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THEME

Valorization and Preservation of Grapevine Genetic Diversity in the Aures Region

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ملخص

تمثل هذه الدراسة أول مسعى شامل لتوصيف وتحديد الأصول الوراثية للكروم في منطقة الأوراس (باتنة، الجزائر) من خلال نهج متكامل يجمع بين التوصيفات الظاهرية والمؤشرات الجزيئية. تم إخضاع خمسة وثلاثين صنفاً للتوصيف الظاهري استناداً إلى أوراق ناضجة، باستخدام 32 واصفاً تابعاً للـ OIV و 13 علاقة قياسية. كانت التحاليل متعددة المتغيرات القائمة على التقييمات الإحصائية بما في ذلك الإحصاءات الوصفية، وتحليل المكونات الرئيسية (PCA)، وتحليل الارتباط، تحليل التباين أحادي الاتجاه، تكرار الصفات النوعية والتجميع الهرمي (HCA) ضرورية لتحليل مجموعة البيانات الظاهرية الواسعة التي تم إنشاؤها. وجدت الدراسة تنوعاً ظاهرياً كبيراً بين الأصناف، مما يعكس استراتيجيات تكيف فريدة واختلافات جينية. أوضح تحليل المكونات الرئيسية (PCA) أن 76.91% من التباين الكلي المفسر بواسطة المكونات الثلاثة الأولى كان مرتبطاً بسمات تمييزية رئيسية متعلقة بزوايا العروق، أعماق الجيوب، أبعاد الأسنان، والحجم الكلي للورقة كعوامل مميزة مهمة بين الأصناف المدروسة. تم تجميع العينات وفقاً للتصنيف الظاهري إلى 18 صنفاً تنتمي إلى 7 مجموعات رئيسية. وسُجل أعلى معامل تشابه بين الصنفين '5 و 'Tazizaouth (J=0.73). في المقابل، تم تمييز عينات أخرى عن جميع هذه المجموعات الرئيسية. وكان ذلك هو الحال بالنسبة لـ '4 و 'Tazogaghth 3 (J= 0.376) والتي أظهرت مسافات كبيرة تدل على تباعدها التام عن الأصناف الأخرى، مما يشير إلى خلفية جينية وخصائص ظاهرية مميزة. واستكمالاً لبيانات النمط الظاهري، كشفت الدراسة عن توصيف جزيئي لـ 41 صنفاً باستخدام 12 مؤشراً جزيئياً (SSR) عن 14 نمطاً وراثياً مميزاً. ومن اللافت أن أربعة أنماط وراثية تعود إلى أصناف محلية جزائرية معروفة: 'Ahmeur Bou Ahmeur'، 'Louali'، 'Tizi Ouinine'، و 'Babari'. بينما تطابقت ستة أنماط وراثية مع أصناف متوسطة شائعة. وبشكل ملحوظ، مثلت أربعة أنماط وراثية أصنافاً جديدة محتملة، مما يشير إلى مادة وراثية فريدة قد تكون خاصة بمنطقة الأوراس. سيتم تسجيل الأنماط الوراثية الجديدة المكتشفة في قاعدة بيانات IVTC للتوثيق والتتبع المناسبين بأسماء تتعلق بالتقاليد المحلية، مثل 'Ichmoul'، و 'Ichmoul Bacha'، 'Bouabane des Aures'، و 'Amer Bouamar'. والأهم من ذلك، في معظم الحالات، أكدت النتائج الجزيئية نتائج الوصف الظاهري. بشكل عام، نجح هذا البحث في استعادة وتوصيف جزء كبير من التنوع المهم لأشجار العنب من منطقة الأوراس، وتحديد موارد جينية قيمة للحفاظ، التحسين الوراثي والزراعة في الحقول المحلية.

الكلمات المفتاحية: *Vitis vinifera* L., الأوراس، التحليلات متعددة المتغيرات، واصفات OIV، العلاقات القياسية، SSR، التنوع الوراثي، المحافظة.

Abstract

This study represents the first comprehensive effort to characterize and identify the grapevine germplasm of Aures region (Batna, Algeria) through an integrated approach combining ampelographic and molecular markers. Thirty-five cultivars were subjected to ampelographic characterization based on mature leaves, using 32 OIV descriptors and 13 ampelometric relationships. Multivariate analyses based on statistical evaluations including descriptive statistics, PCA, correlation analysis, one-way Anova test, the frequency of qualitative traits and HCA were essential for analyzing the extensive ampelographic dataset generated. The study found considerable morphological diversity among the cultivars, reflecting unique adaptive strategies and genetic variations. PCA analysis accounted for 76.91% of the total variation explained by the first three components, with key discriminant traits related to vein angles, sinus depths, tooth dimensions, and overall leaf size among the studied cultivars. Ampelographic-based clustering grouped the grapevines into 18 varieties belonging to 7 major clusters. The highest similarity coefficient was observed between the cultivars 'Tazizaouth 5 and 6' ($J=0.73$). In contrast, other varieties were distinguished from all these main groups. That was the case for 'Tazogaghth 3 and 4' ($J=0.376$) which have large distances indicating that they completely diverge from the other cultivars suggesting a distinct genetic background and ampelographic characteristics. Complementing the phenotypic data, the molecular characterization of 41 cultivars using 12 SSR markers uncovered 14 distinct genotypes. Remarkably, four genotypes corresponded to known autochthonous Algerian varieties: 'Ahmeur Bou Ahmeur', 'Louali', 'Tizi Ouinine' and 'Babari'. Six genotypes matched common Mediterranean varieties. Significantly, four profiles represented putatively novel genotypes, constituting unique germplasm potentially specific to Aures. The novel genotypes discovered will be registered in the *VIVC* Catalogue for proper authentication and traceability with names related to local tradition, named 'Ichmoul', 'Ichmoul Bacha', 'Bouabane des Aures', and 'Amer Bouamar'. More importantly, in most cases, the molecular findings confirmed the results of the ampelographic description. Overall, this research has successfully recovered and characterized a significant fraction of the neglected grapevine diversity from the Aures region, identifying valuable genetic resources for conservation, breeding and on-farm cultivation.

Keywords: *Vitis vinifera* L., Aures, multivariate analyses, OIV descriptors, ampelometric relationships, SSR markers, genetic diversity, conservation.

Résumé

Cette étude représente le premier effort global de caractérisation et d'identification du matériel génétique de la vigne de la région des Aurès (Batna, Algérie) par une approche intégrée combinant des marqueurs ampélographiques et moléculaires. Trente-cinq cultivars ont été soumis à une caractérisation ampélographique basée sur les feuilles matures, en utilisant 32 descripteurs OIV et 13 relations ampélographiques. Les analyses multivariées basées sur des évaluations statistiques, y compris les statistiques descriptives, l'ACP, l'analyse de corrélation, le test ANOVA à un facteur, l'ACH et la fréquence des caractères qualitatifs, a été essentielle pour analyser le vaste ensemble de données ampélographiques générées. L'étude a révélé une diversité morphologique considérable entre les cultivars, reflétant des stratégies adaptatives et des variations génétiques uniques. L'étude a notamment montré que quelques traits ampélographiques suffisaient à distinguer ces vignes pour la première fois. L'ACP a représenté 76,91% de la variation totale expliquée par les trois premières composantes, avec des traits discriminants clés liés aux angles des veines, aux profondeurs des sinus, aux dimensions des dents et à la taille globale des feuilles parmi les cultivars étudiés. Le regroupement ampélographique a permis de classer les vignes en 18 variétés appartenant à 7 groupes principaux. Le coefficient de similarité le plus élevé a été observé entre les cultivars 'Tazizaouth 5 et 6' ($J=0,73$). En revanche, d'autres variétés se distinguent de tous ces groupes principaux. C'est le cas de 'Tazogaghth 3 et 4' ($J=0,376$) qui ont de grandes distances indiquant qu'ils divergent complètement des autres cultivars suggérant un fond génétique distinct et des caractéristiques ampélographiques. En complément des données phénotypiques, la caractérisation moléculaire de 41 cultivars à l'aide de 12 marqueurs SSR a permis de découvrir 14 génotypes distincts. Fait remarquable, quatre génotypes correspondent à des variétés algériennes autochtones connues : 'Ahmeur Bou Ahmeur', 'Louali', 'Tizi Ouinine' et 'Babari'. Six génotypes correspondent à des variétés méditerranéennes communes. De manière significative, quatre profils représentaient des génotypes supposés nouveaux, constituant un germoplasme unique potentiellement spécifique à l'Aurès. Les nouveaux génotypes découverts seront enregistrés dans le catalogue *VIVC* pour une authentification et une traçabilité appropriées, avec des noms liés à la tradition locale, à savoir 'Ichmoul', 'Ichmoul Bacha', 'Bouabane des Aurès' et 'Amer Bouamar'. Plus important encore, dans la plupart des cas, les résultats moléculaires ont confirmé les résultats de la description ampélographique. Dans l'ensemble, cette recherche a permis de récupérer et de caractériser une fraction importante de la diversité négligée de la vigne dans la région des Aurès, en identifiant des ressources génétiques précieuses pour la conservation, la sélection et la culture à la ferme.

Mots clés: *Vitis vinifera* L., Aurès, analyses multivariées, descripteurs OIV, relations ampélographiques, marqueurs SSR, diversité génétique, conservation.

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Wahiba

ACRONYMS AND ABBREVIATIONS

AFLP: Amplified Fragment Length Polymorphism

AI: Artificial Intelligence

AMOVA: Analysis of Molecular Variance

ANN: Artificial Neural Network

ANOVA: Analysis of Variance

BCE: Before Common Era

CNNs: Convolutional Neural Networks

Cos²: Cos Squared

CREA-VE: Research Centre for Viticulture and Oenology

DL: Deep Learning

DNA: Deoxyribonucleic Acid

HCA: Hierarchical Clustering Analysis

IBPGR: International Board for Plant Genetic Resources

ICVV-DNA: Institute of Vine and Wine Sciences - DNA

IPGRI: International Plant Genetic Resources Institute

ITAFV: Institut Technique de l'Arboriculture Fruitière et de la Vigne

KNN: k-Nearest Neighbors

M: Mole

M1: Multiplex 1

M2: Multiplex 2

M. A. R. D: Ministry of Agriculture and Rural Development

ML: Machine Learning

OIV: International Organisation of Vine and Wine

PCA: Principal Component Analysis

PCoA: Principal Coordinates Analysis

NJ: Neighbor-Joining

NTSYS: Numerical Taxonomy System

PAST: Paleontological Statistics

RAPD: Random Amplified Polymorphic DNA

Rel.: Relationship

RFLP: Restriction Fragment Length Polymorphism

SNP: Single Nucleotide Polymorphism

SSR: Simple Sequence Repeat

SVM: Support vector machines

UPGMA: Unweighted Pair Group Method with Arithmetic Mean

UPOV: International Union for the Protection of New Varieties of Plants

VIVC: *Vitis* International Variety Catalogue

Abbreviations

e.g.: For example

et al.: And other authors

etc.: And other more

i.e.: In other words

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INTRODUCTION

INTRODUCTION

1. Overview

Grapevine is considered as one of the most valuable crops, with a long history and deep cultural and economic significance in many regions of the world (Cismaşiu et al., 2023). The current grape varieties are the outcome of prolonged selection, initiated during the domestication process of their wild relative (Ucchesu et al., 2024).

The archaeological and historical evidences suggest that the domestication of the grapevine took place since ancient times, back to between 7000 and 4000 BCE in the Near East (Guasch-Jané, 2019), with evidence of winemaking in Anatolia dating to approximately 6000 BCE (Gorny, 1996). This early cultivation was facilitated by vegetative propagation methods, which allowed for the establishment of vineyards and the spread of grapevine cultivars (Reynolds, 2017). Ancient Egyptian hieroglyphics record the cultivation of grapes (Zohary, 1996), and history attests to the ancient Greeks (Logothetis, 1974), Phoenicians (Greene, 2003), and Romans growing grapes both for table and wine production (Bouby et al., 2013). The growing of grapes would later spread to other regions in Europe, as well as North Africa (This et al., 2006), and eventually in North America (Pinney, 1989). By the 19th and 20th centuries, viticulture expanded globally, with the development of hybrid varieties to improve resistance to diseases like phylloxera and adaptations to diverse climates (Reynolds, 2017).

Algeria is recognized as one of the gene centers for grapes, largely due to its favorable climatic conditions that support a rich biodiversity of grapevine cultivars since ancient times (Isnard, 1951). The country's diverse ecological regions contribute to the genetic diversity and adaptability of grapevines, making it a significant area for grape cultivation. Current Algerian viticulture is related to the long and complex history of the country, which results from a continuous mixture of people and civilizations (Laiadi et al., 2009). However, according to Isnard, (1951), Algerian grapevines remained largely understudied until the 19th century, and even today, only a limited number of studies have focused on their characterization.

It is widely recognized that local grape diversity constitutes a valuable resource to be preserved and maintained both for breeding programs and for marketing of local economic benefit (Pastore et al., 2020). Nevertheless, most of the old cultivars are not preserved in grapevine germplasm banks, highlighting the necessity to counter genetic erosion (El Aou-ouad et al., 2022).

Unfortunately, nowadays, the genetic diversity of local varieties faces a progressive erosion phenomenon due to several reasons, such as climatic variations (Gisbert et al., 2022) and the introduction of commercial high-yield foreign varieties (Güler and Karadeniz, 2023). Indeed, they

should be transferred to the germplasm collection vineyard for biodiversity conservation and possibly for breeding (Khouni et al., 2023).

The exploitation of the local genetic diversity, based on the recovery of old grapevine varieties, has received increasing interest in recent years in order to preserve the genetic resources of different grape-growing regions throughout the world (Urrestarazu et al., 2015; Gisbert et al., 2018; Zombardo et al., 2021; Mendoza et al., 2022; Torres et al., 2022; El Aou-ouad et al., 2022; Jiménez-Cantizano et al., 2023; Saliba et al., 2024).

However, managing grapevine genetic resources remains challenging, due to the use of synonyms and homonyms, the presence of many variants (phenotypes) within cultivars, and the poor documentation of passport data (Tsivelikas et al., 2022).

Traditionally, the identification and discrimination of grapevine varieties has relied on ampelography, which involves describing the morphological characteristics through visual inspection at various growth stages (Galet, 1985). Ampelographic characters are diverse and specific; they are based on the characterization of different organs, such as canes, buds, shoots, leaves, tendrils, berries, and seeds, according to the International Organization of Vine and Wine (OIV, 2001).

Nevertheless, this method could be affected by environmental variations (This et al., 2004) and the subjectivity of the observer (Núñez et al., 2004), which frequently complicates the identification and also leads to incorrect classification, such as cases of identical names for different varieties (homonymy) or different names for the same variety (synonymy), particularly when applied within one single cultivar.

The identification of homonymies is important to avoid the loss of variability (loss of genotypes). On the contrary, the detection of synonymies avoids the maintenance of duplicated materials that do not contribute to increase variability but increase the cost (Villano et al., 2022; Khouni et al., 2023). It is also very important to check the sanitary status of the plants, sanitize them if necessary, and provide suboptimal culture conditions that limit and slow down plant development, without causing physiological damage to the plant material (García-Águila et al., 2007; Gisbert et al., 2018).

To overcome problematic morphological classification, the development of genetic markers is considered a significant advancement and highly reliable approach for variety identification, because they are not affected by environmental conditions, sample type, or the developmental stage, and thereby provide distinctive and reliable information (Arslan et al., 2023).

Genetic markers, such as SSRs microsatellites, are particularly useful for grapevine identification due to their high polymorphism, which allow the creation of unique genetic profiles for each variety (Tessier et al., 1999). These markers have been extensively utilized in grapevine research and viticulture practices in various countries, including Morocco (El-Oualkadi et al., 2009), Tunisia (Ghaffari et al., 2014), France (Magris et al., 2021), Italy (Zombardo et al., 2021), Spain (Jiménez-Cantizano et al., 2023), Portugal (Barrias et al., 2023), Greece (Avramidou et al., 2023), Turkey (Arslan et al., 2023), and Algeria (Khouni et al., 2023).

The Aures region, the focus of our study, boasts a long-standing tradition of grapevine cultivation, as documented by Isnard, (1951). This rich history has led to a proliferation of homonymous, synonymous, and misnamed cultivars, many of which possess unexplored and unknown genetic backgrounds. These cultivars represent a valuable reservoir of genetic diversity that is at risk of being lost to extinction (Rahali et al., 2019).

2. Problem statement and challenges

Algeria is recognized as one of the gene centers for grapes, with favorable climatic conditions that support a rich biodiversity of grapevine cultivars.

In the Aures region, specifically Batna province (Algeria), many grapevine cultivars with unexplored and poorly understood genetic backgrounds exist. These cultivars represent an untapped source of genetic diversity, which could be crucial for future breeding programs aimed at enhancing disease resistance and climate adaptability.

Furthermore, the poor documentation of grapevine diversity, coupled with the presence of synonyms and homonyms in cultivar names, complicates efforts to preserve and manage these resources. This lack of proper identification and classification increases the major problem of genetic erosion, which is primarily driven by factors such as the introduction of high-yielding foreign varieties, climate change, and the limited preservation of traditional cultivars in germplasm banks.

Only a limited number of studies have focused on the ampelographic and molecular characterization of Algerian grapevines and particularly in the Aures (Rahali et al., 2019; Rahali, 2020). This lack of comprehensive research poses a significant challenge to the preservation and effective use of these genetic resources.

Therefore, addressing these challenges is crucial for preserving the genetic diversity of Algerian grapevines and ensuring the sustainability of viticulture in the Aures region.

3. Significance of the study, objectives and contributions

This study holds significant importance for the characterization and preservation of Algerian grapevine genetic resources, especially in the Aures region (Batna, Algeria), which is home to a wealth of local cultivars with unique genetic backgrounds.

By addressing the gaps in our understanding of the ampelographic and genetic diversity of these grapevines, this research contributes to the conservation of biodiversity and provides valuable data that can be used for future breeding programs. The identification, classification, and characterization of local varieties will help safeguard the genetic diversity of grapevines, making them more resilient to challenges such as climate change, pests, and diseases.

The present research explores two main categories of characterization approaches and the primary objectives are the following:

1. **To conduct a comprehensive ampelographic and molecular characterization** of grapevine cultivars from the Aures region, identifying key morphological traits and genetic markers.
2. **To assess the genetic diversity** within local grapevine varieties in the Aures region and compare them with foreign varieties to better understand their uniqueness and potential for breeding programs.

The contributions of this study are multifaceted. First, it provides an essential step toward the identification and the assessment of the genetic diversity of Algerian grapevines in the Aures region, which is increasingly threatened by genetic erosion. Second, the study offers insights into the potential of local varieties cultivated in such historical region. Additionally, the molecular data generated will assist in clarifying the relationships between different cultivars and contribute to reducing errors in cultivar identification, ultimately improving the management and conservation of grapevine diversity.

Overall, this research provides critical information for the sustainable management of grapevine genetic resources in Algeria and the Aures region, ensuring that these unique cultivars can be preserved, studied, and utilized for future generations.

4. Thesis outline

This thesis is structured to provide a comprehensive ampelographic and molecular characterization of grapevine varieties from the Aures region of Algeria.

It is organized in five main sections as follows. The current one is reserved for an introduction to the theme of the thesis, including the overview, problem statement and challenges, and significance of the study, along with its objectives and contributions.

The second section deals with the state-of-the-art review and related works that situate our research topic within the broader academic context where some of the most relevant research works in the ampelographic and molecular characterization were collected during the period (1999 to 2024) and reported using a bibliometric analysis.

In terms of both ampelographic and molecular characterization studies, the sections 3 and 4 detail the materials and methods used including the sampling, trait evaluation, DNA extraction, and data analysis techniques employed. Results will be presented in terms of both ampelographic and molecular findings, with a comparison between local and foreign cultivars. The discussion will interpret these results.

Finally, the conclusion will summarize the research outcomes while also acknowledging the study's limitations, and propose recommendations for future studies on grapevine conservation and sustainable viticulture in the Aures region.

Part 1

A state-of-the-art review and related works

A state-of-the-art review and related works

This section provides a detailed review of existing studies and related works pertinent to the characterization of grapevine varieties, focusing on their ampelographic and molecular traits. It highlights the methodologies employed, the geographic scope of research, and the diversity of varieties studied. This review aims to synthesize the findings of previous investigations, identify gaps in current knowledge, and outline the significance of these studies in understanding and preserving grapevine diversity. It is structured into the following key areas:

1. Ampelographic and Molecular Approaches for Grapevine Characterization: A Bibliometric analysis

Ampelography (from *Ampelos*, ‘vine’ and *grafos*, ‘writing’) is the study of grapevine identification and classification based on morphological characteristics (Galet, 1979), primarily of the shoot, bud, leaf, bunch, berry, and seed, reflecting their qualitative (e.g., color, density, shape) and quantitative (size, number, weight) traits (Bodor-Pesti et al., 2023).

Among the various plant organs used for characterization, leaves are the most prominent due to their extensive range of distinguishable traits. However, other organs, such as the young shoot, bunch, and berry, also play significant roles in the identification and characterization of genotypes. In this approach, linear and angular measurements of plant organs are analyzed using multivariate statistical methods, as described by Bodor-Pesti et al., (2023).

The history, evolution, and development of ampelographic studies have been supported by a standardized set of descriptor lists and manuals, all of which address both inter- and intraspecific morphological variability across grapevine cultivars and varieties.

The foundations of this field trace back to the beginning 19th century, as earlier literature tended to describe morphological characters in qualitative terms, often labeling leaves or bunches as ‘small’ or ‘large’ rather than quantifying them.

Early contributors such as Frege, (1804), Metzger, (1827) and Tersánczki, (1865) who provided numerical data and scales for berry size and petiole length characterization., and later, Goethe, (1876) contributed significantly by proposing the use of the petiole sinus angle as a key parameter in ampelographic characterizations.

At the start of the 20th century, Ravaz, (1902) advanced the quantitative characterization of grapevine leaves by incorporating metric observations of venation and serration patterns. His work

focused on evaluating angles between veins and calculating ratios of vein lengths, providing a novel approach from both ampelographic and statistical perspectives. Notably, Ravaz introduced ten categories for the sum of the angles between veins, with the first category being $\leq 70^\circ$, the second 71° - 80° , and so on, up to the tenth category of 151° - 160° . Later, ampelometry was further developed by Seelinger, (1925) while Moog, (1930) expanded the descriptor list to provide a more comprehensive ampelometric characterization of rootstocks.

Following these foundational contributions, ampelography continued to evolve as an indispensable method for grapevine characterization. The authoritative ampelographic reference is the *Précis d'Ampélographie Pratique* introduced by Galet, (1952), later translated into English as *A Practical Ampelography: Grapevine Identification* (Galet, 1979).

This French ampelographer provided a comprehensive description by employing linear and angular traits, with the description of the ampelometric index which form the basis of traditional morphometry calling ampelometry: 'vine' + 'process of measuring', a method of measuring leaf features for most domesticated and many wild vines (Chitwood, 2021).

Galet, (1951) described several leaf shapes and representative species, such as cordiform (*Vitis cordifolia*, *Vitis cinerea*), cuneiform (*Vitis riparia*), truncate (*Vitis aestivalis*), orbicular (*Vitis vinifera* L. varieties 'Chenin blanc', 'Carignane', and *Vitis labrusca*), and reniform (*Vitis rupestris*).

In 2009, the International Organization of Vine and Wine, one of the leading institutions in the viticulture and oenology sector, compiled a comprehensive list of more than 150 descriptor traits for the purposes of characterization and identification (OIV, 2009).

These descriptors encompass a range of morphological patterns, molecular genetic markers, and phenological traits. Among the morphological traits, the list includes quantitative characteristics alongside linear and angular ampelometric properties (e.g., veins lengths, lateral sinuses length, angles size) and qualitative traits (e.g., color, shape).

In this code, each characteristic in this system is assigned a unique OIV number and classified into three or five categories, with its description provided through specific terms that correspond to these designated categories, which range from 1 to 9. For example, when evaluating a quantitative character such as the main vein (N1) coded OIV 601. It is indicated as very short (up to about 75 mm), short (about 105 mm), medium (about 135 mm), long (about 165 mm) and very long (about 195 mm and more) based on 10 leaves. From a statistical point of view, the 10 leaves could be measured individually and the average value of the 10 leaves' main vein lengths would be typical to the genotype

and this value would be classified as very short or short, etc. The other case is when evaluating a qualitative character such as the shape of blade coded OIV 067 which would be done by visual inspections of 10 leaves and the most frequent value (mode) would be typical to the genotype.

Ampelometry is not limited to analyzing leaf traits; it also involves the highly informative process of reconstructing leaf shapes. Martinez and Grenan, (1999) gave one of the most spectacular graphic reconstructions of the leaf. Building on this approach, Santiago et al. (2005a, 2005b), Martí et al., (2006), Boso et al., (2010), and Beleski and Nedelkovski, (2015) published detailed ampelometric descriptions based on this graphic reconstruction method.

So far, Laiadi et al., (2013) have realized the first and only ampelometric study of 36 grapevine cultivars maintained at the germplasm collection of Tighennif (Mascara). Additionally, the researchers performed the reconstruction of the average leaf of only the most representative varieties using quantitative and qualitative data gathered during their study.

Afterwards, Bounab and Laiadi, (2019), have conducted an ampelographic characterization to complete the previous using the maximum of ampelographic descriptors (108 OIV codes) to confirm the existing synonymies and possible relationships among the accessions in order to preserve the maximum amount of genetic variability for breeding and commercial purposes.

While ampelography provides an accessible and non-invasive means of identifying grapevine cultivars, it has inherent limitations (Carneiro et al., 2024). Environmental factors, such as climate and soil conditions, can affect morphological traits, making it difficult to consistently identify cultivars across regions (Van Heerden et al., 2018).

Additionally, the accuracy of ampelographic classification often depends on the expertise of the observer, which may introduce subjectivity into the process (Núñez et al., 2004). Despite these challenges, it remains an indispensable tool, particularly when combined with molecular approaches (Avramidou et al., 2023).

In recent years, advancements in technology have improved the accuracy and efficiency of ampelographic studies. Geometric morphometrics and AI approaches allow for precise measurement and classification of grapevine features, reducing the subjectivity of traditional methods. Such tools have been instrumental in automating the identification process and supporting large-scale biodiversity assessments of grapevine genetic resources.

Recently, Chitwood, (2021) revisited Galet's (1952) pioneering work on ampelometry, comparing its traditional morphological approach with modern geometric morphometric methods. The

study demonstrated that the investigated samples could be distinctly grouped into two categories based on sinus depth.

Various machine learning algorithms, such as Support Vector Machines (SVM) and k-Nearest Neighbors (KNN), have also been applied to grapevine classification, achieving success rates as high as 96% (Gutiérrez et al., 2018; Xu et al., 2021; Garcia et al., 2022; Abbasi and Jalal, 2024).

Additionally, deep learning models, including Convolutional Neural Networks (CNNs) and vision transformers, have been utilized for the classification of grapevine varieties based on leaf images. These advanced approaches have achieved remarkable accuracy rates ranging from 98% to 100% (Nasiri et al., 2021; Ahmed et al., 2022; Lv et al., 2023; De Nart et al., 2024).

Characterization of grapevines has today been complemented by the use molecular markers, providing a different set of data, which enables more accurate identification and extended characterization (Tomić et al., 2013). The introduction of molecular markers has allowed more accurate identification because they are not affected by environmental conditions, sample type or the developmental stage, and thereby provide distinctive and reliable information (Arslan et al., 2023).

DNA based markers have enabled a new approach to genetic characterization and to the assessment of diversity within an analyzed set of samples, which is important for evaluation of the range and distribution of genetic variability (Villano et al., 2022).

In grapevines, diverse marker techniques, such as RFLP (Bourquín et al., 1993), RAPD (Grando et al., 1995), AFLP (Sensi et al., 1996), SSR (Bowers et al., 1993) and, recently, SNP (Salmaso et al., 2005) have been widely used during recent decades.

Among them microsatellites, or SSR (simple sequence repeat) markers, have become molecular markers of choice, since they offer some advantages over other molecular markers as reported by several authors (Idrees and Irshad, 2014; Grover and Sharma, 2016; Hussain and Nisar, 2020; Sagar et al., 2023; Srivastava et al., 2023; Sharma et al., 2024).

The number of microsatellite loci available has greatly increased in the last few years largely through the establishment of the International Vitis Microsatellite Consortium, leading to the discovery of more than 350 new loci (Papanna et al., 2009). Microsatellite markers, being abundant, multiallelic, and highly polymorphic, provide an efficient and accurate means of detecting genetic polymorphism (Powell et al., 1996). Most importantly, their codominant nature makes them the markers of choice for population genetic analysis to assess genetic organization in germplasm collections (Chen et al., 2015).

In *Vitis*, a large number of markers have been developed by individual groups and these markers have been very successfully applied for genetic studies (Tomić et al., 2013). The suitability of *Vitis* SSR markers for assessing genetic origin and diversity in germplasm collections, cultivar identification, parentage analysis and for genetic mapping is well documented (Bibi et al., 2020; Margaryan et al., 2021; Cretazzo et al., 2022; Arslan et al., 2023; Zinelabidine et al., 2024).

The microsatellite analyses of *Vitis vinifera* L. cultivars from Algeria had begun about 15 years before (Laiadi et al., 2009). The earliest publications suggest the characterization of the genotypes based on morphological and microsatellite (Laiadi et al., 2013; Bounab and Laiadi, 2019), as well as the preservation efforts (Rahali et al., 2019; Khouni et al., 2023).

In the literature summary below (Table 1), a comprehensive and detailed review of some of the most relevant studies related to our research theme is presented. The summary provides clear information about the topics, methodologies, and findings of each study, highlighting the key points of each work.

Table 1: Summary of some publications related to the topic of grapevine ampelographic and/ or molecular characterization during the period (1999 to 2024), the studies were identified from Scopus, Web of Science, and Google scholar.

Author(s) and Year	Geographical Scope	Methodology	Key findings
Martinez and Grenan, (1999)	Northwestern Spain and Northern Portugal	<ul style="list-style-type: none"> • A step-by-step method for average leaf reconstitution based on fundamental parameter of the angles and length as well as notations on the qualitative characters. 	<ul style="list-style-type: none"> • Developed a detailed and systematic method for reconstructing average vine leaves, applicable across the studied varieties. • The reconstructed average leaf effectively captured distinctive traits, enabling variety identification and comparison.
Tomažič and Korošec-Koruza, (2003)	Slovenia	<ul style="list-style-type: none"> • A total of 71 phyllometric parameters were measured, including vein lengths, sinus dimensions, angles, and tooth dimensions. • Ratios and additional calculated parameters were analyzed to assess their stability across samples. • Cluster analysis (UPGMA) and K-means clustering were applied to group cultivars based on stable parameters. 	<ul style="list-style-type: none"> • Identified 25 stable parameters (e.g., angles and ratios) less influenced by environmental factors, making them reliable for cultivar differentiation. • Correctly grouped vines within the same cultivar using the reduced parameter set, improving classification accuracy. • Demonstrated the utility of angles and shape-based parameters (e.g., petiole sinus shape) over size-based metrics for consistent identification.

Snoussi et al., (2004)	Tunisia	<ul style="list-style-type: none"> • Nuclear SSR markers were used to analyze genetic diversity. • Chloroplast microsatellite loci (cpSSR3, cpSSR5, cpSSR10) were employed to evaluate haplotypic diversity. • Genetic parameters such as heterozygosity and allele frequencies were calculated. • Genetic differentiation was assessed using AMOVA and FST statistics. • Genetic similarity was visualized using UPGMA dendrograms. 	<ul style="list-style-type: none"> • Genetic diversity identified 55 distinct genotypes among the cultivated accessions, suggesting the presence of 60 cultivars due to variations in berry color. • Wild and cultivated accessions showed significant genetic differentiation. <p>Hybridization and self-pollination were key contributors to the development of local cultivars.</p> <ul style="list-style-type: none"> • Limited genetic similarity between cultivated and wild grapevines, indicating cultivated varieties were introduced rather than derived from local wild populations.
Santiago et al., (2005a)	Northwestern Spain and Northern Portugal	<ul style="list-style-type: none"> • Ampelographic evaluations through OIV descriptors based on both qualitative and quantitative variables. • Graphic reconstruction of mean leaf of the studied varieties based on the method described by Martinez and Grenan, (1999). 	<ul style="list-style-type: none"> • Five synonymies were confirmed between Spanish and Portuguese cultivars. • Cultivars were distinguished based on leaf shape, vein lengths, and angles as revealed by PCA. • Highlighted the need for preserving and characterizing the grapevine biodiversity in these regions.

Martí et al., (2006)	Spain	<ul style="list-style-type: none"> • Describe by ampelometric descriptors of OIV, (1983) the mature leaves of two red grape varieties. • Graphic reconstruction of mean leaf of the studied varieties based on the method described by Martinez and Grenan, (1999). 	<ul style="list-style-type: none"> • The results showed that most of the linear ampelometric features of ‘Parraleta’ and ‘Moristel’ varieties are significantly influenced by the year-to-year effect, while the angular traits and ratios of the linear features are less variable. • Their results highlighted the differences among the cultivars as ‘Moristel’ was more influenced by the effect of the year as ‘Parraleta’
Santiago et al., (2007)	Northwestern Spain and Northern Portugal	<ul style="list-style-type: none"> • Ampelographic characterization ‘Albariño’, ‘Savagnin Blanc’ and ‘Caíño Blanco’ cultivars based on various characteristics of shoots, adult leaves, clusters, berries, and seeds. • Molecular analysis of the studied cultivars using 6 microsatellite loci. 	<ul style="list-style-type: none"> • The study proved that Albariño, Savagnin Blanc, and Caíño Blanco are three distinct cultivars, both ampelographically and molecularly • Results found that Albariño and Caíño Blanco may be genetically related (shared some genetic markers). • Results showed that Albariño and Caíño Blanco likely share the same geographic origin in the northwestern Iberian Peninsula • This study provided the first comprehensive description of Caíño Blanco, which had limited previous documentation

Laiadi et al., (2009)	Algeria	<ul style="list-style-type: none"> • Genetic characterization 36 Algerian grapevine accessions maintained in the germplasm collection of Skikda (Algeria). • 12 nuclear microsatellite loci and 4 chloroplast loci were analyzed. • Evaluation of genetic diversity parameters like allele numbers, heterozygosity, and identity probabilities were estimated using software such as GENALEX and MEGA. 	<ul style="list-style-type: none"> • The study identified 27 unique genotypes among the 36 accessions, with some synonymous accessions. • Most Algerian cultivars displayed significant genetic diversity, comparable to Mediterranean grapevine accessions. • Genetic relationships suggested some accessions may originate from spontaneous hybridizations and seed propagation. • Close genetic ties among certain clusters reflect local and regional historical exchanges and hybridization events.
Riahi et al., (2010)	Maghreb region (Algeria, Tunisia and Morocco)	<ul style="list-style-type: none"> • Genetic diversity and differentiation were assessed using 20 nuclear microsatellite markers (nSSRs) of a total of 109 grapevine accessions were analyzed, including cultivated and wild vines. • Genetic diversity parameters (alleles per locus, heterozygosity) were calculated. • F-statistics (Fst) were used to measure population differentiation. 	<ul style="list-style-type: none"> • A total of 203 alleles were identified, with an average of 10.15 alleles per locus, showing high genetic diversity. • Cultivated populations exhibited lower differentiation (low Fst values), while wild and cultivated populations displayed significant genetic divergence. • Genetic clusters revealed distinct gene pools corresponding to geographic origins.

		<ul style="list-style-type: none"> • Parent-offspring relationships and genetic clusters were identified using Neighbor-Joining (NJ) tree analysis and related software tools (e.g., GENETIX, GENEPOP). 	<ul style="list-style-type: none"> • Cultivated accessions likely originated from historical introductions rather than direct domestication from local wild populations. • Parent-offspring relationships and synonyms were detected, reflecting historical exchanges of cultivars in the region.
Ateş et al., (2011)	Turkey	<ul style="list-style-type: none"> • Ampelographic characterization of 10 grapevine cultivars (6 autochthonous and 4 hybrid cultivars) using the international grape descriptor lists (IBPGR and its revision). • Measuring various characteristics of shoots, leaves, bunches and berries. • Using UPGMA cluster analysis to assess relationships between cultivars 	<ul style="list-style-type: none"> • The results demonstrated that ampelographic descriptors could effectively separate and identify different cultivars. • Identification of unique characteristics in several autochthonous varieties that were previously under documented. • UPGMA analysis revealed two main clusters at 0.42 similarity level, showing high genetic diversity. • Hybrid cultivars tended to group together in the cluster analysis. • The study helped document and preserve information about local Turkish grape varieties that were at risk of extinction.

Susaj et al., (2012)	Albania	<ul style="list-style-type: none"> • Ampelographic evaluation based on the International Descriptors for Grapevine (IPGRI). • 33 main characters were analyzed, including shoot and leaf morphology, flower type, bunch and berry characteristics, productivity indicators, and resistance to diseases. • Analyses: Observational, descriptive, and dispersive analyses were used to assess variations and establish averages over the study period. 	<ul style="list-style-type: none"> • The grapevine cultivar studied exhibits distinct characteristics in its shoot tips, leaves, and bunches, including medium-sized pentagonal leaves, small and medium-dense bunches, and berries that vary in size and color. • The cultivar demonstrates good yield potential, with moderate juice extraction, high sugar content, and balanced acidity levels in the grape must. • While the leaves are susceptible to certain diseases, the berries show strong resistance to key fungal pathogens, contributing to the cultivar's resilience during fruiting stages. • The cultivar requires cross-pollination with other compatible cultivars for optimal fruit set due to its specific floral characteristics.
Laiadi et al., (2013)	Algeria	<ul style="list-style-type: none"> • Ampelometric measurements of 26 native grapevine varieties maintained in the germplasm collection (Mascara, Algeria) based on the phyllometric method proposed by 	<ul style="list-style-type: none"> • Significant diversity in leaf characteristics (e.g., angles, sinus depth, vein lengths) was observed among the studied varieties.

		<p>Martínez and Grenan, (1999) was used to analyze the leaves quantitatively.</p> <ul style="list-style-type: none"> • Statistical analysis: Principal Component Analysis (PCA) and hierarchical clustering were performed to identify distinguishing characteristics among the varieties. • Average leaf shapes were reconstructed for representative varieties based on quantitative and qualitative data. 	<ul style="list-style-type: none"> • Three main clusters of cultivars were identified, each reflecting similarities in specific traits. • The study highlights the need for further research to explore additional qualitative and quantitative parameters for comprehensive characterization.
Alba et al., (2014)	Italy	<ul style="list-style-type: none"> • Morphological analysis of 26 table grape genotypes using 47 qualitative traits and 23 ampelometric of mature leaves based on OIV, (2009) descriptors. • PCA was used to identify key traits contributing to variability. • Molecular characterization using six microsatellite loci to create genetic profiles and dendrograms for genetic similarity. • Cluster analysis distinguished genotypes based on both morphological and genetic data. 	<ul style="list-style-type: none"> • Significant morphological and genetic variability was observed among the 26 genotypes. • A smaller set of morphological descriptors and two microsatellite markers were sufficient to distinguish all genotypes effectively. • Key traits, such as main vein lengths, petiolar sinus angles, and vein length ratios, were identified as crucial for differentiation. • The study highlights the potential to streamline grapevine biodiversity studies

		<ul style="list-style-type: none"> • Efficiency goal: The study evaluated the possibility of reducing the number of descriptors and markers for streamlined characterization. 	using fewer descriptors and markers, making large-scale characterizations more efficient.
Lamine et al., (2014)	Tunisia	<ul style="list-style-type: none"> • Morphological characterization of the 61 autochthonous grapevine genotypes was performed using 70 descriptors, including shoot, leaf, and fruit traits, based on the OIV standard. • PCA and Hierarchical Cluster Analysis (HCA) were employed to assess phenotypic diversity and identify significant traits contributing to variability. • Analysis of Variance (ANOVA) was used to partition morphological variation within and between groups. • Regression and correlation analyses confirmed the integration of shoot, leaf, and fruit descriptors for classification purposes. • Commercialization assessment: The potential use of genotypes as table or wine grapes was 	<ul style="list-style-type: none"> • Significant phenotypic variation exists within Tunisian autochthonous grapevines, with most of the diversity found within groups rather than between them. • Accessions were successfully clustered into groups based on shared morphological traits, revealing a clear differentiation between wine and table grape varieties. • The findings emphasize the importance of conserving these diverse genetic resources, which are valuable for breeding programs and adaptation to environmental stresses. • Certain genotypes were identified as promising for commercial use, particularly as table grapes, due to their favorable traits such as high sugar content and desirable fruit characteristics.

		evaluated based on characteristics like bunch weight, berry size, sugar content, and acidity.	
Popescu et al., (2015)	Romania	<ul style="list-style-type: none"> • Morphological characterization of nine red and white autochthonous grapevine varieties using standardized descriptors from the OIV, (2009). • Data were analyzed using ANOVA and Duncan's test to identify significant differences among the varieties. 	<ul style="list-style-type: none"> • Red wine varieties showed distinct traits such as heavier berries. However, white wine varieties displayed traits such as larger bunch sizes and higher sugar content. • Significant variability in sugar content and acidity was observed, with autochthonous varieties generally outperforming reference cultivars in specific traits. • Some varieties demonstrated stable yields and consistent quality over the years despite changing environmental conditions.
Benito et al., (2016)	Spain	<ul style="list-style-type: none"> • Ampelographic characterization of 192 wild grapevine accessions using descriptors from the OIV, (2007). • Morphological data were analyzed using correspondence analysis and hierarchical clustering to identify key traits that differentiate wild and cultivated vines. 	<ul style="list-style-type: none"> • Some traits, including berry and bunch size, anthocyanin coloration, and seed shape, were identified as key discriminators between wild and cultivated grapevines. • The study highlights the importance of conserving wild grapevines as a valuable genetic resource for breeding programs and climate change adaptation.

		<ul style="list-style-type: none"> • Predictive models were developed to classify accessions based on significant morphological traits. 	<ul style="list-style-type: none"> • The findings support the use of combined morphological and molecular tools for accurate identification and preservation efforts.
Khalil et al., (2017)	Syria	<ul style="list-style-type: none"> • Ampelographic evaluations based on 42 qualitative and quantitative traits. • Using multivariate statistical analyses (PCA, stepwise-LDA, UHCA) to identify the most discriminant traits. • Genetic analysis using 9 nuclear SSR markers to confirm cultivar distinctness. 	<ul style="list-style-type: none"> • Identified five highly discriminant traits for characterizing grape cultivars: shoot internode length, berry weight, berry elongation, 100-seed weight, and juice titratable acidity. • Local Syrian cultivars showed similarities to internationally grown cultivars, suggesting potential for developing new varieties. • The study highlighted the importance of preserving local Syrian grape genetic resources. • Demonstrated an effective statistical approach for selecting key ampelographic traits to discriminate between cultivars.

Labagnara et al., (2018)	Italy	<ul style="list-style-type: none"> • A total of 85 plant accessions were characterized through ampelographic analysis based on few main ampelometric characters. • Mature leaves from accessions were analyzed using SuperAmpelos software for detailed ampelometric measurements. • PCA was used to identify key traits for genotype differentiation. • Nine microsatellite loci were used to characterize genetic profiles. Comparison with national and international grapevine databases was performed. • Genetic diversity metrics such as allele frequencies, heterozygosity, and probability of identity were calculated. • Cluster analysis and dendrograms were created to explore genetic relationships. 	<ul style="list-style-type: none"> • Out of 85 collected accessions, 42 unique genetic profiles were identified, including 9 new autochthonous genotypes described for the first time. • Ampelometric traits such as vein ratios and angles proved effective for genotype differentiation, emphasizing their importance in grapevine characterization. • High levels of genetic diversity were observed, especially in specific regions like Maratea, which exhibited the most variability. • The study underscores the need to preserve rare and endangered grapevine genotypes, integrating them into germplasm collections like CREA-VE in Arezzo, Italy, for future agronomic and enological evaluations.
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Bounab and Laiadi, (2019)	Algeria	<ul style="list-style-type: none"> • Ampelographic characterization focused on 36 grapevine varieties from the Algerian germplasm collection (Skikda, Algeria) during three-year study period (2015-2017). • Using 108 ampelographic criteria based on OIV descriptors: 44 quantitative and 64 qualitative parameters. • Statistical analysis: PCA, cluster analysis using Euclidean distance and UPGMA method with Jaccard similarity coefficient. 	<ul style="list-style-type: none"> • Results confirmed synonymies between several grape varieties previously identified by Laiadi et al., (2009). • Most significant discriminating factors: length of veins in mature leaves, bunch characteristics (length and weight), density of hairs on young shoots and leaves, berry and seed dimensions. • Results can be used for: Commercialization, breeding programs, evaluation of economically valuable characteristics of Algerian autochthonous grapevine cultivars.
Rahali et al., (2019)	Algeria	<ul style="list-style-type: none"> • A total of 37 grapevines sampled from Babar (Khenchela, Algeria) were analyzed using 12 nuclear microsatellite (SSR) markers. • Genetic profiles were compared with international databases (e.g., CREA and IVVC). 	<ul style="list-style-type: none"> • Thirteen unique genotypes were identified, including three novel autochthonous varieties proposed as 'Babar-Algeria,' 'Amesski-Babar,' and 'Babari,' potentially unique to the Babar region.

		<ul style="list-style-type: none"> • Principal Coordinate Analysis (PCoA) was applied to assess genetic diversity and relationships among genotypes. 	<ul style="list-style-type: none"> • High genetic diversity was observed, with some genotypes closely related to Mediterranean and international cultivars. • The study highlights the need for urgent conservation of these rare and endangered genotypes as valuable resources for breeding, clonal selection, and biodiversity preservation.
Boso et al., (2020)	Spain	<ul style="list-style-type: none"> • Morphometric analysis of grapevine seeds from archaeological sites in Galicia, northwestern Spain, specifically from Roman and Medieval periods. • Measurements included length, width, and shape-related indices of seeds. • Key ratios (e.g., width-to-length, neck width) were calculated to distinguish between wild, cultivated, and archaeological seeds. • ANOVA and PCA were performed to analyze morphometric variation and cluster seeds by morphological traits. 	<ul style="list-style-type: none"> • The study supports the theory that domestication occurred independently in multiple regions, with Galicia being a possible local center of grapevine domestication. • The presence of grapevine seeds in archaeological contexts highlights the importance of viticulture in Roman and Medieval Galicia and its role in shaping the region's agricultural history.

Chitwood, (2021).	USA	<ul style="list-style-type: none"> • A landmark-based approach was employed using 24 homologous points on the leaves, capturing major veins, sinuses, and secondary vein branching. • Pseudo-landmarks were added between primary landmarks to capture the intricate details of curves and serrations. • Generalized Procrustes Analysis (GPA) was applied to align leaf shapes and create mean shapes for each variety. • PCA and Linear Discriminant Analysis (LDA) were used to classify varieties based on shape features. • The methods were compared with the ampelometric system of Galet, (1952) which relies on vein length ratios and angles. 	<ul style="list-style-type: none"> • Leaves were categorized into two major groups: deeply lobed and slightly lobed (entire), with sinus depth emerging as a critical feature for classification. • Using a high number of landmarks and pseudo-landmarks allowed for better capture of fine-scale leaf features, improving the accuracy of variety prediction. • The Procrustes method yielded higher classification accuracy compared to traditional landmark-only or Galet-inspired approaches. • The methods developed offer robust tools for documenting and analyzing grapevine leaf morphology, which is critical for biodiversity preservation and agricultural practices.
Margaryan et al., (2021)	Armenia	<ul style="list-style-type: none"> • Used 25 nSSR markers for genotypic characterization; combined with ampelography for phenotypic studies. 	<ul style="list-style-type: none"> • Identified 221 unique grapevine varieties (66 autochthonous, 49 new Armenian cultivars, 34 foreign).

		<ul style="list-style-type: none"> Genetic diversity, clustering, and parentage analysis. 	<ul style="list-style-type: none"> High genetic variability and heterozygosity observed due to historical introgression of wild species (<i>Vitis sylvestris</i>) into cultivated grapevines. Parentage Analysis identified 62 trios (mother-father-offspring) and 185 half-kinships, revealing extensive genetic relationships.
Cehade et al., (2022)	Lebanon	<ul style="list-style-type: none"> Molecular characterization of 43 grapevine accessions using 9 ISSR (Inter Simple Sequence Repeat). Ampelographic characterization based on 33 morphological traits (OIV, 2009), including leaf, bunch, and berry characteristics, PCA was employed to identify the most discriminating traits. HCA was used to group accessions based on genetic and morphological similarities. A Mantel test assessed the correlation between molecular and morphological clustering. 	<ul style="list-style-type: none"> High genetic diversity was observed among accessions, with 41 unique genetic profiles identified and only one case of synonymy detected. ISSR markers demonstrated their efficiency in differentiating grapevine accessions. Significant variation was observed in traits such as berry shape, bunch weight, and veraison (ripening) dates. PCA identified 12 key traits, including veraison and maturity dates, berry

			<p>dimensions, and bunch density, as the most discriminating.</p> <ul style="list-style-type: none"> • The study underscores the importance of conserving Lebanon's diverse grapevine germplasm, which holds potential for breeding programs and the production of high-quality table and wine grapes.
Gago et al., (2022)	Spain	<ul style="list-style-type: none"> • Ampelographic characterization of 7 grapevine varieties using the OIV descriptors of adult leaves, bunches, and berries. • PCA was used to identify key traits for differentiating genotypes. • Cluster analysis grouped varieties based on leaf morphology, bunch structure, and berry characteristics. • The study linked the morphological traits of each variety to historical records and local viticultural practices to identify synonymies and homonymies. 	<ul style="list-style-type: none"> • The seven genotypes showed significant diversity in leaf, bunch, and berry characteristics, reflecting their historical and geographical origins. • Key traits like berry size, bunch compactness, and leaf sinus depth were critical for distinguishing genotypes. • Several varieties, such as Esclafagerres and Morsí, were linked to historical records, with synonymies and homonymies resolved for more accurate identification. • The study underscores the importance of preserving these rare genotypes to prevent genetic erosion.

Tsivelikas et al., (2022)	Greece	<ul style="list-style-type: none"> • 76 OIV descriptors were used to record leaf, berry, and bunch traits from 96 genotypes representing indigenous Greek landraces. • 13 highly polymorphic microsatellite markers (SSRs) were employed to assess genetic diversity and population structure. • Model-based clustering and UPGMA hierarchical dendrograms were created to identify genetic relationships. • Genetic diversity metrics, including heterozygosity and polymorphic information content (PIC), were calculated. 	<ul style="list-style-type: none"> • High genetic diversity was observed, with a mean of 14.69 alleles per SSR locus and a PIC average of 0.848. • The cultivars displayed significant morphological diversity, particularly in traits related to leaf hair density, berry size, and color. • The cultivars were grouped into three primary clusters, corresponding to eco-geographic groups. • The findings underscore the importance of conserving Greek grapevine germplasm as a valuable genetic resource for breeding programs and sustainable viticulture.
Avramidou et al., (2023)	Greece	<ul style="list-style-type: none"> • 81 OIV descriptors were used for phenotypic characterization of 51 grapevine genotypes, focusing on morphological traits of leaves, berries, and bunches. • Cluster analysis using the Manhattan dissimilarity index was applied to construct dendrograms of cultivar relationships. 	<ul style="list-style-type: none"> • High genetic diversity was observed, with 113 alleles amplified across 13 SSR loci (average of 10.23 alleles per locus). • Most genotypes clustered into cultivar-specific groups, validating the distinctiveness of local Cretan cultivars.

		<ul style="list-style-type: none"> • 13 SSR loci were analyzed for genetic fingerprinting. • Genetic diversity and relationships were assessed through UPGMA clustering and AMOVA, with metrics such as allele frequency, heterozygosity, and probability of identity calculated. • The congruence between ampelographic and SSR-based clustering was tested using the Mantel test, comparing morphological and genetic classifications. 	<ul style="list-style-type: none"> • Differences between ampelographic and genetic clustering highlight the value of using both methods to resolve synonymy and homonymy in grapevine classification.
Güler and Karadeniz, (2023)	Turkey	<ul style="list-style-type: none"> • 37 grapevine genotypes were selected based on morphological characterization based on traits such as bunch weight, berry dimensions, and stalk length. • Two-way ANOVA assessed trait variability by genotype and year. • PCA and hierarchical clustering grouped genotypes based on morphological traits. • Correlation analysis visualized relationships between traits using heatmaps. 	<ul style="list-style-type: none"> • Significant variability was observed in bunch and berry traits, including weight, dimensions, and color properties. • PCA and clustering analyses revealed distinct groups, with berry color (green/yellow, black, rose) being a significant determinant in genotype differentiation.

			<ul style="list-style-type: none"> • The study highlights the potential of these autochthonous genotypes for breeding programs and local grape cultivation.
Khouni et al., (2023)	Algeria	<ul style="list-style-type: none"> • Molecular characterization of 81 grapevine accessions from the germplasm collection (Skikda, Algeria) using 12 SSR markers. • Genetic profiles were compared with databases such as <i>ITVC</i> and <i>CREA</i> Viticulture and Enology. • Genetic diversity metrics (e.g., heterozygosity, allele counts) were calculated. • Synonyms, homonyms, and misnaming were identified through cluster and comparative analyses. 	<ul style="list-style-type: none"> • A total of 43 unique genotypes were identified among the 81 accessions, highlighting significant genetic variability. • Observed and expected heterozygosity values were high (0.864 and 0.850, respectively), indicating the genetic richness of the collection. • Several synonymies and mislabeling issues were resolved, such as identifying overlaps between local and international varieties. • The study underscores the importance of conserving endangered autochthonous varieties and using them for breeding programs.
Mirfateh et al., (2024)	Iran	<ul style="list-style-type: none"> • Morphological characterization of 84 grapevine cultivars based on 69 traits (34 quantitative and 35 qualitative) using descriptors from the OIV, IPGRI, and UPOV. 	<ul style="list-style-type: none"> • Traits such as bunch weight, berry size, and trichome density showed significant variability, highlighting genetic diversity.

		<ul style="list-style-type: none"> • Traits included phenological parameters (e.g., leafing, flowering, and ripening time) and morphological features (e.g., bunch weight, berry size, and trichome density). • Coefficient of variation and cluster analysis (using Ward's method) were applied to group cultivars and identify trait variability. • Pearson correlation and factor analysis were used to study trait interrelations and reduce data complexity. 	<ul style="list-style-type: none"> • The study underscores the potential of these diverse grapevine resources for breeding programs, particularly for drought tolerance and fruit quality. • Findings contribute to the preservation and improvement of local cultivars for sustainable viticulture in Iran.
Mahmoud et al., (2023)	Egypt	<ul style="list-style-type: none"> • Morphological characterization of 10 local Egyptian grapevine cultivars based on 58 attributes of the vine, including shoot, leaf, bunch, and berry traits, using international descriptors (IPGRI, UPOV and OIV). • Nine nuclear SSR markers were used for genotyping. • Phylogenetic relationships were analyzed using the UPGMA method. 	<ul style="list-style-type: none"> • Significant variability was observed among the cultivars in morphological and genetic traits. • SSR markers revealed 24 alleles, with polymorphic information content averaging 0.43, indicating moderate genetic diversity. • Key traits like berry size, shape, and seed presence showed high variability, aiding in cultivar distinction. • Cultivars were grouped into two main clusters based on morphological and genetic

		<ul style="list-style-type: none"> • Correlation matrices, similarity coefficients, and clustering analyses were applied to compare morphological and genetic data. 	data, reflecting their geographical origins and phenotypic traits.
Zinelabidine et al., (2024)	Morocco	<ul style="list-style-type: none"> • Genetic characterization of 60 table grape accessions preserved in the living grapevine collection using 13 SSR and 240 SNP markers. • Genetic profiles were compared with international databases such as the <i>Vitis</i> International Variety Catalogue (IVVC) and the ICVV-DNA database. • Parentage analysis was performed to identify maternal lineages. • Pairwise comparisons, clustering, and likelihood ratio (LOD) analysis determined the genetic relationships and variety identities. 	<ul style="list-style-type: none"> • The study identified 40 unique genetic profiles among the 60 accessions, with 38 matching known varieties and two previously undocumented genotypes. • Several cases of mislabeling, misspellings, and synonyms were resolved, such as distinguishing between local Moroccan varieties and international synonyms. • The study highlights the importance of accurately identifying and managing grapevine genetic resources to support conservation and sustainable viticulture.
Carneiro et al., (2024)		<ul style="list-style-type: none"> • The study is a systematic review of deep learning (DL) and machine learning (ML) techniques applied to grapevine variety identification. • It includes 37 studies conducted globally, with datasets primarily from Portugal, Turkey, and other grapevine-growing regions. 	<ul style="list-style-type: none"> • DL methods outperform ML approaches in classification accuracy and consistency. • Studies using DL achieved up to 100% accuracy with advanced architectures like EfficientNet and Vision Transformers.

	<ul style="list-style-type: none">• The review evaluates ML and DL-based methods for classifying grapevine varieties using plant images, spectra, and hyperspectral imaging.• Studies were selected using databases like Scopus and Web of Science, covering the period 2018–2024.• Classification pipelines, including data acquisition, preprocessing, model training, and evaluation metrics, were systematically analyzed.• Performance metrics for ML and DL methods, including accuracy, F1 score, and AUC, were rescaled for uniformity across studies.	<ul style="list-style-type: none">• ML techniques, such as SVM and ANN, were effective for smaller datasets or spectral data but generally underperformed compared to DL models.• Most datasets focused on grapevine leaves, with limited representation of fruits, seeds, or mixed traits.• Public datasets were sparse and lacked diversity, emphasizing the need for larger and more balanced datasets for real-world applicability.• DL approaches excel in high-throughput, automated identification tasks but require extensive, high-quality training data.• Challenges include addressing environmental variability, ensuring model generalizability, and resolving issues with underrepresented grapevine varieties.
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Bibliometric analysis is a method used to track the development and growth of a specific discipline or field within a specified time frame.

It also reveals emerging themes within the field and how these themes evolve into a structured framework (Kumar et al., 2023; Koç, 2024; Bahar et al., 2024). By examining key characteristics in the literature, one can gain insights into the changes and trends within the field. Data such as the number of publications, authors, journals, geographical distribution, publication types, and titles are analyzed, providing valuable clues about the field's development.

The bibliometric investigation aimed to delve into the scholarly influence and impact of research articles about the domain of grapevine ampelography and molecular characterization. When the literature is examined, studies from Scopus (<https://www.scopus.com/home.uri>), Web of Science (<https://www.webofscience.com/wos/>), and Google scholar (<https://scholar.google.com/>) were identified and only 'Articles' were selected as the study type, and 'English' as the language.

VOSviewer stands out as a powerful tool for visualizing bibliometric maps and conducting cluster analyses (Van Eck and Waltman, 2010). Therefore, for this study, this bibliometric software (version 1.6.20) has been selected as primary tool. Notably, VOSviewer provides diverse visualization tools, including co-occurrence maps, term maps, and network maps (Arruda et al., 2022). These visual tools enable researchers to identify prominent research areas, map author collaboration networks, and trace the progression of research topics over time.

The use of VOSviewer as a bibliometric tool to systematically analyze the literature provides several benefits, including a comprehensive literature analysis that allows us to conduct unprecedented scope investigations (Bekiri, 2023).

The bibliometric analysis map (Figure 1) lists the most frequently used keywords in the literature over the past two decades (1999 to 2024), which are already analyzed in Table (1). The frequency with which a term appears alongside other terms is referred to as co-occurrence, with the color indicating the trend of the research theme of the documents analyzed. The size of the circle is positively correlated with the occurrence of keywords in the title and abstract.

The temporal progression from **blue** nodes (showing early ampelographic studies) to **yellow** nodes (representing advanced genetic analysis and cultivar relationships) demonstrates how the field has advanced. Within this color-coded timeline, we can trace how grapevine research has evolved from basic descriptive work to sophisticated genetic and diversity analyses. Accordingly, 'Diversity' appears as the central keyword and highly interconnected with other terms. This indicates a dominant

Moreover, the clusters formed in the network suggest thematic divisions in the literature. For instance, one cluster revolves around ampelographic analysis, with terms like ‘ampelographic description’, ‘leaf’, ‘grapevine variety,’ and ‘discrimination’, emphasizing the morphological evaluation of grapevines. Another cluster focuses on genetic studies, featuring keywords like ‘SSR’, ‘loci’, ‘genetic variability’, and ‘marker’, showcasing the extensive application of molecular tools in grapevine research.

Figure 1: Bibliometric analysis of publications related to the topic of grapevine ampelographic and molecular characterization during (1999-2024) using Vosviewer: co-occurrence keywords network.

2. Current status of grapevine cultivation in Algeria

The current distribution of Algerian vineyards is the result of a long and complex history, which results from a continuous mixture of people and civilizations (Laiadi et al., 2009). According to Caïd et al., (2019), the history of Algerian grape growing can be divided into two main periods. The first dates back to ancient times, first Phoenician and then Roman. The second, longer and more significant, is that of French colonization.

When the phylloxera (*Daktulosphaira vitifoliae*) ravaged Europe's vineyards at the end of the 19th century (Planchon, 1874), Algeria was considered a prime viticultural area (Pedigo, 2015). All this started with the collapse of vineyards in France, which triggered massive vineyard investments in Algeria in the 1880s (Meloni and Swinnen, 2018).

Algerian viticulture is the fourth largest perennial crop in terms of surface area and is the second most important export product after dates (Bachir, 2023). According to statistics from the Ministry of Agriculture, Rural Development, and Fisheries (M. A. R. D., 2019), grape production in Algeria was 549 833 tons (Table 2). However, there are no reliable statistics that break down grape production by variety. About 91.6% of Algerian production is destined for the fresh market (table grapes) and dried market (raisins), while 8.38% of viticultural production is used for wine production.

Table 2: Evolution of viticultural sector during the years 2018 and 2019 (M. A. R. D., 2019).

	2018			2019			Rate of increase		
	Area	Production	Yield	Area	Production	Yield	2019/2018		
	ha.	q.	q/ha	ha.	q.	q/ha	Area	Production	Yield
Vineyards	66 264	5 709 718	86.2	61 676	5 498 329	89.1	-7	-4	3
Wine	23 577	721 694	30.6	20 294	460 933	22.7	-14	-36	-26
Table grapes	42 656	4 987 524	116.9	41 382	5 037 396	121.7	-3	1	4
Raisins	31	500	16.4	0	0	0	-100	-100	-100

Algeria boasts a large number of native varieties cultivated mainly in mountainous areas (Isnard, 1951). These autochthonous varieties represent a crucial genetic resource, offering potential resilience against diseases and climate change.

Although, vineyards in production are relatively old and are generally managed extensively, with minimal interventions (Toumi, 2006). Production is highest in the central region, which accounts for about 75% of the total output, with approximately 25% coming from the western region, and very little from the eastern part of the country (Khouni, 2023).

Unfortunately, nowadays Algerian viticulture faces several problems, many of these varieties are unknown and destroyed without understanding their importance for the local grape genetic heritage (Khouni et al., 2023).

Several factors had led to a substantial decrease in grapevine diversity, resulting in significant genetic erosion of the gene pool (This et al., 2006). The emergence of the commercial high-yield cultivars grown worldwide such as ‘Chardonnay’, ‘Cabernet Sauvignon’, ‘Syrah’ and ‘Merlot’ (Cardinal) are increasing due to the prevalent market preference for international grape varieties. This transition towards foreign cultivars has led to the decline or even disappearance of old local cultivars (Güler and Karadeniz, 2023).

The presence of synonyms and homonyms further compounds the challenge of identifying minority cultivars (Tympakianakis et al., 2023). On the other hand, sanitary selection of healthy disease-free clones has also induced an erosion in clonal diversity for these major cultivars around the world (Boso et al., 2023).

Interestingly, the knowledge of the existing genetic diversity in vineyards is considered a priority when addressing its conservation and valorization. In order to overcome this situation, germplasm banks have played an important role in the conservation of grapevine diversity (This et al., 2006; Maghradze et al., 2010). Numerous studies on the characterization maintaining of cultivars in germplasm banks are being carried out worldwide (Lopes et al., 1999; Aradhya et al., 2003; Núñez et al., 2004; Yuste et al., 2006; El-Oualkadi et al., 2009; Buhner-Zaharieva et al., 2010; Emanuelli et al., 2013; Maul et al., 2015; Popescu and Crespan, 2018; de Oliveira et al., 2020; Zombardo et al., 2021; Arslan et al., 2023; Zinelabidine et al., 2024).

The ITAFV (Institut Technique de l'Arboriculture Fruitière et de la Vigne) collection of autochthonous varieties constitutes an important reference for the genetic diversity of grapevines in Algeria, preserving numerous autochthonous cultivars, including major and minor ones (Laiadi et al., 2009; Khouni et al., 2023).

This genetic repository plays a crucial role in ongoing efforts to improve grapevine varieties and ensure the sustainability of Algerian viticulture in the face of environmental and economic challenges.

3. The Aures region and its grapevine diversity

The Aures region, located in northeastern Algeria, is a mountainous area characterized by its hilly relief and unique ecosystem features, shaped by geographic isolation and historical inaccessibility

to foreign influences (Rahali et al., 2019). This isolation has allowed the area to preserve its natural environment and biodiversity over centuries.

The Aures is home to a variety of plant species that are either local or particularly well-adapted to its rugged terrain and climate, making it a significant region for studying the ecological, botanical and genetic diversity of Algeria (Lazarova et al., 1988; Beghami et al., 2013; Abdessemed, 2017; Taib et al., 2020).

In fact, in this mountainous region there are opportunities to find old, original local forms, and studying these forms may reveal the existence of great genetic diversity. This is why these forms must be collected, preserved and characterized (Lazarova et al., 1988).

Among its many agricultural treasures, the Aures region boasts a remarkable diversity of grapevine cultivars, which have been cultivated for centuries (Isnard, 1951). This diversity is a reflection of the region's varied microclimates and traditional farming methods, which have allowed numerous grapevine varieties to thrive (Rahali, 2020).

The grapevines of the Aures are not only important for their agricultural value but also for their genetic diversity, which is essential for the sustainability and resilience of viticulture in the face of environmental challenges. This rich grapevine diversity plays a significant role in the local economy and cultural identity of this historical region.

However, Abdelguerfi and Laouar, (1998) noted that grapevines, particularly in mountain regions such as the Aures and other similar areas, have been largely neglected in terms of cultivation and conservation efforts. Indeed, in the Aures region (Batna), there is no interest in viticulture, only 91 hectares of vineyards exist (M. A. D. R., 2019).

These vineyards have been inherited by local families over generations, and today, the remnant plants are found near single houses and are cultivated using primitive methods (Rahali et al., 2019).

The preservation and study of grapevine diversity in the Aures region are crucial for maintaining its viticultural heritage and contributing to the broader conservation of agricultural genetic resources. Therefore, these diverse grapevine varieties should be transferred to germplasm collection vineyards to ensure biodiversity conservation and support future breeding programs (Khouni et al., 2023).

To date, surveying and recovery efforts in unexplored areas such as the Aures remain limited, reflecting the broader lack of attention to viticultural biodiversity in this region. The scarcity of studies and literature on the existing diversity of table grape varieties poses significant challenges in understanding and inferring their genetic and morphological characteristics.

Interestingly, Rahali et al., (2019) conducted the first exploration of grapevine varietal diversity in Babar (Khenchela, Algeria), a region geographically adjacent to Batna province. Their investigation allowed the identification of thirteen distinct genotypes, including the discovery of three novel cultivars unique to Babar region.

Despite this effort, the viticultural biodiversity of the Aures remains largely unexplored and undescribed, both ampelographically and genetically, highlighting the urgent need for further investigation and characterization.

Part 2

Ampelographic characterization of grape varieties (*Vitis vinifera* L.) grown in the Aures region.

Chapter 1

Material and Methods

1. Material and methods

1.1. Plant sampling

A total of 35 table grapevine cultivars were sampled for ampelographic analysis (Appendix 1). These grapevines were collected from traditional vineyards or home gardens maintained by local residents (Chaoui people). The cultivars were identified using names provided by the locals, often based on color, size, or shape. For those that were previously unidentified, we assigned them the names most commonly used by the inhabitants or based on the location where they were found (Table 3).

Table 3: List of cultivars sampled for ampelographic characterization.

Cultivar Number	Grapevine sample name	Name meaning in Amazigh language	Growth location
1	Ait Abdi	Reflecting to the discovery site	Bouzina, Batna
2	Anonymous 1	Not identified	Ichmoul, Batna
3	Anonymous 2	Not identified	Bouzina, Batna
4	Anonymous 3	Not identified	Ichmoul, Batna
5	Anonymous 4	Not identified	Ichmoul, Batna
6	Anonymous 5	Not identified	Ichmoul, Batna
7	Anonymous 6	Not identified	Ichmoul, Batna
8	Anonymous 11	Not identified	Bouzina, Batna
9	Anonymous 12	Not identified	Ichmoul, Batna
10	Amellal 1	White color	Ichmoul, Batna
11	Amellal 2	White color	Ichmoul, Batna
12	Amer bouamar	Name given by local Inhabitants	Bouzina, Batna
13	Ameziane	Small size	Ichmoul, Batna
14	Bouabane	Name given by local Inhabitants	Bouzina, Batna
15	Laadari	Name given by local Inhabitants	Bouzina, Batna
16	Meska 1	Aromatic grape	Ichmoul, Batna
17	Meska 2	Aromatic grape	Ichmoul, Batna

18	Tabarkante 1	Black color	Ichmoul, Batna
19	Tabarkante 2	Black color	Ichmoul, Batna
20	Tasemith	Acid flavor	Ichmoul, Batna
21	Tazizaouth 1	Green color	Ichmoul, Batna
22	Tazizaouth 2	Green color	Ichmoul, Batna
23	Tazizaouth 3	Green color	Ichmoul, Batna
24	Tazizaouth 4	Green color	Ichmoul, Batna
25	Tazizaouth 5	Green color	Ichmoul, Batna
26	Tazizaouth 6	Green color	Ichmoul, Batna
27	Tazizaouth 7	Green color	Ichmoul, Batna
28	Tazizaouth 8	Green color	Ichmoul, Batna
29	Tazizaouth 9	Green color	Ichmoul, Batna
30	Tazizaouth 10	Green color	Ichmoul, Batna
31	Tazizaouth 11	Green color	Ichmoul, Batna
32	Tazogaghth 1	Pink/ red color	Ichmoul, Batna
33	Tazogaghth 2	Pink/ red color	Ichmoul, Batna
34	Tazogaghth 3	Pink/ red color	Ichmoul, Batna
35	Tazogaghth 4	Pink/ red color	Ichmoul, Batna

All these cultivars are unexplored and traditionally planted in 2 different municipalities in the province of Batna in the North-East of Algeria which are geographically distant mountainous grape growing (Ichmoul and Bouzina), to maximize the possibility of diversity among samples (Figure 2).

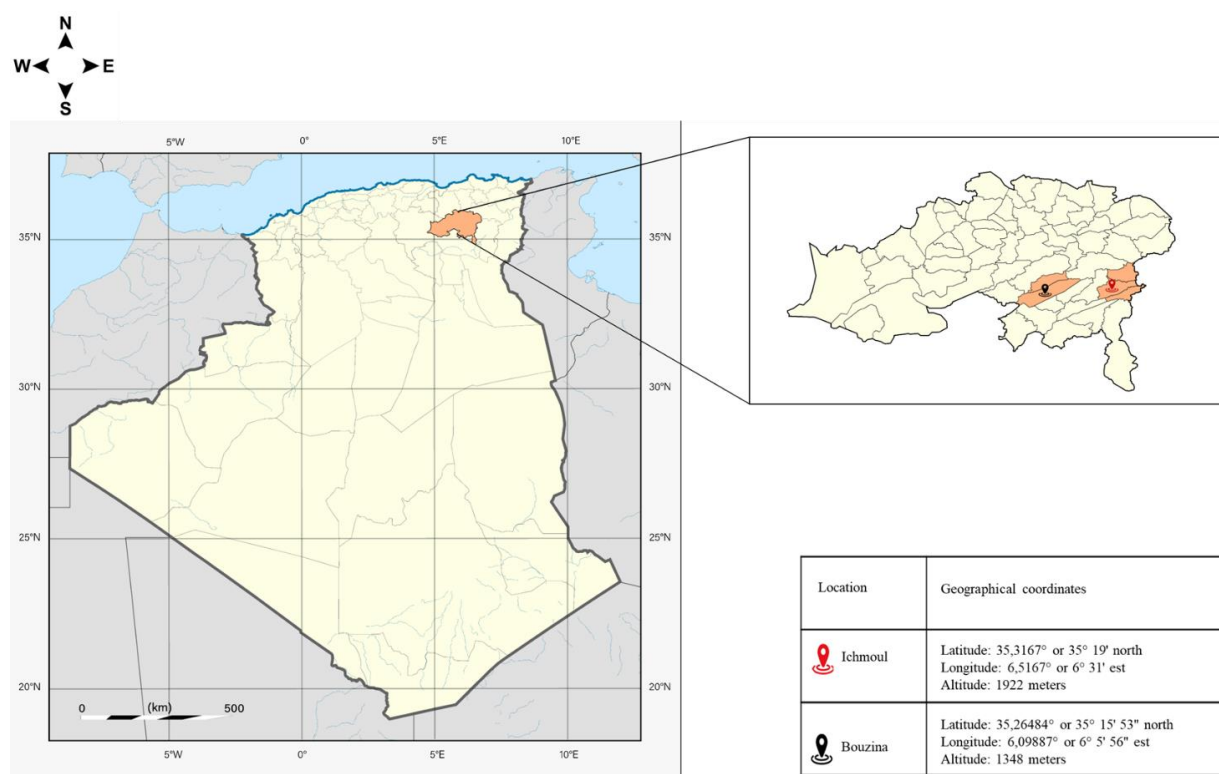


Figure 2: Geographical location of Batna province (Algeria) where the grapevine samples included in the study were recovered.

The study was conducted in the highland region of Batna (Aurès Mountains), specifically within the communes of Ichmoul (~1922 m) and Bouzina (~1348 m) (<https://mapcarta.com/fr>). It is a mountainous area at the foot of the Aures, part of the Atlas range, which offers the best climate in Algeria for grapevine cultivation (Isnard, 1953). The area exhibits a cold semi-arid climate (Köppen BSk), characterized by dry, moderately hot summers and cold, wetter winters with occasional snowfall (Hamzaoui et al., 2025).

According to climate data for Batna, mean daily temperatures range from approximately 4 °C in January to 25 °C in July (<https://fr.climate-data.org/afrique/algerie/batna/batna-3686/>). Annual precipitation ranges between 305 and 326 mm, with most rainfall occurring from January to May, followed by a dry period during midsummer, particularly in July. Relative humidity varies seasonally between 40% and 70%.

1.2. Ampelographic characterization

For each cultivar, eleven mature leaves were sampled according to the guidelines set by the International Organization of Vine and Wine (OIV, 2001). The characteristics of these leaves were defined using a set of 32 OIV codes, which included 18 quantitative traits (OIV 601-618) and 14

qualitative traits (OIV 065, OIV 067, OIV 068, OIV 069, OIV 076, OIV 079, OIV 080, OIV 081-1, OIV 081-2, OIV 082, OIV 083-1, OIV 083-2, OIV 093, and OIV 094) (Appendix 2).

Ampelometric measurements, including vein lengths and angles, were conducted using ImageJ software (<https://imagej.nih.gov/ij/>), with specific calibration for length in centimeters and angles in degrees. For the notation of quantitative traits, the average value was used to represent each parameter, following the full 1 to 9 scale defined by the OIV, (2001) for character description (Appendix 3). For the notation of qualitative traits, the most frequently occurring value was selected as the representative descriptor (Appendix 4).

To refine the method, we incorporated additional measurements based on the approach proposed by Martinez and Grenan, (1999), which considered each side of the leaf (Figure 3).

The aim was to identify the most discriminative traits for cultivar differentiation by correlating the standard OIV codes with these relationships (Appendix 5). These relationships were prioritized as they represent environmentally more stable metrics compared to individual ampelometric measures (Martí et al., 2006).

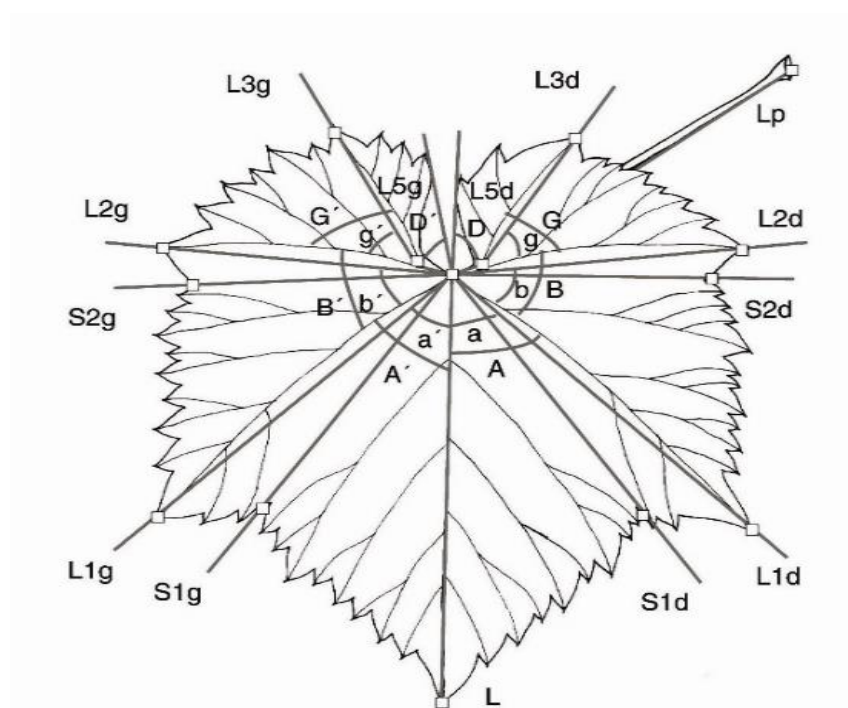


Figure 3: Ampelometric traits recorded on mature leaves of studied cultivars (Martinez and Grenan, 1999).

1.3. Statistical and multivariate evaluations

1.3.1. Quantitative characterization

1.3.1.1. Descriptive statistics

Descriptive statistics were computed for each morphological trait to assess variability within the dataset. Key indicators, including the minimum, maximum, mean, variance, standard deviation, and coefficient of variation (CV%), were determined using the trial version of XLSTAT. The coefficient of variation, expressed as a percentage, serves as a normalized measure of dispersion. It is calculated by dividing the standard deviation of each trait by its mean, providing a standardized comparison of variability across traits.

1.3.1.2. Principal component analysis

Afterwards, a principal component analysis (PCA) was performed using XLSTAT software (trial version) in order to evaluate the most significant characters that contributed to the grapevine cultivar discrimination into distinct groups, according to their morphological traits. In other words, this multivariate approach is used to reduce efficiently the dimensionality of data, representing it in a new space defined by principal components.

The visualization of PCA projection in three-dimensional scatter plots was implemented with the software Numerical Taxonomy System (NTSYS) 2.1v (Rohlf, 2000) based on Cos squared (Cos^2) values of the investigated variables (Appendix 6). These values are important metrics for understanding how well each original variable is represented by the principal components. Variables with high cos^2 values for the first few components are well represented in those components, which supports focusing on those components and possibly ignoring others.

Besides, using PAST 4.13v (Paleontological Statistics) program (Hammer et al., 2001), a graphical representation was created to illustrate the relationships between variables and principal components in multivariate data analysis. Loading plots stand for the correlation between the original variables and the principal components. Each variable is represented as a vector; the direction and length of the vector indicate the strength and direction of the relationship between the variable and the principal component.

1.3.1.3. Correlation analysis

A Pearson correlation analysis was also performed in order to visualize and quantify the relationships between the various ampelographic traits. This analysis provides a comprehensive view

of how different morphological characteristics are interrelated, which is crucial for understanding the overall structure and variability within the grapevine cultivars studied.

1.3.1.4. Analysis of variance of ampelometric characteristics

Following the principal component and correlation analyses of the ampelometric characteristics, univariate ANOVAs were performed using XLSTAT software at a significance level of $\alpha = 0.05$ to the traits identified as discriminant.

This analysis aimed to evaluate these traits individually, determining their contribution to the observed differences among the studied cultivars. Tukey's test was subsequently employed to compare the means of these traits, establishing homogeneous groups and offering a detailed understanding of their role in morphological differentiation. This focused approach highlights the specific ampelometric traits driving the variability across the cultivars.

1.3.2. Qualitative characterization

1.3.2.1. Frequency of qualitative traits

The qualitative traits were assessed to provide a more comprehensive characterization of the studied grapevine varieties (Appendix 2). The frequency distribution of each trait was calculated using Microsoft Excel® 2016 to identify the most prevalent characteristics among the varieties studied.

For this purpose, the occurrence of each trait's categories was recorded and expressed as a percentage relative to the total number of varieties (35). This analysis highlights the diversity or uniformity within the population for each trait. The results of the frequency analysis were visualized using pie charts to facilitate interpretation.

1.4. Ampelographic clustering of studied varieties

Subsequently, all the ampelographic dataset were transformed to create a similarity matrix which were subjected to group analysis in order to cluster the varieties in a phenotypic distance matrix using NTSYS 2.1v software (Rohlf, 2000).

A hierarchical clustering analysis (HCA) was conducted utilizing the acquired similarity matrix. Subsequently, the findings were graphically represented as a dendrogram to elucidate the morphological associations among the cultivars. UPGMA algorithm and JACCARD similarity coefficient were opted for this objective owing to their proficiency in capturing the morphological resemblances between samples.

Chapter 2

Results and Discussion

2. Results and discussion

2.1. Statistical and multivariate evaluations

2.1.1. Quantitative characterization

2.1.1.1. Descriptive statistics

The ampelographic characterization of grapevine cultivars grown in the Aures region (Batna, Algeria) reveals a complex landscape of morphological diversity. Our data covers 35 observations across multiple variables (OIV codes and Rel. variables) with some measurements showing remarkable consistency while others exhibit substantial variation (Table 4).

Overall, the statistical analysis suggests considerable morphological diversity among the studied cultivars, potentially reflecting unique adaptive strategies, genetic variations, or environmental responses specific to Algerian grape varieties. These findings not only provide insights into the morphological complexity of the studied grapevines but also offer valuable baseline data for future research in ampelographic characterization, genetic diversity, and potential breeding strategies.

Table 4: Summary of descriptive statistics for morphological characteristics among studied grapevine cultivars: The minimum (Min), maximum (Max), mean, standard deviation (SD), variance (Var) and variation coefficient (CV%) values.

Variable	Min	Max	Mean	\pm SD	Var	CV (%)
PL	3.572	8.097	5.363	1.144	1.309	21.334
OIV 601	5.583	10.971	8.401	1.209	1.462	14.393
OIV 602	4.795	9.940	7.397	1.050	1.102	14.190
OIV 603	3.306	7.627	5.435	0.844	0.713	15.538
OIV 604	2.164	4.827	3.409	0.592	0.350	17.352
OIV 605	2.403	6.322	4.159	1.199	1.437	28.824
OIV 606	2.735	6.090	4.016	0.943	0.889	23.480
OIV 607	43.340	60.480	50.492	3.867	14.957	7.659
OIV 608	47.457	65.761	53.524	4.532	20.535	8.466
OIV 609	43.466	59.391	50.774	3.920	15.366	7.720
OIV 610	51.545	73.305	62.907	5.468	29.898	8.692
OIV 611	0.917	2.340	1.591	0.395	0.156	24.847
OIV 612	0.461	1.491	0.798	0.246	0.061	30.844
OIV 613	0.676	1.441	0.989	0.211	0.045	21.356
OIV 614	0.450	1.291	0.705	0.181	0.033	25.663
OIV 615	0.573	1.214	0.875	0.157	0.025	17.892
OIV 616	4.318	8.636	6.775	1.344	1.808	19.844
OIV 617	2.701	4.567	3.689	0.471	0.222	12.774
OIV 618	0.658	4.080	2.146	0.668	0.446	31.131

Table 4: Continued.

Variable	Min	Max	Mean	±SD	Var	CV (%)
Rel.1	0.373	0.898	0.645	0.113	0.013	17.497
Rel.2	0.786	0.966	0.886	0.041	0.002	4.603
Rel.3	0.788	0.949	0.881	0.041	0.002	4.689
Rel.4	0.574	0.714	0.647	0.038	0.001	5.913
Rel.5	0.57	0.727	0.648	0.04	0.002	6.129
Rel.6	0.36	0.78	0.566	0.124	0.015	21.959
Rel.7	0.338	0.769	0.559	0.118	0.014	21.1
Rel.8	0.524	0.897	0.747	0.11	0.012	14.749
Rel.9	0.478	0.894	0.736	0.107	0.012	14.587
Rel.10	136.599	181.457	155.582	11.836	140.08	7.607
Rel.11	133.973	175.415	153.999	10.258	105.224	6.661
Rel.14	0.43	0.829	0.641	0.116	0.013	18.091
Rel.15	0.416	0.822	0.633	0.111	0.012	17.49

According to our findings, the minimum and maximum values provide the range of the observed morphological characteristics, indicating the extent of variability within the studied 35 grapevine cultivars. The mean represents the average or central tendency of the characteristics, while the standard deviation and variance describe the dispersion or spread of the data around the mean. Overall, the findings obtained were, in some extent, consistent with the results described in the literature (Martí et al., 2006; Gago et al., 2009; Abiri et al., 2020; Gago et al., 2022) and specifically those found during the first ampelometric study of autochthonous grapevines in Algeria: Germplasm collection of Mascara by Laiadi et al., (2013).

The coefficient of variation (CV%) reveals significant differences in measurement consistency across variables. Some characteristics show remarkable uniformity, while others demonstrate substantial variability. 10 out of 32 characters reached CV values greater than 20.00%, indicating high variation among the cultivars. That was the case for the leaf size dependent parameters (veins lengths and sinuses distances), noting the character OIV 618 which displayed the highest CV (31.13%), followed by the characters OIV 612 (30.84%), OIV 605 (28.82%), OIV 614 (25.66%), OIV 611 (24.85%), OIV 606 (23.48%), Rel.6 (21.96%), OIV 613 (21.36%), PL (21.33%) and Rel.7 (21.1%).

Our results are in correspondence with the earlier researches which showed that leaf size dependent parameters can vary greatly as a result of different environmental conditions (Bodor et al., 2014 and Chitwood et al., 2016) and pruning level/bud load (Bodor et al., 2013).

While the lowest CVs were shown by the ratios between the measured veins lengths Rel.2 (4.6%), Rel.3 (4.69%), followed by the characters Rel.4 (5.91%) Rel.5 (6.13%), also the ratios between the measured angles Rel.11 (6.66%), Rel.10 (7.6%), as well as the traits related to the angles size, noting OIV 607 (7.66%), OIV 609 (7.72%), OIV 610 (8.69%).

Interestingly, several authors argued that the angles between veins and especially the ratios between measured parameters (distances and/or angles) are considered stable within cultivars (Tomažič and Korošec-Koruza, 2003; Martí et al., 2006; Bodor et al., 2013). The fact that some parameters are not affected by different environmental factors is often used as the main criterion for their utilization in cultivar identification (Preiner et al., 2014).

2.1.1.2. Principal component analyses of quantitative traits

The mean values of the quantitative descriptors (OIV parameters and ratios) recorded for the morphological characterization of each cultivar are presented as supplementary (unpublished) data. The findings of the principal component analysis (PCA) based on the ampelometric data of the different cultivars, revealed three components denoted by PC1, PC2, and PC3, collectively capturing 76.91% of the overall leaf shape variation (Table 5).

Table 5: Principal component analysis outcome: Eigenvalues, and percent of variability accounted for the first four principal components on the five studied grape genotypes.

	F1	F2	F3
Eigenvalue	13.771	7.358	3.482
Variability (%)	43.033	22.994	10.882
Cumulative (%)	43.033	66.027	76.910

Our findings are in agreement with those reported in previous studies on grapevine morphological variability. The percentage of total variation explained in our analysis aligns with the values observed by Laiadi et al., (2013) in Algerian cultivars (73.27%), Alba et al., (2014) in Italian varieties (69.9%), and Min et al., (2018) in Chinese wild grape accessions (71%). It also falls within the broader range reported in the literature, being considerably higher than the value observed in Tunisian cultivars (28.61%) by Lamine et al., (2014), and approaching the 88% reported by Zinelabidine et al., (2014) for Algero-Maghrebian grapevine varieties.

The primary component (PC1) is the one that discriminates the most between the cultivars studied with 43.03% of the variance, followed by the second component (PC2), that contributes to 22.99%. Lastly, the third component (PC3) encompasses 10.88%.

A scree plot was developed in PCA based on 18 OIV traits and 13 ampelometric relationships of 35 grapes genotypes as shown in Figure (4). The scree plot showed the estimated eigenvalues and cumulative variations found in grapes genotypes. In the scree plot, the eigenvalues up to the first three factors decreased sharply while the cumulative variation increased sharply up to the first seven factors. Factor F1 had a maximum eigenvalue (13.771) with cumulative variability of 43.033%.

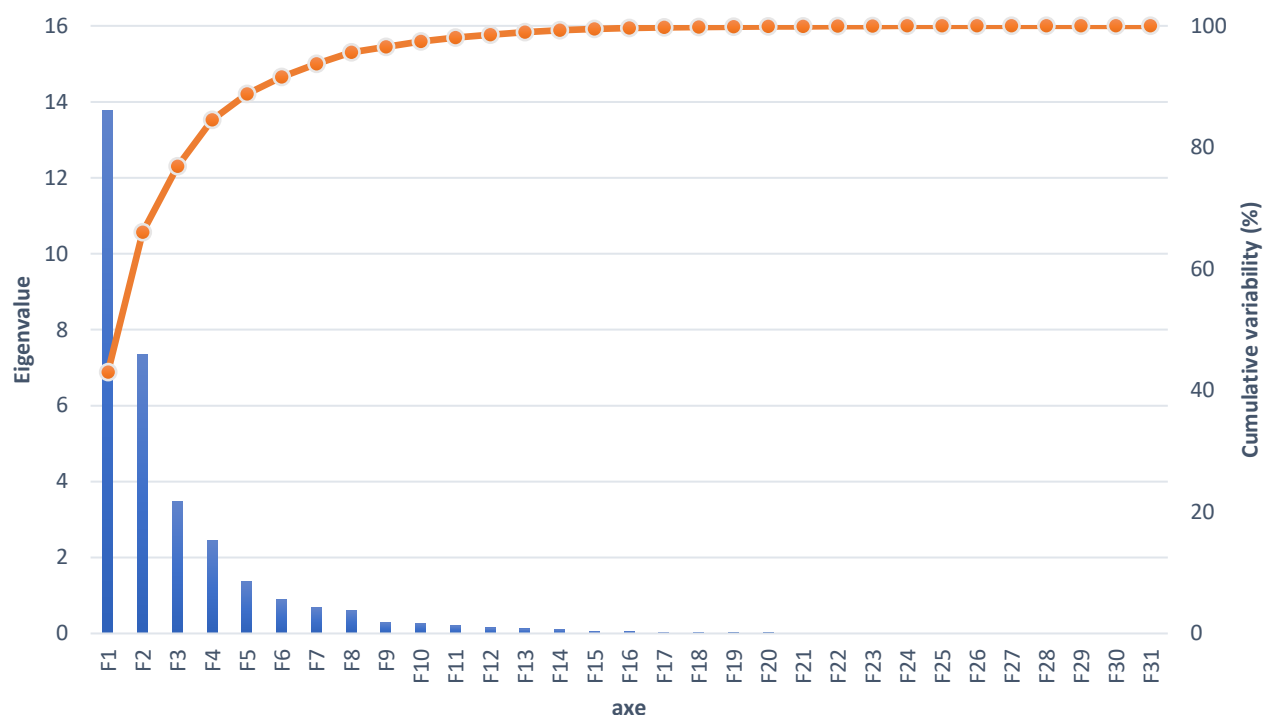


Figure 4: Scree plot of principal components: eigenvalues and cumulative variability.

As expressed in Figure (5), the most distinctive features are represented in the first component (PC1) by the highest weights and positive correlation. These variables are mainly related to the depth of the upper and lower lateral sinuses (OIV 605, OIV 606). Notable is also their corresponding relationships: Rel.14, Rel.6, Rel.7, Rel. 15, Rel.8, and Rel.9.

1) PC1

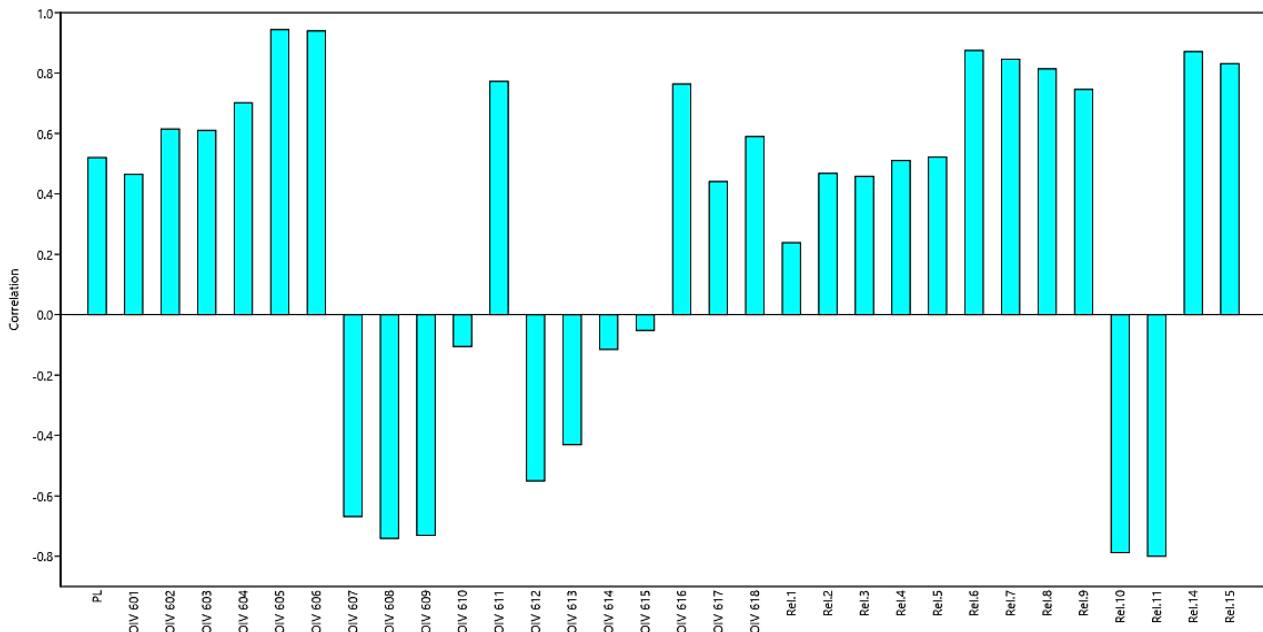


Figure 5: Loading plots from the PCA on the first principal component (PC1).

These findings are in line with those obtained by Zinelabidine et al., (2014) and Gago et al., (2022), which also emphasized the importance of these variables. Although lobing was the primary source of shape variation in the measured leaves as reported by Chitwood, (2021) and Migicovsky, (2022). In the same axis, other variables with the most negative weights and all refer to the angles formed by the main veins (OIV 609, OIV 608, OIV 607) and their relationships are notable: Rel.11 and Rel.10. Similarly, Santiago et al., (2005b) and Gago et al., (2009) reported that the variables related to the angles and the sums of these angles have the greatest weights in PC1.

The second component (PC2) (Figure 6) is expressing the variables related to the lengths and widths of teeth: OIV 615, OIV 614, OIV 613, OIV 612. Notable is also the characters reflected to the size or dimension of the leaf: OIV 601, OIV 603, OIV 602, and OIV 617. As reported by Cunha et al., (2007), certain traits, such as the size of the leaf and the length of teeth compared with their width are discriminant characteristics of the wild grapevine populations located in Portugal.

2) PC2

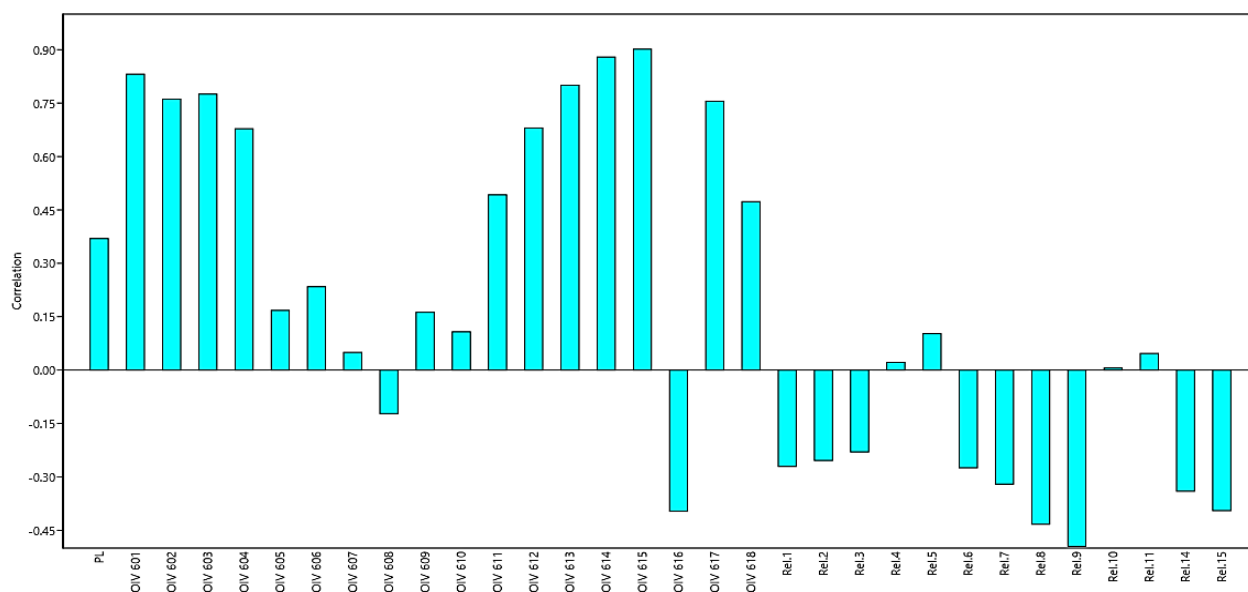


Figure 6: Loading plots from the PCA on the second principal component (PC2).

On the other hand, we observed moderate variations on the third axis (PC3) (Figure 7) based on the following characters: Rel.4, OIV 610, Rel.5, Rel.2, and Rel.3, which are all related to the shape of the leaf. It has been previously proven that leaf shape (predominantly palmate) is critical to the identification of grapevine varieties (Bodor et al., 2013; Diaz, 2017; Chitwood et al., 2014; Chitwood, 2021).

3) PC3

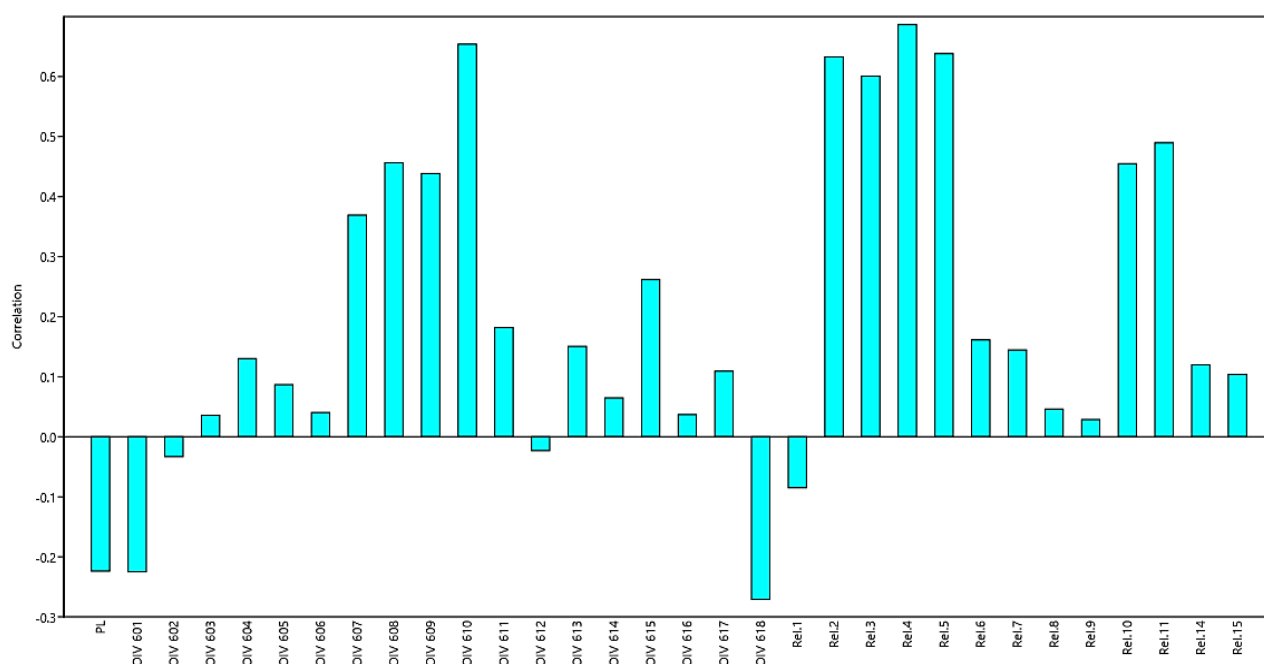


Figure 7: Loading plots from the PCA on the third principal component (PC3).

The Figure (8) illustrates the general distribution of cultivars, according to their behaviors with regards to the morphological characteristics studied along the three axes of the 3-D plot (PC1, PC2, and PC3). Nevertheless, it is difficult to interpret which features of the leaf most strongly contribute to a leaf resembling another along the phenogram due to the diversity of characters that discriminate between our studied cultivars. To address this, the Cos squared (Cos^2) values which indicate the quality of representation of each cultivar on the PCA axes have been calculated and presented in Appendix (7). This complementary table helps clarify the extent to which each axis contributes to the positioning of cultivars in the three-dimensional PCA space.

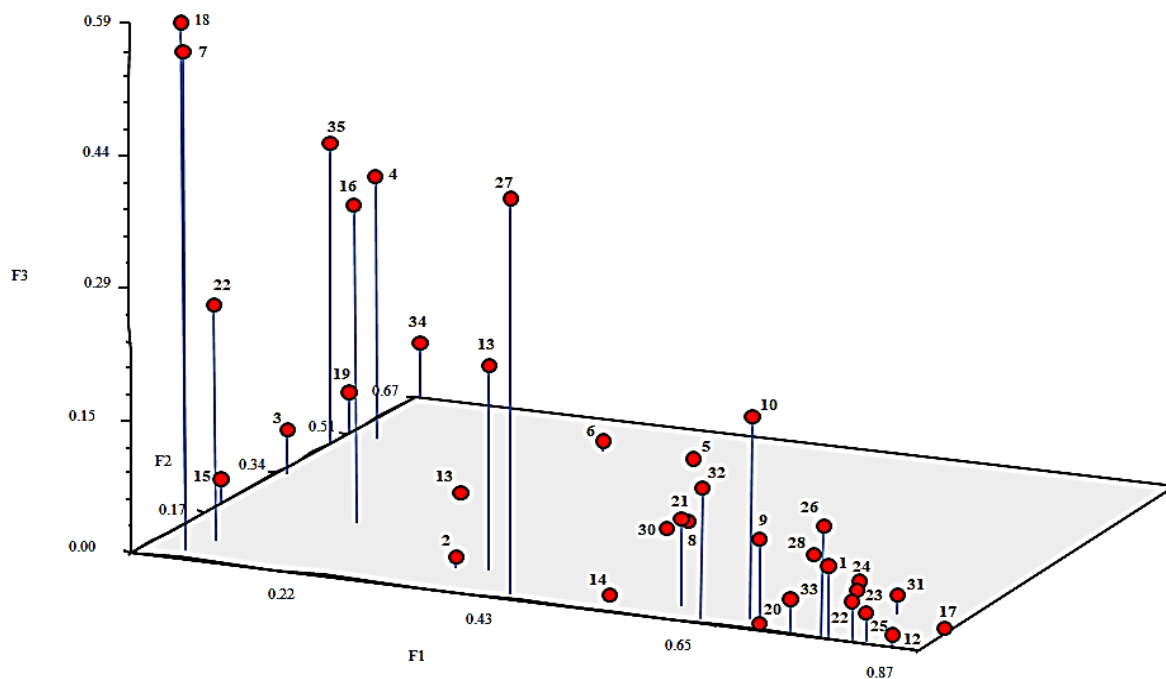


Figure 8: PCA projection performed on 18 ampelometric traits of mature leaves of 35 cultivars in the first three components.

1: 'Ait Abdi'; 2: 'Amellal 1'; 3: 'Amellal 2'; 4: 'A. Bouamar'; 5: 'Ameziane'; 6: 'Anonymous 1'; 7: 'Anonymous 2'; 8: 'Anonymous 3'; 9: 'Anonymous 4'; 10: 'Anonymous 5'; 11: 'Bouabane'; 12: 'Anonymous 6'; 13: 'Laadari'; 14: 'Meska 1'; 15: 'Meska 2'; 16: 'Taberkante 1'; 17: 'Anonymous 11'; 18: 'Anonymous 12'; 19: 'Taberkante 2'; 20: 'Tasemith'; 21: 'Tazizaouth 1'; 22: 'Tazizaouth 2'; 23: 'Tazizaouth 3'; 24: 'Tazizaouth 4'; 25: 'Tazizaouth 5'; 26: 'Tazizaouth 6'; 27: 'Tazizaouth 7'; 28: 'Tazizaouth 8'; 29: 'Tazizaouth 9'; 30: 'Tazizaouth 10'; 31: 'Tazizaouth 11'; 32: 'Tazogaghth 1'; 33: 'Tazogaghth 2'; 34: 'Tazogaghth 3'; 35: 'Tazogaghth 4'.

If we considered the variables related to the depth of lateral sinuses and angles size, the varieties having leaves with deep lateral sinuses and wide angles, such as 'Ait Abdi', 'Anonymous 11', 'Tazizaouth 11', are positioning in the right side of the first axis of the phenogram. This result came in agreement with what was mentioned by Santiago et al., (2005b), whereas the opposite was found by Zinelabidine et al., (2014). Considering the second axis, cultivars with short main veins N1, N2,

and N3 as well as short and narrow tooth of N2 and N4 such as ‘Tazizaouth 9’ and ‘Meska 2’, are in the front of the figure. ‘Tazogaghth 3’, which gathers towards the posterior part of the diagram, is characterized by longest main veins N1, N2, and N3, as well as longest and widest tooth of N2 and N4. Finally, about the third axis, cultivars have the smallest blades, such as ‘Ameziane’ and ‘Anonymous 1’, were situated at the bottom. In contrast, the cultivars with the largest leaves such as ‘Anonymous 2’ and 12’ appear toward the top of the plot. Interestingly, the varieties were not discriminated according to their origin or their growing site, but by the morphology of their leaves.

According to the results of the principal component analysis it can be said that it is a single grapevine variety, ‘Tazizaouth’, presented by several samples. In this sense, leaves of this variety, collected from different sites, can be found very close in the plot because of their similar morphology. This result can be attributed to the fact that there are problems of variety denomination mainly due to synonymy (several names are given to the same variety) and homonymy (the same name is given to several varieties) (El-Oualkadi and Hajjaj, 2019). Actually, this problem is very common among local varieties (Khouni et al., 2023).

Other attractive information could be highlighted from these data. The analysis of the relationships calculated from the basic morphometric parameters shows the reduction of external (environmental) factors in spite of the different factors considered in this work (season weather, growing site, age of the plants).

These relationships were stable over time and seemed to be independent from the environment since they did not vary significantly among the studied years (Martí et al., 2006). For example, ‘A. Bouamar’, one of the native varieties, exhibit large leaves when we consider the descriptors coded OIV 601, OIV 602, OIV 603 and OIV 604. However, when we consider the relationships which reflected to the leaf size (Rel.2, Rel.3, Rel.4, and Rel.5), the leaves appear smaller.

Notably, our results are in correspondence with earlier researches which highlighted that leaf size dependent parameters can show a great discrepancy as a result of different environmental conditions (Bodor et al., 2013; Preiner et al., 2014).

Effectively, this OIV code is reported to be relevant to distinguish cultivars, according to the results of data processing by discriminant analysis. Our results show how few ampelometric traits are sufficient to discriminate grapevine cultivars as reported by previous related works (Bodor et al., 2013; Alba et al., 2014; Labagnara et al., 2018).

In the past, ampelometry has been an important tool in the morphological description of grapevines for the metric calculations of the main characteristics of a "mature leaf" (Galet, 1952). In

fact, analysis of the leaf traits is not the sole purpose of ampelometry. More recently, Martínez and Grenan, (1999) examined the leaf features in even greater details and developed a model that could reconstruct a visual representation of a typical leaf by statistically measuring numerous angles, lengths and tooth numbers for each grape variety. Afterwards, this approach has been applied by many authors. For Algerian varieties, Laiadi et al., (2013) constructed an average leaf of 7 Algerian varieties. Further, Zinelabidine et al., (2014) have studied 71 Algero-Magrebien accessions through the ampelographic construction of their mean leaves following the described approach. The method has advanced over the last few decades from manually obtained measurements of teeth, sinuses and veins to an automated digital method that employs scanned images of leaves to get more accurate results, known as digital morphometrics (Chitwood et al., 2014).

2.1.1.3. Correlations among studied characteristics

To complement the PCA and investigate the relationships between the ampelographic traits for the studied grapevine cultivars, a Pearson correlation analysis was also performed to examine the patterns among the variables.

The correlation analysis of traits in this study was based on large samples as shown in Figure (9). Notably, some of the analyzed traits have not been previously reported in the literature.

The Pearson (r) correlation coefficient, introduced by Pearson, (1900), is the most commonly used measure of correlation. It assesses the linear relationship between two continuous variables, helping to understand how the variables change together (Dehghan et al., 2024). This analysis provides insights into the direction and strength of these relationships, which can be effectively visualized in a correlation matrix using a color-coded plot (Figure 9).

The correlation matrix is symmetrical where variables are listed both vertically and horizontally, with each cell representing the correlation between a pair of variables. The diagonal of the matrix, running from the top-left to the bottom-right, displays perfect correlations of 1, as each variable is perfectly correlated with itself. The values in the matrix range from -1 to 1, where values close to 1 indicate strong positive correlations, values close to -1 indicate strong negative correlations, and values near 0 suggest weak to no correlation. To enhance interpretability, color coding is often used; dark blue represents strong positive correlations, dark red indicates strong negative correlations, and white or lighter colors signify weak or no correlations. This visual aid helps quickly assess the strength and direction of relationships within the data.

Overall, the substantial correlations observed among numerous traits in our study affirm the efficacy of this method in describing leaf morphological variability as also reported by Bodor-Pesti et

al., (2023), highlighting its effectiveness in revealing key patterns between different ampelographic characteristics.

However, a limited number of studies in the literature focus on the correlations between key mature leaf characteristics as defined by OIV traits and the ampelometric relationships.

In line with this, Susaj et al., (2014) identified strong correlation relationships between main mature leaf characters such as length of main veins (OIV 601, OIV 602, OIV 603, OIV 604, OIV 611), length of petiole (PL), length petiole sinus to upper and lower lateral leaf sinuses (OIV 605 and OIV 606, and angles size (OIV 607 and OIV 608). Similarly, Bodor et al., (2017) found a significant correlation between the two halves of the leaves (except for OIV 608). In the other hand, Chitwood et al., (2014) highlighted a significant correlation between their studied traits and those of Galet, (1952) which is highly suggestive of important genetic influences predominating over substantial environmental differences underlying leaf morphology in grapevines.

According to our findings, the correlation coefficients range from $r = -0.74$ to $r = 0.99$, indicating diverse relationships from strong negative to strong positive correlations. Notably, several exceptionally strong positive relationships among the studied variables could be revealed. The strongest correlations ($r = 0.99$) were found between the relationships Rel. 6 and Rel. 14, as well as Rel. 14 and Rel. 15.

Additionally, very strong positive correlations were observed between multiple pairs of variables: OIV 602 and OIV 603, as well as the relationships Rel. 6 and Rel. 7, Rel.8 and Rel.9, Rel. 5 and Rel.15 ($r = 0.98$), Rel.14 and Rel.7 through Rel.9 ($r = 0.94$ to 0.97), Rel.15 and Rel.6 through Rel.9 ($r = 0.96$ to 0.98). These near-perfect correlations suggest these pairs of variables are highly interrelated, potentially indicating shared underlying mechanisms or characteristics.

Other strong positive correlations were found among the following characters, noting OIV 601 and OIV 602 through OIV 604 ($r = 0.85$ to 0.95), OIV 608 and Rel. 10 as well as with Rel.11 ($r = 0.95$ and $r = 0.85$, respectively), OIV 609 and Rel. 10 as well as with Rel.11 ($r = 0.89$ and $r = 0.84$, respectively).

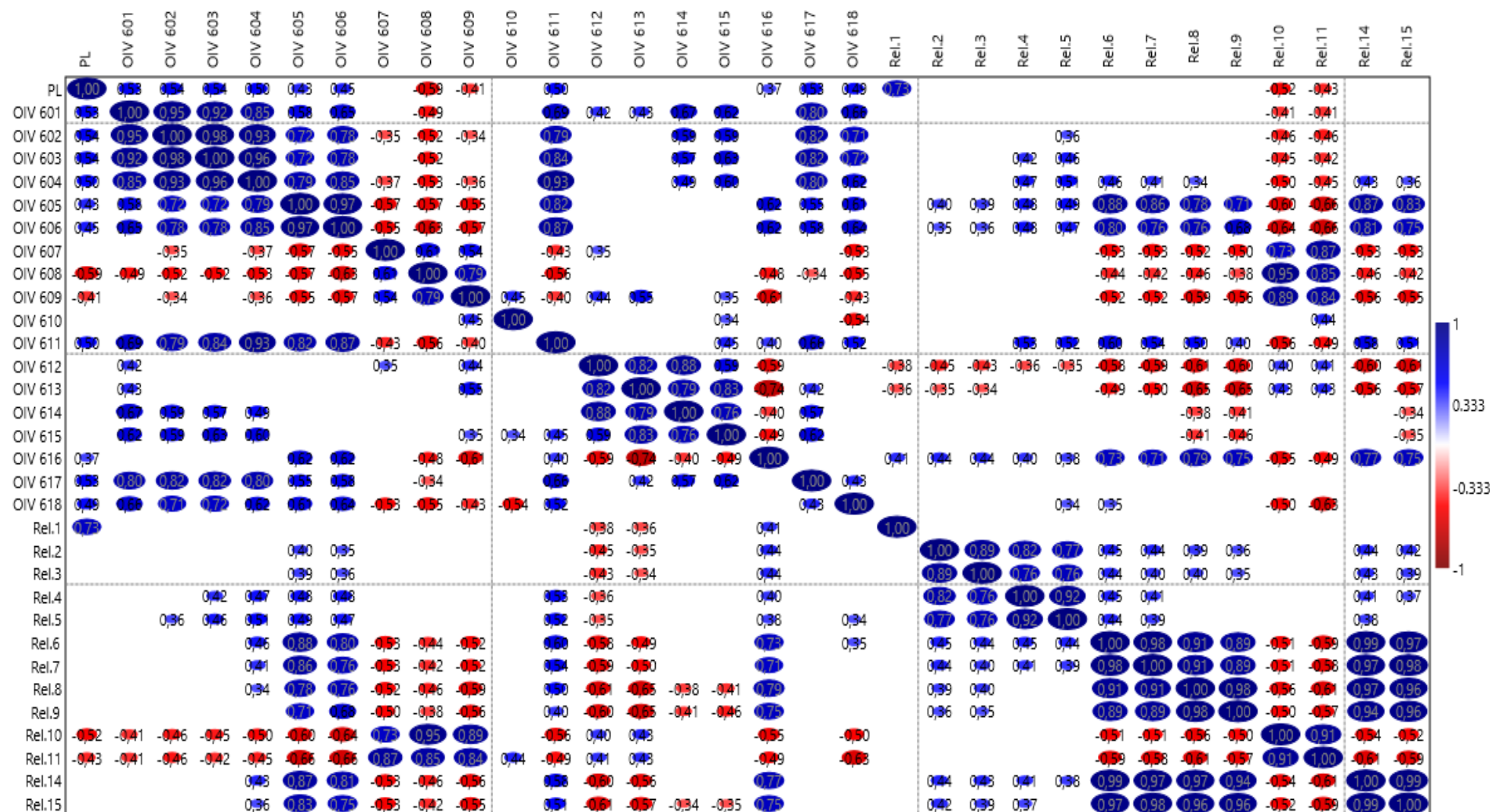


Figure 9: Correlation matrix of phenotypic traits (18 OIV codes and 13 ampelometric relationships) in the studied 35 grapevine cultivars.

Although, some correlation coefficients were moderate to strong. Accordingly, the traits OIV 605 and OIV 606 were prominently associated with other variables. For instance, OIV 605 and PL through OIV 604 ($r = 0.43$ to 0.79), OIV 606 and PL through OIV 604 ($r = 0.45$ to $r = 0.75$), OIV 605 and Rel.6 through Rel.9 ($r = 0.71$ to 0.88), OIV 606 and Rel.6 through Rel.9 ($r = 0.68$ to 0.80), OIV 605 and Rel.14 as well as with Rel.15 ($r = 0.87$ and $r = 0.83$, respectively), OIV 606 and Rel.14 as well as with Rel.15 ($r = 0.81$ and $r = 0.75$, respectively). Also, the trait OIV 611 shows moderate to strong positive correlations with the early numbered variables ($r = 0.50$ to 0.93).

Furthermore, several moderate correlations were identified among other traits, highlighting additional interdependencies. OIV 614 showed moderate positive correlations with OIV 601 through OIV 604 ($r = 0.49$ to 0.67), while OIV 615 similarly correlated with OIV 601 through OIV 604 ($r = 0.59$ to 0.62). OIV 616 demonstrated moderate positive correlations with OIV 605 and OIV 606 ($r = 0.62$). While OIV 617 was moderately to strongly correlated with PL through OIV 606 ($r = 0.53$ to 0.82). Similarly, OIV 618 exhibited moderate to strong correlations with PL through OIV 606, with r values ranging from 0.49 to 0.79 . Finally, PL exhibited moderate correlations with OIV 601 through OIV 606, with coefficients ranging from 0.43 to 0.54 .

Remarkably, moderate but significant negative correlations were observed among the characters. That was the case for those related to the lengths of sinuses and the size of angles, noting: OIV 605 and OIV 607 ($r = -0.57$), OIV 606 and OIV 607 ($r = -0.55$), OIV 605 and OIV 608 ($r = -0.57$), OIV 606 and OIV 608 ($r = -0.63$), OIV 605 and OIV 609 ($r = -0.55$), OIV 606 and OIV 609 ($r = -0.57$). This can be explained by the fact that as the angles increase, there is a concurrent decrease in the length of the petiole sinus to the lateral leaf sinus and therefore the sinuses become deeper.

This phenomenon, has been partially supported by Welter et al., (2007), who highlighted the strong association between leaf angles and the opening or overlapping of leaf sinuses. Couturier et al., (2011) further demonstrated that for two sinuses adjacent to a central vein, the smaller sinus tends to form a larger angle with the vein. This finding suggests a significant genetic component, indicating that intricate morphogenetic processes may play a crucial role in shaping these traits (Chitwood et al., 2014; Demmings et al., 2019).

Remarkably, moderate but significant negative correlations were observed among the characters. That was the case for those related to the lengths of sinuses and the size of angles, noting: OIV 605 and OIV 607 ($r = -0.57$), OIV 606 and OIV 607 ($r = -0.55$), OIV 605 and OIV 608 ($r = -0.57$), OIV 606 and OIV 608 ($r = -0.63$), OIV 605 and OIV 609 ($r = -0.55$), OIV 606 and OIV 609 ($r = -0.57$).

Additionally, OIV 607 was negatively correlated with Rel.6 through Rel.9 ($r = -0.50$ to -0.53) and with Rel.14 and Rel.15 ($r = -0.53$). Other traits, such as OIV 608 and OIV 609, also demonstrated substantial negative correlations. OIV 608 showed correlations with PL through OIV 606 ($r = -0.49$ to -0.63), Rel.6 through Rel.9 ($r = -0.38$ to -0.46), and Rel.14 and Rel.15 ($r = -0.46$ and -0.42 , respectively). OIV 609 exhibited negative correlations with Rel.6 through Rel.9 ($r = -0.52$ to -0.59) and with Rel.14 and Rel.15 ($r = -0.56$ and -0.55 , respectively). Furthermore, OIV 611 was negatively correlated with OIV 607 through OIV 609 ($r = -0.40$ to -0.56).

OIV 612 and OIV 613 showed strong negative correlations with Rel.6 through Rel.9 ($r = -0.58$ to -0.61 and $r = -0.49$ to -0.65 , respectively). OIV 616 displayed negative correlations with OIV 608 ($r = -0.48$) and OIV 609 ($r = -0.61$), as well as with OIV 612 through OIV 615 ($r = -0.40$ to -0.74). OIV 618 exhibited negative correlations with OIV 607 through OIV 610 ($r = -0.43$ to -0.55) and with Rel.10 and Rel.11 ($r = -0.50$ and -0.63 , respectively).

Lastly, Rel.10 and Rel.11 were negatively correlated with multiple traits. Rel.10 showed negative correlations with PL through OIV 606 ($r = -0.41$ to -0.64) and with OIV 616 ($r = -0.55$). Similarly, Rel.11 was negatively correlated with PL through OIV 606 ($r = -0.41$ to -0.66) and with OIV 616 ($r = -0.49$).

On the other hand, certain criteria exhibit either weak correlations, whether negative or positive, or no significant associations. For instance, Rel.14 and Rel.15 showed weak positive correlations with Rel.2 through Rel.5 ($r = 0.38$ to 0.44 and $r = 0.37$ to 0.42 , respectively). Meanwhile, OIV 612 exhibited weak negative correlations with Rel.1 through Rel.5 ($r = -0.35$ to -0.45). In contrast, no significant correlations were observed between PL and OIV 612 through OIV 615, with Rel.2 through Rel.9, or with Rel.14 and Rel.15 ($r = 0$). Additionally, OIV 617 showed no correlation with Rel.1 through Rel.15 ($r = 0$), and Rel.10 and Rel.11 displayed no associations with Rel.1 through Rel.5 ($r = 0$).

2.1.1.4. Analysis of variance of ampelometric characteristics

To elucidate how the variables examined differentiate our studied varieties, an analysis of variance (one-way ANOVA) was also conducted, assessing the significance of variables. Many authors compared primary data with ANOVA within the scope of this study (Khalil et al., 2017; El Fatehi et al., 2021; Cichi et al., 2022). In this sense, the analysis included both OIV traits and ampelometric relationships to capture a comprehensive view of the differences.

The Anova test results illustrated in the following Table 6 and 7 show very high significant results ($\alpha = 0.05$) with $P < 0.0001$ for all ampelometric characteristics analyzed. According to the Tukey HSD test, the averages sharing the same letters do not differ significantly.

The Tukey post-hoc test grouped the 35 cultivars according to the parameter studied, into several groups ranging from 1 to 15 (i.e. Rel. 14 and Rel. 15). The number of homogeneous groups obtained by the HSD test reflected the variability contained in our samples.

The cultivars displayed varying results across different ampelometric characteristics:

In terms of OIV 601 character, the cultivars ‘Anonymous 1’, ‘Ameziane’, ‘Taberkante 2’, ‘Anonymous 5 and 4’, ‘Taberkante 1’, ‘Meska 2’ and ‘Amellal 2’ exhibit short main vein (N1) where the smallest value is observed in ‘Anonymous 1’ with 5.583 cm. Conversely, the cultivars ‘Tazogaghth 1’, ‘Tazizaouth 10, 6 and 9’, ‘Anonymous 12’, ‘Tazogaghth 4’, ‘Anonymous 2’, ‘Tasemith’, ‘A. Bouamar’, ‘Tazizaouth 8’ and ‘Tazogaghth 3’ show long main vein (N1) where the longest vein is observed in ‘Tazogaghth 3’ with 10.971 cm.

In terms of OIV 602 character, the cultivars ‘Anonymous 1’, ‘Ameziane’, ‘Anonymous 5 and 4’, ‘Taberkante 2 and 1’ as well as ‘Ait Abdi’ demonstrate short main vein (N2) where the smallest value is observed in ‘Anonymous 1’ with 4.795 cm. In the other hand, the cultivars ‘Tazizaouth 7’, ‘Tazogaghth 4’, ‘Tasemith’, ‘A. Bouamar’, ‘Tazogaghth 3’ and ‘Tazizaouth 8’ have long main vein (N2) where the longest vein is observed in ‘Tazizaouth 8’ with 9.940 cm.

With regards to the OIV 603 character, the cultivars ‘Anonymous 1’, ‘Ameziane’, ‘Anonymous 4’, ‘Taberkante 2’, ‘Anonymous 5’, ‘Ait Abdi’ and ‘Amellal 2’ unveil short main vein (N3) where the smallest value is observed in ‘Anonymous 1’ with 3.306 cm. Meanwhile, the cultivars ‘A. Bouamar’, ‘Tasemith’, ‘Tazogaghth 4’, ‘Tazizaouth 7’, ‘Tazogaghth 3’ and ‘Tazizaouth 8’ show long main vein (N3) where the longest vein is observed in ‘Tazizaouth 8’ with 7.627 cm.

In terms of OIV 604 character, the cultivars ‘Anonymous 1’, ‘Ameziane’, ‘Anonymous 4’, ‘Taberkante 2’, ‘Ait Abdi’, ‘Anonymous 5’, ‘Amellal 2’, ‘Anonymous 11’, ‘Laadari’, ‘Tazizaouth 11’, ‘Taberkante 1’ and ‘Meska 2’ have short main vein (N4) where the smallest value is observed in ‘Anonymous 1’ with 2.164 cm. In contrast, the cultivars ‘Tazogaghth 1’, ‘Bouabane’, ‘Tazogaghth 2’, ‘Tazizaouth 5’, ‘Tasemith’, ‘Tazogaghth 4’, ‘Anonymous 6’, ‘Tazogaghth 3’ and ‘Tazizaouth 8’ show long main vein (N4) where the longest vein is observed in ‘Tazizaouth 8’ with 4.827 cm.

Regarding the OIV 605 character, the cultivars ‘Ameziane’, ‘Ait Abdi’, ‘Anonymous 11’, ‘Tazizaouth 11’, ‘Anonymous 5 and 1’, ‘Amellal 1’, ‘Bouabane’, ‘Anonymous 4’, ‘Tazizaouth 10’, ‘Taberkante 2’, ‘Meska 2 and 1’, ‘Amellal 2’, ‘Taberkante 1’ and ‘Laadari’ have deep upper lateral leaf sinus where the smallest value is observed in ‘Ameziane’ with 2.403 cm. However, the cultivars ‘Tazizaouth 4 and 7’, ‘Tazogaghth 3 and 2’, ‘Anonymous 3’, ‘Tazizaouth 5’, ‘Tasemith’, ‘Tazizaouth

3, 2 and 1', 'Anonymous 6' and 'Tazizaouth 8' show shallow upper lateral leaf sinus where the greatest value is observed in 'Tazizaouth 8' with 6.322 cm.

In terms of OIV 606 character, the cultivars 'Anonymous 11, 5 and 1', 'Ameziane', 'Anonymous 4', 'Tazizaouth 11', 'Amellal 1', 'Ait Abdi', 'Taberkante 1', 'Meska 1', 'Bouabane', 'Taberkante 2', 'Laadari', 'Tazizaouth 10', 'Amellal 2' and 'A. Bouamar' deep lower lateral leaf sinus where the smallest value is observed in 'Anonymous 11' with 2.735 cm. In the other hand, the cultivars 'Tazizaouth 2', 'Tasemith', 'Anonymous 6', 'Tazogaghth 3' and 'Tazizaouth 8' have shallow lower lateral leaf sinus where the greatest value is observed in 'Tazizaouth 8' with 6.090 cm.

In terms of OIV 607 character, the cultivars 'Anonymous 12', 'Taberkante 2', 'Tazizaouth 6, 3 and 1', 'Anonymous 6', 'Tazizaouth 8', 'A. Bouamar', 'Tasemith', 'Tazogaghth 1', 'Taberkante 1', 'Tazogaghth 2', 'Tazizaouth 4 and 2', 'Anonymous 3 and 2', 'Laadari', 'Tazogaghth 3', 'Tazizaouth 5' and 'Bouabane' demonstrate small angles between N1 and N2 where the smallest value is observed in 'Anonymous 12' with 43.34°. Conversely, the cultivars 'Ameziane', 'Ait Abdi', 'Tazizaouth 10', 'Anonymous 5 and 1', 'Meska 1' and 'Amellal 1' exhibit large angles between N1 and N2 where the largest angle is observed in 'Amellal 1' with 60.48°.

In terms of OIV 608 character, the cultivars 'Anonymous 12', 'Tazizaouth 4', 'Tazogaghth 1', 'Tazizaouth 6, 8, 5 and 2', 'Bouabane', 'Taberkante 2', 'Tazizaouth 9', 'Anonymous 6, 3 and 2', 'Tasemith', 'Tazizaouth 3', 'Tazogaghth 2', 'A. Bouamar', 'Laadari', 'Meska 2', 'Amellal 1 and 2', 'Tazizaouth 7 and 10', 'Ameziane', 'Meska 1', 'Tazogaghth 3 and 4', 'Tazizaouth 1 and 11' display small angles between N2 and N3 where the smallest value is observed in 'Anonymous 12' with 47.46°. However, the cultivars 'Tazogaghth 3 and 4', 'Tazizaouth 1 and 11', 'Ait Abdi', 'Taberkante 1', 'Anonymous 11, 4, 1 and 5' have large angles between N2 and N3 where the largest angle is observed in 'Anonymous 5' with 65.76°.

Regarding the OIV 609 character, the cultivars 'Anonymous 6 and 12', 'Tazogaghth 1', 'Tazizaouth 6', 'Anonymous 2', 'Taberkante 2', 'Tazizaouth 8', 'Meska 2', 'Tazogaghth 4', 'Tazizaouth 3', 'Anonymous 3', 'Tazizaouth 5 and 9', 'Tasemith', 'Tazizaouth 2', 'Amellal 1', 'A. Bouamar', 'Amellal 2', 'Ameziane' and 'Laadari' show small angles between N3 and N4 where the smallest value is observed in 'Anonymous 6' with 43.47°. Meanwhile, the cultivars 'Ameziane', 'Laadari', 'Tazizaouth 1 and 10', 'Anonymous 1', 'Tazizaouth 7', 'Meska 1', 'Bouabane', 'Tazogaghth 3', 'Taberkante 1', 'Tazogaghth 4', 'Anonymous 11 and 5', 'Ait Abdi', 'Tazizaouth 11' and 'Anonymous 4' have large angles between N3 and N4 where the largest angle is observed in 'Anonymous 4' with 59.39°.

In terms of OIV 610 character, the cultivars ‘Anonymous 12’, ‘A. Bouamar’, ‘Laadari’, ‘Amellal 1’, ‘Taberkante 2’, ‘Anonymous 3’, ‘Tazizaouth 1, 3 and 8’, ‘Meska 2’, ‘Amellal 2’, ‘Anonymous 11 and 2’, ‘Ait Abdi’, ‘Ameziane’, ‘Tazogaghth 1’, ‘Tazizaouth 6 and 10’, ‘Meska 1’ and ‘Tasemith’ display small angles between N3 and the tangent between petiole point and the tooth tip of N5 where the smallest value is observed in ‘Anonymous 12’ with 51.55° . Conversely, the cultivars ‘Anonymous 2’, ‘Ait Abdi’, ‘Ameziane’, ‘Tazogaghth 1’, ‘Tazizaouth 6 and 10’, ‘Meska 1’, ‘Tasemith’, ‘Tazizaouth 9, 7, 4 and 11’, ‘Tazogaghth 2’, ‘Anonymous 6’, ‘Tazizaouth 5 and 2’, ‘Anonymous 1 and 5’, ‘Bouabane’, ‘Taberkante 1’, ‘Anonymous 4’, ‘Tazogaghth 4 and 3’ exhibit large angles between petiole point and the tooth tip of N5 where the largest angle is observed in ‘Tazogaghth 3’ with 73.30° .

With regards to the OIV 611 character, the cultivars ‘Anonymous 1’, ‘Ameziane’, ‘Taberkante 2’, ‘Anonymous 5’, ‘Ait Abdi’, ‘Anonymous 4’, ‘Meska 1’, ‘Laadari’, ‘Anonymous 11’, ‘Tazizaouth 11’, ‘Amellal 1’, ‘Anonymous 12’, ‘Amellal 2’, ‘Tazizaouth 10’, ‘Anonymous 2’, ‘Taberkante 1’, ‘Anonymous 1’, ‘Meska 2’ and ‘A. Bouamar’ have short vein N5 where the smallest value is observed in ‘Anonymous 1’ with 0.917 cm. However, the cultivars ‘Tazizaouth 1, 9, 3 and 4’, ‘Tazogaghth 4’, ‘Tazizaouth 2 and 7’, ‘Tazogaghth 2’, ‘Bouabane’, ‘Tazizaouth 6’, ‘Tazogaghth 1’, ‘Tasemith’, ‘Tazizaouth 5’, ‘Anonymous 6’, ‘Tazogaghth 3’ and ‘Tazizaouth 8’ show long vein N5 where the longest vein is observed in ‘Tazizaouth 8’ with 2.340 cm.

Concerning OIV 612 character, the cultivars ‘Taberkante 2’, ‘Tazizaouth 3’, ‘Tazogaghth 2’, ‘Anonymous 3’, ‘Tazizaouth 1 and 4’, ‘Anonymous 6’, ‘Tazogaghth 1’, ‘Tazizaouth 5’, ‘Tasemith’, ‘Anonymous 12’, ‘Tazizaouth 6 and 9’, ‘Taberkante 1’, ‘Anonymous 1’, ‘Tazizaouth 7 and 2’ as well as ‘Ameziane’ display short tooth of N2 where the smallest value is observed in ‘Taberkante 2’ with 0.461 cm. In contrast, the cultivars ‘A. Bouamar’ and ‘Tazogaghth 3’ have medium tooth of N2 where the greatest value is observed in ‘Tazogaghth 3’ with 1.491 cm.

In terms of OIV 613 character, the cultivars ‘Taberkante 2’, ‘Amellal 2’, ‘Anonymous 1’, ‘Tazogaghth 1’, ‘Ameziane’, ‘Anonymous 12’, ‘Tazizaouth 9 and 1’, ‘Meska 2’, ‘Tazizaouth 6’, ‘Tazogaghth 2’, ‘Tazizaouth 3’, ‘Anonymous 3’, ‘Tazizaouth 5, 7 and 4’, ‘Tasemith’, ‘Taberkante 1’ and ‘Tazizaouth 2’ have narrow tooth of N2 where the smallest value is observed in ‘Taberkante 2’ with 0.676 cm. Meanwhile, the cultivars ‘Laadari’, ‘Tazizaouth 11’, ‘Anonymous 4 and 12’, ‘Bouabane’, ‘Tazizaouth 10’, ‘Tazogaghth 4’, ‘A. Bouamar’ and ‘Tazogaghth 3’ have medium tooth of N2 where the greatest value is observed in ‘Tazogaghth 3’ with 1.441 cm.

In terms of OIV 614 character, the cultivars ‘Anonymous 1’, ‘Taberkante 2’, ‘Anonymous 3’, ‘Tazizaouth 4’, ‘Anonymous 12’, ‘Ameziane’, ‘Tazizaouth 3 and 9’, ‘Anonymous 4’, ‘Tazogaghth 1’, ‘Ait Abdi’, ‘Tasemith’, ‘Tazogaghth 2’, ‘Tazizaouth 5, 1, 6 and 2’, ‘Amellal 2’, ‘Taberkante 1’, ‘Tazizaouth 7’, ‘Meska 2’, ‘Anonymous 5’ and ‘Amellal 1’ have short tooth of N4 where the smallest value is observed in ‘Anonymous 1’ with 0.450 cm. In the other hand, the cultivars ‘A. Bouamar’ and ‘Tazogaghth 3’ have medium tooth of N2 where the greatest value is observed in ‘Tazogaghth 3’ with 1.291 cm.

In terms of OIV 615 character, the cultivars ‘Anonymous 1’, ‘Taberkante 2’, ‘Amellal 2’, ‘Ameziane’, ‘Anonymous 12’, ‘Meska 2’, ‘Tazizaouth 9’, ‘Anonymous 3 and 2’, ‘Tazizaouth 4’, ‘Taberkante 1’, ‘Tazizaouth 3 and 5’ as well as ‘Tazogaghth 1’ display narrow tooth of N4 where the smallest value is observed in ‘Anonymous 1’ with 0.573 cm. However, the cultivars ‘Laadari’, ‘Anonymous 6’, ‘Tazizaouth 7’, ‘Anonymous 11’, ‘A. Bouamar’, ‘Tazizaouth 11’, ‘Tazogaghth 4’, ‘Tazizaouth 8 and 10’, ‘Bouabane’ and ‘Tazogaghth 3’ show short to medium tooth of N4 where the greatest value is observed in ‘Tazogaghth 3’ with 1.214 cm.

Concerning the OIV 616 character, the cultivars ‘Tazizaouth 11’, ‘Taberkante 2’, ‘Ait Abdi’, ‘Anonymous 4 and 5’, ‘Tazizaouth 10’, ‘Laadari’, ‘A. Bouamar’, ‘Bouabane’ and ‘Amellal 1’ have medium number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 including the limits, where the lowest number of teeth is observed in ‘Tazizaouth 11’ with 5 teeth. In contrast, the cultivars ‘Tazogaghth 2’, ‘Anonymous 3’, ‘Meska 2’, ‘Tazizaouth 5, 8, 1, 6 and 3’, ‘Tasemith’, ‘Tazizaouth 9 and 7’, ‘Amellal 2’ and ‘Tazizaouth 4’ exhibit large to very large number of teeth where the highest number is observed in ‘Tazizaouth 3’, ‘Tasemith’, ‘Tazizaouth 9 and 7’, ‘Amellal 2’ and ‘Tazizaouth 4’ with 9 teeth.

In terms of OIV 617 character, the cultivars ‘Ameziane’, ‘Anonymous 1’, ‘Taberkante 2’, ‘Anonymous 5’, ‘Ait Abdi’, ‘Laadari’, ‘Anonymous 4 and 11’, ‘Taberkante 1’, ‘Anonymous 3’, ‘Amellal 2’, ‘Anonymous 12’, ‘Tazogaghth 1’, ‘Tazizaouth 4’, ‘Meska 2’, ‘Anonymous 2’, ‘Tazizaouth 9, 11 and 1’, ‘Tazogaghth 2’ and ‘Tazizaouth 2’ display very short to short distance between the tooth tip of N2 and the tooth tip of the first secondary vein of N2, where the smallest value is observed in ‘Ameziane’ with 2.701 cm. Meanwhile, the cultivars ‘Tazizaouth 3’, ‘Amellal 1’, ‘Bouabane’, ‘Tazizaouth 6’, ‘Anonymous 6’, ‘Tasemith’, ‘Tazizaouth 10’, ‘Tazogaghth 4’, ‘Tazizaouth 7 and 8’, ‘A. Bouamar’, ‘Meska 1’ and ‘Tazogaghth 3’ show short to medium distance, where the highest value is observed in ‘Tazogaghth 3’ with 4.846 cm.

Lastly with the OIV 618 character, the cultivars ‘Anonymous 4’, ‘Ameziane’, ‘Taberkante 1’, ‘Meska 1’, ‘Tazogaghth 4’, ‘Bouabane’ and ‘Amellal 2’ demonstrate open petiole sinus except for ‘Anonymous 1’ that have close petiole sinus with an average value of 0.658 cm. However, the cultivars ‘A. Bouamar’, ‘Tazizaouth 1 and 8’ have very wide-open petiole sinus, where the highest value is observed in ‘Tazizaouth 8’ with an average of 4.080 cm.

Table 6: ANOVA results of ampelometric characteristics (OIV traits).

Cultivar	Ait Abdi	Amellal 1	Amellal 2	A. Bouamar	Ameziane	Anonymous 1	Anonymous 2	Anonymous 3	Anonymous 4	Anonymous 5
OIV code										
OIV 601	8.124 ^{df}	8.537 ^{bf}	7.631 ^{dh}	10.563 ^{ac}	5.583 ^{gh}	5.583 ^h	9.429 ^{ad}	7.812 ^{dg}	7.004 ^{eh}	6.713 ^{fh}
OIV 602	6.430 ^{di}	7.793 ^{cg}	6.681 ^{dh}	8.547 ^{ac}	5.188 ^{hi}	4.795 ⁱ	7.642 ^{cg}	7.077 ^{cg}	6.125 ^{fi}	6.048 ^{gi}
OIV 603	4.733 ^{ej}	5.847 ^{bg}	4.773 ^{dj}	6.151 ^{ae}	3.669 ^{ij}	3.306 ^j	5.469 ^{ch}	5.113 ^{ci}	4.217 ^{hj}	4.531 ^{fj}
OIV 604	2.773 ^{gi}	3.260 ^{ch}	2.950 ^{ej}	3.609 ^{bg}	2.255 ^{ij}	2.164 ^j	3.289 ^{ch}	3.200 ^{ci}	2.572 ^{hj}	2.904 ^{fj}
OIV 605	2.501 ^k	2.826 ^{jk}	3.462 ^{gk}	4.093 ^{ei}	2.403 ^k	2.815 ^{ik}	4.022 ^{fj}	5.359 ^{ad}	3.023 ^{ik}	2.711 ^k
OIV 606	3.133 ^{hj}	2.919 ^{ij}	3.656 ^{ej}	3.762 ^{dj}	2.764 ^{ij}	2.754 ^j	4.069 ^{ch}	4.575 ^{be}	2.851 ^{ij}	2.746 ^j
OIV 607	53.60 ^{af}	60.48 ^a	52.77 ^{bg}	48.02 ^{ei}	53.45 ^{af}	57.65 ^{ac}	49.09 ^{di}	48.99 ^{di}	51.09 ^{bh}	56.08 ^{ad}
OIV 608	57.54 ^{ae}	53.50 ^{bf}	53.52 ^{bf}	52.87 ^{cf}	55.53 ^{bf}	63.31 ^{ab}	51.11 ^{cf}	51.11 ^{cf}	60.75 ^{ac}	65.76 ^a
OIV 609	56.78 ^{ac}	50.32 ^{bg}	50.68 ^{bg}	50.67 ^{bg}	51.36 ^{ag}	52.58 ^{af}	46.13 ^{dg}	48.86 ^{cg}	59.39 ^a	56.59 ^{ac}
OIV 610	61.23 ^{ag}	55.25 ^{eg}	60.15 ^{bg}	54.36 ^{fg}	61.55 ^{ag}	67.09 ^{ae}	61.08 ^{ag}	56.27 ^{eg}	71.79 ^{ab}	67.21 ^{ae}
OIV 611	1.204 ^{gi}	1.276 ^{fi}	1.346 ^{ei}	1.566 ^{bi}	0.965 ⁱ	0.917 ⁱ	1.401 ^{di}	1.488 ^{ci}	1.216 ^{gi}	1.204 ^{gi}
OIV 612	0.906 ^{ci}	0.952 ^{cg}	0.809 ^{em}	1.380 ^{ab}	0.716 ^{fn}	0.668 ^{gn}	1.125 ^{bd}	0.527 ^{mn}	0.832 ^{dl}	0.821 ^{dm}
OIV 613	1.117 ^{bh}	1.135 ^{bg}	0.713 ^{jk}	1.339 ^{ab}	0.766 ^{ik}	0.735 ^{ik}	1.036 ^{bi}	0.853 ^{gk}	1.225 ^{af}	1.024 ^{ci}
OIV 614	0.600 ^{ei}	0.691 ^{di}	0.650 ^{di}	1.159 ^{ab}	0.560 ^{gi}	0.450 ⁱ	0.872 ^{cd}	0.532 ^{gi}	0.580 ^{fi}	0.685 ^{di}
OIV 615	0.884 ^{ci}	0.879 ^{ej}	0.657 ^{ik}	1.020 ^{ag}	0.666 ^{ik}	0.573 ^k	0.772 ^{gk}	0.739 ^{hk}	0.916 ^{ci}	0.899 ^{ci}
OIV 616	4.727 ^l	6.273 ^{hk}	9.000 ^a	5.727 ^{il}	6.818 ^{fj}	7.182 ^{di}	6.364 ^{hk}	7.727 ^{ah}	5.000 ^{kl}	5.091 ^{kl}
OIV 617	3.177 ^{dg}	3.847 ^{ae}	3.435 ^{cg}	4.358 ^{ac}	2.701 ^g	2.726 ^{fg}	3.635 ^{bg}	3.424 ^{cg}	3.393 ^{cg}	2.938 ^{eg}
OIV 618	2.008 ^{ci}	2.252 ^{bh}	1.622 ^{ej}	3.112 ^{ab}	1.120 ^{ij}	0.658 ^g	1.886 ^{di}	2.588 ^{be}	1.116 ^{ij}	1.713 ^{ei}

Averages of homogeneous subgroups are displayed.

Table 6: Continued.

Cultivar OIV code	Bouabane	Anonymous 6	Laadari	Meska 1	Meska 2	Taberkante 1	Anonymous 11	Anonymous 12	Taberkante 2
OIV 601	8.611 ^{bf}	8.766 ^{bf}	8.641 ^{bf}	8.319 ^{df}	7.523 ^{dh}	7.090 ^{eh}	8.252 ^{df}	9.273 ^{ad}	6.675 ^{fh}
OIV 602	7.155 ^{cg}	7.945 ^{be}	7.134 ^{cg}	7.127 ^{cg}	7.018 ^{cg}	6.400 ^{ei}	7.035 ^{cg}	7.741 ^{cg}	6.386 ^{ei}
OIV 603	5.761 ^{bg}	5.931 ^{bf}	5.341 ^{ch}	5.177 ^{ch}	5.114 ^{ci}	4.790 ^{ci}	5.006 ^{ci}	5.455 ^{ch}	4.389 ^{gj}
OIV 604	3.849 ^{af}	4.101 ^{ac}	3.010 ^{ej}	3.203 ^{ci}	3.128 ^{cj}	3.061 ^{dj}	2.977 ^{ej}	3.305 ^{ch}	2.688 ^{gj}
OIV 605	2.930 ^{ik}	5.860 ^{ab}	3.580 ^{gk}	3.437 ^{gk}	3.391 ^{hk}	3.520 ^{gk}	2.543 ^k	4.297 ^{dh}	3.263 ^{hk}
OIV 606	3.247 ^{fj}	5.148 ^{ac}	3.298 ^{fj}	3.239 ^{gj}	3.849 ^{di}	3.134 ^{hj}	2.735 ^j	4.247 ^{bg}	3.263 ^{fj}
OIV 607	50.65 ^{bi}	47.52 ^{ei}	49.30 ^{di}	57.82 ^{ab}	51.64 ^{bh}	48.50 ^{ei}	52.48 ^{bh}	43.34 ⁱ	43.37 ⁱ
OIV 608	50.05 ^{df}	50.91 ^{cdef}	53.11 ^{cf}	55.81 ^{bf}	53.50 ^{bf}	57.72 ^{ae}	59.52 ^{ad}	47.46 ^f	50.34 ^{df}
OIV 609	53.39 ^{ae}	43.47 ^g	51.61 ^{ag}	52.68 ^{af}	46.70 ^{dg}	54.12 ^{ae}	56.45 ^{ac}	45.10 ^{fg}	46.20 ^{dg}
OIV 610	70.29 ^{ad}	66.30 ^{af}	54.96 ^{cg}	63.73 ^{ag}	59.60 ^{bg}	71.41 ^{ac}	60.72 ^{bg}	51.55 ^g	55.38 ^{cg}
OIV 611	1.977 ^{ae}	2.207 ^{ab}	1.238 ^{fi}	1.227 ^{fi}	1.514 ^{ci}	1.485 ^{ci}	1.241 ^{fi}	1.342 ^{ei}	1.020 ^{hi}
OIV 612	0.927 ^{ch}	0.583 ⁱⁿ	0.997 ^{cf}	0.942 ^{cg}	0.882 ^{cj}	0.666 ^{gn}	1.033 ^{ce}	0.625 ^{hn}	0.461 ⁿ
OIV 613	1.278 ^{ad}	1.026 ^{ci}	1.143 ^{bg}	1.120 ^{bh}	0.816 ^{hk}	0.947 ^{ek}	1.227 ^{ae}	0.777 ^{ik}	0.676 ^k
OIV 614	0.836 ^{ce}	0.717 ^{dh}	0.872 ^{cd}	0.747 ^{dg}	0.682 ^{di}	0.656 ^{di}	0.746 ^{dg}	0.547 ^{gi}	0.473 ^{hi}
OIV 615	1.195 ^{ab}	0.946 ^{ah}	0.943 ^{ah}	0.874 ^{cj}	0.727 ^{hk}	0.794 ^{ek}	0.991 ^{ah}	0.725 ^{hk}	0.604 ^{jk}
OIV 616	5.727 ⁱⁿ	7.545 ^{ah}	5.364 ^{il}	7.455 ^{bh}	7.909 ^{ag}	7.091 ^{ei}	4.636 ^l	7.273 ^{ch}	7.091 ^{ei}
OIV 617	3.866 ^{ae}	4.025 ^{ad}	3.309 ^{cg}	4.567 ^{ab}	3.606 ^{bg}	3.413 ^{cg}	3.393 ^{cg}	3.468 ^{cg}	2.890 ^{cg}
OIV 618	1.565 ^{fj}	2.176 ^{bh}	2.765 ^{bd}	1.365 ^{hj}	1.883 ^{di}	1.279 ^{hj}	2.173 ^{bh}	2.884 ^{bc}	2.002 ^{ci}

Averages of homogeneous subgroups are displayed.

Table 6: Continued.

Cultivar OIV code	Tasemith	Tazizaouth 1	Tazizaouth 2	Tazizaouth 3	Tazizaouth 4	Tazizaouth 5	Tazizaouth 6	Tazizaouth 7
OIV 601	9.433 ^{ad}	8.472 ^{cf}	8.743 ^{bf}	8.266 ^{df}	8.022 ^{df}	8.778 ^{bf}	9.026 ^{ae}	8.685 ^{bf}
OIV 602	8.527 ^{ac}	7.782 ^{cg}	7.883 ^{cf}	7.452 ^{cg}	7.292 ^{cg}	7.847 ^{cf}	7.994 ^{be}	8.178 ^{ad}
OIV 603	6.163 ^{ae}	5.885 ^{bf}	5.920 ^{bf}	5.411 ^{ch}	5.388 ^{ch}	6.007 ^{bf}	5.826 ^{bg}	6.257 ^{ac}
OIV 604	4.024 ^{ad}	3.674 ^{bg}	3.814 ^{bf}	3.543 ^{ch}	3.483 ^{ch}	3.898 ^{ae}	3.788 ^{bf}	3.783 ^{bf}
OIV 605	5.504 ^{ad}	5.692 ^{ab}	5.654 ^{ac}	5.564 ^{ac}	5.071 ^{af}	5.374 ^{ad}	4.873 ^{bf}	5.208 ^{af}
OIV 606	5.106 ^{ac}	4.955 ^{ab}	5.013 ^{ac}	4.658 ^{be}	4.677 ^{be}	4.967 ^{ab}	4.742 ^{bd}	4.954 ^{bc}
OIV 607	48.07 ^{ei}	46.89 ^{fi}	48.72 ^{ei}	45.98 ^{gi}	48.71 ^{ei}	50.41 ^{ei}	45.34 ^{hi}	51.65 ^{bh}
OIV 608	51.46 ^{cf}	56.56 ^{af}	48.53 ^{ef}	51.88 ^{cf}	47.51 ^f	48.46 ^{ef}	47.65 ^f	54.14 ^{bf}
OIV 609	49.51 ^{cg}	51.79 ^{af}	50.11 ^{cg}	48.69 ^{cg}	47.69 ^{dg}	49.22 ^{cg}	45.90 ^{eg}	52.68 ^{af}
OIV 610	63.84 ^{ag}	57.47 ^{eg}	66.84 ^{ae}	58.84 ^{dg}	64.78 ^{af}	66.75 ^{ae}	62.93 ^{ag}	64.75 ^{af}
OIV 611	2.119 ^{ac}	1.694 ^{ah}	1.835 ^{ag}	1.750 ^{ag}	1.799 ^{ag}	2.139 ^{ac}	1.985 ^{ae}	1.904 ^{af}
OIV 612	0.625 ^{hn}	0.560 ^{ln}	0.713 ^{fn}	0.495 ⁿ	0.575 ^{kn}	0.606 ⁱⁿ	0.637 ^{hn}	0.709 ^{fn}
OIV 613	0.921 ^{fk}	0.790 ^{ik}	0.981 ^{dk}	0.846 ^{gk}	0.880 ^{gk}	0.864 ^{gk}	0.818 ^{hk}	0.868 ^{gk}
OIV 614	0.621 ^{di}	0.637 ^{di}	0.647 ^{di}	0.567 ^{gi}	0.547 ^{gi}	0.635 ^{di}	0.644 ^{di}	0.675 ^{di}
OIV 615	0.864 ^{ej}	0.857 ^{ej}	0.856 ^{ej}	0.796 ^{ek}	0.778 ^{fk}	0.814 ^{ek}	0.923 ^{bi}	0.988 ^{ah}
OIV 616	8.727 ^{ac}	8.273 ^{af}	7.273 ^{ch}	8.636 ^{ad}	8.545 ^{ae}	8.091 ^{af}	8.455 ^{ae}	8.818 ^{ab}
OIV 617	4.095 ^{ad}	3.700 ^{bg}	3.739 ^{bg}	3.780 ^{af}	3.599 ^{bg}	3.746 ^{bf}	3.892 ^{ae}	4.140 ^{ad}
OIV 618	2.732 ^{bd}	3.119 ^{ab}	2.395 ^{bg}	2.463 ^{bf}	2.132 ^{bh}	2.248 ^{bh}	2.480 ^{bf}	2.602 ^{be}

Averages of homogeneous subgroups are displayed.

Table 6: Continued.

Cultivar	Tazizaouth	Tazizaouth	Tazizaouth	Tazizaouth	Tazogaghth	Tazogaghth	Tazogaghth	Tazogaghth
OIV code	8	9	10	11	1	2	3	4
OIV 601	10.653 ^{ab}	9.040 ^{ae}	8.878 ^{ae}	7.962 ^{dg}	8.877 ^{ae}	8.714 ^{bf}	10.971 ^a	9.398 ^{ad}
OIV 602	9.940 ^a	7.431 ^{cg}	7.687 ^{cg}	6.875 ^{ch}	7.813 ^{cg}	7.742 ^{cg}	9.666 ^{ab}	8.523 ^{ac}
OIV 603	7.627 ^a	5.508 ^{bh}	5.571 ^{bh}	5.081 ^{ci}	5.738 ^{bg}	5.850 ^{bg}	6.971 ^{ab}	6.241 ^{ad}
OIV 604	4.827 ^a	3.379 ^{ch}	3.413 ^{ch}	3.036 ^{dj}	3.841 ^{af}	3.857 ^{af}	4.591 ^{ab}	4.074 ^{ac}
OIV 605	6.322 ^a	4.410 ^{ch}	3.034 ^{ik}	2.565 ^k	4.695 ^{bg}	5.325 ^{ae}	5.315 ^{ae}	4.935 ^{bf}
OIV 606	6.090 ^a	4.325 ^{bf}	3.315 ^{fj}	2.874 ^{ij}	4.626 ^{be}	4.995 ^{bc}	5.172 ^{ab}	4.697 ^{be}
OIV 607	47.98 ^{ei}	51.19 ^{bh}	54.78 ^{ae}	52.56 ^{bh}	48.12 ^{ei}	48.68 ^{ei}	49.75 ^{di}	52.61 ^{bh}
OIV 608	47.94 ^{ef}	50.82 ^{df}	55.21 ^{bf}	57.01 ^{af}	47.57 ^f	52.59 ^{cf}	56.06 ^{af}	56.56 ^{af}
OIV 609	46.27 ^{dg}	49.43 ^{cg}	52.33 ^{af}	57.84 ^{ab}	45.87 ^{eg}	48.56 ^{cg}	53.82 ^{ae}	54.27 ^{ad}
OIV 610	59.07 ^{cg}	64.52 ^{af}	63.08 ^{ag}	65.51 ^{af}	62.23 ^{ag}	66.08 ^{af}	73.30 ^a	71.83 ^{ab}
OIV 611	2.340 ^a	1.698 ^{ah}	1.378 ^{ei}	1.263 ^{fi}	2.074 ^{ad}	1.905 ^{af}	2.222 ^{ab}	1.804 ^{ag}
OIV 612	0.800 ^{em}	0.650 ^{gn}	0.866 ^{dk}	1.048 ^{ac}	0.596 ⁱⁿ	0.525 ^{mn}	1.491 ^a	1.175 ^{bc}
OIV 613	0.991 ^{dj}	0.780 ^{ik}	1.310 ^{ac}	1.213 ^{af}	0.764 ^{ik}	0.844 ^{gk}	1.441 ^a	1.338 ^{ab}
OIV 614	0.867 ^{cd}	0.576 ^{fi}	0.813 ^{cf}	0.840 ^{ce}	0.597 ^{ei}	0.630 ^{di}	1.291 ^a	1.020 ^{bc}
OIV 615	1.096 ^{ad}	0.729 ^{hk}	1.116 ^{ac}	1.050 ^{af}	0.830 ^{dk}	0.857 ^{cj}	1.214 ^a	1.069 ^{ae}
OIV 616	8.182 ^{af}	8.727 ^{ac}	5.273 ^{kl}	4.545 ^l	7.364 ^{bh}	7.636 ^{ah}	6.364 ^{hk}	6.455 ^{gk}
OIV 617	4.343 ^{ad}	3.669 ^{bg}	4.108 ^{ad}	3.670 ^{bg}	3.570 ^{bg}	4.397 ^{bg}	4.341 ^a	4.166 ^{ad}
OIV 618	4.080 ^a	1.878 ^{di}	1.969 ^{ci}	2.205 ^{bh}	2.588 ^{be}	2.532 ^{bf}	2.069 ^{ci}	1.462 ^{gj}

Averages of homogeneous subgroups are displayed.

The studied cultivars also demonstrate varying results with regards to the ampelometric relationships of quantitative parameters measured in mature leaves following the method described by Martinez and Grenan, (1999), which are detailed as following:

In terms of the Rel. 2 parameter, the cultivars ‘Ait Abdi’, ‘A. Bouamar’, ‘Anonymous 2’, ‘Laadari’, ‘Tazizaouth 9’, ‘Anonymous 11’, ‘Bouabane’, ‘Anonymous 12’, ‘Meska 1’, ‘Amellal 2’, ‘Tazogaghth 1’, ‘Tazizaouth 10’, ‘Anonymous 1’, ‘Tazizaouth 11’, ‘Tazogaghth 3’, ‘Ameziane’, ‘Tazizaouth 6’, ‘Tazogaghth 2’ and ‘Anonymous 5’ have short to medium distances between the first right lateral vein (L1d) and central vein (L), where the smallest value is observed in ‘Ait Abdi’ with an average of 0.786 cm. However, the cultivars ‘Amellal 2’, ‘Tazogaghth 1’, ‘Tazizaouth 10’, ‘Anonymous 1’, ‘Tazizaouth 11’, ‘Tazogaghth 3’, ‘Ameziane’, ‘Tazizaouth 6’, ‘Tazogaghth 2’, ‘Anonymous 5 and 4’, ‘Tazizaouth 3 and 5’, ‘Anonymous 3’, ‘Tazizaouth 4’, ‘Tasemith’, ‘Tazizaouth 2’, ‘Anonymous 6’, ‘Tazogaghth 4’, ‘Amellal 1’, ‘Taberkante 1’, ‘Meska 2’, ‘Tazizaouth 8, 1 and 7’, ‘Taberkante 2’ have medium to long distances between the first right lateral vein (L1d) and central vein (L), where the greatest value is observed in ‘Taberkante 2’ with an average of 0.966 cm.

In terms of the Rel. 3 parameter, the cultivars ‘Anonymous 2’, ‘Ait Abdi’, ‘A. Bouamar’, ‘Tazizaouth 9’, ‘Bouabane’, ‘Anonymous 12 and 1’, ‘Laadari’, ‘Meska 1’, ‘Tazizaouth 11’, ‘Anonymous 11’, ‘Tazizaouth 10’, ‘Anonymous 4’, ‘Tazogaghth 1, 3 and 2’, ‘Tazizaouth 6 and 2’ as well as ‘Ameziane’ display short to medium distances between the first left lateral vein (L1g) and central vein (L), where the smallest value is observed in ‘Anonymous 2’ with an average of 0.788 cm. In contrast, the cultivars ‘Laadari’, ‘Meska 1’, ‘Tazizaouth 11’, ‘Anonymous 11’, ‘Tazizaouth 10’, ‘Anonymous 4’, ‘Tazogaghth 1, 3 and 2’, ‘Tazizaouth 6 and 2’, ‘Ameziane’, ‘Amellal 2’, ‘Tazizaouth 5’, ‘Taberkante 1’, ‘Tasemith’, ‘Anonymous 6’, ‘Tazogaghth 4’, ‘Tazizaouth 1’, ‘Anonymous 5’, ‘Amellal 1’, ‘Tazizaouth 4’, ‘Anonymous 3’, ‘Tazizaouth 3, 7 and 8’, ‘Meska 2’ and ‘Taberkante 2’ demonstrate medium to long distances between the first left lateral vein (L1g) and central vein (L), where the greatest value is observed in ‘Taberkante 2’ with an average of 0.949 cm.

In terms of the Rel. 4 parameter, the cultivars ‘A. Bouamar’, ‘Anonymous 2’, ‘Ait Abdi’, ‘Anonymous 12’, ‘Meska 1’, ‘Anonymous 11’, ‘Anonymous 4 and 1’, ‘Tazizaouth 9’, ‘Amellal 2’, ‘Tazizaouth 10’, ‘Laadari’, ‘Ameziane’, ‘Tazogaghth 3 and 1’, ‘Anonymous 3’, ‘Tazizaouth 6 and 11’, ‘Taberkante 2’, ‘Anonymous 5’, ‘Tasemith’, ‘Tazizaouth 3’, ‘Bouabane’, ‘Tazogaghth 4’, ‘Taberkante 1’, ‘Tazizaouth 4 and 2’, ‘Tazogaghth 2’, ‘Anonymous 6’ and ‘Tazizaouth 5’ have short to medium distances between the second right lateral vein (L2d) and central vein (L), where the smallest value is observed in ‘A. Bouamar’ with an average of 0.574 cm. However, the cultivars ‘Meska 1’,

‘Anonymous 11’, ‘Anonymous 4 and 1’, ‘Tazizaouth 9’, ‘Amellal 2’, ‘Tazizaouth 10’, ‘Laadari’, ‘Ameziane’, ‘Tazogaghth 3 and 1’, ‘Anonymous 3’, ‘Tazizaouth 6 and 11’, ‘Taberkante 2’, ‘Anonymous 5’, ‘Tasemith’, ‘Tazizaouth 3’, ‘Bouabane’, ‘Tazogaghth 4’, ‘Taberkante 1’, ‘Tazizaouth 4 and 2’, ‘Tazogaghth 2’, ‘Anonymous 6’, ‘Tazizaouth 5’, ‘Amellal 1’, ‘Meska 2’, ‘Tazizaouth 1, 8 and 7’ exhibit medium to long distances between the second right lateral vein (L2d) and central vein (L), where the greatest value is observed in ‘Tazizaouth 7’ with an average of 0.714 cm.

Regarding the Rel. 5 parameter, the cultivars ‘Anonymous 2’, ‘Ait Abdi’, ‘Anonymous 1’, ‘A. Bouamar’, ‘Anonymous 12 and 4’, ‘Tazizaouth 9’, ‘Anonymous 11’, ‘Ameziane’, ‘Laadari’, ‘Tazizaouth 10’, ‘Meska 1’, ‘Tazizaouth 11’, ‘Tazogaghth 3’, ‘Amellal 2’, ‘Tazizaouth 6’, ‘Tasemith’, ‘Tazizaouth 3’, ‘Tazogaghth 1’, ‘Meska 2’, ‘Tazogaghth 4’, ‘Taberkante 2’, ‘Tazizaouth 4’, ‘Tazogaghth 2’, ‘Anonymous 6’, ‘Bouabane’, ‘Anonymous 3 and 5’, ‘Tazizaouth 2’ and ‘Amellal 1’ have short to medium distances between the second left lateral vein (L2g) and central vein (L), where the smallest value is observed in Anonymous 2 with an average of 0.570 cm. Conversely, the cultivars ‘Ameziane’, ‘Laadari’, ‘Tazizaouth 10’, ‘Meska 1’, ‘Tazizaouth 11’, ‘Tazogaghth 3’, ‘Amellal 2’, ‘Tazizaouth 6’, ‘Tasemith’, ‘Tazizaouth 3’, ‘Tazogaghth 1’, ‘Meska 2’, ‘Tazogaghth 4’, ‘Taberkante 2’, ‘Tazizaouth 4’, ‘Tazogaghth 2’, ‘Anonymous 6’, ‘Bouabane’, ‘Anonymous 3 and 5’, ‘Tazizaouth 2’, ‘Amellal 1’, ‘Tazizaouth 1 and 5’, ‘Taberkante 1’, ‘Tazizaouth 8 and 7’ show medium to long distances between the second left lateral vein (L2g) and central vein (L), where the greatest value is observed in ‘Tazizaouth 7’ with an average of 0.727 cm.

With respect to the Rel. 6 parameter, the cultivars ‘Amellal 1’, ‘Tazizaouth 11’, ‘Anonymous 11’, ‘Ait Abdi’, ‘Tazizaouth 10’, ‘Bouabane’, ‘Anonymous 5’, ‘Ameziane’, ‘A. Bouamar’, ‘Meska 1 and 2’, ‘Anonymous 4’, ‘Taberkante 2’, ‘Laadari’, ‘Anonymous 2 and 12’ exhibit short distances between the right lateral upper sinus (S1d) and the first right lateral vein (L1d), where the smallest value is observed in ‘Amellal 1’ with an average of 0.360 cm. Meanwhile, the cultivars ‘Tazogaghth 1’, ‘Tazizaouth 6 and 8’, ‘Tasemith’, ‘Tazizaouth 7, 4 and 5’, ‘Tazogaghth 2’, ‘Tazizaouth 2 and 1’, ‘Anonymous 6’, ‘Tazizaouth 3’ and ‘Anonymous 3’ display long distances between the right lateral upper sinus (S1d) and the first right lateral vein (L1d), where the greatest value is observed in Anonymous 3 with an average of 0.780 cm.

In terms of the Rel. 7 parameter, the cultivars ‘Anonymous 11’, ‘Amellal 1’, ‘Tazizaouth 11’, ‘Ait Abdi’, ‘Tazizaouth 10’, ‘Bouabane’, ‘Anonymous 5’, ‘A. Bouamar’, ‘Ameziane’, ‘Meska 2 and 1’, ‘Amellal 2’, ‘Anonymous 4’ and ‘Laadari’ demonstrate short distances between the left lateral upper sinus (S1g) and the first left lateral vein (L1g), where the smallest value is observed in

‘Anonymous 11’ with an average of 0.338 cm. In contrast, the cultivars ‘Tazizaouth 9’, ‘Anonymous 1’, ‘Tazizaouth 7, 8 and 6’, ‘Tasemith’, ‘Tazogaghth 2’, ‘Tazizaouth 5, 2 and 4’, ‘Anonymous 6’, ‘Tazizaouth 3 and 1’, ‘Anonymous 3’ exhibit long distances between the right lateral upper sinus (S1d) and the first right lateral vein (L1d), where the greatest value is observed in ‘Anonymous 3’ with an average of 0.769 cm.

Concerning the Rel. 8 parameter, the cultivars ‘Amellal 1’, ‘Anonymous 11’, ‘Bouabane’, ‘Tazizaouth 11 and 10’, ‘Laadari’, ‘A. Bouamar’, ‘Anonymous 5’ and ‘Meska 1’ display short distances between the right lateral lower sinus (S2d) and the second right lateral vein (L2d), where the smallest value is observed in ‘Amellal 1’ with an average of 0.524 cm. Meanwhile, the cultivars ‘Tazogaghth 4’, Ameziane, ‘Amellal 2’, ‘Anonymous 12’, ‘Tazizaouth 9 and 7’, ‘Anonymous 1’, ‘Tazizaouth 8’, ‘Tazogaghth 1’, ‘Tazizaouth 6, 1, 5 and 2’, ‘Tasemith’, ‘Tazogaghth 2’, ‘Tazizaouth 3’, ‘Anonymous 6’, ‘Tazizaouth 4’ and ‘Anonymous 3’ show long distances between the right lateral lower sinus (S2d) and the second right lateral vein (L2d), where the greatest value is observed in ‘Anonymous 3’ with an average of 0.897 cm.

With regards to the Rel. 9 parameter, the cultivars ‘Amellal 1’, ‘Anonymous 11’, ‘Tazizaouth 11’, ‘Bouabane’, ‘Tazizaouth 10’, ‘A. Bouamar’ exhibit short distances between the left lateral lower sinus (S2g) and the second left lateral vein (L2g), where the smallest value is observed in ‘Amellal 1’ with an average of 0.478 cm. Conversely, the cultivars ‘Ameziane’, ‘Anonymous 2 and 12’, ‘Amellal 2’, ‘Meska 2’, ‘Tazizaouth 9 and 8’, ‘Tazogaghth 1’, ‘Tazizaouth 7, 5 and 6’, ‘Tasemith’, ‘Tazizaouth 1 and 2’, ‘Tazogaghth 2’, ‘Tazizaouth 4 and 3’, ‘Anonymous 1, 6 and 3’ demonstrate long distances between the left lateral lower sinus (S2g) and the second left lateral vein (L2g), where the greatest value is observed in ‘Anonymous 3’ with an average of 0.894 cm.

In terms of the Rel. 10 parameter, the cultivars ‘Tazizaouth 6’, ‘Anonymous 12’, ‘Taberkante 2’, ‘Anonymous 6’, ‘Tazizaouth 8’, ‘Tazogaghth 1’, ‘Tazizaouth 4, 5, 2 and 9’, ‘Anonymous 2’, ‘Bouabane’, ‘Tasemith’, ‘Tazizaouth 3’, ‘Meska 2’, ‘Tazogaghth 2’, ‘Anonymous 3’, ‘A. Bouamar’, ‘Laadari’ and ‘Amellal 2’ have small to medium angles formed by the right lateral main veins ($A + B + G$), where the smallest value is observed in ‘Tazizaouth 6’ with an average of 136.60° . In the other hand, the cultivars ‘Tazogaghth 3’, ‘Tazizaouth 10’, ‘Taberkante 1’, ‘Amellal 1’, ‘Meska 1’, ‘Tazogaghth 4’, ‘Tazizaouth 11’, ‘Anonymous 11’, ‘Ait Abdi’, ‘Anonymous 1, 4 and 5’ display medium to large angles formed by the right lateral main veins ($A + B + G$), where the greatest value is observed in ‘Anonymous 5’ with an average of 181.46° .

Concerning of the Rel. 11 parameter, the cultivars ‘Anonymous 12’, ‘Taberkante 2’, ‘Tazogaghth 1’, ‘Tazizaouth 6’, ‘Anonymous 6’, ‘Tazizaouth 8, 3 and 4’, ‘Anonymous 3 and 2’, ‘Tazizaouth 2’, ‘Tazogaghth 2’, ‘A. Bouamar’, ‘Tasemith’, ‘Tazizaouth 1 and 5’, ‘Laadari’, ‘Meska 2’, ‘Tazizaouth 9’, ‘Amellal 2’ and ‘Tazizaouth 7’ demonstrate small to medium angles formed by the left lateral main veins ($A' + B' + G'$), where the smallest value is observed in ‘Anonymous 12’ with an average of 133.97° . Conversely, the cultivars ‘Tazizaouth 5’, ‘Laadari’, ‘Meska 2’, ‘Tazizaouth 9’, ‘Amellal 2’, ‘Tazizaouth 7’, ‘Taberkante 1’, ‘Tazogaghth 3’, ‘Bouabane’, ‘Ameziane’, ‘Tazizaouth 10’, ‘Tazogaghth 4’, ‘Ait Abdi’, ‘Tazizaouth 11’, ‘Amellal 1’, ‘Anonymous 11 and 4’, ‘Meska 1’, ‘Anonymous 1 and 5’ have medium to large angles formed by the left lateral main veins ($A' + B' + G'$), where the greatest value is observed in ‘Anonymous 5’ with an average of 175.41° .

In terms of the Rel. 14 parameter, the cultivars ‘Amellal 1’, ‘Anonymous 11’, ‘Tazizaouth 11 and 10’, ‘Bouabane’, ‘Ait Abdi’, ‘Anonymous 5’, ‘A. Bouamar’, ‘Meska 1’ and ‘Laadari’ have short distances between the two right lateral sinuses ($S1d + S2d$) and the first two right main veins ($L1d + L2d$), where the smallest value is observed in ‘Amellal 1’ with an average of 0.430 cm. However, the cultivars ‘Tazogaghth 1’, ‘Tazizaouth 6, 8 and 7’, ‘Tasemith’, ‘Tazizaouth 5’, ‘Tazogaghth 2’, ‘Tazizaouth 4, 2, and 1’, ‘Anonymous 6’ as well as ‘Tazizaouth 3’ display long distances between the two right lateral sinuses ($S1d + S2d$) and the first two right main veins ($L1d + L2d$), where the greatest value is observed in ‘Tazizaouth 3’ with an average of 0.811 cm.

With regards to the Rel. 15 parameter, the cultivars ‘Amellal 1’, ‘Anonymous 11’, ‘Tazizaouth 11’, ‘Bouabane’, ‘Tazizaouth 10’, ‘Ait Abdi’, ‘A. Bouamar’ and ‘Anonymous 5’ show short distances between the two left lateral sinuses ($S1g + S2g$) and the first two left main veins ($L1g + L2g$), where the smallest value is observed in ‘Amellal 1’ with an average of 0.416 cm. Meanwhile, the cultivars ‘Tazizaouth 7, 8 and 6’, ‘Tasemith’, ‘Anonymous 1’, ‘Tazizaouth 5’, ‘Tazogaghth 2’, ‘Tazizaouth 2 and 4’, ‘Anonymous 6’, ‘Tazizaouth 3 and 1’ as well as ‘Anonymous 3’ exhibit long distances between the two left lateral sinuses ($S1g + S2g$) and the first two left main veins ($L1g + L2g$), where the greatest value is observed in Anonymous 3 with an average of 0.822 cm.

Table 7: ANOVA results of ampelometric characteristics (relationships).

Cultivar									
Relationship	Ait Abdi	Amellal 1	Amellal 2	A. bouamar	Ameziane	Anonymous 1	Anonymous 2	Anonymous 3	Anonymous 4
Rel.2	0.786 ^f	0.916 ^{ae}	0.866 ^{af}	0.817 ^{ef}	0.885 ^{af}	0.874 ^{af}	0.827 ^{df}	0.905 ^{ae}	0.893 ^{ae}
Rel.3	0.797 ^{fg}	0.909 ^{ae}	0.897 ^{af}	0.807 ^{eg}	0.896 ^{ag}	0.839 ^{bg}	0.788 ^g	0.917 ^{ad}	0.874 ^{ag}
Rel.4	0.586 ^{bd}	0.688 ^{ac}	0.624 ^{ad}	0.574 ^d	0.633 ^{ad}	0.610 ^{ad}	0.577 ^{cd}	0.640 ^{ad}	0.609 ^{ad}
Rel.5	0.574 ^d	0.683 ^{ad}	0.639 ^{ad}	0.587 ^{cd}	0.622 ^{ad}	0.577 ^d	0.570 ^d	0.674 ^{ad}	0.602 ^{cd}
Rel.6	0.392 ^{ik}	0.360 ^l	0.566 ^{dj}	0.474 ^{hl}	0.465 ^{hl}	0.585 ^{ci}	0.528 ^{el}	0.780 ^a	0.491 ^{gl}
Rel.7	0.387 ^{jl}	0.369 ^{kl}	0.503 ^{gl}	0.478 ^{gl}	0.481 ^{gl}	0.603 ^{ag}	0.531 ^{dk}	0.769 ^a	0.506 ^{fl}
Rel.8	0.676 ^{ej}	0.524 ^k	0.774 ^{ag}	0.620 ^{ik}	0.770 ^{ah}	0.811 ^{ae}	0.745 ^{ci}	0.897 ^a	0.670 ^{fj}
Rel.9	0.668 ^{ci}	0.478 ^j	0.768 ^{af}	0.604 ^{hj}	0.758 ^{bg}	0.866 ^{ab}	0.758 ^{ag}	0.894 ^a	0.687 ^{ci}
Rel.10	171.72 ^{ad}	163.66 ^{ag}	157.49 ^{bk}	154.41 ^{ck}	159.40 ^{bj}	175.37 ^{ac}	147.50 ^{fk}	153.19 ^{dk}	175.57 ^{ab}
Rel.11	164.12 ^{ag}	164.95 ^{af}	156.46 ^{ai}	148.71 ^{bi}	161.27 ^{ah}	171.71 ^{ab}	145.17 ^{di}	144.73 ^{di}	166.90 ^{ad}
Rel.14	0.513 ^{lo}	0.430 ^o	0.653 ^{dk}	0.534 ^{jo}	0.592 ^{hm}	0.676 ^{ci}	0.618 ^{fl}	0.829 ^a	0.563 ⁱⁿ
Rel.15	0.504 ^{ko}	0.416 ^o	0.613 ^{fl}	0.530 ^{jo}	0.594 ^{gm}	0.707 ^{ag}	0.626 ^{ek}	0.822 ^a	0.580 ^{gm}

Averages of homogeneous subgroups are displayed.

Table 7: Continued.

Cultivar Relationship	Anonymous 5	Bouabane	Anonymous 6	Laadari	Meska 1	Meska 2	Taberkante 1	Anonymous 11	Anonymous 12
Rel.2	0.891 ^{af}	0.845 ^{cf}	0.913 ^{ae}	0.832 ^{df}	0.852 ^{bf}	0.933 ^{ad}	0.927 ^{ad}	0.845 ^{cf}	0.849 ^{bf}
Rel.3	0.906 ^{ae}	0.819 ^{cg}	0.902 ^{af}	0.841 ^{ag}	0.861 ^{ag}	0.936 ^{ab}	0.899 ^{ae}	0.864 ^{ag}	0.833 ^{bg}
Rel.4	0.658 ^{ad}	0.668 ^{ad}	0.678 ^{ad}	0.631 ^{ad}	0.603 ^{ad}	0.694 ^{ab}	0.672 ^{ad}	0.604 ^{ad}	0.587 ^{bd}
Rel.5	0.674 ^{ad}	0.673 ^{ad}	0.673 ^{ad}	0.635 ^{ad}	0.638 ^{ad}	0.661 ^{ad}	0.696 ^{ac}	0.612 ^{bd}	0.597 ^{cd}
Rel.6	0.445 ^{hl}	0.417 ^{il}	0.760 ^{ac}	0.515 ^{fl}	0.481 ^{gl}	0.491 ^{gl}	0.573 ^{di}	0.385 ^{kl}	0.534 ^{el}
Rel.7	0.469 ^{gl}	0.405 ^{hl}	0.723 ^{ac}	0.507 ^{fl}	0.501 ^{gl}	0.481 ^{gl}	0.573 ^{ch}	0.338 ^l	0.566 ^{ci}
Rel.8	0.633 ^{hk}	0.558 ^{jk}	0.870 ^{ac}	0.619 ^{ik}	0.643 ^{gk}	0.738 ^{ci}	0.684 ^{dj}	0.547 ^{jk}	0.783 ^{ag}
Rel.9	0.624 ^{gi}	0.572 ^{ij}	0.873 ^{ab}	0.647 ^{ei}	0.634 ^{fi}	0.776 ^{af}	0.659 ^{di}	0.549 ^{ij}	0.767 ^{af}
Rel.10	181.46 ^a	148.65 ^{fk}	141.42 ^{ik}	155.45 ^{bk}	164.41 ^{ag}	150.79 ^{dk}	163.20 ^{ah}	171.34 ^{ad}	137.82 ^k
Rel.11	175.41 ^a	159.54 ^{ah}	142.36 ^{fi}	152.58 ^{ai}	168.22 ^{ac}	152.90 ^{ai}	157.48 ^{ah}	165.58 ^{ae}	133.97 ⁱ
Rel.14	0.523 ^{ko}	0.479 ^{mo}	0.806 ^{ac}	0.558 ^{io}	0.548 ^{io}	0.596 ^{gm}	0.615 ^{fl}	0.452 ^{no}	0.635 ^{el}
Rel.15	0.534 ^{jo}	0.480 ^{lo}	0.787 ^{ab}	0.567 ^{hm}	0.557 ⁱⁿ	0.603 ^{fl}	0.610 ^{fl}	0.425 ^{no}	0.648 ^{cj}

Averages of homogeneous subgroups are displayed.

Table 7: Continued.

Cultivar Relationship	Taberkante 2	Tasemith	Tazizaouth 1	Tazizaouth 2	Tazizaouth 3	Tazizaouth 4	Tazizaouth 5	Tazizaouth 6	Tazizaouth 7
Rel.2	0.966 ^a	0.910 ^{ae}	0.948 ^{ac}	0.910 ^{ae}	0.894 ^{ae}	0.907 ^{ae}	0.897 ^{ae}	0.886 ^{af}	0.956 ^{ab}
Rel.3	0.949 ^a	0.901 ^{af}	0.905 ^{af}	0.896 ^{ag}	0.927 ^{ac}	0.911 ^{ae}	0.897 ^{af}	0.893 ^{ag}	0.927 ^{ac}
Rel.4	0.650 ^{ad}	0.663 ^{ad}	0.709 ^a	0.673 ^{ad}	0.663 ^{ad}	0.672 ^{ad}	0.681 ^{ad}	0.647 ^{ad}	0.714 ^a
Rel.5	0.664 ^{ad}	0.647 ^{ad}	0.692 ^{ac}	0.681 ^{ad}	0.656 ^{ad}	0.665 ^{ad}	0.694 ^{ac}	0.641 ^{ad}	0.727 ^a
Rel.6	0.512 ^{fl}	0.675 ^{af}	0.734 ^{ad}	0.729 ^{ad}	0.769 ^{ab}	0.690 ^{ae}	0.694 ^{ae}	0.619 ^{ah}	0.676 ^{af}
Rel.7	0.521 ^{ek}	0.632 ^{ag}	0.749 ^{ab}	0.704 ^{ad}	0.735 ^{ac}	0.721 ^{ac}	0.686 ^{ae}	0.627 ^{ag}	0.613 ^{ag}
Rel.8	0.750 ^{bi}	0.857 ^{ac}	0.850 ^{ac}	0.857 ^{ac}	0.867 ^{ac}	0.888 ^{ab}	0.851 ^{ac}	0.837 ^{ac}	0.803 ^{af}
Rel.9	0.746 ^{bg}	0.808 ^{ac}	0.843 ^{ab}	0.845 ^{ab}	0.863 ^{ab}	0.857 ^{ab}	0.802 ^{ac}	0.803 ^{ac}	0.791 ^{ad}
Rel.10	140.31 ^{jk}	149.11 ^{fk}	159.08 ^{bj}	147.34 ^{fk}	149.79 ^{ek}	143.38 ^{gk}	143.68 ^{gk}	136.60 ^k	160.00 ^{bj}
Rel.11	139.51 ^{hi}	148.96 ^{bi}	151.40 ^{bi}	147.38 ^{ci}	143.31 ^{ei}	144.44 ^{di}	152.49 ^{ai}	141.18 ^{gi}	156.94 ^{ai}
Rel.14	0.608 ^{fm}	0.751 ^{ae}	0.784 ^{ad}	0.783 ^{ad}	0.811 ^{ab}	0.774 ^{ad}	0.761 ^{ae}	0.710 ^{ah}	0.730 ^{af}
Rel.15	0.613 ^{fl}	0.705 ^{ag}	0.789 ^{ab}	0.764 ^{ad}	0.788 ^{ab}	0.778 ^{ac}	0.736 ^{af}	0.701 ^{ah}	0.691 ^{ai}

Averages of homogeneous subgroups are displayed.

Table 7: Continued.

Cultivar Relationship	Tazizaouth 8	Tazizaouth 9	Tazizaouth 10	Tazizaouth 11	Tazogagth 1	Tazogagth 2	Tazogagth 3	Tazogagth 4
Rel.2	0.934 ^{ad}	0.837 ^{df}	0.874 ^{af}	0.875 ^{af}	0.871 ^{af}	0.887 ^{af}	0.875 ^{af}	0.915 ^{ae}
Rel.3	0.933 ^{ab}	0.815 ^{dg}	0.869 ^{ag}	0.861 ^{ag}	0.890 ^{ag}	0.893 ^{ag}	0.891 ^{ag}	0.904 ^{af}
Rel.4	0.713 ^a	0.616 ^{ad}	0.627 ^{ad}	0.647 ^{ad}	0.638 ^{ad}	0.674 ^{ad}	0.636 ^{ad}	0.671 ^{ad}
Rel.5	0.723 ^{ab}	0.609 ^{cd}	0.637 ^{ad}	0.638 ^{ad}	0.656 ^{ad}	0.670 ^{ad}	0.639 ^{ad}	0.663 ^{ad}
Rel.6	0.653 ^{ag}	0.596 ^{bh}	0.395 ^{jl}	0.365 ^l	0.615 ^{ah}	0.698 ^{ae}	0.548 ^{ek}	0.594 ^{ch}
Rel.7	0.621 ^{ag}	0.597 ^{ag}	0.394 ^{il}	0.386 ^{il}	0.590 ^{bg}	0.681 ^{af}	0.561 ^{cj}	0.563 ^{ci}
Rel.8	0.821 ^{ad}	0.797 ^{af}	0.589 ^{jk}	0.584 ^{jk}	0.831 ^{ac}	0.862 ^{ac}	0.754 ^{bi}	0.767 ^{ah}
Rel.9	0.782 ^{ae}	0.778 ^{ae}	0.600 ^{hj}	0.565 ^{ij}	0.783 ^{ae}	0.846 ^{ab}	0.735 ^{bh}	0.739 ^{bh}
Rel.10	141.74 ^{ik}	147.40 ^{fk}	163.03 ^{ah}	170.68 ^{ae}	142.29 ^{hk}	151.71 ^{dk}	161.59 ^{ai}	164.81 ^{af}
Rel.11	142.65 ^{ei}	155.48 ^{ai}	161.61 ^{ah}	164.12 ^{ag}	140.80 ^{hi}	147.94 ^{ci}	157.66 ^{ah}	162.06 ^{ah}
Rel.14	0.725 ^{ag}	0.680 ^{bi}	0.476 ^{mo}	0.458 ^{no}	0.707 ^{ah}	0.769 ^{ad}	0.635 ^{el}	0.666 ^{dj}
Rel.15	0.691 ^{ai}	0.674 ^{bi}	0.480 ^{lo}	0.461 ^{mo}	0.671 ^{bi}	0.751 ^{ae}	0.634 ^{dk}	0.637 ^{dk}

Averages of homogeneous subgroups are displayed.

2.1.2. Qualitative characterization

The ampelographic traits used in the present characterization could be classified into ‘stable’ or ‘variable’. It has to be mentioned that the grouping of these parameters as “stable” or “variable” depends on the variety, the environmental circumstances and cultivation practices. For that, the frequency of these traits was calculated to better understand their distribution providing a more comprehensive insight into their stability and potential variability across the investigated genotypes.

2.1.2.1. Frequency of qualitative traits

a. Size of blade (OIV 065)

According to OIV 065 character, we distinguished between four groups of grape varieties (Figure 10). The largest group, comprising 48% of the varieties, featured a large blade and included ‘Amellal 1’, ‘Bouabane’, and ‘Laadari’. Varieties with a medium blade followed, making up 37%, with examples such as ‘Amellal 2’, ‘Anonymous 4, and 5’. A very large blade was observed in only 9% of the varieties, including ‘Amer Bouamar’, ‘Tazizaouth 8 and 9’. The smallest category, representing 6%, included varieties with small leaves, such as ‘Ameziane’ and ‘Anonymous 1’.

Notably, this characteristic depends on cultivation practices, climate and soil conditions, development vigor and may be different even within the same variety (Galet, 1979).

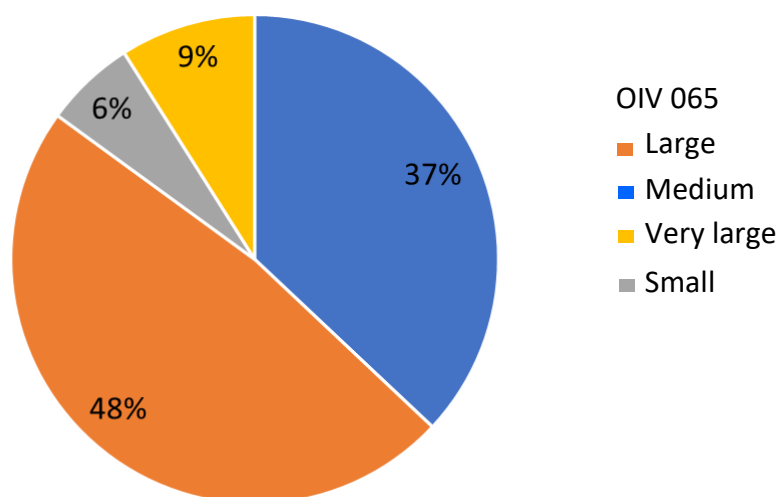


Figure 10: Frequency of size of blade trait in different studied grape varieties.

In comparison to Algerian leaves, Bounab and Laiadi, (2019) reported that the majority of the leaves they studied were characterized by medium-sized blades.

In the other hand, several studies have classified the mature leaf of grapevine varieties into distinct categories based on the size of blade trait. For instance, Alba et al. (2014) described leaves as

small to medium-sized, while Susaj and Susaj (2023) identified them predominantly as medium-sized. On the other hand, Kara et al., (2018) reported varieties with large leaves, whereas in the other study conducted by Kara et al., (2023) observed medium to large leaf blades.

Therefore, these classifications highlight the diversity in leaf morphology across different grapevine varieties, influenced by genetic and environmental factors.

b. Shape of Blade (OIV 067)

According to OIV 067 character, we distinguished between three groups of grape varieties (Figure 11). The majority, exhibited a wedge-shaped blade (51%), including ‘Ait Abdi’, ‘Ameziane’, and ‘Bouabane’. A pentagonal blade shape was observed in 29% of the varieties, such as ‘Amellal 1 and 2’ as well as ‘Meska 1’. Meanwhile, 20% of the varieties, including ‘Tazizaouth 1 to 5’, ‘Tazizaouth 8’, and ‘Tasemith’, featured a kidney-shaped blade.

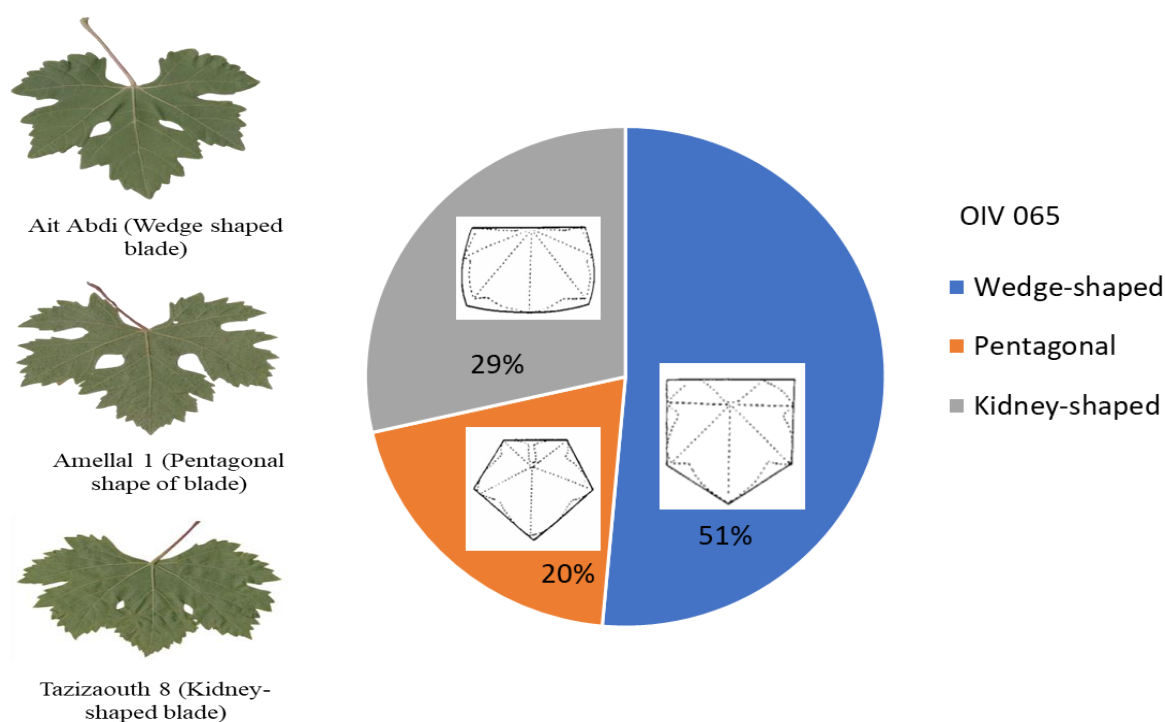


Figure 11: Frequency of shape of blade trait in different studied grape varieties and their representative examples.

According to Diaz, (2017), leaf shapes are critical to the identification of grapevine varieties and their determination remains challenging as were influenced by the environmental conditions (Baumgartner et al., 2020; Chitwood, 2021).

In comparison with the Algerian leaves, Bounab and Laiadi, (2019) found the majority of leaves were evaluated by pentagonal shape of blade. Moreover, our results are in line with other ampelographic studies that identify grapevine varieties based on the shape of blade character (OIV 067) (Panara et al., 2013; Arslan et al., 2018; Kara et al., 2018; Cichi et al., 2022; Susaj and Susaj, 2022; Kara et al., 2023; Hbyaj et al., 2024).

c. Number of lobes (OIV 068)

According to OIV 068 character, we distinguished between three groups of grape varieties based on the number of lobes. The majority of the cultivars studied (91%) had leaves with five lobes, as observed in ‘Amer Bouamar’, ‘Laadari’, and ‘Tazizaouth 1’. Additionally, 6% of the cultivars displayed leaves with three lobes noting ‘Anonymous 2’ and ‘Tazizaouth 4’, while 3% had leaves with seven lobes represented by ‘Tazizaouth 11’ (Figure 12).

Notably, it was indicated in previous studies that the number of lobes could be used as significant parameter in the identification of grapevine varieties.

Our findings are in good agreement in comparison with the Algerian leaves evaluated by Bounab and Laiadi, (2019) where they found the majority of leaves characterized with five lobes.

Additionally, several other studies have reported the presence of five lobes in the majority of leaves they investigated across various grapevine cultivars (Alba et al., 2014; Benito et al., 2016; Vafae et al., 2017; Abiri et al., 2020; Susaj and Susaj, 2022; Hbyaj et al., 2024).

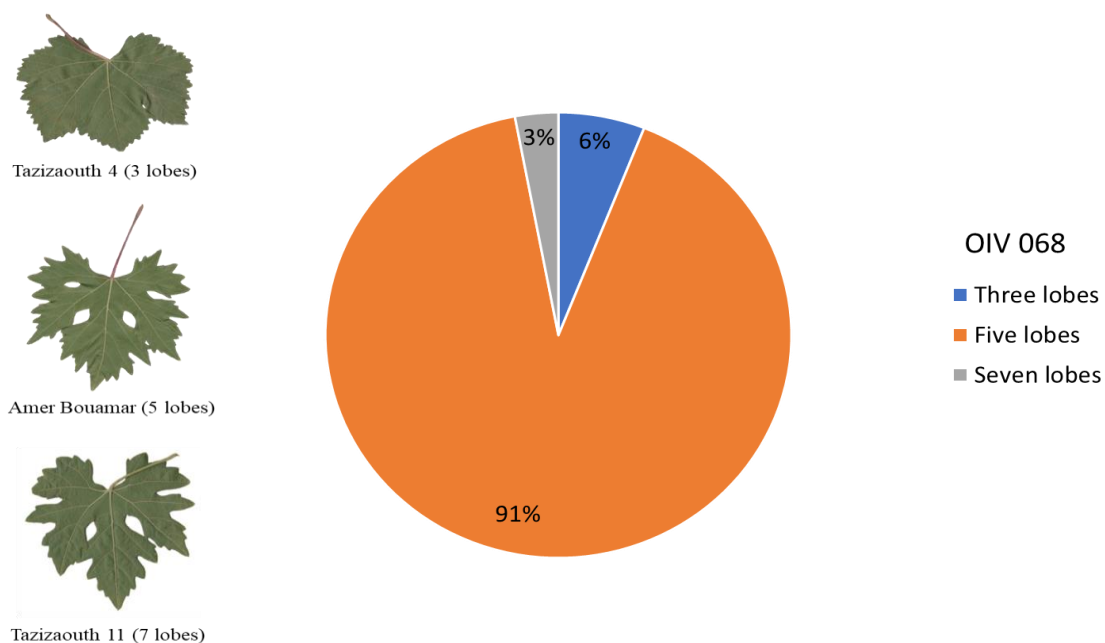


Figure 12: Frequency of number of lobes trait in different studied grape varieties and their representative examples.

d. Color of the upper side of blade (OIV 069)

According to OIV 069 character, we distinguished between two main groups of grape varieties based on the color of the upper side of blade. The distribution of grapevine leaf colors revealed that the vast majority (94%) of the varieties exhibited dark green leaves such as ‘Ait Abdi’, ‘Amer Bouamar’, ‘Ameziane’, ‘Laadari’, while a smaller proportion (6%) showed medium green leaves presented by the varieties ‘Anonymous 2’ and ‘Tazizaouth 11’ (Figure 13).

In comparison with the Algerian leaves, Bounab and Laiadi, (2019) found the majority of leaves were evaluated by medium green color of the upper side of blade. Moreover, our results are in line with other ampelographic studies that identify grapevine varieties based on the color of the upper side of blade character (OIV 069) (Panara et al., 2013; Alba et al., 2014; Arslan et al., 2018; Kara et al., 2018; Susaj and Susaj, 2022; Kara et al., 2023).

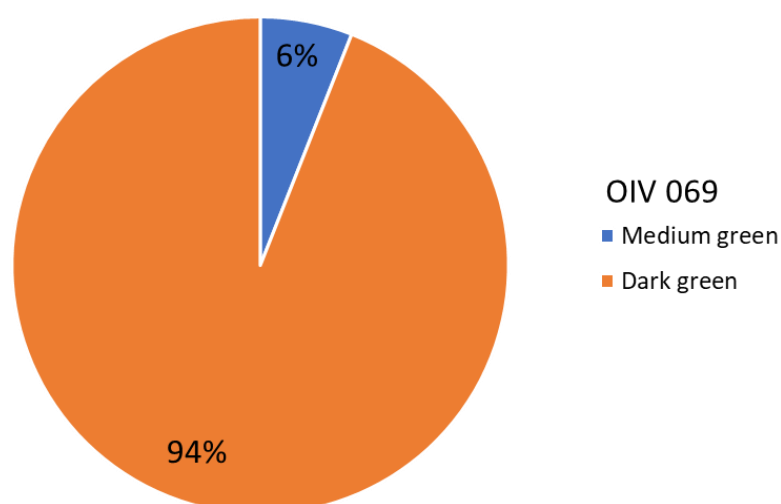


Figure 13: Frequency of color of the upper side of blade trait in different studied grape varieties.

e. Shape of teeth (OIV 076)

According to OIV 076, we distinguished between three groups of grape varieties based on their teeth shape characteristics (Figure 14). The largest group, comprising 54% of the varieties, displayed teeth with a mixture of both sides being straight and both sides convex, including ‘Amellal 1’, ‘Anonymous 1’, ‘Laadari’, and ‘Tazogaghth 3 and 4’. A slightly smaller group, 43%, featured teeth with both sides convex, represented by ‘Ait Abdi’, ‘Amer Bouamar’, and ‘Ameziane’. Only a small proportion, 3%, exhibited an asymmetrical tooth pattern, with one side concave and one side convex, represented by the variety ‘Amellal 2’.

A significant difference was observed when compared to the Algerian leaves evaluated by Bounab and Laiadi, (2019), who characterized the majority of the leaves they studied as having straight teeth on both sides.

Although, these results are in agreement with other ampelographic studies that identify grapevine varieties based on the shape of teeth character (OIV 076) (Panara et al., 2013; Alba et al., 2014; Mdinaradze et al., 2015; Benito et al, 2016; Kara et al., 2018; Kara et al., 2023).

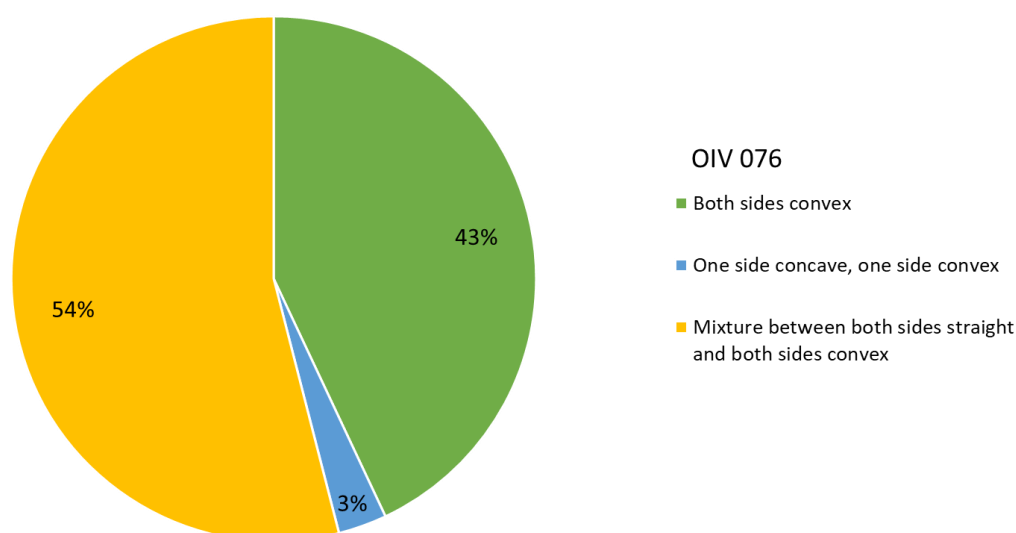


Figure 14: Frequency of shape of teeth trait in different studied grape varieties.

f. Degree of opening / overlapping of petiole sinus (OIV 079)

According to OIV 079 character, which evaluates the degree of openness/overlapping of the petiolar sinus in grape leaves, the analysis revealed two main categories among the studied varieties.

The distribution was nearly even, with 51% of the varieties displayed leaves with open petiolar sinus included ‘Amellal 1 and 2’, ‘Ameziane’ and ‘Anonymous 4’, while the other 49% have leaves showing a very wide open petiolar sinus exemplified by the varieties ‘Amer Bouamar’, ‘Bouabane’ and ‘Laadari’ (Figure 15).

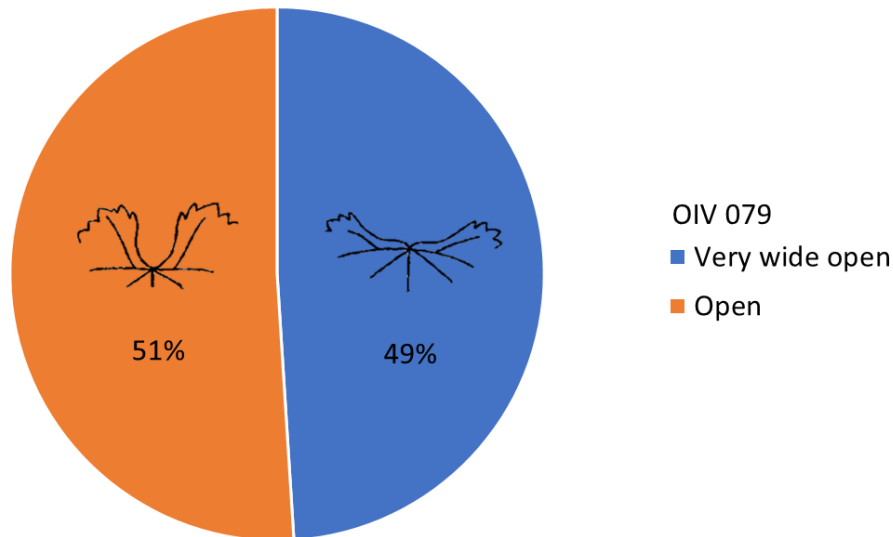


Figure 15: Frequency of degree of opening/ overlapping of petiole sinus trait in different studied grape varieties.

Similar findings were reported by Bounab and Laiadi, (2019), who noted that most Algerian grapevine varieties have leaves with an open petiolar sinus. This observation aligns with previous studies, such as those by Alba et al. (2014), Benito et al., (2016), Stavrakaki and Biniari, (2017); Chehade et al., (2022); Susaj and Susaj, (2022).

The petiolar sinus is highlighted as a key feature for identifying grapevine varieties, particularly due to its angular variation relative to the midvein (Chitwood, 2021). This characteristic has been a long-term focus for ampelographers as it provides valuable identifying information between varieties (Goethe, 1876; Ravaz, 1902; Galet, 1952).

g. Shape of base of petiole sinus (OIV 080)

According to OIV 080 descriptor, which characterizes the base shape of the petiolar sinus in grape leaves, three distinct forms were identified among the studied varieties (Figure 16).

The majority of varieties (63%) displayed a brace-shaped base, represented by the varieties ‘Ait Abdi’, ‘Amellal 1’ and ‘Amer Bouamar’. The second form was the U-shaped base, observed in 29% of the varieties such as ‘Amellal 2’, ‘Ameziane’ and ‘Bouabane’. The least form was the V-shaped base, found in only 8% of the varieties noting ‘Anonymous 1 and 5’, ‘Taberkante 2’.

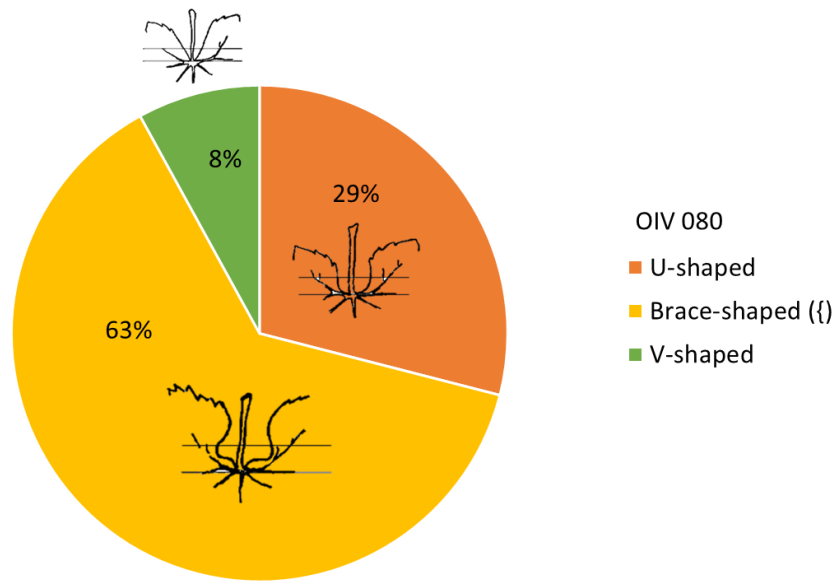


Figure 16: Frequency of shape of base of petiole sinus trait in different studied grapes varieties.

It was found that the shape of base of petiole sinus is an important character for distinguishing the grape cultivars. Ravaz, (1902) established a foundational system for quantifying the shapes of grapevine leaves, placing particular emphasis not only on the angle but also on the shape and contour of the petiolar sinus represented in hand-drawn illustrations.

Our results are consistent with those obtained by Bounab and Laiadi, (2019) for Algerian leaves. Meanwhile, other previous studies have evaluated this trait and obtained similar and/ or different results noting (Alba et al., 2014; Susaj et al., 2014; Benito et al., 2016 and Gisbert et al., 2022).

h. Teeth in the petiole sinus (OIV 081-1)

According to OIV 081-1, which characterizes the presence of teeth in the petiole sinus, we found that the totality of the examined grape varieties showed no teeth in the petiole sinus (100%).

This indicates a complete uniformity (stability) of this ampelographic trait in the studied population.

This trait was not evaluated in the previous ampelographic study of Algerian grapes carried by Bounab and Laiadi, (2019). However, our results are in accordance with other previous studies (Bodor et al., 2013; Alba et al., 2014; Benito et al., 2016; Kara et al., 2018; Kara et al., 2023 and Díaz-Fernández et al., 2024).

i. Petiole sinus base limited by vein (OIV 081-2)

According to OIV 081-2, which characterizes whether the petiole sinus base is limited by veins, we observed that the totality of the examined grape varieties (100%) showed no limitation by veins at the petiole sinus base, representing a completely uniform (stable) trait across the studied population.

This trait was not evaluated in the previous ampelographic study of Algerian grapes carried by Bounab and Laiadi, (2019). However, our results are in accordance with other previous studies (Bodor et al., 2013; Alba et al., 2014; Benito et al., 2016; Kara et al., 2018 and Kara et al., 2023).

j. degree of opening / overlapping of upper lateral sinuses (OIV 082)

According to OIV 082 character, which characterizes the degree of opening/ overlapping of upper lateral sinuses, we distinguished four groups of grape varieties (Figure 17).

The highest proportion (43%) consisted of varieties with slightly overlapped upper lateral sinuses, such as ‘Ait Abdi’, ‘Amellal 1’, and ‘Anonymous 4’. Varieties with strongly overlapped sinuses, including ‘Amellal 2’, ‘Bouabane’, and ‘Laadari’, accounted for 37%. Meanwhile, 17% of the varieties, including ‘Amer Bouamar’, ‘Ameziane’, and ‘Anonymous 1’, had open sinuses. The least represented category, at just 3%, consisted of varieties with closed upper lateral sinuses, exemplified by ‘Tasemith’.

A significant difference was observed when compared to the Algerian leaves evaluated by Bounab and Laiadi, (2019), who characterized the majority of the leaves they studied as having closed upper lateral sinuses. Meanwhile, other previous studies have evaluated this trait and obtained similar and/ or different results noting (Alba et al., 2014; Susaj et al., 2014; Benito et al., 2016; Kara et al., 2023 and Hbyaj et al., 2024).

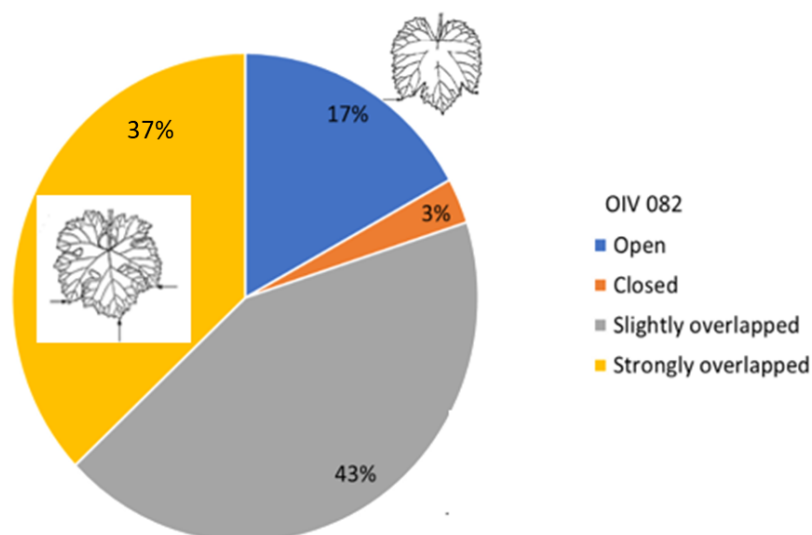


Figure 17: Frequency of degree of opening/ overlapping of upper lateral sinuses trait in different studied grape varieties.

k. Shape of the base of upper lateral sinuses (OIV 083-1)

According to OIV 083-1 character, which characterizes the shape of the base of upper lateral sinuses, we identified two groups of grape varieties (Figure 18).

The majority, comprising 71%, featured V-shaped bases, including varieties such as ‘Amellal 2’, ‘Ameziane’, and ‘Meska 1 and 2’. In contrast, 29% of the varieties exhibited brace-shaped bases, represented by ‘Ait Abdi’, ‘Amellal 1’, and ‘Amer Bouamar’.

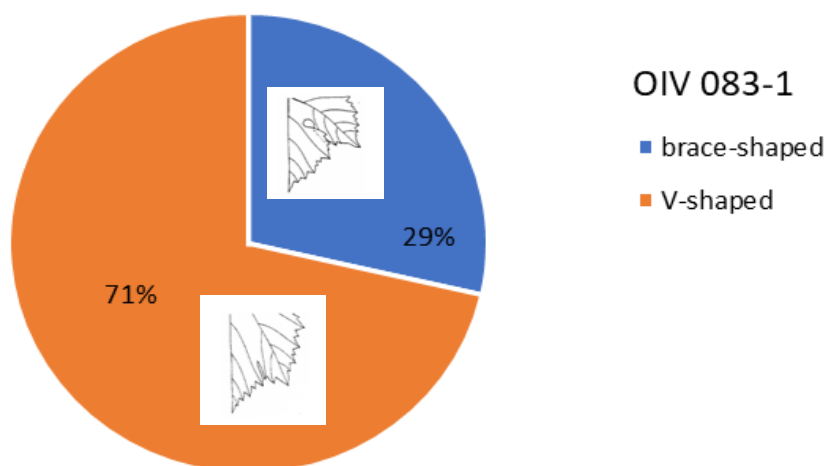


Figure 18: Frequency of shape of the base of upper lateral sinuses trait in different studied grape varieties.

A significant difference was observed when compared to the Algerian leaves evaluated by Bounab and Laiadi, (2019), who characterized the majority of the leaves they studied as having brace-

shaped upper lateral sinuses. Meanwhile, other previous studies have evaluated this trait and obtained similar and/ or different results noting (Bodor et al., 2013; Alba et al., 2014; Benito et al., 2016; Kara et al., 2023 and Díaz-Fernández et al., 2024).

l. Teeth in the upper lateral sinuses (OIV 083-2)

According to OIV 081-2, which characterizes the presence of teeth in the upper lateral sinuses, we found that 100% of the examined grape varieties showed no teeth in the upper lateral sinuses. This finding indicates a complete uniformity (stability) for this ampelographic trait in the studied population.

Our results are consistent with those obtained by Bounab and Laiadi, (2019) for Algerian leaves. Meanwhile, other previous studies have evaluated this trait and obtained similar and/ or different results noting (Bodor et al., 2013; Alba et al., 2014; Benito et al., 2016; Kara et al., 2023 and Díaz-Fernández et al., 2024).

m. Length of petiole compared to length of middle vein (OIV 093)

According to OIV 093 character, which characterizes the length of petiole compared to the length of middle vein, we distinguished between two groups of grape varieties (Figure 19).

The vast majority, comprising 97%, had petioles much shorter than the middle vein, including varieties such as ‘Ait Abdi’, ‘Amellal 1’, ‘Amer Bouamar’, and ‘Ameziane’. In contrast, only 3% exhibited petioles slightly shorter than the middle vein, represented by the variety ‘Amellal 2’.

Our results are consistent with those obtained by Bounab and Laiadi, (2019) for Algerian leaves. Meanwhile, other previous studies have evaluated this trait and obtained similar and/ or different results noting (Bodor et al., 2013; Alba et al., 2014; Akram et al., 2021; Chehade et al., 2022 and Díaz-Fernández et al., 2024).

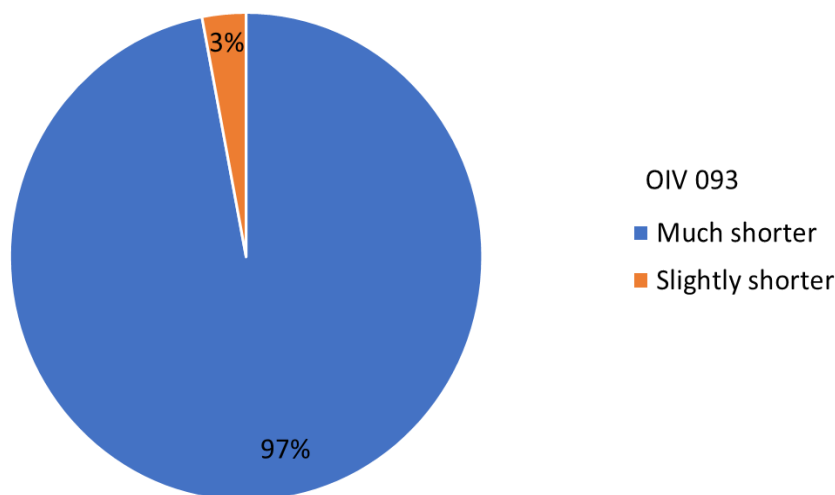


Figure 19: Frequency of length of petiole compared to length of middle vein trait in different studied grape varieties.

n. Depth of upper lateral sinuses (OIV 094)

According to OIV 094 character, which evaluates the depth of the upper lateral sinuses, the examined grape varieties displayed a range of characteristics (Figure 20).

The largest proportion (54%) presented by the varieties that had leaves with medium upper lateral sinuses such as ‘Amellal 2’, ‘Anonymous 4 and 5’ and ‘Laadari’, following with those having leaves with deep upper lateral sinuses (20%) noting the varieties, absent or very shallow upper lateral sinuses (14%), as well as those with shallow upper lateral sinuses (11%).

This data indicates significant diversity in the depth of the upper lateral sinuses across the studied grape population as highlighted in several previous studies noting (Alba et al., 2014; Benito et al., 2016; Akram et al., 2021; Chitwood, 2021; Cichi et al., 2022; Gisbert et al., 2022).

More importantly, the observed distribution is consistent with the ampelographic study carried by Bounab and Laiadi, (2019), who reported comparable sinus depth variations in Algerian grapevines.

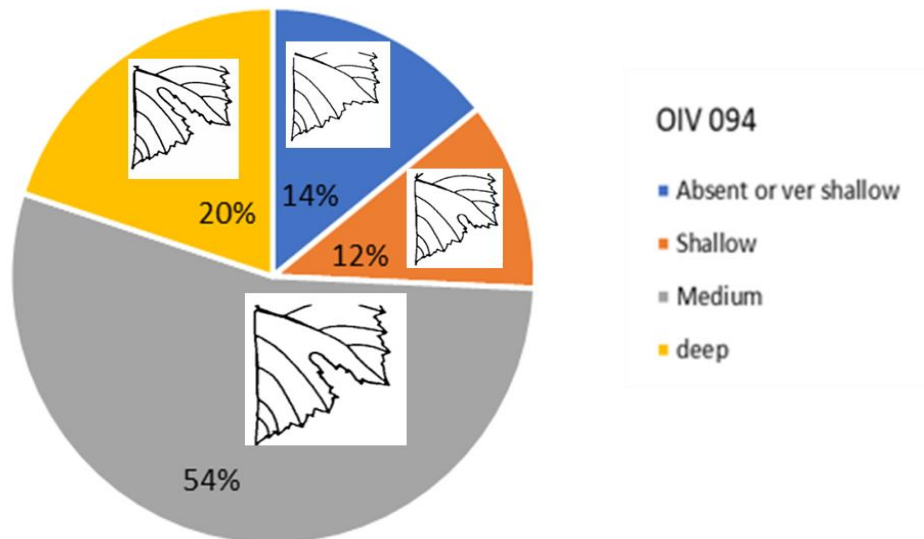


Figure 20: Frequency of depth of upper lateral sinuses trait in different studied grapes varieties.

In brief, the analysis of qualitative trait frequencies on grapevine diversity in the Aures region of Algeria revealed significant morphological variability among 35 cultivars, based on 14 OIV descriptors for mature leaves. For blade size (OIV 065), most cultivars exhibited medium to large blades, with outliers displaying very large blades and others showing smaller blades, while the blade shape (OIV 067) was predominantly wedge-shaped across cultivars. The number of lobes (OIV 068) was typically five, some other varieties standing out with three and seven lobes respectively. The distribution of color of the upper side of blade (OIV 069) revealed that the vast majority of the varieties exhibited dark green leaves while a smaller proportion showed medium green leaves. Tooth shape (OIV 076) displayed notable diversity, with the majority of cultivars displayed teeth with a mixture of both sides being straight and both sides convex. The petiole sinus (OIV 079) was predominantly open, with no cultivars showing overlapping sinuses, possibly an adaptation to enhance leaf ventilation in the arid Aures climate. The base of the petiole sinus (OIV 080) was frequently brace-shaped adding to the morphological distinctiveness in varieties. Notably, the absence of teeth in the petiole sinus (OIV 081-1) and lack of vein-limited petiole sinus bases (OIV 081-2) were consistent across all cultivars, suggesting these traits are less variable between the studied cultivars. For upper lateral sinuses (OIV 082), slightly overlapped configurations were common. The base of upper lateral sinuses (OIV 083-1) was mostly V-shaped. The length of the petiole relative to the middle vein (OIV 093) was generally much shorter for the vast majority of cultivars. The depth of upper lateral sinuses (OIV 094) displayed a range of characteristics varying from absent, shallow, medium and deep, indicating diverse leaf dissection patterns.

2.1.3. Ampelographic clustering of studied varieties

The dendrogram obtained by combinations of all ampelographic parameters scoring (1–9) is presented in Figure (21), where the eighteen quantitative parameters were transferred to qualitative according to OIV, (2001), and the originally fourteen qualitative ones (Appendix 2 and 3). Overall, the clustering analysis based on the JACCARD similarity coefficient revealed the discrimination of 18 grape cultivars grouped into 7 clusters, depending on the threshold used as reference for the distance coefficient ($J=0,532$). This is a remarkable value when the discrimination at cultivar level is considered. It also proves the discriminative potential of the descriptor parameters employed in this study. The analysis identified high similarities between certain cultivars, suggesting potential synonyms, clones and parental relationships in traits such as leaf shape, vein angles and sinus depth. However, some significant differences within groups are also noted.

In more details, the cluster A unites ‘Ait Abdi’ and ‘Anonymous 5’. These 2 cultivars exhibit a short genetic distance ($J=0,626$) and display a remarkable phenotypic similarity, sharing 11 qualitative (e.g., OIV 065, OIV 067, OIV 068, OIV 076, OIV 082, OIV 094) and 15 common morphometric criteria (e.g., OIV 601, OIV 602, OIV 603, OIV 605, OIV 606). This observation has already been substantiated by Principal Component Analysis (PCA). The proximity in genetic distance, despite the fact that these cultivars are found in different geographical areas, strongly suggests a close relationship indicating that they likely originated from a common parent variety and subsequently underwent slight divergence to become distinct cultivars.

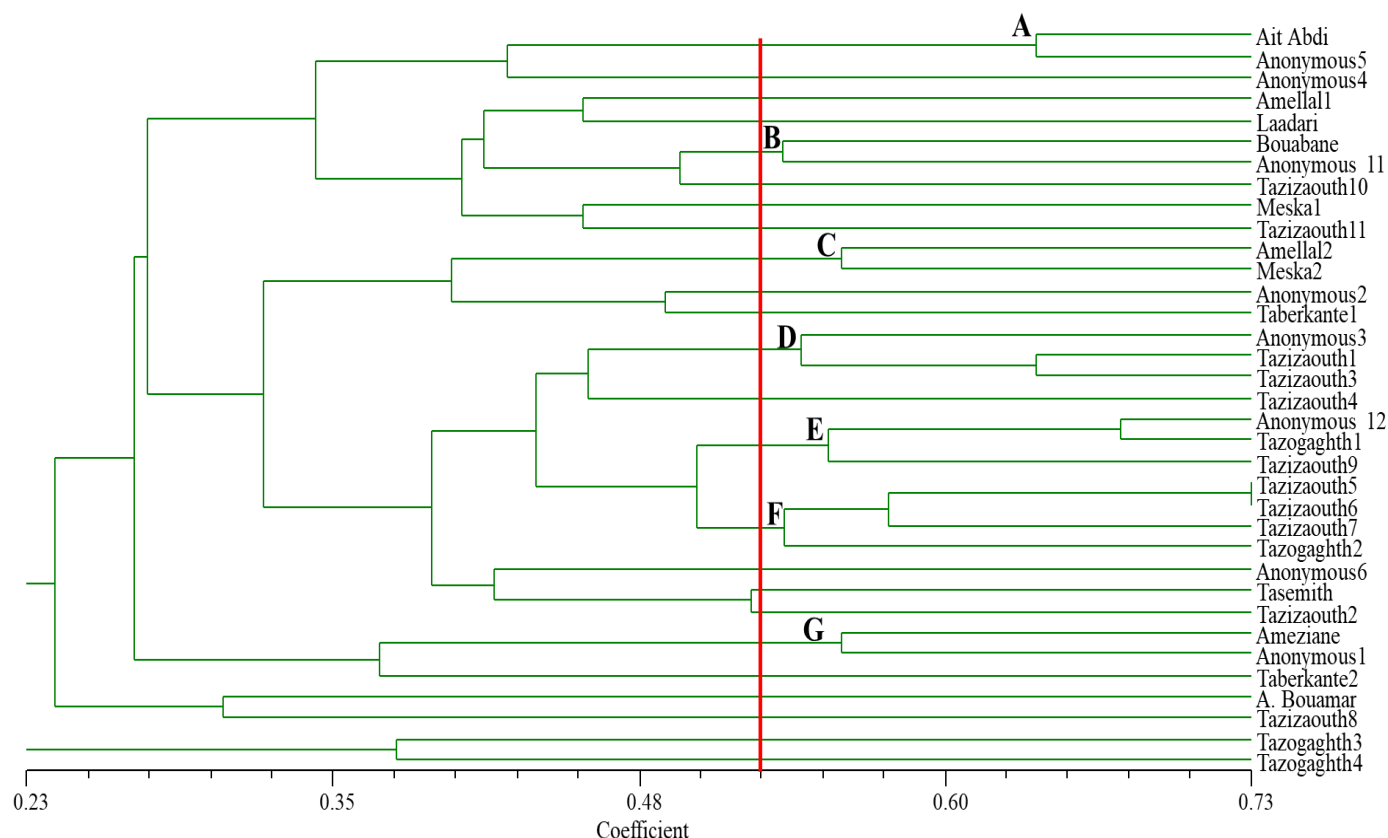


Figure 21: Hierarchical classification of 35 cultivars according of adult leaves constructed from the ampelographic data distance matrix using the UPGMA clustering method and the JACCARD similarity coefficient.

The cluster (B) ($J=0.532$) also includes 2 cultivars ‘Bouabane’ and ‘Anonymous 11’, which exhibit full concurrence in terms of qualitative characteristics. Even though this pair of cultivars is found in the same geographical area, a significant difference was noted with respect to the morphometric characters (e.g., OIV 602, OIV 604, OIV 607, OIV 608, OIV 610, OIV 611, OIV 613, OIV 615, and OIV 616). This difference was clearly appeared in the PCA plot. This variation in morphological characteristics is largely a reflection of genetic variation among cultivars. The cluster C ($J=0.558$) consists of 2 white cultivars, ‘Amellal 2’ and ‘Meska 2’, which have almost similar ampelographic characteristics, sharing 23 characters (OIV 067, OIV 068, OIV 080, OIV 082, OIV 094, OIV 601, OIV 602, OIV 606, OIV 607, OIV 611-616). Given the noticeable degree of similarity in their ampelographic profiles, it is possible that ‘Amellal 2’ and ‘Meska 2’ are synonyms. This hypothesis is already supported by the PCA plot, where these two cultivars are closely regrouped, indicating their strong similarity in the multidimensional space defined by the ampelographic characteristics. The cluster D ($J=0.532$) comprises 3 cultivars, ‘Tazizaouth 1 and 3’ as well as ‘Anonymous 3’, demonstrating high phenotypic similarity with relatively small distances between

them. Besides their morphological similarities, they are recovered from the same viticultural area and they may have originated by the same parent variety. Moreover, a case of homonymy was found between ‘Tazizaouth 1 and 3’ due to the close similarity ($J=0,624$), these cultivars have a common leaf structure sharing all the same characteristics (e.g., OIV 067, OIV 068, OIV 079, OIV 080, OIV 081-1, OIV 081-2, OIV 601-603, OIV 605, OIV 609, OIV 610, OIV 611, OIV 616, OIV 617). These cultivars are also grouped together in the PCA plot reinforcing the evidence of their strong similarity.

The cluster (E) ($J=0,558$) comprises 3 varieties: ‘Anonymous 12’, ‘Tazogaghth 1’ and ‘Tazizaouth 9’. Whereas a possible synonymy was detected between ‘Anonymous 12’ and ‘Tazogaghth 1’ ($J=0,672$), explained by the fact that they completely conform in terms of qualitative characteristics and share 12 similar quantitative characteristics (e.g., OIV 602-606, OIV 608, OIV 609, OIV 616, OIV 617). In the other hand, ‘Anonymous 12’ appears closer to ‘Tazizaouth 9’. Notably, these varieties were adequately positioned according to their relationship in the PCA plot.

The cluster (F) ($J= 0,532$) regrouped a set of 4 cultivars, that are ‘Tazizaouth 5’, 6, and 7’ in addition to ‘Tazogaghth 2’. This cluster is characterized by the highest coefficient of similarity between two cultivars among all clusters ($J=0,73$), namely ‘Tazizaouth 5 and 6’ which are highly coincided: they may be clones as reflected by the single group they formed in the clustering analysis performed with the results of ampelographic characterization. PCA analysis also grouped them together showing indistinguishable leaf morphology. Besides, ‘Tazizaouth 7’ is positioned near these cultivars ($J=0,576$), implying a possible parental relationship. Therefore, all these varieties are coincided with them in 22 features (e.g., OIV 065, OIV 067, OIV 068, OIV 076, OIV 601- 603, and OIV 616 - 618). The remaining cultivar, ‘Tazogaghth 2’, was alone separated as a sub-cluster where the similarity is reduced in a lesser degree in comparison with the previous cultivar ($J= 0,528$).

The cluster (G) ($J=0,558$) consolidates 2 grape cultivars, ‘Ameziane’ and ‘Anonymous 1’, which are characterized by their high similarity in terms of traits such as OIV 601- 606, OIV 609, OIV 610, OIV 611-615. This proximity suggests a close genetic relationship, which could be attributed to their parental status, as emphasized above. It is possible that these cultivars have a common parent or are the result of a similar breeding program. The present case is also well-suited to PCA findings.

Other varieties were distinguished from all these main groups. That was the case for ‘Amer Bouamar’ and ‘Tazizaouth 8’ ($J= 0.308$) in addition to ‘Tazogaghth 3 and 4’ ($J=0.376$) which have large distances indicating that they completely diverge from the other cultivars. They did not correspond to any of the previously defined clusters and could be considered as homonyms. In the

dendrogram, these cultivars are positioned far from the other clusters, suggesting a distinct genetic background and ampelographic profile.

Upon examination of their ampelographic characteristics, they exhibit unique combinations of traits that set them apart from the cultivars in the defined clusters. For instance, they may have different leaf shapes (OIV 067), sinus depths (OIV 094, OIV 605 and OIV 606), or vein angles (OIV 607, OIV 608, OIV 609 and OIV 610) compared to the other cultivars. These distinctive features contribute to their large distances in the dendrogram and their separation from the main groups.

In this study, eighteen basic ampelometric characteristics (OIV, 2001) and thirteen additional relationships inspired from Martinez and Grenan, (1999) were used to assess the phenotypic diversity among the grapevine cultivars grown in two different geographical regions at Batna province. The studied grape cultivars are similar in many characteristics as they differ in many others. The multivariate analysis was efficient to analyze the large data generated in this study by qualitative and quantitative descriptors to identify patterns and relationships among the studied cultivars. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) have been used in several studies to characterize grapevine cultivars, including studies in Spain (Jiménez-Cantizano et al., 2020), Italy (Alba et al., 2014), Greece (Tsivelikas et al., 2022), Turkey (Güler and Karadeniz, 2023), Lebanon (Chehade et al., 2022), Tunisia (Lamine et al., 2014) and Algeria (Bounab and Laiadi, 2019).

Our results suggest how few ampelometric traits are sufficient and allowed for the first time to easily discriminate the Algerian autochthonous grapevines grown in the Aures region. Our findings align with other ampelographic studies utilizing OIV descriptors to characterize and differentiate grapevine cultivars based on adult leaf traits (Tomažič and Korošec-Koruza, 2003; Gago et al., 2009; Rusjan et al., 2012; Bodor et al., 2013; Bodor et al., 2017; El-Oualkadi and Hajjaj, 2019).

These finding were also reported by Santiago et al., (2007) and Chitwood, (2021), where they found that the mature leaf was very informative in discriminating grapevine genotypes, and also it was more stable and objective than other characters. In the other hand, it was observed that certain qualitative parameters lacked representativeness in our study, showing no discernible differences among the grape varieties, consistent with findings reported by Bounab and Laiadi, (2019).

Interestingly, results showed a higher phenotypic distance between some cultivars within each of these clusters that would be expected as homonyms, e.g., ‘Tazogaghth 1, 2, 3 and 4’. With the aim to solve this problem, the genetic distances were further analyzed using SSR markers to distinguish the different varieties.

Despite the efforts to avoid selecting common varieties during vineyard sampling, ‘Tazizaouth’ emerged as the most prevalent genotype recovered among the 35 grapevine samples (11 samples), making it the largest group within our studied cultivars. Notably, this variety holds historical importance, as it is widely domesticated across the region under investigation likely due to its phenotypic plasticity and adaptability to the environmental conditions.

The cultivars called ‘Taberkante’ and ‘Tazogaghth’ were also recovered contributing to the diversity of grapevine genotypes studied. According to local inhabitants, their names originate from the Amazigh language, signifying their characteristic berry color as green for ‘Tazizaouth’, black for ‘Taberkante’ and red for ‘Tazogaghth’.

In addition, it is noteworthy that in the Aures region, grapevines are widely known by the Berber name of '*Hizourin*', meaning grape in Berber dialect (Mercier, 1906). However, it's important to note that growers may mistakenly name local genotypes, often due to inadequate observations and descriptions of variety characteristics, particularly among those with similar phenotypes (Rusjan et al., 2012). In our case, local inhabitants typically name genotypes based on distinguishing features such as berry color, flavor, or size.

Until this time, no historic reference was found for any variety with the local name of ‘Ait Abdi’, and ‘Bouabane’ in the Algerian grapevine genetic resources list and the *Vitis* International Variety Catalogue (IVVC) (Röckel et al., 2024). Regarding the anonymous genotypes further SSR analysis was performed to confirm their identity.

Part 3

**Molecular characterization of grape varieties
(*Vitis vinifera* L.) grown in the Aures region.**

Chapter 3

Material and Methods

3. Material and Methods

3.1. Plant sampling and preparation

A total of 41 table grape individuals were used for molecular characterization. All these cultivars are unexplored and traditionally planted in mountainous grape growing in the province of Batna, located in the North-east of Algeria. Semi-hardwood cuttings, approximately 15-20 cm in length, were collected from each cultivar and marked with a label of identification (Sample 1 to 41) (Table 8).

Prior to arrival at CREA-VE, Research Centre for Viticulture and Enology (Susegana, Italy) for molecular analyses, the cuttings were covered with moist absorbent paper then placed in a plastic box to allow optimal preservation.

Table 8: List of cultivars sampled for molecular characterization.

Sample ID	Grapevine sample Name	Sample name meaning in Amazigh language	Growth location
Sample 1	Tazizaouth 1	Green color	Ichmoul
Sample 2	Anonymous 6	/	Ichmoul
Sample 3	Tazizaouth 5	Green color	Ichmoul
Sample 4	Meska 1	Aromatic grape	Ichmoul
Sample 5	Tazizaouth 13	Green color	Ichmoul
Sample 6	Tazizaouth 3	Green color	Ichmoul
Sample 7	Anonymous 2	/	Ichmoul
Sample 8	Tazizaouth 6	Green color	Ichmoul
Sample 9	Tazizaouth 14	Green color	Ichmoul
Sample 10	Taberkante 2	Black color	Ichmoul
Sample 11	Amellal 1	White color	Ichmoul
Sample 12	Tazizaouth 12	Green color	Ichmoul
Sample 13	Tazizaouth 9	Green color	Ichmoul
Sample 14	Anonymous 7	/	Ichmoul
Sample 15	Anonymous 3	/	Ichmoul
Sample 16	Anonymous 1	/	Ichmoul
Sample 17	Tazizaouth 7	Green color	Ichmoul
Sample 18	Tazizaouth 10	Green color	Ichmoul
Sample 19	Tazizaouth 8	Green color	Ichmoul

Sample 20	Anonymous 8	/	Ichmoul
Sample 21	Tazizaouth 4	Green color	Ichmoul
Sample 22	Tasemith	Acid flavor	Ichmoul
Sample 23	Tazogaghth 2	Pink/ red color	Ichmoul
Sample 24	Tazizaouth 2	Green color	Ichmoul
Sample 25	Amellal 2	White color	Ichmoul
Sample 26	Tazizaouth 11	Green color	Ichmoul
Sample 27	Anonymous 12	/	Ichmoul
Sample 28	Anonymous 5	/	Ichmoul
Sample 29	Tazogaghth 3	Pink/ red color	Ichmoul
Sample 30	Tazogaghth 4	Pink/ red color	Ichmoul
Sample 31	Ameziane	Small size	Ichmoul
Sample 32	Anonymous 4	/	Ichmoul
Sample 33	Meska 2	Aromatic grape	Ichmoul
Sample 34	Meska 3*	Aromatic grape	Ichmoul
Sample 35	Ait Abdi	Reflecting to the discovery site	Bouzina
Sample 36	Anonymous 10*	/	Bouzina
Sample 37	Laadari	Name given by local Inhabitants	Bouzina
Sample 38	Bouabane	Name given by local Inhabitants	Bouzina
Sample 39	Anonymous 9*	/	Bouzina
Sample 40	Amer Bouamar	Name given by local Inhabitants	Bouzina
Sample 41	Anonymous 11	/	Bouzina

Note: The varieties marked with ‘*’ were not subjected to ampelographic characterization.

The difference in sample size between the two characterization approaches noted in our study stemmed from the challenges encountered during sampling, which underscored the precarious state of these genetic resources. Environmental stresses, such as drought, extreme temperatures, and soil degradation, had adversely impacted the health and vigor of certain grapevine individuals from

traditional cultivation sites, rendering them unsuitable for comprehensive ampelographic evaluation such as: ‘Anonymous 8, 9, and 10’, ‘Meska 3’, ‘Tazizaouth 12, 13, and 14’. These cultivars were recently introduced into our samples, and their old age made it challenging to perform reliable phenotypic evaluations, so their molecular characterization was prioritized to capture their genetic profiles before potential loss and to establish their genetic relationships with the other varieties and contribute to our understanding of the genetic diversity.

In the other hand, thirty-three cultivars were subjected to ampelographic and molecular analyses, except for ‘Taberkante 1’ and ‘Tazogaghth 1’ (only ampelographic characterization). For these two cultivars, molecular analysis could not be performed due to the unavailability of viable plant material during the sampling period, as the plants had desiccated from water scarcity.

3.2. DNA Extraction and SSR genotyping

Cambium tissue from wood of a single plant was collected from each cultivar, with repetition in case of inconsistency with previous studies. For each sample, DNA was extracted from lyophilized tissues by grinding the green plant material into a fine powder using a Tissue-Lyser II instrument (Qiagen, Hilden, Germany) and extracting the DNA using a Plant DNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer’s protocol. DNA concentration and quality (e.g., 260/280 and 260/230 ratios) were measured by a Nanodrop 1000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and by gel electrophoresis (1% agarose).

The grapevine varieties analyzed in this study were genotyped using 12 SSR markers; including nine recommended as standard markers for global grapevine research within the GrapeGen06 European project framework (Table 9). These markers (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79) were chosen based on their placement in the linkage groups of *Vitis vinifera* to ensure comprehensive genome coverage and enhance the capacity to differentiate closely related grapevine genotypes (Mahmoud et al., 2023). Additionally, the remaining three markers (VMC6E1, VMC6G1, and VMCNG4b9) from the *Vitis* Microsatellite Consortium were included, following the protocol outlined by Migliaro et al., (2013).

Table 9: Sequences of Simple Sequence Repeat primers (SSRs) used for grapevine genotyping.

Primer		Sequence (5'-3')	Reference
VVS2	Forward	CAGCCCGTAAATGTATCCATC	Thomas and Scott, (1993)
	Reverse	AAATTCAAAATTCTAATTCAACTGG	

VVMD5	Forward	CTAGAGCTACGCCAATCCAA	Bowers et al., (1996)
	Reverse	TATACCAAAAATCATATTCCTAAA	
VVMD7	Forward	AGAGTTGCGGAGAACAGGAT	Bowers et al., (1999)
	Reverse	CGAACCTTCACACGCTTGAT	
VVMD25	Forward	TTCCGTAAAGCAAAAGAAAAAGG	Bowers et al., (1999)
	Reverse	TTGGATTTGAAATTTATTGAGGGG	
VVMD27	Forward	ACGGGTATAGAGCAAACGGTGT	Bowers et al., (1999)
	Reverse	GTACCAGATCTGAATACATCCGTAAGT	
VVMD28	Forward	AACAATTCAATGAAAAGAGAGAGAGAGA	Bowers et al., (1999)
	Reverse	TCATCAATTTTCGTATCTCTATTTGCTG	
VVMD32	Forward	TATGATTTTTTAGGGGGGTGAGG	Bowers et al., (1999)
	Reverse	GGAAAGATGGGATGACTCGC	
VrZAG62	Forward	GGTGAAATGGGCACCGAACACACGC	Sefc et al., (1999)
	Reverse	CCATGTCTCTCCTCAGCTTCTCAGC	
VrZAG79	Forward	AGATTGTGGAGGAGGGAACAAACCG	Sefc et al., (1999)
	Reverse	TGCCCCCATTTTCAAACCTCCCTTCC	
VMC6E1	Forward	CAC TGG CCT GTT GGG AGA TAAT	Migliaro et al., (2013)
	Reverse	CCT TCA ACT GGA AAA GCC TGT C	
VMC6G1	Forward	TGC ATA GTG CTG TAG GCC ATTG	Migliaro et al., (2013)
	Reverse	TCT GTC ATT GCT GTC CCT TTC A	
VMCNG4b9	Forward	CTGGGGAGCATATACACATA CCAG	Migliaro et al., (2013)
	Reverse	CTCTCTCTTCCCGATAGCCACC	

PCR reactions were conducted using forward primers labeled with fluorescent dyes (6-FAM, PET, VIC, or NED). Two multiplex panels of fluorescent-labeled microsatellite loci were employed (Table 10). The direct multiplex PCR method proposed by Migliaro et al., (2013) offers a faster and

more cost-effective alternative to previously used methodologies, while still providing reliable results. This advancement makes SSR DNA analysis more affordable and accessible not only to research institutions but also to a wider range of users.

Simultaneous PCR amplifications were conducted in a final volume of 20 μ L containing 1 \times PCR reaction buffer, 10 ng of genomic DNA, 0.2 mM of each dNTPs, 2 mM MgCl₂, 1.5 U Taq DNA Polymerase (Thermo Fischer Scientific, Waltham, MA). Depending on the locus, primer concentrations ranged from 0.11 to 0.48 μ M. Reactions were performed on a GeneAmp PCR System 9700 using the following profile: a hot start of 95 °C for 5 min, 30 amplification cycles of 45 sec at 95 °C, 1 min at 55 °C, 30 sec at 72 °C, and a final extension step of 30 min at 72 °C. PCR products (0.5 μ L) were mixed with 9.35 μ L of formamide and 0.15 μ L of the GeneScan™ 500 LIZ Size Standard (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Capillary electrophoresis was conducted in an ABI 3130xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Allele calling was performed with GeneMapper 4.0 software (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Allele sizes were recorded in bp and genotypes showing a single peak at a given locus were considered as homozygous.

Table 10: Multiplex panels of Fluorescent-Labeled microsatellite loci used in SSR analysis.

Multiplex	SSR	Primer concentration (μ M)	Labeling	Allele size
M1	VVS2	0,20	6-FAM	123-171
	VVMD7	0,20	6-FAM	227-265
	VrZAG62	0,11	VIC	172-219
	VVMD28	0,24	PET	219-285
	VrZAG79	0,11	NED	236-280
	VMC6E1	0,14	VIC	117-171
M2	VVMD5	0,48	VIC	218-276
	VVMD25	0,16	6-FAM	238-277
	VVMD27	0,20	6-FAM	175-223
	VVMD32	0,30	NED	236-292
	VMC6G1	0,14	PET	169-197
	VMCNG4b9	0,14	NED	138-180

3.3. Varietal identification

Varietal identification is a key step, as we estimate how many varieties analyzed are already in reference databases and how many represent novelties.

Once all the data concerning the presence of varieties with unique profiles and the presence of synonyms and homonyms were examined, the SSR profiles obtained were compared with the CREA Viticulture and Enology molecular database which currently contains about 5000 unique profiles and is constantly updated (partially published in the Italian Grapevine Catalogue, <http://catalogoviti.politicheagricole.it>), literature information, and the *Vitis* International Variety Catalogue (IVC) (Röckel et al., 2024).

In the first comparison, therefore, the data of each unique genotype were loaded and through a simple ‘filter’ operation the comparison of the possible presence or absence was made. If the sample was already found to be present, the final name of the variety was scored, but if no results were obtained, the IVC database (Figure 22) was then used for varietal identification as following:

Access the database: Start by visiting the IVC website (<https://www.vivc.de/>). The database is publicly accessible and free to use.

Database search: The IVC database offers both basic and advanced search functionalities, allowing users to access a wide range of relevant entries, including:

Basic search options:

1. **Species:** Search for grapevine varieties by species (e.g., *Vitis vinifera*, *Vitis labrusca*).
2. **Cultivar name:** Enter a specific cultivar name to locate detailed information on that variety.
3. **Photos:** Find images associated with various cultivars to aid in visual identification.
4. **Pedigree:** Search for the parental lineage of grapevine cultivars and genetic lineage information.
5. **Holding institutions:** Identify institutions that maintain grapevine cultivars, useful to obtain samples or connect with repositories.
6. **Area by countries:** Search for grapevine cultivars associated with specific countries, which is helpful for understanding regional diversity.
7. **Bibliography:** Access references and publications associated with specific cultivars for further study.
8. **History of prime name changes:** Review any name changes for cultivars over time, which can help clarify historical synonyms.

Advanced search options:

1. **Passport data:** Access detailed information on the origin, collector, and location data for cultivars.
2. **Resistance data:** Search for cultivars with resistance traits to certain pests or diseases, which is valuable for breeding programs.
3. **Microsatellites by profile, varieties, and bibliography:** Perform searches based on specific microsatellite marker profiles, which can help in precise genetic identification and comparison with other varieties.
4. **Relationships based on nine microsatellites:** Identify relationships between cultivars based on a standardized set of nine microsatellite markers, useful for genetic diversity studies.



Figure 22: *Vitis* International Variety Catalogue (VIVC) database interface.

3.4. Determining the genetic variability

The presence of samples with identical molecular profiles was evaluated using Cervus 3.0 software (<http://www.fieldgenetics.com>) (Kalinowski et al., 2007) and GenAlEx 6.5 software (Peakall and Smouse, 2006) was used to compute genetic diversity statistics for each SSR locus: the number of different alleles (No alleles), the effective number of alleles (Ne alleles), observed (Ho), expected heterozygosity (He), Shannon's informative index (I), Hardy–Weinberg equilibrium (HW), probability

of null alleles (F null) and probability of identity (PI). These statistics help to determine polymorphism, that is, the information power of the loci used in genotyping.

3.5. Genetic relationships analyses

3.5.1. Principal coordinate analysis (PCoA)

To assess the relationship among the non-redundant genotypes, a principal coordinate analysis (PCoA) was performed to infer the distribution of genetic relationships among the varieties revealed by 12 SSR loci using GenAlEx 6.5 software (Peakall and Smouse, 2006) based on the covariance matrix with data standardization.

3.5.2. Phylogenetic tree construction

Genetic distances between cultivars were calculated as the allele sharing distance (DAS) (Jin and Chakraborty, 1994). The phylogenetic tree based on the distance matrix was constructed using the Neighbour-Joining method (Saitou and Nei, 1987) by POPULATION v.1.2.30 software (<http://bioinformatics.org>, LANGELLA, unpublished) while MEGA v5.2 (Tamura et al., 2011) was used to display it.

Chapter 4

Results and Discussion

4. Results and Discussion

4.1. Molecular Profiling and identification

Molecular analyses using 12 SSR markers identified 14 distinct molecular profiles among the 41 samples analyzed. These profiles were compared with the *VIVC* catalogue (Röckel et al., 2024) and the CREA-Viticulture and Enology molecular database (partially published in the Italian Grapevine Catalogue, <http://catalogoviti.politicheagricole.it>), as shown in Table (11).

Table 11: List of the 41 grapevine samples grouped by their genotype. Cultivar name, country of origin, berry color, true-to-type prime name, and Vitis International Variety Catalogue (*VIVC*) code are reported.

Cultivar Name	Origin	Berry Color	Prime Name (Correspondence by SSR)	<i>VIVC</i> Code
Tazizaouth 1	Tunisia	Blanc	Rassegui	9923
Anonymous 6				
Anonymous 7*				
Tazizaouth 9				
Tazizaouth 3				
Anonymous 3				
Tazizaouth 6				
Tazizaouth 4				
Tazizaouth 5				
Tazizaouth 14*				
Tazizaouth 12*				
Anonymous 8*				
Tazizaouth 7				
Tazizaouth 8				
Tazizaouth 13*				
Anonymous 2				
Tasemith				
Tazogaghth 2				
Tazizaouth 2				
Tazogaghth 3	Algeria	Rouge	Ahmeur Bou Ahmeur	140
Tazogaghth 4				

Anonymous 10*				
Ait Abdi				
Laadari				
Tazizaouth 11	Algeria	Blanc	Tizi Ouinine	24558
Ameziane				
Anonymous 4				
Amellal 2	Greece	Blanc	Zibibbo	8241
Meska 2				
Tazizaouth 10	Algeria	Blanc	Louali	24613
Anonymous 11				
Meska 1	Italy	Blanc	Italia	5582
Taberkante 2	France	Noir	Danugue	3425
Anonymous 1	Algeria	Rouge	Babari	26590
Anonymous 12	Lebanon	Blanc	Regina	122
Meska 3*	Palestine	Blanc	Dabouki	3309
Amellal 1	Algeria	Blanc	Unknown Genotype 1	
Anonymous 5	Algeria	Not identified	Unknown Genotype 2	
Bouabane	Algeria	Not identified	Unknown Genotype 3	
Anonymous 9*				
Amer Bouamar	Algeria	Not identified	Unknown Genotype 4	

Notably, four genotypes were detected in more than one location, aligning with previous studies that identified them as ancient autochthonous varieties: ‘Ahmeur Bou Ahmeur’, ‘Louali’, ‘Tizi Ouinine’ (Laiadi et al., 2009; Khouni et al., 2023) and ‘Babari’ (Rahali et al., 2019). Their discovery uncovers the sustainability and richness of the Algerian gene pool. The SSR profiles also enabled the identification of six foreign genotypes, which corresponded to known Mediterranean varieties, namely from France ‘Danugue’, Italy ‘Italia’, Greece ‘Zibibbo’, Tunisia ‘Rassegui’, Lebanon ‘Regina’, and Palestine ‘Dabouki’. Lastly, the four-remaining corresponded to never-reported genotypes and their corresponding names depending on what is common locally.

About the identified genotypes, the most frequent refers to ‘Rassegui’ and encompasses nineteen samples. This finding aligns with Rahali et al., (2019), who similarly reported ‘Rassegui Blanc’ as a prevalent variety among the identified plants with a close convergence in the genetic profiles proving

to be synonyms. This variety is widespread and known locally as ‘Tazizaouth’ which refers to the green color of its berries in the Amazigh language. The long history of cultivation of ‘Tazizaouth’ dates back to at least the Algerian revolution (1954), as confirmed by local farmers. It is interesting to note that the ‘Rassegui’ variety, despite being listed as of Tunisian origin (Snoussi et al., 2004), is very widespread and abundant in the Aures region according to our results and those found by Rahali et al., (2019). This observation raises questions about the true origin of this variety, suggesting the need for further investigations to trace its history and precise provenance. Such intermixing is unsurprising given the significant exchanges between Algeria and Tunisia over time, as the two countries share borders and have maintained close ties.

The second local variety collected five times from the two areas prospected was ‘Ahmeur Bou Ahmeur’, which is one of the well-known old autochthonous table grape varieties as previously stated by Tessier et al., (1999) and Laiadi et al., (2009). The molecular analyses also revealed the presence of two other local varieties, ‘Tizi Ouinine’ and ‘Louali’, respectively represented by three and two plants. These two varieties are already characterized by ampelographic and molecular approaches (Laiadi et al., 2009; Bounab and Laiadi, 2019; Khouni et al., 2023).

This is the first report that declared the existence of these varieties in this specific area. Furthermore, ‘Babari’, which takes its name from the village of Babar in the neighboring province of Khenchela of northeastern Algeria (Rahali et al., 2019), has never been described in ampelography. This variety has been found very rarely in this region (just one sample), so it is considered a minor variety and has a marked risk of extinction since only isolated plantation has been detected. Some other genotypes correspond to Mediterranean varieties, reflecting the extensive exchange of viticultural heritage. For instance, ‘Danugue’, identified as the true-to-type of ‘Taberkante 2’, has French origin according to the *I*VIC database (Röckel et al., 2024). On the other hand, the cultivars ‘Amellal 2’ and ‘Meska 2’ have been identified as ‘Zibibbo’ of Greek origin (Röckel et al., 2024), while ‘Meska 1’ was identical to the classical Italian genotype ‘Italia’ (Röckel et al., 2024). Regarding the grapevine cultivars of eastern origin, they exhibited unique genotypes each, the one named ‘Anonymous 12’ was identified as the Lebanese variety ‘Regina’, while the Palestinian ‘Dabouki’ was the true-to-type of ‘Meska 3’ (Röckel et al., 2024).

Significantly, four novel genotypes previously unreported and specific to the Aures region were identified among the grapevine varieties analyzed. ‘Unknown genotype 1’ represents the true-to-type genotypic profile of the cultivar ‘Amellal 1’ which translates to white, reflecting the white berry color of this cultivar in the Amazigh language. It is important to note that this ‘Amellal 1’ cultivar is distinct from the ‘Amellal’ genotype previously characterized by Laiadi et al., (2009), despite their similar

names. ‘Unknown genotype 2’ corresponds to the authentic ‘Anonymous 5’ genotype. ‘Unknown genotype 3’ was found to encompass both ‘Bouabane’ and ‘Anonymous 9’ cultivars, which are therefore considered synonyms. Lastly, ‘Unknown genotype 4’ was identified as the true-to-type genotype of ‘Amer Bouamar’.

In order to ensure proper authentication and traceability, registration of these newly discovered varieties in the *ITVC* Catalogue is essential. We propose the following respective cultivar names: ‘Ichmoul’ for ‘Unknown genotype 1’, ‘Ichmoul Bacha’ for ‘Unknown genotype 2’, ‘Bouabane des Aures’ for ‘Unknown genotype 3’, and ‘Amer Bouamar’ for ‘Unknown genotype 4’.

4.2. Genetic diversity assessment

In the current study, 41 cultivars recovered from Batna province were genotyped at 12 SSR loci. The analysis was informative since all the twelve loci analyzed were found to be polymorphic with the total number of amplified alleles (Table 12).

However, the genetic diversity of the 41 cultivars was relatively restricted, as indicated by the low number (84) of different alleles at the 12 SSR loci analyzed. The number of alleles per SSR locus ranged from 4 for VVMD27 to 9 for VMC6E1, with the mean allele number per locus being 7. The most informative locus was VVS2 ($N_e = 5$) while the least informative were VVMD7, VVMD27 and VMC6E1 ($N_e = 2$) with a mean number of effective alleles of 3,8.

Table 12: Statistics on the 12 SSR markers analyzed.

Locus	N. of Obs	No Alleles	N_e Alleles	H_o	H_e	I	HW	PI	F(Null)
VVS2	41	8	5	1.000	0.813	1.992	***	0.038	-0.230
VVMD5	41	7	4	1.000	0.786	1.907	***	0.043	-0.273
VVMD7	41	7	2	0.537	0.587	1.729	***	0.071	0.085
VVMD25	41	6	3	0.951	0.679	1.429	**	0.125	-0.401
VVMD27	41	4	2	0.927	0.661	1.279	***	0.144	-0.403
VVMD28	41	8	4	0.927	0.768	1.777	***	0.069	-0.207
VVMD32	41	7	3	0.829	0.749	1.522	***	0.117	-0.107
VrZAG62	41	6	3	0.902	0.737	1.511	***	0.11	-0.225
VrZAG79	41	7	3	0.927	0.720	1.709	**	0.076	-0.288
VMC6E1	41	9	2	0.537	0.648	1.939	***	0.049	0.171
VMC6G1	41	8	4	0.902	0.763	1.695	***	0.085	-0.183
VMCNG4b9	41	7	4	0.780	0.781	1.541	***	0.115	0.001
Mean		7	3,8	0.852	0.724	1.669		0.038	
Sum		84	46						

N. of obs = number of genotypes analyzed to calculate the statistics; No alleles = number of different alleles; Ne alleles = effective number of alleles; Ho, He = observed and expected heterozygosity; Shannon's information index (I); HW = Hardy–Weinberg equilibrium: ** $P < 0.01$, *** $P < 0.001$; F (Null) = probability of null alleles.

Observed Heterozygosity (Ho) is defined as the number of individuals heterozygous per locus. The lowest observed heterozygosity 0.537 was detected at the VVMD7 and VMC6E1 locus and the highest one was 1.000 at the VVS2 and MD5 locus, where the average observed heterozygosity was (85.2%). The values of observed heterozygosity are higher than those found by some previous studies on grapevines, such as 69.3% in the middle of the Mediterranean basin for 295 genotypes (De Michele et al., 2019), 71.96% in the North African (Maghreb region) for 181 genotypes (Riahi et al., 2012), 74.2% in Central Asia for 1378 genotypes (Riaz et al., 2018), 83.1% in Southern Umbria (Central Italy) for 39 genotypes (Zombardo et al., 2021) and also higher than the result obtained by Rahali, (2019) for 37 genotypes (78.8%).

Expected heterozygosity ranged from 0.813 for VVS2 to 0.587 for VVMD7 with mean expected heterozygosity (72%) which was lower than that recorded for many other collections, such as Moroccan with 76% (El-Ouakadi et al., 2009), Armenian with 78.9% (Margaryan et al., 2021), Georgian with 80.7% (Maghradze et al., 2010), Algerian with 86% (Laiadi et al., 2019), but also similar to other in the Maghreb region: Algerian with 70.4%, Moroccan with 69.5% and Tunisia with 75.0% (Riahi et al., 2010) and Turkish collection with 73.4% (Arslan et al., 2023).

Shannon's information index (I) is an important parameter that reflects the level of polymorphism (Wright, 1949). The highest information index was observed in locus VVS2 (1.992) and the lowest in VVMD27 (1.279) with an average of 1.669.

The value of the total PI determined herein was indeed very low ($6.86 \cdot 10^{-14}$) demonstrating that the 12 microsatellites used were exceedingly powerful for the discrimination of our grapevine cultivars. The highest PI (0.144) was observed for VVMD27, while the lowest (0.038) was for VVS2.

Hardy–Weinberg equilibrium is an important genetic parameter when analyzing identity and parentage, due to the state of gene flow (Štajner et al., 2014). In our study, significant ($P < 0.01$) and highly significant ($P < 0.001$) deviation from the Hardy–Weinberg equilibrium was observed for all SSR analyzed. This deviation can be due to small sample sizes, human manipulation of cultivars (displacements, breeding, clonal propagation), Wahlund effect (substructure in the population), or the presence of null alleles.

In correlation with previous parameters, the frequency of the null allele F (null) was also of particular interest, as it was negative for 9 of the 12 SSRs and positive lower values were found for

VVMD7, VMC6E1, and VMCNG4B9 SSR markers. This parameter is valuable for detecting some allele amplification problems when genotyping or deleting a target sequence.

4.3. Genetic relationships analysis

4.3.1. Principal coordinates analysis (PCoA)

The Principal Coordinates Analysis (PCoA) plot illustrates the relationships among grapevine cultivars based on molecular data. Projections of the PCoA were plotted in a 2-dimension scatter plot (Figure 23). The PCoA 2D projection of the first two principal axes accounted for 33.42% of the total molecular variation. Our findings are in line with what is obtained in the literature, slightly lower than PCoA' results obtained by Rahali et al., (2019) (36.52%), much higher than those of Augusto et al., (2021) (13.09%) and similar to those obtained by Riaz et al., (2018) (32%).

According to our findings, The first axis (Coord. 1) represent the largest proportions of variance in the dataset explaining 18.72 %, while the second axis (Coord. 2) accounts for an additional 14.70%, together capturing key patterns in the dataset.

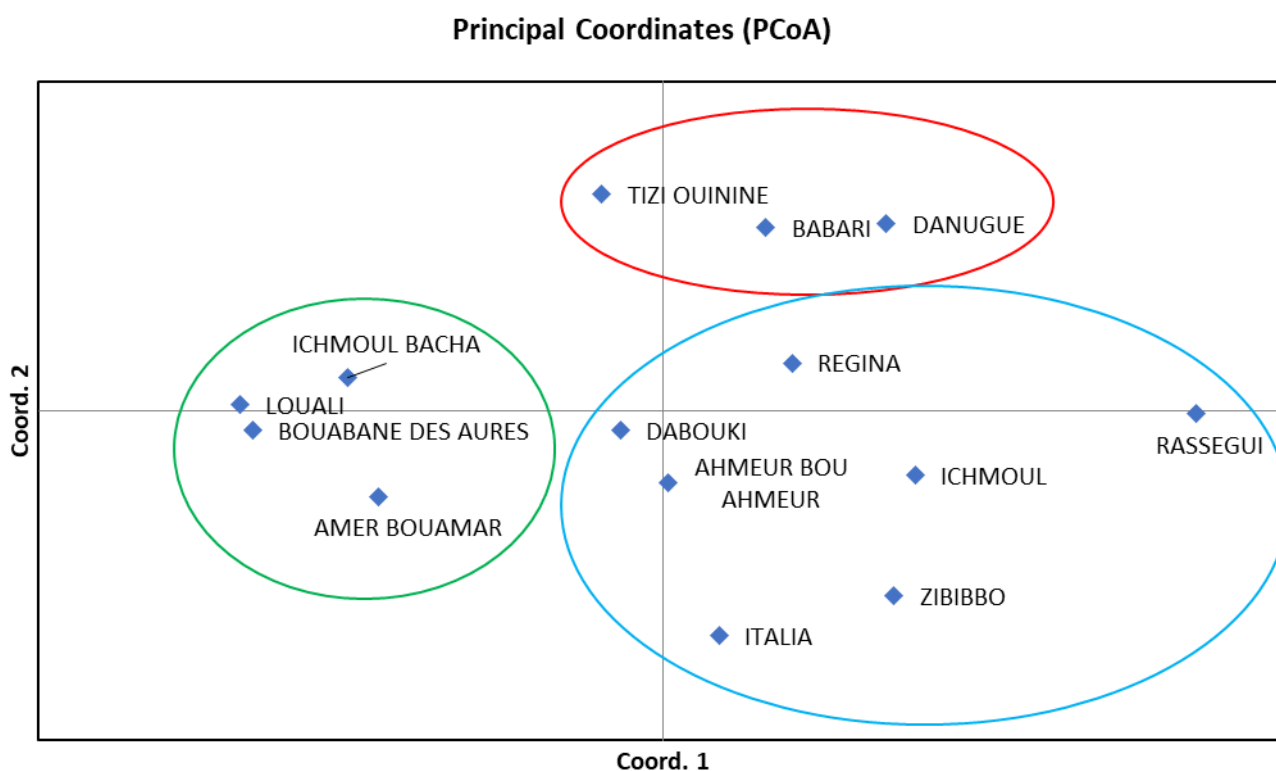


Figure 23: Principal Coordinates Analysis (PCoA) plot revealing genetic diversity and clustering of grapevine cultivars based on 12 SSR markers, with PCoA1 and PCoA2 explaining 18.72% and 14.70% of the molecular variance, respectively.

Remarkably, the cultivars are grouped into three distinct clusters. The red cluster, including ‘Tizi, Ouinine’, ‘Babari’, and ‘Danugue’, likely represents cultivars with closely related molecular

profiles that set them apart from other groups. The green cluster, comprising ‘Ichmoul Bacha’, ‘Louali’, ‘Bouabane des Aures’, and ‘Amer Bouamar’, reflects cultivars with a high degree of genetic similarity. In contrast, the blue cluster, containing ‘Rassegui’, ‘Zibibbo’, ‘Italia’, ‘Ichmoul’, ‘Ahmeur Bou Ahmeur’, ‘Dabouki’, and ‘Regina’, exhibits greater molecular variability, with ‘Rassegui’ positioned farther from the cluster center, suggesting unique genetic traits.

Notably, the closer proximity of the green and blue clusters may reflect some shared molecular characteristics, while the red cluster appears more distinct. These groupings highlight genetic differentiation that could inform conservation strategies, breeding programs, or studies of cultivar origin and adaptation.

4.3.2. Phylogenetic tree

The second method used to evaluate the relationship among the genotypes was a clustering. The cluster analysis of the data yielded two distinct main clusters, as evidenced by the dendrogram constructed through the neighbor-Joining (NJ) method depicting genetic relationships (Figure 24).

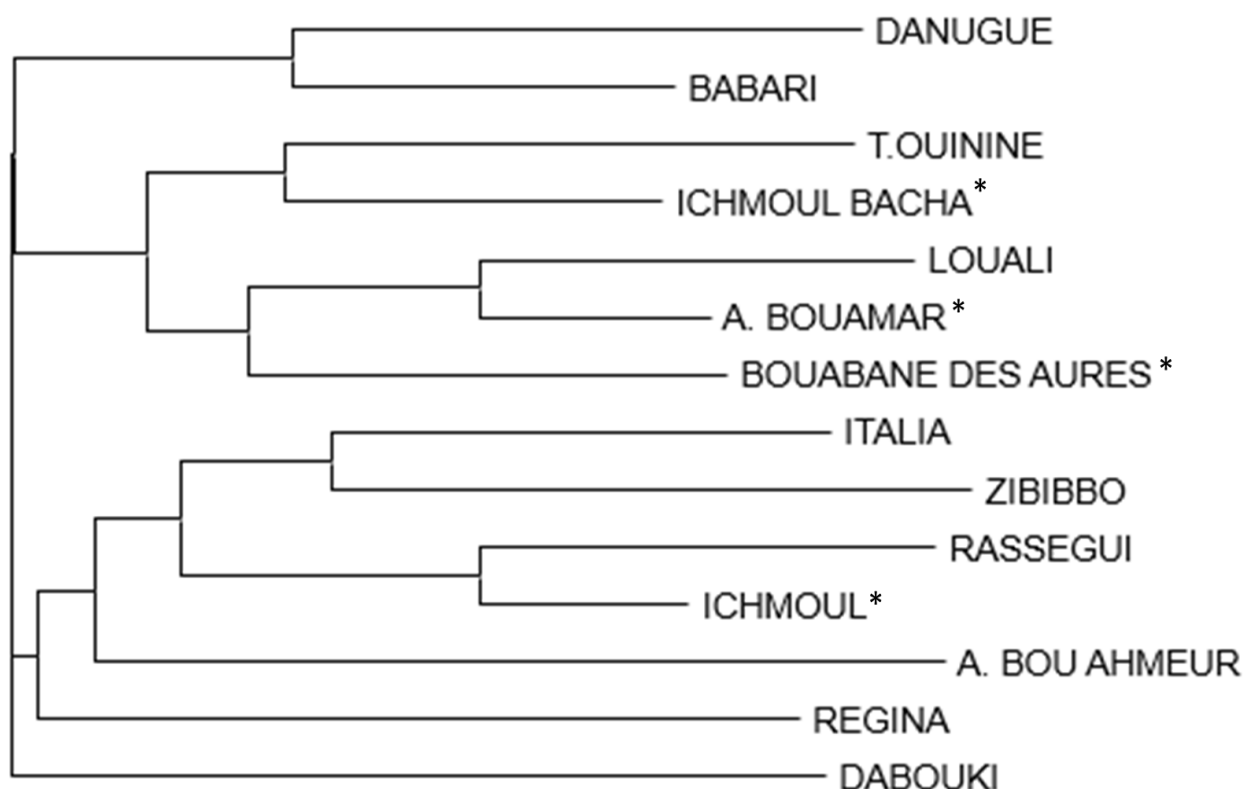


Figure 24: Neighbor-Joining dendrogram illustrating genetic relationships among 14 grapevine genotypes from the Aures region based on 12 microsatellite loci using shared allele distance (DAS) (The unknown genotypes are highlighted with an asterisk (*) and labeled.

Molecular analysis of the data indicates genetic variability among the studied cultivars, with variations in degree observed across different groups and their respective origins. However, the correlation between clustering and growing area where genotypes that were found was not always possible (Labagnara et al., 2018).

Overall, the present findings largely support the results obtained from the ampelographic description for most of the examined grapevine varieties. Furthermore, the molecular clustering results align well with the PCoA findings, indicating a clear separation between the genotypes and confirming the distinct genetic patterns observed in the analyses.

Notably, the first cluster was mainly characterized by local known cultivars, apart from ‘Danugue’. Interestingly, the unknown genotypes were shown to be overall highly close to recognized Algerian varieties recovered as they are grouped together in the same cluster in the dendrogram, potentially indicating a common geographic origin or shared ancestry. However, ‘Unknown genotype 1’ seem to be outlier. In the first cluster, ‘Babari’ demonstrates a close genetic proximity to ‘Danugue’, sharing at least one allele for each locus except for the VVMD28 marker.

The present case is also well-suited to findings obtained by (Rahali et al., 2019). The same goes for ‘Tizi Ouinine’ and Unknown genotype 2’ except for VVMD7, VrZAG62, and VMC6E1 markers. In the ampelographic-based dendrogram, the varieties ‘Babari’ and ‘Danugue’ are already grouped together in the same group. On the other hand, the microsatellite analysis confirmed that the variety ‘Tizi Ouinine’ is the true-to-type of ‘Tazizaouth 11’, ‘Ameziane’, and ‘Anonymous 4’, while ‘Unknown genotype 2’ is the true-to-type of ‘Anonymous 5’. When comparing with the ampelographic data, it has been found that ‘Anonymous 4 and 5’ were grouped together displayed similar leaf characteristics.

Besides, the variety Ameziane was clustered separately but in close proximity with ‘Anonymous 1’ which refers to the ‘Babari’ variety exhibiting together a strong genetic affinity. This present cultivar assignment agreed with grouping based on genetic distances established via SSR markers. In the same subcluster were grouped ‘Louali’, ‘Unknown genotype 4’, and ‘Unknown genotype 3’, where there is an obvious genetic similarity for the variety pairs ‘Louali’ and ‘Unknown genotype 4’ except for VVMD5 marker. On the other hand, ‘Unknown genotype 3’ was situated close to these cultivars, coincided with them showing a possibly a parental relationship.

The second main cluster in the dendrogram is primarily composed of the Mediterranean grape varieties introduced to the region. The grouping of these known varieties in a distinct cluster suggests they share a common genetic origin and heritage, likely reflecting the historical exchange and intermixing of grapevine germplasm across the Mediterranean basin. Within this group, different

subgroups can be discerned based on the genetic similarities revealed by the SSR marker analysis. ‘Zibibbo’ (of Greek origin) and ‘Italia’ (from Italy) cluster closely together indicating a relatively close genetic relationship.

From the SSR profiles in Table (13), we can see that ‘Italia’ and ‘Zibibbo’ share the same alleles at 10 out of the 12 SSR loci analyzed. This high degree of allele sharing suggests they have a shared genetic background and ancestry, despite ‘Italia’ originating from ‘Italy’ and ‘Zibibbo’ having Greek origins (Röckel et al., 2024). Their positioning together in the Mediterranean cluster is expected given their geographic provenances. Regarding ‘Rassegui’ and ‘Unknown genotype 1’ are clustering together in the second major cluster. This close clustering indicates that ‘Rassegui’, despite being reported as a Tunisian variety, shares a high degree of genetic similarity with ‘Unknown genotype 1’, which is new genotype from this study region in Algeria. Their positioning in the same subcluster suggests they likely have a shared genetic background or common ancestral origin.

The SSR profiles in Table (13) show that ‘Rassegui’ and ‘Unknown genotype 1’ share identical alleles at 10 out of the 12 SSR loci analyzed. This genetic closeness is an interesting finding. It raises questions about the precise origins and historical movements of ‘Rassegui’, which may have deeper connections to Algerian viticulture than previously thought. While ‘Ahmeur Bou Ahmeur’ represents a more genetically differentiated local genotype, its inclusion in this Mediterranean cluster suggests it still shares some ancestral ties with the other varieties, potentially due to historical gene flow in the region.

Furthermore, the Lebanese variety ‘Regina’ forms its own distinct subgroup, suggesting it has some degree of unique genetic differentiation compared to the other Mediterranean varieties present, despite its geographic proximity to Greece and Italy. Finally, the Palestinian variety ‘Dabouki’ also forms a nearby but distinct subcluster from ‘Zibibbo’, ‘Italia’ and ‘Regina’. While it groups in the overall Mediterranean cluster, its positioning indicates it has some degree of genetic distinctiveness compared to the other eastern Mediterranean varieties.

Table 13: List of the 14 unique SSR profiles obtained at 12 SSR loci. Allele lengths are expressed in base pairs (bp). Allele lengths for VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79, VMC6E1, VMC6G1 and VMCNG4b9 are provided using the *ITVC* allele sizing.

SSR Profile ID	Grapevine Variety	VVS2		VVMD5		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		VrZAG62		VrZAG79		VMC6E1		VMC6G1		VMCNG4b9	
1	RASSEGUI	143	149	228	242	239	239	249	255	180	195	244	248	250	272	186	188	251	257	141	141	169	177	160	172
2	AHMEUR BOU AHMEUR	135	147	234	240	239	249	255	267	184	195	248	254	252	256	192	204	247	257	165	169	179	193	150	150
3	TIZI OUININE	145	151	228	230	233	239	241	249	186	195	258	260	252	252	188	188	237	251	151	157	177	177	150	158
4	ZIBIBBO	133	149	230	234	249	251	249	249	180	195	244	268	264	272	186	204	247	255	141	143	169	195	158	158
5	LOUALI	133	137	238	242	233	253	241	255	184	186	258	260	252	252	200	204	237	259	141	157	187	191	158	170
6	ITALIA	133	149	234	240	243	247	239	249	180	195	234	244	252	272	192	204	255	257	141	165	187	187	150	158
7	DANUGUE	137	145	230	236	233	239	241	255	195	195	236	244	262	272	188	204	257	257	137	143	177	191	138	166
8	BABARI	145	151	236	242	239	249	241	249	195	195	248	258	252	262	188	202	257	257	143	169	177	191	138	158
9	REGINA	133	135	228	234	239	249	249	255	186	186	234	258	258	272	186	188	243	251	143	167	177	191	150	158
10	DABOUKI	135	151	236	238	247	249	241	245	180	184	258	258	250	272	188	204	247	247	169	175	177	187	158	160
11	ICHMOUL	143	149	240	242	239	243	249	255	180	195	244	248	250	252	188	204	251	257	141	165	177	187	158	160
12	ICHMOUL BACHA	133	151	228	230	249	253	241	249	186	195	258	260	252	252	204	204	237	257	143	165	177	187	158	170
13	BOUABANE DES AURES	143	151	234	240	249	253	241	249	184	195	258	258	252	262	200	204	257	259	141	165	187	197	158	158
14	AMER BOUAMAR	133	137	228	240	249	253	241	255	184	195	258	260	252	252	200	204	257	259	157	165	187	191	150	158

CONCLUSION

CONCLUSION

This research project represents the first comprehensive effort to characterize a significant fraction of the grapevine varieties recovered from the Aures region of Batna (Algeria), employing both ampelographic and molecular approaches.

In our study, the ampelographic characterization was crucial for understanding the phenotypic variability exists among the studied cultivars. This approach was quite helpful to discriminate these cultivars based on mature leaf characteristics taken as prescribed by the descriptor of OIV and ampelometric relationships.

The multivariate study, which employed various statistical analyses (descriptive statistics, PCA, correlation analysis, HCA, and trait frequency), effectively analyzed the large dataset generated in this research using qualitative and quantitative descriptors. It successfully identified the most discriminant traits and their relationships among the studied cultivars.

Our results suggest how few ampelometric traits are sufficient and allowed for the first time to easily discriminate the Algerian autochthonous grapevines recovered from the Aures region.

Overall, the statistical analysis suggests considerable morphological diversity among the studied cultivars, potentially reflecting unique adaptive strategies, genetic variations, or environmental responses specific to Algerian grape varieties.

The coefficient of variation (CV%) reveals significant differences in measurement consistency across variables. Some characteristics show remarkable uniformity, while others demonstrate substantial variability. 10 out of 32 characters reached CV values greater than 20.00%, indicating high variation among the cultivars. That was the case for the leaf size dependent parameters (veins lengths and sinuses distances). While the lowest CVs were shown by the ratios between the measured veins lengths also the ratios between the measured angles as well as the traits related to the angles size.

The PCA capturing 76.91% of the total morphological variation through its first three components (PC1, PC2, and PC3). predominantly influenced by traits related to the depth of lateral sinuses (OIV 605, OIV 606 and their corresponding relationships Rel.14, Rel.6, Rel.7, Rel. 15, Rel.8, and Rel.9), vein angles (OIV 609, OIV 608, OIV 607 and their corresponding relationships Rel.11 and Rel.10), tooth characteristics (OIV 615, OIV 614, OIV 613, OIV 612), and leaf size (OIV 601, OIV 603, OIV 602, and OIV 617) were the most discriminant features among the cultivars studied.

The correlation analysis yielded valuable information about the relationships between key ampelographic traits, with correlation coefficients ranging from $r = -0.34$ to $r = 0.99$, indicating a

spectrum of relationships from strong negative to strong positive correlations. Very strong positive correlations were observed between multiple pairs of variables: OIV 602 and OIV 603, as well as the relationships Rel. 6 and Rel. 7, Rel.8 and Rel.9, Rel. 5 and Rel.15 ($r= 0.98$), Rel.14 and Rel.7 through Rel.9 ($r= 0.94$ to 0.97), Rel.15 and Rel.6 through Rel.9 ($r= 0.96$ to 0.98). These near-perfect correlations suggest these pairs of variables are highly interrelated, potentially indicating shared underlying mechanisms or characteristics. Conversely, negative correlations were noted between traits related to sinus depths (e.g., OIV 605, OIV 606) and vein angles (e.g., OIV 607-609), highlighting their contrasting influence on overall leaf morphology.

The characterization based on qualitative traits provided valuable insights into the varietal identification of the studied cultivars. To further understand the distribution of these traits, their frequency was calculated, offering a deeper perspective on their stability and potential variability across the investigated genotypes. This analysis revealed that while certain ampelographic traits exhibit remarkable stability, others display significant variability, highlighting the diverse phenotypic expression among the studied cultivars.

It is important to note that the classification of these parameters as "stable" or "variable" is influenced by factors such as variety, environmental conditions, and cultivation practices.

Stable traits included the presence/ absence of teeth in the petiole sinus (OIV 081-1), petiole sinus base limited by vein (OIV 081-2), degree of opening/ overlapping of upper lateral sinuses (OIV 082), shape of the base of upper lateral sinuses (OIV 083-1), length of petiole compared to length of middle vein (OIV 093) and depth of upper lateral sinuses (OIV 094), which remained consistent across the studied genotypes.

In contrast, traits such as the size of blade (OIV 065), shape of blade (OIV 067), number of lobes (OIV 068), color of the upper side of blade (OIV 069), shape of teeth (OIV 076), degree of opening/ overlapping of petiole sinus (OIV 079), shape of base of petiole sinus (OIV 080), teeth in the upper lateral sinuses (OIV 083-2) demonstrated considerable variability.

On the other hand, notable findings from the ampelographic clustering allowing the discrimination of studied cultivars into 18 distinct individuals belonging to 7 clusters. The highest similarity coefficient was observed between the cultivars ‘Tazizaouth 5 and 6’ ($J= 0.73$). In contrast, other varieties were distinguished from all these main groups. That was the case for ‘Amer Bouamar’ and ‘Tazizaouth 8’ ($J= 0.308$) in addition to ‘Tazogaghth 3 and 4’ ($J=0.376$) which have large distances indicating that they completely diverge from the other cultivars. They did not correspond to any of the previously defined clusters and could be considered as homonyms. In the dendrogram, these cultivars

are positioned far from the other clusters, suggesting a distinct genetic background and ampelographic profile.

Complementing the phenotypic data, molecular profiling of 41 grapevine cultivars using 12 microsatellite markers uncovered 14 distinct genotypes. The growing areas investigated are rather small, otherwise they resulted rich in terms of biodiversity.

The microsatellite analysis revealed that the 'Tazizaouth' cultivar was the most frequent, referring to 'Rassegui' and encompassing nineteen samples. Interestingly, the true origin of this variety is quite complex, with a lack of references in the literature to its history. Notably, however, this variety holds historical significance, as it is widely domesticated across the region under investigation since at least the Algerian revolution (1954), as confirmed by local farmers, likely due to its phenotypic plasticity and adaptability to local environmental conditions.

Remarkably, four genotypes corresponded to known autochthonous Algerian varieties: 'Ahmeur Bou Ahmeur', 'Louali', 'Tizi Ouinine' and 'Babari', confirming their regional cultivation. Furthermore, six genotypes were found to match widely recognized Mediterranean varieties, highlighting the historical exchange of germplasm over time.

Significantly, four profiles represented novel genotypes, constituting unique resources potentially specific to the Aures. In order to ensure proper authentication and traceability, registration of these newly discovered varieties in the *VIVC* Catalogue is essential. We propose the following respective variety names based on the geographical locations where the genotypes were discovered, as well as the cultural or historical significance of these areas.

Specifically: 'Ichmoul' for 'Unknown genotype 1' which is named after the Ichmoul region, a historically significant area in the Aures mountains known for its traditional viticulture practices, 'Ichmoul Bacha' for 'Unknown genotype 2', similarly named after the Ichmoul region, with the addition of 'Bacha' which refers to a small village within this region, 'Bouabane des Aures' for 'Unknown genotype 3', and 'Amer Bouamar' for 'Unknown genotype 4' were selected to reflect the local nomenclature and the region's deep cultural connection with grape cultivation.

However, further complementary studies are essential to fully characterize these newly described varieties in order to determine their agronomic potential and suitability for use in breeding programs.

More importantly, the findings from the molecular characterization largely support the results obtained from the ampelographic description for most of the examined grapevine varieties indicating consistency between the phenotypic and genotypic data in characterizing these varieties.

Furthermore, the molecular clustering results align well with the PCoA (Principal Coordinate Analysis) findings, indicating a clear separation between the genotypes and confirming the distinct genetic patterns observed in the analyses.

The molecular characterization of grapevine varieties from the Aures region has provided valuable insights into their genetic diversity and relationships. The use of SSR markers has proven effective in distinguishing the cultivars and revealing genetic similarities and differences that traditional morphological methods could not fully uncover.

This study highlights the genetic richness of Algerian grapevines and underscores the importance of preserving these autochthonous varieties for future breeding programs and biodiversity conservation. By integrating molecular data with ampelographic observations, this work contributes to the establishment of a comprehensive framework for the identification, conservation, and sustainable utilization of grapevine genetic resources in the region.

A graphic reconstruction of the 'mean leaf' for each genotype is currently underway to facilitate a comparative analysis of the mature leaves of the genotypes investigated. This approach not only helped in visualizing the morphological differences but also confirmed the accuracy of the ampelographic analysis previously performed.

By examining the mean leaf profile, distinct characteristics such as leaf shape, size, and vein patterns could be clearly distinguished, supporting the validity of the initial ampelographic visualization. Moreover, advancements in modern ampelography, including the application of deep learning and machine learning techniques, are also underway. These emerging technologies hold great potential to further enhance the precision and efficiency of leaf trait analysis, offering new possibilities for high-throughput identification and classification of grapevine varieties.

To build upon the findings of this thesis, several recommendations are proposed:

1. **Germplasm conservation:** The characterized varieties must be transferred to the germplasm collections. These repositories play a pivotal role in conserving these local varieties and supporting future research and breeding efforts.
2. **Holistic characterization frameworks:** Integrate ecological and agronomic studies to better understand the adaptability and resilience of these varieties.
3. **Advanced genomic studies:** Future research should employ high-throughput genomic tools to uncover additional markers and refine the genetic characterization of these cultivars.

4. **Interdisciplinary collaborations:** Future studies should incorporate insights from agronomy, ecology, and socioeconomics to develop holistic strategies for the management and utilization of these genetic resources. Collaboration between researchers, policymakers, and growers will be key to achieving sustainable outcomes.

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APPENDICES

APPENDICES

Appendix 1: Representative morphology of the mature leaf for each studied cultivar.



Ait Abdi



Amellal 1



Amellal 2



Amer Bouamar



Ameziane



Anonymous 1



Anonymous 2



Anonymous 3



Anonymous 4



Anonymous 5



Anonymous 6



Anonymous 11



Anonymous 12



Bouabane



Laadari



Meska 1



Meska 2



Taberkante 1



Taberkante 2



Tasemith



Tazizaouth 1



Tazizaouth 2



Tazizaouth 3



Tazizaouth 4



Tazizaouth 5



Tazizaouth 6



Tazizaouth 7



Tazizaouth 8



Tazizaouth 9



Tazizaouth 10



Tazizaouth 11



Tazogaghth 1



Tazogaghth 2



Tazogaghth 3



Tazogaghth 4

Appendix 2: List of 32 OIV codes used for ampelographic characterization.

Qualitative traits	OIV 065	Mature leaf: size of blade
	OIV 067	Mature leaf: shape of blade
	OIV 068	Mature leaf: number of lobes
	OIV 069	Mature leaf: color of the upper side of blade
	OIV 076	Mature leaf: shape of teeth
	OIV 079	Mature leaf: degree of opening / overlapping of petiole sinus
	OIV 080	Mature leaf: shape of base of petiole sinus
	OIV 081-1	Mature leaf: teeth in the petiole sinus
	OIV 081-2	Mature leaf: petiole sinus base limited by veins
	OIV 082	Mature leaf: degree of opening / overlapping of upper lateral sinus
	OIV 083-1	Mature leaf: shape of base of upper lateral sinuses
	OIV 083-2	Mature leaf: teeth in the upper lateral sinuses
	OIV 093	Mature leaf: length of petiole compared to length of middle vein
	OIV 094	Mature leaf: depth of upper lateral sinuses
Quantitative traits	OIV 601	Mature leaf: length of vein N1
	OIV 602	Mature leaf: length of vein N2
	OIV 603	Mature leaf: length of vein N3
	OIV 604	Mature leaf: length of vein N4
	OIV 605	Mature leaf: length petiole sinus to upper lateral leaf sinus
	OIV 606	Mature leaf: length petiole sinus to lower lateral leaf sinus
	OIV 607	Mature leaf: angle between N1 and N2
	OIV 608	Mature leaf: angle between N2 and N3
	OIV 609	Mature leaf: angle between N3 and N4
	OIV 610	Mature leaf: angle between N3 and the tangent between petiole point
	OIV 611	Mature leaf: length of vein N5
	OIV 612	Mature leaf: length of tooth N2
	OIV 613	Mature leaf: width of tooth N2
	OIV 614	Mature leaf: length of tooth N4
	OIV 615	Mature leaf: width of tooth N4
	OIV 616	Mature leaf: number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 including the limits
	OIV 617	Mature leaf: length between the tooth tip of N2 and the tooth tip of the first secondary vein of N2
	OIV 618	Mature leaf: opening/overlapping of petiole sinus

Appendix 3: Quantitative descriptors recorded for the morphological characterization of 35 grapevine cultivars following the International Organization of Vine and Wine, (2001). Average values of 11 replicates.

	OIV 601	OIV 602	OIV 603	OIV 604	OIV 605	OIV 606	OIV 607	OIV 608	OIV 609	OIV 610	OIV 611	OIV 612	OIV 613	OIV 614	OIV 615	OIV 616	OIV 617	OIV 618
Ait Abdi	1	1	2	3	1	1	6	6	6	7	1	2	3	1	3	4	2	3
Amellal 1	1	2	3	5	1	1	7	6	5	6	1	2	4	1	3	6	3	2
Amellal 2	1	2	2	4	2	2	5	5	5	7	1	2	2	1	1	8	2	3
A. Bouamar	3	3	4	4	2	2	4	5	5	6	1	5	5	4	3	5	4	1
Ameziane	1	1	1	2	1	1	4	6	5	7	1	1	2	1	1	6	1	4
Anonymous 1	1	1	1	2	1	1	6	7	5	7	1	1	2	1	1	7	2	5
Anonymous 2	2	3	3	5	2	2	5	6	5	7	1	3	3	3	2	6	3	3
Anonymous 3	2	2	3	4	4	3	5	5	5	6	1	1	2	1	1	7	2	2
Anonymous 4	1	1	1	3	1	1	5	7	6	8	1	2	4	1	2	4	3	4
Anonymous 5	1	1	2	4	1	1	6	7	6	7	1	2	3	2	3	5	2	3
Bouabane	1	1	3	6	1	1	5	5	6	8	2	3	5	2	4	5	3	3
Anonymous 6	2	2	4	6	4	4	4	5	3	8	2	1	3	2	2	7	3	2
Laadari	2	2	3	4	2	1	5	5	5	6	1	3	4	2	3	5	3	2
Meska 1	2	2	3	4	2	2	6	6	5	7	1	3	4	2	2	7	4	3
Meska 2	1	2	3	4	1	2	5	5	4	6	1	2	2	1	1	8	3	3

Taberkante 1	2	2	2	4	2	2	5	6	6	8	1	2	3	2	2	6	3	4
Anonymous 11	1	2	3	4	1	1	6	7	6	7	1	3	4	2	3	4	3	3
Anonymous 12	2	2	3	5	3	3	4	4	4	5	1	1	2	1	1	7	3	1
Taberkante 2	1	1	2	3	1	2	4	5	4	6	1	1	1	1	1	6	2	3
Tasemith	2	3	4	6	4	4	5	5	5	7	2	1	3	1	2	8	4	2
Tazizaouth 1	2	2	3	5	4	4	4	6	5	6	1	1	2	1	2	8	3	1
Tazizaouth 2	2	2	4	6	4	4	5	4	5	8	2	2	3	1	2	7	3	2
Tazizaouth 3	2	2	3	5	4	3	4	5	5	6	1	1	2	1	2	8	3	2
Tazizaouth 4	1	2	3	5	3	4	5	4	5	7	1	1	2	1	2	8	3	3
Tazizaouth 5	2	2	4	6	3	4	5	5	4	8	2	1	2	1	2	8	3	2
Tazizaouth 6	2	2	4	5	3	4	4	5	4	7	2	1	2	1	2	8	3	2
Tazizaouth 7	2	2	4	6	3	4	5	6	5	7	2	2	2	1	3	8	3	2
Tazizaouth 8	3	4	5	8	6	5	5	5	4	6	2	2	3	2	3	8	4	1
Tazizaouth 9	2	2	3	5	3	3	5	5	5	7	1	1	2	1	1	8	3	3
Tazizaouth 10	2	2	3	5	1	1	6	6	5	7	1	2	5	2	4	5	3	3
Tazizaouth 11	2	2	3	4	1	1	6	6	6	7	1	3	4	2	3	4	3	3
Tazogaghth 1	3	2	3	5	3	3	5	4	4	7	2	1	2	1	2	7	3	2
Tazogaghth 2	2	2	3	6	3	4	5	6	5	7	2	1	2	1	2	7	3	2
Tazogaghth 3	3	4	5	7	4	4	5	6	5	8	2	5	5	5	4	6	5	2
Tazogaghth 4	2	3	4	6	2	4	6	6	6	8	2	4	5	3	3	6	4	4

Appendix 4: Qualitative descriptors recorded for the morphological characterization of 35 grapevine cultivars following the International Organization of Vine and Wine, (2001). Mode values of 11 replicates.

	OIV 065	OIV 067	OIV 068	OIV 069	OIV 076	OIV 079	OIV 080	OIV 081-1	OIV 081-2	OIV 082	OIV 083-1	OIV 083-2	OIV 093	OIV 094
Ait Abdi	5	2	3	7	3	1	2	1	1	3	2	1	1	7
Amellal 1	7	3	3	7	5	3	2	1	1	3	2	1	1	7
Amellal 2	5	2	3	7	4	3	1	1	1	4	3	1	3	5
A. Bouamar	9	2	3	7	3	1	2	1	1	1	2	1	1	7
Ameziane	3	2	3	7	3	3	1	1	1	1	3	1	1	7
Anonymous 1	3	3	3	7	5	3	3	1	1	1	3	1	1	5
Anonymous 2	5	2	3	5	3	1	1	1	1	4	3	1	1	5
Anonymous 3	5	2	2	7	5	1	2	1	1	1	3	1	1	1
Anonymous 4	5	2	3	7	3	3	1	1	1	3	2	1	1	5
Anonymous 5	5	2	3	7	3	1	3	1	1	1	2	1	1	5
Bouabane	7	2	3	7	3	1	1	1	1	4	2	1	1	7
Anonymous 6	7	2	3	7	5	1	2	1	1	4	3	1	1	3
Laadari	7	2	3	7	5	1	2	1	1	4	2	1	1	5
Meska 1	7	3	3	7	3	3	2	1	1	4	3	1	1	5
Meska 2	7	2	3	7	5	1	1	1	1	4	3	1	1	5
Taberkante 1	5	2	3	7	5	3	1	1	1	4	3	1	1	5
Anonymous 11	7	2	3	7	3	1	1	1	1	4	2	1	1	7
Anonymous 12	7	2	3	7	5	1	2	1	1	3	3	1	1	5
Taberkante 2	5	5	3	7	5	3	3	1	1	3	3	1	1	5

Tasemith	7	1	3	7	5	3	2	1	1	2	3	1	1	3
Tazizaouth 1	7	2	3	7	3	1	2	1	1	3	3	1	1	1
Tazizaouth 2	5	2	3	7	5	3	2	1	1	3	3	1	1	1
Tazizaouth 3	5	2	3	7	5	1	2	1	1	1	3	1	1	1
Tazizaouth 4	5	2	2	7	5	3	2	1	1	3	3	1	1	1
Tazizaouth 5	7	2	3	7	5	3	2	1	1	3	3	1	1	3
Tazizaouth 6	7	2	3	7	5	1	2	1	1	3	3	1	1	5
Tazizaouth 7	7	2	3	7	5	1	2	1	1	4	3	1	1	5
Tazizaouth 8	9	5	3	7	5	1	2	1	1	3	3	1	1	5
Tazizaouth 9	9	2	3	7	5	3	2	1	1	3	3	1	1	5
Tazizaouth 10	7	2	3	7	3	3	2	1	1	4	2	1	1	7
Tazizaouth 11	5	2	4	5	3	3	2	1	1	3	2	1	1	5
Tazogaghth 1	7	2	3	7	5	1	2	1	1	3	3	1	1	5
Tazogaghth 2	5	2	3	7	3	3	2	1	1	3	3	1	1	5
Tazogaghth 3	7	2	3	7	3	3	1	1	1	4	1	1	1	5
Tazogaghth 4	7	2	3	7	3	3	1	1	1	4	3	1	1	3

Appendix 5: Relationships of quantitative parameters measured in mature leaves following the method described by Martinez and Grenan, (1999).

Relationship Formula	Description
Rel.1 = PL/L	Ratio between petiole and central vein lengths
Rel.2 = $L1d/L$	Ratio between the first right lateral vein and central vein lengths
Rel.3 = $L1g/L$	Ratio between the first left lateral vein and central vein lengths
Rel.4 = $L2d/L$	Ratio between the second right lateral vein and central vein lengths
Rel.5 = $L2g/L$	Ratio between the second left lateral vein and central vein lengths
Rel.6 = $S1d/L1d$	Ratio between the right lateral upper sinus and the first right lateral vein
Rel.7 = $S1g/L1g$	Ratio between the left lateral upper sinus and the first left lateral vein
Rel.8 = $S2d/L2d$	Ratio between the right lateral lower sinus and the second right lateral vein
Rel.9 = $S2g/L2g$	Ratio between the left lateral lower sinus and the second left lateral vein
Rel.10 = $A + B + G$	Sum of angles formed by the right lateral main veins
Rel.11 = $A' + B' + G'$	Sum of angles formed by the left lateral main veins
Rel.14 = $(S1d + S2d)/(L1d + L2d)$	Ratio between the two right lateral sinuses and the first two main veins
Rel.15 = $(S1g + S2g)/(L1g + L2g)$	Ratio between the two left lateral sinuses and the first two main veins

Appendix 6: Cos squared (Cos^2) values of the investigated variables.

	F1	F2	F3	F4	F5
PL	0.234	0.110	0.078	0.203	0.324
OIV 601	0.202	0.699	0.059	0.010	0.000
OIV 602	0.375	0.578	0.001	0.001	0.002
OIV 603	0.384	0.582	0.002	0.005	0.001
OIV 604	0.499	0.447	0.020	0.001	0.000
OIV 605	0.891	0.032	0.011	0.039	0.000
OIV 606	0.874	0.064	0.004	0.031	0.000
OIV 607	0.425	0.001	0.133	0.008	0.133
OIV 608	0.525	0.012	0.230	0.042	0.001
OIV 609	0.526	0.028	0.206	0.026	0.005
OIV 610	0.011	0.022	0.433	0.207	0.057
OIV 611	0.608	0.219	0.032	0.008	0.010
OIV 612	0.240	0.536	0.002	0.029	0.001
OIV 613	0.192	0.650	0.021	0.016	0.001
OIV 614	0.013	0.775	0.001	0.019	0.000
OIV 615	0.004	0.804	0.069	0.000	0.003
OIV 616	0.638	0.113	0.002	0.004	0.076
OIV 617	0.180	0.551	0.009	0.000	0.056
OIV 618	0.369	0.157	0.074	0.064	0.092
Rel.1	0.064	0.079	0.024	0.372	0.370
Rel.2	0.232	0.065	0.375	0.154	0.045
Rel.3	0.225	0.058	0.332	0.184	0.036
Rel.4	0.287	0.000	0.438	0.166	0.019
Rel.5	0.288	0.005	0.381	0.221	0.021
Rel.6	0.776	0.064	0.026	0.065	0.010
Rel.7	0.723	0.089	0.020	0.094	0.002
Rel.8	0.692	0.160	0.002	0.105	0.005
Rel.9	0.576	0.223	0.001	0.145	0.004
Rel.10	0.603	0.000	0.224	0.024	0.007
Rel.11	0.625	0.002	0.249	0.003	0.068
Rel.14	0.780	0.096	0.014	0.082	0.008
Rel.15	0.708	0.135	0.011	0.116	0.003

Note: Values in bold for each variable correspond to the factor for which the Cos^2 is greatest.

Appendix 7: Cos squared (Cos2) values of the investigated variables.

	F1	F2	F3	F4	F5
Ait abdi	0.768	0.000	0.081	0.058	0.000
Amellal 1	0.324	0.083	0.012	0.444	0.000
Amellal 2	0.016	0.333	0.052	0.105	0.349
A. bouamar	0.036	0.503	0.303	0.005	0.044
Ameziane	0.371	0.569	0.000	0.000	0.005
Anonymous 1	0.273	0.552	0.011	0.098	0.035
Anonymous 2	0.041	0.043	0.551	0.254	0.003
Anonymous 3	0.473	0.326	0.004	0.000	0.008
Anonymous 4	0.676	0.041	0.086	0.097	0.005
Anonymous 5	0.669	0.034	0.222	0.000	0.020
Bouabane	0.205	0.349	0.000	0.056	0.034
Anonymous 6	0.837	0.001	0.012	0.027	0.000
Laadari	0.359	0.087	0.228	0.001	0.131
Meska 1	0.511	0.043	0.004	0.003	0.117
Meska 2	0.000	0.206	0.027	0.274	0.050
Taberkante 1	0.162	0.188	0.360	0.002	0.023
Anonymous 11	0.865	0.066	0.005	0.010	0.009
Anonymous 12	0.047	0.028	0.587	0.036	0.246
Taberkante 2	0.005	0.503	0.051	0.260	0.060
Tasemith	0.688	0.013	0.001	0.030	0.175
Tazizaouth 1	0.586	0.051	0.096	0.003	0.018
Tazizaouth 2	0.791	0.003	0.043	0.063	0.008
Tazizaouth 3	0.725	0.170	0.000	0.012	0.002
Tazizaouth 4	0.714	0.205	0.001	0.001	0.010
Tazizaouth 5	0.805	0.006	0.031	0.001	0.056
Tazizaouth 6	0.761	0.000	0.122	0.005	0.005
Tazizaouth 7	0.417	0.012	0.438	0.027	0.003
Tazizaouth 8	0.643	0.256	0.008	0.036	0.028
Tazizaouth 9	0.061	0.069	0.267	0.140	0.193
Tazizaouth 10	0.455	0.307	0.001	0.111	0.043
Tazizaouth 11	0.796	0.105	0.022	0.005	0.039
Tazogaghth 1	0.625	0.011	0.145	0.000	0.021
Tazogaghth 2	0.724	0.004	0.036	0.080	0.003
Tazogaghth 3	0.005	0.675	0.064	0.181	0.014
Tazogaghth 4	0.001	0.465	0.346	0.074	0.017

Note: Values in bold for each variable correspond to the factor for which the Cos2 is greatest.