

A HOLISTIC APPROACH FOR VITIS: TARGETING GENETIC, AMPELOGRAPHIC AND EPIGENETIC POOLS

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Vitis Vinifera genetic diversity

- The cultivated grapevine (*V. vinifera* L. subsp. *vinifera*) is believed to have been derived from its wild closely related form *V. vinifera* L. subsp. *Sylvestris*.
- Recent data (OPEKEPE, 2019) confirm that 220 cultivars and landraces are currently cultivated in Greece.
- A combined approach using ampelographic and microsatellite markers has been undertaken to study the genetic diversity of **96 genotypes** belonging to **36 *V. vinifera* cultivars**.

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Research Article

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Genetic diversity of Greek grapevine (*Vitis vinifera* L.) cultivars using ampelographic and microsatellite markers

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Abstract

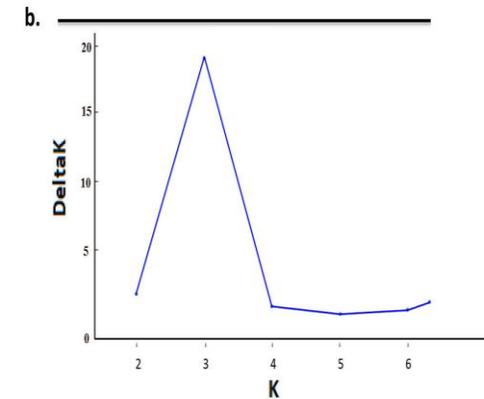
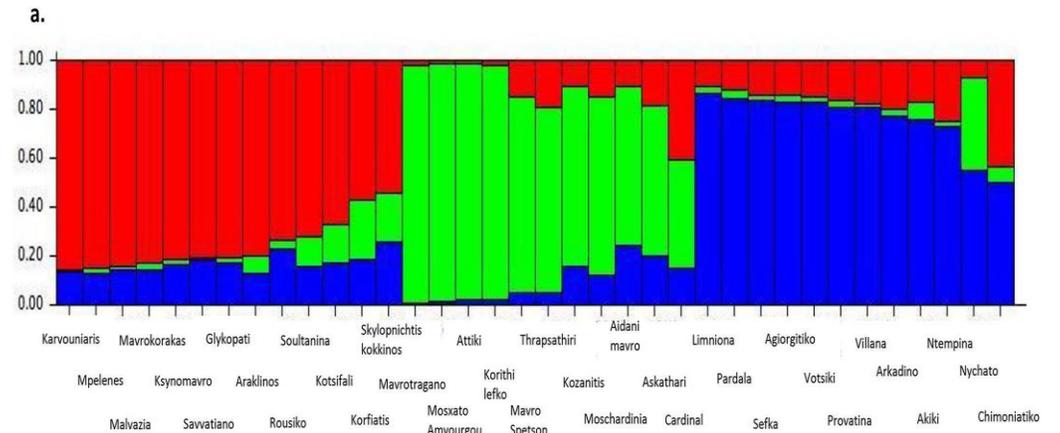
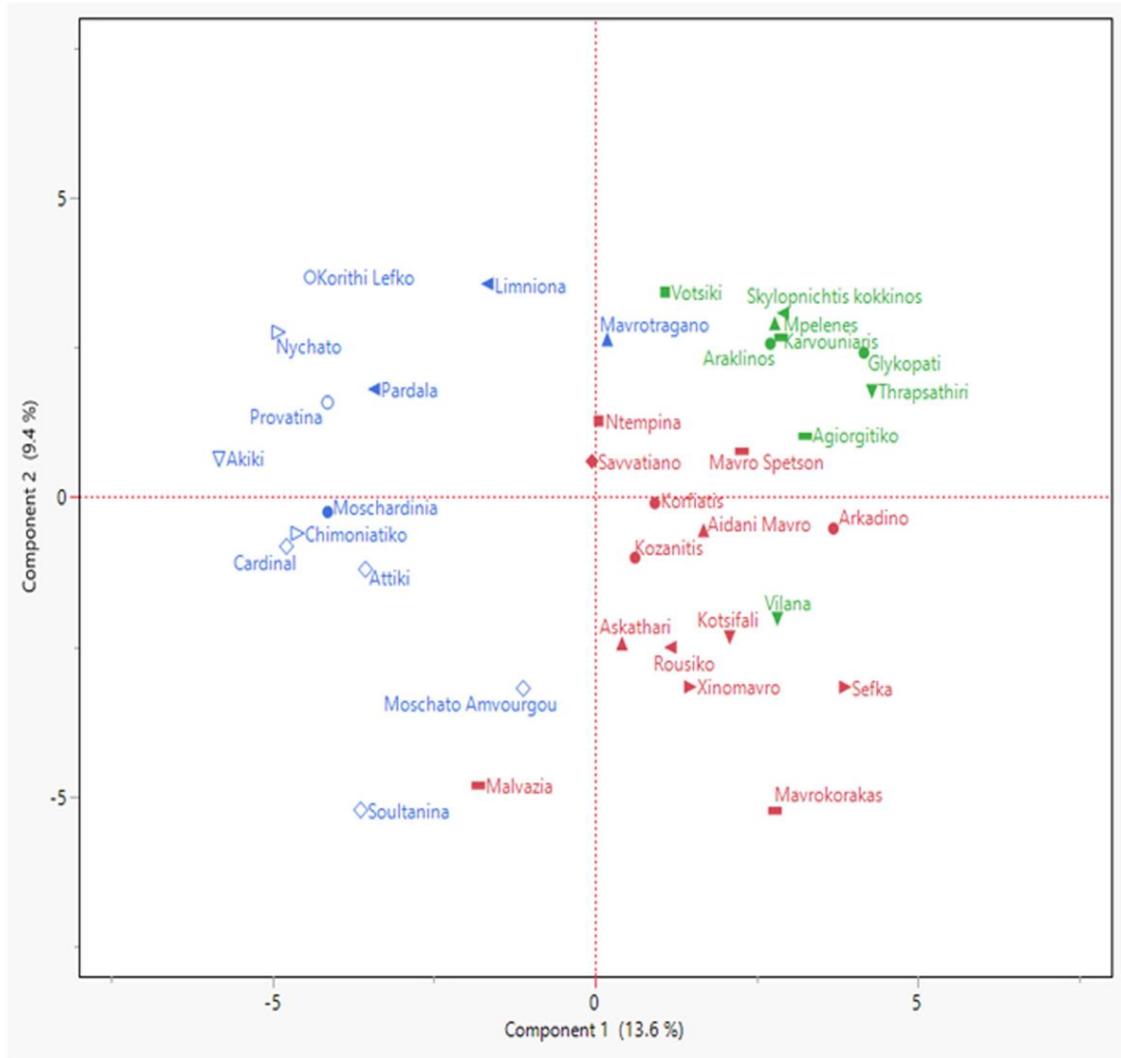
Grapevine (*Vitis vinifera* L.) is a major worldwide crop of high economic importance, tightly interwoven with the traditions and the culture of many civilizations. The Greek vineyard is one of the oldest in the world composed of an ample number of highly diverse indigenous landraces. However, over the last decades the local cultivated grapevine germplasm has undergone a drastic reduction of diversity, due to the established market preferences for international varieties. In the current work a combined approach involving both, ampelographic traits and microsatellite markers has been undertaken, to study the genetic diversity within and among 96 grapevine genotypes belonging to 36 *V. vinifera* subsp. *vinifera* cultivars, predominantly representing autochthonous Greek landraces. Results revealed high genetic diversity for the Greek cultivars yielding a mean number of alleles per locus 14.69 and mean polymorphic information content 0.848. Hierarchical cluster analysis, employing both, ampelographic and microsatellite data, showed a clear distinction based on the origin of the germplasm; Anatolian versus Mediterranean. Principal component analysis, based on the most informative ampelographic traits, coupled to the results from genetic structure analysis further corroborated the proposal of germplasm differentiation on the basis of geographic origin. This information can be further utilized for the reconstitution of the Greek vineyard and can significantly contribute towards a rational conservation and utilization strategy for breeding or for direct cultivation of the Greek indigenous grapevine germplasm.

Introduction

Grapevine (*Vitis vinifera* L.) is a crop of major economic importance that retains fundamental symbolisms for many cultures worldwide. The cultivated grapevine (*V. vinifera* L. subsp. *vinifera*) is believed to have been derived from its wild closely related form *V. vinifera* L. subsp. *sylvestris* (Zohary *et al.*, 2012). Archaeological records suggest that the primate domestication of the grapevine started in the Near East by the late Neolithic period (Zohary, 1996; This *et al.*, 2006) or in the neighbouring Transcaucasia approximately 8000–6000 BC (Levadoux, 1956, cited by Olmo, 1996). However, uncertainty remains about the place and the period of original domestication (This *et al.*, 2006) with different studies to sur-



Ampelographic and microsatellite results



Ampelographic and microsatellite results for Cretan biotypes

- Evidence showed that Crete grapevine cultivars and winemaking date back to 2300 BC.
- In this study **51 genotypes** belonging to **7 different traditional** cultivars, presumed autochthonous to the island of Crete, were selected for their wine-producing potential and classified by **51 ampelographic** descriptors.
- In addition, **5 genotypes** belonging to **2 non-autochthonous** cultivars were included as out-group controls.



Article

Analysis of Wine-Producing *Vitis vinifera* L. Biotypes, Autochthonous to Crete (Greece), Employing Ampelographic and Microsatellite Markers

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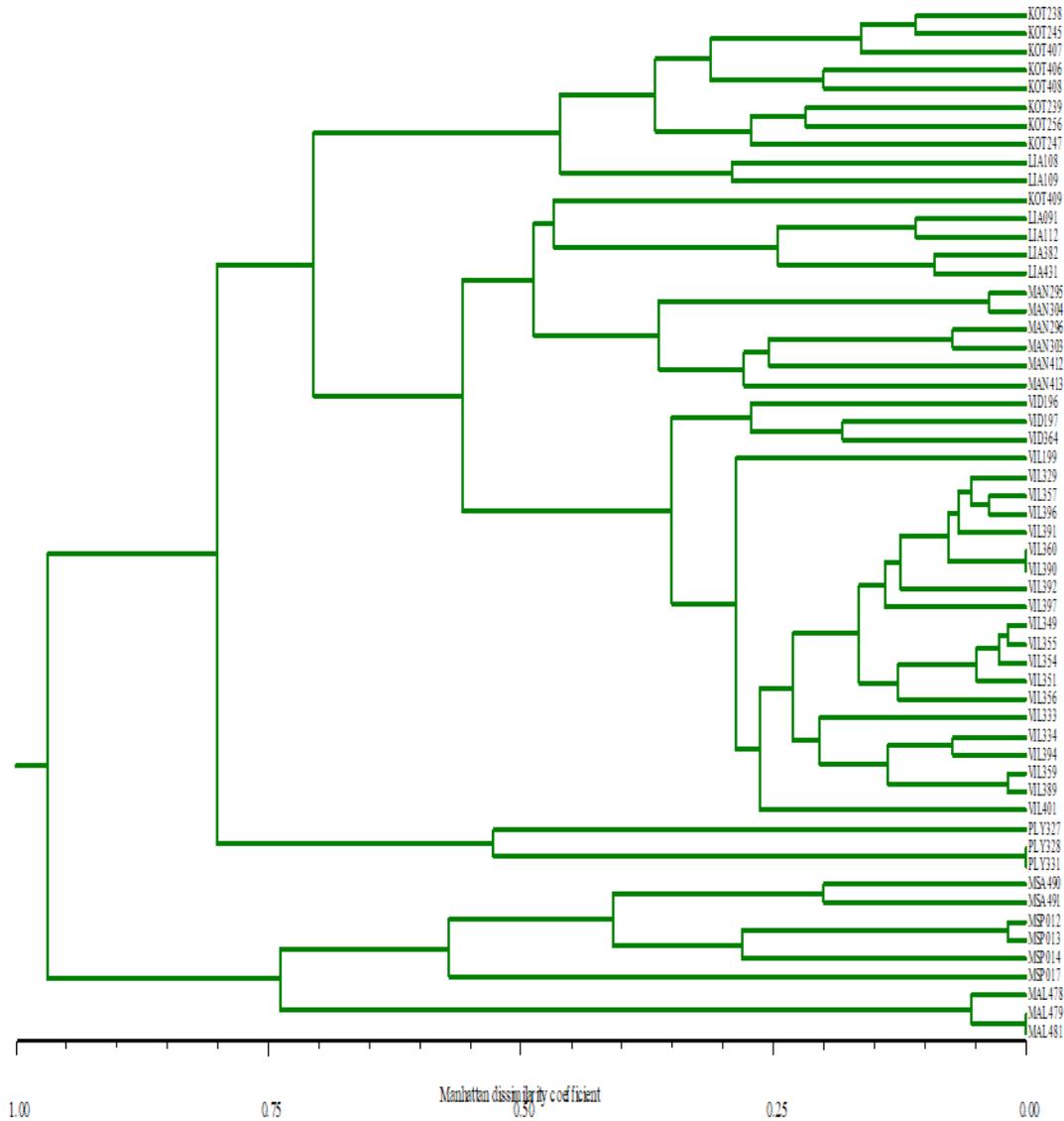
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Abstract: *Vitis vinifera* ssp. *vinifera* (domesticated grapevine) includes thousands of cultivars, which are classified according to their main uses, as wines, fresh fruits or dried raisins and sultanas since ancient times. Evidence showed that Crete grapevine cultivars and winemaking date back to 2300 BC. In this study, fifty-one genotypes belonging to seven different traditional *Vitis vinifera* cultivars, presumed autochthonous to the island of Crete, were selected for their wine-producing potential and classified by 51 ampelographic descriptors. In addition, five genotypes belonging to two non-autochthonous cultivars were included as out-group controls. Subsequently, in order to characterize genetic diversity, establish genetic relationships within and between cultivars and solve accession-labeling problems, genotypes were fingerprinted employing Simple Sequence Repeat (SSR or microsatellite) markers. Four of the autochthonous cultivars namely 'Vidiano', 'Vilana', 'Phyto', and 'Moschato Spinás' are used in the local economy for blanc (white) wine production while the rest, namely 'Kotsifali', 'Liatiko' and 'Mantilarí' for Noir (red) wines. The two cultivars employed as out-group were 'Moschato Samou' and 'Moschato Alexandrias': both white wine producers. Ampelography-based clustering grouped the majority of genotypes along cultivar-specific clusters. All three Moschato cultivars formed a distinct clade pointing to the non-autochthonous origin of 'Moschato Spinás'. A total of one hundred and thirteen (113) SSR alleles were amplified from thirteen (13) SSR loci, with an average number of alleles per locus equal to 10.23 revealing ample genetic polymorphism. The cumulative probability of identity was also quite high (3.389×10^{-16}).

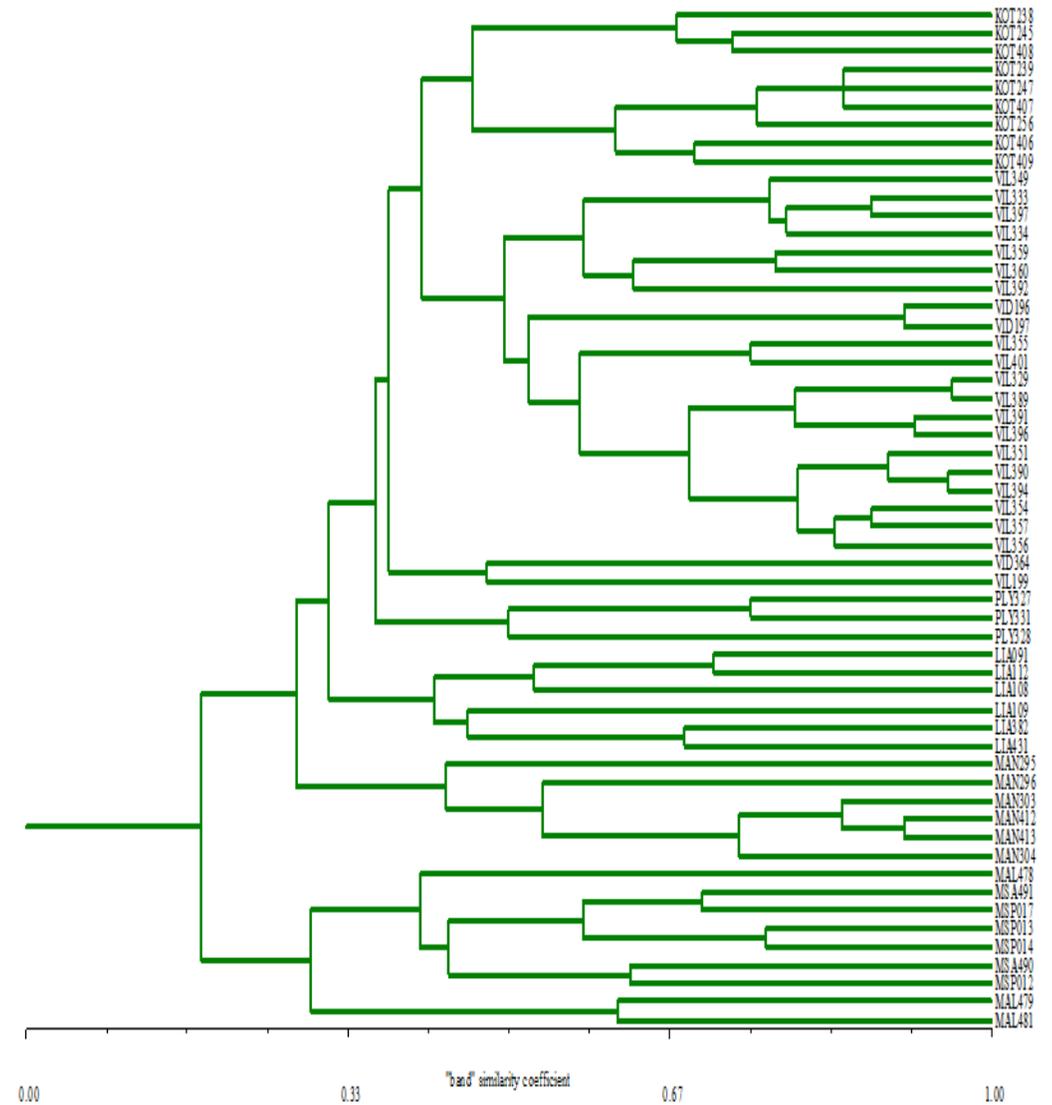


Results

Ampelographic



Microsatellite markers



Results

- Within the island of Crete: (i) old/autochthonous, (ii) widespread non-autochthonous and (iii) locally, on-farm selected hybrids, derived from crosses within and between autochthonous cultivars or outcrosses with other non-autochthonous cultivars.
- Present cultivar assignment based on ampelographic data agreed with grouping based on molecular markers on the basis of forming monophyletic or near monophyletic (cultivar-specific) clusters.
- Nevertheless, topologies between the two similarity dendrograms exhibited some local differences. With the present study, the number of SSR markers employed (a total of thirteen; 13), as well as the degree of SSR polymorphism revealed due to the inherent within cultivar genetic variability allowed for individual genotype discrimination, i.e., clonal differentiation within each cultivar.



Genotyping toolbox

- In this review we presented the most frequently **used molecular markers**, which have been used on *Vitis* germplasm.
- We discussed the scientific progress that led to the new strategies being implemented utilizing **state-of-the-art next generation sequencing** technologies.
- Additionally, we attempted to delimit the discussion **on the algorithms used in phylogenetic analyses** and differentiation of grape varieties.
- Lastly, the **contribution of epigenetics is highlighted to tackle future roadmaps** for breeding and exploitation of *Vitis* germplasm.



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Vitis vinifera genotyping toolbox to highlight diversity and germplasm identification

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The contribution of vine cultivation to human welfare as well as the stimulation of basic social and cultural features of civilization has been great. The wide temporal and regional distribution created a wide array of genetic variations that have been used as propagating material to promote cultivation. Information on the origin and relationships among cultivars is of great interest from a phylogenetics and biotechnology perspective. Fingerprinting and exploration of the complicated genetic background of varieties may contribute to future breeding programs. In this review, we present the most frequently used molecular markers, which have been used on *Vitis* germplasm. We discuss the scientific progress that led to the new strategies being implemented utilizing state-of-the-art next generation sequencing technologies. Additionally, we attempted to delimit the discussion on the algorithms used in phylogenetic analyses and differentiation of grape varieties. Lastly, the contribution of epigenetics is highlighted to tackle future roadmaps for breeding and exploitation of *Vitis* germplasm. The latter will remain in the top of the edge for future breeding and cultivation and the molecular tools presented herein, will serve as a reference point in the challenging years to come.

KEYWORDS

Vitis, biodiversity, genotyping, molecular markers, next generation sequencing, simple sequence repeats, microsatellites, single nucleotide polymorphism

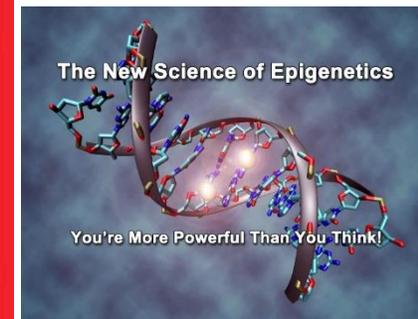
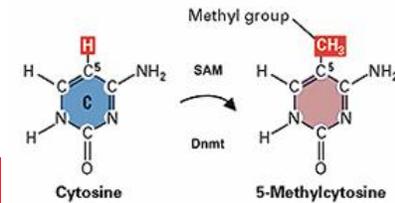
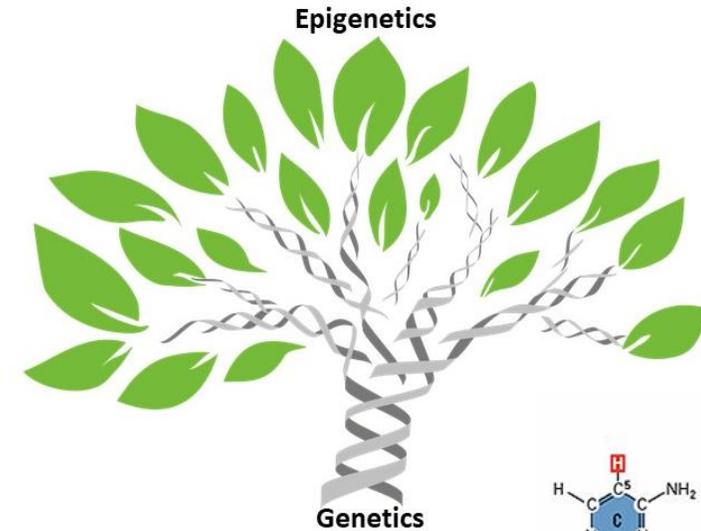


Epigenetic vs Genetics

Epigenetics is defined as mechanisms that regulate gene expression without base sequence alteration.

Main epigenetic mechanisms:

- DNA methylation (addition of a CH₃ in a cytosine)
- Modification of histones (phosphorylation, acetylation)
- mRNAs



Epigenetic transgenerational inheritance

- Knowledge of the regulatory mechanisms involved in adaptive epigenetic responses may help to guide management of genetic resources and plant breeding of *Vitis*.
- Studies have shown that if an environmental stress is maintained long enough epigenetic alterations can reach equilibrium frequencies and transmitted in next generations.
- Potential role in adaptation and evolution.



Epigenetic Changes and Transcriptional Reprogramming Upon Woody Plant Grafting for Crop Sustainability in a Changing Environment

<https://www.frontiersin.org/articles/10.3389/fpls.2020.613004/full>

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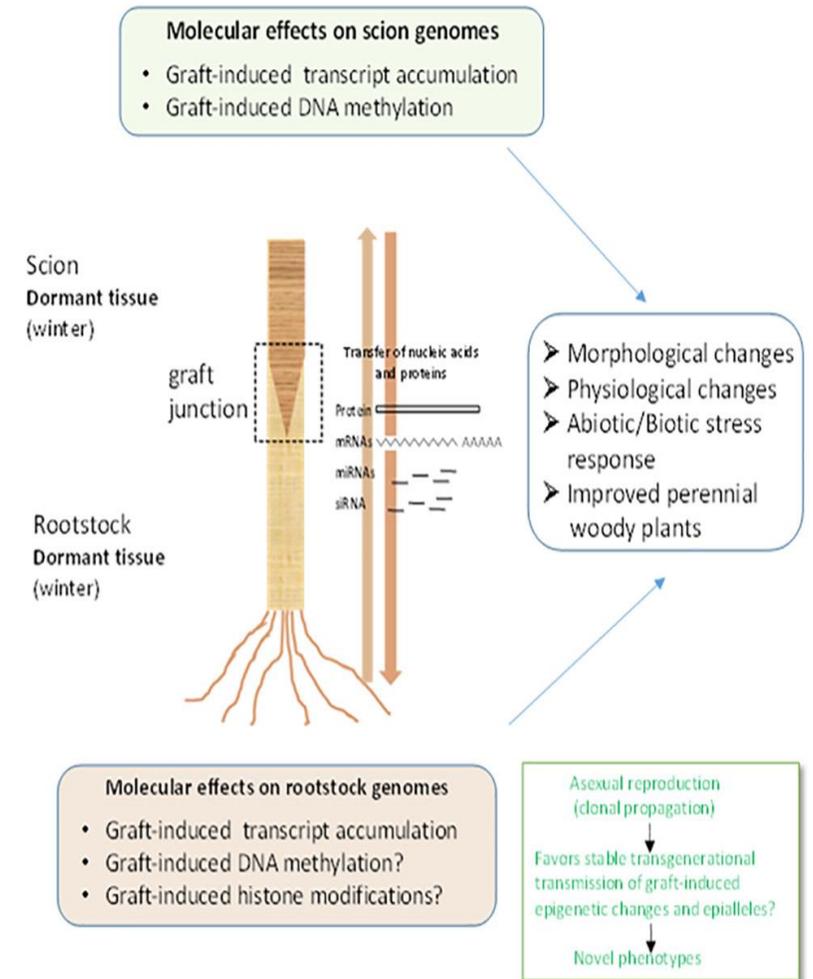
Plant grafting is an ancient agricultural practice widely employed in crops such as woody fruit trees, grapes, and vegetables, in order to improve plant performance. Successful grafting requires the interaction of compatible scion and rootstock genotypes. This involves an intricate network of molecular mechanisms operating at the graft junction and associated with the development and the physiology of the scion, ultimately leading to improved agricultural characteristics such as fruit quality and increased tolerance/resistance to abiotic and biotic factors. Bidirectional transfer of molecular signals such as hormones, nutrients, proteins, and nucleic acids from the rootstock to the scion and vice versa have been well documented. In recent years, studies on rootstock-scion interactions have proposed the existence of an epigenetic component in grafting reactions. Epigenetic changes such as DNA methylation, histone modification, and the action of small RNA molecules are known to modulate chromatin architecture, leading to gene expression changes and impacting cellular function. Mobile small RNAs (siRNAs) migrating across the graft union from the rootstock to the scion and vice versa mediate modifications in the DNA methylation pattern of the recipient partner, leading to altered chromatin structure and transcriptional reprogramming. Moreover, graft-induced DNA methylation changes and gene expression shifts in the scion have been associated



Vitis sylvestris : a new era for genetic and epigenetics

- **Grafting** is an ancient agricultural propagation technique widely used to **improve plant performance**, in terms of yield, quality and resilience to abiotic and biotic stresses. It involves the merging of two genetically different plant parts, the rootstock, and the scion, in such a manner that the two parts join and grow as a single plant.
- Woody plants are clonally propagated by asexual reproduction. This would **favor stable trans-generational transmission of graft-induced epigenetic changes such as DNA methylation**, in woody crops, which may lead to phenotypic variation and ultimately to novel varieties with improved traits.

Woody plants grafting



Adopted from Kapazoglou et al 2021

Epigenetic transgenerational inheritance in *Vitis*: some examples

Multiple examples showed effects of scion/rootstock RNA movement along grapevine grafts as well as graft-induced transcriptional reprogramming with respect to rootstock specificity and plant performance.

- Differential and drought stress-specific accumulation of miRNAs in auto-grafts and reciprocal heterografts of Cabernet Sauvignon and M4, revealed a drought tolerant hybrid (Pagiaroni et al 2017)
- Analysis of the effect of rootstock on Cabernet Sauvignon berries demonstrated that modulation of auxin related genes and the rate of ripening varies according to the rootstock used in the graft (Corso et al., 2016).



Epigenetic transgenerational inheritance in *Vitis*: some examples

- A reference-unbiased super-pangenome of the North American wildgrape species (*Vitis* spp.) reveals genus-wide association with adaptive traits.
- This study highlights how a reference-unbiased super-pangenome can reveal the genetic basis of the adaptive traits from wild relatives, potentially accelerating crop breeding research.

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A reference-unbiased super-pangenome of the North American wild grape species (*Vitis* spp.) reveals genus-wide association with adaptive traits

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Abstract

Capturing the genetic diversity of wild relatives is crucial for improving crops because wild species are valuable sources of agronomic traits that are essential to enhance the sustainability and adaptability of domesticated cultivars. Genetic diversity across a genus can be captured in super-pangenomes, which provide a framework for interpreting genomic variations. Here we report the sequencing, assembly, and annotation of nine wild North American grape genomes, which were phased and scaffolded at chromosome scale. We generated a reference-unbiased super-pangenome using pairwise whole-genome alignment methods, revealing the full resolution of genomic diversity among wild grape species from sequence to gene level. The pangenome graph captured genomic variation between haplotypes within a species and across the different species, and it accurately assessed the similarity of hybrids to their parents. The species selected to build the pangenome ensured a comprehensive representation of the genus, as illustrated by capturing known allelic variants in the sex-determining region and for Pierce's disease resistance loci. Using pangenome-wide association analysis (pan-GWAS), we demonstrated the utility of the super-pangenome by effectively mapping short-reads from genus-wide samples and identifying loci associated with salt tolerance in natural populations of grapes. This study highlights how a





Vitis sylvestris : a new era for studying genetic and epigenetics breeding strategies???

- Collection of various *Vitis sylvestris* sp. populations from different locations.
- Ampelographic description.
- Genetic characterization with microsatellite markers (that is already achieved)
- Epigenetic study and training of plants to resistance to drought or other biotic stress.
- Grafting to local varieties and monitor their characteristics (interaction of genetic and epigenetic pools).





THANK YOU
