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High-Quality Chromosome-Level Genomes Reveal the Structure and Evolution of the *S* and *Z* Self-Incompatibility Loci in *Leymus chinensis*

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Leymus chinensis, a dominant forage species across the Eurasian steppe, combines broad ecological adaptability with excellent forage quality (Huang et al. 2004). However, its gametophytic self-incompatibility (SI) system severely limits seed production and prolongs breeding cycles. In grasses, SI is controlled by two multi-allelic and independently inherited loci, *S* and *Z*; yet their molecular determinants and regulatory mechanisms remain unresolved decades after their discovery (Lundqvist 1954; Hayman 1956). Recent studies indicate that two pollen-specific genes *DUF247* (Domain of Unknown Function 247) and one pistil-specific gene *HPS10* (*Hordeum Pistil-Specific 10*) are required for SI (Kakeda et al. 2008; Manzanares et al. 2016; Lian et al. 2021; Wang et al. 2022; Rohner et al. 2023). Although a chromosome-level genome assembly of *L. chinensis* is available, the *S* and *Z* loci remain incompletely annotated (Li et al. 2023).

Here, we improved the *L. chinensis* assembly and generated full-length transcriptomes of the elite breeding line Lc6 to refine gene models. In parallel, we produced a *de novo* chromosome-scale assembly of *Psathyrostachys juncea*, the Ns-subgenome

progenitor, enabling comparative analyses of subgenome evolution and the origin of the *S* and *Z* loci.

The updated *L. chinensis* assembly yielded two haplotypes of 7.96 Gb comprising 1121 and 1014 contigs (N50 > 40 Mb), respectively (Tables S1 and S2). Chromosome anchoring generated 28 chromosome-level pseudomolecules with an anchoring rate exceeding 96% (Figure 1a,b). Overall, the updated assemblies show improved contiguity and chromosomal completeness compared with the previous reference version. Using the same approach, the *P. juncea* genome was assembled into two haplotypes of 6.33 Gb and 6.38 Gb, consisting of 827 and 229 contigs, respectively (N50 > 120 Mb), with chromosome anchoring exceeding 99%. Benchmarking universal single-copy orthologs (BUSCO) assessments indicated high annotation completeness for both genomes (Table S3, Figure S1).

To infer the sequence of polyploid formation and subgenome divergence, we reconstructed Triticeae phylogeny using representative species. Comparative genomics placed the Ns

Sijie Sun, Jianli Wang, Yu Guan and Hongkui Zhang contributed equally to this work.

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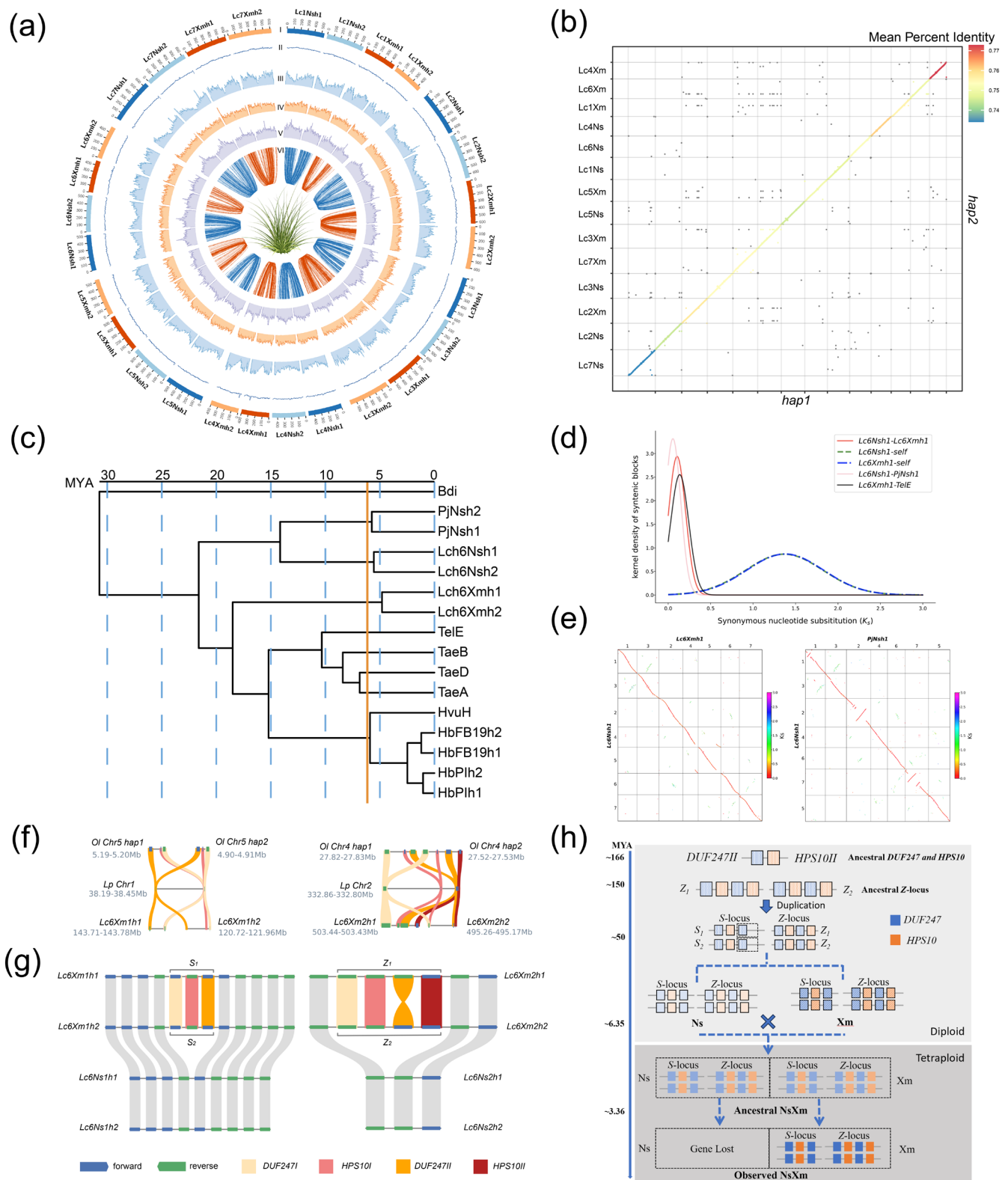


FIGURE 1 | Subgenome evolution and asymmetric distribution of the S and Z loci in *Leymus chinensis*. (a) Circos plot of the *L. chinensis* genome showing subgenome assignments (I), GC distribution (II), gene density (III), *Gypsy* density (IV), *Copia* density (V) and syntenic blocks between haplotypes (VI). (b) Whole-genome haplotype alignment revealing genome-wide heterozygosity. (c) Phylogeny of *L. chinensis* and representative Triticeae species. Orange line indicates polyploidization time (full species name in Table S4). (d) K_s distributions indicating whole-genome duplication and divergence events. (e) Syntenic blocks coloured by K_s values indicating divergence times. (f) Identification of S and Z loci using homology and synteny. Lc, *Leymus chinensis*; Lp, *Lolium perenne*; Ol, *Oryza longistaminata*. (g) Subgenome-asymmetric distribution of S and Z loci. (h) Proposed evolutionary model for the origin and diversification of the S and Z loci in *L. chinensis*.

subgenomes of *L. chinensis* and *P. juncea* within the same clade, while *Thinopyrum elongatum* grouped with wheat ABD subgenomes, supporting the inferred topology (Figure 1c). Ks analyses revealed three peaks (<0.5), indicating that the Xm subgenome diverged from its E-genome ancestor prior to polyploid formation (~8.23 MYA), followed by allotetraploidization at ~6.35 MYA and subsequent gene loss in the Ns subgenome at ~3.36 MYA (Figure 1d,e). These changes likely explain the size disparity observed between the *L. chinensis* Ns subgenome and extant *Psathyrostachys* genomes.

Using homology and synteny, we located *S* and *Z* loci on chromosomes 1Xm and 2Xm in *L. chinensis* (Figure 1f) and additionally identified an *HPS* gene (*HPS10II*) located outside the canonical *Z* locus. PacBio HiFi read mapping and Hi-C contact maps confirmed structural integrity (Figure S2). After manual curation, DUF247I/II and HPS10 proteins showed clear haplotype discrimination, with stamen-specific *DUF247* and pistil-specific *HPS10* expression in the Xm subgenome (Figure S3). Synteny analyses revealed conserved flanking gene order as in most grasses (Figure 1g). Notably, neither *DUF247* nor *HPS10* was detected in the Ns subgenome, suggesting evolutionary loss and subgenome-asymmetric distribution.

To explore the origin of *S* and *Z* loci, we retrieved the *DUF247* genes from identified gene families. *Brachypodium distachyon* possesses more homologues within *DUF247II-Z*, suggesting an ancient origin of the *Z* locus (Figure S4). Phylogenetic analyses of *DUF247* proteins from 101 *L. chinensis* accessions resolved four clades, with *DUF247II-Z* showing greater diversity (Figure S5). Ks dating across species placed the emergence of *HPS10II* at ~191 MYA (Table S5), followed by *DUF247I/II-Z* at ~150 MYA, whereas *DUF247I/II-S* arose more recently at ~50 MYA. This relative temporal order is supported by the consistent increase in 4DTV values. Taken together with the synteny patterns and chromosomal rearrangements following the ρ -WGD (Wang et al. 2026), these results support a model in which an ancestral *Z* locus duplicated to generate the *S* locus, followed by selective loss of SI genes from the Ns subgenome after polyploidization (Figure 1h).

We now have high-quality chromosome-level genomes for the allotetraploid *L. chinensis* and its Ns donor *P. juncea*. Our analyses reveal asymmetric subgenome evolution and show that the *S* and *Z* loci are exclusively retained on the Xm subgenome in *L. chinensis*, whereas the *Z* locus has a more ancient origin. These results provide a clear target for gene editing to overcome SI, boosting seed yield and speeding breeding.

Author Contributions

Y.Z., Y.X., Y.C., and X.C. conceived and designed the research. J.W., Z.S., Y.G., S.S., H.Z., Y.Z., S.L., M.Z., Z.L., S.Z., L.M., X.Z., and D.Z. provided or collected plant materials. S.S. and H.Z. performed the data analyses. Y.G. and Y.Z. carried out the experiments. S.S., H.Z., Y.Z., and Y.X. wrote and revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequencing data, genome assemblies and annotations have been deposited in the National Genomics Data Center (NGDC) under the accession number PRJCA053464 and figshare (<https://doi.org/10.6084/m9.figshare.30971629>).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section, comprising Figures S1–S4 and Tables S1–S5.