

Journal Pre-proof

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PII: S2468-0141(25)00058-5

DOI: <https://doi.org/10.1016/j.hpj.2025.01.007>

Reference: HPJ 742

To appear in: *Horticultural Plant Journal*

Received Date: 21 October 2024

Revised Date: 18 December 2024

Accepted Date: 23 January 2025

Please cite this article as: Zhang Z, Liu W, Zhang T, Chen Z, Zhang M, Yao J, Liu Y, Li J, Fan T, Zhao R, Qiao S, Zhang Y, Zhou L, Dong Y, Xu Y, Liu R, Tian L, Zhou Y, Xu X, Population genomics of *Vitis pseudoreticulata* reveals the genetic basis of fungal resistance in grapevine, *Horticultural Plant Journal*, <https://doi.org/10.1016/j.hpj.2025.01.007>.

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Population genomics of *Vitis pseudoreticulata* reveals the genetic basis of fungal resistance in grapevine

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Abstract

Grapevine cultivation is facing significant challenges from fungal diseases. Wild *Vitis* species exhibit extraordinary fungal resistance, offering valuable genetic resources for grapevine improvement, yet their genetic basis of fungal resistance remains largely unexplored. In this study, we assembled a haplotype-resolved, chromosome-level genome of *Vitis pseudoreticulata*, an East-Asian wild grapevine species known for its exceptional fungal resistance. The genome size for *V. pseudoreticulata* haplotype 1 and 2 were 520.32 and 511.58 Mb, with contig N50 values of 27.31 and 21.33 Mb, respectively. Fungal response genes were significantly expanded in this genome, accounting for approximately 10% of the expanded gene families and showing functional enrichment within these families, which provide a genetic foundation for its fungal resistance. Population genomic analyses identified genes under selection in *V. pseudoreticulata* were enriched for functions related to responses to fungus, auxin, heat, and salt stress, potentially contributing to the strong fungal resistance, rapid growth, and adaptation to hot and humid subtropical environment of this species. Key candidate genes, such as *FER*, *TDX*, and *PAT10* exhibited low intraspecies genetic diversity and high interspecies genetic divergence, likely playing important roles in these processes. Over half (54.37%) of the genes under selection were specific to *V. pseudoreticulata*, highlighting the uniqueness of its resistance and adaptation. Notably, a 3.7 Mb homozygous inversion, specifically identified in *V. pseudoreticulata*, encompasses genes related to anther dehiscence and pollen acceptance, potentially contributing to its reproductive success. The reference genome, variation map, and candidate genes identified in this study provide valuable genetic resources for breeding grapevine cultivars and rootstocks with enhanced fungal resistance.

Keywords: grape breeding; fungal resistance; structural variation; subtropical adaptation; Viticulture

1. Introduction

Grape, one of the most important fruit crops, is facing significant challenges from fungus diseases (Gessler et al., 2011; Qiu et al., 2015; Bois et al., 2017; Li et al., 2021). Grapevine cultivation relies heavily on fungicides to prevent fungus infections, which account for approximately 70% of all fungicide use across only 8% of total crop areas in Europe (Feechan et al., 2013). The extensive utilization of fungicides has raised serious health risks to

consumers and led to serious environmental pollution (Pimentel, 2005; Zubrod et al., 2019; Fan et al., 2024). The limited fungal resistance of domesticated grapevine is primarily inherited from their wild progenitor, the Eurasian grapevine species (*V. vinifera* subsp. *sylvestris*), which is highly susceptible to fungal diseases (Wang et al., 1998; Wan et al., 2007). And the domestication process has further weakened its resistance by reducing its genetic diversity and the number of disease-resistance genes (Doebley et al., 2006; Zhang et al., 2017; Zhou et al., 2017; Gaut et al., 2018; Huang et al., 2022; Long et al., 2024; Wang et al., 2024c). There is a growing demand for breeding grapevine cultivars with improved fungal resistance (Qiu et al., 2015).

Grapevine wild relatives have, on the contrary, demonstrated strong resistance to various fungal diseases, such as the highly destructive downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*; Cadle-Davidson et al., 2011; Feechan et al., 2013; Pap et al., 2016; Zhang et al., 2024b), which represent ideal genetic resources for the breeding of fungal-resistant grapevine cultivars. Besides, while the cultivating of grapevine, particularly in subtropical regions, requires substantial costs and extensive human management, numerous wild grapevine species have naturally adapted to the hot and humid subtropical climate through long-term evolution (Ma et al., 2018b). Integrating the resistance and adaptive genes from these wild grapevine species offers a promising solution for improving domesticated grapevine (Zhang et al., 2019; Morales-Cruz et al., 2023). A high-quality reference genome is an effective tool for mining the genetic potential of crop wild relatives (Jayakodi et al., 2020). Along with the improvement of genome sequencing technology, chromosome level genome has been achieved for several domesticated grapevine cultivars (Zhang et al., 2023; Djari et al., 2024; Liu et al., 2024), which significantly improved the estimated heritability of some important agronomic traits, and successfully reveals the polygenic basis of seedlessness in grapevine (Wang et al., 2024b). Genomes of some wild grapevine species were also reported in previous studies (Cheng et al., 2023; Cochetel et al., 2023; Li et al., 2024; Wang et al., 2024a; Zhang et al., 2024a), which have significantly promoted our understanding of their disease resistance and adaptation. However, most of these wild grapevine genomes remain highly fragmented, thereby significantly hindered their full utilization in breeding. Zhang et al. (2024a) reported a high-quality, haplotype-resolved, chromosome-level genome of *V. rotundifolia*, providing valuable insights into its adaptation to the saline coastal environment. However, this study alone is not sufficient to capture the vast diversity of wild grapevine species, particularly in relation to the genetic resources with fungal resistance. The report of additional high-quality fungal-resistant wild grapevine genomes is essential for fully harnessing the genetic potential of wild grapevine fungal resistance and adaptive traits.

The eastern Chinese grapevine (*V. pseudoreticulata*), one of the most widely distributed wild grapevine species in subtropical regions of East Asia, stands out for its remarkable fungal resistance (Wan et al., 2007; Li et al., 2010), vigorous growth, and excellent adaptation to subtropical climates. The majority of studies involving *V. pseudoreticulata* were related to its fungal resistance, identifying some genes, such as *EIRP1* (Yu et al., 2013), *VpPR-10* (Xu et al., 2014; Ma et al., 2018a), *VpPR4-1* (Dai et al., 2016), *CDPKs* (Hu et al., 2021), *VpRH2* (Wang et al., 2017), that confer resistance to powdery mildew or downy mildew. However, all of these studies relied on the genome of domesticated grapevine as reference due to the lack of a genome for *V. pseudoreticulata*, which may induce reference bias and potentially reduce the efficacy of analyses (Günther and Nettelblad, 2019). A high-quality genome of *V. pseudoreticulata* would significantly advance our understanding of this valuable wild grapevine genetic resource. Besides fungal resistance, the other superior characteristics of *V. pseudoreticulata*, such as its fast-growing nature, broad adaptability to subtropical regions, and the underlying genetic bases of these traits, remain largely unexplored. Wild grapevine species with fast-growing nature could potentially be exploited as rootstock to expand the adaptability, quality, and productivity of domesticated grapevine

(Warschefsky et al., 2016; Marín et al., 2023).

In this study, leveraging the newly sequenced whole genome sequencing data (HiFi, HiC, and ONT), and population resequencing data of *V. pseudoreticulata*, we intend to, 1) assemble a high-quality, haplotype-resolved, chromosome-level genome of *V. pseudoreticulata* to facilitate the exploration of this valuable wild grapevine resource; 2) unravel the genetic basis for its fungal resistance; 3) investigate the driving factor behind its fast-growing nature; and finally 4) conduct extensive population genomic analyses to identify signals associated with its adaptation to subtropical climate. These superior traits are highly desirable in domesticated grapevine, and the related genetic resources could be used to improve grapevine cultivars and rootstocks.

2. Materials and methods

2.1. Plant materials and sequencing

Young leaves from a *V. pseudoreticulata* sample (A000790) were collected from Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Science, Henan, China, for HiFi, HiC, ONT, and Transcriptome sequencing (Table S1) in Wuhan Benagen Technology Co., Ltd (Wuhan, China). HiFi reads were sequenced on PacBio Revio platform in a circular consensus sequencing (CCS) mode, using 15–20 kb SMRT cell library. HiC and transcriptome sequencing was performed on the Illumina NovaSeq 6000 platform with 150 bp paired-end reads. ONT reads were sequenced on an Oxford Nanopore PromethION (R9.4) sequencer. Whole-genome resequencing of this sample, along with 11 additional *V. pseudoreticulata* samples from six populations (Table S1), was conducted on the Illumina NovaSeq 6000 platform with 150 bp paired-end reads by Personalbio (Shanghai, China). Additionally, 52 whole-genome resequencing data from five grapevine species, reported in previous studies, were downloaded from National Center of Biotechnology Information (NCBI) (Table S2). For comparative genomic analyses, genome assemblies of 13 grapevine accessions (primarily haplotype-resolved genomes, except for *V. vinifera* ssp. *vinifera* ‘PN40024’ which is a highly homozygous inbred line; Table S2) from previous studies were included. All the raw sequencing data newly generated in this study have been deposited in NCBI under BioProject number PRJNA1164911, and in the National Genomics Data Center (NGDC) Genome Sequence Archive (GSA) (<https://ngdc.cnbc.ac.cn/gsa/>) under BioProject number PRJCA030739.

The potential distribution of the wild grapevine species was predicted with Maxent v3.4.4 (https://biodiversityinformatics.amnh.org/open_source/maxent), based on their occurrences data from the Global Biodiversity Information Facility (GBIF; www.gbif.org; doi: 10.15468/dl.mmdtbd) and nineteen bioclimatic variable data (averaged for the years 1970–2000 at resolution of 5 min) obtained from Worldclim2 (Fick and Hijmans, 2017).

2.2. Genome assembly, evaluation, and annotation

The HiC reads were filtered using fastp v0.23.4 (Chen et al., 2018) with default parameters. HiFi, ONT, and filtered HiC reads of *V. pseudoreticulata* were then collectively assembled into haplotigs using Hifiasm v0.19.8-r603 (Cheng et al., 2021) with default parameters. The haplotigs were initially scaffolded using RagTag v2.1.0 (Alonge et al., 2022) with the genome of a domesticated grapevine *V. vinifera* ssp. *vinifera* ‘PN40024’ (Shi et al., 2023) as a reference. Simultaneously, the filtered HiC reads were anchored to the haplotigs with Juicer v1.6 (Durand et al., 2016) and transformed into a HiC contact map using 3D-DNA v190716 (Dudchenko et al., 2017). The HiC contact map was visualized in Juicebox v2.17.00 (Robinson et al., 2018) and manually adjusted to generate the final chromosome scaffolds. The k-mer-based consensus quality (QV) scores of the *V. pseudoreticulata* genome, which represent the log-scaled probability of error E at each base ($-10\log_{10}E$), were

calculated using Merqury v1.3 with the k-mers counted by Meryl v1.4.1 (Rhie et al., 2020) based on resequencing data. The mapping rate of resequencing data was calculated using samtools v1.19.2 (Daneczek et al., 2021), based on BAM files generated by mapping the reads to the genome using BWA-mem algorithm (Li, 2013). Genome assembly completeness was assessed by the proportion of detected complete Benchmarking Universal Single-Copy Orthologs (BUSCO) using BUSCO v5.2.2 (Manni et al., 2021) based on the embryophyta_odb10 datasets. As a complement, the proportion of intact LTR retrotransposons (LTR Assembly Index, LAI) was also used to evaluate the completeness of the *V. pseudoreticulata* genome. LTR retrotransposons were identified using LTRharvest (Ellinghaus et al., 2008) in GenomeTools v1.6.2 and LTR_FINDER v1.0.7 (Xu and Wang, 2007), and the resulting data were used as input to estimate LAI using LTR_retriever v3.0.1 (Ou et al., 2018; Ou and Jiang, 2018) with default parameters.

Telomeric repeat units of the *V. pseudoreticulata* genome were identified using TIDK (v0.2.0) with telomere repeat unit of TTTAGGG previously reported in grapevine (Castro et al., 2021) in a window of 100 bp (-s TTTAGGG -w 100; <https://github.com/tolkit/telomeric-identifier>). Whole-genome tandem repeats were identified using TRF (Benson, 1999), and the results were converted to gff3 format using TRF2GFF (<https://github.com/Adamtaranto/TRF2GFF>). The centromere regions of *V. pseudoreticulata* were predicted based on the distribution of 107 bp tandem repeat unit reported in a previous study (Shi et al., 2023). Genes were annotated following the pipeline developed by Shi et al. (2023) (<https://github.com/unavailable-2374/Genome-Wide-Annotation-Pipeline>). The repetitive sequences in the *V. pseudoreticulata* genome were annotated using RepeatMasker v4.1.5 (<http://www.repeatmasker.org>) based on the pan-*Vitis* repeat database constructed by Shi et al. (2023). The newly assembled genome of *V. pseudoreticulata* and its annotations have been deposited in Zenodo (<https://doi.org/10.5281/zenodo.15009359>).

2.3. Gene family expansion-contraction analyses

To detect the expanded and contracted gene families in the *V. pseudoreticulata* genome, 13 previously published grapevine genomes (Table S2) were included in our analyses. Orthogroups were identified using OrthoFinder v2.5.5 (Emms and Kelly, 2019) with default parameters. The Orthogroup statistics were used as input for detecting expanded and contracted gene families using CAFE5 v5.1.0 (Mendes et al., 2020).

2.4. Divergence time estimation

The single-copy genes shared among 13 grapevine accessions were used to estimate divergence time using MCMCTree v4.10.7 (Yang, 2007). To minimize the influence of human domestication, only one of the two domesticated grapevine cultivars, 'PN40024' (out of 'Cabernet Sauvignon' and 'PN40024'), was included in this analysis. The CDS region of each single copy gene was aligned using MAFFT v7.525 (Katoh and Standley, 2013), trimmed with trimAl v1.4.rev15 (Capella-Gutiérrez et al., 2009) to exclude sites with more than 25% missing genotypes, and then concatenated for divergence time estimation. Two calibration points were applied to constrain the divergence time following previous studies (Wan et al., 2013; Cochetel et al., 2023): one constrains the crown age of *Vitis* to 27.32–29.32 million years ago (Ma), and the other constrains the crown of subg. *Vitis* to 16.82–18.82 Ma.

2.5. Structural variation analyses

To identify structural variations (SVs) in the genome of *V. pseudoreticulata* that may have contributed to its adaptation, we aligned its two haplotypes to the genomes of 'PN40024' and *V. rotundifolia* using Minimap2

v2.28-r1209 (Li, 2018), and identified genome rearrangement regions using SyRI v1.7.0 (Goel et al., 2019). The synteny of these three genomes and the SVs were plotted using plotsr v1.1.5 (Goel and Schneeberger, 2022). Given that the other 11 grapevine genomes included in this study were highly fragmented, with contig N50 of 0.31–1.99 Mb, we have just selected the three highest-quality genomes (N50 > 21 Mb; including *V. pseudoreticulata*) for accurate SV detection. The genome region of *V. pseudoreticulata* identified as an SV when compared with both ‘PN40024’ and *V. rotundifolia* was defined as a specific SV for *V. pseudoreticulata* (only haplotype 1 was compared here). SVs, when shared, are typically between closely related species (inherited from a common ancestor), given the low probability for two independent SVs to arising at identical genomic region. Therefore, if an SV is specific to *V. pseudoreticulata* compared to its closely related ‘PN40024’ and *V. rotundifolia*, it is highly likely to be also specific to this species within the grapevine genus.

2.6. Variant calling and filtering

The raw resequencing data were filtered using fastp v0.23.4 (Chen et al., 2018) with default parameters, and the quality of filtered reads were evaluated using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The filtered reads were mapped to haplotype 1 of *V. pseudoreticulata* with the BWA-MEM algorithm (Li, 2013) implemented in bwa v0.7.17 (Li and Durbin, 2009). Genomic variants calling was performed using the GATK v4.0.1.2 (McKenna et al., 2010) pipeline. HaplotypeCaller was used to detect variants, and generate general variant calling files (gvcf). The gvcf files were then combined by CombineGVCFs into a single gvcf file, and subjected to joint genotyping with GenotypeGVCFs. Next, we used vcftools v0.1.17 (Danecek et al., 2011) to filter the SNPs, treating those with genotype quality score (GQ) < 20, or read depth (DP) < 4 or > 1 000 as missing, and removing all SNPs with a genotype missing rate > 30% (--minGQ 20 --minDP 4 --maxDP 1000 --max-missing 0.7). To reduce the computation burden of some analyses, we have pruned the SNP data based on linkage disequilibrium (LD) with 50 SNPs windows, 5 SNPs steps, and an r^2 threshold of 0.2 (--indep-pairwise 50 5 0.2) in plink v1.9 (Purcell et al., 2007).

2.7. Phylogenetic tree inference

The Maximum Likelihood (ML) phylogenetic tree was constructed using IQtree v2.1.4 (Minh et al., 2020) based on 3 760 593 SNPs (with a missing rate < 10%) from all 64 accessions with GTR+I+G model. The resulting phylogenetic tree was visualized and modified with ggtree v3.4.4 (Yu et al., 2017) in R v4.2.2 (<https://www.r-project.org>).

2.8. Population genetic structure analyses

To investigate how each grapevine population and individual was separated from one another, principal component analysis (PCA) was performed using plink v1.9 (Purcell et al., 2007) for 58 accessions (six accessions from the outgroup *V. rotundifolia* were not included). PCA results and most statistical data in this study were plotted using ggplot2 (Hadley, 2016) in R v4.2.2 (<https://www.r-project.org/>), unless otherwise indicated. Nucleotide diversity (π) was calculated with 10 kb non-overlapping slid window using Vcftools. Genetic divergence (Dxy) was assessed with 10 kb non-overlapping sliding windows (> 50 SNPs) using popgenWindows.py script from genomics_general (https://github.com/simonhmartin/genomics_general.git). The genetic structure of the populations was explored using the Maximum Likelihood clustering program ADMIXTURE v1.3.0 (Alexander et al., 2009) with postulated ancestral populations (K) values ranging from 2 to 8. Population demographic history of the East-Asian grapevine species was estimated using SMC++ v0.0.0 (Terhorst et al., 2017) based on the SNPs with a

genotype missing rate < 20%. For *V. pseudoreticulata*, we have further estimated demographic history for each chromosome based on the SNPs with a genotype missing rate < 30%.

2.9. Population branch statistical analyses and selective sweep analyses

To identify selection signals in the subtropical lineage, population-specific selection outliers were detected in the three subtropical species using PBSscan v2020.03.16 (Hämälä and Savolainen, 2019) with temperate *V. amurensis* as the paired focal populations and *V. riparia* as outgroup. Genes in the regions with the highest 5% PBS values were classified as selected genes. The signals shared between these three subtropical grapevine species were expected to be relevant to their subtropical adaptation.

Another software, SweeD v4.0.0 (Pavlidis et al., 2013) was used to detect positive selection signals within a species, without reference to other species, capturing all the signals that may contribute to the adaptation of a species. Candidate selective sweeps were detected using the composite likelihood rate (CLR) test from SweeD with a 10 kb sliding window. The highest 5% CLR scores were considered candidate loci under a selective sweep, and regions within a 5 kb flanking distance from these loci were considered as candidate regions under a selective sweep. Genes overlapping these regions were classified as positively selected genes.

2.10. Gene ontology analyses

To examine whether any functional classes of genes were over-represented in gene sets of interest, we performed gene ontology (GO) analysis using the R package clusterProfiler v4.3.1.9 (Wu et al., 2021). *P*-values for GO terms were calculated using Fisher's exact test. GO terms with *p*-values lower than 0.05 and *q*-value lower than 0.2 (default setting of clusterProfiler) were considered significantly enriched. A new database was constructed for GO enrichment analyses using DIAMOND v2.1.4 (Buchfink et al., 2021) based on all the *Vitis* species genes that have been annotated with GO information and the UniProt SwissProt database (Bateman et al., 2023). All the genes in haplotype 1 of *V. pseudoreticulata* were blasted against this newly constructed database using DIAMOND as the background.

3. Results

3.1. Haplotype-resolved, chromosome-level genome of *V. pseudoreticulata*

To assemble and annotate the genome of *V. pseudoreticulata*, we sequenced 23.86 Gb of PacBio HiFi long reads (45×, with an average length of 19.2 kb), 56.86 Gb of HiC reads (110×), 25.98 Gb of Oxford Nanopore reads (50×, with an average length of 102.85 kb), and 20.82 Gb of transcriptome reads (Table S1). The newly assembled, phased, chromosome-level genome of *V. pseudoreticulata* was 520.32 Mb and 511.58 Mb, with contig N50 of 27.31 Mb and 21.33 Mb, for haplotypes 1 and 2 respectively (hereafter abbreviated as hap1 and hap2; Fig. 1, A), making it one of the highest-quality wild grapevine genomes. The contigs were well resolved into 38 chromosomes (2n = 38; Fig. S1, A) in the HiC contact heatmap. Telomeres, which ranged from 200 bp (Chr1_hap2) to 19 547 bp (Chr2_hap1) in length, were detected on 37 out of 38 chromosomes, with two telomeres for 33 chromosomes and one telomere for four chromosomes (Table S3). Centromeres were detected on all 38 chromosomes, ranging from 580 kb (Chr15_hap1) to 3.5 Mb (Chr17_hap1) in length (Table S3). In total, a telomere-to-telomere (T2T) assembly was achieved for 21 of the 38 *V. pseudoreticulata* chromosomes (Table S3). The assembly consensus quality value (QV) for *V. pseudoreticulata* genome was 42.95 (42.93 for hap1, and 42.97 for hap2, respectively; estimated using Merqury v1.3 based on resequencing data), indicating over 99.99% accuracy. The mapping rate of resequencing data to the *V. pseudoreticulata* genome was 97.67% (97.22% for hap1,

and 97.25% for hap2, respectively). About 97.8% and 98.0% of the Embryophyte universal conserved genes (embryophyta_odb10) were detected in hap1 and hap2 of *V. pseudoreticulata* genome, respectively. The LTR Assembly Index (LAI) for the two haplotypes was 18.25 for hap1, and 17.21 for hap2, respectively. These results collectively suggest the high quality and completeness of the newly assembled *V. pseudoreticulata* genome.

About 65% of the *V. pseudoreticulata* genome was composed of repetitive sequences, with the majority identified as retroelements (Class I TEs) and DNA transposons (Class II TEs; Fig. S1, B; Table S4). The centromere regions were predominantly composed of 107 bp tandem repeat unit (Fig. 1, A), which was consistent with a previous study (Shi et al., 2023). Additionally, we found a few chromosomes mainly composed of 157 bp (Chr3 hap1 and hap2), 105 bp (Chr7 hap1), and 106 bp repeat units (Chr12 hap1; Fig. S1, C). The density of Gypsy, the largest repetitive family, was relatively higher around the centromere regions, while on the contrary, the density of Copia and DNA TEs was relatively lower in these regions (Fig. 1, A). The proportion of major repetitive sequences in the *V. pseudoreticulata* genome was similar to those in the domesticated grapevine (*V. vinifera* ssp. *vinifera* 'PN40024') and the wild grapevine *V. rotundifolia* (Fig. 1, B).

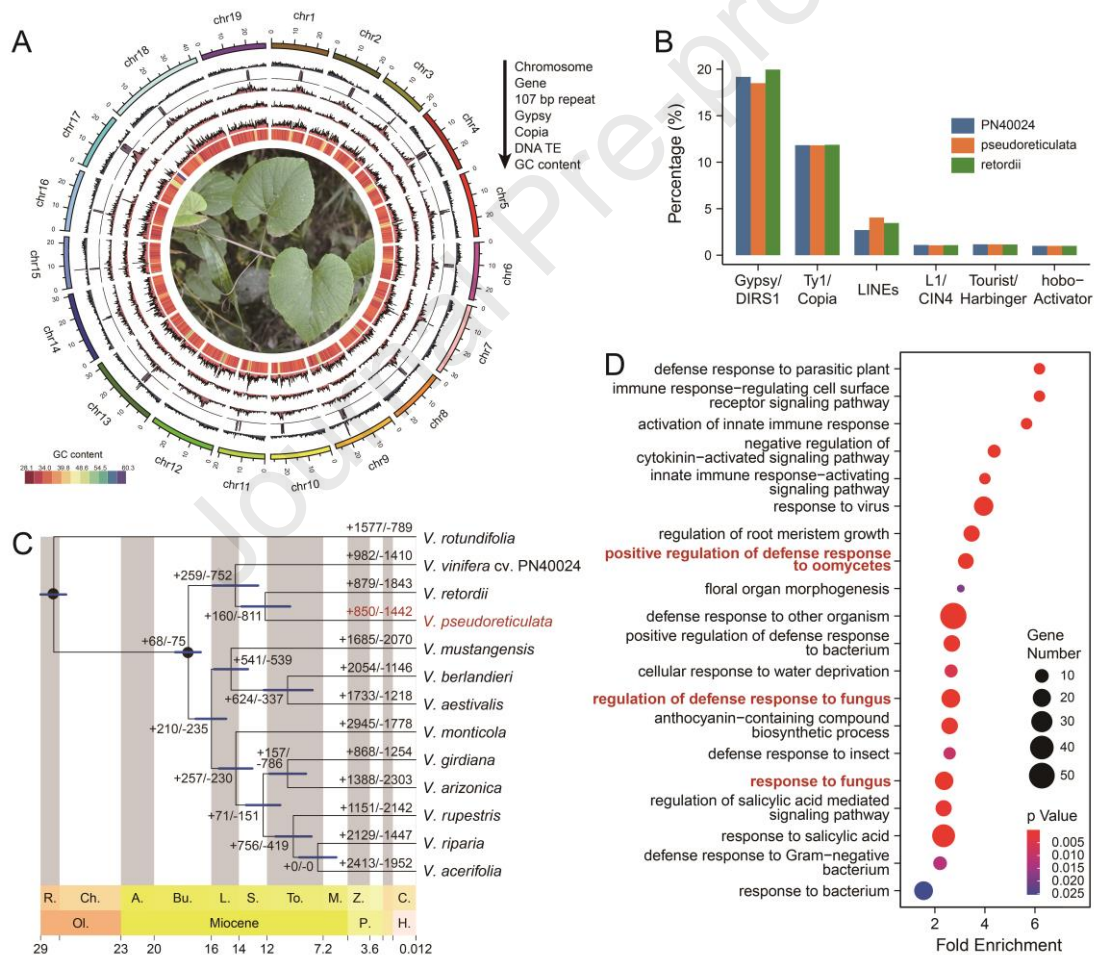


Fig. 1 Genome structure of *Vitis pseudoreticulata*

(A) Density of genes, 107 bp centromere repeats, Gypsy, Copia, DNA TE, and GC content across the genome. (B) Proportions of major TE families among *V. pseudoreticulata*, 'PN40024', and *V. rotundifolia* genomes. (C) Divergence time estimates and gene family expansion-contraction among grapevine species. Solid circles indicate calibration points, and bars at each node represents the 95% Highest Posterior Density (HPD) intervals. Numbers adjacent to branches represent the count of expanded (+) and contracted (-) gene families. (D) GO enrichment results of the expanded gene families. For clarity, only a subset of GO terms potentially important for breeding is plotted.

A total of 33 843 and 32 815 genes were annotated in hap1 and hap2 of *V. pseudoreticulata* genome, respectively, using a combination of *de novo*, homology-based, and transcript-based approaches. The gene density was generally higher towards both ends of chromosomes, while relatively lower in centromere regions (Fig. 1, A). Based on 3 799 single copy genes detected across the 13 *Vitis* genomes in this study, the crown age of *Vitis* was estimated to be 28.27 million years ago (Mya; 95%HPD 27.31–29.30 Mya; Fig. 1, C and Fig. S1, D), which was consistent with a previous study (Cochetel et al., 2023). And *V. pseudoreticulata* was estimated to have diverged from the common ancestor with *V. rotundifolia* around 11.76 Mya (95% HPD 9.83–13.61 Mya).

3.2. Fungus response gene families were significantly expanded in *V. pseudoreticulata* genome

During the evolutionary process of *V. pseudoreticulata*, 850 orthogroups encompassing 3 095 genes (Fig. 1, C), were detected to have expanded in its genome using CAFE5 v5.1.0 (Mendes et al., 2020). These genes were involved in diverse biological processes (Fig. S2, A; Table S5), with the most genes related to defense response to fungus (GO:0050832). Compared with the whole genome, genes related to response to fungus (including GO:1902290, GO:1900150, and GO:0009620) were also significantly enriched in the expanded gene families (Fig. 1, D). The expanded fungus response genes tend to accumulate in specific chromosomes, such as Chr18, 16, and 13 (Fig. S2, B). Besides, these expanded gene families were enriched in some other biological processes important for breeding, such as response to bacterium (GO:0009617), and response to virus (GO:0009615; Fig. 1, D). Additionally, 45 genes related to response to heat (GO:0009408, GO:0034605, and GO:0070370) and stomatal movement (GO:0090333, GO:0010118, and GO:0010119) were also detected in the expanded gene families. The expansion of genes related to response to fungus, bacterium, heat, etc., likely provided a genetic foundation for the resistance and adaptability of *V. pseudoreticulata* in the subtropical climate.

A total of 1 442 gene families contracted in the genome of *V. pseudoreticulata*, of which 501 gene families retained some genes. For the rest 941 contracted gene families, their genes were either completely lost in *V. pseudoreticulata*, or newly evolved in other grapevine species. In total, 729 genes were detected in these contracted gene families of *V. pseudoreticulata*, which were primarily related to some intracellular process (Fig. S2, C; Table S5), including snRNA processing (GO: 0016180), protein complex oligomerization (GO: 0051259), proteolysis (GO: 0006508), and DNA integration (GO: 0015074).

3.3. Structural variations potentially confer the fast-growing nature of *V. pseudoreticulata*

The genome of *V. pseudoreticulata* displayed high synteny with both ‘PN40024’ and *V. rotundifolia*, except in the centromere and adjacent regions (Fig. 2, A and Fig. S2, D). Approximately 84.85% of the genome in haplotype 1 of *V. pseudoreticulata* was in synteny with its haplotype 2, while only 80.61% and 76.89% were in synteny with the genome of *V. rotundifolia* and ‘PN40024’, respectively (Fig. 2, B). Contrarily, the proportion of not aligned regions (NOTAL), large structural variations (from INV to INVDP), and SNPs increased gradually from the comparison with *V. pseudoreticulata* hap2, *V. rotundifolia*, to ‘PN40024’ genomes. This was consistent with the expectations that the longer species diverged from each other, the less synteny they would share, and the more mutations they would accumulate. Exceptionally, a higher proportion of inversions were detected between *V. pseudoreticulata* hap1 and *V. rotundifolia*, instead of between *V. pseudoreticulata* hap1 and ‘PN40024’, likely due to the larger divergence in the corresponding regions, which were difficult to be aligned with ‘PN40024’. For example, around the centromere region of *V. pseudoreticulata* Chr5 and Chr8, while a large inversion was detected with *V. rotundifolia*, most of the corresponding regions could not be aligned with ‘PN40024’ (Fig. 2, A). The higher proportion of insertions (INS)

and deletions (DEL) between the two haplotypes of *V. pseudoreticulata*, could also probably be explained with a similar reason. Additionally, structural variations (including insertions and deletions) involved about ten times more nucleobases than SNPs in the genome of *V. pseudoreticulata* (Fig. 2, B), suggesting that these variations likely played a more important role in the evolution of *V. pseudoreticulata*.

Most of the structural variations (SVs) in the *V. pseudoreticulata* genome (4 464 out of 5 690 SVs; 78.47%) were smaller than 10 kb, with only nine SVs exceeding 1 Mb. In terms of total length, inversions were the largest SVs in our dataset (Fig. 2, B), and were relatively longer. Inversions accounted for 63.89% of SVs larger than 100 kb, with the 15 longest all being of inversions. The largest SV identified was a homozygous inversion (3.7 Mb) in *V. pseudoreticulata*, located on Chr6 (9 750 233–13 447 967 bp of hap1), which was specific to this species in this study when compared with the genome of *V. retordii* and 'PN40024' (Fig. 2, C). This inversion most probably occurred 11.76 Mya, following the divergence of *V. pseudoreticulata* and *V. retordii*, as supported by divergence time estimation (Fig. 1, C). Notably, genetic diversity in this inverted region was overall lower than the other genome regions in *V. pseudoreticulata* (Fig. 2, C). A total of 73 genes were annotated in this inversion, including four genes (homologous with *APY6* and *SEC8*) related to some important reproductive process, such as anther dehiscence (GO:0009901), pollen exine formation (GO:0010584), and acceptance of pollen (GO:0060321), and one gene (homologous with *SNL3*) related with response to abscisic acid (GO:0009737; Table S6). This suggests that the inversion could have played a role in shaping specific traits related to reproduction and stress response in *V. pseudoreticulata*.

Some other specific SVs were also detected in the genome of *V. pseudoreticulata*, either individually in one haplotype (e.g. inversion in Chr14_hap2: 13 217 026–14 342 863 bp and Chr16_hap2: 6 546 711–7 459 883 bp), or homozygous between its two haplotypes (e.g. Chr2_hap1: 3,245,258–3,384,738 bp and Chr5_hap1: 14 211 044–14 456 829 bp). The specific SVs in haplotype 1 of *V. pseudoreticulata* genome involved 165 genes, some of which may have played important roles in its adaptation to subtropical climate. These genes included nine related to development (flower development, pollen exine formation, etc.), eight related to reproduction, six related to defense response, and four related to response to heat (Table S6). Notably, genes related to meristem development and root meristem growth were significantly enriched among the specific SVs of *V. pseudoreticulata* (Fig. S2, E). Together with seven genes related to hormone signaling pathway (e.g. auxin, abscisic acid), these genes may have contributed to the fast-growing nature of *V. pseudoreticulata*, which is likely advantageous for its survival in subtropical climates and valuable for rootstock breeding.

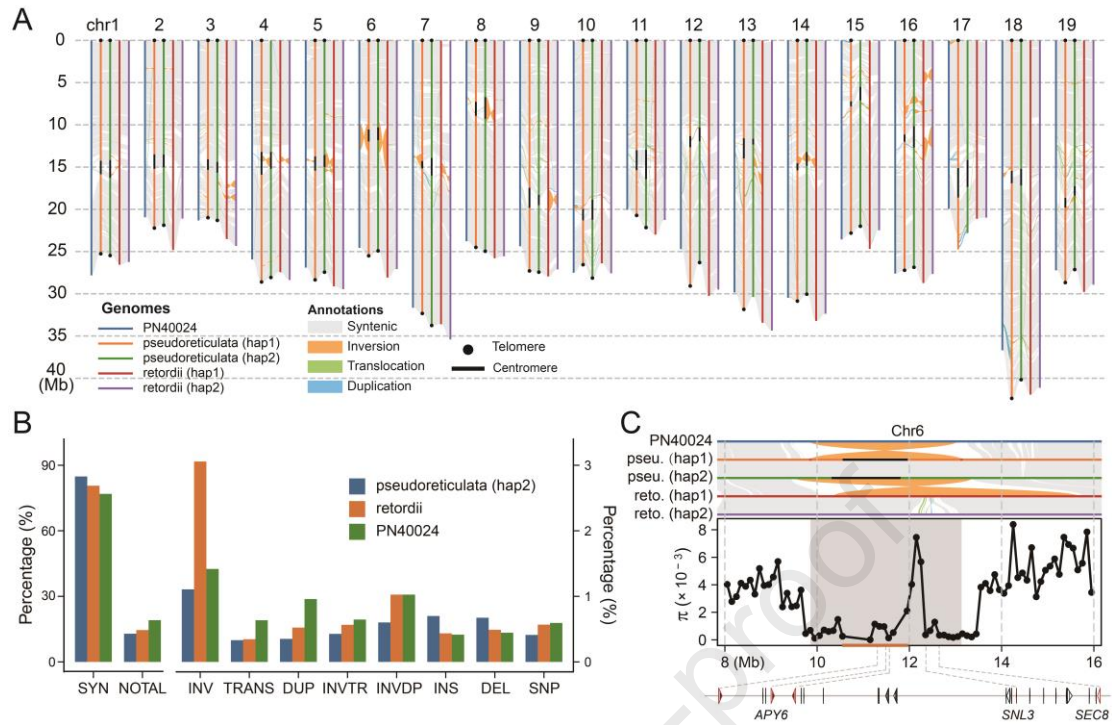


Fig. 2 Structure variations in the genome of *Vitis pseudoreticulata*

(A) Synteny relationship among *V. pseudoreticulata*, 'PN40024', and *V. retordii*. (B) Proportions of synteny (SYN), not aligned (NOTAL), inverted (INV), translocated (TRANS), duplicated (DUP), inverted translocated (INVTR), inverted duplicated (INVDP), insertion (INS), deletion (DEL), single nucleotide polymorphism (SNP) nucleobase in hap1 of *V. pseudoreticulata* genome as compared to its hap2, 'PN40024', and *V. retordii*. (C) The largest inversion on Chr6 of *V. pseudoreticulata*, along with genetic diversity and gene arrangement in this region. The abbreviations "pseu." and "reto." represent *V. pseudoreticulata* and *V. retordii*, respectively.

3.4. *Vitis pseudoreticulata* is genetically distinct among subtropical grapevine species

To explore the genetic diversity of *V. pseudoreticulata* at population scale, we sequenced 18.15–26.79 Gb (35×–50×) of pair-end resequencing data from 12 *V. pseudoreticulata* samples (Table S1). Combined with resequencing data from five other wild grapevine species obtained from previous studies, a total of 33 532 228 high-quality SNPs (genotype quality >20, missing rate < 30%) were identified and used for subsequent analyses.

Phylogenetic analysis showed that *V. pseudoreticulata* and the two other subtropical species (*V. adenoclada* and *V. retordii*) were grouped in a fully supported clade (SH-aLRT support = 100% and ultrafast bootstrap support = 100%), which was sister to the temperate clade composed of *V. amurensis* (Fig. 3, A). Samples from the same species were grouped in a monophyletic clade in Maximum Likelihood phylogenetic tree, shared the same ancestry in Admixture results (middle panel of Fig. 3, A), and clustered together in PCA results (Fig. 3, B). These indicated that the five grapevine species were genetically distinct from each other. The three subtropical grapevine species exhibited relatively higher genetic diversity, with *V. pseudoreticulata* showing the highest, as compared with the temperate *V. amurensis* (right panel of Fig. 3, A and Fig. S3, A). The predicted most suitable distribution range of *V. pseudoreticulata* overlapped with that of the other two subtropical species (*V. retordii* and *V. adenoclada*), while it was clearly distinct from the temperate grapevine species (*V. amurensis*; Fig. 3, C). Population demographic analysis revealed that the effective population size of *V. pseudoreticulata* gradually decreased since 100 000 generations ago, reaching its lowest point around 9 000 generations ago (approximately 27 000 years ago, assuming 3 years per generation) during the last glacial maximum, and then recovered slightly (Fig. 3, D). Different chromosomes of *V. pseudoreticulata* have undergone different demography histories, with

Chr6 showing the lowest and Chr2 having the highest effective population size after the last glacial maximum. Compared to the other East-Asian grapevine species, *V. pseudoreticulata* had a generally larger effective population size (Fig. S3, B). The three subtropical grapevine species had larger effective population size as compared with the temperate *V. amurensis*, and displayed distinct demography pattern. These findings highlight the uniqueness of *V. pseudoreticulata* and suggest its potentially superior adaptive fitness.

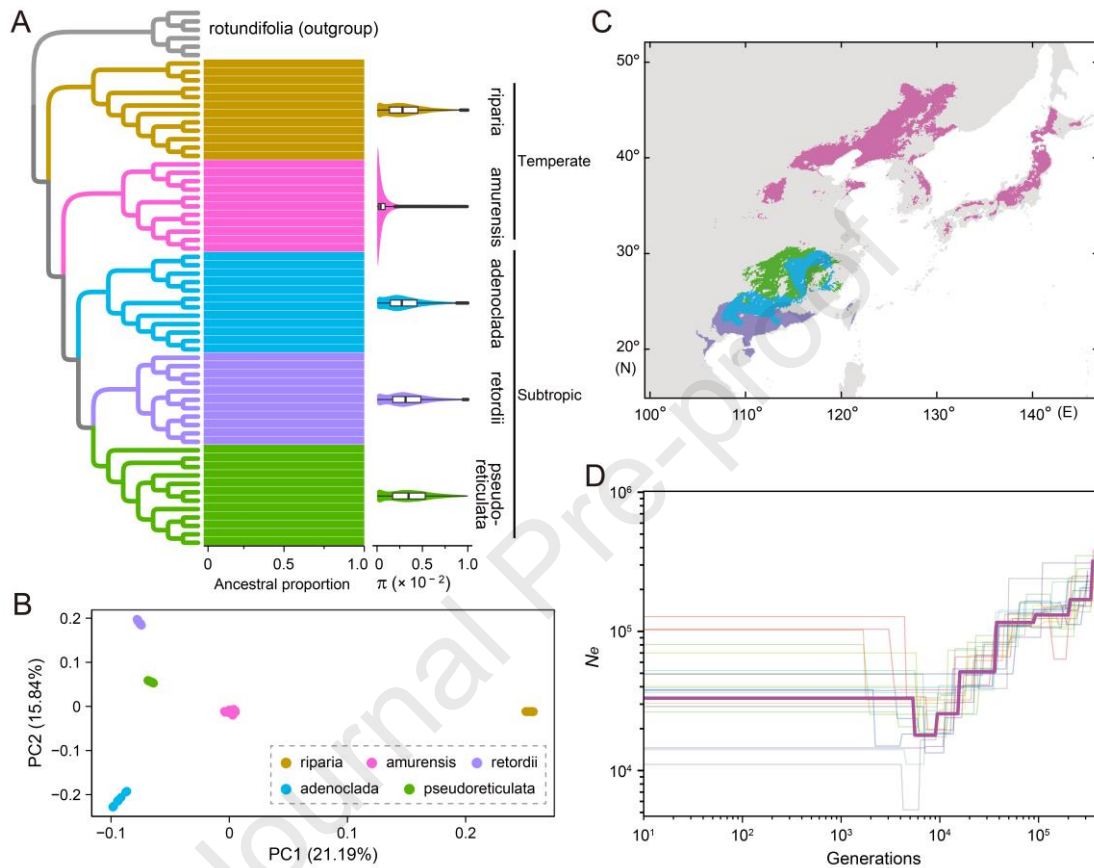


Fig. 3 Population structure of *Vitis pseudoreticulata*

(A) Maximum Likelihood phylogenetic tree (left), Admixture results (middle), and genetic diversity (right) for the five grapevine species. All major clades received 100% SH-aLRT support and 100% ultrafast bootstrap support. For genetic diversity, only the values between 0–0.01 are plotted to better highlight the difference between species, as values above 0.01 were mostly outliers (see Fig. S3, A for the full distribution). (B) PCA results. (C) Predicted most suitable distribution range of *V. pseudoreticulata*, two other subtropical species (*V. retordii* and *V. adenoclada*), and one temperate (*V. amurensis*) grapevine species using Maxent. Color of species is consistent with those in panel A. (D) Population demographic history of *V. pseudoreticulata*. The thick line represents estimation for the complete genomes, and the thin line represents estimation for each chromosome.

3.5. Genes under selection potentially promoted subtropical climate adaptation of *V. pseudoreticulata*

Genes under mutual selection among the three subtropical grapevine species are expected to have played a key role in subtropical adaptation of *V. pseudoreticulata*, due to their shared adaptation to similar climate conditions. Based on population branch statistical (PBS) analyses using PBScan v2020.03.16 (Hämälä and Savolainen, 2019), a total of 3 072 genes were detected in the selection outliers (top 5%) of subtropical *V. pseudoreticulata* population (upper panel of Fig. 4, A). For these genes, 22.0% (675) were shared among the selection outliers of the three subtropical grapevine species (Fig. S3, C and Fig. 4, B). These shared genes were mostly related to embryo development (GO:0009793), flower development (GO:0009908), response to salt stress (GO:0009651), and response to water deprivation (GO:0009414), etc. (Fig. S3, D; Table S7). Some of these genes,

such as *PAT10* (left panel of Fig. 4, C; Zhou et al., 2013), *SAC6* (Fig. S3, E; Zhong and Ye, 2003), *SIS8* (Fig. S3, E; Gao and Xiang, 2008), which are related to response to salt stress, showed relatively low intraspecies genetic diversity but high interspecies genetic divergence as compared to *V. amurensis*. This pattern indicates that these genes may have been subjected to strong selective pressures, potentially playing a critical role in enhancing the adaptive fitness of *V. pseudoreticulata*. Another gene, *TDX* (Fig. S3, F), related to heat acclimation (Lee et al., 2009), was also identified in the selection outliers of *V. pseudoreticulata* and showed relatively low intraspecies genetic diversity but high interspecies genetic divergence, which may have promoted its adaptation to hot climate. These shared genes likely contributed to the adaptation of *V. pseudoreticulata* and the other two subtropical grapevine species to their subtropical environments.

Furthermore, positive selection signals within *V. pseudoreticulata* population, were detected using a likelihood-based selective sweep detection method in SweeD (Pavlidis et al., 2013). A total of 1 920 genes were detected in the positively selected regions (top 5%) of *V. pseudoreticulata* genome (lower panel of Fig. 4, A and Fig. S4, A). These genes involved diverse biological processes (Table S8). Interestingly, in addition to some intracellular process, the biological process with the most genes was related to defense response (GO:0006952 with 36 genes) and defense response to fungus (GO:0050832 with 17 genes). It implies that the subtropical diseases, particularly the fungus diseases, were probably the most serious threat to *V. pseudoreticulata*. However, since *V. pseudoreticulata* have well adapted to its environment in the long evolutionary history, we would expect that these fungus response genes may convey higher resistance. And, some of the well-known fungal resistance genes, such as *RPV1* (Feechan et al., 2013) and *PMR5* (Vogel et al., 2004) genes, were exactly detected in the positively selected genes of *V. pseudoreticulata*. The versatile *FER* gene on Chr14 (right panel of Fig. 4, C), which is simultaneously related to defense response to fungus, pollen tube reception, post-embryonic development, root development, stomatal movement, and some hormone signaling pathways (Deslauriers and Larsen, 2010; Kessler et al., 2010; Yu et al., 2012, 2018) displayed relatively low intraspecies genetic diversity and high interspecies genetic divergence with *V. amurensis*, suggesting its important role in the fungal resistance of *V. pseudoreticulata*. Additionally, 10 genes related to response to heat (GO:0009408), 10 genes related to response to auxin (GO:0009733), and enormous genes related to the development of embryo, flower, root, pollen (GO:0009793, GO:0009908, GO:0048364, GO:0009555), etc. (Fig. S4, B) were also detected in the positively selected regions of *V. pseudoreticulata*, which have potentially contributed to its adaptation. Unexpectedly, only 48 genes were shared among the positively selected regions of the three subtropical grapevine species (Fig. 4, D and Fig. S4, A). These shared genes were involved in biological processes that are potentially important for breeding, including defense response to fungus (GO:0050832), flower development (GO:0009908, GO:0048444), and regulation of auxin polar transport (GO:2000012), etc.

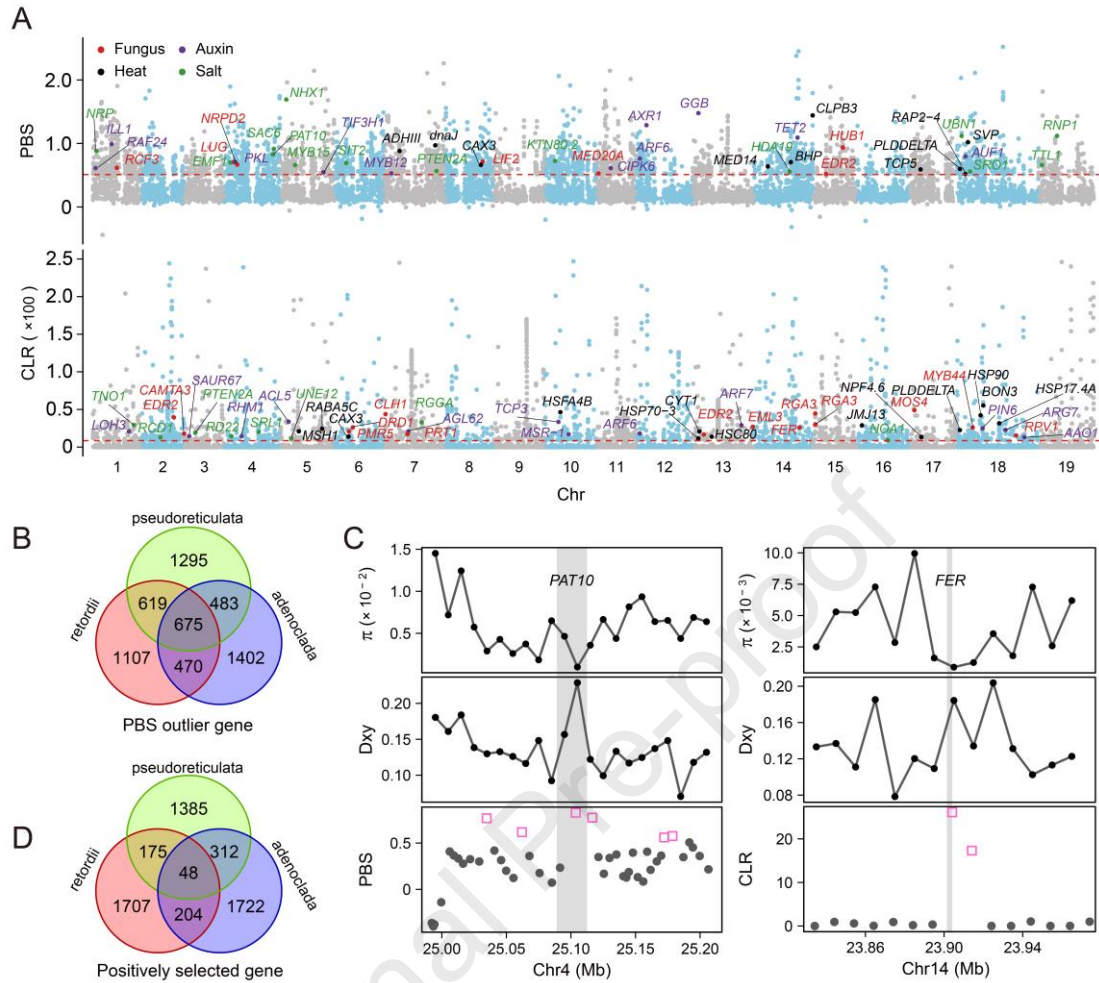


Fig. 4 Population selection signals of *Vitis pseudoreticulata*

(A) PBS (upper) and SweeD (lower) results of *V. pseudoreticulata*. For SweeD results, the windows with CLR values above 250 are not plotted, as no target genes need to be marked in this region. The red lines represent top 5% threshold. (B) Venn diagram showing the overlap of genes in the selection outliers of the three subtropical grapevine species in PBS analyses. (C) Genetic diversity, genetic divergence (compared with *V. amurensis*), CLR, and PBS (compared with *V. amurensis*) of two representative genes related to response to salt stress (*PAT10*) and defense response to fungus (*FER*) in *V. pseudoreticulata* genome. Pink box points represent values within the top 5% of PBS (left) or SweeD (right) results. (D) Venn diagram of positively selected genes in the three subtropical grapevine species in SweeD analyses.

Combining the selection signals from both PBS and SweeD analyses, we found that more than half of the genes under selection (54.37%; 2 632 unique genes in a total of 4 841 selected genes) were specific to *V. pseudoreticulata*. And, some biological processes of breeding interest, such as response to fungus, heat, auxin, and salt stress were frequently detected throughout analyses, though the number and composition of involved genes varied greatly. These genes, scattered throughout the genome (Fig. S4, C), may have collectively shaped the resistance, adaptability, and uniqueness of *V. pseudoreticulata*.

4. Discussion

Grapevine cultivation is facing significant challenges from fungal diseases (Price et al., 2015), particularly in humid subtropical regions (Bois et al., 2017; Solairaj et al., 2022). Wild grapevine species that have long adapted to these subtropical environments provide valuable genetic resources for the breeding of fungal-resistant cultivars. However, the scarcity of high-quality wild grapevine genomes has significantly hindered efforts in grapevine breeding.

In this study, we have newly assembled a phased chromosome-level genome of *V. pseudoreticulata* using HiFi, HiC, and ONT data, which may greatly facilitate the mining of resistant genetic resources in this species. Through population genomics analyses of 12 *V. pseudoreticulata* accessions, we have generated a detailed variation map for this grapevine species, uncovering novel selection signals in its genome that were not detectable in previous single-accession studies. The genome of *V. pseudoreticulata* showed high synteny, and shared similar genome size, gene content, and TE composition with the other grapevine species, suggesting that some other factors may have contributed to its adaptation.

Structural variations (SVs) play an important role in species adaptation (Zhou et al., 2019; Kou et al., 2020; Merot et al., 2020). In this study, SVs in *V. pseudoreticulata* affected a significantly larger proportion of its genome compared to SNPs and small indels, suggesting their more prominent role in the adaptation. Among the specific SVs of *V. pseudoreticulata*, genes related to meristem development were notably enriched, indicating that these SVs might be related to its fast-growing nature. Due to the limited availability of high-quality wild grapevine genomes, the SVs identified as specific to *V. pseudoreticulata* in this study will need to be verified in future studies. When species more closely related to *V. pseudoreticulata* are included, some of the SVs maybe no longer appear specific, but new, potentially more interesting findings may also emerge. The inclusion of SVs can significantly enhance the accuracy of identifying agronomic trait loci in crops (Zhou et al., 2022; Liu et al., 2024). Therefore, we recommend incorporating SVs in future detailed studies of *V. pseudoreticulata*, such as genome-wide association studies, to more precisely locate genes or loci associated with its superior traits.

Fungal diseases are a major threat not only to grapevine, but also to many other crops, such as melon (Cui et al., 2022) and wheat (Lu et al., 2020). Though fungal resistance has been reported in many wild grapevine species, little information is available regarding their resistance at population level. In this study, fungal resistance genes were significantly expanded in the genome of *V. pseudoreticulata*, which provided a solid foundation for it to be one of the most fungal-resistant wild grapevine species. Dozens of fungus response genes were under positive selection, or located in the branch-specific selected regions of *V. pseudoreticulata*, which may be directly related to its enhanced fungal resistance. Some of these genes are homology to previously confirmed fungal resistance genes in grapevine, such as *RPV1*, *RGA3*, *PMR5*, etc. (He et al., 2013; Yu et al., 2013; Hu et al., 2021), while some were newly detected in this study. These findings, combined with previous studies, supported the notion that fungal resistance is a multi-genes trait, which sometimes even involving genes related to salicylic acid, jasmonic acid, and other pathways (Weng et al., 2014). Further in-depth studies, particularly with the help of our new genome, are necessary to elucidate the full network of fungal resistance in *V. pseudoreticulata*. Fungal resistance of grapevine usually varies between species, or even individuals (Wan et al., 2007; Cadle-Davidson et al., 2011; Feechan et al., 2013; Merdinoglu et al., 2018). In this study, the majority of genes with adaptative fitness were specific in each species (only 48 were shared), suggesting that the adaptation of these grapevine species to similar environments or diseases may be mediated by different genes. This highlights the importance of maintaining the diversity of resistant and adaptative genes among the wild grapevine species for sustainable industry, particularly when diseases are continually evolving (Qiu et al., 2015; Kunova et al., 2021). Heat stress represents another challenge for grapevine cultivation, particularly in subtropical regions (Hu et al., 2016; Liu et al., 2018). In this study, some genes related to response to heat and stomatal movement were under positive selection, suggesting their potential role in adapting *V. pseudoreticulata* to hot climates. Given its strong adaptation to the subtropical climate, these genes detected in this study may have potential to enhance the subtropical resilience of domesticated grapevine. While this study has identified a substantial number of candidate genes of breeding interest, detailed experimental validation is needed to identify which genes have played a key role in the fungal

resistance and subtropical adaptation of *V. pseudoreticulata*. The reference genome, variation map, and candidate genes identified in this study provided valuable genomic resources for breeding grapevine and rootstocks with enhanced fungal resistance and subtropical adaptation.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by National Natural Science Foundation of China for Excellent Young Scientists Fund Program (Overseas) to Yongfeng Zhou, and Shenzhen Polytechnic University Research Project (Grant No. 6024330001K) to Ling Tian.

Supplementary materials

Supplementary data to this article can be found online at <https://doi.org/>

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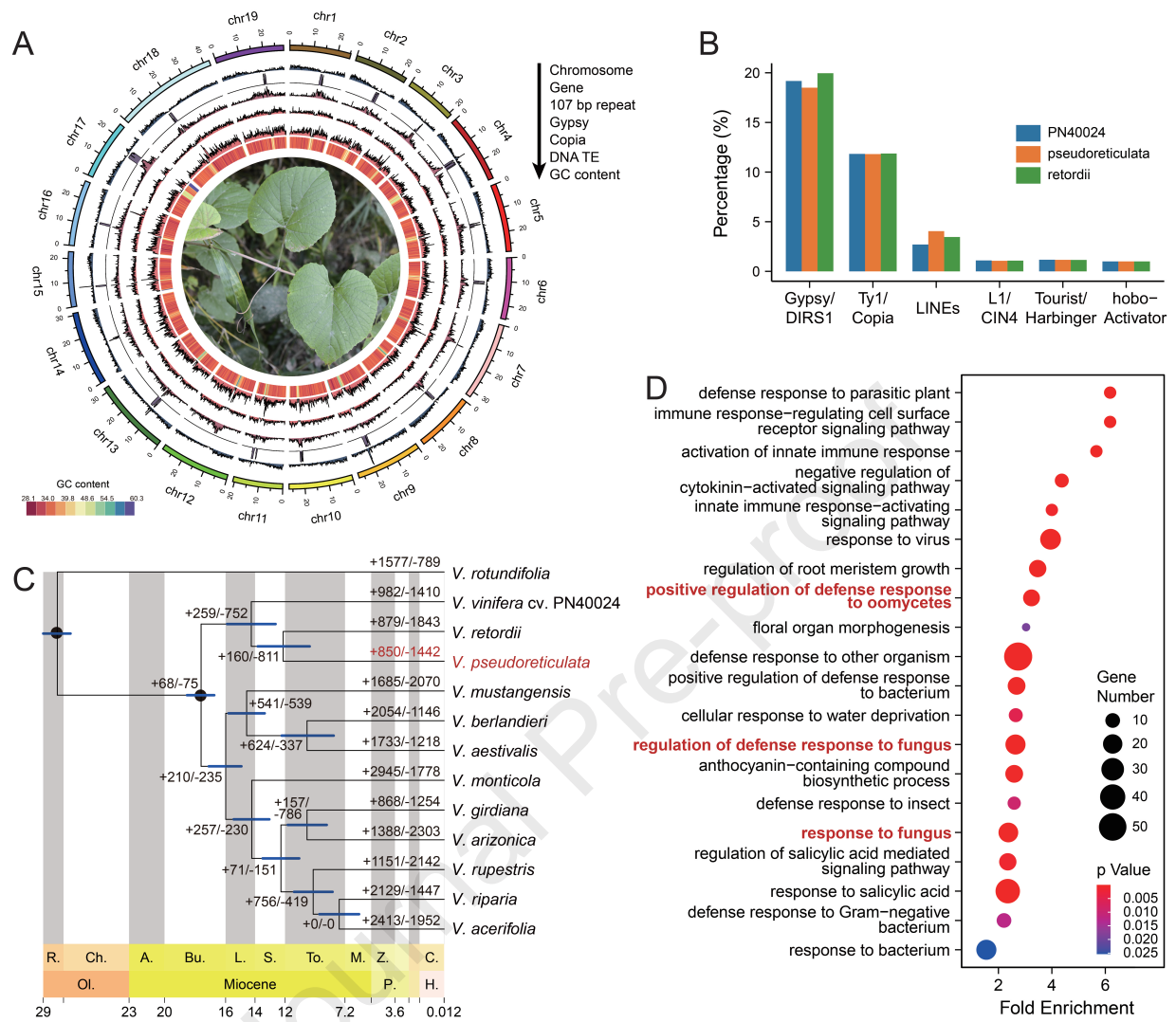
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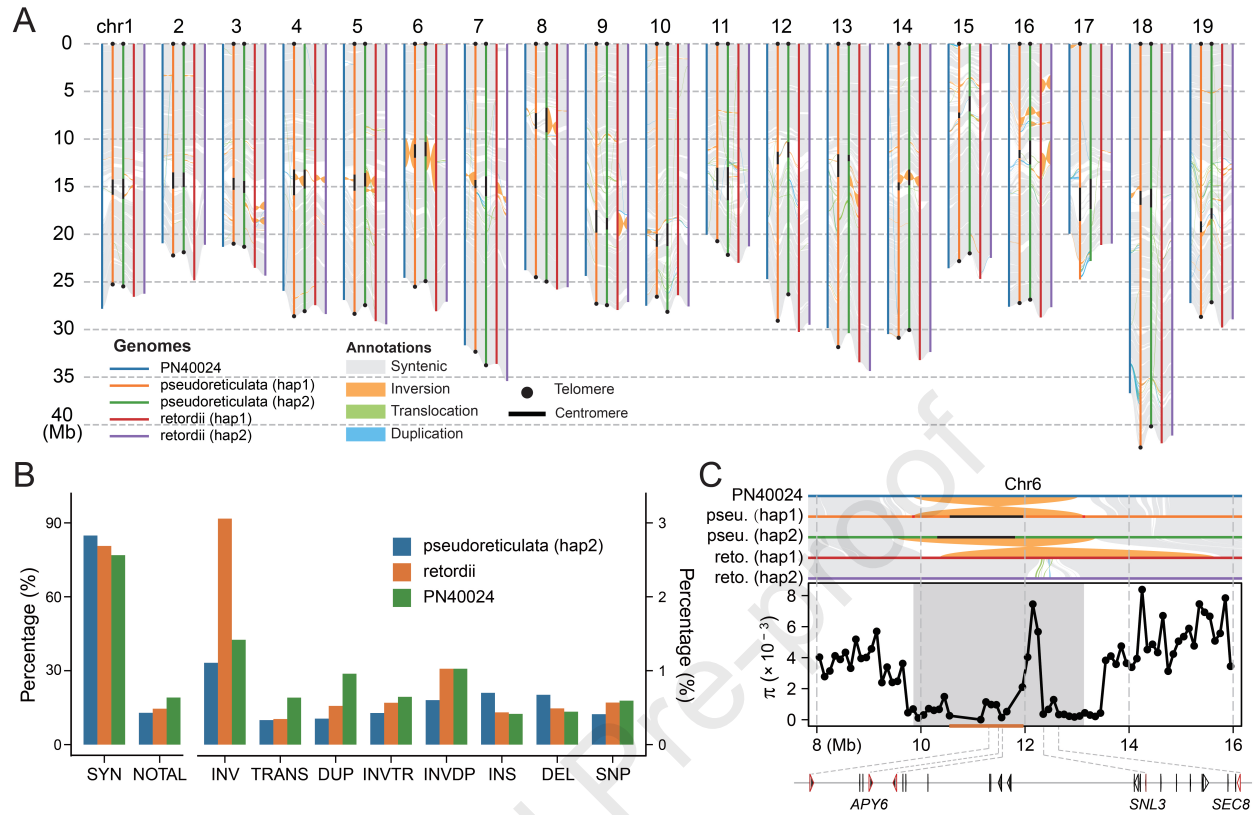
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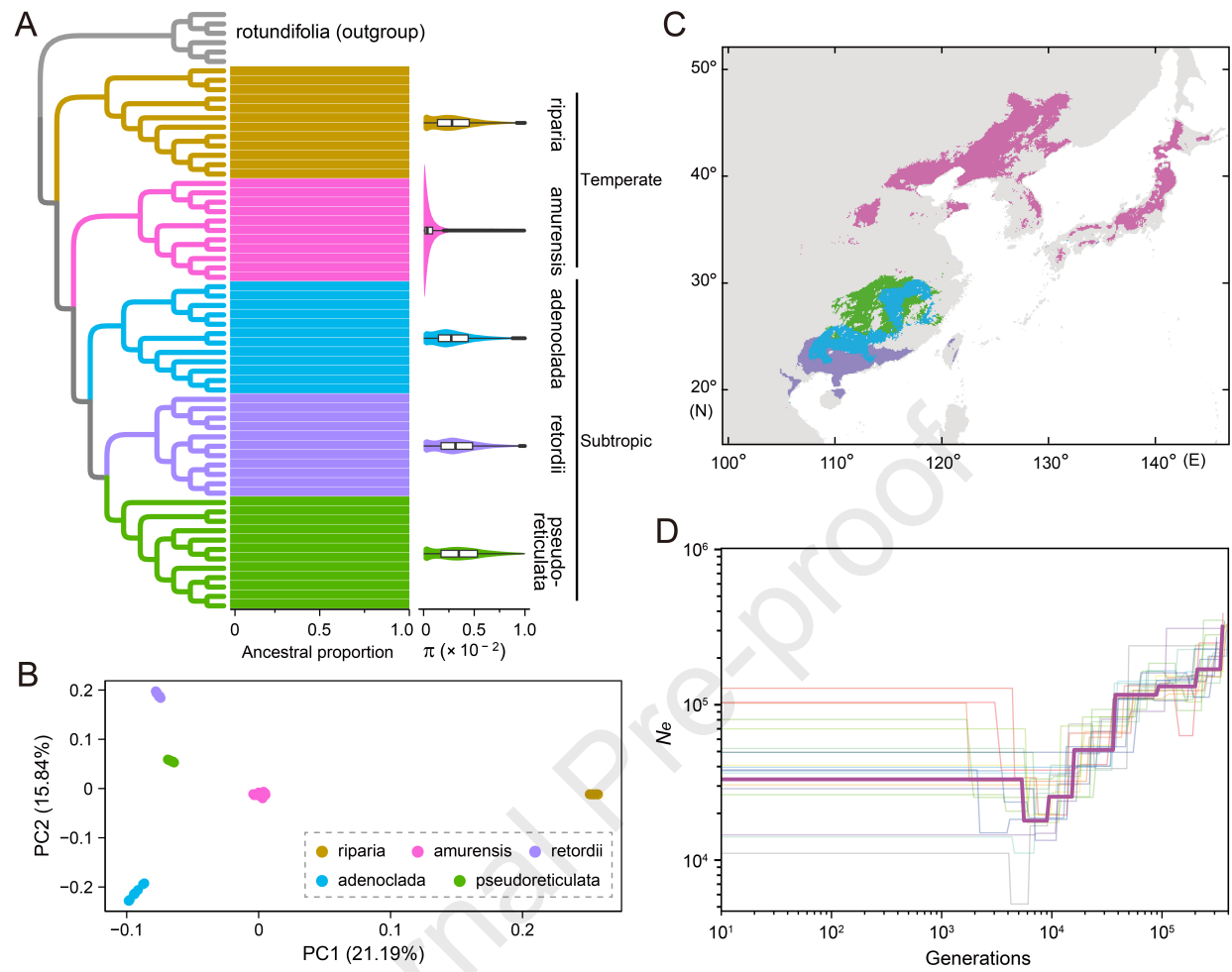
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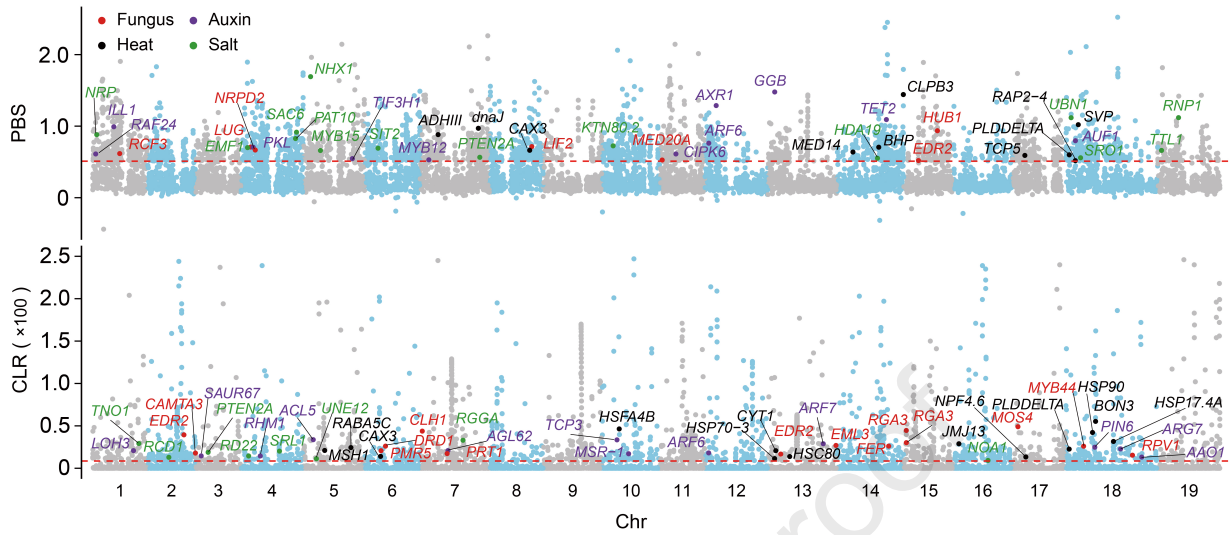
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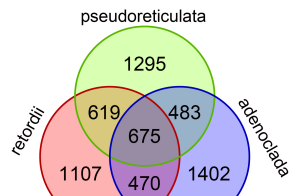




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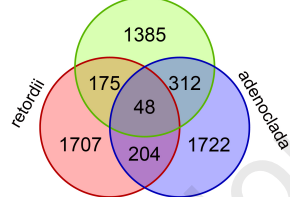
B



PBS outlier gene

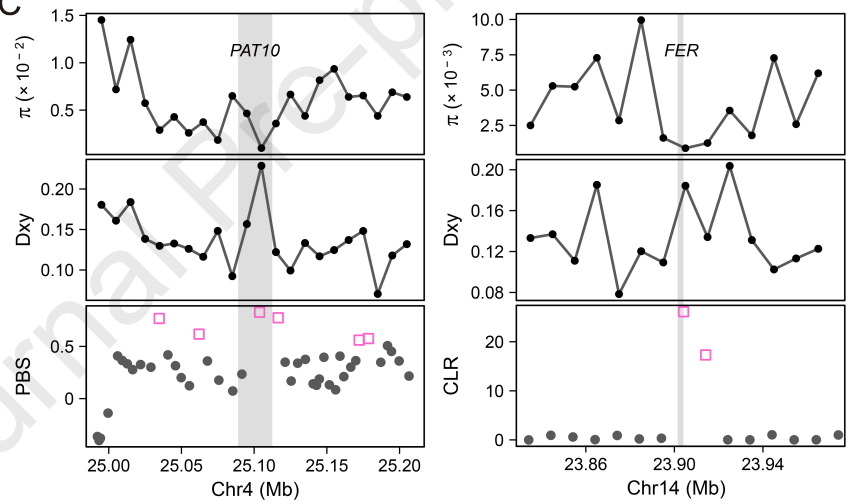
pseudoreticulata

D



Positively selected gene

C



Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.