

10.1071/FP16437\_AC

© CSIRO 2017

Supplementary Material: *Functional Plant Biology*, 2017, 44(7), 739–750.

## Supplementary Material

### Molecular characterisation and expression profiling of *calcineurin B-like (CBL)* genes in Chinese cabbage under abiotic stresses

Hee-Jeong Jung<sup>A</sup>, Md. Abdul Kayum<sup>A</sup>, Senthil Kumar Thamilarasan<sup>A</sup>, Ujjal Kumar Nath<sup>A</sup>, Jong-In Park<sup>A</sup>, Mi-Young Chung<sup>B</sup>, Yoonkang Hur<sup>C</sup> and Ill-Sup Nou<sup>A,D</sup>

<sup>A</sup>Department of Horticulture, Sunchon National University, 255 Jungang-ro, Suncheon, Jeonnam 57922, South Korea.

<sup>B</sup>Department of Agricultural Education, Sunchon National University, 255 Jungang-ro, Suncheon, Jeonnam 57922, South Korea.

<sup>C</sup>Department of Biology, College of Biological Sciences and Biotechnology, Chungnam National University, Daejeon, South Korea.

<sup>D</sup>Corresponding author. Email: nis@sunchon.ac.kr

**Table S1. List of primers used in RT-PCR and Realtime PCR**

Gene name	Forward primer (5' → 3')	Reverse primer (5' → 3')	Product size (bp)
<i>BrCBL1-1</i>	TTCCGAGGACACGAAGACC TCGTAAACAAGAACCCATCA	AATCGCTCCACTCTAGTTT TTGCAAACACTCGCTGGTC	493 150
<i>BrCBL1-2</i>	ACTGCCTTAGTGTGAGTGAGG ACTGGAGTGGAGCGATTG	ATCGCTCCACTCCAGTTATC CGGAACCTGGTTTGGTCGT	453 184
<i>BrCBL2-1</i>	TGCATTAAATCATGGCGC AAGCAGACACAAAACATGATGG	AGCTTGAAACGTAGTCGTG TCAACCTGAGAGTGGAACACA	660 151
<i>BrCBL2-2</i>	TTATCAATCATGACGCAG ACAAGGAGGAGTTCAGTTGG	TTCTTGAGAAGGCTAGGATGT GCATTGGATGGAATACAGAGA	614 151
<i>BrCBL3-1</i>	ATTGATATAGGCAAATCTCTG ACCAAACATGACGGAAGGA	TTATCGATCCTTCGTCATG GCCAAATTGCGCTGCTCTAGT	470 183
<i>BrCBL3-2</i>	GAAGGAAGAACGACAATG TTTGGGTTCGGTCTTGACTC	CATTGGTCCAAGAACGATC CTTGAATCCTGACGCCACTT	631 116
<i>BrCBL4-1</i>	ACCATTACGGTACAGGAAG TGGAAAGATTGTGTCAGAAGA	TAAATACGGTAACGTCATGTT GCATGGATTACTCAGAAATATAGGTT	502 152
<i>BrCBL4-2</i>	AAGTGGAGGCTTGTATGAA AGATGGAATCGCGCTTCTT	GAAGCTGAGTCTTGTATCG CTTGCAGTCAGCTCCATAAAC	618 98
<i>BrCBL4-3</i>	CGTTACGGCAGCAGAACGTTG TGACGGAAAATTGATGTAGA	GGTTTGCAACTCCAGTT ATTTTGGCAACCTGCATAGA	562 186
<i>BrCBL5</i>	CGGCTATATGATACTCGTCAGACTGG GAGGTGAAAGAGATGATAATAGACG	AAGGATGCCTGGCCACAAAGTTCT GCCACAAAGTTCTCCCATTC	205 152
<i>BrCBL7</i>	TCGACTCGAACTCAGCCTGAAACA GAAGCAAATGGTGGTTCTACTC	AGAGAGCACGAGCAAACCTCTCAA CGCATCAACTCCTCAAATGTC	307 100
<i>BrCBL8</i>	TTGAAGCGAACAAAGCATCCTCGC GGAAACGAAACGGTGTGATTG	TTGAGGTAGGGTAAAGTCATG CCGGTTCCATCAAGGTATAA	563 124
<i>BrCBL9-1</i>	CGAATAAGCTTCTTCTTGTTCG AACGTTAGCAACCAAGCTTAC	GTGGTCGTTATGTCTCTCAG CTCATGACCAGGAAACTCTCTG	635 149
<i>BrCBL9-2</i>	CAATCGTAGTAGTAAGCTTCTC AGCTCTTCTCGTTTACTTGTCA	TGGTCGTTATGTCCCTCAG GAGGCTAGCTTGACAGGGTTC	638 100
<i>BrCBL10-1</i>	GACTGGACGAACGTTCCCTCA GCATCCCAGCTTGTGAAG	AGAAGTTCATCTGACAGCATIC ACAGCTAACATGATCACCAACT	551 159
<i>BrCBL10-2</i>	GCTCCATATGGTGAGAACCTCT CCTTACCTCAAGGATGTGACG	GAGTCATATTCTTCAGCAAGG GGAAACTGACAAAATCCAAAGC	376 103
<i>BrCBL10-3</i>	GCTTGATTACAAGGAAGAG AGCATCCGACCTTGTGAAG	TGTTGTTGTCTCAGTCCTCA GCTACCTCACATTACAACCTCA	495 167
<i>BrActin</i>	ATGCCGAGGCTGATGACAT GGAATCCACGAGACAACCTATAA	AGCCTCGGTAAAGAAGAACCG TCCGCAATACCAGAGAACATAG	410 116

**Table S2.** Sequence relatedness of *Brassica rapa* CBL genes and *Arabidopsis thaliana* CBL genes amino acid sequence

<i>BrCBL7 (u)</i>	45	59	58	37	33	47	40	38	46	37	45	46	58	60	60	54	38	38	37	28	$\begin{smallmatrix} 10 \\ 0 \end{smallmatrix}$			
<i>BrCBL8 (v)</i>	53	52	53	62	43	46	35	92	52	52	53	54	53	46	50	49	61	61	61	39	38	$\begin{smallmatrix} 10 \\ 0 \end{smallmatrix}$		
<i>BrCBL9-1 (w)</i>	89	60	60	60	47	50	41	51	97	56	<b>90</b>	90	59	52	57	53	61	60	58	40	45	52	$\begin{smallmatrix} 10 \\ 0 \end{smallmatrix}$	
<i>BrCBL9-2 (x)</i>	89	59	60	59	46	50	40	51	96	55	<b>90</b>	89	59	52	57	53	61	59	57	40	45	52	$\begin{smallmatrix} 10 \\ 0 \end{smallmatrix}$	
<i>BrCBL10-1 (y)</i>	53	57	55	52	42	46	41	53	54	85	53	54	56	50	57	48	53	52	50	43	37	53	53	$\begin{smallmatrix} 10 \\ 0 \end{smallmatrix}$
<i>BrCBL10-2 (z)</i>	53	57	59	54	39	51	49	51	54	81	53	54	57	52	58	53	55	55	52	42	44	51	54	$\begin{smallmatrix} 10 \\ 0 \end{smallmatrix}$
<i>BrCBL10-3 (ab)</i>	52	54	56	52	42	48	46	51	54	80	53	53	57	50	57	45	54	52	50	41	37	50	53	$\begin{smallmatrix} 10 \\ 0 \end{smallmatrix}$

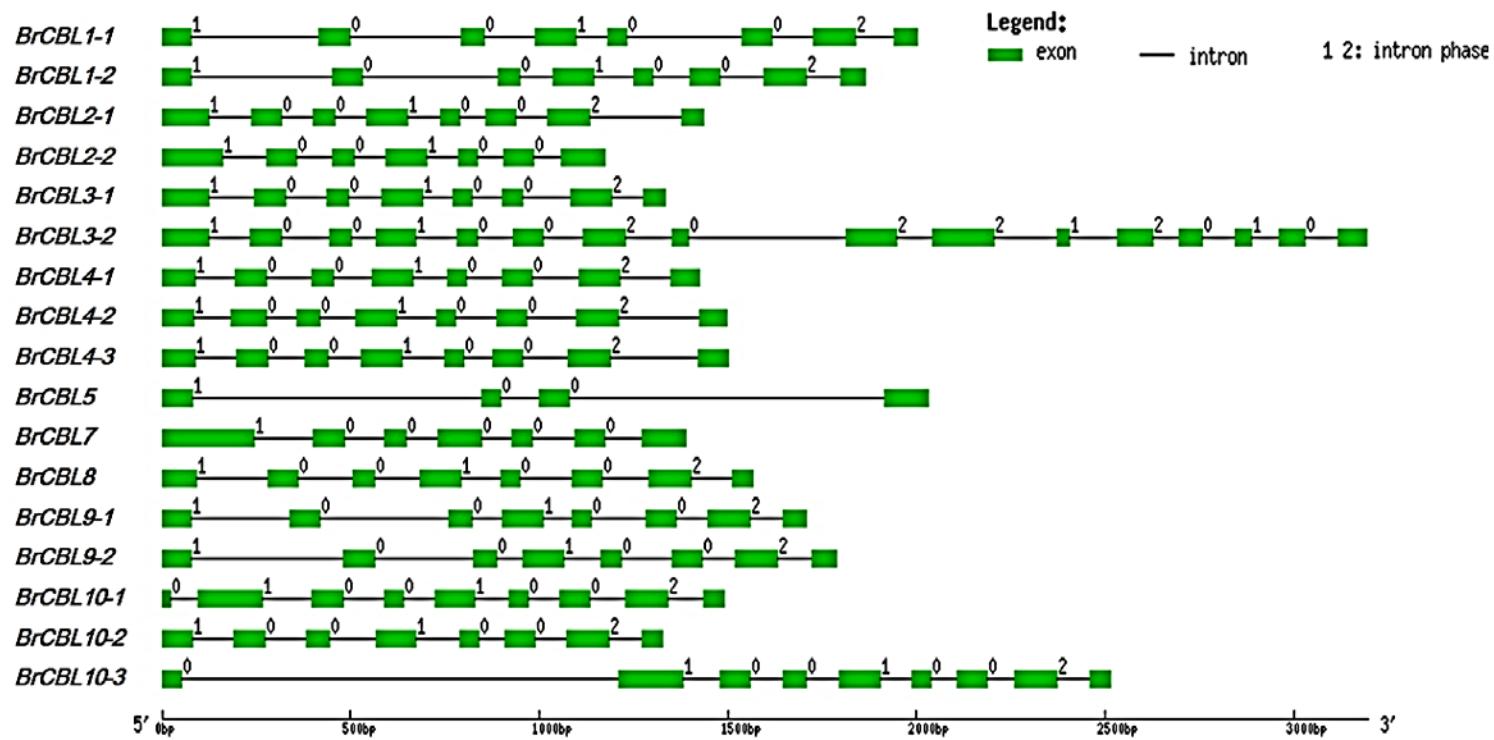
### N-terminal region

	10	20	30	40	50	60	70	80	90	100	110	120
BrCBL4-1	-	MGCAPS	KKKTNALRPPG	YEDPDLLIASVT-	-	-	-	PFTVQEVEALYELFKLSSII	DDGLIHKEEFQIALFRNRNK	-	-	-
BrCBL4-2	-	MCCSLS	KKKKNAIRPPGYEDPDLLIASVTW-	-	-	-	TIVTVAEVEALYELFKLSSII	DDGLIHKEEFQIALFRNRNK	-	-	-	
BrCBL4-3	-	MCCSLS	KKKKNAIRPPGYEDPDLLIASVT-	-	-	-	FTAAEVEVLYELFKLSSII	EDGLIHKEEFQIALFRNRNK	-	-	-	
BrCBL3	-	MIAFVKR	FSLKRTKHPRGYEDPQVIASETP-	-	-	-	FTVNEIEALHNLFKKLSTSII	NDGLIHKEEFQIALFRNSNMQ	-	-	-	
BrCBL1-1	-	-	-	-	-	-	HEDPVK	LASETAESVSEVEALYELFKSISSSVVDDGLINKKEFQIALFKRKRE	-	-	-	
BrCBL1-2	-	-	-	-	-	-	HEDPVK	LASETAESVSEVEALYELFKSISSSVVDDGLINKKEFQIALFKRKKE	-	-	-	
BrCBL9-1	-	-	-	-	-	-	HENPVK	LASETAESVSEVEALYELFKSISSSVVDDGLINKKEFQIALFKRKKE	-	-	-	
BrCBL9-2	-	-	-	-	-	-	HENPVK	LASETAESVSEVEALYELFKSISSSVVDDGLINKKEFQIALFKRKKE	-	-	-	
BrCBL1-1	-	-	-	-	-	-	HENPVK	LASETAESVSEVEALYELFKSISSSVVDDGLINKKEFQIALFKRKKE	-	-	-	
BrCBL2-1	-	-	-	-	-	-	LGDP	ELLARDT	TVFSVSEIEALYELFKKISSAVIDDGLINKKEFQIALFKTNKE	-	-	
BrCBL2-2	-	-	-	-	-	-	LGDP	ELLARDT	TVFSVSEIEALYELFKKISSAVIDDGLINKKEFQIALFKTNKE	-	-	
BrCBL3-1	-	-	-	-	-	-	PGDPE	LLARDT	TVFSVSEIEALYELFKKISSAVIDDGLINKKEFQIALFKTNKE	-	-	
BrCBL3-2	-	-	-	-	-	-	PGDPE	LLARDT	TVFSVSEIEALYELFKKISSAVIDDGLINKKEFQIALFKTNKE	-	-	
BrCBL7	MSSLPILSSPSATN	OPETKSAAVNRCNS	ESTVPGSSM	EAPEPTTVV	STRTQ	PEEEQSG	GTVHN	LSIILAR	DTVFSVSEIEALYDLYRK	KISSAVIDDGLINKKEFQIALFKTNKE	-	-
BrCBL10-2	-	-	-	-	-	-	SCTWISM	FSVNE	VEALYELFKKLS	CSIIIDDGLIHKEEIRIALFCAPYGE	-	-
BrCBL10-3	-	-	-	-	-	-	-	-	-	-	-	-
BrCBL10-1	-	-	-	-	-	-	-	-	-	-	-	-
BrCBL5	-	-	-	-	-	-	-	-	-	-	-	-

### C-terminal region

	130	140	150	160	170	180	190	200	210	220	230	240	250									
BrCBL4-1	NLFAD	RIFDV	DVKRNGVIE	FGEFVRSLGV	VHPNAPVHEKIKFA	KFLYDLRQT-	-	GFIER	EEELKEMVIAL	I	HESELVLSED	LIEVMVDKA	FEADRKNDGKIDLD	WFDVSKNP	SLIKNMTLPY	LKD						
BrCBL4-2	NLFAD	RIFDV	DVKRNGVIE	FGEFVRSLGV	VHPNAPVHEKIKFA	NFLYDLRQT-	-	GFIER	EEELKEMVIAL	I	HESELVLSED	LIEVMVDKA	FEADRKNDGKIDLD	DEWKEFV	SMNP	SLIKNMTLPY	LKD					
BrCBL4-3	NLFAD	RIFDV	DVKRNGVIE	FGEFVRSLGV	VHPNAPVHEKIKFA	NFLYDLRQT-	-	GFIER	EEELKEMVIAL	I	HESELVLSED	MVMDRA	FEADRKNDGKIDLD	DEWKEFV	SMNP	SLIKNMTLPY	LKD					
BrCBL8	NLFAD	RIFDV	DVKRNGVIE	FGEFVRSLGV	VHPNAPVHEKIKFA	KFLYDLRQT-	-	GFIER	EEELKEMVIAL	I	HESELVLSED	MVMDRA	FEADRKNDGKIDLD	DEWKEFV	SMNP	SLIKNMTLPY	LKD					
BrCBL1-1	NIEFAD	RIFDV	DVKRNGVIE	FGEFVRSLGV	VHPNAPVHEKIKFA	KFLYDLRQT-	-	GFIER	EEELKEMVIAL	I	HESELVLSED	MVMDRA	FEADRKNDGKIDLD	DEWKEFV	SMNP	SLIKNMTLPY	LKD					
BrCBL1-2	NLFAN	RIFDM	DVKRKGVID	FGDFVRSLNV	VHPNASLED	KIDFTRFLYDMDC-	-	GFIER	QEVK	KMLIAL	CESEM	KLADETE	EIILDKT	FECLAD	VNC	NDGKIDLE	WSDFVNKNP	SLLKIMTL	YLRLD			
BrCBL9-1	NLFAN	RIFDM	DVKRKGVID	FGDFVRSLNV	VHPNASLED	KIDFTRFLYDMDC-	-	GFIER	QEVK	KMLIAL	CESEM	KLADETE	EIILDKT	FECLAD	VNC	NDGKIDLE	WSDFVNKNP	SLLKIMTL	YLRLD			
BrCBL9-2	NLFAN	RIFDL	DVKRKGVID	FGDFVRSLNV	VHPNASLED	KIDFTRFLYDMDC-	-	GFIER	QEVK	KMLIAL	CESEM	KLADDT	EVILDQ	TFEAD	VDRDGKIG	KTEWSDFV	IKPSSLK	IMTL	YLRLD			
BrCBL2-1	NLFAD	DRVFD	DVKRKGVID	FGDFVRSLNV	VHPNASLED	KIDFTRFLYDMDC-	-	GFIER	QEVL	KMLIAL	CESEM	KLADDT	EVILDQ	TFEAD	VDRDGKIG	KTEWSDFV	IKPSSLK	IMTL	YLRLD			
BrCBL2-2	NLFAD	DRVFD	DVKRKGVID	FGDFVRSLNV	VHPNASLED	KIDFTRFLYDMDC-	-	GFIER	QEVL	KMLIAL	CESEM	KLADDT	EVILDQ	TFEAD	VDRDGKIG	KTEWSDFV	IKPSSLK	IMTL	YLRLD			
BrCBL2-3	SLEAD	DRVFD	DVKHNGILG	FEEFARALS	SVFHPNAP	I	DDKKI	HFSQFLYDLRQ	-	QGFI	ERQELK	CMVVA	TLAESGMNL	KDTVIED	IIDKTFEEAD	T	KHDGKIDKEEWR	RSLVLRHPS	LLKIMTL	QLYLK		
BrCBL3-1	SLEAD	DRVFD	DVKHNGILG	FEEFARALS	SVFHPNAP	I	DDKKI	HFSQFLYDLRQ	-	QGFI	ERQELK	CMVVA	TLAESGMNL	-	-	-	-	-	-	-		
BrCBL3-2	SLEAD	DRVFD	DVKHNGILG	FEEFARALS	SVFHPNAP	I	DDKKI	HFSQFLYDLRQ	-	QGFI	ERQEVK	CMVVA	TLAESGMNL	-	-	-	-	-	-	-		
BrCBL7	SLEFS	DRVFD	DVKHNGILG	FEEFARALS	SVFHPNAP	I	DDKKI	HFSQFLYDLRQ	-	QGFI	ERQEVK	CMVVA	TLAESGMNL	-	-	-	-	-	-	-		
BrCBL10-2	NLFLD	DRVFD	DTEKKNGVIE	FEEFIH	ALSTF	HFMYAPIE	EKIDFA	FRFLYDLRQT-	-	GYIE	REEV	HCMVAA	ILMESEM	ILSDELLTM	I	DKTFAC	ACAD	KDKGKISKEE	WRKVYV	VLKHP	PTLLKNMTLPY	LKD
BrCBL10-3	NLFLD	DRVFD	DTEKKNGVIE	FEEFIH	ALSTF	HFMYAPIE	EKIDFA	FRFLYDLRQT-	-	GYIE	REEV	HCMVAA	ILMESEM	ILSDELLTM	I	DKTFAC	ACAD	KDKGKISKEE	WRKVYV	VLKHP	PTLLKNMTLPY	LKD
BrCBL10-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	260	270	280	290	300	310	320	330	340	350	360	370	380									
BrCBL4-1	IHGT	TPPS	FISSCED	EELI	QNL	YF	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL4-2	IKG	TPSF	VSSCED	EELI	QNL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL4-3	IKA	TPSF	VLSSE	EELI	QNL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL8	VTLA	EFSV	ELD	EVDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL1-1	ITTT	TPSF	VNFSE	DE	IAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL1-2	ITTT	TPSF	VNFSE	EV	DE	IAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL9-1	ITTT	TPSF	VNFSE	EV	DE	IAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL9-2	ITTT	TPSF	VNFSE	EV	DE	IAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL2-1	ITTT	TPSF	VNFSE	EV	DE	EDT	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL3-1	ITTT	TPSF	VHFQS	QV	SE	EDT	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL3-2	KIPEN	SIAD	QTVWQ	SLE	MLR	I	A	D	K	I	D	E	W	E	W	R	H	P	L	T		
BrCBL7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL10-2	VTTIA	FFPS	FI	FNTE	EVED	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL10-3	M	TIA	FFPS	FI	FNTE	EVED	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL10-1	VTTIA	FFPS	FI	FNTE	EVED	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	390	400	410	420	430	440	450	460	470	480	490											
BrCBL4-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL4-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL4-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL1-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL1-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL9-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL9-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL2-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL2-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL3-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL3-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL10-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL10-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL10-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
BrCBL5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

**Fig. S1.** Multiple alignment of all BrCBL proteins and Identical amino acids are asterisks. N and C terminal are indicated in figure.

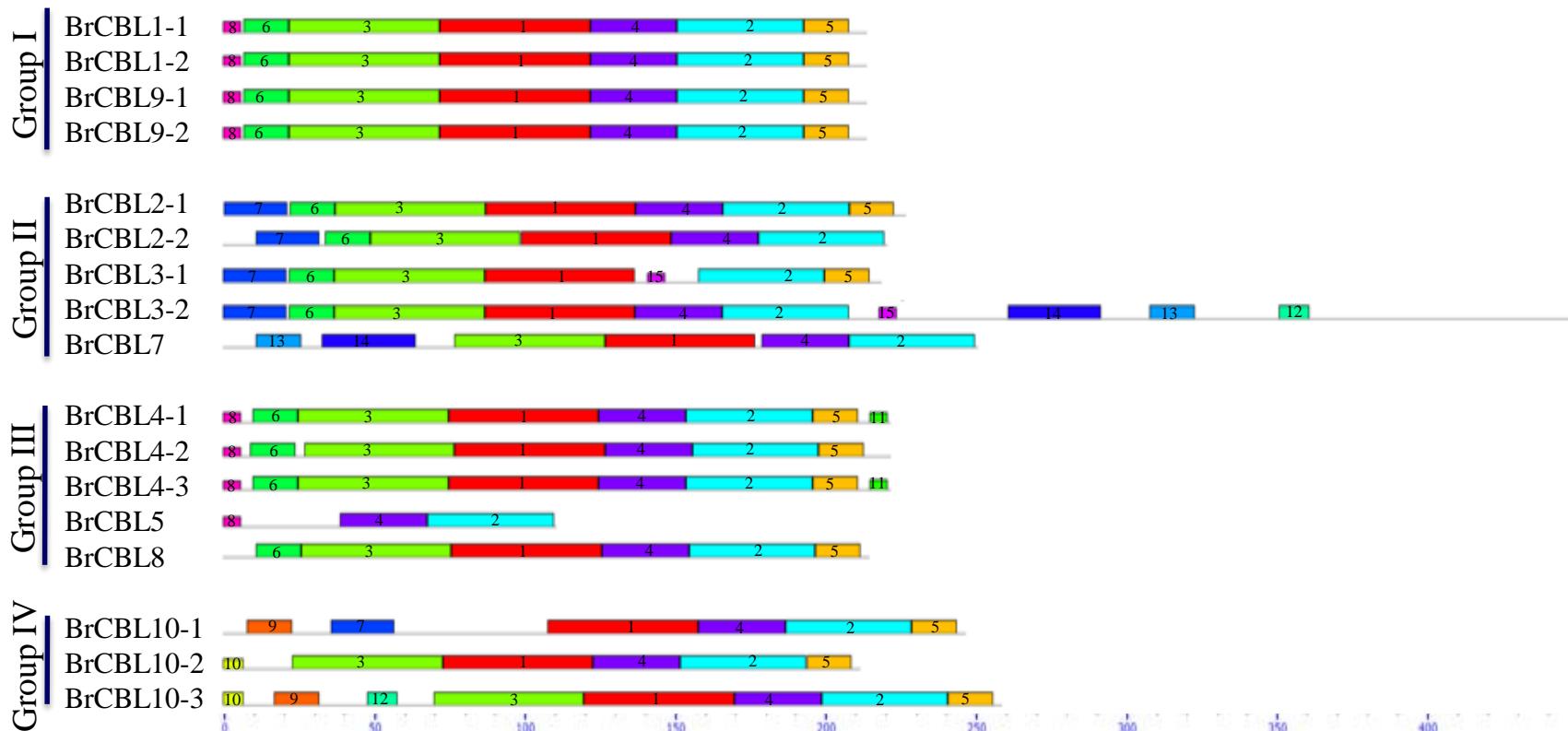


**Fig. S2.** Exon- intron structure of the 17 *CBL* genes in *Brassica rapa*. Horizontal bars indicate exons; horizontal lines indicate introns.

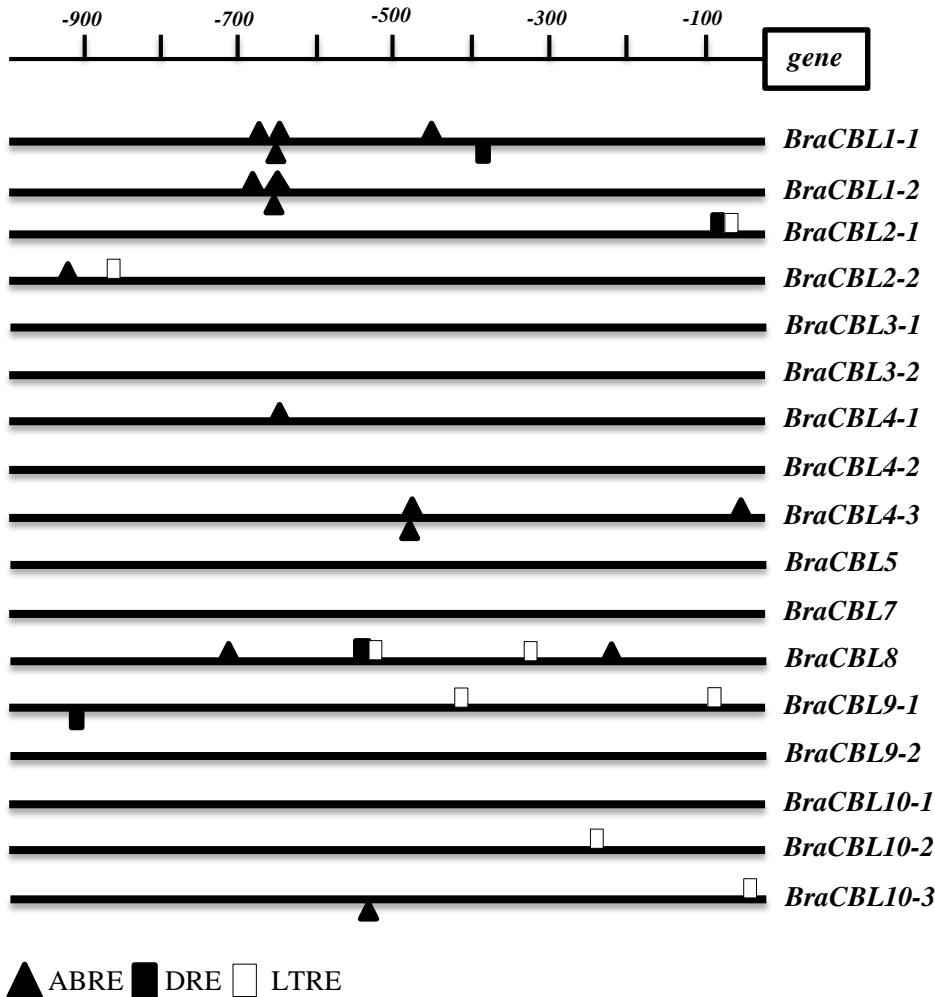
### Motif logo



### Motif distribution



**Fig. S3.** Schematic representation of motif compositions in the BrCBL protein sequences. Different motifs, numbered 1–15, are displayed in different colored boxes. The names of all members are displayed on the left-hand side, while the length of motif is shown in scale at bottom of the figure and motif logo is shown in top of the figure.



**Fig. S4.** Putative, ABRE, DRE and LTRE core sequences in the 1-kb promoter regions of the stress-inducible *BrCBL* genes. The lines represent promoter sequences. The elements located in the forward strand (sense strand of the gene) and the reverse strand, were indicated above and below the lines respectively.