Use phyllometric parameters to discriminate the Moroccan Native *vitis vinifera* cultivars

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Abstract—The aims of this study is to use the phyllometric parameter to identify the autochthonous cultivars in north east of Morocco. Eighty six phyllometric parameters were measured/calculated on 585 leaf samples from thirty nine grapevine cultivars, gathered during several years, from different traditional vineyard in Northwestern of Morocco. The leaves were scanned and images were analyzed using SuperAmpelo. Principal component analysis was performed to discriminate different accessions of cultivars. The results show a heterogeneous distribution of different accessions. This can be explained by the existence of the problem of synonymy and the homonyms frequently encountered in local varieties. In this study we tried to value these native cultivars by using the phyllometric approach. These varieties could be a valuable tool for improving local economies.

**Keyword**—Phyllometry, Vitis vinifera, Morocco, discriminant analysis, cultivar identification.

I. INTRODUCTION

The grapevine is one of the oldest known fruit species in the world and therefore researchers have always wanted to learn more [1], [2]. It is important to mention that it has an economic importance, with a total area of 7.726 mha and global production up to 750 mql over the world (http://www.oiv.int/en/databases-and-statistics/statistics).

According to [3], the origin of the genus *Vitis* is located in Eurasia and then spread to the rest of the world. In North Africa Viticulture became consolidated under Roman influence [4], [5]. The grapevine has always occupied an important place in the traditional Mediterranean landscape, with its presence under these two wild and cultivated forms [6]. The culture of grapevine is well rooted in the traditions of the Maghreb populations in general and Moroccan in particular. In Morocco, according to the statistics of the Ministry of Agriculture, the culture of grapevine has an area of 33 787 ha with a production of 346656 T of the grape table in 2017/2018. Study of plant morphology, mainly leaves, buds, and cluster morphology (also called ampelography) until it is the last means of detecting vine cultivars [7]. This method is still used for identification [8], [9], [10] particularly during the collection of data plants in situ.

Many ampelographic studies have been made of grapevine cultivars from all over the world, but only a few have described those of Algeria and Morocco [11], [12], [13], [14]. Recently, many of the Maghrebi cultivars have now been profiled by nuclear and chloroplast microsatellite analysis [15], [16], [17], [18], [19]. Ampelometric methods can also be a useful tool in cultivar identification, as they are less expensive, do not require special equipment and can give relevant results if managed properly and the resulting data are analyzed appropriately. Leaf descriptors have generally been used as effective tools for characterization of grapevine cultivars [20], [21], [22], [23], [24], [25], [26], [27]. For these reasons, they can still be the method of choice, especially for research groups without the resources or knowledge required for molecular analyses. Computer-aided survey systems for phyllometry, such as SUPERAMPELO [28] and GRA.LE.D [29] and some others have been developed as solutions for the time-consuming limitations of phyllometric research. The aim of this research is the use the phyllometric parameters for valorization of autochthonous cultivars, which could represent a valuable instrument for improving the local economies.

II. MATERIAL AND METHODS

**Plant material**

The plant materials consisted of samples prospected in Northwestern of Morocco (Fig 1 and Table 1), all of them were classified as minor or endangered varieties. The total number of accessions studied was 39 (Table 1). Each accession consisted of five replications.
Fig. 1 Location of the stations prospected on the map

Table 1: Geographical coordinates of sampling sites

| Station     | Locality                      | Longitude (N) | Latitude (W) | Altitude (AL) | Varieties                                                                 |
|-------------|-------------------------------|---------------|--------------|----------------|---------------------------------------------------------------------------|
| EL Aachayich|                               | 3512377       | 00519568     | 359 m          | Echabel; Ineb Byad; Mouska; Bezoul El Awda; Maticha; Taferyalt khal; Taferyalt Byad; Sbiyae Bnat; Fekass                  |
| Bni Hamdillah|                              | 3502009       | 00519870     | 303 m          | Ineb Byad; Dibani; Ineb Nhal; Fekas Khal; Aferyal Byad; Aferyal Khal; Fekas Byad; Dibi                              |
| Mokrissat   |                               | 3554627       | 00520687     | 562 m          | Dibani; Sanso; Boukhanzir; Taferyalt Khal                                 |
| Zoumi       |                               | 3449690       | 00516783     | 648 m          | Bezoul El Awda; Boukhanzir; Rjiyil Dib; Zbarjel; Taferyalt Khal            |

Based on the phenological stages of [30], sampling of adult leaves was done between fruit set and veraison. We selected 15 leaves per varietals. Several studies show that this is a sufficiently large and representative number of samples [31], [32], [33], [34], [35], [36]. The leaves selected were between the 7th and 12th nodes, counted from the base of the primary branch following the recommendations of the [35]. The leaves were dried in herbarium. Indeed, the measurements were made on the sheets scanned using Super Ampelo software. This software measures different quantitative characteristics of the sheet and also calculates different parameters such as distances, angles, ratios, and descriptors of the OIV (International Office of Vine and Wine) (Figure 2). The software provides a total of 125 numeric values for each sheet. But only 86 characters were used, eliminating redundancy between characters. Principal Component Analysis (A.C.P) was done using SPSS Version 10 software.
III. RESULTS AND DISCUSSION

Averages of the distances between remarkable points

We calculated the average for the leaf parameters of the different trees from the automatic measurements performed by the SuperAmpelo software. Originally, the software measures 86 parameters for each sheet. This total number seems to be high compared to previous studies on grapevine cultivars as [37] (43 descriptors); [38] (50 descriptors); [39] (61 descriptors) and [40] (71 descriptors).

The averages per tree and per variety were calculated. Based on the measurements made using the SuperAmpelo software, the variables relating to the distances between remarkable points of the sheet were 31 variables. The averages obtained show significant variations not only between trees of different varieties but also between trees of the same variety. For example, the parameter OP (petiole length) showed in the tree Taferyalt Kahla 8 the highest value compared with Taferyalt Kahla 5 and Feryal khal 2 which showed a lower value in trees of this variety.

The Fekas tree showed a value of the lowest petiole length among all trees. The Feryal Khal 2 tree showed the highest values of OS distances (Petiolar sinus distance at the upper right chest) and OS1 (Petiolar sinus distance at left chest) and BN2 (tooth base located at the end of N2) and BN21 (Base of the tooth at the end of N2 '). View the symmetry of the leaf of the vine one can think that certain distances like (OS, OS1, OI1, OI, OI1, HN2, HN21, HN4, HN41, BN2, BN21, O4N5 and O41N51) will bring the same information. Thus, the parameters OS, OI, HN2, HN4, BN2, O03 and O4N5 on the right side would be respectively symmetrical with the parameters OS1, OI1, HN21, HN41, BN21, O031 and O41N51 on the left side. However, we note that the values on the right side are different from the values on the left side.

Mean angles

We calculated the average values of the angles for the leaves of the different trees according to the SuperAmpelo software data. The number of angle variables measured is very important, 20 variables. The averages obtained show significant variations not only between trees of different varieties but also between trees of the same variety. Thus, for example, the parameter AL (Angle (α) between N1 and N2 measured at the first bifurcation), it has shown that the value of the highest angle (α) is observed in the tree Taferyalt Khal 3 and on the contrary, the Taferyalt Kahla 8 tree shows the lowest value in this variety.

The Ineb Byad1 tree recorded the largest angle value (α) of all varieties, whereas the Zharjel tree showed the lowest angle value (α). The sum of the angles showed a slight difference between the trees. Like the distances, the parameters of the angles also have their symmetrical parameters. This is the case of angles (α', β', ɤ' and ɛ'). The study of the characters relating to the angles (α', β', ɤ' and ɛ') shows a wider opening in Aferyal Byad2 with 75.96°. For the angle (β'), the maximum is observed in the Maticha mferqa tree with 20.73° followed by Aferyal Byad2 with 17.74° and Ineb Nhal with 17.62°. As for the angle (ɤ'), its maximum is reached at Bezoul El awda1 with 62.089°. However, the minimum value of the variable (ɤ') varies between 40.91° for Boukhanzir1 and 44.562° for Boukhanzir3. We obtained an angle π (angle of opening of the petiole measured between SP and SP ') very open in trees Bezoul El awda2 and Zharjel with respectively 88.56° and 82.8° which reflects on the degree of opening of the petiolar sinus. The low values are those obtained by Ineb byad1 with 16.05° and Aferyal Byad2 with 17.58°.

Averages of the ratios between variables
We calculated the average ratios for the leaves of different trees. The ratio variables were calculated automatically by the software used. The number of ratios calculated is very important, 16 ratios. The averages obtained show significant variations not only between trees of different varieties but also between trees of the same variety. The parameter ALBEOSOI (The ratio of the sum of the angles AL + BE and the sum of the distance between the sinuses of the petiole and OS at the top right and the sinus of the petiole and bottom right OI) showed the same value (0.08) in 11 trees. Likewise for parameter ALBEOSOI1 (Ratio between the sum of the angles AL1 + BE1 and the sum of the distance between the sinuses of the petiole and OS1 upper left and the sinus of the petiole and lower left OI1) which shows a value of 0.05 at 18 trees and usually the values of the ratios are closer to each other.

Principal component analysis

We conducted a principal component analysis on the 39 trees surveyed. The result is shown in Figure 3. From the figure we see a very heterogeneous distribution of accessions. Some accessions of the same variety are grouped together, as is the case for some accessions of the Taferyalt variety. However the other accessions are not grouped around the same variety. This result is explained by the existence of the problem of synonymy and homonymies frequently encountered in local varieties. Synonyms, homonyms, similar names, and possible sampling and / or errors collection, proper identification can be complicated.

The leaves of the vine have five main veins that start from the petiolar point. The relative dimensions of the ribs relative to each other and the angles that separate them are at the origin of a number of elementary forms of limbs: wedge-shaped, cordate, pentagonal, circular and reniform. But the enormous variability of other traits such as lobes, teeth, petiole sinus, hairiness, pigmentation, makes the leaves the organs of choice for variety differentiation [41].

In a similar study [42], on the analysis of the efficacy of discrimination of certain phyllometric parameters of indigenous V. vinifera cultivars in Croatia and with a similar methodological approach, they obtained results which go in the same direction as those we got. According to [43], the size of the leaf reflects the vigor of the plant. Among the feet studied, the foot Ineb byad1 has the longest leaf with a value of 62.43 cm followed by Fekas khal and Mouska with respectively 58.77 and 58.58 cm. The rest of the feet express intermediate values.

Ampelography remains the main and unavoidable tool for the identification of grape varieties [44], but the morphological characters are influenced by environmental factors, such as soil properties [45], water availability and salinity [46], the nature of the rootstock, the level of nutrition [47] and the health status of the plant [48]. Thus,
it must be remembered that this method of morphological characterization is not sufficient but must be completed by molecular characterization.

IV. CONCLUSION
This study represents a model for the analysis of discriminant of certain phyllometric parameters for the discrimination of native *Vitis vinifera* cultivars. The result of the Principal component analysis has been satisfying for some accessions that group together author of the same variety. The use of morphological parameters remains the first step in cultivar characterization. But we need to be further elucidated by studying the Moroccan grapevine with molecular methods.

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