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Ampelometric and Ampelographic Characterization of Leaves of Indigenous "*Vitis vinifera* ssp. *Vinifera*" in the North of Morocco †

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Abstract: Morocco, with its Mediterranean climate and its various potentialities, conceals an important space for the extension of viticulture in particular that of traditional grape varieties, which has undergone profound upheavals linked to economic, social and environmental constraints, which have had a negative impact on genetic diversity. The present study aimed to evaluating the richness of the local phylogenetic heritage of the traditional vine in the North-West of Morocco. For this, we carried out a characterization of a collection of 1617 leaves, taken from 162 vine plants and belonging to 27 different traditional varieties. Thereafter, we use an ampelometric and ampelographic approach with the SUPER AMPELO software. The ANOVA test revealed the most discriminating parameters, that is the angles, the depth of the lateral sinuses in relation to the lengths of the ribs and the relationships between all the parameters. In this sense, the qualitative parameters (OIV Codes) confirmed the presence of morphological diversity within the grape varieties studied, the study of general averages made it possible to specify the varieties with the large values of distance/angles and of ratio and we have shown the presence of a great intra-varietal diversity in addition to that which is inter-varietal. The analysis in principal components allowed to grouping the grape varieties in 5 groups according to their expressions by the quantitative parameters and it confirmed the hypothesis of the influence of the external environment in addition to the gene pool on the grape varieties. This leads us to provide more efforts to maintain inter and intra-varietal variability and to fight against genetic erosion and the threat of changes environmental.

Keywords: *Vitis vinifera L.*; ampelographic and ampelometric description; traditional agroecosystems

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1. Introduction

For centuries, man has not stopped domesticating animal or plant species. The vine is one of his great successes, a wild liana which over time has undergone very significant development and adaptation at the genetic, physiological and organoleptic levels, as well as varietal diversification [1]. Nowadays, most cultivated varieties are all of the *Vitis vinifera* species, representing more than 10,000 grape varieties [1,2]. Thus, the products of the vine would occupy the first economic place at the world level [3], explaining its place among the most cultivated fruit trees in the world with 7,528 million ha of area and a grape production of 691 million quintals [4].

Over time, the vine has shown a great capacity to develop on rich soils but also on steeply sloping, rocky and poor soils. The vine has always occupied an important place in

the traditional Mediterranean landscape, by its presence in these two wild and cultivated forms [5]. The culture of this species is well rooted in the traditions of the Maghreb peasant populations in general and Moroccan in particular. However, the introduction of new wine and table grape varieties by globalization has generated a genetic erosion of the wine heritage [6–8]. This has led to a regression or even the disappearance of a large number of indigenous grape varieties that contain genes for adaptation and resistance to biotic and abiotic constraints.

Faced with the almost total ignorance of our wine heritage and the absence of a descriptive catalog of these grape varieties, it is imperative to seek to recognize the local phylogenetic heritage, given the phytogenetic wealth that the country conceals in this area. In order to identify the diversity of traditional grape varieties in the traditional agroecosystems of mountain of the Rif and their possible conservation, we characterized the traditional grape varieties according to different approaches (ampelographic and ampelometric) recommended by the OIV (International Organization of the Vine and Wine).

2. Experiments

2.1. Sampling:

The surveys took place in 13 traditional vineyards (Figure 1), located in the region of Tanger Tétouane Al-Hoceima. This is the northwest section of the Rif arc, which many authors refer to as "Jbala country". Due to the energetic aspect of its relief, this ensemble is characterized as much by the originality of its environment (mountain, Mediterranean and Atlantic) as by the specificity of its role in both the ancient and contemporary history of the Kingdom. In this region, there are mainly four homogeneous zones: The Tangérois; The coast and the Mediterranean basins; The Bas Loukkos basin; and the Jbala containing precisely the sampling sites [9].

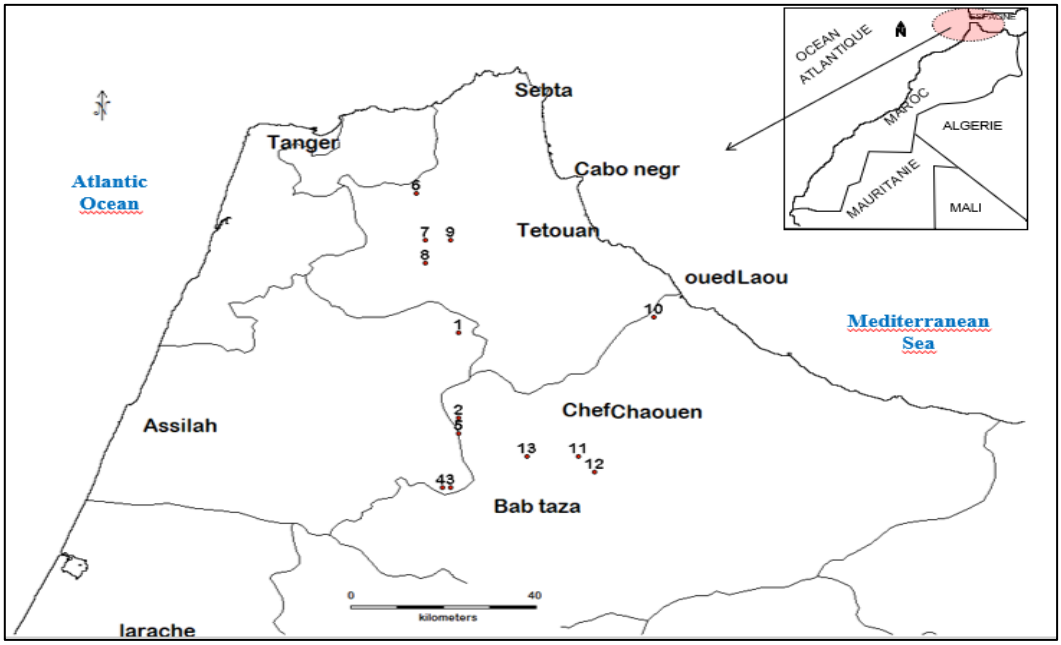

Figure 1. Location of the surveyed sites.

Sampling was carried out at the time of fruiting. In each prospected vineyard, with the help of the farmer, we collected samples of leaves for each named and recognized grape variety. Ainsi, pour chaque pied, nous avons récolté 10 à 20 feuilles situaient entre le 7eme et 12eme nœud, dénombrées à Thus, for each plant, we collected 10 to 20 leaves located between the 7th and 12th node, counted from the base of the primary branch according to the recommendations of the OIV [10].

2.2. In the Laboratory:

After drying and organizing the leaves harvested in a herbarium, they were scanned and the images obtained are processed by the Super Ampelo measurement software, which is a program designed to help catalog the genetic material of the vine by ampelographic and ampelometric characters according to the descriptors of the OIV [11].

3. Results

Although the area of distribution of the vine within the traditional agroecosystems of mountain of the Rif is very restrained, our study revealed a very important diversity made up of 27 traditional grape varieties: Ainab byed (AIB), Aineb Noir (AIN), Albaydi (ALB), Labyed(LBY),Alfasi (ALF), Alkouz (ALK),Babour Hmara (BABH), Boukhanzir (BKH), Boukwanes (BKW), Bezul laawda (BZA), Bezul laawda byed (BZAB), Bezul laawda hmer (BZAH),Chouikhi (CH), Douak (DAK), Eddibani (EDD), Feqqas (FKS), Hmar Bouaamar (HBA), Houmrani (HMR), Jenyani (JNY), Lechehab (LCH), Matecha (MTCH), Sbiyae Lebnat (SBLB), Taferyalt (TFR), Taferyalt byed (TFRB), Taferyalt Sfar (TFRJ), Taferyalt Lakhal (TFRN), Valenci (VAL).

3.1. Qualitative Characterization of the Adult Leaf

3.1.1. Shape of Blade

According to OIV 067, we distinguish between 5 groups of grape varieties (Figure 2): grape varieties with a pentagonal shape with a proportion of 83%, While 8% have a wedge shape, 6% with a circular leaf and only 1% which have heart-shaped leaves.

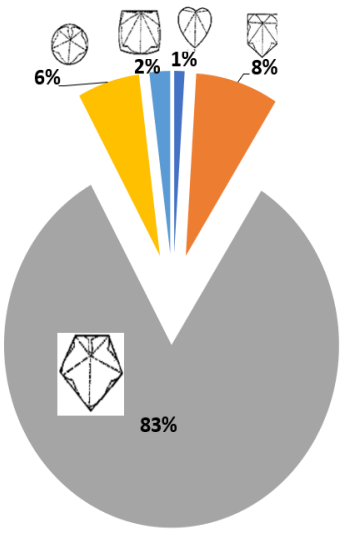

Figure 2. Shape of blade.

3.1.2. Number of Lobes

According to OIV 068, 96% of the grape varieties studied have leaves with five lobes, 2% (Figure 3).

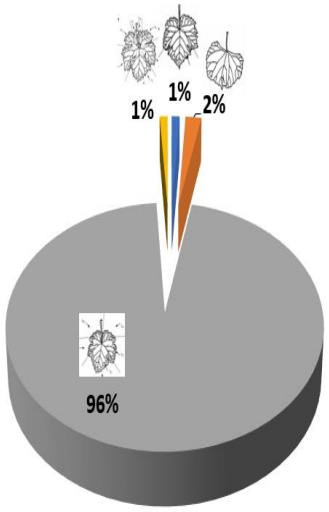

Figure 3. Number of lobes.

3.1.3. Shape of Teeth

According to OIV 076, we noticed that the majority of grape varieties have rectilinear toothed leaves observed on both sides (99%). However, only 1% who had two-sided convex teeth (Figure 4).

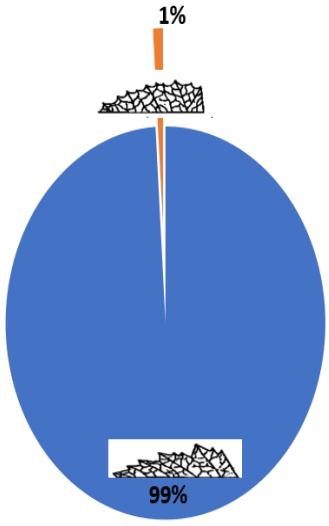

Figure 4. Shape of teeth.

3.1.4. Degree of Opening of Petiole Sinus

Concerning OIV-079, it was observed that on the sampled grape varieties, 57% presented leaves with a closed petiole sinus; while 31% have leaves with half overlapping sinuses and 12% have leaves with open petiolar sinuses (Figure 5).

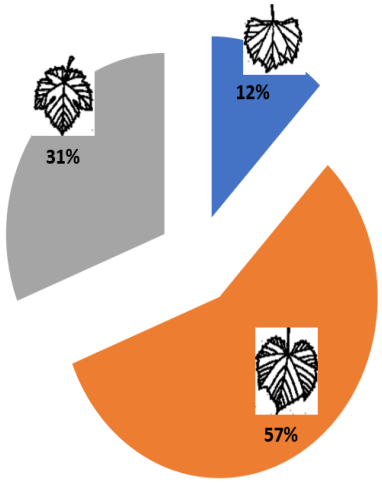

Figure 5. Degree of opening of petiole sinus.

3.1.5. Shape of Sinus

Taking into account the OIV-083-1, we noted the presence of two forms of sinus in the grape varieties studied with 96% of individuals having U-shaped petiole sinuses and 4% V-shaped (Figure 6).

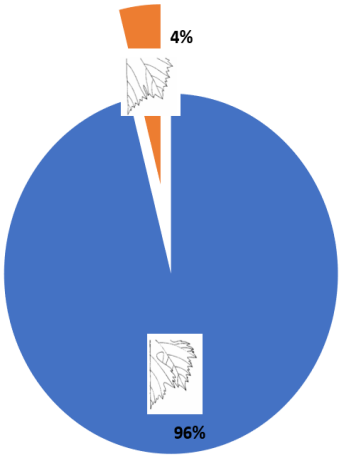

Figure 6. Shape of Sinus.

3.1.6. Teeth in the Petiole Sinus

Regarding OIV 081-1, it was noted that 94% of the grape varieties studied are characterized by the absence of teeth in the petiolar sinus against 6% having the particularity of the presence of teeth (Figure 7).

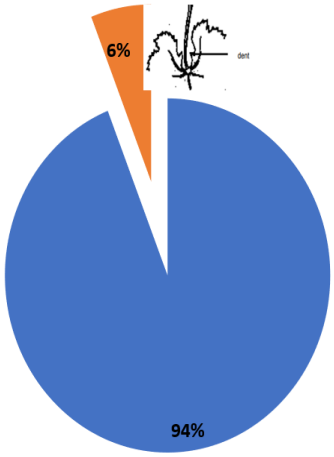

Figure 7. Teeth in the petiole *sinus*.

3.2. Quantitative Characterization of the Adult Leaf

The analysis of variance made it possible to specify the importance of the variability of the 86 ampelometric parameters of the leaves likely to differentiate the grape varieties. In this context, the analysis of variance, for the 73 most descriminants parameters out of 86 measured, showed a highly significant difference between the different grape varieties ($p < 0.0001$) and which is the result of a significant morphological diversity within and between the different individuals that make up these grape varieties. This difference is expressed more with very high F for the characteristics related to the size and the base of the teeth (HD, BD), shape of teeth (HBD1), petiole opening angle (PI) and surface (Area) (Table 1).

Table 1. ANOVA analysis of the various ampelometric measured parameters.

Variable	Fisher's F	Pr > F	Variable	Fisher's F	Pr > F	Variable	Fisher's F	Pr > F	Variable	Fisher's F	Pr > F
HD1	185.648	**<0.0001**	ON41	6.595	**<0.0001**	ON1	5.042	**<0.0001**	ANGA1	3.319	**<0.0001**
BD1	44.433	**<0.0001**	LUPIC	6.206	**<0.0001**	OI	4.973	**<0.0001**	HN21	3.25	**<0.0001**
BD	34.627	**<0.0001**	O41N51	6.069	**<0.0001**	BN4	4.97	**<0.0001**	LULA	3.162	**<0.0001**
HBD1	16.126	**<0.0001**	O31N41	5.933	**<0.0001**	OS1	4.969	**<0.0001**	HD	3.032	**<0.0001**
RS1	10.574	**<0.0001**	FN2	5.746	**<0.0001**	OI1	4.895	**<0.0001**	AL1	2.995	**<0.0001**
RI	9.696	**<0.0001**	ON21	5.71	**<0.0001**	ON3	4.813	**<0.0001**	GA	2.981	**<0.0001**
RS	9.316	**<0.0001**	O4N5	5.684	**<0.0001**	HN2	4.64	**<0.0001**	ALBEOSOI1	2.935	**<0.0001**
PI	9.061	**<0.0001**	BN41	5.615	**<0.0001**	ALBEGA	4.232	**<0.0001**	GA1	2.909	**<0.0001**
BN2	8.962	**<0.0001**	BN21	5.574	**<0.0001**	ALBE	4.131	**<0.0001**	OO3	2.815	**<0.0001**
Area	8.043	**<0.0001**	ON2	5.463	**<0.0001**	TA	4.066	**<0.0001**	OO31	2.779	**<0.0001**
MU	7.622	**<0.0001**	O3N4	5.344	**<0.0001**	ALBEOSOI	4.041	**<0.0001**	ALBEGA1	2.707	**<0.0001**
RI1	7.612	**<0.0001**	HBD	5.262	**<0.0001**	SPSP1	3.942	**<0.0001**	OM	2.53	**<0.0001**
LAM	7.578	**<0.0001**	N4N41	5.242	**<0.0001**	ET	3.887	**<0.0001**	OM1	2.409	**<0.0001**
LUXLA	7.424	**<0.0001**	ON4	5.19	**<0.0001**	ANGA	3.878	**<0.0001**	BE	2.38	**<0.0001**
N2N21	7.393	**<0.0001**	ON31	5.171	**<0.0001**	TA1	3.826	**<0.0001**	ALBE1	2.121	**<0.0001**
LA	7.092	**<0.0001**	OS	5.124	**<0.0001**	AL	3.715	**<0.0001**	DE	2.059	**<0.0001**
LU	6.886	**<0.0001**	N3N31	5.067	**<0.0001**	HBN21	3.701	**<0.0001**	HBN4	1.659	**0.001**
OP	6.79	**<0.0001**	FN21	5.047	**<0.0001**	ET1	3.455	**<0.0001**	HBN2	1.503	**0.009**
BE1	1.336	0.047	R51	1.063	0.35	R5	0.965	0.553	R31	0.899	0.691
R21	1.087	0.305	R41	0.98	0.519	R2	0.909	0.671	RP	0.895	0.7
R4	0.895	0.699	R3	0.876	0.737	HN4	0.832	0.815	HN41	0.82	0.833
HBN41	0.65	0.982									

4. Discussion

To get an idea of the structure of the variation in the various parameters measured, a principal component analysis was performed on a matrix encompassing the 27 traditional grape varieties, taking into account the 73 variables considered in the study (Figure 8).

Most of the variance expressed by axis 1 is explained by the correlation with the variables related to the following distances; leaf width (LA), leaf length (LU), length of veins and ribs (N1, N2, N3, N4 and N5), distance between vein tips (N2-N21, N3-N31 and N4-N41), leaf length including petiole (LUPIC), surface (Area) and petiole length (OP). Axis 2; as for him, it is correlated with the variables related to the following angles: angle between I and I '(MU), angle between S and S1 (LAM), angle between N2 and N2' (DE), angle between N1 and N2 (OM), angle between N2 and N3 (ET), angle between N3 and N4 (TA), the sum of the angles AL + BE (ALBE) and the sum of the angles AL1 and BE1 (ALBE1). This axis is also correlated with the ratio between the sum of the angles AL + BE and between the sum of the petiolar sinuses OS and OI (ALBEOSOI).

Considering the number of grape varieties studied, we carried out two projections; one made up solely of "Taferyalt", grape varieties which is more representative of the sites; and a second projection, encompassing the rest of grape varieties without "Taferyalt".

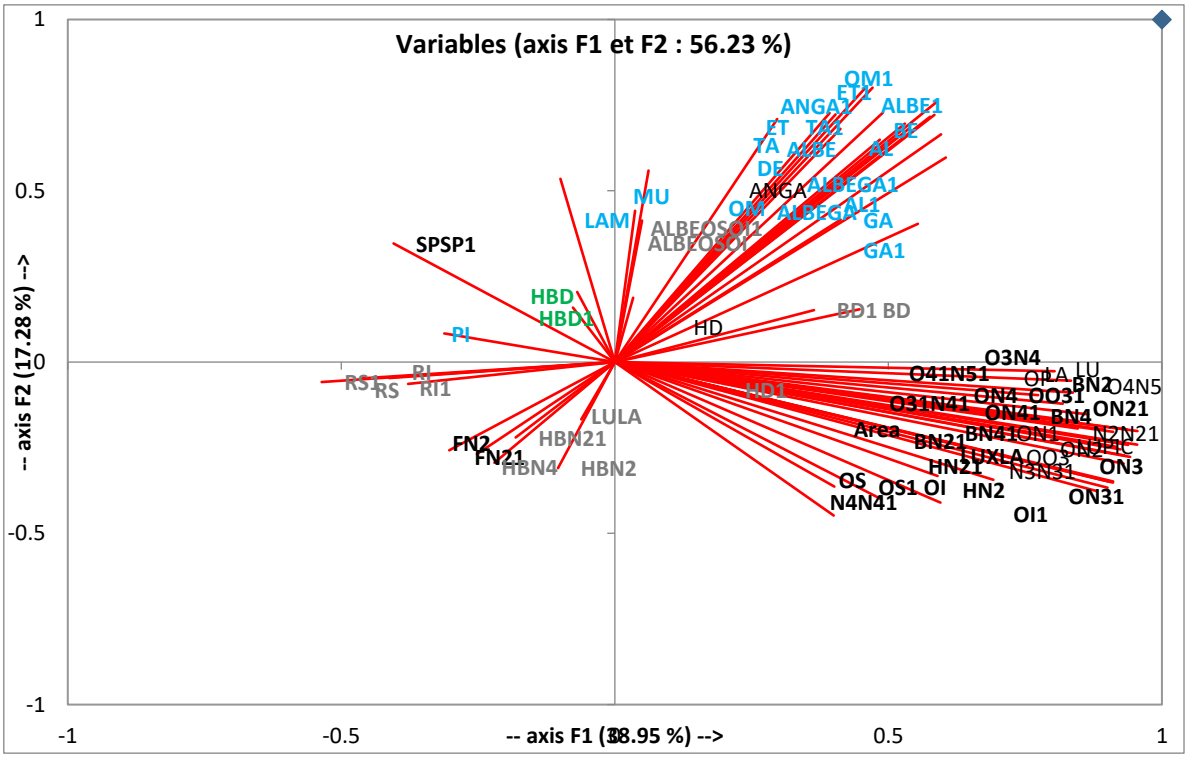

Figure 8. Projection of the variables on the plan [1,2].

The PCA grouping together all varieties with the exception of "Tafaryalet" (Figure 9), showed the existence of three categories depending on the variation of distances and angles: **Category 1;** constitutes the pole of the positive side of axis 1, which groups LBY (100%), VAL (100%), MATCH (100%), BZAB (100%), then CH (75%), FKS (60%), ALB (50%), BZA (50%); this category is distinguished by large corner sheets. **Category 2;** constitutes the intermediate pole which includes HBA (100%), SBLB (100%), BKH (100%), ALF (100%), JNY (100%), DAW (100%); AIB (100%), LCH (100%), ALK (100%), FKS (40%), BZA (50%), ALB (50%), EDD (40%) and CH (25%), this category is distinguished by medium sized corner sheets. **Category 3;** constitutes the pole of the negative side of axis 1

which includes AIN (100%), BABH (100%), BZAH (100%), BKW (100%), HMR (100%) and EDD (60%) and this category is distinguished by sheets of angles of small size.

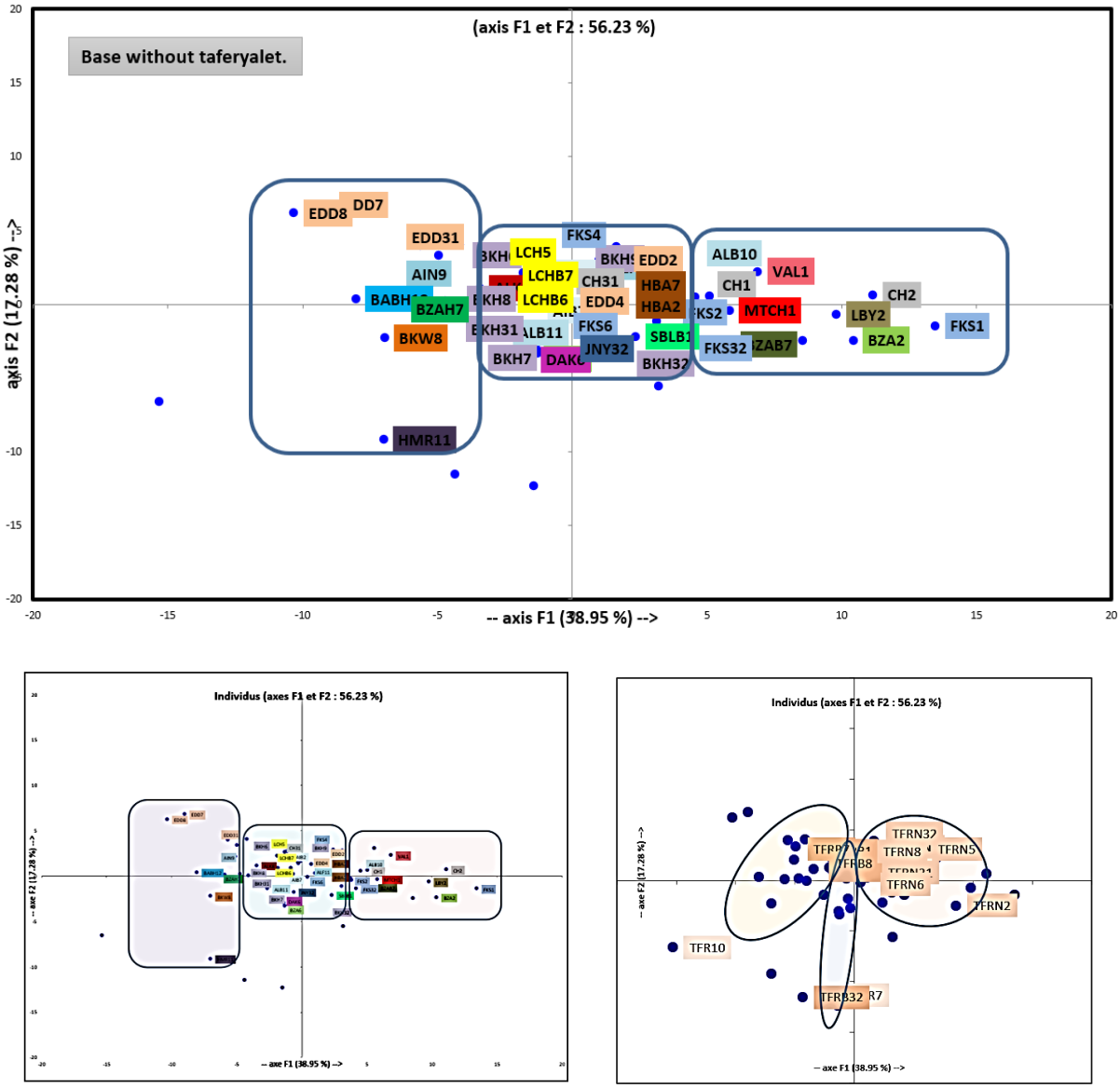

Figure 9. Projection of grape varieties without Taferyalet.

However, the FKS, BZA, ALB and CH grape varieties are divided between categories 1 and 2 and the EDD grape variety between categories 2 and 3.

Regarding the second projection of the ACP with the "Tafaryalet" grape varieties (Figure 10), we also made a distinction between 3 categories: **Category 1;** constitutes the pole of the positive side of axis 1, by grouping TFRN (100%) and TFRJ (100%), **Category 2;** constitutes the intermediate division regrouping TFR (100%) and **Category 3;** constituting the pole of the negative side of axis 1 comprising TFRB (100%).

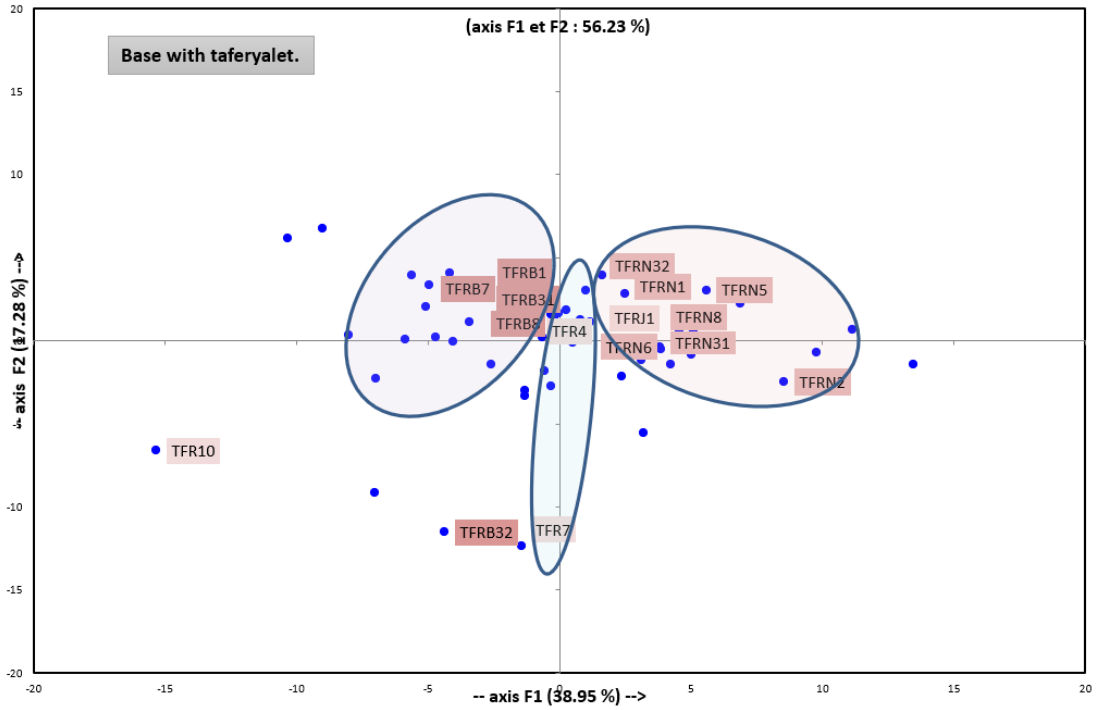

Figure 10. Projection of grape varieties with Taferyalet.

Although the study was based only on the morphological characters of the leaf, a significant intra and inter-grape variability has been demonstrated. The interest and importance of this variability are particularly interesting insofar as they have been demonstrated over a small and relatively delimited territory.

5. Conclusions

This study constitutes a contribution to the characterization of the traditional grape varieties of the vine in the traditional agroecosystems of the Rif mountain using the ampelographic and ampelometric parameters. These approaches are complementary and allow a better understanding of the diversity of local varieties of the vine. This study allowed us to collect preliminary data concerning the phenotypic characteristics of traditional grape varieties.

From the ampelographic approach, it emerges that the majority of the quantitative and qualitative parameters used demonstrate the existence of intra- and inter-grape variability within the individuals studied. As a result, it remains the main and essential tool for identifying grape varieties [12], but the morphological characters are influenced by environmental factors, such as soil properties [13], water availability and salinity [14], the nature of the rootstock [15], the level of nutrition [16] and the health of the plant [17], without forgetting the problem of synonymy and homonymy, especially in the case of vernacular names, may differ for the same variety depending on the region of sampling. For this, it is of major importance to complete the present study by a molecular characterization.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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Conflicts of Interest: The authors declare no conflict of interest.

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