Figure 1: Root and shoot growth (cm) of foxtail millet seedlings under PEG and Salt stress.

a. Root and shoot growth (cm per plant) of foxtail millet seedlings under PEG stressed conditions (± SD).

b. Root and shoot growth (cm per plant) of foxtail millet seedlings under NaCl stressed conditions (± SD).
Figure 2: Malondialdehyde (MDA) content (µmol/g FW) of foxtail millet leaf and root samples under PEG and NaCl stress (±SD).

a. MDA content in foxtail millet under PEG stress

b. MDA content in foxtail millet under NaCl stress
Figure 3: Expression analysis of *SiAKRI* gene in foxtail millet leaf and root samples under PEG and Salt stress by qRT-PCR.

a. Expression of *SiAKRI* gene under PEG stress by comparative CT

![Fold change 2-ΔΔCT vs Concentration of PEG (%)](image1)

b. Expression of *SiAKRI* gene under NaCl stress by comparative CT

![Fold change 2-ΔΔCT vs Concentration of NaCl (mM)](image2)

In qRT-PCR reactions, 1µg of cDNA from root and leaf samples was used for both gene specific and relative control (actin) reactions. Relative expression of *SiAKRI* gene was determined by triplicate measurements of three independent biological replicates. The *SiAKRI* expression data was normalized against rice actin. The fold change value was calculated following the comparative CT method.
Figure 4: PCR amplified product of *SiAKR1* gene on 1% agarose gel

Lane M = 1 Kb DNA Marker
Lane 1 = PCR amplified *SiAKR1* gene product

~933bp
Figure 6: Agarose gel showing the *SiAKR1* gene amplified plasmid PCR products from the (TA) positive recombinant clones.

Lane M = 1 Kb DNA Marker
Lane 1 to 4 = PCR amplified products of Positive recombinant plasmids (TA: *SiAKR1*).
Figure 7: Restriction analysis of positive recombinant plasmid DNA, gel showing released ~1 Kb of SiAKRI fragment with EcoRI and BamHI.

Lane M = 1 Kb DNA marker  
Lane 1 = undigested TA:SiAKRI plasmid  
Lane 2 = RE digested TA:SiAKRI plasmid
Figure 8: Nucleotide and deduced amino acid sequences of SiAKR1 gene. Comprises 933 bp, and encodes 310 amino acid residues with 34.5 kDa molecular weight.

**Foxtail millet AKR1 nucleotide sequence**

```
ATGGCGAAGC ATTTCTGCTG CACACCGGC GCAAGATCC CCTCGGTCGG GCTCGGCACC 60
TGGCAGTCCG ACCCCCGGTCT CGTGCTCAAC GGCCTCTCAC CCGCGCTCAA GGGGGGTAAC 120
CGACACATCG ATGCACGGCC AGTTTACCGC AGATGACGCT ATGACACCTCT GAGGGACCA 180
AAATATATAG ATGGTCCGAG AGGGGATGTG CAGTGCTTCT GATGAATCTT ATCAAGAGAA 240
TGCTTCTGCT TGGTACGCTG TCTCCAGCTG TGGATGATTT CAGCAGTCAA GGGGGGACCA 300
CGAGCAATCA CTTTGGATCT CCTTCTTATC CATTGGCCAT TGGATGATTT CAGCAGTCAA GGGGGGACCA 360
```

**Foxtail millet AKR1 amino acid sequence**

```
MAKHFVLNTG AKIPSVGLGT WQSDPGVVGN AVYAAVKAGY RHIDCARVYG NEKEIGLALK 60
KLFEEGVVKR EDLFITSKLW NDHHAPEDVP EALNESLNDL QLDYLDLYLI HWPFRVKKGT 120
NISPENFITP DIPATWAAME KLYDAGKAHA IGVSNFSSKK LGDLLAVARV PPAVDQVECH 180
PGWQQTTLHN FCQTTGVHLT AYSPLGSFPT TWMNGNVLKK PVIISIAEKI GKTFAQVALR 240
WNIQMGHSV L PKSTNEERIK QNLDVYDWSI PDDLAKFSE IKQARLLRGN FIVGPQSYK 300
THEELMDGEI 310
```
Figure 9: Protein BLAST analysis of SiAKR1 protein

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**Figure 10:** Multiple sequence alignment of *SiAKR1* gene with other species AKR proteins: Positions of Loop A, B and C in AKR contribute to substrate binding site; DxxxY, K and Y residues form catalytic tetrad; Yellow colour highlighted text indicates the Aldoketo_reductase_1 and 3 conserved sites.
**Figure 11: Phylogenic tree of *SiAKR1* protein.** Phylogenetic tree was derived for *SiAKR1* and other reported AKR family protein sequences using Tree explorer software. Bootstrap values obtained with 1000 repetitions are indicated at all branches.
Figure 12: **Conserved domain analysis of SiAKR1 protein.** AKR conserved domains were found at 7 to 263 amino acid residues.
Figure 13: Predict-protein motif analysis of SiAKR1 protein: Image shown the different protein motifs distribution in SiAKR1 amino acid sequence.
Figure 14. Predict sub-cellular localization of SiAKR1 protein. This report showed the cytoplasm localization of SiAKR1 protein with a probability of 99.52%.

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Figure 15: Agarose gel showing the *SiAKR1* gene amplified plasmid PCR products from the (pRSET-A:*SiAKR1*) positive recombinant clones

Lane M = 1 Kb DNA Marker
Lane 1 to 5 = PCR amplified products of Positive recombinant plasmids (pRSET-A:*SiAKR1*)
Figure 16: Restriction analysis of positive recombinant plasmid DNA, gel showing released ~1 kb of SiAKRI fragment with BamHI and XhoI.

Lane M = 1 Kb DNA marker
Lane 1 = undigested pRSET-A:SiAKRI plasmid
Lane 2 = RE digested pRSET-A:SiAKRI plasmid
Figure 17: Agarose gel showing the SiAKRI gene amplified plasmid PCR products from the (pYES2:SiAKRI) positive recombinant clones

Lane M = 1 Kb DNA Marker
Lane 1 to 4 = PCR amplified products of Positive recombinant plasmids (pYES2:SiAKRI)
Figure 18: Restriction analysis of positive recombinant plasmid DNA, gel showing released ~1 kb of SiAKR1 fragment with BamHI and XhoI.

Lane M = 1 Kb DNA marker
Lane 1 = undigested pYES2:SiAKR1 plasmid
Lane 2 = RE digested pYES2:SiAKR1 plasmid
Figure 19: Agarose gel showing the SiAKR1 gene amplified plasmid PCR products from the (pRT101:SiAKR1) positive recombinant clones

Lane M = 1 Kb DNA Marker
Lane 1 to 3 = PCR amplified products of positive recombinant plasmid clones (pRT101:SiAKR1)
Figure 20: Restriction analysis of *SiAKR1* gene having pRT101 plasmid with *PstI* restriction enzyme.

Lane M = 1 Kb DNA marker
Lane 1 = RE digested pRT101:*SiAKR1* plasmid
Lane 2 = Undigested pRT101:*SiAKR1* plasmid
Figure 21: Agarose gel showing the *SiAKR1* gene amplified plasmid PCR products from the (pCAMBIA2301:*SiAKR1*) positive recombinant clones

Lane M = 1 Kb DNA Marker
Lane 1 to 4 = PCR amplified products of Positive recombinant clones plasmids (pCAMBIA2301:*SiAKR1*)
Figure 22: Agarose gel showing the *NptII* gene amplified PCR products with *NptII* gene specific primers from the positive recombinant *E. coli* clones (pCAMBIA2301:SiAKR1)

Lane M = 1 Kb DNA Marker
Lane 1 to 5 = PCR amplified products of *NptII* in Positive *E. coli* DH5α recombinant clones (pCAMBIA2301:SiAKR1)
Figure 23: SDS-PAGE analysis of SiAKR1 protein overproduction in recombinant *E.coli* BL21 cells.

**Lane M** - Protein marker (kDa)

**L1** - total protein lysate of recombinant *E.coli* BL21 cells without IPTG induction (pRSET-A::SiAKR1::BL21).

**L2** - total protein lysate of recombinant *E.coli* BL21 cells obtained after 8 hours post induction with 1mM IPTG (pRSET-A::SiAKR1::BL21).

**L3** - total protein lysate of empty vector containing recombinant *E.coli* BL21 cells without IPTG induction (pRSET-A::BL21).

**L4** - total protein lysate of empty vector containing recombinant *E.coli* BL21 cells obtained after 8 hours post induction with 1mM IPTG (pRSET-A::BL21).
**Figure 24:** Growth analysis of recombinant *E. coli* BL21 DE3 cells harbouring *SiAKR1* gene on LB liquid medium with different supplements.  
**a.** PEG stress (6%)  
**b.** NaCl stress (6%);  
**c.** CuSO₄ stress (100 mM). OD₆₀₀ was recorded at 1 hour interval up to 10 hours values are mean of five replicates.

**a. Dehydration stress (6% PEG)**

![Graph a. Dehydration stress (6% PEG)](image)

**b. Salt stress (6% NaCl)**

![Graph b. Salt stress (6% NaCl)](image)

**c. Heavy metal stress (100 mM CuSO₄)**

![Graph c. Heavy metal stress (100 mM CuSO₄)](image)
Figure 25: SDS-PAGE analysis of SiAKR1 protein overproduction in recombinant S. cerevisiae W303A cells.

Lane M - Protein marker (kDa)
L1 - total protein lysate of recombinant S. cerevisiae W303A cells obtained after 8 hours post induction with 2% Galactose (pYES2::SiAKR1::W303A)
L2 - total protein lysate of recombinant S. cerevisiae W303A cells without 2% Galactose induction (pYES2::SiAKR1::W303A).
L3 - total protein lysate of empty vector containing recombinant S. cerevisiae W303A cells without 2% Galactose induction (pYES2::W303A).
L4 - total protein lysate of empty vector containing recombinant S. cerevisiae W303A cells obtained after 8 hours post induction with 2% Galactose (pYES2::W303A).
Figure 26: Growth analysis of recombinant *S. cerevisiae* W303A cells harbouring *SiAKR1* gene on YPD liquid medium with different supplements. 26 a) PEG stress (6%); 26 b) NaCl stress (6%); 26 c) CuSO₄ stress (100 mM). OD600 was recorded at 1 hour interval up to 10 hours values are mean of five replicates.

a. Dehydration stress (6% PEG)

b. Salt stress (6% NaCl)

c. Heavy metal stress (100 mM CuSO₄)