

Supplemental information

**A near-complete genome assembly of the allotetraploid *Cenchrus fungigraminus* (JUJUNCAO) provides insights into its evolution and C4 photosynthesis**

**Huakun Zheng, Baiyu Wang, Xiuting Hua, Ruiting Gao, Yuhao Wang, Zixin Zhang, Yixing Zhang, Jing Mei, Yongji Huang, Yumin Huang, Hui Lin, Xingtian Zhang, Dongmei Lin, Siren Lan, Zhongjian Liu, Guodong Lu, Zonghua Wang, Ray Ming, Jisen Zhang, and Zhanxi Lin**

**A near complete allotetraploid genome provides insight into the evolution and C<sub>4</sub> photosynthesis characteristics of *Cenchrus fungigraminus* (JUNCAO)**

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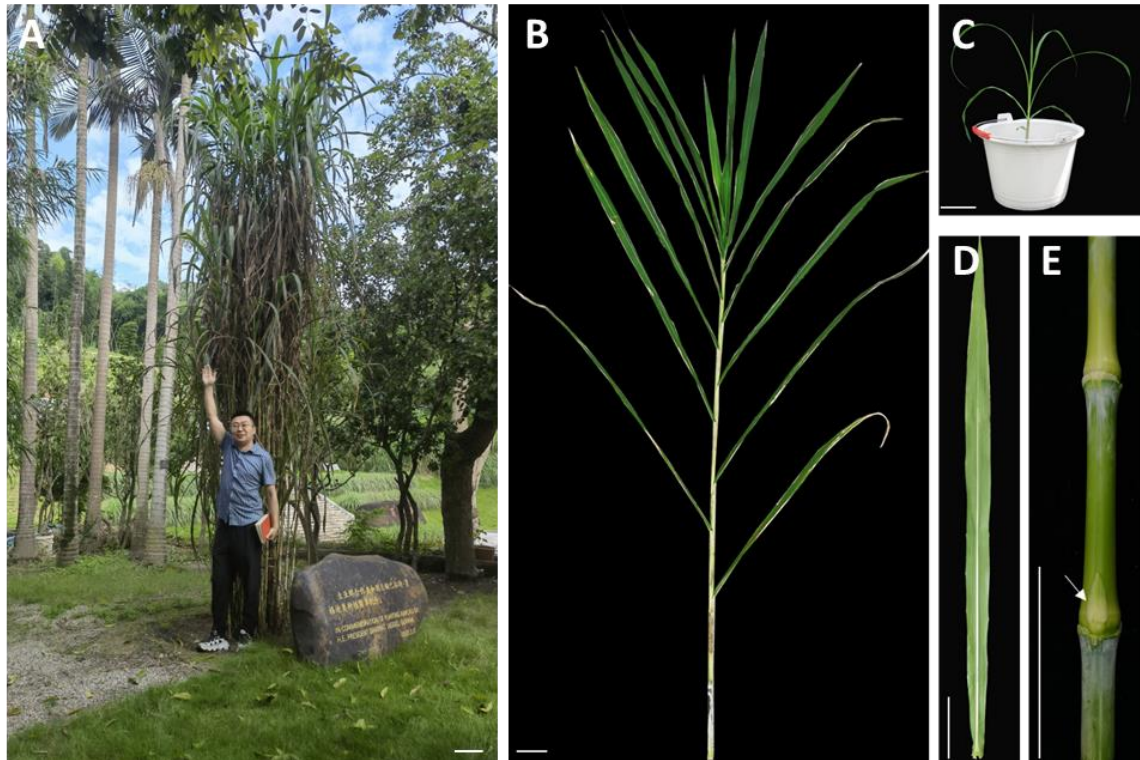
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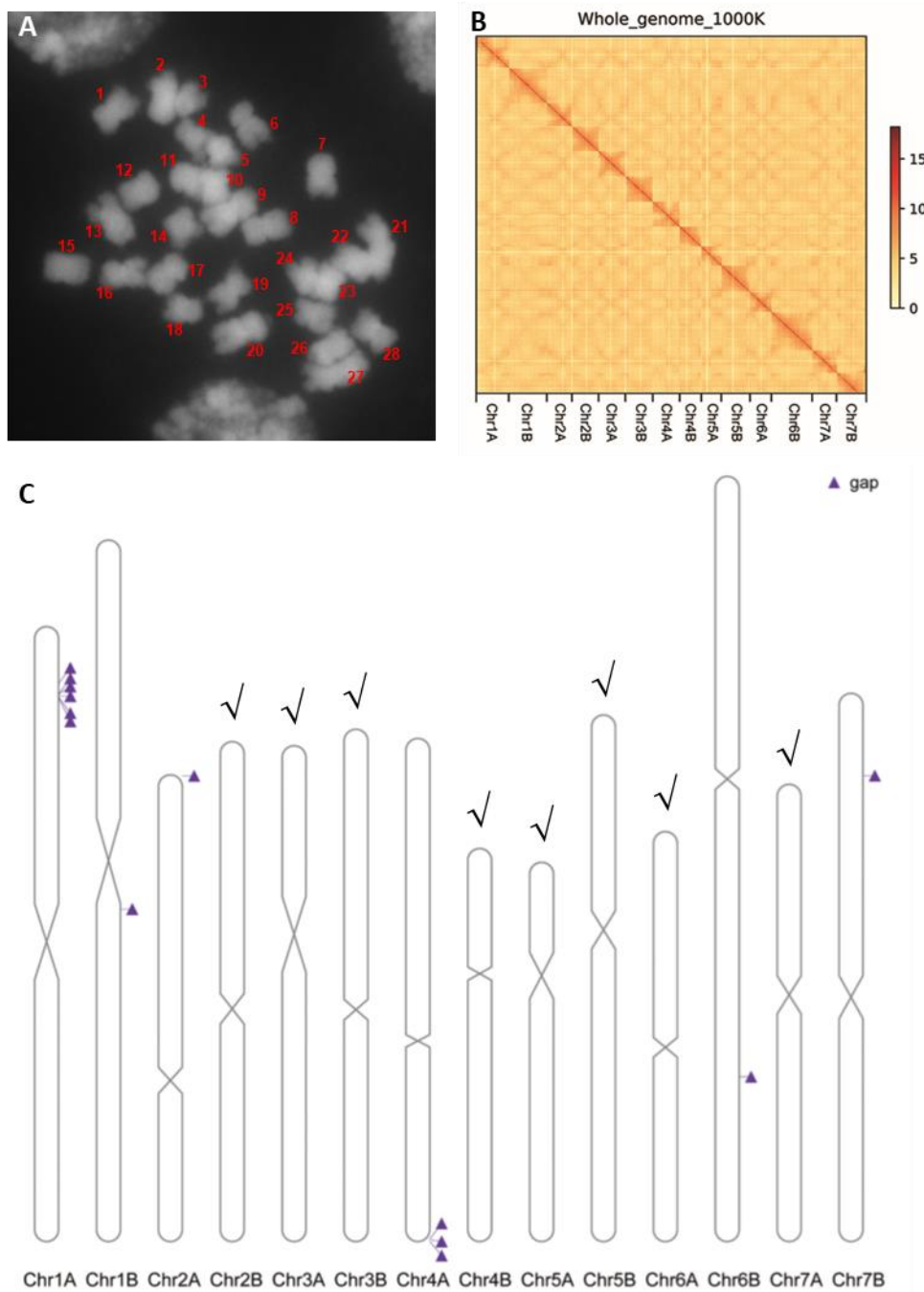


**Figure S1. *Cenchrus fungigraminus* (JUJUNCAO).**

**(A)** The JUJUNCAO plants in the field. Scale bar: 30 cm.

**(B)** Model of JUJUNCAO. Scale bar: 10 cm.

**(C-E)** Seedling (C), leaf (D) and stem (E) of JUJUNCAO. The arrow in (E) indicates the bud on a stem. Scale bar: 10 cm.

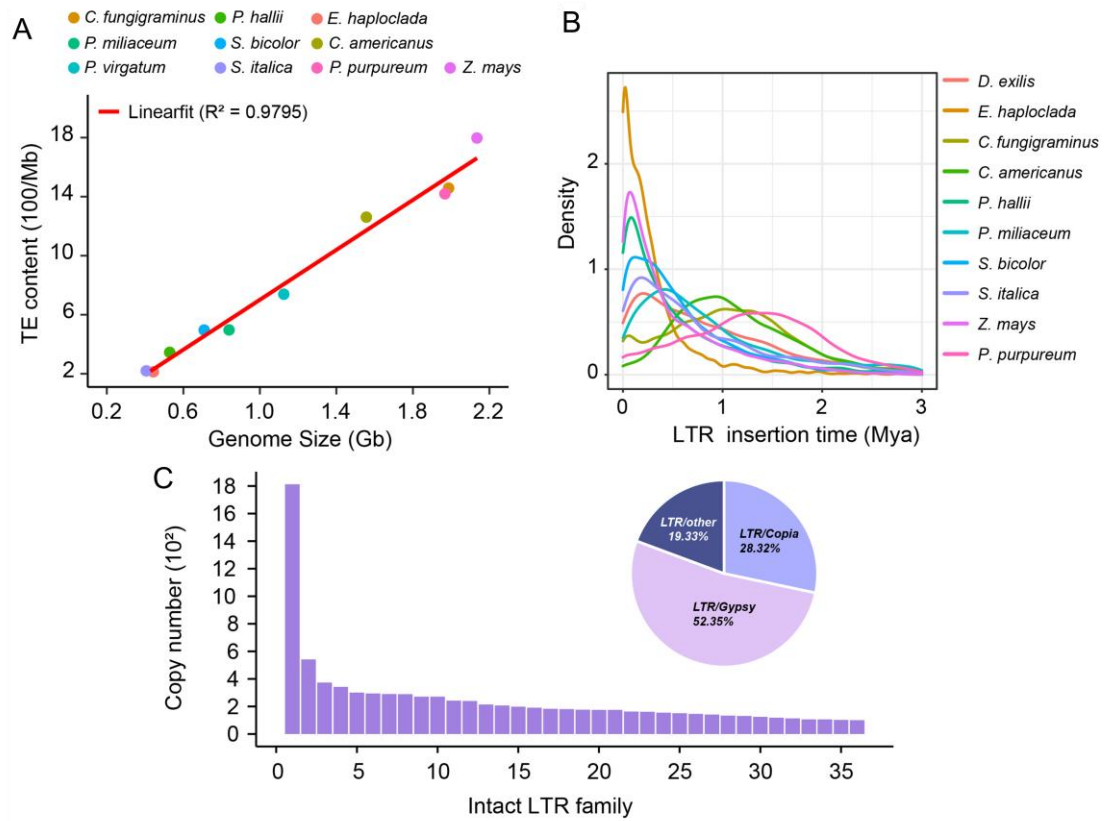


**Figure S2. The JUJUNCAO genome consists of 14 chromosomes.**

**(A)** The microscopic karyotype of JUJUNCAO.

**(B)** The Hi-C chromatin interaction map for the 14 pseudo-chromosome of JUJUNCAO genome.

**(C)** The 14 assembled chromosomes of the JUJUNCAO genome. “✓” indicates the telomere to telomere chromosomes; “▲” indicates the gap on assembled chromosomes.

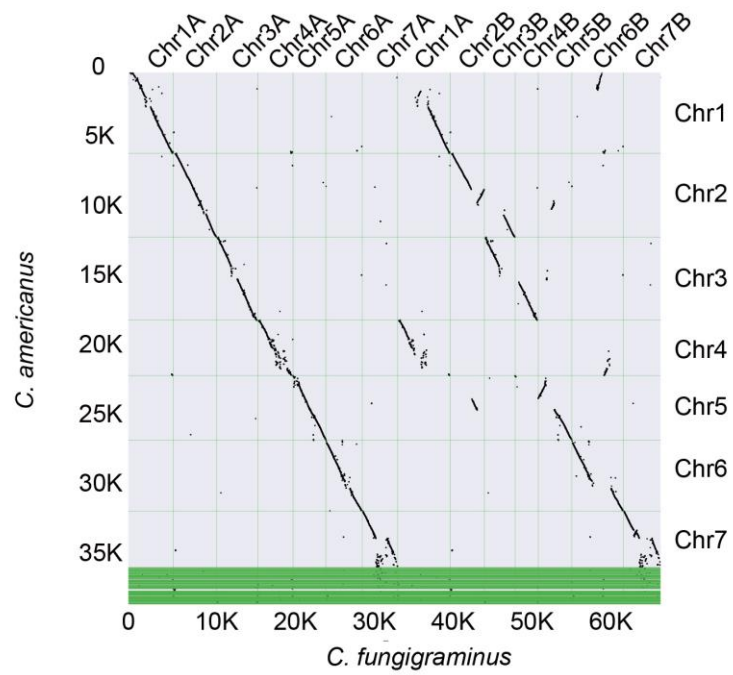
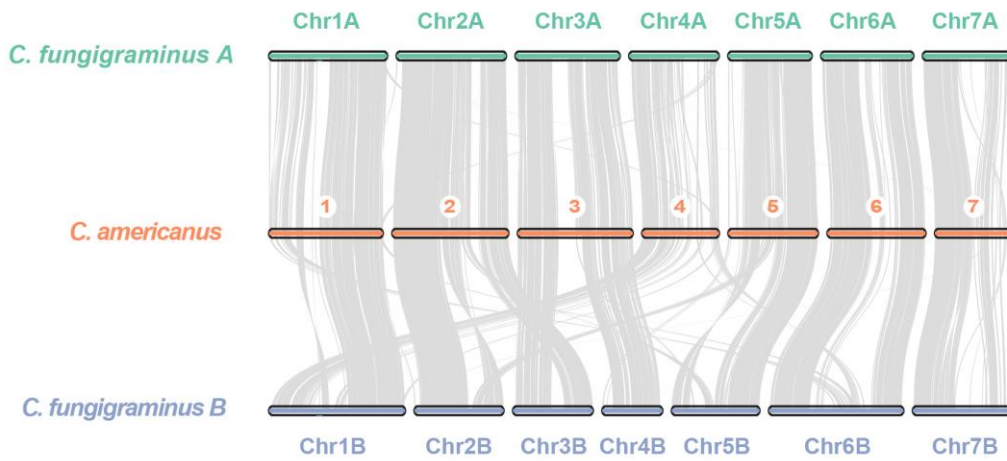


**Figure S3. Characteristics of TE sequences in JUJUNCAO genome.**

**(A)** Genome size expansion is highly correlated with TE amplification bursts ( $R^2 = 0.9795$ ). The red line shows the linear relationship between genome size and TE content.

**(B)** Intact LTR retrotransposon insertion time of *D. exilis*, *E. haploclada*, *C. fungigraminus*, *C. americanus*, *P. hallii*, *P. miliaceum*, *S. bicolor*, *S. italica*, *Z. mays*, *P. purpureum*, X-axis represents LTR retrotransposon insertion time, Y-axis represents density.

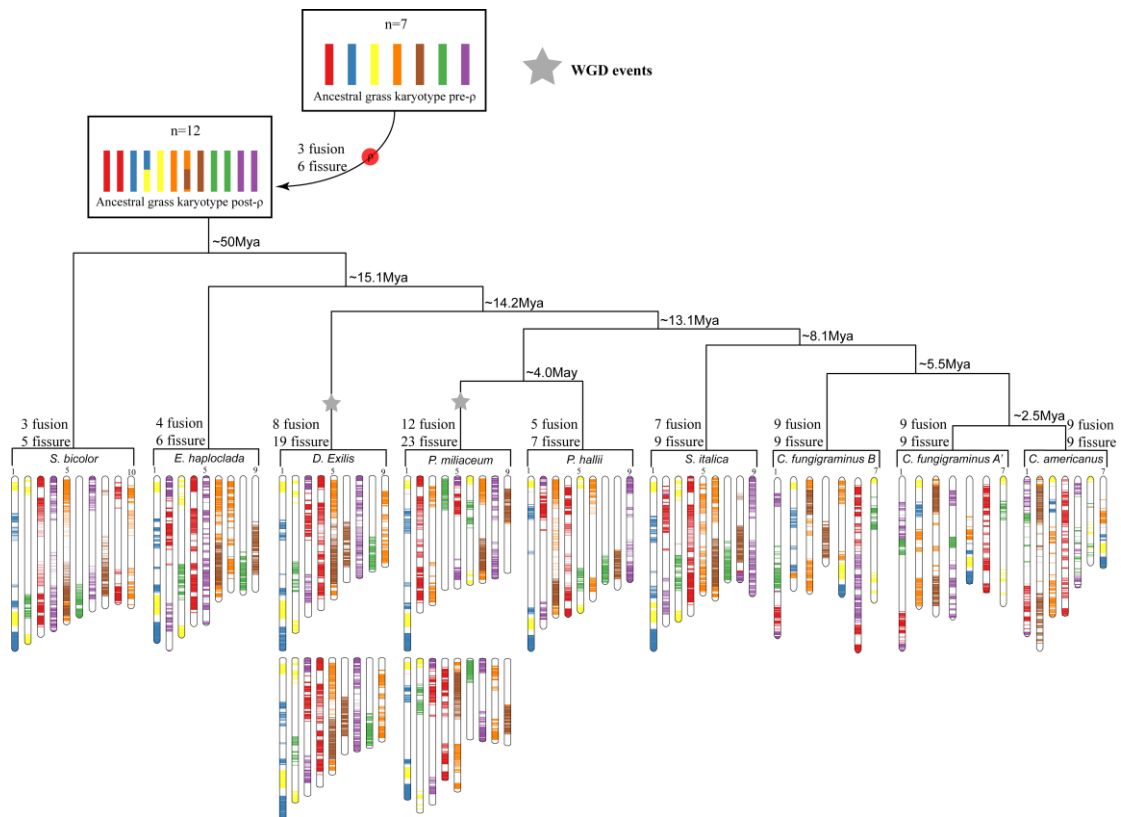
**(C)** Classification of intact LTR retrotransposons in the JUJUNCAO genome, LTR families with more than 100 copies are shown.

**A****B**

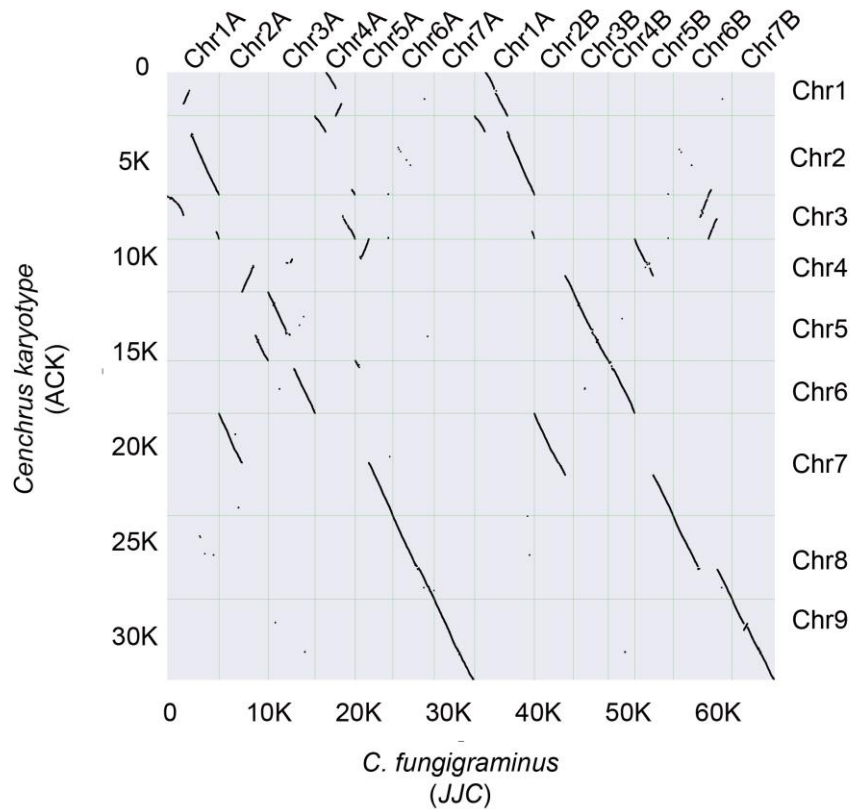
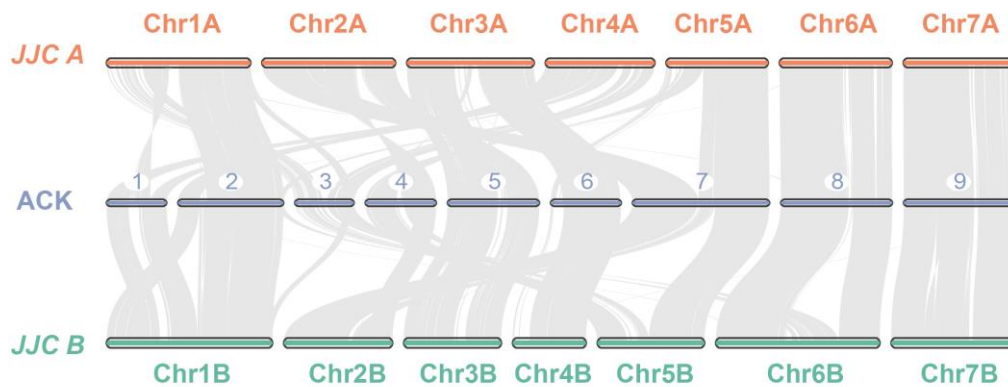
**Figure S4. Comparative genomic analysis of the A and B subgenomes of *C. fungigraminus* and *C. americanus*.**

(A) Dot-plot of alignments.

(B) Syntenic blocks



**Figure S5. Chromosome evolution of JUJUNCAO and species of Panicoideae.** Evolutionary scenario of chromosomes in *Z. mays*, *S. bicolor*, *E. haploclad*, *D. exilis*, *P. miliaceum*, *P. hallii*, *S. italica*, *C. americanus*, *C. fungigraminus* A and B, from the seven chromosomes of ancestral grass karyotype (AGK) pre-p and the 12 protochromosomes of the AGK post-p. Polyploidization events are indicated by stars, along with shuffling events (fusions and fissions).

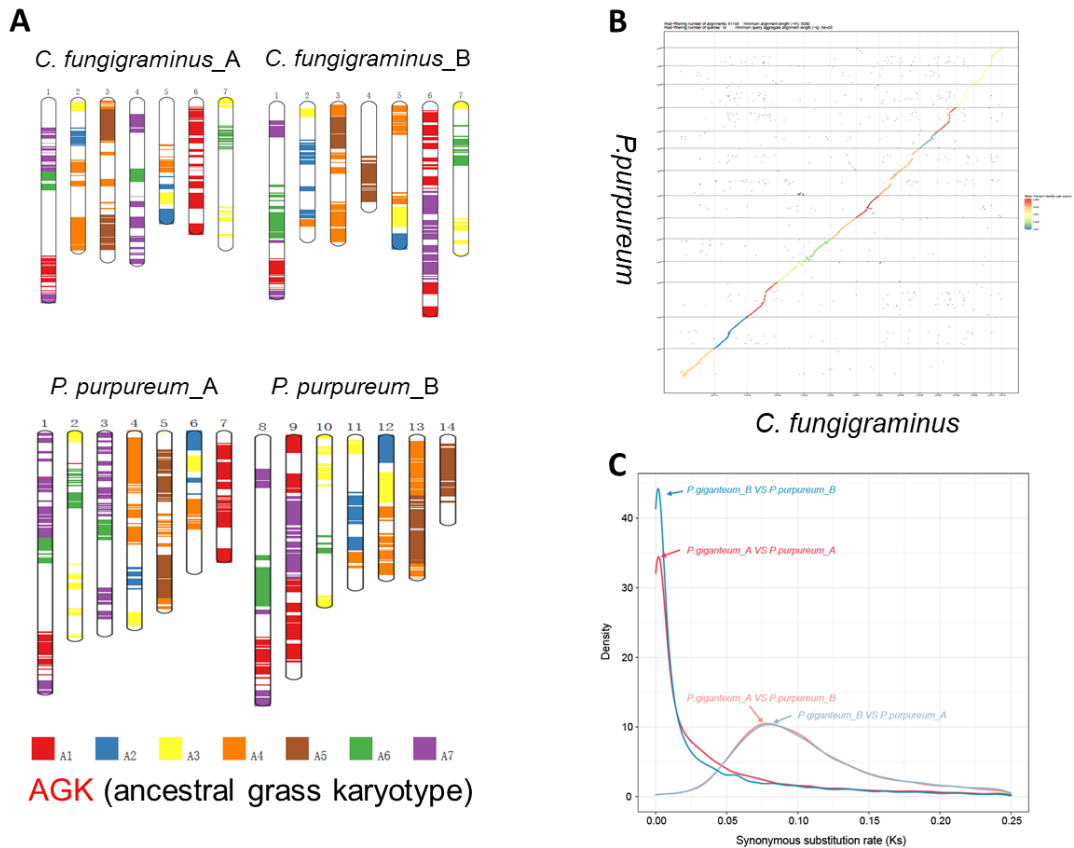
**A****B**

**Figure S6. Comparative genomic analysis of the A and B subgenomes of *C. fungigraminus* and ancestral *Cenchrus* karyotype (ACK).**

(A) Dot-plot of alignments.

(B) Syntenic blocks



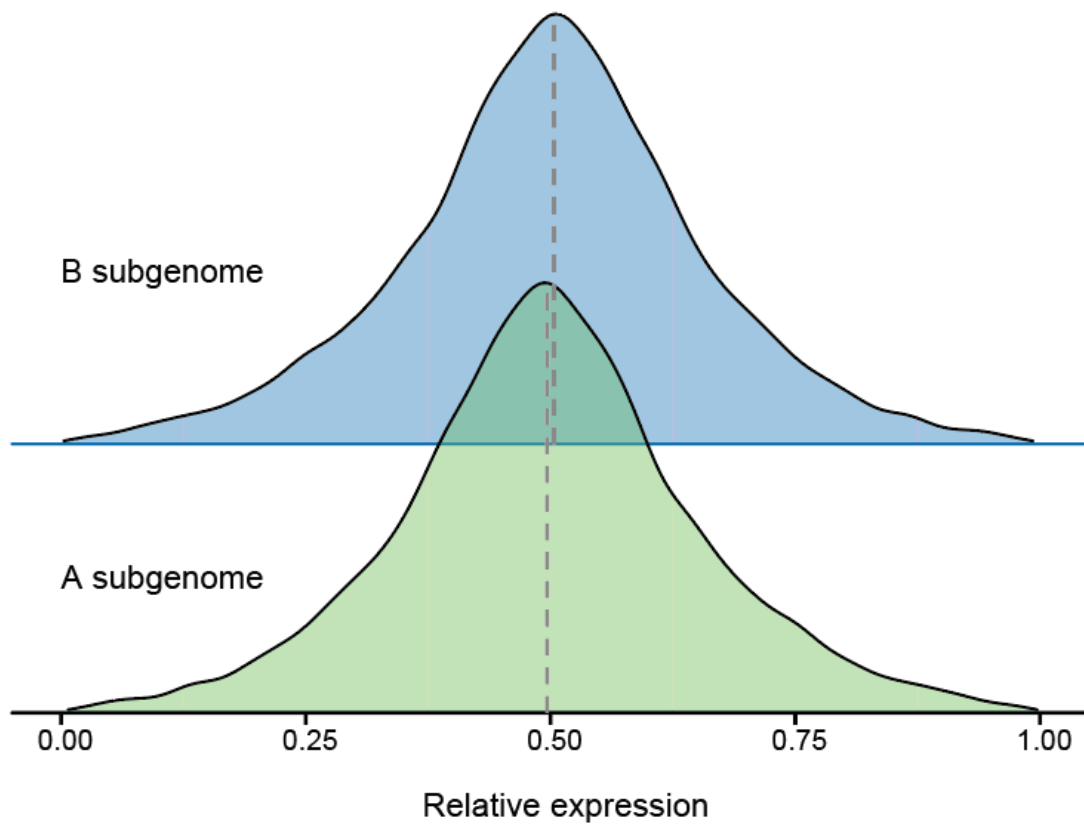


**Figure S7. The chromosome structure and evolution between JUJUNCAO and *P. purpureum*.**

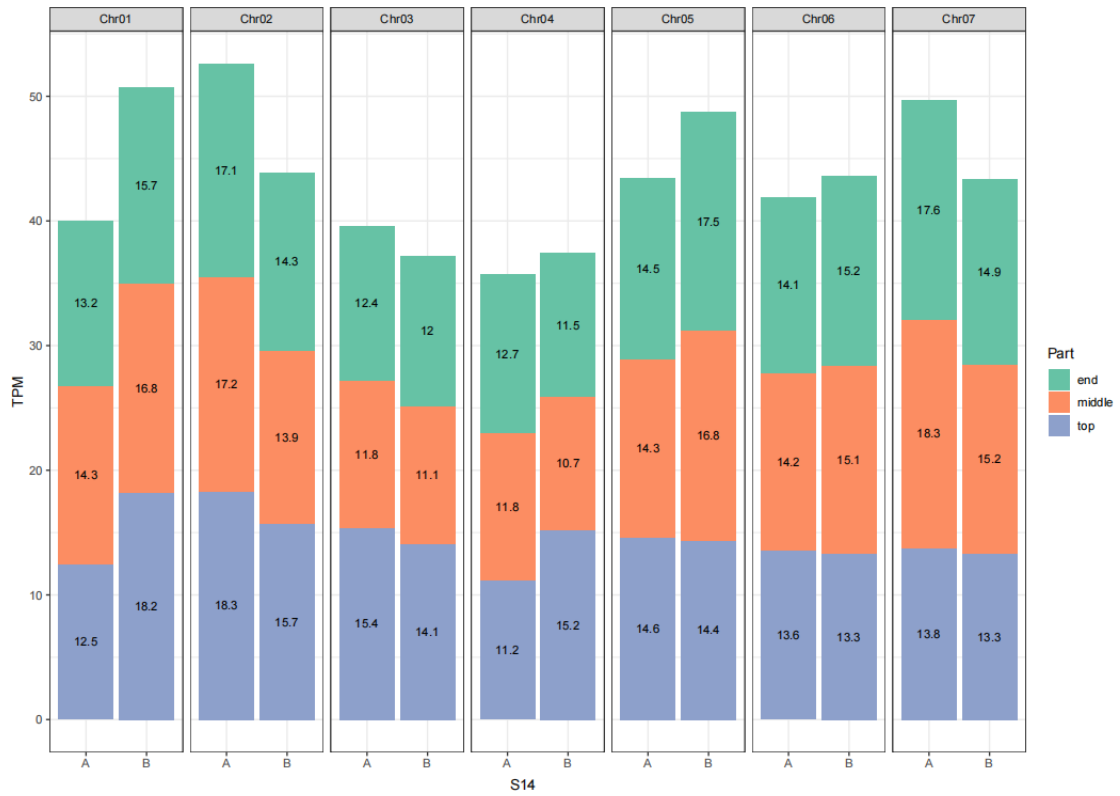
(A) Comparison of chromosome structure between JUJUNCAO and *P. purpureum*.

(B) Graphical alignment of JUJUNCAO chromosomes with *P. purpureum* chromosomes.

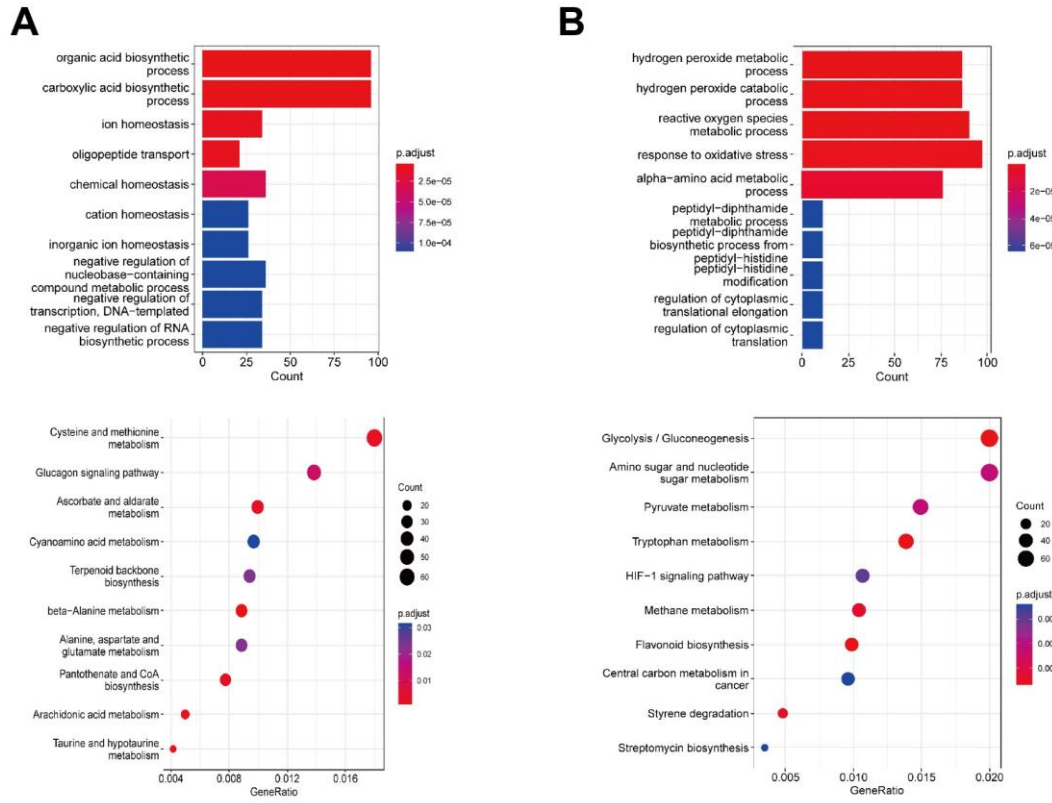
(C) Distributions of Ks between JUJUNCAO and *P. purpureum*.



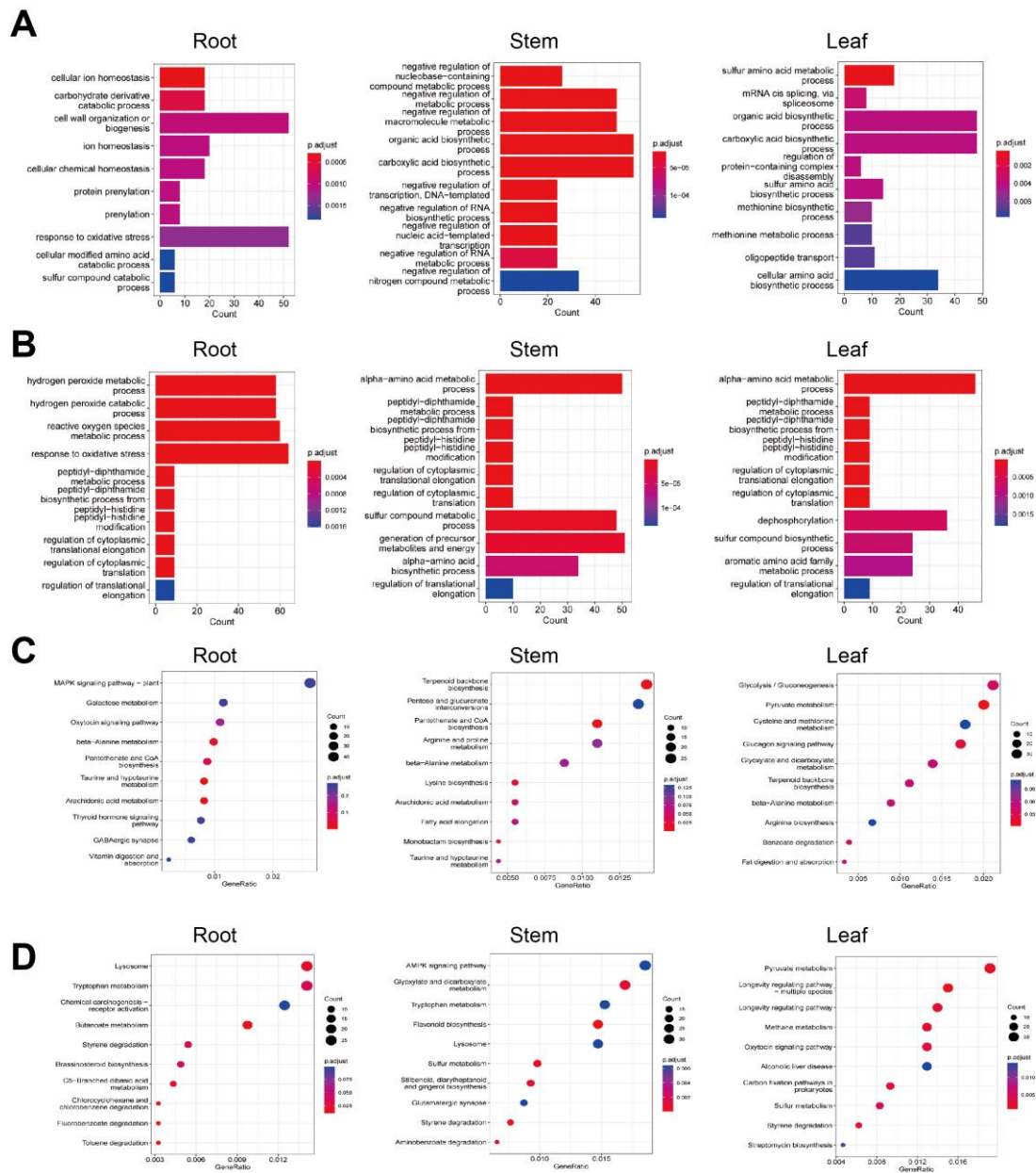
**Figure S8. Comparison of the overall expression level and natural selection feature between the two subgenomes.** The overall expression level between the two subgenomes of JUJUNCAO.



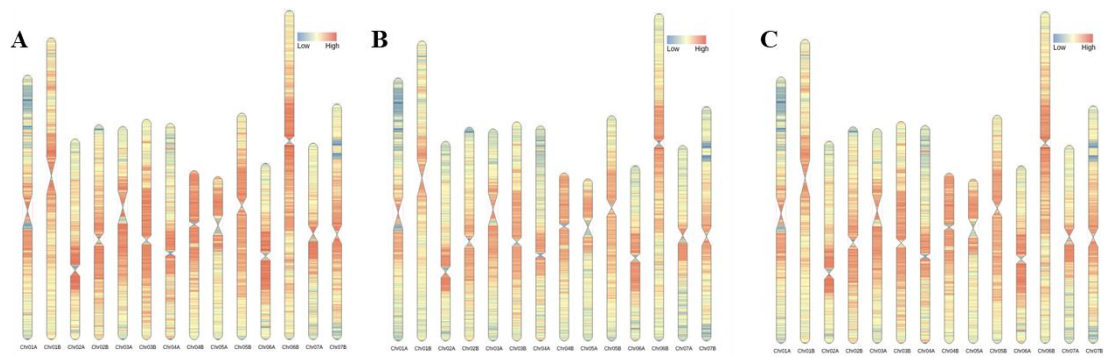
**Figure S9. The distribution of differentially expressed homeologous genes between A and B subgenomes. TPM (Transcript per million).**



**Figure S10. GO (top) and KEGG (bottom) enrichment of subgenome A-biased genes (A) and subgenome B-biased genes (B).**

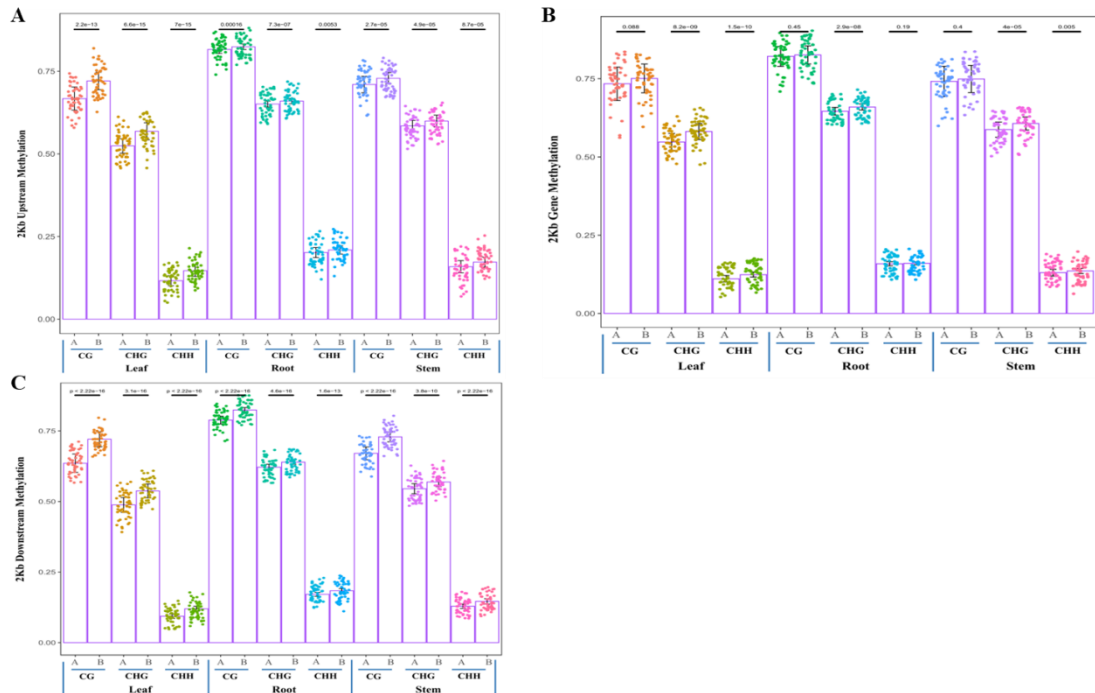


**Figure S11. GO (A,B) and KEGG (C,D) enrichment of A subgenome-biased genes (A,C) and B subgenome biased genes (B,D).**



**Figure S12. Distribution of methylation through the chromosomes from different tissues**

- (A) The distribution of all methylation sites on chromosomes in the leaf.
- (B) The distribution of all methylation sites on chromosomes in the stem.
- (C) The distribution of all methylation sites on chromosomes in the root.

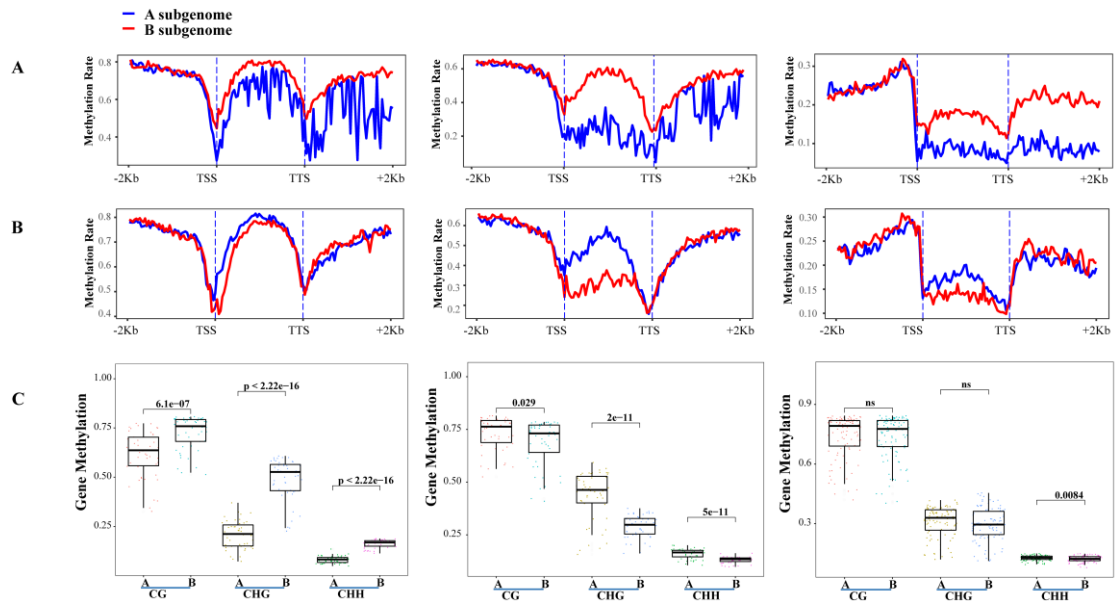


**Figure S13. The comparison of different methylation types of subgenomes A and B in different tissues.** The 2 Kb upstream, gene-body and 2 Kb downstream of A and B subgenomic genes were divided into 50 bins on average. Each point represents the average methylation ratio in a bin.

(A) Comparison of 2 Kb upstream methylation ratio of the genes of A and B subgenomes.

(B) Comparison of gene-body methylation ratio of the genes of A and B subgenomes.

(C) Comparison of 2 Kb downstream methylation ratio of the genes of A and B subgenomes.



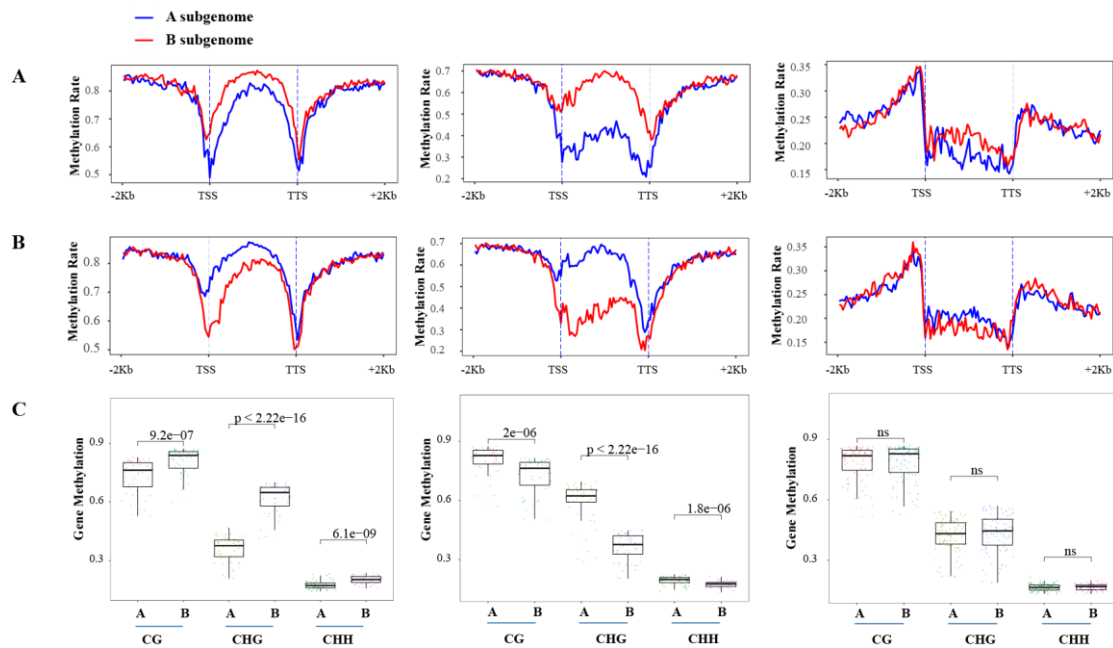
**Figure S14. DNA methylation divergence of the allotetraploid in the leaf.**

(A) The methylation rates of subgenome A-biased homeologous gene pairs (Fold>2) between the two subgenomes in the leaf.

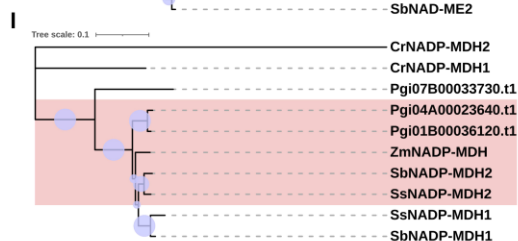
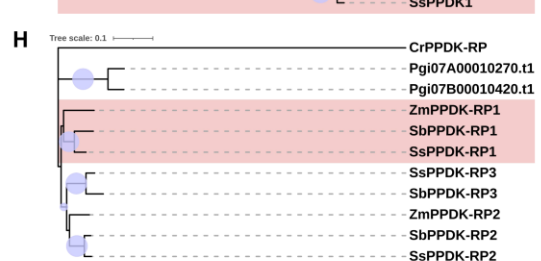
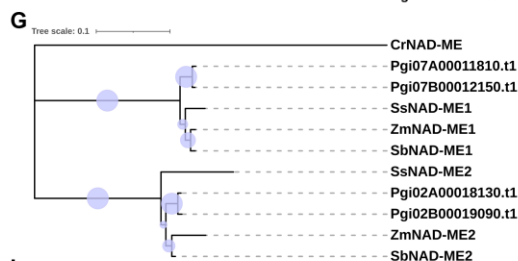
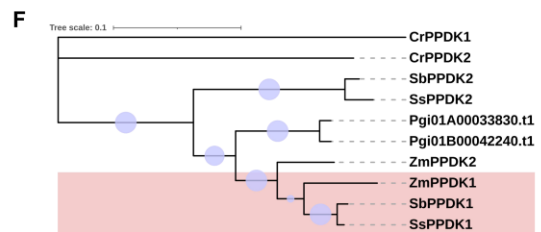
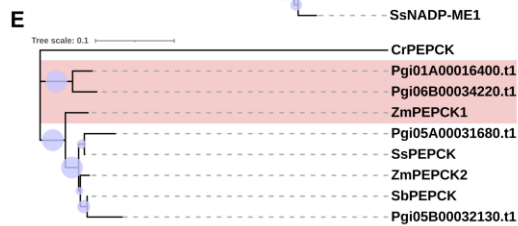
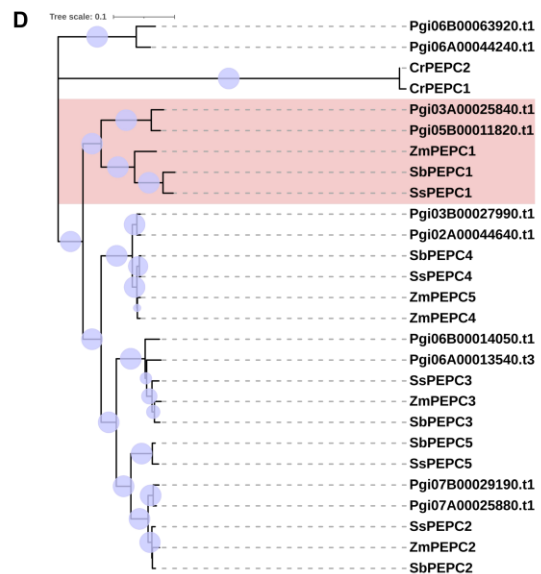
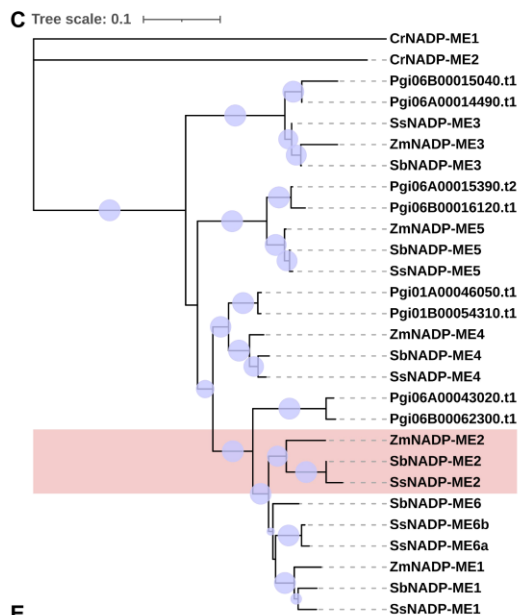
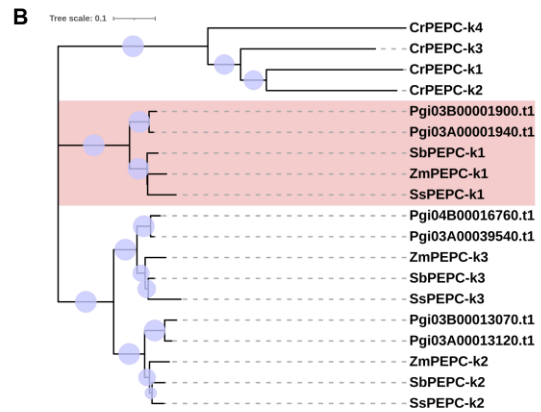
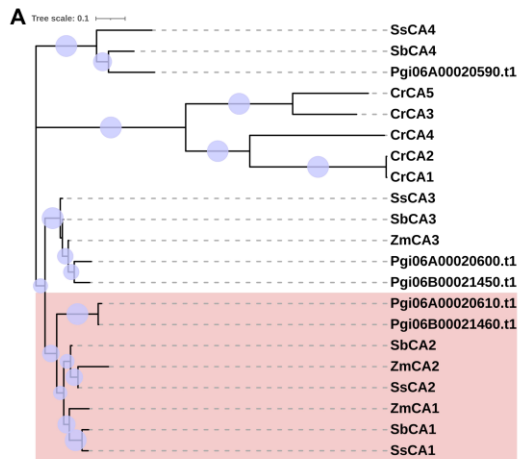
(B) The methylation rates of subgenome B-biased homeologous gene pairs (Fold>2) between the two subgenomes in the leaf.

(C) Comparison of methylation rate in gene-body of A and B subgenomes in the leaf. The left panel shows the methylation rate of subgenome A-biased homeologous gene pairs between the two subgenomes (fold >2); The middle panel shows the methylation rate of subgenome B-biased homeologous gene pairs between the two subgenomes; The right panel shows the methylation rate of inconspicuous differentially expressed gene pairs (with 0-2 times changes) from the two subgenomes.





**Figure S15. DNA methylation divergence of the allotetraploid in the root.**  
 (A) The methylation rates of subgenome A-biased homeologous gene pairs (Fold>2) between the two subgenomes in the root.  
 (B) The methylation rates of subgenome B-biased homeologous gene pairs (Fold>2) between the two subgenomes in the root  
 (C) Comparison of methylation rate in gene-body of A and B subgenomes in the root. The left panel shows the methylation rate of subgenome A-biased homeologous gene pairs between the two subgenomes (fold >2); The middle panel shows the methylation rate of subgenome B-biased homeologous gene pairs between the two subgenomes; The right panel shows the methylation rate of inconspicuous differentially expressed gene pairs (with 0-2 times changes) from the two subgenomes.



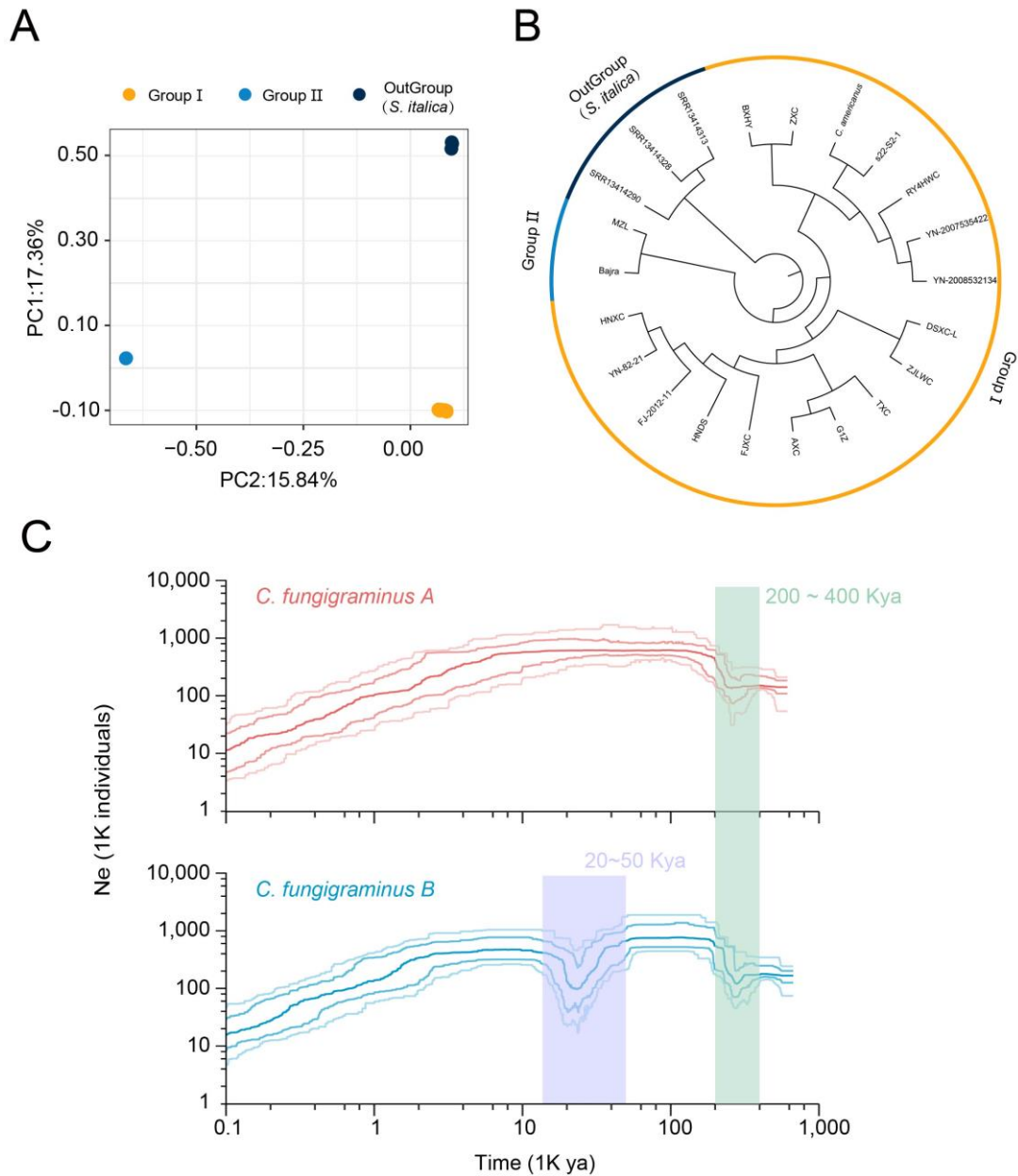
**Figure S16. Phylogenetic analysis of genes encoding C<sub>4</sub> enzymes and their non-C<sub>4</sub> isoforms.** Cf indicates *C. fungigraminus*, Ss indicates *Saccharum spontaneum*, Sb indicates *Sorghum bicolor*, Zm indicates *Zea mays*, Os indicates *Oryza sativa*, and Cr indicates *Chlamydomonas reinhardtii*. Purple circles indicate bootstraps. Genes in the pink clade encode proteins in the C<sub>4</sub> pathway. Tree scale: 0.1.

Phylogenetic analysis of CA **(A)**; PEPC-k **(B)**; NADP-ME **(C)**; PEPC **(D)**; PEPCK **(E)**; PPK (F); NAD-ME **(G)**; PPK-RP **(H)**; and NADP-MDH**(I)** proteins.

	root	stem	leaf
CfCA1_A	1	17	71
CfCA1_B	11	28	196
CfCA3_A	9	13	5
CfCA3_B	8	51	9
CfCA4_A	0	0	0
CfNAD-ME1_A	4	3	3
CfNAD-ME1_B	4	5	6
CfNAD-ME2_A	3	3	2
CfNAD-ME2_B	6	4	1
CfNADP-MDH2_A	0	13	34
CfNADP-MDH2_B	0	8	32
CfNADP-MDH1_B	0	8	34
CfNADP-ME2_A	4	11	20
CfNADP-ME2_B	4	16	34
CfNADP-ME3_A	0	0	0
CfNADP-ME3_B	0	0	0
CfNADP-ME4_A	3	0	0
CfNADP-ME4_B	8	3	0
CfNADP-ME5_A	12	3	2
CfNADP-ME5_B	15	13	20
CfPEPC1_A	0	69	476
CfPEPC1_B	0	137	769
CfPEPC2_A	18	15	7
CfPEPC2_B	67	38	38
CfPEPC3_A	11	9	6
CfPEPC3_B	17	13	23
CfPEPC4_A	66	20	9
CfPEPC4_B	63	23	16
CfPEPC5_A	0	28	207
CfPEPC5_B	0	0	15
CfPEPC-k1_A	12	10	21
CfPEPC-k1_B	14	36	21
CfPEPC-k2_A	86	138	459
CfPEPC-k2_B	39	36	159
CfPEPC-k3_A	26	127	112
CfPEPC-k3_B	50	145	100
CfPPDK1_A	0	26	203
CfPPDK1_B	0	25	252
CfPPDK-RP1_A	2	19	79
CfPPDK-RP1_B	7	68	236
CfPEPCK1_A	0	1	43
CfPEPCK1_B	1	1	8
CfPEPCK2_A	8	1	0
CfPEPCK2_B	5	1	2



**Figure S17. Expression pattern of carbon fixation genes in root, stem and leaf tissue of JUJUNCAO.**



**Figure S18. Population characteristics and evolution of JUJUNCAO.**

**(A)** Principal component analysis with PC1 and PC2.

**(B)** The phylogenetic tree was constructed based on genome-wide SNP, with different colors representing different groups.

**(C)** Historical effective population sizes ( $N_e$ ) for *Cenchrus* population estimated using *C. fungigraminus A* and *B* as the ancestor genome. Nucleotide substitution rate  $\mu = 6.5e-9$  and generation time  $gt = 1$  were used for analysis.

**Table S1. Estimation of the JUJUNCAO genome using flow cytometry**

<b>Sample</b>	<b>Reference (Ref)</b>	<b>Signal of Ref</b>	<b>Signal of Sample</b>	<b>Ratio</b>	<b>Estimated Genome size (Gb)</b>
JUJUNCAO-1	Tomato	24.72	58.76	2.38	2.09
JUJUNCAO-2	Tomato	25.23	59.86	2.37	2.09
JUJUNCAO-3	Tomato	25.25	58.81	2.33	2.05

**Table S2. Statistics of Hi-C mapping of the JJJUNCAO genome**

<b>Statistics of mapping</b>	
Clean Paired-end Reads	815,147,702
Clean Paired-end Reads Rates (%)	100%
Unmapped Paired-end Reads	58,095,120
Unmapped Paired-end Reads Rates	7.12%
Paired-end Reads with singleton	293,148,296
Paired-end Reads with singleton Rates (%)	35.96%
Multi Paired-end Reads	0
Multi Paired-end Reads Rates	0.00%
Unique Paired-end Reads	222,332,443
Unique Paired-end Reads Rates (%)	27.27%
<b>Statistics of valid reads</b>	
Unique Paired-end Reads	222,332,443
Dangling End Paired-end Reads	35,538,486
Dangling End Paired-end Reads Rates (%)	15.98%
Self Circle Paired-end Reads	67,508
Self Circle Paired-end Reads Rates (%)	0.03%
Dumped Paired-end Reads	12,745
Dumped Paired-end Reads Rates (%)	0.01%
Valid interaction Paired-end Reads	174,777,070
Valid interaction Paired-end Reads Rate (%)	78.61%
Lib Valid interaction Paired-end Reads	166,760,242
Lib Valid interaction Paired-end Reads Rate (%)	75.00%
Lib Dup (%)	25.00%

**Table S3. BUSCO analysis of the completeness of the *C. fungigraminus* genome.**

<b>Description</b>	<b>Number</b>	<b>Percentage</b>
<b>Complete BUSCOs (C)</b>	<b>1588</b>	<b>98.4%</b>
<b>single-copy BUSCOs (S)</b>	<b>322</b>	<b>20.0%</b>
<b>duplicated BUSCOs (D)</b>	<b>1266</b>	<b>78.4%</b>
<b>Fragmented BUSCOs (F)</b>	<b>7</b>	<b>0.4%</b>
<b>Missing BUSCOs (M)</b>	<b>17</b>	<b>1.2%</b>
<b>Total BUSCO groups searched</b>	<b>1614</b>	<b>100.0%</b>



**Table S4. Summary of assembly, and annotation statistics for four *Cenchrus* Species**

<b>Species</b>	<b><i>C. fungigraminus</i></b>	<b><i>C. americanus</i></b>	<b><i>C. purpureus</i> cv. <i>Purple</i></b>	<b><i>P. purpureus</i></b>
Ploidy	2n=4x=28	2n=2x=14	2n=4x=28	2n=4x=28
Assembly size (Gb)	1.98	1.58	1.97	2.07
Number of contig	815	208,873	2,059	1956
Contig N50 (bp)	134,074,806	18,180	1,829,308	2,900,000
Longest contig (bp)	162,376,048	282,900	15,071,384	20,190,000
BUSCO assessment	98.40%	-	97.83%	97.80%
Percentage of repeat elements	73.38%	77.2%	66.32%	60.47%
GC content	47.11%	47.90%	46.95%	46.95

**Table S5. BUSCO analysis of the annotation of the *C. fungigraminus* genome.**

<b>Description</b>	<b>Number</b>	<b>Percentage</b>
<b>Complete BUSCOs (C)</b>	<b>1501</b>	<b>93.0%</b>
<b>single-copy BUSCOs (S)</b>	<b>212</b>	<b>13.1%</b>
<b>duplicated BUSCOs (D)</b>	<b>1289</b>	<b>79.9%</b>
<b>Fragmented BUSCOs (F)</b>	<b>10</b>	<b>0.6%</b>
<b>Missing BUSCOs (M)</b>	<b>103</b>	<b>6.4%</b>
<b>Total BUSCO groups searched</b>	<b>1614</b>	<b>100.0%</b>

**Table S6. Repeat contents of JUJUNCAO were compared with other grass species**

	<i>Z.mays</i>	<i>S.italica</i>	<i>P. miliaceum</i>	<i>S.bicolor</i>	<i>C. fungigraminus</i>		
					<i>AB</i>	<i>Sub A</i>	<i>Sub B</i>
Total Repeat Fractions	81.75	48.63	55.08	69.67	68.87	66.39	70.75
Class I :	52.50	18.56	23.35	45.59	35.69	33.14	38.45
Retrotransposon							
LTR							
Retrotransposon							
Ty3/Gypsy	31.98	11.60	19.07	38.78	22.40	19.33	25.37
Ty1/Copia	19.86	5.31	3.26	5.70	11.25	11.92	10.86
Other	0	0.07	0.04	0.18	0.26	0.23	0.3
Non-LTR							
Retrotransposon							
LINE	0.66	1.58	0.98	0.93	1.78	1.66	1.92
SINE	0	0	0	0	0	0	0
Class II :	2.43	5.47	1.99	8.68	4.51	4.25	4.79
DNA transposon							
CACTA	1.58	3.37	1.21	3.56	2.73	2.60	2.89
Mutator	0.32	0.79	0.29	0.68	1.27	1.14	1.40
hAT	0.19	0.53	0.23	0.51	0.22	0.23	0.2
PIF/Harbinger	0.18	0.56	0.06	2.79	0.14	0.13	0.15
Helitron	0.16	0.18	0.20	0.28	0.14	0.14	0.14
TcMar-							
Stowaway	0	0.04	0	0	0.01	0.01	0.01
Other	0	0	0	0.86	0	0	0
Tandem Repeat	0	0	0	2.56	0	0	0
Unknown	26.82	24.60	29.74	4.46	28.67	29.00	27.51

**Table S7. The centromeric repeats identified from JUJUNCAO genome**

<b>Chr</b>	<b>Start</b>	<b>End</b>	<b>Length (bp)</b>	<b>Monomer</b>
Chr01A	18,253,776	19,139,182	885,406	<i>CEN156</i>
Chr01A	75,007,454	95,409,560	20,402,106	<i>CEN137, CEN156</i>
Chr01B	75,121,515	98,234,246	23,112,731	<i>CEN156</i>
Chr02A	79,096,377	86,197,020	7,100,643	<i>CEN137</i>
Chr02B	68,090,932	76,337,386	8,246,454	<i>CEN156</i>
Chr03A	40,718,343	61,191,030	20,472,687	<i>CEN137, CEN156</i>
Chr03B	73,050,604	78,621,099	5,570,495	<i>CEN156</i>
Chr04A	80,088,409	83,465,161	3,376,752	<i>CEN137</i>
Chr04B	32,009,996	35,621,487	3,611,491	<i>CEN156</i>
Chr05A	24,377,613	36,892,929	12,515,316	<i>CEN137, CEN156</i>
Chr05A	45,359,011	46,301,987	942,976	<i>CEN137</i>
Chr05B	52,902,785	63,299,058	10,396,273	<i>CEN148, CEN156</i>
Chr06A	55,670,148	61,016,392	5,346,244	<i>CEN156</i>
Chr06B	79,034,047	84,664,505	5,630,458	<i>CEN156</i>
Chr07A	51,781,816	62,044,859	10,263,043	<i>CEN137, CEN156</i>
Chr07B	76,324,440	87,934,803	11,610,363	<i>CEN156</i>

**Table S8. Depth of the cytosine among three sequence contexts**

<b>Tissue</b>	<b>Depth of all CpG</b>	<b>Depth of all CHG</b>	<b>Depth of all CHH</b>
Leaf	16.9217	18.3554	19.0562
Stem	16.9264	18.4428	19.1824
Root	15.1407	16.2506	17.3339

**Table S9. The comparison and de-repetition of BS-seq**

<b>Tissue</b>	<b>Mapping efficiency (%)</b>	<b>De-repetition retention rate (%)</b>
Leaf	69.60	74.46
Stem	68.70	75.09
Root	63.60	75.63

**Table S10. Methylation information of the three tissue**

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<b>Tissue</b>	<b>methC of all CpG (%)</b>	<b>methC of all CHG (%)</b>	<b>methC of all CHH (%)</b>
Leaf	63	44.3	2.1
Stem	63.5	47.2	2.3
Root	73.3	53	2.4

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**Table S11. Members of the C<sub>4</sub> related gene**

<b>Name</b>	<b>Gene ID</b>	<b>Abbreviation ID</b>
	Pgi06A00020610	CfCA1_A
	Pgi06B00021460	CfCA1_B
<b>Carbonic anhydrase</b>	Pgi06A00020600	CfCA3_A
	Pgi06B00021450	CfCA3_B
	Pgi06A00020590	CfCA4_A
	Pgi07A00011810	CfNAD-ME1_A
<b>NAD-malic enzyme</b>	Pgi07B00012150	CfNAD-ME1_B
	Pgi02A00018130	CfNAD-ME2_A
	Pgi02B00019090	CfNAD-ME2_B
	Pgi04A00023640	CfNADP-MDH2_A
<b>NADP-malate dehydrogenase</b>	Pgi01B00036120	CfNADP-MDH2_B
	Pgi07B00033730	CfNADP-MDH1_B
	Pgi06A00043020	CfNADP-ME2_A
	Pgi06B00062300	CfNADP-ME2_B
	Pgi06A00014490	CfNADP-ME3_A
<b>NADP-malic enzyme</b>	Pgi06B00015040	CfNADP-ME3_B
	Pgi01A00046050	CfNADP-ME4_A
	Pgi01B00054310	CfNADP-ME4_B
	Pgi06A00015390	CfNADP-ME5_A
	Pgi06B00016120	CfNADP-ME5_B
	Pgi03A00025840	CfPEPC1_A
	Pgi05B00011820	CfPEPC1_B
	Pgi07A00025880	CfPEPC2_A
	Pgi07B00029190	CfPEPC2_B
<b>Phosphoenolpyruvate carboxylase</b>	Pgi06A00013540	CfPEPC3_A
	Pgi06B00014050	CfPEPC3_B
	Pgi02A00044640	CfPEPC4_A
	Pgi03B00027990	CfPEPC4_B
	Pgi06A00044240	CfPEPC5_A
	Pgi06B00063920	CfPEPC5_B
	Pgi03A00001940	CfPEPC-k1_A
	Pgi03B00001900	CfPEPC-k1_B
<b>PEPC kinase</b>	Pgi03A00013120	CfPEPC-k2_A
	Pgi03B00013070	CfPEPC-k2_B
	Pgi03A00039540	CfPEPC-k3_A
	Pgi04B00016760	CfPEPC-k3_B
	Pgi01A00033830	CfPPDK1_A
<b>Pyruvate orthophosphate dikinase</b>	Pgi01B00042240	CfPPDK1_B
	Pgi07A00010270	CfPPDK-RP_A
<b>PPDK regulatory protein</b>	Pgi07B00010420	CfPPDK-RP_B
<b>Phosphoenolpyruvate carboxykinase</b>	Pgi01A00016400	CfPEPCK1_A



	Pgi06B00034220	CfPEPCK1_B
	Pgi05A00031680	CfPEPCK2_A
	Pgi05B00032130	CfPEPCK2_B
<b>Aspartate transaminase</b>	Pgi03A00002400	CfAspAT_A
	Pgi03B00002380	CfAspAT_B
<b>Alanine transaminase</b>	Pgi02A00024740	CfAlaAT_A
	Pgi02B00025890	CfAlaAT_B
<b>Probable sodium/metabolite cotransporter 2</b>	Pgi06A00020290	CfBASS2_A
	Pgi06B00021230	CfBASS2_B
<b>2-oxoglutarate/malate transporter 1</b>	Pgi04A00009500	CfOMT1_A
	Pgi01B00009320	CfOMT1_B
<b>Dicarboxylate transporter 2</b>	Pgi04A00029510	CfDiT2_A
	Pgi01B00029590	CfDiT2_B
<b>Triose/phosphate transmembrane antiporter</b>	Pgi01A00026190	CfPPT_A
	Pgi01B00023890	CfPPT_B
<b>Sodium/proton antiporter 1</b>	Pgi07A00029340	CfNHD1_A
	Pgi07B00032820	CfNHD1_B

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**Table S12. Quality control and mapping statistics of resequencing data**

genus names	sample	clean reads	Clean Base (bp)	GC Content (%)	Mapping rate		
					Sub A	Sub B	AB
<i>Pennisetum</i>	YN-2007535422	156,605,216	23,490,782,400	47	72.70%	80.45%	98.01%
<i>Pennisetum</i>	YN-2008532134	150,295,676	22,544,351,400	47	72.53%	80.24%	97.92%
<i>Pennisetum</i>	RY4HWC	219,117,450	32,867,617,500	47	72.18%	79.90%	97.68%
<i>Cenchrus</i>	<i>Cenchrus fungigraminus</i> (JUJUNCAO)	35,610,000	5,341,500,000	47	71.02%	79.05%	97.63%
<i>Pennisetum</i>	G1Z	253,614,512	38,042,176,800	47	70.74%	78.39%	95.66%
<i>Pennisetum</i>	BXHY	146,492,930	21,973,939,500	47	70.88%	78.95%	95.60%
<i>Pennisetum</i>	ZXC	251,433,182	37,714,977,300	46	70.71%	78.80%	95.45%
<i>Pennisetum</i>	DSXC-L	229,195,698	34,379,354,700	47	70.57%	78.83%	95.40%
<i>Pennisetum</i>	ZJLWC	216,187,006	32,428,050,900	47	70.28%	78.67%	95.06%
<i>Pennisetum</i>	FJXC	159,663,548	23,949,532,200	47	69.17%	77.60%	93.78%
<i>Pennisetum</i>	YN-82-21	158,911,116	23,836,667,400	47	69.32%	77.49%	93.74%
<i>Pennisetum</i>	HNXC	248,139,874	37,220,981,100	47	68.99%	77.49%	93.58%
<i>Pennisetum</i>	TXC	258,405,408	38,760,811,200	46	68.96%	77.51%	93.55%
<i>Pennisetum</i>	FJ-2012-11	144,233,242	21,634,986,300	47	69.01%	77.37%	93.53%
<i>Pennisetum</i>	HNDS	205,998,212	30,899,731,800	47	68.48%	76.75%	92.70%
<i>Pennisetum</i>	AXC	225,487,608	33,823,141,200	47	67.87%	76.33%	91.89%
<i>Cenchrus</i>	Bajra	227,346,622	34,101,993,300	48	72.69%	48.43%	74.41%
<i>Pennisetum</i>	s22-S2-1	50,240,552	7,536,082,800	54	62.48%	62.38%	73.48%
<i>Pennisetum</i>	MZL	265,395,788	39,809,368,200	48	71.90%	47.05%	73.35%
<i>Setaria</i>	SRR13414328	76,141,500	11,421,225,000	46	9.16%	8.72%	18.25%
<i>Setaria</i>	SRR13414313	81,423,842	12,213,576,300	47	14.14%	14.67%	16.95%
<i>Setaria</i>	SRR13414290	81,800,876	12,270,131,400	45	13.49%	14.16%	15.83%

**Table S13. Sequence of probes for the three centromeric satellite repeats**

<b>Repeat</b>	<b>Oligomer sequence</b>
<b><i>CEN137</i></b>	ATTCGGGGTACCAAAGTTGTGAAGCATCC
<b><i>CEN148</i></b>	ACCTTCGGCATGTAAATAACTTGTGTTTCG
<b><i>CEN156</i></b>	TTGCGAAAATGGTTTCGCAACAAAACATCC