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Unveiling the viticultural heritage of Rachaya, Lebanon: genetic, ampelographic and chemical profiling of local grape varieties

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Abstract. Located in the Western Bekaa region of Lebanon, Rachaya is renowned for its long history of viticulture and ideal Mediterranean climate for grapevine cultivation. Despite the region's significance in grape production, knowledge about the local grape varieties remains limited, making it essential to characterize these indigenous cultivars for a better understanding of the area's viticultural landscape. To address this, we conducted a comprehensive analysis of five local grapevine varieties: 'Obeidy', 'Kassoufi', 'Fodde', 'Halawani', and 'Souri', which were compared to a set of two international varieties. Our study encompassed genetic analysis using 22 SSR markers, morphological assessment based on 15 OIV descriptors related to leaves, bunches, and berries, and chemical profiling of various polyphenol classes in wine, with untargeted chemical discrimination carried out through a High-Resolution Mass Spectrometry-based metabolomics workflow. This detailed evaluation of the local grapevine gene pool revealed substantial diversity, showing that Lebanese grape varieties are genetically unique, with two out of the five genetic profiles previously undocumented in international databases. Additionally, these varieties exhibited distinct chemical traits. These findings are crucial for valorizing and promoting the sustainable use of these grape varieties, particularly as Lebanon's wine production sector continues to expand.

1. Introduction

Viticulture in Lebanon has a deep history, rooted in ancient agricultural traditions. The Mediterranean climate, with its long, dry summers and mild, wet winters, provides an ideal environment for grapevine cultivation [1]. One area of particular interest is the Rachaya district, located in the Western Bekaa Valley at the foot of Mount Hermon. The district sits at an elevation ranging from approximately 900 to 1,600 meters above sea level, providing an ideal climate for grapevine cultivation. It comprises a group of villages, with some of the most important ones being Rachaya Al Wadi, Kfarmichki, Bakka, Ain Arab, and Mdoukha. These villages are known for their agricultural heritage, particularly in viticulture, and play a key role in Lebanon's wine production landscape [2]. The area's unique terroir combining soil, altitude, and microclimate, has supported grapevine growth for centuries [3]. Rachaya's high-altitude vineyards benefit from cool nights and warm days, which enhance the development of distinctive grape flavors [4]. Despite this, the area remains underexplored in terms of its contribution to Lebanon's viticulture, especially local grapevine varieties that have adapted over time to the environment. Mount Hermon, also known as Jabal al-Shaykh, forms the southern tip of the Anti-Lebanon Mountains and is renowned for its rich cultural and natural heritage [5,6]. The mountain supports diverse ecosystems and is recognized as an Important Plant Area in Lebanon, home to many endemic and threatened species, including over 100 unique plant species [7,8]. Grapevines are central to the cultural identity of the Mount, with local products widely produced, such as arak alcoholic drink and

molasses. On the other hand, the area faces multiple challenges from habitat fragmentation, and overuse of resources to climate change, which threaten its unique ecosystems and the genetic diversity of its species [9,10].

Lebanon's grapevine germplasm represents a valuable genetic resource developed over millennia. However, many local varieties remain poorly documented and underutilized [1,3,11]. Like other marginal areas such as Hadchit and Btorram villages in northern Lebanon, which have revealed a diverse genetic diversity and unique genetic profiles conserved by the local farmers [12,13], Rachaya district is expected to harbor a similar rich genetic diversity. Indeed, farmers play a crucial role in conserving their traditional varieties in their farming system and family backyards. Yet, this genetic wealth is at risk with the global focus on the use of international varieties [14]. Hence, characterizing and conserving traditional grapevine varieties growing in Rachaya is essential to ensure their sustainable use and explore their potential value for wine production and other applications.

In this context, the objectives of this study are bifold: (1) to characterize five common traditional varieties growing in Rachaya district using SSR markers and OIV descriptors; and (2) to evaluate the chemical composition of the monovarietal wines produced from these varieties. This comprehensive analysis will contribute to a better understanding of Lebanon's local grapevines potentialities within a sustainable utilization approach.

2. Materials and methods

2.1. Plant material

Based on our surveys and engagement with local communities, five grapevine varieties were selected for study as they are the most common in the vineyards of Rachaya district. To minimize environmental variation, all accessions were chosen from a single vineyard in the village of Kfarmichki (33.515946, 35.7673256). The vernacular names of these accessions are linked to distinctive traits: 'Obeidy' and 'Halawani' are named in reference to their high sugar content; 'Kassoufi' is derived from an Arabic word meaning "involved in eating" (likely for processing); 'Fodde', meaning "silver," refers to its shiny white berry skin; and 'Souri', which in Lebanese Arabic could refer either to Syria or the town of Sour (Tyre) in southern Lebanon, as both geographic references share the same name. According to farmers, these landraces were selected for their specific uses in Arak production, Lebanon's national drink, or for making grape molasses, a regional specialty. For comparison, vines of Chardonnay and Viognier were included as international varieties.

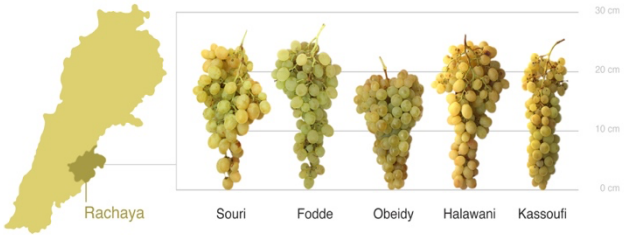

Figure 1. Location of Rachaya district in Lebanon with the respective grape varieties selected for this study.

Molecular and ampelographic characterization of the Lebanese varieties has been performed as described in Merheb et al. [15] and Laucou et al[16].

2.2. Microvinification and untargeted (LC-HRMS) analysis

Grapes at maturity were harvested during the growing season of summer 2023 and microvinifications were carried out at Chateau St Thomas winery, West Bekaa, Lebanon. Fermentions were done following the white wine microvinification protocol as follows. After manual destemming and crushing, the grapes were pressed using a manual press, and the must was sulfited with potassium metabisulfite (50mg/L). The must was then cold-settled to remove solids before fermentation. A commercial strain of *Saccharomyces cerevisiae* was rehydrated and inoculated at a concentration of 20g/hL, along with its activator. Temperature and density were monitored throughout fermentation, and bottling was performed when the wine reached a density of 995 for 4 days making sure all the sugar was transformed into alcohol. The wine then underwent cold stabilization to precipitate tartrates. Following stabilization, the wine was racked and sulfited with a final addition of 3 mL/L sulfur dioxide. The bottles were capped, and the wine was stored for six months at the winery until analyses were performed.

Analysis was operated on a UHPLC-DAD-HRMS system. Separations were performed using a Vanquish UHPLC-DAD (Thermo Fischer Scientific, San José, CA, USA) on a (10 × 1 mm i.d.) Acquity HSST3 column (Waters, Milford, MA, USA; 1.7 µm), operated at 35 °C following the protocol and data analysis procedures previously described by Leborgne et al [17]. Annotation was performed manually by comparing retention times and mass spectra accuracy with a mass tolerance of 5 ppm based on the previous experience of the group with the specific instrumentation mass resolution and in accordance with the four levels of annotation described by Summer et al [18].

2.3. Statistical analysis

Principal component analysis (PCA) was performed in order to study the degree of similarity among accessions at the genetic and morphological level. All statistical results were run with 'corrplot' and 'factextra' packages for R studio v.3.6.2. Results of chemical analysis were processed using Compound Discoverer version 3.1

software for PCA that was generated using centered and scaled values [15,17].

3. Results

SSR analysis conducted on the five traditional varieties of Rachaya generated five different genetic profiles, well differentiated from Chardonnay and Viognier profiles, thus validating the reliability of this study (Table1). Comparison of the five Rachaya traditional varieties with both databases of Domaine de Vassal and *Vitis* International Variety Catalogue (VIVC) allowed to identify two profiles that do not have any correspondence in the databases; while the three others matched with other accessions of the databases, but with cultivars of Lebanese origin, obtained from Lebanon at Domaine de Vassal grape collection.

Table 1. Genetic identity of genotyped varieties as compared to international databases in addition to VIVC code and country of origin.

Variety Name	Identification according to Vassal and VIVC databases	VIVC Code	Country of Origin
Obeidy	Obeidi	8646	Lebanon
Kassoufi	Inab el Mir	5523	Lebanon
Souri	Zeini baladi	921	Lebanon
Halawani	Unidentified	-	-
Fodde	Unidentified	-	-
Chardonnay	Chardonnay blanc	2455	France
Viognier	Viognier	13106	France

Molecular analysis indicated a significant degree of genetic variation. With the set of 22 nuclear SSR markers, a total of 96 alleles and a mean number of 4.36 alleles per marker were detected among the five grapevine varieties. The highest number of alleles identified was 6 in VMC4f3, VVIh54, VVIp60, and VVIv37, while the lowest was at 1 with VVIn73. PCA analysis based on 22 SSR markers covered 41.48% of the total variability and demonstrated clear genetic differentiation among the grapevine varieties. All varieties were distinctly separated from each other, indicating substantial genetic diversity within the dataset. Notably, the international varieties clustered on one side of the PCA plot (right), while the Lebanese varieties occupied a distinct, opposite side (Figure 2).

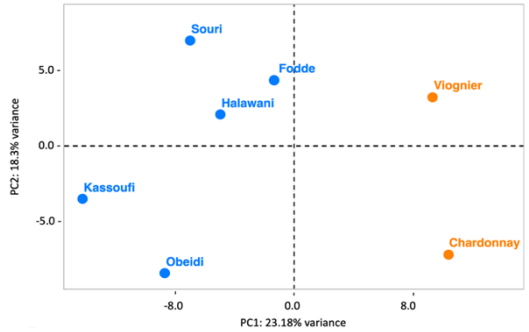

Figure 2. Scatter plot constructed based on the 22 SSR markers with the first two principal component axes showing the genetic distribution of studied grapevines from Rachaya, West Bekaa, Lebanon.

In terms of ampelography based on the OIV descriptors, all the varieties studied shared common morphological traits, which included same teeth size and form, absence of pigmentation on the lower leaf veins, lack of pilosity on the upper leaf surface and pedicel, and a green-yellow berry skin color. PCA analysis based on ampelographic descriptors (Figure 3) clusters the two international varieties 'Chardonnay' and 'Viognier' close to the two varieties of Rachaya 'Obeidy' and 'Fodde', sharing dense clusters and globose berries. While the remaining three varieties exhibited looser clusters and berry shape varying between obovoid ('Kassouf') and narrow ellipsoide ('Souri' and 'Halawani').

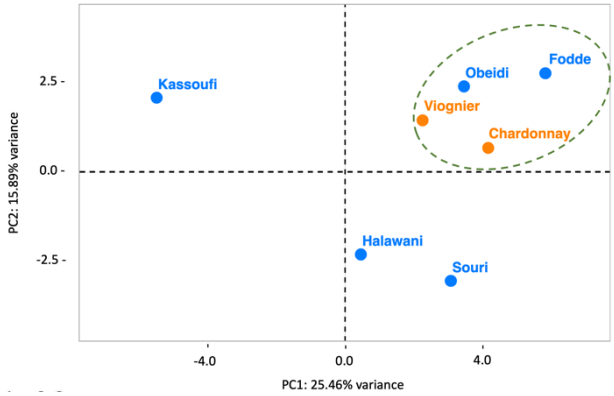

Figure 3. Scatter plot constructed based on the OIV descriptors on the basis of the first two principal component axes showing the morphological distribution of studied grapevines from Rachaya, West Bekaa, Lebanon.

The analysis of micro-fermented wine samples using untargeted metabolomics via UHPLC-HRMS revealed 235 different compounds in the wines. PCA plot on UHPLC-HRMS analyses revealed a scattered scheme indicating distinct chemical profiles (Figure 3).

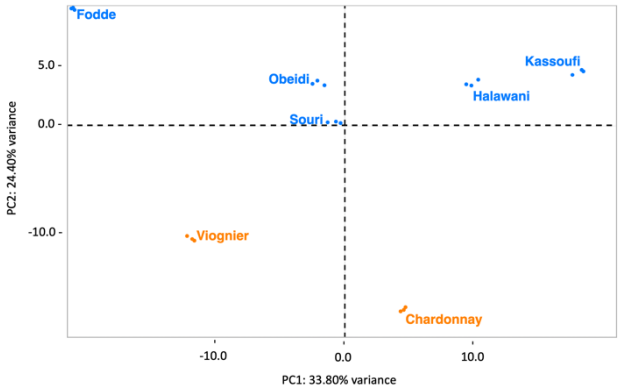

Figure 3. Scatter plot constructed on the basis of the first two principal component axes showing the chemical profiles distribution of wines of the studied grapevines from Rachaya, West Bekaa, Lebanon. Each point corresponds to one injection of the sample. Each sample was characterized three times.

'Obeidy' and 'Souri' were positioned closely together, while the other varieties of the district were distributed across various angles of the plot. 'Fodde', in particular, was distinctly separated from all other samples. Additionally, the international varieties clustered in the lower part of the scatter plot, while the regional varieties were grouped in the upper part.

4. Discussion

The results of this study indicate that Lebanon possesses significant genetic diversity in grapevine varieties, particularly within traditional landraces. The grapevines from the Rachaya region were examined for the first time using three approaches: genetic, morphological, and chemical analyses. These complementary methods provide a comprehensive view of the varieties, offering a well-rounded understanding of their unique characteristics. By integrating these techniques, we can more effectively identify and characterize the diversity within these grapevine populations, shedding light on their potential for both conservation and utilization [19-21].

The identification results confirmed that farmers in the Western Bekaa region have successfully conserved traditional Lebanese varieties over generations ('Inab el Mir,' 'Obeidi' and 'Zeini baladi'). The presence of two unmatched genetic profiles among the local varieties highlights the rich tradition of grapevine cultivation in this region, similar to findings from other areas in Lebanon [12,13,15]. These results confirms that the Bekaa Valley, is a historical center of grapevine diversity that is conserved by local farmers.

Genetic analysis using 22 SSR markers reinforced the uniqueness of the Lebanese varieties compared to international ones. The PCA revealed a clear separation between local and international varieties, suggesting limited genetic overlap and strong population structure. This genetic distinction highlights the uniqueness of the Lebanese grapevine gene pool, shaped by local environmental conditions and long-standing cultural practices. The genetic diversity observed is particularly important for conservation and utilization efforts, especially as genetic diversity is increasingly valued in global viticulture for enhancing resilience and sustainability.

The morphological analysis, based on OIV descriptors, provided additional insights into the similarities and differences among the varieties. Notably, the international varieties 'Chardonnay' and 'Viognier' were morphologically grouped with the Lebanese varieties 'Obeidy' and 'Fodde,' suggesting that these Lebanese varieties may also be well-suited for wine production. Obeidy, in fact, is already being used for wine in Lebanon [1]. This morphological similarity, combined with the genetic findings, emphasizes the potential of these varieties for wine production and their suitability for further development.

The untargeted metabolomics analysis of micro-fermented wines revealed distinct chemical profiles for each mono-varietal wine. The PCA showed that Lebanese varieties exhibited considerable chemical diversity, with 'Obeidy' and 'Souri' positioned closely together, reflecting shared metabolic traits. 'Fodde', on the other hand, was chemically distinct from all other varieties, highlighting its unique composition. The separation between international and Lebanese varieties in terms of their chemical profiles further reinforces the uniqueness of the local varieties. This chemical distinctiveness could contribute to unique sensory characteristics in the wines, potentially offering a novel product in the growing Lebanese wine industry.

Across both genetic and chemical analyses, a clear distinction was observed between international varieties and those cultivated locally. Lebanese varieties, both genetically and chemically, are distinct from their international counterparts, suggesting that they offer unique traits for wine production. This genetic and chemical uniqueness positions Lebanese varieties as potential candidates for producing differentiated wines that stand out in the global wine market, contributing to the region's competitive edge.

5. Conclusion

This study offers a first evaluation of the genetic, morphological, and chemical diversity of few grapevine varieties in the Western Bekaa area of Lebanon, highlighting the rich genetic heritage of the Rachaya area and their potential for wine production. While some varieties have shown promise for wine, further genetic validation and chemical analysis are needed to fully explore Lebanon's genetic diversity. The local terroir, including soil and climate, likely influences the unique traits of varieties, which warrants additional investigation. The identified genetic diversity provides valuable opportunities for breeding programs, particularly for enhancing climate adaptability, given the high-altitude cultivation. Future efforts should focus on the sustainable conservation of these traditional varieties, as their preservation is both culturally and economically significant, especially in the context of growing global interest in indigenous varieties and their potential in the modern wine industry.

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