



VITICULTURE SHORT COMMUNICATIONS

Georgian wild grapevine germplasm is confirmed to be genetically distinct from Mediterranean germplasm

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ABSTRACT

The wild progenitor of the cultivated grapevine, *Vitis vinifera* subsp. *sylvestris* is a key reservoir of genetic diversity and adaptive traits. Georgia, one of the primary centres of grapevine domestication, still hosts remaining populations of wild grapevines that preserve unique genetic lineages. In this study, the genetic diversity and structure of 23 Georgian wild grapevine accessions were investigated using 18k SNP loci and compared with those of 112 additional wild genotypes from the Mediterranean basin. Genetic analyses revealed that wild Georgian grapevine populations from eastern Georgia constitute a single, highly diverse genetic pool with limited population stratification; however, cluster analysis detected geographically associated substructures, particularly within Kakheti. In the context of the Mediterranean basin, genetic analysis revealed a genetic distinctiveness of the Georgian grapevine population. Clustering and PCA consistently identified Georgian samples as a coherent and genetically distinct group, showing limited overlap with Mediterranean accessions. Population structure analyses supported three ancestral populations, with the Georgian group forming an independent cluster (POP2), while North African and Iberian Peninsula (POP1) and Western–Central European samples constituted the others. A minimum spanning network revealed three main populations, with the North African group acting as a genetic bridge between Georgian and Western–Central European wild grapevine samples. TreeMix analysis indicated a clear tree-like divergence among the three populations, with POP1 and POP2 forming sister groups and POP3 diverging earlier. Georgian samples showed the highest genetic drift, and no evidence of admixture or post-divergence gene flow was detected. Altogether, these results emphasise the importance of preserving Georgian wild grapevine germplasm for future conservation and breeding strategies.

KEYWORDS: *Vitis vinifera* subsp. *sylvestris*, SNP, genetic diversity, population structure, gene flow

INTRODUCTION

The Epic of Gilgamesh, one of the oldest literary texts dated back to the 18th century BCE, mentions the social importance of grapes and wine. After millennia, nothing has changed, as suggested by economic data. In 2023, global grape (*Vitis vinifera* L.) production reached 72.4 million tons, with wine consumption totalling 221 million hectolitres and a market value of approximately 36 billion euros (www.fao.org, www.oiv.int). This species belongs to the *Vitaceae* family and the *Vitis* genus. Today, within this genus, *V. vinifera* is the most widespread species (Wan *et al.*, 2013). It's thousands of cultivars originated from the wild progenitor, *Vitis vinifera* subsp. *sylvestris*, through crosses and vegetative propagation (Myles *et al.*, 2011; This *et al.*, 2006). Prior to purposeful cultivation, which began around 11,000 years ago (Dong *et al.*, 2023), humans exerted unconscious selection on wild populations, leading to significant changes in phenotypic traits (McGovern, 2003). Differences include: i) flower sex, with wild populations being dioecious and cultivated ones typically hermaphroditic; ii) seed morphology, with wild grapes having spherical seeds with a small beak, while domesticated varieties exhibit pyriform seeds; iii) berry size, which is larger in cultivated varieties; iv) leaf size as a consequence of cluster size modification aimed at supplying a greater amount of photosynthates (This *et al.*, 2006); v) changes in several technological characteristics of the grapes (Maghradze *et al.*, 2021).

Genetic analysis, along with archaeological findings, suggests that the Southern Caucasus region and Western Asia were primary centres of grapevine domestication, playing a crucial role in grapevine diffusion through human migration routes: eastward to India and China, northward through the Balkans, and westward via the North African coast (Dong *et al.*, 2023). This geographic expansion triggered introgression between *sylvestris* and *sativa* individuals. However, some grapevine germplasm, including the Georgian one, has preserved its original traits, maintaining the characteristics of the local breeding. Georgian wild grapevines exhibit high heterozygosity and unique genetic variability, with alleles that are either absent or less common in other regions (De Lorenzis *et al.*, 2015; Imazio *et al.*, 2013). This genetic diversity has historically been the key source for varietal improvement and adaptation to changing environmental conditions (Sargolzaei *et al.*, 2021). Moreover, this high variability could also be a source of resistance to various pathogens, including *Plasmopara viticola* and phytoplasmas (Bitsadze *et al.*, 2024; Quaglino *et al.*, 2016; Ricciardi *et al.*, 2024; Toffolatti *et al.*, 2018).

Despite the importance of preserving this heritage, as demonstrated by various National (such as the Shota Rustaveli National Science Foundation's grant titled Wild Grapevine of Georgia: Research and Preservation and the National Wine Agency's grant titled Research on Georgian Grapes and Wine Culture) and European (such as the GrapeGen06 – Management & Conservation of Grapevine Genetic Resources project and Cost Action FA1003

– East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding) research programs, the erosion of Georgian wild grapevine germplasm is real (Ekhvaia & Akhalkatsi, 2010). Considering the current distribution of wild individuals in Georgia, predominantly in the higher eastern regions, such as Kartli, Mtskheta-Mtianeti, and Kakheti, and fragmented in the western regions, the causes could vary. The loss of its natural habitats (riverbanks and flooded forests) due to human activities, the biotic pressure exerted by pathogens introduced from North America in the late 19th century, and the potential competition with non-*vinifera* *Vitis* species are just a few examples (Cola *et al.*, 2025).

This genetic heritage can be explored through molecular markers, which have been introduced to overcome the limitations of phenotypical analysis, such as environmental growth conditions (Nadeem *et al.*, 2018). For effective genetic applications in *Vitis*, molecular markers must be polymorphic, reproducible, and provide publicly available data for comparative studies (Cabezas *et al.*, 2011; Tympakianakis *et al.*, 2023). Single Nucleotide Polymorphisms (SNPs) are among the most commonly used molecular markers, owing to their high frequency and broad distribution throughout the genome (Broccanello *et al.*, 2018). Thanks to their abundance across the genome and ease of detection, SNP markers are particularly well-suited for identifying cases of synonymy and homonymy, as well as for population studies, parentage, and kinship analyses (De Lorenzis, 2024; Laucou *et al.*, 2018). Among the available approaches for SNP detection, high-throughput genotyping chip arrays represent the most commonly used and efficient method. In *V. vinifera*, the Vitis18kSNP genotyping chip array has been widely adopted for large-scale genotyping studies. Developed by the GrapeReSeq consortium (Laucou *et al.*, 2018), the Vitis18kSNP genotyping chip array provides advanced tools for the genetic analysis of the *Vitis* genus. This genotyping chip array is the result of extensive sequencing of selected genotypes, including *V. vinifera* subsp. *vinifera* (43 individuals), *V. vinifera* subsp. *sylvestris* (4), *Vitis cinerea* (3), *Vitis berlandieri* (3), *Vitis aestivalis* (3), *Vitis labrusca* (3), *Vitis lincecumii* (1), and *Muscadinia rotundifolia* (5). The Vitis18kSNP chip includes 13,537 SNPs specifically targeting *V. vinifera* and 4,510 SNPs for broader analysis across various *Vitis* species, for a total of 18,047 loci (Laucou *et al.*, 2018).

Although numerous population genetic studies have been conducted on grapevine cultivars using SNP markers, comparatively few have focused on wild grapevine accessions (De Lorenzis *et al.*, 2015; D'Onofrio, 2020; Marinov *et al.*, 2024; Ramos-Madrugal *et al.*, 2019). This study aims to investigate the genetic variability and relationships between Georgian wild grapevines and wild genotypes across the Mediterranean basin using the Vitis18kSNP genotyping chip array.

MATERIALS AND METHODS

1. Plant materials

To investigate the genetic variability and relationships among *V. vinifera* subsp. *sylvestris* individuals in one of the two primary centres of domestication (Georgia), a sample set (#1) of 23 wild grapevine accessions, previously genotyped using the Vitis18kSNP array (De Lorenzis *et al.*, 2015), was analysed. These samples, coming from regions with the highest concentration of wild grapevines, including Inner Kartli (7), Lower Kartli (4), and Kakheti (12) (Figure S1), are now part of the ampelographic Jighaura collection (FAO code GEO038) at the Scientific-Research Center of Agriculture, Georgia. To further explore the genetic relationship between Georgian wild population and the ones across the Mediterranean basin, sample set #1 was subsequently expanded with SNP profiles of 112 accessions (sample set #2) from Switzerland (CHE), Germany (DEU), Algeria (DZA), Spain (ESP), France (FRA), Georgia (GEO), Greece (GRC), Hungary (HUN), Morocco (MAR), Slovakia (SVK), Tunisia (TUN), and Turkey (TUR) (Table 1), and previously genotyped by Ramos-Madrigal *et al.* (2019). Sample set #2 accounted for 135 genotypes (Table 1).

2. Data analysis

2.1. Sample set #1

In sample set #1, genetic distance and variability among genotypes were assessed. To explore genetic relationships among individuals, a dendrogram based on Nei's genetic distance has been generated. Genotype data were imported using the *poppr* package (Kamvar *et al.*, 2014) in R software (R Core Team, 2021), and pairwise genetic distances were computed with the *nei.dist* function. Hierarchical clustering was performed using the Neighbour-Joining (NJ) algorithm,

and node support was evaluated through 1,000 bootstrap replicates using the *aboot* function in *poppr*. Bootstrap values greater than 80 % were displayed on the resulting dendrogram. (Nei, 1972; Kamvar *et al.*, 2014; R Core Team, 2021). To explore genetic relationships within and between geographic groups, a Principal Components Analysis (PCA) was conducted using the *adeget* package (Jombart, 2008) in R, and the results were displayed in a two-dimensional scatter plot. Finally, ancestral population structure was inferred using the *lea* package in R (Frichot & François, 2015), which estimates individual ancestry proportions. The optimal number of clusters (K) was selected based on the cross-entropy criterion, choosing the value associated with the lowest cross-entropy score.

2.2. Sample set #2

SNP allelic profiles from sample sets #1 and #2 were merged using the R function *merge*, retaining only the loci shared between the two datasets. Clustering, PCA, and structure analysis were carried out on sample set #2 following the same procedures described for sample set #1. Additionally, on the three populations inferred by structure analysis (assignment probability $q \geq 0.80$), a minimum spanning network (MSN) and directional migration rates were estimated. An MSN was built to visualise genetic relatedness among individuals using the *poppr* package in R, starting from the populations obtained by structure analysis. Genetic distances were computed as bitwise genetic distances (percentage dissimilarity) using the *bitwise.dist* function, and these distances were then used to construct the network with *poppr.msn* function. (Keenan *et al.*, 2013). To infer population relationships and test for potential historical admixture among groups, the software TreeMix v1.13 (Pickrell & Pritchard, 2012) was applied to the populations obtained by structure analysis. TreeMix was

TABLE 1. List of the 135 wild grapevine (*V. vinifera* subsp. *sylvestris*) accessions analysed in this study. All genotypes were previously characterised using the Vitis18kSNP genotyping chip array.

Country	Number of accessions
Switzerland	1
Germany	14
Algeria	6
Spain	3
France	41
Georgia	35
Greece	1
Hungary	2
Morocco	10
Slovakia	7
Tunisia	11
Turkey	4
Total	135

first run assuming a purely bifurcating population tree and then allowing for one and two migration events. All analyses were performed using blocks of 500 SNPs to account for linkage disequilibrium. The resulting trees were visualised in R. To independently test for admixture, f_3 -statistics were calculated using the *threepop* function implemented in TreeMix. The f_3 -statistic was computed for all the possible combinations of the populations. Standard errors were estimated using blocks of 500 SNPs. Positive f_3 values indicate that the target population is consistent with a simple branching model, whereas significantly negative values provide evidence of admixture.

RESULTS

1. Genetic population study of Georgian wild germplasm

Genetic diversity among 23 Georgian wild grapevine accessions was assessed using a high-throughput genotyping system based on the Vitis18kSNP genotyping chip array in De Lorenzis *et al.* (2015). After excluding loci that were monomorphic or failed to amplify in all samples, the final dataset retained 15,317 loci. A circular dendrogram (Figure 1A) was constructed to assess the genetic relationships

among the genotypes, revealing a similarity range between approximately 78 % and 99 %. A total of 22 genotypes were identified and grouped based on their geographic origin. “Delisi 06” and “Ninotsminda 11” shared the same SNP profile, which is likely the result of a sampling error, as samples originate from two different regions, Inner Kartli for the first and Kakheti for the second individual. Four statistically significant nodes (bootstrap ≥ 80 %) delineate distinct clusters: one grouping samples from Lower Kartli, one comprising the majority of samples from Inner Kartli, and two separating the samples originating from the Kakheti region. To evaluate genetic diversity within the Georgian wild grapevine populations, a PCA was conducted based on SNP profiles (Figure 1B). The first two principal components (PC) accounted for 20 % of the total genetic variation, with PC1 explaining 11 % and PC2 explaining 9 %. PCA did not result in a clear separation of the wild grapevine samples according to their population of origin. Samples from the three populations largely overlapped in the bidimensional space defined by the first two PCs. However, a subset of five individuals from the Kakheti population was distinctly separated from the rest, showing high scores along both PC1 and PC2. Additionally, a slight differentiation can be observed along PC2 between the samples from the Lower Kartli region and those from Inner Kartli and Kakheti.

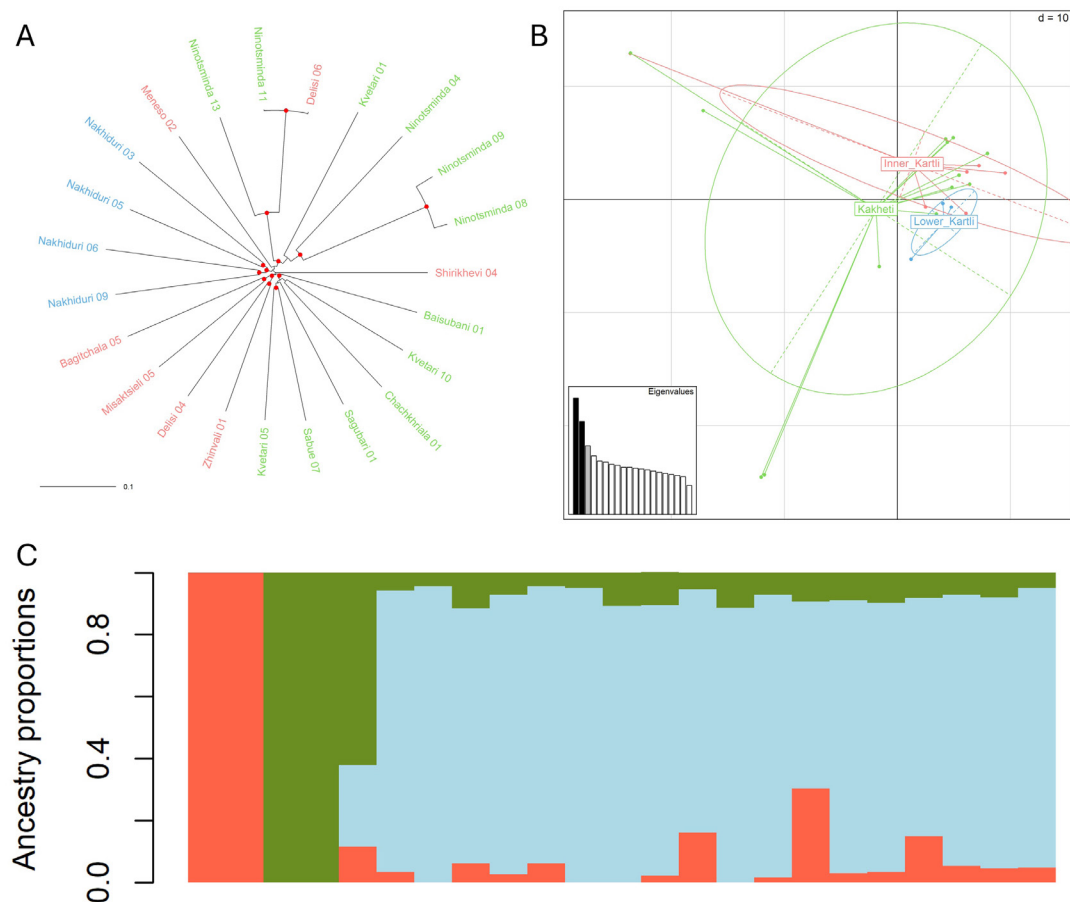


FIGURE 1. Overview of the genetic relationships among 23 wild grapevine accessions from sample set #1 (Georgia), based on Vitis18kSNP genotyping data as inferred by hierarchical clustering (A), principal component analysis (B), and structure analysis (C). In A and B, genotypes are colour-coded according to their region of origin. In A, nodes supported by bootstrap values greater than 80 % are marked with a red dot.

A structure analysis was performed to explore the hierarchical population structure. To determine the optimal number of populations, values of K ranging from 1 to 10 were tested. The most likely number of populations was $K = 3$. The membership probabilities of each accession for the three clusters ($K = 3$) are provided in Table S1. As a result, the structure analysis identified three distinct groups (Figure 1C). The first cluster (cyan) comprised 15 samples with membership values higher than 0.8, including 6 of the 7 individuals from Inner Kartli, 6 of 12 from Kakheti, and 3 of 4 from Lower Kartli. The second cluster (red) included the “Ninotsminda 08” and “Ninotsminda 09” accessions, both from the Kakheti region. The third cluster (green) encompassed a shared genetic profile represented by the “Delisi 06” and “Ninotsminda 11” samples. This result likely reflects a clustering artefact caused by the inclusion of two individuals with identical genotypes in a relatively small dataset. Four out of the 23 individuals were identified as admixed (membership values lower than 0.8), with the highest incidence (three of them) observed in the Kakheti region.

2. Genetic population study of Mediterranean basin wild germplasm

To evaluate the genetic similarity between Georgian wild genotypes (sample set #1) and those from regions associated with the grapevine domestication process across the Mediterranean basin, a second dataset, referred to as sample set #2, was assembled. This dataset comprised 135 genotypes and 7,998 SNPs. Genetic relationships among individuals were visualised using a circular dendrogram, with similarity values ranging from 65 to 100 % (Figure 2A). The analysis revealed 129 unique genetic profiles and 6 pairs of individuals sharing the SNP profile. The circular dendrogram clustered most accessions according to their geographic origin, and this pattern is further supported by the significance of several internal nodes (bootstrap ≥ 80 %). All Georgian samples, derived from both studies, form a coherent cluster, which also includes a limited number of accessions from France, Hungary, Germany, Algeria, Greece, and Turkey. Another well-supported cluster is composed of the samples from Spain and Morocco, while a separate significant cluster groups the German and Slovenian accessions.

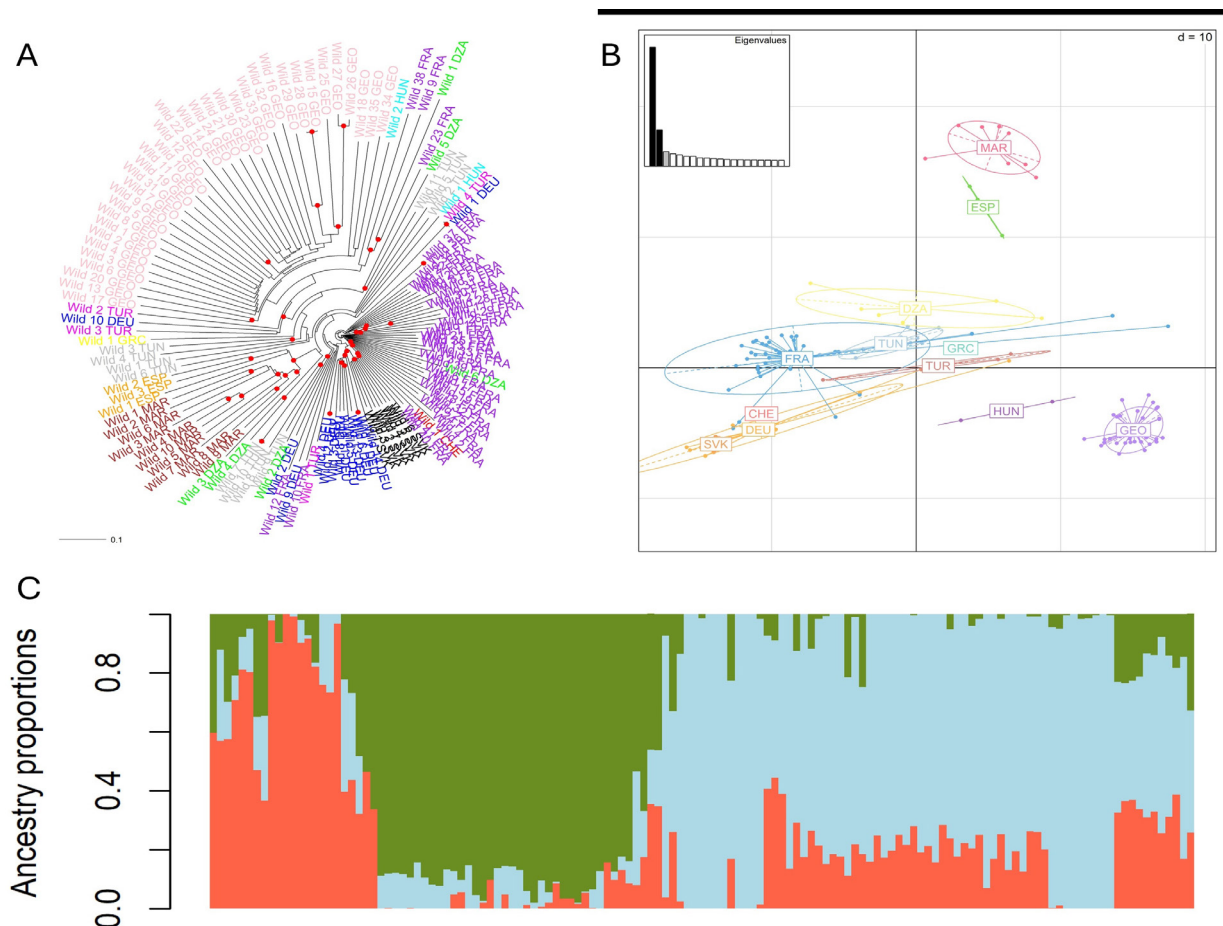


FIGURE 2. Overview of the genetic relationships among 135 wild grapevine accessions from sample set #2 across the Mediterranean basin as inferred by hierarchical clustering (A), principal component analysis (B), and structure analysis (C). In A and B, genotypes are colour-coded and labelled according to their country of origin: Switzerland (CHE), Germany (DEU), Algeria (DZA), Spain (ESP), France (FRA), Georgia (GEO), Greece (GRC), Hungary (HUN), Morocco (MAR), Slovakia (SVK), Tunisia (TUN), and Turkey (TUR). In A, nodes supported by bootstrap values greater than 80 % are marked with a red dot.

To evaluate the genetic diversity in sample set #2, a PCA (Figure 2B) was performed on the SNP profiles. The first two PC accounted for 24 % of the total genetic variation, with PC1 explaining 18 % and PC2 explaining 6 %. The distribution of samples across the four PCA quadrants reveals substantial overlap among populations. Nonetheless, certain patterns of separation are evident. Specifically, PC1 differentiates Georgian samples from most other accessions, with the exception of two non-Georgian samples (from France) that cluster in a similar PC1 range. While PC2 differentiates samples from Morocco and Spain. The French population exhibits the highest level of variability, with some accessions positioned markedly distant from the main cluster of French samples.

The cross-entropy analysis identified three ancestral populations as the optimal value to adequately represent the genetic structure of the sample set #2. The membership probabilities of each accession for the three clusters ($K = 3$) are provided in Table S2. Eighty-two samples, representing approximately 61 % of the dataset, exhibited membership values greater than 0.8 and were thus assigned to a specific ancestral population (Figure 2C). The remaining 39 % of samples were considered admixed. The 82 assigned samples were distributed as follows: 10 belonged to ancestral population 1 (red), including 8 samples from Morocco

and 2 from Spain; 37 to ancestral population 2 (green), comprising 1 sample from Switzerland, 11 from Germany, 18 from France, and 7 from Slovakia; and 35 to ancestral population 3 (cyan), consisting of the entire Georgian population. Populations composed entirely of admixed individuals (*i.e.*, with no sample showing a membership value above 0.8) included Algeria, Greece, Hungary, Tunisia, and Turkey.

Genetic relatedness between Georgian wild samples and those from the Mediterranean basin was then visualised using an MSN (Figure 3). Only samples with an assignment probability greater than 80 % to a specific population were included in the analysis. This network connects the three main populations through nodes, each representing one or two individuals, and edges, where the thickness reflects the genetic distance. This analysis indicated that the North African population (POP1, in red) serves as a central connector, linking the Georgian population (POP2, in cyan) from one site to the other site, the POP3 (in green), accounting for Western and Central European samples.

The TreeMix analysis illustrates the evolutionary relationships and divergence history among the three structure-defined populations, based on the amount of accumulated genetic drift (Figure 4). The tree structure

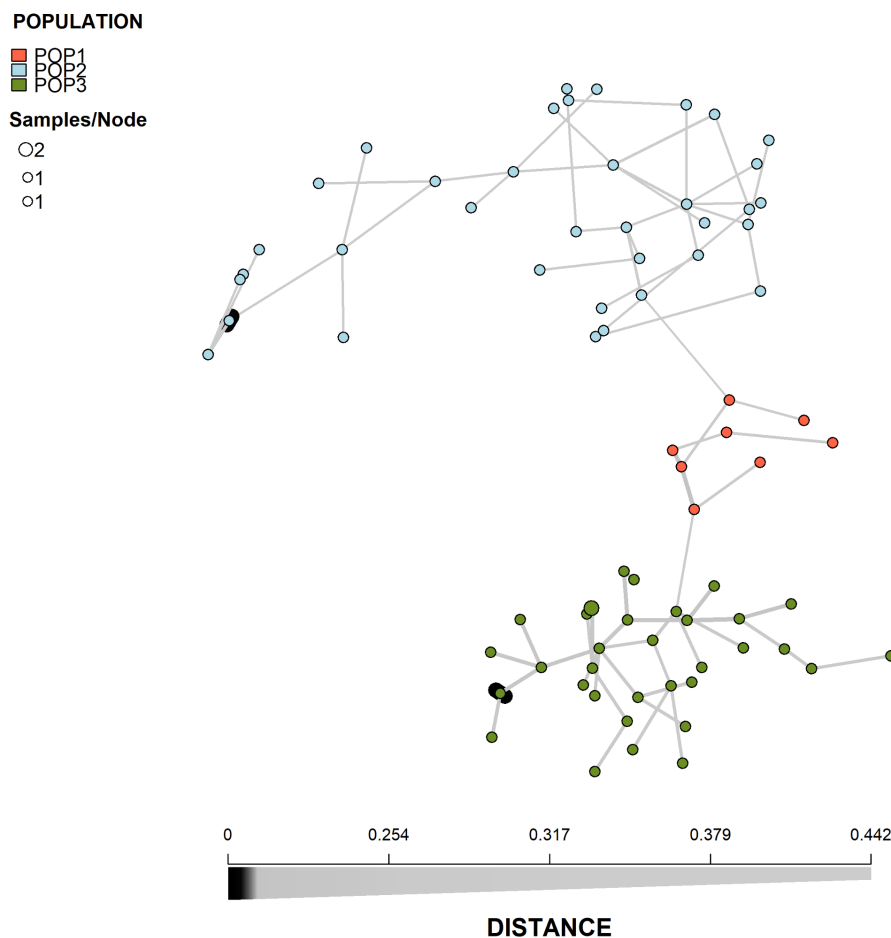


FIGURE 3. MSN based on Vitis18kSNP genotyping data, showing genetic relationships among the three grapevine populations identified through structure analysis (assignment probability $q \geq 0.80$).

reveals that POP1 and POP2 are the most closely related populations, acting as sister groups sharing a more recent common ancestor. The initial divergence separated the POP3 lineage from the lineage that later gave rise to both POP1 and POP2. Looking at the branch length, which represents the drift parameter, the branch leading to POP2 is significantly longer than the others, suggesting that this population has accumulated a significantly greater amount of genetic drift. On the contrary, POP3 is the population showing the shorter branch length. Finally, the absence of additional migration arrows in the model indicates that the history of these three populations is well explained by a pure tree-divergence model, without the need to postulate significant post-separation mixing or gene flow events. The absence of migration arrows in the TreeMix graph is fully consistent with the results of the f_3 -statistics (Table 2). All tested f_3 values were positive and highly significant (Z-scores > 13), providing no evidence of admixture for any of the three populations, indicating that each population is better explained as an independent branch of the population tree rather than as a mixture between the other two.

DISCUSSION

The Vitis18kSNP array is a widely used and reliable tool for studying the cultivated compartment of grapevine, particularly for assessing synonyms, homonyms, genetic

diversity, population structure, kinship, and association studies (De Lorenzis *et al.*, 2019; D’Onofrio *et al.*, 2021; Laucou *et al.*, 2018; Vervalle *et al.*, 2022). However, to date, it has been rarely applied to the wild compartment (De Lorenzis *et al.*, 2015; Mercati *et al.*, 2021; Ramos-Madrigal *et al.*, 2019), and in the few studies where it has been used, wild grapevine has seldom been the primary focus. This study provides new insights into the genetic diversity and relationships of wild grapevine populations, with a particular focus on the Georgian wild grapevine germplasm and its relationship to other populations in the Mediterranean basin. Using a high-throughput genotyping system based on the Vitis18kSNP chip array, combined with hierarchical clustering, PCA, structure analysis, MSN, and migration rates, this study investigated the complexity of grapevine wild genetic diversity.

1. A single genetic pool across wild grapevine populations in eastern Georgia

The analysis of Georgian wild grapevine germplasm revealed a substantial genetic diversity, despite the geographic isolation of these populations. This high level of diversity is consistent with previous findings that emphasised the genetic richness of Georgian wild grapevines (De Lorenzis *et al.*, 2015; Imazio *et al.*, 2013; Riaz *et al.*, 2018).

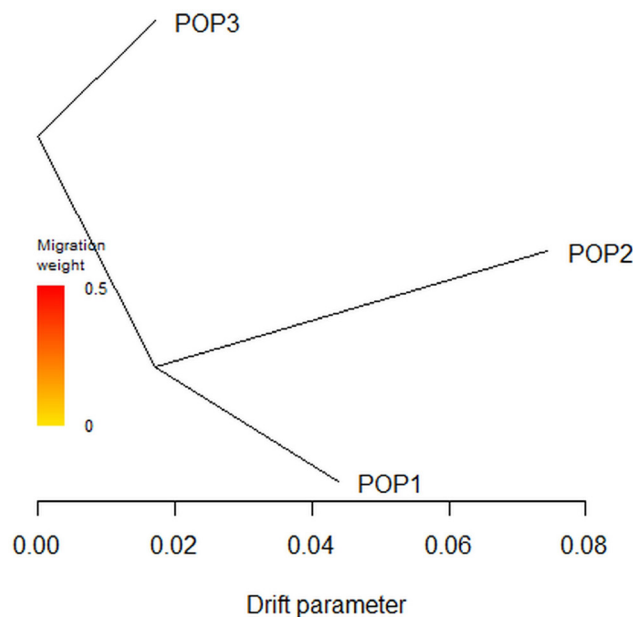


FIGURE 4. Maximum-likelihood population tree inferred with TreeMix on the tree grapevine wild populations identified through structure analysis (assignment probability $q \geq 0.80$).

TABLE 2. Results of the f_3 -statistics for the three tree grapevine wild populations identified through structure analysis (assignment probability $q \geq 0.80$).

Target	Source 1	Source 2	f_3	Standard error	Z-score
POP1	POP2	POP3	0.0259322	0.00161145	16.0924
POP2	POP1	POP3	0.0581924	0.00263031	22.1237
POP3	POP1	POP2	0.0352671	0.00259144	13.6091

Given that all samples came from neighbouring regions of eastern Georgia (Kakheti, Inner Kartli, and Lower Kartli), no clear geographic structure was observed among the 23 wild grapevine accessions. Among the analyses performed, only the cluster analysis was able to capture the genetic differentiation between populations based on their geographic origin, where samples were clustered according to their geographic origin, and samples from Kakheti were split into two groups. The separation of the Kakheti accessions into two distinct subgroups can be explained by the geographic location of the sampled populations within the region. These two populations are located in distant areas of Kakheti, separated by a mountain range, the Gomboris Kedi, which likely limited gene flow and contributed to their genetic divergence. From an agro-climatic point of view, the three regions belong to the same classes of the Köppen classification (Cfa and Dfb), showing very similar values of temperature and precipitation (Table S3). Furthermore, the precipitation pattern along the years is similar for all three regions, with the absolute maximum in spring, a secondary maximum in autumn, the main minimum in winter, and a secondary minimum in summer (Cola *et al.*, 2022). Temperature levels translate into comparable levels of thermal resources for grapevine, as represented by the Winkler Index class (Table S3).

Both PCA and structure analyses indicated that most individuals belong to a single genetic pool, with limited population stratification. This suggests a high degree of gene flow and shared ancestry among populations in these neighbouring regions. Structure analysis identified one predominant cluster comprising most samples from all three regions, supporting the hypothesis of a common genetic background. At the same time, the relatively wide range of genetic similarity observed in the dendrogram (78 %–99.5 %), together with the tendency of samples from the same geographic region to cluster together, indicates a high level of genetic diversity within this pool. These findings suggest that, although the populations are not clearly structured according to geography, a certain degree of local genetic cohesion persists. This pattern is consistent with a scenario in which wild grapevines from Kakheti and Kartli derive from a common genetic background, while maintaining regional genetic signatures likely shaped by limited gene flow, historical connectivity, and local adaptation within a contiguous area.

However, given the limited number of accessions and their narrow geographic coverage, these patterns should be interpreted with caution and not extrapolated to the full diversity of Georgian wild grapevines. Rather, they likely reflect the genetic relationships among the specific localities sampled in this study. Expanded sampling across broader areas will be needed to evaluate whether the subtle geographic signals observed in this study are consistent across the species' wider distribution.

2. Genetic distinctiveness of Georgian wild grapevines within a Mediterranean context

To contextualise the Georgian material within a broader phylogeographic framework, Georgian SNP genotyping data have been merged with a dataset from a previously published study that surveyed wild grapevine accessions across the Mediterranean basin, including additional Georgian accessions (Ramos-Madrugal *et al.*, 2019). In all the analyses performed (clustering, PCA, and structure analysis), the Georgian samples, regardless of the study they originated from, consistently grouped together as a single, well-defined cluster, distinct from the Mediterranean genotypes. This consistent pattern demonstrated the robustness and portability of high-density SNP markers and confirmed their suitability for comparative population studies based on datasets generated in different laboratories, using different platforms, as well as the distinctiveness of the Georgian grapevine population from the Mediterranean basin ones. Analysis of the remaining populations revealed that samples from the Iberian Peninsula and Morocco consistently clustered together in both the PCA and structure analyses, and samples from France, Germany, and Slovakia belong to a unique ancestral population (POP3), indicating a close genetic relationship among samples from these countries. In Dong *et al.* (2023), the wild compartment was divided into two main groups: the eastern ecotype, including samples from Israel (Syl-E1) and from the Southern Caucasus (Syl-E2), and the western ecotype, including samples from Central–Eastern Europe (Syl-W1) and from the Iberian Peninsula and France (Syl-W2). While our results confirm the clear genetic distinction between Georgian (POP2) and European (POP1 and POP3) populations and, within the European group, the existence of two distinct subpopulations, they diverge from those reported by Dong *et al.* (2023), particularly regarding the assignment of the French accessions. In our work, the French population clustered with those from Central–Eastern Europe (POP3), whereas Dong *et al.* (2023) reported a different pattern. In their analysis, the French population clustered together with some samples from the Iberian Peninsula. It is important to note, however, that our dataset included only three samples from the Iberian Peninsula, and the Dong *et al.* (2023) dataset did not include any samples from Morocco. These differences in geographic representation may partly explain the observed discrepancies between the two studies. Differences in sample size and composition between studies are sufficient to generate the contrasting placements (Puechmaille, 2016). The three populations identified through structure analysis were used to generate a representation of genetic relationships, via MSN, and a model-based inference of population splits using TreeMix. The MSN provides a direct visualisation of the genetic structure of the wild grapevine populations analysed in this study. The most evident pattern is the partitioning of individuals into three clearly separated clusters, confirming that the populations identified through the structure analysis represent genetically distinct groups. The dispersion of nodes within each cluster reflects

internal genetic variability. Broader clusters such as POP2 and POP3 indicate higher levels of within-population diversity. This pattern is consistent with the deep ancestry of the Caucasian wild germplasm, recognised as one of the centres of grapevine domestication (Dong *et al.*, 2023; Magris *et al.*, 2021), and with the recovery and long-term maintenance of high genetic diversity in multiple glacial refugia across Europe (Hewitt, 1999). The apparent centrality of the North African population in the MSN should be interpreted as a distance-based pattern and not as evidence of admixture, which is explicitly rejected by TreeMix and f_3 -statistics. TreeMix reconstructs the historical relationships among these populations and identifies the Georgian population (POP3) as the earliest diverging lineage, indicating that the genetic separation between the Caucasian and European pools represents the oldest divergence event. POP1 and POP2 diverge from a common ancestral node at a later evolutionary stage. Their separation likely reflects range fragmentation associated with Pleistocene climatic oscillations and subsequent post-glacial recolonisation processes. Although direct evidence for the distribution of wild grapevines during the last glacial maximum is lacking, insights can be drawn from the extensive body of work by Hewitt (1999), which summarises the dynamics of several temperate tree species during the last ice age. These species, including oaks, beech, alder, and fir, retreated into geographically isolated refugia such as the southern peninsulas of Europe, and recolonised northern Europe in a predominantly south-to-north and east-to-west pattern. Following this framework, POP1 (North Africa/Iberian Peninsula) may represent a southwestern refugial lineage that persisted in the Iberian–North African region, whereas POP2 (Western/Central Europe) may correspond to populations that expanded from more eastern or central refugia and diversified during the recolonisation of Europe after the glaciation.

It is crucial to recognise that the complex biogeographic history of *Vitis vinifera* subsp. *sylvestris* cannot be fully captured by a purely tree-like model. The fact that approximately 39 % of the samples (including individuals from France, Germany, and Spain) are classified as admixtures (with ancestry from two or three ancestral pools) reflects the existence of a reticulate (not tree-like) evolutionary network and the presence of contact zones and continuous gene flow along post-glacial expansion pathways.

CONCLUSION

This study provides new insights into the genetic landscape of *V. vinifera* subsp. *sylvestris*, with a focus on Georgian wild grapevines. The results confirm that the Georgian population represents a genetically distinct and diverse lineage within the Mediterranean gene pool. These findings underscore the critical value of Georgian wild grapevines as a reservoir of genetic variation and their potential contribution to breeding programs aimed at increasing resilience to climate change and pathogens. Given the ongoing erosion of wild germplasm in Georgia, our data further highlight the urgency of targeted conservation strategies.

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REFERENCES

- Bitsadze, N., Kikilashvili, S., Chipashvili, R., Mamasakhlisashvili, L., Maghradze, T., Kikvadze, M., Toffolatti, S. L., De Lorenzis, G., Failla, O., Ocete Rubio, R., & Maghradze, D. (2024). Resistance to downy mildew in wildy growing Eurasian *Vitis vinifera* L. grapevines. *Journal of Plant Pathology*, 106(4), 1759–1771. <https://doi.org/10.1007/s42161-024-01728-7>
- Broccanello, C., Chiodi, C., Funk, A., McGrath, J. M., Panella, L., & Stevanato, P. (2018). Comparison of three PCR-based assays for SNP genotyping in plants. *Plant Methods*, 14(1), 28. <https://doi.org/10.1186/s13007-018-0295-6>
- Cabezas, J. A., Ibáñez, J., Lijavetzky, D., Vélez, D., Bravo, G., Rodríguez, V., Carreño, I., Jermakow, A. M., Carreño, J., Ruiz-García, L., Thomas, M. R., & Martínez-Zapater, J. M. (2011). A 48 SNP set for grapevine cultivar identification. *BMC Plant Biology*, 11(1), 153. <https://doi.org/10.1186/1471-2229-11-153>
- Cola, G., De Lorenzis, G., Failla, O., Kvaliashvili, N., Kikilashvili, S., Kikvadze, M., Mamasakhlisashvili, L., Mdinaradze, I., Chipashvili, R., & Maghradze, D. (2025). The Status of Wild Grapevine (*Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi) Populations in Georgia (South Caucasus). *Plants*, 14(2), 232. <https://doi.org/10.3390/plants14020232>
- Cola, G., Mariani, L., Failla, O., Brancadoro, L., & Maghradze, D. (2022). *Climate analyses for modern Georgian viticulture: A practical handbook for viticulturists*. National Wine Agency of Georgia, Tbilisi.
- De Lorenzis, G. (2024). From ancient to modern grapevine cultivars: a lesson from cultivars that made the history of viticulture. *Acta Horticulturae*, 1385, 47–58. <https://doi.org/10.17660/ActaHortic.2024.1385.7>
- De Lorenzis, G., Chipashvili, R., Failla, O., & Maghradze, D. (2015). Study of genetic variability in *Vitis vinifera* L. germplasm by high-throughput Vitis18kSNP array: The case of Georgian genetic resources. *BMC Plant Biology*, 15, 154. <https://doi.org/10.1186/s12870-015-0510-9>
- De Lorenzis, G., Mercati, F., Bergamini, C., Cardone, M. F., Lupini, A., Mauceri, A., Caputo, A. R., Abbate, L., Barbagallo, M. G., Antonacci, D., Sunseri, F., & Brancadoro, L. (2019). SNP genotyping elucidates the genetic diversity of *Magna Graecia* grapevine germplasm and its historical origin and dissemination. *BMC Plant Biology*, 19, 7. <https://doi.org/10.1186/s12870-018-1576-y>
- Dong, Y., Duan, S., Xia, Q., Liang, Z., Dong, X., Margaryan, K., Musayev, M., Goryslavets, S., Zdunić, G., Bert, P.-F., Lacombe, T., Maul, E., Nick, P., Bitskinashvili, K., Bisztray, G. D., Drori, E., De Lorenzis, G., Cunha, J., Popescu, C. F., ... & Chen, W. (2023). Dual domestications and origin of traits in grapevine evolution. *Science*, 379(6635), 892–901. <https://doi.org/10.1126/science.add8655>

- D'Onofrio, C. (2020). Introgression Among Cultivated and Wild Grapevine in Tuscany. *Frontiers in Plant Science*, *11*. <https://doi.org/10.3389/fpls.2020.00202>
- D'Onofrio, C., Tumino, G., Gardiman, M., Crespan, M., Bignami, C., de Palma, L., Barbagallo, M., Mугanu, M., Morcia, C., Novello, V., Schneider, A., & Terzi, V. (2021). Parentage Atlas of Italian Grapevine Varieties as Inferred From SNP Genotyping. *Frontiers in Plant Science*, *11*, 605934. <https://doi.org/10.3389/fpls.2020.605934>
- 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>
- Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*(8), 925–929. <https://doi.org/10.1111/2041-210X.12382>
- Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, *68*(1–2), 87–112. <https://doi.org/10.1111/j.1095-8312.1999.tb01160.x>
- Imazio, S., Maghradze, D., De Lorenzis, G., Bacilieri, R., Laucou, V., This, P., Scienza, A., & Failla, O. (2013). From the cradle of grapevine domestication: molecular overview and description of Georgian grapevine (*Vitis vinifera* L.) germplasm. *Tree Genetics & Genomes*, *9*, 641–658. <https://doi.org/10.1007/s11295-013-0597-9>
- Jombart, T. (2008). *adeigenet*: A R package for the multivariate analysis of genetic markers. *Bioinformatics (Oxford, England)*, *24*, 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, *2*, e281. <https://doi.org/10.7717/peerj.281>
- Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). *diveRsity*: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, *4*(8), 782–788. <https://doi.org/10.1111/2041-210X.12067>
- Laucou, V., Launay, A., Bacilieri, R., Lacombe, T., Adam-Blondon, A. F., Bérard, A., Chauveau, A., de Andrés, M. T., Hausmann, L., Ibáñez, J., Le Paslier, M.-C., Maghradze, D., Martínez-Zapater, J. M., Maul, E., Ponnaiah, M., Töpfer, R., Péros, J.-P., & Boursiquot, J.-M. (2018). Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10K genome-wide SNPs. *PLOS ONE*, *13*(2), 1–27. <https://doi.org/10.1371/journal.pone.0192540>
- Maghradze, D., Kikilashvili, S., Gotsiridze, O., Maghradze, T., Fracasseti, D., Failla, O., & Rustioni, L. (2021). Comparison between the Grape Technological Characteristics of *Vitis vinifera* Subsp. *sylvestris* and Subsp. *sativa*. *Agronomy*, *11*(3), 472. <https://doi.org/10.3390/agronomy11030472>
- Magris, G., Jurman, I., Fornasiero, A., Paparelli, E., Schwöpe, R., Marroni, F., Di Gaspero, G., & Morgante, M. (2021). The genomes of 204 *Vitis vinifera* accessions reveal the origin of European wine grapes. *Nature Communications*, *12*(1), 7240. <https://doi.org/10.1038/s41467-021-27487-y>
- Marinov, L., Magris, G., Di Gaspero, G., Morgante, M., Maletić, E., Bubola, M., Pejić, I., & Zdunić, G. (2024). Single nucleotide polymorphism (SNP) analysis reveals ancestry and genetic diversity of cultivated and wild grapevines in Croatia. *BMC Plant Biology*, *24*(1), 975. <https://doi.org/10.1186/s12870-024-05675-4>
- McGovern, P. E. (2003). *Ancient Wine*. Princeton University Press. ISBN 9780691070803.
- Mercati, F., De Lorenzis, G., Mauceri, A., Zerbo, M., Brancadoro, L., D'Onofrio, C., Morcia, C., Barbagallo, M. G., Bignami, C., Gardiman, M., de Palma, L., Ruffa, P., Novello, V., Crespan, M., & Sunseri, F. (2021). Integrated Bayesian Approaches Shed Light on the Dissemination Routes of the Eurasian Grapevine Germplasm. *Frontiers in Plant Science*, *12*. <https://doi.org/10.3389/fpls.2021.692661>
- Myles, S., Boyko, A. R., Owens, C. L., Brown, P. J., Grassi, F., Aradhya, M. K., Prins, B., Reynolds, A., Chia, J.-M., Ware, D., Bustamante, C. D., & Buckler, E. S. (2011). Genetic structure and domestication history of the grape. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(9), 3530–3535. <https://doi.org/10.1073/pnas.1009363108>
- Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., & Baloch, F. S. (2018). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, *32*(2), 261–285. <https://doi.org/10.1080/13102818.2017.1400401>
- Nei, M. (1972). Genetic distance between populations. *American Naturalist*, *106*, 283–292. <https://doi.org/10.1086/282771>
- Pickrell, J. K., & Pritchard, J. K. (2012). Inference of Population Splits and Mixtures from Genome-Wide Allele Frequency Data. *PLoS Genetics*, *8*(11), e1002967. <https://doi.org/10.1371/journal.pgen.1002967>
- Puechmaille, S. J. (2016). The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*(3), 608–627. <https://doi.org/10.1111/1755-0998.12512>
- Quaglino, F., Maghradze, D., Casati, P., Chkhaidze, N., Lobjanidze, M., Ravasio, A., Passera, A., Venturini, G., Failla, O., & Bianco, P. A. (2016). Identification and Characterization of New ‘*Candidatus* Phytoplasma solani’ Strains Associated with Bois Noir Disease in *Vitis vinifera* L. Cultivars Showing a Range of Symptom Severity in Georgia, the Caucasus Region. *Plant Disease*, *100*(5), 904–915. <https://doi.org/10.1094/PDIS-09-15-0978-RE>
- Ramos-Madrigal, J., Runge, A. K. W., Bouby, L., Lacombe, T., Samaniego Castruita, J. A., Adam-Blondon, A.-F., Figueiral, I., Hallavant, C., Martínez-Zapater, J. M., Schaal, C., Töpfer, R., Petersen, B., Sicheritz-Pontén, T., This, P., Bacilieri, R., Gilbert, M. T. P., & Wales, N. (2019). Palaeogenomic insights into the origins of French grapevine diversity. *Nature Plants*, *5*(6), 595–603. <https://doi.org/10.1038/s41477-019-0437-5>
- R Core Team. (2021). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria.
- Riaz, S., De Lorenzis, G., Velasco, D., Koehmstedt, A., Maghradze, D., Bobokashvili, Z., Musayev, M., Zdunic, G., Laucou, V., Walker, M. A., Failla, O., Preece, J. E., Aradhya, M., & Arroyo-Garcia, R. (2018). Genetic diversity analysis of cultivated and wild grapevine (*Vitis vinifera* L.) accessions around the Mediterranean basin and Central Asia. *BMC Plant Biology*, *18*(1), 137. <https://doi.org/10.1186/s12870-018-1351-0>
- Ricciardi, V., Crespan, M., Maddalena, G., Migliaro, D., Brancadoro, L., Maghradze, D., Failla, O., Toffolatti, S. L., & De Lorenzis, G. (2024). Novel loci associated with resistance to downy and powdery mildew in grapevine. *Frontiers in Plant Science*, *15*. <https://doi.org/10.3389/fpls.2024.1386225>
- Sargolzaei, M., Rustioni, L., Cola, G., Ricciardi, V., Bianco, P. A., Maghradze, D., Failla, O., Quaglino, F., Toffolatti, S. L., & De Lorenzis, G. (2021). Georgian Grapevine Cultivars: Ancient Biodiversity for Future Viticulture. *Frontiers in Plant Science*, *12*. <https://doi.org/10.3389/fpls.2021.630122>

- This, P., Lacombe, T., & Thomas, M. R. (2006). Historical origins and genetic diversity of wine grapes. *Trends in Genetics : TIG*, 22(9), 511–519. <https://doi.org/10.1016/j.tig.2006.07.008>
- Toffolatti, S. L., De Lorenzis, G., Costa, A., Maddalena, G., Passera, A., Bonza, M. C., Pindo, M., Stefani, E., Cestaro, A., Casati, P., Failla, O., Bianco, P. A., Maghradze, D., & Quagliano, F. (2018). Unique resistance traits against downy mildew from the center of origin of grapevine (*Vitis vinifera*). *Scientific Reports*, 8(1), 12523. <https://doi.org/10.1038/s41598-018-30413-w>
- Tympakianakis, S., Trantas, E., Avramidou, E. V., & Ververidis, F. (2023). *Vitis vinifera* genotyping toolbox to highlight diversity and germplasm identification. *Frontiers in Plant Science*, 14. <https://doi.org/10.3389/fpls.2023.1139647>
- Vervalle, J. A., Costantini, L., Lorenzi, S., Pindo, M., Mora, R., Bolognesi, G., Marini, M., Lashbrooke, J. G., Tobutt, K. R., Vivier, M. A., Roodt-Wilding, R., Grando, M. S., & Bellin, D. (2022). A high-density integrated map for grapevine based on three mapping populations genotyped by the *Vitis*18K SNP chip. *Theoretical and Applied Genetics*, 135(12), 4371–4390. <https://doi.org/10.1007/s00122-022-04225-6>
- Wan, Y., Schwaninger, H. R., Baldo, A. M., Labate, J. A., Zhong, G. Y., & Simon, C. J. (2013). A phylogenetic analysis of the grape genus (*Vitis* L.) reveals broad reticulation and concurrent diversification during neogene and quaternary climate change. *BMC Evolutionary Biology*, 13(1), 141. <https://doi.org/10.1186/1471-2148-13-141>