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Grapevine Varieties of the Aures Region of Algeria: An Exploration of Ampelographic Characteristics and Physiological Performance

Wahiba Yahiaoui1,* , and Ziane Laiadi²

^{1,2}Laboratory of Genetic, Biotechnology and Valorization of Bioresources (LGBVB), University of Biskra, BP 145 RP, Biskra 07000, Algeria.

Email: wahiba.yahiaoui@univ-biskra.dz

1. Introduction

The Aures region of northeastern Algeria is renowned for its viticulture practices and harbors several local grape varieties that have been cultivated by the local Amazigh (Berber) communities for generations (Isnard, 1951). However, many of these ancient varieties remain poorly characterized and underutilized, leading to the risk of genetic erosion and potential loss of valuable genetic resources (Rahali et al., 2019). In fact, these autochthonous varieties may hold the key to developing resilient, high-quality grapes suited to both traditional and modern viticultural practices. Interestingly, comprehensive morphological characterization and evaluation of autochthonous grapevine varieties are crucial steps towards their conservation (Ocete Rubio et al., 2014), valorization, and sustainable utilization (Fanelli et al., 2021). Furthermore, understanding the physiological performance of these varieties, particularly in terms of their propagation potential and growth dynamics (Tzortzakis et al., 2020) is essential for their successful integration into breeding programs and commercial cultivation. According to Sabir and Sabir, (2018); Muttulani, (2022), factors such as rooting ability, acclimatization success, and shoot growth patterns are among the more reliable parameters to evaluate in vitro development and multiplication of vine genotypes. This study aims to investigate five previously undescribed autochthonous table grape varieties from the Aures region, focusing on their ampelographic characteristics and physiological performance during early growth stages. By employing a combination of morphological analysis and physiological assessments, we seek to elucidate the unique features of these varieties and their potential for cultivation and breeding programs.

Our research objectives include:

- 1. Conducting a detailed ampelographic description of the five varieties using standardized methods.
- 2. Analyzing the morphological relationships among the varieties through multivariate statistical techniques.
- 3. Evaluating the physiological performance of these varieties during early growth stages, including rooting ability, acclimatization success, and shoot growth dynamics.

This comprehensive approach will provide valuable insights into the genetic diversity and agronomic potential of these understudied grapevine varieties, contributing to their conservation and potential utilization in future viticultural practices.

2. Material and Methods

2.1. Plant sampling for ampelographic description

Five autochthonous table grape varieties grown in the Aures region of Batna, Algeria are used in this study (Figure 1). These varieties are largely unknown, neglected, and have never been described before. The sampling was performed during the summer season (August-September 2023) when the leaves were fully developed and representative of the plant's morphology.

Figure 1. Morphology of the mature leaf for each studied cultivar

Briefly, for each variety, 10 mature leaf samples were collected for the ampelographic description following the method described by Martinez and Grenan, (1999) (Figure 2). The selected leaves were taken from the middle part of the shoots to ensure uniformity and consistency of the analysis (Galet, 1985).

Veins lengths and angles are measured utilized ImageJ software [\(https://imagej.nih.gov/ij/\)](https://imagej.nih.gov/ij/) with specific calibration settings, and the 15 ampelometric relationships where then taken into account for the morphologic description (Table S1).

2.2. Multivariate study of ampelographic traits

To comprehensively analyze the ampelographic traits of the five autochthonous table grape varieties, a multivariate study was conducted. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed on the recorded ampelometric parameters for each variety using the XLSTAT program (trial version).

Principal component analysis (PCA) was employed to reduce the dimensionality of the dataset and identify the key traits that contribute most significantly to the variation among the grape varieties. PCA facilitated the visualization of patterns and relationships within the data.

Besides, to define the relatedness between the grape varieties based on leaf morphology descriptive characteristics, the Euclidean dissimilarity index was employed. This index quantified the dissimilarities between the varieties, providing a measure of how different each variety is from the others.

Subsequently, hierarchical cluster analysis (HCA) was performed using the Ward clustering algorithm. This method aimed to group the grape varieties based on their ampelographic traits, identifying clusters of varieties with similar morphological characteristics. The hierarchical clustering produced a dendrogram, illustrating the degree of similarity between the varieties and their potential classification.

2.3. Plant sampling for physiological study

The correct choice of samples is the first step in the successful production of grapevine fruiting cuttings. Hardwood cuttings of the previously described grape cultivars were sampled during dormancy period (January 2024). Cuttings were collected from various cane segments and were 15 to 20 cm long and 5 to 10 mm wide, with a minimum of three nodes N0, N1, and N2.

These cuttings were selected to obtain fruit-bearing cuttings according to the method originally described by Mullins, (1966) and later modified by Ollat et al., (1998); Santa Maria, (2004)

then Antolín et al., (2010), which ensured that adventitious root formation preceded bud burst. According to Mullins and Rajasekaran, (1981) the production of fruiting cutting is founded on two basic procedures: pre-rooting and pruning.

2.4. Protocol implementation

2.4.1. Experimental Design 1: hydroponic culture system

For this experimental design, 10 replications of cuttings were used for each variety. Initially, all cuttings basis were treated with an aqueous solution of indolebutyric acid (IBA). As IBA is not soluble in water, therefore, it is necessary to first dissolve an amount of 4 g in 25 mL of ethanol (96 %), then continue the volume to 1 L by distilled water and applied by immersion of the segment bases during 24 hours (Daskalakis et al., 2018).

Subsequently, a hydroponic system using tap water served as the culture medium (Sabir and Sabir, 2018). This set up allowed us to quantify the variations in rooting performance of hardwood cuttings among the studied genotypes at different times intervals. Plastic container with a volume of 10 L was filled with tap water and utilized for this purpose. Cuttings were carefully inserted into predrilled polystyrene supports and placed in the container ensuring that a single node (N0) was completely submerged and only one cutting was planted per cell. To maintain warmth, the container was heated through a cool water conduit, maintaining the temperature between 25-27 °C. Following this, the cuttings were subjected to a cold storage treatment at 4 °C for 60 days until they were transplanted into the growth chamber. The water reservoir in the system was recirculated with an air pump. The water in the culture was changed with four days interval.

2.4.2. Design of Experiment 2: acclimatization in growth chamber

At the end of the first experiment, rooted cuttings were removed from the container then washed with running tap water to remove any residual then potted into a mixture of peat and sand $(2:1, 1)$ v/v) in 2 L plastic pots then were transplanted from March to May 2024 in the growth chamber under controlled conditions of 16 h at 25.0 ± 0.9 °C and 8 h darkness at 20.0 ± 1.0 °C.

2.5. Measurement of growth parameters

- The measurements relating to root development in the first experiment were obtained sixty (60) days after the cuttings were placed in water (hydroponic cultivation system).

- The parameters relating to the second experiment were measured 2 months after the cuttings were transferred to the growth chamber.

a. Rooting rate (%)

Rooting rate is calculated by the percentage of cuttings with visible root primordia or emerged roots in relation to the total number of cuttings per cultivar.

Number of cuttings with visible
Rooting rate =
$$
\frac{\text{root primordia or emerged roots}}{\text{Total number of cuttings}} \times 100
$$

b. Root length (cm)

Root length is obtained by image analysis of each rooted cutting using ImageJ software [\(https://imagej.nih.gov/ij/\)](https://imagej.nih.gov/ij/) (Gordillo et al., 2022). The sum of all root lengths for each cutting was calculated, and the mean value of these sums across all cuttings of the same variety was taken as the final root length value for that variety.

c. Acclimatization rate (%)

Acclimatization in a growth chamber refers to the ability of plants (here cuttings) to adapt and

survive in the controlled conditions of the growth chamber after being initially prepared or grown in another environment (Morales et al., 2014). This process involves the plants adapting to the temperature, water stress and other specific conditions of the growth chamber (Kizildeniz

et al., 2015; Martínez-Lüscher et al., 2016; Kizildeniz et al., 2018; Antolín et al., 2022). During this period, plants must adjust their physiology to survive and continue to grow in the new conditions (Antolin et al., 2010).

The acclimatization rate is calculated by dividing the number of plants that survive and show signs of continued growth by the total number of plants transferred, then multiplying by 100 to obtain a percentage.

Acclimatization rate (%) = $\frac{\text{Number of plants referenced}}{\text{Total number of transferred plants}} \times 100$

d. Shoot growth

The elongation of the aerial shoots and the appearance of new leaves indicate that the cutting for each cultivar is acclimatized to the new controlled conditions in the growth chamber. To provide specific stem growth values for each vine variety, we measured stem height over a

period of eight weeks (2 months).

2.6. Data analyses

For each physiological parameter (rooting rate, root length, acclimatization rate, and shoot growth), descriptive statistics were calculated allowing for direct comparison across the five cultivars. Rooting and acclimatization rates were expressed as percentages for each variety. Root length was presented as mean \pm standard error to account for variability within each variety.

Shoot growth was analyzed over an eight-week period. Weekly measurements were recorded and presented as mean \pm standard error for each variety. Growth trends were examined to identify varietal differences in growth rates and patterns.

3. Results and Discussion

3.1. Ampelographic description of studied varieties

3.1.1. Principal component analyses of quantitative traits

PCA performed taking into account the 15 ampelometric relationships considering leaf measurements based on vein lengths and angles showed that the first three PCs account for 97.7 % of the total variation (Table 1). However, the first two PCs were found to be significant and sufficient in explaining the majority of variation and accounted for 91.8 % (Figure 3).

Most of the variance expressed by the first component (F1) explained 61.2 % of the variables mainly related to the angles formed by the main veins were Rel.10 and Rel.11 with most positive weight. Similarly, Gago et al., (2009) and Laiadi et al., (2013) reported that the variables related to the angles have the greatest weight in PC1. In the same axis, the variables with most negative weight, all of which refer to the depth of the upper and lower lateral sinuses, noting Rel.6, Rel.7, Rel.8, Rel.14, Rel.15.

These findings are in line with those obtained by Zinelabidine et al., (2014) and Gago et al., (2022). However, the parameters Rel.9 and Rel.12 are more represented in F2. Notably, a strong negative correlation was revealed between the depth of the upper and lower lateral sinuses and the angles size. This remarkable finding was well described by Couturier et al., (2011). Such relationship may indicate a strong genetic component in which more detailed morphogenetic processes would take place. With regard to the second component (F2), the most important discriminant parameters, accounting for 30.5 %, were related to leaf size and shape, specifically Rel. 3, Rel. 4, and Rel. 5. This finding is consistent with the result reported by Laiadi et al., (2013). However, the parameter Rel. 2 show greater representation in F2.

The biplot visualization of the PCA outcomes (Figure 3) reveals the interrelations among the 15 ampelometric relationships assessed across the 10 leaves per grapevine cultivar. A notable pattern emerges, as shown by the circle in red where grape varieties Anounymous 1, 3, and 5 grouped together in the center of the biplot, indicating a higher degree of similarity. These varieties are characterized by their deep lateral sinuses and large angles. In contrast, grape varieties Anonymous 2 and 4 are positioned at a distinct location, suggesting that they exhibit a different ampelometric profile compared to the former group. Anonymous 2 variety having leaves with small blade and the largest angles appears at the bottom of the graph. In the other hand, Anounymous 4 exhibits a strong negative association with PC1 and a moderate positive association with PC2, placing it far to the left of the graph. This cultivar is characterized by their large leaf blade and shallow lateral sinuses with small angles.

Figure 3. Visualization of PCA outcomes illustrating the interrelations among the various ampelometric parameters assessed across the 10 leaves per grapevine cultivar

3.1.2 Ampelographic clustering of studied varieties

The dendrogram (Figure 4) illustrates the hierarchical relationships among the five grape varieties based on their ampelometric relationships inspired by Martiez and Grenan, (1999). Notably, the varieties grouping here by this clustering were came compatible to those obtained by PCA (Figure 3). The dendrogram identifies two distinct clusters: the first one comprising the varieties Anonymous 5, 3, 1 and 2. The second one consisting of variety Anonymous 4. The clustering pattern reveals that varieties 5 and 3 are the most similar forming a tight subgroup characterized by deeper lateral sinuses and larger angles. Anonymous 1 joins this subgroup next, sharing the same traits but maintaining some other distinct features. Anonymous 2, while part of this cluster, stands as the most distant member, notable for its small leaf blades and largest angles. The clustering aligns with the PCA results, where varieties 5, 3, and 1 grouped centrally in the biplot, while Anonymous 2 occupied a distinct position.

This pattern indicates a shared genetic background among these four varieties, with enough variation to distinguish them as separate cultivars. Lastly, Anonymous 4 stands out as the most distinct variety, forming its own cluster that confirms its unique characteristics observed in the PCA (large leaf blade, shallow lateral sinuses, small angles).This gradation of similarity within the cluster suggests a spectrum of ampelometric characteristics, likely reflecting subtle variations in leaf morphology as described in several studies characterizing grape leaf morphology in Algeria (Laiadi et al., 2013), Tunisia (Lamine et al., 2014), Spain (Cervera et al., 2001), Greece (Avramidou et al., 2023) and Turkey (Ates et al., 2011).

Figure 4. Hierarchical classification of the studied varieties using ampelometric relationships recorded for the 5 grape genotypes

3.2. Physiological study and assessment of growth performance

3.2.1. Rooting rate

Table (2) shows the rooting rates, expressed as percentages, for the five vine varieties studied. The rooting rate represents the proportion of cuttings that actually developed roots during the first experiment, providing crucial insights into each variety's propensity for vegetative propagation. Anonymous 3 exhibits the highest rooting success at 90 %, closely followed by Anonymous 5 at 80 %, indicating that these two varieties can be classified as easy- to-root. Their high rooting rates suggest a strong genetic predisposition for root initiation, making them valuable candidates for efficient propagation in viticultural practices. Anonymous 2 and 4 demonstrate moderate rooting capabilities at 70 % and 60 % respectively, placing them in an intermediate category. These varieties, while not as prolific as Anonymous 3 and 5, still show a reasonable capacity for root development. Indeed, according to Sabir and Sabir, (2018) all the cuttings have more than 60 % rooting rate which can be commercially acceptable. In stark contrast, Anonymous 1 displays a markedly low rooting rate of 30 %, categorizing it as difficult-to-root. This poor performance in root initiation suggests that Anonymous 1 may require specialized propagation techniques or treatments to overcome its inherent challenges in vegetative reproduction. Notably, Galavi et al., (2013) highlighted that the better performance of the hardwood cuttings could be due to the presence of auxin, which can promote mobilization of nutritional reserves to the region of root formation. The significant variability observed among these cultivars, ranging from 30 % to 90 %, underscores the substantial genetic differences in rooting ability within the studied grapevine varieties. Differences in rooting success attributable to genotype are another factor of interest (Buck et al., 2022).

Variety	Anonymous	Anonymous	Anonymous	Anonymous	Anonymous
Rooting rate $(\%)$	ΟU	v	90	60	80

Table 2. Rooting rate of vine cuttings (%)

3.2.2. Root length

Table (3) presents the average root length data for the five grapevine varieties, offering valuable insights into the vigor and quality of their root systems. Anonymous 3 demonstrates superior root development, producing the longest roots with an average length of 6.5 ± 0.4 cm. This exceptional root growth suggests that Anonymous 3 not only initiates roots easily but also exhibits strong root vigor, potentially leading to better nutrient absorption and anchorage. Following closely is Anonymous 5, with an average root length of 5.7 ± 0.3 cm, indicating another variety with robust root development. Anonymous 1 shows moderate root length at 5.2 \pm 0.3 cm, which is noteworthy given its low rooting rate, suggesting that while it struggles with root initiation, it can produce reasonably long roots when successful. Anonymous 4 and Anonymous 2 exhibit shorter root lengths at 4.3 ± 0.2 cm and 3.8 ± 0.1 cm respectively. indicating less vigorous root development. The considerable variation in root length among these varieties, ranging from 3.8 cm to 6.5 cm, highlights significant differences in their root growth potential. This variability could be attributed to genetic factors influencing root elongation and overall root system as reported by Köse et al., (2023). The longer roots observed in Anonymous 3 and 5 may confer advantages in water and nutrient uptake, potentially resulting in more robust and resilient plants. Conversely, the shorter root lengths in Anonymous 2 and 4 might indicate a need for more attentive care during the growth stages to ensure successful establishment.

Table 3. Average root length of rooted vine cuttings (cm)

Variety	Anonymous	Anonymous	Anonymous	Anonymous	Anonymous
Rooting length (cm)	5.2 ± 0.3	3.8 ± 0.1	6.5 ± 0.4	4.3 ± 0.2	5.7 ± 0.3

3.2.3. Acclimatization rate

Table (4) presents the acclimatization rates for the five grapevine varieties, offering crucial insights into their ability to adapt to new growing conditions after the rooting phase. This parameter is particularly important as it reflects the varieties' resilience and potential for successful establishment in field conditions. Grapevines adjust dynamically and acclimatize to controlled environmental factors, such as temperature, water stress and light incidence (Antolin et al., 2010; Salazar-Parra et al., 2012; Bettoni et al., 2015; Gallo et al., 2020; Rafique et al., 2023). Notably, a high rate of acclimatization indicates that the cuttings are successfully adjusting to the new conditions and continue to grow (Reinhart and Biasi, 2018). Similarly, in our case, Anonymous 3 continues to exhibit superior performance with the highest acclimatization rate of 78 %, demonstrating not only excellent rooting ability but also remarkable adaptability to environmental changes. This suggests that Anonymous 3 possesses robust stress tolerance mechanisms, making it a promising candidate for various cultivation scenarios. Anonymous 2 shows a surprisingly high acclimatization rate of 71 %, despite its moderate rooting performance, indicating strong resilience once rooted.

This disparity between rooting and acclimatization success in Anonymous 2 highlights the complexity of plant establishment processes and suggests that initial rooting vigor doesn't always predict subsequent adaptability.

Anonymous 4 and 5 both display moderate acclimatization rates of 50 %, indicating average stress tolerance and adaptability. Interestingly, Anonymous 5, which showed strong rooting performance, doesn't maintain the same level of superiority in acclimatization, suggesting potential sensitivity to environmental changes despite initial vigor. Anonymous 1 struggles the most with acclimatization, showing the lowest rate of 33 %, which aligns with its poor rooting performance. This consistently low performance across both rooting and acclimatization phases indicates that Anonymous 1 may require specialized care and optimized conditions throughout the propagation and establishment process. The significant variation in acclimatization rates among these varieties, ranging from 33 % to 78 %, underscores the importance of considering not only rooting ability but also post-rooting adaptability when selecting grapevine varieties for propagation and cultivation. These findings have important implications for nursery management and early-stage vineyard establishment, suggesting that tailored post-rooting care strategies may be necessary to maximize the survival and early growth of different grapevine varieties.

Table 4. Acclimatization rate of rooted vine cuttings (%)

Variety	Anonymous	Anonymous	Anonymous	Anonymous	Anonymous
Acclimatization rate $(\%)$	33		7c	50	50

3.2.4. Shoot growth

Table (5) and Figure (5) present the shoot growth rates of the five grapevine varieties over an eight-week period, providing valuable insights into their vegetative vigor and growth patterns. This data reveals distinct varietal differences in growth dynamics and overall shoot development as reported in several studies. Anonymous 5 consistently demonstrates superior shoot growth, reaching a maximum length of 17.0 ± 1.3 cm by week 8. This exceptional performance indicates robust vegetative vigor and suggests a strong capacity for rapid canopy development, which could be advantageous for early season growth and potentially earlier fruit production. Anonymous 1 and Anonymous 3 show similar growth trajectories, achieving final shoot lengths of 15.8 ± 1.2 cm and 15.2 ± 1.1 cm respectively. Their comparable performance, despite differing rooting and acclimatization rates, highlights the complex relationship between early propagation success and subsequent vegetative growth. Anonymous 2 exhibits moderate but steady growth, reaching 14.0 ± 1.1 cm by week 8, indicating a balanced growth pattern that may reflect good resource allocation between root and shoot development. Anonymous 4 consistently displays the slowest growth rate, attaining only 11.5 ± 1.0 cm by the end of the study period, suggesting it may be a naturally slower-growing variety or one that prioritizes root development over shoot growth in early stages. These results are superior to the ones showed by Patil et al., (2001) and inferior to those values found by Shah et al., (2021) with vine multiplication in different culture media. Notably, Warmund et al., (1986) suggest reduced shoot growth may have occurred due to the size of cuttings and late planting date. In the other hand, all varieties exhibit a sigmoidal growth curve, characterized by an initial lag phase (weeks 1-2), followed by a period of rapid, almost exponential growth (weeks 3-6), and concluding with a deceleration phase (weeks 7-8). This pattern is typical of many plant species and reflects the physiological changes occurring during growth stages (Cao et al., 2019). The timing and magnitude of these growth phases vary among the varieties, with Anonymous 5 showing the steepest acceleration and maintaining higher growth rates throughout, while Anonymous 4 demonstrates a more gradual increase and earlier deceleration. In contrast, slower-growing varieties like Anonymous 4 might benefit from practices that promote vegetative growth, such as adjusted fertilization or pruning strategies.

The final heights of Anonymous 1 and 3, despite their different early propagation performances, suggest that initial rooting and acclimatization success may not always predict long-term growth potential.

This comprehensive analysis of shoot growth dynamics provides crucial information for optimizing cultivation practices, it also underscores the importance of considering both early propagation success and subsequent growth patterns when selecting grapevine varieties for specific viticultural objectives or growing conditions.

Variety		Anonymous 1 Anonymous 2 Anonymous 3 Anonymous 4			Anonymous 5
Week					
	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.6 ± 0.2
2	1.2 ± 0.3	0.8 ± 0.2	1.0 ± 0.2	0.5 ± 0.2	1.5 ± 0.3
3	2.5 ± 0.4	1.7 ± 0.3	2.1 ± 0.3	1.1 ± 0.4	3.0 ± 0.5
$\overline{4}$	4.8 ± 0.6	3.5 ± 0.5	4.2 ± 0.5	2.4 ± 0.4	5.5 ± 0.7
5	8.0 ± 0.8	6.2 ± 0.7	7.3 ± 0.7	4.5 ± 0.6	9.0 ± 0.9
6	11.5 ± 1.0	9.5 ± 0.9	10.8 ± 0.9	7.2 ± 0.8	12.8 ± 1.1
7	14.2 ± 1.1	12.3 ± 1.0	13.6 ± 1.0	9.8 ± 0.9	15.5 ± 1.1
8	15.8 ± 1.2	14.0 ± 1.1	15.2 ± 1.1	11.5 ± 1.0	17.0 ± 1.3

Table 5. Growth rate of grape cuttings (cm/week) during the eight weeks of study

 Figure 5. Growth rate of grape cuttings (cm/week) during the eight weeks of study

4. Conclusion

Our study provides a comprehensive characterization of five autochthonous grapevine varieties from the Aures region of Algeria, revealing significant diversity in both morphological traits and physiological performance. The ampelographic analysis, utilizing principal component analysis and hierarchical clustering, effectively differentiated the varieties based on leaf morphology, with Anonymous 3, 5, and 1 showing similarities, while Anonymous 2 and 4 exhibited distinct characteristics. The physiological assessments revealed substantial varietal differences in rooting ability, acclimatization success, and early shoot growth.

Anonymous 3 consistently demonstrated superior performance across most parameters, suggesting its potential as an easily propagated and vigorous variety. In contrast, Anonymous 1 showed challenges in rooting and acclimatization, indicating a need for specialized propagation techniques. Interestingly, initial rooting success did not always predict subsequent growth performance, as evidenced by the variable relationships between rooting rates, acclimatization success, and shoot growth patterns among the varieties. This highlights the complex interplay of genetic and physiological factors influencing early grapevine development. These findings contribute valuable insights to the field of viticulture, offering a foundation for optimizing propagation protocols and early growth management strategies for these unique varieties. Furthermore, this study underscores the importance of characterizing and preserving autochthonous grapevine varieties, which may harbor valuable traits for future breeding programs and adaptation to changing environmental conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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