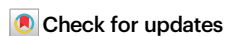


Pan-genome analysis uncovers extensive structural variations in Africa's indigenous legume crop cowpea

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Cowpea (*Vigna unguiculata* L. Walp.) is an Africa-originated legume providing a vital source of protein for millions across resource constrained areas of Africa, Asia and Latin America. Understanding genetic diversity in cowpea is pivotal to its genetic improvement. Here, we assemble 20 high-quality genomes representing cowpea global diversity and construct a graph-based pan-genome to characterize genetic variation. The pan-genome comprises 29,557 orthogroups, with approximately 73% conserved across all accessions. Extensive structural variations (SVs) are uncovered, affecting thousands of coding sequences and gene expression. Thousands of SVs are found under selection between two cowpea subspecies, highlighting their roles in subspecies divergence. We pinpoint key SVs underlying variation in pod length and seed number per pod. Two SV clusters appear to modulate pod length through altering expression and function of *VuWAK* and *VuGA2ox2* genes. These findings provide insights into cowpea genetic diversity and establish a fundamental resource for cowpea genetic improvement.

Africa is facing an unprecedented hunger and malnutrition crisis due to food shortages (FAO)¹. Indigenous crops, with their adaptation to local environments, play a pivotal role in ensuring regional food security. Among these, cowpea (*Vigna unguiculata* L. Walp., 2n = 22) is the second most widely cultivated legume in Africa, owing to its resilience in hot and drought-prone environments². As a legume crop with efficient nitrogen fixation, cowpea provides a critical source of protein for hundreds of millions of people across sub-Saharan Africa, East Asia and Latin America^{3,4}.

Substantial variation in morphological traits has been observed in cowpea⁵. As a result, two major cowpea subspecies have been identified, including the grain-type *V. unguiculata* ssp. *unguiculata* (Vu), predominantly used for dry seed production in Africa, and the vegetable-type *V. unguiculata* ssp. *sesquipedalis* (Vs), favored for its uniquely tender and elongated pods in East Asia⁶. The two subspecies show marked differences in a range of agronomic traits, including plant architecture, growth habit and pod morphology. Improving cowpea productivity through breeding relies on the effective

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exploration and utilization of cowpea genetic diversity. However, the underlying genetic diversity in cowpea remains largely untapped⁷.

Genetic variation is the raw material upon which human selection acts to improve crop productivity⁸. The assembly of reference genomes and subsequent re-sequencing studies have reported millions of sequence variations in cowpea by mapping short-read sequences to the cowpea reference genome^{6,9,10}. Evidence suggests a single reference genome is inadequate to fully capture genetic variation within a species^{11–13}. Reference-based genome approaches often overlook gene content variation and structural variations (SVs), which are increasingly recognized as key drivers of trait variation and crop evolution^{14,15}. Characterizing these types of variation and their impact is critical for cowpea improvement and breeding. Large structural variants have been reported in cowpea through a recent comparison of six cowpea genomes assembled using short read sequencing¹⁶. While informative, the genotypes selected represent only a subset of the global diversity of cowpea germplasm (Supplementary Fig. 1).

Here, we assemble 20 high-quality cowpea genomes representative of global cowpea diversity and construct a pan-genome to characterize gene content variation and SVs. Leveraging the graph-based cowpea pan-genome, we explore the potential roles of SVs in sub-species divergence and the genetic regulation of key agronomic traits. This study deepens our understanding of genetic diversity in cowpea and offers a valuable resource to accelerate its improvement and breeding.

Results

High-quality de novo genome assemblies and annotation of cowpea accessions

To capture the diversity in cowpea, SNP variation within 9609 accessions were assessed from a global collection sourced from five major global genebanks (see Methods). A total of 20 accessions representing the genetic diversity of this collection were selected for genome sequencing (Fig. 1a, b and Supplementary Figs. 1–3). Six previously published high-quality cowpea genomes (NJ, A147, G98, G323, IT97K-499-35, and FC6)^{6,10,17–19} were also utilized to develop a globally diverse cowpea pan-genome consisting of 26 genomes in total. These 26 accessions included six accessions from the Vs subspecies and 20 accessions from the Vu subspecies (Fig. 1c and Supplementary Data 1).

Cowpea is a predominantly self-pollinating, cleistogamous crop with low heterozygosity (Supplementary Table 1). A total of 408.23 gigabases (Gb) of PacBio circular consensus sequencing reads were generated for the 20 cowpea accessions with an average depth of 36× for each accession (Supplementary Data 2). Each genome was assembled individually using Hifiasm. The resulting genomes ranged from 557.03 to 606.59 megabases (Mb) in size, with contig N50 values > 24.24 Mb (Table 1). Genome completeness assessments revealed that the 20 newly generated genomes captured an average of 98.55% of benchmarking universal single-copy orthologues (BUSCO)²⁰ with assembly consensus quality values (QV)²¹ ≥ 59.65 (Table 1). Genome error rates were assessed using the Clipping Reveals Assembly Quality²² (CRAQ) analysis, generating regional assembly quality indicators (R-AQI) and overall assembly quality indicators (S-AQI) averaged at 98.83 and 98.40, respectively, for the 20 genomes (Supplementary Data 1). These quality metrics indicated the 20 assembled genomes are high-quality. A telomere-to-telomere (T2T) genome of the cowpea elite cultivar FC6 was used as a reference in the subsequent analyses^{6,23}. Reference-guided scaffolding for the 20 selected accessions achieved an average anchoring rate of 95.75% (Table 1). To evaluate chromosome anchoring and orientation, three represent accessions (Vung05, Vung14, and Vung17) were selected and subjected to Hi-C sequencing. Collinearity analysis between the Ragtag scaffold assemblies and the Hi-C anchored assemblies showed high concordance and no obvious structural inconsistencies (Supplementary Fig. 4), supporting the reliability of the assemblies. Compared to the previously published

cowpea pan-genome¹⁶, our assembled genomes exhibit a longer contig N50 and a higher BUSCO value (Supplementary Table 2). A high degree of genome consistency was observed among the 26 cowpea accessions (Fig. 1d) with an average synteny relationship index²⁴ value as high as 0.94.

To minimize the annotation discrepancies caused by different pipelines, the 26 cowpea genomes were annotated using a standardized approach integrating ab initio gene prediction, homology-based searches and RNA-seq evidence. An average of 29,297 protein-coding genes were annotated for these assemblies, with a mean BUSCO completeness of 98.70% (Table 1). These annotated genes were well supported by expression and homology evidence. Over 90% of the annotated genes had RNA-seq matches (Supplementary Table 3). An average of 87.09% of annotated genes showed matches with the known functional databases, including Pfam²⁵, SwissProt²⁶, GO²⁷, and KEGG²⁷ (Supplementary Table 4). Transposable elements (TEs) accounted for an average of 42.26% of the genome (range: 39.61–44.72%; Fig. 1e and Supplementary Fig. 5), and their abundance increased with genome size ($R = 0.61$, $P = 0.04$; Supplementary Fig. 6). Taken together, these data suggested consistent and robust gene annotations.

Pan-genome construction and diversity analysis in cowpea

To investigate gene content variation in cowpea, we conducted a pan-genome analysis of the 26 genomes. A gene-based pan-genome analysis demonstrated that cowpea has a closed pan-genome, as the number of orthogroups plateaued around 19 genomes (Fig. 2a). A total of 29,557 orthogroups were identified in the developed cowpea pan-genome. The cowpea pan-genome was found to be relatively conserved, comprising 21,620 core orthogroups (73.2%) shared among all 26 genomes. By contrast, 7867 dispensable orthogroups (26.6%) were found between 2 and 25 genomes, while 70 private orthogroups (0.3%) appeared in only one genome (Fig. 2b and Supplementary Fig. 7). On average, core genes accounted for 78.7% of genes in the cowpea genome (Fig. 2c). The high proportion of core genes in the pan-genome and individual genomes of cowpea mirrors a pattern observed in cultivated soybean and mung bean^{28,29}.

Core genes exhibited longer coding DNA sequences with lower nucleotide diversity (π) and non-synonymous to synonymous substitution ratios (K_a/K_s) compared to dispensable and private genes (Fig. 2d–f), suggesting their greater functional conservation. Expression analysis revealed that core genes had the highest mean expression levels, with dispensable genes showing moderate expression and private genes the lowest (Fig. 2g). Further functional enrichment analysis showed that core genes were enriched in fundamental biological processes, including nucleobase-containing compound metabolism, cellular processes, and cellular component organization (Fig. 2h). By contrast, dispensable and private genes were enriched with stress-related functions, such as response to biotic stimuli, response to external stimuli, and secondary metabolic processes (Fig. 2i). This indicates the fast-evolving dispensable genes could play a pivotal role in environmental adaptation in cowpea.

Genomic landscape of structural variations in cowpea

Given the critical roles that SVs play in regulating agronomic traits and adaptation³⁰, we investigated the genomic landscape of SVs in cowpea using our high-quality genomes. By comparing the 25 genomes with the FC6 reference, we identified a total of 62,591 non-redundant SVs, including 29,625 insertions (INS), 25,489 deletions (DEL), 368 inversions (INV), 4096 duplications (DUP), and 3013 translocations (TRANS) (Fig. 3a). These SVs were unevenly distributed across 11 chromosomes with an enrichment in heterochromatin regions ($P = 1.56 \times 10^{-8}$, Wilcoxon test, Supplementary Fig. 8 and Supplementary Table 5). An average of 9358 SVs were detected in each genome with Vu accessions containing more SVs than Vs accessions, probably due to the closer

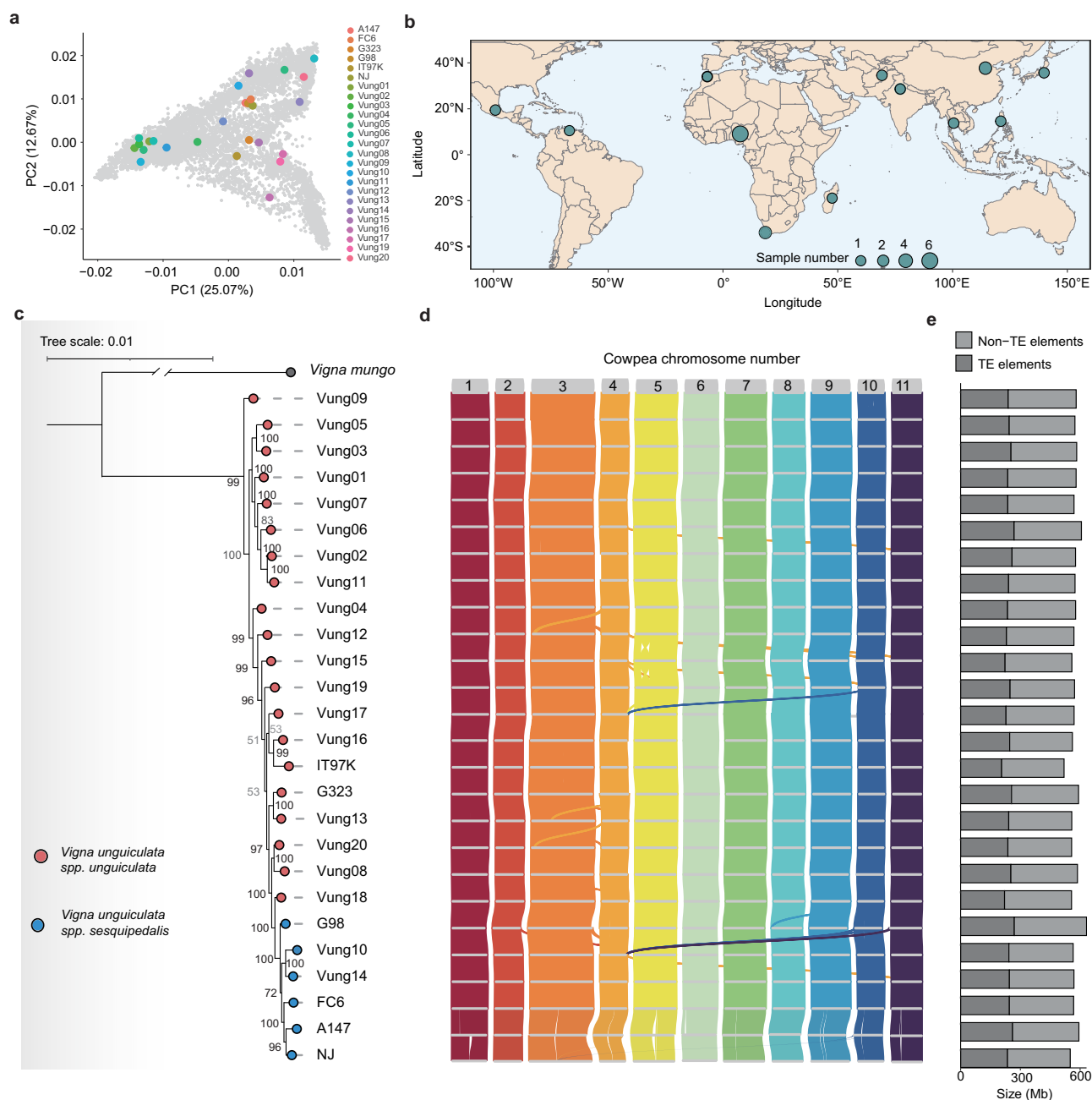


Fig. 1 | Geographic distribution and genomic feature analysis of cowpea accessions. **a** Principal component analysis (PCA) based on single-nucleotide polymorphisms (SNPs) of cowpea accessions. Each dot represents an accession, with colored dots indicating cultivars selected for pan-genome analysis. **b** Geographic distribution of cultivated cowpea accessions, with dot size proportional to sample size. **c** Phylogenetic tree of 26 representative cowpea accessions,

using *Vigna mungo* as the outgroup. Red solid dots represent *Vigna unguiculata* ssp. *unguiculata*, and blue solid dots represent *Vigna unguiculata* ssp. *sesquipedalis*. **d** Genomic collinearity among pan-genome accessions. **e** Genome size (Mb) and proportions of non-repetitive (light grey) and repetitive (dark grey) sequences for each cowpea accession.

relationship between Vs and the FC6 reference (Fig. 3b and Supplementary Fig. 9). The number of pan-SVs has not reached a plateau with the 26 genomes, indicating that more SVs could be detected when extra genomes were included (Fig. 3c). We randomly selected 20 SVs located within genic region for PCR validation, all of which were confirmed (Supplementary Fig. 10 and Supplementary Table 6), demonstrating the high accuracy of the SV detection.

These SVs covered 329.2 Mb of genomic sequence, equivalent to >50% of the reference genome. Significant differences in variation length were observed among the five types of SVs, with DUP, INV, and TRANS being longer than DEL and INS (Fig. 3d and Supplementary

Fig. 11a). INV, which accounted for only 0.6% of the total SV number, contributed to 44.13% of the total SV length (Supplementary Table 7). There were 26 SVs larger than 1 Mb, each encompassing multiple genes. They included three TRANSs, seven DUPs, and 16 INVs (Supplementary Table 8). For example, a 4.8 Mb inversion containing 85 genes was detected on chromosome 10 (Fig. 3e, Supplementary Fig. 12 and Supplementary Data 3). These 85 genes included a cluster of homologs of the powdery mildew resistance gene *RUN1*³¹ (*Vu10G0620*, *Vu10G0623*, *Vu10G0625*–*Vu10G0627*, *Vu10G0630*, *Vu10G0641* and *Vu10G0642*), a seed maturation-related gene *VuMYB118* (*Vu10G0599*)³², and a petal morphogenesis gene *VuASI* (*Vu10G0648*)³³.

Table 1 | Statistics of assemblies and annotation of cowpea accession genomes

Sample ID	Assembly size (Mb)	Anchored rate (%)	Contig N50 (Mb)	GC content (%)	TE content (%)	Gene number	Annotation completeness (BUSCO, %)	QV
Vung01	580.03	95.73	29.26	39.33	40.82	29,683	98.7	63.63
Vung02	577.16	96.08	26.39	36.70	44.72	29,521	98.8	63.79
Vung03	582.98	95.81	43.20	40.90	43.39	28,839	98.8	63.85
Vung04	577.57	95.68	34.14	42.13	40.82	28,726	98.7	64.36
Vung05	574.07	97.40	35.00	38.55	42.51	28,736	98.7	64.72
Vung06	606.59	93.84	25.92	37.37	44.27	30,459	98.8	61.19
Vung07	569.46	96.02	40.46	36.00	41.60	29,244	98.8	64.15
Vung08	586.79	92.71	30.27	34.38	43.01	33,180	98.6	59.65
Vung09	580.20	95.34	24.80	38.18	41.16	30,779	98.8	63.71
Vung10	565.12	95.50	24.24	40.64	42.89	28,328	98.9	63.74
Vung11	574.56	96.63	32.79	35.98	41.92	28,826	98.8	63.79
Vung12	569.10	95.27	26.88	37.15	40.46	30,605	98.6	63.36
Vung13	558.21	96.98	30.05	38.30	43.28	28,603	98.8	63.99
Vung14	569.13	96.04	33.02	41.22	43.05	29,115	98.9	63.96
Vung15	558.53	96.47	40.31	36.28	39.96	30,008	98.6	63.55
Vung16	561.33	96.17	26.85	38.26	43.89	28,911	98.6	63.17
Vung17	569.87	94.87	31.84	35.83	39.99	31,599	98.8	62.82
Vung18	557.03	96.60	25.60	37.27	39.61	29,792	98.7	63.71
Vung19	572.19	95.54	27.50	39.78	43.39	29,386	98.9	63.43
Vung20	557.49	96.27	32.89	36.25	42.58	29,485	98.9	63.81
FC6*	567.48	95.60	46.12	33.61	43.14	29,940	98.8	63.42
IT97K**	519.44	91.15	–	32.82	39.87	27,781	97.5	–
G323**	592.27	93.31	–	33.81	43.08	27,679	98.7	–
G98**	632.31	89.87	–	34.28	42.62	28,372	98.8	–
NJ**	550.31	99.37	–	33.41	42.78	27,610	98.7	–
A147**	593.98	90.80	–	33.83	43.93	31,708	98.6	–

*The full PacBio and ONT raw reads of FC6 were downloaded from the National Genomics Data Center under accession CRA007957.

**The genome assemblies of these five cultivars were accessed from previous publications^{6,10,17–19} and re-analyzed in this study.

Three haplotypes of this SV were identified, which showed a clear separation alongside the phylogenetic tree (Fig. 3f and Supplementary Fig. 13). Most of the cowpea SVs were low-frequency variation, with over 60% of them present in only one or two accessions (Supplementary Fig. 11b).

To understand the potential functional impact of SVs, SVs were annotated based on their location relative to the protein-coding genes. Although the majority (62%) of SVs were located in the intergenic region, a total of 18,946 genes (including gene bodies and their 5-kb flanking regions) overlapped with 24,220 SVs (Fig. 3g and Supplementary Fig. 14). SVs caused marked alteration of gene coding sequence with 900 high-impact gene-disrupting events identified, including transcript ablations, frameshift mutations, and gain or loss of stop codons (Supplementary Table 9). Further tests demonstrated that SVs also affected expression of their overlapping genes ($P \leq 2.2 \times 10^{-16}$, Wilcoxon test) (Supplementary Fig. 15). Gene ontology enrichment showed that the genes which overlapped with SVs were enriched in biological processes related to environmental adaptation, such as response to stress, cell communication, signal transduction, and secondary metabolic process (Supplementary Data 4).

The observed enrichment of SVs in heterochromatic regions prompted an investigation into the factors shaping their distribution. We found TEs were enriched in SV surrounding regions in cowpea ($P \leq 2.2 \times 10^{-16}$, Wilcoxon test; Fig. 3h). A sliding window test showed that the number of SVs in each window was positively correlated with the number of TEs, particularly long terminal repeat retrotransposon (LTR) and terminal inverted repeat transposons (TIR) (SV versus TE: $R = 0.26$, $P = 3.78 \times 10^{-10}$; SV versus LTR: $R = 0.44$, $P \leq 2.2 \times 10^{-16}$;

SV versus TIR: $R = 0.12$, $P = 0.01$) (Fig. 3i and Supplementary Fig. 16). Further investigation determined that LTR was the dominant type of TE surrounding all four types of SVs (Supplementary Fig. 17). The evidence indicates that TE movement is a major driver of SV formation. SVs were classified into TE-derived and non-TE-derived groups according to their overlap with TEs to evaluate their differences. Although TE-derived SVs were significantly longer than non-TE-derived SVs, they were less likely to co-locate with genes (Fig. 3j). In addition, a significant negative correlation was observed between SV number and recombination rate in cowpea ($R = 0.56$, $P \leq 2.2 \times 10^{-16}$) (Supplementary Fig. 18a), indicating that high recombination rates may facilitate SV purging given the generally deleterious effects of SVs on gene function. SV number were significantly influenced by recombination rate, especially INS and DEL (Supplementary Fig. 18b). Overall, our evidence indicated that movement of TEs and recombination could influence SV distribution.

The cowpea pan-genome uncovers structural variations associated with subspecies divergence

The cowpea pan-genome presents an excellent opportunity to investigate the roles of SVs in the divergence of two cowpea subspecies. To enable accurate genotype calling in the resequencing samples, a graph-based pan-genome of 26 genomes was constructed. The 775.55-Mb cowpea pan-genome incorporated extensive genetic variation, including 5,412,029 single-nucleotide polymorphisms (SNPs, 1 bp), 545,048 insertions and deletions (indels, 2–49 bp), and 69,245 SVs (≥ 50 bp), with a strong overlap between the SVs identified by the pan-genome method and those detected by SyRI (Supplementary Fig. 19).

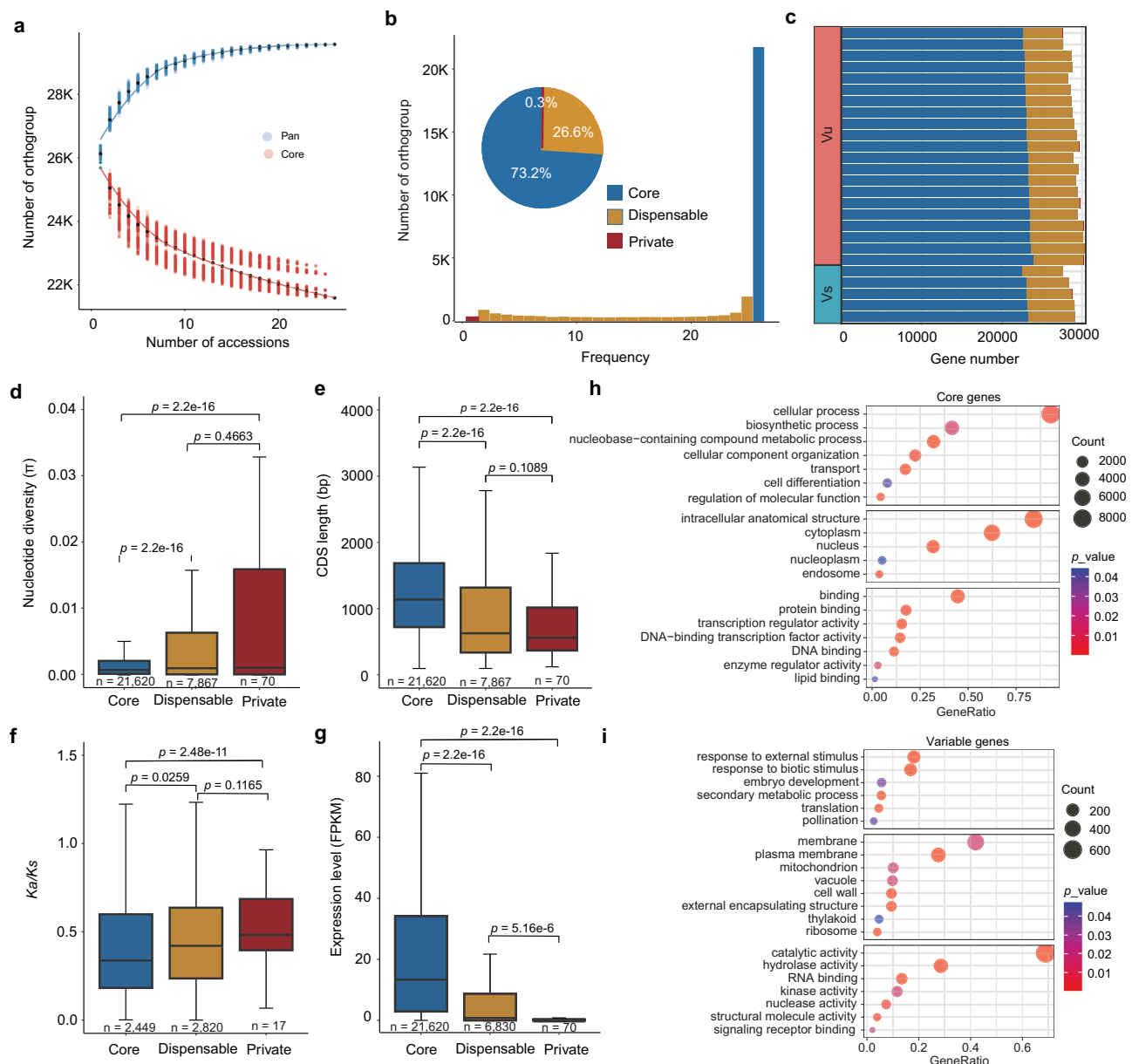


Fig. 2 | Pan-genome analysis of 26 cowpea accessions. **a** Counts of pan and core gene families, based on 1000 random samplings per count. **b** Composition of the pangenome. The histogram shows the number of orthogroups, and the pie chart illustrates the proportions of core, dispensable, and private orthogroups. **c** Composition of individual genomes, with each row representing an accession. **d–f** Comparison of nucleotide diversity (π), gene length, and K_a/K_s ratio among core, dispensable, and private genes. **g** Expression levels of core, dispensable, and private genes, measured in fragments per kilobase of transcript per million mapped

reads (FPKM). **h** Gene Ontology term enrichment for core genes. **i** Gene Ontology term enrichment for variable (dispensable and private) genes. The dot size indicating gene count and color representing the P value. In box plots, boxes span the 25th to 75th percentiles, with central lines at the median and whiskers extending to $1.5 \times$ the interquartile range. Statistical significance was determined using two-sided Wilcoxon tests, with sample sizes (n) indicated for each group. Source data are provided as a Source Data file.

Short-read sequences of 619 diverse cowpea accessions from previous studies^{6,10} were mapped to the graph-based cowpea pan-genome, identifying 61,612 SVs and 4,248,171 SNPs. Population structure analysis identified 89 Vu accessions and 235 Vs accessions with group membership $>70\%$ as group representatives for subsequent analyses (Supplementary Fig. 20).

These racial representatives were subsequently employed to identify genomic regions underlying subspecies divergence through an SV-based genome-wide association study (SV-GWAS) with racial identity as the phenotype, resulting in the identification of 126 genetic loci (Fig. 4a and Supplementary Data 5). A complementary method integrating a Pi-ratio, fixation index (F_{ST}), and a cross-population

composite likelihood ratio (XP-CLR) was also employed to screen genomic regions under selection between the Vs and Vu subspecies (Fig. 4b, and Supplementary Fig. 21). A total of 299 genomic regions covering 30.06 Mb sequences and 1851 protein-coding genes were identified (Fig. 4b and Supplementary Data 6). Combining the two approaches resulted in the identification of 2374 non-redundant candidate genes associated with cowpea subspecies divergence (Supplementary Data 7). These candidate genes were enriched in various biological processes, including responses to various stimuli (external, biotic, stress, light), cellular processes (programmed cell death, protein modification, secondary metabolism, catabolic processes), and regulation of molecular function (Supplementary Fig. 22 and

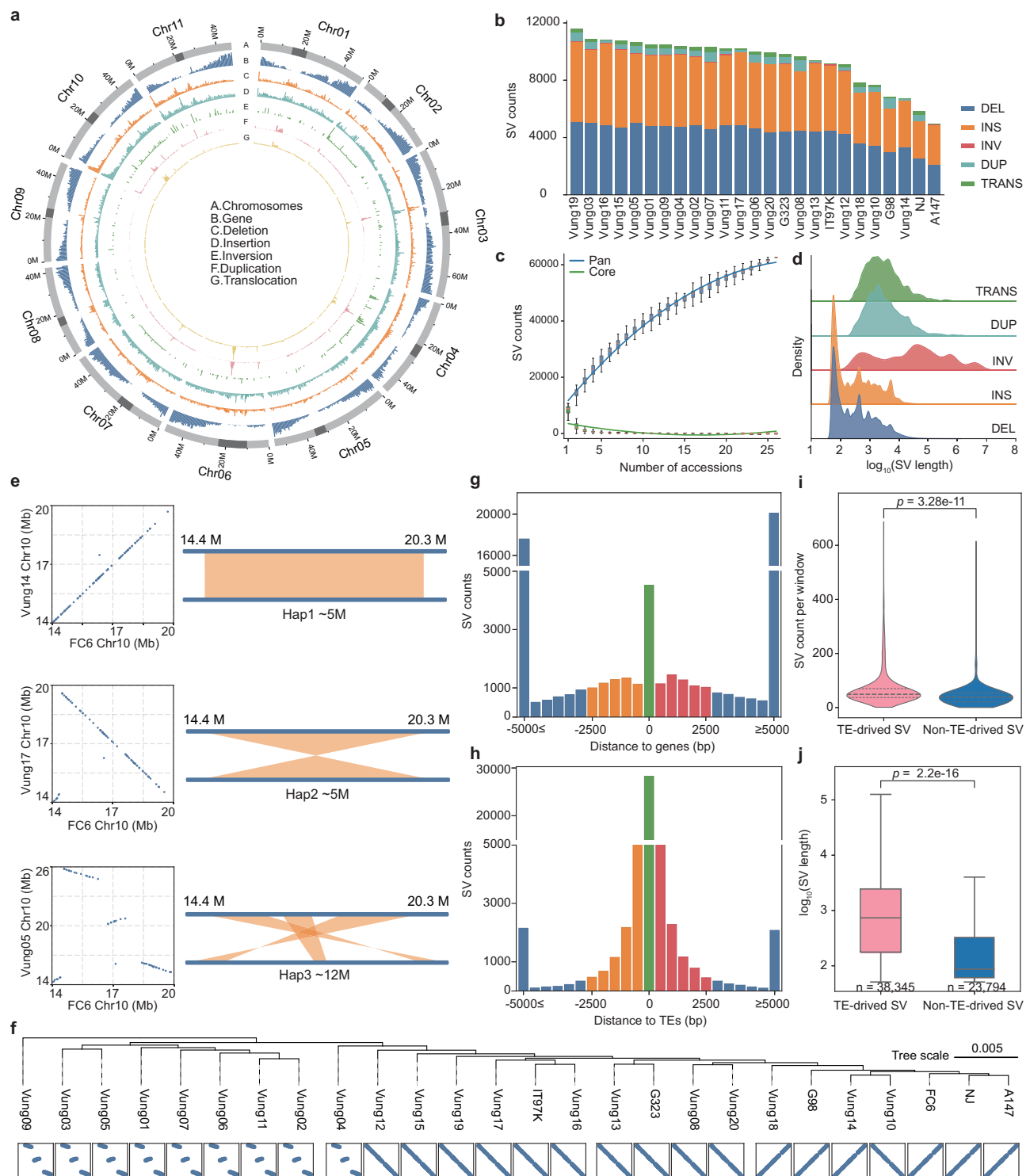


Fig. 3 | Structural variant landscape and pan-SV analysis in cowpea.

a Distribution of SVs in the FC6-T2T reference genome. (A) Chromosomes, (B) Gene density, (C) Deletions, (D) Insertions, (E) Inversions, (F) Duplications, (G) Translocations. **b** Composition of SVs across 25 cowpea accessions. **c** Numbers of pan and core SVs identified in different number of genomes, based on 1000 random sampling per genome count. **d** Length distribution of the five types of SVs. **e** Example of a complex inversion event on chromosome 10. Left: Dot plot showing genome alignment to FC6-T2T. Right: Genomic collinearity of the inverted region.

f Illustration of inversion types across 26 accessions. **g, h** SVs distribution relative to genes (**g**) and transposable elements (TEs) (**h**). **i** Violin plots showing numbers of TE-derived SVs and non-TE-derived SVs within 1-Mb windows ($n = 549$ windows). **j** Comparison of SVs length between TE-derived ($n = 38,345$) and non-TE-derived ($n = 23,794$) SVs. In box plots, boxes span the 25th to 75th percentiles, with central lines at the median and whiskers extending to $1.5 \times$ the interquartile range. Statistical significance was determined using a two-sided Wilcoxon test. Source data are provided as a Source Data file.

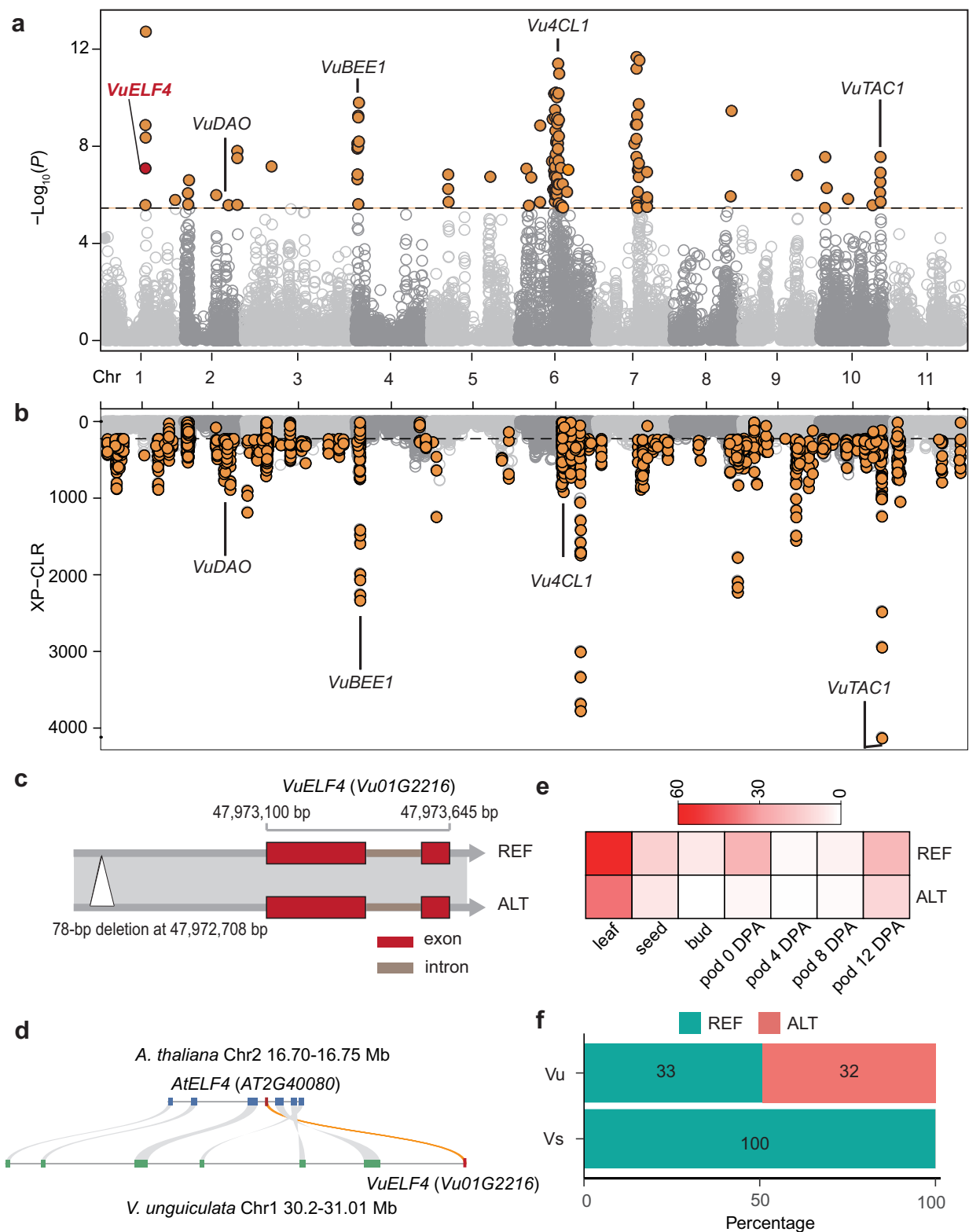


Fig. 4 | Identification of structural variants underlying divergence between *V. unguiculata* subsp. a Manhattan plot showing SV-based GWAS of subspecies identity in cowpea. Colored dots represent significant loci, and the horizontal line indicates the genome-wide significance threshold. Statistical significance was assessed using 0.05/SV number. **b** Manhattan plot displaying XP-CLR scores between Vs and Vu, with horizontal dotted lines indicating the top 5% threshold.

Regions identified by at least two of XP-CLR (top 5%), Pi-ratio (bottom 5%), and F_{ST} (top 5%) are highlighted in orange. **c, d** Example of an identified candidate gene underlying subspecies divergence: gene structure of *VuELF4* and its synteny with the homologous *AtELF4* in *Arabidopsis thaliana*. **e** Expression levels of *VuELF4* in different tissues and their comparison between REF and ALT genotype accession. **f** Frequency of the 78-bp deletion in *VuELF4* across Vu and Vs populations.

Supplementary Data 8), indicating that the divergence between the two subspecies involves multiple complex biological processes. Comparing these results with previous SNP-based selective sweep analyses reveals that 21.5% of the identified candidate genes overlap (expected overlap ≈ 76 genes; hypergeometric test, $P < 1 \times 10^{-100}$; Supplementary Fig. 23a, b)¹⁰, supporting a strong concordance between the two studies. Functional annotation of the non-overlapping genes shows an enrichment of biological functions related to environmental adaptation, supporting their role in racial divergence (Supplementary Fig. 23c).

Multiple candidate genes in the differentiated regions were associated with the distinct agronomic traits between the two cowpea subspecies (Fig. 4a, b). *VuDAO* (*Vu02G1064*), encoding a 2-oxoglutarate-dependent dioxygenase, is a candidate for regulating pod development and length through auxin biosynthesis³⁴. *VuBEE1* (*Vu04G0211*) is a basic helix-loop-helix type transcription factor, controlling photoperiod flowering in *Arabidopsis thaliana*³⁵. *VuTAC1* (*Vu10G1353*), encoding a tiller angle control protein, regulates lateral branch angles through influencing polar auxin transport in peach³⁶. Likewise, *Vu4CL1* (*Vu06G0638*), encoding a 4-coumarate CoA ligase, is a crucial gene in lignin biosynthesis in *Arabidopsis* and may contribute to plant architecture and erect growth habit³⁷. Interestingly, the cowpea homolog of the *Arabidopsis thaliana* circadian rhythm and flowering regulator gene *EARLY FLOWERING 4* (*AtELF4*)³⁸, *VuELF4* (*Vu01G2216*), lies within a differentiated region at 47.97 Mb on chromosome 1 (Fig. 4c, d). A 78-bp deletion in the *VuELF4* promoter, encompassing the core promoter element TATA-box and the light-responsive GA-motif, was identified, implying an alteration in gene expression (Fig. 4c). Expression analysis showed that *VuELF4* is predominantly expressed in cowpea leaves, and this deletion reduces its expression level (Fig. 4e), potentially leading to delayed flowering³⁹. This deletion was present in the 50% Vu accessions, but was fixed in the Vs accessions (Fig. 4f), suggesting a potential role in environmental adaptation.

Allele frequency analyses of SVs identified 578 and 109 SVs that were fixed in the Vu and Vs subspecies group, respectively (Supplementary Fig. 24 and Supplementary Data 9). A total of 89 genes were identified that overlapped with these fixed SVs. Functional enrichment of these genes showed they were related to flower development, responsiveness to endogenous stimulus, lipid metabolic processes, and transport (Supplementary Data 10), suggesting they may be critical in the adaptation of the two subspecies to different environmental conditions and human selection pressure. These fixed SVs provide critical resources for functional studies to elucidate molecular mechanisms underlying cowpea subspecies divergence.

Structural variations underlying key cowpea agronomic traits

To investigate the role of SVs in genetic regulation of agronomic traits in cowpea, we conducted SV-based GWAS of pod length (PL) and seed number per pod. Eight QTL associated with pod length were identified, located on chromosome 3 (*PL 3.1*), chromosome 5 (*PL 5.1*, *PL 5.2* and *PL 5.3*), chromosome 8 (*PL 8.1* and *PL 8.2*), chromosome 9 (*PL 9.1*), and chromosome 10 (*PL 10.1*) (Fig. 5a, Supplementary Fig. 25a and Supplementary Table 10). *PL3.1* and *PL9.1* overlapped with pod length QTL reported in a previous study⁶, while the other QTLs are located outside previously reported pod-length regions. Six of these QTL that were significant related to pod length were further validated using a haplotype-based analysis (Fig. 5b and Supplementary Fig. 26). Accessions carrying more favorable alleles of the six loci exhibited longer pods (Fig. 5c and Supplementary Fig. 27), indicating potential pyramiding effects on regulating pod length.

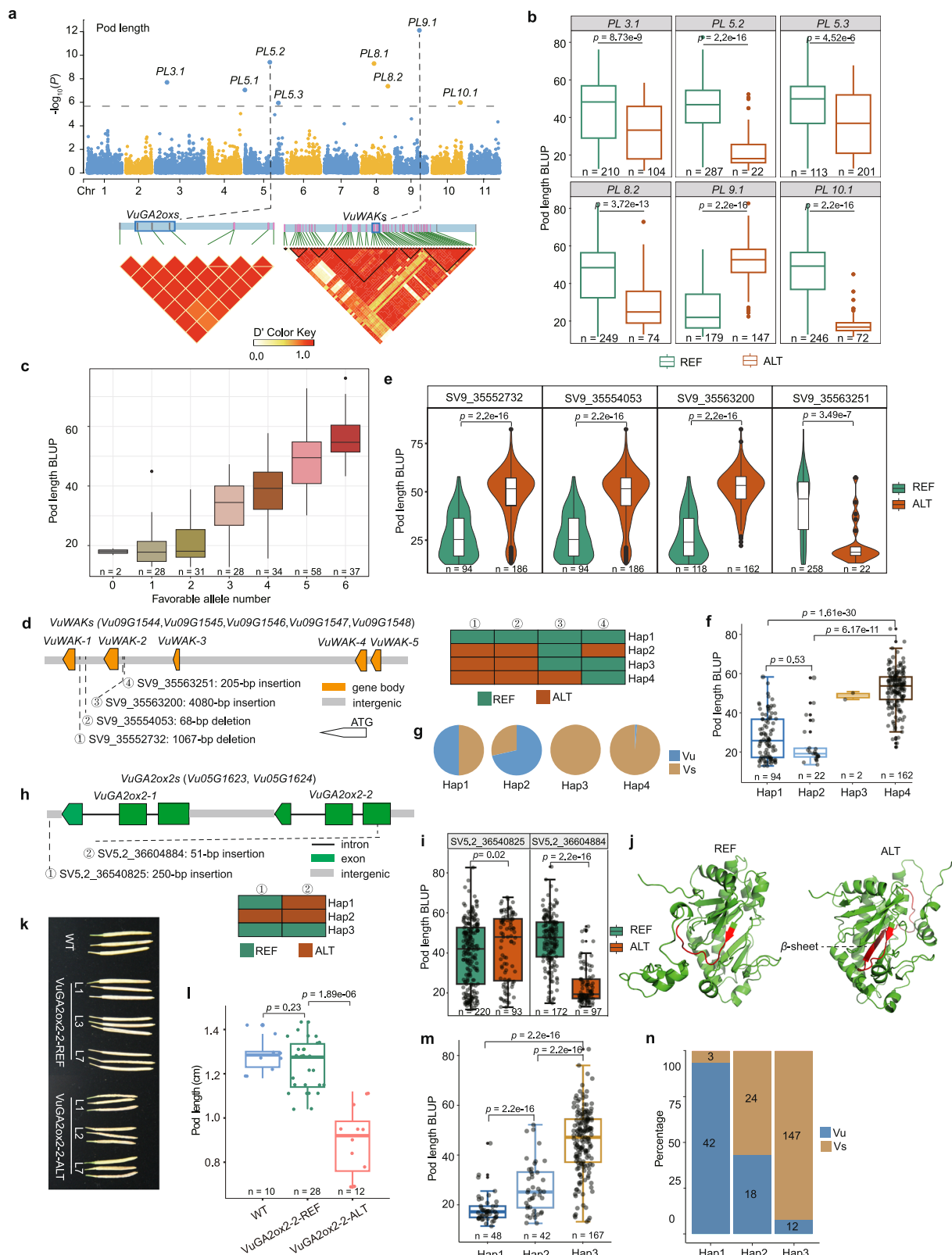
Candidate genes and SVs in these pod length QTL were examined to identify possible causal variations. A tandem duplication consisting of five *Wall-Associated Receptor Kinase* (*VuWAK*) genes, involved in the regulation of cell shape and size and affect silique length in *Arabidopsis*

*thaliana*⁴⁰, was found in *PL9.1* with the highest significance signal in GWAS analysis (Fig. 5d). Two deletions (SV9:35552732, 1067 bp; SV9:35554053, 68 bp) in the promoter of *VuWAK-1* and two insertions (SV9:35563200, 4080 bp; SV9:35563251, 205 bp) in the promoter of *VuWAK-2* were identified, each having significant effects on pod length (Fig. 5d, e). Two *VuWAK* genes were predominantly expressed in pods, and genotypes carrying different alleles of these SVs exhibited variable expression levels of the two *WAK* genes during pod development (Supplementary Fig. 28). Luciferase reporter assays showed that the SV9:35552732 deletion in the promoter region of *VuWAK-1* markedly enhanced promoter activity, whereas the SV9:35563251 insertion in the *VuWAK-2* promoter reduced promoter activity (Supplementary Fig. 29).

Four haplotypes (Hap1-4) were defined by the four SVs due to high LD among these physically close SVs (Fig. 5d). Significant differences in pod length were observed among these four types of haplotypes with Hap4 accessions displaying the longest pods (Fig. 5f). Subspecies Vs exhibits significantly longer pods than subspecies Vu, reflecting human-mediated selection for longer pods for fresh consumption (Supplementary Fig. 30). Hap3 and Hap4 were mainly found in the Vs population, while more than half of Hap1 and Hap2 were present in Vu accessions (Fig. 5g). These results suggest that the four SVs may contribute to pod length difference between Vu and Vs through altering expression of the two *WAK* genes.

Additionally, a tandem duplication comprising two *VuGA2ox2* genes (*Vu05G1623* and *Vu05G1624*) was found at the second highest significance loci (*PL5.2*) (Fig. 5h). *GA2ox2* encodes a key enzyme that catalyzes the deactivation of bioactive gibberellins, which negatively regulates pod length in *Arabidopsis thaliana*^{41,42}. Two SVs including a 250-bp insertion in the downstream of *Vu05G1623* and a 51-bp insertion in the third exon of *Vu05G1624* were significantly correlated with pod length (Fig. 5i). Alignment of the *Vu05G1624* sequence in the pan-genome and its ortholog in related *Vigna* species demonstrated that this 51-bp DNA fragment is absent in nearly all Vs accession, but present in all Vu accessions and related *Vigna* species (Supplementary Fig. 31). Protein structure modelling showed the absence of this 51-bp sequence in *VuGA2ox2-2* led to the deletion of a β -sheet in 3D structure of the encoded protein, which reduced its binding affinity to bioactive gibberellins (Fig. 5j and Supplementary Table 11). Consistently, overexpression of *VuGA2ox2-2* containing the 51-bp insertion (*VuGA2ox2-2-ALT*) in *Arabidopsis thaliana* significantly reduced pod length, whereas overexpression of the reference type (*VuGA2ox2-2-REF*) showed no significant effect, suggesting that the insertion may reduce overall gibberellin levels and consequently inhibit pod elongation (Fig. 5k, l). Three haplotypes combining the two SVs showed significant effects on pod length regulation (Fig. 5m). The distinct subspecies composition in the three haplotypes supports the combination of two SVs underlying the variation of pod length between the two subspecies (Fig. 5n).

Seed number per pod is one of the key yield components of grain cowpea⁴³. Our SV-based GWAS of seed number per pod identified three QTLs, including *seed number per pod 4*, which overlaps with a previous reported QTL⁶. While the previous study could not pinpoint a causal gene, our graph-based pan-genome provided the resolution to identify a high-confidence candidate. The strong association signal on chromosome 4, which harbored *Vu04G0313* encoding a pentatricopeptide repeat (PPR) protein (Fig. 6a, b and Supplementary Fig. 25b). *VuPPR* is a homolog of the *EMBRYO-DEFECTIVE* gene *AtEMB1586*⁴⁴, that controls embryo development and fertility rate in *Arabidopsis thaliana* (Supplementary Fig. 32). A 83-bp deletion was found in the exon of *VuPPR* causing a premature stop codon at the 134th amino acid (Fig. 6c). Accessions with the deletion exhibited significantly lower seed number per pod (Fig. 6d). The two cowpea subspecies had pronounced differences in allelic composition of this SV (Fig. 6e). The deletion was predominately found in Vu accessions, consistent with their lower seed number per pod (Fig. 6f). These results



suggest that the 83-bp deletion in *VuPPR* may influence variation of seed number per pod in cowpea.

Discussion

Genetic improvement of cowpea holds the key to ensuring food security in the sub-Saharan region, which in turns relies on effective utilization of genetic diversity within global germplasm^{1,4,11}. In this

study, our construction of the graph-based cowpea pan-genome using 20 new de novo assemblies and six existing genomes represents a paradigm shift from single-reference genomics to a population scale understanding of cowpea. Our study systematically characterizing gene content variation and structural variations. We found that approximately 73% of orthogroups in cowpea pan-genome are core genes, indicating a relatively conserved gene composition in cultivated

Fig. 5 | Structural variants associated with pod length in cowpea. **a** Manhattan plot displaying SV-based GWAS results for pod length, with linkage disequilibrium heatmaps of the top two significant loci. The horizontal line denotes the genome-wide significance threshold. **b** Box plots validating effects of identified significant loci on pod length. **c** Box plots illustrating the effects of different combinations of favorable alleles on pod length. **d** Gene structure of *VuWAK* genes and locations of four SVs. *VuWAK*s include *VuWAK-1* (*Vu09G1544*), *VuWAK-2* (*Vu09G1545*), *VuWAK-3* (*Vu09G1546*), *VuWAK-4* (*Vu09G1547*) and *VuWAK-5* (*Vu09G1548*). **e** Effects of the four SVs in *VuWAK-1* and *VuWAK-2* on pod length. **f** Box plots showing effects of combined haplotypes of the four SVs in *VuWAK-1* and *VuWAK-2* on pod length. Each dot represents an accession. **g** Pie charts depicting the subspecies composition of the combined haplotypes of the four SVs in *VuWAK-1* and *VuWAK-2*. **h** Gene structure of *VuGA2ox2s* and locations of two SVs. *VuGA2ox2s* include *VuGA2ox2-1* (*Vu05G1623*) and *VuGA2ox2-2* (*Vu05G1624*). **i**, Effects of the two SVs in *VuGA2ox2* genes on pod length. **j** Changes of protein structure led by the 51-bp insertion in

VuGA2ox2-2. Three-dimensional protein structure was modeled by AlphaFold3. Regions colored in red correspond to the 51-bp insertion and 5 amino acid flanking regions. **k** Representative siliques from wild-type (WT), *VuGA2ox2-2*-REF, and *VuGA2ox2-2*-ALT overexpression lines in *Arabidopsis thaliana*. **l** Pod length measurements of WT (control) and transgenic lines. Each dot represents an individual transgenic line. Pod length was calculated as the mean of five siliques sampled from the middle portion of each plant. Each line represents a biological replicate, with at least ten independent transgenic lines included. **m** Box plots showing the effects of combined haplotypes of the two SVs in *VuGA2ox2* genes. Each dot represents an accession. **n** The subspecies composition of combined haplotypes of the two SVs in *VuGA2ox2* genes. In box plots, boxes represent the 25th to 75th percentiles, with central lines at the median and whiskers extending to 1.5× the interquartile range. Significance was determined by two-sided Wilcoxon tests, with sample sizes (*n*) indicated for each group. Source data are provided as a Source Data file.

cowpea. This high core gene proportion in cowpea is comparable to other self-pollinating legumes like soybean and mungbean, yet stands in stark contrast to the highly fluid pan-genomes of outcrossing cereals such as maize^{13,28,29,45}, possibly reflecting unique features of legumes in terms of its cleistogamous mating system and specific TE composition³⁰. However, the dispensable genome, which comprises 26.6% of orthogroups, is significantly enriched in stress-responsive and secondary metabolic pathways. This suggests that while the core genes remains rigid to maintain fundamental biological processes, the accessory genes act as a dynamic repository of genetic variation, possibly facilitating cowpea's renowned resilience in the semi-arid tropics.

While SNPs have long been the focus of cowpea genetic studies⁵, our identification of 62,591 non-redundant SVs reveals a hidden layer of diversity, affecting over 50% of the reference genome. We observed that SV distribution is positively correlated with TE density (specifically LTRs) and negatively correlated with recombination rates. Consistent with the patterns observed in soybean¹¹ and *Brassica oleracea*⁴⁶, these SVs in cowpea are predominantly localized in intergenic regions. This distribution is primarily attributed to strong purifying selection against deleterious mutations in genic regions, which limits SV accumulation in functional sequences⁴⁷. Conversely, the high density of TEs in intergenic spaces facilitates SV formation via unequal crossing over⁴⁸. This genomic landscape aligns with the sheltering hypothesis, where low-recombination heterochromatic regions serve as refugia for large SVs and TE insertions that might otherwise be purged in high-recombination regions^{14,49,50}. The discovery of a 4.8 Mb inversion on chromosome 10, which sequesters a cluster of powdery mildew resistance (*RUN1*) and seed maturation (*VuMYB118*) genes, suggests that SVs play a critical role in maintaining adaptive gene complexes that are protected from crossover-mediated disruption^{51,52}.

The divergence between *Vu* and *Vs* cowpea provides a compelling model for human-mediated selection for distinct agricultural niches⁵³. Our SV-based GWAS and selection scans identified over 2300 candidate genes underlying subspecies divergence, highlighting pronounced genetic differentiation. The fixation of a 78-bp deletion in the *VuELF4* promoter in *Vs* accessions highlights the role of circadian rhythm regulation in subspecies divergence. By reducing the expression of this light-responsive regulator, this SV may have facilitated the delayed flowering and environmental adaptation required for vegetable-type cultivation in East Asia³⁸.

Structural variations have been shown to be major sources of gene functional variation and play a critical role in the genetic regulation of agronomic traits in a range of species^{54–57}. We identified two distinct structural mechanisms driving the elongated pod phenotype in *Vs*. First, SVs in the promoters of *VuWAK* genes likely modulate expression to alter cell shape and size⁵⁸. Second, a 51-bp deletion in the exon of *VuGA2ox2-2*, which fixed in the *Vs* subspecies, results in a structural alteration of the protein. This modification potentially

reduces the enzyme's affinity for bioactive gibberellins, thereby increasing hormonal levels to promote pod elongation^{41,42}. Compared to previous studies^{6,59}, our SV-based GWAS analysis identified novel genetic loci and possible causal mutations controlling important traits, emphasizing that SVs could complement SNPs in genetic analysis. The identified SVs in the cowpea pangenome and their associations with key agronomic traits offer a valuable foundation for genetic improvement through the pyramiding of favorable alleles.

Our study confirms a closed cowpea pan-genome with a high proportion of core genes, consistent with previous report¹⁶. More importantly, we constructed a graph-based pan-genome that captures complex structural variations underrepresented in earlier short-read-based studies¹⁶, and further explored their functional relevance through SV-based genome-wide association analyses (SV-GWAS) and candidate gene analysis, representing a substantial advance. However, limitations remain. The absence of wild *Vigna* limits our ability to capture genetic variation lost during domestication. In addition, although the statistical associations identified are robust, high-throughput functional validation, such as CRISPR-based approaches, will be essential to establish causality for candidate genes.

In conclusion, our study establishes a comprehensive, graph-based pan-genomic resource for cowpea, providing a fundamental framework for understanding its global genetic diversity. We demonstrate that SVs are key drivers of subspecies divergence and agronomic trait variation, influencing key biological processes and gene functions. These resources constitute an essential molecular toolkit to accelerate the development of climate-smart varieties, ensuring food security in resource-constrained regions.

Methods

Sample selection and sequencing

The 20 cowpea accessions were selected from a large diversity analysis of 9609 accessions from multiple genebanks and collections globally⁶⁰, including the United States Department of Agriculture (USDA), International Institute of Tropical Agriculture (IITA), Australian Grains Genebank (AGG), the Japanese National Agriculture and Food Research Organization (NARO), the Mozambique Genebank and other previously established collections including the UCR mini-core⁶¹. The accessions were sequenced using the DArTseq™ reduced-representation sequencing platform, resulting in the identification of 2302 high quality SNPs. A maximum likelihood phylogeny was subsequently constructed with IQ-TREE (v2.2.2.3)⁶².

Based on phylogenetic relationships, geographic origin, and subgroup clustering information, 20 representative cowpea accessions were selected for genome sequencing. These accessions originated from 11 countries, encompassing Venezuela, Mexico, Thailand, India, Philippines, Afghanistan, Japan, South Africa, Nigeria, Madagascar, and Morocco, representing a broad geographical distribution across the Americas, Asia, and Africa. Genomic DNA isolated from

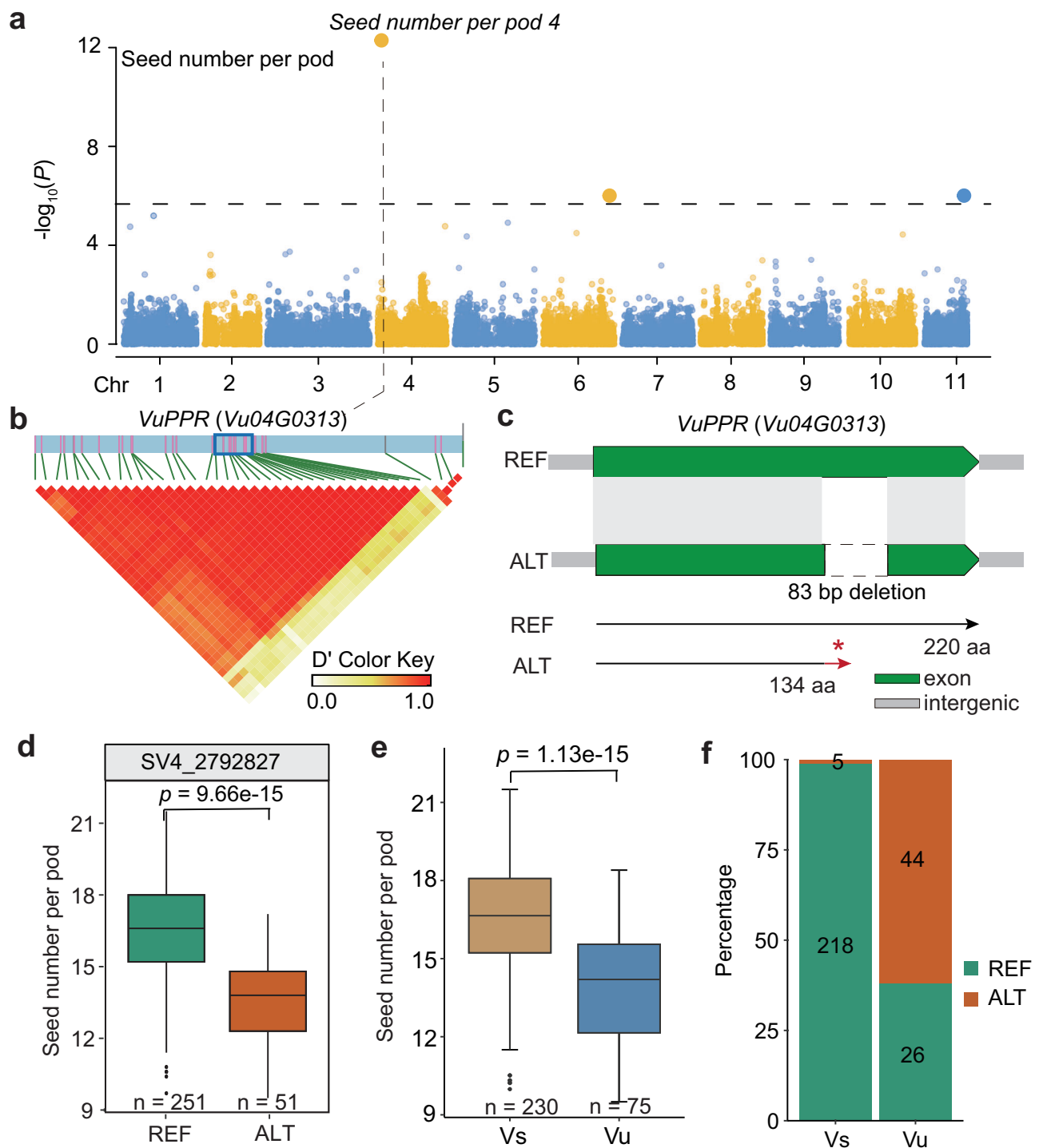


Fig. 6 | GWAS of structural variants linked to seed number per pod in cowpea. **a** Manhattan plot displaying SV-based GWAS results for the seed number per pod. The horizontal line denotes the genome-wide significance threshold. **b** Linkage disequilibrium heatmap surrounding the candidate gene *VuPPR*. **c** Gene structure of *VuPPR* and the position of the 83-bp deletion. **d** Effects of the 83-bp deletion in *VuPPR* on seed number per pod. **e** Comparison of seed number per pod between Vu

and Vs. **f** Allele frequency differences for the 83-bp deletion between Vu and Vs. In box plots, boxes represent the 25th to 75th percentiles, with central lines at the median and whiskers extending to 1.5× the interquartile range. Significance was assessed using two-sided Wilcoxon tests, with sample sizes (*n*) indicated for each group. Source data are provided as a Source Data file.

young leaves of the 20 cowpea accessions were used to construct PacBio SMRT libraries for each sample, following the recommended standard protocols. In brief, high-quality genomic DNA was first mechanically sheared into fragments of approximately 15–20 kb using g-TUBE devices. The resulting DNA fragments underwent a series of enzymatic reactions for DNA damage repair (nick repairing) and end

repair, followed by the ligation of SMRTbell adapters to both ends of the double-stranded DNA. To ensure high library purity, the ligation products were treated with exonucleases to selectively degrade failed or incompletely ligated fragments. Subsequently, the libraries were subjected to size selection using the BluePippin system with a target threshold of >10 kb to enrich for long-insert fragments. The final

SMRTbell libraries were assessed for size distribution and concentration using the Agilent 4200 TapeStation and Qubit Fluorometer, respectively. Sequencing was performed on the PacBio Sequel II platform in Circular Consensus Sequencing mode using 8 M SMRT Cells. A 30-h movie time was utilized to ensure sufficient passes for each molecule, generating a total of 15.01–24.45 Gb of high-fidelity (HiFi) reads ($Q \geq 20$) per accession.

Young leaf tissues were collected from Vung05, Vung14, and Vung17 for Hi-C sequencing. Samples were cross-linked with 1% for maldehyde and digested using Dpn II. DNA ends were biotin-labeled and proximity-ligated to capture spatial interactions. Following streptavidin-mediated enrichment and library preparation, sequencing was performed on the Illumina NovaSeq 6000 platform using a PE150 strategy. Valid interaction pairs were identified for chromosome-level scaffolding analysis.

Genome assembly, annotation and quality assessment

We assembled the genomes of 20 cowpea accessions sequenced with HiFi reads. Briefly, HiFi reads were firstly assembled using Hifiasm (v0.20.0-r639) with default parameters⁶³. The resulting assemblies were anchored and oriented onto the chromosomes of the FC6 reference genome using the reference-guided scaffolding tool RagTag⁶⁴ (v2.1.0) with default settings. Structural accuracy of these assemblies was assessed using HiFi reads and Hi-C data of Vung05, Vung14 and Vung17, which detected no evidence of structural inconsistencies or mis-assemblies. The quality of genome assemblies was evaluated using BUSCO²⁰ (v5.7.1) with the *fabales_odb10* lineage dataset, Clipping Reveals Assembly Quality²² (CRAQ, v1.0.9-alpha), and Merquy²¹ (v1.4.1).

TEs were annotated using the Extensive de novo TE Annotator (EDTA, v2.2.0), which integrates multiple TE prediction tools⁶⁵. Briefly, long terminal repeat retrotransposons (LTR-RTs) were initially identified using LTR_FINDER_parallel (v1.0.7) and LTR_HARVEST (v1.1), followed by quality filtering and refinement with LTR_retriever (v2.9.4)^{66–68}. Terminal inverted repeat (TIR) elements were detected using TIR-Learner (v3.0), while Helitron elements were annotated with HelitronScanner⁶⁹ (v1.1). The remaining TE components were identified using RepeatModeler (v2.0.5) and further annotated through homology-based searches with RepeatMasker (v4.1.5), incorporating the TE library generated by EDTA^{70,71}. The combined results from all tools were integrated to generate the final comprehensive TE annotation. Gene structures were predicted for each genome assembly using the BRAKER3 pipeline⁷² (v3.0.8), which integrates ab initio gene predictions, transcript evidence, and homologous protein evidence. To improve gene model prediction accuracy, GeneMark-ETP (v1) was first trained using both transcript and protein evidence, followed by training of AUGUSTUS (v3.5.0) based on the GeneMark-ETP predictions⁷³. Protein homology evidence was derived from the UniProt90 database²⁶. Publicly available cowpea RNA-seq data from Wu et al.⁶ (NCBI accessions: PRJNA970477 and PRJNA954189) were used to support gene annotation. These datasets include roots, seeds, leaves, flower buds, and pods at 0, 4, 8, and 12 days after anthesis for the two cowpea subspecies. The reads were aligned to the each soft-masked genomes using HISAT2⁷⁴ (v2.2.1).

The quality of gene annotation was assessed using BUSCO²⁰ (v5.7.1) with the *fabales_odb10* dataset. Functional annotation of predicted protein-coding sequences was performed using BLASTP searches against the Swiss-Prot database. Conserved protein domains were identified using InterProScan⁷⁵ (v5.45-80.0). Gene Ontology (GO) terms (<http://geneontology.org/>) and KEGG pathway annotations (<https://www.genome.jp/kegg/>) were assigned using eggNOG-mapper²⁷.

Phylogenetic tree and collinearity analysis

Six previously published cowpea genomes^{6,10,17–19} (NJ, A147, G98, G323, IT97K-499-35, and FC6) with comparable quality were combined with

the 20 assembled genomes for following genome analysis. To investigate the phylogenetic relationships among the 26 cowpea accessions, *Vigna mungo* was used as an outgroup⁷⁶. OrthoFinder⁷⁷ was used to group protein-coding genes into orthogroups and a total of 14,174 single-copy orthologous genes were identified. A maximum likelihood (ML) phylogenetic tree was constructed based on the concatenated sequences of single-copy orthologous genes using the RAxML⁷⁸ software package (v8.2.12) with 1000 bootstrap replicates. Pairwise genome synteny analysis was performed using the JCVI⁷⁹ package (v1.4.22). The syntenic relationship index (SRI) was calculated to quantify the level of synteny between two genomes (<https://github.com/Yujiaxin419/SRI-Pipeline>).

Gene-based pan-genome analysis

To construct the gene-based pan-genome, protein sequences from 26 cowpea accessions were analyzed using OrthoFinder⁷⁷ (v2.5.5), which applies the Markov Cluster Algorithm (MCL) to group protein-coding genes into orthogroups. The resulting orthogroups were categorized as core (present in all 26 genomes), dispensable (present in 2–25 genomes), or private (unique to a single genome). The nonsynonymous to synonymous substitution ratio (Ka/Ks) for each orthogroup category was estimated using KaKs_Calculator⁸⁰ (v2.0) with the Yang–Nielsen (YN) method. Nucleotide diversity (π) was calculated with vcftools⁸¹ (v0.1.16). Wilcoxon rank-sum tests were performed to assess statistically significant differences among the gene categories.

SV detection

To identify SVs across the 26 assembled cowpea genomes with high resolution, a whole-genome alignment-based approach was employed. Specifically, 25 genome assemblies were aligned to the FC6 reference genome using Minimap2⁸² (v2.28) with default parameters. The resulting whole-genome alignments were analyzed using SyRI⁸³ (v1.7.0) to detect SVs larger than 49 bp. To merge SVs across all genomes, we applied SURVIVOR⁸⁴ (v1.0.7) with the following parameters: 1000 11 0 0 50, which allows a maximum distance of 1000 bp between breakpoints for merging SVs across samples.

Graph-based pan-genome construction and population SV genotyping

To eliminate bias introduced by the reference genome, when performing SV genotyping on resequenced samples^{6,10}, we constructed a graph-based pan-genome using Minigraph-Cactus⁸⁵ (v2.9.1) with default parameters, incorporating all 26 assembled cowpea genomes. Briefly, minigraph was first used to generate a draft variation graph from all 26 assemblies, and the draft graph was subsequently refined and fully aligned using Cactus to produce a whole-genome multiple alignment graph. This graph represents all shared and accession-specific sequences, including structural variants, insertions, deletions, and presence–absence regions across the 26 genomes. Re-sequencing reads were mapped to the graph genome using vg giraffe⁸⁶ (v1.63.1). Mapping coverage was analyzed using vg pack⁸⁷ (v1.63.1), and SV genotyping was performed with vg call⁸⁸ (v1.63.1). Genotype data for each sample were subsequently indexed using tabix (v1.21, <https://github.com/tabixio/tabix>). Finally, all individual genotype VCF files were merged using bcftools⁸⁹ (v1.21) for downstream analysis. ODGI⁹⁰ (v0.9.4) is used for graph-based pangenome visualization.

Selective sweep analysis

To minimize potential misclassification caused by subspecies assignment, we retained only individuals with an ancestral component greater than 70% under the ADMIXTURE population structure analysis^{91,92} at $K=2$. This commonly used threshold effectively excludes highly admixed individuals while preserving sufficient sample size for group comparison^{5,93}. As a result, 72 accessions were retained for the Vu group and 283 accessions for the Vs group. To eliminate bias

due to unequal sample sizes, we randomly selected 72 accessions from the Vs group to match the Vu group for downstream analyses.

Selective sweeps potentially associated with artificial selection were identified by integrating three approaches: XP-CLR (v1.1.2), nucleotide diversity ratio (π_{Vu}/π_{Vs}), and F_{ST} . Due to the limited number of SVs, XP-CLR analysis was conducted using only SNP data. XP-CLR⁹⁴ was run with a 50-kb window size, a 10-kb step size, and a maximum of 200 SNPs per window. Nucleotide diversity (π) and fixation index (F_{ST}) were calculated using vcfTools⁸¹ (v0.1.16) with a sliding window of 50 kb and a step size of 10 kb. For each method, the top 5% of scores was used as the threshold to define candidate selective sweep regions. Regions identified by at least two of the three methods were considered as putative selective sweeps. Overlapping regions were determined using bedtools⁹⁵ (v2.31.1).

Genome-wide association studies

For the SV-based genome-wide association study (SV-GWAS), only biallelic structural variants were retained for analysis. To account for population structure and relatedness among samples, a kinship matrix was included as a covariate. GWAS was performed using the R package rMVP⁹⁶. Phenotypic data for pod length and grain number per pod were obtained from a previously published study⁶. The best linear unbiased predictions (BLUPs) across multiple years were calculated using the lme4⁹⁷ package and used as phenotypic inputs for the SV-GWAS. Both general linear models (GLM), mixed linear models (MLM), and the Fixed and random model Circulating Probability Unification (FarmCPU) model were evaluated for association testing. The FarmCPU model was ultimately selected as the optimal model due to its better control of false positives and higher statistical power. The genome-wide significance threshold was determined using the Bonferroni correction based on the effective number of independent SVs, as calculated by the genetic type I error calculator. A uniform threshold of $0.05/N$ was applied, resulting in a final significance cutoff of 2.14×10^{-6} . It is worth noting that FarmCPU iteratively uses significant markers as covariates, which frequently results in fragmented or interrupted peaks in Manhattan plots around true causal loci. PopLDdecay⁹⁸ (v3.43) was used to generate linkage disequilibrium heatmaps.

To identify genomic regions associated with divergence between Vu and Vs, we performed a binary-trait GWAS. Accessions with group membership >70% were selected as racial representatives (72 Vu and 283 Vs), and their racial identity was assigned as a numeric trait (Vu = 1, Vs = 0). Association analysis was conducted in rMVP using a general linear mixed model, with the centered kinship matrix and the first five principal components as covariates.

RNA-seq analysis

The RNA-seq raw data were retrieved from the study by Wu et al.⁶. The dataset includes transcriptomes of seeds, leaves, flower buds, and pods collected at 0, 4, 8, and 12 days after anthesis from three cowpea accessions representing two subspecies. To quantify the expression of genes in this study, RNA-seq reads from different tissues were trimmed using the fastp⁹⁹ (v0.24.0) program. The clean reads were then mapped against the reference gene models using HISAT2⁷⁴ (v2.2.1). The featureCounts¹⁰⁰ (v2.0.6) package was used for estimating FPKM values.

Protein structure and binding site prediction

The three-dimensional structure of the protein was predicted using AlphaFold3¹⁰¹. To identify potential ligand-binding sites, DeepSite (available at <https://playmolecule.com/deepsite/>) was employed with default parameters.

Dual-luciferase assay

The synthetic 2000-bp upstream fragments as the promoter of the two *VuWAK1* alleles and three *VuWAK2* alleles were ligated into luciferase (LUC) expression vector pGreenII 0800-LUC, and the empty vector

and CaMV 35S promoter were used as the negative and positive control, respectively. These constructs were introduced into *Agrobacterium tumefaciens* GV3101, which was then used to transiently transform *Nicotiana benthamiana* leaves via infiltration. After 2 days of dark incubation and 1 day of light exposure, total protein was extracted. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega), and signals from Firefly LUC and Renilla luciferase (REN) were detected with a Synergy H1 multimode reader (BioTek).

Vector construction, plant transformation and phenotypic characterization

The coding sequences of GA2OX2-2-REF and GA2OX2-2-ALT alleles were cloned into the binary vector pCambia1301 under the control of the CaMV 35S promoter. The constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 and used to transform wild-type *Arabidopsis thaliana* (Col-0) via the floral dip method¹⁰². Transgenic lines were selected on hygromycin-containing medium, and positive transformants were confirmed by PCR with gene-specific primers (Supplementary Table 12). To measure silique length, mature siliques from the middle portion of the main inflorescence stem were collected.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw sequence and the pan-genome assembly data have been deposited in the National Genomics Data Center (NGDC; <https://ngdc.cncb.ac.cn/gsa/>) under accession PRJCA051570 and PRJCA044967. The genotype and pan-genome annotation data can be accessed at Figshare [<https://doi.org/10.6084/m9.figshare.30931271>] and [<https://doi.org/10.6084/m9.figshare.32260974>]. The previously released transcriptome data used in this study are available at NCBI under accession PRJNA970477 and PRJNA954189⁹. The previously released resequencing data used in this study are available at NCBI under accession PRJNA889224⁶ and PRJNA890023¹⁰. Source data are provided with this paper.

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Y.T., E.M. and D.J. designed this project and coordinated the research activities. A.C., S.P., E.M., D.J. and Y.Y. collected and provided plant materials. S.P. prepared the sequenced samples. X.Z. and H.W. provided constructive feedback throughout the research process. S.S., C.W., Yanbo Wang, Yinzi Wang, T.D., Y.Z. and S.P. performed data analyses. S.S. wrote the manuscript. Y.T., E.M., H.W., S.P. and D.J. revised the manuscript. All authors read and approved the manuscript.

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