Article

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Mehdi Trad & Mounira Harbi

Towards a bio-morphometric approach for the discrimination between wild and domesticated vines under Mediterranean environment

Abstract

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All the traditional vines, constituting the subspecies *Vitis vinifera* subsp. *vinifera*, were domesticated from the wild *V. vinifera* subsp. *silvestris*. Shape and morphometry of the seed provides information to differentiate between wild and cultivated vine populations. Measurements were made on grape seeds for the assessment of genetic diversity and for the discrimination between the two subspecies according to the Stummer index. Morphometric analysis was carried out on 340 seeds from 34 ecotypes (22 wild and 12 cultivated) originated from the north of Tunisia. In the wild vine population, seed length (L) varied between 3.2 and 5.2 mm, beak length (LS) between 0.4 and 1.7 mm and breadth (B) between 1.7 and 3.2 mm. The Stummer index (SI) was comprised between 51.43 and 88.57. Among the twenty-two wild ecotypes, seven confirmed their belonging to the spontaneous morphotype and four had 95% chance to belong to the cultivated morphotype (EC.30, EC.33, CPN5-2000 and CPN6-2000). In the cultivated vine pool, seed length ranged from 3.8 to 5.2 mm, stalk length from 0.8 to 1.4 mm and the total width from 2.3 to 3.2 mm. The Stummer index varied between 56.10 and 65.00. Among the twelve cultivars, five confirmed their belonging to the cultivated morphotype and three showed 95% chance to belong to the spontaneous type (Bidh El Hmem, Chaaraoui and Meski Rafraf). The results allowed us to relate a probable genetic proximity between spontaneous and some cultivated vines and to retrace the evolution pathway of local vine gene pool.

Key words: Discriminant function analysis, genetic variability, morphometry, plant selection; Stummer index; *Vitis vinifera*.

Introduction

Most botanists regard the wild ancestral grape *V. silvestris* as the primitive form of the cultivated grape because of the close morphological resemblance and free gene flow between them (Heywood & Zohary 1991). Since the 19th century, many researches have been carried out to clarify the evolution of species in agriculture (Zohary & Spiegel-Roy 1975; Zohary & Hopf 1988; Mc Govern & al. 1996; Terral & al. 2010).

Grapevine is one of the most important crops in the Mediterranean region and has been the subject of a number of studies aiming to discriminate between wild and cultivated vines. Discrimination between wild and cultivated accessions used biometric approach based on the length/width ratio of the seed. This ratio, designated by the Stummer index (Stummer 1911), was the basis of the work of Marinval (1988) to establish a date of appearance of viticulture in the second Iron Age (Marinval 1988; Buxo & Capdevila 1996; Bouby & Marinval 2001). Since the work of Stummer (1911) it has been generally accepted that domestication has caused changes in the dimensions and some other morphological characteristics of the seed, so that in general it was possible to distinguish cultivated from wild vines. Stummer (1911) first and Mangafa & Kotsakis (1996) later, described seeds of wild vines as small, robust, with a rounded outline, or cordate, with short stalks. They were almost flat ventrally with sharp angles and a strongly developed chalaza. On the other hand, seeds of domesticated vines were described as large, elongated, oval or pyriform, with a longer stalk. They were more rounded ventrally and less sharply sculptured. Schieman (1953) has completed the Stummer studies by reporting that the width/length index varies with the grape variety. In the last few years, several studies have been carried out using the berry seed of the vine to classify it as cultivated or spontaneous. This provides proposals and hypotheses on vine evolution and phyllogenetic relationships between cultivated and spontaneous populations (El Oualkadi & al. 2011; Ucchesu & al. 2016; Ardenghi & al. 2017). For the description of the corpus of the seed, these researches considered qualitative criteria primarily related to the stalk, the chalaza or the fossete. Mangafa and Kotsakis (1996) proposed four formulas following the application of the four-dimensional measurement of the seed. The effectiveness of each formula varies from one vine to another. These formulas show good aptitude to recognize the seeds of the cultivated vines from those of the spontaneous vines while being only exploitable in archeobotany for Greek vines.

Tunisian gene pool of the *Vitis* genus is mostly concentrated in the north side of the country. Despite the abundance of introduced cultivars for commercial purposes, many farmers still preserve autochthonous cultivars with a strong faculty of adaptation to marginal soil and changing climate conditions. Considered as a significant part of the local vine heritage, wild grapevines were encountered in isolated forms or in groupings, in humid locations, along permanent water courses in the down side of hills and mountains showing a large polymorphism and a great morphological variability (Harbi & al. 2010; Trad & al. 2017). Their number remains limited as human activities are rapidly eroding the size of wild grapevine populations. *Vitis vinifera* subsp. *silvestris* Hegi, is a unique and valuable genetic resource for the improvement of cultivated grapevines regarding their genetic tolerance to salinity (Raymond & al. 2008), their resistance to many virus diseases and their high adaptation potential to different soil types and climates (Ocete & al. 1995; Arnold & al. 1998).

In the present work, we propose to test the accuracy and reliability of the two main methods used, namely the Stummer index and the application of the Mangafa and Kotsakis formulas for the characterization of Tunisian autochthonous vine seeds, their shapes and their dimensions, to specify their attribution to either wild (*Vitis vinifera* subsp. *silvestris*) or cultivated (*Vitis vinifera* subsp. *vinifera*) vine. Data results will help to explore the course of development and domestication of grapevines and to retrace the history of viticulture in Tunisia.

Materials and methods

Plant material.- During the two seasons 2014 and 2015, lots of grapes were picked from about 34 vines (22 wild and 12 cultivated) growing under Mediterranean conditions in the north of Tunisia. Grape clusters were collected in different says after anthesis, depending on the geographic origins of the vine plants: Nefza, Outchtata, Tabarka, Ain Draham, Cap Negro and Sejnane for spontaneous vines, and Rafraf for domesticated ones (Fig. 1). The group of local cultivars is mainly table type but also include a dual-use cultivar (Table 1). All these accessions are actually part of the national germplasm collection maintained in the vineyards of the Horticulture laboratory in the National Institute for Agricultural Research (INRAT, Tunisia) and Herbarium specimens have been deposited in the institute. A leaf comparison is provided to show variability between the grapevine genetic resources studied (Fig. 2). Seeds derived from both cultivated and wild vines were examined for their morphometric data.

Seed measurements.- Morphometric analysis covered 340 seeds from the 34 ecotypes. The twelve autochthonous vines are representative of the varietal assortment of local cultivars growing under Mediterranean conditions in the North of Tunisia. Depending on the availability of grape clusters, especially for wild vines, a minimum of 5 bunches per clone were collected. From each vine cep was collected one grape bunch. Seeds were first removed from the berry, dried at room temperature and cleaned finally from pulp trash to finally constitute a batch from which ten seeds per clone were randomly picked. The following measurements were taken: length (L), breadth (B), length of the stalk (LS), placement of chalaza, i.e. the distance from the base of chalaza to the tip of the stalk (PCH) (Fig. 3) (Mangafa & Kotsakis 1996). Then, the following indices were calculated: breath to length (B/L), length of stalk to length (LS/L) and placement of chalaza to length (PCH/L). The diagnostic features of the seeds considered these dimensions as reference to Negrul (1960) who thought that all these parameters are the most important characteristics of the seed. Two morphometric approaches were tested: the Stummer Index (IS) and the four formulas proposed by Mangafa & Kotsakis (1996). These are formulas based on four measurements performed on the grape seed. From these four measurements were calculated:

The Stummer index: SI = B/L 100

An ecotype whose Stummer index varies between 58.37 and 61.89 has 95% chance of belonging to the cultivated type. An ecotype whose Stummer index varies between 62.72 and 68.58 has 95% chance of belonging to the spontaneous type.

The indices: LS/L and PCH/L used in the formulas of Mangafa and Kotsakis according to:

$$Fomula\ 1:\ -0.3801 + (-30.2 \times LS/L) + (0.4564 \times PCH) - (1.386 \times L) + (2.88 \times PCH/L) + (9.4239 \times LS)$$

Values smaller than -0.2 indicate wild seeds and values bigger than 0.8 indicate cultivated ones. Values from -0.2 to 0.2 indicate seeds which have a 64.7% chance of being wild while values from 0.2 to 0.8 indicate seeds which have a 76.2% chance of being cultivated.

$$Formula\ 2: 0.2951 + (-12.64 \times PCH/L) - (1.6416 \times L) + (4.5131 \times PCH) + (9.63 \times LS/L)$$

Values smaller than -0.2 indicate wild seeds and values bigger than 0.9 indicate cultivated ones. Values from -0.2 to 0.4 indicate seeds which have a 90.1% chance of being wild while values from 0.4 to 0.9 seeds which have a 63.8% chance of being cultivated.

$$Formula\ 3{:}\ -7.491 + (1.7715 \times PCH) + (0.49 \times PCH/L) + (9.56 \times LS/L)$$

Values smaller than 0 indicate wild seeds and values bigger than 0.9 indicate cultivated ones. Values from 0 to 0.5 indicate seeds which have a 93.3% chance of being wild while values from 0.5 to 0.9 seeds which have a 63.3% chance of being cultivated.

$$Formula\ 4{:}\ 0.7509 + (-1.5748 \times L) + (5.297 \times PCH) - (14.47 \times PCH/L)$$

Values smaller than -0.9 indicate wild seeds and values bigger than 1.4 indicate cultivated ones. Values from -0.9 to 0.2 indicate seeds which have a 91% chance of being wild while values from 0.2 to 1.4 seeds which have a 76.5% chance of being cultivated.

Statistics.- For each variable, means, standard deviation, univariate ANOVA were calculated to assess differences (p <0.05). Discriminant function analysis was considered based on linear combinations of the predictor variables to provide the best discriminations between groups. Wilks' Lambda was used to test for significant differences between groups and summary of canonical discriminant functions was displayed with the eigenvalues and structure matrix. Classification statistics were launched to assess the how well the discriminant function works.

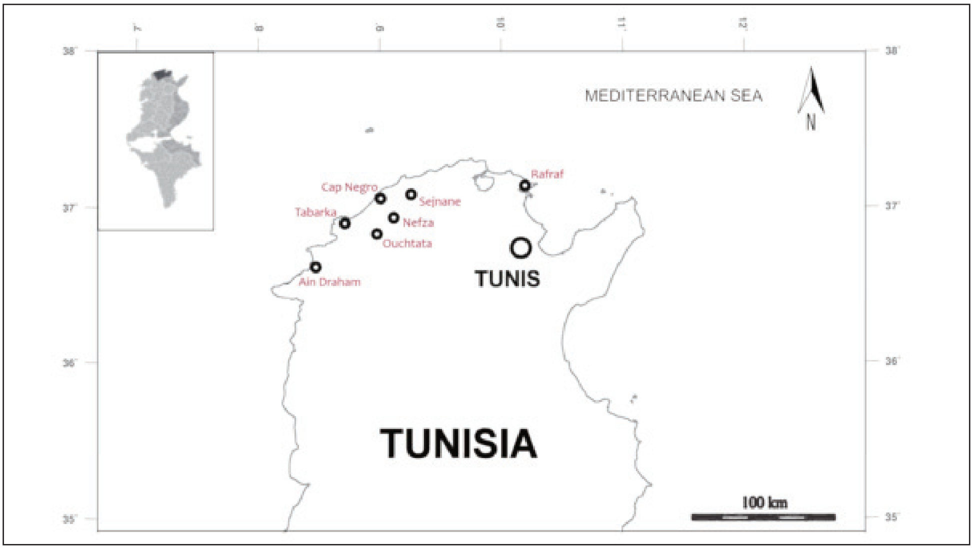

Fig. 1. Origin sites of the wild *Vitis vinifera* subsp. *silvestris* and the cultivated *V. vinifera* subsp. *vinifera* grapes collected from the north of Tunisia.

Table 1. General description and metric data of the seed in wild and cultivated grapes. (Means ± SD). L: total length, LS: length of the beack, PCH: placement of chalaza, B: total breadth, W: weight of 100 seeds. L, LS, PCH and B are expressed in (mm); W is expressed in (g)

				Berry		*Seed*				
Ecotype	Code	Origin	Main use	Shape [a]	Colour [a]	L	LS	PCH	B	W
Wild										
01	EC.01	Nefza	-	Round	Blue-black	3.5 ± 0.2	1.0 ± 0.1	2.1 ± 0.2	1.8 ± 0.1	5.88
03	EC.03	Nefza	-	Round	Blue-black	3.6 ± 0.2	0.5 ± 0.1	1.8 ± 0.1	3.1 ± 0.1	9.70
08	EC.08	Nefza	-	Round	Green-yellow	4.3 ± 0.1	0.9 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	7.61
09	EC.09	Nefza	-	Round	Green-yellow	4.5 ± 0.2	1.0 ± 0.2	2.4 ± 0.1	2.6 ± 0.1	7.43
10	EC.10	Nefza	-	Round	Blue-black	3.8 ± 0.1	0.6 ± 0.1	1.8 ± 0.1	2.1 ± 0.2	8.37
13	EC.13	Nefza	-	Round	Blue-black	4.0 ± 0.2	0.7 ± 0.1	2.0 ± 0.1	2.6 ± 0.2	8.17
14	EC.14	Nefza	-	Round	Blue-black	4.3 ± 0.1	0.8 ± 0.1	1.9 ± 0.1	2.9 ± 0.1	6.57
15	EC.15	Nefza	-	Round	Blue-black	3.8 ± 0.2	0.9 ± 0.2	1.6 ± 0.1	2.5 ± 0.2	7.20
26	EC.26	Ain Draham	-	Round	Dark red-violet	3.5 ± 0.2	0.8 ± 0.1	1.7 ± 0.1	3.1 ± 0.1	7.85
30	EC.30	Tabarka	-	Round	Dark red-violet	4.6 ± 0.2	1.3 ± 0.1	2.4 ± 0.1	2.9 ± 0.2	8.44
33	EC.33	Tabarka	-	Round	Blue-black	4.2 ± 0.2	1.3 ± 0.1	2.3 ± 0.2	2.6 ± 0.1	8.45
35	EC.35	Tabarka	-	Round	Green-yellow	4.4 ± 0.3	1.3 ± 0.3	2.4 ± 0.3	2.8 ± 0.2	5.72
45	EC.45	Sejnane	-	Round	Blue-black	3.9 ± 0.1	0.9 ± 0.1	2.0 ± 0.1	2.7 ± 0.1	5.44
46	EC.46	Sejnane	-	Round	Blue-black	3.3 ± 0.2	0.6 ± 0.2	1.6 ± 0.1	2.6 ± 0.2	6.60
Cap Negro 5-2000	CPN5-2000	Cap Negro	-	Round	Blue-black	4.3 ± 0.2	1.1 ± 0.2	2.3 ± 0.2	2.6 ± 0.1	5.36
Cap Negro 6-2000	CPN6-2000	Cap Negro	-	Round	Blue-black	4.6 ± 0.1	1.4 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	6.13
Cap Negro I	CPN-I	Cap Negro	-	Round	Dark red-violet	3.9 ± 0.2	0.9 ± 0.2	1.9 ± 0.1	2.8 ± 0.2	7.50
Cap Negro III	CPN-III	Cap Negro	-	Round	Dark red-violet	3.8 ± 0.3	1.5 ± 0.1	2.6 ± 0.2	2.4 ± 0.2	7.13
Cap Negro IV	CPN-IV	Cap Negro	-	Round	Dark red-violet	3.9 ± 0.1	0.8 ± 0.1	1.9 ± 0.1	2.7 ± 0.1	7.41
Ouchtata 3	OCT3	Ouchtata	-	Round	Green-yellow	3.7 ± 0.1	0.9 ± 0.1	2.0 ± 0.1	2.4 ± 0.1	6.63
Ouchtata 16	OCT16	Ouchtata	-	Round	Green-yellow	3.7 ± 0.1	0.7 ± 0.1	1.6 ± 0.2	2.6 ± 0.0	6.54
Ouchtata 17-2000	OCT17-2000	Ouchtata	-	Round	Blue-black	3.7 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	2.4 ± 0.1	6.70
Cultivated										
Bidh El Hmem	BHM	Rafraf	Table	Obovate	Green-yellow	4.1 ± 0.1	1.0 ± 0.1	2.0 ± 0.1	2.8 ± 0.2	7.23
Tebourbi	TEB	Rafraf	Table	Narrow elliptic	Green-yellow	4.2 ± 0.1	1.4 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	4.92
Bazzoul Khadem	BZK	Rafraf	Table	Narrow elliptic	Dark red-violet	4.7 ± 0.2	1.3 ± 0.2	2.5 ± 0.1	2.6 ± 0.2	5.11
Meski Rafraf	MKR	Rafraf	Table/wine	Ovate	Green-yellow	4.4 ± 0.1	1.2 ± 0.2	2.4 ± 0.2	2.8 ± 0.1	6.13
Chaaraoui	CHR	Rafraf	Table	Elliptic	Green-yellow	3.7 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	2.4 ± 0.1	4.45
Marsaoui	MRS	Rafraf	Table	Round	Green-yellow	5.0 ± 0.2	1.1 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	9.31
Farrani	FRN	Rafraf	Table	Oblate	Green-yellow	4.3 ± 0.1	1.0 ± 0.6	2.0 ± 0.1	2.6 ± 0.2	6.03
Boukhasla	BKS	Rafraf	Table	Round	Dark red-violet	4.3 ± 0.3	1.4 ± 0.2	2.3 ± 0.2	2.6 ± 0.2	7.67
Beldi	BLD	Rafraf	Table	Round	Green-yellow	3.9 ± 0.1	1.3 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	4.62
Essifi	ESF	Rafraf	Table	Round	Green-yellow	4.1 ± 0.1	1.2 ± 0.1	2.4 ± 0.2	2.3 ± 0.2	6.98
Hammemi	HMM	Rafraf	Table	Obtuse-ovate	Green-yellow	4.1 ± 0.1	1.1 ± 0.1	2.2 ± 0.2	2.5 ± 0.2	7.11
Souabaa Eljia	SBE	Rafraf	Table	Arched	Green-yellow	4.8 ± 0.2	1.3 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	7.04

[a] Descriptors for grapevine (IPGRI, UPOV, OIV 1997)

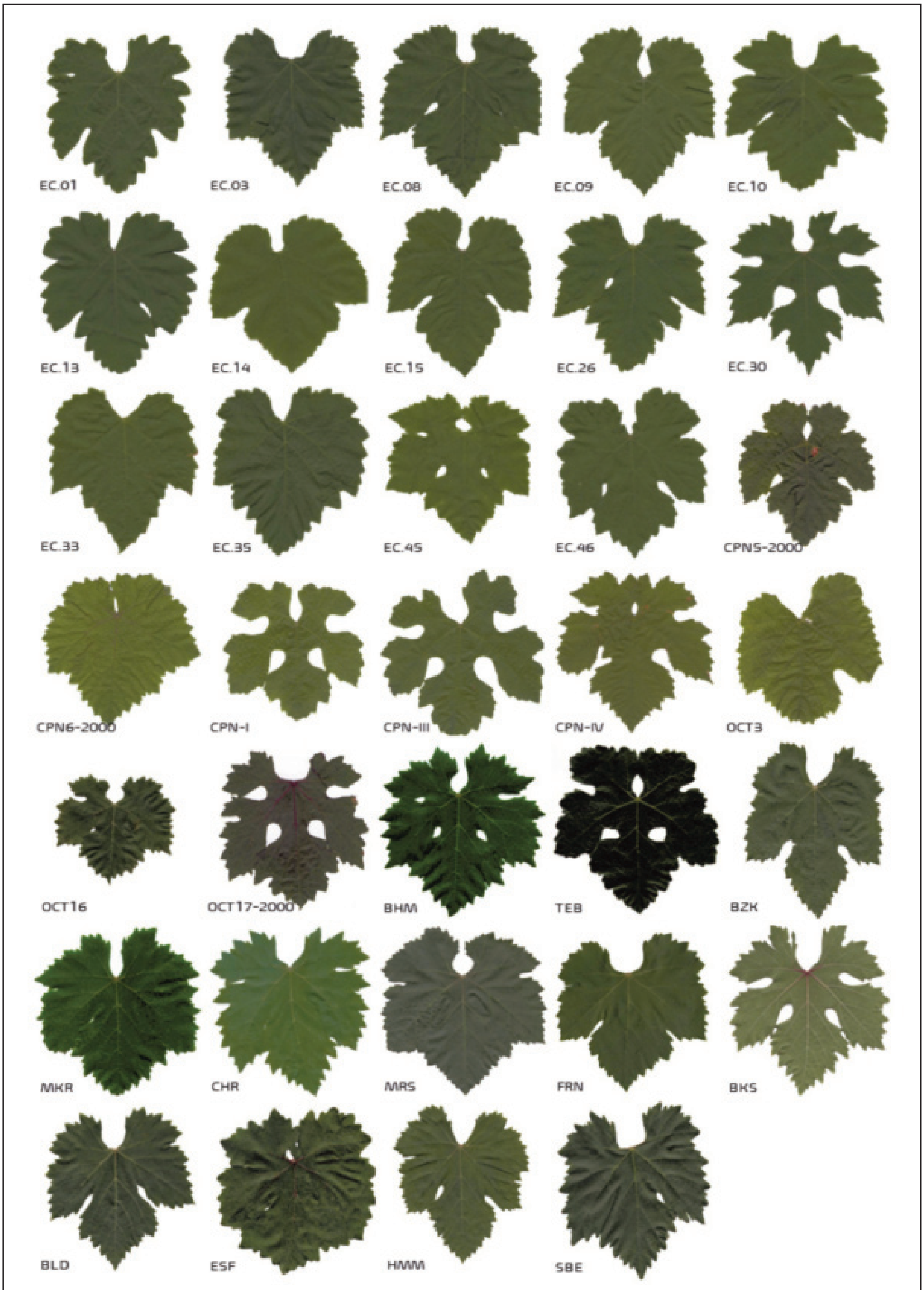

Fig. 2. Leaf variation of the spontaneous and domesticated grapevines composing the vineyard collection of the National Institute of Agricultural Research.

Multivariate analysis was tested using principal component analysis (PCA) to layout groups of vines with homogeneous metric characters of the seed. All these statistics were performed using PC software package SPSS (IBM® SPSS® Statistics, version 20.0.0).

Results

General description of the berry.- Among the 22 spontaneous grapevines, five produce green-yellow berries (Table 1). These ecotypes are originated from Nefza (2), Ouchtata (2) and Tabarka (1). Other ecotypes develop small bunches with round dark coloured berries which correspond to the general feature of wild grapes encountered in other locations from the Mediterranean basin (Levadoux 1956; Ocete & al. 1999; Cunha & al. 2007). The white-berry vines come close to *V. sativa* and could be considered as autochthonous (Levadoux 1956; Imazio & al. 2015). Local grape cultivars developed large berries with a wide range of forms, green yellow coloured for most of them, and are clearly differentiated from wild vines (Table 1). 'Meski Rafraf' is the single cultivar used for dual purposes (table and wine). The other cultivars are mainly for fresh consumption. 'Meski Rafraf' belongs to the group of Muscats. This group is widely distributed in Mediterranean area and well appreciated for the typical berry flavour with high sugar rate and low acid content (Snoussi & al. 2004; Trad & al. 2017). Characters describing the berry can therefore provide a preliminary differentiating criterion between wild and domesticated grapevines.

Seed morphometry of Vitis *grapes*.- The analysis of the 340 seeds from vines growing under Mediterranean environment showed a large variability in shape and size (Table 1). Wild grapes were characterized by spherical seeds with a small beak while cultivated grapes developed pyriform-shaped seed with a well-developed beak (Fig. 3). In wild vines, size of the seed ranged between 3.3 and 4.6 mm in length and between 1.8 and 3.1 mm in breadth. Placement of chalaza ranged between 1.4 and 2.6 mm. In *V. vinifera* subsp. *vinifera*, length of the seed varied between 3.7 and 5.0 mm and the breadth between 2.3 and 2.9 mm. Placement of chalaza varied between 2.0 and 2.6 mm.

Fig. 3. (**A**) General appearance and dimensions of a grape seed. L: total length, LS: length of the beak, PCH: placement of chalaza, B: total breadth (From Mangafa & Kotsakis 1996). **(B)** Seed from cultivar 'Beldi'. **(C)** Seed from wild 'EC.46'. Left: dorsal side, right: ventral side. (Binocular × 10).

Differences between the 34 genotypes were highly significant ($p < 0.01$) for all metric parameters. The longest seed was found in cultivar 'Marsaoui' while the largest seed was identified in EC.03 and EC.26. The wild 'Cap Negro III' showed the longest beak paradoxically to most other wild genotypes for which the beak is very small. In wild populations, the length of the beak ranged between 0.5 and 1.5 mm, while it was comprised between 1.0 and 1.7 mm in domesticated grapes. Most of the wild ecotypes developed seeds with short beak compared to cultivated vines. The beak is generally considered to be a valuable characteristic, as under the effects of cultivation its length increases proportionally faster than the length of the corpus (Negrul 1960).

The average width/length ratio was 0.66 in wild seeds and reached 0.63 in domesticated grape seeds. The weight, expressed as gram per 100 seeds, was comprised between 5.36 and 9.70 g in wild vines and between 4.45 and 9.31 g in cultivated vines. In general, wild ecotypes produced heavier seeds compared to domesticated grapevines. Disparity between wild and cultivated grapes was particularly observed in seed/berry ratio. This proportion is more significant in wild grapes which produce small berries with a large seed in a tiny pulp.

Discriminant function analysis (DFA).- The test of equality of group means indicates that Stummer index and formula 4 are the most discriminating variables (Table 2). The smaller the Wilks' Lambda is, the more important the independent variable to the discriminant function (Wilks' Lambda = 0.063 and 0.099 respectively for IS and F4). Additionally, Examination of the F test leads to exclude the (F4) formula. F test value is high for Stummer index compared to the other four formulas proposed by Mangafa & Kotsakis (1996). So, the Stummer index seems to have more influence and to provide the best discrimination between wild and cultivated grape genotypes.

The summary of canonical discriminant functions displayed in Table 3 indicates that with eigenvalue of 21.08, most of the variance (67.3 %) in the dependant variable is explained by function 1 which is strongly correlated with the index of Stummer (Table 4). Thus, it becomes possible to formulate the discriminant function (DF) basing on the unstandardized discriminant function coefficients presented in Table 5.

Classification statistics showed that the model adopted correctly classified more than 68% of the genotypes. Overall, 68.6% of the grape genotypes are correctly classified. In charred grape seeds, Mangafa & Kotsakis (1996) recommended the use of the second and third formulae as more appropriate for the identification of archaeological grapevines.

Classification of Vitis *ecotypes*.- In the following, the classification will take into account the Stummer index. The Stummer index (SI) varied from 51.43 to 89.46 (variance = 80.4) for wild grapes while it was between 54.21 and 67.45 (variance = 14.7) for cultivated grapes (Fig. 4). Among the 22 wild ecotypes, seven confirmed their belonging to the spontaneous morphotype (EC.13, EC.14, EC.15, EC.35, CPN-III, OCT3 and OCT17-2000) and four had 95% chance to belong to the cultivated type (EC.30, EC.33, CPN5-2000 and CPN6-2000). The confirmed spontaneous types originated for most of them from Nefza, while the four ecotypes, presumably belonging to the cultivated type, originated from Tabarka and Cap Negro respectively i.e. 34 km away and even closer to the coast.

Table 2. Tests of equality of group means.

	Wilks' Lambda	F	df1	df2	Sig.
Index of Stummer	0.063	30.797	33	68	0.000
Formula 1	0.155	11.248	33	68	0.000
Formula 2	0.152	11.541	33	68	0.000
Formula 3	0.134	13.362	33	68	0.000
Formula 4	0.099	18.681	33	68	0.000

Table 3. Summary of canonical discriminant functions – *Eigenvalues.*

Eigenvalues				
Function	Eigenvalue	% of variance	Cumulative %	Canonical correlation
1	21.09	67.3	67.3	0.977
2	5.00	16.0	83.2	0.913
3	3.03	9.7	92.9	0.867
4	1.73	5.5	98.4	0.796
5	0.50	1.6	100.0	0.572

Table 4. Summary of canonical discriminant functions – *Structure matrix.*

	Function				
	1	**2**	**3**	**4**	**5**
Index of Stummer	0.802*	0.464	0.283	-0.169	0.182
Formula 4	-0.523	0.795*	0.170	0.153	-0.207
Formula 2	-0.398	0.265	0.762*	0.198	0.388
Formula 3	-0.445	0.332	0.748*	0.036	0.363
Formula 1	-0.401	0.480	0.449	0.300	0.564*

Pooled with-in groups correlations between discriminating variables and standardized canonical discriminant functions. Variables ordered by absolute size of correlation within function.
*: largest absolute correlation between each variable and any discriminant function.

Table 5. Canonical discriminant function coefficients.

	Function				
	1	**2**	**3**	**4**	**5**
Index of Stummer	0.336	0.197	0.119	-0.024	-0.011
Formula 1	-0.697	1.531	-3.524	0.341	4.980
Formula 2	3.721	-2.963	6.012	12.734	-6.385
Formula 3	-3.707	1.128	-1.062	-12.240	3.753
Formula 4	-0.487	3.082	0.351	2.066	-4.345
(Constant)	-23.862	-6.031	-7.424	5.246	-2.722

Unstandardized coefficients.

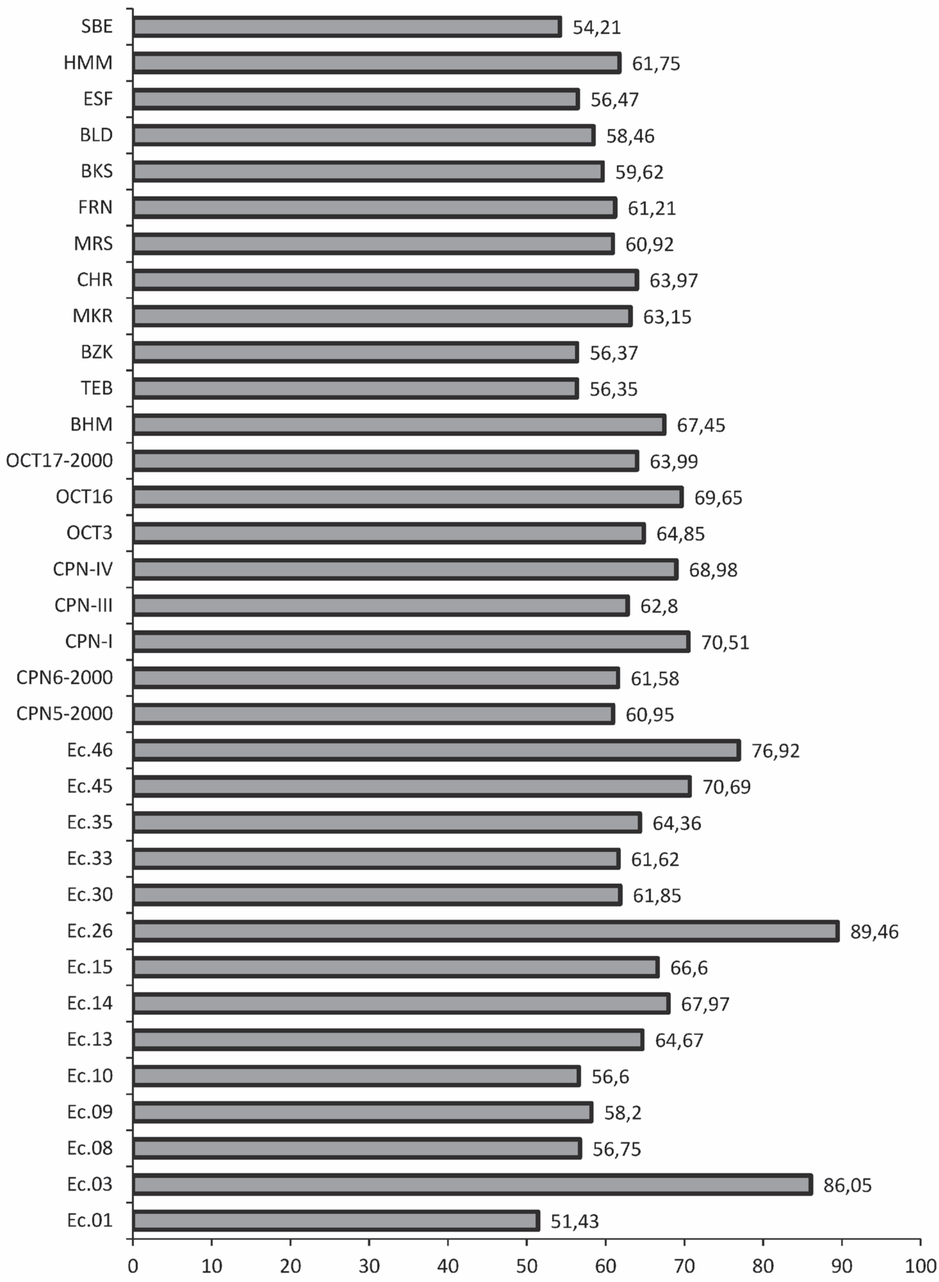

Fig. 4. Stummer index (SI) for the 34 wild and cultivated Tunisian grape ecotypes.

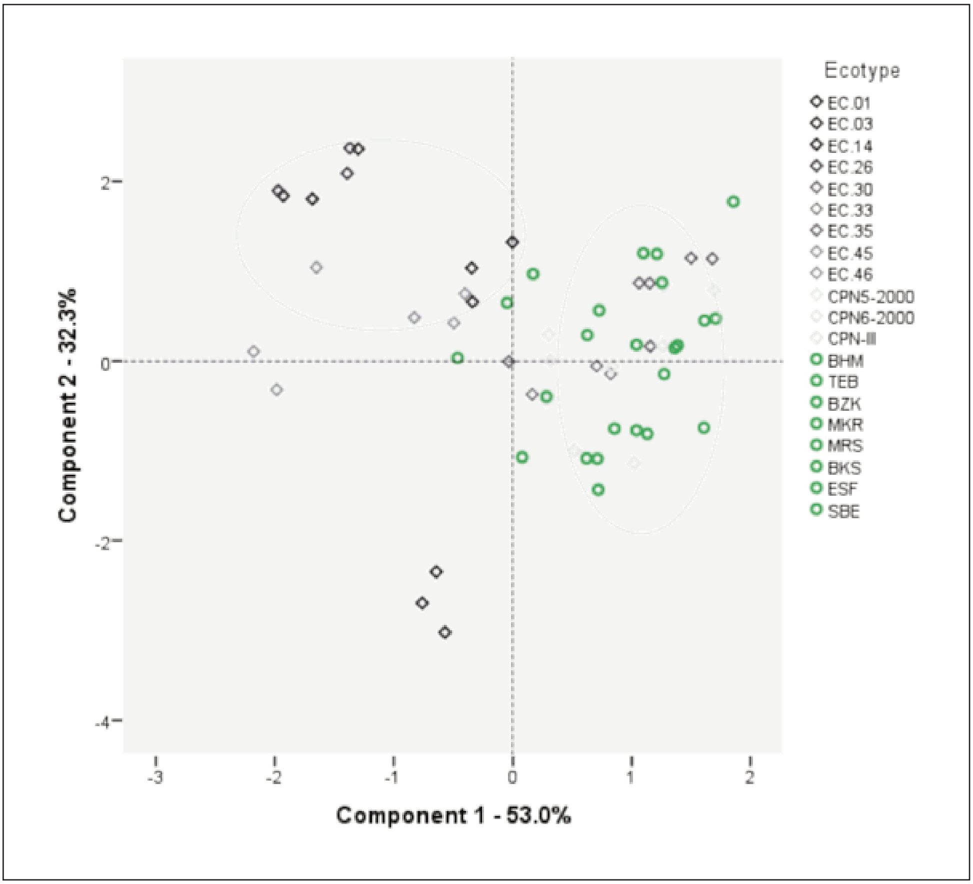

Fig. 5. PCA showing bi-dimensional discrimination between wild and domesticated grapes on the basis of morphometry and Stummer index of the seed.

From the twelve traditional varieties cropped in Rafraf, five confirmed their belonging to the cultivated morphotype (Beldi, Boukhasla, Farrani, Hammemi and Marsaoui) and three showed 95% chance of belonging to the spontaneous type (Bidh El Hmem, Chaaraoui and Meski Rafraf). The formers are definitely moving away from the *silvestris* type while the evolutionary process lasted even longer in the last cultivars (Levadoux 1956). The other unclassified grapevines could belong to either subspecies.

Principal component analysis (PCA).- Two components were extracted using PCA after varimax rotation and Kaiser Normalization. 85% of the total variance was explained by the two axes. The dispersion of the ecotypes according to axes 1 and 2 emphasized two main groups (Fig. 5): the first group is composed by cultivars ‘Tebourbi’, ‘Bazzoul Khadem’, ‘Meski Rafraf’, ‘Marsaoui’, ‘Boukhasla’, ‘Essifi’ and ‘Souabaa Eljia’. They are all varieties with long seeds. The second group comprises wild ecotypes 'EC.03', 'EC.14', 'EC.26' and 'EC.46' characterized by large seeds with high Stummer index which provides information in relation with shape and curves of the seed.

Component 1 explained 53% of the variance and was well correlated with placement of chalaza, beak length and total length of the seed. This component assembled the group of local grapevines characterized by a long seed and unambiguous chalaza (Fig. 3). This axis would be a dimension axis. Component 2 explained 32% of the variance and was correlated with total breadth and Stummer index. This second component grouped four among the twelve wild ecotypes (EC.03, EC.14, EC.26 and EC.46) characterized by a large seed with high SI value. This axis would be a form axis.

The PCA classification of the collected local ecotypes reinforced their characterization and allowed the recognition of ecotypes of spontaneous vines originating from the north of Tunisia. Placement of chalaza, beak length and total length of the seed were the most predictive features for the discrimination between grape populations as they are not influenced by the year of sampling.

Discussion

Morphological data of grape seeds are important for the identity of cultivars, history, biogeography and mechanisms of grapevine domestication (Terral & al. 2010). In biological populations, shape of the seed is less variable and less subject to environmental factors than size (Mangafa & Kotsakis 1996). Morphometric analysis of grape seeds from Mediterranean locations in Tunisia has allowed us to attribute a small proportion of them to the wild *V. vinifera* subsp. *silvestris* and an equivalent proportion to the domesticated *V. vinifera* subsp. *vinifera*. This joins the idea of Bouby & al. (2010) who stated that the number of seeds attributed to the wild type is comparable to that attributed to the cultivated one whatever the discriminating method applied.

Classification based on the Stummer index showed an inter-reliability and morphological connection between seeds from wild and domesticated grapevines. In wild populations, the SI was comprised between 51 and 89. Wild grapes picked from different locations in Portugal showed seeds with SI higher than 75 in three ecotypes and between 65 and 75 in three other vines (Cunha & al. 2007). Indexes lower than 65 were not detected unlike the values reported here for some ecotypes. Schiemann (1953), working on seven Austrian grapevines, indicated an SI between 54 (cv. Sylvaner) and 65 (cv. Elbling). Such results strongly agree with those identified for local cultivars with SI ranging between 54 and 67.

Studies on seeds were carried out to allow discrimination between wild and cultivated grapes (Kislev 1988; Stummer 1911; Mangafa & Kotsakis 1996; Marinval 1997; Terral & al. 2010) and to distinguish the genetic diversity of local resources. Tunisian wild vines were clearly discriminated from cultivated vines by large spherical seeds deprived of stalk in most accessions. This genetic differentiation between wild and local cultivated populations was proved by Snoussi & al. (2004) using the genotyping of several vine accessions. Genotypic analysis of Tunisian cultivars and wild accessions at nine nuclear microsatellite loci showed that both sets of samples maintain high levels of genetic variation (Snoussi & al. 2004). This genetic diversity pattern was later observed by Oualkadi & al. (2011) when prospecting 18 sites with 168 individual wild vines collected from the north of Morocco in comparison with populations picked from Algeria, France and Tunisia. In their study, it was demonstrated that some ecotypes from Ouchtata and Tabarka (Tunisia) were clustered with French group of wild grapevines.

The organization of genetic diversity is reminiscent of historical genetic structure originating from natural evolution, domestication and modern plant breeding (Aradhya & al. 2003; Lacombe & al. 2003). Such a structure could generally be described based on the amount and pattern of distribution of genetic diversity within and between different geographic or genetic groups. Aradhya & al. (2003) showed that measures of within-group genetic diversity merged three wild grape genotypes from Tunisia along with an accession from Corsica. These authors revealed that Tunisian grape cultivars showed close affinity to the wild progenitor subsp. *silvestris* reflecting the origin and domestication gait of many of the autochthonous cultivars. This was confirmed later by Zoghlami & al. (2009) who expected first that *V. silvestris* with small and fragmented populations would have low levels of genetic variation. Levadoux (1956) reported that foreign cultivated vines were introduced to North Africa and crosses with local populations lead to the genesis of more advanced genotypes holding the specificity of the region. According to the same author, the most advanced forms of vine seem to have appeared after the forms that come closest to spontaneous types. 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 a conclusion already made by Snoussi & al. (2004). According to the authors, table grape cultivars are either female or hermaphroditic self-compatible plants and most of the seeds produced by hermaphrodites result from selfing events. It is therefore possible to speculate that many of the current table grape cultivars derived from a few original ones that were imported to Tunisia as plants or raisins (Snoussi & al. 2004). The low gene flow between the wild grapevine populations and the set of Tunisian autochthonous grapevine cultivars was also revealed by Zoghlami & al. (2009). Wild grapes occur in natural closed landscape of forests, whereas the cultivated forms occur very far from the former. This constitutes a physical barrier against successful characterization of parentage between both grapevine sub-species (Zoghlami & al. 2009; 2013) taking into consideration also that the pollen cannot be dispersed far enough (Di Vecchi & al. 2007). Gristina & al. (2017) and Barbagallo & al. (2018, 2019) revealed that Sicily and its surrounding islands in the Mediterranean basin represent underexplored hotspots of genetic diversity for grapevine. These important reservoirs of potentially valuable genotypes shed light on the migration phenomenon of cultivars.

The study of *silvestris* vine has raised many prospects, in particular using them in future breeding programs to improve rusticity and disease resistance of local and introduced grape varieties. Considering that wild vines disappeared more quickly in hot and humid enough regions (Levadoux 1956), and in view of actual climate context, conservation efforts are required to maintain the genetic integrity and survival of the remnant populations (Cunha & al. 2007; Zoghlami & al. 2013). Wild grape germplasm is a potential source of unique alleles and is important for the improvement of both wine and table grapes (Aradhya & al. 2003). The spontaneous vine *V. vinifera* subsp. *silvestris* could represent a valuable gene pool to enrich diversity within the Tunisian germplasm of local vines since intensive cultivation of few grapevine varieties in extensive areas has drastically decreased genetic variability and has increased the risk of epidemic diseases (Arnold & al. 1998; Cunha & al. 2007; Harbi & al. 2010).

Conclusions

The morphometric features of the seed showed a clear difference in shape and size between wild and cultivated grapevines. Among the twenty-two wild vines, seven confirmed their belonging to the spontaneous type and are mainly originated from highlands of Nefza. Among the twelve native varieties, five confirmed their belonging to the cultivated morphotype and three suggest the wild grape. Some cultivars are definitely in more advanced form compared to others. Propagation by seedling probably contributed to the birth of these wild type cultivated forms. Certainly, the vegetative propagated varieties are and are likely to change considerably during sexual reproduction. Though, it is not to exclude the possibility that some cultivars derived from ancestral events of local domestication or cross hybridization with native wild plants.

Wild grapevines provide reservoir of useful traits and makes them particularly important sources of germplasm for breeding programs. Geometric morphometry suggests rich perspectives on the genetic and geographical characterization of cultivars. Morphometry of the seed from local grape populations showed a high level of polymorphism between wild and cultivated types. The certainty of this morphometric tool should be confronted to molecular techniques to verify the existence of a field of interaction between the two analytical methods.

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Address of the authors:
Mehdi Trad & Mounira Harbi,
Laboratory of Horticulture, National Institute of Agricultural Research, University of Carthage. INRAT, Rue Hédi Karray 2049 Ariana, Tunisia. E-mail: mh.trad@yahoo.com