38	+	+	+	-	+	-	479	no hit	+
39(4)	+	+	-	+	-	+	129	no hit	+
40	+	+	-	+	-	+	290	no hit	+
43	+	+	-	+	-	+	607	B. napus partial RT gene for reverse transcriptase from Ty3-gypsy-like retroelement 21G42-04	+
46	+	+	+	+	-	+	234	ARATH Copia-like retroelement pol polyprotein	-
54(1)	-	+	+	+	+	+	435	A. thaliana mRNA for putative trehalose-6-phosphate synthase, complate cds	+
54(2)	+	-	-	-	-	-	416	no hit	+
54(3)	+	+	+	+	-	+	269	Brassica oleracea Contig C, complete sequence	-
72	+	+	+	+	-	+	408	ARATH Putative retroelement pol polyprotein.	-

H, *Hap* II digestion; M, *Msp* I digestion; +, band present; -,band absent

*, Southern blot analysis was performed (+) or not performed (-). Tissue specific band pattern consistent with MSAP was appeared only in No. 38.

Table 5. Percentages of methylated cytosines in CCGG sites

band name	locus*	organ	rates of methylsted cytosine (%)	
			outer cytosine	inner cytosine
4(4)	41	leaf	0	54.5
		stamen	0	40
		pistil	10	40
12	39	leaf	9.1	36.4
		stamen	0	72.2
		pistil	0	45.5
13	151	leaf	0	0
		stamen	0	0
		pistil	0	0
19**	230	leaf	0	58.3
		stamen	0	91.7
		pistil	0	50
19	235	leaf	0	0
		stamen	0	0
		pistil	8.3	8.3
54(1)	113	leaf	80	100
		stamen	90	100
		pistil	70	100
54(1)**	119	leaf	20	100
		stamen	30	100
		pistil	10	100
54(1)	131	leaf	10	100
		stamen	0	100
		pistil	0	100

* indicates position of inner C

** indicates CCGG sites recognized in our MSAP analysis.

One possible explanation for the difference between the results of MSAP and bisulfite sequencing is that MSAP analysis is not quantitative and may detect a small difference of methylation levels as DNA polymorphism.

The sequences showing polymorphism between different organs detected by MSAP were mostly genes, 44.8% of the determined sequences. This percentage is much higher than the percentage of genic regions in the Brassica genome (Rabinowicz *et al*., 2005), suggesting that the regions of genes are subjected to organ-specific methylation more frequently than other regions. The ChIP-on-chip or bisulfite sequencing analyses have revealed that about one-third of expressed genes are methylated in their ORFs in *A. thaliana* (Zhang *et al*., 2006, Zilberman *et al*., 2007, Cokus *et al*., 2008, Lister *et al*., 2008). The present study showed that expression levels of genes whose ORFs were methylated organ-specifically did not differ between organs, suggesting low involvement of ORF methylation in the control of gene expression. Zhang *et al*. (2006) have reported that about 5% of genes methylated in promoter regions showed organ-specific expression. Methylation in the promoter region may be more important than that in the coding region for tissue-specific gene regulation.

The majority of differences of DNA methylation statuses between different organs in *B. rapa* detected by MSAP analysis were the differences between leaves and stamens/pistils. Differences in the methylation status among different organs or between different developmental stages have been found in several plants. HPLC analysis has revealed less methylation in immature tomato tissues, stems, leaves, and roots than in seeds, mature leaves, and fruits (Messeguer *et al*., 1991). Immunohistochemical analysis has detected change of methylation statuses during plant development in *Silene latifolia* (Zluvova *et al*., 2001). Different methylation patterns during development of *A. thaliana* have been reported, and differences in methylation between seedlings and adult plants

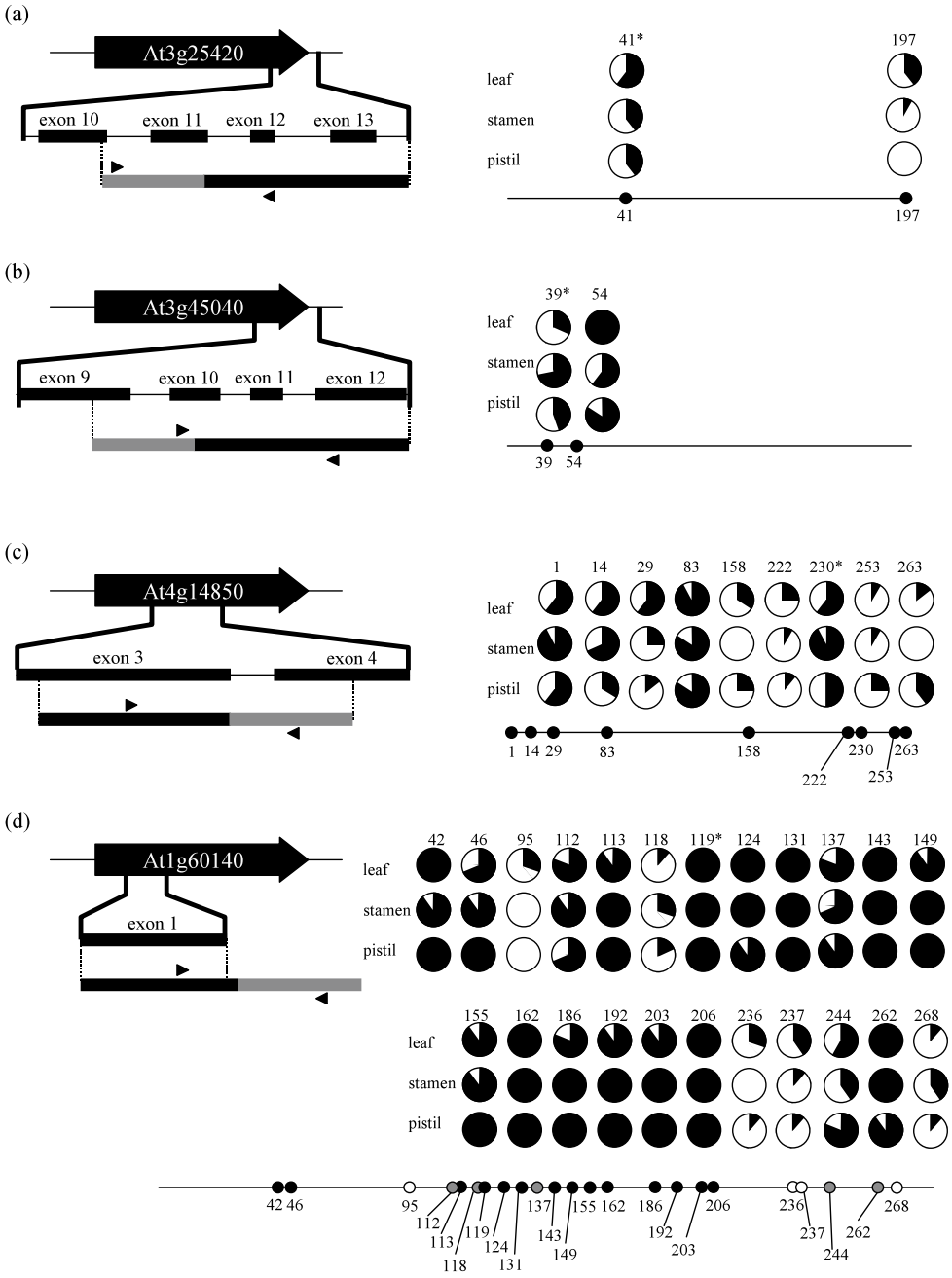

Figure 2. Differences of methylation levels between organs analyzed by bisulfite sequencing.
Analyzed gene structures are shown at the left. Large arrows indicate homologous genes in *A. thaliana*, and middle bars indicate the structure of the gene in *A. thaliana*. Lower bars represent sequences obtained by MSAP analysis (gray) and suppression PCR (black). Arrowheads show primers used in bisulfite-sequencing analysis. Rates of methylated cytosines are shown at the right. Small circles and numbers indicate methylated cytosines and their positions, respectively, and numbers with * indicate the recognition site in MSAP analysis. Black, gray, and white circles represent cytosines in CG, CNG, and CHH contexts, respectively. For cytosines differentially methylated between organs, proportions of methylated cytosine are shown as black parts of the pie chart. (a), (b), (c) and (d) indicate putative serine carboxypeptidase I (MSAPt-4(4)), putative protein (MSAPt-12), hypothetical protein (MSAPt-19), and putative trehalose-6-phosphate synthase (MSAPt-54(1)) respectively. Cytosines methylated in more than three clones in each organ were regarded as methylated cytosines.

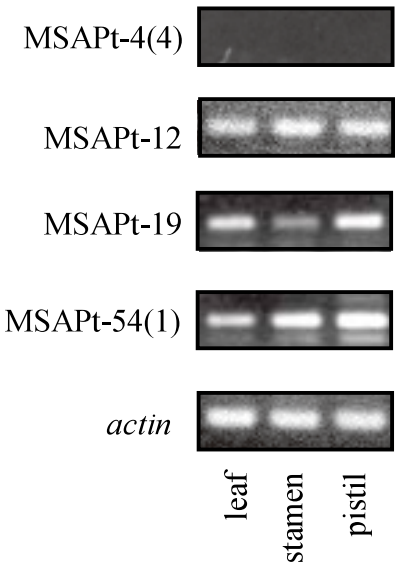

Figure 3. RT-PCR of genes showing polymorphic bands in MSAP, MSAPt-4(4), -12, -19, and -54(1).

have also been identified in rice (Ruiz-Garcia *et al*., 2005, Sha *et al*., 2005). Differences of DNA methylation patterns between different organs or tissues revealed in these previous studies and in the present study suggest the presence of some mechanisms controlling tissue-specific methylation. Since methylation patterns are the results of *de novo* methylation, demethylation, and the maintenance of existing methylation (Hsieh, 2000), these mechanisms may function during plant development. *A. thaliana* has *DRM2* as *de novo* methylation enzyme and *DME* and its paralogs as DNA demethylation enzymes (Cao and Jacobsen 2002, Morales-Ruiz *et al*., 2006, Penterman *et al*., 2007). In *B. rapa*, orthologs of these genes have not been identified, but genes involved in maintenance of DNA methylation have been characterized (Fujimoto *et al*., 2006). Interestingly, expression levels of *BrCMT* and *BrMET1a* have been shown to change drastically throughout stamen development (Fujimoto *et al*., 2006), and may be involved in tissue specificity of DNA methylation. Further investigations are required for elucidation of organ- and tissue-specific control of DNA methylation. Since the organs in *B. rapa* are much larger than those in *A. thaliana*, it may be advantageous to use *B. rapa* rather than *A. thaliana*.

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Brassica rapa における MSAP 法による器官特異的な DNA のメチル化の解析

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要　約

DNA のメチル化は遺伝子の転写制御に重要なエピジェネティックな変化である。本研究では、*Brassica rapa* の葉、雄しべ、雌しべの DNA のメチル化レベルの違いについて MSAP 法を用いて調べた。DNA のメチル化は葉と雄しべ / 雌しべで最も違っていた。DNA のメチル化が異なっていた配列の半分は遺伝子領域であったが、遺伝子の発現量は3つの器官で違いが見られなかった。MSAP 法で見出された領域の内、遺伝子領域が占める割合は、ゲノム中に占める遺伝子領域の割合よりも高かったことから、*B. rapa* において、器官特異的な DNA のメチル化の違いは遺伝子領域に起こりやすい可能性が示された。

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