

Article

Chloroplast Genomes of *Vitis flexuosa* and *Vitis amurensis*: Molecular Structure, Phylogenetic, and Comparative Analyses for Wild Plant Conservation

Ji Eun Kim ¹ , Keyong Min Kim ², Yang Su Kim ³, Gyu Young Chung ⁴, Sang Hoon Che ⁵ and Chae Sun Na ^{1,*} 

¹ Wild Plant Seed Office, Baekdudaegan National Arboretum, Bongwha 36209, Republic of Korea; jekim803@koagi.or.kr

² Arboretum Education Office, Baekdudaegan National Arboretum, Bongwha 36209, Republic of Korea

³ Department of General Affairs, General Affairs Team, Gangeung-Wonju National University, Gangeung 25457, Republic of Korea

⁴ Department of Forest Science, Andong National University, Andong 36729, Republic of Korea

⁵ Forest Bioresources Department, Baekdudaegan National Arboretum, Bongwha 36209, Republic of Korea

* Correspondence: chaesun.na@koagi.or.kr

Abstract: The chloroplast genome plays a crucial role in elucidating genetic diversity and phylogenetic relationships. *Vitis vinifera* L. (grapevine) is an economically important species, prompting exploration of wild genetic resources to enhance stress resilience. We meticulously assembled the chloroplast genomes of two Korean *Vitis* L. species, *V. flexuosa* Thunb. and *V. amurensis* Rupr., contributing valuable data to the Korea Crop Wild Relatives inventory. Through exhaustive specimen collection spanning diverse ecological niches across South Korea, we ensured comprehensive representation of genetic diversity. Our analysis, which included rigorous codon usage bias assessment and repeat analysis, provides valuable insights into amino acid preferences and facilitates the identification of potential molecular markers. The assembled chloroplast genomes were subjected to meticulous annotation, revealing divergence hotspots enriched with nucleotide diversity, thereby presenting promising candidates for DNA barcodes. Additionally, phylogenetic analysis reaffirmed intra-genus relationships and identified related crops, shedding light on evolutionary patterns within the genus. Comparative examination with chloroplast genomes of other crops uncovered conserved sequences and variable regions, offering critical insights into genetic evolution and adaptation. Our study advances the understanding of chloroplast genomes, genetic diversity, and phylogenetic relationships within *Vitis* species, thereby laying a foundation for enhancing grapevine genetic diversity and resilience to environmental challenges.

Keywords: chloroplast; conservation; genetic diversity; genome; *Vitis*



Citation: Kim, J.E.; Kim, K.M.; Kim, Y.S.; Chung, G.Y.; Che, S.H.; Na, C.S. Chloroplast Genomes of *Vitis flexuosa* and *Vitis amurensis*: Molecular Structure, Phylogenetic, and Comparative Analyses for Wild Plant Conservation. *Genes* **2024**, *15*, 761. <https://doi.org/10.3390/genes15060761>

Academic Editor: Zhiqiang Wu

Received: 3 May 2024

Revised: 5 June 2024

Accepted: 6 June 2024

Published: 10 June 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Food security has emerged as a global concern owing to climate change and population growth, and crop wild relatives (CWRs) are considered a principal solution to this problem. CWRs are wild plants closely related to crops and serve as potential wild ancestors or allied species. During domestication, crops undergo a substantial reduction in genetic variation, making them more susceptible to biotic and abiotic stresses and decreasing their capacity to withstand these stresses and reproduce [1]. In contrast, CWRs harbor a wealth of genetically valuable traits that allow them to adapt to diverse habitats and avoid genetic bottlenecks associated with domestication [2]. Therefore, CWRs are a valuable resource for increasing the genetic diversity of crops. This genetic improvement is essential for sustaining crop yields and food supply amidst ongoing and future climatic challenges.

Vitaceae is a family of flowering plants comprising ~950 species distributed across 16 genera [3]. Predominantly composed of climbing plants, this family is primarily found in the tropical regions of Asia, Africa, Australia, the Neotropics, and the Pacific Islands,

with a few genera also present in temperate regions [3,4]. Among them, the genus *Vitis* L. is well known to include the most cultivated grape species, *V. vinifera* L. [5], and is the most economically important resource of fruits, wine, and raisins [6,7]. However, *V. vinifera* has historically faced challenges in terms of productivity owing to its susceptibility to pests, diseases, and abiotic stresses such as cold [5]. To address these limitations, the use of genes from wild plants has proven effective in enhancing biotic and abiotic tolerance and resistance [8–10]. Consequently, introducing wild genetic resources into breeding programs is crucial for the long-term sustainability and competitiveness of the grape industry in response to global environmental changes.

The chloroplast, a distinctive organelle in plant cells, plays a crucial role in essential metabolic pathways such as photosynthesis [11]. Generally, the size of the chloroplast genome ranges from 120 kb to 160 kb in angiosperms [12]. The chloroplast genome consists of four main structural components: a large single-copy (LSC, 80–90 kb) region, a small single-copy (SSC, 16–27 kb) region, and two inverted repeat (IR, 20–28 kb) regions [13,14]. Most angiosperm chloroplast genomes evolve slower than nuclear genomes and are primarily maternally inherited [15,16]. In contrast, chloroplast genomes in gymnosperms are typically paternally inherited [17]. Therefore, chloroplast genomes play a major role in classification, differentiation, and evolutionary processes. Recently, with advancements in sequencing technology and genetic information databases, there has been extensive research and analysis of chloroplast genomes of numerous plants [18–21]. Particularly, in recent years, the use of chloroplast genome sequences for species barcoding has emerged as a powerful approach in biological research. This approach offers several advantages, including rapid and cost-effective species identification, making it a valuable asset for various fields such as biodiversity conservation and agriculture [21,22]. This rapidly advancing field has potential for enhancing our understanding of plant biology and for devising effective strategies to conserve and sustainably manage plant biodiversity.

The aim of this study was to complete the chloroplast genomes of two *Vitis* species distributed in South Korea and contribute to the Korea Crop Wild Relatives (KCWRs) inventory. First, we assembled the chloroplast genomes, elucidated their basic structure, analyzed their genetic features, and explored the diversity of chloroplast genes through comparison among different crops. Subsequently, through phylogenetic analysis, we ascertained the overall trends within the genus and analyzed the phylogenetic relationships and positions relative to closely related crops. Overall, our results improve our knowledge of the chloroplast genome, genetic diversity, and phylogenetic relationships within *Vitis* species, thereby providing valuable perspectives for genetic diversity and wild plant conservation.

2. Material and Methods

2.1. Plant Materials

Six wild plants species belonging to the genus *Vitis* L. are distributed throughout South Korea. We collected the seeds of *V. flexuosa* Thunb. and *V. amurensis* Rupr. (Figure 1). These plants exhibit differences in terms of not only morphological features, such as fruit and leaf characteristics, but also their distribution in South Korea. The distribution analysis in the Republic of Korea revealed that *V. amurensis* is mainly distributed in the inland, whereas *V. flexuosa* is predominantly found in the southern and Jeju-do regions. Some of the seeds were stored in the Seed Bank at Baekdudaegan National Arboretum, Bongwha, South Korea, whereas others were grown into plants for use as materials. *Vitis flexuosa* samples were collected from Haan-dong, Gwangmyeong-si, Gyeonggi-do, Republic of Korea (37°27'33.1" N, 126°52'9.4" E) (seed bank accession number: B0021148). *Vitis amurensis* samples were collected from Hachu-ri, Inje-eup, Inje-gun, Gangwon-do, Republic of Korea (38°1'52.7" N, 128°17'16.0" E) (seed bank accession number: B002034).

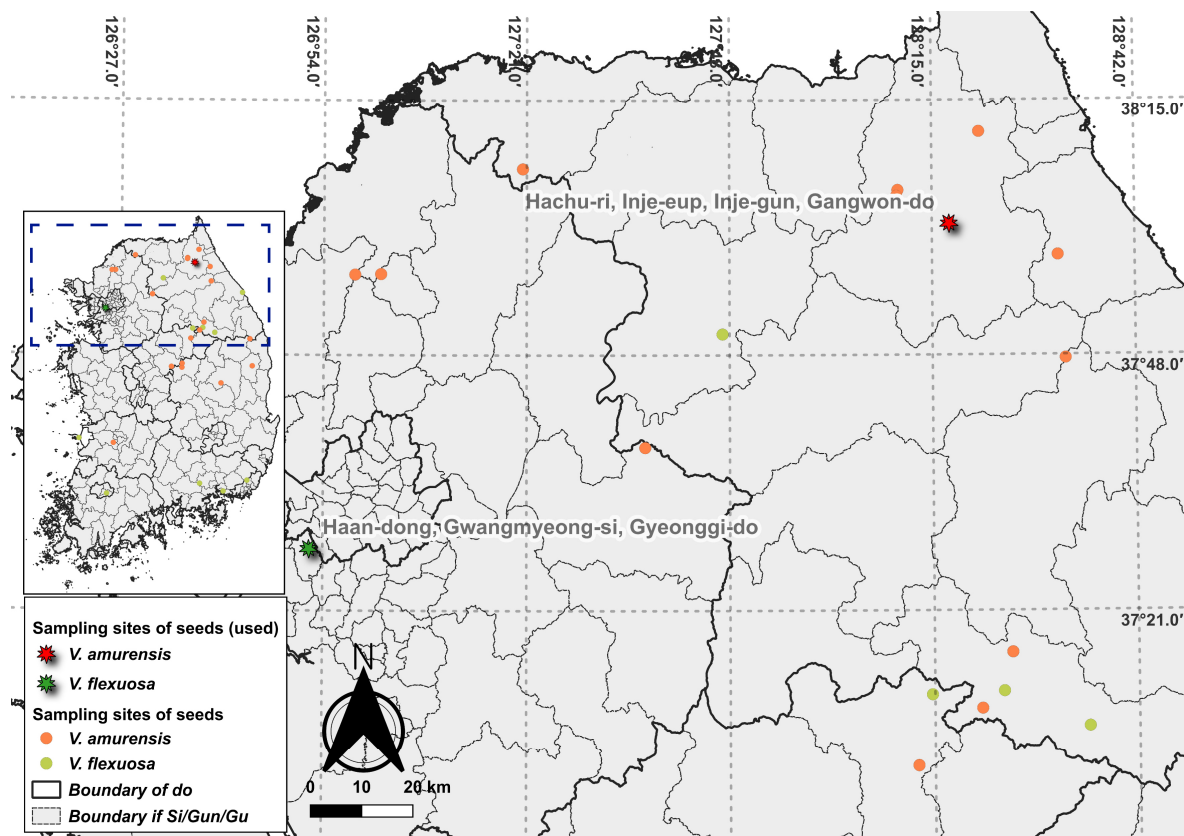


Figure 1. Seed sampling sites for *Vitis flexuosa* and *V. amurensis* in South Korea. The indicated colors and shapes identify seeds of each species that were used or unused.

2.2. DNA Extraction, Sequencing, Assembly, and Annotation

Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA quantity was measured using a NanoDrop 2000 (Thermo, Wilmington, DE, USA) and a Qubit 3.0 Fluorimeter (Invitrogen, Carlsbad, CA, USA). Next-generation paired-end sequencing was performed on a NovaSeq platform using TruSeq NanoDNA (Illumina, San Diego, CA, USA). We eliminated adapter sequences and low-quality reads (Phred score > 20), and then assembled the chloroplast genomes de novo using CLC Assembly Cell v4.2.1 (CLC Inc., Aarhus, Denmark). The genes were annotated using GeSeq [23] and Geneious Prime v2023.2.1 (Biomatters Ltd., Auckland, New Zealand) [24]. Finally, a circular map of the complete chloroplast genome was generated using CPGView [25]. The data supporting the findings of this study are available in GenBank, under accession numbers PP191159 and PP191162.

2.3. Analysis of Relative Synonymous Codon Usage Bias and Repeats

Relative synonymous codon usage (RSCU) was identified from protein-coding genes using the MEGA11 software [26]. According to Sharp and Li (1986), RSCU indicates the probability of selecting a particular codon among synonymous codons for the same amino acid [27]. An RSCU < 1 indicates less usage, RSCU = 1 suggests unbiased usage, and RSCU > 1 indicates high usage [28]. Additionally, RSCU values below 0.6 signify very low usage, whereas those exceeding 1.6 suggest very high usage [29–31]. Simple sequence repeats (SSRs) were identified using MISA [32], with mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide sets of 10, 5, 4, 3, 3, and 3, respectively. A long repetitive sequence was identified, including forward (F), palindromic (P), reverse (R), and complement (C) repeats, with a set range from a minimum size of 20 bp to a maximum size of 50 bp, while setting a Hamming distance of 3 using REPuter [33].

2.4. Chloroplast Genome Structure and Comparative Analyses

We downloaded the chloroplast genomes of nine *Vitis* species from the NCBI GenBank (<https://www.ncbi.nlm.nih.gov/>, accessed on 23 November 2023) for these analyses. The chloroplast genome structure was determined using IRscope [34] to visualize and analyze the LSC, SSC, and IR region boundaries. The chloroplast sequences were aligned with those of *Ampelopsis japonica* (Thunb.) Makino, from the same subfamily (GenBank accession number: MK547541), using LAGAN alignment [35] within mVISTA [36,37].

2.5. Analysis of Divergence Hotspots

We used DnaSP v5 [38] to calculate nucleotide diversity (π) of the chloroplast sequences and identify the hotspots. Chloroplast genome sequences were aligned using MAFFT v7.490 [39] in Geneious Prime v2023.2.1 [24]. The step size for DnaSP v5 was set to the midpoint of 200 bp and a window size of 600 bp.

2.6. Phylogenetic Analysis

To explore phylogenetic relationships within the genus, we downloaded the chloroplast genomes from the NCBI GenBank. Additionally, the division of species characteristics was based on GRIN-Global [40], in which landraces refer to cultivated species. For phylogenetic analysis, protein-coding genes were selected using PhyloSuite v1.2.3 [41]. The sequences were concatenated into a single alignment and subsequently used to construct a phylogenetic tree employing the maximum likelihood (ML) method with IQ-TREE [42]. The best-fit model (K3Pu + F + I) was automatically selected, and 1000 ultrafast bootstrap replicates were employed to assess branch support.

3. Results

3.1. Features of the Complete Chloroplast Genomes

The complete chloroplast genomes of the two *Vitis* species exhibited considerable similarities in terms of gene composition, total length, and GC content. Specifically, *V. flexuosa* (GenBank: PP191159) and *V. amurensis* (GenBank: PP191162) have chloroplast genomes of 160,986 and 161,013 bp, respectively (Figure 2). The chloroplast genome has a quaternary structure consisting of LSC regions of 89,206–89,238 bp, IR regions of 26,360–26,354 bp, and SSC regions of 19,060–19,067 bp. Overall, the GC content of the two chloroplast genomes was 37.38% (Table 1).

Table 1. Summary of complete chloroplast genomes of *V. flexuosa* and *V. amurensis*.

Attribute	<i>V. flexuosa</i>	<i>V. amurensis</i>
GenBank no.	PP191159	PP191162
Total length (bp)	160,986	161,013
LSC length (bp)	89,206	89,238
IR length (bp)	26,360	26,354
SSC length (bp)	19,060	19,067
GC content (%)	37.38	37.38
Total genes	133 (114)	133 (114)
Protein-coding genes	88 (80)	88 (80)
tRNA genes	37 (30)	37 (30)
rRNA genes	8 (4)	8 (4)

The number in parentheses represents the number of unique genes, excluding duplicates.

In total, 133 genes were annotated, including 88 protein-coding, 37 tRNA, and 8 rRNA genes in each genome, of which 8 protein-coding (*ycf1*, *ycf2*, *ycf15*, *rps7*, *rps12*, *rpl2*, *rpl23*, and *ndhB*), 7 tRNA (*trnV*^{GAC}, *trnR*^{ACG}, *trnN*^{GUU}, *trnL*^{CAA}, *trnI*^{GAU}, *trnI*^{CAU}, and *trnA*^{UGC}), and 4 rRNA (*rrn16*, *rrn23*, *rrn4.5*, and *rrn5*) genes were duplicated in the IR regions. Nine protein-coding (*rps16*, *atpF*, *rpoC1*, *petB*, *petD*, *rpl16*, *rpl22*, *ndhB*, and *ndhA*) and six tRNA (*trnK*^{UUU}, *trnG*^{UCC}, *trnL*^{UAA}, *trnV*^{UAC}, *trnI*^{GAU}, and *trnA*^{UGC}) genes contained one intron

each, whereas three protein-coding genes (*rps12*, *ycf3*, and *clpP*) contained two introns each (Table 2). *rps12* is a trans-spliced gene with its 5' exon located in the LSC region and 3' exon located in the IR region. *matK* is located in the intron of *trnK^{UUU}*.

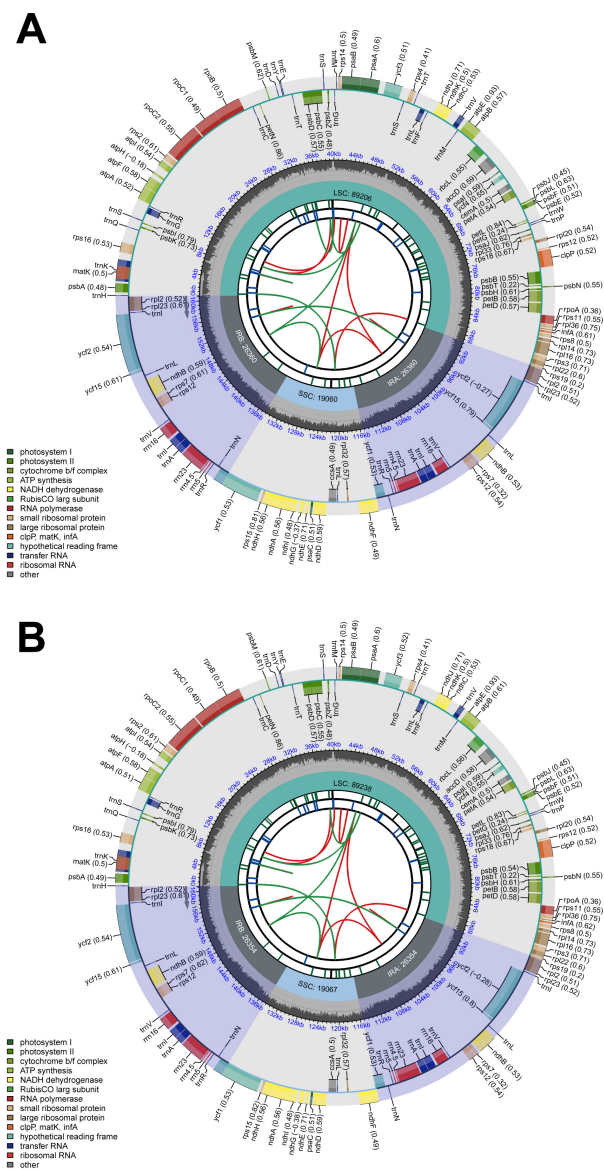


Figure 2. Circular maps representing the complete chloroplast genomes of (A) *V. flexuosa* and (B) *V. amurensis*. The center boundary shows the length of each region. Each gene has been color-coded to distinguish its functionality. Genes inside and outside the circle are transcribed in clockwise and counterclockwise directions, respectively, as indicated by the arrows.

Table 2. List of annotated genes in the chloroplast genomes of *V. flexuosa* and *V. amurensis*.

Group of Genes	Gene Symbols
Photosystem I	<i>psaA</i> , <i>psaB</i> , <i>psaC</i> , <i>psaI</i> , <i>psaJ</i> , <i>ycf3</i> **, <i>ycf4</i>
Photosystem II	<i>psbA</i> , <i>psbB</i> , <i>psbC</i> , <i>psbD</i> , <i>psbE</i> , <i>psbF</i> , <i>psbH</i> , <i>psbI</i> , <i>psbJ</i> , <i>psbK</i> , <i>psbL</i> , <i>psbM</i> , <i>psbN</i> , <i>psbT</i> , <i>psbZ</i>
Cytochrome b/f complex	<i>petA</i> , <i>petB</i> *, <i>petD</i> *, <i>petG</i> , <i>petL</i> , <i>petN</i>
ATP synthase	<i>atpA</i> , <i>atpB</i> , <i>atpE</i> , <i>atpF</i> *, <i>atpH</i> , <i>atpI</i>
Rubisco	<i>rbcL</i>
NADH dehydrogenase	<i>ndhA</i> *, <i>ndhB</i> 2 *, <i>ndhC</i> , <i>ndhD</i> , <i>ndhE</i> , <i>ndhF</i> , <i>ndhG</i> , <i>ndhH</i> , <i>ndhI</i> , <i>ndhJ</i> , <i>ndhK</i>
Proteins of large ribosomal subunits	<i>rpl14</i> , <i>rpl16</i> *, <i>rpl22</i> *, <i>rpl20</i> , <i>rpl22</i> , <i>rpl23</i> 2, <i>rpl32</i> , <i>rpl33</i> , <i>rpl36</i>

Table 2. Cont.

Group of Genes	Gene Symbols
Proteins of small ribosomal subunits	<i>rps11, rps12² **, rps14, rps15, rps16 *, rps18, rps19, rps2, rps3, rps4, rps7², rps8</i>
RNA polymerase	<i>rpoA, rpoB, rpoC1 *, rpoC2</i>
Acetyl-CoA carboxylase	<i>accD</i>
C-type cytochrome synthesis gene	<i>ccsA</i>
Envelope member protein	<i>cemA</i>
Protease	<i>clpP **</i>
Maturase	<i>matK</i>
Translational initiation factor	<i>infA</i>
Ribosomal RNAs	<i>rrn16, rrn23, rrn4.5, rrn5</i>
Transfer RNAs	<i>trnA^{UGC} *, trnC^{GCA}, trnD^{GUC}, trnE^{UUC}, trnF^{GAA}, trnG^{GCC}, trnG^{UCC} *, trnH^{GUG}, trnI^{CAU}, trnI^{GAU} *, trnK^{UUU} *, trnL^{CAA}, trnL^{UAA} *, trnL^{UAG}, trnM^{CAU}, trnN^{GUU}, trnP^{UGG}, trnQ^{UUG}, trnR^{ACG}, trnR^{UCU}, trnS^{GCU}, trnS^{GGA}, trnS^{UGA}, trnT^{GGU}, trnT^{UGU}, trnV^{GAC}, trnV^{UAC} *, trnW^{CCA}, trnY^{GUA}, trnfM^{CAU}</i>
Conserved ORFs	<i>ycf1², ycf15², ycf2²</i>

Gene² = number of copies of a multi-copy gene; Gene * = genes containing a single intron; Gene ** = genes containing two introns; ORF = open reading frame.

3.2. Codon Usage Bias

RSCU was calculated from the protein-coding genes of the chloroplast genomes of each species. These genes contained 64 codons encoding 21 amino acids (Figure 3). The codon count ranged from 21,503 (*V. flexuosa*) to 21,541 (*V. amurensis*). Among the amino acids, leucine (Leu), encoded by UUA, UUG, CUU, CUC, or CUA, was the most abundant amino acid with 2258 codons (10.50–10.48%), whereas tryptophan (Trp), encoded by UGG, was the least abundant amino acid with 386 codons (1.80–1.79%). Although these species exhibit similar RSCU patterns, there are subtle differences among them in terms of the stop codons, particularly in the ratio of UAA (1.39 and 1.46%) to UGA (0.83% and 0.75%).

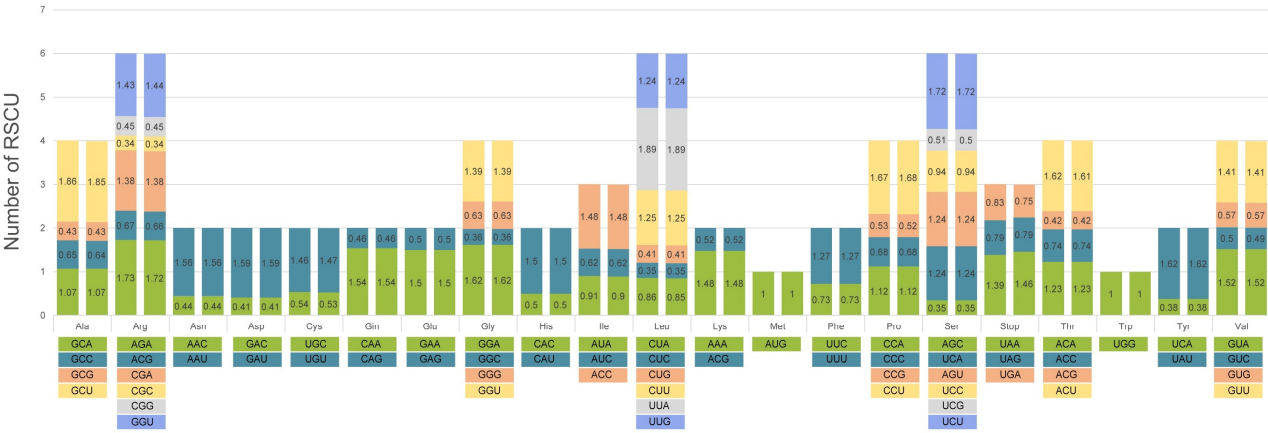


Figure 3. Relative synonymous codon usage for amino acids in the protein-coding regions of the chloroplast genomes of the two *Vitis* species. For each amino acid, the left bar represents *V. amurensis* and the right one represents *V. flexuosa*.

In both species, 30 codons showed RSCU values greater than 1, whereas 32 codons exhibited values less than 1. The UUA codon had the highest RSCU value (1.89), whereas the CGC codon had the lowest (0.34). Methionine (Met) and Trp showed unbiased codon use (RSCU = 1). Notably, codons ending in A or U bases (GUU, AGA, GGA, UUA, CCU, UCU, ACU, and UAU) showed very high biases (RSCU > 1.6), whereas 20 codons showed very low biases (RSCU < 0.6).

3.3. SSRs and Long-Repeat Sequences

In total, 86 SSRs were identified in the chloroplast genome of *V. flexuosa*, whereas 87 SSRs were identified in that of *V. amurensis*. These included 62 and 63 SSRs in the LSC regions of *V. flexuosa* and *V. amurensis*, respectively; 10 in the IR regions; and 14 in the SSC regions (Figure 4). Mono-nucleotides constituted the majority of the SSR motifs, accounting for 65.1–66.3% of the total repeats, ranging from 56 to 57. Additionally, 9 di-nucleotide (di-), 9 tri-nucleotide (tri-), 9 tetra-nucleotide (tetra-), and 9 penta-nucleotide repeat counts were recorded; however, no hexa-nucleotide motifs were identified. Among the identified repeat types, the mono-nucleotide repeat unit (A/T) was the most abundant, comprising 55–56 repeats.

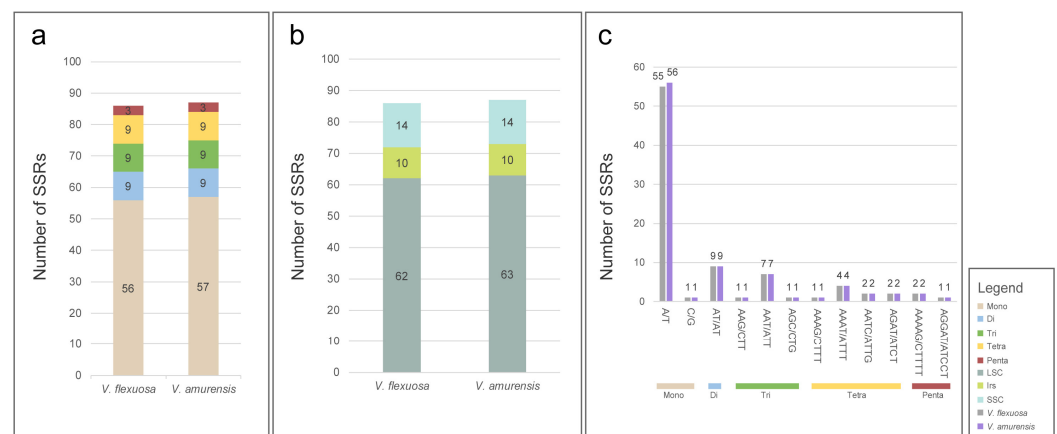


Figure 4. Simple sequence repeats (SSRs) in the chloroplast genomes. X-axes: species and types of SSRs. Y-axes: number of SSRs. (a) Number and types of SSRs. (b) Identification of SSRs in each region. (c) Frequency of SSRs in different repeat class types.

In this study, 49 repeats were identified in each chloroplast genome and categorized into four types: 1 complementary repeat, 17 forward repeats, 23 palindromic repeats, and 8 reverse repeats (Figure 5). The sizes of the complex repeats varied from 20 bp to over 40 bp, with the majority falling within 30–39 bp.

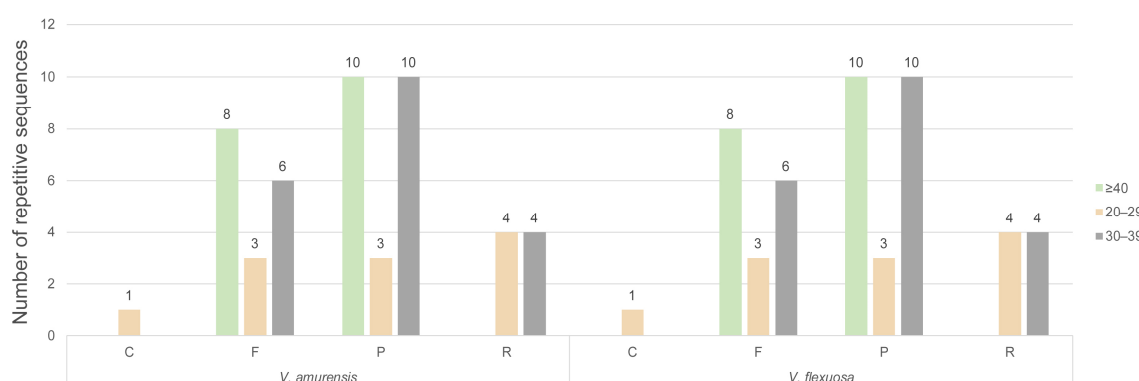


Figure 5. Number of long-repeat sequences in the *Vitis* chloroplast genomes (F: forward repeats; R: reverse repeats; P: palindromic repeats; C: complementary repeats).

3.4. Comparison of the Chloroplast Genome Sequences

We aimed to identify the characteristics of the chloroplast genomes shared by *Vitis* species. Eleven chloroplast genomes from nine crops were downloaded from GenBank. We compared the positions of the boundaries of the LSC/IRb/SSC/IRa regions and adjacent genes (Figure 6). The lengths of the IR regions in the genus ranged from 26,312 bp to 26,374 bp, showing similarities. Although changes in the length of the IR region were

minimal, variations in the positions of boundary expansions and contractions were variable. *Vitis labrusca* and *V. amurensis* (MN389556) had an LSC–IRb boundary (JLB) located outside *rps19*, whereas in the remaining species, it was positioned within the gene. In five species, the IRb–SSC boundary (JSB) was located 19 bp from *ndhF*, whereas in the other five species, it was located 40 bp from *ndhF*. Interestingly, in *V. flexuosa*, the IRb–SSC boundary was located at 38 bp from *ndhF*. Furthermore, the SSC–IRa boundary (JSA) was located within *ycf1*. In five species, it was positioned between the boundaries of 4456 bp and 1136 bp, whereas in the other five species, it was positioned between the boundaries of 4446 bp and 1116 bp. In *V. flexuosa*, it was located between the boundaries of 4559 bp and 1123 bp. The IRa–LSC boundary (JLA) was located 11 bp from *trnH* in almost all species, whereas in *V. labrusca*, it was positioned at a distance of 73 bp.

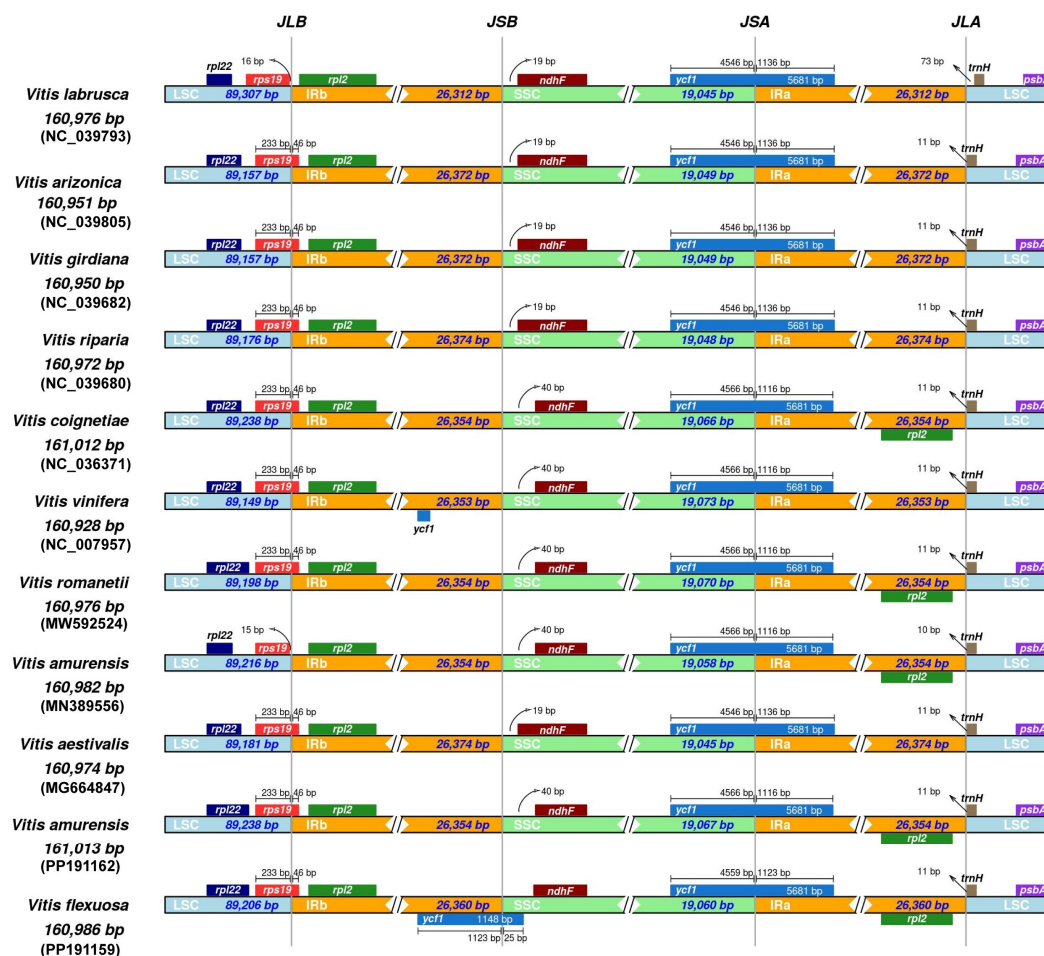


Figure 6. Comparison of boundary positions in LSC, SSC, and IR regions of *Vitis*. Genes are represented with boxes, and gaps between the genes and boundaries are indicated with the number of bases.

To analyze the variability in DNA sequences in these species compared with those in other crops, we compared 11 chloroplast genomes using mVISTA, with *A. japonica* as a reference (Figure 7). The coding regions exhibited sequences conserved among these species. In contrast, the non-coding regions showed high variability. Among these, high variability was identified in several regions of *trnQ*^{UUG}-*rps16*, *psbC*-*rps14*, *trnT*^{UGU}-*rps4*, and *ndhI*-*ndhG*.

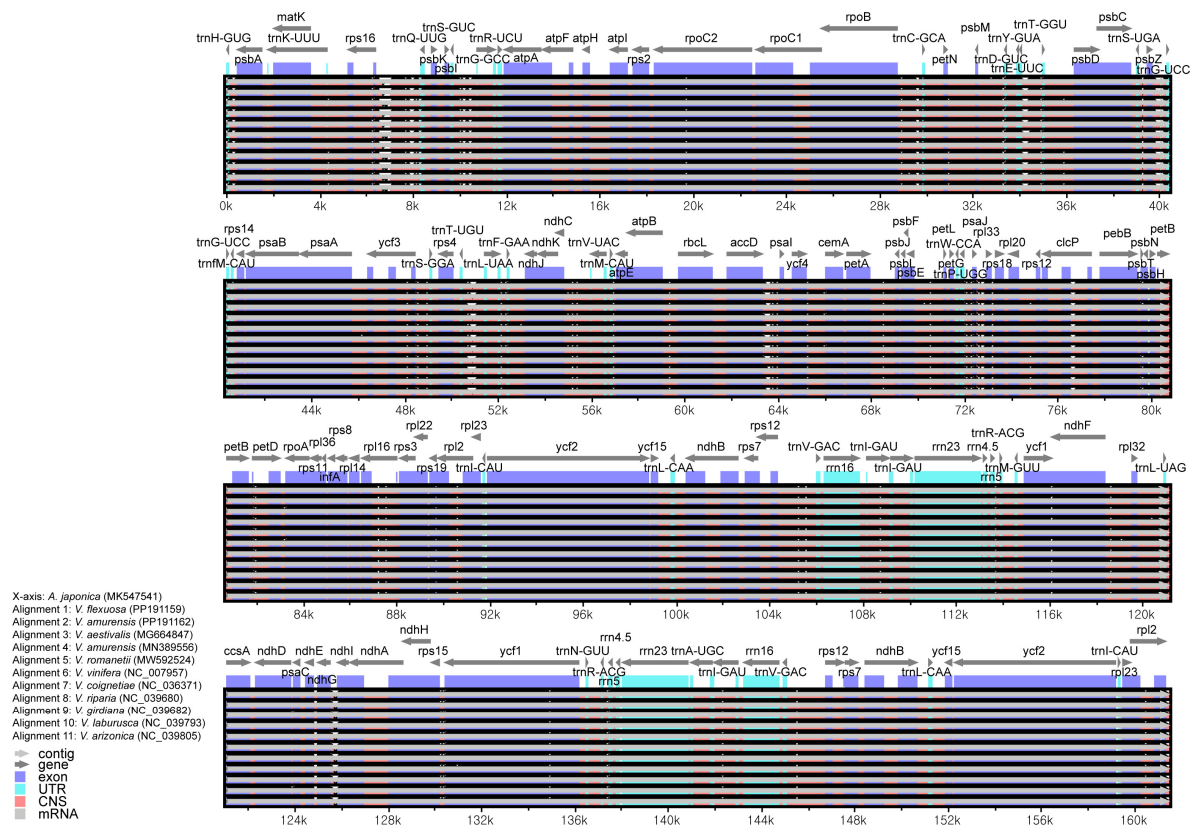


Figure 7. Comparison of three *Vitis* chloroplast genomes using the mVISTA program. Gray arrows indicate the direction and location of genes. Genomic regions, including non-coding (CNS) and coding regions, are depicted with red, light blue, and blue blocks.

3.5. Divergence Hotspots as Potential Markers

We used DnaSP v5 to analyze P_i (Figure 8). The P_i values of the 11 chloroplast genomes were in the range of 0–0.01145. *psbI-trnS^{GCU}-trnG^{UCC}*, *trnS^{UGA}-psbZ-trnG^{GCC}-trnM^{CAU}*, and *ycf1-ndhF* exhibited higher values (>0.006) than other regions. These are included in the LSC and IRb–SSC and distributed in non-coding gene regions, except for *ycf1-ndhF*.

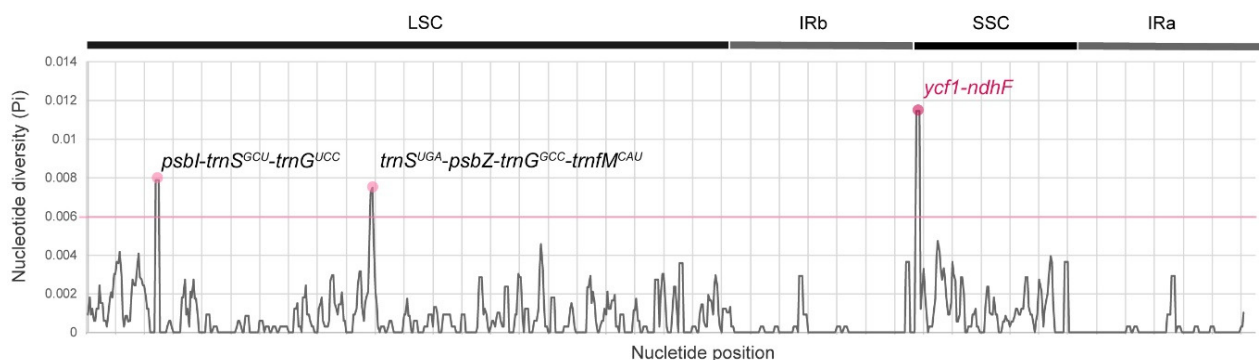


Figure 8. Sliding window analysis of chloroplast genomes of *Vitis*.

3.6. Phylogenetic Relationships within *Vitis* Species

We constructed an ML phylogenetic tree consisting of 53 chloroplast genomes, which included 51 *Vitis* species (42 CWRs, 8 landraces, and 1 modern cultivar), along with two species of *Parthenocissus* Planch., which served as an outgroup (Figure 9). Based on our findings, we aimed to reconfirm the overall phylogenetic relationships within the

genus, identify closely related crops with the two species, and integrate them into the KCWRs inventory.

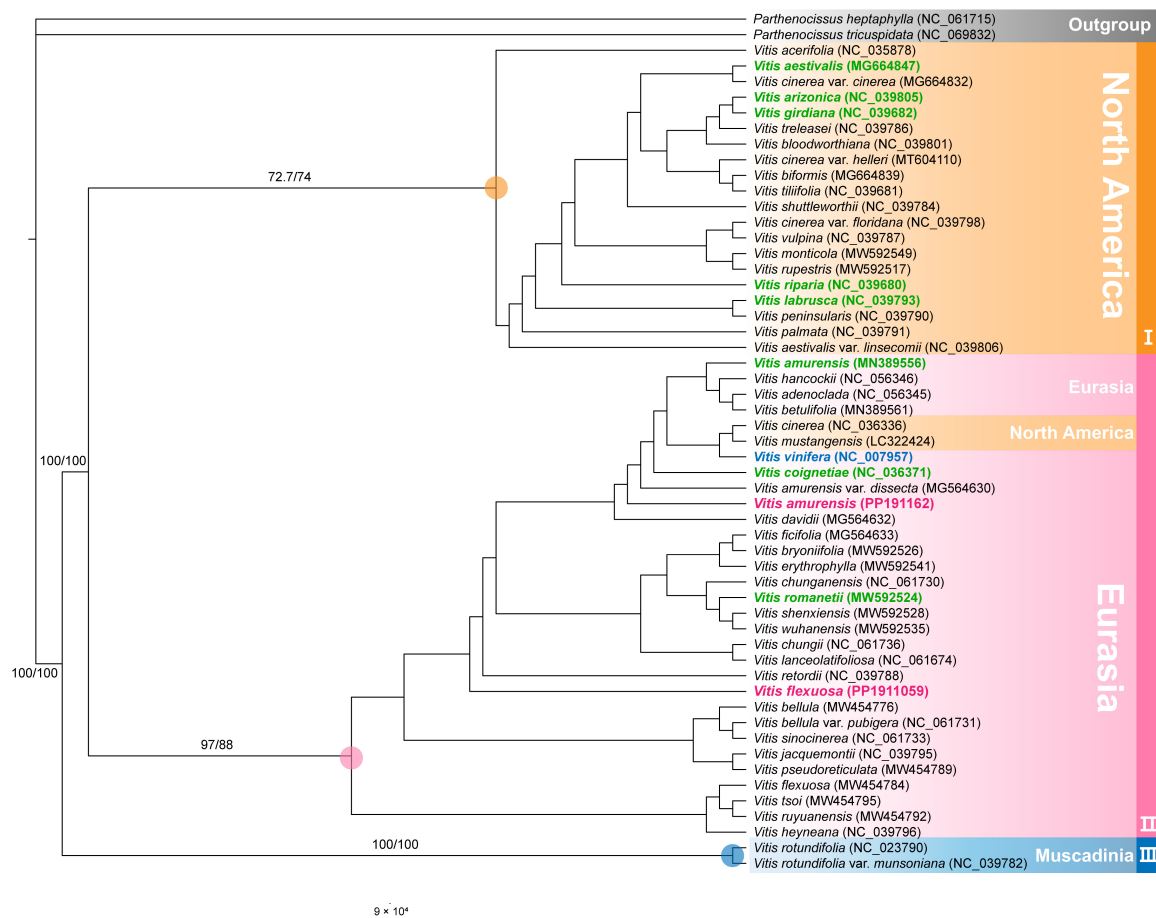


Figure 9. Phylogenetic tree constructed from *Vitis* chloroplast sequences, and bootstrap values based on 1000 replicates are displayed on each node. The tip label colors represent different categories: black for crop wild relatives (CWRs), green for landraces (native and also-cultivated), pink for subjects in this study, and blue for modern cultivars (criteria by GRIN-Global).

The phylogenetic analysis revealed that the genus branches into three major clades, forming distinct clades within the genus, with some exceptions. This genus is divided into two subgenera, *Vitis* and *Muscadinia* (Planch.) Rehder, belonging to *Vitis* (subgenus). This clade was supported by bootstrap values of 72/77.4, 97/88, and 100/100. Both *V. flexuosa* and *V. amurensis*, whose chloroplast genomes were assembled in the present study, were included in clade 2, supported by a bootstrap value of 97/88.

4. Discussion

4.1. Chloroplast Genome Characteristics in the Two *Vitis* Species

The sizes of the complete chloroplast genomes of *V. flexuosa* and *V. amurensis* determined in this study (160,986 and 161,013 bp) were consistent with the genome size of angiosperms [12]. These genomes exhibit a quadruple structure, with LSC regions of 89,206 and 89,238 bp, IR regions of 26,360 and 26,354 bp, and SSC regions of 19,060 and 19,067 bp [13,14]. The observed similarity in genome sequence information within the genus suggests a shared genetic makeup among these species [18–20]. These results not only affirm the conservation of the chloroplast genome in *Vitis* but also support the maternal inheritance of chloroplasts, based on the length of the LSC, IR, and SSC regions [15,16].

The RSCU values indicate that most optimal synonymous codons end with A or U in higher plants [43]. The investigation of codon usage bias is essential for understanding the

patterns of codon usage in closely related species [44,45]. This knowledge is valuable for exploring genetic evolution, deciphering gene expression characteristics, and providing guidance for breeding [46,47].

SSRs, also known as microsatellites, are repetitive DNA sequences that constitute a substantial proportion of higher eukaryotic genomes. These include one or a few bases that are repeated in tandem several times [48]. The distribution of SSRs in several chloroplast genomes is non-random and primarily characterized by mono-nucleotides, with A/T bases constituting the majority [49–51]. Long-repeat sequences are classified into four types with variations in size ranging from 20 to over 40 base pairs. This sequence information is used to identify phylogenetic relationships among species and serves as a molecular marker for analyzing genetic diversity [52–54].

4.2. Comparative Analysis of Chloroplast Genomes of *Vitis* Species with Those of Other Crops

Fluctuations in IR regions due to expansion and contraction are common occurrences in genome evolution. These variations contribute to shifts in the length of chloroplast genomes, and they can be useful for studying phylogenetic relationships and taxonomic classifications [55,56]. The IR region lengths ranged from 26,312 to 26,374 bp. Notably, several *Vitis* species exhibited variations in the boundary positions of the IRs, which were distinct from those of other species. Specific species such as *V. labrusca* and *V. flexuosa* were distinguished from other species in terms of their boundary positions. However, the overall changes in genome size were not significantly affected by the contraction and expansion of the IR regions. The mVISTA results, with *A. japonica* as the reference, showed conserved sequences within the coding regions; in contrast, the non-coding regions exhibited higher variability. This is common in the chloroplast genomes of most angiosperms [57,58] and genera [47]. DNA polymorphisms (Pi) ranged from 0 to 0.01145, and the nucleotide diversity was calculated for 11 chloroplast genomes. These nucleotide positions, primarily situated in the LSC and IRb–SSC regions, were predominantly composed of non-coding genes, with the exception of *ycf1-ndhF*. These non-coding genes exhibit higher variability and potentially greater discriminatory power, and when combined with coding gene markers, they could enhance identification accuracy and reliability. Identified as hotspot regions, these areas have the potential to serve as molecular markers for phylogenetic analyses and as DNA barcodes, thereby providing valuable genetic information for taxonomic studies.

4.3. Phylogenetic Relationships between Other Crops and CWRs of *Vitis* Species

We performed an ML phylogenetic analysis with 53 chloroplast genomes to reaffirm the overall phylogenetic relationships within the genus, identify crops closely related to the two species, and select them as CWRs. The phylogenetic analysis revealed that the genus branches into three major clades (North America, Eurasia, and Muscadinia) and is divided into two subgenera (*Vitis* and *Muscadinia*). The *Vitis* subgenus consists of groups from North America and Eurasia [3,59]. In a previous study, groups from Europe and Asia formed a sister group constituting the Eurasian clade. Moreover, they have been distinguished from the North American groups [60,61]. Furthermore, in this study, supporting inferences for this content were found at the IR boundary positions for each crop in each clade, with similar positions marked for the crops included in each clade. This clear partitioning of the main origins, maintaining geographical distribution, was further confirmed through phylogenetic analysis of the chloroplast genomes and genome structure in this study. In addition, some species distributed in North America identified within the Eurasian lineage may have undergone transitions, such as migration from North America to Eurasia through North Atlantic land bridges or long-distance dispersal [62]. *V. flexuosa* and *V. amurensis* formed clade 2 along with four crops, indicating a closer relationship with the four crops than with the others, with a supported bootstrap value of 97/88. In addition, we identified a high potential for the utilization of the two species as CWRs for these four crops (*V. vinifera*, *V. amurensis*, *V. coignetiae*, and *V. rotundifolia*). In the case of *V. amurensis* (PP191162 and MN389556), genetic distance was observed despite belonging to the same species. For

example, PP191162 was collected in South Korea, whereas MN389556 was collected in China. These results suggest that geographical distance may contribute to genetic variation within species, highlighting the importance of considering geographic factors in genetic analyses. The major aims of this analysis were to elucidate the phylogenetic relationships within the genus, identify related crop species, and ultimately establish KCWRs. This study not only advances our understanding of the phylogenetic relationships within the genus but also elucidates the potential for utilizing these species as valuable genetic resources for crop improvement.

5. Conclusions

We comprehensively analyzed the chloroplast genome of *Vitis* species, confirming its genetic characteristics and demonstrating its potential as a CWR for crop improvement. The chloroplast genomes of *V. flexuosa* and *V. amurensis* were determined to be 160,986 and 161,013 bp, respectively, similar to those of common angiosperms. Analysis of chloroplast genomes, including gene composition, codon usage bias, SSRs, and long repeats, provided valuable insights. These findings not only contribute to our understanding of the genetic features of these genomes but also offer the potential for developing molecular markers and DNA barcodes. The phylogenetic analysis revealed three distinct clades within the genus, supported by two subgenera, and highlighted the close relationship of the two species with specific crops. In summary, our study provides insights into enhancing and improving the genetic diversity of crops facing challenges due to climate change and population growth. Additionally, it confirms the potential of the two species analyzed to serve as KCWRs. Following this comprehensive analysis, research could be pursued in different directions to further promote the utilization of *Vitis* species as valuable genetic resources for crop improvement. For example, future studies should investigate the functional significance of specific genes identified in the chloroplast genomes of *V. flexuosa* and *V. amurensis*. The genetic information obtained can be utilized to develop breeding strategies aimed at enhancing genetic diversity.

Author Contributions: Conceptualization, J.E.K., K.M.K., S.H.C. and C.S.N.; methodology, G.Y.C. and Y.S.K.; investigation: G.Y.C.; formal analysis, J.E.K. and K.M.K.; writing—original draft preparation, J.E.K. and K.M.K.; writing—review and editing, J.E.K. and K.M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the “R&D Program for Forest Science Technology (Project No. 2021400B10-2425-CA02)” supported by the Korea Forest Service (Korea Forestry Promotion Institute).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data used are provided in the manuscript and are deposited in the NCBI GenBank (<https://www.ncbi.nlm.nih.gov/>, accessed on 23 November 2023) with accession numbers PP191159 and PP191162.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Jarvis, A.; Lane, A.; Hijmans, R.J. The effect of climate change on crop wild relatives. *Agric. Ecosyst. Environ.* **2008**, *126*, 13–23. [CrossRef]
2. Coyne, C.J.; Kumar, S.; von Wettberg, E.J.; Marques, E.; Berger, J.D.; Redden, R.J.; Ellis, T.N.; Brus, J.; Zablatzká, L.; Smýkal, P. Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. *Legume Sci.* **2020**, *2*, e36. [CrossRef]
3. Wen, J.; Lu, L.M.; Nie, Z.L.; Liu, X.Q.; Zhang, N.; Ickert-Bond, S.; Gerrath, J.; Manchester, S.R.; Boggan, J.; Chen, Z.D. A new phylogenetic tribal classification of the grape family (Vitaceae). *J. Syst. Evol.* **2018**, *56*, 262–272. [CrossRef]
4. Soejima, A.; Wen, J. Phylogenetic analysis of the grape family (Vitaceae) based on three chloroplast markers. *Am. J. Bot.* **2006**, *93*, 278–287. [CrossRef]

5. Wan, Y.; Schwaninger, H.R.; Baldo, A.M.; Labate, J.A.; Zhong, G.-Y.; Simon, C.J. A phylogenetic analysis of the grape genus (*Vitis* L.) reveals broad reticulation and concurrent diversification during neogene and quaternary climate change. *BMC Evol. Biol.* **2013**, *13*, 141. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Ma, Z.-Y.; Wen, J.; Ickert-Bond, S.M.; Chen, L.-Q.; Liu, X.-Q. Morphology, structure, and ontogeny of trichomes of the grape genus (*Vitis*, Vitaceae). *Front. Plant Sci.* **2016**, *7*, 704. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Sivan, A.; Rahimi, O.; Lavi, B.; Salmon-Divon, M.; Weiss, E.; Drori, E.; Hübner, S. Genomic evidence supports an independent history of Levantine and Eurasian grapevines. *Plants People Planet* **2021**, *3*, 414–427. [\[CrossRef\]](#)
8. Li, G.R.; Ji, W.; Wang, G.; Zhang, J.X.; Wang, Y.J. An improved embryo-rescue protocol for hybrid progeny from seedless *Vitis vinifera* grapes × wild Chinese *Vitis* species. *In Vitro Cell. Dev. Biol. Plant* **2014**, *50*, 110–120. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Dangl, G.S.; Mendum, M.L.; Yang, J.; Walker, M.A.; Preece, J.E. Hybridization of cultivated *Vitis vinifera* with wild *V. californica* and *V. girdiana* in California. *Ecol. Evol.* **2015**, *5*, 5671–5684. [\[CrossRef\]](#)
10. Alexandrov, E. Interspecific hybrids of vines (*Vitis vinifera* L. × *Muscadinia rotundifolia* Michx.) with increased resistance to biotic and abiotic factors. *Sci. Pap. Ser. Manag. Econ. Agric. Rural Dev.* **2016**, *16*, 39–44.
11. Zoschke, R.; Bock, R. Chloroplast translation: Structural and functional organization, operational control, and regulation. *Plant Cell* **2018**, *30*, 745–770. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Palmer, J.D. Comparative organization of chloroplast genomes. *Annu. Rev. Genet.* **1985**, *19*, 325–354. [\[CrossRef\]](#)
13. Jansen, R.K.; Raubeson, L.A.; Boore, J.L.; DePamphilis, C.W.; Chumley, T.W.; Haberle, R.C.; Wyman, S.K.; Alverson, A.J.; Peery, R.; Herman, S.J. Methods for obtaining and analyzing whole chloroplast genome sequences. *Methods Enzymol.* **2005**, *395*, 348–384. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Jiang, H.; Tian, J.; Yang, J.; Dong, X.; Zhong, Z.; Mwachala, G.; Zhang, C.; Hu, G.; Wang, Q. Comparative and phylogenetic analyses of six Kenya *Polystachya* (Orchidaceae) species based on the complete chloroplast genome sequences. *BMC Plant Biol.* **2022**, *22*, 177. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Corriveau, J.L.; Coleman, A.W. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *Am. J. Bot.* **1988**, *75*, 1443–1458. [\[CrossRef\]](#)
16. Birky Jr, C.W. Uniparental inheritance of mitochondrial and chloroplast genes: Mechanisms and evolution. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11331–11338. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Feng, M.; Kong, H.; Lin, M.; Zhang, R.; Gong, W. The complete plastid genome provides insight into maternal plastid inheritance mode of the living fossil plant *Ginkgo biloba*. *Plant Divers.* **2023**, *45*, 752. [\[CrossRef\]](#)
18. Xie, H.; Jiao, J.; Fan, X.; Zhang, Y.; Jiang, J.; Sun, H.; Liu, C. The complete chloroplast genome sequence of Chinese wild grape *Vitis amurensis* (Vitaceae: *Vitis* L.). *Conserv. Genet. Resour.* **2017**, *9*, 43–46. [\[CrossRef\]](#)
19. Fu, P.; Tian, Q.; Li, R.; Wu, W.; Song, S.; Lu, J. The complete chloroplast genome sequence of *Vitis piasezkii*. *Mitochondrial DNA Part B Resour.* **2019**, *4*, 1034–1035. [\[CrossRef\]](#)
20. Xiang, J.; Wei, L.; Cheng, J.; Li, M.; Cui, P.; Wu, J. The complete chloroplast genome sequence of *Vitis champinii*. *Mitochondrial DNA Part B Resour.* **2020**, *5*, 2855–2856. [\[CrossRef\]](#)
21. Li, H.; Xiao, W.; Tong, T.; Li, Y.; Zhang, M.; Lin, X.; Zou, X.; Wu, Q.; Guo, X. The specific DNA barcodes based on chloroplast genes for species identification of Orchidaceae plants. *Sci. Rep.* **2021**, *11*, 1424. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Jiang, S.; Chen, F.; Qin, P.; Xie, H.; Peng, G.; Li, Y.; Guo, X. The specific DNA barcodes based on chloroplast genes for species identification of Theaceae plants. *Physiol. Mol. Biol. Plants* **2022**, *28*, 837–848. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Tillich, M.; Lehwark, P.; Pellizzer, T.; Ulbricht-Jones, E.S.; Fischer, A.; Bock, R.; Greiner, S. GeSeq—versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* **2017**, *45*, W6–W11. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**, *28*, 1647–1649. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Liu, S.; Ni, Y.; Li, J.; Zhang, X.; Yang, H.; Chen, H.; Liu, C. CPGView: A package for visualizing detailed chloroplast genome structures. *Mol. Ecol. Resour.* **2023**, *23*, 694–704. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Sharp, P.M.; Li, W.-H. An evolutionary perspective on synonymous codon usage in unicellular organisms. *J. Mol. Evol.* **1986**, *24*, 28–38. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Feng, J.-l.; Wu, L.-w.; Wang, Q.; Pan, Y.-j.; Li, B.-l.; Lin, Y.-l.; Yao, H. Comparison analysis based on complete chloroplast genomes and insights into plastid phylogenomics of four *Iris* species. *Biomed. Res. Int.* **2022**, *2022*, 2194021. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Wong, E.H.; Smith, D.K.; Rabadan, R.; Peiris, M.; Poon, L.L. Codon usage bias and the evolution of influenza A viruses. Codon usage biases of influenza virus. *BMC Evol. Biol.* **2010**, *10*, 253. [\[CrossRef\]](#)
30. Shi, S.-L.; Xia, R.-X. Codon usage in the Iflaviridae family is not diverse though the family members are isolated from diverse host taxa. *Viruses* **2019**, *11*, 1087. [\[CrossRef\]](#)
31. Shi, S.-L.; Liu, Y.-Q.; Xia, R.-X.; Qin, L. Comprehensive analysis of codon usage in *Quercus* chloroplast genome and focus on *psbA* gene. *Genes* **2022**, *13*, 2156. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Beier, S.; Thiel, T.; Münch, T.; Scholz, U.; Mascher, M. MISA-web: A web server for microsatellite prediction. *Bioinformatics* **2017**, *33*, 2583–2585. [\[CrossRef\]](#) [\[PubMed\]](#)

33. Kurtz, S.; Choudhuri, J.V.; Ohlebusch, E.; Schleiermacher, C.; Stoye, J.; Giegerich, R. REPuter: The manifold applications of repeat analysis on a genomic scale. *Nucleic Acids Res.* **2001**, *29*, 4633–4642. [[CrossRef](#)] [[PubMed](#)]
34. Amiryousefi, A.; Hyvönen, J.; Poczai, P. IRscope: An online program to visualize the junction sites of chloroplast genomes. *Bioinformatics* **2018**, *34*, 3030–3031. [[CrossRef](#)] [[PubMed](#)]
35. Brudno, M.; Do, C.B.; Cooper, G.M.; Kim, M.F.; Davydov, E.; Green, E.D.; Sidow, A.; Batzoglou, S.; Program, N.C.S. LAGAN and Multi-LAGAN: Efficient tools for large-scale multiple alignment of genomic DNA. *Genome Res.* **2003**, *13*, 721–731. [[CrossRef](#)] [[PubMed](#)]
36. Mayor, C.; Brudno, M.; Schwartz, J.R.; Poliakov, A.; Rubin, E.M.; Frazer, K.A.; Pachter, L.S.; Dubchak, I. VISTA: Visualizing global DNA sequence alignments of arbitrary length. *Bioinformatics* **2000**, *16*, 1046–1047. [[CrossRef](#)] [[PubMed](#)]
37. Frazer, K.A.; Pachter, L.; Poliakov, A.; Rubin, E.M.; Dubchak, I. VISTA: Computational tools for comparative genomics. *Nucleic Acids Res.* **2004**, *32* (Suppl. S2), W273–W279. [[CrossRef](#)] [[PubMed](#)]
38. Librado, P.; Rozas, J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **2009**, *25*, 1451–1452. [[CrossRef](#)]
39. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [[CrossRef](#)]
40. Postman, J.; Hummer, K.; Ayala-Silva, T.; Bretting, P.; Franko, T.; Kinard, G.; Bohning, M.; Emberland, G.; Sinnott, Q.; Mackay, M. GRIN-Global: An international project to develop a global plant genebank information management system. *Int. Symp. Mol. Markers Hort.* **2009**, 859. [[CrossRef](#)]
41. Nguyen, L.-T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [[CrossRef](#)] [[PubMed](#)]
42. Zhang, D.; Gao, F.; Jakovlić, I.; Zou, H.; Zhang, J.; Li, W.X.; Wang, G.T. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol. Ecol. Resour.* **2020**, *20*, 348–355. [[CrossRef](#)] [[PubMed](#)]
43. Kawabe, A.; Miyashita, N.T. Patterns of codon usage bias in three dicot and four monocot plant species. *Genes Genet. Syst.* **2003**, *78*, 343–352. [[CrossRef](#)] [[PubMed](#)]
44. Athey, J.; Alexaki, A.; Osipova, E.; Rostovtsev, A.; Santana-Quintero, L.V.; Katneni, U.; Simonyan, V.; Kimchi-Sarfaty, C. A new and updated resource for codon usage tables. *BMC Bioinform.* **2017**, *18*, 391. [[CrossRef](#)] [[PubMed](#)]
45. Parvathy, S.T.; Udayasuriyan, V.; Bhadana, V. Codon usage bias. *Mol. Biol. Rep.* **2022**, *49*, 539–565. [[CrossRef](#)] [[PubMed](#)]
46. Talat, F.; Shahdparvar, S.; Anarjan, M. Comparative bioinformatics analyses of the chloroplast genomes of *Vitis vinifera* with two caucasica subspecies of grape fruit. *J. Phylogen. Evol. Biol.* **2018**, *6*, 2. [[CrossRef](#)]
47. Zhang, L.; Song, Y.; Li, J.; Liu, J.; Zhang, Z.; Xu, Y.; Fan, D.; Liu, M.; Ren, Y.; He, J. Identification, comparative and phylogenetic analysis of eight *Vitis* species based on the chloroplast genome revealed their contribution to heat tolerance in grapevines. *Sci. Hortic.* **2024**, *327*, 112833. [[CrossRef](#)]
48. Katti, M.V.; Ranjekar, P.K.; Gupta, V.S. Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Mol. Biol. Evol.* **2001**, *18*, 1161–1167. [[CrossRef](#)] [[PubMed](#)]
49. George, B.; Bhatt, B.S.; Awasthi, M.; George, B.; Singh, A.K. Comparative analysis of microsatellites in chloroplast genomes of lower and higher plants. *Curr. Genet.* **2015**, *61*, 665–677. [[CrossRef](#)]
50. Ren, F.; Wang, L.; Li, Y.; Zhuo, W.; Xu, Z.; Guo, H.; Liu, Y.; Gao, R.; Song, J. Highly variable chloroplast genome from two endangered Papaveraceae lithophytes *Corydalis tomentella* and *Corydalis saxicola*. *Ecol. Evol.* **2021**, *11*, 4158–4171. [[CrossRef](#)]
51. Zhu, M.; Feng, P.; Ping, J.; Li, J.; Su, Y.; Wang, T. Phylogenetic significance of the characteristics of simple sequence repeats at the genus level based on the complete chloroplast genome sequences of *Cyatheaceae*. *Ecol. Evol.* **2021**, *11*, 14327–14340. [[CrossRef](#)]
52. Cipriani, G.; Marrazzo, M.T.; Di Gaspero, G.; Pfeiffer, A.; Morgante, M.; Testolin, R. A set of microsatellite markers with long core repeat optimized for grape (*Vitis* spp.) genotyping. *BMC Plant Biol.* **2008**, *8*, 127. [[CrossRef](#)] [[PubMed](#)]
53. Doulati-Baneh, H.; Mohammadi, S.; Labra, M. Genetic structure and diversity analysis in *Vitis vinifera* L. cultivars from Iran using SSR markers. *Sci. Hortic.* **2013**, *160*, 29–36. [[CrossRef](#)]
54. Bibi, A.C.; Gonias, E.D.; Doulis, A.G. Genetic diversity and structure analysis assessed by SSR markers in a large collection of *Vitis* cultivars from the island of Crete, Greece. *Biochem. Genet.* **2020**, *58*, 294–321. [[CrossRef](#)] [[PubMed](#)]
55. Yan, M.; Dong, S.; Gong, Q.; Xu, Q.; Ge, Y. Comparative chloroplast genome analysis of four *Polygonatum* species insights into DNA barcoding, evolution, and phylogeny. *Sci. Rep.* **2023**, *13*, 16495. [[CrossRef](#)]
56. Zhang, H.; Huang, T.; Zhou, Q.; Sheng, Q.; Zhu, Z. Complete chloroplast genomes and phylogenetic relationships of *Bougainvillea spectabilis* and *Bougainvillea glabra* (Nyctaginaceae). *Int. J. Mol. Sci.* **2023**, *24*, 13044. [[CrossRef](#)]
57. Nazareno, A.G.; Carlsen, M.; Lohmann, L.G. Complete chloroplast genome of *Tanaecium tetragonolobum*: The first Bignoniaceae plastome. *PLoS ONE* **2015**, *10*, e0129930. [[CrossRef](#)] [[PubMed](#)]
58. Li, C.; Cai, C.; Tao, Y.; Sun, Z.; Jiang, M.; Chen, L.; Li, J. Variation and evolution of the whole chloroplast genomes of *Fragaria* spp. (Rosaceae). *Front. Plant Sci.* **2021**, *12*, 754209. [[CrossRef](#)]
59. Moore, M.O. Classification and systematics of eastern North American *Vitis* L. (Vitaceae) north of Mexico. *SIDA Contrib. Bot.* **1991**, *14*, 339–367.
60. Xu, G.; Xu, W. Complete chloroplast genomes of Chinese wild-growing *Vitis* species: Molecular structures and comparative and adaptive radiation analysis. *Protoplasma* **2021**, *258*, 559–571. [[CrossRef](#)]

61. Liang, Z.; Duan, S.; Sheng, J.; Zhu, S.; Ni, X.; Shao, J.; Liu, C.; Nick, P.; Du, F.; Fan, P. Whole-genome resequencing of 472 *Vitis* accessions for grapevine diversity and demographic history analyses. *Nat. Commun.* **2019**, *10*, 1190. [[CrossRef](#)] [[PubMed](#)]
62. Liu, X.-Q.; Ickert-Bond, S.M.; Nie, Z.-L.; Zhou, Z.; Chen, L.-Q.; Wen, J. Phylogeny of the *Ampelocissus*–*Vitis* clade in Vitaceae supports the New World origin of the grape genus. *Mol. Phylogenet. Evol.* **2016**, *95*, 217–228. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.