

MutS HOMOLOG1 silencing mediates *ORF220* substoichiometric shifting and causes male sterility in *Brassica juncea*

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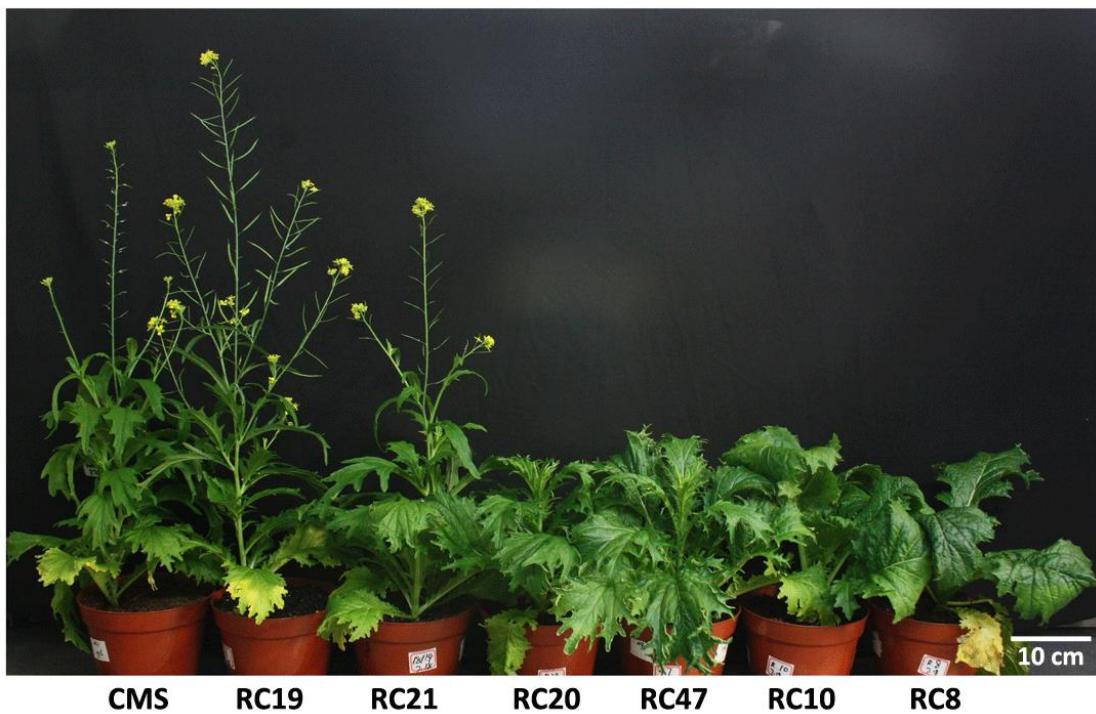


Figure S1 Candidate revertant lines of *B. juncea*.

Figure S2 Comparison of mitochondrial DNA from CMS and revertant lines of *B. juncea*.

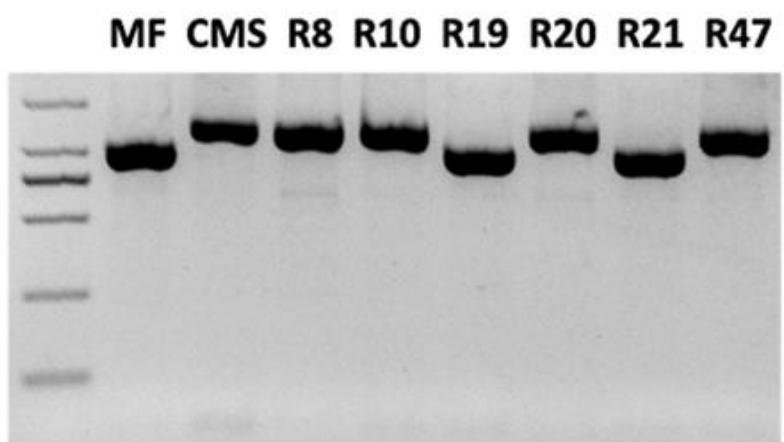


Figure S3 Mitochondrial genome rearrangement of the *atpA* gene.



Figure S4 Schematic diagram of ORF220 gene construction.

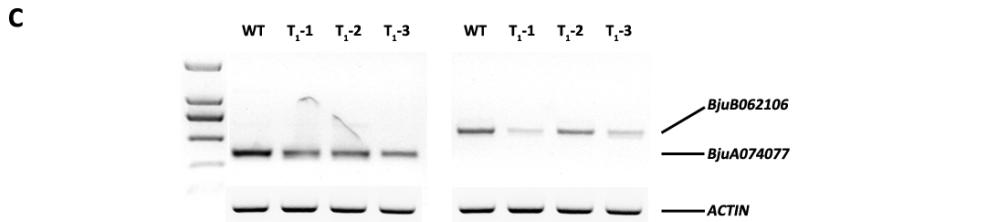
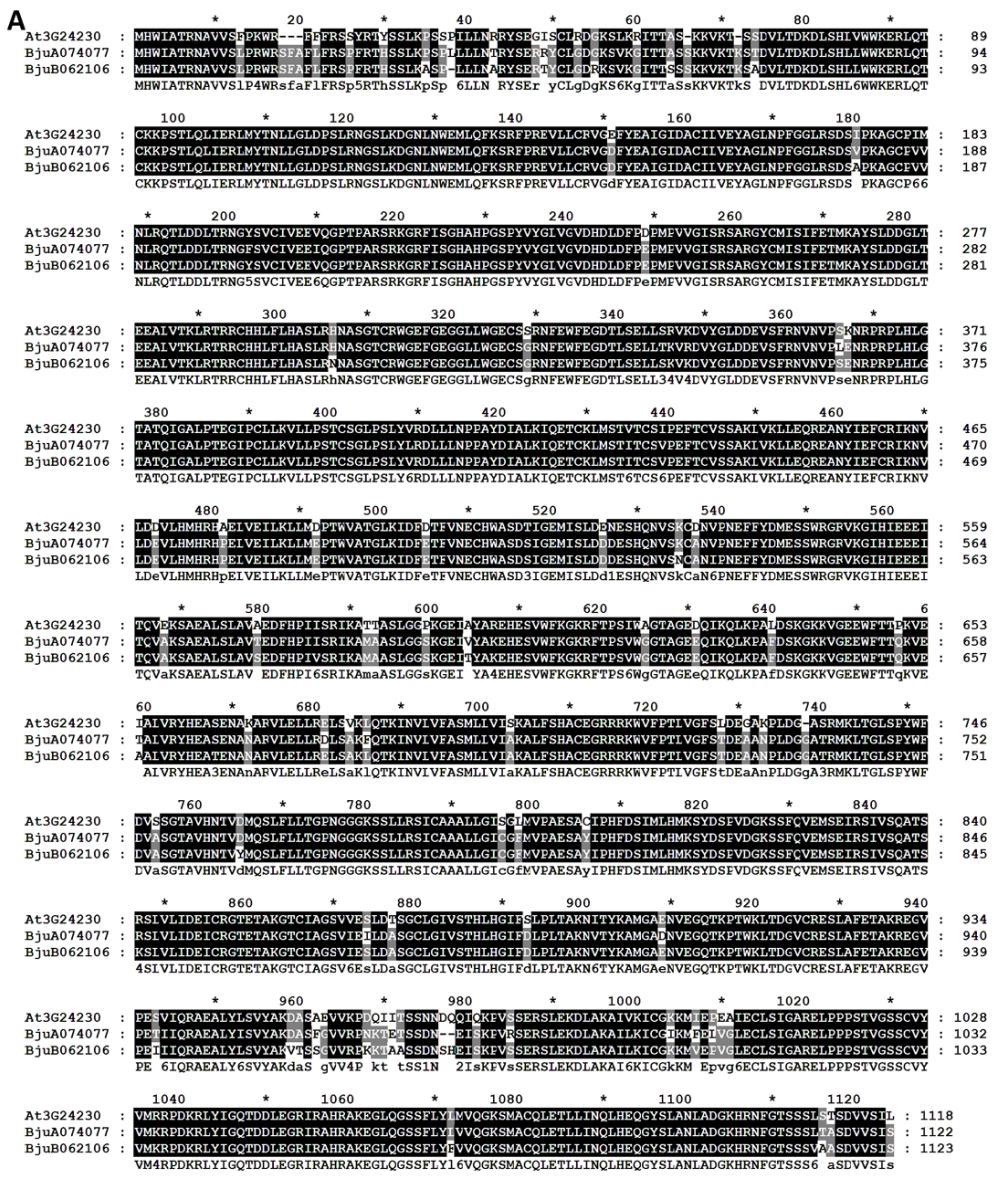


Figure S5 *MSH1* from *B. juncea* and comparison with its ortholog in *A. thaliana*.

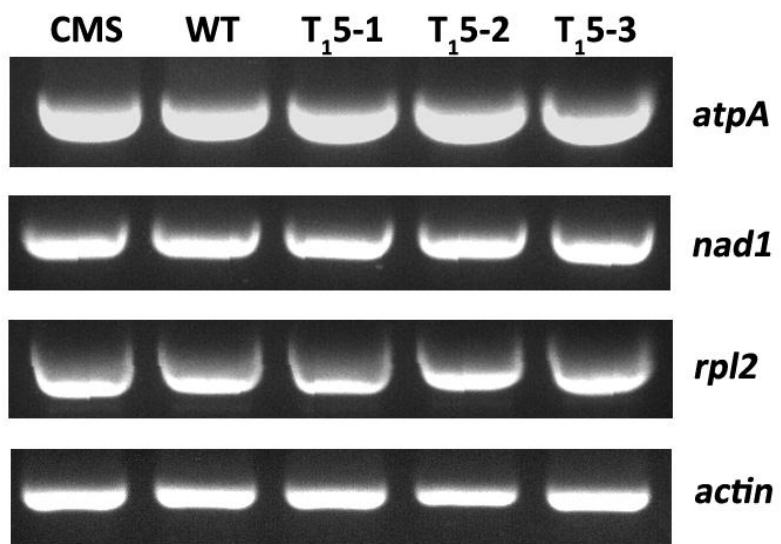


Figure S6 Substoichiometric shifting of mitochondrial genes in MSH1-RNAi lines relative to wild type.

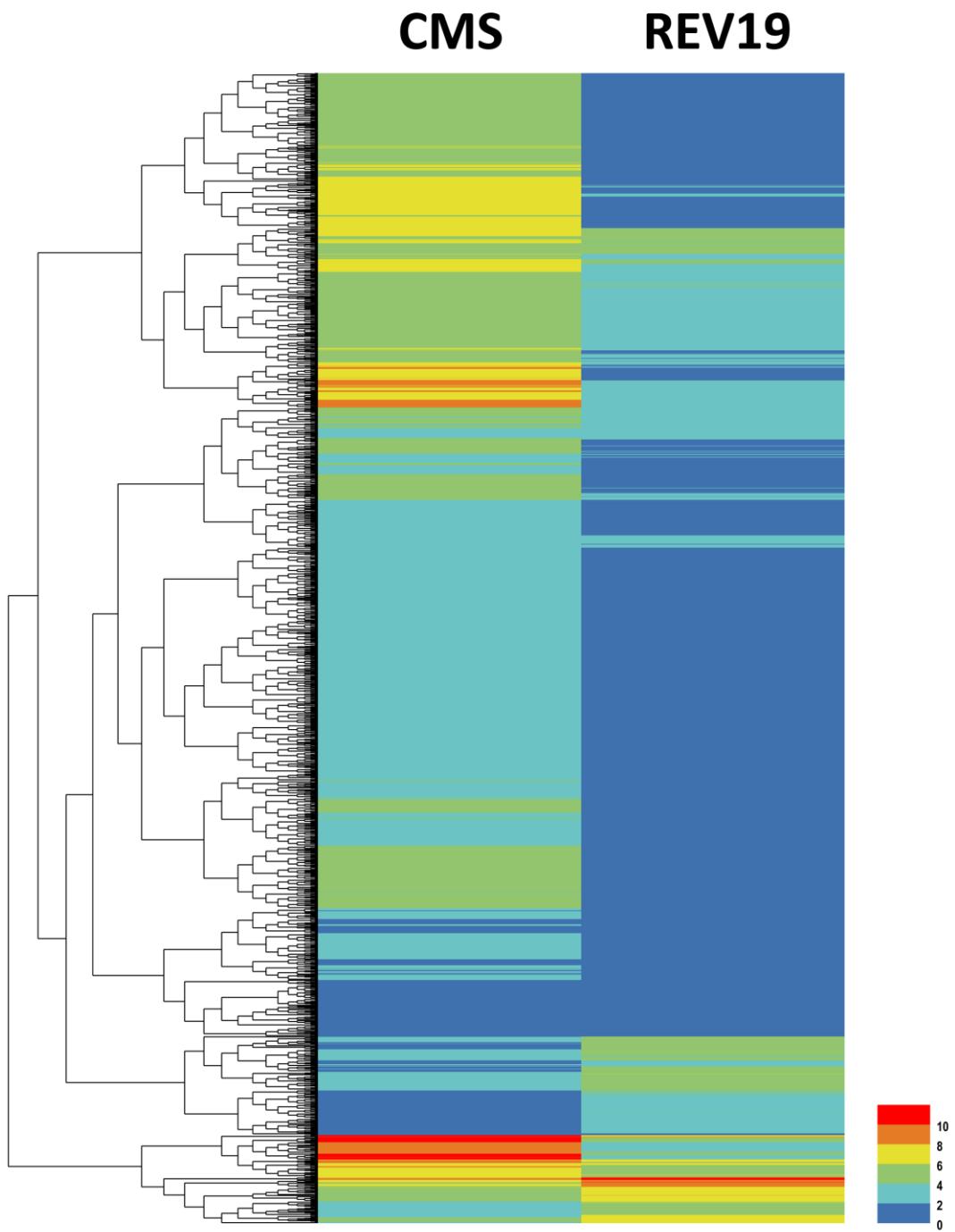


Figure S7 Genes differentially expressed between CMS and REV19 lines of *B. juncea* by RNA-seq.

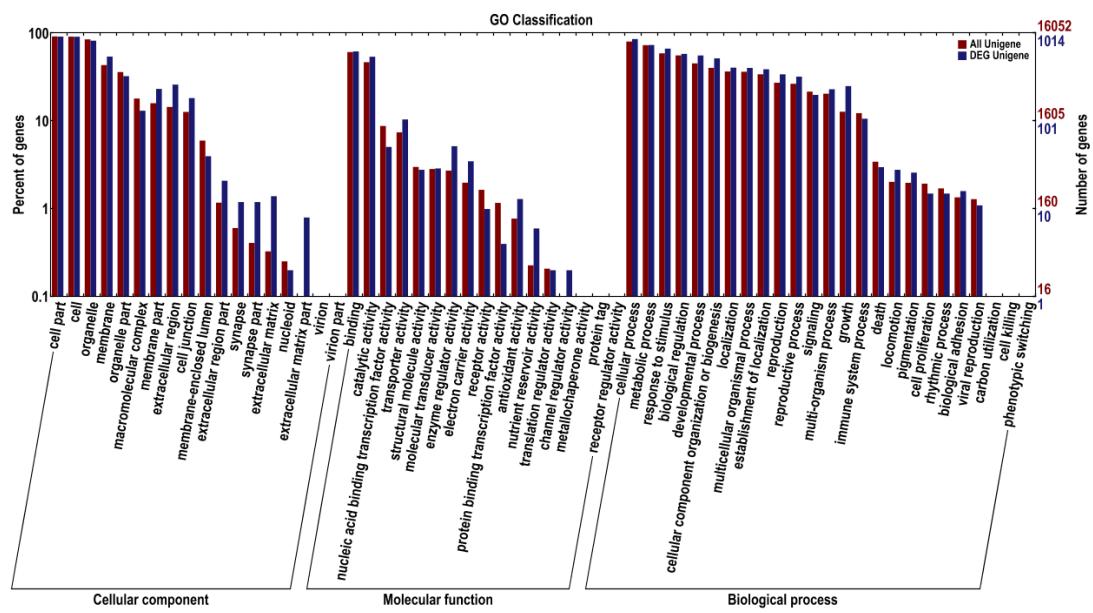


Figure S8 Gene Ontology (GO) enrichment analysis of differentially expressed genes.

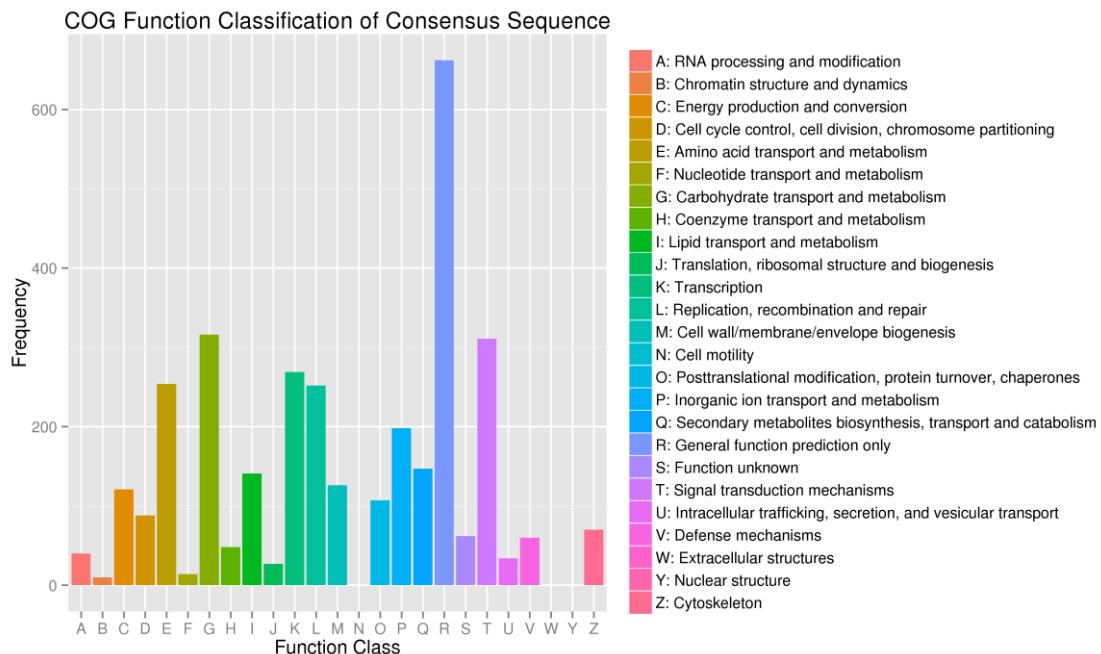


Figure S9 Clusters of Orthologous Groups (COG) analysis of differentially expressed genes.

Table S1 Primers used in this study

Gene	Forward primer	Reverse primer
ORF220-SSS	ATGCCTCACTGGATAAATTCACTT	TCATCGATAGAGATCGAGAATTTCG
<i>atpA</i>	GCTGCTTACAGGAGTTAGCC	GTCCAATCGCTACATAGACA
<i>ACTIN</i>	CGCCGAGCGGGAAATCGTC	GGAAAGTGCTGAGGGATGC
<i>ORF220-Construction</i>	CGAAACCAACTGCTTCACA	TTCGTTCATCCTGGCTCTCT
<i>AP3 promoter</i>	CAGTAACTGTGGCCAACTTAGT	ATTCTTCTCTTTGTTAACCTTT
<i>mt-targeted</i>	CCATGGCTTCGGAGGGCTCT	CGCTCGGGAGGTAGCGTACTG
<i>RT-MSH1-A</i>	AAGAATACGTGCGCATAGGG	AAGCAAAAACGGCATACATTG
<i>RT-MSH1-B</i>	GCTTCATCGGACAATAGCCAC	CCTGCCGTTGCAATATCTC
<i>Q-ORF220</i>	GGAGATTCGAAGCCTACAGC	ATCGAGGATCTCGTTCATCC
<i>Q-AMS</i>	TACCGGGAGGATTAGTGGAG	TCATGTTAACCGTTGCGGTT
<i>Q-AP3</i>	GGTGTGTTGATGCTAGGG	CAACATCAGAAACCGTTGG
<i>Q-C2H2</i>	ACAACACGATGAGGGCAATA	AAGCATTCCCTGAATTGGAC
<i>Q-DYT1</i>	CATGGGAAGTGAACTTGTGG	TTTAGGTATCGGTCCAAGCC
<i>Q-GH3</i>	AAGCGCAACACATTACTCG	ACTTCGCGTCCTGAGTTT
<i>Q-MYB99</i>	GAGGTTGGACTCAGAAAGGG	GCTTGCTCCACGTGTTCTTA
<i>Q-MYB103</i>	CACGGTGGAATACGTACAGC	CCACCACTTCTCCCTTCAAT
<i>Q-MS1</i>	CTCATGGGAAGTTGGCCTT	GCAACGGAGGTTAACAGAGGG
<i>Q-MS2</i>	GCGGTTAAGAACGAGGGTGT	TCTGTTGCAACCCAATGTT
<i>Q-PI</i>	GCTGCAACACAAGAGATGG	GGTTGAATCGGCTGAACCTCT
<i>Q-SHN1</i>	CTGACATCTCAGCCTCTCA	AGACTTGCAGCATTTCCTCA
<i>Q-SPL</i>	GTACTTCAACGAGGGACAA	GATGTAGCCACTGGAAACGA
<i>Q-SPL8</i>	GCTCGATTCACTTCCAACA	TACAGCCTGCTCACGAAATC
<i>Q-WUS</i>	CCAAGAGAGCGGTAACAACA	AGGATTCTGATCTGCTCCGT
<i>Q-4CL3</i>	CACCGTTATCACCACAGAGG	GTGCCAGAGGAGAAAGGAAG
<i>Q-25S</i>	CGGTTCCCTCGTACTAGGTTGA	CCGTCGTGAGACAGGTTAGTTT

Table S2 Statistic of candidate revertant event from CMS *B. juncea*

CMS plant number	Seed number	Sterile	Fertile
1	5	5	0
2	5	5	0
3	4	4	0
4	4	4	0
5	3	3	0
6	3	3	0
7	3	3	0
8	2	2	0
9	2	2	0
10	2	2	0
11	2	2	0
12	2	2	0
13	1	1	0
14	1	1	0
15	1	1	0
16	1	1	0
17	1	1	0
18	1	1	0
19	7	5	2
20	1	1	0
21	1	1	0
22	1	1	0
From No. 23 - No.39	0	0	0
Total	53	51	2

Table S3 Statistic of fertility of ORF220 expression in *Arabidopsis*

	<i>ORF220</i>		<i>mt target-ORF220</i>	
	Sterile	Fertile	Sterile	Fertile
35S promoter	0	20	17	42
AP3 promoter	1	13	28	0