MULTIVARIATE ANALYSIS AND CLUSTERING REVEAL HIGH MORPHOLOGICAL DIVERSITY IN TUNISIAN AUTOCHTHONOUS GRAPES (*Vitis vinifera*) : INSIGHTS INTO CHARACTERIZATION, CONSERVATION AND COMMERCIALIZATION

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Abstract

Aim : To characterize autochthonous grapevine cultivars from a national germplasm collection, to estimate the phenotypic diversity among and within the cultivars, and to identify the traits that contributed to cultivar heterogeneity.

Methods and results : Seventy major ampelographic descriptors comprising shoot, leaf and fruit traits were investigated to determine the overall degree of polymorphism among 61 autochthonous Tunisian grapevine genotypes. Based on the correlation values obtained between the characters, all descriptors must be considered for the characterization/clustering of the grapes, of which 12 descriptors were identified as the most important. Based on principal component analysis (PCA) and hierarchical cluster analysis (HCA), all cultivars were discriminated and high morphological variation was observed among the accessions. ANOVA demonstrated that most of the morphological variation was found within (89.31 %) rather than between the groups (10.69 %). The Khalt Bouchemma Gabès, Blanc 3 and Blanc 2 genotypes were identified as the barycentres of the groups, representing all the morphological variation observed within autochthonous grapes in Tunisia. These genotypes exhibited all the required characteristics to be introduced into the market and commercialized as table grapes and stand out as the most promising for commercial cultivation.

Conclusion : The detailed ampelographic description presented herein highlighted clear morphological differentiation between Tunisian autochthonous grapevines, investigated for the first time using 70 OIV descriptors, and allowed us for the first time to easily split the Tunisian autochthonous grapevine accessions into wine and table grapes. Numerical analyses showed that the number of morphological traits that are effectively contributing to the characterization of the cultivars could be reduced to 12.

Significance and impact of the study : In this investigation, we highlight the importance of importance of breeding programs, commercialization and evaluation of economically valuable characteristics of the highly diverse autochthonous grapevine cultivars from Tunisia.

Key words : *Vitis vinifera* L., OIV morphological descriptors, principal component analysis, analysis of variance (ANOVA), commercialization

Résumé

Objectif : Caractériser les cépages de vignes autochtones à partir d'une collection nationale, estimer la diversité phénotypique entre les cultivars et identifier les caractères morphologiques qui ont contribué à l'hétérogénéité des cultivars.

Méthodes et résultats : Soixante-dix descripteurs ampélographiques majeurs de caractères des pousses, des feuilles et des fruits ont été utilisés afin de déterminer le degré de polymorphisme chez 61 génotypes de vignes autochtones tunisiennes. En se basant sur les valeurs de corrélation entre les caractères utilisés, tous les descripteurs doivent être intégrés dans la caractérisation des vignes autochtones, dont 12 parmi eux ont été identifiés comme les plus importants. Sur la base de l'analyse des composantes principales (ACP) et de la classification ascendante hiérarchique (CAH), tous les cultivars ont été discriminés et une forte variation morphologique a été observée entre les accessions. L'étude ANOVA montre que la variation morphologique intra-groupe (89,31 %) était plus élevée que celle d'inter-groupes (10,69 %). Les génotypes Khalt Bouchemma Gabès, Blanc 3 et Blanc 2 ont été identifiés comme les barycentres des groupes soutenant toute la variation morphologique au sein de raisins autochtones en Tunisie. Ces génotypes présentaient toutes les caractéristiques nécessaires leur permettant d'être introduits sur le marché et commercialisés comme raisin de table et se distinguent comme les plus prometteurs pour la commercialisation.

Conclusion : La description ampélographique détaillée présentée ici pour la première fois en utilisant 70 descripteurs OIV a mis en évidence la différenciation morphologique claire entre les vignes autochtones tunisiennes et a permis pour la première fois de distinguer les vignes de table et les vignes de cuve. Des analyses numériques ont montré que le nombre de descripteurs morphologiques qui contribuent efficacement à la caractérisation des cultivars pourrait être réduit à 12.

Signification et impact de l'étude : Nous avons mis en évidence l'importance de la sélection clonale, de la conservation et de l'évaluation des caractéristiques à valeur économique des divers cépages de vignes autochtones de la Tunisie.

Mots clés : *Vitis vinifera* L., descripteurs morphologiques OIV, analyse des composantes principales, analyse de la variance (ANOVA), commercialisation

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INTRODUCTION

In Tunisia, viticulture is very ancient and the first historical record of grapevines dates back to 6000 BC (Zohary and Hopf, 2000). Autochthonous grapes are often grown by poor farmers in marginal, low-input and drought-stressed environments. These genetic resources, which may represent valuable reservoirs of interesting genes for crop improvement such as adaptation to biotic and abiotic stresses (Brush, 1995), represent a small population with a high risk of extinction due to the introduction of commercial high-yield foreign varieties (Hjalmarsson and Ortiz, 2000). All these factors contribute to the need for a detailed description and evaluation of the Tunisian grapevine genetic resources.

Initial efforts to identify genetic diversity in Tunisian grapevine cultivars were mainly based on molecular tools (Zoghlami *et al.*, 2001 ; 2009). Despite the use of molecular markers, knowledge of the phenotype given by morphological and agronomical descriptors is still important for breeding programs, conservation and commercialization of new varieties (Franco *et al.*, 2005 ; Gonçalves *et al.*, 2008 ; Laurentin, 2009).

The description of the morphological characteristics is the usual methodology accepted from a legal point of view for patenting and registration of varieties (Badenes, 1991). In fact, ampelography is the first step in grapevine identification and selection and for resolving different classification problems (Martinez de Toda and Sancha, 1997). The complete characterization as well as the conservation of autochthonous cultivars is of great importance to prevent the loss of diversity (Rodrigues *et al.*, 2008).

In the last years, morphological data have been used to resolve the complex problem of the definition and classification of crop accessions using multivariate statistical analyses such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Manjunatha

Table 1 - List of the 61 Tunisian autochthonous grapevine accessions from the collection of the CBBC[a].

No.	Cultivars	Origin	No.	Cultivars	Origin
1	Asli Hadab	Rafraf	32	BKB Gabes	Hencha
2	Asli Dar Slimane	Rafraf	33	Djebbi	Hencha
3	Châaraoui	Djebba	34	Kahli Sfax	Baddar
4	Hencha H1	Rafraf	35	Musc d'Alexandrie	Kerkennah
5	Khamri Tozeur	Rafraf	36	Khédhiri 1	Baddar
6	Sakasly Baddar	Rafraf	37	Arich Dressé	Mornag
7	Muscat Rafraf	Déguache	38	BKB Sfax	Baddar
8	Farrani	Djebba	39	Khalt s1	Kerkennah
9	Bidh el Hamem Sfax	Tozeur	40	Sakasly Djerba	Rafraf
10	Bahbahi Djebba	Balta	41	Tounsi Djerba	Kerkennah
11	Chaouche Djerba	Tozeur	42	Khédhiri 2	Balta
12	Hamri Kerkennah	Rafraf	43	Arich Djerba	Rafraf
13	Marsaoui	Tozeur	44	Bezzoul el Khadem Rafraf	Kerkennah
14	Saouadi S4	Djerba	45	Khédhiri 3	Kerkennah
15	Amokrane	Djerba	46	Khalt Abiadh	Mornag
16	Beldi Baddar	Balta	47	Razaki Rafraf	Baddar
17	Kahli Kerkennah	Baddar	48	Turky	Rafraf
18	Medina	Rafraf	49	Beldi Local Rafraf	Djebba
19	Sfaxi s2	Djerba	50	Bidh el Hamem Baddar	Nafta
20	Blanc 1	Baddar	51	Guelb Sardouk s3	Nafta
21	Arbia	Djerba	52	Khalt Bouchemma Gabès	Nafta
22	Beldi Rafraf	Balta	53	Razegui	Djebba
23	Jerbi Déguache	Gabes	54	El Biodh	Baddar
24	Mahdaoui	Mornag	55	Bidh el Hamem Rafraf	Djebba
25	Blanc 2	Baddar	56	Khalt Mdaouar	Nafta
26	Beldi Sayeb	Gabes	57	Balta 1	Sfax
27	Dattier de Beyrout	Baddar	58	Balta 4	Sfax
28	Hencha H2	Djebba	59	Balta 2	Djebba
29	Meski local	Mornag	60	Akhal Mguergueb	Sfax
30	Blanc 3	Gabes	61	Balta 3	Djebba
31	Arich Ahmar	Gabes			

[a]Centre of Biotechnology of Borj Cédria, Tunisia

et al., 2007; Aghaei *et al.*, 2008). These methods assist in the successful management of plant genetic resources and contribute to the determination of the agronomical value of the accessions in the germplasm collection (bank).

Morphological characterization continues to be the first step for the description and classification of germplasm accessions and statistical methods like PCA are useful tools for screening the accessions of a collection (Badenes *et al.*, 2000). PCA transforms the original variables into a limited number of uncorrelated new variables. As reported by Martínez-Calvo *et al.* (2008), this method allows the visualization of differences among individuals, the identification of groups, and the identification of relationships among individuals and variables.

Keeping in mind the importance of morphological characteristics in varietal identification and registration, the characterization of the worldwide *Vitis* materials has been homogenized by the use of standardized OIV/IPGRI descriptors (Soylemezoglu *et al.*, 2001; Santiago *et al.*, 2007). These descriptors allow the inventory of the grapevine genetic resources with passport, primary and secondary descriptors, bibliography and photos.

In this study we provide, for the first time, an inventory of the Tunisian autochthonous grapevine genetic resources using 70 major OIV descriptors (OIV, 2007). By applying a multivariate analysis to these data, we characterized the autochthonous grapevine cultivars from the Tunisian national germplasm collection, estimated the phenotypic diversity among and within the cultivars, and identified the traits that contributed to cultivar diversity.

MATERIALS AND METHODS

1. Plant material

Sixty-one Tunisian autochthonous grapevine genotypes were included in this study (Table 1). They were collected from different parts of the country and are kept at the repository of the Centre of Biotechnology of Borj Cédria, Tunisia (CBBC).

2. Data collection

Seventy major morphological traits, selected from the OIV list (OIV, 2007) and comprising shoot (12 descriptors), leaf (39 descriptors) and fruit (19 descriptors) descriptors, were measured and used to design a numbered-data matrix (Table 2). Ten specimens per accession were individually evaluated for their morphological diversity across the 70 descriptors. Measurements were performed by the same two persons to avoid errors due to individual variation.

3. Statistical analysis based on morphological diversity using shoot, leaf and fruit descriptors

Morphological data were analyzed by multivariate analysis, clustering and ANOVA analysis using XLSTAT software (Addinsoft, www.xlstat.com); PCA was performed to identify accession groups and to determine the axes and the characters significantly contributing to the variation. In this procedure, the similarity matrix was used to generate eigenvalues and scores for the accessions. The first two principal components, which accounted for the highest variation, were then used to plot two-dimensional scatter plots. HCA was carried out using Ward's minimum variance method as a clustering algorithm (Williams, 1976) and squared Euclidean distances as a measure of dissimilarity (Ward, 1963).

Among the groups identified based on different classifications (shoot, leaf and fruit), we determined the cultivars corresponding to the barycentre, which is a geometrical measurement allowing the concentration of a set of cultivars onto one that best expresses the inherent morphological diversity per variation class.

4. Evidence for integrating shoot, leaf and fruit descriptors

Regression analysis was applied to set up evidence for integrating shoot, leaf and fruit descriptors in distinguishing between autochthonous grape cultivars. Thus, the correlation between Euclidean distances calculated from shoot-leaf, shoot-berry and leaf-berry descriptors was determined from the linear regression plot (Rousset, 1997). A positive correlation is expressed by a positive R^2 value. The significance of this correlation was tested by a Mantel test using 10000 permutations (Mantel and Valand, 1970).

5. Perspectives for the commercialization of Tunisian autochthonous cultivars: table or wine cultivars?

To assess the vocation of the Tunisian grapevine accessions as table or wine cultivars, the following characters were determined according to the OIV descriptor list (Table 2): bunch weight, 100-berry weight, and sugar content, pH and acidity of the must.

RESULTS

1. Morphological diversity based on individual characteristics

1.1 Shoot characteristics

Twelve descriptors were used for the characterization of the shoots. The discrimination between the accessions under investigation revealed that 53.76 % of the variation (Table 3) was explained by the first three axes of the PCA plot (Figure 1a): the first axis was defined by the distribution and the intensity of the anthocyanin coloration on the bud scales (OIV 015 and OIV 015-2, respectively). These two descriptors were strongly correlated (0.940). The second axis was defined by both the anthocyanin coloration of the tip (OIV 003) and the form of the tip (OIV 001).

Based on shoot descriptors, the accessions clustered into three main groups (C1, C2 and C3) as revealed in the dendrogram (Figure 1b). Group C1 was the largest and comprised the 31 accessions with the highest density of prostrate hairs on the tip (OIV 004), as inferred from the morphological data matrix. Group C2 contained the 16 accessions with the highest distribution and intensity of

Table 2 - Ampelographic characteristics based on OIV descriptors for the 61 Tunisian autochthonous grapevine cultivars (GENRES-081, 2001 ; OIV, 2007). From 1 to 61 : analyzed cultivars (see Table 1 for accession names).

Description of the character	OIV values for the 61 grapevine accessions																																																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
form of tip	7	7	7	5	5	5	7	5	7	7	5	7	7	7	7	5	5	7	7	5	5	5	1	5	5	5	1	7	7	5	7	7	5	1	7	5	1	5	7	5	7	7	5	5	5	1	5	1	5	1	5	5	5	1	1	7	5	5	1	7	5
anthocyanin coloration of tip	1	1	1	3	5	1	1	1	5	3	1	1	1	3	5	1	3	1	1	5	5	1	3	1	3	3	3	1	1	3	3	3	3	5	1	3	3	1	3	3	5	1	5	3	1	3	3	1	1	7	1	1	1	1	5	7	1	1	3	3	1
density of prostrate hairs on tip	5	5	3	3	3	5	1	3	5	3	7	5	3	1	1	3	3	1	5	1	1	7	1	5	1	7	1	5	5	5	3	5	3	1	7	5	1	1	5	1	5	3	1	3	7	1	1	7	5	3	7	5	7	5	3	3	5	7	3	5	5
attitude (habit)	3	3	5	1	3	1	3	3	3	3	1	3	3	3	1	1	1	5	3	3	3	1	3	3	1	1	1	1	3	5	3	1	3	5	1	5	1	1	3	3	1	5	3	3	3	3	1	3	3	1	5	5	3	1	1	3	3	5	3	1	3
intensity of anthocyanin coloration on the bud scales	1	1	1	1	5	5	1	1	1	1	3	1	1	1	5	1	1	1	1	3	1	1	1	1	5	1	1	3	1	1	1	3	3	1	1	1	1	1	1	7	3	1	1	1	1	1	1	3	1	1	5	5	1	1	1	3	1	1	1	5	1
distribution of the anthocyanin coloration on the bud scales	1	1	1	1	5	5	1	1	1	1	5	1	1	1	5	1	1	1	1	5	1	1	1	1	5	1	1	5	1	1	1	5	5	1	1	1	1	1	1	9	5	1	1	1	1	1	1	5	1	1	9	5	1	1	1	5	1	1	1	5	1
fertility of basal buds (basal buds 1-3)	1	1	1	1	1	5	1	1	1	1	5	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	5	5	1	5
color of ventral side of internodes	2	2	2	2	2	2	2	1	2	2	2	2	2	1	2	1	1	1	1	2	1	1	2	1	2	2	2	2	2	1	3	2	2	1	2	2	2	1	2	2	1	2	2	3	1	2	1	1	2	1	2	5	1	2	1	1	2	1	2	2	1
color of dorsal side of internodes	2	2	2	3	2	1	3	1	2	1	1	2	2	2	2	1	1	2	2	2	1	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	3	1	1	2	1	2	2	1	2	5	2	2	2	2	2	2	2	2	2
number of consecutive tendrils	1	1	1	1	1	1	2	2	1	1	2	2	2	2	1	1	1	1	2	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	2	1	1	1	2	1	2
length of tendrils	3	3	3	5	3	3	5	3	3	5	7	3	3	3	3	3	3	3	3	1	3	3	3	3	1	1	7	3	3	3	3	3	1	1	3	5	3	5	3	3	3	5	3	3	3	3	3	3	3	3	3	5	1	3	1	3	3	5	3	3	3
time of bud burst	1	1	1	1	7	5	1	3	1	3	3	1	1	1	1	1	1	1	1	1	7	1	1	1	1	3	3	3	5	5	1	1	1	3	3	3	5	7	5	5	3	3	5	7	5	5	5	1	5	5	3	5	5	5	5	5	5	5	5	5	5
colour of upper surface	3	3	2	4	1	4	1	1	1	2	3	1	1	1	4	2	4	3	1	4	3	2	4	1	3	2	3	1	3	2	3	4	4	3	3	2	3	3	3	4	3	2	3	4	3	3	3	3	2	3	4	5	3	2	3	4	4	3	2	4	3
density of prostrate hairs between veins on lower side of leaf	5	3	1	1	1	1	1	1	1	3	9	1	1	1	1	5	3	1	1	1	1	5	1	1	1	9	1	5	1	3	1	1	5	1	1	5	1	1	3	1	5	1	1	1	3	1	1	7	3	1	3	5	9	1	1	1	1	3	3	1	3
density of erect hairs between veins on lower side of leaf	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1
density of erect hairs on veins	1	1	3	1	3	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	1	5	1	5	1	1	1	1	1	1	1	1	1
density of prostrate hairs on veins	3	3	1	1	1	1	1	1	1	1	5	1	1	1	1	3	1	1	1	1	1	7	3	3	1	7	1	1	1	3	1	1	3	1	7	5	1	1	5	1	3	1	1	1	5	1	1	5	3	1	3	5	7	3	1	1	3	3	1	1	3
shape of blade	3	1	2	3	3	1	3	5	2	2	1	2	3	2	3	3	2	2	3	2	3	3	1	3	3	3	3	1	2	2	3	2	2	2	2	2	4	3	2	2	2	3	2	2	3	3	2	3	3	3	2	5	2	3	2	2	2	1	3	2	2
number of lobes	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	4	2	2	2	2	2	3	2	2	4	3	2	2	4	2	3	4	2	2	2	3	4	3	4	3	2	4	2	2	2	2	2	3	5	2	2	2	3	4	4	2	2	2
anthocyanin coloration of main veins on blade upper side	1	1	1	2	5	3	2	3	1	4	3	5	1	3	4	1	3	2	1	4	4	5	1	4	5	2	1	1	2	3	5	2	5	2	4	5	2	2	1	1	1	1	3	2	1	3	2	1	1	1	4	5	3	5	1	3	2	1	1	1	1
profile	4	4	4	1	5	4	1	1	5	1	1	1	1	4	1	1	1	1	1	4	4	4	1	4	1	1	3	4	4	1	1	4	3	4	5	3	4	1	4	1	4	1	1	3	4	1	3	3	1	1	3	5	4	1	3	1	1	1	1	1	1
blistering of upper side	3	1	1	1	3	5	3	1	1	1	3	3	1	3	3	1	1	1	1	7	3	3	1	3	3	3	3	1	1	3	1	1	3	1	1	3	1	1	1	1	1	1	1	1	1	1	3	1	1	3	3	5	3	1	3	1	1	1	1	1	1
shape of teeth	2	2	3	2	2	2	2	4	2	2	5	2	2	2	2	1	2	2	2	2	2	4	2	3	2	3	3	4	5	2	3	2	3	3	2	3	3	2	3	4	2	4	4	2	3	3	3	2	2	2	2	5	3	3	4	3	2	2	5	4	2
general shape of petiole sinus	2	2	1	2	1	3	2	1	1	2	4	2	1	2	2	1	1	2	2	2	2	2	1	2	2	3	2	3	2	2	2	1	2	2	2	2	2	2	2	2	2	2	3	1	4	2	2	1	4	1	1	5	1	2	1	1	2	1	2	6	1
opening/overlapping of petiole sinus	3	3	3	3	1	5	3	1	1	3	5	3	1	3	5	1	1	3	3	3	5	3	1	3	5	7	3	3	3	5	5	1	5	3	3	3	3	3	3	3	3	3	3	3	5	3	3	3	7	3	3	5	3	5	3	1	3	1	3	7	1
shape of base of petiole sinus	1	1	1	3	1	2	1	1	1	1	3	1	1	3	1	1	2	1	3	3	3	1	1	3	3	3	3	1	1	3	3	1	3	3	1	1	1	3	1	2	2	2	2	3	2	3	1	3	3	3	1	5	1	1	3	2	2	3	2	3	2
tooth at petiole sinus	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	2	1	1	1
petiole sinus limited by veins	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	5	1	1	1	1	1	1	1	1	1
presence of teeth at the base of the upper leaf sinuses	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	2	1	1
length of vein N1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	3	3	3	3	3	3	3	1	3
length of vein N2	3	3	3	3	5	3	3	3	5	5	5	3	5	3	3	1	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3	5	1	3	3	3	3	3	1	1	3	3	3	3	5	3	3	3	3	3	3	5	3	3	3	5	5	5	3	1	3
length of vein N3	3	3	3	3	5	3	5	5	5	5	5	3	5	3	5	1	3	5	5	3	3	5	3	5	3	3	5	3	5	3	3	5	3	3	5	3	3	3	3	5	3	3	3	3	5	3	5	3	3	5	3	5	3	3	3	5	5	5	3	3	5
length of vein N4	5	5	5	5	7	7	7	7	7	7	7	5	7	5	7	1	1	3	5	5	5	7	1	7	5	5	7	3	5	5	5	7	3	5	5	5	3	5	5	5	5	3	5	5	3	5	5	3	7	5	5	5	5	5	5	3	3	1	1	3	5
length from petiole sinus to upper leaf sinus	3	3	3	1	7	1	3	5	7	3	3	3	1	3	1	1	1	3	3	3	3	5	1	5	3	3	5	1	3	3	3	3	3	3	3	3	5	5	3	1	1	3	3	3	5	5	3	3	3	3	1	5	3	3	3	5	3	1	5	3	3
length from petiole sinus to lower leaf sinus	3	3	3	3	5	1	3	5	3	3	3	7	1	3	1	1	1	3	5	1	1	3	1	5	3	3	5	3	5	3	3	3	3	1	3	3	5	3	1	1	1	5	3	3	3	3	3	3	3	3	1	5	3	3	3	5	3	3	3	3	5
angle between N1 and N2 measured at the first ramification	5	5	5	7	3	7	5	5	3	5	5	5	3	5	3	1	3	3	7	5	5	5	5	7	5	7	3	3	7	7	7	3	7	5	5	5	5	5	3	5	5	5	5	5	7	7	5	3	7	3	3	5	3	5	5	5	3	5	5	5	3
angle between N2 and N3 measured at the first ramification	5	5	5	3	3	7	5	3	3	5	5	5	5	5	3	1	5	3	5	7	7	7	5	7	5	7	7	7	5	7	7	3	7	5	3	7	5	7	3	5	5	5	5	7	5	5	3	5	7	5	5	5	7	5	5	5	3	3	5	7	3
angle between N3 and N4	5	7	5	7	3	5	7	3	5	5	5	5	5	5	5	1	5	5	5	5	5	5	3	5	5	7	3	7	5	5	7	5	7	7	3	7	7	5	5	7	7	7	3	5	7	5	3	5	5	7	7	5	5	7	7	3	5	5	5	5	3
angle between N3 and the tangent between petiole point and N5 tooth tip	7	9	3	7	9	7	7	5	7	5	9	9	5	7	9	1	7	7	7	7	5	7	5	7	7	7	9	7	7	9	9	9	9	7	7	7	9	7	9	5	9	7	7	5	7	7	7	7	7	7	7	5	7	9	7	3	7	7	7	9	7
length of teeth N2	3	3	3	5	5	3	3	1	5	3	1	3	3	3	1	1	5	5	1	3	5	3	3	1	3	1	3	5	3	1	3	3	1	3	3	3	3	3	1	1	5	3	3	3	3	3	3	1	1	1	3	5	1	3	3	3	5	3	1	1	1
width of teeth N2	5	3	3	5	5	5	3	3	5	3	3	3	3	1	3	1	3	3	1	3	5	3	3	1	3	3	5	7	5	3	3	3	3	3	3	3	3	3	1	5	5	3	3	3	5	3	3	3	3	3	1	5	1	3	3	3	5	3	3	3	3
length of teeth N4	1	1	3	3	3	3	5	5	3	3	1	1	3	1	3	1	3	1	1	3	1	1	1	1	3	1	3	3	1	1	1	3	1	3	3	1	3	3	1	1	1	1	1	3	3	3	1	1	1	1	1	5	1	1	3	1	1	1	1	1	1
width of teeth N4	3	3	3	3	3	5	5	3	3	3	3	3	3	1	1	1	3	1	1	3	1	3	1	1	1	3	3	5	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	3	3	3	3	3	1	3	3	3
teeth number between tooth tip and tooth tip of N2 first secondary vein	5	3	3	5	7	3	5	3	5	5	7	7	3	5	5	1	5	3	9	5	7	7	5	3	7	3	3	5	3	3	5	5	5	5	3	5	7	5	5	3	3	3	3	3	5	7	5	5	5	3	3	5	5	3	9	3	5	5	5	3	5
length between N2 tooth tip and tooth tip of first secondary N2 vein	3	3	5	5	3	5	5	3	5	5	5	3	3	3	3	1	5	3	7	3	5	5	3	3	3	3	3	5	5	3	3	5	5	3	3	5	3	3	3	3	3	5	3	3	5	3	3	3	3	3	3	5	3	3	3	3	5	5	3	3	3
vein N3, length from petiole sinus to vein N4	3	3	5	3	7	3	5	5	5	5	3	3	5	3	3	1	3	1	5	3	5	3	5	3	5	5	3	1	3	3	3	3	3	3	5	3	3	3	3	1	3	3	3	3	5	5	3	5	5	3	3	5	3	3	3	7	1	5	3	1	3
length of vein N5	1	3	1	1	3	1	1	1	1	3	3	3	1	3	3	1	1	3	3	3	1	3	1	3	1	1	3	3	1	3	3	1	1	1	3	1	1	1	1	1	1	1	3	1	3	3	3	3	3	1	3	5	3	3	3	1	3	1	3	3	3
density of prostrate hairs between main veins (lower side)	5	5	1	1	1	5	1	1	1	1	7	1	1	3	1	1	1	1	3	1	1	5	1	3	1	5	1	3	1	3	1	1	5	1	3	3	1	1	1	3	1	5	1	1	1	1	1	1	5	1	3	5	1	1	1	1	1	1	1	1	1
density of erect hairs on main veins (lower side)	5	7	3	1	3	7	1	3	5	1	5	3	1	1	5	1	3	1	3	1	1	3	3	1	1	1	1	7	1	1	1	3	7	1	3	3	1	1	1	5	1	3	1	1	1	3	1	1	3	1	5	5	3	1	1	3	3	1	3	3	1
degree of resistance to *Plasmopara*	9	7	9	7	9	3	3	7	7	1	1	5	5	7	5	1	1	5	5	7	5	1	1	5	1	1	5	7	5	5	5	5	1	7	5	1	9	1	1	5	7	7	5	3	5	5	5	5	5	5	7	5	5	5	3	5	5	5	5	3	7
degree of resistance to *Oidium*	5	7	9	9	9	9	9	9	9	3	1	9	9	5	9	1	7	9	9	5	7	3	7	5	7	1	5	7	7	5	9	9	7	5	7	9	9	5	3	5	7	7	9	9	5	7	9	7	9	9	5	5	7	3	9	5	7	5	7	9	7
sex of flower	3	3	4	3	3	3	3	3	3	3	4	3	3	3	3	1	4	3	4	4	3	3	4	3	4	3	3	3	3	3	3	3	3	4	3	3	3	3	3	4	3	3	3	4	4	4	3	4	3	4	3	5	3	3	4	4	3	3	4	3	4
length	5	3	7	3	5	7	3	7	5	7	3	7	7	7	7	1	5	5	7	5	7	7	7	5	5	7	7	7	5	5	9	7	7	5	3	7	3	7	3	3	7	7	9	7	7	7	5	7	7	7	5	5	7	7	7	5	5	7	7	7	5
density	1	1	3	3	1	1	1	3	5	7	3	5	5	1	7	1	3	5	5	1	5	5	5	5	1	3	1	5	1	3	5	5	5	5	5	1	5	1	1	3	1	3	7	9	7	5	5	5	3	1	1	5	5	1	5	5	5	3	5	1	7
length of peduncle	3	3	3	3	7	5	3	5	3	5	3	3	3	1	3	1	1	3	1	1	3	3	5	1	1	7	1	5	3	3	5	5	5	3	1	3	1	3	3	3	7	3	3	1	3	5	3	3	3	5	3	5	5	1	3	3	5	3	3	5	1
shape	1	1	2	2	1	2	2	1	2	3	2	3	3	1	2	1	1	3	2	1	3	3	2	3	2	1	1	3	2	1	1	3	2	3	2	2	3	1	3	3	2	3	3	1	1	1	1	2	3	1	1	5	2	1	1	3	3	1	1	1	2
number of wings	2	1	2	2	3	3	3	2	2	3	2	3	2	2	3	1	3	2	2	3	3	3	3	2	2	3	3	3	3	2	3	3	3	3	3	3	2	3	3	2	3	3	3	1	3	2	2	3	2	2	3	5	3	3	2	2	3	3	3	3	2
length	5	5	7	7	7	7	7	5	7	7	7	7	7	7	7	1	7	5	7	5	7	5	7	7	5	7	7	5	5	7	7	7	5	5	7	5	7	7	5	7	5	5	9	9	5	7	7	7	5	7	7	5	9	7	7	7	5	7	7	5	7
width	5	3	5	3	5	3	7	5	5	7	7	5	7	7	7	1	5	7	7	5	5	3	7	5	5	7	5	5	5	5	5	3	7	5	7	5	7	5	5	5	5	5	7	7	5	7	7	7	5	7	5	5	5	5	7	5	7	5	7	5	7
shape	2	2	2	2	2	3	5	2	3	1	5	2	2	5	2	1	2	1	6	2	4	2	2	2	2	2	2	2	5	2	2	8	1	2	2	2	1	4	2	4	2	2	2	4	2	1	7	1	2	1	2	5	7	2	1	4	1	7	2	4	1
color of skin	1	1	1	1	5	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	5	3	1	1	1	1	1	1	5	1	1	1	5	5	1	1	5	1
color of flesh	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1
degree of firmness of flesh	1	5	5	1	5	5	5	5	9	5	5	9	5	5	5	1	5	5	5	5	9	5	5	3	5	5	5	5	9	5	5	5	5	5	5	5	5	5	5	9	5	5	5	9	9	9	5	5	5	5	1	5	5	5	5	9	5	5	5	5	5
particular flavor	1	1	4	5	5	4	2	5	5	4	5	4	1	5	4	1	4	4	5	4	4	4	3	4	4	4	3	4	2	4	4	4	4	4	2	5	4	4	1	5	1	1	4	4	4	5	5	4	4	4	4	5	5	1	5	4	5	4	5	4	1
presence of seeds	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	3	3	3	3	3	3	3	3	3
single bunch weight	3	3	5	5	5	3	3	5	3	5	3	5	3	5	3	1	3	5	3	5	3	5	5	5	5	5	5	5	5	5	5	5	3	5	3	5	5	5	5	3	5	5	5	3	3	5	5	5	5	3	5	5	5	5	5	3	5	5	5	3	5
single berry weight	3	3	5	5	5	5	5	5	5	5	5	5	5	7	3	1	5	3	5	5	3	3	5	5	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	5
sugar content (%)	7	7	5	5	7	5	5	5	7	5	5	7	5	3	3	1	3	5	5	5	3	7	5	5	5	7	5	5	5	5	5	5	3	5	5	7	5	5	5	5	7	7	5	3	5	5	5	7	5	5	5	5	5	3	5	5	5	3	5	3	5
total acid content	3	3	3	3	1	1	3	3	1	3	1	1	3	3	1	1	1	5	3	3	3	1	1	3	3	1	3	3	3	3	3	1	3	1	3	3	3	1	1	1	3	3	3	3	3	3	3	3	3	3	3	5	3	1	3	1	3	1	3	1	3
pH of must	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	3	3	3	3	3	5	3	3	5	3	3	3	3	3	1	3	3	3

*The 12 most important morphological descriptors for the classification of the accessions

anthocyanin coloration on the bud scales (OIV 015 and OIV 15-2, respectively). Group C3 included 14 accessions that are grouped by the form of the tip (OIV 001), which varied from "half open to open".

The variance components within and between the individual groups (C1, C2 and C3) detected with ANOVA showed that most of the morphological variation was partitioned within (62.98 %) rather than between (37.02 %) the groups. These were both significant at $p<0.01$ (Table 4). Per variation class, the accessions Asli Hadab, Khalt Bouchemma Gabès and Khalt Abiadh (Sd1, Sd2 and Sd3, respectively) were identified as the barycentres of the groups C1, C2 and C3, respectively.

1.2 Leaf characteristics

Thirty-nine OIV descriptors were used for the characterization of the leaves (Table 2). According to the PCA plot (Fig. 2a), the first three principal components accounted for 28.61 % of the total variation (Table 3). The variables with the greatest weight in the first principal component were the opening (OIV 079-1) and the general shape (OIV 079) of the petiolar sinus and the angle between veins N2-N3 at the first ramification (OIV 608). The second axis was defined by three characters : the length from the petiolar sinus to the upper leaf sinus (OIV 605), the length from the petiolar sinus to the lower leaf sinus (OIV 606) and the shape of the blade (OIV 067). The characters OIV 079-1 and OIV 079 were the most correlated descriptors (0.738 %). Based on leaf descriptors, the 61 studied cultivars grouped into three major groups : C1' (19 genotypes), C2' (39 genotypes) and C3' (3 genotypes), as illustrated in the dendrogram (Fig. 2b).

The variance components within and between the individual groups (C1', C2' and C3') detected with ANOVA showed that most of the morphological variation was partitioned within (85.58 %) rather than between (14.42 %) the identified groups (Table 4). Per variation class, the accessions Khalt Bouchemma Gabès, Arich Djerba and Chaouche Djerba (Ld1, Ld2 and Ld3, respectively) were identified as the barycentres of the groups C1', C2' and C3', respectively.

Further observations of leaf descriptors showed that the shape of the blade was either wedge-shaped or pentagonal (OIV 067) with open petiole sinus (OIV 079-1). From a disease sensitivity point views, the leaves of the accessions Châaraoui, Khamri Tozeur and Arich Dressé exhibited high resistance to both *Oidium* (OIV 455) and *Plasmopara* (OIV 452), while the accessions Khédhiri 1, Sakasly Baddar, Muscat Rafraf, Akhal Mguergueb, Bidh el Hamem Rafraf and Bezzoul el Khadem Rafraf displayed lower resistance to *Plasmopara* but high resistance to *Oidium*.

1.3 Fruit characteristics

Nineteen ampelographic descriptors were used for the description of the fruits. The discrimination between all cultivars revealed that the first three axes of the PCA plot explained 37.32 % of the variation (Table 3). The highest loadings on the first PCA axis corresponded to berry length (OIV 220) and bunch density (OIV 204) (Figure 3a). The variables with the highest loadings on the second PCA axis were color of the berry flesh (OIV 230) and total acid content of the must (OIV 506). The color of the berry skin (OIV 225) and color of the berry flesh (OIV 230) were the most correlated characters. Three major groups were determined using cluster analysis : group C1" (29 genotypes), C2" (14 genotypes) and C3" (18 genotypes) (Fig. 3b).

The variance components within and between the individual groups (C1", C2" and C3") detected with ANOVA showed that most of the morphological variation was partitioned within (79.12 %) rather than between (20.88 %) the groups (Table 4). Per variation class, the

Table 3 - Estimates of variances (eigenvalues), cumulative variance and eigenvectors of the first three principal components (F1, F2, F3) for shoot, leaf, fruit and total descriptors evaluated on 61 Tunisian autochthonous grapevine accessions.

	Shoot descriptors			Leaf descriptors			Fruit descriptors			Total descriptors		
	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
Eigenvalue	2.298	1.856	2.298	5.187	3.319	2.652	2.731	2.354	2.006	6.052	4.704	4.492
Variability (%)	19.146	15.470	19.146	13.301	8.510	6.799	14.373	12.389	10.560	8.646	6.721	6.418
% cumulated	19.146	34.616	53.762	13.301	21.810	28.610	14.373	26.762	37.322	8.646	15.366	21.784

Table 4 - Variance decomposition for optimal classification based on shoot, leaf, fruit and total descriptors (significant at $p<0.01$).

	Shoot descriptors		Leaf descriptors		Fruit descriptors		Total descriptors	
	Absolute	Percentage	Absolute	Percentage	Absolute	Percentage	Absolute	Percentage
Intra group variation	17.318	62.98%	55.546	85.58%	21.046	79.12%	106.277	89.31%
Inter group variation	10.179	37.02%	9.356	14.42%	5.556	20.88%	12.723	10.69%
Total variation	27.497	100.00%	64.902	100.00%	26.601	100.00%	119.001	100.00%

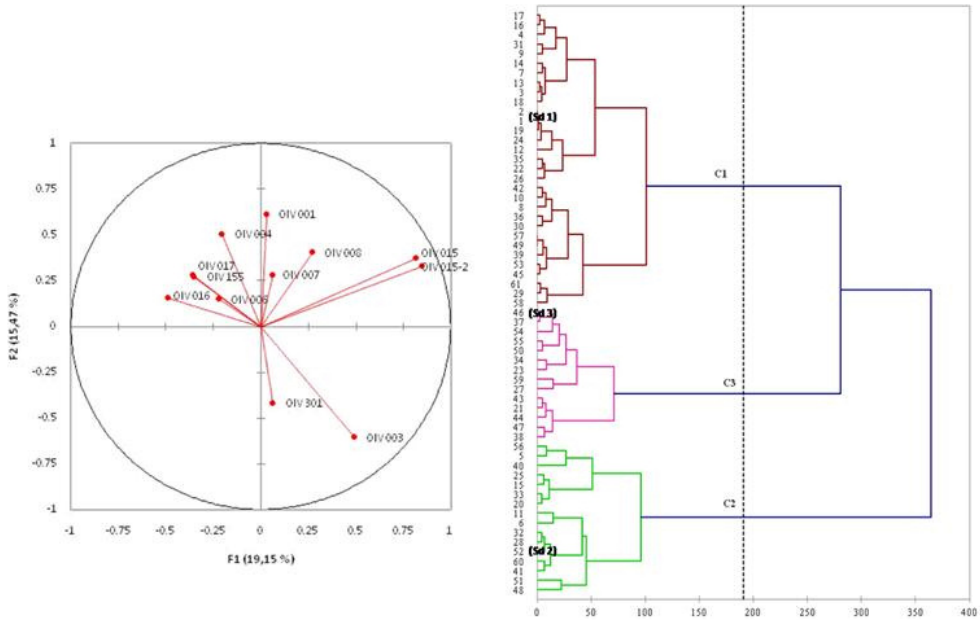

Figure 1 - Shoot descriptor analysis. (a) Principal component analysis (PCA) plotted along the first two axes and (b) UPGMA dendrogram of Euclidean distance illustrating the genetic relationships among the 61 studied grapevine cultivars based on shoot descriptors. Sd1, Sd2 and Sd3 : barycentres of the groups.

accessions Blanc 3, Arbia and Balta 2 (Fd1, Fd2 and Fd3, respectively) were identified as the barycentres of the groups C1", C2" and C3", respectively.

The bunch density character (OIV 204) varied from loose to dense and the cluster analysis showed that the accessions with the lowest bunch density were grouped together. Across all accessions, whatever the size or the shape of the bunch or the berry, the weight of a bunch did not exceed 500 g and the weight of a single berry ranged between 2 and 5 g. The sugar content of the must (OIV 505) varied from low (Saouadi, Amokrane, Arbia, Djebbi) to high (Asli Hadab, Asli Dar Slimane, Beldi Rafraf, Beldi Sayeb, Khédhiri 1, Tounsi Djerba, Khédhiri 2, Turky). A low total acid content of the must (OIV 506) characterized all the studied accessions and most of the accessions displayed low ph (OIV 508). Considerable variation was observed for the fruit shape (OIV 223). The intensity of the skin color (OIV 225) was quite diverse, ranging from very light green-yellow to dark red-violet, with medium to firm flesh (OIV 235).

2. Statistical evidence for integrating all morphological data in discriminating between Tunisian autochthonous grapevines

Different dendrograms were obtained and different relationships between the accesssions were observed when based upon shoot, leaf or berry criteria (Figures 1b ; 2b and 3b). Therefore, we performed statistical analysis to see whether or not all morphological data ought to be integrated in the characterization of Tunisian grapes. Thus, Euclidean distances calculated for shoot/berry, shoot/leaf and leaf/berry were compared by Mantel tests (Rousset, 1997) using 10000 permutations (XLSTAT software). As shown in Figure 4, low correlation values were obtained between shoot and berry descriptors (r=0.023) and between leaf and berry descriptors (r=0.027). However, a higher significant correlation value was obtained between shoot and leaf descriptors (r=0.125). Based on these results (Table 5), all descriptors ought to be integrated in clustering Tunisian autochthonous grapevines.

3. Morphological diversity based on total characters

The use of all the 70 ampelographic descriptors (Table 2) yielded a high number of morphotypes and permitted the discrimination of all cultivars. High morphological variation was recorded among the studied accessions. The majority of the descriptors were significantly correlated, though with very heterogeneous values for the coefficients of correlation.

The first three PCA axes accounted for 21.78 % of the total variation (Table 3 ; Fig. 5a). Besides, 12 descriptors out of 70 were identified as the most useful morphological descriptors for the classification of the accessions. These were the following : the shape of petiolar sinus (OIV 079), the opening/overlapping of the petiolar sinus (OIV 079-1), the density of prostrate hairs between the main veins (lower side) (OIV 084), the angle between N2 and N3 measured at the first ramification (OIV 608), the intensity and distribution of anthocyanin coloration on the bud scales (OIV 015-2 and OIV 015, respectively), the intensity of berry skin color (OIV 225), the width of teeth N2 of mature leaf (OIV 613), the sugar content of must (OIV 505), the anthocyanin coloration of shoot tip (OIV 003), the density

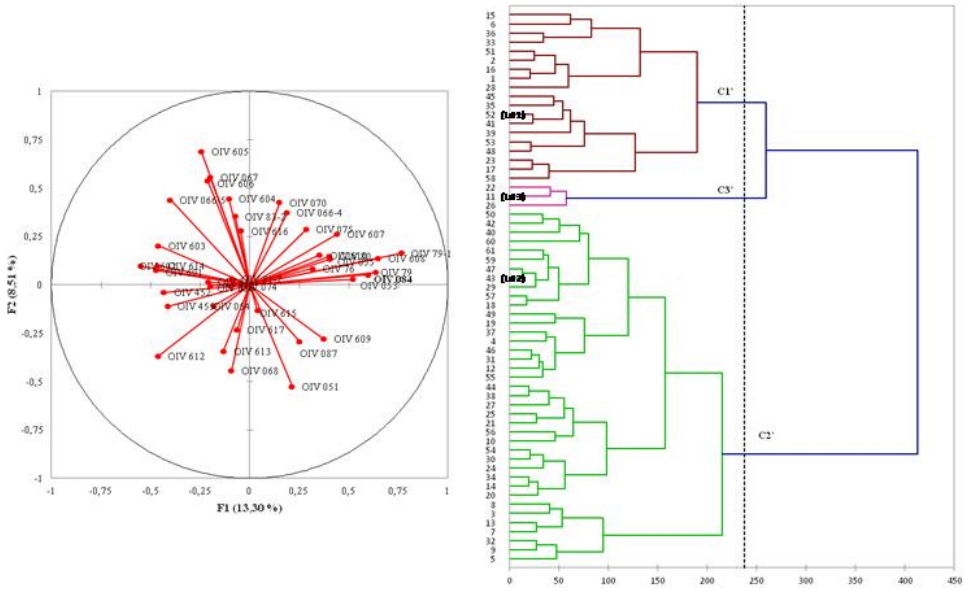

Figure 2 - Leaf descriptor analysis. (a) Principal component analysis (PCA) plotted along the first two axes and (b) UPGMA dendrogram of Euclidean distance illustrating the genetic relationships among the 61 studied grapevine cultivars based on leaf descriptors. Ld1, Ld2 and Ld3 : barycentres of the groups.

of prostrate hairs on shoot tip (OIV 004) and the sex of flower (OIV 151) (Table 2).

Using all descriptors, three major groups were identified by the cluster analysis : groups C1"' (14 accessions), C2"' (36 accessions) and C3"' (11 accessions) (Figure 5b). The variance components within and between the individual groups (C1"', C2"' and C3"') detected with ANOVA showed that most of the morphological variation was partitioned within (89.31 %) rather than between (10.69 %) the groups (Table 4). These were both significant at $p<0.01$. Per variation class, the accessions Khalt Bouchemma Gabès, Blanc 3 and Blanc 2 (Td1, Td2 and Td3, respectively) were identified as the barycentres of the groups C1"', C2"' and C3"', respectively. Consequently, these accessions are representative of all the morphological variation within autochthonous grapes in Tunisia.

4. Perspectives for the commercialization of Tunisian autochthonous cultivars : table or wine cultivars ?

The question that was dealt with in this section was how these accessions ought to be introduced into the market : are they wine or table cultivars ?

The three genotypes that were identified as the barycentres of the groups (Fig. 5b) were considered to determine the use and analyzed for the following characters : weight of the bunch (g), weight of 100 berries (g), must sugar content (brix), must organic acid composition (titratable acidity ; TA) and must pH (Table 6). The brix and TA parameters were the most compulsory criteria that ought to be considered when distinguishing between table and wine grapes. As shown in Table 6, the sugar content of the analyzed accessions (between 14.2 and 17.1 brix) was lower than that usually reported for wine grapes (between 24 and 26 brix) and closer to values reported for table grapes (between 17 and 19 brix)".

DISCUSSION

The present study revealed the morphological diversity within a collection of autochthonous grapevine germplasms grown in different geographical regions of Tunisia using 70 morphological descriptors. These were used to estimate the phenotypic diversity among and within the cultivars, to identify the traits contributing to the heterogeneity, to classify them using PCA and HCA and to see how these accessions ought to be commercialized.

The use of ampelographic descriptors comprising shoot, leaf and fruit traits yielded a high number of morphotypes and permitted the discrimination of all cultivars. This discrimination was found to be higher than those reported previously using isozyme (Ben Abdallah *et al.*, 1998) and molecular markers (Zoghlami *et al.*, 2001 ; 2009) for the same varieties. In fact, morphological markers assume higher degree of genomic coverage (Veteläinen *et al.*, 2005) and most individual phenotypic markers are multigenic. Therefore, variation at more than one locus is being analyzed.

Conversely, the set of OIV descriptors used here allowed for the first time to easily split the Tunisian autochthonous grapevine accessions into wine and table grapes. These findings were not clearly attained when using molecular tools.

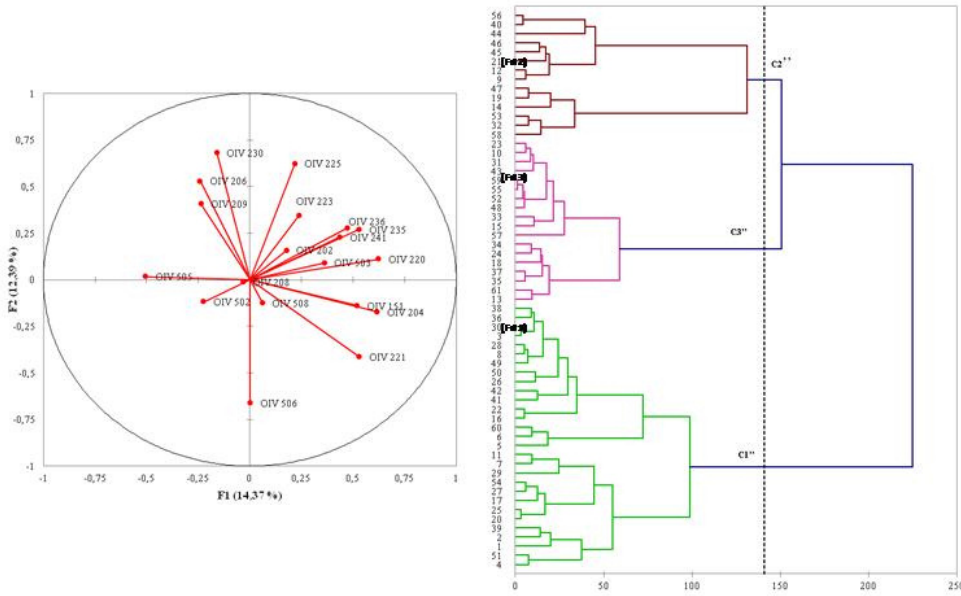

Figure 3 - Fruit descriptor analysis. (a) Principal component analysis (PCA) plotted along the first two axes and (b) UPGMA dendrogram of Euclidean distance illustrating the genetic relationships among the 61 studied grapevine cultivars based on fruit descriptors. Fd1, Fd2 and Fd3 : barycentres of the groups.

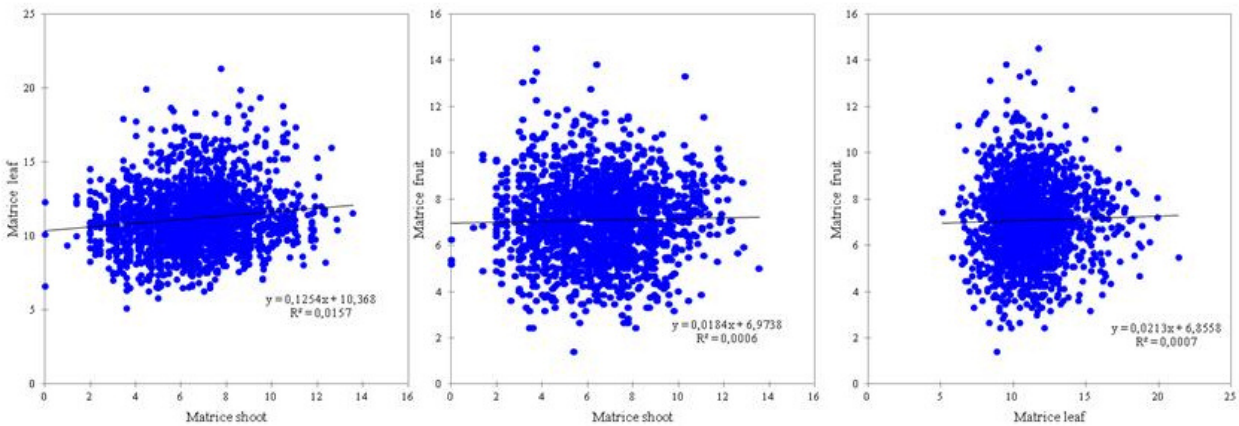

Figure 4 - Linear regression between shoot/leaf, shoot/fruit and leaf/fruit Euclidean distances in 61 grapevine genotypes.

Several authors have analyzed morphological diversity in crop plants (Bozokalfa *et al.*, 2009 ; Jesus *et al.*, 2009 ; Aghaee *et al.*, 2010 ; Sarıkamış *et al.*, 2010). In grapevine, morphological investigations have been carried out on Brazilian (Leão *et al.*, 2011), Croatian (Sladonja *et al.*, 2007), Egyptian (Hassan *et al.*, 2011), Georgian (Ekhvaia and Akhalkatsi, 2010), Italian (Muganu *et al.*, 2009 ; Alba *et al.*, 2011), Portuguese (Cunha *et al.*, 2009), Spanish (Santiago *et al.*, 2007) and Turkish grapevines (Ates *et al.*, 2011).

Multivariate analyses based on morphological characters provide information allowing the breeder to improve populations by selecting from specific geographical regions (Souza and Sorrells, 1991). The multivariate techniques have been applied in viticulture with several objectives : morphological and agronomical descriptions (Coelho *et al.*, 2004 ; Borges *et al.*, 2008), management (Intrieri *et al.*, 2001) and disease resistance (Nascimento *et al.*, 2006).

Because grapevine shoot is an important character for the description of cultivars, many studies were conducted using such descriptors (Santiago *et al.*, 2007 ; Zdunic *et al.*, 2008 ; Barth *et al.*, 2009 ; Sabir *et al.*, 2009). A thorough knowledge of the quantitative aspects of shoot development is therefore necessary to understand the determination of

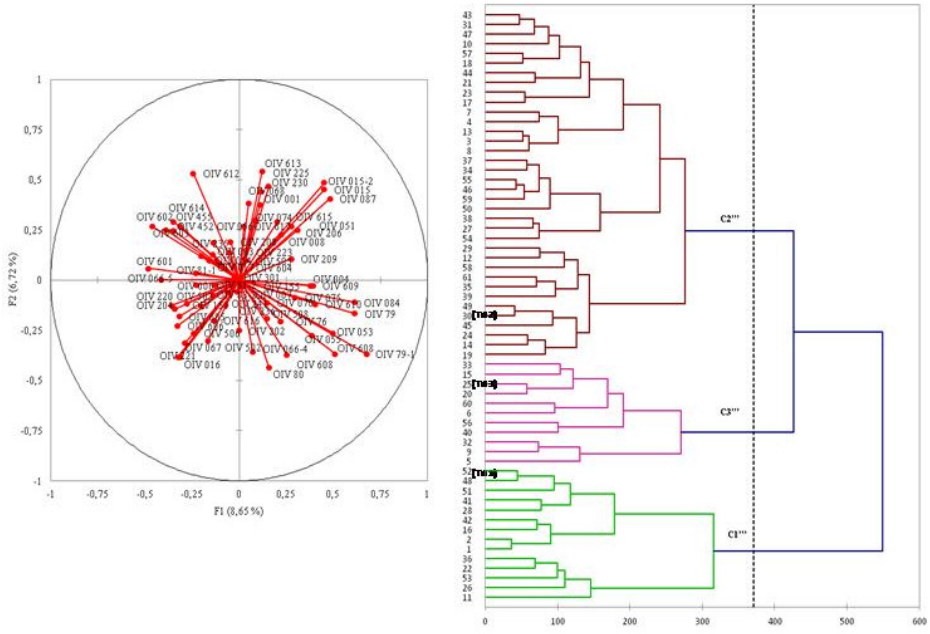

Figure 5 - Total descriptor analysis. (a) Principal component analysis (PCA) plotted along the first two axes and (b) UPGMA dendrogram of Euclidean distance illustrating the genetic relationships among the 61 studied grapevine cultivars based on total descriptors. Td1, Td2 and Td3 : barycentres of the groups.

crop quality and productivity as a function of environment. In our case, the highest amount of variation was attained using shoot descriptors (PCA ; 53.76%) (Table 3 and Figure 1a). The number of shoot descriptors (n=12) was higher than those reported previously : 4 descriptors (Santiago *et al.*, 2007 ; Alba *et al.*, 2011), 5 descriptors (Sabir *et al.*, 2009), and 9 descriptors (Muganu *et al.*, 2009).

The tip of the young shoot was open for all the cultivars. This character allows the differentiation among the *Vinifera* and other *Vitis* species. Additionally, the coloration of the shoot tip, which defines the second axis of the PCA plot, seems to be an important character in distinguishing between the grapevine cultivars (Morton, 1979). Moreover, this character may vary in relation to exposure to light (Kara, 1990). The accessions Asli Hadab, Khalt Bouchemma Gabès and Khalt Abiadh were established as being the barycentres of the identified groups (Figure 1b). This means that using shoot descriptors all the morphological variation could be explained by these accessions.

Leaf descriptors have been generally used as powerful tools for identifying grapevine genotypes (Santiago *et al.*, 2007 ; Celik *et al.*, 2008 ; Sabir *et al.*, 2009 ; Gago *et al.*, 2009 ; Harbi-Ben Slimane *et al.*, 2010). Thirty-nine leaf descriptors were used in a set of 61 autochthonous grapes. This number was higher than those reported previously by Sabir *et al.* (2009), Santiago *et al.* (2007) and Gago *et al.* (2009) using 12, 17 and 19 descriptors, respectively. According to the PCA plot (Figure 2a), although using 39 descriptors, the first three principal components accounted for only 28.61 % of the total variation.

The discrimination between all grapevines, based on fruit descriptors, revealed that the first three axes of the PCA plot explained 37.32 % of the variation using only 19 ampelographic descriptors (Fig. 3a). The number of descriptors was higher than those used by Ates *et al.*, 2011 (6 descriptors), Alba *et al.*, 2011 (11 descriptors) and Leão *et al.*, 2011 (12 descriptors). It was observed that all accessions had low acidity values, which is one characteristic of cultivated grapevine (Navarro *et al.*, 2001 ; Liu *et al.*, 2006).

Based on the differences in clustering that occurred with shoot, leaf or berry descriptors (Figures 1b, 2b and 3b) and the correlation values registred between shoot and berry (r=0.023), leaf and berry (r=0.027) and shoot and leaf descriptors (r=0.125) (Table 5), all descriptors ought to be integrated in clustering for the discrimination of the Tunisian autochthonous cultivars. Significant correlations were detected between all descriptors (Figure 4), confirming the results of Ocampo *et al.* (2006), who found positive correlations between all morphological traits.

Therefore, a total number of 70 major OIV descriptors has been used to discriminate among autochthonous grapes. This number appears to be high if compared to previous studies in grapevine cultivars : Leão *et al.*, 2011 (12 descriptors) ; Sabir *et al.*, 2009 (17 descriptors) ; Cunha *et al.*, 2009 (22 descriptors) ; Alba *et al.*, 2011 (30 descriptors) ; Muganu *et al.*, 2009 (34 descriptors) ; Ekhvaia and Akhalkatsi, 2010 (43 descriptors) ; Sladonja *et al.*, 2007 (50 descriptors) ; and Celik *et al.*, 2008 (61 descriptors).

As inferred from the PCA plot (Figure 5a), when using the 70 descriptors to draw a final cluster scheme, the

Table 5 - Correlation coefficients (r) between shoot/leaf, shoot/berry and leaf/berry matrices of the 61 Tunisian autochthonous grapevine cultivars (significant at p<0.01).

	Shoot/Leaf	Shoot/Berry	Leaf/Berry
r(AB)	0.125	0.023	0.027
p-value (bilateral)	<0.0001	0.318	0.238
alpha	0.05	0.05	0.05

Table 6 - Weight of the bunch, weight of 100 berries, sugar content (brix), pH and tartaric acid (TA) content of the identified barycentres (as obtained using total descriptors).

Barycentres	Weight of the bunch (g)	Weight of 100 berries (g)	Brix	pH	TA
Khalt Bouchemma Gabès	153.3	511.6	17.1	3.8	4.9
Blanc 2	65.5	257.5	15.7	3.4	6.1
Blanc 3	246.8	384.7	14.2	3.4	9.3

eigenvalues of the first, second and third axis of the principal components accounted for 8.65, 15.37 and 21.78 % of the total variation, respectively (Table 3).

The relative magnitude of the first three PCA eigenvectors showed that 12 descriptors out of 70 were identified as the most important morphological descriptors for the classification of the accessions. These were OIV 079, OIV 079-1, OIV 084, OIV 608, OIV 015-2, OIV 015, OIV 225, OIV 613, OIV 505, OIV 003, OIV 004, and OIV 151 (Table 2). These descriptors ranked within the primary descriptor list established by the OIV for the characterization of cultivars (OIV, 2012). Iezzoni and Pritts (1991) mentioned that associations between traits uncovered by PCA may correspond to a genetic linkage between loci controlling traits or a pleiotropic effect.

To access the grapevine fresh fruit market, we must characterize the cultivar as table or wine grapes. The overall flavor is one of the most important qualities for establishing a continuous consumer preference. Flavor composition has been defined as a complex quality attribute, in which the mix of sugars, acids, and volatiles plays a primary role (Baldwin, 2002). Among the flavor metabolites already mentioned, sugar and organic acid compositions, which are measured through total soluble solids (TSS) and titratable acidity (TA), are most commonly associated with the taste of fruits, including table grapes (Shiraishi *et al.*, 2010). In our case, the accessions Khalt Bouchemma Gabès, Blanc 2 and Blanc 3 were identified as table grapes (Table 6), according to their flavor composition and bunch weight, which meet the market requirements for table grapes previously set up by the United Nations Economic Commission for Europe (UNECE) (Norme CEE-ONU FEV-19, 2010). These indicate that table grapes must have a refractive index of at least 16 to 19 brix and a minimum bunch weight of 75 g. Based on the aforementioned statements, Khalt Bouchemma Gabès possess the required characteristics to be introduced into the market as table grapes. Their overall flavor composition that is associated with their taste (Baldwin, 2002 ; Shiraishi *et al.*, 2010) may therefore strongly satisfy consumer preference.

CONCLUSION

The detailed ampelographic description presented in this study highlights clear morphological differentiation between 61 Tunisian autochthonous grapevines characterized for the first time using 70 OIV descriptors.

Morphological traits were shown to enable cultivar comparison and classification in germplasm collection. Numerical analyses showed that the number of morphological traits that are effectively contributing to the characterization of the cultivars could be reduced to 12. Furthermore, three cultivars out of 61 were found to represent the morphological variability observed in Tunisian grapes, representing the barycentres of the individual groups in cluster analysis.

Finally, the autochthonous Tunisian grapes ought to be classified as table grapes according to the standards of the UNECE (Normes CEE-ONU FEV-19, 2010).

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