iVES OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2024.58.4.8087

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Plastid genomics of wild grapevines (*Vitis vinifera* subsp. *sylvestris*) of Georgia the cradle of viticulture

Ia Pipia[1,*], Levan Ujmajuridze[2], Londa Mamasakhlisashvili[2], Álvaro Hernández[3], Vazha Tabidze[1]

[1] Institute of Molecular Genetics, Agricultural University of Georgia, 0159, Tbilisi, Georgia.
[2] LEPL Scientific-Research Center of Agriculture, 0159, Tbilisi, Georgia.
[3] Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

*correspondence:
i.pipia@agruni.edu.ge

Associate editor:
Thierry Lacombe

Received:
15 April 2024

Accepted:
21 September 2024

Published:
12 November 2024

This article is published under the **Creative Commons licence** (CC BY 4.0).

Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.

ABSTRACT

The species *Vitis vinifera* (common grape) is dividing into two sub-species: *Vitis vinifera* subsp. *vinifera* (cultivated grapevines) and *Vitis vinifera* subsp. *sylvestris* (wild grapevines). *Vitis vinifera* subsp. *vinifera* is widely utilised for table fruits and serves as the primary source for the production of grape-related beverages, including wine and vinegar. Wild grapevines (*Vitis vinifera* subsp. *sylvestris*) are of great interest as they are considered the progenitors of cultivated varieties and are key to understanding the grapevine domestication process in general. To unlock the molecular mechanisms of grapevine domestication, genome-based studies are widely carried out. In this study, the complete chloroplast genome of two Georgian wild grapevine samples is subject to Illumina sequencing and *in silico* genome assembly, followed by gene annotation. According to the results, each analysed chloroplast genome is 160.928 bp in length, comprises a total of 128 genes (83 protein coding, 37 tRNA, 8 rRNA) and belongs to the genetically unique 'Rkatsiteli' haplotype (AAA). A comparative genomic study reveals the presence of certain InDels and SNPs in the chloroplast genomes.

KEYWORDS: *Vitis vinifera* subsp. *sylvestris*, plastid DNA, next-generation sequencing, genome

INTRODUCTION

Georgia, a country located in the South Caucasus, is recognised as the "*cradle of viticulture*" for several reasons, some of which are explained here. Georgia is home to over 500 indigenous grapevine cultivars (Ketskhoveli *et al*., 2012). Morphological and ampelographic characteristics of grapevine pips discovered at the Shulaveri archaeological site in southeastern Georgia (6000 BC) indicate that they belong to *Vitis vinifera* L. subsp. *sativa* (Maghradze *et al*., 2020). The analysis of ancient organic compounds found in pottery fabrics from Georgia dating back to the early Neolithic era has provided the earliest archaeological evidence from the Near East of grape wine and viniculture in around 6000–5800 BC (McGovern, 2017). According to many researchers, the South Caucasus–including Georgia and adjacent areas–is the geographic region where grapevines were most likely first domesticated (McGovern 2003; Barnard *et al.*, 2011; Myles *et al.*, 2011; Arroyo García *et al*., 2013; Bouby *et al*., 2021). Georgia has an ancient tradition of Qvevri wine-making, a technology that was designated as a national monument of intangible cultural heritage by UNESCO in 2013 (UNESCO, n.d.). Georgia (specifically Colchis, the western part of the country), is one of the few refugia to still exist in the world (Sękiewicz *et al*., 2022). Wild grapevines can be found in their natural habitats in Georgia, where they are typically sporadically distributed and grown in forestry regions and riverbanks up to 1200 m above sea level (Ramishvili, 2001; Arroyo García *et al*., 2013). The extensive morphological diversity of wild grapevines found in Georgia represents a significant gene pool that played a role in the domestication process of grapevines (Ekhvaia *et al.*, 2010). Notably, the neotype of the wild grapevine was found in East Georgia (Alazani River basin, Jumaskure) and was described in the paper of Ferrer-Gallego and co-authors (Ferrer-Gallego *et al.*, 2019).

Over recent years, next-generation plastid DNA genomics has become a potent and more accessible tool for plant phylogenetics. In the past decade, numerous scientific studies have utilised advanced plastid DNA technologies to emphasise the significance of Georgian grapevines in the history of world viticulture (Pipia *et al.*, 2012, Pipia *et al*, 2023; Tabidze *et al.*, 2014). It is a well-known fact that sharing DNA haplotypes between native and cultivated populations enables identification of the geographic area in which ancestral native populations were brought under domestication. Such a concurrence of plastid haplotypes has been found in a worldwide set of cultivated grapevines and in South Caucasian (especially Georgian), wild grapevines, highlighting the role of this geographic region in the grapevine domestication process in general (Pipia *et al.*, 2012, Pipia *et al*, 2023).

The main goals of the present research were to i) perform next-generation sequencing of the complete plastid genomes of Georgian wild grapevines (*Vitis vinifera* subsp. *sylvestris*), ii) carry out *in silico* genome assembly and gene annotation of the analysed plastomes, and iii) reconstruct the picture of mutational relationships and conduct comparative analyses among genomes of the studied grapevine samples, along with phylogeographical analysis.

MATERIALS AND METHODS

The wild grapevine samples of Georgia, especially the young green leaves, were provided by the National Centre for Grapevine and Fruit Tree Planting Material Propagation of Scientific research Center of Georgia (Mtskheta, Georgia). DNA isolation for subsequent next-generation sequencing (NGS) was conducted using young green leaves by standard CTAB protocol (Lodhi *et al.*, 1994). The construction of shotgun Nextera genomic library and HiSeq2500 Sequencing was carried out at the facilities of Roy J. Carver Biotechnology Center, University of Illinois in Urbana-Champaign (USA). The SOAPdenovo package (http://soap.genomics.org.cn/soapdenovo.html) was utilised for the assembly of plastid DNAs. For data analysis, the multiple sequence alignment softwares BLAST and Mafft were used. Detection of SNPs and InDels, along with the construction of a phylogenetic tree, was performed to reconstruct mutational relationships between the analysed plastomes and to perform a phylogeographical analysis. All sequenced and *in silico* assembled genomes were deposited in the appropriate databases (DDBJ) and are now available at the NCBI with the GenBank numbers provided in Table 1.

RESULTS AND DISCUSSION

In this study, plastid genomes of two Georgian wild grapevines (*Vitis vinifera* subsp. *sylvestris*) underwent NGS, following by subsequent genome assembly, gene annotation, and phylogenetic analyses. One sample of wild grapevine belonged to West Georgia and was named 'Chkumi' (male plant), while the other, named 'Ninotsminda' (female plant), was from the East Georgia. The names of each sample correspond to the toponimic names of the geographic areas where they had been found in nature.

In silico genome assembly for each sequenced plastome was conducted using the SOAPdenovo package. Following the genome assembly, a gene annotation study was performed for both analysed plastomes using the GESEQ v.1.42 software and BLAST v.2.6.0 searches. It was revealed that each complete chloroplast genome encoded for a total of 128 genes, comprising 83 protein coding genes, 37 transfer RNA genes (tRNA), and 8 ribosomal RNA genes (rRNA) [Tables 1, 2 and 3]. All these genes, along with their corresponding products (proteins, tRNAs, rRNAs) are accessible on NCBI under the aforementioned GenBank numbers (see Table 4).

For the construction of chloroplast genome maps, we used a comprehensive online tool (Chloroplot, https://irscope.shinyapps.io/chloroplot/) to visualise the plastid genomes. This tool extracts information from GenBank to generate detailed maps of chloroplast genomes, highlighting all types of genes (protein-coding, tRNA, rRNA), SSC regions, etc. (Zheng *et al.*, 2020). Figure 1 displays the Chloroplot map for the 'Chkumi' sample. The genome size is 160.928 bp long with a 37 % GC content, and comprises all the genes listed in Tables 1, 2, and 3.

TABLE 1. All Protein coding genes (83) detected in the complete chloroplast genomes of Georgian wild grapevines 'Chkumi' and 'Ninotsminda'.

Gene	Genome position (bp)	Gene	Genome position (bp)	Gene	Genome position (bp)
*psb*A	412..1473	*psa*I	63739..63849	*rpl*22	88320.. > 88808
*mat*K	2016..3524	*ycf*4	64269..64829	*rps*19	88907..89185
*rps*16	5154..6330	*cem*A	65676..66365	*rpl*2	89256..90744
*psb*K	8630..8815	*pet*A	66593..67555	*rpl*23	90763..91044
*psb*I	9221..9331	*psb*J	68613..68735	*ycf*2	91372..98274
*atp*A	11770..13293	*psb*L	68885.. > 69001	*ndh*B	99934..102145
*atp*F	13359..14663	*psb*F	69024..69143	*rps7*	102466..102933
*atp*H	15164..15409	*psb*E	69153..69404	*ndh*F	115534..117783
*atp*I	16311..17054	*pet*L	70713..70808	*rpl*32	118989..119162
*rps*2	17268..17978	*pet*G	70987..71100	*ccs*A	120526..121494
*rpo*C2	18196..22398	*psa*J	71929..72060	*ndh*D	121764.. > 123266
*rpo*C1	22569..25380	*rpl*33	72503..72703	*psa*C	123402..123647
*rpo*B	25412..28624	*rps*18	72896..73201	*ndh*E	123894..124199
*pet*N	30570..30659	*rpl*20	73487..73840	*ndh*G	124433..124963
*psb*M	31893..31997	*rps*12	74626..74739, 146275..147072	*ndh*I	125339..125842
*psb*D	36092..37153	*clp*P	74882..76916	*ndh*A	125919..128139
*psb*C	37101..38522	*psb*B	77360..78886	*ndh*H	128141..129322
*psb*Z	39228..39416	*psb*T	79074..79181	*rps*15	129426..129692
*rps*14	40561..40863	*psb*N	79241..79372	*ycf*1	130000..135681
*psa*B	40995..43199	*psb*H	79457..79696	*rps7*	147126..147593
*psa*A	43225..45477	*pet*B	79815..79820, 80509..81150	*ndh*B	147914..150125
*ycf*3	46174..48150	*pet*D	81339..81346, 82081..82555	*ycf*2	151785..158687
*rps*4	49185..49790	*rpo*A	82767..83762	*rpl*23	159015..159296
*ndh*J	52836..53312	*rps*11	83843..84259		
*ndh*K	53407..54084	*rpl*36	84375..84488		
*ndh*C	54138..54500	*inf*A	84612..84845		
*atp*E	56754..57155	*rps*8	84967..85371		
*atp*B	57152..58648	*rpl*14	85541..85909		
*rbc*L	59428..60855	*rpl*16	86037..87508		
*acc*D	61466..62992	*rps*3	87679..88335		

1. Haplotypes and InDels

In the first step of the genome analyses, the haplotype of the samples of 'Chkumi' and 'Ninotsminda' plastoms was determined: both plastomes belong to the so-called "Rkatsiteli haplotype" (AAA) (Table 4). This haplotype represents one of the four previously detected previously detected universal plastid haplotypes (Chkhaveri-Pinot noir haplotype [GTA], Meskhuri Mtsvane-Chardonnay haplotype [ATA], Saperavi-Cabernet-Sauvignon haplotype [ATT], Rkatsiteli haplotype [AAA]) found in both cultivated and wild grapevines. All these haplotypes were derived from single nucleotide substitutions (SNPs) in the *trn*H-*psb*A, and *rpl*16 dataset of plastid genomes. In each haplotype, the first letter of the three-letter acronym represents the specific nucleotide at position 205 bp of the chloroplast genome. Two other positions correspond to nucleotides at two regions of *rpl*16 intron (86.715 and 86.721 bp). Interestingly, the assessment of plastid polymorphism in an additional plastid genome region, *acc*D-*psa*I, did not change the haplotype distribution within the set of cultivated and wild grapevine plastoms (Pipia *et al.*, 2012). Based on the chronogram outlining the primary lineages of *Vitaceae*, we found that the Chkhaveri-Pinot-like genomes (GTA haplotype) originated 5 to 4 million years ago (Ma). In contrast, the lineages of 'Saperavi' (ATA haplotype), 'Meskhuri Mtsvane' (ATA haplotype), and 'Rkatsiteli' (AAA haplotype) are estimated to have emerged 3 to 1 Ma ago (Zecca *et al.*, 2019). As both samples in the present study were identified as members of the AAA

TABLE 2. All tRNA genes (37) detected in the complete chloroplast genomes of Georgian wild grapevines 'Chkumi' and 'Ninotsminda'.

Gene	Genome position (pb)	Gene	Genome position (pb)
*trn*H-GUG	2..76	*trn*M-CAU	56446..56518
*trn*K-UUU	1721..1755,4257..4293	*trn*W-CCA	71231..71304
*trn*Q-UUG	8212..8283	*trn*P-UGG	71470..71545
*trn*S-GCU	9474..9561	*trn*I-CAU	91209..91283
*trn*G-GCC	10569..10599,11281..11340	*trn*L-CAA	99281..99363
*trn*R-UCU	11501..11572	*trn*V-GAC	105502..105573
*trn*C-GCA	29696..29766	*trn*I-GAU	107584..107625,108570..108604
*trn*D-GUC	33112..33188	*trn*A-UGC	108668..108704,109508..109543
*trn*Y-GUA	33632..33717	*trn*R-ACG	113309..113383
*trn*E-UUC	33770..33844	*trn*N-GUU	113984..114057
*trn*T-GGU	34761..34834	*trn*L-UAG	120328..120407
*trn*S-UGA	38773..38865	*trn*N-GUU	136002..136075
*trn*G-GCC	40108..40179	*trn*R-ACG	136676..136750
*trn*fM-CAU	40321..40394	*trn*A-UGC	140516..140551,141355..141391
*trn*S-GGA	48823..48909	*trn*I-GAU	141455..141489,142434..142475
*trn*T-UGU	50091..50165	*trn*V-GAC	144486..144557
*trn*L-UAA	51122..51156,51675..51724	*trn*L-CAA	150696..150778
*trn*F-GAA	52050..52123	*trn*I-CAU	158776..158850
*trn*V-UAC	55624..55679,56234..56272		

TABLE 3. All rRNA genes (8) detected in the complete chloroplast genomes of Georgian wild grapevines 'Chkumi' and 'Ninotsminda'.

Gene	Genome position (bp)
rRNA 16S	105793..107283
rRNA 23S	109701..112503
rRNA 4.5S	112611..112713
rRNA 5S	112930..113050
rRNA 5S	137009..137129
rRNA 4.5S	137346..137448
rRNA 23S	137547..140349
rRNA 16S	142776..144266

haplotype, the 'Rkatsiteli' plastid genome (GenBank# AB856289.1) belonging to the same AAA haplotype was used as the reference genome for *in silico* genome assembly.

The chloroplast genomes for both analysed samples were 160.928 bp in length (Table 4). Generally, the chloroplast genome can be divided into four specific regions: large single copies (LSC), small single copies (SSC), and two inverted repeats. SSCs contain only single gene copies, while inverted repeats emphasise identical genes but in opposite or reverse orientations (Zheng *et al.*, 2020; Turudić *et al.*, 2022). In both the samples 'Chkumi' and 'Ninotsminda' three InDels were found (Table 5). In particular, 33 bp duplication at the 6.658-6.691 bp position, 18 bp duplication at the 19.527-19.544 bp position, and 54 bp deletion at the 30.133-30.186 bp position of the chloroplast genome. This finding was expected, as we had detected these InDels in the chloroplast genomes of three other Georgian wild grapevines in previous work, (Pipia *et al.*, 2023). It seems these InDels are generally characteristic of the 'Rkatsiteli' haplotype.

2. Comparative genomics

The comparative genomic approaches BLAST and Mafft revealed 100 % coincidences between the genomes of the Georgian cultivar 'Rkatsiteli' and the wild grapevines 'Chkumi' and 'Ninotsminda'. There were only two exceptions: a single insertion (one A) at the 160.845 bp genome position in both wild grapevine genomes, and one SNP (T to G) in 'Chkumi' genome at the 8.436 bp position (Table 6). These results are consistent with the results of our previous studies, which revealed practically the complete identity of 'Rkatsiteli' and Georgian wild grapevines, namely 'Borjomi', 'Dusheti' and 'Qsovrisi' (Pipia *et al.*, 2023). From a phylogenetic point of view, genome identities of this kind are crucial, as they indicate the sharing of haplotypes between cultivated and wild samples in a specific geographic location. Such information can provide valuable insights into the potential geographic origin of the domestication process.

Once the identities of cultivated and wild plastoms had been determined, comparative analyses were performed to further substantiate the hypothesis that the South Caucasus, particularly Georgia, is one of the possible centres of domestication. In particular, the Georgian cultivars 'Rkatsiteli' and 'Ojaleshi' (the same haplotype as Rkatsiteli–AAA, unpublished data), 'Saperavi' (ATT haplotype) and 'Meskhuri Mtsvane' (ATA haplotype) and the wild grapevines of the present research, along with genomes of earlier work ('Dusheti', 'Qsovrisi',

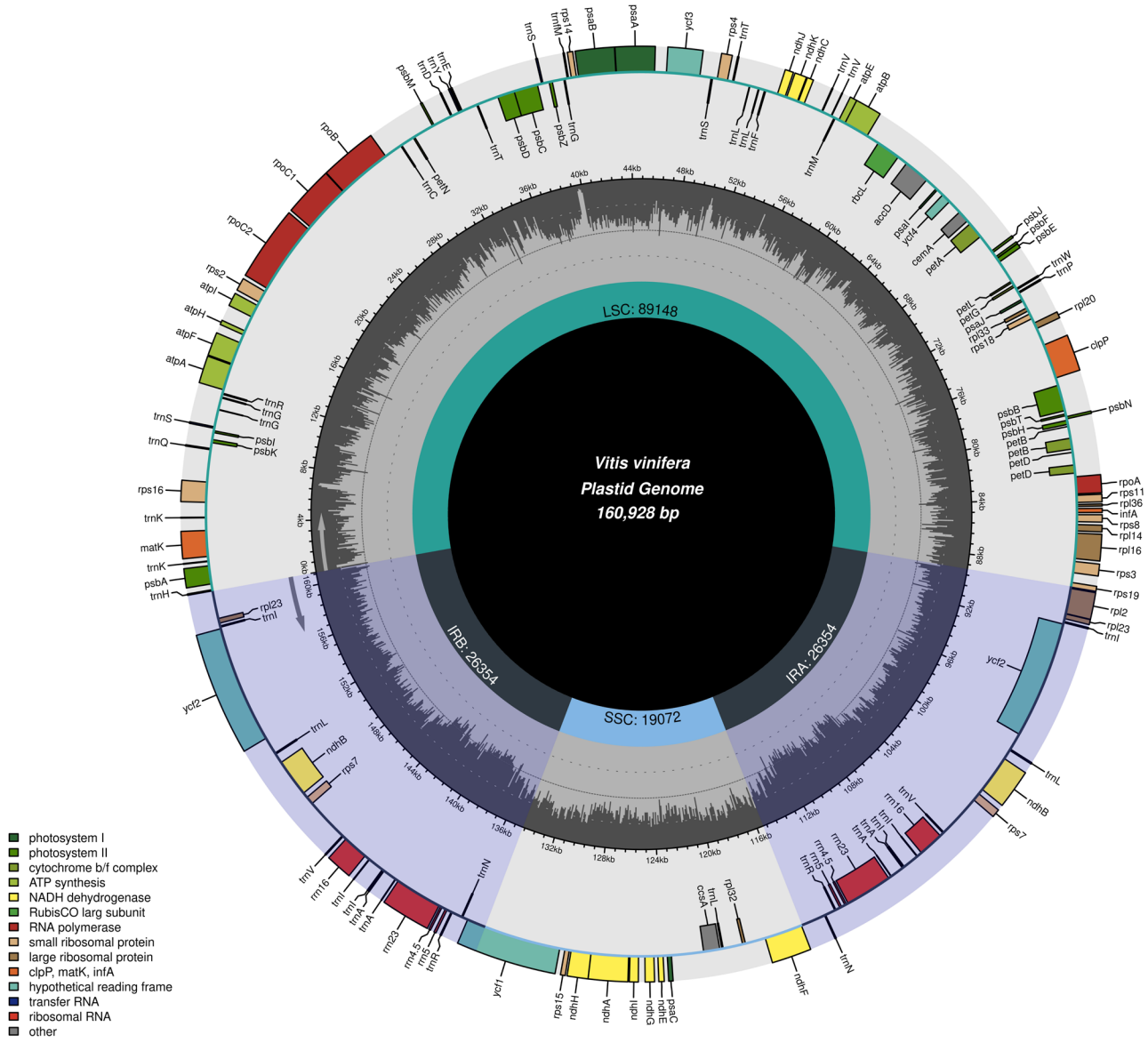


FIGURE 1. Circular chloroplast genome map of Georgian wild grapevine (*Vitis vinifera* subsp. *sylvestris*) 'Chkumi'.

TABLE 4. Genome length, SNP positions, haplotypes and GenBank numbers of analysed plastoms of Georgian wild grapevines. Nucleotide positions are given according to 'Maxxa' plastid DNA (Jansen *et al.*, 2006).

Wild grapevine sample	Genome Length (bp)	SNP position (bp)			Haplotype	GenBank #
		86721	86715	86721		
Chkumi	160.928	A	A	A	AAA	LC687362.1
Ninotsminda	160.928	A	A	A	AAA	LC687362.1

TABLE 5. The long size InDels of the analysed wild grapevine of 'Chkumi' and 'Ninotsminda'. Nucleotide positions are given according to Tabidze *et al.* (2014).

Nucleotide position	Locus	InDel length	InDel type
6.658-6.691	*rps*16-*trn*Q-UUG intergenic spacer	33 bp	Duplication
30.133-30.186	*trn*C-GCA-*pet*N intergenic spacer	54 bp	Deletion
19.527-19.544	*rpo*C2 gene	18 bp (6 amino acid)	Duplication

TABLE 6. Location of one SNP and one insertion in wild grapevines 'Chkumi' and 'Ninotsminda' calculated using Mafft software.

Chkumi	8421	attgatcattacatag**g**aattcaattaagatatttatgaaag	8461
Ninotsminda	8421	attgatcattacatat**t**aattcaattaagatatttatgaaag	8461
Rkatsiteli	8421	attgatcattacatat**t**aattcaattaagatatttatgaaag	8461
Chkumi	160831	ttcttcgtctttacaaaaaaaaaaaaaaata	160860
Ninotsminda	160831	ttcttcgtctttacaaaaaaaaaaaaaaata	160860
Rkatsiteli	160831	ttcttcgtctttac-aaaaaaaaaaaaaata	160859

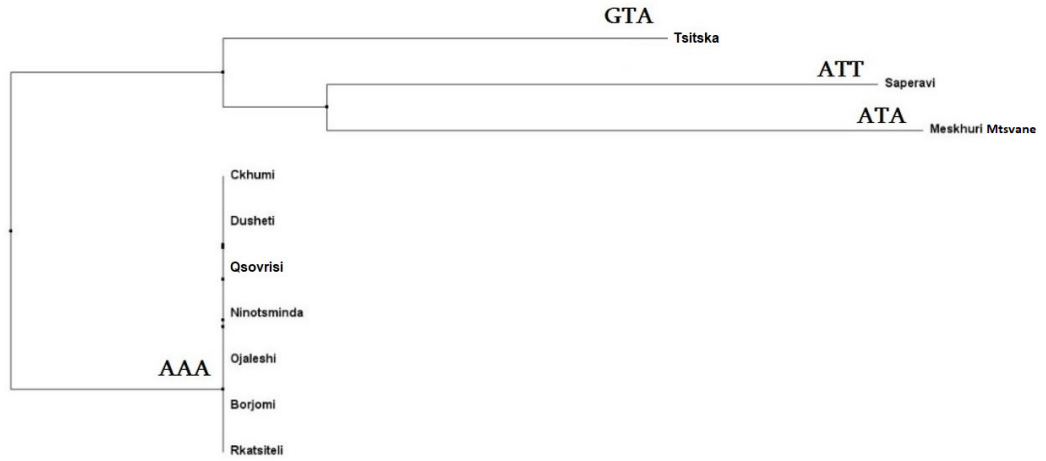


FIGURE 2. Complete chloroplast genome phylogeny of Georgian grape cultivars 'Rkatsiteli', 'Ojaleshi', 'Tsitska', 'Saperavi', 'Meskhuri Mtsvane' (*Vitis vinifera* subsp. *vinifera*) and Georgian wild grapevine samples (*Vitis vinifera* subsp. *sylvestris*) conducted by maximum likelihood (ML) analysis of MEGA software. Names of wild grapevines correspond to their geographic origin.

'Borjomi'), were subject to Mafft alignment. All chloroplast genomes used in this analysis were retrieved from the NCBI database (#AB856289.1, LC714846.1, AB856291.1, LC495883, LC523806, LC494572). The alignment with the inclusion of the abovementioned samples did not reveal any additional SNPs or gaps beyond those identified in the comparison of 'Rkatsiteli' and 'Chkumi' and 'Ninotsminda': *i.e.*, the picture was exactly the same (Only a gap at 160.845 and SNP at 8435 bp, see above). The comparative genomic study uncovered the complete plastid genome identity of cultivated 'Rkatsiteli' and 'Ojaleshi' plastomes, with the exception of one additional A at the 160.842 bp position in the 'Ojaleshi' genome. Simultaneously, the complete plastome identity of the two aforementioned cultivated Georgian varieties ('Rkatsiteli' and 'Ojaleshi') and the Georgian wild grapevines ('Chkumi', 'Ninotsminda', 'Borjomi', 'Dusheti', 'Qsovrisi') was determined, with the exception of one SNP. All these findings are well depicted in Figure 2, which shows a phylogenetic tree of Georgian cultivated and wild grapevines. The tree highlights two general clades: one consisting of the Georgian cultivated varieties 'Rkatsiteli' and 'Ojaleshi' along with Georgian wild grapevines, and the other shown as separated sub-groups, each completed with the Georgian grape cultivar belonging to the corresponding haplotype ('Tsitska'–GTA haplotype, unpublished data), Saperavi–ATT haplotype, 'Meskhuri Mtsvane'–ATA haplotype).

CONCLUSIONS

The main findings of the present research can be summarised as follows:

- the Georgian wild grapevines (*Vitis vinifera* subsp. *sylvestris*) 'Chkumi' and 'Ninotsminda' belong to the Rkatsiteli haplotype (AAA). The sharing of haplotypes between Georgian cultivated and wild grapevines within one geographic region emphasises the potential role of Georgia in the grapevine domestication process;
- *in silico* genome assembly and genome annotation revealed that the plastoms of the Georgian wild grapevines (*Vitis vinifera* subsp. *sylvestris*) 'Chkumi' and 'Ninotsminda' are 160.928 bp long, encompassing 128 genes (83 protein coding, 37 tRNA, 8 rRNA);
- phylogenetic relationships between the analysed plastoms align with their haplotype diversity, including one clade representing the 'Rkatsiteli' haplotype (AAA) and three other distinct clades representative of three other haplotypes;
- to gain a deeper understanding of the potential role of Georgian grapevines in the domestication process, the analysis of a larger number of sylvestris samples from the "*cradle of viticulture*" is recommended.

ACKNOWLEDGEMENTS

The authors kindly thank Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [grant number: FR-19-14378] for supporting this research. We also extend our gratitude to Prof. David Maghradze for providing samples of wild Georgian grapevines.

REFERENCES

Arroyo García, R. A., & Revilla, E. (2013). The current status of wild grapevine populations (*Vitis vinifera* ssp *sylvestris*) in the Mediterranean Basin. In The Mediterranean Genetic Code - Grapevine and Olive. https://doi.org/10.5772/52933

Barnard, H., Dooley, A. N., Areshian, G., Gasparyan, B., & Faull, K. F. (2011). Chemical evidence for wine production around 4000 BCE in the Late Chalcolitic Near Eastern highlands. *Journal of Archaeological Science, 38*, 977-984.

Bouby, L., Wales, N., Jalabadze, M. M., Rusishvili, N., Bonhomme, V., Ramos-Madrigal, J., Evin, A., Ivorra, S., Lacombe, T., Pagnoux, C., Boaretto, E., Gilbert, M. T. P., Bacilieri, R., Lortkipanidze, D., & Maghradze, D. (2021). Tracking the history of grapevine cultivation in Georgia by combining geometric morphometrics and ancient DNA. Vegetation History and Archaeobotany, 30(1), 63–76. https://doi.org/10.1007/s00334-020-00803-0

Ekhvaia, J., & Akhalkatsi, M. (2010). Morphological variation and relationships of Georgian populations of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi. Flora - Morphology, Distribution, *Functional Ecology of Plants, 205*(9), 608-617. https://doi.org/10.1016/j.flora.2009.08.002

Ferrer-Gallego, P. P., Ferrer-Gallego, R., Laguna, E., & Pipia, I. (2019). Proposal to conserve the name *Vitis sylvestris* C.C. Gmel in (*Vitaceae*) against *V. sylvestris* W. Bartram, *TAXON, 68*(2), 409-410. https://doi.org/10.1002/tax.12043

Jansen, R. K., Kaittanis, C., Lee, S. B., Saski, C., Tomkins, J., Alverson, A. J., & Daniell, H. (2006). Phylogenetic analyses of *Vitis* (*Vitaceae*) based on complete chloroplast genome sequences: Effects of taxon sampling and phylogenetic methods on resolving relationships among Rosids. *BMC Evolutionary Biology, 6*(1), 32.

Ketskhoveli, N., Ramishvili, M., & Tabidze, D. (2012). Georgian ampelography (2nd ed.). Tbilisi.

Lodhi, A., Ye, G., Weeden, N.F., & Reisch, B.I. (1994). A simpleand efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Molecular Biology Reporter, 12*, 6-13. https://www.doi.org/10.1007/BF02668658

Maghradze, D., Melyan, G., Salimov, V., Chipashvili, R., Iñiguez, M., Puras, P., Melendez, E., Vaca, R., Ocete, C., Rivera, D., Obón, C., Valle, J. M., Rodriguez-Miranda, A., Failla, O., & Ocete, R. (2020). Wild grapevine (*Vitis sylvestris* C.C.Gmel.) wines from the Southern Caucasus region. *OENO One, 54*(4). https://doi.org/10.20870/oeno-one.2020.54.4.3720

McGovern, P. E., Jalabadze, M., Batiuk, S., Callahan, M. P., Smith, K. E., Hall, G. R., Kvavadze, E., Maghradze, D., Rusishvili, N., Bouby, L., Failla, O., Cola, G., Mariani, L., Boaretto, E., Bacilieri, R., This, P., Wales, N., & Lordkipanidze, D. (2017). Early Neolithic wine of Georgia in the South Caucasus. PNAS, 114(48), E10309–E10318. https://doi.org/10.1073/pnas.1714728114

McGovern, P. E. (2003). Ancient Wine. Princeton, NJ: University Press.

Myles, S., Boyko, A. R., Owens, Ch. L., Brown, P., Grassi, F., Aradhya M. K., Prins, B., Reynolds, A., Chia, J-M., Ware, D., Bustamante C. D., Buckler E. (2011). Genetic structure and domestication history of the grape. *PNAS*, *108*(9), 3535-3531. https://doi.org/10.1073/pnas.1009363108

Pipia, I., Gogniashvili, M., Tabidze, V., Beridze, T., Gamkrelidze, M., Gotsiridze, V., Melyan, G., Musayev, M., Salimov, V., Beck., J., and Schaal, B. (2012). Plastid DNA sequence diversity in wild grapevine samples (*Vitis vinifera* subsp. *sylvestris*) from the Caucasus region. *Vitis*, *51*(3), 119-124.

Pipia, I., Lacombe, T., Kotorashvili, A., Kotaria, N., & Tabidze, V. (2023). Complete chloroplast genome sequencing of *Vitis vinifera* subsp. *sylvestris* – wild ancestors of cultivated grapevines. *Vitis*, *62*(4). https://doi.org/10.5073/vitis.2023.62.193-201

Ramishvili, R. (2001). History of Georgian grape and wine. Tbilisi (in Georgian).

Sękiewicz, K., Danelia, I., Farzaliyev, V., Gholizadeh, H., Iszkuło, G., Naqinezhad, A., Ramezani, E., Thomas, P. A., Tomaszewski, D., Walas, Ł., & Dering, M. (2022). Past climatic refugia and landscape resistance explain spatial genetic structure in Oriental beech in the South Caucasus. *Ecology and Evolution*. Advance online publication. https://doi.org/10.1002/ece3.9320

Tabidze, V., Baramidze, G., Pipia, I., Gogniashvili, M., Ujmajuridze, L., Beridze, T., Hernandez, A. G., & Schaal, B. (2014). The complete chloroplast DNA sequence of eleven grape cultivars. OENO One, *Journal International des Sciences de la Vigne et du Vin (JISVV), 48*, 99-109.

Turudić, A., Liber, Z., Grdiša, M., Jakše, J., Varga, F., & Šatović, Z. (2022). Chloroplast genome annotation tools: Prolegomena to the identification of inverted repeats. *International Journal of Molecular Sciences*, *23*(18), 10804. https://doi.org/10.3390/ijms231810804

UNESCO. (n.d.). Ancient Georgian traditional Qvevri wine-making method. Retrieved from https://ich.unesco.org/en/RL/ancient-georgian-traditional-qvevri-wine-making-method-00870

Zheng, S., Poczai, P., Hyvönen, J., Tang, J., & Amiryousefi, A. (2020). Chloroplot: An online program for the versatile plotting of organelle genomes. *Frontiers in Genetics*, *11*. https://www.doi.org/10.3389/fgene.2020.576124

Zecca, G., Grassi, F., Tabidze, V., Pipia, I., Kotaria, N., Kotorashvili, A., Beridze, T. (2019). Dates and rates in grape's plastomes: evolution in slow motion. *Current Genetics*, *66*, 123-140. https://www.doi.org/10.1007/s00294-019-01004-7