

Sugarcane *ScDREB2B-1* Confers Drought Stress Tolerance in Transgenic *Nicotiana benthamiana* by Regulating the ABA Signal, ROS Level and Stress-related Gene Expression

Yufeng Chen¹, Zhu Li¹, Tingting Sun¹, Dongjiao Wang¹, Zhoutao Wang¹, Chang Zhang¹, Youxiong Que¹, Jinlong Guo¹, Liping Xu^{1,*} and Yachun Su^{1,2,*}

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

² Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture and Rural Affairs/Guangxi Key Laboratory of Sugarcane Genetic Improvement/Sugarcane Re-search Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China

* Correspondences: xlpmail@126.com (L.X.); syc2009mail@163.com (Y.S.); Tel.: +86-591-8385-2547 (L.X. & Y.S.)

Supplementary Figures and Tables

Figure S1. Nucleic acid sequence alignment of the cDNA and DNA sequences of *ScDREB2B-1* gene. The red arrow was the ORF sequence.

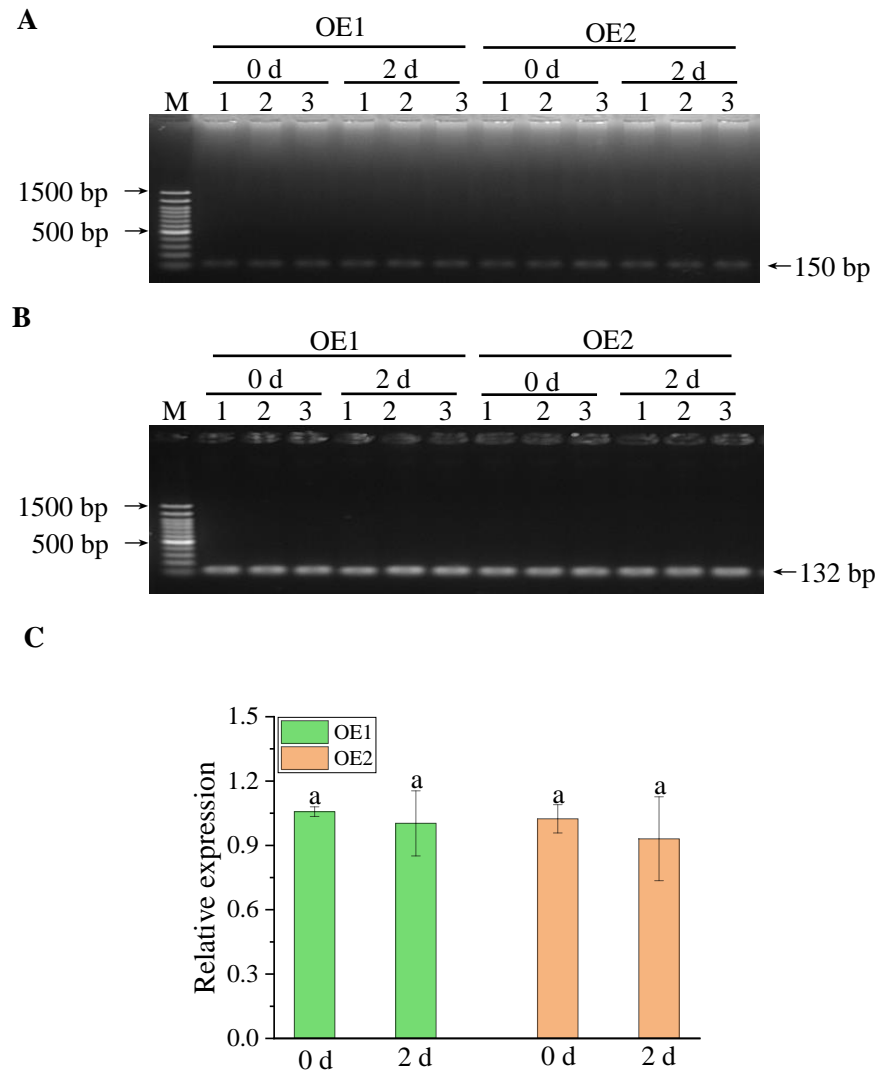


Figure S2 Detection of the *ScDREB2B-1* gene in two transgenic *Nicotiana benthamiana* lines under drought stress for 2 d. (A) RT-PCR analysis of the *NbEF1α* gene with the primers *NbEF1α*-F/R (Table S1); (B) RT-PCR analysis of the *ScDREB2B-1* gene with the primers RT-qPCR-*ScDREB2B-1*-F/R (Table S1). M: 100 bp-III DNA marker; OE1 and OE2: different transgenic *N. benthamiana* lines overexpressed *ScDREB2B-1*. 1-3: PCR amplification products of different duplicates transgenic *N. benthamiana* plants overexpressed *ScDREB2B-1*. (C) The relative expression level analysis of the *ScDREB2B-1* gene by RT-qPCR method. *NbEF1α* was used as the internal reference gene. All data points were means \pm standard error ($n=3$). Significant differences were found between different letter substitutes on the column calculated by Duncan's new multiple range test (P -value < 0.05).

Table S1 Primers used in this study

Primer name	Primer sequences (5'-3')
pMD19-T- <i>ScDREB2B-1</i> -F	TAGTGTGTGCGCTTGACCA
pMD19-T- <i>ScDREB2B-1</i> -R	GCAGTGTTCCTTCCACTT
RT-qPCR- <i>ScDREB2B-1</i> -F	ACACAATGTGGGTACCGTGG
RT-qPCR- <i>ScDREB2B-1</i> -R	ATAGGCCCTAGCTGCATCCT
Promoter- <i>ScDREB2B-1</i> -F	CGTGAACCTGTGCCAATG
Promoter- <i>ScDREB2B-1</i> -R	CTCCCGTAAATTCAAATGG
pGBKT7- <i>ScDREB2B-1</i> -F	CATGGAGGCCGAATTCATGGGTTTCAAGAAGGGCTG
pGBKT7- <i>ScDREB2B-1</i> -R	GGATCCCCGGGAATTCCTTTAGCTGCTCCAGACCCT
CD3689- <i>ScDREB2B-1</i> -F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGTTTCAAGAAGGGCTG
CD3689- <i>ScDREB2B-1</i> -R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTTAGCTGCTCCAGACCCT
<i>NbEF1α</i> -F	TGCTGCTGTAACAAGATGGATGC
<i>NbEF1α</i> -R	GAGATGGGGACAAAGGGGATT
<i>NbRbohA</i> -F	ACACACGCCATCAGAACTCCA
<i>NbRbohA</i> -R	CCCACCCAACCAAAATACGC
<i>NbRbohB</i> -F	GTTTGCCAGCCACCACCTAAT
<i>NbRbohB</i> -R	AAGAGCAGAACGAGCATCACC
<i>NbERD</i> -F	CACTGATAAGAACTATGCGTTCAC
<i>NbERD</i> -R	CTAAGCTAATCACATTCAGCGAG
<i>NbLEA</i> -F	GGATCTAATTGACAAGGCGAAG
<i>NbLEA</i> -R	CTCGCCGCTATAAGAGAGAG
<i>NbNCED</i> -F	CGACCCACGAGTCCAGATTTC
<i>NbNCED</i> -R	GAGCCTAGCAATTCCTCCGAGTG

Table S2 PCR amplification systems and programs used in this study

Test name	The PCR system	The PCR program
Cloning of <i>ScDREB2B-1</i> gene	10 × Ex Taq Buffer, 2.0 µL; dNTP mixture (2.5 mM), 1.6 µL; Primer-F/R (10 µM), each 1.0 µL; Ex Taq enzyme (5 U/µL), 0.1 µL; cDNA template, 1.0 µL; ddH ₂ O, 13.7 µL.	94 °C, 4 min; 94 °C, 30 s, 65 °C-55 °C, 30 s, 72 °C, 2 min, 10 cycles (each cycle lowers 1 °C); 94 °C, 30 s, 53 °C, 30 s, 72 °C, 2 min, 25 cycles; 72 °C, 10 min. 50 °C, 2 min;
RT-qPCR analysis	SYBR Green PCR Master (ROX), 10.0 µL; ddH ₂ O, 7.0 µL; Primer-F/R (10 µM), each 1.0 µL; cDNA template (20-fold dilution), 1.0 µL.	95 °C, 10 min; 95 °C, 15 s, 60 °C, 1 min, 40 cycles; 95 °C, 15 min; 60 °C, 1 min; 95 °C, 15 s, 60 °C, 15 s.
Promoter cloning	10 × Ex Taq Buffer, 2.5 µL; dNTP mixture (2.5 mM), 2.0 µL; Primer-F/R (10 µM), each 1.0 µL; Ex Taq enzyme (5 U/µL), 0.125 µL; DNA template, 1.0 µL; ddH ₂ O, 17.375 µL.	94 °C, 4 min; 94 °C, 30 s, 55 °C, 30 s, 72 °C, 3 min 30 s, 35 cycles; 72 °C, 10 min.
Construction of <i>ScDREB2B-1</i> entry vector	10 × Ex Taq Buffer, 2.5 µL; dNTP mixture (2.5 mM), 2.0 µL; Primer-F/R (10 µM), each 1.0 µL; Ex Taq enzyme (5 U/µL), 0.125 µL; cDNA template (pM19-T- <i>ScDREB2B-1</i> plasmid), 1.0 µL; ddH ₂ O, 17.375 µL.	94 °C, 4 min; 94 °C, 30 s, 71 °C, 30 s, 72 °C, 1 min, 35 cycles; 72 °C, 10 min.
Target gene detection in transgenic plants	ddH ₂ O, 9.5 µL; 2 × Buffer, 12.5 µL; Primer-F/R (10 µM), each 1.0 µL; DNA template, 1.0 µL.	94 °C, 4 min; 94 °C, 30 s, 60 °C, 30 s, 72 °C, 1 min 30 s, 35 cycles; 72 °C, 10 min.

Table S3 The amino acid sequences of 51 DREB proteins from A1 to A6 subgroups of DREB subfamily used for phylogenetic tree analysis

Subgroups	Protein name	Amino acid sequence
A1	AtCBF3	MNSFSAFSEMFGSDYESSVSSGGDYIPTLASSCPKKPAGRKKFRETRHPHYRGVRR RNSGKWVCEVREPNNKTRIWLGTFTQTAEMAARAHDVAALALRGRSACLNFA DSAWRLRIPESTCAKDIQKAAAEALAFQDEMCDATTDHGFDMETLVEAIYT AEQSENAFYMHDEAMFEMPSLLANMAEGMLLPSPVQWNHNHEVDGDDDD VSLWSY
A1	HvCBF3	MDMGLEVSSSSPSSSSVSSSPEHAARRASPAKRPAGRTKFRETRHPVYRGVRRRG NTERWVCEVRVPGKRGARLWLGTYATAEVAARANDAAMLALGGRSATCLNF ADSAWLLAVPSALSADLADVRRAAVEAVADFQRREAADGSLAIAVPKEASSGA PSLSPSSGSDSAGSTGTSEPSANGVFEGPVVMDSEMFRDLDFPEMDLGSYYMSLA EALLMDPPPTATIIHAYEDNGDGADVRLWSYSVDM
A1	OsDREB1A (LOC_Os09g350 30.1)	MCGIKQEMSGESSGSPCSSASAERQHQTWVTPPKRPAGRTKFRETRHPVFRG VRRRGNAGRWCVRVPGRRGCRLWLGTFTDAEGAARAHDAAAMLAINAGG GGGGGACCLNFADSAWLLAVPRSYRTLADVRHAAVEAEVEDFFRRRLADDALS ATSSSTTPSTPRTDDEESAATDGDESSSPASDLAFELDVLSDMGWDLYYASLA QGMLMEPPSAALGDDGDAILADVPLWSY
A1	OsDREB1B	MEVEEAAYRTVWSEPPKRPAGRTKFRETRHPVYRGVRRRGGRPGAAGRWWCE VRVPGARGSRWLWGTFTATAEAAAARAHDAAALALRGRAACLNFAADSAWRMP PVPASAAAGARGVRDAVAVAVEAFQRQSAAPSSPAETFANDGDEEEDNKDV LPVAAAIEVFDAGAFELDDGFRFGMDAGSYASLAQGLLVEPPAAGAWWED GELAGSDMPLWSY
A1	ZmDREB1.2 (GRMZM2G069 082_P01)	MDMGRHQLQLQHAASSSTSASSSSEQDKPLCCSGPKKRPAGRTKFRETRHPVF RGVRRRGAAGRWCVRVPGRRGARLWLGTYLGAEEAAAARAHDAAAMLALGR GAACLNFPDSAWLLAVPPPPALSGGLDGARRAALEAVAEFQRRRFGAAAAD ATSGTSPSSSSSATKPAPIERVPVEASETVALDGAVFEPDWFGMDLDLYAS LAEGLLVEPPPPPPPAAWDHGDCCDSGADVALWSY
A1	ZmDREB1.3 (GRMZM2G069 146_P01)	MCPTKKGMTGEPSSPCRSASASTLPEHHQTVWTSPPKRPAGRTKFRETRHPVFR GVRRRGSAGRWCVRVPGRRGCRLWLGTFTDAEAAAARAHDAAAMLALAGA AACCLNFADSAWLLAVPASCASLAEVRHAAVEAEVEDFLRHQVVPEDDALAA TPSSPSEDGNTSDGGESSDSSPPTGASPFEDVFNDMSWDLHYASLAQGLLVE PPSAVTAFMDEGFADVPLWSY
A1	SsAP2/ERF-027	MCPIKKEMIGDSSSPCSSASSEHQTWVWTSQPKRPAGRTKFRETRHPVFRGVRRR GNAGRWCVRVPGRRGCRLWLGTFDAAEAAAARAHDAAMLAIAAGAGRACL NFADSAWLLAVPASYASLAEVRHAAVEAEVEDFLRREVVPDPEDDALSATSTP PSSPSSDDGSTSDGESSDYSSPAATGAVSAFELDVFNDSWDLHYASLAQGM LVEPPSAVTAFMDEGFADAARVAACYGVLVLTSRDVAVAAAPLGAPKPQLET RILHSPLALGLYLPRAEPTSEQQRTQLGSLRPGRAVTGRACLFRDRKNKYVARA YNLLLTKAATSLAVRDASDDDIGAPTRHGCAGGAEASSVRPPTSWARGQT LFIERGEPAKPKGSDVLSAFPGRSRSIPSKWPFQNTSGRQQDCGSGIGTA ATHSPHAVQVRELPGCRCPLCDTDTVGGFSIAAHKMLPSRAARPPGAEMVLA RNTFLFIHS

Continued Table S3

Subgroups	Protein name	Amino acid sequence
A1	SsAP2/ERF-028	MCPIKKEMIGDSSSPCSSASSEHQTIVWTSPPKRPAGRTKFRETRHPVFRGVRRRG NAGRWWCEVRVPGRRCRLWLGTFDAAEAAAARAHDAAMLAIAGAGRACLN FADSAWLLAVPASLASLAEVRHAVTEAVEDFLRREVVPDPEDDALSATSSTPPS SPSSDDGSTSDGGESSDYSSPAATGAVSAFELDVFNDMMSWDLYYASLAQGMLV EPPSAVTAFMDEGFADSKMCPIKKEMSAESSASASSWPSSSPSTSSEHHQTIVWTS PPKRPAGRTKFRETRHPVFRGVRRRGNAGRWWCEVRVPGRRCRLWLGTFTD AEAAAARAHDAAMLAIAGAGACLNFAADSAWLLAVPVSYASLADVRRRAVAEAV EDFLQRRDGEAAAAGDTNARSATSSSAPSTSGNEDDAATDGEESPATDTEDSS FQLDVFNDMMSWDLYYASMAQGMLMELPSAVPAFGDDGDANVADVLSWSY*
A1	SsAP2/ERF-029	MCPIKKEMIGDSSSPCSSASSEHQTIVWTSPPKRPAGRTKFRETRHPVFRGVRRRG NAGRWWCEVRVPGRRCRLWLGTFDAAEAAAARAHDAAMLAIAGAGRACLN FADSAWLLAVPASLASLAEVRHAAEAVEDFLRREVVPDPEDDALSATSSTPPP SPSSDDGSTSDGGESSDYSSPATGAVSPFELDVFNDMMSWDLYYASLAQGMLV EPPSAVTAFMDEGFADVPLWSY*
A2	ZmDREB2A (GRMZM2G006 745_P01)	MTLDQNHAMPMQPPALQPRKKRPRRSRDGPTSVAAVIQRWAERNKHLEYE ESEEAKRPRKAPAKGSKKGCMKGKGGPDNTQCGYRGVRQRTWGKWVAEIRE PNRVDRLWLGTFTPTAEDAARAYDEAARAMYGDLARTNFPQDATTSAQAAL ASTSAQAAPTAVEALQTGTSCESTTTSNYSDIASTSHKPEASDISSSLKAKCPAGS CGIQEGTPSVADKEVFGPLEPITNLPDGGDGFDIGEMLRMMESDPHNAGGADA GMGQPWCLDELDSSVLESMLQPQPEPEPFLMSEEPDMFLAGFESAGFVEGLERL N
A2	OsDREB2A (LOC_Os01g071 20.1)	MERGEGRRGDCSVQVRKKRTRRKSDGPDZIAETIKWWKEQNQKLQEENSSRK APAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGRRLWL GSFPTALEAAHAYDEAARAMYGPTARVNFADNSTDANSCTAPSMLMMSNGP ATIPSDEKDELESPPFIVANGPAVLYQPDKKDVLERVVPEVQDVKTEGSNGLKR VCQERKNMEVCESEGIVLHKEVNISYDYFNVHEVVEMIIVELSADQKTEVHEEY QEGDDGFSLFSY
A2	LcDREB3a	MTVHQMEAAAAAALPYAPFEIPALQPRKKRPRRSRDGPNSVSETIRRWKEVN QQLEHDPQGAKRARKPPAKGSKKGCMQKGKGPENTQCGFRGVRQRTWGKW VAEIREPNRVSRWLWLGTFTPTAEVAAQAYDEAARAMYGPLARTNFPQLQDAQAP AVAVPAAIEGVVRGASASCESTTTSNHSDVASSSHNKQPQAQAPEISSQSDVLE STQSVVLESTQSVVLESVRHYGQQETVPDAGSSIAIRSTYEEDVFEPLPISSLPDGE ADGFDIEELLRLMEADPVEVEPTTGGCWNEFQDAGANTGGSWNANTGVEMG QQEPLYLDGLDQGMLEGMLQSDYPYPMWISEDRAHNPFAFHDAEMSEFFEGL MGAYDQVSVRPLDSSRKRKSRSRGDSRSVAETIAKWKEYNEHLYSGKDDGRS TRKAPAKGSKKGCMKGKGGPQNYECNYRGVRQRTWGKWVGEIREPNRGSRL WLGTFTSTAQEAALAYDEAARAMYGPCARLNFPNISDYASVKESFKDSFLAASSS
A2	VuDREB2A	CSSTTSDTTTTTTSNRSEVCAAEADAKENVLPALDKGNHSDCHKAYEYASPTSR MKLEPKDDEAGEGIIHHAEQAENVNEDQMDFSWIDALDFNDDYSKSFNDEL FQVDELLGLIENNPVDDSGLMQGLDFGQTGFPGESNPQVDDTSSSFFYQLQNP DAKLLGSLPHMEQTPSGFDYGLDFMKTVPEDYDGGGEEPQFLNLDDVLNHD SKEMVASKE

Subgroups	Protein name	Amino acid sequence
A2	OsDREB2B (LOC_Os05g27930.1)	MTVDQRTTAKAIMPPVEMPPVQGRKKRPRRSRDGPTSAETIKRWAEELNNQQELDPQ GPKKARKAPAKGSKKGCMKGKGGPENTRCDFRGVRQRTWGWVVAEIREPNQQSRLW LGTFTPTAEAAACAYDEAARAMYGPMARTNFGQHHAPAASVQVALAAVKCALPGGG LTASKSRTSTQGASADVQDVLTGGLSACESTTTTINNQSDVVSTLHKPEEVSEISSPLRAP PAVLEDGSNEDKAESVTYDENIVSQQRAPPEAEASNGRGEVFEPELEPIASLPEDQGDYC FDIDEMPLRMMEADPTNEGLWKGDKDGSDAILELGQDEPFYYEGVDPGMLDNLLRSDE PAWLLADPAMFISGGFEDDSQFFEGL
	ZmDREB2.2 (AC209257.4_FG P006)	MGGPDNTRHNYRGVRQRRWGKWVAEIREPNRGKRHWLGTFDNPVDAAVAYDRAA VSIHGAHAHLNFPSCAATTADASLEHEAKPMVAAAALGGGGAEAVSQRQEDDDPHD IAMYIDFDAVFDMPVFCCHGKREDQCHEGFDGDAVHSPLWALGD MDHRLPPVAMQVAAMQRQRQQQQQQQFVHHLQVHQQQGTHHQLPPAPPPPQQQQ HQNSGGVGSRAAGGRRCCPLRQSRKGCMKGKGGPDNQCPYRGVRQRTWGWVVA EIREPNRGTRLWLGTFGSAFEAACAYDNAARKLYGDCAHLNLQLPPPAVAAMAAGG
A2	SsAP2/ERF-006	GGPAVVVSSPSPDTVAAGPAAAAGGHNRRHQYLQQQQQQQAAMAAAPMMMMQY SSSYSADASSNSGSFSNSYSSSSPVTTAAVAASPTYNNHHQMFQMTPPPSSCSGVMM APAVPQAQGCCHVNTTTTTTTTTMEMQRHQQMIRELAAAPLHQEPDDFADFMTWLPEA EDFGLQGFQEVPPPEVFDEAAGGIWDHTAATWSTPTMMIDSAAGAAQHQQ MVSHMDMLIRSRSNKQIDRTRQTGGGLIRNRMLCTSYYEEHHVKSTLVRAKNAVRWIF TPRIHAALITMTTNQRARLSLFLYVLTLTGLEKARHHRTYAETHAFPAVTTFKPTPCLA DHPRRRPARAHSTKPPAPLVTLSSTPTPTPIPIPMESYGRKRAWKKGPTRGKGGPQNAA CEYRGVRQRTWGWVVAEIREPNKRTRLWLGSFATAEEAALAYDEAARRLYGPD AFLN
A2	SsAP2/ERF-020	LPHLRASVSAAAHQRLRLWPASARGAAAAAVPAYGLNLNAQHNHVIHQRLQELK NGGSPAKPPPPQPARQVAPVHHLPAAASTSPCSTVTTHAVAALPPPMSCFHAEQAVA AAAMTTADDDAEPCGGACPPGADKPQLDLREFLQQIGVLKTDDEGMTATAKASYH GDAADAGCFGGNGEFDWDALAADLNDIAGAHGGAIGVNNGGFQMDDLHEVDQFGT CLPIPVWDV*
A2	SsAP2/ERF-051	MRRKSTGPDSIAETIKWWKEQNQKLQDESGSRKAPAKGSKKGCMTGKGGPENVCVY RGVRQRTWGWVVAEIREPNRGRRLLWLSFPTAVEAAHAYDEAAKAMYGPKARVNFS ENSADANSCTGTSALSLASSVPAATLQRSDEKVETEVESEVTEVHEVKTEGNDLGSVH VACKTVDVQSEKSVLHKEGEVSVDYFNVEEVVEMIIELNADKKIEAHEEYHDGDDGFS LFAY
A2	SsAP2/ERF-107	MTLDQNQAMPMQPPALLPGRKKRPRRSRDGPTSAAVIQRWAEHNKQLEHDPEGAK RPRKAPAMGSKKGCMKGKGGPDNTQCGYRGVRQRTWGWVVAEIREPNRVNRLWL TFPTSEDAARAYDEAARAMYGDLARTNFPQRQAATYAQAALASTSVQAAPTAVEALR PGTSCESTTTSNHSDIASTSHKPEASDSSSLKAEWPEALEAGSSGIQAGTPSVADKVFGT LEPITNLPDGGDGGVTNLPDGGDDGFDVDEMLRMMEADPHNEGGADAGMGQPWCL DGLDSSVLESMLQSEPEPFMLMSEPEMFLAGFESPSSFFEGLERLK*
A3	SbERF7 (Sb01g040280.1)	MVKNPGSNGVLTATAFSDNKLARPESGVVGNGKAAARPYKGVMRMRWSGWSVSEIRA PNQKRRIWLGSYATPEAAARAYDAALLCLKGSDAVLNFPATSTSSASASSSHRRADKD DDPAAGGGMSPRSIQRAAAAAAIDAADAGGISADDRCSSACAMTPTSASLSSTQGSS DHVRQEQAHTTTSPAAASTGSPPEGEELWTDLEAFASPKFMDLVDTGAAAPFSSTWEE PEDDGELMRLWSFC

Continued Table S3

Subgroups	Protein name	Amino acid sequence
A3	SbERF40 (Sb03g042060.1)	MMRRAEPVGEAADAERRRGRGGYKGVRRRRWGWKWWSEIRVPGTRERLWLGSYAT PEAAAVAHDTAVYFLRGGTGAGAGTGAGVAGGGDVAALNFPERAAAAYGTGA GAGAAGRLSPRSVQRVASDAGMAADAQLVAARESAPAPALAHARTGIGIGSA HDGGASAHARPGAGREQPAVSGEINVDDMEILM MVKSAQLQDTAVDGAAGNAVAARQQGAGAPAGYSGGGKRQYKGVMRMSWG SWVSEIRAPNQKTRIWLGSYSTAEAAARAYDAALLCLKGSAADLNFPVHLPFHIP A
A3	SbERF55 (Sb04g034290.1)	AAMSPKSIQRVAAAAANATCSPLPAAAPYSASTAVNDGATPPCSYYGDASSG VSSPETGNADLCHDGMDMAGDADFAALADIEAFFQSPKMEYGMMDPCSTFFA PAPMATDAANEWEEEGEINLWSFSSLN MDDGAGASRSDGEGSPRLPAERRYKGVFRKWGRWVSEIRMPNSRERIWLGSYSS AEKAARAFDAAAVCLRGSRAGSLNFPESPPNVRHIPGAMLTPEQIQAEARHAN Q
A3	SbERF82 (Sb07g022265.1)	QLLPSPPVASPAASSSSQPAAGGASSDRTTSLMPPSTYYGSGAAVCGDDEALDWS FMDALPSMPASSMGMGNSGADIVPALDDFMYGSPHPVMPPEEVTQDMIDDDD DHTFISDDLWRF MSRAASGGGAGTGRGGGGGAERAHRCRYRGVRRRAWGWKWWSEIRVPGTRERLW L
A3	SbERF94 (Sb09g020690.1)	GSYAAPEAAAVAHDAACLLRGGGGRAAGQAHLNFPDRAACYIVYGGSHGH GPPLSPRSVQRVASDAGMAADAQIVDARAAAAALAAPTTRVQPAAFAGIGAAQ GGAQGAACAPPPYSDGASSSSSTYWSTPSASASRTSSSTGSEQPLVFGDISVDDIEIL V MSSSPETEAGSSSGGKKFKGVRRKRWGWKWWSEIRLPNSRERIWLGSYDAPDKAAR AFDAAFVCLRGGRGAAGADLNFPDSPPPCRAGGCSSDPREVQAAALSHANRAAVT A
A3	SsAP2/ERF-056	AQQAAAFIMDDAEADGGSAAPWDYYSVAHDAGGVLGAAATSATEVVAPVR ADGSIDWRPIMAHPPPLFSPTGWGSNAYDFLQVPPPAAVADEDMDDGIHGATA SLWSFDRDSYFRH MVKTAVAASSNDAAAARHGGSGKQRTYKGVMRMSWGSWVSEVRAPGQKTRIW L
A3	SsAP2/ERF-071	GSHSTADAAARAYDAALLCLKGSAAAPDLNFPRLRPLFDLPPAGAMSPKAIQVRA AAAAASSATPFAPCAENNGSACTDGDGDITPAWSSSSPARDDVSSPESTVSSSED LSGD MNDGAGASRSDGEGSPRLPAERRYKGVFRKWGRWVSEIRMPNSRERIWLGSYSS AEKAARAFDAAAVCLRGSRAGSLNFPESPPNVRHIPGALLTPEQIQAEARHANQ Q
A3	SsAP2/ERF-097	QLPSPPVASPASSSSQPAPAPPAGGASSDRTALSMPTYYSSGDAVCGDDEALDWS FMDALPSSMPASSVGMGNSADIVPALDDFMYGSPHQVMPPSEEATHDMIDSDDD HTFISDDLWRF*
A4	SbERF46 (Sb04g022943.1)	MENHHLQQQQQQQQPAAAATASSPQYRGVRRRKWGWKWWSEIRQPGTKVRVW LGSFDSAEEAAVAHDVAALCLRGPRDAQLNFPGSAGWLPRPPSTD PADIRAAAA EAAERVRRPALVGTSAAAAEPGAGSRVHAASASRLDLAVGDEFDDDLASPRL WTEMAEAMLLDPPKWGPDGSDGSDGSGSQHWPQGSWLDAC MATKQFRMAAAYSGTSMHEQPLRCITTRWRWPWRLLYKSAVITRESTLHLHHRSE A
A4	SsAP2/ERF-069	ASYLLSSS GRALPHSMENHQQQQQPAAAASSPQYRGVRRRKWGWKWWSEIRQPGT KVRVWLGSFDSAEEAAVAHDVAALCLRGPRDAQLNFPGSAGWLPRPASTDPADI RAAAAEAAERVRRPALVGTAAAAEPGSVPASASGRDLAVGDEFDDDLASPR LWTEMAEAMLLDPPKWGPDGSDGSEGSQGHWPQGSWLDGC*

Continued Table S3

Subgroups	Protein name	Amino acid sequence
A5	SbERF6 (Sb01g029065.1)	MAQELQLETSSSASATTTSSCTTSCCSSTVTDSSSSSPAAANAAPAPRKRQA AEAEAEAEAEAEAEAVEEEEEEGCAGKTTAPAAKKRKRSEGGKHTYRQV MRAWGKWVSEIREPRKKSRIWLGTFTAEEMAARAHDVAALAIKGRAATA YLNFPDLAGVLPRAASAAPKDVQAAAAALAAFTSPSSPSSEPGDDAGAPA APCDAREEPAAAAKGGAAPEETAEEEREPVPLPPPVSQPGTPSSSGVEEER QLFDLPDLLLLDIRDGFSGCFPPMWAPLDTDVEEVVNAELRLEEPLLWE MEREQEAEAGTAQQLLGRRVRADTRHPVYRGIRYRGKWVSEIREPRKSN RIWLGTYPAPEMAAAAAYDAAALALRGAEALNFPGAAMSRPAPASCPD DIRAAAAAAAAAAVIGRSHSPQVGGEAAAGGCGASTWSSGAGAQQQVP EHRAGDRRIVDEDDVFQVPRLLAGMAEGLMMSPPRLVGPATDGAVLLEE DGSEDGVVSLWDHS
A5	SbERF14 (Sb01g049400.1)	MAMSELSSDTSSSYSSDPSTSTSPRAAAPCSAAAASVSGKKRPRSNGGGES GSAPAYRGVIRMRAWGKWVSEIREPRKKSRIWLGTFTCPPEMAARAHDVAA LSIKGARAVLNFPDLARHLPRPASLAPRDVQAAAAARAALMHMHSDGAG SGSGSGSSSPSSSSSSSTRDAADTDGAAAHDEPEPEPEPQRQHERQPVEM AAELVFDELAPLWVEDVVEFGGPPSDHTWTPCDGLDAAVGFHLPLLLW DY
A5	SbERF33 (Sb03g002630.1)	MAEQPLPRPHSPSTPAAVQVQVQPGPAAASHQAAPSSGSAPASPRSPSPLL LLLQGGDGGTSSAGAAAASSVATAPSTTMATSSGEPSPRSSGKHAFYRGIR CRSGKWVSEIREPRKARRIWLGTYPTEMAAAAAAYDVAARALRGADAVLN FPGAIAASRAAPASAPADIRAAAAAAAAAAAAQLEYAQSSQGTAAANHPPPA AARQDHRWHHQQQQHGVMMMSGGASATADAAASLYNTPQQQQQQQEI GGDEFMDDEAIFEMPQLLRNMAAGMMMSPPRLSPDTSDESPDPSEAGESL WSYHDP
A5	SbERF51 (Sb04g031960.1)	MAMDDSSSGSEPEPTSSSSVEAPASPTASSSDSASAAGSDSKKRRRTKDGH HPTYRGVIRMRAWGKWVSEIREPRKKSRIWLGTFTAEEMAARAHDVAAL AIKGRAAHLNFPDELAGEPRPATAAPKDVQAAAAALAAADFPATPANA AGADDDCGPDASAASDDVSASASPPPATTLNPDALFDLPDLLLLDLRH GASSCQLLSCAPSWYDDVCFSGAAAGAFRLLEEPLLWEY
A5	SbERF52 (Sb04g032940.1)	MEADALLHAPTTSSSCSDSGGGSCVINGAQERLSKTGGKPHKNVKKRRTA SSPPAAPGGVAVAPEATVDDDCGGGGGRQRKRGSAGTRHPTFRGVRMR VWGKWVSEIREPRKKSRIWLGTFTAEEMAARAHDVAALAIKGRAAHLNF PHLAHELPRPASTSPADIQAAAAKAAAAAAAAAADADVEQCESSHHIAE TPSSSSSSAANSEEVAAASNSEESVLLFDLFDLPDLLLLDLRDGLWSPIWEAAA PAAAAEEYDGDLSLEPLLWAHDHCWMDAAAVPVQPD
A5	SbERF53 (Sb04g032960.1)	MDAPAQRAMAVDTGNAPTTSCSSSSSTSSSSSVNDAPQEVVPKNSCKGTN KRKRASSPDSQEQEVEANGSTNNGHHQGESSSSCCSTDDTEASVGDDKA EEAAAATATRSSRSGYKHPYRGVRRRSWGKWVSEIREPRKKSRIWLGTFT TAEMAARAHDVAALAIKGRAAHLNFPDRAHELPRPASTSPADIQAAAA QAAAGAADVQCDAPPSPSPSPSSSAELLPLSSPAADADASPEQAAAAT MTTTHGDGGGGQGGQGGQGESALFDLPDLLLLDLRDGLWWPPVWPAAAM AAEEYDGCDDVGMHDEPLLWAE
A5	SbERF69 (Sb06g024530.1)	

Continued Table S3

Subgroups	Protein name	Amino acid sequence
A5	SbERF70 (Sb06g024540.1)	MEHEQVVSPQVSEGCCTCSSSFSNTTTTSGGGGSLNASSPSSSDDSGGGG NKGTKRPRRDLKHPTYRGVVRMTWGKWWSEIREPRKKSRIWLGTFDNPE MAARAHDAAAVAIKGRAAHLNFPDLAHELPRAAASAAPKDVQAAAAAL AAATVVVAASPAVVPSSCGHDDADAETEDLPPPERAMPECESVNQAQL EQLGGDIDSGLGFTFLDVPDALLDFGHMLSPLPLPSYCGSPWDDIADDLC FEEPLLLWEH
A5	SbERF71 (Sb06g025890.1)	MSDRGGPASPRSGKHPFYRGIRSRSGKWWSEIREPRKTRRIWLGTFTAE MAAVAYDVAARALRGADAALNFPHLAASRPAPASTSADDIRAAAAEA AASLLLQQEQQQQPADHHQPPARGGAPGRGIAPAEAGAAQQQTGGSS AAAWAQQEGGSGAGNPYFLDEEALFETPQYLRNMAAGMMMSPPRFGR NSSDDSPDHPSSSDAGDSLWSYRDP
A5	SsAP2/ERF-067	MEEERALSPVSDACTTCSSSGGTGPNSPIASSSQSLDDTSGGGAGGSSSRK RPRRELKHPTYRGVVRMRAWGKWWSEIREPRKKSRIWLGTFTDTPEMAAR AHDVAALAIKGRAAHLNFPPEMSHELPRAAASAAPEDVRAAAAALAAAME TRESVAPPASSDSIHGARNEDNEEPAPSSSSGNDADDSAQSEEPAAAPPSE HSMLADGHILDALFELPDVLLFEGFALPPPLTSSVAALGALTTRKGERD MRAIPQYPGTARQPGH TLGSLFASLSYPLQHQQYVHLCSSRPE* MAVDTSNAPTTSCSSSSSTSSSSSVNDAPQEVVPKNCKGTNKRKRRTSSP DSQEVEANGSTNNGHRVEESSSCCSTEASVGNKEAEEAAAATATRSRS GYKHPSYRGVRRRSWGKWWSEIREPRKKSRIWLGTFTAEEMAARAHDV AALAIKGRAAHLNFPERAHHELPRPASTSPADIQAAAAQAAAAAEVQCD APPSPAPSPSSSAELLPSSPAADADASPEQAAADCCCPEAAAAATTTTTTH GGDGGGQVQETALFDLPDLLLLDLRDGLWWPPVWPAAAMAAEEYDCC DVVGMHEPELLWAE*
A5	SsAP2/ERF-075	MLLPDETNIKQPESNVGSVLQTDHRPSEKQKQRETL LGVVISSKLSGFTRSP SSHSPRHQVSPQLSEGCTCSSSNTSSLNASSPSSSDDSGSVKQGTKKRPRR DLKHPTWGKWWSEIREPRKKSRIWLGTFD DPESAARAHDAAAVAIKGR AAHLNFPDLAHELPRAAASAAPKDVQAAAAALAAATVAAAASPVVIPSC AHAHGHGDADTETEELPPPERAMPEECETENQAQLEQLGGDIDIGLGC TFLDVPDALLDFGYMLSPLPLSSYCGSPWDDIADDLCFDEPLLLWEH* MQFIQAQLHLQRNPGLGPRAQPMKPAVPVPPAPAPQRPVKLYRGVRQR HWGKWVAEIRLPRNRTRLWLGTFTAEQAALAYDQAAAYRLRGDAARL NFPDNAESRAPLDPVDAKLQAICATIAAASSSSKNSKAKSKAMPINAS VLEAAAASPSNSSSDEGSGSGFGSDDEMSSSSPTPVVAPPVADMGQLDFS EVPWDEDESFVLRKYPSYEIDWDALLSN
A5	SsAP2/ERF-076	MAATTIDWHGRNAALYGVADRDSKELVRALAPPMQQAAPTISFAYPCS GVEQQSAAAAAGSFLGAGGSSGLLTPAQILQLQSRLQFLRRPAAGGATL AAVTTQQMQRQGVQPAPAPLTSRPAVSKLYRGVRQRHWGKWVAEIRL PRNRTRLWLGTFTDTEEAALAYDSAAFRLRGDSARLNFPELRRGGQHL GPPLHAAVDAKLHAICNGTTDVVVPLPQSQSQSQSQSQSHATTATATT PSSLSSASPHVKSEPGCSGSESSFSADGDVSSTGSSDVVPEMQLLDFSEAP WDESESFHLRKYPSLEIDWDSILIS
A6	ZmDBF1 (AAM80486.1)	
A6	SbERF8 (Sb01g044410.1)	

Continued Table S3

Subgroups	Protein name	Amino acid sequence
A6	SbERF21 (Sb02g023230.1)	MAAAIDLSGEELMRALEPFIRDASSAPHGSSPLLHPHHQPLSPSSPFS FHHAAYGGYPFAAAAAEGAGQLSPAQMQYIARLHLQRQSQQS SVLGPRAPMKASAAAAPTTPRPQKLYRGVRQRHWGKWVAEIRLP RNRTRLWLGTFTDAEEAALAYDQAAYRLRGDAARLNFPDNAASR GPLHASVDAKLQTLQCQNIASKKGAKKHAASASAAAAAATSSSAPT SNCSSSPSSDDASSSCCLESAAESSCSPSPSPSPSPESSTVPQMQLDFSE APWDEAAGFALT KYPSY EIDWDSLLAAN MAAAIDMYKYNAHQIASSSPSDEELAKALEPFITSASSSSPYHRYSS SPSMSQDSYMP TSYTSFATSPLTPAATSSSSSPFSQLPPLYSSPYAAP SMVGQMGLNQLGPAQIQQIQAFMFQQQQQQQRLHAAFLGPR A6
A6	SbERF49 (Sb04g027660.1)	AQPMKQSGSPPLAPAQSKLYRGVRQRHWGKWVAEIRLPKNRTRL WLGTFTDAEDAALAYDKAAFRLRGDMARLNFPALRRDGAHLGPR LHASVDAKLTAICQSLTGSKNGGSSGDESAGSPDPSPKCSASASTEG EEESGSAGSPSP TLAAPPVPEMAKLDFT EAPWDETETFHRLKYP SWEI DWDSILS MAAAINLPGPSEDLMRAMESFMQEDAAPSPLPMPAAQYPPATPT HLSPAQM QFIQAQLHLQRNPGLGPRAQPMKPAVPIPPAQQRPAQK LYRGVRQRHWGKWVAEIRLPNRNTRLWLGTFTDAEEAALAYDQA AYRLRGDAARLNFPD NAASRAPLDAVD AKLQAICATIAAASSKG GARAQSKAMPINAPVLEATAACPSNSSSDEGSGSDEEMSSRTTTTP VVVAPPVVADMGQLDFSEAPWDEAESFVL RKYPSY EIDWDALLSN MDASLRTLPPAGSSFPGEVRS AVSSLLLSPGGTSALD TVFSHLPPPV TIPPLGSSVYYRQCELLRHFAASQTHQPATATAAACSSSSSSSAASA SFQFQPQAPPDDAAAAAAML RQKLYRGVRQRQWGKWVAEIRLPQ A6
A6	SbERF80 (Sb07g020090.1)	NRVRVWLGT YDSPETA AHAYDRAAYRLRGEYARLNFP GVM DAPD TDCPDHLRQLRAAVDAKIQAIRARMARKRARARKQREEKESARSG SGANSSKPAAARPVASEGAATTTTTTTTTSETSTTPYGS PDGVL SVSAV SAEGDCPLERMPSFDPELIW EMLNF MAAIDLYTNQLSSSSSSSDQELMKALEPFIRSASSPTSSTSTTTSPFSYS YPYCSALPQDSYYLPATSSYTSFPPPPPAPTAATSFSQLPPLPQSSSYA SPAAASYPTSSSADAASGLAALNHLGPAAQI HQIQAQLLAQH QEQ A6
A6	SbERF98 (Sb09g029070.1)	QQQQRGLLA AAAFLGPRATQPMKHAGAPSASSAKLFRGVRQRHW GKWVAEIRLPNRNTRLWLGTFD SAEDAALAYDKAAFRLRGDAARL NFP SLRRGGVHLAGPLDASVDAKLTAICQGLTAE PASSKAADTDA STTATAAPDSPKASASTTTTEGDES VHSAGSPSSLPAFFQPPPPQ QHPEMVSLDFTEAPWDESAALHLNKYPSWEIDWDSILS MAATTIDWHGSNAALPAALYGVADSKELVRALAPPMEQAAPTISF SYPCSGVEHQSAAGSFLGAGGGSGLLTLAQILQVQSRLQFLRRPAA A6
A6	SbERF103 (Sb10g007780.1)	AGGGALAAVATQQMKRQGVPHAPAPAPLPARPAVSKLYRGVRQR HWGKWVAEIRLPNRNTRLWLGTFTDAEEAALAYDSAAFRLRGDSA RLNFP ELRRGGQH LGPPLHAAVDAKLHAICNGTL PQSQSNATAAT ATATTTTTPSFSSASPHVKSEPGCSGSESSFSADGDV SSTGSSDV

Continued Table S3

Subgroups	Protein name	Amino acid sequence
A6	SsAP2/ERF-092	MAAAINLPGPSEDLMRAMESFMQDDAAPSPLAMPPPPRAPRLPL PAAGAHGHGHGAQQYPATTHLSPEQMQFIQAQLHLQRNPGLGP RAQPMKPAVPVPPQQRQPQKLYRGVRQRHWGKWVAEIRLPRNRT RLWLGTFTDAEEAALAYDQAAYRLRGDAARLNFPDNAASRAPL DPAVDAKLQSIATIAAASSSSKGARAKSKAMPINAPVLEAPSNSS SDEGSGSGSGSGSDDEMCSSSATPVVVAPPVVADMGQLDFSEVPW DEAESFVLRKYPSYEIDWDALLSN*
A6	SsAP2/ERF-098	MAAAINLPGPSEDLMRAMESFMQDDAAPSPLAMPPPPPPRAPPL PLPAAGAHGHGHGAQQYPATTHLSPEQMQFIQAQLHLQRNPGL GPRAQPMKPAVPVPPQQRQPQKLYRGVRQRHWGKWVAEIRLPRN RTRLWLGTFTDAEEAALAYDQAAYRLRGDAARLNFPDNAASRA PLDPAVDAKLQSIATIAAASSSSKGARAKSKAMPINAPVLEAPSN SSSDEGSGSGSGSGSDDEMCSSSATPVVAAPPVVTDMGQLDFSEVPW DEAESFVLRKYPSYEIDWDALLSN*