Supporting Information

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SI Text

Analysis of the Evolutionary Conservation of the AS Events. How the AS events in the genes that we studied change the function, or lower the expression level, is unknown. Indeed, the functions of AS isoforms are currently poorly characterized in plants, with only a relatively small number (probably <100 genes) with functional characterization. The vast majority of the events we assayed are intron retention (IR), which is the most common type of AS in plants, with most of those causing premature stop codons that would result in the production of truncated proteins or potentially lower the level of gene expression by being degraded by nonsense-mediated decay. Evolutionary conservation can provide some clues as to the possibility of functionality.

We analyzed conservation of the AS events between *Brassica* and *A. thaliana* using publicly available EST data. We found that 24 events, including 18 IR events, were conserved between the two species (Table S5). However, the *Arabidopsis* EST collections are not comprehensive with regards to AS and thus there could be additional conserved events. Also we analyzed AS events in duplicated genes in *Brassica*, many of which probably originated during one of the ancient whole-genome duplication events in its lineage. We found 13 additional events that are conserved between the duplicated genes, all of which are IR. An additional eight AS events (including seven IR) show differential regulation in different organ types or under stress conditions (Table S2); developmental or stress-regulation of AS events has been suggested as evidence of true AS events (1). We also evaluated AS conservation across the four total accessions of the diploids *B. rapa* and *B. oleracea* that we studied. There were 33 additional conserved events, including 30 IR events, which were not found in the other analyses described above. Thus, there are a total of 68 IR events, of 72 that were assayed, that show evolutionary conservation, or developmental or stress regulation.

SI Materials and Methods

Plant Materials and Nucleic Acid Extractions. Plants included *B. napus* (canola cultivar Sentry summer rape; a gift of Peter McVetty, University of Manitoba, Winnipeg, MB, Canada) (2), *B. rapa* (Chinese cabbage cultivar MU525B; West Coast Seeds), and *B. oleracea* (cauliflower cultivar Semences; Rennies). Plants were grown from seed in growth chambers at 20 ± 0.5 °C under a 16-h day length (3) with 50% humidity for 2 wk (4). Plants were rotated to minimize edge effects. After 2 wk and 1 d, two different organ types, leaf and cotyledon, were collected from plants, frozen in liquid nitrogen, and stored in −80 °C until RNA extraction. As biological replicates, two sets of tissue from different plants were collected. Several plants were used per replicate. For the cold treatment on 2-wk-old seedlings, 20 °C was ramped to 4 °C so as not to induce cold shock, and the plants were then maintained at 4 °C for 24 h (5). For the heat treatment on the 2-wk-old seedlings, 20 °C was ramped to 38 °C and then maintained at 38 °C for 24 h (6). After the heat and cold treatments, the cotyledons and leaves were collected, frozen in liquid nitrogen, and stored at −80 °C. Stressed plants were collected on the same day as the nonstressed plants. All of the tissues were collected at about the same time of day to minimize circadian effects among samples.

Seeds of the resynthesized *B. napus* lines and their diploid parents were obtained from the Arabidopsis Biological Resource Center (ABRC accession nos. CS29001, CS29002, CS29003, and CS29008). The diploid parents were mostly or completely homozygous because they were created by self-pollination for five and eight generations in *B. oleracea* and *B. rapa*, respectively, followed by microspore culture to produce a doubled-haploid stock (7). The synthetic allotetraploid was created by Lukens et al. (6) by crossing doubled-haploid *B. rapa* (IMB218A) and *B. oleracea* (TO1000C) followed by spontaneous chromosome doubling or colchicine doubling. The lines were propagated to the fifth generation by single-seed descent (8). The resynthesized allopolyploids and their diploid parental lines were grown under the same conditions as the natural *B. napus*. Two organ types, cotyledons and leaves, were collected after 2 wk and 1 d at about the same time of day as the natural polyploids.

The cetyltrimethylammonium bromide (CTAB) method was used to extract total genomic DNA from young leaves (9). RNA was extracted using the method described by Chan et al. (10). The quantity of RNA and DNA was estimated using a spectrophotometer, and the quality of RNA was checked on 1.5% agarose gels after the DNaseI treatment. The RNA was treated with DNaseI (New England BioLabs) to remove the residual DNA.

Gene Choice, Sequence Alignments, and Assessment of Homeologs. Genes, putative functions, and accession numbers are listed in Table 1 and Table S1. Gene choice was done as follows: All available *B. oleracea* ESTs from the National Center for Biotechnology Information (NCBI; as of January 2009) were clustered using CD-Hit. Clusters containing multiple ESTs were retained and randomly selected for further analysis. The homologous gene from *A. thaliana* for each *B. oleracea* cluster was identified using BLAST searches. The TAIR database (http://www.arabidopsis.org/) was used to help identify AS events in the *Brassica* ESTs. Genes with no evidence of AS in *Brassica* were discarded. Also, genes with small alternative donor or alternative acceptor events, which would be difficult to separate from completely spliced transcripts on agarose gels after RT-PCR, were discarded.

Sequence alignments and phylogenetic analysis: *B. rapa*, *B. oleracea*, and *B. napus* ESTs and genomic sequences were retrieved from NCBI by BLAST searches. Sequencher 4.9 software was used to align the ESTs/cDNA of each gene to its genomic regions and make contigs to authenticate the identified AS. By comparing ESTs of *B. napus*, *B. rapa*, and *B. oleracea*, the SNP sites in *B. napus* ESTs were identified. Phylogenetic trees were constructed to verify homeologs and other paralogs in the gene families using maximum likelihood with default parameters in Molecular Evolutionary Genetic Analysis (MEGA) 4.1 (11).

RT-PCR Analysis of AS. PCR primers were designed with the Primer3 analyzer tool (12). All primers were designed to amplify both homeologs from *B. napus* and the corresponding genes from *B. rapa* and *B. oleracea* (Table S3), but not paralogous genes that were created by other duplication events. By sequencing all RT-PCR products from *B. napus* and some from *B. rapa* and *B. oleracea*, the amplification of the homeologous genes, and not the paralogous genes, was confirmed.

DNaseI-treated RNA (1 μg) was used to synthesize the first-strand cDNA. The cDNA was made from 1 μg of total RNA in a final reaction volume of 20 μL using oligo(dT) primers and M-MLV Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol. Oligo(dT) will prime mostly on polyadenylated transcripts to assay mostly fully synthesized transcripts. To confirm the absence of genomic DNA contamination, a parallel reaction without the reverse transcriptase enzyme was performed. For PCR analysis, 1 μL of cDNA or genomic DNA was amplified with 1 unit Paq5000 DNA polymerase (Stratagene), 1× Paq5000 reaction buffer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, and 0.25 μM of each primer in a final reaction volume

of 20 µL. The cycling conditions were 94 °C for 4 min; 34 cycles of 94 °C for 24 s, 60 °C for 27 s, and 72 °C for 1 min with a final extension period at 72 °C for 8 min. To ensure all reagents were free of DNA contamination, a negative control with water instead of template was used. Electrophoresis runs were used to resolve the amplified fragments on agarose gels (1.5%) and stained with ethidium bromide for visualization. By confirming the presence or absence of the AS band on agarose gels, one could compare the pattern of AS in *B. oleracea*, *B. rapa*, and *B. napus*. However, sequencing was necessary to determine whether one or both homeologs showed AS in *B. napus*. Some of the assayed genes did not show AS in the organ types or conditions that were used in this study, perhaps because of the presence of AS in different organ types or growth conditions; those genes were removed from this study because they were uninformative about AS.

Sequencing of AS Bands from the Polyploids and Homeolog-Specific Analysis of AS. RT-PCR bands were cut and purified by GenElute Gel Extraction Kit (Sigma-Aldrich). The purified DNA was reamplified by 20 cycles of PCR and precipitated by adding 1/10th vol of 3M sodium acetate (pH 5.2) and 2.5 vol of cold absolute ethanol. The mixture was incubated at −20 °C overnight. The nucleic acids were recovered by centrifugation, and the pellets were washed with 70% (vol/vol) ethanol. Finally, the air-dried pellets were dissolved in ddH_2O, and the quantities of the nucleic acids were measured with a spectrophotometer. Intron-specific primers (Table S3) were used to sequence the alternatively spliced bands that contain whole or partial intronic regions by using BigDye Terminator v3.1 (Applied Biosystems) sequencing chemistry.

After trimming the low-quality bases at the end of the chromatograms, the obtained sequences were aligned to the contig that contained *B. rapa*, *B. oleracea*, and *B. napus* sequence alignments. By assessing the SNPs sites, one could infer whether one or both homeologous genes were present in the AS band. The existence of double peaks in the chromatograms at polymorphic sites represented the case of AS in both homeologs. If a single peak corresponding to either the *B. oleracea* or *B. rapa*-specific nucleotide was present in the sequence, it was scored as presence of one homeolog. Several SNPs were assayed for each gene. If only one of the homeologous genes was present in the AS band, then sequencing of the major splice form was required to determine if both homeologs were expressed. If both of the homeologous genes were present in the major RT-PCR band, then one could conclude that both homeologs are expressed, and the lack of AS in one homeolog was not due to lack of expression of that homeolog. However, if only one of the homeologous genes was present in the major band, one can conclude that homeolog silencing or loss occurred. Finally, the presence of only one of the homeologous genes in genomic DNA would indicate gene deletion or recombination.

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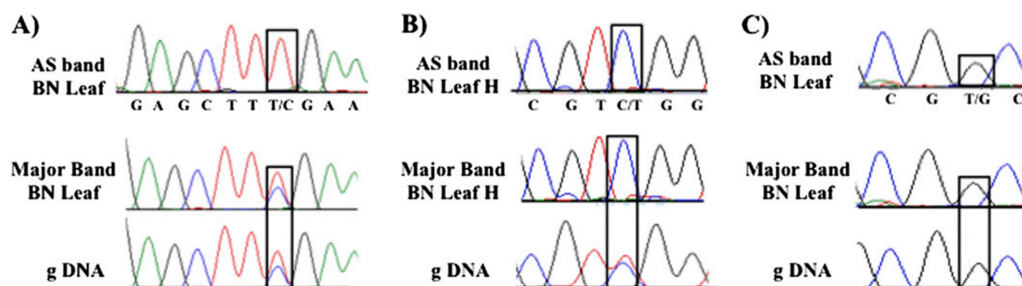

Fig. S1. Distinguishing among AS of only one homeolog, homeologous gene silencing, or homeologous gene loss/chromosome rearrangements cases in natural *B. napus* (BN). Chromatograms show sequencing of RT-PCR products from the AS band, major band, and PCR from genomic DNA, each containing one SNP site. Multiple SNPs were evaluated per gene. (*A*) Gene 17 showed only one peak at a SNP site in the AS band from leaf but two peaks in the major form band (fully spliced form) and in genomic DNA, indicating AS in only one homeolog. (*B*) Gene 9 showed one peak in both the AS form and major form in leaf under heat stress, but two peaks in genomic DNA, indicating silencing of one homeolog. (*C*) Gene 28-1 showed only one peak corresponding to one of the homeologs in the AS band, major form band, and in genomic DNA, indicating loss or rearrangement of one homeolog.

A)

	Leaf	Leaf Heat	Leaf Cold	Cotyl.	Cotyl. Heat	Cotyl. Cold	Leaf Synth. (I)	Cotyl. Synth. (I)	Leaf Synth. (II)	Cotyl. Synth. (II)
AC	51	50	52	51	45	49	39	39	42	42
A	10	11	11	11	12	12	15	15	14	14
C	10	10	8	10	12	10	14	14	14	14
A*	2	3	2	3	3	3	1	1	1	1
C*	1	1	2	1	2	1	7	7	4	4
A♦	3	3	3	3	3	3	2	2	2	2
C♦	1	1	1	1	1	1	0	0	0	0
NI	4	3	3	2	4	3	3	3	4	4
nd	0	0	0	0	0	0	1	1	1	1
Total	**82**	**82**	**82**	**82**	**82**	**82**	**82**	**82**	**82**	**82**

Fig. S2. AS events in natural and resynthesized *B. napus* lines. (*A*) AC, AS in both the A and C homeologs; A, AS only in the A homeolog; C, AS only in the C homeolog; A*, only expression of the A homeolog; C*, only expression of the C homeolog; A♦, loss of the C homeolog; C♦, loss of the C homeolog (note that the preceding two symbols are different from Table 1). Cotyl., cotyledon; synth., resynthesized allopolyploid; NI, no AS isoform present; nd, not determined. (*B*) Distribution of AS status from *A*. (*C* and *D*) Overlaps in AS status among the three genotypes.

Table S1. Sequence accession numbers, locations of AS, and types of AS

Gene pair	*Brassica* gene accession no.	Location of AS	Type of AS
1	EX092731	Intron 4	IR
2	BZ430218	Intron 3	IR
3	EX102573	Intron 3	IR
4	EX101076	Intron 3	IR
5	EE524832	Intron 1	IR
6-1	EX091462	Intron 2	IR
6-2	EV094871	Intron 8	IR
7	EV101436	Intron 3	IR
8	EE428842	Intron 1	IR
9	EX094209	Intron 4	IR
10	EH416211	Intron 1	IR
11	EX044606	Intron 2	IR
12	ES967018	Exon 2	ES
13	EX112955	Intron 2	IR
14	EE467429	Intron 1	IR
15	CX273025	Intron 1	IR
16	EV040303	Intron 2	IR
17	GR439896	Exon 3	ES
18	EE455614	Intron 2	IR
19	EX118242	Intron 3	IR
20	EX088617	Intron 4	ES
21	EX087677	Intron 1	IR
22	EH425494	Intron 1	IR
23	EV226178	Intron 11	IR
24	DU830597	Intron 7	IR
25	CX266395	Intron 8	IR
26	CO750639	Intron 2	IR
27	EV155331	Intron 1	IR
28-1	EV194328	Intron 4	IR
28-2	FG570383	Intron 2	IR
29	EX027867	Intron 6	ES
30	DU833471	Intron 2	IR
31	EE411222	Intron 4	IR
32	CB686408	Intron 3	IR
33	EE517739	Intron 2	IR
34	DY005808	Intron 1	IR
35	EX118932	Intron 2	IR
36	EX089805	Intron 2	IR
37	EV043623	Intron 7	IR
38	EX093589	Intron 1	IR
39-1	EX091499	Exon 2	ES
39-2	EX091499	Intron 2	AP
40	EV069552	Intron 3	IR
41	DN963102	Intron 2	IR
42	EV133401	Intron 8	IR
43	CN736572	Intron 2	IR
44	EX023963	Intron 3	IR
45-1	EX090312	Intron 3	IR
45-2	EX090312	Intron 3	AA
46	CN830429	Intron 1	AA
47	EE454130	Intron 2	IR
48	EV226263	Intron 1	IR
49	EX093729	Intron 2	IR
50	FG553573	Intron 2	IR
51	DY006239	Exon 2	ES
52	ES932238	Intron 1	IR
53	EX112152	Intron 1	IR
54	EX091109	Intron 1	IR
55	EV056008	Intron 2	IR
56	EX087897	Intron 5	IR
57	CX281473	Intron 1	IR
58-1	EX087358	Intron 1	IR

Table S1. Cont.

Gene pair	*Brassica* gene accession no.	Location of AS	Type of AS
58-2	EX087358	Intron 3	IR
59	ES901878	Intron 8	IR
60	ES906610	Intron 1	IR
61	EV183065	Intron 4	IR
62-1	EV029046	Intron 1 and 2	IR
62-2	EV029046	Intron 1	IR
63	EE465989	Intron 2	IR
64-1	EX090185	Intron 1 and 2	IR
64-2	EX090185	Intron 2	IR
65	DC844896	Intron 1	IR
66	EX133829	Intron 1	IR
67-1	EX086361	Intron 2	IR
67-2	EX086361	Exon 3	ES
68	EX111656	Intron 3	IR
69	EV050778	Intron 2	IR
70	EX086611	Intron 3	IR
71	EX071437	Intron 1	IR
72	EX125049	Intron 8	IR
73	EX095546	Intron 3	IR
74	EX123946	Intron 1	IR

Gene pair numbers are the same as in Table 1. Locations of AS are relative to the homologous gene in *A. thaliana*. Types of AS: IR, intron retention; ES, exon skipping; AA, alternative acceptor; AP, alternative position.

Table S2. AS patterns in homeologs of *B. napus* in leaves and cotyledons and under heat and cold stresses

Gene pair	Leaf	Leaf heat	Leaf cold	Cotyl	Cotyl heat	Cotyl cold
1	AC	AC	AC	AC	AC	AC
2	AC	AC	AC	AC	AC	AC
3	AC	A	A	A	A	A
4	AC	AC	AC	AC	A	A
5	AC	AC	AC	AC	AC	AC
6-1	C	C	AC	C	C	C
6-2	C	C	AC	C	C	C
7	AC	AC	AC	AC	AC	AC
8	AC	AC	AC	AC	AC	AC
9	AC	A*	AC	A*	A*	A*
10	AC	AC	AC	AC	AC	AC
11	NI	NI	NI	NI	NI	NI
12	AC	AC	AC	AC	AC	AC
13	AC	AC	AC	AC	AC	AC
14	AC	AC	AC	AC	AC	AC
15	AC	AC	C*	AC	C*	AC
16	A	A	A	A	A	A
17	A	A	A	A	A	A
18	AC	AC	AC	AC	AC	AC
19	AC	AC	AC	AC	C	AC
20	AC	AC	AC	AC	AC	AC
21	AC	AC	AC	AC	AC	AC
22	AC	AC	AC	AC	AC	AC
23	AC	AC	AC	AC	AC	AC
24	AC	AC	AC	AC	AC	AC
25	AC	AC	AC	AC	AC	AC
26	A–	A–	A–	A–	A–	A–
27	AC	AC	AC	AC	C	AC
28-1	A–	A–	A–	A–	A–	A–
28-2	A–	A–	A–	A–	A–	A–
29	A	A	A	A	A	A
30	A	A	A	A	A	A
31	C–	C–	C–	C–	C–	C–
32	C	C	C	C	C	C
33	A	A	A	A	A	A
34	AC	AC	AC	AC	AC	AC
35	AC	AC	AC	AC	AC	AC
36	AC	AC	AC	AC	AC	AC
37	AC	AC	AC	AC	AC	AC
38	A	A	A	A	A	A
39-1	AC	AC	AC	AC	AC	AC
39-2	C	C	C	C	C	C
40	C	C	C	C	C	C
41	AC	AC	AC	AC	AC	AC
42	AC	AC	AC	AC	AC	AC
43	A*	A*	A*	A*	A*	A*
44	NI	NI	NI	NI	NI	NI
45-1	AC	AC	AC	AC	AC	AC
45-2	AC	AC	AC	AC	AC	AC
46	AC	AC	AC	AC	AC	AC
47	C	C	C	C	C	C
48	AC	AC	AC	AC	AC	AC
49	A	A	A	A	A	A
50	AC	AC	AC	AC	AC	AC
51	A*	A*	A*	A*	A*	A*
52	AC	AC	AC	AC	AC	AC
53	AC	AC	AC	AC	AC	AC
54	AC	AC	AC	AC	AC	AC
55	AC	AC	AC	AC	AC	AC
56	AC	AC	AC	AC	AC	AC
57	C*	C*	C*	C*	C*	C*
58-1	NI	AC	NI	NI	AC	NI

Table S2. Cont.

Gene pair	Leaf	Leaf heat	Leaf cold	Cotyl	Cotyl heat	Cotyl cold
58-2	NI	NI	AC	NI	NI	AC
59	AC	AC	AC	AC	AC	AC
60	AC	AC	AC	AC	AC	AC
61	AC	AC	AC	AC	AC	AC
62-1	AC	AC	AC	AC	AC	AC
62-2	AC	AC	AC	AC	AC	AC
63	AC	AC	AC	AC	AC	AC
64-1	AC	AC	AC	AC	AC	AC
64-2	C	C	C	C	C	C
65	A	A	A	A	A	A
66	A	A	A	A	A	A
67-1	AC	AC	AC	AC	AC	AC
67-2	AC	AC	AC	AC	AC	AC
68	C	C	C	C	C	C
69	C	C	C	C	C	C
70	C	C	C	C	C	C
71	AC	AC	AC	AC	AC	AC
72	AC	AC	AC	AC	AC	AC
73	AC	AC	AC	AC	AC	AC
74	A	A	A	A	A	A

A is the homeolog derived from *B. rapa*, and C is the homeolog derived from *B. oleracea*. AC, AS in A and C homeologs; A, AS in A homeolog only; C, AS in C homeolog only; A*, no expression of the C homeolog; C*, no expression of the A homeolog; A−, loss of the C homeolog; C−, loss of the A homeolog; NI, no AS isoform present.

Table S3. Primers used for PCR reactions

Gene no.	F primers	R primers	Intron- or exon-specific primers
1	AAATTCTGGGTGGTGAAGCA	AAATTCTGGGTGGTGAAGCA	TTTTGGTTCTTTACKTGAA
2	CAACGGATCAGTCAATTTGCAG	TAATCTCGGGAAGTAACGGATG	AAAGAAGACGATCCAAGTCAGA
3	CAACTGTGAAGTTGGTGTCTTTTC	CGATTCTCTGGACCACAACTTG	TGATATTGTCCACTTGC
4	GCTCTCGGTTACGGTTGAG	CACTTGCTGCTGCTAGGAAA	GTTGCKACTAGTTTTTGTGTTAGCTCT
5	AACAGCGACGATGAAGAAGACT	CTTCTTGTTGTTGTTCTCCCAA	GTTTCCMAATTTAGAAAGTT
6-1	CGGGGTCTTTCTCCTTCG	AAACAGCCTTGAACCTATCTGC	CCAGGGAAATGCAAAGAGAA
6-2	AGATCACCTGTTTCTGCAACCT	TCTCTTACGCTGACCAAGCA	GTAAGAAAAGGTCAAATGAGG
7	TCAAGGGTGTGTCCTTCTTATG	TGTGATGAAGCTTCTTGTGTTC	GTAAGYTTAAACACATACA
8	CCGTGAACTCCGTGCTCTCAT	GGATGCTTCGTTTTCACGTCCT	TTTTTTGTGTACTGCATGTGC
9	TGCTGAGTTTAGGTTTCAATATGGT	ATCTCCTCCGTTTCCAGGC	MWGGATTGCAGAYAAGTCACA
10	CAAGAAGATATTCGAGAAGAGTAGTGTT	TTGATCCGGGAAATTCGATAG	TGAAGCTTGCAAGACCTTTG
11	CTAGCACCGAGAGATTGGAACAG	GAGACACAAGCCAAGGACTTCAA	GTATATAAAATCTCATAATAGCTTT
12	GCGCGTGTCCTGCACTAAATA	CGAAAGTCTCGTTCCAATGACTT	TGTCCTGCACTAAATACTTCGAC
13	ACTTCTCCATCGCTTCTTCCTC	TTTCTCCACAAATCTATGCACCA	CTAATATAACATAGCCTAGG
14	TCTTCTGTCTGTGTCTGATTCTACG	GGAGATTCCATGGGGTAATGTT	TTAAGCAAGATCGCGTG
15	AGGGAATATCTACTCGGTGAAGC	TGCTTCTTTAGCTCGGAGACC	TGACGAAAGTGATTGGCTTTT
16	TGAGAAGCAGCGAAAGAGAGGA	GCAAGTAGCGGAACAACAAAGG	CTGCCTATCGCAATTCACAC
17	CACTCCGACAACGCCACA	TTTAACGGTGCTGACTCCTTGAC	GATAACGAAGCTCAAAGGAA
18	TCCTCCGTTGTACTATGGTCCT	GCAGCTTCACGATACTTGGG	CATCCATGGAYAGGGTTGAT
19	CCACCACTAAGGGAGGATGA	CATCCCTGTATTTCTTGATGCTT	TAGCTTGTCAAGTGGCATGG
20	CCTCACACACACATGGATCG	AACAATTTTCACTGCCACCAT	CCACGCTATAAAATATATAG
21	TTCTTCGCTGCAGACAGATCC	AACATCTCAGCGTGGTCTCGT	CCGTTTGTGGAGGTGAAGAA
22	AGAGCTCGCGTTATCTCCTG	CCTCCACCTCCACAGTTCAT	CTCGACGATAATATCGCACCGA
23	AAGTTTGGATTCATCTGGGGAA	TAATGAGAGCCTGCAAATAAAGAAAAG	GCATAAATTAACAATGGGTTGC
24	TTACTTGTTCGCAATCTCCGG	GGCTTCTTTCTGTTCTCCTCTG	CTGGTCAAAGTCGAAGTTAGTACA
25	AAGTTCAGCTGGTCCATCTTCGG	GCTAAAGTTGAAGCAGCTTGAG	CAGCCAAAGAAGAAGGGATG
26	GGAACACTCGTCTGTACGTTGGA	AATGTCTCGCGTCATCAGCATCACGG	TACACATCCCTTCTTCTTTG
27	ATCCCTCATCGACTTCTCATCCTTGA	GAGTACCGGTGCTTCGTGGG	GGATCAGAYCATCGGAAAYC
28-1	CTTGAGCGTCTTTTCAGCAGATACG	CGTCATCAGCATCACGAGGAT	—
28-2	CGAGATGTTGATATGAAGCGTGA	CCATCAACGTCCCTTCCG	—
29	GGCCTGGTGCTATGCTTATG	CCAAGAATAAGATCAAAGCCATC	AAACAGAAGARACACATCAATAGC
30	TTGTGTGTGAGAAAGGCATGT	CCAGTTATAGGACCATAGATTTTGGA	GTAATGTTATCTGCTGATAATGATGT
31	AATGGGAATTGATCTGAAGACGT	CCTCCAATACCAGAAAGCGAAC	TTATAAGAGTAGCCTCCTGGAGTTGA
32	AACCCGAAGCAGAAGCAA	ACATGAAGGGCACATGGTC	GTAGGGAAACACAACAACAC
33	TGATCCTGACGAGAGGCTTAAT	AAGACCTCCATTGAGCTGATTC	CAAGGTTCTAAACACAGTTCTT
34	TCTTCGTGAAAACCCTCACC	CAAACACAATCAGAGAGCAAGAAC	GTGTTGAAACTGAGCTCTGC
35	ACGTTCAAGCAAGCATGTGTAG	CCTTGTCCTCCTCTCTTAATGG	GAGAGAGTTTGGATATAACAAAC
36	GATCGATAACGGTGATTCCG	GACGGTTGTACATCACTCGG	CTTTATCTTAGGTTGTCGATTGAG
37	TGCTCTTAGAACAGGCACTGA	GCTCAAAGCAAATGCAACAGAGT	CTGTTAACAACGTATTATACC
38	GGAGGACTCATGGGAACACTCT	TCGGTGAGTAATGGTAAAGTGG	TCAATCAAGAAACTCACTTC
39	TGTCGGGATAAACGCGATG	CGGAAGCAACCCACTTCTAA	—
40	GAGGTTGGGATCAAATCAGTT	CTTTTATCTTTACGCCACTTCTCTT	ACCTCACCACTAGCAAATTTTC
41	CTCGTCCTTCTTCGATTCTCAG	CATGGACAAGCCAGAAGAGAGA	GTTATCGTTTCGCTGAAATG
42	CGGATGGGACTGTCTGACAAAG	AGTGCGGTGCAGTCTGTTATCG	GTAAGATTCTTTAAATTCCTG
43	GGTGGTCTTATCCAGCAGCATCT	AGGGAGGCTAAAGGGAAGTTTGA	ATCAATCATCCCATTATGAC
44	TTACCCGTGCTTCGACCCTAA	CGTACTGCTTCTCCACGGCA	TATGTCAAATTCAAGTTCTTTC
45-1	GTGTCCTCCGATTCGTCAGACT	CTTCTACGCTCACCATCGTCCT	—
45-2	GTGTCCTCCGATTCGTCAGACT	CTTCTACGCTCACCATCGTCCT	AAAACGAGACAAAGCTCACAC
46	GTTACGGTTCCTCTCGTTGG	GTCTGAGGATGAAACTGTCTGC	GCTTGGCGTTTTAAATTACAG
47	CCCTGTGGTTTCTGTTTCGT	CTTCTACCACGTCCCCATTG	ACAGGGTAAGTTTCATTTC
48	AGTACCGGTGCTTCGTGGTA	CTGAGCCTCGTTGACGGAA	GTCCGTTACACGAGAGATCG
49	CTCGACGAATCCGTCTCTGTC	ATCAGCTGCAACACCCCATT	ATATCCTTTCTTATTGCTTTGG
50	GAGGAAGAGGACGAGAAGGACT	GCAGTCTTCCCCATACCAGA	AGAGCCAAAGAAAATTGCAGC
51	GGATCGAATAGAATCCACGAGT	CCTTCTTCAGCTGTACCAGTATATCT	—
52	GTCGGCAAAGGCTTCGAG	GAGCATTCTCTGGCCAACCT	CTCTCTGCGAGATTACCAAA
53	ACGCAAGCAAAACGAGCAG	TGACCGGAGAGGAGGAAGAG	CCTGAGAGAGATAACGTTTAT
54	TGATTGGTGGGCTTGGCC	AGCCACCACAGCCTTAAGGGAC	AAGGTTCTGTATTATTCCTC
55	CCTTTTAGGTATATAAACTCTGACGA	CTTCCCTTACTGCAGCTTCTT	AATTGTAGTTGCTGGTACC
56	CCGGTAGATCGATCCAGCTA	CTTTCTGTGATGTGAGATCACAC	GTTAAGTGTGATAATAAGCATAC
57	GCGTGTCGTGACTCTTGAATC	ATATCAACCTTACCGAAATGGTTTA	GTAACTCCTCGAAAGTTTGTT
58	CGGAATCTCTTCTTCCGTTTCC	TCACCTTGCCACCTTTGCTA	—
59	CCTAAAGAGCCTAGAATTCCGCGA	CCACCTTCTGGTTCTTCTTCCTCAAC	CTGAAACCAAAGAGAAGCAAATTCA
60	GATAACCCCAGGAAGGCTACT	AGTCCTCAAGAATCTCAGCATACC	GAAAGTCAGATTCTTTGATAGG

Table S3. Cont.

Gene no.	F primers	R primers	Intron- or exon-specific primers
61	AGCCAAGGAATTCTCCAAGGCC	GCGTCCTGGATCTGTTTCATGTTA	TCAATGTTTCTGCCAAAACACACATC
62-1	CTTCCGTTCAACAACAGTCAGATG	GCGCCACCCAATTTCCA	GATAAAAAGGTTTCTCCTTTAC
62-2	CTTCCGTTCAACAACAGTCAGATG	GCGCCACCCAATTTCCA	GTAAGAGACCCTTCTAAATCC
63	CAGAGTGCACCAGACTTCACAC	CACTCCCCAATCTTTCCTCA	CTATTGTACAAAGTAAACCATG
64-1	GAAGGTGAAGTCCTTGGTATGG	CTCTTTCTCTTGAGTGAATCTCC	TAATTATTGTATCTGTTGCAGG
64-2	GAAGGTGAAGTCCTTGGTATGG	CTCTTTCTCTTGAGTGAATCTCC	CTGATAGATAAGTTAGAACC
65	CACGGAATCAACCGAAAGTT	GACAGAAACAAGGCCAAAGC	TAAATTCACCATCGACCCAT
66	CCTCCCCTCTCCAAGAAACTC	ATTCATCATCTCCCTTACAAATAACTC	TTTCTCGGGTTTGCATAGGA
67-1	GACCATGGAACCCTTCAAAAC	TGAAGCAAGTAACCTCCAATTCC	AAGTGTTGCATCTGGTTTAC
67-2	GACCATGGAACCCTTCAAAAC	TGAAGCAAGTAACCTCCAATTCC	—
68	CTTCGTTCACCGTCGTACCT	TAGCCCAATCATTTCGATCC	CTACAACAAACACACACCACGTA
69	GATGAGCGACGGAGACGTT	CCTTGGGTGGTTTGATTCCT	GATGTAACCTTTCTTTCCTT
70	AATGTACATGGAGGATAGGACTCTTC	CTCCAAAGTCACGAGCTTTGTTA	TGCAAGTAAAGCCTTCTGTG
71	GAGTAAGATGCTGCGCTGTTAA	CTCCCTGAAGCTAAACTCCACT	GACGCAATCAAGTGACCAAG
72	GATGGAGGTGATGAGTAGACAAA	ACACATCCCATAAAGGATTGG	ATCACATCTTATTTGTGGCAGT
73	AACGGAGGCATCACCTTTGA	AGAGGCAGCGATCATCATTTTG	GGTAATATTTTCTTCTTTTAC
74	AGCCCTGGGAGATCGGTTC	CACTGCTTCCTTCCCACATGAG	AAAGACCAAAAGAATGTAAATTCAG

Table S4. Presence (yes) or absence (no) of AS events in the diploids *B. rapa* and *B. oleracea*

Gene no.	B. rapa 1	*B. rapa* 2	*B. oleracea* 1	*B. oleracea* 2
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes
5	Yes	Yes	Yes	Yes
6-1	Yes	Yes	Yes	Yes
6-2	Yes	Yes	Yes	Yes
7	Yes	Yes	Yes	Yes
8	Yes	Yes	Yes	Yes
9	Yes	Yes	Yes	Yes
10	Yes	Yes	Yes	No
11	Yes	Yes	No	Yes
12	Yes	Yes	Yes	Yes
13	Yes	Yes	Yes	Yes
14	Yes	Yes	Yes	Yes
15	Yes	Yes	Yes	Yes
16	Yes	Yes	No	No
17	Yes	Yes	Yes	Yes
18	Yes	Yes	Yes	Yes
19	Yes	Yes	Yes	Yes
20	Yes	Yes	Yes	Yes
21	Yes	Yes	Yes	Yes
22	Yes	Yes	Yes	Yes
23	Yes	Yes	Yes	Yes
24	Yes	Yes	Yes	Yes
25	Yes	Yes	Yes	Yes
26	Yes	Yes	Yes	Yes
27	Yes	Yes	Yes	Yes
28-1	Yes	Yes	Yes	Yes
28-2	Yes	Yes	Yes	Yes
29	Yes	Yes	No	No
30	Yes	Yes	No	Yes
31	Yes	Yes	Yes	Yes
32	Yes	Yes	Yes	Yes
33	Yes	Yes	No	No
34	Yes	Yes	Yes	Yes
35	Yes	Yes	Yes	Yes
36	Yes	Yes	Yes	Yes
37	Yes	Yes	Yes	Yes
38	Yes	Yes	No	No
39-1	No	No	No	No
39-2	No	No	Yes	Yes
40	Yes	Yes	Yes	Yes
41	Yes	Yes	Yes	Yes
42	Yes	Yes	Yes	Yes
43	Yes	Yes	Yes	Yes
44	Yes	Yes	No	No
45-1	Yes	Yes	Yes	Yes
45-2	Yes	Yes	Yes	Yes
46	Yes	Yes	Yes	Yes
47	Yes	Yes	Yes	Yes
48	Yes	Yes	Yes	Yes
49	Yes	Yes	Yes	Yes
50	Yes	Yes	Yes	Yes
51	Yes	Yes	Yes	Yes
52	Yes	Yes	Yes	Yes
53	Yes	Yes	Yes	Yes
54	Yes	Yes	Yes	Yes
55	Yes	Yes	Yes	Yes
56	Yes	Yes	Yes	Yes
57	Yes	Yes	Yes	Yes
58-1	No	No	No	No

Table S4. Cont.

Gene no.	B. rapa 1	*B. rapa* 2	*B. oleracea* 1	*B. oleracea* 2
58-2	No	No	No	No
59	Yes	Yes	Yes	Yes
60	Yes	Yes	Yes	Yes
61	Yes	Yes	Yes	Yes
62-1	Yes	Yes	Yes	Yes
62-2	Yes	Yes	Yes	Yes
63	Yes	Yes	Yes	Yes
64-1	Yes	Yes	Yes	Yes
64-2	Yes	Yes	Yes	Yes
65	Yes	Yes	No	No
66	Yes	Yes	Yes	Yes
67-1	Yes	Yes	Yes	Yes
67-2	Yes	Yes	Yes	Yes
68	Yes	Yes	Yes	Yes
69	No	No	Yes	Yes
70	Yes	Yes	Yes	Yes
71	Yes	Yes	Yes	Yes
72	Yes	Yes	Yes	Yes
73	Yes	Yes	Yes	Yes
74	Yes	Yes	Yes	Yes

B. rapa 1 is Chinese cabbage cultivar MU525B; *B. rapa* 2 is a doubled-haploid line IMB218A that was a parent of the resynthesized allopolyploids (6); *B. oleracea* 1 is cauliflower cultivar semences; and *B. oleracea* 2 is a doubled-haploid line TO1000C that was a parent of the resynthesized allopolyploids (1).

1. Lukens LN, et al. (2006) Patterns of sequence loss and cytosine methylation within a population of newly resynthesized *Brassica napus* allopolyploids. *Plant Physiol* 140:336–348.

Table S5. Evolutionary conservation of AS events between species and between duplicated genes in *Brassica*

Gene no.	*Brassica* gene accession no.	Conservation between *Brassica* and *Arabidopsis*	AS event found in another *Brassica* duplicate	AS event conserved among all four *B. rapa* and *B. oleracea* accessions
1	EX092731	—	No	Yes
2	BZ430218	DP	n/a	Yes
3	EX102573	—	No	Yes
4	EX101076	—	No	Yes
5	EE524832	SP	Yes	Yes
6-1	EX091462	DP	No	Yes
6-2	EV094871	DP	No	Yes
7	EV101436	SP	No	Yes
8	EE428842	SP	n/a	Yes
9	EX094209	—	No	Yes
10	EH416211	SP	No	No
11	EX044606	SP	No	No
12	ES967018	SP	n/a	Yes
13	EX112955	SP	No	Yes
14	EV117694	SP	No	Yes
15	CX273025	DP	Yes	Yes
16	EV040303	DP	No	No
17	GR439896	SP	No	Yes
18	EE455614	SP	Yes	Yes
19	EX118242	DP	No	Yes
20	EX088617	SP	n/a	Yes
21	EX087677	—	No	Yes
22	EH425494	DP	n/a	Yes
23	EV226178	DP	n/a	Yes
24	DU830597	SP	No	Yes
25	CX266395	DP	Yes	Yes
26	CO750639	DP	Yes	Yes
27	EV155331	SP	Yes	Yes
28-1	EV194328	SP	No	Yes
28-2	FG570383	DP	No	Yes
29	EX027867	SP	n/a	No
30	DU833471	DP	n/a	No
31	EE411222	—	No	Yes
32	CB686408	—	n/a	Yes
33	EE517739	DP	n/a	No
34	DY005808	—	n/a	Yes
35	EX118932	DP	No	Yes
36	EX089805	—	No	Yes
37	EV043623	DP	No	Yes
38	EX093589	—	n/a	No
39-1	EX091499	DP	n/a	No
39-2	EX091499	SP	n/a	No
40	EV069552	—	No	Yes
41	DN963102	—	No	Yes
42	EV133401	SP	n/a	Yes
43	CN736572	DP	n/a	Yes
44	EX023963	—	No	No
45-1	EX090312	SP	n/a	Yes
45-2	EX090312	SP	n/a	Yes
46	CN830429	—	n/a	Yes
47	EE454130	DP	n/a	Yes
48	EV226263	SP	Yes	Yes
49	EX093729	—	Yes	Yes
50	FG553573	SP	Yes	Yes
51	DY006239	DP	No	Yes
52	ES932238	SP	No	Yes
53	EX112152	SP	n/a	Yes
54	EX091109	SP	Yes	Yes
55	EV056008	DP	Yes	Yes
56	EX087897	—	Yes	Yes
57	CX281473	—	No	Yes
58-1	EX087358	—	Yes	No

Table S5. Cont.

Gene no.	*Brassica* gene accession no.	Conservation between *Brassica* and *Arabidopsis*	AS event found in another *Brassica* duplicate	AS event conserved among all four *B. rapa* and *B. oleracea* accessions
58-2	EX087358	—	No	No
59	ES901878	—	n/a	Yes
60	ES906610	—	n/a	Yes
61	EV183065	DP	No	Yes
62-1	EV029046	—	Yes	Yes
62-2	EV029046	—	Yes	Yes
63	EE465989	—	n/a	Yes
64-1	EX090185	DP	No	Yes
64-2	EX090185	DP	Yes	Yes
65	DC844896	—	n/a	No
66	EX133829	—	Yes	Yes
67-1	EX086361	DP	No	Yes
67-2	EX086361	DP	No	Yes
68	EX111656	—	n/a	No
69	EV050778	—	Yes	Yes
70	EX086611	—	No	Yes
71	EX071437	—	Yes	Yes
72	EX125049	—	n/a	Yes
73	EX095546	DP	n/a	Yes
74	EX123946	—	No	Yes

SP, an AS event in the same position in *Brassica* and *Arabidopsis*; DP, an AS event in a different position in the homologous gene between *Brassica* and *Arabidopsis*; dashes indicate no shared AS event was found; n/a, no duplicate was found in *Brassica* (excluding homeologs in *B. napus*).