

Association of SNP in a novel DREB2-like gene SiDREB2 with stress tolerance in foxtail millet [*Setaria italica* (L.)]

Charu Lata, Sarita Bhutty, Ranjit P Bahadur, Manoj Majee, and Manoj Prasad

Supplementary Material

Figure S1. Sequence alignment of full length SiDREB2 protein with other DRE-binding transcription factors: *Sorghum* SbDREB2 (ACA79916), maize ZmDREB2A (ACG47772), *Buchloe* BdDREB (ABP52086), *Cynodon* CdDREB1 (AAS46284), *Zoya* ZjDREB1 (ACV42429), *Festuca* FaDREB2A (CAG30547), *Poa* PpDREB (AAS59530), oat AsDREB2 (ABS11171), barley HvDREB1 (AAY25517), wheat DREB6, rice OsDREB2A (A2WL19.2), OsDREB2 (NP_001042107), *Phyllostachys* PeDREB2 (ABY19375), tomato SiDREB (AAN77051). Identical amino acid residues at the DNA binding domain are coloured red, strongly similar in green and weakly similar in blue. Predicted secondary structures, helix (H), sheet (S), and coil (C) are also shown.

Figure S2. Southern gel blot analysis of *SiDREB2* cDNA. 10 µg of foxtail millet genomic DNA was digested with *Pvu*II and *Hind*III and fractioned in a 1% agarose gel. The *SiDREB2* ORF (705 bp) was used as a probe.

Figure S3. Genome organization of *SiDREB2*. The 1,103 bp genomic clone as well as cDNA clone was PCR amplified using the primer pair, SiDREB2 FL_F and SiDREB2 FL_R and electrophoresed on 0.8% agarose gel.

```

          10      20      30      40      50      60      70      80      90
          |      |      |      |      |      |      |      |      |
          CCCCCCCCCCHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
SiDREB -----MRRKSTGPDZIAETIKWKEQNQKLQDESGS-----RKAPAKGSKKGCMAKGGPENGNCPYRGV
1GCCAArabidopsis -----K-----HYRGV
ACA799161 -MELGDATAGQGAQGDAAAGALVRKKRMRKSTGPDZIAETIKWKEQNQKLQDESGS-----RKAPAKGSKKGCMTGKGGPENVNCVYRGV
ACG477721 MEELGDA--GGPQGD--ASGALVRKKRMRKSTGPDZIAETIKWKEQNQKLQDESGS-----RKAPAKGSKKGCMMGKGGPENVNCVYRGV
ABP520861 -MERVEVQGGDSSGD-----QVRKKRMRKSTGPDZIAETIKWKEHNQKLQEYGA-----RKAPAKGSKKGCMAKGGPDNGNCVYRGV
AAS462841 -MERVEVLGGSSAG-----QVRKKRMRKSTGPDZIAETIKWKEHNQKI HED-----RKAPAKGSKKGCMAKGGPENGNCA YRGV
ACV42429 -MERVDGQGGEDAGVQ-----AQVRKKRMRKSTGPDZIAETIKWKEHNQKLQEYGT-----RKAPAKGSKKGCMAKGGPENRACAYRGV
CAG305471 -----MSRKKKVRRRSTGPDVSAEIIKKWKEQNQKLQEENGS-----RKAPAKGSKKGCMAKGGPENSNCKYRGV
AAS595301 -----MSRKKKVRRRSTGPDVSAEIIKKWKEQNQKLQEENGS-----RKAPAKGSKKGCMAKGGPENSNCKYRGV
ABS111711 -----KRGDCSV-----PERKKKVRRRSTGPDVSEIIKKWKEQNQKLQEENGS-----RKAPAKGSKKGCMAKGGPENSNCKYRGV
AAZ255171 -METGGSKREGDCPG-----QERTKKVRRRTTGPDSVAETIKWKEQNQKLQEENGS-----RKAPAKGSKKGCMAKGGPENSNCA YRGV
AAZ13289 -METGGSKREGDCPG-----QERKKKVRRRSTGPDVSAEIIKKWKEENQKLQENGS-----RKAPAKGSKKGCMAKGGPENSNCA YRGV
A2WL192 -MERGEGRR--GDCSV-----QVRKKRTRRKSSTGPDZIAETIKWKEQNQKLQEENSS-----RKAPAKGSKKGCMAKGGPENSNCA YRGV
NP_0010421071 -MERGEGRR--GDCSV-----QVRKKRTRRKSSTGPDZIAETIKWKEQNQKLQEENSS-----RKAPAKGSKKGCMAKGGPENSNCA YRGV
ABY193751 -MERGDGKR--GECGS-----QERKKKMRKSTGPDZIAETIKWKEQNQKLKEENAS-----RKAPAKGSKKGCMAKGGPENSNCDYRGV
AAN770511 -MIMDEAANMVCV-----LDYSRKKRSRSDRRTKNVEETLAKWKEYNKLDNEGKPKVPRKPAKGSKKGCMRGGPENWRCKYRGV
Prim. cons. MMRGDKGRGGDCSGDAASGAQVRKK22RKRSTGPDZIAETIKWKEQNQKLQEENGSKPVRRKAPAKGSKKGCMAKGGPENSNCA YRGV

```

```

          100      110      120      130      140      150      160      170      180
          |      |      |      |      |      |      |      |      |
          SSCCCCSSSSSSCC CCCCSSSSCCCCCHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
SiDREB RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPKARVNFENSADANSCTALSLLASSVPAA----ALHGFNE-
1GCCAArabidopsis RQRPWGGKFAEIRDPAKNGARVWLGFETAEDAALAYDRAAFMRGSRALLNPLRV-----
ACA799161 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPKARVNFENSADANSCTALSLLASSVPAA----TLQRSDEK
ACG477721 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPKARVNFENSADANSCTALSLLASSVPAA----ALQRSDEK
ABP520861 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADGNSGCTALSLLASNPPA----AQRSEDK-
AAS462841 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNIPPA----SQRSDDK-
ACV42429 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADATSGCMSALSLLASNPPA----DQSDDK-
CAG305471 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----VSYPSDE-
AAS595301 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----VSYPSDE-
ABS111711 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----VSYPSDE-
AAZ255171 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----VSHPSDG-
AAZ13289 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----ASHPSDG-
A2WL192 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFADNSTDANSCTALSLLASNPPA----ATIPSE-
NP_0010421071 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFADNSTDANSCTALSLLASNPPA----ATIPSE-
ABY193751 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----ALNQSDA-
AAN770511 RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----ALNQSDA-
Prim. cons. RQRTWGWKVAEIREP--NRGKRLWLGSFPTAVEAAHAYDEAAKAMYGPTARVNFESSADANSCTALSLLASNPPA----ALNQSDA-

```

```

          190      200      210      220      230      240      250      260      270
          |      |      |      |      |      |      |      |      |
          CCCCCCCCCC CCCCCCCC CCCCCCCCCCCCCCCCCSSCC CCCCCCCCCCCCCCCCCHHHHH
SiDREB -KDESVETE-----VHEVKAEA--NDDLGSIHVECKSVEVLQS--EEIIVLQKGENVSYDYFNVEEVV
1GCCAArabidopsis -----
ACA799161 VETEVESESE-----VHEVKTG--NDDLGSVHVACTVVDVIQS--EKSVLHKAGEVSYDYFNVEEVV
ACG477721 VETEVESETE-----VHEVKTG--NDDLGSVHVACTVVDVIQS--EKSVLHKAGEVSYDYFNVEEVV
ABP520861 --DEVESLETE-----VHEVKMEV--NDDLRSIHVESKTLVEVFQS--EESALRKEKRDVNFVDFHVEDVL
AAS462841 --DEVESVETE-----VHEVKMEV--NDDMRSHVVERKTLEVFQS--EESVLRKEKGDVSYDYFHVEDVL
ACV42429 --DEVESVETE-----VHALKVEV--NDDLGSIHMERKTMEVFQS--EEIILRKEKGDVSYDYFHVEDVL
CAG305471 -KDESESPSLMSDAPTATLHRSDA--KDEPESAGTLVHEVKTEV--SNDRSIREERKTVEVFQP--EGKALHKEEKVSYDYFNVEEVV
AAS595301 -KDESESPSLMSDAPTATLHRSDA--KDEPESAGTLVHEVKTEV--SNDRSIREERKTVEVFQP--EGKALHKEEKVSYDYFNVEEVV
ABS111711 -KDESESPSLMSDAPTATLHRSDA--KDEPESAGTLVHEVKTEV--SNDRSIREERKTVEVFQP--EGKALHKEEKVSYDYFNVEEVV
AAZ255171 -KDESESPSLMSDAPTATLHRSDA--KDEPESAGTLVHEVKTEV--SNDRSIREERKTVEVFQP--EGKALHKEEKVSYDYFNVEEVV
AAZ13289 -KDESESPSLMSDAPTATLHRSDA--KDEPESAGTLVHEVKTEV--SNDRSIREERKTVEVFQP--EGKALHKEEKVSYDYFNVEEVV
A2WL192 -KDELESPFFIVANGP--AVLYQPK--KDVLERVVEVQDVKTEG--SGLKRVCCQERKTMEVCES--EGIVLHKEVNI SYDYFNVEEVV
NP_0010421071 -KDELESPFFIVANGP--AVLYQPK--KDVLERVVEVQDVKTEG--SGLKRVCCQERKTMEVCES--EGIVLHKEVNI SYDYFNVEEVV
ABY193751 -KDELESPFFIVANGP--AVLYQPK--KDVLERVVEVQDVKTEG--SGLKRVCCQERKTMEVCES--EGIVLHKEVNI SYDYFNVEEVV
AAN770511 ALHEADTPLSSVQDEGTAMDIVEPTSIDETLKSQDCLDKLNMDE--MFDVDELLAMLDSPTVFTKDYNSDGKHNMMVSDSQQEPNAV
Prim. cons. VKDEVESSPSEVS22PTAVLHRSASIDKDELES2GTVVHEVKTEVKS2DLGSIHEERKTLEVFQS2YEGKVLHKEGNVSYDYFNVEEVV

```

```

          280      290      300
          |      |      |
          HCCCCCCCCCCCCC CCCCCCCCCSSCC

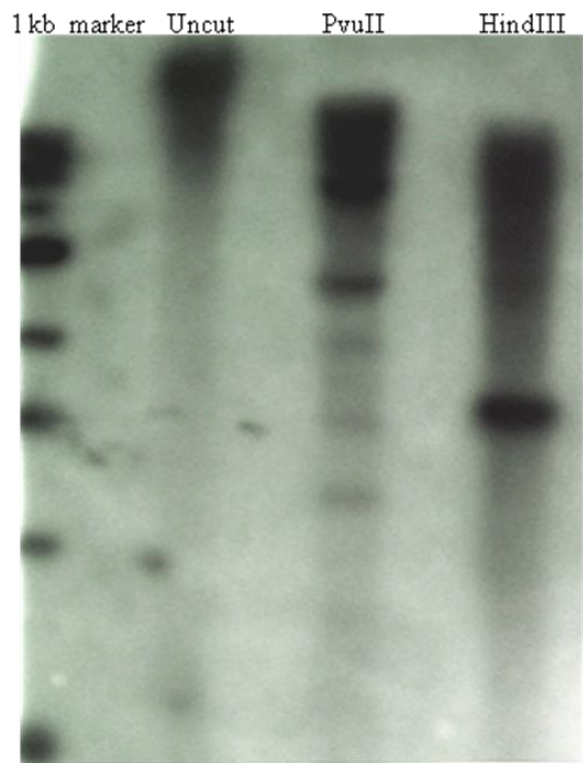
```

```

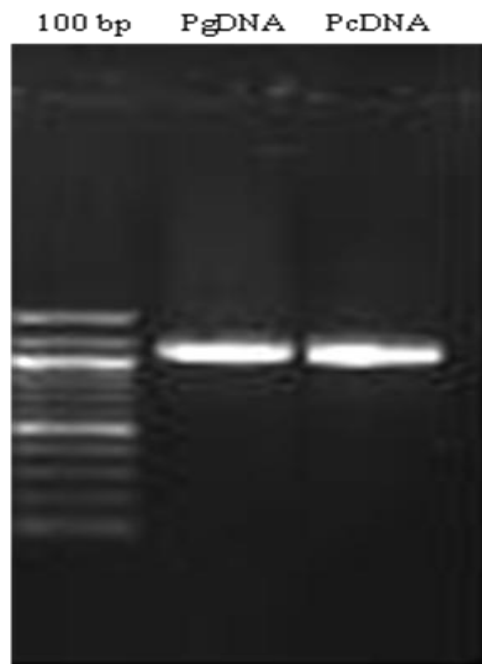
SiDREB EMI I IELNADKKIEVH--EECLGGDDGFSLFAY-----
1GCCAArabidopsis -----
ACA799161 EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
ACG477721 EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
ABP520861 EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
AAS462841 EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
ACV42429 EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
CAG305471 DMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
AAS595301 DMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
ABS111711 DMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
AAZ255171 DMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
AAZ13289 DMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
A2WL192 EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
NP_0010421071 EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
ABY193751 KMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----
AAN770511 DPMTVDYGFDFLKPGRQ--EDLNFSDDLAFIDLDSELV
Prim. cons. EMI I IELNADKKIEAN--EYHDGDDGFSLFAY-----

```

Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3