

Fine mapping of *BoVl* conferring variegated leaf in ornamental kale **(*Brassica oleracea* var. *acephala*)**

Supplementary information: Figure S1-S6 and Table S1-S4.

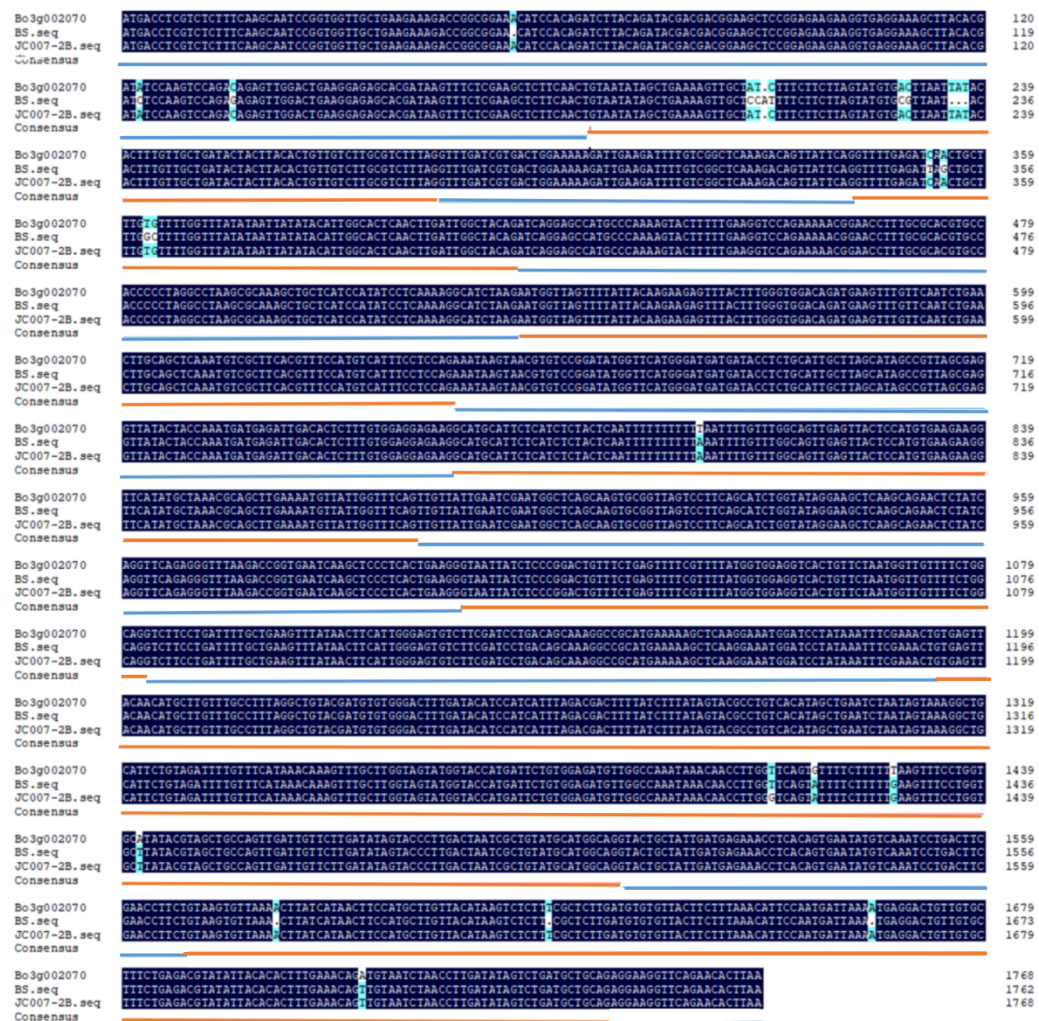
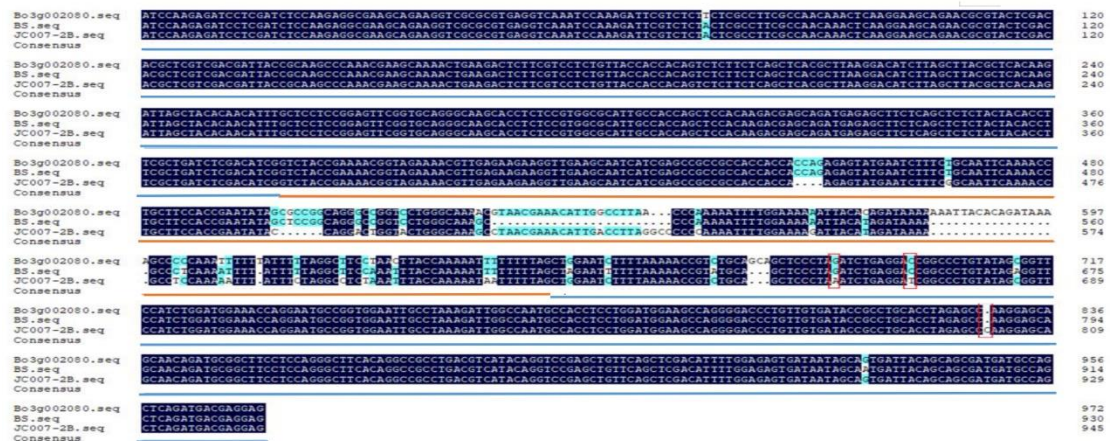


Figure S1. Alignments of nucleotide sequences of *Bo3g002070* in ‘BS’ and ‘JC007-2B’. Note: *Bo3g002070* is the reference genome sequence of cabbage; Blue underline indicates exon; Red underline indicates intron.

Bo3g002090.seq	ATGGCAGCGCCGCGCACGCCCTTAGTCACCGTCCAAGGACTCGACGGAGACATGACAACCGACCAATCCACCACCGTCTGCTACCCGAGCTCATGACCGCGCCGTTGACACAGAC	120
BS.seq	ATGGCAGCGCCGCGCACGCCCTTAGTCACCGTCCAAGGACTCGACGGAGACATGACAACCGACCAATCCACCACCGTCTGCTACCCGAGCTCATGACCGCGCCGTTGACACAGAC	120
JC007-2B.seq	ATGGCAGCGCCGCGCACGCCCTTAGTCACCGTCCAAGGACTCGACGGAGACATGACAACCGACCAATCCACCACCGTCTGCTACCCGAGCTCATGACCGCGCCGTTGACACAGAC	120
Consensus	ATGGCAGCGCCGCGCACGCCCTTAGTCACCGTCCAAGGACTCGACGGAGACATGACAACCGACCAATCCACCACCGTCTGCTACCCGAGCTCATGACCGCGCCGTTGACACAGAC	
Bo3g002090.seq	ATCGTCAACTTCGTCCACGCCAAATCTCAAAACAACAGCGCTCAGCCTTACGCCGTCTCCAAAAAGCCGGTCACAGACCTCCGCCGAGTCATGGGAAACCGGACGCCGCTTCCCGT	240
BS.seq	ATCGTCAACTTCGTCCACGCCAAATCTCAAAACAACAGCGCTCAGCCTTACGCCGTCTCCAAAAAGCCGGTCACAGACCTCCGCCGAGTCATGGGAAACCGGACGCCGCTTCCCGT	240
JC007-2B.seq	ATCGTCAACTTCGTCCACGCCAAATCTCAAAACAACAGCGCTCAGCCTTACGCCGTCTCCAAAAAGCCGGTCACAGACCTCCGCCGAGTCATGGGAAACCGGACGCCGCTTCCCGT	240
Consensus	ATCGTCAACTTCGTCCACGCCAAATCTCAAAACAACAGCGCTCAGCCTTACGCCGTCTCCAAAAAGCCGGTCACAGACCTCCGCCGAGTCATGGGAAACCGGACGCCGCTTCCCGT	
Bo3g002090.seq	ATCCCTCGTGTTCCTCCGCGGGAACACACCGCCGCGCAAGCGGCTTCGGAACATGTCGCGGTGGTCGGATGTTCCCTCCGACTAAGATCTGGAGACGTGGCACCCTCGCGTC	360
BS.seq	ATCCCTCGTGTTCCTCCGCGGGAACACACCGCCGCGCAAGCGGCTTCGGAACATGTCGCGGTGGTCGGATGTTCCCTCCGACTAAGATCTGGAGACGTGGCACCCTCGCGTC	360
JC007-2B.seq	ATCCCTCGTGTTCCTCCGCGGGAACACACCGCCGCGCAAGCGGCTTCGGAACATGTCGCGGTGGTCGGATGTTCCCTCCGACTAAGATCTGGAGACGTGGCACCCTCGCGTC	360
Consensus	ATCCCTCGTGTTCCTCCGCGGGAACACACCGCCGCGCAAGCGGCTTCGGAACATGTCGCGGTGGTCGGATGTTCCCTCCGACTAAGATCTGGAGACGTGGCACCCTCGCGTC	
Bo3g002090.seq	AACGTCAACATGAAGAGGACGCGATCGTCTCGGCAATCGCCGCGCACCGCGGTTCCCGCGCTCGTGTATGGCAGCTGGCCACAGATCGAGAATGTCCCGAGATGCTCTTGTGTATAGC	480
BS.seq	AACGTCAACATGAAGAGGACGCGATCGTCTCGGCAATCGCCGCGCACCGCGGTTCCCGCGCTCGTGTATGGCAGCTGGCCACAGATCGAGAATGTCCCGAGATGCTCTTGTGTATAGC	480
JC007-2B.seq	AACGTCAACATGAAGAGGACGCGATCGTCTCGGCAATCGCCGCGCACCGCGGTTCCCGCGCTCGTGTATGGCAGCTGGCCACAGATCGAGAATGTCCCGAGATGCTCTTGTGTATAGC	480
Consensus	AACGTCAACATGAAGAGGACGCGATCGTCTCGGCAATCGCCGCGCACCGCGGTTCCCGCGCTCGTGTATGGCAGCTGGCCACAGATCGAGAATGTCCCGAGATGCTCTTGTGTATAGC	
Bo3g002090.seq	GACTCAGCTGAGGCCGTTGAGAAGACTAACGCCCAATCAAGGTTTTGAAGCAGATCGGTGCTTATGATGACGCCGAGAAGGCGAAGGATAGTATGGGATTAGGTCTGGTGTGTATAG	600
BS.seq	GACTCAGCTGAGGCCGTTGAGAAGACTAACGCCCAATCAAGGTTTTGAAGCAGATCGGTGCTTATGATGACGCCGAGAAGGCGAAGGATAGTATGGGATTAGGTCTGGTGTGTATAG	600
JC007-2B.seq	GACTCAGCTGAGGCCGTTGAGAAGACTAACGCCCAATCAAGGTTTTGAAGCAGATCGGTGCTTATGATGACGCCGAGAAGGCGAAGGATAGTATGGGATTAGGTCTGGTGTGTATAG	600
Consensus	GACTCAGCTGAGGCCGTTGAGAAGACTAACGCCCAATCAAGGTTTTGAAGCAGATCGGTGCTTATGATGACGCCGAGAAGGCGAAGGATAGTATGGGATTAGGTCTGGTGTGTATAG	
Bo3g002090.seq	ATGAGGAACCGTCTTACATTTCTAGGAAGGTCCTCTGTGTGTGACGGAACCGAGGAGCTAAGATGTGAAAGCGTTTAGGAACATTACTGGCGTTGAGCTTTGTACGTTGAGAGG	720
BS.seq	ATGAGGAACCGTCTTACATTTCTAGGAAGGTCCTCTGTGTGTGACGGAACCGAGGAGCTAAGATGTGAAAGCGTTTAGGAACATTACTGGCGTTGAGCTTTGTACGTTGAGAGG	720
JC007-2B.seq	ATGAGGAACCGTCTTACATTTCTAGGAAGGTCCTCTGTGTGTGACGGAACCGAGGAGCTAAGATGTGAAAGCGTTTAGGAACATTACTGGCGTTGAGCTTTGTACGTTGAGAGG	720
Consensus	ATGAGGAACCGTCTTACATTTCTAGGAAGGTCCTCTGTGTGTGACGGAACCGAGGAGCTAAGATGTGAAAGCGTTTAGGAACATTACTGGCGTTGAGCTTTGTACGTTGAGAGG	
Bo3g002090.seq	CTTAAGTTGTGAGGCTAGCTCTGGTGGTCACTTGGGAGGTTTGTTATTTGGACTAAGCTGCTTTTGAAGGCTTGAGCTATCTATGGATCTGTTGAGAGCCGCTGAGATGAAG	840
BS.seq	CTTAAGTTGTGAGGCTAGCTCTGGTGGTCACTTGGGAGGTTTGTTATTTGGACTAAGCTGCTTTTGAAGGCTTGAGCTATCTATGGATCTGTTGAGAGCCGCTGAGATGAAG	840
JC007-2B.seq	CTTAAGTTGTGAGGCTAGCTCTGGTGGTCACTTGGGAGGTTTGTTATTTGGACTAAGCTGCTTTTGAAGGCTTGAGCTATCTATGGATCTGTTGAGAGCCGCTGAGATGAAG	840
Consensus	CTTAAGTTGTGAGGCTAGCTCTGGTGGTCACTTGGGAGGTTTGTTATTTGGACTAAGCTGCTTTTGAAGGCTTGAGCTATCTATGGATCTGTTGAGAGCCGCTGAGATGAAG	
Bo3g002090.seq	AAGGGTTATGCTTGCCTCGTGGAGATGGTGAAGCTGATTGGCGAGGATTATTAATCGGATGAGGTTGAGAGCTGGTGTGAAGCGGATTAAAGAGGATGCGAAGAGAGCTGTGATG	960
BS.seq	AAGGGTTATGCTTGCCTCGTGGAGATGGTGAAGCTGATTGGCGAGGATTATTAATCGGATGAGGTTGAGAGCTGGTGTGAAGCGGATTAAAGAGGATGCGAAGAGAGCTGTGATG	960
JC007-2B.seq	AAGGGTTATGCTTGCCTCGTGGAGATGGTGAAGCTGATTGGCGAGGATTATTAATCGGATGAGGTTGAGAGCTGGTGTGAAGCGGATTAAAGAGGATGCGAAGAGAGCTGTGATG	960
Consensus	AAGGGTTATGCTTGCCTCGTGGAGATGGTGAAGCTGATTGGCGAGGATTATTAATCGGATGAGGTTGAGAGCTGGTGTGAAGCGGATTAAAGAGGATGCGAAGAGAGCTGTGATG	
Bo3g002090.seq	AAGAAGAATCCGTTGAAGAATCTTAATGTGATGCTTAAGTTGAATCCTTATGCTAAGACCGGGAAGAGGATGCTTTGTTGCTGAAGCGCAGAGGTTAAGTCTAAGAAGGAGAGCTC	1080
BS.seq	AAGAAGAATCCGTTGAAGAATCTTAATGTGATGCTTAAGTTGAATCCTTATGCTAAGACCGGGAAGAGGATGCTTTGTTGCTGAAGCGCAGAGGTTAAGTCTAAGAAGGAGAGCTC	1080
JC007-2B.seq	AAGAAGAATCCGTTGAAGAATCTTAATGTGATGCTTAAGTTGAATCCTTATGCTAAGACCGGGAAGAGGATGCTTTGTTGCTGAAGCGCAGAGGTTAAGTCTAAGAAGGAGAGCTC	1080
Consensus	AAGAAGAATCCGTTGAAGAATCTTAATGTGATGCTTAAGTTGAATCCTTATGCTAAGACCGGGAAGAGGATGCTTTGTTGCTGAAGCGCAGAGGTTAAGTCTAAGAAGGAGAGCTC	
Bo3g002090.seq	GAGAAGAAGAGGAGGCGCTCTAAGGTAACCTAACCGCTCTCTCTTCTCTAAGCCCTTTTTTTT...ATGTGGGTTACAGTTTGTATTATCTTGTGAATTCAGACACATTACATT	1198
BS.seq	GAGAAGAAGAGGAGGCGCTCTAAGGTAACCTAACCGCTCTCTCTTCTCTAAGCCCTTTTTTTT...ATGTGGGTTACAGTTTGTATTATCTTGTGAATTCAGACACATTACATT	1200
JC007-2B.seq	GAGAAGAAGAGGAGGCGCTCTAAGGTAACCTAACCGCTCTCTCTTCTCTAAGCCCTTTTTTTT...ATGTGGGTTACAGTTTGTATTATCTTGTGAATTCAGACACATTACATT	1198
Consensus	GAGAAGAAGAGGAGGCGCTCTAAGGTAACCTAACCGCTCTCTCTTCTCTAAGCCCTTTTTTTT...ATGTGGGTTACAGTTTGTATTATCTTGTGAATTCAGACACATTACATT	
Bo3g002090.seq	TAGATTAGTTTTGTATAACTCATGTTCTGTCTGAACATTTCTATAGGTTTTTTTAAATCTTTTTTGTCTTTAGAAATCCCAAAGCTGGACTCATTTCTCAAACTCGTTTGTCTTTGTTA	1318
BS.seq	TAGATTAGTTTTGTATAACTCATGTTCTGTCTGAACATTTCTATAGGTTTTTTTAAATCTTTTTTGTCTTTAGAAATCCCAAAGCTGGACTCATTTCTCAAACTCGTTTGTCTTTGTTA	1320
JC007-2B.seq	TAGATTAGTTTTGTATAACTCATGTTCTGTCTGAACATTTCTATAGGTTTTTTTAAATCTTTTTTGTCTTTAGAAATCCCAAAGCTGGACTCATTTCTCAAACTCGTTTGTCTTTGTTA	1318
Consensus	TAGATTAGTTTTGTATAACTCATGTTCTGTCTGAACATTTCTATAGGTTTTTTTAAATCTTTTTTGTCTTTAGAAATCCCAAAGCTGGACTCATTTCTCAAACTCGTTTGTCTTTGTTA	
Bo3g002090.seq	CTCTAGGAGCAATCAAGCCATTAAAGCAGCAGGAAAAGCGTGGTACCAGACTATGATCTCCGACAGTGACTACACAGAGTTTGATAAATTCACCAAGTGGCTCGGAGCCAGTCAGTAA	1438
BS.seq	CTCTAGGAGCAATCAAGCCATTAAAGCAGCAGGAAAAGCGTGGTACCAGACTATGATCTCCGACAGTGACTACACAGAGTTTGATAAATTCACCAAGTGGCTCGGAGCCAGTCAGTAA	1440
JC007-2B.seq	CTCTAGGAGCAATCAAGCCATTAAAGCAGCAGGAAAAGCGTGGTACCAGACTATGATCTCCGACAGTGACTACACAGAGTTTGATAAATTCACCAAGTGGCTCGGAGCCAGTCAGTAA	1438
Consensus	CTCTAGGAGCAATCAAGCCATTAAAGCAGCAGGAAAAGCGTGGTACCAGACTATGATCTCCGACAGTGACTACACAGAGTTTGATAAATTCACCAAGTGGCTCGGAGCCAGTCAGTAA	

Figure S2. Alignments of nucleotide sequences of *Bo3g002090* in ‘BS’ and ‘JC007-2B’. Note: *Bo3g002090* is the reference genome sequence of cabbage; Blue underline indicates exon; Red underline indicates intron.

a



b



Figure S3. Nucleotide and amino acid sequence alignments of *Bo3g002080* in ‘BS’ and ‘JC007-2B’. (a) Nucleotide sequences alignments of *Bo3g002080* in ‘BS’ and ‘JC007-2B’. Blue underline represents exon; Red underline represents intron. (b) Amino acid sequence alignment of *Bo3g002080* in ‘JC007-2B’ and ‘BS’. ‘Bo3g002080’ is the reference genome sequence of from non-variegated phenotype cabbage. Red boxes represent base substitutions.

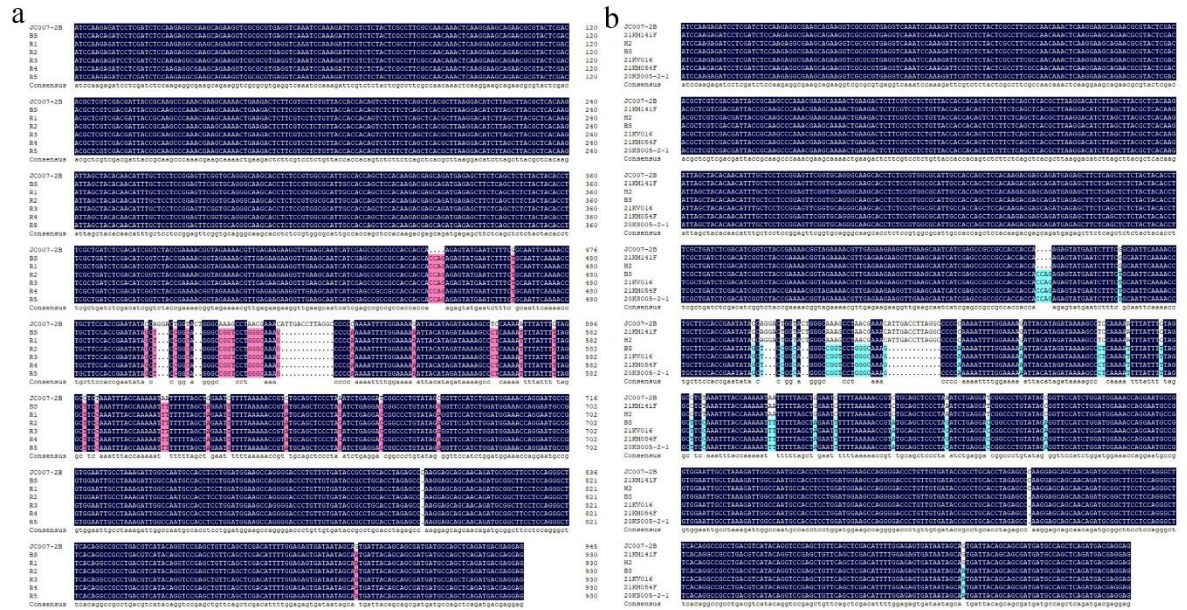


Figure S4. Nucleotide sequences alignments of *Bo3g002080* in two parents, F₂ recombinant plants and DH line plants. (a) Nucleotide sequences alignments of *Bo3g002080* among two parents and recombinant plants. R1-R5 represent the five recombinant plants that identified by Q3I-8 and Q3I-15; (b) Nucleotide sequences alignments of *Bo3g002080* among two parents and DH lines. ‘JC007-2B’, ‘H2’ and ‘21KM141F’ were with variegated leaf, while the leaf phenotype of ‘BS’, ‘21KV016’, ‘21KM054F’ and ‘20KS005-2-1’ was normal.

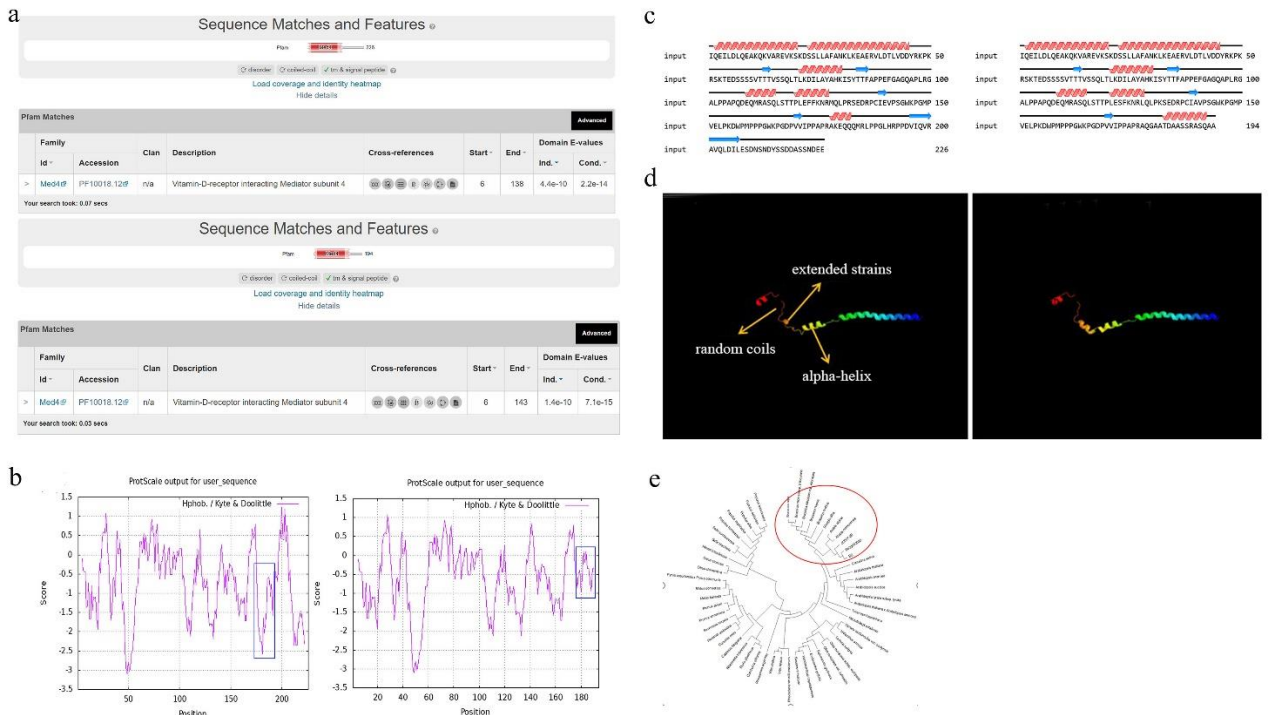


Figure S5. Hydrophobicity and the protein structure of the candidate gene. (a) Conserved domain analysis, the upper figure was the conserved domain analysis of 'BS', and the down figure was the conserved domain analysis of 'JC007-2B'; (b) Hydrophobicity analysis of 'BS' and 'JC007-2B' protein. The left figure was the hydrophobicity analysis of 'BS', and the right figure was the hydrophobicity analysis of 'JC007-2B'; (c) The predicted secondary structure of 'BS' and 'JC007-2B' protein. The left figure was the predicted secondary structure of 'BS', and the right figure was the predicted secondary structure of 'JC007-2B'. The red asterisk represents the position of 132 amino acids, and the green asterisk represents the position of 179 amino acids; (d) The predicted secondary structure of 'BS' and 'JC007-2B' protein. The left figure was the predicted secondary structure of 'BS', and the right figure was the predicted secondary structure of 'JC007-2B'; (e) Phylogenetic tree of *B03g002080*, 'BS' and 'JC007-2B' in ornamental kale and other species. *Brassica rapa* (RID43011.1); *Brassica cretica* (KAF2535840.1); *Brassica oleracea* var. *oleracea* (XP013611601.1); *Brassica napus* (KAH0908033.1); *Arabis alpina* (KFK24735.1); *Brassica rapa* subsp. *trilocularis* (VVB12997.1); *Camelina sativa* (XP010490754.1); *Tarenaya hassleriana* (XP010553019.1); *Prunus armeniaca* (CAB4304152.1); *Capsella rubella* (XP006287800.1); *Carpinus fangiana* (KAE8099520.1); *Arabidopsis arenosa* (CAE6085689.1); *Arabidopsis suecica* (KAG7553896.1); *Arabidopsis thaliana* (AAP40461.1); *Arabidopsis lyrata* subsp. *Lyrata* (XP020879558.1); *Camelina sativa* (XP010423809.1); *Arabidopsis thaliana* x *Arabidopsis arenosa* (KAG7549282.1); *Helianthus annuus* (XP022039233.1); *Sesamum indicum* (XP011077385.1); *Citrus clementina* (XP006428900.1); *Rehmannia glutinosa* (ALO61893.1); *Salix brachista* (KAB5519807.1); *Salix dunnii* (KAF9664990.1); *Olea europaea* subsp. *Europaea* (CAA3013307.1); *Durio zibethinus* (XP022748477.1); *Olea europaea* var. *Sylvestris* (XP022846688.1); *Sinapis alba* (KAF8088602.1); *Populus trichocarpa* (XP_002322847.1); *Populus deltoides* (KAH8485176.1); *Salix suchowensis* (KAG5253153.1); *Prunus armeniaca* (CAB4270352.1); *Populus euphratica* (XP011048421.1); *Handroanthus impetiginosus* (PIN03719.1); *Nicotiana attenuata* (XP019238085.1); *Lactuca sativa* (XP023762142.1); *Citrus sinensis* (XP006480577.1); *Malus baccata* (TQE13352.1); *Populus tomentosa* (KAG6744068.1); *Populus alba* (XP034910512.1); *Pyrus ussuriensis* x *Pyrus communis* (KAB2597909.1); *Malus domestica* (RXI07622.1); *Cucumis sativus* (XP004142109.1); *Corchorus olitorius* (OMO73465.1); *Cynara cardunculus* var. *Scolymus* (XP024990234.1); *Hevea brasiliensis* (XP021666870.1); *Cucumis melo* (XP008449288.1); *Rhododendron williamsianum* (KAE9447899.1); *Prunus avium* (XP021822650.1); *Vitis vinifera* (XP002273310.1); *Benincasa hispida* (XP038882754.1); *Vitis riparia* (XP034693987.1); *Vitis vinifera* (RVW98737.1); *Erythranthe guttata* (XP_012842735.1).

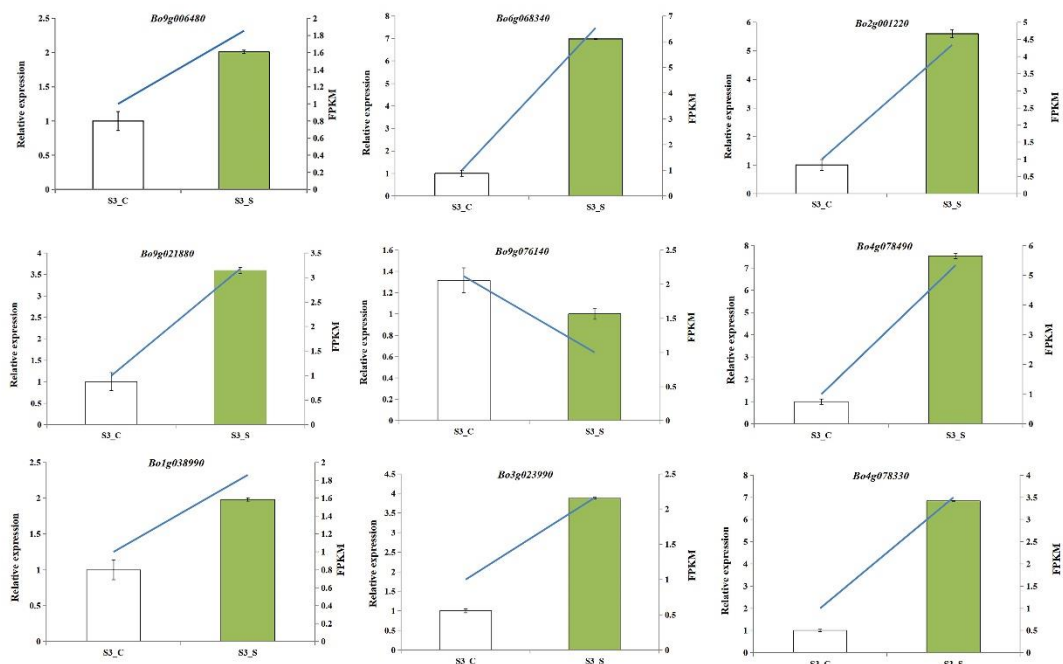


Figure S6. The validation and expression analysis of another 9 randomly selected genes using qRT-PCR.

Table S1 Data statistics and quality control of RNA-Seq.

Sample	Raw Data	Valid Data	Valid Ratio (reads)	Q20%	Q30%	GC content%
S3_S_1	378, 777, 42	373, 854, 02	98.70	99.67	96.23	47
S3_S_2	478, 043, 84	472, 213, 44	98.78	99.64	96.11	47
S3_S_3	504, 932, 20	499, 029, 14	98.83	99.68	96.25	46.5
S3_C_1	521, 933, 28	516, 489, 84	98.96	99.77	96.46	46.5
S3_C_2	574, 185, 18	567, 177, 70	98.78	99.64	95.92	46.5
S3_C_3	487, 531, 74	483, 011, 34	99.07	99.76	96.12	47

Table S2 Reference genome alignment reads statistics.

Sample	Valid reads	Mapped reads	Unique Mapped reads	Multi Mapped reads	PE Mapped reads Pair-end
S3_S_1	373,854,02	334,514,59 (89.48%)	245,067,02 (65.55%)	894,475,7 (23.93%)	297,076,06 (79.46%)

S3_S_2	472,213,44	419,359,98 (88.81%)	309,063,02 (65.45%)	110,296,96 (23.36%)	372,903,16 (78.97%)
S3_S_3	499,029,14	445,226,67 (89.22%)	328,172,86 (65.76%)	117,053,81 (23.46%)	402,526,62 (80.66%)
S3_C_1	516,489,84	459,860,60 (89.04%)	336,564,87 (65.16%)	123,295,73 (23.87%)	418,153,94 (80.96%)
S3_C_2	567,177,70	501,622,41 (88.44%)	367,100,13 (64.72%)	134,522,28 (23.72%)	444,724,58 (78.41%)
S3_C_3	483,011,34	429,720,63 (88.97%)	310,603,44 (64.31%)	119,117,19 (24.66%)	391,313,86 (81.02%)

Table S3 Primer sequences for *Bo3g002070*, *Bo3g002080*, *Bo3g002090*.

Gene	Primers	Sequences (5' to 3')
<i>Bo3g002070</i>	2070-1-L	ATGACCTCGTCTCTTTCAAGCAAT
	2070-1-R	CTTCTCCTCCACAAAGAGTGTCAAT
	2070-2-L	CGGATATGGTTCATGGGATGA
	2070-2-R	TTCGAAATTTATAGGATCCATTTCC
	2070-3-L	CGGATATGGTTCATGGGATGA
	2070-3-R	ATAAGTGTTCCTGAACCTTCCTCTGC
<i>Bo3g002080</i>	2080-1-L	GGGATATTAAATCGTATTGGTCTGG
	2080-1-R	TGACTTACTCCTCGTCATCTGAGC
	2080-2-L	TAAACGCACAGCCACACTCCT
	2080-2-R	TCCAAATAACAAACCTCCCAAGAT
<i>Bo3g002090</i>	2090-1-L	AAGCGTTTAGGAACATTACTGGCG
	2090-1-R	ATAACCAAAATCTTATATGAGACGACAA

Table S4 Primers used to validate expression patterns determined by transcriptome profiling, and primers for the qRT-PCR of candidate genes.

Markers	Forward primer sequences (5'→3')	Reverse primer sequence (5'→3')	Gene
CAD	GCCGTA CTGTCTCTGCTTAG	TCCTCTGTTTCCTTCATGCTC	<i>Bo7g116770</i>
PSAO	GCTCTCACTTCTCAGTTCTGG	ATCCGATTTGTCCGAAGGTG	<i>Bo8g112500</i>
LHCA4	CTTAAC TCGCTCCTACGCTC	TCACGAACCCTAAGAATGCC	<i>Bo3g134510</i>
PSBQ	GGATAGATGGTCCTCTTCCAAC	CTTCCTTTGGTCCCGATGTC	<i>Bo1g158910</i>
LHCB4	GAAAGGTGGAGCTAGTGGATG	GGGTACAAACGCTTCTCAGAG	<i>Bo2g001220</i>
LHCB6	AGAATTTCTGCTGTATCCCGG	ACTTTCAACCTCTCCAGCTTC	<i>Bo8g066580</i>
PSAE	AATCCTACTGGTTCAAGAGCG	TCTTCAATCTCGTCCAGTGC	<i>Bo8g054500</i>
PSAN	ATGTTGATGGGTCTAGTGGAAG	GAGGTATTCATCGAAGACGCTA G	<i>Bo3g102750</i>
GLK1	AGGAAAAGGGTCTTGAGGTTT	GGTGAGTGCTTACAGGTACATG	<i>Bo7g003410</i>
YBo3g002070	TCGTCTCTTTCAAGCAATCCG	CTGTCTGGACTTGGATATCGTG	<i>Bo3g002070</i>
YBo3g002080	CAAAC TCAAGGAAGCAGAACG	GTGGTAACAGAGGACGAAGAG	<i>Bo3g002080</i>
YBo3g002090	TGGTCATCTTGGGAGGTTTG	GCCAAATCAGCATTCACCATC	<i>Bo3g002090</i>
Actin	GGTCGTGACCTTACTGATTACCT CA	GAAGTCTCCATCTCCTGCTCGT	<i>Bo1g116200</i>