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Chromosome-Level Genome Assembly and Population Genomic Analyses Reveal Geographic Variation and Population Genetic Structure of Prunus Tenella

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Abstract: *Prunus tenella* is a rare and precious relict plant in China. It is an important genetic resource for almond improvement and an indispensable material in ecological protection and landscaping. However, the research of molecular breeding and genetic evolution has been severely restricted, due to the lack of genome information. In this investigation, we created a chromosome-level genomic pattern of *P. tenella*, 231Mb in length with a contig N50 of 18.1 Mb by Hi-C techniques and high-accuracy PacBio HiFi sequencing. The present assembly predicted 32088 protein-coding genes, and an examination of the genome assembly indicated that 94.7% among all assembled transcripts were alignable to the genome assembly; most (97.24%) were functionally annotated. By phylogenomic genome comparison, we found that *P. tenella* is an ancient group that diverged approximately 13.4 million years ago (Mya) from 13 additional closely related species and about 6.5 Mya from the cultivated almond. Collinearity analysis revealed that *P. tenella* is highly syntenic and has high sequence conservation with almond and peach. However, this species also exhibit many presence/absence variants. Moreover, a large inversion at the 7,588 kb position of chromosome 5 was observed, which may have a significant association with phenotypic traits. Lastly, population genetic structure analysis in eight different populations indicated a high genetic differentiation among the natural distribution of *P. tenella*. This high-quality genome assembly provides critical clues and comprehensive information for the systematic evolution, genetic characteristics, and functional gene research of *P. tenella*. Moreover, it provides a valuable genomic resource for in-depth study in protecting, developing, and utilizing *P. tenella* germplasm resources.

Keywords: *Prunus tenella*; genome; assembly; almond; population genetic structure

1. Introduction

Wild almond (*Prunus tenella*) is one of the oldest relict plants left from the Tertiary Miocene epoch and is mainly distributed in China and Kazakhstan[1]. In China, wild almond is an endangered and rare species with economic, scientific, and cultural importance and is scattered only in the northern mountainous areas of Xinjiang province[2]. It is considered to be at danger of extinction due to natural and human-caused disturbances, and as a result is on the list of nationally significant protected wild plants.

As a relatively wild species of cultivated almond, wild almond has adapted to extremely harsh environments, displaying impressive cold and drought tolerance[3]. For example, it can grow normally in the hills and mountains in Tacheng and Altay at extremely low temperatures reaching -35° C. Additionally, wild almond possesses exceptional agronomic traits and unique genomic

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characteristics, making them highly valuable for diverse applications in various fields, such as food processing and medicine[2]. Additionally, these characteristics provide precious genetic materials for studying the adaptive evolution of the wild almond genome and allow for the genetic improvement of cultivated almond, thus improving the resistance of cultivated almond to biotic and abiotic stresses and improving yield.

With the increased attention to wild resources, many desirable traits have been mined and applied to agricultural varieties[4–8]. Moreover, many breakthrough varieties have also benefited from the discovery of wild-breeding genetic resources[9–12], especially for wheat, rice, soybean, and other economically important food crops[13–16]. Together, these lines of evidence have shown that introducing wild gene resources has greatly improved disease resistance, insect resistance, and the growth of cultivated varieties[17–21].

In *P. tenella*, some genes such as self-incompatibility-related genes SBPI, Petcullin1, SFB, SSK1, S-RNas, and the cold resistance-related gene AlsCBF1-A were identified by homologous cloning technology[22–24]. Chloroplast DNA (cp DNA), ISSR, and SSR markers are only a few of the molecular markers that were applied for examining population structure and diversity in the past[25,26]. Yet, a thorough knowledge of the economically significant genetic features has been severely hampered by a paucity of genomic resources. More research into the evolutionary adaptations and the development process of distinct features is required before the genetic characteristics of wild almonds can be properly analyzed.

Obtaining high-quality reference genome sequences is the key to revealing allelic variation, genetic relationship, and evolutionary history[27–31]. The present paper describes a highly accurate chromosome-scale standard genome of *P. tenella* assembled from scratch using Hi-C technique and lengthy PacBio SMRT reads. Additionally, the genetic variation and geographical differentiation of 130 individual plants from 8 wild natural populations were assessed using genome-wide high-resolution molecular markers, which provided important basis for advancing our understanding of origin, formation, and geographical distribution profile of *P. tenella*.

2. Results

2.1. De novo assembly of the wild almond genome

After quality assessment (GC distribution statistics, quality value Q20, Q30 assessment) and filtering, 29.55 Gb clean data were obtained for genome size assessment and assembly. The sequencing depth totaled approximately 137.00×, with a GC content of approximately 38.15%. Additionally, the proportion of Q20 was greater than 97.19%, and the proportion of Q30 was greater than 91.81%. Based on kmer distribution analysis, the sample genome size was estimated at around 215.65 Mb, consisting of 35.76% repeat sequences and 0.47% heterozygosity. A kmer distribution of k = 21 was observed (Supplementary Figure S1).

Using PacBio SMRT single molecule sequencing, 34.6 GB high-accuracy HiFi reads were obtained for de novo genome assembly. The average length of ccs produced was more than 1,765 bp, and the longest was 34,337 bp. After assembly and deduplication, a contig level assembly of 231 M sequences was obtained, a contig-level assembly of 231 M sequences was obtained, comprising 3,073 contigs (longest = 35.9 Mb) with a 18.1 Mb contig N50 (Table 1). After sequencing quality control, 36.05 Gb Hi-C Fastq clean data were obtained by 3D de novo assembly with the proportion of Q30 was more than 93.10% and the effect rate of 32.5%. Finally, 231 Mb of sequences were anchored onto 8 pseudochromosomes (Figure 2), covering 479 scaffolds (longest = 44.8 Mb, scaffold N50 = 25.6 Mb) (Table 1). A 89.7% mounting rattion have been estimated (Table 2). The BUSCO results showed that more than 2009 (94.7%) genes could be compared to the lineal homologous database, of which 62.5% were single-copy, and 32.7% were duplication (Table 3).

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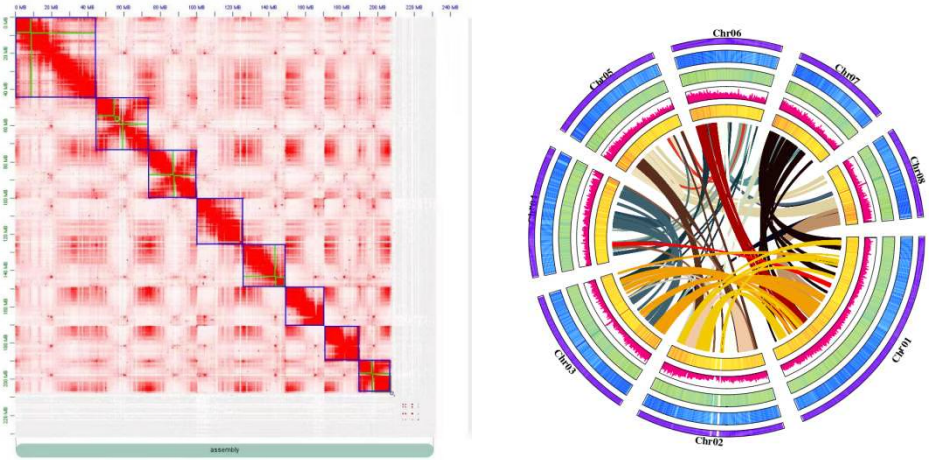
Figure 1. Morphological characteristics of the *Prunus tenella.* A) Flower, B) Fruit, and C) Seed.



(**A**) (**B**)

Figure 2. Hi–C assisted assembly of *Prunus tenella genome* pseudomolecules. **A.** high-resolution (100 kb) Hi-C interaction heat map among eight chromosomes. **B.** Chromosome characteristics of the Prunus genome. Following the outermost ring are the following items: Pseudochromosomal visualization of gene density, GC content, repeat content, SNP density, and gene collinearity from a genome assembly.

Table 1. Statistical findings of the assembled *Prunus tenella* genome.

Term	Contig number	Contig size (bp)	Scaffold number	Scaffold size (bp)
N90	5	270515	9	1233122
N80	12	8938573	7	19226261
N70	11	10302541	6	21538263
N60	15	14010243	5	23141640
N50	1	18100976	4	25637364
Max length (bp)		35886393		44825466
Total size (bp)		231191648		231208648
Total number		513		479
Average length		450665.98		482690.29
Number >= 10kb		513		479

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Table 2. Statistical findings of chromosomal level assembly of *Prunus tenella.*

Chr ID	Length (bp)
Chr1	44825466
Chr2	29024987
Chr3	26387986
Chr4	25637364
Chr5	23141640
Chr6	21538263
Chr7	19226261
Chr8	17664456
Total chromosome level contig length	207446423
Total contig length	231208648
Chromosome length/Total length	89.7%

Table 3. Findings of *Prunus tenella* genome intgerality assessment by BUSCO.

Library	eudicotyledons_odb10
Fragmented BUSCOs (F)	48
Missing BUSCOs (M)	64
Complete and duplicated BUSCOs (D)	42
Complete and single-copy BUSCOs (S)	1967
Complete BUSCOs (C)	2009
Total BUSCO groups searched	2121
Summary	94.7%

2.2. Functional annonations, gene prediction, and repetitive sequences

To further describe the wild almond genome, we categorized all sequences that recur using a combination of de novo and homology-based methods. We estimated that transposable elements made up 28.97% of the genome, with TIRs making up to 7.56% and non-TIRs making up to 3.04%. Table 4 shows that only long terminal repeat (LTR) retrotransposons were found. The present assembly projected a total of 32088 protein-coding genes, of which 97.24% had at least one public database functional annotation (Table 5).

Table 4. Statistical findings of repeated sequences TE annotations in *Prunus tenella* genome.

Class	Length (bp)	Type	Sub-Class	(%)
retrotransposons	8454829	Ty1/Copia	LTR	3.66%
	18247107	Ty3/Gypsy		7.89%
	15756928	unknown		6.82%
	-	LINE	Non-LTR	-
	-	unknown		-
DNA transposons	1845596	CACTA	TIR	0.80%
	7818414	Mutator		3.38%
	4181524	PIF/Harbinger		1.81%
	278071	Tc1/Mariner		0.12%
	3345229	hAT		1.45%
	7038992	helitron	Non-TIR	3.04%
Total	66966690			28.97%

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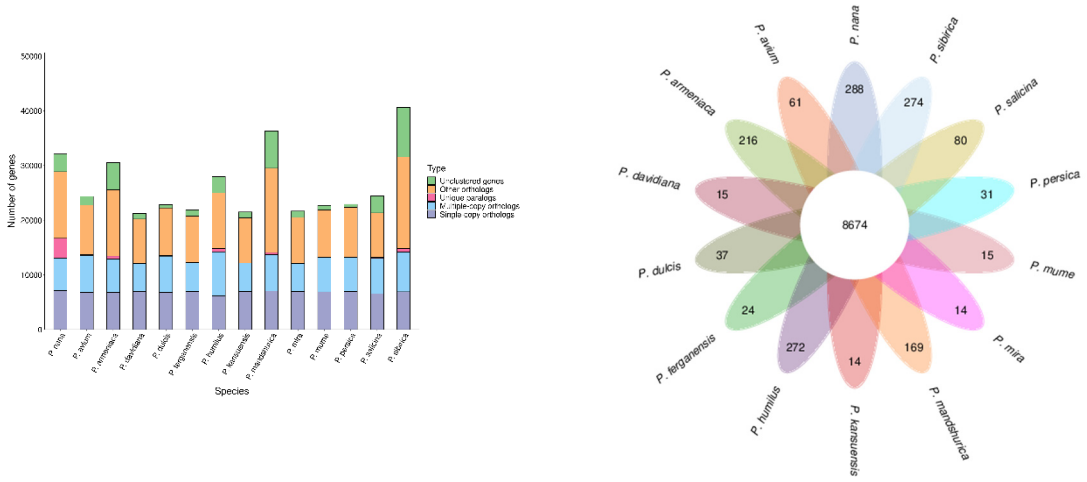
Table 5. Protein-coding genes-related functional annotations in *P. tenella* genome.

Database	Gene numbers	(%)
GO	10761	33.54
KEGG	11435	35.64
KOG	19251	59.99
Swissprot	19449	60.61
Pfam annotation	22815	71.1
Nr annotation	31202	97.24

2.3. Synteny analysis, and genome evolution and phylogeny

We utilized 10,184 gene families from related species, including, P. avium, P. armeniaca, P. kansuensis, P. ferganensis, P. dulcis, P. davidiana, P. humilus, P. mandshurica, P. mira, P. mume, P. persica, P. salicina, P. sibirica. Among them, 8,674 were common to all species, while 288 were unique to wild almonds (Figure 3).



(**A**) (**B**)

Figure 3. Number of homologous genes in species and gene family clustering. A) Homologous gene statistics. B) Gene family clustering petal map. .

The period of divergence was then approximated by comparing the protein sequences from every single-copy gene families common to those species, and the resulting phylogenetic trees were created. Bootstrap values greater than 90% provided strong evidence for the association between the species (Figure 4). Indeed, all seven species of the peach genus are on the same branch; among them, wild almond is an ancient group that diverged approximately 13.4 million years ago (Mya). Furthermore, wild almond almost diverged about 6.5 Mya from the cultivated almond. A total of 2679 genes involved in expansion and contraction were identified by gene family analysis, among which 1581 genes in 105 gene families were significantly expanded, and 26 genes in 23 gene families were significantly contracted. Together, these data indicate that the wild almond gene family has undergone a significant contraction (2256 genes) relative to other species (486-1613 genes) in the peach genus, which may be related to natural selection.

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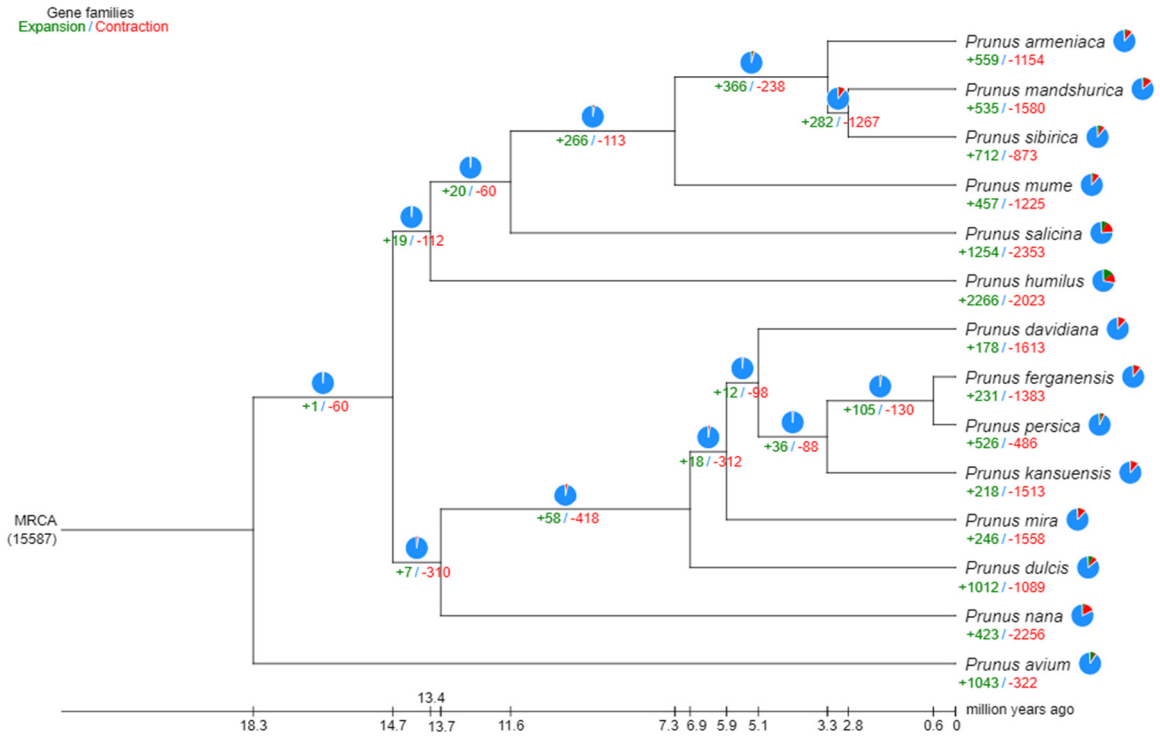

Figure 4. Divergence time and phylogenetic correlation among species. The percentage of conserved (blue), contracted (red), and expanded (green) gene families among all gene families in the 14 species is shown as a pie chart.

Next, synteny analysis was performed to further understand genes' position relationship on homologous chromosomes and the variation of genome structure. Coding gene and genome-wide collinearity analysis showed a highly linear relationship between wild almond, cultivated almond, and Peach. Meanwhile, 1,540,264 SNPs, 105,280 deletions, and 155,863 insertions (including absence/presence variations) sequences were identified when compared with almond (Supplementary Table S1). Compared with Peach, the 1,574,620 SNP, 106,957 deletions, and 161,747 insertions (including absence/presence variations) sequences were identified (Supplementary Table S2). These structural variations are the main source of genomic variation and may have a significant association with phenotypic traits. In addition, a large inversion（7588kb) was identified on chromosome 5, which is valuable for further understanding gene regulation and epigenetic inheritance in wild almond (Figure 5).

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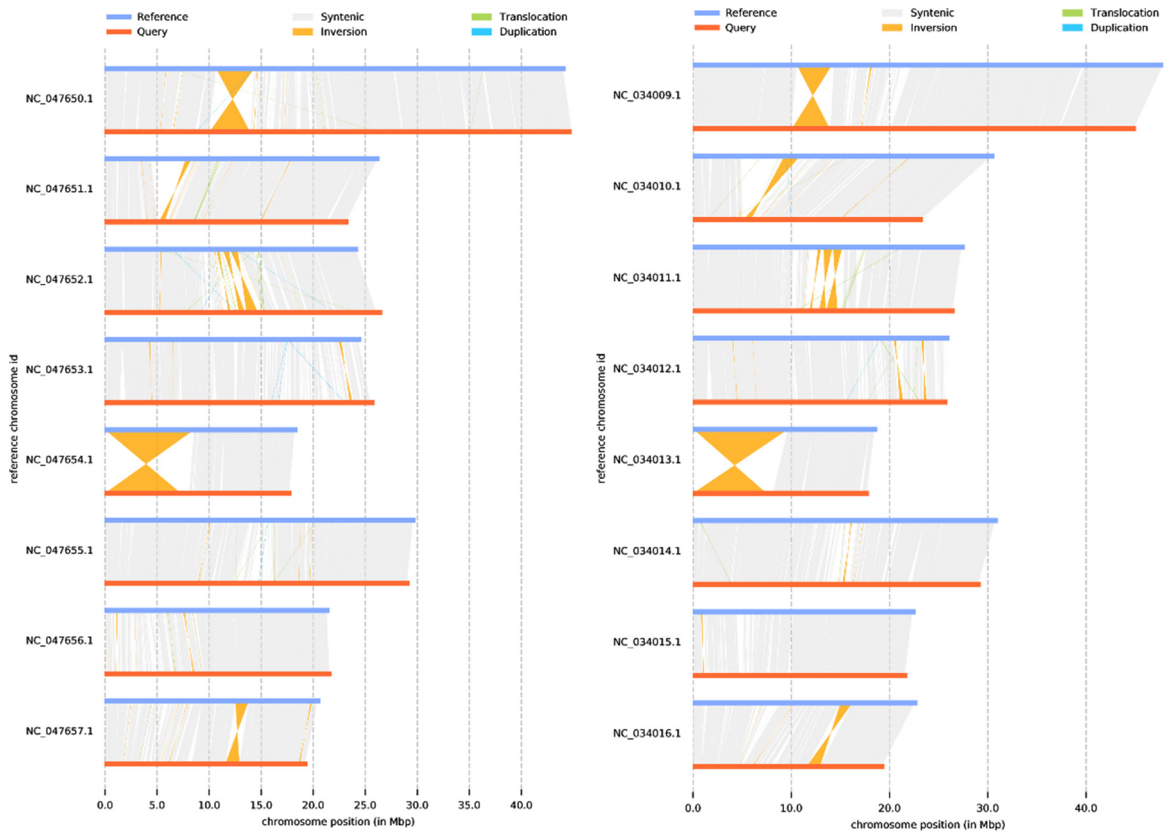

A. *Prunus dulcis* vs *Prunus tenella* B. *Prunus persica* vs *Prunus tenella*

Figure 5. Genome structure map. The grey area is the collinear relationship area, yellow is the inversion area, green is the translocation area, and blue is the area where reduplication occurs.

2.4. Population genetic structure and genetic diversity analysis

Using the Illumina HiSeq X Ten platform, 512.5 Gb of clean PE150 paired-end data (approximately 15 average sequencing depth) was generated from 130 separate specimens gathered from the native area in Xinjiang Province, revealing the genetic variants in wild almond across different geographic populations. Based on the genetic distances derived from the genotypes at all the SNP sites of the eight subpopulations in the three areas, a maximum-likelihood and neighbor-joining (Figure 6A) phylogenetic tree was created using the SNPs/genotypes. Some individuals within the subgroups were grouped in other subgroups, but overall, the groupings from the three locations exhibited strong genetic isolation and constituted distinct groups within the phylogenetic tree. The phylogenetic tree's conclusion was confirmed by principal component analysis. Tacheng, Yuoli, and Yumin samples clustered together in a distinct subgroup of the PCA (Figure 6B).

Structure analysis results also showed that the 130 samples were mainly from three ancestral populations, consistent with their distribution areas. The samples from the Tacheng are a pure population, while the TuoLi and Yumin samples are hybridized populations with slight levels of admixture (K = 3; Figure 6C). Genetic diversity analysis showed that Expected_heterozygous_number, Observed_allele_number, Nei_diversity_index, and Shnnon_Wiener_index were 0.17-0.29, 1.31-1.49, 0.18-0.30 and 0.25-0.43, respectively (Supplementary Table S3). Compared with other populations, the genetic diversity of the three populations in Yumin was relatively high, while the Tacheng population was relatively low.

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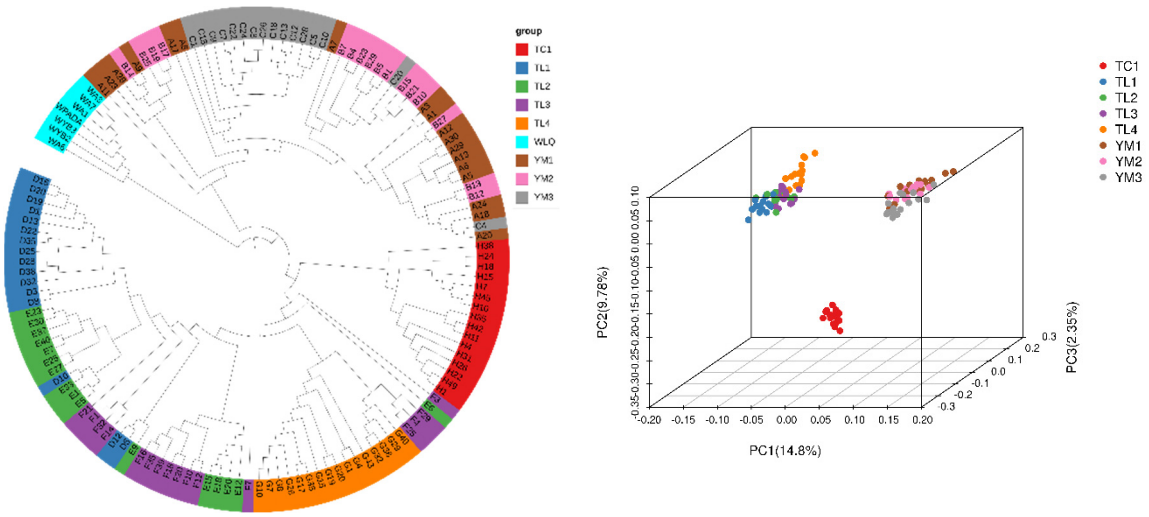
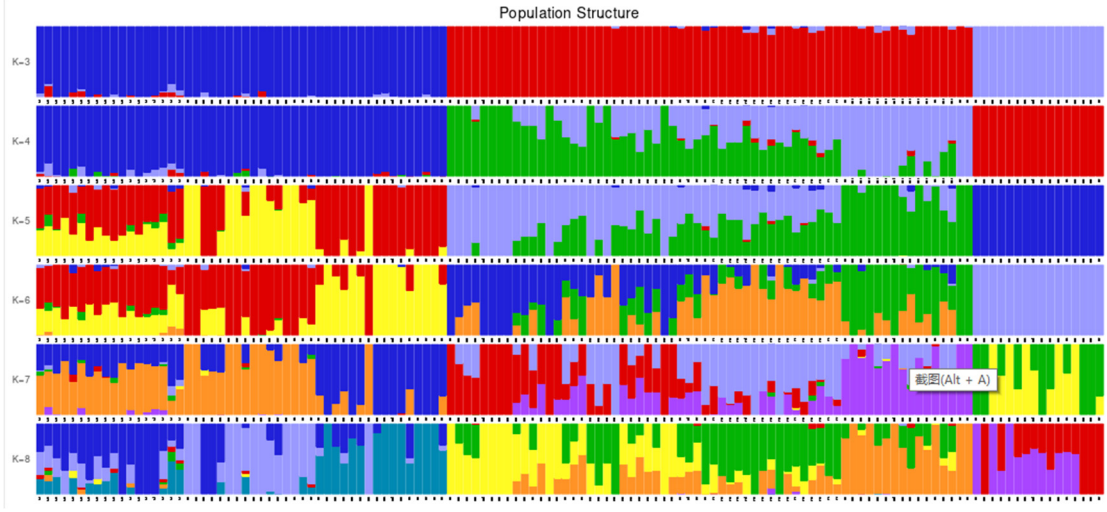


A. Results of the NJ evolutionary tree analysis B. PCA 3D clustering plot



C. Distribution of population genetic structure

Figure 6. Phylogeny and population structure of different populations. (A) phylogenetic tree; (B) PCA three-dimensional cluster diagram of samples; (C) Admixture sample clustering results corresponding to K values (3-8).

2.5. Genome-wide selection signatures analysis of differentiation

To understand the genetic differentiation among populations, we conducted selective sweep analyses and calculated all pairs of Fst values between 8 sampling populations in three different regions. The results showed that the genetic differentiation between Tacheng and Yumin populations was 0.29-0.32. The genetic differentiation between Tacheng and Tuoli was 0.28-0.3, and the genetic differentiation between Tuoli and Yumin was 0.21-0.27. The inter-population genetic differentiation within the region was low, with the differentiation coefficient falling between 0.05 and 0.1 in the four sample populations of Tuoli and between 0.05 and 0.09 in the three sample populations of Yumin. These results indicate that there had been strong genetic differentiation and geographical variation among different geographical regions of wild almond, which may be due to the obstruction of gene flow caused by geographical isolation.

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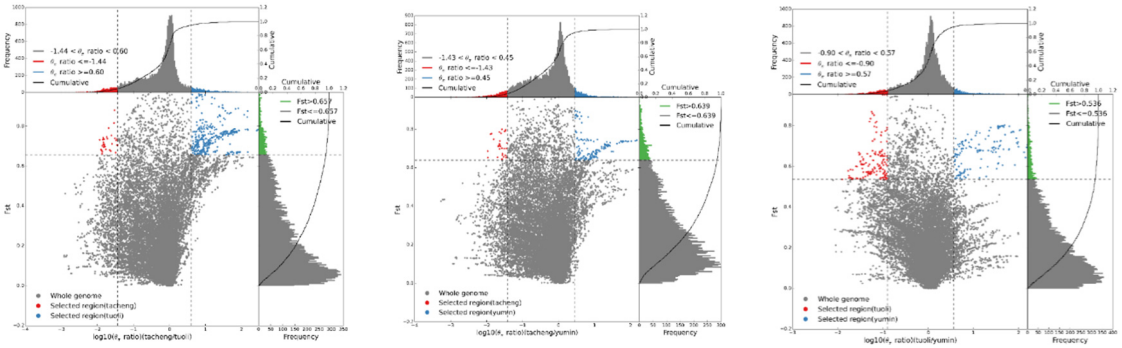

A. TC_vs_TL B. TC_vs_Yumin C. TL_vs_Yumin

Figure 7. Schematic of selection signatures between populations. A.)Selective sweep analysis of Tacheng Vs. Tuoli; B.) Selective sweep analysis of Tacheng Vs. Yumin; C.) Selective sweep analysis of Tuoli Vs. Yumin; The ratio of π in the abscissa and Fst in the ordinate corresponding to the frequency distribution diagram above and the frequency distribution diagram on the right, respectively. The dot plot in the middle represents the corresponding Fst and π ratios in different windows. The blue and red areas at the top are the top 5% of the region selected by π, the green area at the right is the top 5% of the region selected by Fst, and the blue and red areas in the middle are the intersection of Fst and π, which are the candidate sites.

3. Discussion

Through the use of various sequencing methods, we assembled the first complete reference genome for wild almond in this work. The *Prunus genus* has several commercially and ecologically significant species in forestry and agriculture, and these data are essential for learning more about wild almonds and the genus as a whole. These findings will also aid in the development of genome-enabled wild almond breeding initiatives. Last but not least, wild almond's status as a relict species makes it a useful model for studying the genetic basis of population formation, evolution, and adaptation to environmental effects under conditions of geographic isolation.

Given the high quality of our wild almond genome assembly, high-depth PacBio long-read, and whole genome re-sequencing data, we now have a comprehensive understanding of the genome of the wild almond. Single-copy, multi-copy, and species-specific gene families were obtained, the evolutionary status was inferred, and the genome's evolutionary history was traced, laying the foundation for further exploration and research. Additionally, we found many variation sites, including SNPs, insertions, deletions, and inversion. Many of these variants may be associated with phenotypic traits, which will help understand the phylogenetic evolution of wild almond. Moreover, these various sites can be used as important molecular markers for germplasm identification, genetic analysis, functional gene extraction, and assisted breeding.

Through the analysis, we also found some different characteristics of the wild almond genome relative to other species of the same genus. Compared with other tree species, wild almond had relatively more endemic gene families（288）, while cultivated almond and peach had relatively few, only 31-37, which may be related to the earlier differentiation time of wild almond. More gene families were contracted in wild almond, which might be related to natural selection caused by the extreme natural environment. The evolutionary status inferred by the phylogenetic tree shows that the wild almond is an independent branch, most closely related to cultivated almond. Importantly, this observation indicates that wild almond has the potential utilization value of providing genetic resources for cultivated almond, and this lays the foundation for further exploration and research.

Population genetic structure can be used to analyze the evolutionary dynamics of a population by describing gene transmission, gene frequency change, and genotype distribution[32–35]. Based on the SNP information derived from the whole-genome re-sequencing data, thousands of single SNP markers can be used for the fine-scale description of genetic structure[36–38]. The results showed that compared with the Tacheng （Nei's = 0.18; Ho = 0.16）and Tuoli（Nei's = 0.23-0.26； Ho = 0.16-0.22 ）populations, the Yumin variety（Nei's = 0.26-0.3； Ho = 0.17-0.22）has relatively high genetic

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diversity. This observation is consistent with the study on genetic diversity using chloroplast sequences[4]. Additionally, the results indicated a high genetic differentiation among the natural distribution of wild almond, as the pairwise genetic differentiation (Fst) in a different region is 0.23-0.32, especially within the Tacheng and Yumin group where Fst reached 0.29 to 0.32, values much higher than wright's high differentiation coefficient[39–41]. However, there is little differentiation between subgroups within the Tuoli and Yumin group (0.05-0.1). These results suggest that geographical isolation is an important factor affecting the genetic evolution of wild almonds. This higher differentiation may result from long-term natural selection without gene flow.

In summary, we assembled the first chromosome-level genome of *P. tenella* and assessed the genetic variation and geographical differentiation of 8 natural populations, which laid a solid foundation for further research on genetic improvement and formation mechanism of important characters in the future.

4. Materials and Methods

4.1. Utilized materials

P. tenella sample materials used for genome assembly were obtained from the germplasm conservation nursery of Xinjiang Academy of Forestry Sciences, Xinjiang, China (Figure 1). Fresh leaves were utilized for Hi-C library development, PacBio HiFi sequencing, and Illumina sequencing. To aid in genome assembly and annotation, fruit, leaf, root, and stem tissues were taken for RNA-seq study.

The fresh leaves used for whole genome re-sequencing were collected from Yumin County, Tuoli County, and Tacheng City, Xinjiang, China. A total of 8 wild almond populations were collected, including 3 from Yumin County, 4 from Tuoli County, and 1 from Tacheng City. Approximately 15-18 samples were collected from each population. In addition, 7 cultivated almond samples were collected for population evolution analysis.

4.2. Genome sequencing and transcriptome sequencing

The experiments were carried out in accordance with Illumina's recommended methodology. The ultrasonic shock was used to physically fragment the qualifying genomic DNA into fragments (350 bp), and then end restoration, adding A, an adapter, and target fragment picking and PCR were used to generate the tiny fragment sequencing library. By using bridge PCR, the library was transferred to the sequencing chip. An Illumina sequencer performed double-ended 150 bp (PE 150) library sequencing.

DNA capture and purification, cyclization, end repair, endonuclease digestion, cell cross-linking, and on-machine sequencing were all necessary steps for HI-C sequencing to be completed. The mRNA was utilized to synthesize full-length cDNA with the help of the SMARTerTM PCR cDNA Synthesis Kit, which was then used to generate sequencing libraries. Using the PacBio system, we sequenced the whole transcriptome.

Library sequencing, library quality testing, library creation, and sample quality testing were all carried out as per Illumina's recommended approach for re-sequencing a variety of population samples. In order to prepare the DNA for sequencing, it was first physically fragmented (using ultrasonic waves), then purified, the ends were mended, the 3' end was augmented with A, and the sequencing joint was linked. Finally, agarose gel electrophoresis was used to determine the optimal fragment size, and PCR amplification was carried out to form the sequencing library.

Transcriptome sequencing of the stem, root, leaf, and fruit tissues was performed on the NovaSeq 6000 platform.

4.3. Assurance of Sequencing Data Quality

Low-quality sequences and duplicated readings in the sequencing data were removed using stringent filtering algorithms that were optimized for the particular platform utilized to assure data integrity and accuracy. Filtering criteria included the following actions for Illumina Hi-Seq data:

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Firstly, polyG tails were removed. Secondly, paired reads less than 100bp in length were discarded. Thirdly, read pairs containing more than 10% of bases that are the same as the next base were removed. Fourthly, read pairs with over 50% low-quality bases (quality score less than 10) were discarded. The last step was to clean the data of read pairings with a typical quality rating below 20. The Hi-C sequencing results went through a comparable filtering procedure as Illumina Hi-Seq Data before being processed in 3D. With the default settings of the pbccs pipeline, subreads from the PacBio HiFi long readings were filtered and corrected immediately. Approximately 2,000 PacBio HiFi (CCS) reads were randomly selected from the sequencing data and compared with the NT library to evaluate whether the sequencing data contained contamination.

4.4. Heterozygosity and genome size estimation

Heterozygosity and genome size were analyzed before HiFi library construction and sequencing. From the Illumina data, Jellyfish v.2.2.10[42] examined frequency distributions of quality-filtered short fragments (21-mers). Then, based on Jellyfish's results, genome escope2[2] was used for genome analysis. This strategy obtained genomic information of *P. tenella* (Supplementary Figure 1), like heterozygosity, genome size, and proportion of repeat sequences.

4.5. Genome assembly

Following correction and filtering, HiFi CCS reading could be used in the de novo assembly using hifiasm (v0.14-r312) with default parameters. Purge haplotigs was used to remove redundant haploids[43]. In 2017, Dudchenko et al[44]. used the 3D de novo assembly (3D-DNA) software for scaffolding the haploid contigs. The Hi-C readings could be aligned within the draft genome 3D-DNA and Juicebox v1.9.8 was used for the candidate assembly. Assembly Tools (JBAT) [45] was utilized for reviewing the candidate assembly and corrected artificially. The eudicotyledons_odb10 database have been employed in conjunction with BUSCO v3.0.2 (Benchmarking Universal Single-Copy Orthologs)[46] algorithm for assessing genome integrality and gene annotation. A combination of the BWA-MEM method and HISAT2 (v2.1.0)[47] was utilized for mapping small reads filteration obtained by Illumina and the assembled transcripts to the assembly.

4.6. Repetitive element annotations

To annotate the TEs or transposable elements[48], the EDTA genome annotation pipeline was utilized. TEs include retrotransposons and DNA transposons. RepeatModeler was used to identify DNA transposons, including long interspersed nuclear elements (LINEs) of the terminal inverted repeats (TIRs) and retrotransposons, and long tandem repeats (LTRs), as well as helitrons found in DNA transposons. To do this, we used Repbase and RepeatMasker (v4.0.7) and Repbase with the optimal settings to generate a de novo repeat library for repeat sequence identification[49,50].

4.7. Functional annonations and gene prediction

The StringTie (v1.3.5) and HISAT2 (v2.1.0) pipeline was approached for maping the RNA-seq data within the fruits, leavers, stems, and roots were mapped to the genome. Gene prediction together with de novo transcripts assembly were conducted through Trinity [51]. PASA (v2.4.1) pipeline transdecoder4 have also been applied to annotate the transcripts-relevant coding regions[52]. Exonerate v2.2.0 carried out homolog predictions. GlimmerHMM (v3.0.4) and the protein sequences of *Prunus dulcis*, *Prunus mira*, *Prunus persica*, *Prunus armeniaca*, *Prunus mume*, and *Prunus salicina* could also be mapped to the genome [53]. For de novo gene speculation, genes from the PASA results were trained by AUGUSTUS (v3.3.3) and SNAP[54,55]. In order to combine the gene models, EVidenceModeler (v1.1.1) was approached[56]. The predicted protein sequences have been compared to the EuKaryotic Orthologous Groups (KOG), Nr databases, Pfam, SwissProt, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO) to infer possible functions for the protein-coding genes.

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4.8. Phylogenetic and gene family analysis

Thirteen closely related species were selected for phylogenetic and gene family analysis along with *P.tenella*. Additionally, *P.avium* was selected as the outgroup. The genome database for Rosaceae (www.rosaceae.org), and the NCBI database (www.ncbi.nlm.nih.gov) was used for getting the protein sequences of these species. Alignment quality was ensured by excluding sequences with lengths < 100 bp. OrthoFinder (v2.5.2) have been deployed for identifying single-copy homologous genes and classifying families, using the settings "-M msa -S diamond -T raxml-ng"[57]. RaxML[58] have been approached for estimating and evaluating the phylogenetic connection tree of 14 species using 100 bootstrap repetitions. Time to diverge was computed using PAML's MCMC tree[59]. CAFE (v3.1) have been approached for examining relevant growth patterns and gene families-related declines described by Han et al[60]. By counting the number of ancestral gene families on each branch of the phylogenetic tree, we were able to determine the rate at which gene family sizes shrank or grew. Cafetutorial_clade_and_size_filter.py was used to filter gene families characterized by very high variations in gene copy numbers in an effort to decrease prediction mistakes. Exact data on the contraction and expansion gene families of 14 species was utilized using the script cafetutorial_report_analysis.py, and this data was then analyzed. For selected gene families, we used Fisher's exact test to analyze GO functional enrichment.

4.9. Whole-genome synteny analysis

Almond and peach were selected for whole genome replication (WGD) analysis. Fourfold synonymous (degenerative) third-codon transversion (4DTv) values and synonymous mutation distributions for each synonymous site (Ks) were calculated to analyze the genome replication events. YN substitution model was used to calculate the 4DTv rates based on fourfold degenerate sites. KaKs_Calculator (v2.0)[61] with default parameters was used to calculate Ks values. The minimap2 software was used for genome-wide comparison, and syri software was used to identify collinear regions between the two genomes, structural rearrangements (inversion, translocation, and duplication), local variations (SNP, indel, and CNV), and unaligned regions. The nucmer (4.0.0beta2) program in MUMmer4[62] was used to determine whether similar gene pairs on chromosomal were adjacent in different species.

4.10. Single-nucleotide polymorphism (SNP) calling

Trimmomatic v0.36 was used to eliminate adaptors and low-quality sequences during the preprocessing phase. Every sample's clean reads have been planned using Burrows-Wheeler Aligner to the wild almond standard genome. Next, Picard (http://broad insti tute. github.io/picard/) have been employed for identifying and aligning PCR duplicated sample findings. SNP sites in re-sequencing people from diverse geographical regions were identified using GATK v4 (Genome Analysis Toolkit) for SNP recalling. Each genome's VCF files were generated using variant calling with GATK Hap-lotypeCaller, and then the VCF files for all 137 genomes were combined to create a single VCF file. just SNPs that had a Hardy-Weinberg Equilibrium < 0.001, Minor allele frequency > 0.05, and genotype missing rate 10% for each were kept for further study, narrowing the analysis down to just biallelic variation sites.

4.11. Phylogenetic analysis

A phylogenetic tree was generated using the distance matrix produced by MEGA-CC software (MEGAX)[63] and 1000 bootstrap repetitions to assess the phylogenetic connection of various individuals in order to study the evolutionary links between different populations. In addition, the SMARTPCA application included in the EIGENSOFT software (https://github. com/chrch ang/eigen soft) was utilized to carry out principal component analysis (PCA) and ascertain the subpopulations' clustering status [64].

4.12. Population genetic structure and genetic diversity analysis

In order to learn about the genetic makeup of populations, including their variety, structure, and differentiation, Nucleotide diversity was assessed by dividing each population into 10 kb chunks and analyzing a 100 kb window[65]. Using a Bayesian-based strategy, the K-values (the hypothesized number of populations) ranged between 1 – 10 in ADMIXTURE[66]. The optimal K-value was determined using cross-validation statistics across five separate studies. Bar graphs of the Q matrix for each K-value were made with the aid of the R package Pophelper (http://royfrancis.github.io/pophelper). Fixation index (FST) and nucleotide diversity ratios (π) were computed using VCF methods to identify genomic areas possibly experiencing natural selection sweeps throughout the adaptation process.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Supplementary Table S1. Variation type Statistics (*Prunus dulcis* vs *Prunus tenella*). Supplementary Table S2. Variation type Statistics (*Prunus persica* vs *Prunus tenella*). Supplementary Table S3. Genetic diversity parameters of different populations. Supplementary Figure S1. The kmer distribution of k=21.

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Data availability: The whole genome sequence data reported in this paper have been deposited in the Genome Warehouse in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences / China National Center for Bioinformation, under accession number GWHCBGA00000000 that is publicly accessible at https://ngdc.cncb.ac.cn/gwh.The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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