

A K-mer-based GWAS approach empowering gene mining in polyploids

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Supplementary Notes

Supplementary Note 1. Challenges in genotyping for polyploids and motivation for developing KMERIA Method.

1. Challenges in genotyping for autopolyploids

Genome-wide association studies (GWAS) play a critical role in molecular breeding by identifying genetic variants associated with important agronomic traits, enabling marker-assisted selection (MAS) and accelerating the development of improved crop varieties with desirable characteristics¹⁻⁵. However, GWAS has yet to be effectively applied to autopolyploids due to a major factor: genotyping. Two main challenges contributing to this issue are the difficulty of aligning reads in regions with high sequence similarity among homologous chromosomes and the inefficiency of existing genotyping models.

Alignment challenge. Autopolyploid genomes typically exhibit high sequence similarity among homologous chromosomes, which leads to frequent multi-mapping of sequencing reads when aligning resequencing data. This ambiguity often results in inaccurate alignments and biases in read distribution, especially in repetitive regions. Additionally, the reliance on single reference genomes exacerbates these issues, as non-reference alleles may be underrepresented or misaligned, introducing reference bias. The reliance on single reference genomes exacerbates these issues, as non-reference alleles may be underrepresented or misaligned, introducing reference bias (**Figure 1a**).

Genotyping challenge. The Genome Analysis Toolkit (GATK) is one of the most widely used tools for genotype calling, originally designed for diploid genomes. However, it also includes specific parameters for polyploid genotyping, enabling its adaptation to polyploid datasets. GATK's `--ploidy` parameter in HaplotypeCaller functionality involves calculating the posterior probability of each genotype by analyzing the pileup of sequencing reads at a given locus and considering the expected allele dosage distribution within the sample. This process leverages Bayesian inference to estimate prior probabilities for all possible genotypes^{6,7}. This method struggles to handle the exponential increase in possible genotype combinations. For each potential genotype $g' \in \mathcal{G}$, GATK must compute the likelihood $Pr(D|g)$. As $|\mathcal{G}|$ grows exponentially with P and A , the computational burden becomes unsustainable for high-ploidy species (**Figure 1b-c, Formula 1 and Formula 2**). Additionally, the allele dosage in polyploid genomes introduces substantial complexity. Unlike diploids, where allele ratios are straightforward, polyploids can exhibit various allele ratios depending on ploidy levels and genotype combinations. Insufficient sequencing depth can obscure these ratios, resulting in misclassification of genotypes or failure to detect low-frequency alleles.

Formula 1: This formula calculates the total number of possible genotype combinations $|\mathcal{G}|$

at a given locus can be represented by:

$$|G| = \binom{P + A - 1}{P} = \frac{(P + A - 1)!}{P! \cdot (A - 1)!}$$

Where P represent genome ploidy, A denote the allele number, and $|G|$ is the set of all possible genotype combinations.

Formula 2: GATK's core genotype calling algorithm is based on a Bayesian model:

$$Pr(g|D) = \frac{Pr(D|g) \cdot Pr(g)}{\sum_{g' \in G} Pr(D|g') \cdot Pr(g')}$$

Where D represent the read base pileup at the reference base, g denote given genotype, and $Pr(g)$ is the prior probability of seeing genotype.

A simple example illustrates the explosion of genotype combinations with increasing genome ploidy, As ploidy increases, the number of potential genotypes grows exponentially, complicating computational analyses and genotyping accuracy.

Tetraploid ($P = 4, A = 2$)

$$|G| = \binom{4 + 2 - 1}{4} = \binom{5}{4} = 5$$

For hexaploid $P = 6$ with $A = 3$ alleles:

$$|G| = \binom{6 + 3 - 1}{6} = \binom{8}{6} = 28$$

2. Developing KMERIA Method for autopolyploids.

K-mer-based GWAS offers several significant advantages over traditional approaches, particularly in polyploid genomes. By capturing a wide range of genetic variations, including presence-absence variations (PAVs), structural variations (SVs), and copy number variations (CNVs), which provides comprehensive and unbiased coverage of genetic diversity. Unlike conventional genotyping methods, it does not rely on a high-quality reference genome, thereby reducing reference bias and enabling analyses in diverse genomic contexts. Importantly, sequence variations that influence phenotypes often lead to changes in k-mer abundance, which can serve as an effective proxy for genotypes. This feature is particularly valuable for estimating allele dosages in polyploid genomes, where traditional genotyping

faces significant challenges in polyploids. Additionally, constructing k-mer-based population genotypes simplifies the genotyping process, reducing computational complexity and resource demands. Historically, population genetics for polyploids has been difficult, due to both the technical difficulty of accurately genotyping loci as well as the assumptions of diploidy in commonly used analyses. k-mer-based methods are advantageous for this purpose, as they do not rely on accurately assessing genotype likelihood at each locus. Instead, the count data that they produce implicitly takes dosage into account, as different genotypic combinations are represented as different k-mer counts. Together, these advantages make k-mer-based GWAS a powerful and efficient tool for uncovering genetic associations in complex and polyploid genomes. Therefore, we developed KMERIA, a tool specifically designed for k-mer-based genotyping and GWAS in autopolyploid.

We further compared the genotyping efficiency of the KMERIA strategy with the GATK (-ploidy) pipeline in polyploid genomes using resequencing data from 100 tetraploid potato dataset⁸. The analysis was conducted on a 28-core Linux server with 196 GB RAM. Detailed parameters for the workflows are provided below. The comparison revealed that the GATK (-ploidy) pipeline required approximately 35,413 h Real time to perform population-level genotyping for 100 samples. In contrast, KMERIA method completed the same task with a Real time of only 38.9~82.3 hour, achieving at least 430-fold faster processing compared to the GATK (-ploidy) pipeline (**Table S1**). In terms of memory usage, the peak memory consumption during the execution of both strategies was comparable. These results highlight the exceptional efficiency of the KMERIA genotyping approach, significantly reducing computational costs while maintaining robust performance.

Command lines of GATK(-ploidy) and KMERIA used for genotyping test.

(1) GATK best practice pipeline (-ploidy) :

```
$ bwa index $REF
```

```
$ samtools faidx $REF
```

```
$ java -jar ${picardpath}picard.jar CreateSequenceDictionary R=$REF O=ref.dict
```

```
$ bwa mem -t 8 ${REF} ${FQ1} ${FQ2} | samtools view -@8 -b - > ./${sample}.bam
```

```
$ java -jar ${picardpath}picard.jar AddOrReplaceReadGroups \
```

```
    I=${sample}.bam \
```

```
    O=${sample}.AddOrReplaceReadGroups.bam \
```

```
    RGID=${sample} \
```

```

RGLB=lib1 \

RGPL=illumina \

RGPU=unit1 \

RGSM=${sample}

$ java -jar ${picardpath}picard.jar MarkDuplicates \

    I=${sample}.sorted.AddOrReplaceReadGroups.bam \

    O=${sample}.MarkDuplicates.bam \

    M=${sample}.MarkDuplicates.matrices

$ java -jar ${picardpath}picard.jar SortSam \

    I=${sample}.MarkDuplicates.bam \

    O=${sample}.SortSam.bam \

    SO=coordinate

$ java -jar ${picardpath}picard.jar SetNmMdAndUqTags \

    I=${sample}.SortSam.bam \

    O=${sample}.tagged.bam \

    R=${REF}


$ gatk --java-options "-Xmx40G -XX:ParallelGCThreads=8" \

    HaplotypeCaller \

    -R ${REF} \

    -I ${sample}.tagged.bam \

    -O ${sample}.g.vcf.gz \

    --sample-ploidy 4 \

    -OVI \

    -contamination 0 \

    --emit-ref-confidence GVCF

```

#Make Joint vcf

```
$gatk --java-options "-Xmx40G -XX:ParallelGCThreads=8" CombineGVCFs \
```

```
(echo $samples) \
```

```
-O $out.samples.vcf \
```

```
-R $REF
```

(2) KMERIA pipeline:

```
$ kmeria count -k 31 -t 8 ${sample}_1.fastq.gz ${sample}_2.fastq.gz -o ${sample}.kc
```

```
$ kmeria dump -c 5 -C 1000 ${sample}.kc -o ${sample}.kc.tsv
```

```
$ pigz -p 8 ${sample}.kmc.tsv
```

```
$ kmeria kctm2 -i ${sample}.kc.list -o kmer_abundance_matrix
```

```
$ kmeria flt -i kmer_abundance_matrix -t 8 -c 500 -s 0.8 -p 4 -d \
```

```
sample_depth_file -o kmer_genotype_matrix
```

Reference

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Supplementary Tables

Table S1. Statistics of alignment accuracy in polyploids using *Saccharum hybrid* genome

Items	count	Percentage (%)
Total reads	20,000,240	100
Low-quality reads (MAPQ<1)	11,384,068	56.92
High-quality reads (MAPQ≥1)	8,616,172	43.08
Correctly aligned reads	4,264,454	49.49
Incorrectly aligned reads	4,351,718	50.51
MAPQ Distribution		
0	11,384,068	56.92
1-9	203,524	1.02
10-29	1,642,525	8.21
30-59	3,679,313	18.4
≥60	3,090,810	15.45

Table S2. Summary of Synthetic Autopolyploid populations and Real Datasets with Variable Ploidy Levels

Data types	Datasets	Ploidy	Population size	Heritability (h^2)	QTL number
Synthetic	population1	4	200	50%	10
	population2	4	200	50%	100
	population3	4	200	80%	10
	population4	4	200	80%	100
	population5	4	400	50%	10
	population6	4	600	50%	10
	population7	4	800	80%	10
Real	Sweet potato*	6	294	Zhang, X. <i>et al.</i> Refining polyploid breeding in sweet potato through allele dosage enhancement. <i>Nat. Plants</i> (2024) doi:10.1038/s41477-024-01873-y.	
	Potato*	4	137	Li, H. <i>et al.</i> Genomic basis of divergence of modern cultivated potatoes. Preprint at https://doi.org/10.21203/rs.3.rs-3968149/v1 (2024).	
	Alfalfa*	4	176	Zhang, F. <i>et al.</i> Evolutionary genomics of climatic adaptation and resilience to climate change in alfalfa. <i>Mol. Plant</i> 17 , 867–883 (2024).	
	Rice*	2	400	Wei, X. <i>et al.</i> A quantitative genomics map of rice provides genetic insights and guides breeding. <i>Nat. Genet.</i> 53 , 243–253 (2021).	

Table S3. Computing efficiency of GATK and KMERIA in polyploid genotyping

Genotyping strategy	Species	Ploidy	Population size	Sequencing depth	Elapsed time (hour)	Peak RAM (GB)	Limitations
GATK (-ploidy)	Potato	4	100	56×	~35,413	38.5	Ploidy <= 6
KMERIA	Potato	4	100	56×	38.9 - 82.3	60.5	<32-mer
GATK (-ploidy)			Real Time (Min)	CPU time (Min)	Peak RAM (GB)		CPU Number
Map to Reference (BWA-MEM)				1,082	8,454	38.5	8
Picard AddOrReplaceReadGroups				187	308	1.03	8
Picard MarkDuplications				172	190	16.5	8
Picard SortSam				106	111	0.57	8
GATK HaplotypeCaller (-ploidy)				19,931	36,466	1.3	8
Total time in 1 sample (Min)					21248		
Total time in 100 samples (Min)					~2124800		
KMERIA			Real Time (min)	CPU time (min)	Peak RAM (GB)		CPU Number
k-mer abundance (KMC)				15	53	60.5	8
k-mer abundance (KMERIA)				41	170	64.2	8
k-mer genotype matrix				836	2360	1	8
Total time in 100 samples (Min)					2336 - 4936		
Genotyping strategy			Time Cost (hour)	Memory Usage (Gb)			
GATK (-ploidy)				35,413	38.5		
KMERIA				39	60.5		

Table S4. Information of the 295 *S. spontaneum* samples used in this study

#ID	Sample name	Province, Country	Total pairs	Total reads	Ploidy	Total bases (bp)	Depth (haploid genome size)
ss_001	yunnan82-17	Yunnan, China	960005490	995544313	10	1.49E+11	186.6645587
ss_002	yunnan82-44	Yunnan, China	960310322	998461975	10	1.50E+11	187.2116203
ss_003	yunnan83-202	Yunnan, China	960140452	995984657	9	1.49E+11	186.7471232
ss_004	yunnan84-258	Yunnan, China	960035792	996291876	10	1.49E+11	186.8047268
ss_005	yunnan82-33	Yunnan, China	960280504	997662757	10	1.50E+11	187.0617669
ss_006	fujian88- I -4	Fujian, China	960192850	996767590	10	1.50E+11	186.8939231
ss_007	fujian89- I -4	Fujian, China	960060344	996542655	10	1.49E+11	186.8517478
ss_008	fujian88- I -7	Fujian, China	960040416	997185022	10	1.50E+11	186.9721916
ss_010	sichuan79- II -16	Sichuan, China	960095544	997610793	9	1.50E+11	187.0520237
ss_011	sichuan88-22	Sichuan, China	960277970	998116066	10	1.50E+11	187.1467624
ss_012	sichuan88-8	Sichuan, China	960010166	998029272	10	1.50E+11	187.1304885
ss_013	sichuan92-50	Sichuan, China	960265746	998532988	9	1.50E+11	187.2249353
ss_014	yunnan76- II -32	Yunnan, China	960165858	996011383	9	1.49E+11	186.7521343
ss_015	yunnan75- II -17	Yunnan, China	960006972	997059178	9	1.50E+11	186.9485959
ss_016	yunnan75- II -43	Yunnan, China	960001340	996580560	10	1.49E+11	186.858855
ss_017	yunnan82-25	Yunnan, China	960021014	998929119	8	1.50E+11	187.2992098
ss_018	yunnan82-26	Yunnan, China	960008678	999695920	8	1.50E+11	187.442985
ss_019	yunnan82-48	Yunnan, China	960268308	997444412	10	1.50E+11	187.0208273
ss_020	yunnan82-50	Yunnan, China	960089676	997036083	9	1.50E+11	186.9442656
ss_021	yunnan82-58	Yunnan, China	960216928	997259853	9	1.50E+11	186.9862224

ss_022	yunnan82-59	Yunnan, China	960233828	997373279	9	1.50E+11	187.0074898
ss_023	yunnan83-173	Yunnan, China	960265858	996588367	9	1.49E+11	186.8603188
ss_024	yunnan83-190	Yunnan, China	960224758	996462862	9	1.49E+11	186.8367866
ss_025	yunnan83-196	Yunnan, China	960260376	996660221	9	1.49E+11	186.8737914
ss_026	yunnan83-205	Yunnan, China	960109894	997292948	9	1.50E+11	186.9924278
ss_027	yunnan83-206	Yunnan, China	945642296	981025398	9	1.47E+11	183.9422621
ss_028	yunnan83-225	Yunnan, China	960152764	996179207	9	1.49E+11	186.7836013
ss_029	yunnan83-240	Yunnan, China	960248628	997177206	9	1.50E+11	186.9707261
ss_030	guangdong-6	Guangdong, China	938436448	973725587	9	1.46E+11	182.5735476
ss_031	guangdong-12	Guangdong, China	960116834	996238795	10	1.49E+11	186.7947741
ss_032	guangdong-17	Guangdong, China	960040744	996571955	10	1.49E+11	186.8572416
ss_033	guangdong-60	Guangdong, China	960165410	996987451	10	1.50E+11	186.9351471
ss_034	guizhou78- II -012	Guizhou, China	960202236	996760513	9	1.50E+11	186.8925962
ss_035	jiangxi79-17	Jiangxi, China	960284104	997363783	10	1.50E+11	187.0057093
ss_036	jiangxi79-27	Jiangxi, China	960312246	996674993	9	1.50E+11	186.8765612
ss_037	sichuan74- I -14	Sichuan, China	960152370	997047400	10	1.50E+11	186.9463875
ss_038	sichuan76- II -16	Sichuan, China	960134830	997170150	10	1.50E+11	186.9694031
ss_040	sichuan79- I -19	Sichuan, China	960113904	998295249	9	1.50E+11	187.1803592
ss_041	sichuan79- I -27	Sichuan, China	960114558	997292731	10	1.50E+11	186.9923871
ss_042	sichuan79- I -4	Sichuan, China	960254246	997913493	9	1.50E+11	187.1087799
ss_043	sichuan88-4	Sichuan, China	960098368	997056057	9	1.50E+11	186.9480107
ss_044	sichuan92-12	Sichuan, China	960133024	997293024	10	1.50E+11	186.992442
ss_045	sichuan92-14	Sichuan, China	960074492	996645295	9	1.49E+11	186.8709928

ss_046	sichuan92-16	Sichuan, China	960056160	996713350	10	1.50E+11	186.8837531
ss_047	sichuan92-38	Sichuan, China	960021342	995608588	9	1.49E+11	186.6766103
ss_048	yunnan76- I -024	Yunnan, China	960034726	995912910	9	1.49E+11	186.7336706
ss_049	yunnan75- II -35	Yunnan, China	960035446	996849482	9	1.50E+11	186.9092779
ss_050	yunnan76-III-20	Yunnan, China	960107868	996939861	10	1.50E+11	186.9262239
ss_051	yunnan82-103	Yunnan, China	960148910	996941018	9	1.50E+11	186.9264409
ss_052	yunnan82-12	Yunnan, China	960269916	996804548	9	1.50E+11	186.9008528
ss_053	yunnan82-20	Yunnan, China	960104150	997337441	9	1.50E+11	187.0007702
ss_054	yunnan82-34	Yunnan, China	960019778	996535638	9	1.49E+11	186.8504321
ss_055	yunnan83-189	Yunnan, China	960009328	996896294	9	1.50E+11	186.9180551
ss_056	yunnan83-207	Yunnan, China	960083086	997109244	9	1.50E+11	186.9579833
ss_057	yunnan83-213	Yunnan, China	908263298	943277391	9	1.41E+11	176.8645108
ss_058	yunnan83-231	Yunnan, China	871008732	904962226	9	1.36E+11	169.6804174
ss_059	yunnan83-235	Yunnan, China	895237314	929663590	9	1.39E+11	174.3119231
ss_060	yunnan84-247	Yunnan, China	960092410	997092688	9	1.50E+11	186.954879
ss_061	yunnan84-259	Yunnan, China	898454468	932550548	9	1.40E+11	174.8532278
ss_062	yunnan84-264	Yunnan, China	832973660	864907918	9	1.30E+11	162.1702346
ss_063	yunnanheqing	Yunnan, China	960184612	995057827	9	1.49E+11	186.5733426
ss_064	yunnankaiyuan	Yunnan, China	899701484	933749979	10	1.40E+11	175.0781211
ss_065	fujian88- I -13	Fujian, China	895980464	932397446	10	1.40E+11	174.8245211
ss_066	fujian88- I -12	Fujian, China	853069838	888408068	10	1.33E+11	166.5765128
ss_067	fujian89- I -7	Fujian, China	960148248	998946145	11	1.50E+11	187.3024022
ss_068	guangdong-8	Guangdong, China	960066760	996975189	9	1.50E+11	186.9328479

ss_069	guangdong-67	Guangdong, China	946966788	983066987	9	1.47E+11	184.3250601
ss_070	sichuan79- II -10	Sichuan, China	960198594	995977774	10	1.49E+11	186.7458326
ss_071	yunnan82-132	Yunnan, China	960282976	996987293	10	1.50E+11	186.9351174
ss_072	yunnan82-136	Yunnan, China	960078212	997678860	9	1.50E+11	187.0647863
ss_073	yunnan83-193	Yunnan, China	960250086	997741230	9	1.50E+11	187.0764806
ss_074	fujian92- I -12	Fujian, China	960303768	998489887	10	1.50E+11	187.2168538
ss_075	guangdong-45	Guangdong, China	960180208	999396568	9	1.50E+11	187.3868565
ss_076	guangdong-50	Guangdong, China	960195766	997027711	9	1.50E+11	186.9426958
ss_077	guangdong-74	Guangdong, China	960145784	996860483	10	1.50E+11	186.9113406
ss_078	yunnan75- I -6	Yunnan, China	960079392	996443133	9	1.49E+11	186.8330874
ss_079	yunnan75- II -8	Yunnan, China	960124134	996839161	9	1.50E+11	186.9073427
ss_080	yunnan76- I -012	Yunnan, China	960279530	997113078	10	1.50E+11	186.9587021
ss_081	yunnan82-84	Yunnan, China	960071392	997039913	9	1.50E+11	186.9449837
ss_082	yunnan83-163	Yunnan, China	960285630	998516705	9	1.50E+11	187.2218822
ss_083	yunnan83-201	Yunnan, China	960186952	998003920	9	1.50E+11	187.125735
ss_084	yunnan83-208	Yunnan, China	960313122	996839215	9	1.50E+11	186.9073528
ss_085	yunnan84-248	Yunnan, China	960269216	997052507	9	1.50E+11	186.9473451
ss_086	yunnan84-251	Yunnan, China	960134992	996347608	10	1.49E+11	186.8151765
ss_087	yunnan84-256	Yunnan, China	960095118	996472211	9	1.49E+11	186.8385396
ss_088	hainan92-14	Hainan, China	960240962	996796050	10	1.50E+11	186.8992594
ss_089	guangdong-58	Guangdong, China	960012350	997253070	9	1.50E+11	186.9849506
ss_090	yunnan83-200	Yunnan, China	960242740	997606887	9	1.50E+11	187.0512913
ss_091	yunnan87-40	Yunnan, China	960271042	997182489	9	1.50E+11	186.9717167

ss_092	guangdong-3	Guangdong, China	960144404	997244552	9	1.50E+11	186.9833535
ss_093	guizhou78- II -09	Guizhou, China	960237996	996688812	9	1.50E+11	186.8791523
ss_094	yunnan75- II -28	Yunnan, China	960060650	996623954	9	1.49E+11	186.8669914
ss_095	yunnan76-III-13	Yunnan, China	960035072	996317799	9	1.49E+11	186.8095873
ss_096	yunnan82-145	Yunnan, China	960305194	996525990	9	1.49E+11	186.8486231
ss_097	yunnan87-26	Yunnan, China	960076498	997554194	9	1.50E+11	187.0414114
ss_098	yunnan83-159	Yunnan, China	960258656	997302779	9	1.50E+11	186.9942711
ss_099	guangdong-2	Guangdong, China	960198968	997545481	10	1.50E+11	187.0397777
ss_100	yunnan83-157	Yunnan, China	960285936	998674971	10	1.50E+11	187.2515571
ss_101	yunnan83-166	Yunnan, China	960226394	997882324	9	1.50E+11	187.1029358
ss_102	fujian88- I -3	Fujian, China	893084686	927545584	10	1.39E+11	173.914797
ss_103	fujian79- II -4	Fujian, China	960186348	999373358	10	1.50E+11	187.3825046
ss_104	fujian92- I -11	Fujian, China	960094052	997206062	10	1.50E+11	186.9761366
ss_105	fujian92- I -19	Fujian, China	960300920	999273360	10	1.50E+11	187.363755
ss_106	sichuan79- I -16	Sichuan, China	960033566	997692002	10	1.50E+11	187.0672504
ss_107	sichuan79- I -21	Sichuan, China	960001580	997041518	10	1.50E+11	186.9452846
ss_108	sichuan79- II -6	Sichuan, China	960103444	996568398	10	1.49E+11	186.8565746
ss_109	sichuan88-18	Sichuan, China	960234766	997122387	10	1.50E+11	186.9604476
ss_110	sichuan88-34	Sichuan, China	960049354	997594776	10	1.50E+11	187.0490205
ss_111	sichuan88-48	Sichuan, China	960088860	996588784	10	1.49E+11	186.860397
ss_112	sichuan92-17	Sichuan, China	960277256	997666309	9	1.50E+11	187.0624329
ss_113	sichuan92-28	Sichuan, China	960018858	997695915	9	1.50E+11	187.0679841
ss_114	sichuan92-31	Sichuan, China	960065508	997171651	10	1.50E+11	186.9696846

ss_115	sichuan92-46	Sichuan, China	960177532	997273683	9	1.50E+11	186.9888156
ss_116	sichuan92-5	Sichuan, China	960177454	996634574	10	1.49E+11	186.8689826
ss_117	sichuanfushun-5	Sichuan, China	960226824	997743736	10	1.50E+11	187.0769505
ss_118	sichuanjianyang-8	Sichuan, China	960187438	997714583	10	1.50E+11	187.0714843
ss_119	sichuanneijiang-36	Sichuan, China	960027088	997340217	9	1.50E+11	187.0012907
ss_120	sichuanneijiang-4	Sichuan, China	960198748	996012582	10	1.49E+11	186.7523591
ss_121	sichuanneijiangbadi	Sichuan, China	959466264	995277943	9	1.49E+11	186.6146143
ss_122	sichuanzizhong-3	Sichuan, China	960076314	997232343	9	1.50E+11	186.9810643
ss_123	sichuanzizhong-9	Sichuan, China	960180506	996089412	9	1.49E+11	186.7667648
ss_124	yunnan75- I -10	Yunnan, China	960197176	997120154	10	1.50E+11	186.9600289
ss_125	yunnan75- II -2	Yunnan, China	960082254	998051299	10	1.50E+11	187.1346186
ss_126	yunnan82-24	Yunnan, China	1145048022	1189675597	10	1.78E+11	223.0641744
ss_127	yunnan82-7	Yunnan, China	960164318	996073334	9	1.49E+11	186.7637501
ss_128	yunnan82-70	Yunnan, China	960134600	996166492	9	1.49E+11	186.7812173
ss_129	fujianhuian	Fujian, China	960227582	997907686	10	1.50E+11	187.1076911
ss_130	sichuan79- I -20	Sichuan, China	960304108	996404398	10	1.49E+11	186.8258246
ss_131	sichuan92-18	Sichuan, China	960075200	996872103	10	1.50E+11	186.9135193
ss_132	sichuan92-30	Sichuan, China	960091340	997102422	10	1.50E+11	186.9567041
ss_133	sichuan92-34	Sichuan, China	960191088	998077147	10	1.50E+11	187.1394651
ss_134	sichuanneijiang-1	Sichuan, China	960244960	995982689	9	1.49E+11	186.7467542
ss_135	sichuanneijiang-44	Sichuan, China	960156670	994316101	9	1.49E+11	186.4342689
ss_136	guangxige-1	Guangxi, China	960046518	996274451	10	1.49E+11	186.8014596
ss_137	fujian79- II -18	Fujian, China	960306036	998469480	10	1.50E+11	187.2130275

ss_138	guangdong-55	Guangdong, China	960078182	996748478	9	1.50E+11	186.8903396
ss_139	guangdong-71	Guangdong, China	960303338	996508780	10	1.49E+11	186.8453963
ss_140	guangdong-75	Guangdong, China	960153734	995674914	10	1.49E+11	186.6890464
ss_141	guizhou78- II -04	Guizhou, China	960267754	996591011	10	1.49E+11	186.8608146
ss_142	hainan92-41	Hainan, China	960091272	997322813	10	1.50E+11	186.9980274
ss_143	sichuan79- I -6	Sichuan, China	960024248	996543144	10	1.49E+11	186.8518395
ss_144	sichuan92-49	Sichuan, China	960185862	996465586	10	1.49E+11	186.8372974
ss_145	yunnan75- I -21	Yunnan, China	960194134	995678257	9	1.49E+11	186.6896732
ss_146	yunnan75- I -4	Yunnan, China	942512476	978023000	9	1.47E+11	183.3793125
ss_147	yunnan75- II -27	Yunnan, China	960137596	997302711	10	1.50E+11	186.9942583
ss_148	yunnan82-134	Yunnan, China	960120496	998526022	10	1.50E+11	187.2236291
ss_149	yunnan82-77	Yunnan, China	960213284	996968256	10	1.50E+11	186.931548
ss_150	yunnan82-79	Yunnan, China	934750972	970302623	10	1.46E+11	181.9317418
ss_151	yunnan83-167	Yunnan, China	888194178	921695407	10	1.38E+11	172.8178888
ss_152	yunnan83-169	Yunnan, China	897452716	931240470	9	1.40E+11	174.6075881
ss_153	yunnan83-171	Yunnan, China	960291120	996223395	10	1.49E+11	186.7918866
ss_154	yunnan83-185	Yunnan, China	960216390	996155813	10	1.49E+11	186.7792149
ss_155	yunnan-4	Yunnan, China	960061876	996019124	10	1.49E+11	186.7535858
ss_156	yunnanzhedianbaihuacao	Yunnan, China	960144192	996004432	10	1.49E+11	186.750831
ss_157	fujian87- I -10	Fujian, China	960282010	998486868	10	1.50E+11	187.2162878
ss_158	fujian87- I -11	Fujian, China	960053118	997793250	10	1.50E+11	187.0862344
ss_159	fujian89- I -21	Fujian, China	955010804	992970371	10	1.49E+11	186.1819446
ss_160	guangdong-46	Guangdong, China	960094530	995441995	10	1.49E+11	186.6453741

ss_161	sichuan79- I -31	Sichuan, China	960181608	995790231	9	1.49E+11	186.7106683
ss_162	sichuan92-33	Sichuan, China	960185676	996582947	10	1.49E+11	186.8593026
ss_163	sichuan92-42	Sichuan, China	960032362	997059795	10	1.50E+11	186.9487116
ss_164	sichuan92-7	Sichuan, China	949303174	985598810	10	1.48E+11	184.7997769
ss_165	India-1	India	946227108	981620484	10	1.47E+11	184.0538408
ss_166	hainan92-23	Hainan, China	960094270	995972877	10	1.49E+11	186.7449144
ss_167	hainan92-72	Hainan, China	960263408	996430553	10	1.49E+11	186.8307287
ss_168	guangdong-21	Guangdong, China	960088068	996596930	10	1.49E+11	186.8619244
ss_169	guangdong-48	Guangdong, China	960172770	997514339	10	1.50E+11	187.0339386
ss_170	guangdong-78	Guangdong, China	960200882	996901931	9	1.50E+11	186.9191121
ss_171	hainan92-2	Hainan, China	960082026	996121421	10	1.49E+11	186.7727664
ss_172	hainanhaikou	Hainan, China	960078748	998053005	9	1.50E+11	187.1349384
ss_173	yunnan82-120	Yunnan, China	960151938	996692972	10	1.50E+11	186.8799323
ss_174	yunnan82-83	Yunnan, China	960204768	996431537	9	1.49E+11	186.8309132
ss_175	yunnan83-160	Yunnan, China	960002836	999171363	10	1.50E+11	187.3446306
ss_176	yunnan83-181	Yunnan, China	960045668	996643031	7	1.49E+11	186.8705683
ss_177	yunnan84-249	Yunnan, China	960273126	997101008	10	1.50E+11	186.956439
ss_178	guangdong-40	Guangdong, China	960030384	996457097	10	1.49E+11	186.8357057
ss_179	guangdong-53	Guangdong, China	960020036	997037963	10	1.50E+11	186.9446181
ss_180	hainan92-46	Hainan, China	960173380	996672255	10	1.50E+11	186.8760478
ss_181	sichuan79- I -8	Sichuan, China	960099616	997330416	10	1.50E+11	186.999453
ss_182	sichuan79- II -11	Sichuan, China	960113706	998727260	10	1.50E+11	187.2613613
ss_183	yunnan75- II -12	Yunnan, China	960060658	998973623	10	1.50E+11	187.3075543

ss_184	yunnan75-II-7	Yunnan, China	960141988	996748516	10	1.50E+11	186.8903468
ss_185	yunnan76-III-39	Yunnan, China	960250134	995396566	10	1.49E+11	186.6368561
ss_186	yunnan82-86	Yunnan, China	960296776	997128710	10	1.50E+11	186.9616331
ss_187	yunnan83-184	Yunnan, China	960160608	996678437	10	1.50E+11	186.8772069
ss_188	hainan92-18	Hainan, China	960027502	996143635	10	1.49E+11	186.7769316
ss_189	hainan92-61	Hainan, China	960177316	996862419	10	1.50E+11	186.9117036
ss_190	hainan92-16	Hainan, China	960226578	997413604	10	1.50E+11	187.0150508
ss_191	fujianxianyou-1	Fujian, China	960224346	997526620	10	1.50E+11	187.0362413
ss_192	guangdong-23	Guangdong, China	960069674	996765962	10	1.50E+11	186.8936179
ss_193	guangdong-47	Guangdong, China	960141142	997364767	10	1.50E+11	187.0058938
ss_194	sichuan79-I-17	Sichuan, China	960053592	996616737	10	1.49E+11	186.8656382
ss_195	yunnan76-II-25	Yunnan, China	960274404	995932637	9	1.49E+11	186.7373694
ss_196	yunnan75-II-21	Yunnan, China	960240296	997747836	10	1.50E+11	187.0777193
ss_197	yunnan75-II-23	Yunnan, China	960139926	997535127	10	1.50E+11	187.0378363
ss_198	yunnan76-III-17	Yunnan, China	960152384	996339092	10	1.49E+11	186.8135798
ss_199	yunnan82-141	Yunnan, China	960044002	1026830050	10	1.54E+11	192.5306344
ss_200	yunnan83-210	Yunnan, China	960093026	996101658	10	1.49E+11	186.7690609
ss_201	yunnan87-27	Yunnan, China	960273228	996479062	10	1.49E+11	186.8398241
ss_202	yunnan87-30	Yunnan, China	960161754	996307058	10	1.49E+11	186.8075734
ss_203	yunnan-7	Yunnan, China	960005882	996987166	10	1.50E+11	186.9350936
ss_204	guangdong-33	Guangdong, China	960126492	997140101	10	1.50E+11	186.9637689
ss_205	guizhou78-I-31	Guizhou, China	960131542	995922261	10	1.49E+11	186.7354239
ss_206	hainan92-3	Hainan, China	960098776	995846140	10	1.49E+11	186.7211513

ss_207	hainan92-7	Hainan, China	960275118	996209572	10	1.49E+11	186.7892948
ss_208	sichuan79- II -9	Sichuan, China	960229192	996462760	10	1.49E+11	186.8367675
ss_209	yunnan75- I -8	Yunnan, China	960005648	995788306	10	1.49E+11	186.7103074
ss_210	yunnan75- II -26	Yunnan, China	960194000	996007334	10	1.49E+11	186.7513751
ss_211	yunnan76- I -003	Yunnan, China	960051296	996473569	10	1.49E+11	186.8387942
ss_212	yunnan82-116	Yunnan, China	960285876	996212559	9	1.49E+11	186.7898548
ss_213	yunnan82-67	Yunnan, China	960166404	997891848	10	1.50E+11	187.1047215
ss_214	hainan92-56	Hainan, China	960280856	995827998	10	1.49E+11	186.7177496
ss_215	hainan92-24	Hainan, China	909292302	942660655	10	1.41E+11	176.7488728
ss_216	fujian79- II -9	Fujian, China	960055782	995330667	10	1.49E+11	186.6245001
ss_217	hainan-3	Hainan, China	960126142	996621676	10	1.49E+11	186.8665643
ss_218	hainan92-52	Hainan, China	960213830	995757841	10	1.49E+11	186.7045952
ss_219	yunnan82-111	Yunnan, China	960133360	995830381	10	1.49E+11	186.7181964
ss_220	yunnan87-29	Yunnan, China	960128640	996931294	10	1.50E+11	186.9246176
ss_221	yunnan-1	Yunnan, China	960174438	995628153	10	1.49E+11	186.6802787
ss_222	fujianxianyou-2	Fujian, China	960029518	995796854	10	1.49E+11	186.7119101
ss_223	guangdong-41	Guangdong, China	960261564	996023247	10	1.49E+11	186.7543588
ss_224	guizhou-26	Guizhou, China	960207240	995686208	10	1.49E+11	186.691164
ss_225	yunnan-8	Yunnan, China	960204222	995313919	10	1.49E+11	186.6213598
ss_226	yunnan-2	Yunnan, China	960052178	996294754	10	1.49E+11	186.8052664
ss_227	guizhou78- I -11	Guizhou, China	960109516	996015060	10	1.49E+11	186.7528238
ss_228	yunnan82-112	Yunnan, China	960183490	994671485	10	1.49E+11	186.5009034
ss_229	hainan92-5	Hainan, China	960124824	996274351	10	1.49E+11	186.8014408

ss_230	yunnan75- II -34	Yunnan, China	960287460	995742359	10	1.49E+11	186.7016923
ss_231	guangxige-4	Guangxi, China	960272618	996199786	10	1.49E+11	186.7874599
ss_232	fujian87- I -4	Fujian, China	960058276	998349872	10	1.50E+11	187.190601
ss_233	guangdong-31	Guangdong, China	960109658	997291527	10	1.50E+11	186.9921613
ss_234	guangxi87-17	Guangxi, China	960113748	996160606	10	1.49E+11	186.7801136
ss_235	hainan92-60	Hainan, China	960022408	995650395	10	1.49E+11	186.6844491
ss_236	guangxi92-6	Guangxi, China	960267430	996229809	10	1.49E+11	186.7930892
ss_237	hainan92-8	Hainan, China	960155362	995769866	10	1.49E+11	186.7068499
ss_238	guangxi87-13	Guangxi, China	960095418	997509757	10	1.50E+11	187.0330794
ss_239	guangxi86-8	Guangxi, China	960061222	996011735	10	1.49E+11	186.7522003
ss_241	yunnan75- II -4	Yunnan, China	960230514	996774178	10	1.50E+11	186.8951584
ss_242	yunnan82-14	Yunnan, China	960131546	995604960	10	1.49E+11	186.67593
ss_243	yunnan82-149	Yunnan, China	960193674	998047547	8	1.50E+11	187.1339151
ss_244	guangxitianlin	Guangxi, China	960172018	996015997	10	1.49E+11	186.7529994
ss_245	guangxi87-14	Guangxi, China	795359912	823917141	10	1.24E+11	154.4844639
ss_246	guangxi92-16	Guangxi, China	960156952	996254867	10	1.49E+11	186.7977876
ss_247	hainan92-34	Hainan, China	960260588	995875670	10	1.49E+11	186.7266881
ss_248	guangxi87-23	Guangxi, China	960146156	995635733	10	1.49E+11	186.6816999
ss_249	guangxi87-1	Guangxi, China	960124776	996328159	10	1.49E+11	186.8115298
ss_250	hainan92-63	Hainan, China	960123416	995952454	10	1.49E+11	186.7410851
ss_251	fujian89- I -17	Fujian, China	960131962	996358822	10	1.49E+11	186.8172791
ss_252	fujianputian	Fujian, China	960023598	998141781	10	1.50E+11	187.1515839
ss_253	guangdong-29	Guangdong, China	960010266	997574353	10	1.50E+11	187.0451912

ss_254	guangdong-36	Guangdong, China	960296100	995224971	10	1.49E+11	186.6046821
ss_257	guangdonghuanzhou	Guangdong, China	960199354	995886734	10	1.49E+11	186.7287626
ss_258	guizhou78- II -08	Guizhou, China	960266830	995562156	10	1.49E+11	186.6679043
ss_259	hainan92-31	Hainan, China	960047274	996346259	10	1.49E+11	186.8149236
ss_260	guangxi87-25	Guangxi, China	960153912	995610419	10	1.49E+11	186.6769536
ss_261	fujian87- I -14	Fujian, China	960122484	997654057	10	1.50E+11	187.0601357
ss_262	hainanyanglan-9	Hainan, China	960082722	995817304	10	1.49E+11	186.7157445
ss_263	yunnanmanhao-1	Yunnan, China	960221416	996256419	10	1.49E+11	186.7980786
ss_264	hainanyacheng-1	Hainan, China	960191732	995599373	10	1.49E+11	186.6748824
ss_265	guangdong-28	Guangdong, China	960276842	995912434	10	1.49E+11	186.7335814
ss_266	guangdong-44	Guangdong, China	960203348	995660085	10	1.49E+11	186.6862659
ss_267	yunnan76-III-18	Yunnan, China	960196562	995719482	10	1.49E+11	186.6974029
ss_268	yunnan87-47	Yunnan, China	960203828	997747073	10	1.50E+11	187.0775762
ss_269	hainan92-39	Hainan, China	960152656	994450564	10	1.49E+11	186.4594808
ss_270	guangdong-81	Guangdong, China	810894794	840428572	10	1.26E+11	157.5803573
ss_271	Vietnam-3	Vietnam	860907378	892709697	10	1.34E+11	167.3830682
ss_272	hainan92-66	Hainan, China	891961378	924784056	10	1.39E+11	173.3970105
ss_273	hainan-2	Hainan, China	960123332	995223064	10	1.49E+11	186.6043245
ss_274	hainan92-50	Hainan, China	958895498	994840620	10	1.49E+11	186.5326163
ss_275	yunnan82-110	Yunnan, China	868298192	900223889	10	1.35E+11	168.7919792
ss_276	Thailand S.SP2003-1	Thailand	938229318	972943696	10	1.46E+11	182.426943
ss_277	hainan92-47	Hainan, China	960093832	996103762	10	1.49E+11	186.7694554
ss_278	Thailand S.SP98T-266	Thailand	960023808	995993664	10	1.49E+11	186.748812

ss_279	fujian89- I -19	Fujian, China	960278314	998831204	10	1.50E+11	187.2808508
ss_280	guangxi87-51	Guangxi, China	933560202	968955001	9	1.45E+11	181.6790627
ss_281	guangxige-3	Guangxi, China	905980994	939612947	10	1.41E+11	176.1774276
ss_282	guangxi85-13	Guangxi, China	873702930	906259521	10	1.36E+11	169.9236602
ss_283	guangxi87-53	Guangxi, China	960252250	996275147	10	1.49E+11	186.8015901
ss_284	guangxi92-1	Guangxi, China	872795038	905954251	10	1.36E+11	169.8664221
ss_285	guangxi83-25	Guangxi, China	960018394	993929221	10	1.49E+11	186.3617289
ss_286	guangdong-80	Guangdong, China	807284552	837126545	10	1.26E+11	156.9612272
ss_287	sichuanzizhong-2	Sichuan, China	960120270	996845198	10	1.50E+11	186.9084746
ss_288	yunnan82-63	Yunnan, China	841669026	874925394	10	1.31E+11	164.0485114
ss_289	yunnan82-65	Yunnan, China	960192252	997980105	10	1.50E+11	187.1212697
ss_290	yunnan82-16	Yunnan, China	960047652	995915726	10	1.49E+11	186.7341986
ss_291	guangxi87-16	Guangxi, China	948290954	983679593	10	1.48E+11	184.4399237
ss_292	hainan92-32	Hainan, China	960186624	995895467	10	1.49E+11	186.7304001
ss_293	guangxi87-46	Guangxi, China	960140418	997111252	10	1.50E+11	186.9583598
ss_294	guangxi87-21	Guangxi, China	960095850	995769879	10	1.49E+11	186.7068523
ss_300	sichuan79- II -1	Sichuan, China	957185050	993406389	10	1.49E+11	186.2636979

Table S5. Summary of HiFi sequencing data of 14 *S. spontaneum* samples

#ID	Sample name	Species	Total bases (Gb)	Total reads	Average length	Read N50	Coverage (×)*
Ss01	fujian88-1-4	<i>Saccharum spontaneum</i>	1.89062E+11	8,966,743	21087.26	21123	236.33
Ss02	fujian89-1-7	<i>Saccharum spontaneum</i>	1.76851E+11	8,432,657	20969.515	20959	221.06
Ss03	guangdong8hao	<i>Saccharum spontaneum</i>	1.75635E+11	8,340,730	21068.38	21160.5	219.54
Ss04	guangxi86-8	<i>Saccharum spontaneum</i>	1.96999E+11	10,015,304	19671.735	19675	246.25
Ss05	guangxi87-51	<i>Saccharum spontaneum</i>	1.79756E+11	8,819,374	20378.73	20499	224.69
Ss06	hainan92-5	<i>Saccharum spontaneum</i>	1.85699E+11	8,740,643	21265.705	21390	232.12
Ss07	sichuan79-1-19	<i>Saccharum spontaneum</i>	1.79681E+11	8,835,174	20345.1	20431.5	224.60
Ss08	sichuan88-22	<i>Saccharum spontaneum</i>	1.76605E+11	8,249,409	21407.825	21547	220.76
Ss09	yunnan82-149	<i>Saccharum spontaneum</i>	1.7244E+11	8,518,010	20276.995	20216.5	215.55
Ss10	yunnan82-132	<i>Saccharum spontaneum</i>	1.83138E+11	8,878,792	20733.845	20872	228.92
Ss11	yunnan82-48	<i>Saccharum spontaneum</i>	1.87128E+11	9,549,627	19623.315	19674.5	233.91
Ss12	yunnan82-84	<i>Saccharum spontaneum</i>	1.78919E+11	8,777,899	20385.225	20390.5	223.65
Ss13	yunnanheqinggeshoumi	<i>Saccharum spontaneum</i>	1.79806E+11	8,612,785	20875.1	21024.5	224.76
Ss14	yunnankaiyuangeshoumi	<i>Saccharum spontaneum</i>	1.99708E+11	10,155,612	19672.355	19750	249.64

Table S6. Summary statistics of 14 *S. spontaneum* assemblies

Basic information				Genome Assemblies			
#ID	Sample name	Species	Assembly level	Assembly size (bp)	Contig Number	N50	BUSCO (%)
Ss01	fujian88-1-4	<i>Saccharum spontaneum</i>	contig	8,368,781,140	23,298	2,484,927	99.4
Ss02	fujian89-1-7	<i>Saccharum spontaneum</i>	contig	9,324,246,601	31,424	1,791,182	99.4
Ss03	guangdong8hao	<i>Saccharum spontaneum</i>	contig	7,939,614,880	41,205	1,519,160	99.3
Ss04	guangxi86-8	<i>Saccharum spontaneum</i>	contig	7,813,376,143	39,129	1,802,156	99.3
Ss05	guangxi87-51	<i>Saccharum spontaneum</i>	contig	7,440,054,966	25,708	2,319,968	99.4
Ss06	hainan92-5	<i>Saccharum spontaneum</i>	contig	7,603,310,039	32,243	1,834,047	99.2
Ss07	sichuan79-1-19	<i>Saccharum spontaneum</i>	contig	8,146,733,135	49,353	997,139	99.5
Ss08	sichuan88-22	<i>Saccharum spontaneum</i>	contig	8,502,472,194	54,238	733,173	99.3
Ss09	yunnan82-149	<i>Saccharum spontaneum</i>	contig	6,518,384,450	48,694	1,048,187	99.7
Ss10	yunnan82-132	<i>Saccharum spontaneum</i>	contig	7,640,365,253	34,854	1,694,461	99.4
Ss11	yunnan82-48	<i>Saccharum spontaneum</i>	contig	7,952,106,007	46,680	1,729,590	99.5
Ss12	yunnan82-84	<i>Saccharum spontaneum</i>	contig	8,760,448,060	42,012	1,741,544	99.5
Ss13	yunnanheqinggeshoumi	<i>Saccharum spontaneum</i>	contig	7,900,885,789	44,016	1,668,255	99.3
Ss14	yunnankaiyuangeshoumi	<i>Saccharum spontaneum</i>	contig	7,763,673,639	40,054	1,388,620	99.5

[illegible]

Table S8. Gene annotation summary of 14 *S. spontaneum* genomes

#ID	Gene count	Average gene length	Mean exon length	Mean intron length	Exon count per gene
Ss01	296879	4258.90	258.276434733761	476.620432977118	4.50
Ss02	332535	4137.80	257.911032538294	482.551230347918	4.50
Ss03	284471	4270.80	256.180503576529	475.951527218628	4.60
Ss04	282072	4198.10	255.99808676193	473.834880199484	4.60
Ss05	271315	4250.30	259.562839833236	471.819379975999	4.50
Ss06	282165	4264.40	256.634093211438	478.16698323183	4.60
Ss07	284006	4210.00	253.384902801347	485.922434245784	4.60
Ss08	286578	4022.60	255.081441625994	479.159906807914	4.50
Ss09	231354	4266.80	256.153671182786	480.48988261438	4.60
Ss10	274973	4283.70	257.330337757576	473.387055589189	4.60
Ss11	283350	4116.80	257.027205816367	469.058037888225	4.60
Ss12	307442	4222.30	257.766694571148	472.064220383254	4.60
Ss13	280316	4242.30	256.212414788489	474.778225466561	4.60
Ss14	283978	4178.50	257.134973741674	472.39069359695	4.60

Table S9. Summary statistics of 14 *S. spontaneum* monoploid assemblies.

Basic information				Genome Assemblies		
#ID	Sample name	Species	Assembly level	Assembly size (bp)	Scaffold N50	BUSCO (%)
Ss01	fujian88-1-4	<i>Saccharum spontaneum</i>	chromosomal level	764,821,631	95,582,447	94.2
Ss02	fujian89-1-7	<i>Saccharum spontaneum</i>	chromosomal level	768,876,700	96,397,687	92.6
Ss03	guangdong8hao	<i>Saccharum spontaneum</i>	chromosomal level	766,201,751	95,549,994	94.6
Ss04	guangxi86-8	<i>Saccharum spontaneum</i>	chromosomal level	766,964,079	96,446,034	94
Ss05	guangxi87-51	<i>Saccharum spontaneum</i>	chromosomal level	760,308,127	94,716,395	93.2
Ss06	hainan92-5	<i>Saccharum spontaneum</i>	chromosomal level	762,389,088	94,784,936	94
Ss07	sichuan79-1-19	<i>Saccharum spontaneum</i>	chromosomal level	765,443,347	95,251,182	92.1
Ss08	sichuan88-22	<i>Saccharum spontaneum</i>	chromosomal level	764,610,058	96,552,627	91.9
Ss09	yunnan82-149	<i>Saccharum spontaneum</i>	chromosomal level	758,969,491	94,516,644	93.2
Ss10	yunnan82-132	<i>Saccharum spontaneum</i>	chromosomal level	764,322,881	96,342,861	93.3
Ss11	yunnan82-48	<i>Saccharum spontaneum</i>	chromosomal level	772,800,286	96,697,331	95.1
Ss12	yunnan82-84	<i>Saccharum spontaneum</i>	chromosomal level	766,935,651	95,890,998	94.3
Ss13	yunnanheqinggeshoumi	<i>Saccharum spontaneum</i>	chromosomal level	766,618,634	95,890,998	94.5
Ss14	yunnankaiyuangeshoumi	<i>Saccharum spontaneum</i>	chromosomal level	762,672,373	95,213,323	91.7

Table S10. Summary statistics of genetic variants between biallelic and multi-allelic

	SNPs	Indels	SVs			Total Num
	1 (bp)	1-50 (bp)	50-1000 (bp)	1000- 10,000 (bp)	>10000 (bp)	
Biallelic	8,240,591	1,397,711	89,775	25,700	2,785	118,260
Multiallelic	244,454	1,927,163	104,499	34,014	20,360	158,873

Table S11. Summary statistics of SVs between biallelic and multi-allelic

	Biallelic			Multiallelic	
	Insertions	Deletions	Divergent SVs	SVs	Total
SV count	103,972	11,515	3,065	158,873	277,425
SV length (bp)	134,655,486	16,036,251	10,937,970	2,516,809,543	2678439250
SV influenced genes	24,253	8,868	2,832	26,479	
Total influenced genes			25,092	26,479	

Table S12. Phenotypic data of 290 *S. spontaneum* accessions used in this study

#ID	Accession Name	Brix (%)	Apparent purity (%)	Tiller Number
ss_001	yunnan82-17	11.75	39.41	NA
ss_002	yunnan82-44	12.8	38.35	11.33333333
ss_003	yunnan83-202	16.4	38.11	NA
ss_004	yunnan84-258	16.02	36.68	NA
ss_005	yunnan82-33	17.32	48.77	6.33333333
ss_006	fujian88- I -4	16.07	23.01	7.66666667
ss_007	fujian89- I -4	15.17	49.58	14.66666667
ss_008	fujian88- I -7	19.1	47.06	17.33333333
ss_010	sichuan79- II -16	20.39	51.28	7.66666667
ss_011	sichuan88-22	13.41	30.61	11
ss_012	sichuan88-8	18.86	51.95	7.66666667
ss_013	sichuan92-50	18.67	48.88	12.66666667
ss_014	yunnan76- II -32	17.53	47.17	5.33333333
ss_015	yunnan75- II -17	17.13	59.89	8.66666667
ss_016	yunnan75- II -43	16.44	7.1	4.66666667
ss_017	yunnan82-25	12.21	21.36	24.33333333
ss_018	yunnan82-26	10.21	27.22	9
ss_019	yunnan82-48	15.52	31.13	9
ss_020	yunnan82-50	18.43	43.92	15
ss_021	yunnan82-58	16.66	42.81	11
ss_022	yunnan82-59	17.97	20.69	15.33333333
ss_023	yunnan83-173	13.32	44.97	5
ss_024	yunnan83-190	19.21	47.16	10
ss_025	yunnan83-196	12.68	42.66	11.33333333
ss_026	yunnan83-205	13.63	38.81	11.66666667
ss_027	yunnan83-206	14.63	42.22	12
ss_028	yunnan83-225	12.95	38.86	10

ss_029	yunnan83-240	15.89	29.59	7.333333333
ss_030	guangdong-6	14.87	35.73	10.66666667
ss_031	guangdong-12	17.44	49.55	12
ss_032	guangdong-17	14.35	38.82	13.33333333
ss_033	guangdong-60	17.89	52	22.66666667
ss_034	guizhou78- II -012	18.83	50.74	6.66666667
ss_035	jiangxi79-17	17.92	46.09	11
ss_036	jiangxi79-27	18.29	44.7	10.66666667
ss_037	sichuan74- I -14	19.64	43.83	12.33333333
ss_038	sichuan76- II -16	16.86	36.73	9.66666667
ss_040	sichuan79- I -19	17.65	51.51	10.33333333
ss_041	sichuan79- I -27	18.97	54.19	9
ss_042	sichuan79- I -4	20.2	51.58	NA
ss_043	sichuan88-4	19.05	45.44	14.33333333
ss_044	sichuan92-12	12.47	45	8.333333333
ss_045	sichuan92-14	14.45	39.18	11.66666667
ss_046	sichuan92-16	17.39	46.48	18.66666667
ss_047	sichuan92-38	19.07	55.23	10.33333333
ss_048	yunnan76- I -024	17.52	50.24	9
ss_049	yunnan75- II -35	16.35	43.94	7.333333333
ss_050	yunnan76-III-20	14.43	32.41	9
ss_051	yunnan82-103	12.13	40.63	6.66666667
ss_052	yunnan82-12	11.82	35.79	11.33333333
ss_053	yunnan82-20	11.19	33.7	14
ss_054	yunnan82-34	12.59	41.6	10
ss_055	yunnan83-189	13.02	43.78	6.333333333
ss_056	yunnan83-207	13.87	42.58	6.333333333
ss_057	yunnan83-213	16.11	48.56	11.66666667
ss_058	yunnan83-231	13.99	34.77	6

ss_059	yunnan83-235	15.81	36.09	17.666666667
ss_060	yunnan84-247	16.93	35.42	14
ss_061	yunnan84-259	17.41	39.14	11.333333333
ss_062	yunnan84-264	15.12	28.77	10
ss_063	yunnanheqing	14.93	59.76	8.666666667
ss_064	yunnankaiyuan	15.81	42.58	7.666666667
ss_065	fujian88- I -13	15.7	22.79	9.666666667
ss_066	fujian88- I -12	15.15	22.7	13
ss_067	fujian89- I -7	15.86	32.29	8
ss_068	guangdong-8	14.96	46.52	16
ss_069	guangdong-67	14.33	46.63	12
ss_070	sichuan79- II -10	16.61	50.26	13.333333333
ss_071	yunnan82-132	14.06	42.66	8.666666667
ss_072	yunnan82-136	15.07	44.96	13
ss_073	yunnan83-193	16.95	43.74	11
ss_074	fujian92- I -12	17.3	42.49	14.333333333
ss_075	guangdong-45	9.98	28.4	6.333333333
ss_076	guangdong-50	15.81	53.61	19.666666667
ss_077	guangdong-74	13.92	29.8	7.333333333
ss_078	yunnan75- I -6	13.9	30.37	8
ss_079	yunnan75- II -8	16.74	38.63	8
ss_080	yunnan76- I -012	15.41	51.87	16.333333333
ss_081	yunnan82-84	12.06	30.11	12.333333333
ss_082	yunnan83-163	16.12	50.97	7
ss_083	yunnan83-201	15.94	28.88	15.333333333
ss_084	yunnan83-208	12.56	20.36	15.666666667
ss_085	yunnan84-248	14.74	41.39	5
ss_086	yunnan84-251	17.27	48.64	11
ss_087	yunnan84-256	15.32	38.14	9.666666667

ss_088	hainan92-14	16.98	46.1	5.666666667
ss_089	guangdong-58	18.02	51.88	11
ss_090	yunnan83-200	11.79	29.56	10.66666667
ss_091	yunnan87-40	11.64	35.94	8.333333333
ss_092	guangdong-3	14.64	54.3	14.33333333
ss_093	guizhou78- II -09	17.83	56.96	7.666666667
ss_094	yunnan75- II -28	15.53	43.42	16.33333333
ss_095	yunnan76-III-13	14.54	52.16	7.666666667
ss_096	yunnan82-145	16.83	31.6	15
ss_097	yunnan87-26	13.6	37.98	6.666666667
ss_098	yunnan83-159	16.39	47.4	9.333333333
ss_099	guangdong-2	17.47	49.16	14.33333333
ss_100	yunnan83-157	15.73	45.3	8
ss_101	yunnan83-166	17.44	45.73	7.333333333
ss_102	fujian88- I -3	16.52	22.63	16.66666667
ss_103	fujian79- II -4	17.27	33.13	10.33333333
ss_104	fujian92- I -11	16.03	21.37	12.66666667
ss_105	fujian92- I -19	15.81	21.54	7.333333333
ss_106	sichuan79- I -16	18.95	47.32	13.66666667
ss_107	sichuan79- I -21	17.05	39.6	8.666666667
ss_108	sichuan79- II -6	14.8	42.39	13.33333333
ss_109	sichuan88-18	18.14	48.02	12
ss_110	sichuan88-34	19.27	48.51	15
ss_111	sichuan88-48	17.78	40.17	9.333333333
ss_112	sichuan92-17	15.67	39.27	7
ss_113	sichuan92-28	16.77	44.08	23.66666667
ss_114	sichuan92-31	15.19	50.62	8.333333333
ss_115	sichuan92-46	15.61	59.03	11.33333333
ss_116	sichuan92-5	15.96	53.36	7.666666667

ss_117	sichuanfushun-5	17.44	45.9	13.66666667
ss_118	sichuanjianyang-8	18.05	43.16	21.66666667
ss_119	sichuanneijiang-36	18.49	42.73	9.333333333
ss_120	sichuanneijiang-4	19.36	42.65	7.666666667
ss_121	sichuanneijiangbadi	18.3	42.39	6.666666667
ss_122	sichuanzizhong-3	16.31	46.62	7.333333333
ss_123	sichuanzizhong-9	16.88	41.18	11
ss_124	yunnan75- I -10	15.44	28.27	12.33333333
ss_125	yunnan75- II -2	13.78	31.72	11
ss_126	yunnan82-24	11.77	32.57	15.33333333
ss_127	yunnan82-7	15.03	30.89	7.666666667
ss_128	yunnan82-70	13.49	37.21	11
ss_129	fujianhuian	14.57	22.12	8.666666667
ss_130	sichuan79- I -20	14.11	41.24	13.33333333
ss_131	sichuan92-18	13.98	39.02	9.666666667
ss_132	sichuan92-30	15.11	47.78	12
ss_133	sichuan92-34	13.59	41.11	12.66666667
ss_134	sichuanneijiang-1	17.67	41.68	11.33333333
ss_135	sichuanneijiang-44	18.71	48.65	10.66666667
ss_136	guangxige-1	15.93333333	31.7944205	8.333333333
ss_137	fujian79- II -18	13.97	32.87	19.33333333
ss_138	guangdong-55	18.93	28.34	12.66666667
ss_139	guangdong-71	17.29	41.82	13.33333333
ss_140	guangdong-75	15.78	41.61	11.66666667
ss_141	guizhou78- II -04	15.23	52.22	7.333333333
ss_142	hainan92-41	16.58	42.42	10
ss_143	sichuan79- I -6	12.81	38.31	10
ss_144	sichuan92-49	18.32	54.12	10.66666667
ss_145	yunnan75- I -21	13.67	23.14	NA

ss_146	yunnan75- I -4	15.12	52.96	10.33333333
ss_147	yunnan75- II -27	16.66	40.43	13
ss_148	yunnan82-134	13.99	47.06	9.666666667
ss_149	yunnan82-77	11.68	21.76	13.33333333
ss_150	yunnan82-79	12.06	37.33	9.666666667
ss_151	yunnan83-167	16.2	40.62	10.66666667
ss_152	yunnan83-169	16.69	41.26	11.33333333
ss_153	yunnan83-171	16.39	45.15	7
ss_154	yunnan83-185	18.77	45.09	8.333333333
ss_155	yunnan-4	17.83	51.33	12.33333333
ss_156	yunnanzhedianbaihuacao	15.61	30.62	13.66666667
ss_157	fujian87- I -10	11.93	29.21	NA
ss_158	fujian87- I -11	11.99	20.54	17.66666667
ss_159	fujian89- I -21	10.83	15.01	7.333333333
ss_160	guangdong-46	17.31	59.38	7.333333333
ss_161	sichuan79- I -31	17.42	50.43	5.333333333
ss_162	sichuan92-33	12.42	31.19	11.66666667
ss_163	sichuan92-42	14.87	42.83	8
ss_164	sichuan92-7	19.66	64	10.66666667
ss_165	India-1	17.21	53.52	15
ss_166	hainan92-23	15.84667	40.20265	6.666666667
ss_167	hainan92-72	15.40667	35.20556	10
ss_168	guangdong-21	17.65	58.58	13
ss_169	guangdong-48	17.41	44.61	11.66666667
ss_170	guangdong-78	17.23	54.85	8.666666667
ss_171	hainan92-2	17.56	38.52	5.666666667
ss_172	hainanhaikou	11.69	24.94	15.66666667
ss_173	yunnan82-120	11.75	29.46	7
ss_174	yunnan82-83	10.7	32.03	9.666666667

ss_175	yunnan83-160	14.47	32.02	7
ss_176	yunnan83-181	21.07	56.17	8.666666667
ss_177	yunnan84-249	14.12	41.2	8.666666667
ss_178	guangdong-40	15.37	40.41	8
ss_179	guangdong-53	17.71	46.26	18.33333333
ss_180	hainan92-46	13.69	34.49	10
ss_181	sichuan79- I -8	15.71	23.38	11.66666667
ss_182	sichuan79- II -11	13.1	24.19	14.66666667
ss_183	yunnan75- II -12	15.37	42.79	6.666666667
ss_184	yunnan75- II -7	14.93	45.76	7.333333333
ss_185	yunnan76-III-39	14.93	33.4	5.333333333
ss_186	yunnan82-86	11.08	22.76	9.666666667
ss_187	yunnan83-184	17.57	40.52	7.333333333
ss_188	hainan92-18	18.96	51.01899	10.33333333
ss_189	hainan92-61	16.64667	43.72079	8.333333333
ss_190	hainan92-16	14.96667	43.87304	14.33333333
ss_191	fujianxianyou-1	16.17	25.4	10.66666667
ss_192	guangdong-23	16.53	34.29	10.66666667
ss_193	guangdong-47	16.71	36.52	11.33333333
ss_194	sichuan79- I -17	17.15	47.84	9.666666667
ss_195	yunnan76- II -25	16.25	38.78	9
ss_196	yunnan75- II -21	14.74	31.53	5.666666667
ss_197	yunnan75- II -23	16.93	39.44	6
ss_198	yunnan76-III-17	15.91	47.23	10.66666667
ss_199	yunnan82-141	12.89	40	11.66666667
ss_200	yunnan83-210	16.41	46.72	7
ss_201	yunnan87-27	13.19	39.06	9
ss_202	yunnan87-30	12.27	40.28	9.666666667
ss_203	yunnan-7	17.22	4.83	12

ss_204	guangdong-33	17.25	45.77	12.33333333
ss_205	guizhou78- I -31	14.46	40.71	9
ss_206	hainan92-3	19.73	43.71	9
ss_207	hainan92-7	15.5	26.73	6
ss_208	sichuan79- II -9	16.01	44.17	10
ss_209	yunnan75- I -8	14.28	35.38	11.66666667
ss_210	yunnan75- II -26	19.05	45.44	7.666666667
ss_211	yunnan76- I -003	15.21	55.98	14.66666667
ss_212	yunnan82-116	13.08	38.42	10
ss_213	yunnan82-67	11.6	33.73	9
ss_214	hainan92-56	12.66667	39.39018	11.66666667
ss_215	hainan92-24	15.47333	27.44036	8.333333333
ss_216	fujian79- II -9	15.69	40.13	7
ss_217	hainan-3	14.29	40.69	10.33333333
ss_218	hainan92-52	14.56	31.95	6.666666667
ss_219	yunnan82-111	11	18.85	8
ss_220	yunnan87-29	15.39	44.15	9.666666667
ss_221	yunnan-1	18.15	53.57	10.66666667
ss_222	fujianxianyou-2	15.44	22.87	8.666666667
ss_223	guangdong-41	16.98	49.97	7.333333333
ss_224	guizhou-26	13.9	30.71	8
ss_225	yunnan-8	12.81	36.17	25.33333333
ss_226	yunnan-2	16.03	48.98	9
ss_227	guizhou78- I -11	13.64	25.56	9.333333333
ss_228	yunnan82-112	13.81	35.77	7.666666667
ss_229	hainan92-5	15.95	36.82	15.33333333
ss_230	yunnan75- II -34	17.83	52.19	7.666666667
ss_231	guangxige-4	15.06	45.48663347	NA
ss_232	fujian87- I -4	15.08	23.3	16.66666667

ss_233	guangdong-31	15.13	15.74	13.66666667
ss_234	guangxi87-17	18.02666667	46.85838018	9.666666667
ss_235	hainan92-60	12.9	40.74509	8
ss_236	guangxi92-6	17.34	37.20239331	12.66666667
ss_237	hainan92-8	18.4	46.62218	9.666666667
ss_238	guangxi87-13	16.46	33.66798906	9
ss_239	guangxi86-8	11.75333333	23.92324731	9.666666667
ss_241	yunnan75- II -4	17.09	54.73	12.33333333
ss_242	yunnan82-14	12.68	43.44	13
ss_243	yunnan82-149	13.82	21.08	8
ss_244	guangxitianlin	19.85333333	56.26194762	16.66666667
ss_245	guangxi87-14	16.61333333	42.45064406	9.333333333
ss_246	guangxi92-16	12.46	18.91841894	11
ss_247	hainan92-34	18.7	52.28883	8.333333333
ss_248	guangxi87-23	15.36	42.3085612	7
ss_249	guangxi87-1	16.07333333	35.90361676	4.333333333
ss_250	hainan92-63	15.53333	33.16693	8.666666667
ss_251	fujian89- I -17	14.41	35.22	10.66666667
ss_252	fujianputian	11.92	25.26	11.66666667
ss_253	guangdong-29	12.8	11.05	NA
ss_254	guangdong-36	16.76	49.38	NA
ss_257	guangdonghuanzhou	16.44	28.37	11.66666667
ss_258	guizhou78- II -08	16.35	57.25	7.666666667
ss_259	hainan92-31	16.23	42.59	6
ss_260	guangxi87-25	14.40666667	51.61600648	12.33333333
ss_261	fujian87- I -14	12.21	20.97	12.33333333
ss_262	hainanyanglan-9	14.86	26.63	7
ss_263	yunnanmanhao-1	17.05	49.17	8
ss_264	hainanyacheng-1	14.77	36.47	9

ss_265	guangdong-28	15.17	30.76	10.33333333
ss_266	guangdong-44	18.12	49.7	8.333333333
ss_267	yunnan76-III-18	13.52	34.38	11.66666667
ss_268	yunnan87-47	12.63	25.33	9
ss_269	hainan92-39	12.34	47.53	9
ss_270	guangdong-81	16.91	52.91	5.333333333
ss_271	Vietnam-3	16.68	27.67	0
ss_272	hainan92-66	14.93333	39.03041	6.666666667
ss_273	hainan-2	13.79	46.55	8
ss_274	hainan92-50	15.39	32.05	8
ss_275	yunnan82-110	15.69	53.09	12
ss_276	Thailand S.SP2003-1	16.08	51.77	11
ss_277	hainan92-47	13.79	49.07	7.666666667
ss_278	Thailand S.SP98T-266	11.49	18.87	5
ss_279	fujian89- I -19	12.14	28.47897	13.33333333
ss_280	guangxi87-51	16.45333333	41.2657658	6.666666667
ss_281	guangxige-3	16.54666667	48.07843272	7.333333333
ss_282	guangxi85-13	12.71333333	28.09	7
ss_283	guangxi87-53	18.78666667	42.86518275	14.33333333
ss_284	guangxi92-1	16.22666667	35.09789852	9.333333333
ss_285	guangxi83-25	15.82	42.89	8.333333333
ss_286	guangdong-80	16.18	41.88	9
ss_287	sichuanzizhong-2	17.73	37.43	9.333333333
ss_288	yunnan82-63	12.17	27.37	6.333333333
ss_289	yunnan82-65	13.99	29.46	12
ss_290	yunnan82-16	15.65	40.09	7
ss_291	guangxi87-16	13.65333333	40.54476563	6
ss_292	hainan92-32	17.27333	52.75046	7.666666667
ss_293	guangxi87-46	10.45333333	9.332018495	5

ss_294	guangxi87-21	18.57333333	60.38360912	6.333333333
ss_300	sichuan79- II -1	20.98	53.84	9.333333333

Table S13. Comparison of k-mer mappability between linear-based alignment and graph-based alignment

Item	aligned k-mers	unaligned k-mers	mapping ratio (%)
linear-based alignment	40,074	72,733	35.52439122
graph-based mapping	107,279	5,528	95.09959488
Total associated k-mers	112,807		

Supplementary Figures

Benchmarking Misalignment in Polyploid Genomes Using Short Reads

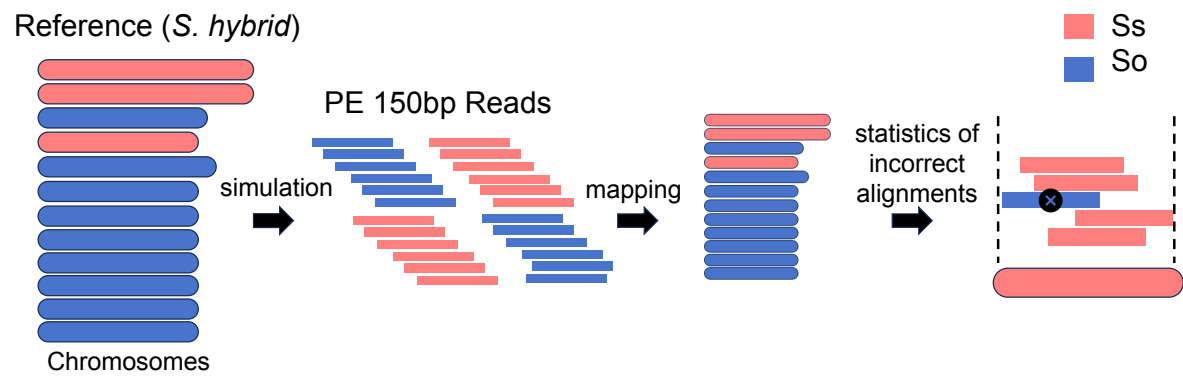


Figure S1. Benchmarking misalignment in polyploid genomes using short reads.

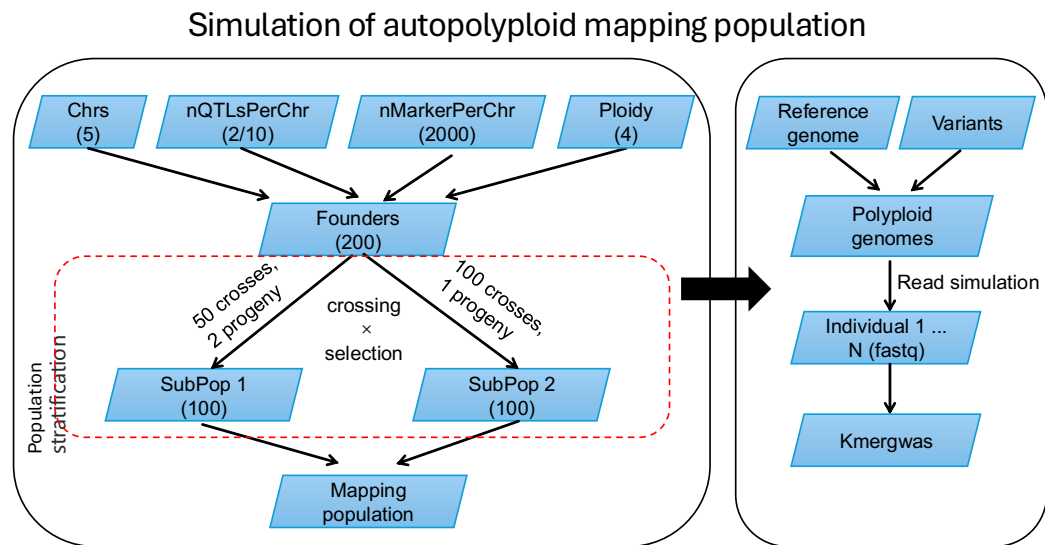


Figure S2. The pipeline of simulated autopolyploid population used for the KMERIA.

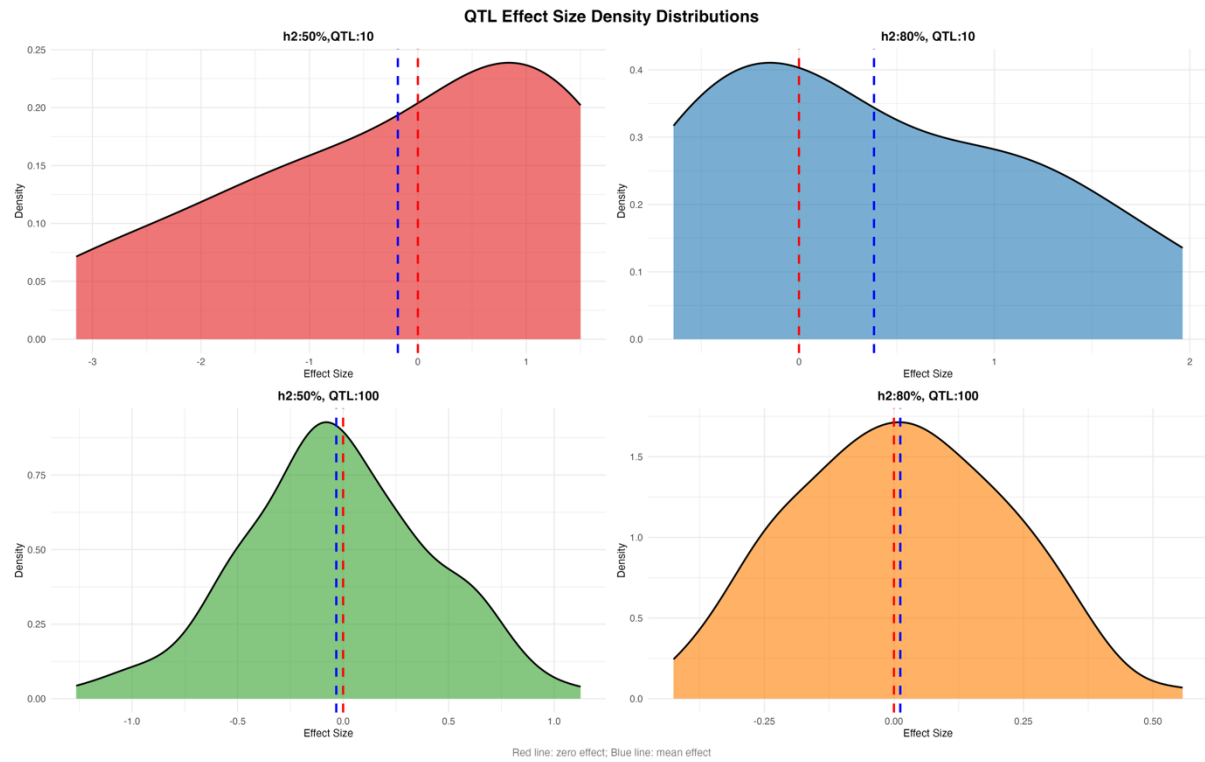


Figure S3. Simulation of quantitative traits governed by 10 and 100 QTNs, respectively, with QTN effect sizes conforming to a geometric (gaussian) distribution.

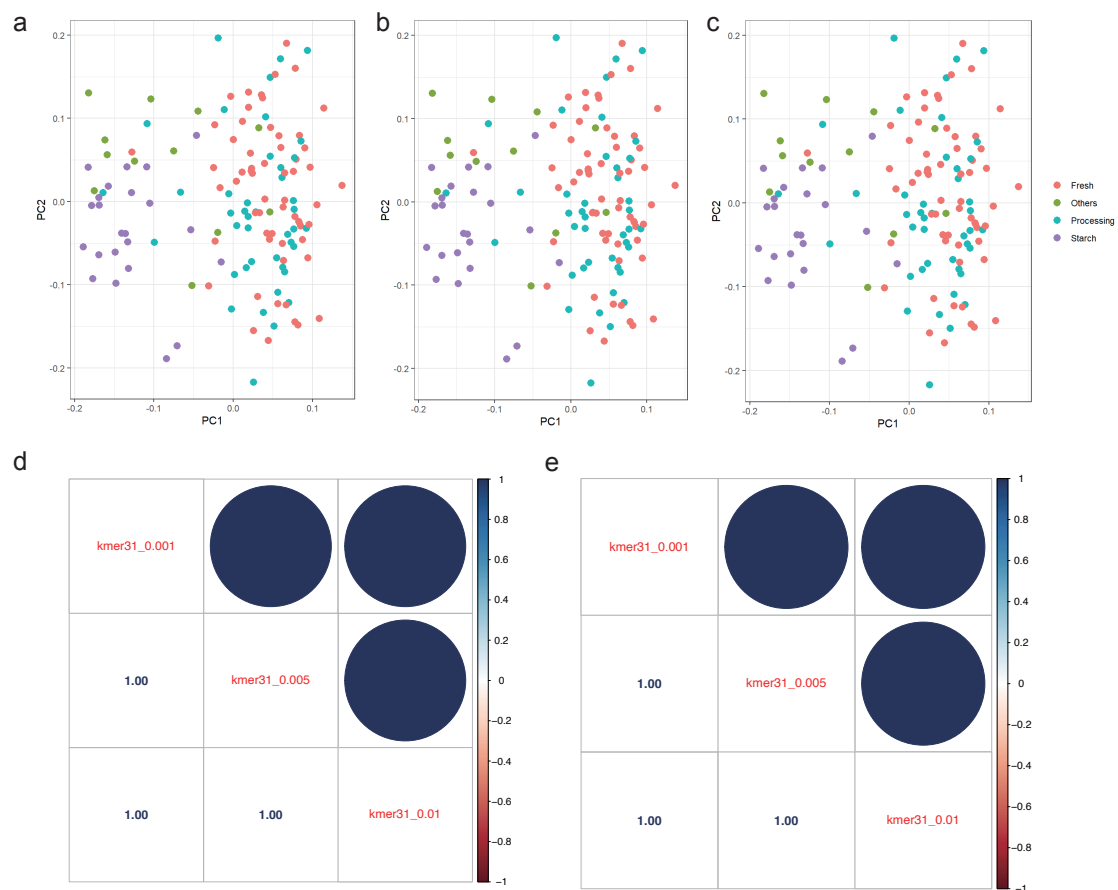


Figure S4. Impact of k-mer random sampling rates on population structure (Q) Inference in tetraploid potato population.

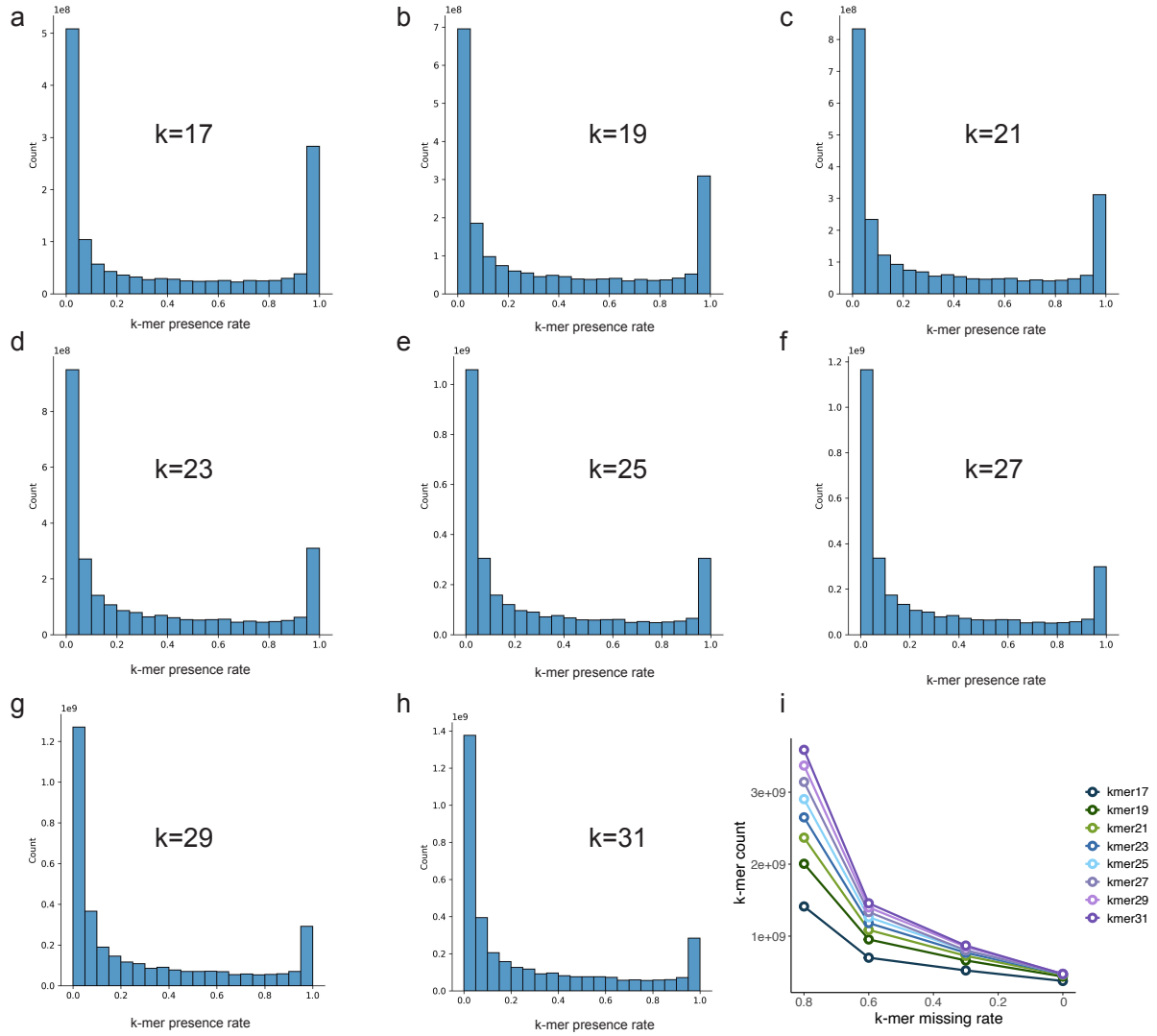


Figure S5. Distribution analysis of k-mer presence frequencies across varying k-mer sizes in a tetraploid potato population, visualized through comparative histogram profiling.

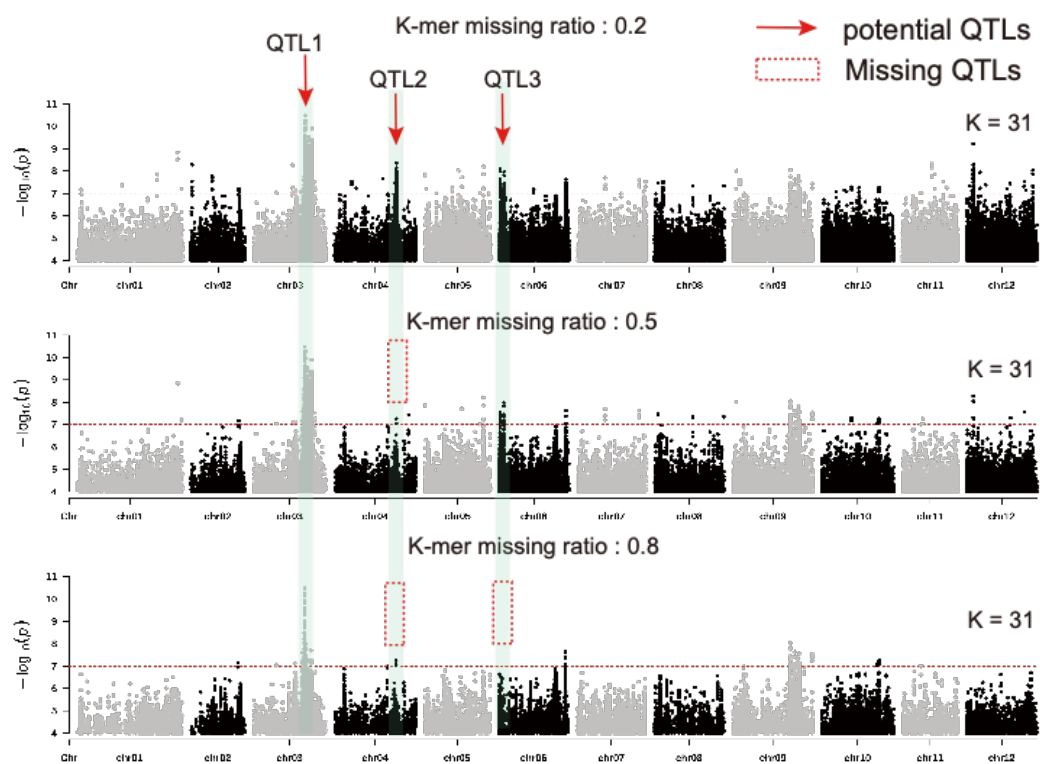


Figure S6. Assessment of genotype missingness impact on GWAS sensitivity using k-mer datasets (K = 31) with progressive missingness gradients (20%, 50%, 80%).

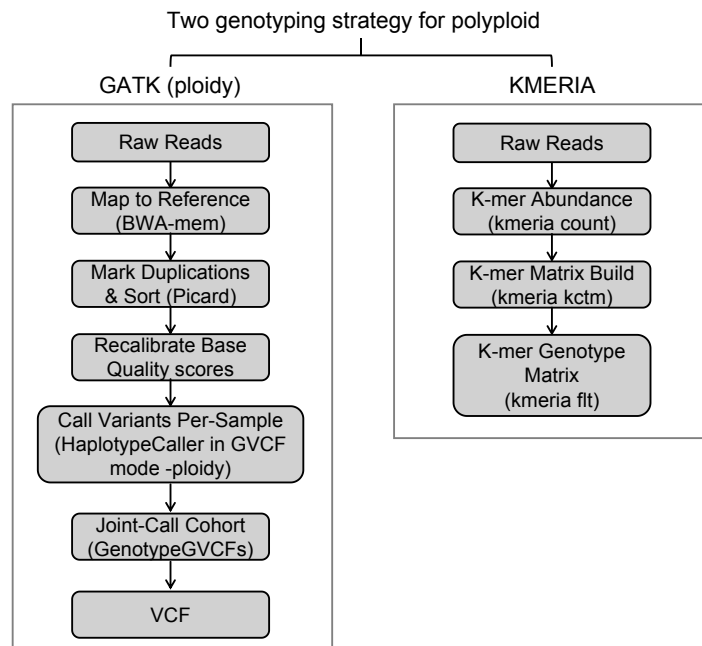


Figure S7. The genotyping pipeline between GATK (ploidy-aware mode) and KMERIA for polyploid population analysis.

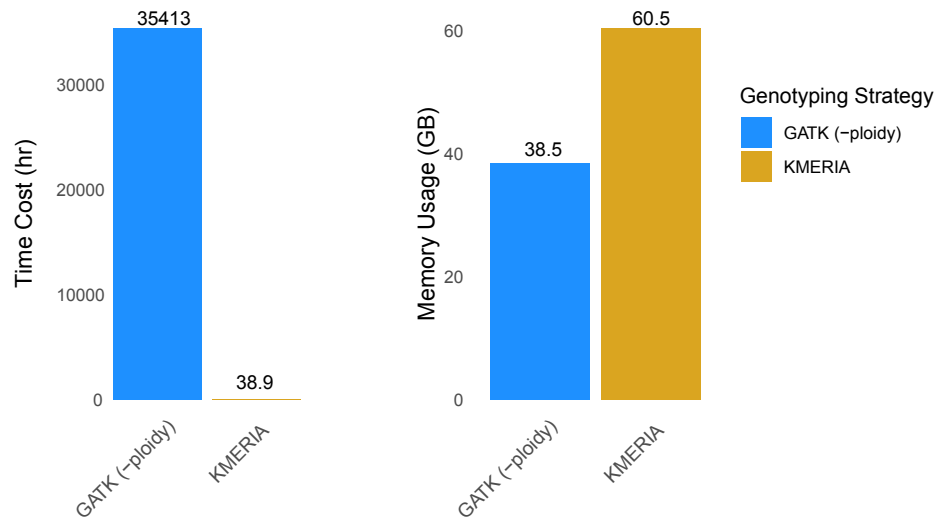


Figure S8. Comparative benchmarking of computational efficiency (time cost) and memory utilization between GATK (ploidy-aware mode) and KMERIA pipelines for tetraploid potato population genotyping, evaluated using 100 re-sequenced accessions with 8 CPUs.

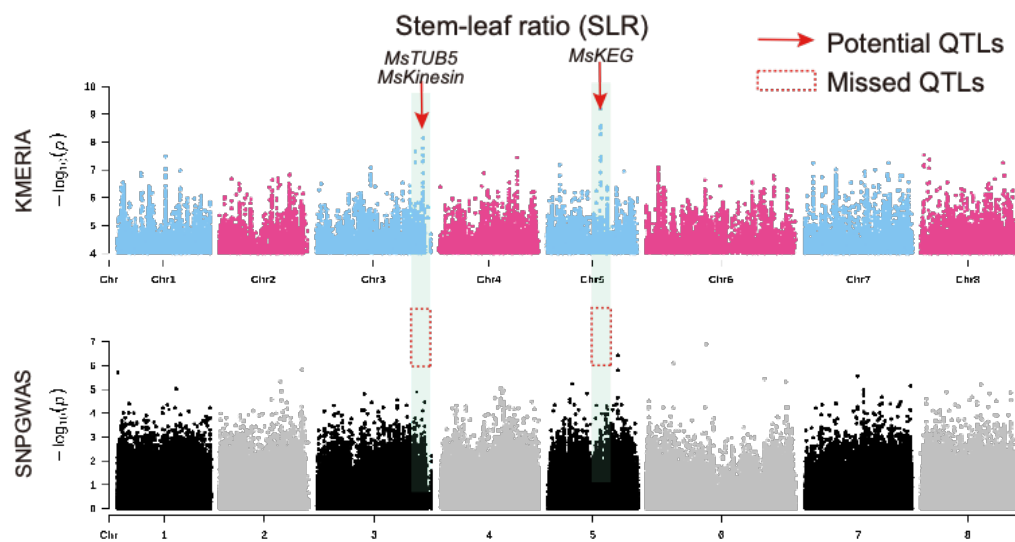


Figure S9. Power comparison between KMERIA and GWASpoly for tetraploid genome-wide association studies, evaluated using resequencing data from alfalfa population for Stem-leaf ratio (SLR) trait.

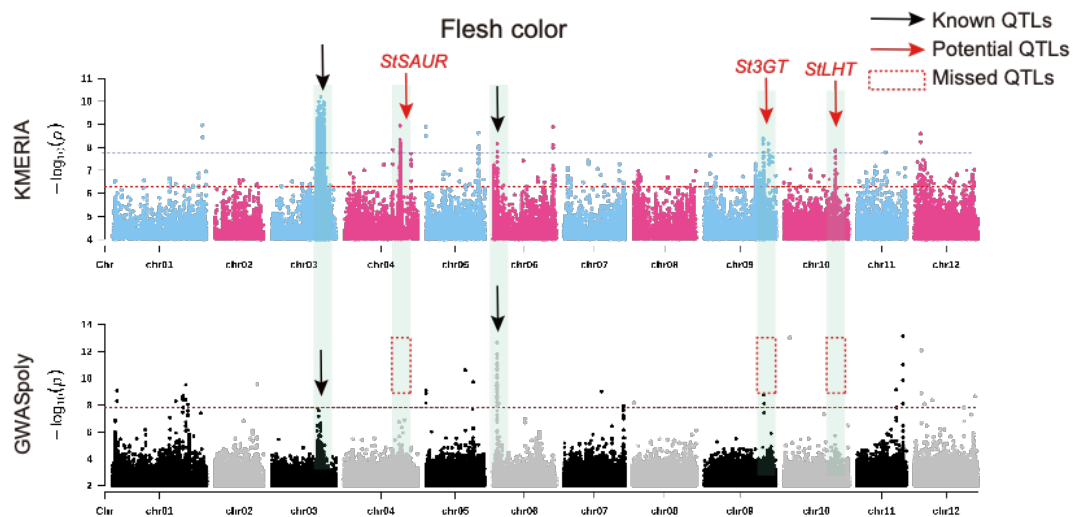


Figure S10. Power analysis of KMERIA versus GWASpoly for tetraploid genome-wide association studies, evaluated using resequencing data from a potato population for Flesh color trait.

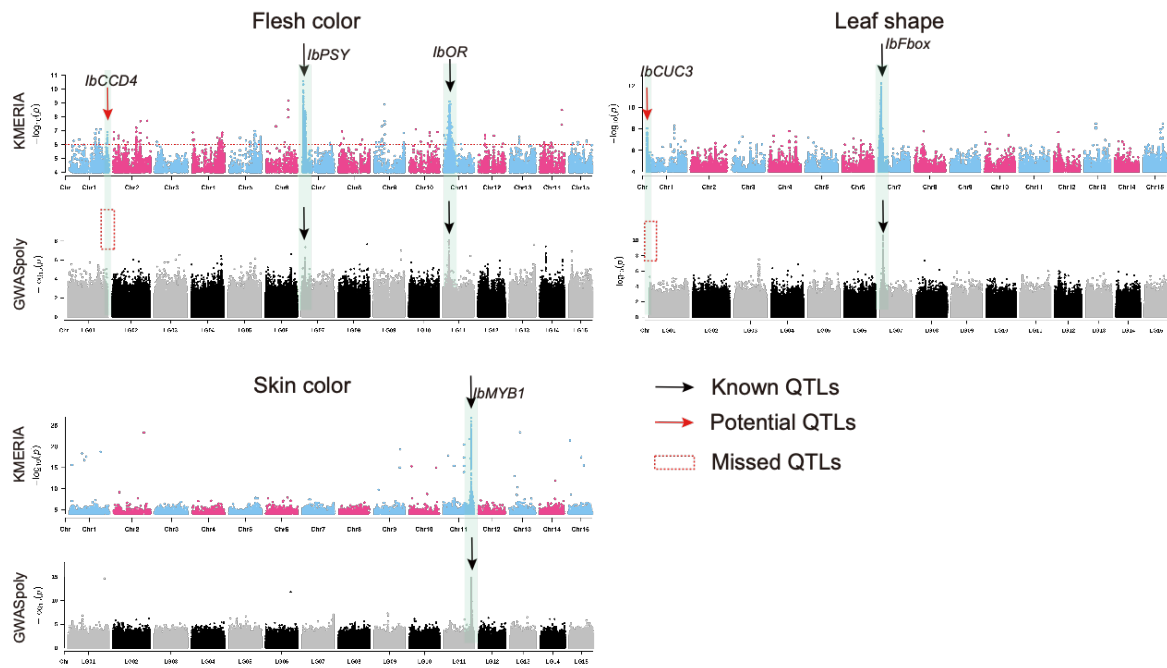


Figure S11. Power analysis of KMERIA versus GWASpoly for hexaploid genome-wide association studies, evaluated using resequencing data from a sweet potato population across three agronomic traits (Flesh color, Leaf shape, and Skin color).

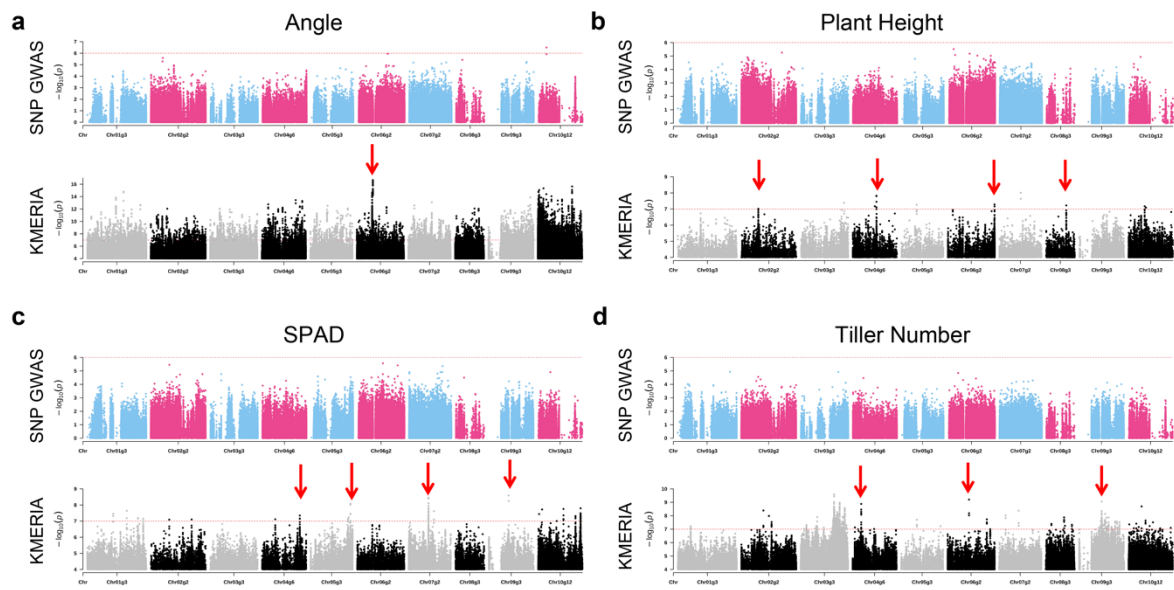


Figure S12a-d. Power analysis of KMERIA versus SNP-based GWAS for cultivated sugarcanes (*S. hybrid*), evaluated using resequencing data from a 603 re-sequenced data across four agronomic traits (Tiller angle, Plant height, SPAD, and Tiller number).



Figure S13. Phylogenetic relationship NJ tree of *S. spontaneum* germplasm accessions derived from Mash distance matrices of k-mer sets.

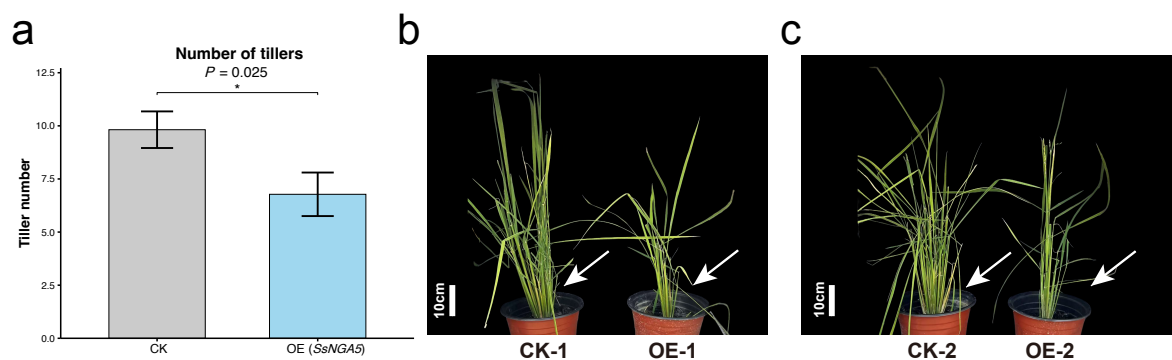


Figure S14. Phenotypic effects of *SsNGA5* overexpression in rice. a. Comparison of CK-type (n=11) and *SsNGA5*-OE (n=9) lines after 3 months growth under standard laboratory conditions ($P=0.025$, Student's t-test). b-c. Representative samples showing tiller number differences between CK-type and *SsNGA5*-OE plants. Arrows indicate tillers. Scale bar, 10 cm.