

Sugarcane calcineurin B-like (*CBL*) genes play important but versatile roles in regulation of responses to biotic and abiotic stresses

Su Wei-hua ¹, Huang Long ¹, Ling Hui ¹, Mao Hua-ying ¹, Huang Ning ¹, Su Ya-chun ¹, Ren Yong-juan ¹, Wang Dong-jiao ¹, Xu Li-ping ¹, Khushi Muhammad ² & Que You-xiong ^{1,3,*}

¹ Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China

² Department of Genetics, Hazara University, Mansehra, Pakistan

³ Guangxi Collaborative Innovation Center of Sugarcane Industry, Guangxi University, Nanning 530005, China

*Corresponding author. E-mail: queyouxiong@126.com

Supplementary tables

Table S1 Primers used in this study

Primer	Forward primer (5'-3')	Reverse primer (5'-3')	Strategy
<i>ScCBL2-1</i>	ATGTTGCAGTGCCTGGATG	GGTATCATCGACCTGAGAAT	Gene cloning
<i>ScCBL3-1</i>	GCCTGCCCAACTCGATTCT	ACTCGAATCCAGCATGCAAC	Gene cloning
<i>ScCBL4</i>	GTGCTTGCTTTGGTCGCTT	GTTGCATCCTTCAGTGCGTT	Gene cloning
<i>qScCBL2-1</i>	TGAGGAGGCAGACACAAG	CGACCTGAGAATGGAAGACA	qRT-PCR analysis
<i>qScCBL3-1</i>	ATGGTTGTTGCGACACTTGC	AGCAAGGATGGATGCCGAAT	qRT-PCR analysis
<i>qScCBL4</i>	GCAAGCAGACATGAACGACG	GTGTTCCCTTCCCTCCAGCA	qRT-PCR analysis
<i>CAC</i>	ACAACGTCAGGCAAAGCAAA	AGATCAACTCCACCTCTGCG-3	qRT-PCR analysis
<i>CUL</i>	TGCTGAATGTGTTGAGCAGC	TTGTGCGCGCTCCAAGTAGTC	qRT-PCR analysis
<i>gScCBL2-1</i>	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATGTTGCAGTGCCTGGATGG	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTCG</u> GTATCATCGACCTGAGAAT	Gateway entry vector construction and RT-PCR analysis
<i>gScCBL3-1</i>	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATGGTGCAGTGCCTGGACGG	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTCG</u> TTATCATCGACTTGAGAGT	Gateway entry vector construction and RT-PCR analysis
<i>gScCBL4</i>	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATGGGGTGTGTCTCCTCCAA	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTCG</u> AACTCTTCATCACTAGCTC	Gateway entry vector construction and RT-PCR analysis
<i>NtHSR201</i>	CAGCAGTCCTTTGGCGTTGTC	GCTCAGTTTAGCCGCAGTTGTG	qRT-PCR analysis
<i>NtHSR203</i>	TGGCTCAACGATTACGCA	GCACGAAACCTGGATGG	qRT-PCR analysis
<i>NtHSR515</i>	TTGGGCAGAATAGATGGGTA	TTTGGTGAAAGTCTTGCGTC	qRT-PCR analysis
<i>NtPR-1a/c</i>	AACCTTTGACCTGGGACGAC	GCACATCCAACACGAACCGA	qRT-PCR analysis
<i>NtPR2</i>	TGATGCCCTTTTGGATTCTATG	AGTTCCTGCCCCGCTTT	qRT-PCR analysis
<i>NtPR3</i>	CAGGAGGGTATTGCTTTGTTAGG	CGTGGGAAGATGGCTTGTTGTC	qRT-PCR analysis
<i>NtEFE26</i>	CGGACGCTGGTGGCATAAT	CAACAAGAGCTGGTGGCTGGATA	qRT-PCR analysis
<i>NtAccdeaminase</i>	TCTGAGGTTACTGATTTGGATTGG	TGGACATGGTGGATAGTTGCT	qRT-PCR analysis
<i>NtEF1-α</i>	TGCTGCTGTAACAAGATGGATGC	GAGATGGGGACAAAGGGGATT	qRT-PCR analysis

Note: attB1 and attB2 adapters were underlined in the primer *gScCBLs*.

Table S2 The amplification reaction procedures for cloning of sugarcane *ScCBLs*

Gene name	Pre-denaturation	Denaturation	Annealing	Extension	Cycles	Extension
<i>ScCBL2-1</i>	94 °C 4 min	94 °C 1 min	55 °C 30 s	72 °C 80 s	35	72 °C 10 min
<i>ScCBL3-1</i>	94 °C 4 min	94 °C 30 s	55 °C 30 s	72 °C 2 min	35	72 °C 10 min
<i>ScCBL4</i>	94 °C 4 min	94 °C 30 s	55 °C 30 s	72 °C 2 min	35	72 °C 10 min

Table S3 GenBank Accession Numbers used in this study

Species name	Gene name	GenBank Accession Numbers	Species name	Gene name	GenBank Accession Numbers		
<i>Arabidopsis thaliana</i>	AtCBL1	AAC26008	<i>Oryza sativa</i>	OsCBL1	DQ201195		
	AtCBL2	AAC26009		OsCBL2	DQ201196		
	AtCBL3	AAC26010		OsCBL3	DQ201197		
	AtCBL4	AAC26110		OsCBL4	DQ201198		
	AtCBL5	CAB80951		OsCBL5	DQ201199		
	AtCBL6	AAG28400		OsCBL6	DQ201200		
	AtCBL7	AAG10059		OsCBL6	DQ201201		
	AtCBL8	AAL10300		OsCBL8	DQ201202		
	AtCBL9	AAL10301		OsCBL9	DQ201203		
	AtCBL10	AAO72364		OsCBL10	DQ201204		
<i>Zea mays</i>	ZmCBL1	EU907931	<i>Sorghum bicolor</i>	SbCBL1	FJ901259		
	ZmCBL2-1	EU907932		SbCBL2	FJ901264		
	ZmCBL2-2	EU907933		SbCBL3	FJ901265		
	ZmCBL3	EU907934		SbCBL4	FJ901261		
	ZmCBL5	EU907935		SbCBL5	FJ901263		
	ZmCBL6	EU907936		SbCBL6	FJ901262		
	ZmCBL8	EU907937		SbCBL7	FJ901260		
	ZmCBL10	EU907938		SbCBL8	FJ901266		
	<i>Saccharum</i> spp. (ROC22)	SsCBL1		KC800815.1	<i>Saccharum</i> spp. (GT28)	SoCBL1	KC800815
		ScCBL2-1		KX013374		SoCBL3	KC800816
ScCBL3-1		KX013375	SoCBL5	KC800817			
ScCBL4		KX013376	SoCBL6	KC800818			
SsCBL6		KC800818.1	SoCBL9	KC800819			

Supplementary figures

AtCBL2MSQCVDGKHLCTSVLGCDFDLDYK.QSGGLGDPPELLARDTVFS.VSEIEALYELFK	55
AtCBL3MSQCIDGFKHVCSSFFRCFDIDYK.QSGGLGDPPELLARETVFS.VSEIEALYELFK	55
ScCBL2-1MLQCLDGVKHLGLVLLKCCDIDLK..QPKGLEDPVLARETVFS.VSEVEALYELFK	54
ScCBL3-1MVQCLDGVQRLLAVLLKCCDVELK..QPRGLEDPQVLARETVFS.VSEVEALYELFK	54
AtCBL1MG.CFHSKAAK...EFRGHEDPVKLASETAFS.VSEVEALFELFK	40
AtCBL9MG.CFHSTAAR...EFPDHENPVKLASETAFS.VSEVEALYELFK	40
AtCBL10	MEQVSSRSSSLTVGEQFCVAFIPFFFAIIDVLVSSVQCFCDCRSTSP...RTCQHADLERLARESCFS.VNEVEALYELFK	76
AtCBL4MG.CSVSKKKKKNAMRPPGYEDPELLASVTTFE.VEEVEALYELFK	44
ScCBL4MG.CVSSKGSK...RPPGYVDPNILAKETTFE.VNEVEALYELFK	40
AtCBL8MLAFVK.CFSLKRAK...HPRGYEDPHVLASETTFE.VNEIEALHDLFK	44
AtCBL5MG.CVCSKQLE...GRRQEDISLLASQTFE.EAEVEVLHGLFI	39
AtCBL6MMMQLDGLKHLALILLTCCDAD...PPKVRQNPKDVARGTVET.VNEIEALYELFK	53
AtCBL7MDSTRNSASSNS...TGCFTDQKKRKLALYEVEKLLSGVDCQRNEGN	43
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AtCBL2	KISSAVIDDGLINKEEFQALAFKTNKKESLFADRVFDLFDTKHNGILGEEEFARALSVFHPNAPIDDKIHFSFQLMDDLKQ	135
AtCBL3	KISSAVIDDGLINKEEFQALAFKTNKKESLFADRVFDLFDTKHNGILGEEEFARALSVFHPNAPIDDKIHFSFQLMDDLKQ	135
ScCBL2-1	KISSAVIDDGLINKEEFQALAFKTNKKESLFADRVFDLFDTKHNGILGEEEFARALSVFHPNAPIDDKIHFSFQLMDDLKQ	134
ScCBL3-1	KISSAVIDDGLINKEEFQALAFKTNKKESLFADRVFDLFDTKHNGILGEEEFARALSVFHPNAPIDDKIHFSFQLMDDLKQ	134
AtCBL1	SISSSVDDGLINKEEFQALAFKSRKRENIIFANRIEFDLFDVKKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	120
AtCBL9	SISSSVDDGLINKEEFQALAFKSRKRENIIFANRIEFDLFDVKKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	120
AtCBL10	KLSCSIIDDGLIHKEELRLALFQAPYGENLFLDRVFDLFDLDEKKNVIEEETHALSVFHPNAPIDDKIHFSFQLMDDLKQ	156
AtCBL4	KLSSSIIDDGLIHKEEFQALAFKRNRRNRLFADRVFDLFDVKKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	124
ScCBL4	KISYSIIKDGLIHKEEFQALAFKRNRRNRLFADRVFDLFDLKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	120
AtCBL8	KLSTSIINDGLIHKEEFLALFRNGSMQNLFADRVFDFDRKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	124
AtCBL5	KLTSCLSNNDLLTKKFKQFILKNTKRRSLSAERIEFDLFDLKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	119
AtCBL6	SIS...KNGLIDKEQFQVLVLFKMNTRSLFADRVFDLFDLKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	129
AtCBL7	VVEGVTCTYYGEMNKEQFHVAIFQTDKNESLFSERIEFDLFDLKRNGVIDEGDFVRSINVFHPNAPIDDKIHFSFQLMDDLKQ	123
Consensuske r f fd g f f l fhp k f l d	
AtCBL2	QGFIERQEVKQMVVATLAESGMNLDKTVIEDIIDKTFEEADTKHDGKIDKEEWRSLVLRHPSLLKNMTLQYLKDIITTTFF	215
AtCBL3	QGFIERQEVKQMVVATLAESGMNLDKTVIEDIIDKTFEEADTKHDGKIDKEEWRSLVLRHPSLLKNMTLQYLKDIITTTFF	215
ScCBL2-1	QGFIERQEVKQMVVATLAESGMNLDKTVIEDIIDKTFEEADTKHDGKIDKEEWRSLVLRHPSLLKNMTLQYLKDIITTTFF	214
ScCBL3-1	QGFIERQEVKQMVVATLAESGMNLDKTVIEDIIDKTFEEADTKHDGKIDKEEWRSLVLRHPSLLKNMTLQYLKDIITTTFF	214
AtCBL1	TGFIERQEVKQMLIALCESEMKLADDEIIEIIDKTFEADTVNQDGKIDKLEWSDVFNKNPSLLKIMTLPYLRDITTTFF	200
AtCBL9	TGFIERQEVKQMLIALCESEMKLADDEIIEIIDKTFEADTVNQDGKIDKLEWSDVFNKNPSLLKIMTLPYLRDITTTFF	173
AtCBL10	TGFIEREVQQMVSAIILLESMDMLSDLELTMIDKVVSS.....SLSVSRKKTVL.....	194
AtCBL4	TGFIEREELKEMVVALHESELVLSDEMTIEVMVDKAFVQADRNDGKIDIDEWKDFVSLNPSLTKNMTLPYLKDIRNTRFF	204
ScCBL4	TGFIEREELKEMVVALHESELVLSDEMTIEVMVDKAFVQADRNDGKIDIDEWKDFVSLNPSLTKNMTLPYLKDIRNTRFF	200
AtCBL8	TGFIERPEHLKVMGALLGHTDLELSEESIIEAVEQTMLEVDITNKDGKIDEEWKELVAKNPSLTKNMTLPYLKDIRNTRFF	204
AtCBL5	TGFIERPEEVKEMIIDVLEESLMLSESIIDSIIVSKTFEEADTKHDGKIDIDEWKELVAKNPSLTKNMTLPYLKDIRNTRFF	199
AtCBL6	QGFIERQEVKQMVVATLAESGMNLDKTVIEDIIDKTFEEADTKHDGKIDKEEWRSLVLRHPSLLKNMTLQYLKDIITTTFF	209
AtCBL7	QGFIERQGVKQLVVATLAESGMSQSDIEIIESIIDKTFVQADTKHEGMIDIEEWMDLVFRHPLLLKNMTLQYLKDIITTTFF	203
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AtCBL2	SFVFHQSQVEDT.....	226
AtCBL3	SFVFHQSQVEDT.....	226
ScCBL2-1	SFVFHQSQVDDT.....	225
ScCBL3-1	SFVFHQSQVDDN.....	225
AtCBL1	SFVFHSEVDEIAT.....	213
AtCBL9	173
AtCBL10	194
AtCBL4	SFVSSCEEEEMELQNVS	221
ScCBL4	SFVLNSGASDEEL....	213
AtCBL8	SFVLDSEVED.....	214
AtCBL5	TFLR.....	203
AtCBL6	NVVFHTIVTDTPSELDG	226
AtCBL7	SFVLHSQVEDT.....	214
Consensus	

Figure S1 Sequence homology analysis of sugarcane and *A. thaliana* CBLs. Sequences highlighted in dark blue, red, and light blue indicate homology 100%, $\geq 75\%$, and $\geq 50\%$ homology, respectively. GenBank accession numbers were as follows: AtCBL1 (AAC26008); AtCBL2 (AAC26009); AtCBL3 (AAC26010); AtCBL4 (AAC26110); AtCBL5 (CAB80951); AtCBL6 (AAG28400); AtCBL7 (AAG10059); AtCBL8 (AAL10300); AtCBL9 (AAL10301); AtCBL10 (AAO72364); ScCBL2-1 (KX013374); ScCBL3-1 (KX013375); ScCBL4 (KX013376).

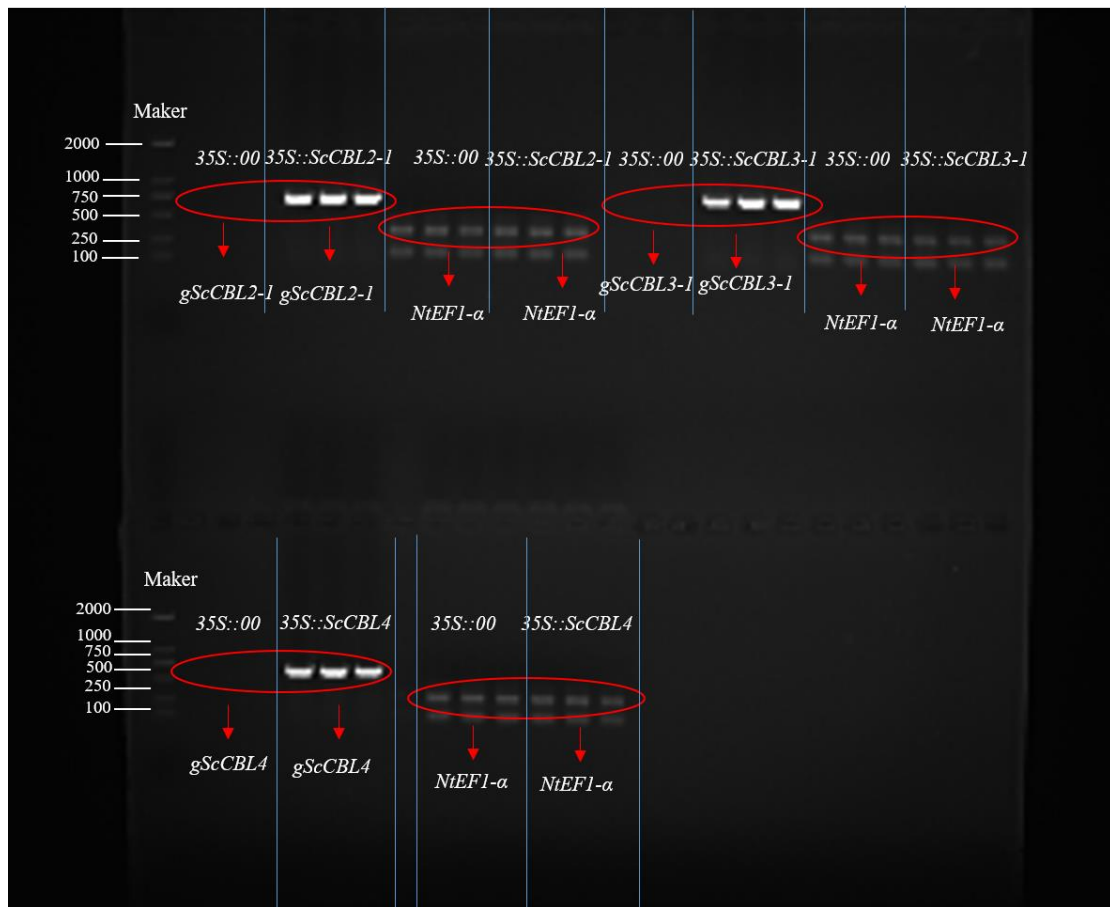


Figure S2 RT-PCR analysis of *ScCBLs* in the *N. benthamiana* leaves at 24 h post-infiltration. Note: 35S::00 stands for the control templet, 35S::ScCBL2-1, 35S::ScCBL3-1, and 35S::ScCBL4 stand for the templet of instantaneous expression *ScCBL2-1*, *ScCBL3-1*, and *ScCBL4*, respectively. *gScCBL2-1*, *gScCBL3-1*, *gScCBL4* and *NtEF1-α* represented the primer used in the RT-PCR.