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Characterization of Tunisian Grapevine (*Vitis vinifera* L.) Cultivars Using Leaves Morphological Traits and Mineral Composition

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Abstract

The diversity of the local grapevine varietal set of Tunisia was remarkably noted in areas of Sfax, Kerkennah, Gabès, Tozeur, Nafta, Dégache, Zarzis and Djerba. Some varieties showed interesting characteristics concerning the production quality, its lateness and its fitness to take on tree, and its adaptation to various Tunisian pedoclimatic conditions. In an attempt to explore the genetic diversity of 30 autochthonous grapevine (Vitis vinifera L.) cultivars, the study of foliar morphology (colour, number of lobes, shape of blade, shape of the teeth, size) and the determination of leaves composition on major mineral elements (Na, K, Ca, Mg, Cu, Zn, Fe, Mn) have been carried out in order to assess the inter-cultivars variability and its structuring in this collection. Data analysis by unweighted pair-group method with arithmetic mean (UPGMA) clustering have proved that some of these parameters were designed suitable to discriminate significantly few cultivars (ARCz, TURm, SABm, MITm…). Comparison of UPGMA dendrograms of morphological and mineral content parameters using the Mantel test indicated no significant correlation (r = 0.036). The recorded variability could be explained by genetically differentiations and environmental factors influences.

Key words: *Vitis vinifera* L., foliar morphology, mineral composition, genetic diversity, UPGMA clustering.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and most important perennial crops in the world. In Tunisia, viticulture was introduced by the Phoenicians, the founders of Carthage, who brought with them their taste for wine (Greene & Kehoe [1]). Much later, with the expansion of the Muslim culture, wine production was forbidden and grapevines were grown to produce table grapes and raisins. Currently, grapevines are grown throughout Tunisia mainly for fruit production (Harbi Ben Slimane [2]).

Several native grapevine (*Vitis vinifera* L.) genotypes, highly appreciated for their organoleptic characteristics and commercial potential, are still cultivated in Tunisia, from the Kroumirie-Mogods mountains (North-West, humid climate) to the desert region of Rjim-maâtoug (South-West, arid climate) (Zoghlami & al. [3]). However, their substitution by newly bred cultivars has caused a rapid reduction in the number of local genotypes. Developing viticulture requires the conservation of autochthonous varieties that have evolved several mechanisms enabling them to cope with the local bioclimatic and edaphic conditions (Ben Abdallah & al. [4]).

The survey of the genetic distinctness of Tunisian grapevine cultivars can provide information to enhance the evaluation, the conservation and the improvement of this phytogenetic patrimony. The present study was interested to a preliminary assessment of the genetic diversity and its structuring among 30 Tunisian grapevine (*Vitis vinifera* L.) genotypes using morphological descriptors of mature leaves and their composition on major mineral elements.

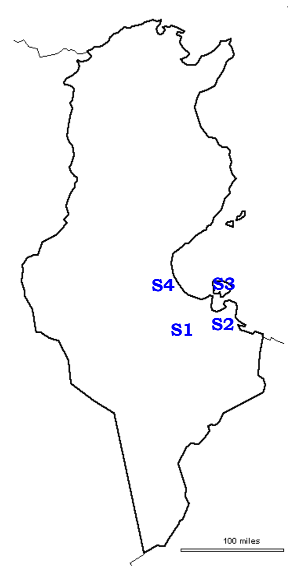
Materials and Methods

Plant material

The present study was carried out on a set of 30 grapevine accessions, corresponding to 24 different cultivars. The list of these genotypes and their locations are given in Table 1.

Table 1. Cultivar names, codes and collection sites of 30 Tunisian grapevine accessions.

Cultivar name	Code	Collection site
Beldi	BLDm	Médenine (33°20' N, 10°29' E), S1
Razzagui	RAZm	Médenine (33°20' N, 10°29' E), S1
Siper Abiadh	SABm	Médenine (33°20' N, 10°29' E), S1
Superior Italie	SITm	Médenine (33°20' N, 10°29' E), S1
Muscat d'Italie	MITm	Médenine (33°20' N, 10°29' E), S1
Meski	MESm	Médenine (33°20' N, 10°29' E), S1
Cardinal	CARm	Médenine (33°20' N, 10°29' E), S1
Tounsi	TONm	Médenine (33°20' N, 10°29' E), S1
Mguargueb	MGBm	Médenine (33°20' N, 10°29' E), S1
Arbi	ARBm	Médenine (33°20' N, 10°29' E), S1
Dalia	DALm	Médenine (33°20' N, 10°29' E), S1
Akhal	AKHm	Médenine (33°20' N, 10°29' E), S1
Akhal Twil	AKTm	Médenine (33°20' N, 10°29' E), S1
Turki	TURm	Médenine (33°20' N, 10°29' E), S1
Muscat d'Alexandrie	MEAz	Zarzis (33°30' N, 11°06' E), S2
Razzagui	RAZz	Zarzis (33°30' N, 11°06' E), S2
Meski	MESz	Zarzis (33°30' N, 11°06' E), S2
Cardinal	CARz	Zarzis (33°30' N, 11°06' E), S2
Aricha	ARCz	Zarzis (33°30' N, 11°06' E), S2
Nab Jmel	NAJz	Zarzis (33°30' N, 11°06' E), S2
Mguargueb	MGBd	Djerba (33°48' N, 10°50' E), S3
Superior Seedless	SUSd	Djerba (33°48' N, 10°50' E), S3
Sakasli	SAKd	Djerba (33°48' N, 10°50' E), S3
Tounsi	TONd	Djerba (33°48' N, 10°50' E), S3
Meski	MESg	Chénini Gabès (33°51' N, 10°03' E), S4
Bazzoul Kalba	BAKg	Chénini Gabès (33°51' N, 10°03' E), S4
Médina	MEDg	Chénini Gabès (33°51' N, 10°03' E), S4
Korkobbi	KORg	Chénini Gabès (33°51' N, 10°03' E), S4
Mlouhi Mkarkeb	MMKg	Chénini Gabès (33°51' N, 10°03' E), S4
Saoudi	SADg	Chénini Gabès (33°51' N, 10°03' E), S4



Map of Tunisia showing the sites of collection. Sites:

S1 = Médenine
S2 = Zarzis
S3 = Djerba
S4 = Chénini Gabès

Morphological description

Mature leaves were sampled between fruit setting and veraison from 2 or 3 randomly selected plants of each accession. They were collected from the sixth to the ninth node of fruiting branches. The morphological leaf descriptions were made following the list of descriptors of IPGRI, UPOV & OIV [5] and also according to Galet [6]. Five descriptors were performed: colour, number of lobes, shape of blade, shape of the teeth and size.

Mineral content analysis

A total of 30 adult leaves per accession were taken from lower, middle and upper regions of the plant, bulked together and transported directly to the laboratory. Leaves were taken out from 1, 2 or 3 individuals depending on number of individuals per accessions available. They have been oven-dried for 48 h at 70 ˚C and ground to pass through a 1 mm diameter sieve. Four grams (4 g) of dried leaves were calcinated for 6 hours at 500 ˚C and then digested by boiling with 5 mL of concentrated HCl and 3 ml H_2O. Each sample was filtered and brought up to a final volume of 100 mL with deionised water. The concentrations of the main mineral elements (Na, K, Ca, Mg, Cu, Zn, Fe and Mn) were determined using an atomic absorption spectrophotometer (SHIMADZU AA 6800).

Data analysis

A statistical analysis of the collected data, from morphological and mineral analysis, was carried out using XLSTAT 4.03 program (Addinsoft Inc.). From the raw data matrix constructed from the recorded values of each analysis, a dissimilarity matrix was performed according to "Euclidean distance". This matrix was then subjected to a cluster analysis using the unweighted pair-group method with arithmetic mean (UPGMA) (Sneath & Sokal [7]), showing the relationships among the 30 studied grapevine genotypes.

The comparison between morphological and mineral traits was performed for all the accessions. The Mantel's test (Mantel [8]) was used to see the correlation significance between the two data sets using the XLSTAT 4.03 software (Addinsoft Inc.).

Results and Discussions

Ampelography

The evaluation of the ampelographic descriptors (Table 2) shows that the characters color (1, 3, 4), shape of blade (1, 2, 3, 4, 5) and shape of teeth (1, 2, 3, 4, 5) are variable and can be used for cultivars differentiation. The character related to leaf size show less variability represented by 2-3 different descriptor categories. Most cultivars had mature leaves with 3 lobes except MMKg had leaves with 4 lobes.

The relationships between the 30 Tunisian studied grapevine cultivars based on their morphological characteristics were reflected in the UPGMA dendrogram shown in Figure 1.

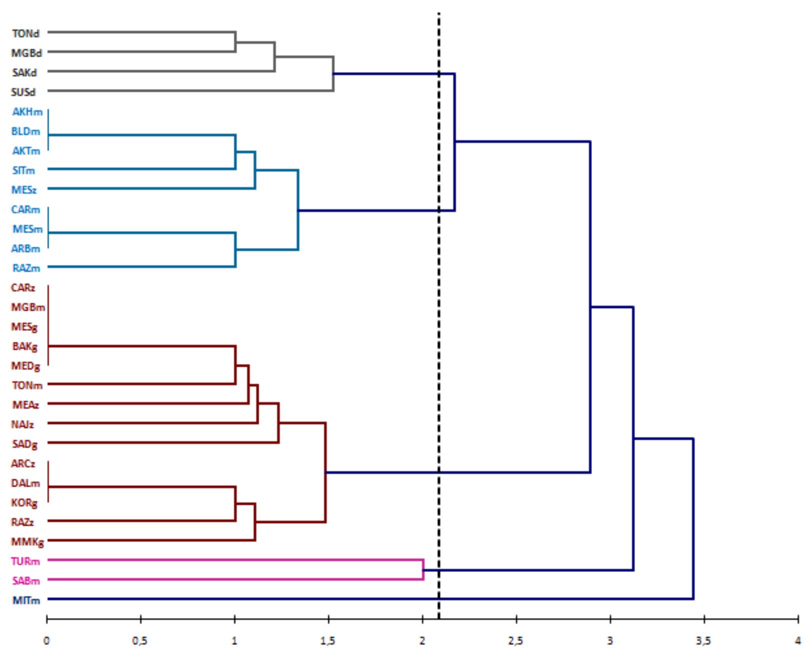


Fig 1. UPGMA dendrogram of Euclidean distance illustrating the genetic relationships among the 30 studied grapevine cultivars based on morphological characteristics.

At a dissimilitude level of 2.1, five clusters were revealed. The first cluster enclosed 4 cultivars from Djerba (TONd, MGBd, SAKd, SUSd). The second cluster consisted of 9 cultivars and can be subdivided into 2 subclusters at a dissimilitude level of 1.3. The first one contained the cultivars MESz, originated from Zarzis, and SITm, AKHm, BLDm and AKTm from Médenine. The second included the cultivars CARm, MESm, ARBm and RAZm from Médenine. The major third cluster was more heterogeneous. It is made of 14 cultivars: 6 cultivars were from Chénini Gabès (MESg, BAKg, MEDg, SADg, KORg, MMKg), 5 cultivars from Zarzis (CARz, MEAz, NAJz, ARCz, RAZz) and 3 other cultivar from Médenine (MGBm, TONm, DALm). These cultivars were subdivided into 2 subgroups. The forth cluster consisted just of TURm and SABm originated from Médenine. Finally, MITm cultivar is the merely member of the fifth cluster of the dendrogram. Our Data shows that various local cultivars exhibited significant morphological variability. Similar findings were obtained in previous works (Zaki & al. [9]; Harbi Ben Slimane [10], [2]) that were interested to the characterization of Tunisian autochthones cultivars based on ampelographic traits.

Observing this dendrogram several homonyms were revealed. For instance, the accessions 'MESm' from Médenine, 'MESz' from Zarzis and 'MESg' from Chénini Gabès, which are all known as 'Meski, but show different foliar characteristics, as well as the case of the two introduced accessions 'CARm' from Médenine and 'CARz' from Zarzis both named 'Cardinal', the two 'Tounsi' accessions, 'TONd' from Djerba and 'TONm' from Médenine and the two 'Mguargueb' accessions 'MGBd' and 'MGBm' from Djerba and Médenine, respectively.

In fact, several studies showed the existing of confusion in Tunisian grapevine nomenclature. Several putative synonyms and homonyms cases were reported (Zaki & al. [9]; Harbi-Ben Slimane [10], [2]; Zoghlami & al. [3], [11]; Snoussi & al. [12]).

The expression of morphological characters is influenced by environmental factors, individual plant biology, and life history. Besides, it is known that juvenile plants are nearly impossible to identify because within 4 or 5 years, they do not exhibit the typical morphological traits of adult plants. Some genetically related cultivars are morphologically very similar and difficult to differentiate by visual comparison (Aradhya & al. [13]). On the other hand, intravarietal clones can differ considerably in phenotype even though they have virtually identical DNA profiles (Vignani & al. [14]; Franks & al. [15]).

Muscats, known as 'Meski' in Tunisia, are a large widely distributed group of grape varieties appreciated for their special berry flavor, and have been used both for fruit and wine production (Sammoud & Askri [16]). Our results showed that the cultivars 'Meski' (MESz, MESm, MESg), 'Muscat d'Alexandrie' (MEAz) from Zarzis and 'Muscat d'Italie' (MITm) from Médenine showed different foliar characteristics and seems to be distinct from each other. Snoussi & al. [12] reported that Meski cultivars from Tunisia represent different genotypes that cannot be easily associated to either 'Muscat of Alexandria' or 'Moscato Bianco', which found to having related genotypic profiles (Crespan & Milani [17]).

Besides, dealing with relationships between Tunisian cultivars and commercial table grape varieties, we found that the commercial table grape 'Cardinal' (CARz) showed a high foliar similarity with cultivar 'Meski' (MESg), 'Bazzoul kalba' (BAKg), 'Medina' (MEDg) and 'Mguargueb' (MGBm). Snoussi & al. [12] reported that there is a little but significant genetic differentiation between local Tunisian cultivars and commercial table grape ones. Besides, higher differentiation between cultivated and wild Tunisian accessions could be revealed (Snoussi & al. [12]). Thus, Tunisian local cultivars are generally not related to the still existent grapevine wild populations, suggesting that most of them could derive from materials introduced in the region in different historical times. This is supported by historical data, since Tunisia has always been the crossroads of traders, and the area of choice for the settlement of various civilizations. Possible cases of cross pollination or local domestication could be occurred (Snoussi & al. [12]).

Table 2. Ampelographic values and foliar composition on mineral elements of the 30 studied accessions of Tunisian grapevine.

Cultivars	Morphological Traits					Mineral Composition							
	C	NL	SB	ST	S	Na	K	Ca	Mg	Cu	Zn	Fe	Mn
BLDm	3	3	2	2	2	0.038	1.426	1.618	0.146	0.00030	0.0015	0.027	0.004
RAZm	4	3	2	3	2	0.083	0.746	2.363	0.303	0.00033	0.0025	0.029	0.011
SABm	1	3	3	5	2	0.089	1.091	0.914	0.147	0.00057	0.0015	0.026	0.003
SITm	3	3	2	1	2	0.049	0.937	0.876	0.183	0.00049	0.0017	0.015	0.002
MITm	3	3	5	5	2	0.073	0.999	1.338	0.233	0.00007	0.0019	0.020	0.011
MESm	3	3	2	3	2	0.084	1.124	1.087	0.190	0.00039	0.0026	0.019	0.011
CARm	3	3	2	3	2	0.074	1.335	1.637	0.253	0.00032	0.0024	0.020	0.005
TONm	3	3	2	5	3	0.093	1.199	0.931	0.119	0.00015	0.0011	0.049	0.002
MGBm	3	3	2	5	2	0.058	1.596	1.297	0.130	0.00036	0.0014	0.022	0.003
ARBm	3	3	2	3	2	0.063	0.867	3.761	0.305	0.00045	0.0035	0.101	0.010
DALm	4	3	2	5	3	0.060	1.292	2.383	0.233	0.00044	0.0020	0.087	0.005
AKHm	3	3	2	2	2	0.052	0.723	1.517	0.165	0.00038	0.0027	0.029	0.005
AKTm	3	3	2	2	2	0.042	1.512	1.598	0.215	0.00029	0.0019	0.017	0.003
TURm	1	3	3	3	2	0.064	1.394	1.591	0.326	0.00018	0.0030	0.040	0.016
MEAz	3	3	2	4	2	0.063	0.688	3.361	0.339	0.00093	0.0015	0.070	0.009
RAZz	4	3	2	5	2	0.065	0.605	3.626	0.350	0.00139	0.0022	0.055	0.006
MESz	4	3	2	2	2	0.065	0.660	3.496	0.296	0.00134	0.0021	0.107	0.007
CARz	3	3	2	5	2	0.057	0.418	4.433	0.396	0.00107	0.0012	0.077	0.004
ARCz	4	3	2	5	3	0.057	2.582	1.480	0.277	0.00188	0.0018	0.033	0.004
NAJz	3	3	3	5	2	0.077	0.448	2.371	0.392	0.00088	0.0016	0.066	0.013
MGBd	4	3	4	2	3	0.036	0.437	4.336	0.559	0.00039	0.0013	0.031	0.009
SUSd	3	3	4	3	3	0.020	0.463	3.040	0.280	0.00065	0.0015	0.040	0.006
SAKd	4	3	3	3	3	0.032	0.270	2.600	0.230	0.00067	0.0006	0.033	0.005
TONd	4	3	3	2	3	0.048	0.369	3.707	0.546	0.00044	0.0016	0.051	0.005
BAKg	3	3	2	5	2	0.054	0.566	2.677	0.356	0.00039	0.0018	0.060	0.014
MESg	3	3	2	5	2	0.119	0.673	2.776	0.279	0.00056	0.0015	0.062	0.003
MEDg	3	3	2	5	2	0.047	0.993	4.874	0.495	0.00056	0.0025	0.071	0.007
KORg	4	3	2	5	3	0.049	1.428	3.742	0.512	0.00112	0.0039	0.053	0.005
MMKg	4	4	2	5	3	0.014	0.645	2.578	0.275	0.00041	0.0014	0.020	0.003
SADg	3	3	1	5	2	0.040	0.953	2.750	0.341	0.00082	0.0022	0.054	0.003

C: Color, **NL**: Number of Lobes, **SB**: Shape of Blade, **ST**: Shape of Teeth, **S**: Size.
Na: Sodium, **K**: Potassium, **Ca**: Calcium, **Mg**: Magnesium, **Cu**: Copper, **Zn**: Zinc, **Fe**: Iron, **Mn**: Manganese.

Mineral composition

Among the 8 major elements studied (Table 3), the calcium was the major element. Its content ranged from 0.87 to 4.87 mg/100 g, registered in SITm and MEDg cultivars, respectively. Potassium was the second major mineral. Its highest content (2.58 mg/100g) was registered in the ARCz cultivar and the lowest value (0.27 mg/100g) was detected in SAKd. The magnesium content has middling levels, with an average of 0.3 mg/100g. All the remaining elements had levels less than 0.12 mg/100g. Copper was detected only in traces.

Based on the UPGMA clustering analysis (Figure 2) dressed following Euclidean distance, the accessions were broadly grouped into 4 clusters at a dissimilarity level of 1.2, whereby the individuals in anyone cluster are more closely related than the individuals in different clusters. The First cluster had 11 accessions and can be subdivided into two sub groups at 0.7 level of dissimilarity. It was interesting to note that this cluster contains accessions that are all originated from Médenine and characterised by high foliar content on potassium and slight amounts of calcium and magnesium. The second cluster is made just by the cultivar ARCz from Zarzis which contains the highest foliar contents on potassium (2.58 mg/100g) and copper (0.0019 mg/100g). The third cluster was more heterogeneous and enclosed 9 cultivars: 4 cultivars from Zarzis (MESz, MEAz, RAZz, CARz), 2 cultivars from Djerba (TONd, MGBd), 1 from Médenine (ARBm) and the last one (KORg) from Chénini Gabès. These accessions are characterised by their high foliar contents of calcium and magnesium. Finally, the forth cluster, which is also heterogeneous, included 4 cultivars from Chénini Gabès (MMKg, MESg, BAKg, SADg), 2 cultivars from Djerba (SAKd, SUSd), 2 cultivars from Médenine (RAZm, DALm) and 1 cultivar from Zarzis (NAJz). These accessions contained moderate quantities of all minerals.

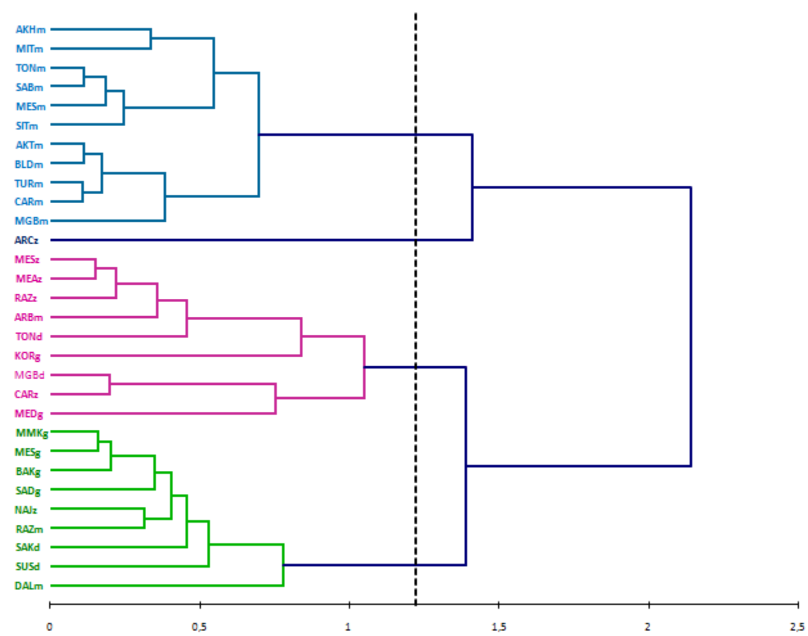


Fig 2. UPGMA dendrogram of Euclidean distance illustrating the genetic relationships among the 30 studied grapevine cultivars based on mineral content.

Dendrogram clustering showed a strong relationship between cultivars grouping/diversity and geographical origin. Recorded variability could be explained by both genetic and environmental conditions, such as: position of leaves on the branches, rootstock, nature of the soil, culture and climatic conditions, irrigation, salinity, etc (Bouillet [18]).

Earlier, several works were interested in studying the origin of these differences. Among the most reported factors, we noted the effect of the physicochemical composition of the ground on the plant content of mineral elements (Wallace [19]).

In fact, plant nutrition depends on soil mineral nutrients, but also on its pH which has a strong influence on the assimilation of these biogenic salts (Vilain [20]) and on its fertilization (Christensen [21]). Another component of the grounds having an extreme importance is water. In fact, any deficiency or excess of water can play a determining role on roots function, culture production and tissue mineral contents (Keller [22]). The role of the mode of irrigation has been evidenced on the growth and the mineral nutrition of the grapevine (Paranychianakis & al. [23]). Moreover, the effect of the rootstock on the content of mineral elements has been reported on many researches (Spring & al. [24]).

Comparison between morphology and mineral content analyses

To provide an objective comparison, matrices of cophenetic values, generated from morphological and mineral content data sets were compared using the Mantel's test (Figure 3). No significant correlation was found between the two kind of parameters (r = 0.036, p = 0.463>0.05) after doing 10000 random permutations using XLSTAT program. We believe that correlation between them could be improved if there was more morphological markers analyzed as was previously reported by other researchers (Harbi Ben Slimane [10], [2]) or more mineral content parameters were used.

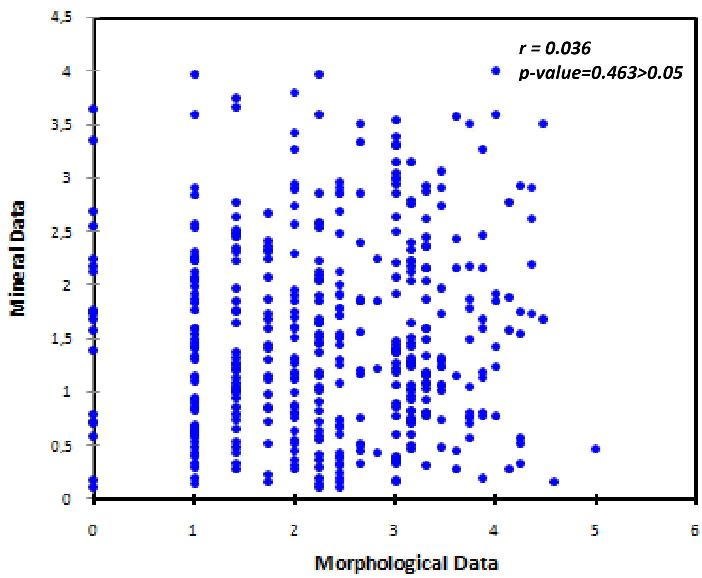


Fig 3. Correlation between the two cophenetic matrices (based on morphological and mineral parameters) of 30 grapevine accessions using Mantel's test.

Conclusions

The studied Tunisian grapevine accessions displayed a considerable level of variability based on foliar morphology and mineral content analysis. In fact, some of these parameters were designed suitable to discriminate significantly some cultivars, such as: ARCz, TURm, SABm, MITm…. Cluster analysis has proved to be an effective method in grouping accessions using both morphology and mineral content parameters. The correlation between the two systems was not significant. Molecular analysis could be for great interest in order to ascertain the extent of genetic diversity of local grapevine germplasm for further use and valorization.

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References

1. J.A. GREENE, D.P. KEHOE, Mago the Carthaginian on agriculture: archeology and the ancient sources In: Proceedings of the Cogress International des Estudes pheniciennes et puniques, Tunis, Tunisia, 1991, pp. 11-16.
2. M. HARBI-BEN SLIMANE, Ampélographie des vignes autochtones cultivées et spontanées de Tunisie, Volume I. INRAT/IPGRI CWANA ISBN 92-9043-502-x (2001).
3. N. ZOGHLAMI, A. MLIKI, A. Ghorbel, Evaluation of genetic diversity among Tunisian grapevines by RAPD markers. *Vitis*, 40, 31-37 (2001).
4. F. BEN ABDALLAH, F. CHIBANI, A. FNAYOU, A. GHORBEL, J.M. BOURSIQUOT, Caractérisation biochimique des variétés tunisiennes de vigne. *J. Int. Sci. Vigne Vin*, 32, 17-25 (1998).
5. IPGRI, UPOV, OIV, Descripteurs de la vigne (*Vitis* spp). Union internationale pour la Protection des Obtentions Végétales, Genève, Suisse/Office International de la Vigne et du Vin, Paris, France/Institut International des Ressources Phytogénétiques, Rome, Italie, 1997, 62 p.
6. P. GALET, Précis d'Ampélographie pratique. Imprimerie JF Impression, eds. 7, Saint Jean de Védas, 1998, 235 p.
7. P.H.A. SNEATH, R.R. SOKAL, Numerical taxonomy. Freeman, San Francisco, USA (1973).
8. N. MANTEL, A technique of disease clustering and a generalized regression approach. *Cancer Res*., 27, 209-220 (1967).
9. Z. ZAKI, M.L. KCHOUK, A. DOUIK, A. BEN SALEM, A. GHORBEL, M. ANNABI, Electronic imagery: a new method for grapevine identification. *Acta. Hort*., 441, 317-324 (1997).
10. M. HARBI-BEN SLIMANE, Etude de la variabilité génétique des vignes autochtones cultivées et spontanées de Tunisie. Thèse de doctorat. Université de Tunis II, Faculté des Sciences de Tunis, Tunis, Tunisie (1999).
11. N. ZOGHLAMI, L. RIAHI, V. LAUCOU, T. LACOMBE, A. MLIKI, A. GHORBEL, P. THIS, Origin and genetic diversity of Tunisian grapes as revealed by microsatellite markers. *Sci. Hort*., 120, 479-486 (2009).
12. H. SNOUSSI, M. HARBI BEN SLIMANE, L. GARCIA-RUIZ, J.M. MARTINEZ-ZAPATER, R. ARROYO-GARCIA, Genetic relationship among cultivated and wild grapevine accessions from Tunisia. *Genome*, 47, 1211-1219 (2004).
13. M.K. ARADHYA, G.S. DANGL, B.H. PRINS, J.M. BOURSIQUOT, A.M. WALKER, C.P. MEREDITH, C.J. SIMON, Genetic structure and differentiation in cultivated grape *Vitis vinifera* L. *Genet. Res. (Camb)*, 81, 179-192 (2003).
14. R. VIGNANI, J.E. BOWERS, C.P. MEREDITH, Microsatellite DNA polymorphism analysis of clones of Vitis vinifera 'Sangiovese'. *Sci. Hort*., 65, 163-169 (1996).
15. T. FRANKS, R. BOTTA, M.R. THOMAS, J. FRANKS, Chimerism in grapevines: implication for cultivar identity ancestry and genetic improvement. *Theor. Appl. Genet*., 104, 192-199 (2002).
16. S. SAMMOUD, F. ASKRI, Variétés de vigne en Tunisie, Bouslama, eds., Tunis (1987).
17. M. CRESPAN, N. MILANI, The Muscats: a molecular analysis of synonyms homonyms and genetic relationships within a large family of grapevine cultivars. *Vitis*, 40(1), 23-26 (2001).
18. JP. BOUILLET, Quelques données sur le diagnostic foliaire. Unité de recherche sur la productivité des plantations industrielles U. R. 2P. I, 1996, 22 p.
19. T. WALLACE, The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms. Chapter III: Methods of Determining Mineral Deficiencies in Crops (1943).
20. M. VILAIN, La production végétale, Volume 1. Les composantes de la production. Agriculture d'Aujourd'hui, Sciences, Techniques, Application. Coll. Technique et Documentation, Paris, Lavoisier, 1993, 438 p.
21. P. CHRISTENSEN, Foliar fertilization in vine mineral nutrient management programs, In P. Christensen, D.R. Smart, eds., Proceedings of the Soil Environment and Vine Mineral Nutrition Symposium American Society of Enology and Viticulture, Davis, CA, 2005, pp 83-90.
22. M. KELLER, Deficit Irrigation and Vine Mineral Nutrition. *Am. J. Enol. Vitic*., 56(3), 267-283 (2005).
23. N.V. PARANYCHIANAKIS, M. NIKOLANTONAKIS, Y. SPANAKIS, A.N. ANGELAKIS, The effect of recycled water on the nutrient status of Sultanina grapevines grafted on different rootstocks. *Agricultural Water Management*, 81, 185-198 (2006).
24. J.L. SPRING, J. AERNY, J.P. RYSE, Influence du porte-greffe sur l'alimentation minérale du greffon Premiers résultats d'un essai avec du Chasselas et du Gamay dans le bassin lémanique. Revue Suisse de Viticulture, Arboriculture Horticulture, Suisse. 6, 321-327 (1999).