

Numerical taxonomy of the genus *Glycyrrhiza* in the Hyrcanian region

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Noticeable variation was observed in a number of morphological characters in the *Glycyrrhiza* specimens in various habitats in north of Iran. To study these variations, two taxa related to the Hyrcanian region from all four known taxa of this genus in Iran, including *Glycyrrhiza echinata*, and *G. glabra* var. *glabra* were studied by numerical taxonomy using morphological characters in three provinces, Golestan, Mazandaran, and Gilan. A total of 15 populations were studied in terms of 60 quantitative and qualitative characters of vegetative and reproductive organs, and the obtained data were analyzed by SPSS software after encoding. Based on the PCA analysis, 11 components created about 98% of diversity, indicating the separation of relatively numerous characters for these taxa. Among these traits, the best one was the length of the pedicle. In the WARD cluster analysis, the populations of each taxon are located in a separate cluster and their position in each cluster is also significantly aligned with the longitude, which can be attributed to the significant influence of climate conditions, including relative humidity from west to east on the variety of peculiarities in these taxa. Based on the results of this study, taxonomic treatment of the studied taxa in Flora Iranica confirmed.

Keywords: *Fabaceae*, *Glycyrrhiza*, Iran, morphometric, numerical taxonomy**مطالعه تاکسونومی عددی جنس شیرین بیان در منطقه هیرکانی***

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خلاصه

تنوع قابل ملاحظه در صفات ریختی متعددی در جنس شیرین بیان (باقلائیان) مشاهده گردید. جهت بررسی چگونگی این تغییرات، دو آرایه مربوط به منطقه هیرکانی شامل: *G. echinata* و *Glycyrrhiza glabra* var. *glabra* از مجموع سه آرایه شناخته شده از این جنس در ایران (گونه سوم *G. aspera* در غرب و مرکز کشور، در منطقه رویشی ایران-تورانی پراکنش دارد)، در هر سه استان گلستان، مازندران و گیلان مورد مطالعه ریخت‌شناسی به روش تاکسونومی عددی قرار گرفتند. در مجموع، ۱۵ جمعیت از لحاظ ۶۰ صفت کمی و کیفی مربوط به اندام‌های رویشی و زایشی مورد مطالعه دقیق قرار گرفته و داده‌های به دست آمده، پس از کدبندی با استفاده از آنالیزهای آماری توسط نرم‌افزار SPSS ارزیابی شدند. براساس آنالیز PCA، تعداد ۱۱ مؤلفه حدود ۹۸ درصد تنوع را ایجاد کردند که نشان‌دهنده قدرت تفکیکی صفات نسبتاً متعددی برای این آرایه‌هاست. از میان این صفات، بهترین صفت طول دمگل بوده است. براساس نتایج، وضعیت رده‌بندی دو آرایه مطالعه شده در فلورا ایرانیکا تایید شد. در آنالیز خوشه‌بندی به روش WARD، جمعیت‌های هر آرایه در خوشه جداگانه قرار گرفته و جایگاه آن‌ها در هر خوشه نیز به طور معنی‌داری در راستای طول جغرافیایی مرتب شده است و این می‌تواند مربوط به تاثیر قابل توجه شرایط اقلیمی از جمله میزان رطوبت نسبی از غرب تا شرق روی تنوع ویژگی‌های ریختی در این آرایه‌ها باشد. براساس نتایج مطالعات متعدد، تفاوت‌های ریختی در این آرایه‌ها به طور معنی‌داری با تفاوت در مواد مؤثره گیاهی همراه است و از این نظر مطالعات تنوع ریختی در بررسی‌های آنالیز مواد مؤثره دارویی جایگاه ویژه می‌یابد.

واژه‌های کلیدی: ایران، باقلائیان، تاکسونومی عددی، شیرین بیان، مورفومتري

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Introduction

The name of *Glycyrrhiza* L. due to its very sweet root is derived from the Greek word Glucose and Rizza, respectively, with the meaning of sweet and root (Xian Zhang *et al.* 2012). The most important characteristics of the licorice are gastric ulcer healing and gastric cancer treatments (Valiollahpor *et al.* 2006).

Based on the APG IV classification system, the genus of licorice is classified in the *Angiosperm* division, the *Eudicots* class, the *Rosids* subclass, the *Fabales* order, and the *Fabaceae* family (Ghahremaninejad & Nejad Falatoury 2016). The genus *Glycyrrhiza* includes 30 species in the world (Vibha *et al.* 2009) dispersed in the temperate and hot temperate regions, particularly in the Mediterranean area (Barghi & Siljak-Yakovlev 1990). The Hyrcanian region is the important area in distribution map of the three *Glycyrrhiza* taxa in Iran and the most of variable habitats are in three northern provinces. According to Flora Iranica (Rechinger 1984), there are three *Glycyrrhiza* species in Iran, including *G. aspera* Pall., *G. echinata* L., and *G. glabra* L. with two varieties *G. glabra* L. var. *glabra* (the autonym) with smooth legume and *G. glabra* var. *glandulifera* (Waldst. & Kit.) Boiss. with glandular-bristly legume. *Glycyrrhiza aspera* and *G. glabra* var. *glandulifera* not identified among the *Glycyrrhiza* materials in this study and not yet reported in flora Iranica from the north of the country.

Noticeable variation was observed in a number of morphological characters in the *Glycyrrhiza* specimens collected from north of Iran. Significant variation in Glycyrrhizin was observed in a number of wild populations of the *Glycyrrhiza* species in Iran (Haji-Mehdipour *et al.* 2008). The results of phenetic relationships study among natural population accessions

of *G. glabra* varieties in central Zagros region confirmed that separation of the varieties based on the single qualitative character in the main Flora and literatures (Sharifi-Tehrani *et al.* 2012). In the cyto-morphological studies of the genus *Glycyrrhiza* in Iran, clustering by morphological characters indicated distinctness species. The biosystematics studies about the genus *Glycyrrhiza* are limited in the world, and are very little in Iran too (Sheidai *et al.* 2008). To survey about morphological variation in the northern populations of *Glycyrrhiza* in Iran and for prepared the base of pharmacological study about how change contains extracted of them, two taxa related to the Hyrcanian region, including *G. echinata*, and *G. glabra* var. *glabra* from all four known taxa of this genus in Iran, studied morphologically.

Materials and Methods

For present study, samples totally consisted of 15 populations of habitats in three provinces include Gilan, Mazandaran, and Golestan on the margin of the Caspian Sea in the north of Iran (Fig. 1) evaluated. The specimens were identified based on important flora including: Flora Iranica (Rechinger 1984), Flora of Iraq (Townsend 1974), Flora of China (Bojian & Larsen 2010), Flora of Turkey (Chamberlain 1970), Flora Europaea (Yeo 1968), and Flora of the USSR (Grigor' ev & Vassil' chenko 1948). The herbarium specimens of the material studied in this paper are deposited at the Herbarium of Mazandaran University (HUMZ), Babolsar (Iran). Fifteen populations of *G. glabra* var. *glabra* and *G. echinata* were evaluated morphologically. The populations studied using the relevant codes are shown in figure 1 and table 1.



Fig. 1. The situation of the studied populations of *Glycyrrhiza* localities in Hyrcanean area in North of Iran.

Morphometric characters were considered to study populations. For the selection of characters, previous studies and related flora and results from personal experiences were used (Sheidai & Fadaie 2005, Sheidai *et al.* 2011). A total of 60 vegetative and reproductive characteristics, including 24 qualitative and 36 quantitative characters, were studied on 15 selected populations (Table 2). These characters were examined for the first time. Values (for quantitative features) and states (for qualitative features) were evaluated for the attributes obtained from all organs (including leaf, flower, fruit etc.).

All characteristics were encoded as 0, 1, or 2 and first entered in Excel software and then in SPSS software (Initial statistical evaluation of qualitative and quantitative analysis was conducted by SPSS Ver. 16 software). The ANOVA method was used to analyze the qualitative characters and all the traits were analyzed in the Least Significant Different (LSD) test. WARD and Average Linkage methods were used for cluster analysis based on morphological characteristics to determine the relationship between taxa and populations. In order to determine the proximity between species and populations, in most of the morphological characteristics, Principal Component Analysis (PCA) was used. Factor analysis was performed based on PCA to identify the most variable morphometric characters between species and populations.

Results

Based on the obtained data from the principal component analysis (PCA), the first 11 components generated a total of 98.002% of the total diversity, which the first five components contributed 76.181% of the variation.

In the first component, with 43.565% of the total variation, the characters include legume apex shape, legume tissue, legume indumentum, seed number in legume, legume number in apical raceme, legume length, angle of aggregate legume with stem axis, apical aggregate legume length, basal aggregate legume length, plant height, leaflet apex shape, leaf indumentum, corolla color, vexillum apex, vexillum shape, keel shape, inflorescence length, inflorescence shape, calyx shape, calyx indumentum, sepal apex shape, pedicle length, pedicle indumentum, mature aggregate legume shape, and stem indumentum respectively have the highest level of correlation to create the diversity. Among the second component created 10.081% of the total variance, with leaf indumentum, keel length, and calyx upper lobe length characters, respectively played the most role in creating diversity. In the third component, with 8.75% of the total variation, the apical leaflet length in apical leaf, the number of the leaflet at apical leaf, and petiole width characters had the most roles for creating diversity.

The last six components (6–11th components), also showed 21.821% of the total variation. Variable

characters included lowest aggregate legume width, wing length, and calyx upper lobe length.

- Analysis of morphological features and studying phenetic relationships

Morphological characteristics of 15 populations of two species, includes four populations of *G. glabra* and 11 populations of *G. echinata* have been studied based on 60 qualitative and quantitative characters. Dendrogram from cluster analysis using WARD method represent two main clusters, grouped according to 98/002% of diversity (Fig. 2). The main cluster divided finally into four main groups with 98 diversity including two main clusters. The main cluster 1 consists of all four populations of *G. glabra*, in which the subcluster 1-1 consist of Gorgan Agricultural Research Station, and Kordkouy (G. Ta. and G. Ko.) populations and the subcluster 1-2, including the Manjil and Totekabon (G. Mn. and G. To.) populations. Two populations in each of these subclusters are close to the environmental, geographic and other habitats, and have enough distance between the another subclusters too. The main cluster (2) contains the population of *G. echinata* and is divided into

two subclusters. The subcluster 2-1 contains the populations of *G. echinata* from Kiyakola (E. Ki.), Joybar (E. Ju.), and Sari University of Agricultural Sciences (E. Da.). The subcluster 2-2 is divided into two secondary subclusters. The secondary subcluster 2-2-1 consists of *G. echinata* population from Gorgan airport (E. Fo.), Ghaemshahr (E. Le.), Fereydunkenar (E. Fe.), Rasht (E. Ra.), and Bablosar (E. Ba.). The secondary subcluster 2-2-2 includes the *G. echinata* populations from Sari, Firouzkandeh (E. Fi.), Mah-forouz-mahalleh (E. ma.), and Kochesfahan (E. Ku.).

Based on results of analyses on morphological characters, among all the studied populations of *G. glabra* species, Manjil (G. Mn.), and Totekabon (G. To.) populations are morphologically most proximate and Kordkouy (G. Ko.), and Gorgan (G. Ta.) populations have the least proximity. Among the *G. echinata* populations, the highest morphological proximity is found among Firouzkandeh (E. Fi.), and Mah-forouz-mahalleh (E. Ma.), are located in Sari.

Table 1. Characteristics of populations in *Glycyrrhiza* species studied

Taxon	Po. No.	Po. co.	Locality (Iran)	Alt. (m)	Hb. No.
<i>G. echinata</i>	1	E. Da	Mazandaran province: Sari Husbandry University	-4 m	4003
<i>G. echinata</i>	2	E. Ju	Mazandaran province: Jooybar, Shahnekola village	-14 m	4004
<i>G. echinata</i>	3	E. Ki	Mazandaran province: Kiakola	-2 m	4005
<i>G. echinata</i>	4	E. Ba	Mazandaran province: Babolsar	-16 m	4006
<i>G. echinata</i>	7	E. Fo	Golestan province: Gorgan airport road	-5 m	4007
<i>G. echinata</i>	8	E. Le	Mazandaran province: Qaemshahr, Paeinlamok village	3 m	4008
<i>G. echinata</i>	10	E. Fi	Mazandaran province: Sari, Firoozkande village	-9 m	4010
<i>G. echinata</i>	16	E. Fe	Mazandaran province: Fereidoonkenar, sea beach	-34 m	4016
<i>G. echinata</i>	18	E. Ra	Gilan province: Rasht	49 m	4018
<i>G. echinata</i>	21	E. Ku	Gilan province: Kuchesfahan village	23 m	4020
<i>G. echinata</i>	11	E. Ma	Mazandaran province: Sari, Mahforoozmahalle village	-1m	4011
<i>G. glabra</i>	5	G. Ko	Golestan province: Kordkooy	23m	4021
<i>G. glabra</i>	6	G. Ta	Golestan province: Gorgan Agricultural Research Station	515 m	4022
<i>G. glabra</i>	19	G. Mn	Gilan province: Manjil	358 m	4023
<i>G. glabra</i>	20	G. To	Gilan province: Roodbar, Totekabon	157 m	4019

Abbreviations: Po. No. = Population number, Po.co = Population code, Alt. (m) = Altitude (m), Hb. No. = Herbarium No. (In HUMZ)

Table 2. Morphological characters with their abbreviations and coding in studied *Glycyrrhiza* populations

No.	Character	Coding		
		0	1	2
1	Aggregate legume shape (A.L.S.)	Ovate	Clustered	
2	Legume tissue (L.T.)	Woody	Leathery	
3	Legume indumentum (L.I.)	Spiny (echinate)	Glaber (inermis)	
4	Seed number in legume (S.N.L.)	1-3	4-5	
5	Legume number in apical raceme (L.N.A.)	15-30	31-60	61-100
6	Legume number in basal raceme (L.N.B1)	15-30	31-60	61-100
7	Legume number in middle raceme (L.N.M.)	15-30	31-60	61-100
8	Legume Length (L.L.)	Less than 1cm	2-5 cm	
9	Legume width (L.W.)	Less than 1 cm	1 cm and more	
10	Angle of aggregate legume with stem axis (A.A.A)	About 45 degree	60-90 degree	
11	Legume apex shape (L.A.S.)	Lanceolate	Aristate	
12	Apical aggregate legume length (A.A.L.)	1-5 cm	5-10 cm	11-15 cm
13	Basal aggregate legume length (B.A.L.)	1-5 cm	5-10 cm	11-15 cm
14	Apical aggregate legume width (A.A.W.)	1-2 cm	2-5 cm	
15	Lowest aggregate legume width (L.A.W.)	1-2 cm	2-5 cm	
16	Basal leaflet length at apical leaf (B.L.A.)	1-2 cm	2-5 cm	
17	Apical leaflet length in apical leaf (A.L.A.)	1-2 cm	2-5 cm	
18	Basal leaflet width in apical leaf (B.L.W.)	1-2 cm	2-5 cm	
19	Apical leaflet width in apical leaf (A.L.W.)	1-2 cm	2-5 cm	
20	Leaflets number at lowest leaf (L.N.L.)	1-10	11-20 cm	
21	Leaflet number at apical leaf (L.N.A1)	1-10	11-20 cm	
22	Plant height (P.H.)	Less than 0.5 cm	0.5-1.5 cm	
23	Petiole length (P.L.)	Less than 2 cm	2-5 cm	
24	Petiole width (P.W.)	Up to 1 mm	1-2 mm	
25	Leaflet apex shape (L.A.S1)	Mucronate	Emarginate	
26	Leaf indumentum (L.I1)	Tomentose	Glandular tomentose	
27	Corolla color (C.C.)	Violate	Purple and white	
28	Vexillum apex (V.A.)	Acuminate	Mucronate	
29	Vexillum shape (V.S.)	Subrhomboid-elliptic	Narrowly elliptic	
30	Vexillum length (V.L.)	Less than 5 mm	5-10 mm	
31	Vexillum width (V.W.)	Less than 2 mm	2-5 mm	
32	Wing shape (W.S.)	Lanceolate	Ovate-elliptic	
33	Wing length (W.L.)	Less than 2 mm	2-4 mm	
34	Wing width (W.W.)	Less than 2 mm	2-4 mm	
35	Keel shape (K.S.)	Narrowly elliptic	Linear	
36	Keel width (K.W.)	Less than 2 mm	2-4 mm	
37	Keel Length (K.L.)	0.15-2 mm	2-4 mm	
38	Stamen length (S.L.)	Less than 2 mm	4-8 mm	
39	Inflorescence density (I.D.)	Aggregated	Non aggregated	
40	Inflorescence length (I.L.)	Less than 3 mm	3-9 mm	
41	Number of flowers in inflorescence (F.N.I.)	0-30	31-40	
42	Inflorescence shape (I.S.)	Compound cluster	Cluster	
43	Peduncle length (P.L1)	2 cm	2-5 cm	
44	Stipule width (S.W.)	Less than 2 mm	2-4 mm	
45	Stipule length (S.L1)	Less than 2 mm	2-4 mm	
46	Stipule shape (S.S.)	Triangular (acute)	Lanceolate-triangular	
47	Calyx shape (C.S.)	Triangular	Cup-shaped	
48	Calyx upper lobe length (C.U.L.)	Less than 2 mm (rarely 2 mm)	2-4 mm	
49	Calyx lower lobe length (C.L.L.)	Less than 2 mm	2-4 mm	
50	Calyx indumentum (C.I.)	Tomentose	Glandular tomentose	
51	Sepal apex shape (S.A.S.)	Pungent	Mucronate	
52	Pediceal length (P.L2)	Basifixed (without pedicel)	1-3 mm	
53	Pediceal indumentum (P.I1)	Tomentose	Glaber	
54	Rachis length (R.L.)	Less than 5 cm	6-10cm	
55	Rachis indumentum (R.I.)	Tomentose	Glaber	
56	Mature legume shape (M.L.S.)	Spherical	Clustered	
57	Mature legume density (M.L.D.)	Aggregated	Non-aggregated	
58	Vein indumentum (V.I.)	Tomentose	Glaber	
59	Petiole indumentum (P.I2)	Glandular tomentose	Tomentose	
60	Stem indumentum (S.I.)	Glandular tomentose	Tomentose	

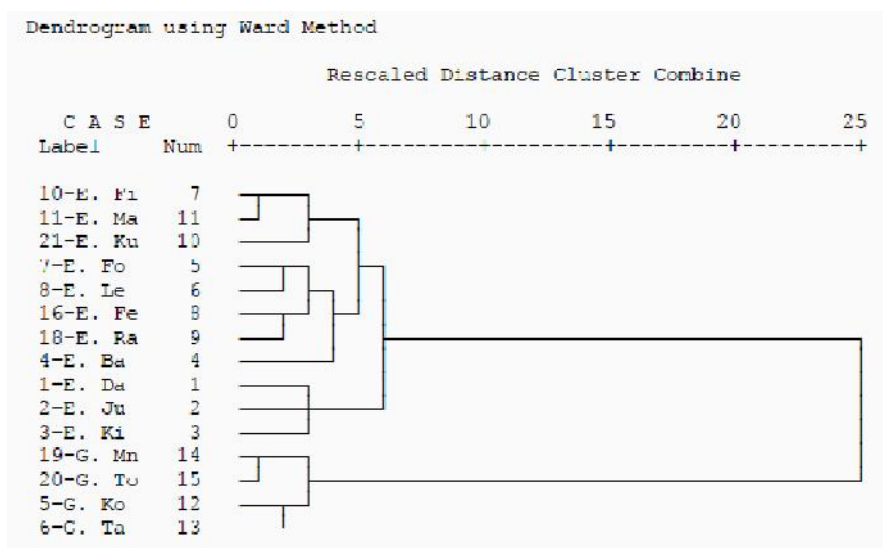


Fig. 2. The clustered analysis on morphological characters using WARD method.

- Classification according to PCA

In ordination analysis, based on the first two components (Fig. 3), the populations related to *G. glabra* are located at the beginning of the initial branch and *G. echinata* populations stand independently in the secondary branch. This indicates the proximity between the populations of each species and the enough distant for separation from other species in a genus that can be morphologically identified. Among them, the populations of Kordkooy (*G. Ko*) and Gorgan Agricultural Research Station (*G. Ta.*) are very close together and with a slight distance, the Manjil (*G. Mn.*) and Totekabon (*G. To.*) populations are close to each other too. *Glycyrrhiza glabra* populations have a remarkable distance from the studied populations of *G. echinata*.

Discussion

In the WARD cluster analysis, the populations of each taxon are located in a separate cluster and showed that the concept of their taxa and classification of them was accurate and the variations were observed in morphological characters in *Glycyrrhiza* specimens in beginning of this study cannot be considered as

change species or varieties borders. In other hand infra-specific variation in populations of the taxa confirmed because of position and their distance in cluster. By consider of geographical characteristic of populations and their position in each cluster indicated significantly aligned with the longitude, which can be attributed to the significant influence of climate conditions, including relative humidity from west to east on the variety of peculiarities in these taxa. Sharifi-Tehrani *et al.* (2012) also obtained similar results from cluster analysis of morphological data that can separate two varieties of *G. glabra* on their geographical location of populations.

Noticeable results of Hayashi *et al.* (2003) in the leaves of the morphologically intermediate-type plants of *G. uralensis* and *G. glabra*, collected in Kazakhstan, showed both specific component of these species and suggesting that the intermediate-type plant is a hybrid of *G. glabra* and *G. uralensis*. Therefore, closely correlation between morphological characteristic and active component of these medicinal species can be useful for evaluated analysis of constituent in pharmacological study.

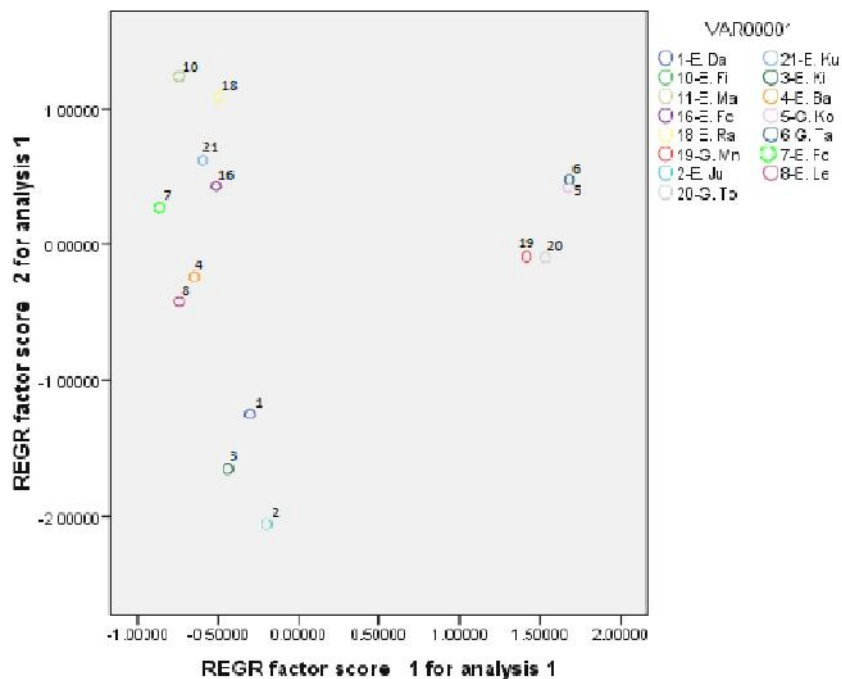


Fig. 3. Ordination of studied *Glycyrrhiza* populations using the first two factors of PCA analysis on morphological characters.

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