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Morphometric variation and taxonomic identification of thirteen wild rose populations from Tunisia

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Abstract – Thirteen populations of wild roses (*Rosa* L.) growing in northern and central Tunisia have been used for studies on the discrimination between accessions and populations. Thirty-eight morphological characters related to the branches, prickles, leaves and corymbs were measured on the collected accessions to study the phenotypic diversity among and within species. Principal component and hierarchical cluster analyses (PCA and HCA) separated rose accessions into two distinctive groups and eight subgroups. A taxonomic interpretation of the morphological variability has shown that Tunisian rose populations belong to two sections (*Synstylae* and *Caninae*) of the genus *Rosa*. Moreover, they have been identified as seven separated taxa: *R. sempervirens* L., *R. sempervirens* var. *submoshata* Rouy., *R. sempervirens* var. *prostrata* Lindl. belonging to *Synstylae* section and *R. canina* L., *R. agrestis* Savi., *R. micrantha* Smith. and *R. dumetorum* Thuill. belonging to *Caninae* section. PCA and HCA proved that morphological characters used in taxonomic identification such as styles form, leaf and leaflets length, number of flowers by corymb, leaflet serration, presence of glands in leaflet, peduncle, receptacle and sepal have a high value of discrimination, and have been very successful in morphological identification.

Keywords: *Caninae* section, morphometry, *Synstylae* section, taxonomic differentiation, wild roses.

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Introduction

Roses are native to diverse habitats within the northern temperate hemispheres (Europe, Asia, the Middle East, North Africa and North America) and have been introduced and naturalized through the world. They have been planted around human habitats for at least two millennia for the ornamental and medicinal values of their flowers (flowering landscape, cut flowers, perfume, oil and rose water) (Hummer and Janick 2009). The genus *Rosa* L. is characterized by an enormous phenotypic plasticity that has challenged botanists. Indeed, the taxonomic treatment of this highly diverse subgenus is complicated and it is caused by an enormous phenotypic, genotypic and ecological variability and plasticity due to some evolutionary process, such as hybridization, introgression, etc. (De Cock 2008). Rose species exhibit a highly diverse ploidy level from diploid to octoploid, while decaploidy was observed in *R. prealucens* (Jian et al. 2010) and pentaploidy (2n = 5x = 35) in species from section *Caninae*. Those species evolved a mode of reproduction unique in the plant kingdom a distinctive unbalanced meiosis termed 'canina meiosis'(Klasterska and Natarajan 1974, Kovarik et al. 2008). The evidence for apomixis in wild *Rosa* L. is extremely limited and confined to the section *Caninae* (Dickinson et al. 2003). Czapik (1994) mentioned that apomictic taxa are highly polymorphic with numerous microspecies leading to a difficult and controversial taxonomic treatment. The classification system adopted by Rehder (1940), divided this genus in four subgenera, three of which are monotypic; *Hulthemia* (Dumort) Focke, *Platyrhodon* (Hurst) Rehder and *Hesperhodos* Cockerell. *Rosa*, the fourth subgenus, contains about 95% of all species (about 200 species) and was subdivided into 10 sections: *Pimpinellifoliae* (DC.) Ser, *Gallicanae* (DC.) Ser, *Caninae* (DC.) Ser, *Carolinae* Crép, *Cinnamomeae* (DC.) Ser, *Synstylae* (DC.), *Chinensis* (DC.) Ser, *Banksiae* Lindl, *Laevigatae* Michx. and *Bracteatae* Thory., each of the four latter sections have only one to three species (Ritz et al. 2005). Rehder's system was widely accepted and is still used as a basis for modern treatises (Bailey 1963, Wisseman 2003, Nybom 2009).

During recent years, wild roses have acquired great importance in research, not only in genetic but in other domains. In fact, rose hips, the fruits of the rose plant are in many European countries used in food products (Gao et al. 2005) and for medicinal purposes (Warholm et al. 2003, Rein et al. 2004, Boskabady et al. 2006). Indeed, rose hips are rich in phyto-nutrients such as phenols, carotenoids (lycopene), vitamins (C, A, E and P) (Erenturk et al. 2005, Olsson et al. 2005, Barros et al. 2010) and carbohydrates (Demir and Ozcan 2001), as well as other bioactive compounds that have beneficial medical effects. Recently, anti-inflammatory properties (Winther et al. 1999), anti-ulcerogenic (Gurbuz et al. 2003), anti-oxidant (Gao et al. 2005, Ozmen et al. 2005) and anti-mutagenic activities have been demonstrated (Karakaya and Kavas 1999).

In Tunisia, native rose bushes used to grow in the ravines, in brush fields and hedges and in the forest from the North to the Tunisian Dorsal (Jebel Chaâmbi) (Pottier-Alapetite 1979). But in the last ten years, climate changes and the programs of rural development and the expansion of the cities have threatened the biodiversity in Tunisia and caused the risk of the extinction of some species particularly *Rosa* accessions from northern and central humid zones. Those populations were subjected to genetic erosion and the information about the species is limited. In this way it is urgent to prospect the Tunisian rose populations, to identify and essentially to conserve them for subsequently valorisation.

In this work we aim to study the variability within the genus *Rosa* accessions growing wild in the northern and the central part of Tunisia. To accomplish this we have analysed a plant material collection from 13 populations. The main objectives of this work were: 1) evaluation of morphometric variability and 2) to place the identified groups in a hierarchical classification.

Materials and methods

Plant material

Field collections of rose populations were carried out in April and May 2007–2010. The collection sites were selected according to the Flora of Tunisia (Pottier-Alapetite 1979). From five provinces, nine localities were chosen to cover the geographical range of wild roses. In the Northwest regions of Tunisia, nine populations were found to be naturally distributed in seven localities. In each of the localities Tabarka, Aïn Drahem, Tebaba, Wechtata, Djeba and Kessra we found one type of population but in the Teboursouk locality we found three types. In the Northeast we found two populations in one locality (Sidi Median from Zaghouan). In the Center we found also two populations in the locality of Haffouz (Kairouan). The latitude longitude and altitude of each location were determined using the geographical positioning system (GPS) using a Magellan Explorist (Tab. 1).

Morphological characters

Studies were based on vegetative and floral characters. In each population five accessions were sampled. Thirty samples of one year old branches of 100 cm of length (we respect all different positions and directions) were collected randomly in the spring (April–May). Thirty samples of leaves, inflorescences and flowers in full bloom were taken to measure morphological characters (qualitative and quantitative characters). All voucher specimens are deposited in the High Institute of Agronomy of Chott-Meriem Botanic Laboratory (Herbarium ISACH) and each one was assigned a corresponding number and code RR110-RR123.

Twenty one vegetative characters that are associated with the branches, prickles, leaves and leaflets and gland density on those organs were defined. Seventeen floral characters that are associated with the corymb and with all other flower parts, and gland density on those parts were also defined (Fig. 1).

Taxonomic differentiation

Hierarchical classification was based on Baily's, Crépin's and Maire's classifications (Boulenger 1924, 1932, 1937, Crépin 1869, Bailey 1963, Maire 1980, Silvestre and Montserrat 1998). In addition to the characters already established and to define sections, we are also interested in styles (styles exerted beyond the mouth of receptacle, long or short, exerted styles free or connate into a column), in stipules (free, adnate only at the base or adnate more than one half of their length) and in the kind of inflorescence (solitary or corymbose).

Tab. 1. Province, locality, species, corresponding code and eco-geographical parameters of sites harvest.

Province	Locality	Species	Code	Number of samples	Bioclimatic stage	Latitude	Longitude	Altitude (m)
Jandouba (Northwest)	Tabarka	*R. sempervirens*	Tab	10	Humid	36°52’	8°43’	91
	Ain Drahem	*R. sempervirens*	AinD	10	Humid	36°40’	8°40’	290
Beja (Northwest)	Tebaba	*R. sempervirens*	Teb	10	Subhumid	36°57’	8°57’	114
	Wechtata	*R. sempervirens*	Wech	10	Subhumid	36°54’	9°08’	73
	Teboursouk	*R. canina*	Tebs1	5	Subhumid	36°29’	9°18’	228
		R. agrestis	Tebs2	5	Subhumid	36°29	9°18’	228
		R. sempervirens	Tebs3	10	Subhumid	36°28’	9°16’	290
	Djeba	*R. sempervirens*	Djb.	10	Subhumid	36°28’	9°05’	298
Seliana (Transition zone between the Northwest and the Northeast)	Kessra	*R. canina*	Kess	5	Semi-arid	35°50’	9°22’	942
Zaghouan (Northeast)	Sidi Median	*R. sempervirens*	Zagh1	10	Semi-arid	36°20’	10°20’	335
		R. canina	Zagh2	5	Semi-arid	36°20’	10°04’	228
Kairouan (Center)	Haffouz	*R. micrantha*	Hafz1	10	Semi-arid	35°38’	9°39’	264
		R. agrestis	Hafz2	5	Semi-arid	35°38’	9°39’	264

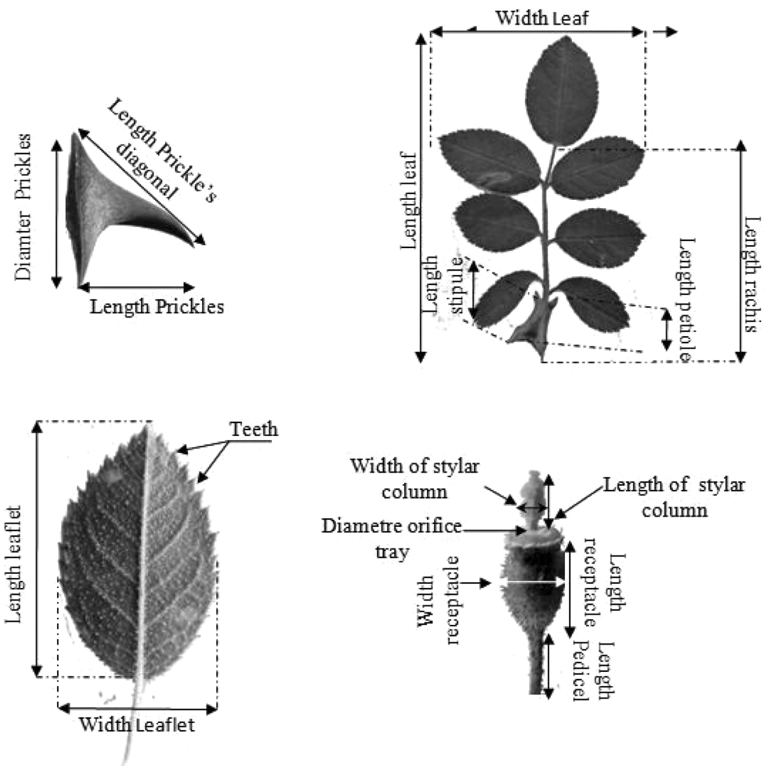

Fig. 1. Morphological and floral characters of wild roses (*Rosa* L.) evaluated in this study.

Statistical analysis

The data were analysed using analysis of variance (ANOVA) and the significance of the differences between means were determined at $p < 0.05$ using Duncan's multiple range test. To evaluate the approximation or the removal of species all the data were subjected to principal components analysis (PCA) and hierarchical clusters analysis (HCA) (Crisci and Lopez Armengol 1983, Cottin 1988) using the SPSS 16 software (Statistical Package for the Social Sciences) and Excel 2007 software, allowing classification of individuals into groups (Molina Cano 1977).

Results

Morphological diversity

Mean values of morphological characters studied are reported in Tabs. 2, 3, 4. Data show large variability among accessions for almost characters. Among the examined characters diameter and length of prickles (Dia. Pr, L. Pr) were highly correlated with the length of prickle's diagonal (L. Pr. dig) (75 and 88%, respectively) (data not reported). The length of leaf (L. Lf) was highly correlated with the width (W. Lf) and the area of the leaf (Lf. a) and with the length of rachis (L. ra) (84, 81 and 73%, respectively). The number of flowers per corymb (Nb. Fl. co) was correlated with the length of corymb (L. co) (78%), and this last

Tab. 2. Mean values of vegetative characters related to branches and prickles of each rose accessions growing wild in Tunisia. For the abbreviations of rose accessions (codes) see Tab. 1; Dia. Br – diameter of branches, Dt. Pr – density of prickles on a branch length of 10 cm, Dia. Pr – diameter of prickle's base, L. Pr – length of prickle, L. Pr. dig – length of prickle's diagonal. Values with the same letters within the same column are not different at $p > 0.05$.

Accessions	Dia. Br (cm)	Dt. Pr	Dia. Pr (mm)	L. Pr (mm)	L. Pr. dig (mm)	L. Pr/ Dia. Pr
Hafz2	0.6±0.1cd	6.2±6.8e	6.8±1.5d	4.8±0.6a	6.1±0.9ab	0.7±0.1a
Tebs2	0.5±0.2b	15.6±4.4cd	7.5±1.4e	6.0±0.7bcd	7.9±0.9e	0.7±0ai
Hafz1	0.7±0.1cd	25.1±6.5e	8.6±1.5g	6.8±0.9d	8.7±1.1f	0.8±0.1ab
Kess	0.6±0.1c	11.9±5.3b	6.7±1.0d	6.2±0.9cd	7.0±1.0cd	0.9±0.1bc
Tebs1	0.6±0.2c	13.1±3.7c	8.2±1.3ef	11.0±3.2f	10.8±2.2g	1.5±0.3g
Zagh2	0.7±0.2d	36.5±13.9f	7.9±1.7ef	9.2±2.7e	10.3±2.2g	1.2±0.3ef
Tebs3	0.4±0a	23.3±4.0e	4.9±0.7ab	6.3±1.8cd	6.5±1.1bcd	1.3±0.3f
Djb	0.5±0.1a	17.3±3.4d	4.7±0.7ab	4.9±1.3a	5.9±0.8ab	1.0±0.3cde
Teb	0.4±0.1a	16.5±5.3d	5.2±0.8bc	5.0±1.0a	6.2±1.0ab	0.9±0.1cd
Tab	0.4±0.1a	18.3±3.7d	5.7±0.7c	6.2±1.4cd	7.2±0.6d	1.0±0.2de
AinD	0.4±0.1a	24.2±5.6e	5.2±0.8bc	5.4±1.6abc	6.5±1.0bc	1.0±0.3cde
Wech	0.6±0.1bc	16.8±5.2d	5.6±0.7c	6.5±1.9d	7.0±0.9cd	1.1±0.3ef
Zagh1	0.4±0.1a	21.7±6.9e	4.5±0.6a	5.1±1.0ab	5.7±0.8a	1.1±0.2ef

character was correlated with the length of pedicel (L. ped) and of stylar column (L. sty) (86 and 75%, respectively). This last character (L. sty) was negatively correlated with the length of sepal (L. s), the number of lobes per sepal (Nb. lob. s) and number of lobulated sepals (Nb. s. lob) (87, 71 and 70%, respectively). The presence of glands in pedicel, receptacle and sepal (Gl. ped, Gl. rep, Gl. s) was positively correlated with the length of corymb (L. co) (74%) and the length of stylar column (L. sty) (95%) and negatively correlated with the diameter of prickles (Dia. Pr) (71%), number of lobulated sepals (Nb. s. lob), number of lobes per sepal (Nb. lob. s) and length of sepal (L. s.) (78, 91 and 72%, respectively).

Principal component analysis and hierarchical cluster analysis

The 38 morphological characters were used for the PCA and HCA. The HCA based on the *Euclidean* distances between groups indicated two groups of accessions: A and B, with six and seven accessions, respectively, identified by their floral and vegetative characters with a dissimilarity > 20 (Figs. 2, 3).

When the dissimilarity was higher than 16, the A group was divided into two sub groups, A_1 (Hafz1, Hafz2 and Tebs2) and A_2 (Zagh2, Tebs1 and Kess). With a dissimilarity > 5, subgroup A_1 was further divided into two subgroups A_{11} (Tebs2, Hafz2) and A_{12} (Hafz1) and the second subgroup A_2 was divided in two subgroups A_{21} (Tebs1, Kess) and A_{22} formed by one accession (Zagh2).

The B group with dissimilarity > 8 was divided into 2 subgroups: B_1, B_2. When the dissimilarity was higher than two, the subgroup B_1, was divided in three subgroups B_{11} (Tebs3,

Tab. 3. Mean values of vegetative characters related to leaf, leaflet, gland and bristle of each rose accessions growing wild in Tunisia. For the abbreviations of rose accessions (codes) see Tab. 1; Nb. Lft – number of leaflets, L. Lf – length of leaf, W. Lf – width of leaf, L. ra – length of rachis, L. Lft – length of leaflet, W. Lft – width of leaflet, L. pet – length of petiole, L. st – length of stipule, Lf. a – leaf area, Nb. Pr. ra – number of prickles on the rachis, Lft. ser – leaflet serration (number of teeth in 1 cm of leaflet margin), Gl. uLft – gland present (glandular; 5) or absent (eglandular; 0) on upper side leaflet, Bs. uLft – with (pubescent; 5) or without (glabrous; 0) bristle on upper side leaflet. Values with the same letters within the same column are not different at $p > 0.05$.

Accessions	Nb. Lft	L. Lf (cm)	W. Lf (cm)	L. ra (cm)	L. Lft (cm)	W. Lft (cm)	L. Lft/ W. Lft	L. pet (cm)	L. st (cm)	Lf. a (cm^2)	Nb. Pr. ra	Lft. ser	Gl. uLft	Bs. uLft
Hafz2	6.8± 0.6e	8.4± 0.9cde	6.7± 1.0d	4.6± 0.5cde	3.8± 0.6de	2.2± 0.4cd	1.7± 0.0d	1.1± 0.3a	1.1± 0.1bc	25.0± 7.5cd	4.9± 2.0de	10.2± 2.2d	0a	0a
Tebs2	6.0± 1.0cd	8.5± 1.1cde	6.0± 0.8c	4.9± 0.6e	3.6± 0.5d	2.5± 0.4ef	1.4± 0.1c	1.7± 0.4cd	0.9± 0.1b	24.9± 7.1cd	6.5± 2.3f	11.5± 2.1e	0a	0a
Hafz1	7.0± 0e	8.0± 1.1bcd	5.9± 0.8c	5.0± 0.8e	2.1± 0.4a	1.9± 0.2b	1.0± 0.2a	1.3± 0.2b	0.8± 0.1a	24.7± 6.0cd	5.3± 1.5e	9.2± 1.2c	5b	5b
Kess	5.2± 0.6a	7.4± 0.7ab	5.4± 0.6c	4.3± 0.5abc	3.0± 0.4c	2.3± 0.2de	1.3± 0.1b	2.2± 0.3f	1.7± 0.32f	18.9± 4.7b	1.7± 1.5a	6.9± 1.7b	0a	5b
Tebs1	5.8± 1.0cd	7.8± 0.8bc	3.1± 0.6a	4.7± 0.5de	5.6± 0.5j	2.0± 0.6bc	2.8± 0.4g	1.9± 0.5e	1.0± 0.1bc	18.0± 2.9b	3.6± 1.3bc	7.4± 1.6b	0a	5b
Zagh2	6.7± 0.6e	6.9± 1.3a	4.3± 1.1b	4.4± 0.8bcd	2.5± 0.5b	1.4± 0.3a	1.7± 0.2d	1.6± 0.3c	1.0± 0.2bc	12.9± 5.5a	6.5± 2.7f	9.3± 3.2c	0a	0a
Tebs3	5.6± 0.9bc	8.5± 1.6cde	7.1± 1.5def	4.4± 0.9bcd	4.1± 0.7ef	2.2± 0.2bcd	1.9± 0.3e	1.7± 0.3c	1.1± 0.2c	24.7± 8.8cd	3.8± 1.5bc	7.4± 1.2b	0a	0a
Djb	5.3± 0.7ab	8.6± 1.2de	7.5± 1.2ef	4.4± 0.6bcd	4.2± 0.6f	2.6± 0.3f	1.6± 0.1d	1.9± 0.4de	1.1± 0.1c	30.7± 8.0e	3.7± 1.2bc	7.7± 0.8b	0a	0a
Teb	5.2± 0.7ab	8.5± 0.9cde	6.9± 0.8de	4.0± 0.5a	4.6± 1.3gh	2.1± 0.3bcd	2.1± 0.3f	1.5± 0.3c	1.0± 0.1b	21.6± 8.6bc	3.8± 1.4bc	5.8± 1.0a	0a	0a
Tab	5.2± 0.6ab	9.4± 0.4f	8.9± 1.5g	4.0± 0.6ab	5.2± 0.8i	2.5± 0.4ef	2.1± 0.2f	1.6± 0.2c	1.1± 0.2c	28.8± 10.5de	2.9± 1.2b	5.8± 1.1a	0a	0a
AinD	5.3± 0.7ab	8.8± 0.9ef	7.8± 0.7f	4.3± 0.5abc	4.3± 0.5fg	2.5± 0.3ef	1.7± 0.2d	1.9± 0.2de	1.4± 0.2d	25.4± 5.4cd	3.7± 1.2bc	5.8± 0.8a	0a	0a
Wech	5.2± 0.6ab	10.5± 1.5g	9.7± 1.5h	4.9± 0.7e	5.3± 0.8ij	2.5± 0.4ef	2.1± 0.2f	1.9± 0.2e	1.5± 0.2de	30.3± 9.8e	4.4± 1.5cd	5.5± 1.1a	0a	0a
Zagh1	6.1± 1.0d	10.4± 1.8g	9.3± 1.6gh	5.4± 0.8f	5.0± 1.0hi	2.5± 0.6ef	2.0± 0.3ef	1.9± 0.3de	1.6± 0.2ef	42.5± 17.1f	4.8± 1.4de	7.3± 1.2b	0a	0a

Tab. 4. Mean values of floral characters of each rose accessions growing wild in Tunisia. For the abbreviations of rose accessions (codes) see Tab. 1; Nb. Flco – number of flowers per corymb, L. co – length of corymb, L. ped – length of pedicel, Nb. Lft. Blt – number of leaflet in flowering branchlets, L. rep – length of receptacle, W. rep – width of receptacle (medium), L. sty – length of stylar column, W. sty – width of stylar column, Dia. rep. o – diameter of receptacle orifice, Nb. s. lob – number of sepal lobulated, Nb. lob. s – number of lobes per sepal, L. s – length of sepal, W. s – width of sepal, L. p – length of petal, W. p – width of petal, Gl. ped – glandular (5) or eglandular (0) pedicel, Gl. rep – glandular (5) or eglandular (0) receptacle, Gl. S – glandular (5) or eglandular (0) sepals. Values with the same letters within the same column are not different at $p > 0.05$.

Accession	Nb. Flco	L. co (cm)	L. ped (cm)	Nb. Lft. Blt	L. rep (cm)	W. rep (cm)	L. sty (mm)	W. sty (mm)	Dia. rep. o (mm)	Nb. s. lob	Nb. lob. s	L. s (cm)	W. s (cm)	L. p (cm)	W. p (cm)	Gl. ped	Gl. rep	Gl. s
Hafz2	2.8± 1.1a	3.7± 0.8a	1.5± 0.7a	5.1± 0.5a	0.9± 0.1e	0.5± 0.0de	2.6± 0.2a	1.9± 0.0a	4.6± 0.7c	2.5± 0d	7.1± 0.9f	2.6± 0.6g	0.5± 0.4c	2.1± 0.2ef	2.4± 0.2e	0a	0a	0a
Tebs2	2.8± 1.1a	3.7± 0.7a	1.5± 0.7a	5.1± 0.4a	0.9± 0.1e	0.5± 0de	2.3± 0.2a	2.7± 0a	5.1± 0.7e	2.5± 0d	7.0± 0.9f	2.6± 0.6g	0.6± 0.4c	2.1± 0.2ef	2.4± 0.1e	0a	0a	0a
Hafz1	2.9± 1.1a	3.6± 0.9a	1.2± 0.5a	7.0± 0c	1.1± 0.1g	0.4± 0c	1.9± 0.2a	2.6± 0a	4.2± 1.2bd	2.5± 0d	5.0± 0.6d	1.9± 0.3e	0.6± 0.3d	2.0± 0.2de	2,0± 0.2d	0a	0a	0a
Kes	2.9± 1.5a	4.0± 1.2a	1.3± 0.8a	6.6± 0.7b	1.0± 0f	0.5± 0de	2.3± 0.3b	2.6± 0.3e	5.1± 0.4e	2.4± 0d	6.1± 1.2e	1.7± 0.1d	0.5± 0ab	1.7± 0.2b	2.0± 0.2cd	0a	0a	0a
Tebs1	2.9± 1.4a	4.0± 1.2a	1.3± 0.8a	6.6± 0.7b	1.0± 0f	0.5± 0de	2.3± 0.2b	2.6± 0.2e	5.1± 0.4e	2.4± 0.1d	6.1± 1.1e	1.7± 0.1d	0.5± 0ab	1.7± 0.1b	2.0± 0.2cd	0a	0a	0a
Zagh2	3.0± 1.2a	4.8± 1.0a	1.4± 0.6a	5.0± 0a	1.1± 0h	0.5± 0e	2.6± 0.4c	4.0± 0.3f	5.2± 0.3e	2.5± 0d	5.2± 2.3d	2.1± 0.2f	0.4± 0a	1.9± 0.2cd	1.9± 0.2bc	0a	0a	0a
Tebs3	4.7± 1.7bc	8.3± 2.3c	2.7± 1.4b	5.0± 0a	0.8± 0d	0.4± 0a	5.6± 0.5d	1.8± 0.2c	3.9± 0.3a	0± 0a	0± 0a	1.3± 0.2c	0.5± 0ab	2.0± 0.1cd	2.3± 0.1de	5b	5b	5b
Djb	4.1± 1.7ab	8.0± 1.7c	3.2± 1.2c	5.0± 0a	0.7± 0bc	0.4± 0a	5.6± 0.7de	1.6± 0.2b	3.7± 0.4a	0.2± 0.4b	0.2± 0.4a	1.1± 0.1b	0.5± 0ab	1.7± 0.2b	1.8± 0.2b	5b	5b	5b
Teb	8.4± 5.5d	12.2± 3.1e	4.6± 1.3d	5.0± 0a	0.7± 0.1ab	0.4± 0bc	6.1± 0.7fg	1.6± 0.2b	4.0± 0.5ab	1.1± 1.0c	0.8± 0.8b	0.9± 0.1a	0.4± 0.1a	1.9± 0.2cd	2.0± 0.2bcd	5b	5b	5b
Tab	6.0± 3.2c	9.5± 2.8d	3.7± 1.2c	5.0± 0a	0.7± 0c	0.4± 0bc	6.0± 0.8f	1.6± 0.1b	3.8± 0.3a	0± 0a	0± 0a	1.3± 0.1c	0.6± 0b	2.1± 0.3f	2.3± 0.2e	5b	5b	5b
AinD	8.1± 4.3d	12.3± 2.9e	3.4± 0.7c	5.0± 0a	1.0± 0f	0.5± 0d	6.3± 0.6g	2.1± 0.2d	4.3± 0.3c	2.5± 0.0d	2.2± 0.5c	1.5± 0.1c	0.5± 0ab	2.4± 0.2g	2.7± 0.2f	5b	5b	5b
Wech	7.9± 3.5d	10.5± 2.5d	3.5± 0.8c	5.0± 0a	0.7± 0a	0.5± 0ac	5.9± 0.7ef	1.7± 0.3bc	4.2± 0.3b	0.4± 0.8b	0.3± 0.6ab	0.9± 0.1a	0.5± 0ab	1.6± 0.21a	1.6± 0.1a	5b	5b	5b
Zagh1	3.9± 2.3ab	6.4± 2.3b	2.2± 1.0b	5.0± 0a	0.7± 0a	0.4± 0b	5.6± 0.6de	1.6± 0.2b	4.3± 0.4bd	0± 0a	0± 0a	1.1± 0.1b	0.5± 0a	1.8± 0.1bc	1.9± 0.1bc	5b	5b	5b

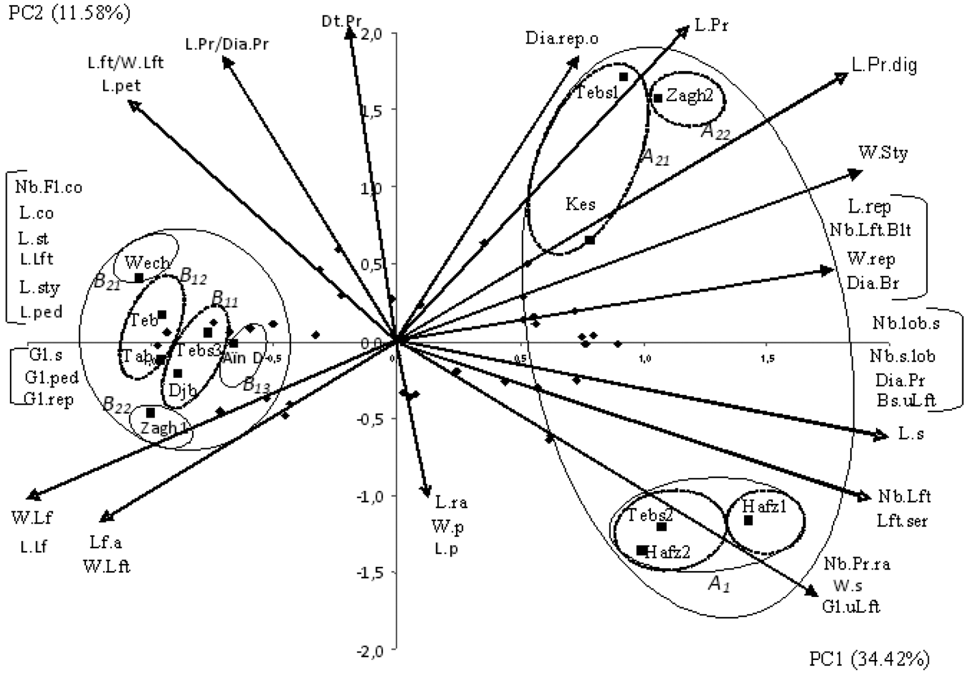

Fig. 2. Repartition of the vegetative and floral characters on the two principal axes of the principal components analysis. Identification of the groups and subgroups corresponding to the 13 Tunisian rose accessions studied. For the abbreviations of rose accessions (■) and the characters (◆) see Tabs. 1, 2, 3, 4.

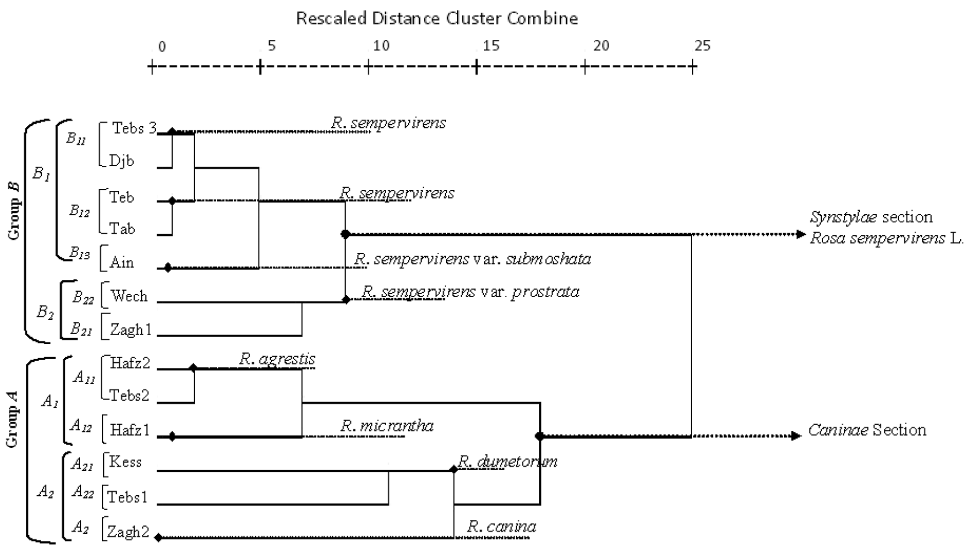

Fig. 3. Dendogram of the 13 Tunisian rose accessions studied, obtained by cluster analysis based on the Euclidean distances relating to the floral and vegetative characters. For the abbreviations of rose accessions see Tab. 1.

Djb), B_{12} (Tab, Teb), and B_{13} (AinD). The subgroup B_2 was divided in 2 subgroups B_{21} (Wech) and B_{22} (Zagh1).

The PCA horizontal axis explained 34.42% of the total variance and the vertical axis 11.58%. The groups A and B clearly stand out, forming separate groups in PCA (Fig. 2) and a deep dichotomy in HCA (Fig. 3).

The horizontal axis (PC1) was positively correlated with groups A_1 and A_2 and negatively correlated with group B. The variables most highly correlated were diameter of prickles (Dia. Pr), the presence of glands and of bristles on the upper side of leaflet (Gl. uLft, Bs. uLft), length of receptacle (L. rep), number of lobulated sepals and of lobes per sepal (Nb. s. lob, Nb. lob. s), the length and the width of sepals (L. s, W. s) (On-line Supplement Tab. 1). This axis 1 was moderately correlated with Dia. Br, L. Pr. dig, Lft. ser, Nb. Lft. Blt, W. rep, W. sty.

The width of leaf (W. Lf), the length of leaflet (L. Lft), the lengths of corymb, peduncle and stylar column (L. co, L. ped, L. sty) and the presence of glands in pedicel, receptacle and sepal (Gl. ped, Gl. rep, Gl. s) were highly negatively correlated with PC1.

The vertical axis (PC2) was positively correlated with subgroup A_2 and negatively with A_1. It was positively correlated with the length of prickle and the ratio of length and diameter of prickle (L. Pr, L. Pr/Dia. Pr) (On-line Supplement Tab. 1, Fig. 2) and negatively with the width of sepal (W. s) and the presence of glands in the upper side of leaflet (Gl. uLft).

All accessions from group A were correlated with those characters: Dia. Br, Dia. Pr, L. Pr. dig, W. rep, L. rep, W. s, L. s, W. sty, Gl. uLft, Bs. uLft, Lft. ser, Nb. lob. s, Nb. s. lob, Nb. Lft. blt, (Figs. 2, 3) and were characterized by large branches (Dia. Br = 0.5–0.7 cm), dotted with a large prickle having a long diagonal (Dia. Pr = 6.7–8.6 mm; L. Pr. Dig = 6.1–10.8 mm), leaves with a high number (Nb. Lft = 5.2–7.0) of short leaflets (L. Lft = 2.1–5.6 cm) with high serration (6.9–11.5). Leaves on flowering branchlets were formed by a high number of leaflets (Nb. Lft. Blt = 5 to 7), short corymbs (L. co = 3.6–4.8 cm) with few flowers (Nb. Fl. co = 2.8–3.0). A short pedicel (L. ped = 1.2–1.5 cm) bearing a flower also having a short column stylar (L. sty = 1.8–2.6 mm), but a long receptacle (L. rep = 0.9–1.1 cm), long sepal (1.7–2.6 cm) with many appendix (Nb. slob = 2.4–2.5; Nb. Lobs = 5.0–7.1). Receptacle, pedicle and sepals are all eglandular.

The subgroupA_1, was represented by Hafz1, Hafz2 and Tebs2, all characterized by the highest values for the characters related to the serration of leaflet and sepals and the high values for length and wide of sepals (Lft. ser, Nb. Lob. s, Nb. s. Lob, L. s, W. s). This subgroup was divided into two subgroups:

Subgroup A_{11}: Hafz2 and Tebs2. Those accessions have eglandular and glabrous leaflets with short petiole, rachis with numerous prickles, short corymb (L. co = 3.7 cm) with the lowest number of flowers (Nb. Fl. co = 2.8), the flower has a long petal, the longest sepal longer than petal (L. s = 2.6 cm, L. p = 2.1 cm). Hafz2is characterized by highly serrated sepal (Nb. lob. S = 7.1), the narrowest and the shortest scattered and shorter prickles (Dt. Pr = 6.2, L. Pr = 4.8mm), the lowest number of flowers by corymbs (Nb. Fl. co = 2.8), while Tebs2 is characterized by rachis with the highest number of prickles (Nb. Pr. ra = 6.5), and highly serrated leaflet (Lft. ser = 11.5).

Subgroup A_{12}: Hafz1 include accessions with a robust bush exhaling an apple scent. It differs in having its highest number of leaflets in bloomy branchlets (Nb. Lft. Blt = 7.0), in the largest thorny branches (Dia. Br = 0.7 cm, Dt. Pr = 25.1), with the shortest leaflet (L. Lft

= 2.1 cm), glandular and pubescent (Gl. uLft = 5, Bs. uLft = 5), flowers in the shortest corymb (L. co = 3.6 cm), dotted with the shortest pedicel (L. ped = 1.2 cm), the longest receptacle (L. rep = 1.1 cm), a high number of lobulated sepals (Nb. s. lob = 2.5) and of lobes per sepal (Nb. lob. s = 5.0).

The subgroup A_2 reported in the positive section for PC1 and PC2 (Fig. 2), was correlated with diameter of branches and of prickle's base (Dia. Br, Dia. Pr), lengths of prickle and of their diagonals (L. Pr, L. Pr. dig), number of leaflets in flowering branchlets (Nb. Lft. Blt), length and width of receptacle (L. rep, W. rep), width of styles (W. sty), and number of lobulated sepals (Nb. slob). This subgroup is divided into two subgroups:

Subgroup A_{21}: Kess and Tebs1. Those accessions were mainly characterized by an eglandular (Gl. uLft = 0) and pubescent leaflet (Bs. uLft = 5) and a long receptacle (L. rep = 1.0 cm). Those accessions were characterized by bristly leaflets with low serration (Lft. ser = 6.9 and 7.4, respectively) with a rachis with few prickles. Kess accession was characterized by the longest petiole (L. pet = 2.2 cm) and rachis with the lowest number of prickles (Nb. Pr. Ra = 1.7). Tebs1 accession is characterized by the longest prickle and the longest prickle diagonal (L. Pr = 11.0 mm; L. Pr. dig = 10.8 mm), the narrowest leaf (W. Lf = 3.1 cm), the longest leaflet (L. Lft = 5.6 cm), and the highest ratios for leaflet and prickle (L. Lft/W. Lft = 2.8; L. Pr/Dia. Pr = 1.5).

Subgroup A_{22}: Zagh2 is an upright shrub, characterized by the largest thorny branches (Dia. Br = 0.7 cm, Dt. Pr = 36.5), rachis with numerous prickles (Nb. Pr. ra = 6.5), leaflets eglandular (Gl. uLft = 0), glabrous (Bs. uLft = 0), and doubly serrated (Lft. ser = 9.3), flower with the longest (L. rep = 1.2 cm) and the broadest (W. rep = 0.5 cm) receptacle, the largest receptacle orifice (Dia. rep. o = 5.2 mm) and the widest style (W. sty = 4.0 mm).

The group B was represented by Zagh1, Wech, AinD, Tab, Teb, Djb and Tebs3, which were correlated with the length and diameter of prickle ratio (L. Pr/Dia. Pr), the length of stipule (L. st), the number of flowers per corymb (Nb. Flco), the length and the width of leaf (W. Lf, L. Lf), the length of leaflet, pedicel and stylar column (L. Lft, L. ped, L. sty) (Figs. 2, 3). All accessions from group B were characterized mainly by a long stylar column (L. sty. = 5.6 to 6.3 mm. They were also characterized by slender branches (Dia. Br = 0.4–0.6 cm), long (8.5–10.5 cm) and width (9.7–6.9 cm) leaves, mainly with lanceolate (L. Lft = 4.1–5.3 cm and W. Lft = 2.1–2.6 cm), eglandular and glabrous leaflets (Gl. uLft = 0, Br. ulft = 0), the lowest number of leaflets by leaves in flowering branchlets (Nb. Lft. Blt = 5), long corymbs (L. co = 6.4–12.3 cm) with many small flowers (Nb. Fl. co = 3.9–8.4), long and glandular pedicel (L. ped = 2.2–4.6 cm). Receptacle (L. rep = 0.7–1.0 cm) and sepals (L. s = 0.9–1.5 cm) were glandular too but short. Group B was divided into 2 subgroups: B_1, B_2. The subgroup $B_1$was divided in three subgroups: B_{11}, B_{12} and $B_{13.}$

Subgroup B_{11} is represented by Tebs3 and Djb accessions which were characterized mainly by a medium number of flowers (Nb. Fl. co = 4.1–4.7), and short leaflet (L. Lft = 4.1–4.2 cm) with high leaflet serration (Lft. ser = 7.4–7.7). Djb accession presents a medium density for prickles, the broadest leaflet (W. Lft = 2.6 cm), a low number of lobultated sepals and of lobes per sepal (Nb. s. lob = Nb. lob. s = 0.2), flower with the shortest receptacle (L. rep = 0.7 cm), having the lowest diameter for the receptacle orifice (Dia. rep. o = 3.7 mm). Tebs3 accession has a flower with a sepal with entire edges (Nb. lob. s = 0, Nb. s. lob = 0) and the narrowest branches (Dia. Br = 0.4 cm) but with high density of prickles (Dt. Pr = 23.3).

Subgroup B_{12} is represented by Tab and Teb accessions, characterized by prickles with a medium density (16.5–18.3) and a prickle base of medium diameter (Dia. Pr = 5.2–5.7 mm), the shortest rachis (L. ra = 4.0 cm) and short petiole (L.pet = 1.5–1.6 cm). Its flowers having the shortest and the narrowest receptacle (L. rep = 0.7 cm, W. rep = 0.4 cm). Teb is characterized by the longest pedicel (L. ped = 4.6 cm), the highest number of flowers by corymb (Nb. Flco = 8.4), the shortest (L. s = 0.9 cm) and narrowest (W. s = 0.4 cm), low number of lobulated sepals (Nb. s. lob = 1.1) and low lobes per sepal (Nb. lob. s = 0.8). In contrast, Tab has a flower with long pedicel (L. ped = 3.7 cm) and sepals with entire edges (Nb. s. lob = 0, Nb. lob. s = 0). Subgroup B_{13} is represented by AinD accession, and it was characterized by thorny branches with a high number of prickles (Dt. Pr = 24.2), the longest corymbs (L. co = 12.3 cm), the broadest receptacle (W. rep = 0.5 cm), the longest stylar column (L. sty = 6.3 mm), the highest number of lobulated sepals (Nb. s. lob = 2.5), the longest and widest petals (L. p = 2.4 cm, W. p = 2.7 cm).

The subgroup B_2 was divided in two subgroups each of them with one accession (B_{21}; Wech and B_{22}; Zagh1) characterized by long (L. Lf = 10.5; 10.4 cm, respectively) and broad leaves (W. Lf = 9.3; 9.7 cm, respectively), ratio of length and diagonal of prickles tends towards 1 (L. Pr/Dia. Pr = 1.1, which means the length and the diagonal of prickles are equal) and the shortest receptacle (L. rep = 0.7 cm). Wech accession has the longest (L. Lf = 10.5 cm) and the broadest (W. Lf = 9.7 cm) leaf, the broadest receptacle (W. rep = 0.5 cm), the lowest number of leaflets (Nb. Lft = 5.2), a low leaflet serration (Lft. ser = 5.5), the shortest sepal (L. s = 0.9 cm) with low number of lobulated sepals (Nb. s. lob = 0.4), and lobes per sepal (Nb. lobs = 0.3), the shortest and narrowest petal (L. p = 1.6 cm, W. p = 1.6 cm). Zagh1 accession was characterized by the lowest diameter (Dia. Pr = 4.5 mm), and length of diagonal prickles (L. Pr. dig = 5.7 mm). The highest length of rachis (L. ra = 5.4 cm) and the highest leaf area (Lf. A = 42.5 cm^2). A medium length for pedicel (L. ped = 2.2 cm) and corymb (L. co = 6.4 cm), with few flowers (Nb. Fl. co = 3.9), sepal with entire edges (Nb. s. lob = Nb. lob. s = 0).

Discussion

Accessions from group A were characterized by a large branches dotted with long prickles, leaves, in general, with seven short leaflets, the margins of which were highly serrated, leaves in flowering branchlets with five to seven leaflets, short corymb with few flowers, pedicel short too, bearing flowers having short styles but long receptacles, sepals also long with many appendices. The receptacle, the pedicel and the sepals of those accessions were eglandular. All this characteristics described species of section *Caninae*, according to the taxonomy of roses (BAKER 1871, BOULENGER 1937, BAILEY 1963, MAIRE 1980).

In this group, Hafz2 and Tebs2 accessions are characterized by big flowers, leaflet presumed with few glands in the under surface but eglandular in the upper surface, a glabrous pedicel. According to BAKER (1871) and MAIRE (1980) those accessions taxonomically should belong to *Rosa agrestis* Savi. Hafz2 and Tebs2, The slight variability within the two taxa can be related to the effect of the environment, indeed accession of Tebs2 was collected from a river bank but Hafz2 was collected from an arid region.

The Hafz1 accession is a robust bush that gives off a smell like apples. Leaves with seven short leaflets, glandular and pubescent in both sides, flowers clustered in short corymb with short pedicel. Flowers with the broadest sepal are dotted with many appendices. This accession presents the same characteristics of *R. villosa* L. but differs by a glabrous pedicel and receptacle (Bailey 1963). Maire (1980) describes *R. micrantha* Smith. with glandular leaflets on both sides but did not give any information about the coloration and the scent of glands of leaflets. Nevertheless, this accession may be a hybrid, specific to the Tunisian region, of *R. canina* L. and *R. villosa*.

Accessions Tebs and Kess were characterized by an eglandular leaflets on both sides with glabrous pedicel, receptacle and sepal. Those characteristics correspond to *R. canina* taxa (Maire 1980), in addition those accessions present a pubescent leaflet on both sides and bristles are oppressed in the upper side of leaflets which characterize *R. canina* ssp. *dumetorum* Thuill. (syn *R. dumetorum* Thuill.), (syn *R. corymbifer*a Borkh.) (Le Floc'h et al. 2010). The slight variability within the two species can be related to the effect of the environment, indeed accession of Tebs1 was collected from a river bank.

Zagh2 accession is an upright shrub, characterized by the largest thorny branches, glabrous leaflets, and doubly serrated edge. Zagh2 accession should belong to *Rosa canina* ssp. *vulgaris* Gams (syn *R. caninasensu strict*) because of its glabrous leaflets (Maire 1980).

Accessions from group B are characterized by the longest stylar column, this character was a systematic determinant character only for *Synstylae* section, indeed the styles connate into a slender exerted column (Crépin 1869, Boulenger 1932, Bailey 1963, Maire 1980, Silvestre and Montserrat 1998). They were also characterized by slender branches, leaves, generally, with five lanceolate and glabrous leaflets, leaves of flowering branchlets with five leaflets also. They have a long corymb with many small flowers. The pedicel, receptacle and sepals were glandular. Those characters characterized *Rosa sempervirens* L. (Silvestre and Montserrat 1998). In this group five subgroups were defined with HCA and we defined two varieties.

Tebs3 and Djb accessions were characterized mainly by a medium number of flowers and short leaflet with high leaflet serration. In addition, the stylar column was densely pubescent throughout the column length and reached the length of the internal stamens, the calyx and the floral pedicel were glandular; all those characters should indicate *R. sempervirens* var. *genuina* Rouy. (syn *R. sempervirens*).

Teb and Tab accessions are characterized by a medium density of prickles, prickle base with medium diameter, the shortest rachis and a short petiole, and by flowers that have the shortest and the narrowest receptacle. According to Maire (1980) those accessions present the characteristics of the variety *genuina* but they differ by the globular form of the receptacle. Thus, we identified them as the form f. *scandens* (Maire 1980). So Teb and Tab accessions should be *R. sempervirens* var. *genuina* f. *scandens* (Mill) Batt.

The accessions Tebs3, Djb, Teb and Tab were collected from the North East of Tunisia (longitude: 36°28'–36°57'; latitude: 9°05'–8°57'). Indeed, their descriptions suggest *R. sempervirens* and the slight morphological differences can be related to the effect of environment and we can consider those accessions as *R. sempervirens* ecotypes.

AinD accession was distinguished by its biggest flowers with the largest style and lobulated sepals. This accession presents the characteristics of the *R. sempervirens* var. *submoshata* Rouy. (Silvestre and Montserrat 1998).

Wech and Zagh1 accessions presented a prostrate habit, which characterize *R. sempervirens* var. *prostrate* (DC) Desv. (De Candole 1815, Gore 1832, Boulenger 1932).

As a result of this study, we can conclude that among the vegetative and floral characters studied, those showing the highest discriminating value were the sizes of prickle, leaf and leaflet, leaf area, lengths of style, pedicel and receptacle, sizes of sepal, petal, and receptacle, pedicel and sepal gland density. Moreover, those discriminant characters are used in the taxonomic identification of the genus *Rosa*. The study revealed considerable phenotypic diversity in the Tunisian wild rose population. Accessions from the 13 populations studied belong to two different sections. The first one is section *Caninae*, into which the accessions from Teboursouk1, 2, Kessra, Haffouz1, 2 and Zaghouan2 were grouped, being related by some cases of synonymy but differing in some morphological traits such as the presence or absence of bristles and glands in leaflet. Thus, this morphological criterion subdivided the six *Caninae* accessions into four species such as *R. canina* (Zagh2), *R. agrestis* (Hafz2, Tebs2), *R. micrantha* (Hafz1) and *R. dumetorum* (Tebs1, Kess). The second section is *Syntylae*, which is represented by accessions from Tebaba, Aïn Drahem, Wechtata, Tabarka, Djeba, Teboursouk3 and Zaghouan1, identified as *R. sempervirens.* Indeed, the morphologic analyses prove the presence of four ecotypes of *R. sempervirens* such as: ecotypeTebs 3, ecotype Djb, ecotype Teb and ecotype Tab and two varieties; *R. sempervirens* var. *submoshata* (AinD) and *R. sempervirens* var. *prostrata* (Zagh1, Wech). Phenotypic variation for quantitative characters is most likely non-genetic in origin. The most reliable estimates of genetic structure should therefore come from qualitative characters, especially when environmental effects can be minimized. We are now in the process of developing suitable molecular markers through DNA fingerprinting with microsatellite DNA hybridization SSR. These markers will eventually provide an additional data set on those accessions.

Determination key

A. Exerted styles, connate into a column usually long as stamens (L. sty = 5.6–6.3 mm)…...	Section: *Synstylae*
Leaves usually with 5 leaflets, lanceolate, long and slender branches.....	*Rosa sempervirens*
1. Climbing shrub	
1.1. Stylar column densely pubescent throw the column length and reach the length of the internal stamens, receptacle, calyx and floral pedicel were glandular..	*R. sempervirens* (**Tebs3**, **Djb**, **Teb** and **Tab**)
1.2. The same characters than as for var. *genuina* but sepals were lobulated with many appendix……...	*R. sempervirens* var. *submoshata* (**AinD**)
2. A prostrate habit...	*R. sempervirens* var. *prostrate* (**Zagh1** and **Wech**)
B. Style reaching only the mouth of the receptacle and stigmas forming a sessile head over it (L. sty = 1.8–2.6 mm), leaves of flowering branches usually with 7 leaflets (5.0–7.0), short pedicel with long and smooth receptacle (L. rep = 0.9–1.1 cm), glabrous sepals with many appendices...	Section *Caninae*
1. Leaflet pubescent and glandular on both sides, copiously pinnate......	*R. micrantha* (**Hafz1**)
2. Leaflet glandular and pubescent in under side and longer than 2 cm.....	*R. agrestis* (**Hafz2** and **Tebr2**)
3. Leaflet eglandular...	*R. canina* (**Zagh2**)
4. Leaflet pubescent in both sides with bristles oppressed in the upper side of leaflet...	*R. dumetorum* (**Tebr1** and **Kess**)

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