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A taxonomic review of *Rubus* L. (Rosaceae) in the Northern Iran based on the analysis of quantitative morphological characters

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Abstract

The genus *Rubus* is highly variable and frequently occurring in Iran, particularly in North regions. It has a global distribution on all climates except the Antarctic region. Ten morphological quantitative characters in seven species of *Rubus* in N Iran were studied. Based on the UPGMA cluster analysis of quantitative characters, the similarity of individuals was measured from 0.04 to 1. Our results showed high morphological variation among the populations. The northern Iranian *Rubus* populations were segregated from each other based on analysis of the quantitative morphological data. Quantitative morphological characters of the species were variable in different climatic conditions. Two species i.e. *R. sanctus* and *R. persicus* showed high rate of morphological variability in comparison to other species. Our results indicates that the morphology does not reflect the high rate of hybridization in *Rubus*, which is frequently reported by other researchers.

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Introduction

The family Rosaceae consists of ca. 90 genera and about 2520 species, distributed cosmopolitan with a higher dispersion in temperate regions of the northern hemisphere (Stevens, 2010; Khatamsaz, 1992). The genus *Rubus* (Rosaceae; subfamily Rosoideae) includes shrubs or rarely perennial herbaceous plants. Due to its hybridization, polyploidy, agamospermy, and loss of the concept of species, genus *Rubus* is one of taxonomically most complex taxa (Alice & Campbell, 1999). This complex taxonomy has resulted in broad disagreement about the number of species in the genus, with different estimates ranging from 600 (Thompson, 1995) to several thousand (Jennings, 1988). The genus is divided into 12 subgenera of which only few species have been domesticated (Focke, 1910-1914; Jennings, 1988; summarized in Tab. 1). Three subgenera, *Idaeobatus*, *Rubus*, and *Malachobatus*, include the most species of *Rubus*. *Rubus* subg. *Idaeobatus* contains the "raspberries" that are distributed in the northern hemisphere, mainly Asia, Africa, Europe, and N America, whereas the subgenus *Rubus* (blackberries) includes species found in Eurasia, and N America.

Table 1. Subgenera and number of species of *Rubus* in the world (Focke, 1910, 1911, 1914).

Subgenus	Number of species
R. subg. *Anoplobatus* (Focke) Focke	6
R. subg. *Chamaebatus* (Focke) Focke	5
R. subg. *Chamaemoras* (Hill) Focke	1
R. subg. *Comaropsis* (Rich.) Focke	2
R. subg. *Cylactis* (Raf.)Focke	14 (4 series)
R. subg. *Dalibarda* (L.) Focke	5
R. subg. *Dalibardastrum* Focke	4
R. subg. *Idaeobatus* (Focke) Focke	117 (9 sections)
R. subg. *Lampobatus* Focke	10
R. subg. *Malachobatus* (Focke) Focke	115 (7 sections)
R. subg. *Orobatus* (Focke)	19
R. subg. *Rubus* L. (= *Eubatus* Focke)	132 (6 sections)

The ploidy level of this interesting group of plants ranges from diploid to dodecaploid, mostly apomictic and highly heterozygous (Thompson, 1995). Polyploidy and hybridization prevails in the subgenus *Rubus*. Only subgenera *Idaeobatus, Dalibarda*, and *Anoplobatus* are predominantly diploid, whereas *Dalibardastrum, Malachobatus*, and *Orobatus* are exclusively polyploid (Thompson, 1995, 1997). Hybridization occurs in *Rubus* between closely related species (Kraft *et al*., 1996; Naruhashi, 1979, 1990; Steele & Hodgdon, 1963, 1970) and, in some cases, between subgenera (Alice *et al*., 1997; Gustafsson, 1942; Jennings, 1978; Weber, 2003).

Taxonomy of the genus in Iran has long been disputable. Parsa in his Flora de l' Iran (1943), reported 11 species of *Rubus*.). Flora Iranica described seven species for Iran, all of them from subgenus *Rubus* (Riedl, 1969). As most recent taxonomic treatment of the genus, Khatamsaz described eight species from Iran, most of them from N Iran (Khatamsaz, 1992; Tab. 2).

Several studies on the genus *Rubus* have shown close but variable relationships between the taxa at infra-generic level, resulted in complexity of classification (Gustafsson, 1942, Alice and Campell, 1999).

In Iran, a comprehensive study has been done on *Rubus*. Resources, full description and key to identify the species of raspberry, not provide. Therefore, this study intends to review the taxonomy of the genus in the N Iran, by performing an extensive collection of specimens belonging to populations of different species growing in wild. We here aim to use morphological markers to delimit the species boundaries by providing complete and accurate species, try to resolve the taxonomic problems of the genus *Rubus* L. in N Iran.

Table 2. Number, distribution and vegetative form of *Rubus* species in Iran according to Khatamsaz (1992).

Species	Distribution in Iran	Distribution in world	Vegetative form
R. saxatilis L.	North west	Iran, Europe, Turkey, Soviet	Herbaceous
R. caesius L.	North, West, Center	Iran, Europe, Turkey, Soviet, Iraq	shrub
R. hirtus Waldst. & Kit.	North	Iran, Europe, Turkey, Soviet	shrub
R. sanctus Schreber	North, West, Center, East	Iran, Europe, Soviet, Pakistan, Afghanistan, Turkmenistan	shrub
R. discolor Weihe & Nees.	North, North west	Iran, Europe, Turkey, Soviet, Lebanon	shrub
R. persicus Boiss	North	Iran, Soviet	shrub
R. hyrcanus Juz.	North	Iran, Soviet	shrub
R. dolichocarpus Juz.	North	Iran, Soviet	shrub

Materials and methods

Plant materials

Morphologic examination was performed on 111 specimens belonging to seven species of *R.* subg. *Rubus* collected from their natural habitat in N Iran (Guilan and Mazandaran provinces; Fig. 1). Herbarium specimens from healthy plants with no contamination by fungi and insects were collected and each specimen was placed in a separate envelope. For each specimen, latitude, longitude, and altitude were recorded with a GPS (Tab. 3) (Martin, *et al.* 2011).

Table 3. *Rubus* species studied and collection sites.

Species	Collection sites
R. caesius	Guilan, Astara, Abasabad. 38º 46´ 05´´ N, 48º 89´ 46´´E, 280 m. Guilan, Langroud, Otaghvar. 37º 06´ N, 50º 06´ E, 80 m. Guilan, Langroud, Otaghvar. 37º 06´ N, 50º 06´E, 76 m.
R. discolor	Guilan, Talesh, Ghorogh. 37º 83´ N, 48º 96´ E, 92 m. Guilan, Astara, Khotbeh sara, 38º 46´ 45´´ N, 48º 90´ 427´´E, 272 m. Guilan, Rudsar, 36º 96´ 67´´ N, 50º 56´ 67´´ E, 76 m. Mazandaran, Tunkabon, 36º 81´ 05´´ N, 50º 87´ 88´´ E, 175 m.
R. dolichocarpus	Guilan, Lahijan, Khokehab-e Loonak, 37º 00´ 30´´ N, 49º 51´ 48´´E, 1021 m. Guilan, Astara, Khalkhal, 37º 37´ N, 48º 32´ E, 778 m. Guilan, Fuman, 34º 35´ 16´´ N, 41º 08´ 79´´E, 338 m.
R. hirtus	Mazandaran, Noshahr, kheyroudkenar Forest, 36º 27´ N, 51º 33´ E, 603 m. Guilan, Astara, Khalkhal, 37º 37´ N, 48º 32´ E, 778 m. Guilan, Astara, Khalkhal, 37º 37´ N, 48º 32´ E, 545 m. Guilan, Siahkal, 37º 00´ 30´´ N, 49º 51´ 48´´ E, 100 m.
R. hyrcanus	Guilan, Astara, Khalkhal, 37º 37´ N, 48º 32´ E, 711 m. Guilan, Astara, Khalkhal, 37º 37´ N, 48º 32´ E, 634 m. Guilan, Astara, Khotbeh sara, 38º 46´ 45´´N, 48º 90´ 27´´E, 272 m. Guilan, Talesh, Chobar. 38º 25´ 91´´N, 48º 92´ 27´´E, 89 m. Guilan, Langroud, Otaghvar. 37º 06´ N, 50º 06´ E, 70 m.
R. persicus	Guilan, Astara, Khalkhal, 37º 37´ N, 48º 32´ E, 731 m. Guilan, Talesh, 37º 55´ 08´´N, 48º 03´ 71´´E, 80m. Mazandaran, Noshahr, kheyroudkenar Forest, 36º 27´ N, 51º 33´ E, 603 m. Guilan, Astara, Gardeneh-e Heyran, 38º 90´ 73´´ N, 48º 02´ 20´´ E, 317 m, 494 m and 541 m. Guilan, Astara, 25 Kilometer to Gardeneh-e Heyran, 38º 90´ 70´´ N, 48º 02´ 18´´ E, 89 m. Guilan, Talesh, Chobar. 38º 25´ 91´´ N, 48º 92´ 71´´ E, 89 m.
R. sanctus	Guilan, Talesh, 37º 55´ 08´´ N, 48º 03´ 71´´ E, 80 m. Mazandaran, Noshahr, kheyroudkenar Forest. 37º 34´ N, 52º 47´ E, 623 m. Guilan, Langroud, Leilakouh, 37º 011´ 72´´N, 50º 09´ 09´´ E, 5 m. Guilan, Rasht, inside of Rasoul-e Akram Hospital, 38º 27´ N, 49º 27´ E, 0 m. Mazandaran, Behshahr, 36º 70´ 98´´ N, 53º 53´ 73´´ E, 200 m. Mazandaran, Babolsar, 36º 69´ 98´´ N, 52º 64´ 47´´ E, 109 m.

Fig. 1. *Rubus* sampling localities in N Iran.

Quantitative morphological characters

10 quantitative characters based on published literature and observations collected during the study were considered. *Rubus* herbarium specimens were examined using a stereomicroscope and each specimen was imaged. The quantitative characters was include: Stipule length, stipule width, number of leaflets, petiole length, terminal leaflet width, terminal leaflet length, terminal petiolule length, latitude of stems spine and internode length. Quantitative characters were measured with a millimeter ruler.

To determine the affinity of the genotypes and grouping them on the basis of morphological results, cluster analysis was carried out based on UPGMA method using Jaccard's coefficient (Sokal and Michener. 1958) and then dendrogram was plotted using software NTSYSpc, ver. 2.02. In this type of analysis, people of similar characters are placed together in a single cluster. As a result of clustering, people who are in a cluster have high similarity and those who are placed in separate clusters are more heterogeneous (large differences).

Results

Diagnostic characters of Rubus species in Northern Iran

1. *R. caesius*

It is a species with a very low altitude, short and runner. Pale green stems, thin, round (not polygonal), needles spine, slightly curved and short with the maximum length of 1.2 mm and a too low density were observed. The leaves are tri-leaflets, very small,

with the maximum length of 40-50 mm, broad and short stipule with a length of 7-8 mm. It is a late flowering species with late fruit. Sepals are broad, nearly erect on the fruit and petals are white. Sepals and pedicel has red glandular multicellular trichomes. The fruit is dusty black and carpel is large and small numbers. This species often, seen in shady and moist areas of the N Iran as single colonies and rarely in combination with other species of *Rubus*.

2. *R. hirtus*

Shoot is not polygonal, pale green and thin. Young shoots has multicellular glandular and red-purple needle trichome with high density. Needle spines are straight, thin and have a maximum length of 6 mm. The leaves are tri-leaflets (Maximum length 130 mm) and the upper and lower surfaces possess abundant trichome, long and filiform stipule with the length of approximately 16 mm. Sepals are very elongate and taper, fully erect on the fruit, white petals with a depression at the apex and freshly black druplets. This is a rare species in N Iran and could be seen in moist, shady forest floor.

3. *R. Sanctus*

It is a plant often with excessive height, specifically dusty stem (presence of star and felt trichomes) and polygonal and thick. Spines with a broad triangular base and a curved tip with the maximum length of 7 mm are the remarkable characters of this species. The leaves are tri-leaflets (Maximum length 70 mm) with considerable thickness; midrib on the adaxial surface has a curved spine. Sepals are broad and perfectly hung over the fruit, pink-red and thick petals; filament is pink with considerable thickness. This species is one of the major species on the street margin and widely seen in most habitats of N Iran.

4. *R. discolor*

It is a plant often with excessive height with reddish-brown polygon and thick stems. The spines are needles, straight with maximum length 6 mm, thin on top and slightly wider at the base. Leaves have 3-5 leaflets with approximately 80 mm in length; midrib on the adaxial level has a spine and filiform and long stipule with 15 mm in length. Sepals are broad and perfectly hung over the fruit; petals are pale pink, with white filament. It is desirable species distribution in N Iran.

5. *R. persicus*

Stems are reddish-brown, particularly polygon with considerable thickness. The hook-shaped spines (maximum length 6 mm) with a very high density in the shoots are the remarkable characters of this species. The leaves are usually 5 leaflets (length of about 90 mm); leaflets are marked with an elongate tip (acuminate) and midrib on the adaxial level has spine. Sepals are elongate and perfectly hung over the fruit and petals are white. It has aroma fruit with very pleasant flavors and widely seen in the varied habitats of N Iran.

6. *R. hyrcanus*

Stems are pale green and polygon and few thin and fragile and curved needle spines with maximum length 5 mm. The leaves are tri-leaflets; large leaflets (maximum length 140 mm), pale green and thin and midrib on the adaxial surface has spine. Stipule is long and filiform about 16 mm. Pedicel often is lack of spine; sepals are elongate and perfectly hung over the fruit and petals are white, elongate and thin. It is a sciophyte species (shade plant) with a very limited distribution in N Iran.

7. *R. dolichocarpus*

It is a plant with very high heights, and dark green–brown shoot, and polygon and has straight needle spine with a triangular base (maximum length of 5 mm high). The leaves are tri-leaflets; large leaflets (maximum length 160 mm) and midrib on the adaxial surface has spine. Sepals are elongate and perfectly hung over the fruit and petals are white. The number of carpel is large with low water and dry. This species with considerable distribution were found in the rainy areas of N Iran.

Morphological analysis and taxon proximity

The similarity of the varieties and genotypes on the dendrogram based on Jaccard's coefficient is variable

from 0.04 to 1. This similarity is indicating the presence of a very high morphological variation among the populations (Fig. 2). *Rubus* populations of N Iran based on morphological data were specifically distinct in similarity coefficient 0.52.

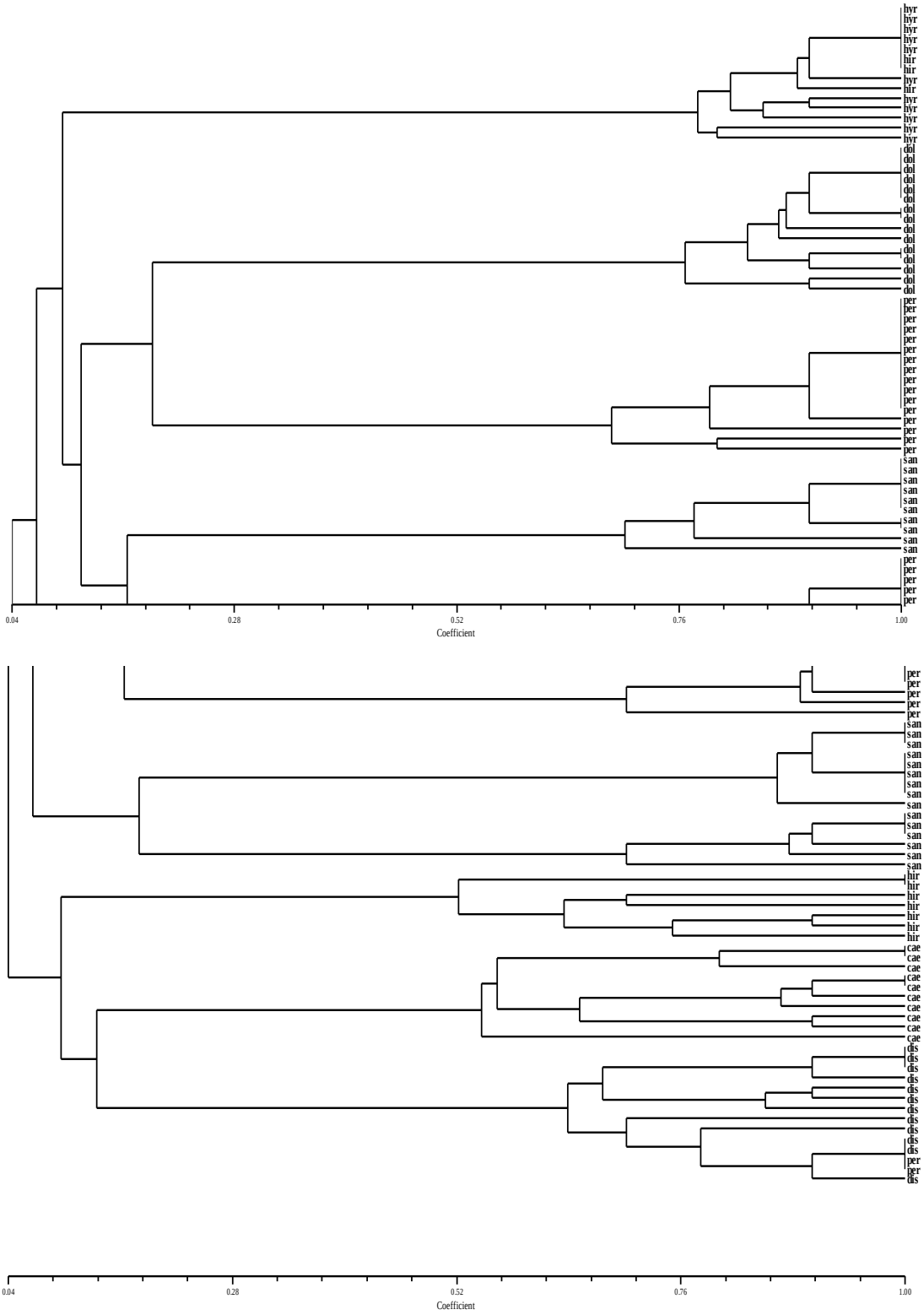

Fig. 2. Cluster analysis of 111 genotypes of *Rubus* L., calculated based on morphologic markers. The UPGMA method was the grouping criterion.

Quantitative morphological characters such as trichome density and shoots spine, internode and leaflets length under different climatic conditions in the species, especially in the two species *R. Sanctus* and *R. persicus* was variable. The morphological characters of two species because of widespread distribution in all habitats and climates have changed and the population was grouped according to climatic conditions. Some population belonging to two species *R. Sanctus* and *R. persicus* located in similarity coefficient about 0.15 in a cluster. The main reason for the close proximity of these two species can be mentioned as following:

Taxon belonging to two above species has been sampled from a range of ecological and climatic area (West Guilan). On the other hand, synchronization of flowering and consequently the pollination in the two species can be an increasing factor to occurrence of interspecific hybridization. Consequently, the morphological structure of the population is heterogeneous and the population will develop an interspecific morphological characters.

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