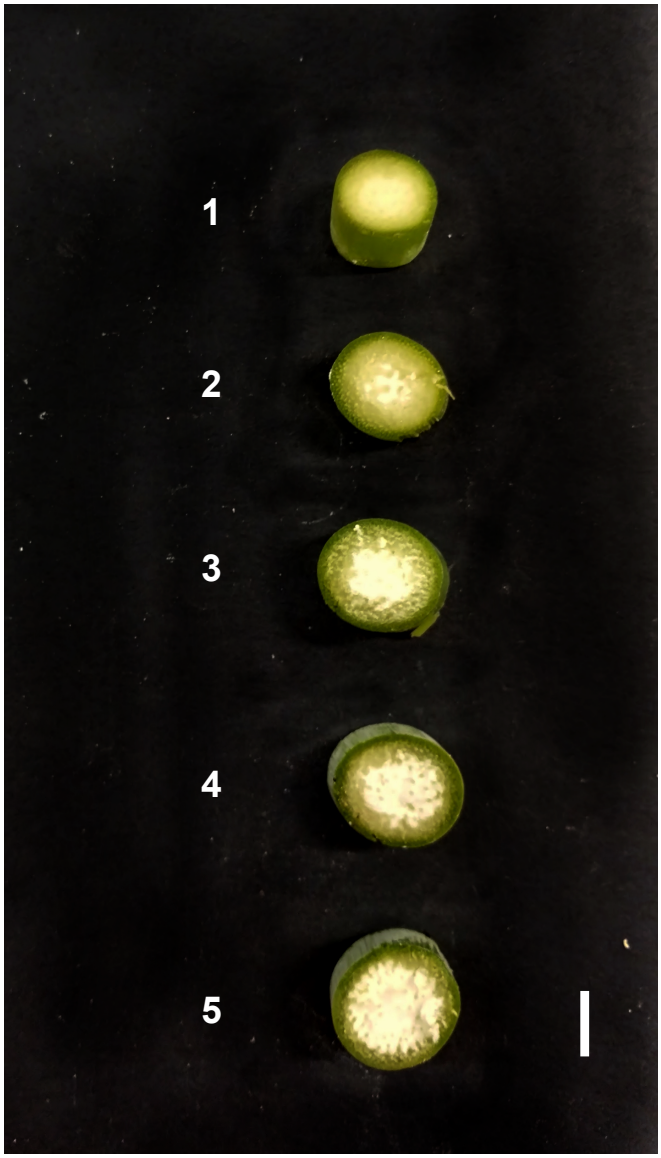
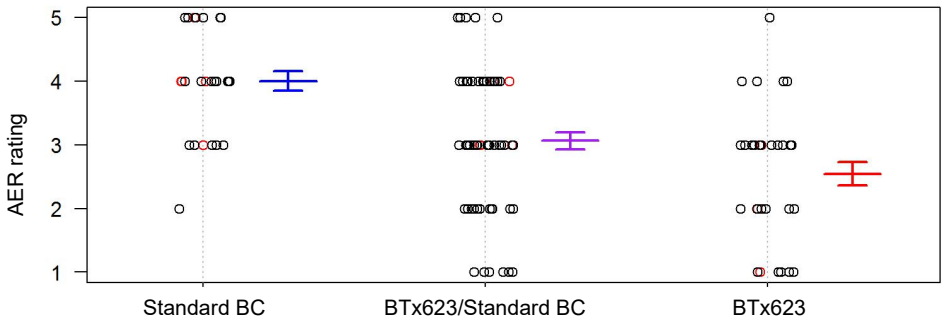
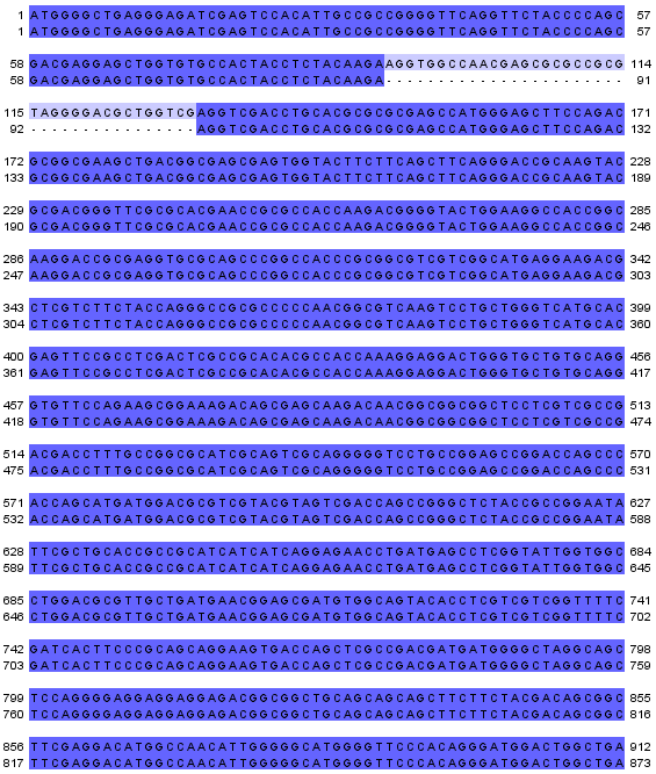


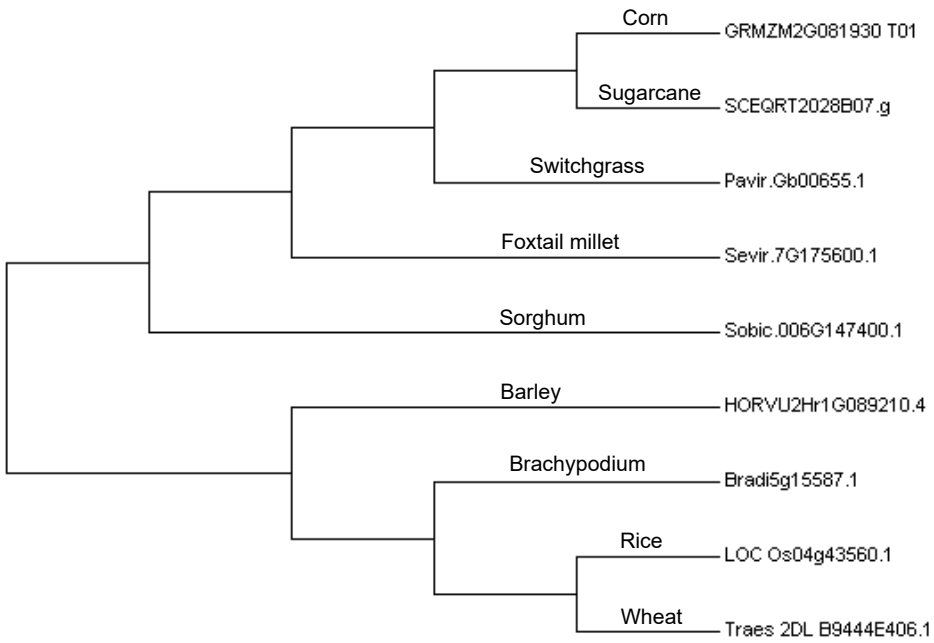

**Supplemental figure 1.** Stem aerenchyma rating scale, 1 (no aerenchyma) to 5 (high aerenchyma)



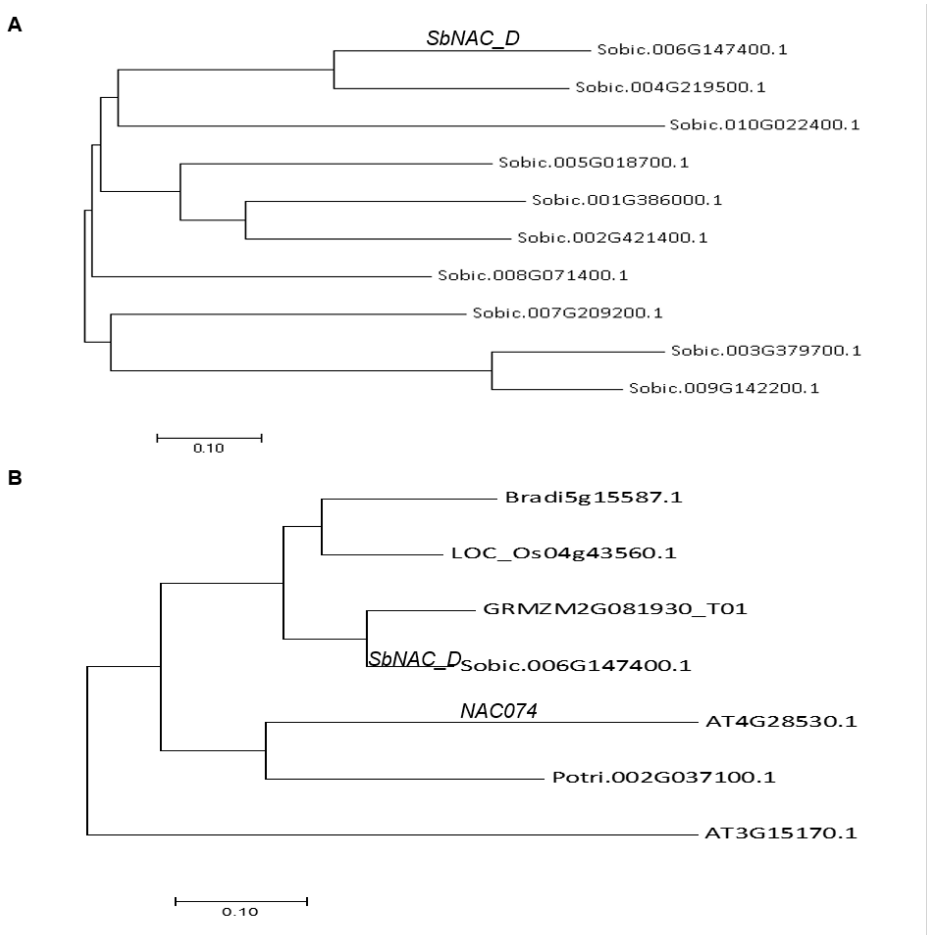

**Supplemental figure 2.** Phenotype-genotype plot of stem aerenchyma ratings in the F2 popultion BTx623*Standard Broomcorn at the marker with the highest LOD score in the QTL interval. The open circles represent AER ratings of individuals in the population. The average AER ratings of individuals genotyped as Standard BC at this marker were higher than the average rating of individuals genotyped as BTx623. Heterozygous individuals had intermediate phenotypes.



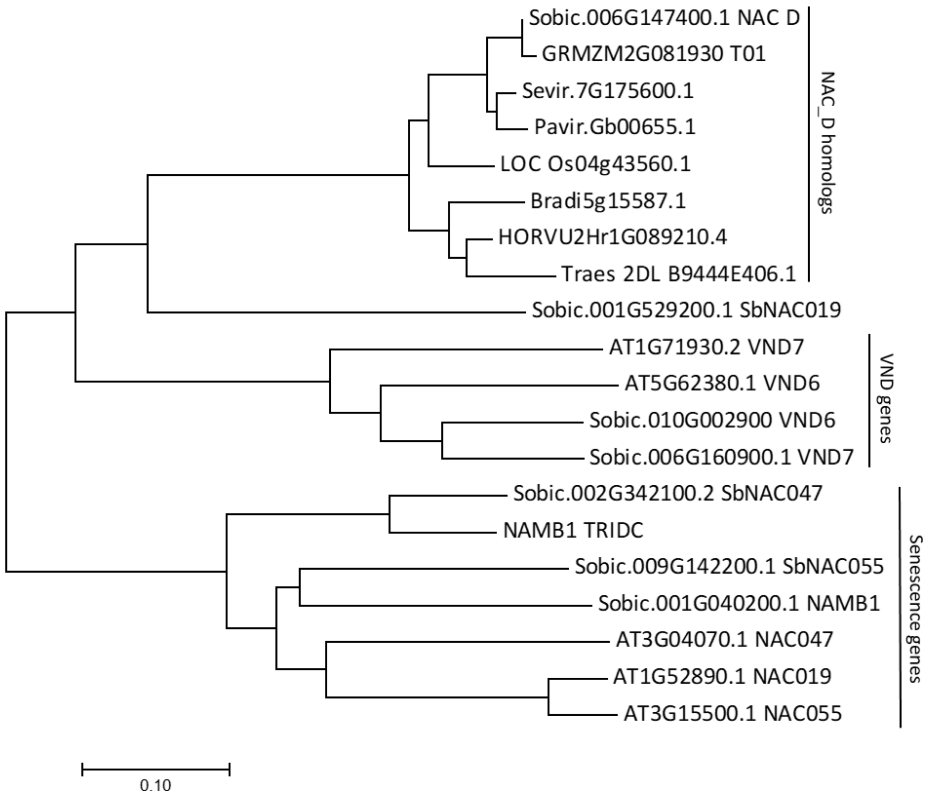
**Supplemental figure 3.** Transcript sequences of the candidate gene Sobic.006G147400 obtained from Sanger sequencing (top) and from Phytozome (bottom). The first exon of the Sobic.006G147400 transcript sequence available on Phytozome contains a 39 nucleotide deletion because those bases were misannoted as an intron, likely because of the stop codon mutation (at 115 nt) in BTx623.



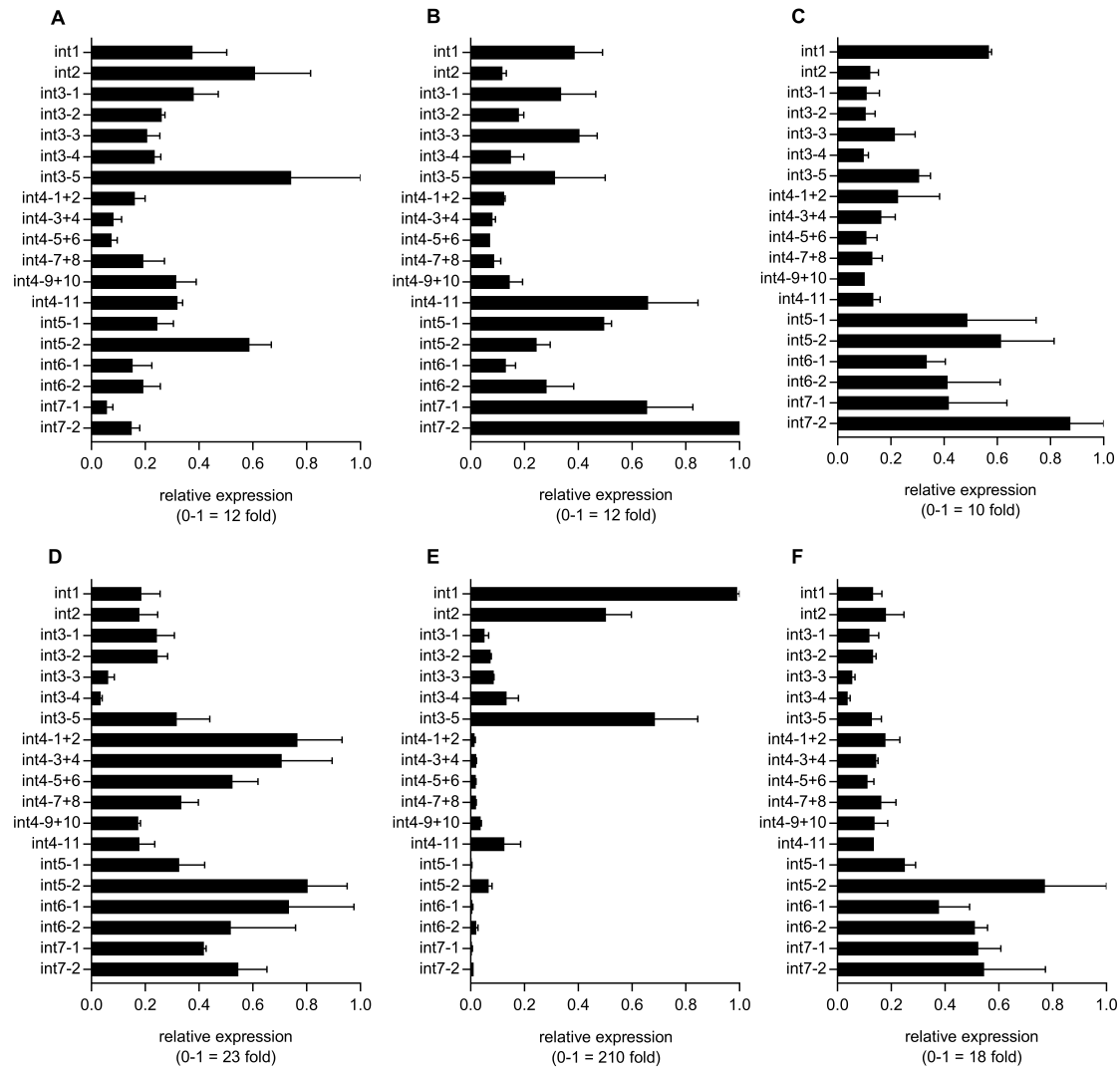

**Supplemental figure 4.** Phylogenetic analysis of grass homologs of SbNAC_D. Unroote phylogenetic analysis grouped SbNAC_D homolgs into C4 (i.e. sorghum, corn) and C3 (i.e. barley, rice) grasses. Relationships were inferred using hte Neighbor-Joining method (Jones et al., 1992). All positions containing gaps and missing data were eliminiated. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).




**Supplemental figure 5.** Phylogenetic relationships of SbNAC_D to (A) SbNAC_D paralogs in the sorghum genome and (B) SbNAC_D orthologs in other plant species. Relationships were inferred using the Neighbor-Joining method (Jones et al., 1992). All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).




**Supplemental figure 6.** Phylogenetic relationships of SbNAC_D homologs in grasses to NAC-family VND genes involved in vascular differentiation and NAC-family genes involved in senescence. Relationships were inferred using the Neighbor-Joining method (Jones et al, 1992). All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 (Kumar et al, 2016).




**Supplemental figure 7.** Expression of genes in the NAC_D QTL interval during the development of internodes of R07020 stems: (A) Sobic.006g147450, (B) Sobic.006g147500, (C) Sobic.006g147600, (D) Sobic.006g147800, (E) Sobic.006g147900, (F) Sobic.006g148000. Internodes were numbered from the apex of the plant downward. Whole internodes were collected from the top two nacent, unelongated internodes below the shoot apex (int1, int2). The next internodes (int3) was elongating and was sectioned into five 1-cm sections from the top down (int3-X). Internode 4 was also divided into 1-cm sections (int4-X). Adjacent pairs of sections from internode 4 were averaged for simplicity and since their expression values were very similar. From internode 5 (int5), internode 6 (int6), and internode 7 (int7), the top 2 cm and the bottom 2 cm of each internode were collected. Expression of the genes in the NAC_D QTL interval was not well correlated with AER formation. Relative expression was calculated via the $2^{\wedge-\Delta\Delta Ct}$ method relative to the internode section with the highest expression (Pfaffl et al., 2004). Expression values are the average of 3 biological replicates. Fold change in expression between the minimum and maximum values on the y axis were calculated based on SbUBC normalized values according to $FC = 2^{\Delta Ct(max)-\Delta Ct(min)}$. No expression could be detected from Sobic.006g147700 in R07020 tissues.

**Supplemental Table 1.** 2 LOD QTL intervals from three biparental mapping populations

| Population | CHR | 2-LOD interval (CHR_Mb) | Peak position (Mb) | LOD score |
|---|---|---|---|---|
| BTx623/IS3620c | 6 | 6_50820292 - 6_51044545 | 50.8 | 30.1 |
| BTx623/R07007 | 6 | 6_48273018 - 6_51329068 | 50.8 | 43.6 |
| BTx623/Std Broomcorn | 6 | 6_50954277 - 6_51787450 | 51.5 | 7.37 |

**Supplemental Table 2.** Genes in the fine-mapped QTL region

| Gene name | Description | Location |
|---|---|---|
| Sobic.006G147400 | No apical meristem (NAM) protein (NAM) | Chr06:50896169..50898604 forward |
| Sobic.006G147450 | Threonine aldolase | Chr06:50902561..50905501 forward |
| Sobic.006G147500 | Predicted protein | Chr06:50914667..50916048 forward |
| Sobic.006G147600 | Threonine aldolase | Chr06:50922630..50925976 reverse |
| Sobic.006G147700 | Threonine aldolase | Chr06:50929529..50933978 reverse |
| Sobic.006G147800 | Predicted protein | Chr06:50941894..50945361 forward |
| Sobic.006G147900 | Predicted protein | Chr06:50948145..50951111 forward |
| Sobic.006G148000 | Myb protein | Chr06:50959448..50962420 reverse |

**Supplemental Table 3.** Coding region sequence variants in genes in the NAC_D QTL interval among the parental lines used in QTL mapping. Deleterious alleles are highlighted in red. Variant calls were taken from publicly available whole genome sequence data (Phytozome v12.1.6). The effect of each amino acid change was determined by PROVEAN analysis (Choi et al. 2012).

| Gene ID | Position (Chr_Mb) | BTx623 | IS3620c | R07007 | Standard Broomcorn | Rio | SC170 | Amino acid change | Effect |
|---|---|---|---|---|---|---|---|---|---|
| Sobic.006G147400 | 6_50896283 | **T** | C | C | C | **T** | **T** | X39Q | Deleterious |
| Sobic.006G147450 | 6_50903023 | A | A | A | C | C | C | T95P | Neutral |
| Sobic.006G147500 | | | | | | | | | No variants |
| Sobic.006G147600 | 6_50924009 | A | **G** | A | A | A | **G** | S218P | Deleterious |
| Sobic.006G147600 | 6_50924916 | G | G | T | T | G | T | P128Q | Neutral |
| Sobic.006G147700 | 6_50931496 | T | T | T | **A** | T | T | K267I | Deleterious |
| Sobic.006G147700 | 6_50932111 | G | G | G | C | G | G | Q164E | Neutral |
| Sobic.006G147700 | 6_50932356 | G | G | G | C | G | G | L122V | Neutral |
| Sobic.006G147700 | 6_50932467 | C | C | C | **G** | C | C | F293L | Deleterious |
| Sobic.006G147800 | 6_50944184 | T | C | C | C | C | C | F208S | Neutral |
| Sobic.006G147800 | 6_50944189 | G | A | A | A | A | A | A210T | Neutral |
| Sobic.006G147800 | 6_50944209 | A | C | C | C | C | C | L216F | Neutral |
| Sobic.006G147800 | 6_50944295 | T | T | **C** | **C** | **C** | **C** | I245T | Deleterious |
| Sobic.006G147800 | 6_50944541 | C | C | C | C | A | A | T327L | Neutral |
| Sobic.006G147800 | 6_50944760 | T | T | **A** | **A** | **A** | **A** | V400D | Deleterious |
| Sobic.006G147800 | 6_50944922 | T | T | C | C | C | C | L454S | Neutral |
| Sobic.006G147800 | 6_50945034 | C | C | A | A | A | A | S491R | Neutral |
| Sobic.006G147800 | 6_50945071 | C | C | **A** | **A** | C | **A** | P504T | Deleterious |
| Sobic.006G147900 | 6_50948305 | G | G | C | C | G | C | A11P | Neutral |
| Sobic.006G147900 | 6_50948336 | T | T | C | C | T | C | F21S | Neutral |
| Sobic.006G147900 | 6_50948375 | T | T | C | C | T | C | V32A | Neutral |
| Sobic.006G147900 | 6_50948380 | G | G | T | T | G | T | A36S | Neutral |
| Sobic.006G147900 | 6_50948386 | G | G | T | T | G | T | A38S | Neutral |
| Sobic.006G147900 | 6_50950605 | C | C | G | G | C | G | L111V | Neutral |
| Sobic.006G147900 | 6_50950719 | A | A | G | G | G | G | N149D | Neutral |
| Sobic.006G147900 | 6_50950813 | C | C | C | C | C | **T** | S180F | Deleterious |
| Sobic.006G148000 | 6_50960848 | T | G | T | G | G | G | Y238S | Neutral |

**Supplemental Table 4.** Expression genes in the D-locus in the internode subtending the peduncle was analyzed during development in RIL49 (NAC-d1) and RIL392 (NAC_D). RNA was extracted from the internode from RIL49 and RIL392 prior to aerenchyma formation (TP1) and 7-10 days later (TP2) when aerenchyma were visible. Gene expression was analyzed by qRT-PCR and are reported as fold-induction.

| Gene ID | RIL | NAC_D allele | Fold change in expression between TP1 – TP2 | p-value (NS, not significant) |
|---|---|---|---|---|
| Sobic.006G147400 | RIL49 | *SbNAC_d1* | 25 | $p < 0.05$ |
| Sobic.006G147400 | RIL392 | *SbNAC_D* | 1010 | $p < 0.05$ |
| Sobic.006G147450 | RIL49 | *SbNAC_d1* | - | NS |
| Sobic.006G147450 | RIL392 | *SbNAC_D* | - | NS |
| Sobic.006G147500 | RIL49 | *SbNAC_d1* | - | NS |
| Sobic.006G147500 | RIL392 | *SbNAC_D* | - | NS |
| Sobic.006G147600 | RIL49 | *SbNAC_d1* | - | NS |
| Sobic.006G147600 | RIL392 | *SbNAC_D* | - | NS |
| Sobic.006G147700 | RIL49 | *SbNAC_d1* | - | NS |
| Sobic.006G147700 | RIL392 | *SbNAC_D* | - | NS |
| Sobic.006G147800 | RIL49 | *SbNAC_d1* | - | NS |
| Sobic.006G147800 | RIL392 | *SbNAC_D* | - | NS |
| Sobic.006G147900 | RIL49 | *SbNAC_d1* | - | NS |
| Sobic.006G147900 | RIL392 | *SbNAC_D* | - | NS |
| Sobic.006G148000 | RIL49 | *SbNAC_d1* | - | NS |
| Sobic.006G148000 | RIL392 | *SbNAC_D* | 8 | $p < 0.005$ |

**Supplemental Table 5.** Sobic.006G147400 DNA and cDNA sequencing primers

| Type | Genotype | Forward primer (5' → 3') | Reverse primer (5' → 3') |
|---|---|---|---|
| DNA template | IS3620c, Tx2910, BTx3297, RTx2909, Rio, BTx623 | CAGCAGCGGTTTCTTTTGCT | TCAGCTGAACAGTCAGAAACCTT |
| DNA template | Tx7000 | CCTGGCATGACACTACAGCA | AGTCAGCTGAACAGTCAGAAAC |
| DNA template | All remaining genotypes | CAGGCTGCAAGAGCGAGATA | TCGATCAGTCCTCTCAGCCA |
| DNA Sequencing | - | CCTGCAGGCTTCCTCGTCCCTATAAA | GCTCGTTGGCCACCTTCTTGTAGAGG |
| DNA Sequencing | - | CCTCTACAAGAAGGTGGCCAACGAGC | CAGCCCTCTGCACCATGTGATGCAC |
| DNA Sequencing | - | GTGCATCACATGGTGCAGAGGGCTG | GGTGACGAGTCTTGTGCGTTGGGATC |
| DNA Sequencing | - | GATCCCAACGCACAAGACTCGTCACC | AAGACGAGCGTCTTCCTCATGCC |
| DNA Sequencing | - | CCGCATCATCATCAGGAGAACCTG | CAGGTTCTCCTGATGATGATGCGG |
| DNA Sequencing | - | GGCATGAGGAAGACGCTCGTCTT | CCTTTTGGATTGGAGAGGTGCACTC |
| cDNA template | BTx623, IS3620c | CAGGCTGCAAGAGCGAGATA | CCTTCACAATGCCCAGTCCA |
| cDNA sequencing | - | CAGGCTGCAAGAGCGAGATA | TCTTTCCGCTTCTGGAACAC |
| cDNA sequencing | - | GTGTGCCACTACCTCTACAAG | ACATCGCTCCGTTCATCAG |
| cDNA sequencing | - | CGAGTGGTACTTCTTCAGCTTC | CCTTCACAATGCCCAGTCCA |

**Supplemental Table 6.** qPCR primers

| Gene ID | gene name | Forward primer (5' → 3') | Reverse primer (5' → 3') |
|---|---|---|---|
| Sobic.006G147400 | *SbNAC_D* | AGAGTGCACCTCTCCAATCC | GCAAATGAAAATGACACCTCCT |
| Sobic.007G172100 | *SbXCP1* | GTGAAGAACTCGTGGGGACC | ATGCGATTCAGAGCTCGTCG |
| Sobic.001G526600 | *SbUBC* | CATGCTGCACATTCGCATAG | AGAGACATGGTCCACAAGAAC |