

Study on fine mapping of QTL for juice yield traits of sweet sorghum (*Sorghum dochna*)

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Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

Table S1. List of primers used in this experiment

Name	Forward primer	Reverse primer	Location	bp	Type	Source
sam06092a	TCTTCATAGCCG-TACGTTG	ATAACTGCCCTC-CTCTCG	32030207	163	SSR	Li et al. (2009)
sam52086c	ACAGGGAATGGCT-TAAAGTT	AAATAAAGAGA GACCACCAATG	39532829	171	SSR	Li et al. (2009)
sam52086b	AAAGCTAGCACA-CGCATC	TTTAAGCCATT CCCTGTG	39532983	176	SSR	Li et al. (2009)
sam58007	TGCCTAAGTAAA-CCACCCTA	GTTCCAAAATAA CCAGGACA	40827992	161	SSR	Li et al. (2009)
sam79818b	ATGGAATTCACAG-TTGGTATG	TCAACGCATG-TAATTCACAC	44766658	175	SSR	Li et al. (2009)
sam34761	CTCATCCTCCATT-GCTTATC	GGACACAATTA GAGGATCGA	46169563	106	SSR	Li et al. (2009)
sam74922	TACTACGCCTATC-GATCGTT	AGATGAAGGTCC GAGAGG	46622574	137	SSR	Li et al. (2009)
sam57212	CCTGCCAAGGTA-CATCAC	ATCCGAG-CAAACACACAC	46905059	150	SSR	Li et al. (2009)
sam51942	CGTCTCTTGATC-GATTTCG	TACACGCACTG-GTTCTCC	48297321	147	SSR	Li et al. (2009)
Xtxp45	CTCGGCGGCTCC-CTCTC	GGTCAAAGCGCT CTCCTCCTC	49285366	199	SSR	Bhatramakki et al. (2000); Kong et al. (2000)
sam33545	CCATGACCTG-CAAAATCTAT	CGGTTATGT-GCGTTTCAT	49670812	168	SSR	Li et al. (2009)
sam79728	CAAGGTCGTCGAGA-TCAC	ACTAGTACTAC ATGGCAGGCA	49965614	199	SSR	Li et al. (2009)
sam68840	AGGATGGCTGGA-GAGATC	TACAGCGTTACTA CTGGCTTC	50316061	142	SSR	Li et al. (2009)
sam73036	GACTCCTGGTG-GCTCAG	ATCCCACCCGAG ACAG	50622447	189	SSR	Li et al. (2009)
Xtxp317	CCTCCTTTTCCTC-CTCCTCCC	TCAGAATCCTAGC CACCGTTG	50757123	162	SSR	Bhatramakki et al. (2000); Kong et al. (2000)
Xtxp265	GTCTACAGGCGTG-CAAATAAAA	TTACCATGCTACCC-CTAAAAGTGG	51179133	212	SSR	Bhatramakki et al. (2000); Kong et al. (2000)
sam46240	TTCTTTCGTCTCT-CTCCTC	CGAACGAGAACA-GAATGC	51526326	147	SSR	Li et al. (2009)
F51-5	TGCGAACGAGAA-CAGAAATGCG	TCGTCTT-GTCTTCGTTCTCTGG	51527242	241	SSR	this work
F51-8	TGACAGAAAAAG-TACGGACAAACG	CAAAGTCAACTGGT-GTCGAGG	51588218	240	SSR	this work
F51-9	AGAGGCTCAT-GTTGGGTTTCA	ACTGATTTTGGCT GAGATAATTCCT	51711901	211	SSR	this work
F51-11	ATCGGTTGCGCT-AAGGAAGA	CGTCCATGATTG-GAGGGGC	51805774	221	SSR	this work
11F/20F	ATCGGTTGCGCT-AAGGAAGA	CCGTGTGGAGGG ATCCATAA	51806543	1136	SNP	this work
33F/45	CGAACAAGGCCTAA-GCGAGA	TCGTTTCGTCAA TTCCGACCA	51807486	529	SNP	this work
F51-21	CATGTTTTCTCTT-GCGGTGGT	CTGAGACCACGA CGGTGTAAT	51808395	417	SSR	this work
Xtxp97	CAAATAAACGGTG-CACACTCA	GTATGATTGGAGA CGAGACGG	51812449	130	SSR	Bhatramakki et al. (2000); Kong et al. (2000)
F51-22	ATACAGTTGTTCGT-CATGCAGGT	CTGATGAACGGA GCGATGTG	51815810	508	SSR	this work
F51-28	TGTGGAGGCAAAGTA-CACATCT	TCCTATACGCCTG-GATTGGT	51819007	1649	InDel	this work

<https://doi.org/10.17221/48/2021-CJGPB>

Table S1 to be continued.

Name	Forward primer	Reverse primer	Location	bp	Type	Source
F51-29	TGTTCAATATGCA-CTGGCGTGT	GCCTAAGCCTTTC-CTAAGCCCCT	51824378	1559	InDel	this work
29F/46R	TGTTCAATATGCA-CTGGCGTGT	TGGACTACTGGA-GCCATCGG	51824378	2260	SNP	this work
48F/23R	CAAATGGCGAGA-CACGAACG	CAGCCAAACCAAA-TGGCACATA	51827757	1777	SNP	this work
F51-14	TGTGTGATGGCTT-GCTTTGC	GTAGCCCAGAG-CCACATAACC	51841971	112	SSR	this work
sam27739	TCGCCTGTAGGA-TAGCAG	AGGCGAGAGCTA-GAGGAC	51868646	123	SSR	Li et al. (2009)
F51-27	AGAGCTTTGCGA-GTTGCGA	GACCCGTTTCCAA-AATGCCG	51892868	228	SSR	this work
F51-12	TTGTCCCCGAC-GAGTTTTGT	TCACCGTCGCA-CTACACTTT	51920628	248	SSR	this work
F51-7	TGTCTCGAC-TCAGTTCGTGTT	TAAACTAACCTAG-CC-CCAGCG	51984427	228	SSR	this work
F52-12	ACCCGAACAGCC-CTAGTCTTTA	CACATAGGCAGT-TTGGCACAT	52011353	208	SSR	this work
M8/E2-720	TGAGTCCAAA-CCGGACT	GACTGCGTACGAA-TTTGC	52111385	720	SRAP	this work
sam49940	ACTCCATAACAGA-TTCTCTCTCTCTCT	TATTGTACATGC-TCCAATT	52251648	145	SSR	Li et al. (2009)
6-2snp	GCAGAGTGACAT-TCGAACACT	AGCAGATGC-CAAACCTCAGTCC	52408577	1300	SNP	this work
sam56942	GGCACAGT-TATTTCTTCCAC	GTCCACTACTTCA-GTGTTTGC	53391566	149	SSR	Li et al. (2009)
sam36845	GGTGCATTGTA-CCTGTGT	GTGTGTATTGTA-CCCGCTG	53730282	122	SSR	Li et al. (2009)
sam79056a	GATTCAAGAAA-CGCCCAC	TGGTATTATTAA-CGCCGC	54309900	225	SSR	Li et al. (2009)
sam44008	AAGGCTCTTTC-GTGGATC	CTACTTTACCA-CCGGCCT	59113643	157	SSR	Li et al. (2009)
28F/18R	TGTGGAGGCAAA-GTACACATCT	GTGCTCGTAGGCC-AGATACG	51819007	942	InDel	this work
11F/20F	ATCGGTTGCGC-TAAGGAAGA	CCGTGTGGAGGG-ATCCATAA	51805774	1156	InDel	this work
33F/45	CGAACAAGGCC-TAAGCGAGA	TCGTTTCGTCAA-TTCCGACCA	51806910	529	InDel	this work
29F/46R	TGTTCAATATGCA-CTGGCGTGT	TGGACTACTGGA-GCCATCGG	51824379	2261	InDel	this work
48F/23R	CAAATGGCGAGA-CACGAACG	CAGCCAAACCAAA-TGGCACATA	51827757	1777	InDel	this work
22F/55	ATACAGTTGTTCGT-CATGCAGGT	TTGCTGATGACTC-TTGGTG	51815810	810	InDel	this work
22F/39R	ATACAGTTGTTCGT-CATGCAGGT	TACTTCTTCAGCT-TCAGGGACC	51815165	1152	InDel	this work
49/39R	AGATCGATGGT-GTCTTGCGGCG	TACTTCTTCAGCTT-CAGGGACC	51816317	355	InDel	this work
28F/18R	TGTGGAGGCAAA-GTACACATCT	GTGCTCGTAGGCC-AGATACG	51819007	942	InDel	this work

Table S2. List of SRAP primers used in this experiment

Forward primer	Sequence (5'–3')	Reverse primer	Sequence (5'–3')
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTAAC
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTGCA
Me6	TGAGTCCAAACCGGACA	Em6	GACTGCGTACGAATTCAA
Me7	TGAGTCCAAACCGGACG	Em7	GACTGCGTACGAATTCAC
Me8	TGAGTCCAAACCGGACT	Em8	GACTGCGTACGAATTCAT
Me9	TGAGTCCAAACCGGAGG	Em9	GACTGCGTACGAATTCTA
Me10	TGAGTCCAAACCGGAAA	Em10	GACTGCGTACGAATTGTC
F11	GTAGCACAAGCCGGAGC	R11	CGCACGTCCGTAATTAAC
F12	GTAGCACAAGCCGGACC	R12	CGCACGTCCGTAATTCCA
F13	CGAATCTTAGCCGGATA	R13	CGTAGCGCGTCAATTATG
F14	CGAATCTTAGCCGGAGC	R14	CGTAGCGCGTCAATTAAC
F15	CGAATCTTAGCCGGCAC		
F16	CGAATCTTAGCCGGAAT		

<https://doi.org/10.17221/48/2021-CJGPB>

Table S3. Leaf midrib colour and juice yield data of the different species

Variety	Main vein colour	Juice yield	Pole weight	Juicy	Flowering period
Xinliang52	B	0.181	1138.0	206.0	7.15
W455	L	0.473	206.7	97.7	7.20
W452	L	0.375	792	297.7	–
07-27	L	–	–	–	–
232EB	L	0.393	197.0	77.4	7.30
2731B	B	0.206	296.0	61.1	7.20
314B	L	0.397	204.0	80.9	7.17
7050B	L	0.444	430.0	191.0	8.07
8110-1	L	–	–	–	–
B35	L	0.448	1050.0	470.0	7.29
BJ-166	L	0.408	493.0	201.0	7.22
BJ-285	L	–	–	–	–
BJ-299	L	–	–	–	–
BJ-320	L	–	–	–	–
BJ-338	L	–	–	–	–
GW0686	L	0.370	495.0	183.2	8.09
GW3614	L	0.384	321.0	123.3	7.18
GW3627	L	0.500	283.0	141.5	8.08
GW3968	L	0.516	1036.0	534.8	8.06
GW3998	L	0.396	570.0	225.7	8.12
GW4000	L	–	–	–	–
HAOA	L	0.462	442.0	204.0	7.29
JS105	B	0.051	670.0	34.3	7.27
JS108	L	0.446	518.0	231.0	7.19
M-81E	L	–	–	–	–
MN-2735	B	0.194	247.0	48.0	7.16
MN-3461	L	0.390	541.0	210.9	8.12
MN-3506	L	0.488	804.0	392.6	7.29
MY-10	B	0.302	804.0	243.2	8.02
MY-11	B	0.281	554.0	155.4	7.25
MY-14	L	–	–	–	–
MY-15	L	–	–	–	–
MY-16	B	–	–	–	–
MY-2	L	0.469	1300.0	609.4	8.14
MY-3	L	0.530	1452.0	768.9	8.12
MY-4	L	–	–	–	–
MY-5	L	–	–	–	–
MY-8	L	–	–	–	–
P39-3	–	–	–	–	–
SOY	B	0.378	688.0	259.8	7.22
SUG	L	0.467	776.0	362.1	7.22
TAM428B	L	0.319	139.0	44.4	7.26
TS185	LB	0.377	378.0	142.5	7.31
Tx-623B	L	0.455	228.0	103.8	7.25

Table S3 to be continued.

Variety	Main vein colour	Juice yield	Pole weight	Juicy	Flowering period
W453	L	0.501	939.0	470.2	7.22
w454	B	0.358	315.0	112.7	7.23
W456	L	–	–	–	8.10
W457	B	0.230	422.0	97.2	7.20
W461	B	0.176	291.0	51.2	7.16
Ao1	-	0.313	311.0	97.4	7.18
Ao2	LB	0.374	216.0	80.8	7.25
GZ No.1	L	–	–	–	–
GZ No.2	B	0.234	773.0	181.2	7.25
Jing 7B	L	0.384	237.0	91.0	7.26
Jing 852	L	0.325	108.0	35.1	8.12
Jinchang B	L	0.395	100.0	39.5	8.15
Jiutian 1B	L	–	–	–	–
Keller	L	0.496	977.0	484.7	8.17
Kaoli	L	–	–	–	–
Rey	L	–	–	–	–
Leo	L	0.442	1029.0	454.5	8.01
LT No.3	L	0.441	1220.0	538.0	8.10
Green Energy 1	L	0.526	533.0	280.2	7.28
Green Energy 2	L	–	–	–	–
Green Energy 3	L	–	–	–	–
Rome	L	–	–	–	–
Neinong 1	L	–	–	–	–
Sanchisan	L	0.435	348.0	151.3	7.28
Siping	B	0.371	277.0	102.7	7.23
Itari	L	0.495	540.0	267.5	7.19
YuantiaNo1	L	0.460	610.0	280.7	7.29

B – white pulse; L – wax pulse; LB – wax veins and white veins

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<https://doi.org/10.17221/48/2021-CJGPB>

Supplementary data 1

The specific fragment produced by the Me8/Em2-2 primer pair was sequenced and found to be 712 bp in size. The fragment sequence is as follows: CCTCTAAGCAGTTTCTTCTTTTCCTCAGGAAGTAC-CAATTTCTTATGGTTTAAGTCATTGCCTTCTTTCTTGATCTCCAAGCCCAGGCAACGAACCCGT-TACCCAAACGTGTCTCCTTTTGTATCTGAGTTTGAGGAGCGTCACTGCTGACCAATCTGTTGC-CTTCTCATTGACCACCTTACCCCTTTATACGTGGCTTCTTTTTCCTCATGCACTTGGCGATATCT-GTTGCTTATAAACATGGTCCAAGAATCGGTTTTTCTTTTCACTTTGCTTCGTTTCGCTGTTGC-CCTCATCCTTATAATTACGTGTCACACACAGGTCTCGATCTTCCCCCATATATACACAGAGCG-GTAGGAGCGATGGACCATGAGATGGAGCAGAAGGGATGTCTCTGCCGGATCAAGAACTGCGC-CATCGAGCTCTTCTCTGCTATGGAGGAGGATCTGGAGGTGGACGACGAGGACTCGTGGGAC-CTCGTTGGAAGGGACCTTCGGCTAAAGGCCACCTTCCTGTACATTGACCTCAGCCGTGTGATCAC-CTTCTGCGAGGGCGAAGAGCATAAGAAGGCGCTCACTGTCCTTGCCAACAAGTTCTTCTACTC-CATGGATGAGGTGAGGATCCTTTCCCTCTTTTACGACATTTTCATAATTTTTCATAATTTTCACAATA-CAAAGCACGTCTTGGTCCATTGCAAATTCGTACGCAGTC

Using Blast software to compare the identity in the GenBank database, bases 365 to 621 are highly consistent with bases 1 to 257 of the unknown functional mRNA protein of sorghum, and the identity is as high as 99% (Figure S1). After the BLAST comparison of the mRNA sequence of this unknown functional protein, it was found that it has a certain homology region with the oxygen evolving enhancer mRNA protein sequence on a variety of plant chloroplasts (Figure S2).

```
>\[ref|XM\_002522780.1\] G Ricinus communis Oxygen-evolving enhancer protein 3-1, chloroplast precursor, putative, mRNA
Length=675

GENE ID: 8280704 RCOM_0181530 | Oxygen-evolving enhancer protein 3-1, chloroplast precursor, putative [Ricinus communis]

Score = 65.9 bits (159), Expect = 4e-10, Method: Compositional matrix adjust.
Identities = 41/105 (39%), Positives = 63/105 (60%), Gaps = 5/105 (4%)
Frame = +1

Query 8   KGCLCRINKCAIELFSAMEEDLEVDDESDWDLVGRDLRLKATFLYIDLRSVITFCGESEH 67
          KG + RI+ C +L + EDL D+ + V + LRLK+TF+Y D +VI+ +
Sbjct 352 KGSMYRIRKCGFDLLAL--EDL--IGPDTLNYVRKYLRLKSTFMYYDFDKVISAAPVSD- 516

Query 68   KKALTVLANKFFYSMDLSDAVESRSLPLTQVRYSDTADALREVV 112
          K++LT LANK F + +EL DA ++LP T++ Y T L+EV+
Sbjct 517 KQSLTDLANKLFDNFEELEDASRRKNLPETELSYQGTKVILQEV 651

>\[ref|XM\_002446757.1\] G Sorghum bicolor hypothetical protein, mRNA
Length=826

GENE ID: 8056018 SORBIDRAFT_06q022890 | hypothetical protein [Sorghum bicolor]
(10 or fewer PubMed links)

Score = 459 bits (508), Expect = 1e-125
Identities = 256/257 (99%), Gaps = 0/257 (0%)
Strand=Plus/Plus

Query 365   ATGGACCATGAGATGGAGCAGAAGGGATGTCTCTGCCGGATCAAGAACTGCGCCATCGAG 424
          |||
Sbjct 1     ATGGACCATGAGATGGAGCAGAAGGGATGTCTCTGCCGGATCAAGAACTGCGCCATCGAG 60

Query 425   CTCTTCTCTGCTATGGAGGAGGATCTGGAGGTGGACGACGAGGACTCGTGGGACCTCGTT 484
          |||
Sbjct 61    CTCTTCTCTGCTATGGAGGAGGATCTGGAGGTGGACGACGAGGACTCGTGGGACCTCGTT 120

Query 485   GGAAGGGACCTTCGGCTAAAGGCCACCTTCCTGTACATTGACCTCAGCCGTGTGATCACC 544
          |||
Sbjct 121   GGAAGGGACCTTCGGCTAAAGGCCACCTTCCTGTACATTGACCTCAGCCGTGTGATCACC 180

Query 545   TTCTGCGAGGGCGAAGAGCATAAGAAGGCGCTCACTGTCTTGCCTGCAACAAGTTCTTCTAC 604
          |||
Sbjct 181   TTCTGCGAGGGCGAAGAGCATAAGAAGGCGCTCACTGTCTTGCCTGCAACAAGTTCTTCTAC 240

Query 605   TCCATGGATGAGGTGAG 621
          |||
Sbjct 241   TCCATGGATGAGCTGAG 257
```

Figure S1. BLAST results of M8E2-J712 with sorghum hypothetical mRNA protein

Using the BLAST software, 712 bp was compared with the sorghum genome sequence. The results showed that the M8E2-J712 had the highest sequence identity between the bases 29909752~29910438 on chromosome 6 of a two-colour sorghum (Figure S3). Approximately located near base 29910095 on chromosome 6.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
NW_002994235.1	Sorghum bicolor chromosome 6 genomic scaffold, whole genome shot	1253	1253	96%	0.0	99%	
NC_012875.1	Sorghum bicolor chromosome 6, whole genome shotgun sequence >	1253	1253	96%	0.0	99%	
NW_002994239.1	Sorghum bicolor chromosome 4 genomic scaffold, whole genome shot	130	130	35%	5e-26	76%	
NC_012873.1	Sorghum bicolor chromosome 4, whole genome shotgun sequence >	130	130	35%	5e-26	76%	

The specific fragment produced by the Me8/Em2-1 primer pair was sequenced and found to be 727 bp in size. The fragment sequence is as follows: CCTCTGAACAGTTTCTTTCTTTCTTCTTCTTTCTTTCTTTCTCAGGAAGTACCAATTTCTTATGGTTTAAGTCATTGCCTTCTTTCTTGTATCTCCAAGCCCAGGCAACGAACCCGTTACCCAAACGTGTCTCCTTTTGTTTATCTGAGTTTGAGGAGCGTCACTGCTGACCAATCTGTTGCCTTCTCATTGACCACTTTACCCCTTTATACGTGGCTTCTTTTTCTTCATGCACTTGGTGATATCTGTTGCTTATAAACATGGTCCAAGAGTCGATTTTTCTTTCACTTTGCTTCGTTTCGCTGTTGCCCTCATCCTTATAATTACGTGTACACACAGGTCTCGATCTTCCC-CATATATACACAGAGCGGTAGGAGCGATGGACCATGAGATGGAGCAGAAGGGATGTCTCTGCGGATCAAGAAGTGCGCCATCGAGCTCTTCTCTGCTATGGAGGAGGATCTGGAGGTGGACGACGAGGACTCGTGGGACCTCATTGGAAGGGACCTTCGGCTAAAGGCCACCTTCTGTACATTGACCTCAGCCGTGTGATCACCTTCTGCGAGGGGCGAAGAGCATAAGAAGGCGCTCACTGTCCTTGC-CAACAAGTTCTTCTACTCCATGGATGAGGTGAGGATCCTTTCCCCTCTTTTACAACATTTTCAT-AATTTTTTCATAATTTTACAATACAAAGCACGTCTTGGTCCATTGCAAATTCGTACGCAGTCA

Using the BLAST software, the 727 bp was compared with the sorghum genome sequence. The results showed that the M8E2-J727 had the highest sequence identity between bases 29909723 and 29910438 on chromosome 6 of the sorghum (Figure S5). Approximately located near base 29910081 on chromosome 6.

<https://doi.org/10.17221/48/2021-CJGPB>

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>[ref|XM_002446757.1] [G] Sorghum bicolor hypothetical protein, mRNA
Length=826

GENE ID: 8056018 SORBITDRAFT_06g022890 | hypothetical protein [Sorghum bicolor]
(10 or fewer PubMed links)

Score = 455 bits (504), Expect = 1e-124
Identities = 255/257 (99%), Gaps = 0/257 (0%)
Strand=Plus/Plus

Query 379 ATGGACCATGAGATGGAGCAGAAGGGATGCTCTGCCGGATCAAGAACTGCGCCATCGAG 438
      |||
Sbjct 1 ATGGACCATGAGATGGAGCAGAAGGGATGCTCTGCCGGATCAAGAACTGCGCCATCGAG 60

Query 439 CTCTTCTCTGCTATGGAGGAGGATCTGCAGGTGGACGACGAGGACTCGTGGGACCTCATT 498
      |||
Sbjct 61 CTCTTCTCTGCTATGGAGGAGGATCTGCAGGTGGACGACGAGGACTCGTGGGACCTCGTT 120

Query 499 GGAAGGGACCTTCGGCTAAAGGCCACCTTCCTGTACATTGACCTCAGCCGTGTGATCACC 558
      |||
Sbjct 121 GGAAGGGACCTTCGGCTAAAGGCCACCTTCCTGTACATTGACCTCAGCCGTGTGATCACC 180

Query 559 TTCTGCGAGGGCGAAGAGCATAAGAAGGCGCTCACTGTCTTGTCCCAACAAGTTCTTCTAC 618
      |||
Sbjct 181 TTCTGCGAGGGCGAAGAGCATAAGAAGGCGCTCACTGTCTTGTCCCAACAAGTTCTTCTAC 240

Query 619 TCCATGGATGAGGTGAG 635
      |||
Sbjct 241 TCCATGGATGAGCTGAG 257
```

Figure S4. BLAST results of M8E2-J727 with the sorghum hypothetical mRNA protein

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
NW_002994235.1	Sorghum bicolor chromosome 6 genomic scaffold, whole genome shc	1306	1306	98%	0.0	99%	
NC_012875.1	Sorghum bicolor chromosome 6, whole genome shotgun sequence >	1306	1306	98%	0.0	99%	
NW_002994239.1	Sorghum bicolor chromosome 4 genomic scaffold, whole genome shc	124	124	35%	2e-24	76%	
NC_012873.1	Sorghum bicolor chromosome 4, whole genome shotgun sequence >	124	124	35%	2e-24	76%	

Figure S5 BLAST results of M8E2-J727 with the sorghum genomic sequences