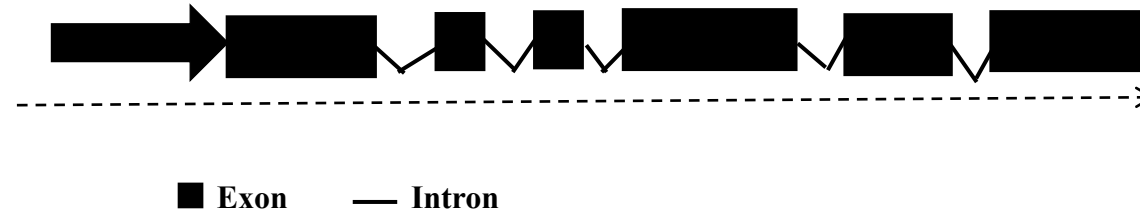


(A) Gene structure of Arabidopsis *KOR* (AT5G49720)

Chromosome-05

20197391

20200468

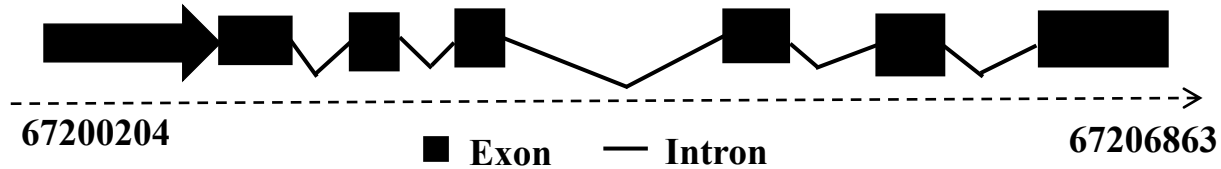


(B) Gene structure of Sorghum *KOR1* / *SbKOR1* (Sb01g036480)

Chromosome-01

67200204

67206863



WT 620 aa

thl1 Q46*

Supplementary Figure S1: Gene structure of *AtKOR* (AT5G49720) and *SbKOR1* (Sb01g036480) with 6 exons and 5 introns.

CLUSTAL O(1.2.4) multiple sequence alignment

```

ATKOR      --MYGRDPWGGPLEINTADSATDDDRSRNLNDLDRAALSRLPDETTQSWLLGP---TEQK 55
SB01G036480 MSMYGRDPWGGPLETCHPDSATDDDRSRNL-DIDRGAMSRTLDETQQSWLLAGPGDQARK 59
              *****          *****  *:*:*:*  *****          :*

```

```

ATKOR      KKKYVDLGCIIIVSRKIFVWTVGTLVAAALLAGFITLIVKTVPRHHPKTPPPDNYTIALHK 115
SB01G036480 KKKYVDIGCLVVSRLKLFVWTLGVLLAAAVFAGVVAGIAKAIPRRHRPPPPDDYTVALHK 119
              *****:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      ALKFFNAQKSGKLPKHNNVSWRGNSGLQDGKGETGSFYKDLVGGYYDAGDAIKFNFPMA 175
SB01G036480 ALMFFNAQKSGKLPKHNNIPWRGNMCKDGLSDP-AVRRSLVGGYYDAGGAVKFNFPAAF 178
              **  *****:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      AMTMLSWSVIEYSAKYEAAGELTHVKELIKWGTDFLKTFFNSTADSIDDLVSQVSGNTD 235
SB01G036480 SMTLLSWSVIEYSAKYDAVGELGHVRDIIKWGSDYFLKTFNSTADSIDRVIAQVGSAA 238
              :*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      DGNTDPNDHYCWMRPEDMDYKRPVTTGNGGCSDLAAEMAAALASASIVFKDNKEYSKKL 295
SB01G036480 PGSTQPNDDQYCWMRPEDIDYPRPVVECH-ACSDLAAEMAAALAAASIVFKDNKAYSQKL 297
              *:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      HGAKVVYQFGRTRRGYSAGTAESSKFYNSSMYWDEFIWGGAWMYATGNVTYLNLTQP 355
SB01G036480 HGATTLFQFARERRGRYSAGGSDATKFYNSTSYWDEFVWGSSWMYLATGNSSYLTATHP 357
              ***..*:*:*:*  *****  :*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      TMAKHAGAFWGGPYGVFSWDNKLAGAQLLLSRLRLFLSPGYPYEEILRTFHNQTSIVMC 415
SB01G036480 KLAKHAGAFWGGPDYGVFSWDNKLTGAQVLLSRLRLFLSPGYPYEEMLRAFHNQTSIIMC 417
              .:*:*:*:*:*:*  *****:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      SYLPPIFNKFNRTNGGLIELNHGAPQLQYSVNAAFLATLYSDYLDAAADTPGWYCGPNFY 475
SB01G036480 SYLPPIFKSFNRTRGGLIQLNHGKQPLQYVVNAAFSLASVFSYDLEAADTPGWYCGPHFY 477
              *****:*  *****  *****:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      TSVLRDFARSQIDYILGKNPRKMSYVVGFGTKYPRHVHHRGASIPKNKVYNCKGGWKWR 535
SB01G036480 IEVLRSFARTQIEYILGKNPLKMSYVVGFGNHYPKHVHHRGASIPKNGVHYGCKGGWKWR 537
              .***.*:*:*:*:*  *****  .:*:*:*:*:*:*:*:*:*:*:*:*

```

```

ATKOR      DSKKPNPNTIEGAMVAGPDKRDGYRDVRMNYNYTEPTLAGNAGLVAALVALSGEEEATGK 595
SB01G036480 DTKKPNPNIIVGAMVAGPDRHDFKDKARKNYNYTEATLVGNAGLVAALVALSGEGH---G 594
              *:*:*:*:*  *****:*:*:*:*  *****  *:*:*:*:*

```

```

ATKOR      IDKNTIFSAPVPLFPPTPPPPAPWKP 621
SB01G036480 VDKNAMFSAPVPMFPPSPPPPPAPWKP 620
              :*:*:*:*:*:*:*:*:*:*:*:*:*

```

CLUSTAL O(1.2.4) multiple sequence alignment

```

ATKOR      --MYGRDPWGGPLEINTADSATDDDRSRNLNDLDRAALSRLPDETTQSWLLGPTEQKKK 58
th12      MSMYGRDPWGGPLEICHPDSATDDDRSRNL-DIDRGAMSRTLDETQ----- 45
              *****          *****  *:*:*:*  *****

```

```

ATKOR      YVDLGCIIIVSRKIFVWTVGTLVAAALLAGFITLIVKTVPRHHPKTPPPDNYTIALHK 118
th12      ----- 45

```

```

ATKOR      FFNAQKSGKLPKHNNVSWRGNSGLQDGKGETGSFYKDLVGGYYDAGDAIKFNFPMA 178
th12      ----- 45

```

```

ATKOR      MLSWSVIEYSAKYEAAGELTHVKELIKWGTDFLKTFFNSTADSIDDLVSQVSGNTDDGN 238
th12      ----- 45

```

```

ATKOR      TDPNDHYCWMRPEDMDYKRPVTTGNGGCSDLAAEMAAALASASIVFKDNKEYSKKL 298
th12      ----- 45

```

```

ATKOR      KVVYQFGRTRRGYSAGTAESSKFYNSSMYWDEFIWGGAWMYATGNVTYLNLTQPTMA 358
th12      ----- 45

```

```

ATKOR      KHAGAFWGGPYGVFSWDNKLAGAQLLLSRLRLFLSPGYPYEEILRTFHNQTSIVMC 418
th12      ----- 45

```

```

ATKOR      PIFNKFNRNTNGGLIELNHGAPQLQYSVNAAFLATLYSDYLDAAADTPGWYCGPNFY 478
th12      ----- 45

```

```

ATKOR      LRDFARSQIDYILGKNPRKMSYVVGFGTKYPRHVHHRGASIPKNKVYNCKGGWKWR 538
th12      ----- 45

```

```

ATKOR      KPNPNTIEGAMVAGPDKRDGYRDVRMNYNYTEPTLAGNAGLVAALVALSGEEEATGK 598
th12      ----- 45

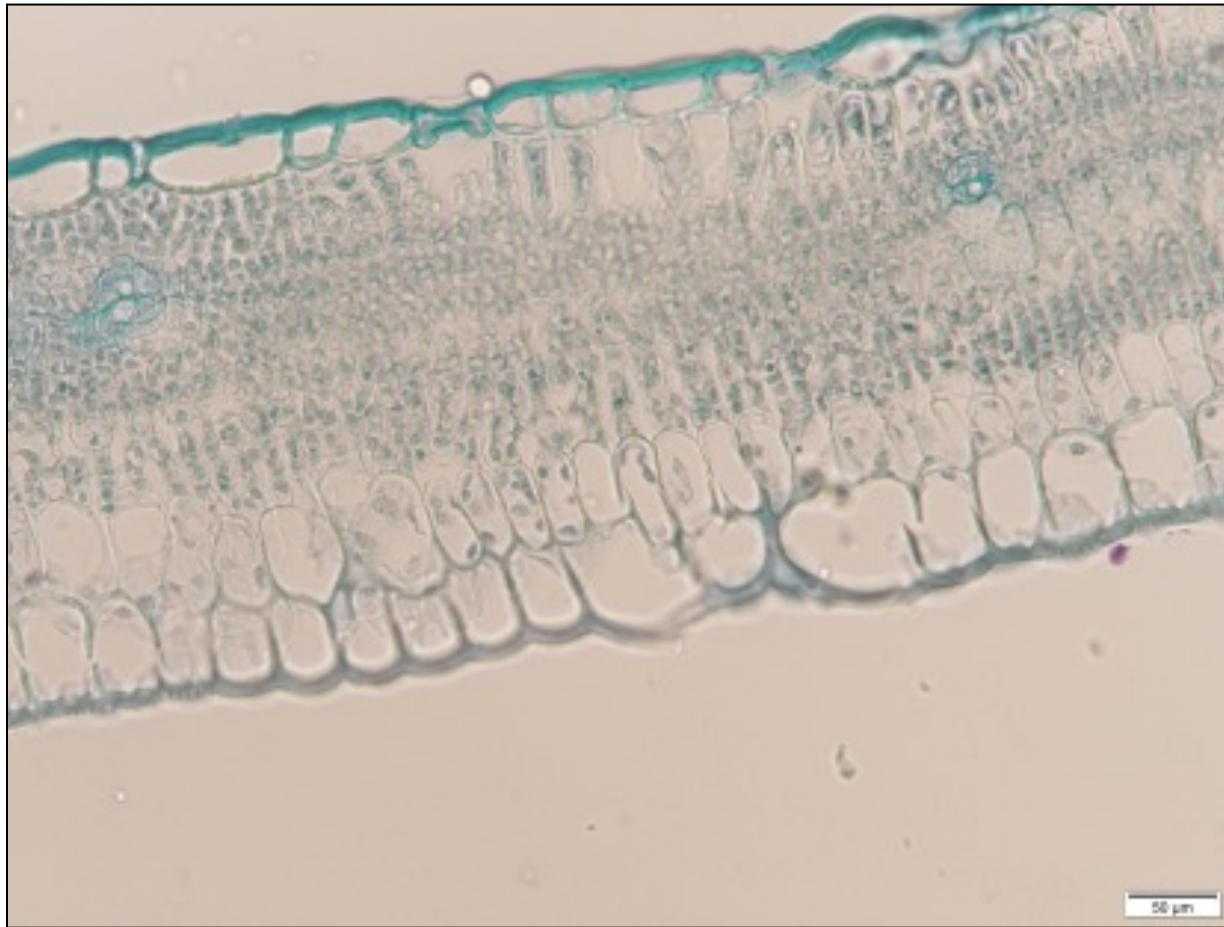
```

```

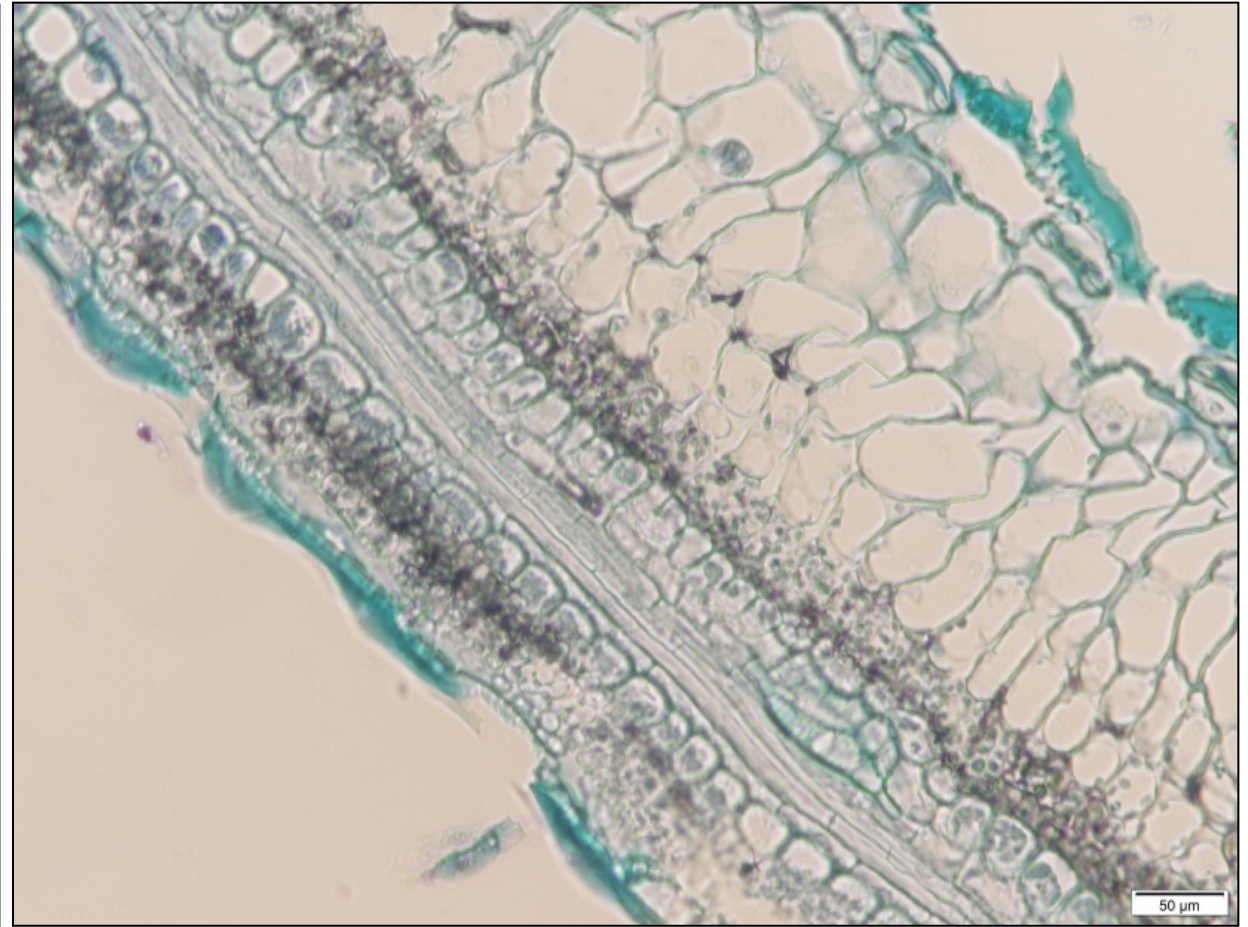
ATKOR      NTIFSAPVPLFPPTPPPPAPWKP 621
th12      ----- 45

```

Supplementary Figure S2: Alignment of AtKOR1 and Sb01G036480 (SbKOR1) proteins.



BTx623



thl1

Supplementary Figure S3. Leaf cross section of BTx623 and *thl1* (Scale bar: 50 μm)



Complementation construct with native promoter



Over expression construct

Supplementary Figure S4. Vector construction maps.

Supplementary Table S1: Identification of mutant locus using BSaseq.

Mutant	Mutation	Gene ID (Sorghum)	Gene ID (Arabidopsis)	Gene function	Nature of the mutation
<i>thl1</i>	16QK6341 to 6352	Sb01g036480	AT5G49720.1	glycosyl hydrolase 9A1	Chr01:60010687;Q46*;STOP_GAINED;homo
<i>thl2</i>	16QK6353 to 6364	Sb01g036480	AT5G49720.1	glycosyl hydrolase 9A1	Chr01:60010937;SPLICE SITE DONOR;;homo
<i>thl3</i>	16QK6365 to 6376	Sb01g036480	AT5G49720.1	glycosyl hydrolase 9A1	Chr01:60010784;W78*;STOP_GAINED;homo

Supplementary Table S2: Primers used for experiments and sequence

Primer number	Primer description	Primer sequence
1465	1465_ACoa_Over_AscI F	gggcGGCGCGCCATGTTAGAACTAACGAGCAAAAG
1466	1466_ACoa_Over_PacI R	taaaAATTAATTTTCAGACACACCGGTGTGGAGAGTC
1520	1520_SbKOR1_AscI F	gcttGGCGCGCCATGAGCATGTACGGGAGGGA
1521	1521_SbKOR1_PacI F	ggccTTAATTAATTATGGTTTCCATGGGGCCG
1522	1522_At_KOR1prom_SBF1	gcttCCTGCAGG TTTAGAGCTTGTAAGATCCTACAAC
1523	1523_At_KOR1prom_AscI	gcttGGCGCGCC ATATTTTTGGCCAACTATGATTGAGCG
1590	1590_Atkori2-1_F_AscI	gcttCGATGCTTTCAAGGCCAAAGAAC
1591	1591_Atkori2-1_R_PacI	ggccCGTCATCATCCTAAGACTCCGCC
2274	2274_Kor_genotyping_F	GTTGTTGGATGGATCATTCAAGG
2275	2275_Kor_genotyping_R	CAAAGACTCTTTGAGAGATCTGG
2276	2276_rsw_genotyping_F	GCTGGGACAACAAGCTTGCTGG
2277	2277_rsw_genotyping_R	CCATCCAGGAGTATCAGCAGC
2303	2303_SbKOR1_AscI-FP	aagggttGGCGCGCCgATGAGCATGTACGGGAGGGACCCG
2304	2304_SbKOR1_PacI-RP	ggccggTTAATTAATTATGGTTTCCATGGGGCCGGTGGC
2305	2305_At_KOR1prom_SBF1-FP	gttccCCTGCAGGcgatgctttcaaggccaaagaac
2306	2306_At_KOR1prom_AscI-RP	caaggccGGCGCGCCgatgatgctctctgataaagc
708	708_qPCRSbKOR-F	GATGAGACCAGAGGACATCGACT
709	709_qPCRSbKOR-R	CTTGCAAACCTGGAAGAGAGTGGT