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Inter- and Intraspecific Morphological Variation of Four Iranian Rose Species

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ABSTRACT

There are about 200 rose species in the world, but only a few of them have contributed to the breeding pool of today's modern roses. In Iran there are 14 wild rose species with a few of them endemic to the region. In the present investigation 14 populations representing *Rosa canina* L. and *R. iberica* Stev. from the section *Caninae* and *R. foetida* Herrmann and *R. hemisphaerica* Herrmann from the section *Pimpinellifoliae* were studied. A multivariate statistical analysis was performed on 48 quantitative and qualitative morphological characters to investigate inter- and intraspecific variation. Cluster analysis indicated that inter- and intrasectional variation exists. Factor analysis and ordination based on principal component analysis revealed that intraspecific variation was present in both quantitative and qualitative characters. Traits such as presence or absence of hair on pedicle, prickle on sepal and hip shape were useful in the classification of these roses. Interspecific and intersectional relationships were comparable to the Rehder classification of rose.

Keywords: cluster analysis, ordination, principal component analysis, *Rosa*
Abbreviations: OTUs, operational taxonomic units; **PCA**, principal component analysis; **UPGMA**, unweighted paired group mean average; **WARD**, minimum variance spherical clusters

INTRODUCTION

The genus *Rosa* is one of the most economically important genera within ornamental horticulture in terms of economy and cultural history with humankind. *Rosa* consists of about 200 species distributed in the Northern Hemisphere (Wissemann 2003). However, classification in *Rosa* is problematic due to the wide variation in phenotypic characters. In the best classification, *Rosa* species are grouped taxonomically into four subgenera, three of which are monotypic and include only 1 or 2 species: Hulthemia (Dumort.) Focke, Platyrhodon (Hurst) Rehder, and Hesperhodos Cockerell (Wissemann 2003). The fourth subgenus, *Rosa*, harbors about 95% of all species distributed into 10 to 12 sections (Rehder 1940; Klastersky 1968; Gudin 2000). Phylogenetic investigations on the genus have been carried out by Wu *et al*. (2001) and Matsumoto *et al*. (1998, 2000). The natural distribution of the genus is separated into three major geographical areas: North America, East Asia and Europe/West Asia. The European/West Asian region is dominated by the members of section *Caninae* (DC.) Ser., the dogroses, which play an essential role in the production of rootstocks for ornamental rose propagation (Grant 1971; Wissemann 2000). Their hips are an excellent source of vitamins A, B_3, C, D and E, as well as flavonoids, fructose, malic acid, tannins and zinc. The fruits are commonly used to make jam and fruit juice (Uglla *et al*. 2003). The dried fruits and roots are excellent for making tea and for medicinal uses (Ercisli 2005).

There are reports of the diversity and number of rose species in Iran, i.e. Flora of Pakistan (Nasir 1972), Flora of Turkey (Davis 1985) and Flora Iranica (Zielhnski 1982). However, based on the Flora of Iran (Khatamsaz 1992), there are 14 species (*R. persica* Michx. ex Juss, *R. hemisphaerica* Herrmann, *R. foetida*. Herrmann, *R. spinosissima* L., *R. elimatica* Boiss & Hausskn, *R. villosa* L., *R. beggeriana* Schrenk, *R. webbiana* Wall, *R. orientalis* Dupont, *R. pulverulenta* M.B., *R. canina* L., *R. iberica* Stev., *R. boissieri* Crépin, *R. moschata* Herrmann) and 8 natural occurring hybrids of roses present in different regions of the country. Although these species grow wildly across Iran, they are concentrated mainly in the northwest and west (Alborz and Zagros mountains), especially in the Kandovan mountain and Chaloos Valley (**Fig. 1**). There are also some rare reports about the existence of some rose species in the desert regions of the southeast.

Fig. 1 Distribution map of 4 *Rosa* species. I = *R. iberica*, F = *R. foetida*, C = *R. canina*, H = *R. hemisphaerica*.

Despite advanced knowledge about roses in central

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Table 1 *Rosa* population code, locality, altitude and collector.

Species	Sp. code	Locality and altitude	Collector
R. iberica	I1	West Azarbayjan,Oroomye 1340 m	Koobaz, Khatamsaz & Zarshenas
	I2	Mazandaran, 25 km kandovan Road 2100 m	Koobaz, Khatamsaz, Kermani & Hosseini
	I3	Semnan, Parvar Strait, Karor Village 1900 m	Koobaz ,Khatamsaz & Hosseini
	I4	Mazandaran, Shahrestanak 2060 m	Koobaz, Khatamsaz & Jokar
R. canina	C1	Hamedan, Alvand climbs 2700	Koobaz, Hosseini & Ahangar
	C2	Khorasan Razavi, Mashhad, Torghabe 1020 m	Koobaz and Khatamsaz
	C3	West Azarbayjan, Oroomye, Sangar Road	Koobaz, Khatamsaz & Zarshenas
	C4	Mazandaran, Shahrestanak 2060 m	Koobaz, Khatamsaz & Jokar
R. hemisphaerica	H1	West Azarbayjan, Oroomye 1340	Koobaz & Khatamsaz
	H2	Mazandaran, Rine 2260 m	Koobaz, Khatamsaz & Kermani
R. foetida	F1	Hamedan (1), Tooyserkan 2340	Koobaz, Khatamsaz & Jokar
	F2	Hamedan (2), Zarinrood 1850 m	Koobaz, Hosseini & Akbari
	F3	West Azarbayjan, Oroomye, Oshnavye	Koobaz & Khatamsaz
	F4	Tehran, Damavand, Mara Village 1500 m	Koobaz, Khatamsaz & Kermani

Europe, there is not systematic and ecologic information about east European and Asian rose species (Wissemann 2003). However, there are a few reports about roses in North America, such as the monograph by Lewis (1957) and the report by Joly *et al*. (2000). Although, there is literature dealing with classification of *Rosa* in Iran, reports on the biosystematics of these roses is extremely limited with only partial classification of herbarium samples of some species by Khatamsaz (1992).

In the present investigation 14 populations (from different ecological and geographical regions) representing four rose species were studied. They correspond to two distinctly different sections; *Pimpinelifoliae*, the most primitive section, and *Caninae*, the most advanced section (Khatamsaz 1992), present in Iran. Qualitative and quantitative characters were documented to explore inter- and intraspecific variation and classify the species.

MATERIALS AND METHODS

Plant material

Fourteen populations representing four *Rosa* species; *R. canina*, *R. foetida*, *R. hemisphaerica* and *R. iberica* were selected for morphological characterization. Details of the localities and codes are presented in **Table 1**. For each population, 3 to 6 individuals were studied. For each individual, 5 measurements per trait were recorded and means of quantitative traits were calculated (for example: for one population of a species four individuals were found and the diameter of 5 mature flowers per individual were measured = 20 measurements). The identity of specimens collected in the present investigation were confirmed with the assistance of Mrs. Khatamsaz (botanist) and an identification key which was reported by her (Khatamsaz 1992).

The specimens were deposited in the Herbarium of the Agricultural Biotechnology Research Institute of the Ministry of Agriculture of Iran.

In total, 48 quantitative and qualitative morphological characters were selected based on traits documented in the Flora of Iran (Khatamsaz 1992) and also our own field studies (**Table 2**). In phonetic analysis, the means of quantitative characters were used, but for qualitative characters, binary/multistate codes were applied. Variables were standardized (mean=0, variance=1) for multivariate statistical analyses (Chatfield *et al*. 1995; Sheidai *et al*. 2000).

Morphometry

In order to identify species with morphological similarities, cluster analysis using UPGMA (unweighted paired group mean average) and WARD (minimum variance spherical clusters) (Everitt 1986) as well as ordination based on principal component analysis (PCA) were performed (Sneath and Sokal 1973). The squared Euclidean distance was used as the dissimilarity coefficient in cluster analysis of morphological data.

To determine the most variable morphological characters among the species or populations, factor analysis based on principal component analysis was performed (Sheidai *et al*. 2000). Invariable characters were omitted before factor analysis. For multivariate statistical analyses the software NTSYS (Rohlf 1988) version 2.02 e (1997) and SPSS version 11.5 (2002) were used.

RESULTS AND DISCUSSION

Intraspecific variations

Cluster analysis and ordination of *R. canina* populations produced similar results (**Fig. 2**); two clusters were formed in both analyses, populations from Oroomye and Mazandaran (C3 and C4) which had comparable ecological positions showed greatest similarity in morphological characters and formed the first group. The other two populations (C1 and C2) formed the second group (**Fig. 2**). Factor analysis revealed that the first 3 factors embraced 100% of the total variation (**Table 3**). Characters such as form of prickles, glands on sepal, color of hips, leaflet length and width, petal length, petal width and flower diameter, which were the first factor analysis, comprised about 43.95% of the total variance and possessed the highest correlation (>70%). These factors separated Oroomye and Mazandaran populations from the others (**Fig. 2**).

The form of surplus to stipule, base of leaflet, hair on top of leaflet, hair on hypanthium and form of inflorescence showed the highest correlation with the second and third factor analyses (**Table 3**). Therefore, these are the most variable morphological characters among the *R. canina* populations.

Cluster analysis and ordination based on PCA factors of *R. iberica* populations produced similar results (**Fig. 3**). Populations from Oroomye and Mazandaran (I1, I2 and I4) showed similarities and formed the first group, but the Semnan population (I3) was distinctly separated from the other three populations. Factor analysis of morphological characters revealed that the first three factors embraced about

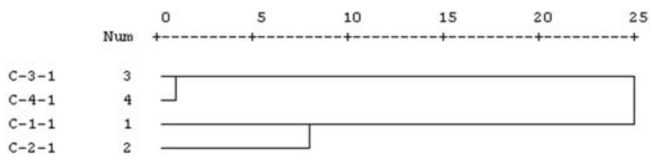

Fig. 2 Cluster analysis (WARD) of *Rosa canina*. Population codes as in **Table 1**.

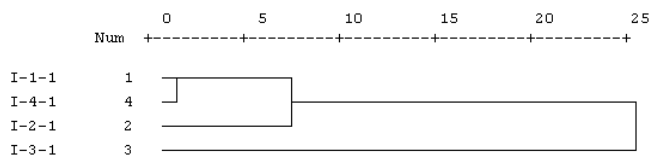

Fig. 3 Cluster analysis (WARD) of *Rosa iberica*. Population codes as in **Table 1**.

Table 2 Morphological characters and their coding key.

Character code	Description of the character	Coding key for the character
Qualitative characters		
1	Form of srhub	(0) erect (1) patent (2) uprepent (3) inclining
2	Color of branch	(0) reddish yellow (1) reddish green (2) red (3) brown (4) green (5) brownish green
3	Form of prickles	(0) equal (1) unequal
4	Base of prickle	(0) wide (1) narrow (2) wide or narrow
5	Form of surplus to stipule	(0) with surplus (1) without surplus
6	Base of leaflet	(0) roundish (1) cuneate (2) roundish or cuneate
7	Hair on adaxial surface of leaflet	(0) few hairs (1) glabrous (2) hairy
8	Hair on abaxial surface of leaflet	(0) few hairs (1) glabrous (2) hairy
9	Glands on adaxial surface of leaflet	(0) few glands (1) glabrous (2) glandular
10	Glands abaxial surface of leaflet	(0) few glands (1) glabrous (2) glandular
11	Leaflet shape	(0) ovate (1) obovate (2) ovate or ovate
12	Leaflet tip	(0) acute or obtuse (1) obtuse or truncate (2) obtuse or round (3) acute or apiculate
13	Leaflet margin	(0) serrate or double-serrate (1) serrate (2) double-serrate
14	No. of leaflets	(0) 5 (1) 7 (2) 5 or 7
15	Prickles on petiole	(0) prickly (1) glabrous
16	Hair on petiole	(0) hairy (1) glabrous
17	Glands on petiole	(0) glandular (1) glabrous
18	Form of inflorescence	(0) singular (1) single or double (2) panicle (3) singular, triplet or fivelet (4) corymb or raceme
19	Glands on pedicel	(0) glandular (1) glabrous
20	Hair on pedicel	(0) hairy (1) glabrous
21	Involucre	(0) has involucre (1) does not have involucre
22	Hair on hypanthium	(0) hairy (1) glabrous
23	Glands on hypanthium	(0) glandular (1) glabrous
24	Prickles on hypanthium	(0) prickly (1) glabrous
25	Form of sepal	(0) entire (1) dentate (2) dentate rarely entire (3) lanceolate
26	Hair on sepal	(0) hairy (1) glabrous
27	Prickles on sepal	(0) has prickles (1) glabrous
28	Glands on sepal	(0) glandular (1) glabrous
29	Sepal permanency	(0) deciduous (1) not deciduous
30	Sepal shape	(0) erect (1) reflexed (2) some are erect and some are reflexed (3) not erect and not reflexed
31	Edge of sepal	(0) entire (1) has lobe (2) lanceolate
32	Color of hips	(0) Brownish purple (1) red (2) blackish purple (3) reddish orange (4) brownish orange (5) yellow (6) brownish red
33	Prickles on hips	(0) prickly (1) glabrous
34	Hair on hips	(0) hairy (1) glabrous
35	Glands on hips	(0) glandular (1) glabrous
36	Hip shape	(0) roundish (1) roundish or ovate (2) apiculate
37	Petal color	(0) yellow (1) white (2) pink or white
38	Petal shape	(0) emarginated (1) truncate
39	Color leaf	(0) green (1) dark green (2) green attend to blue
40	State of achenes in hypanthium	(0) center (1) attached to wall
Quantitative characters		
41	Leaflet length	cm
42	Leaflet width	cm
43	Pedicel length	cm
44	Hip length	cm
45	Hip width	cm
46	Flower diameter	cm
47	Petal length	cm
48	Petal width	cm

100% of the total variation, in which glands on sepal, prickles on hypanthium, hair on sepal, prickles and hair on petiole possessed the highest correlation (>0.70). Therefore, these characters were considered the most variable morphological characters among *R. iberica* populations.

Cluster analysis and ordination based on PCA analysis for *R. foetida* populations is shown in **Fig. 4**. Two main clusters/groups were formed; populations of Hamedan (2), Oroomye and Damavand (F2, F3 and F4) formed the first group and Hamedan (1) (F1) formed the second group. Factor analysis revealed that the first 3 factors embraced 100% of the total variance. Characters such as glands on hypanthium, leaflet length, leaflet width, prickles on sepal, hair on hips and leaflet tip possessed the highest correlation (>70%). Therefore, these characters are considered the most variable morphological characters among *R. foetida* populations.

Only two populations for *R. hemisphaerica* were found, it was not possible to perform a cluster analysis, however, the two populations were different in some qualitative and quantitative characters such as color of branch, hair on the abaxial side of the leaflet, glands on the adaxial side of the leaflet, form of leaflet and leaflet tip.

In conclusion, factor analysis of morphological characters revealed that floral characteristics were more valuable than vegetative characteristics in determination of intraspecific variation and could be used as important tools in separating the populations (Nybom *et al*. 1997). Moreover, both quantitative and qualitative characteristics may generally be independent from geographical districts and environmental conditions, i.e. **Fig. 4** demonstrates that F1 and F2 populations of *R. foetida* which were from close geographical regions belonged to two different groups, whereas the F2 and F4 populations, both from different geographical districts, fitted in the same group. However, in order to accentuate the above statement, populations collected from different regions have been cultivated in the rose germplasm collection at Agricultural Biotechnology Research Institute of Iran (ABRII) and will be studied in a common environment in further investigations.

Table 3 Factor loading (showing high correlation) of morphological characters in *Rosa canina* populations with character code as in **Table 2**.

Character code	**Factor**		
	1	**2**	**3**
1	.437	.600	-.670
2	.705	.490	.513
3	.901	.421	.103
4	-.864	.406	.298
5	.375	.914	-.155
6	-.675	.737	-.039
7	.176	.892	.416
8	.421	-.795	-.437
11	.107	.522	-.846
14	.604	-.114	.789
15	.437	.600	-.670
16	-.864	.406	.298
17	-.604	.114	-.789
18	.176	.892	.416
20	.370	-.871	.323
22	.176	.892	.416
28	.864	-.406	-.298
29	.604	-.114	.789
32	.718	.587	-.375
35	-.176	-.892	-.416
41	.855	.170	-.491
42	.782	-.519	-.344
43	.873	-.102	.477
44	.575	-.452	.682
45	-.752	.044	.658
46	.983	-.171	.072
47	.980	.167	.107
48	.896	.404	-.184

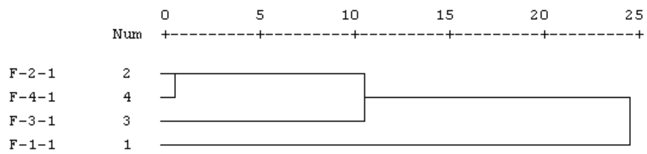

Fig. 4 Cluster analysis (WARD) of *Rosa foetida*. Population codes as in **Table 1**.

Interspecific relationship

The investigated species belonged to two different sections of subgenus Rosa: *R. canina* and *R. iberica* are in the section *Caninae* and *R. foetida* and *R. hemisphaerica* are in the section *Pimpinelifoliae*.

The phenogram obtained from cluster analysis and ordination plot based on PCA analysis of 14 populations showed two main clusters (**Figs. 5, 6**). *Rosa canina* and *R. iberica* formed the first sub-cluster and *R. hemisphaerica* and *R. foetida* formed the second sub-cluster, distinctly separating species according to the section.

Important qualitative characters separating *R. iberica* and *R. canina* were presence/absence of glands on the abaxial surface of the leaves (**Fig. 7**), hair and glands on hypanthium, hair on sepals and hips, form of leaflet tip (**Fig. 8**) and form of prickles. The quantitative characters that significantly separated the two species included leaflet length, pedicel length, flower diameter, petal length and petal width (**Fig. 5**).

In *R. hemisphaerica* and *R. foetida*, a few quantitative characters such as pedicel and hips length were significantly different. The qualitative characters separating *R. hemisphaerica* from *R. foetida* included the presence of glands and prickles on hips, presence of hair on sepal and hypanthium, edge of sepal and shape of leaflet (**Fig. 8**).

Variation in the quantitative characters may be due to the environment in which these plants grow. Ecological characters such as temperature, amount and frequency of rain and other climatic conditions may have an effect on inducing inter- and intraspecific variation (Nybom *et al.* 1997). It is possible that altitude and latitude may influence phenotype, for example the H1 population in Oroomye (1340 m in altitude) and the F1 population in Hamedan 1 (2147 m in altitude) were expected to be very different, but showed great similarities, which is misleading and this similarity could be due to these species growing in significantly different latitudes. Further investigations, characterizing propagates of individuals of all populations in a common environment will help determine the extent of morphological variability that can be attributed to genetic differences and the environment.

Roberts (1977) studied the morphological characters and showed that there was 79% similarity between *R. hemisphaerica* and *R. foetida*. Zielinski (1982) also reported that *R. foetida* was an intermediate between *R. hemisphaerica* and *R. kokanica* Regel. The observed similarity between *R. hemisphaerica* and *R. foetida* in the present investigation is comparable to previous reports.

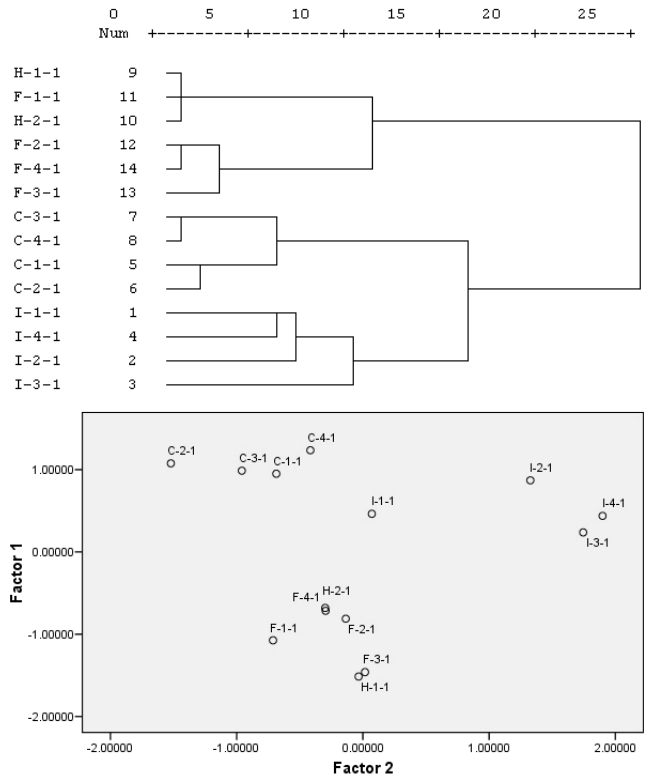

Fig. 5 Cluster analysis (WARD) and ordination of *Rosa* species. Population codes as in **Table 1**.

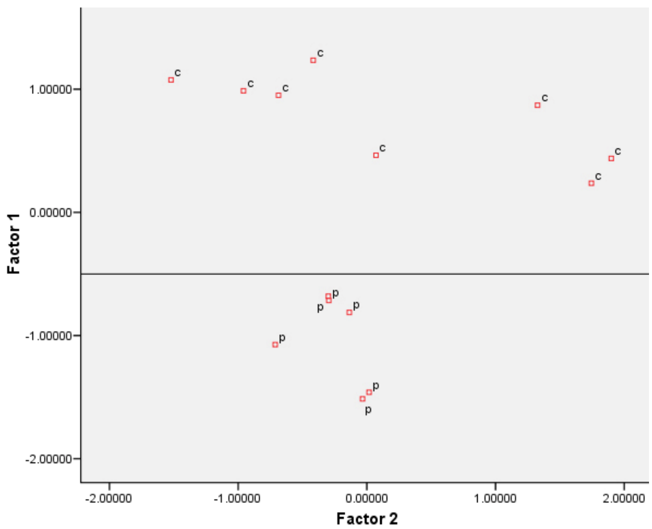

Fig. 6 Ordination of *Rosa* sections. C = section Canineae, P = section Pimpinellifolieae.

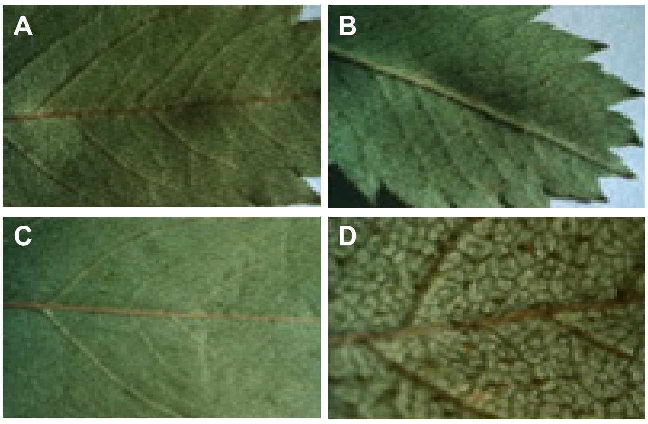

Fig. 7 The abaxial surface of the leaflet for (A) *Rosa hemisphaerica*, (B) *R. foetida*, (C) *R. canina* and (D) *R. iberica.* A & B both have hair and a few glands on the main vein, C is glabrous, and D is glandular and hairy.

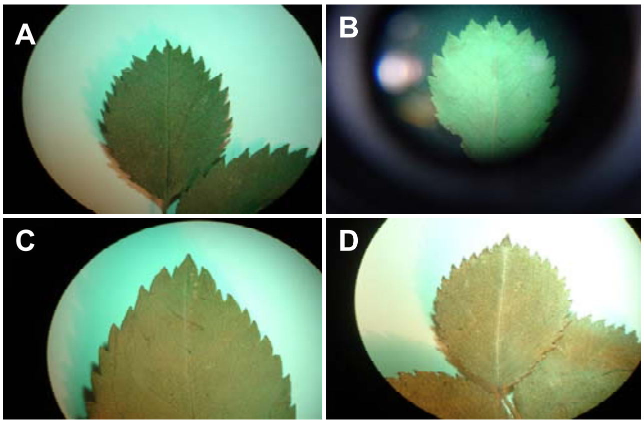

Fig. 8 Leaflet tip and leaflet form for (A) *Rosa hemisphaerica*, (B) *R. foetida*, (C) *R. canina* and (D) *R. iberica.* A and D have a round tip, B is acute, and C is apiculate.

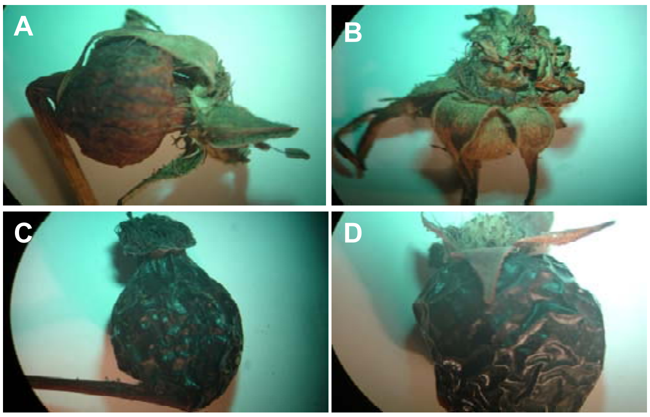

Fig. 9 Hip shape and sepal stability for (A) *Rosa hemisphaerica*, (B) *R. foetida*, (C) *R. canina* and (D) *R. iberica*. A, B and D have permanent sepals but C is deciduous. Hip shape is roundish in A and B but is urceolate in C and D.

Intersectional relationships

The intersectional relationship between *Caninae* and *Pimpinellifoliae* was investigated by multivariate statistical analysis. **Fig. 5** reveals a separation of the two sections and supports the classification by Rehder (1949). Khatamsaz (1992) and Rehder (1949) separated the two sections according to the form of sepal and petal color respectively, whereas our investigation revealed that other morphological characters, especially qualitative characters, such as hair on pedicle, prickles on sepal and hip shape, could also be used in the classification of these roses (**Fig. 9**). The OTUs (operational taxonomic units) versus their predicted group membership (sections) demonstrated 100% correct classification for the sections studied. A similar study by Aryavand (2002) also revealed that the phenetic analysis on OTUs of 12 taxa of the genus *Bromus* using UPGMA, WPGMA, clustering and factorial analysis could separate the taxa.

In conclusion, the morphological characteristics, especially the floral qualitative traits, were able to define the inter- and intraspecific variation in *R. canina*, *R. iberica*, *R. hemisphaerica* and *R. foetida* of Iran. The observed variation could be due to the general cross pollination and self incompatibility of roses (Cole and Melton 1986; Ueda and Akimoto 2001). The vast geographical distance between the populations, results in a low rate of gene transfer across the populations and species by natural means. Therefore, the diversity, may allow introduction of new genetic resources in the breeding pool of roses in a designed breeding program. Although, further study is needed to suggest a comprehensive key to separate different rose species across Iran according to their morphological characteristics, below is our suggested primary key for the investigated species.

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Primary key

Pedicles hairy; sepals prickly; hips roundish …………………………………………… Sect. *Pimpinelifoliae*
Leaflets roundish; sepals hairy, edge of sepal smooth; hypanthia hairy; hips glandular and prickly ……………… *R. foetida*
Leaflets obovate; sepals smooth, edge of sepal lanceolate; hypanthia smooth; hips smooth ……………… *R. hemisphaerica*
Pedicles glabrous; sepals hairy; hips urceolate …………………………………………… Sect. *Caninae*
Leaflets roundish; hips hispid; hypanthia hispid …………………………………………… *R. iberica*
Leaflet apiculate; hips smooth; hypanthia smooth …………………………………………… *R. canina*

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