

# Cold-induced modulation and functional analyses of DRE-binding transcription factor gene, *GmDREB3*, in soybean (*Glycine max* L.)

Ming Chen<sup>1</sup>, Zhaoshi Xu<sup>1</sup>, Lanqin Xia<sup>1</sup>, Liancheng Li<sup>1</sup>, Xianguo Cheng<sup>2</sup>, Jianhui Dong<sup>1</sup>, Qiaoyan Wang<sup>1</sup>, Youzhi Ma<sup>1,\*</sup>

<sup>1</sup> National Key Facility for Crop Gene Resources and Genetic Improvement (NKFCRI)/Key Laboratory of Crop Genetics and Breeding, Ministry of Agriculture, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), 12<sup>#</sup> Zhongguancun South Street, Beijing 100081, China

<sup>2</sup> Institute of Natural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, 12<sup>#</sup> Zhongguancun South Street, Beijing 100081, China

**\* Corresponding author:**

Name: You-Zhi Ma

Address: Institute of Crop Science, Chinese Academy of Agricultural Sciences, 12<sup>#</sup> Zhongguancun South Street, Beijing 100081, China

Telephone: 86-10-68918789

Fax: 86-10-68918789

e-mail: mayzh@mail.caas.net.cn

A.

GmDREB3	.....	0
GmDREB2	.....MEEAGL.G	7
GmDREB1	.....MEDR...D	5
GhDBF1	.....MELG....	4
PpDBF1	.....MVEKVVVSSLRRKRGGN	17
RAP2.1	.....	0
RAP2.10	FFETSSLLRFRVPLLSWPTCSSISPSACNRFVNITSFFFFIFKISLDRFFCGF	55
Consensus		

		***	
GmDREB3	.....MAKPSS.....EKPEEHSDS.KYYKGVRRKRWGKWVSEI	33	
GmDREB2	DCCSSNTTITRKSEKR.....KQQHQQQE..KPYRGIRMRKRWGKWVAEI	49	
GmDREB1	HCCSNNSTMITTTKKRTGRRSPTSDKLNQHREKQSMKPYRGIRMRKRWGKWVAEI	60	
GhDBF1	DCCLTSSPAS..GEKR.....KLHRTQQEKPFYRGIRMRKRWGKWVAEI	45	
PpDBF1	LLAPLKRNVSSIAKKSST.....PKSGKPVGSPKVYKGVRRMRTWGKWVSEI	63	
RAP2.1	MRKRRQPPQE...EVP.....NHVATR...KPYRGIRRRKRWGKWVAEI	37	
RAP2.10	LKSPEKTTMETATEVATVTVSTPAVT..VAAVATRKRDKPYKGVRRMRTWGKWVAEI	108	
Consensus			k g r r wgkwv ei

GmDREB3	RLPNSRQRIWLGSYDTPEKAARAFDAAMFCLRCRNAKENFDPNPPDIAG.....	82
GmDREB2	REPnkRSRIWLGSYATPVAAARAYDTAVFHLRCPSARLNFPPELLSQDDD.....	98
GmDREB1	REPnkRSRIWLGSYTTTPVAAARAYDTAVFYLRCP TARLNFPPELLFQDDDQEGSD.	114
GhDBF1	REPnkRSRIWLGSYTTTPVAAARAYDTAVFYLRCP SARLNFPDLIFQEDE.....	94
PpDBF1	REPnkRSRIWLGSFPTAEMAAYDAAVCLRCRSVKLNFPDSPPRCAARCNSPR	118
RAP2.1	REPnkRSRIWLGSYTTDIAAARAYDVAVFYLRCP SARLNFPDLLLQEEEDHLS...	89
RAP2.10	REPnkRSRIWLGSYSTPEAAARAYDTAVFYLRCP SARLNFPPELLAGVTVTGGG..	161
Consensus	r p n r r wlgs t aa a d a lrg nfp	

GmDREB3	.....GTSMTPSQIQIAAQAQFANAGPHEG.....HSGRPEHPPM	116	
GmDREB2	.....VSTQQQGKMSADSIKQKATQVG.....ARV	123	
GmDREB1	.....S...VQHGAAGNMSADSIKQKATQVG.....ARV	140	
GhDBF1	.....LRDISAASIRKKATEVG.....AKV	114	
PpDBF1	EVQAAATAAAVACIPSATALTVANFQSPAQTLESSPLHSSPSSSDMSSGDEDSGE	173	
RAP2.1	.....AATTADMPAALIREKAAEVG.....ARV	112	
RAP2.10	.....G...GGVNGGGDMSAAYIRKAAEVG.....AQV	187	
Consensus			a

GmDREB3	ESPSPSVSEGTIQTDSVPTLNGSVTDLFTP.....VGSSGYASDYGIFPGFDDF	166
GmDREB2	DALQTALQOSS.....STHSISSSHV..	144
GmDREB1	DALQTALHHHAP.....STNSLN.....	158
GhDBF1	DALQTSLHHASA.....SSSESNPTR..	136
PpDBF1	DSVQSFVAEGSQISPSEVVPVLEWVKVEFGDKETVAAGDVDFDCDYFIFSEFAAD	228
RAP2.1	DALLASAAPS.....MAHSTP.....	128
RAP2.10	DALEAAGAGGNRHHHHHQHQRGNHDYVDNHSD...YRINDDLMECSKKEGFKRC	238
Consensus		

LWSY motif

GmDREB3	SGDFYVPEMPN.VNYGEENGEFIVDESFLWNF	198
GmDREB2	..SYEKPDLENYPKPED.....	159
GmDREB1	...LKPDLNEFPKLEELQD.....	174
GhDBF1	..VFRKPDLENYPKPED.....	153
PpDBF1	SLLACSYNSKGRTEYLPPIETHDAALYDSTLWFF	261
RAP2.1	..PVIKPDLENYPKPED.....ESGDI.....	144
RAP2.10	NGSLERVDLNLKLPDPETSDDD.....	259
Consensus		

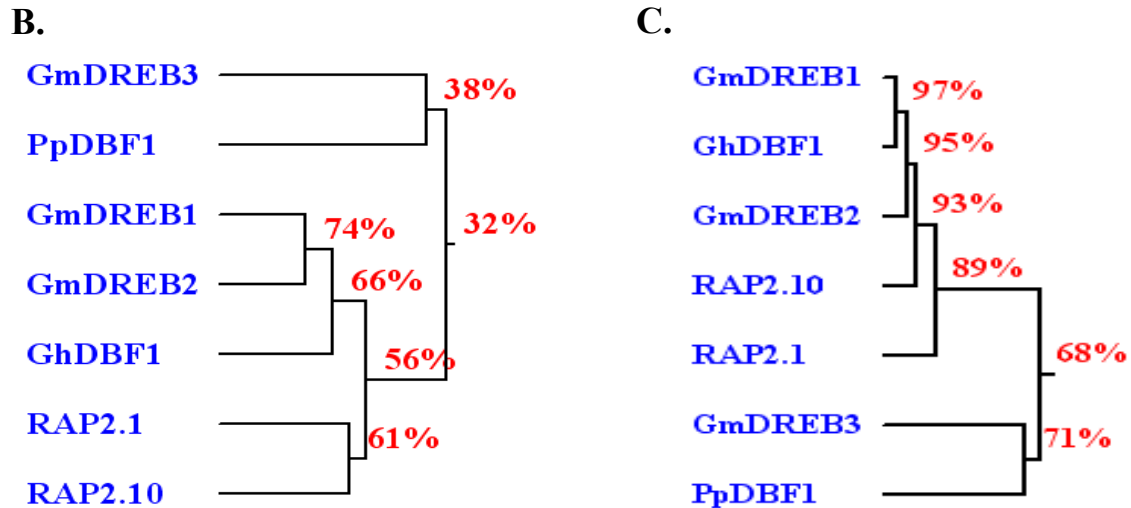


Figure S1. Amino acid sequence alignment of GmDREB3 and other members in the A-5 subgroup. A, Comparison of deduced amino acid sequence of GmDREB3 with DREB proteins from soybean, moss plant, cotton and *Arabidopsis*. The beeline above the sequences represents the conserved AP2 domain. Asterisks indicate putative nuclear localization signals. The pane shows the LWSY motifs in the C terminal of GmDREB3 and PpDBF1. Accession numbers for the DREB proteins used are as follows: GmDREB3, DQ208969; GmDREB1, AF514908; GmDREB2, DQ208968; GhDBF1, AY174160; PpDBF1, DQ202211; RAP2.1, AF003094; RAP2.10, AF003103. Homologies of the full-length amino acid (B) and AP2 domains (C) sequences in the A5 subgroup are shown with % values.

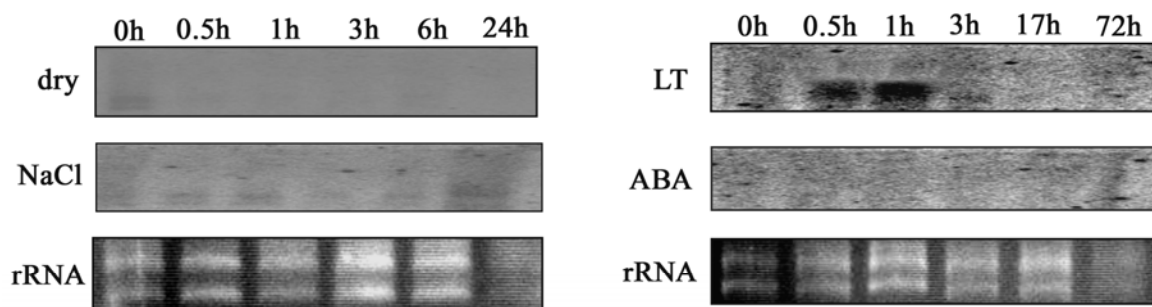


Figure S2. Expression pattern of the *GmDREB3* gene in soybean under different abiotic stresses including drought (dry), 200 mM NaCl (NaCl), low temperature 4°C (LT), and 200  $\mu$ M ABA treatment (ABA). rRNA was used as the RNA control.

```

1 .....GTTACGAGTAATCAGTTACACG. . TACATACATAATTT
      |  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
1 ATGTTGGCCAGGCATATAAAGTGAAACACGTTATTATTTATATGATCGCATATAAAAGAA

37 TGAGTAACTTTTTT. . . TTTGGTGAATATAATTATGAGTAGATTTTTACTGTTGTGTAAC
   |  |  |  |  ||  |  ||  |  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
61 TTGGAAGGTCTTGTAAGTTTCGAGAATGTATGTTGGTTAAAGTATAAATACATTGGTAG

94 TTAGCTTTA. . . . . CTTGTTATCTTGTATCTAGTTGG. . . TACTGCTGATACCTTG
   |||  |||  |  ||  |  |  ||  ||  |  ||  ||  ||  ||  ||  ||  ||  ||
121 TTAAC TTCATGGTAAACTCTACCGCGTCACCTTTAGTCGAAGGATTTTCATTATCTTTTG

143 ATTTTAATAGTAGTATAATTTTGTCTTTTGCCGACCCTCTCCAGAAAAAAGAAACCCT. C
     ||  |  |  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
181 ATCAAATCAATAATATAATATAATGTCATGTGTATTAATTTTTTATATTCTAATTTTAAT

202 ATTCCATTTTATATAATT. AAACCTTTTTTATTTATTAATTTTACTTATTAATAATAAAC
     |||  |||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
241 ATTTCATTAATATAATTTAAAAAATGAATATATTTACAAAATGATCCTGAATTAATT. T

261 ATGAAGTCCTATACATATTAAGGGAAAATAAAAGGGATTAGTTGATGTGAGGAAAGTGA
     |  ||  |  |  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
300 GTAAAATTTAAACTAATTTGCATCTCATCCGATCTCAGTGGCTCACGCATTCTCCGTCA

321 AAACGATGGAATGCATTGCACCAG. GTGGTTGTGGTCATACGGGGCAT. . TCTGAATCGG
     ||  ||  |  |  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
360 TCGCGTCTTCTCCCTCTCTGCCGACACCTTCGCCGCCGATCCCGTCTTGGTCTCCACCGC

378 GATTAGAAAGGTCATGATCGAATCAGATTATGACAATGGGAGAACATAAGCATCACACAT
     |  ||  ||  ||  |  |  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
420 CGCCAAAATCAAAGAGACGCGGT. . GGCTCTGGCGGCGCTCCAAGACCCAGCTCCAGTGG

438 GACGTAAAATATACACGTGGGGCATATGTCTCCAACCCTGTCTCTTTACTCTTCAGCTAC
     |  ||  ||  |  |  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
478 CTCCTATGATTCCCCCTCGGGACCCGTCCTCTGGTCTGTTTCTTCTGGTCTCCTACGTT

498 TTTTTCTTAGAATGTTTAATACTTCTACGATTAATAATTAATTTTCTGTT. GTTAAACAA
     ||  |  |  |  ||  |  |  |  |  |  |  |  ||  ||  ||  ||  ||  ||
538 TTCTACACATGTTGCGCACCGTCTTCGTCGTCGTAAGCTCGTTTTCTTTTCTTCTTCTTCTA

557 GTAAAATAGGTG. GAGAAAAAAACCATTTGATAGCGAGATTAATAATATGCTACTTGATA
     |  |  |  |  |  |  |  |  |  |  ||  ||  ||  ||  ||  ||  ||
598 CCACGCCATTTCCGCGTTCATGTCTTTTCTGTGGCTGGAGTTCTCGCGATCCTCTTACC

```

616 GTAGTTTTTTGATATTTTTATGGTAGCTAAGAATTTTAGTAGTAAGATCAAAAATAAAAAA  
| | | | | | | | | | | | | | | | | | | |  
658 ACGCTCCCGTTCTCCGTTATGTTTGGTTACAGGTTCTGG. . . . . ACCGCAGTACG. . .  
  
676 TTAATTTCGTGATAAAAATCAATTCAAAAATGTCCGATCAATGTATTATAATATTTTCGAT  
| | | | | | | | | | | | | | | | | | | |  
708 TGGCGGCGCAAGGCGAGTGCCTGCCACTCGTGCTCAACTGCCAGATTGCGCTGCTCGGGT  
  
736 AACATGTGATAATTCTCGCATCACTTACTACATTTCAAAATAAATTAATTACAAATGCA.  
| | | | | | | | | | | | | | | | | | | |  
768 GCAACTTGGTTTGCCACGTGGCGGTGTCT. . . TGTTGCATTTTTTTTACAGGTGGGTGTAA  
  
795 TAAAAAATTGCAAGAACTATATAATAAAAATCATTAGAATAATTTATACCAATTT.  
| | | | | | | | | | | | | | | | | | | |  
825 CGGGATTGGCGGTGGGTTCGTCATTCCTGCT. CAACTGTGCTATTTTGTTCCTTTTC  
  
854 . ACATTTTTATCCTTTTACGAAATGGTAAACTGAATCCCATCGATTGATGGATGCAAGG  
| | | | | | | | | | | | | | | | | | | |  
884 TAAATTTTTATCACGTTGTGAGAATGGTA. . . GCATTGC. TCGATCGTTC. TGCTCCT  
  
913 CAGCGATTTTTTTTTTAACAATTTATGAAAATGTTTAATTAATCTTGACATAATAAAAAAC  
| | | | | | | | | | | | | | | | | | | |  
938 CTGTCTTACGCGCTGGAAAAGAAGCACATAGTAATAAATTGAAGTCCA. ATAAAAGCAC  
  
973 GGGCACTGATATGCTTAATAAAAATATTACTGTATACTGAAAAGTATTCAACCTCATGAA  
| | | | | | | | | | | | | | | | | | | |  
997 AGATTCATTGAAGGTTAAGTCATGAGTACCGTTGCCAATGATGGCTTGAAGGTTTTAGG  
  
1033 ATTTA. . . TTTCTTTTGTAATAAA. . TATTTAAAATTTAAGTTCATACTGATAGATGT  
| | | | | | | | | | | | | | | | | | | |  
1057 TTCTAAACCATCATGTATGAGTAAAAATGTCTTTTGAAGATCTGACATGGATACAAAC  
  
1088 TTAAATTTTATCTTGTTCCTAATAAATAATATACTAATTATTCTAAAAATAATTTAA  
| | | | | | | | | | | | | | | | | | | |  
1117 ACGAATTTGAGATATATAATGTTGGATGAAAAAGCT. . CTAAACAAAACATACATAAAA  
  
1148 TGTAGTAATAATTCCTTAATGTTGAAGTAGTAGTATTAATGTTTTTTTTCTAGAGTT  
| | | | | | | | | | | | | | | | | | | |  
1175 TGCAGTTACACACGAGATATTGC. ATGATGGGAGCATTTTATATTATTCATTTTGAGTGA  
  
1208 ACTTTCTGTAGATACTGAATAAATAATAGTATTTGATTCTTA ACTATCAAAATCACTTCA  
| | | | | | | | | | | | | | | | | | | |  
1234 ATTTTTTAAAATTAATAATTATA. . . AATATTTAAATATTTAT. . . AAAATTAACA



```

1920 TAAGTTTTCAGTTCTCACAAATAAATAAAAAAAAAATTGTTTTTCGT. GTGGAAACAAAAAAA
    | | |||| | | | | | | | | | | | | | | | | | | | | | | | | | |
1924 TTAATTTT. ATTAATTACTACTAGGTAGCTGGTTTTTAATAGGTAGTAGGTAGGTGTAAT

1979 CAATTCAAAAACAAACATATGATG
    | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1983 CGTTGTCTTCCTGCAGATATG. . .

```

**Figure S3. Promoter sequence alignment of *GmDREB3* (upper line) and *GmDREB2* (low line) in the soybean genome. The promoter sequence of *GmDREB2* was searched from Scaffold 26 in the Soybean genome project ([www.phytozome.net/soybean.php](http://www.phytozome.net/soybean.php)). The results of alignment indicated that the promoter of *GmDREB3* shared 43.5% identity with the promoter of *GmDREB2*.**